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# PRE-HARVEST APPLICATION OF SALICYLIC ACID MAINTAIN THE RIND TEXTURAL PROPERTIES AND REDUCE FRUIT ROT AND CHILLING INJURY OF SWEET ORANGE DURING COLD STORAGE

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Trees of citrus cvs 'Lane Late' and 'Valencia Late' oranges were sprayed ten days before anticipated harvest with salicylic acid (SA) at different concentrations (2, 3, 4, 6, 8, 9 mM). Fruits were harvested and stored at 5°C for 93 days to investigate the effects of SA on fruit rot, chilling injury and quality relating parameters such as rind and fruit firmness as well as sugar and organic acid contents. Fruits were analyzed before storage and after 31,62 and 93 days of storage and found that SA at 8 mM and 9 mM reduced fruit rot from 16.93% to 6.06% and 12.78% to 5.12% in 'Lane Late' and 'Valencia Late', respectively. Chilling injury was significantly reduced at 8mM and 9mM treatments. Textural properties relating to rind puncture, rind tensile and fruit firmness forces showed that treated fruits were significantly firmer than those of control. Maintained contents of SSC, TA, individual sugars and organic acids in treated fruit with higher doses after 93 confirmed preliminary findings such as SA has anti-senescent effect. Our research suggests that pre-harvest spray of SA can be used effectively to minimize the postharvest/storage losses of sweet oranges cultivars.
**Keywords:** Chilling injury, fruit rot, Lane Late, organic acid, sugar, Valencia late

## INTRODUCTION

Among various citrus species, sweet orange (*Citrus sinensis* Osbeck.) occupies prominent position in citrus cultivation of the world (FAOSTAT, 2011). Presently postharvest losses of sweet oranges have been found to be 40% of the total production (Ansari and Feridoon, 2008). Occurrence of postharvest chilling injury and decay has been fond as the main reason for these losses (Baldwin, 1993). Pre- and postharvest applications of fungicides are mostly used to protect citrus fruit against fungus and other different pathogens. However, the use of fungicide should be reduced because pathogens become resistant and residual effects of these fungicides in food and environment are harmful for human health. Therefore, it is very important to introduce new strategies to control the postharvest losses during prolonged cold storage.

Sweet oranges can be stored for two months at 4°C with very little loss of fruit quality (Davis and Albrigo, 1998). Citrus fruit undergo internal quality changes during long-term storage. These changes include increase in TSS:TA ratio, reduction in citric acids contents which directly affect the fruits quality as well as nutritional status of fruits. Some harmless compound such as salicylic acid (SA) has been reported to induce resistance against different abiotic stresses, infections and pathogen attack (Hayat, 2010). SA is simple phenolic compound which commonly produced in abundance amount by the plants (Abdelal, 1983). Some physiological and biochemical activities of plants are also regulated by SA (Arberg, (1981). It is also evidence that SA improved the antioxidant capacity of plants to regulate the plants growth in oxidative stress (Senaratna *et al*., 2000). Preharvest treatment of SA increased the endogenous polyamines in 'Ponkan' mandarin and reduced the degradation of antioxidants in 'Cara Cara' navel orange fruit (Zheng and Zhang, 2004; Huang *et al.*, 2008). Literature also describes the beneficial effects of SA treatment on different fruit due to ethylene inhibition. Zhang *et al*. (2003) reported that softening of kiwifruit was delayed by treatment of acetylsalicylic acid. Elwan and El-Hamahmy (2009) found that SA treatment ($10^{-6}$M) reduced the peroxidase and increased invertase activities of paper leaves and fruits. It is also well known that SA plays important role against fungi and diseases (Singh 1978: Rainsford, 1984; Li *et al*., 1998). Mycorrhizal fungi improve stress tolerance of orange (Zou *et al*., 2011; Wu and Zou, 2012). Walker (1988) recommended that foliar spray of 100 ppm phosphorus acid and 100 ppm acetyl salicylic acid is effective to control the phytophthora root-rot of citrus. Treatment of SA suppress the postharvest diseases in mango (Zainuri *et al.*, 2001), pear (Cao *et al.*, 2006) and sweet cherry (Chan and Tian, 2008).

Chilling injury (CI) of fruit also is an important postharvest storage disorder which occurs at low, non-freezing temperatures, and its severity is directly related to storage temperature and duration of exposure (Henriod *et al.*, 2005). The SA being as bioactive compounds is reported as safe for human health (Hooper and Cassidy, 2006). Recently it has been found that preharvest spray of SA reduce the CI in peaches and pomegranates (Wang *et al.*, 2006; Sayyari *et al.*, 2009). Sporadic information is available in the literature where efforts have been made to control the decay of stored fruit by the treatment of SA. However, very little is known about the effect of preharvest application of SA on 'Lane Late'(Ahmad, 2013) but information about response of cultivar especially 'Valencia Late' oranges relating to textural properties, fruit rot and chilling injury during cold storage are still lacking. Therefore, study was carried out to investigate the effects of preharvest spray SA on textural properties, fruit rot and chilling injury during cold storage of 'Lane Late' and 'Valencia Late' oranges.

## MATERIALS AND METHODS

***Plant material*:** Two separate experiments were carried out in commercial orchard located at Gingin, Western Australia (Latitude 31° 21'S; Longitude 155° 55'E) with wet winters and hot, dry summers climate. All the cultural practices including irrigation, fertilizer application and insect and weed control were kept uniform in both varieties.

**Experiment 1: Effect of preharvest application of SA on the shelf life and quality of 'Lane Late' sweet oranges**

Thirteen year old uniform 'Lane Late' oranges trees grafted on 'Troyer' rootstock planted at 7.50 m row to row and 2.70 m plant to plant distance with north-south row direction were selected for the experimental purposes. Single tree was considered as experimental unit replicated three times. SA at 2.0, 4.0, 6.0 or 8.0 mM containing 1% (v/v) ethanol was prepared and sprayed on the trees and control was sprayed with distal water only on 3rd October, 2009. All experimental trees were sprayed using (selecta Trolleypak Mk II, Victoria, Australia). In this experiment the nozale (Chierici Titisri, Rubiera Italy) with 250 KPa pressure was used. The experiment was laid out according to Randomized Complete Block Design (RCBD).

Ten fruit from each tree were harvested after ten days of spray and placed in separate bags and brought in the Horticulture Lab, School of Environment and Agriculture, Curtin University, WA, according to the treatments for analysis. Hundred fruit from each tree were also harvested and transferred in the Lab same way and stored at 5°C for 31, 62 and 93 days of storage. Ten fruit per replication were removed after 31, 62 and 93 days after fresh analysis (DAFA) and analysed as fresh analysis. Incidences of fruit rot and chilling injury were noted visually on daily bases. Following observations were recorded.

***Rind texture*:** Texture properties of rind such as rind hardness, tensile force and fruit firmness were determined using a texture analyser (TA Plus, AMETEK Lloyed Instruments Ltd Hampshire, UK) interfaced to a personal computer with Nexygen®software.

***Rind puncture test*:** A uniform piece of rind (2.5 cm wide and 0.6 cm thick) was removed from the every fruit with a Slicer (Zyliss Easy slic 2″ folding Mandolin slicer, Swiss) to determine the puncture test. Ten fruit each of replication were tested and average was calculated. The rind sample was placed on to the flat plate of texture analyser. A cylinder probe (4.00 mm diameter) was used with the speed of 50mm/min. Hardiness is the maximum force of the first penetration when the rind sample is contacted to the probe at 70% of the rind sample thickness.

***Rind tensile strength test*:** A rind sample section of 2.5 cm wide x 5.0 cm length x 0.6 cm thick was removed from each fruit using the slicer to give uniform sections. A sample was held using two clamps. One clamp was fixed to the base of the machine while another one was attached to the moveable load cell. The rind sample was subjected to axial tensile loading with rind deflection of 10.0 mm at the crosshead speed of 100 mm/min and preloads of 10N. The rind tensile strength force was calculated at the maximum load and limit points where the rind deflection occurred. Rind samples were collected from the ten fruits from the each replication and average was calculated and expressed as newton (N).

***Fruit compression test (fruit firmness)*:** The fruit with height of about 8.5 cm were used for each compression test. Each fruit was placed between two flat plates with the stem axis perpendicular to the plate. The crosshead speed was 200mm/min. This test was completed at strain of 25% of the fruit height. Ten fruit of each replication were used and average was calculated and expressed in newton (N).

***Soluble solids contents (SSC), titrable acidity (TA) and SCC:TA ratio*:** SSC of juice were determined using an infrared refractometer (Atago-Palette PR101, Atago Co. Ltd, Itabashi-Ku, Tokyo, Japan) at 20ºC and expressed as percentage. Fruit juice was titrated against 0.1 N NaOH solution using phenolphthalein as an indicator to pH 8.2. TA was determined as percent citric acid and SSC:TA ratio was calculated by dividing SSC with corresponding TA values.

***Individual organic acids and sugars*:** Organic acids and individual sugars were determined by following the method outlined by Singh *et al*. (2009)**.** Freshly extracted one mL juice was diluted with 19 mL mQ water. The diluted juice was centrifuged at 3000 × *g* for 10 min using a centrifuge (Eppendorf Centrifuge 5810R, Hamburg, Germany ). A part of supernatant was filtered through a 0.22 µm nylon True™ syringe filter [ Alltech Associates (Australia) Ltd., NSW, Australia] and the filtrate was collected in glass vial and used for high-performance liquid chromatography (HPLC) analysis. The concentrations of predominant organic acids mainly citric acid, malic acid, famaric acid and individual sugars were determined by using a high-performance liquid chromatography (HPLC) system (Waters, Milford, MA, USA). An aliquot (20 µL) of the extract was injected using an auto sampler (Waters 717 plus Milford, MA, USA) maintained at 25°C. Separation of sugars was performed isocratically with millipore water as a mobile phase flowing at 1 mL $min^{-1}$ using a fast carbohydrate, column, 100 x 7.8 mm i.d. (Bio Rad Laboratories, Hercules, CA, USA) maintained at 60°C and attached to a refractive index detector (Waters 2414, Milford, MA, USA). Organic acids were isocratically eluted Aminex 87 X–H column, 300 x 7.8 mm i.d. (Bio Rad Laboratories) at 45°C and were deducted by UV- absorbance detector (Waters 2487) at 214 nm. Mobile phase consisted of two solvents, 5 mM sulphuric acid and water, delivered in a 50:50 proportion at a flow rate of 0.3 mL $min^{-1}$. Chromatographic peaks were identified by comparing retention times with those of standards and by spiking samples with pure compounds while quantification was carried out using the external standard method. The data were collected and processed with Breeze® 3.30 software (Waters, Milford, MA, USA). The concentrations of different sugars (fructose, glucose, sucrose) and organic acids (citric and malic ) in the fruit were expressed as g 100 $mL^{-1}$ on fresh juice basis.

***Incidence of fruit rot and CI*:** Fruit rot and CI sheets were prepared according to treatments and replications. Fruit rot incidence was noted on daily bases and incidence of CI was noted weekly bases. Affected fruit were removed from the storage and noted on the sheets daily and weekly. Percentages were calculated on 31, 62 and 93 days of storage and presented in table for statistical analysis.

***Statistical data analysis*:** Statistical analysis was performed with Genstat release 11.1 (VSN International Ltd., Hemel Hempstead, UK). Least significant difference (Fisher's LSD) were calculated following significant F test ($P \leq 0.05$). Effects of different treatments on various parameters and their interactions were assessed with ANOVA. To ensure validity of statistical analysis all the assumptions of ANOVA were checked.

**Experiment 2: Effect of pre-harvest spray of SA on the shelf life and quality of 'Valencia Late' oranges**

Experiment 2 was conducted to reconfirm the results of experiment 1 and to investigate the influence of higher concentration on 'Valencia Late' oranges. Thirteen year old uniform 'Valencia late' oranges trees grafted on 'Troyer' root stock were selected. The plant to plant and line to line distance and layout design was the same as in experiment 1. SA at 0, 3.0, 6.0 and 9.0 mM containing 1% (v/v) ethanol was prepared and sprayed on the trees and control was sprayed with distal water only following the same method described earlier for experiment 1 on 3rd November, 2009. Fruit were harvested after ten days of spray. Storage method, and observations recorded were the same as described in experiment 1.

## RESULTS

**Texture profile analysis:**

***Rind puncture test*:** Concentration of SA, storage period and

**Table1. Effect of salicylic acid pre-harvest spray on the fruit peel strength properties of 'Lane Late' sweet orange**

| Storage period (days) | Rind puncture force (N ) | | | | | |
|---|---|---|---|---|---|---|
| | Salicylic acid (mM) | | | | | |
| | **0** | **2** | **4** | **6** | **8** | **Mean (T)** |
| 0 | 24.59 | 24.87 | 24.60 | 25.71 | 28.31 | 25.61 |
| 31 | 22.00 | 22.23 | 23.50 | 24.59 | 25.46 | 23.55 |
| 62 | 19.25 | 19.52 | 19.34 | 19.78 | 23.27 | 20.23 |
| 93 | 16.21 | 18.57 | 18.93 | 19.47 | 19.74 | 18.58 |
| Mean (SP) | 20.51 | 21.30 | 21.59 | 22.39 | 24.19 | |
| LSD $P \leq 0.05\%$ | T = 0.35, ST = 0.31, T x SP = 0.70 | | | | | |
| | **Rind tensile force (N)** | | | | | |
| 0 | 59.63 | 60.04 | 60.78 | 61.99 | 63.20 | 61.13 |
| 31 | 58.24 | 59.97 | 60.13 | 60.62 | 61.48 | 60.12 |
| 62 | 57.38 | 57.85 | 58.35 | 59.50 | 61.58 | 58.93 |
| 93 | 49.47 | 51.21 | 57.27 | 57.41 | 60.94 | 55.46 |
| Mean (SP) | 56.18 | 57.52 | 59.18 | 59.88 | 601.80 | |
| LSD $P \leq 0.05\%$ | T = 0.51, SP = 0.46, T x SP = 1.03 | | | | | |
| | **Fruit compression force (N)** | | | | | |
| 0 | 243.1 | 247.3 | 238.0 | 245.3 | 273.8 | 249.5 |
| 31 | 230.8 | 246.4 | 252.2 | 266.2 | 274.6 | 254.0 |
| 62 | 197.7 | 226.7 | 233.3 | 231.3 | 242.6 | 226.3 |
| 93 | 202.1 | 218.2 | 228.7 | 228.3 | 237.6 | 223.0 |
| Mean (SP) | 218.4 | 234.7 | 238.1 | 242.8 | 257.1 | |
| LSD $P \leq 0.05\%$ | T = 20.15, SP = 18.03, T x SP = NS | | | | | |

T = Concentrations of SA, SP = Storage period, n = 30 (10 fruit x 3 replicates)

their interaction showed significant ($P \leq 0.05$) results (Table 1). Pre-harvest spray of SA resulted in highest peel puncture strength (PPS) after 93 days of storage, while the lowest was noted in control. Higher concentrations exhibited less decrease than lower concentration. 'Valencia Late' oranges also showed similar response relating to rind puncture strength as 'Line Late' oranges. Treatments showed higher force to puncture the rind as compared to control but no significant difference was noted between the treatments (Table 2). A significant different was also found between the storage period as rind puncture strength decreased significantly after 31, 62 and 93 days after fresh analysis.

***Rind tensile test*:** Pre-harvest spray of SA significantly ($P \leq 0.05$) improved the retention of peel tensile strength (PTS) during 93 days of storage (Table 1). The increase in storage duration resulted in decline of PTS. At harvest, pre-harvest spray of SA at 8 mM resulted in highest PTS. Control fruit exhibited maximum decrease(16.21N) during the 93 days of storage, while pre-harvest spray of SA with 2 4 6 and 8 mM resulted in lower decreases regularly. Concentrations of SA and storage periods significantly influenced the rind tensile strength in 'Valencia Late' as in 'Lane Late' (Table 2). Similar trend was noted relating to treatment and storage

***Fruit compression force (Fruit firmness)*:** Pre-harvest spray exhibited significantly ($P \leq 0.05$) improved the retention of fruit firmness strength (FFT) than control (Table 1). A significant greater FFT was found at 8 mM than 2 mM concentration. The increase in storage period resulted decline in FFT. Fruit compression force significantly influenced by the concentration and storage periods (Table 2), but interaction was non-significant. The treatments and fresh fruit analysis showed significantly higher force values as compared to control and extended period of storage as in 'Lane Late'. Interaction showed that more force was required to compress the fruit at fresh storage (0) rather those which were analysed at 62 and 93 DAFA.

***SSC, TA and SSC:TA ratio*:** Concentrations (4, 6 and 8 mM) of SA significantly ($P \leq 0.05$) resulted in greater values of SSC% (13. 43)after 93 days of storage than control (12.68) (Table 3). Extended storage period declined the SSC from 13.92% to 13.15% after 93 days. TA% was non-significant (Table 2B). SSC: TA ratio (Table 3) after 93 days of storage was significantly lower than those which analysed fresh (FA) and 31days after storage. The SSC of 'Valencia Late' oranges influenced by the concentrations, storage period and relating to the interactions was the same as in 'Lane Late' (Table 4). However, the SSC was lower in 'Valencia Late' as compared to the 'Lane Late' oranges. TA was also influenced by treatments and storage periods (Table 4). Treatments (concentrations) influenced significantly SSC% but non-significant responses were recorded relating to TA% and SSC:TA ratio. Results relating

**Table 2. Effect of salicylic acid pre harvest spray on the peel and fruit strength properties of 'Valencia Late' sweet orange**

| Storage period (days) | Rind puncture force (N ) | | | | |
|---|---|---|---|---|---|
| | Salicylic acid (mM) | | | | |
| | **0** | **3** | **6** | **9** | **Mean (T)** |
| 0 | 43.12 | 43.55 | 44.23 | 43.63 | 43.63 |
| 31 | 33.51 | 33.93 | 36.88 | 37.10 | 35.35 |
| 62 | 36.80 | 33.42 | 34.33 | 36.49 | 35.26 |
| 93 | 30.37 | 32.23 | 32.59 | 35.72 | 32.98 |
| Mean (SP) | 36.20 | 35.78 | 37.01 | 38.23 | |
| LSD $P \leq 0.05\%$ | T = NS , SP = 2.13, T x SP = NS | | | | |
| | **Rind tensile force (N)** | | | | |
| 0 | 228.90 | 232.30 | 233.50 | 269.70 | 241.10 |
| 31 | 238.40 | 152.8 | 220.60 | 219.40 | 207.80 |
| 62 | 226.40 | 207.30 | 220.00 | 228.20 | 220.50 |
| 93 | 159.50 | 182.70 | 193.40 | 187.70 | 180.80 |
| Mean (SP) | 213.30 | 193.80 | 216.90 | 226.20 | |
| LSD $P \leq 0.05\%$ | T = 13.41 , SP = 13.41, T x SP = 26.81 | | | | |
| | **Fruit compression force (N)** | | | | |
| 0 | 228.90 | 232.30 | 233.50 | 269.70 | 241.10 |
| 31 | 238.40 | 152.8 | 220.60 | 219.40 | 207.80 |
| 62 | 226.40 | 207.30 | 220.00 | 228.20 | 220.50 |
| 93 | 159.50 | 182.70 | 193.40 | 187.70 | 180.80 |
| Mean(SP) | 213.30 | 193.80 | 216.90 | 226.20 | |
| LSD $P \leq 0.05\%$ | T = 13.41, SP = 13.41, T x SP = 26.81 | | | | |

T= Concentrations of SA, SP = Storage period, n= 30 (10 fruit x 3 replicates).

**Table 3. Effect of salicylic acid pre harvest spray on SSC, TA and SSC:TA ratio of 'Lane late' sweet orange fruit**

| Storage period (days) | SSC (%) | | | | | |
|---|---|---|---|---|---|---|
| | Salicylic acid (mM) | | | | | |
| | **0** | **2** | **4** | **6** | **8** | **Mean (T)** |
| 0 | 13.42 | 13.33 | 13.95 | 14.31 | 14.58 | 13.92 |
| 31 | 13.63 | 13.75 | 13.68 | 13.73 | 14.00 | 13.76 |
| 62 | 12.96 | 13.60 | 13.63 | 13.77 | 13.96 | 13.58 |
| 93 | 12.99 | 12.68 | 13.26 | 13.40 | 13.43 | 13.15 |
| Mean (SP) | 13.25 | 13.34 | 13.63 | 13.80 | 13.99 | |
| LSD *P* ≤ 0.05% | T = 0.24 , SP = 0.22, T x SP = NS | | | | | |
| | **TA (%)** | | | | | |
| 0 | 1.25 | 0.92 | 0.91 | 0.92 | 0.94 | 0.99 |
| 31 | 0.85 | 0.92 | 0.91 | 0.90 | 1.00 | 0.92 |
| 62 | 0.94 | 0.92 | 0.96 | 0.93 | 0.99 | 0.95 |
| 93 | 0.95 | 0.95 | 0.96 | 0.97 | 0.96 | 0.96 |
| Mean (SP) | 1.00 | 0.93 | 0.94 | 0.93 | 0.97 | |
| LSD *P* ≤ 0.05% | T = NS , SP = NS, T x SP = NS | | | | | |
| | **SSC: TA ratio** | | | | | |
| 0 | 12.08 | 14.39 | 15.28 | 15.50 | 15.52 | 14.55 |
| 31 | 15.91 | 14.84 | 15.04 | 15.15 | 13.91 | 15.97 |
| 62 | 13.74 | 14.73 | 14.16 | 14.71 | 14.10 | 14.29 |
| 93 | 13.68 | 14.35 | 13.82 | 13.72 | 14.02 | 13.72 |
| Mean (SP) | 13.85 | 14.33 | 14.57 | 14.77 | 14.39 | |
| LSD *P* ≤ 0.05% | T = NS , SP = 0.79, T x SP = NS | | | | | |

T= Concentrations of SA, SP = Storage period, n= 30 (10 fruit x 3 replicates)

**Table 4. Effect of salicylic acid pre harvest spray on SSC, TA and SSC:TA ratio the fruit peel hardness of 'Valencia Late' sweet orange**

| Storage period (days) | SSC (%) | | | | |
|---|---|---|---|---|---|
| | Salicylic acid (mM) | | | | |
| | **0** | **3** | **6** | **9** | **Mean (T)** |
| 0 | 14.27 | 12.18 | 12.71 | 13.21 | 13.09 |
| 31 | 10.20 | 10.28 | 10.88 | 9.34 | 10.17 |
| 62 | 10.23 | 10.28 | 11.01 | 9.54 | 10.26 |
| 93 | 10.65 | 11.28 | 12.02 | 1062 | 11.14 |
| Mean (SP) | 11.33 | 11.00 | 11.66 | 10.68 | |
| LSD *P* ≤ 0.05% | T = 0.54 , SP = 0.54, T x SP = 1.09 | | | | |
| | **TA (%)** | | | | |
| 0 | 0.83 | 1.00 | 0.96 | 0.92 | 0.93 |
| 31 | 1.05 | 1.15 | 1.07 | 1.21 | 1.12 |
| 62 | 1.12 | 1.20 | 1.07 | 1.19 | 1.14 |
| 93 | 1.09 | 1.08 | 1.00 | 1.11 | 1.07 |
| Mean (SP) | 1.028 | 1.112 | 1.028 | 1.112 | |
| LSD *P* ≤ 0.05% | T = 0.049 , SP = 0.047, T x SP = 0.099 | | | | |
| | **SSC: TA ratio** | | | | |
| 0 | 0.83 | 1.00 | 0.96 | 0.92 | 0.93 |
| 31 | 1.05 | 1.15 | 1.07 | 1.21 | 1.12 |
| 62 | 1.12 | 1.20 | 1.07 | 1.19 | 1.14 |
| 93 | 1.09 | 1.08 | 1.00 | 1.11 | 1.07 |
| Mean (SP) | 1.028 | 1.112 | 1.028 | 1.112 | |
| LSD *P* ≤ 0.05% | T = 0.047 , SP = 0.048, T x SP = 0.098 | | | | |

T= Concentrations of SA, SP = storage period, n = 30 (10 fruit x 3 replicates) to SSC:TA ratio significant in treatments, storage periods and as well as in the interactions between both (Table 4). Increased storage of 93 days resulted significant decline in SSC: TA ratio (1.07) at 93 DAFA.

***Individual sugars*:** Glucose, fructose and sucrose contents significantly influenced by concentrations of SA, storage periods and their interactions at $P \leq 0.05\%$ (Table 5). Pre-harvest spray of SA at 6 and 8 mM significantly ($P \leq 0.05$) improved the glucose contents during 93 days of storage as compared to lower concentration and control. A significant difference was also found in storage period such as fresh fruit showed minimum (1.86%) of glucose where as higher levels (2.33% and 2.32%) were recorded in fruit which were analysed after 62 and 93 DAFA, respectively. Analysis at 0 days (fresh) showed more increase after 31 days than 62 and 93 days after storage. Fructose also showed statistically increasing trend relating to higher concentration and extending storage periods (Table 5), but no interaction was found between both. Sucrose contents were significantly influenced by the concentration and storage periods, but non-significant results were recorded relating to their interaction (Table 5). Control fruit showed significantly less sucrose as compared to treatments. Non-significant difference was found between the treatments but a increasing trend was observed relating to higher doses. Storage period also reduced the sucrose contents in fruit of 62 and 93 days after storage. Glucose, fructose and sucrose contents were also significantly influenced by pre-harvest spray of SA and storage periods in 'Valence Late' (Table 6). Concentrations and storage periods showed similar increasing trend as in 'Lane Late' oranges.

***Organic acids*:** Pre-harvest spray of SA significant *($P \leq 0.05$)* increased the citric acid than control. (Table 7). No difference was found relating to storage period and interaction. Statistically no difference was found in malic acids. Citric acid and malic acid was also influenced by the treatments, storage periods and with interaction of both (Table 7 and 8) but the trend was similar like 'Lane Late' oranges. Comparison of both cultivars showed that 'Valencia Late' had more citric contents as compared to 'Lane Late' oranges. No changes were observed in fumaric acid contents.

***Fruit rot and CI during storage*:** Fruit rot percentage was also significantly influenced by the concentration, storage periods and their interactions at $P \leq 0.05\%$ (Table 9). No significant difference was found between last three higher doses (4, 6 and 8 mM) but significantly less fruit rot percentages (1.72, 1.72 and 1.51, respectively) were noted in these concentration as compared to control (4.23%) and 2 mM dose (2.17%) in total three months. Interaction showed that higher differences were found between the fruit rot percentage in fruit of 62 DAFA and 93 DAFA at control and lowest dose. Fruit CI also showed the same trend as in fruit rot percentages (Table 9).

**Table 5. Effect of salicylic acid pre harvest spray on individual sugars in 'Lane Late' sweet orange fruit juice**

| Storage period (days) | Glucose (g 100 mL$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Salicylic acid (mM) | | | | | |
| | **0** | **2** | **4** | **6** | **8** | **Mean (T)** |
| 0 | 1.93 | 1.43 | 1.93 | 1.99 | 2.05 | 1.86 |
| 31 | 2.18 | 2.14 | 2.14 | 2.27 | 2.44 | 2.23 |
| 62 | 2.24 | 2.23 | 2.39 | 2.35 | 2.44 | 2.33 |
| 93 | 2.22 | 2.23 | 2.39 | 2.35 | 2.44 | 2.32 |
| Mean (SP) | 2.14 | 2.00 | 2.21 | 2.24 | 2.34 | |
| LSD $P \leq 0.05\%$ | T = 0.09 , SP = 0.08, T x SP = 0.19 | | | | | |
| | **Fructose (g 100 mL$^{-1}$)** | | | | | |
| 0 | 1.98 | 1.97 | 1.54 | 1.98 | 2.08 | 1.91 |
| 31 | 2.08 | 2.05 | 2.04 | 2.09 | 2.91 | 2.11 |
| 62 | 2.35 | 2.36 | 2.04 | 2.28 | 2.57 | 2.32 |
| 93 | 2.06 | 2.18 | 2.35 | 2.46 | 2.57 | 2.33 |
| Mean (SP) | 2.12 | 2.14 | 1.99 | 2.20 | 2.38 | |
| LSD $P \leq 0.05\%$ | T = 0.24 , SP = 0.22, T x SP = NS | | | | | |
| | **Sucrose (g 100 mL$^{-1}$)** | | | | | |
| 0 | 7.44 | 7.63 | 7.18 | 7.39 | 7.67 | 7.46 |
| 31 | 6.36 | 7.23 | 7.32 | 7.52 | 7.54 | 7.20 |
| 62 | 6.62 | 7.13 | 7.34 | 7.20 | 7.15 | 7.09 |
| 93 | 6.47 | 6.92 | 7.19 | 7.20 | 7.30 | 7.02 |
| Mean (SP) | 6.72 | 7.23 | 7.26 | 7.33 | 7.42 | |
| LSD $P \leq 0.05\%$ | T = 0.31 , SP = 0.28, T x SP = NS | | | | | |

T= Concentrations of SA, SP = storage period, n = 30 (10 fruit x 3 replicates)

**Table 6. Effect of salicylic acid pre harvest spray on individual sugars of 'Valencia Late' sweet orange**

| Storage period (days) | Glucose (g 100 mL$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | Salicylic acid (mM) | | | | |
| | **0** | **3** | **6** | **9** | **Mean (T)** |
| 0 | 1.76 | 2.01 | 2.72 | 2.72 | 2.30 |
| 31 | 2.03 | 2.60 | 2.60 | 2.49 | 2.43 |
| 62 | 2.13 | 2.66 | 2.51 | 2.60 | 2.47 |
| 93 | 2.67 | 2.78 | 2.74 | 2.88 | 2.77 |
| Mean (SP) | 2.15 | 2.51 | 2.64 | 2.67 | |
| LSD $P \leq 0.05\%$ | T = 0.22, SP = 0.22, T x SP = NS | | | | |
| | **Fructose (g 100 mL$^{-1}$)** | | | | |
| 0 | 2.00 | 1.99 | 2.09 | 2.01 | 2.02 |
| 31 | 1.81 | 1.99 | 2.16 | 2.15 | 2.03 |
| 62 | 1.81 | 1.87 | 2.14 | 2.12 | 1.98 |
| 93 | 1.65 | 1.79 | 2.13 | 2.14 | 1.93 |
| Mean (SP) | 1.82 | 1.91 | 2.13 | 2.10 | |
| LSD $P \leq 0.05\%$ | T = 0.064, SP = 0.064, T x SP = 0.129 | | | | |
| | **Sucrose (g 100 mL$^{-1}$)** | | | | |
| 0 | 6.54 | 6.79 | 6.49 | 6.77 | 6.65 |
| 31 | 5.52 | 6.29 | 6.45 | 6.55 | 6.20 |
| 62 | 5.91 | 6.05 | 6.15 | 6.20 | 6.08 |
| 93 | 5.74 | 6.00 | 6.26 | 6.15 | 6.04 |
| Mean (SP) | 5.93 | 6.34 | 6.41 | 5.93 | |
| LSD $P \leq 0.05\%$ | T = 0.25, SP = 0.25, T x SP = NS | | | | |

T = Concentrations of SA, SP = storage period, n = 30 (10 fruit x 3 replicates)

**Table 7. Effect of salicylic acid pre harvest spray on organic acid contents in 'Lane Late' sweet orange fruit juice**

| Storage period (days) | Citric acid (g 100 mL$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Salicylic acid (mM) | | | | | |
| | **0** | **2** | **4** | **6** | **8** | **Mean (T)** |
| 0 | 0.17 | 0.20 | 0.18 | 0.18 | 0.18 | 0.18 |
| 31 | 0.15 | 0.19 | 0.19 | 0.17 | 0.17 | 0.17 |
| 62 | 0.17 | 0.17 | 0.19 | 0.17 | 0.17 | 0.18 |
| 93 | 0.16 | 0.18 | 0.16 | 0.18 | 0.18 | 0.17 |
| Mean (SP) | 0.16 | 0.18 | 0.18 | 0.18 | 0.17 | |
| LSD $P \leq 0.05\%$ | T = 0.007 , SP = 0.006, T x SP = 0.015 | | | | | |
| | **Malic acid (g 100 mL$^{-1}$)** | | | | | |
| 0 | 0.53 | 0.04 | 0.05 | 0.06 | 0.06 | 0.05 |
| 31 | 0.57 | 0.05 | 0.06 | 0.06 | 0.06 | 0.06 |
| 62 | 0.06 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| 93 | 0.03 | 0.04 | 0.03 | 0.04 | 0.05 | 0.04 |
| Mean (SP) | 0.05 | 0.04 | 0.05 | 0.05 | 0.05 | |
| LSD $P \leq 0.05\%$ | T = NS , SP = NS, T x SP = NS | | | | | |

T = Concentrations of SA, SP = storage period, n = 30 (10 fruit x 3 replicates)

**Table 8. Effect of salicylic acid pre harvest spray on organic acids of 'Valencia Late' sweet orange**

| Storage period (days) | Citric acid (g 100 mL$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | Salicylic acid (mM) | | | | |
| | **0** | **3** | **6** | **9** | **Mean (T)** |
| 0 | 0.55 | 0.65 | 0.64 | 0.70 | 0.63 |
| 31 | 0.92 | 0.84 | 0.86 | 0.92 | 0.89 |
| 62 | 0.61 | 0.64 | 0.64 | 0.64 | 0.63 |
| 93 | 0.99 | 0.75 | 0.68 | 0.70 | 0.78 |
| Mean (SP) | 0.77 | 0.72 | 0.70 | 0.74 | |
| LSD $P \leq 0.05\%$ | T = 0.047 , SP = 0.047, T x SP = 0.095 | | | | |
| | **Malic acid (g 100 mL$^{-1}$)** | | | | |
| 0 | 0.33 | 0.21 | 0.35 | 0.26 | 0.29 |
| 31 | 0.17 | 0.19 | 0.16 | 0.17 | 0.17 |
| 62 | 0.23 | 0.16 | 0.16 | 0.17 | 0.18 |
| 93 | 0.28 | 0.25 | 0.17 | 0.19 | 0.22 |
| Mean (SP) | 0.25 | 0.20 | 0.21 | 0.20 | |
| LSD $P \leq 0.05\%$ | T = 0.03 , SP = 0.03, T x SP = 0.06 | | | | |

T = Concentrations of SA, SP = storage period, n = 30 (10 fruit x 3 replicates)

**Table 9. Effect of salicylic acid pre harvest spray on fruit rot and chilling injury of 'Lane Late' sweet orange in three month storage**

| Storage period (days) | Fruit rot (%) | | | | | |
|---|---|---|---|---|---|---|
| | Salicylic acid (mM) | | | | | |
| | **0** | **2** | **4** | **6** | **8** | **Mean (T)** |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 31 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 62 | 2.18 | 0.88 | 0.85 | 0.70 | 0.71 | 1.06 |
| 93 | 14.75 | 7.80 | 6.03 | 6.16 | 5.35 | 8.02 |
| Mean (SP) | 4.23 | 2.17 | 1.72 | 1.72 | 1.51 | |
| LSD $P \leq 0.05\%$ | T = 1.02 , SP = 0.91, T x SP = 2.04 | | | | | |
| | **Chilling injury (%)** | | | | | |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 31 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 62 | 0.55 | 0.00 | 0.00 | 0.00 | 0.00 | 0.11 |
| 93 | 4.63 | 1.56 | 1.86 | 1.60 | 1.25 | 2.18 |
| Mean (SP) | 1.30 | 0.39 | 0.47 | 0.40 | 0.31 | |
| LSD $P \leq 0.05\%$ | T = 0.29 , SP = 0.26, T x SP = 0.58 | | | | | |

T = Concentrations of SA, SP = storage period, n = 30 (10 fruit x 3 replicates)

**Table 10. Effect of salicylic acid pre harvest spray on fruit rot and chilling injury the of 'Valencia Late' sweet orange**

| Storage period (days) | Fruit rot (%) | | | | |
|---|---|---|---|---|---|
| | Salicylic acid (mM) | | | | |
| | **0** | **3** | **6** | **9** | **Mean(T)** |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 31 | 1.11 | 0.56 | 0.00 | 0.00 | 0.42 |
| 62 | 1.67 | 1.67 | 2.22 | 0.56 | 1.53 |
| 93 | 11.09 | 5.11 | 5.69 | 4.56 | 6.61 |
| Mean (SP) | 3.47 | 1.83 | 1.98 | 1.28 | |
| LSD $P \leq 0.05\%$ | T = 0.74 , SP = 0.74 T x SP = 1.49 | | | | |
| | **Chilling injury (%)** | | | | |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 31 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 62 | 5.00 | 0.00 | 0.00 | 0.00 | 1.25 |
| 93 | 6.35 | 4.33 | 3.33 | 2.85 | 4.22 |
| Mean (SP) | 2.84 | 1.08 | 0.83 | 0.71 | |
| LSD $P \leq 0.05\%$ | T = 1.51 SP = 1.51, T x SP = NS | | | | |

T = Concentrations of SA, SP = storage period, n = 30 (10 fruit x 3 replicates)

Fruit rot and CI were reduced in higher doses treatments and increased in extended storage periods such as 93 DAFA as in 'Lane Late' oranges (Table 10). However, less fruit rot % and more fruit CI were noted in 'Valencia Late' oranges at end of storage (93 DAFA).

## DISCUSSION

Higher values of rind puncture strength, rind tensile strength and fruit compression strength in higher doses and in treated fruits indicated that these fruit were firmer than those of non-treated and those which were treated with lower doses. It is because the softening of the citrus fruit occurs due to the changes in turgor pressure and development of senescence (Baldwin, 1993). The softening of citrus fruit could be due to water loss after harvest, aging of the fruit and increased levels of malonic acid (Monselise, 1977). It indicated that fruit without treatments of SA were more close to aging or scenes than those which were treated with higher concentrations of SA. Similar results were found in kiwifruit by Zhang *et al.* (2003) where, the application of acetylsalicylic acid reduced the softening process of kiwifruit by maintaining higher endogenous SA levels. Overall this characteristic is beneficial for citrus industry because firmer fruit is less susceptible to mechanical injury during transportation and storage.

The lower SSC values relating to extended storage period indicated that fruit was already matured because the commercial harvest date is July-August in Australia but these were harvested in October and November then stored in cold temperature. It means that senesce relating processes may be started because during senesces or fomentation consume the total soluble solids in oranges (Supraditareporn and Pinthong, 2007). Higher SSC contents TA contents in higher concentration of SA than lower concentrations and fresh in cv. 'Lane Late' and 'Valencia Late' indicated that higher doses of SA worked as anti-senescent agent and maintained SSC and TA contents. It could not perform clear picture but proved superiority of higher doses to reduce the senescence process. The significantly higher SSC in treated fruit of both cultivars than control support the finding of Elwan and El-Hamahmy (2009) where, contents of sugar were the lowest in leaves and highest in fruit when paper plants were sprayed with salicylic acid (SA). It may be ascribed that salicylic acid had crucial role in the energy status of the plant, translocation and storage of assimilation. In present study we did not measure the SSC from the leaves but we recommend measuring SSC from leaves to more conformation.

The significantly higher values of glucose, fructose and sucrose in treated fruit (especially in higher doses) in both cultivars indicated the influence of SA regarding the individual sugars. It is because the spray of SA regulated the carbohydrate metabolism in both source and sink tissues of citrus plants. Ehness and Roitsch (1997) reported that carbohydrate metabolism in both source and sink tissue of plants is regulated by plant hormones those increased the invertase activity. The hydrolysis of sucrose by invertase regulates the levels of some plant hormones like indole-3-acetic acid, salicylic acid and jasmonic acid (LeClere *et al.,* 2003). This information conform the relationship between SA and invertase activity. It can be concluded that higher individual sugars in treated fruit was may be due to the translocation of more photo assimilates to fruit. No reference was found in citrus to confirm the present results but the more sugars in agronomic crops such as wheat and maize by the spray of SA are reported by Arfan *et al.* (2007) and Gunes *et al*. (2007). Higher citric acid in lane late treated fruit have the same explanation as in TA because SA treatment showed non senescent function. The results of citric acid in Valencia were haphazard which indicated that SA could not maintain its superiority due to some unknown reasons.

Decay or fruit rot in storage is responsible for most of the postharvest losses. SA with the concentration of 8 mM showed minimum fruit rot of 1.52 and 1.28% in cv 'Lane Late' and 'Valencia Late' respectively as compared to control where the fruit rots were 4.23% and 3.47%, respectively. This showed the superiority of SA against fungus attack. The pre-harvest spray of SA provide the tolerance to fruit against pathogen or fungi is well known (Singh 1978; Rainsford, 1984; Walker, 1988; Li *et al.*, 1998:). In present study the fruit treated with SA were firmer as measured by different textural properties such as rind puncture, tensile and fruit firmness of control fruit was more susceptible to fungus and disease pathogens. Similar results were also reported on different fruit crops such as pre-harvest treatment of SA inhibited the infection of several postharvset fugal diseases in melon fruit (Huang *et al.,* 2000) and suppressed postharvest anthracnose in mango fruit (Zainuri *et al*., 2001).

The reduction of chilling injury from 1.3% to 0.31 % and 2.84% to 0.71 % in 'Lane Late' and 'Valencia Late' indicated that pre-harvest pray of SA may have developed some defence system in oranges peel against the CI. It is because; application of exogenous SA could significantly induce resistance against a variety of biotic and abiotic stresses (Dat *et al.,* 1998; Kang *et al*., 2003; Chan and Tian, 2006). Similar results to present study were also found in pomegranate where preharvest spray of 2 mM SA reduced the CI effectively (Sayyar, 2009). The use of fungicide should be discouraged because they have some side effects. Fruit are treated with fungicide to minimize the losses by fungus attach but fungicide leave their toxic effects or produce toxic component in produce. These products become harmful instead of curing or improving the health. Therefore, it is need of the time to find out some alternate which could be harmless for human health and protect the crop against the pathogen, So, the use of salicylic acid could be achieved the goal.

***Conclusions*:** Sweet orange cultivars (Lane Late and Valencia Late) showed similar response to Pre-harvest spray of SA and doses of 8 mM and 9mM are beneficial to minimize the fruit rot, CI and to maintains the quality of fruit for 93 days at 5°C.

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