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Use of salicylic acid for alleviation of chilling injury and quality assurance of guava fruits during storage

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ABSTRACT

The efficacy of salicylic acid in alleviating chilling injury and its role in maintaining the quality of guava fruit was studied. Fruits of guava cv. Allahabad Safeda were subjected to salicylic acid (SA) and 5-sulfosalicylic acid (SSA) treatments @ 1 mM and 2 mM and stored at 5°C for 12 days. Post-cold storage, fruits were transferred to supermarket conditions for a three-day shelf life simulation period. Observations were taken at 3+3 days (3 days at 5°C followed by three days at supermarket conditions) up to 12+3 days. Our results revealed that fruits treated with SSA or SA @ two mM showed significantly low weight loss, chilling injury, malondialdehyde content, and increased phenylalanine ammonia-lyase activity. The treatments also delayed the yellowing of fruit skin and respiratory peak. In addition, SSA and SA treatments helped maintain approximately 40% higher firmness than control fruits. SSA and SSA treatment also retarded the loss in total antioxidant capacity and maintained the fruit quality during storage.

Keywords: Guava, salicylic acid, sulfosalicylic acid, chilling injury, firmness

INTRODUCTION

Guava (*Psidium guajava* L.) is an important fruit crop widely cultivated in tropical and sub-tropical regions worldwide. It is considered as 'poor man's apple' or 'the apple of tropics' due to its low cost of production, high nutritional value and availability at cheap rates throughout the year. High rate of ripening is manifested by a very short shelf life of around 3 days under ambient conditions (Madhav *et al.*, 13) with high disease incidence. Under low temperature storage, it is susceptible to chilling injury manifested by surface pitting, skin and flesh browning (Gonz´alez-Aguilar *et al.*, 9). Studies are available on use of modified atmospheres (Antala *et al.*, 2), edible coatings (Madhav *et al.*, 13) and fungicide application to extend the storage life of guava. Salicylic acid is an endogenous hormone that can be alternatively applied exogenously on fruits reduce lipid peroxidation (Huang *et al.*, 10) and chilling injury and enhanced shelf life (Barman and Asrey, 5). The present investigation was conducted to determine the effect of postharvest application of salicylic acid and its derivative, 5-sulfosalicylic acid (SSA) in alleviating chilling injury and maintaining guava fruit cv. Allahabad Safeda quality during low temperature storage.

MATERIALS AND METHODS

Uniform sized sound fruits of guava cv. Allahabad Safeda were harvested at mature green stage from the orchard located at Indian Agricultural Research Institute (IARI), New Delhi. The harvested fruits were randomly divided into 5 groups, each group containing 210 fruits, and immersed into solutions of salicylic acid (1 and 2 mM), 5-sulfosalicylic acid (1 and 2 mM) and distilled water for 5 min. Three replicates of 70 fruits per replicate were used for each treatment. Fruits were air-dried at room temperature (20±2°C) and stored at 5°C under 80-90% RH. After an interval of every 3 days of cold storage, fruits were transferred to supermarket conditions (20±2°C) for 3 days before conducting quality analysis.

To determine weight loss the fruit during storage, both treated and untreated (control) fruits were weighed at different sampling intervals. The percent difference between fruit weight at commencement of experiment and fruit weight at the time of measurement was taken as the weight loss. The firmness (N) of fruit was measured using a Stable Microsystems (UK) texture analyzer with a 6 mm probe up to a depth of 5 mm at a crosshead speed of 50 mm min^{-1}. Three fruits per replication were subjected to firmness test with each fruit punctured on either side.

A five point hedonic scale was used to calculate the chilling injury (CI) index on basis of the necrosis level of the fruit tissue and presence of black spots on the fruit skin (Gonzalez-Aguilar *et al.*, 9). The scale used was: 1, 0–20% of damaged area in the fruit; 2, 20–40% of damaged area in the fruit; 3, 40–60% of damaged area in the fruit; 4, 60–80% of damaged area in the fruit; and 5, ≥80% damaged area. The

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chilling injury index was calculated by multiplying the number of fruits scoring similar score of the hedonic scale with the corresponding scale number and dividing the resultant by the total number of fruits. Hunter Colour Lab Scan XE was used to determine guava fruit peel color by measuring the hue angle using a* and b* colour coordinates. Auto gas analyzer (Checkmate 9900, Dansensor, Denmark) was used to measure the gas in the headspace and calculate respiration rate (mL CO_2 kg^{-1} h^{-1}).

Total antioxidant (AOX) capacity in terms of cupric reducing antioxidant capacity method was determined (Apak *et al.*, 3) and expressed as μmol Trolox g^{-1} FW. Phenylalanine ammonia-lyase (PAL) activity (μmol cinnamic acid h^{-1} g^{-1} FW) was determined by the method of Khan and Vaidyanathan (11) while malondialdehyde (MDA) content was measured according to Kumar *et al.* (12) and expressed as μmol^{-1} FW.

Control and treated fruits were subjected to sensory evaluation by a panel of 15 semi-trained judges during storage for appearance, colour, texture, odour, taste and overall acceptability at each interval on a 9 point hedonic scale (1: dislike extremely; 5: neither like nor dislike; and 9: like extremely) (Amerine *et al*., 1)

Data were analyzed by two way analysis of variance (ANOVA) using SAS 9.3. Further, a multiple comparison was done by using Tukey's HSD test. All other statistical analysis was done using MS Excel 2007. Replicated data obtained for different treatments were subjected to statistical analysis and the treatment means along with letter grouping based on Tukey's HSD test was also worked out and is given in different tables for various parameters.

RESULTS AND DISCUSSION

A progressive loss in weight of the guava fruits was observed during storage irrespective of the treatment applied (Table1) with significantly higher ($p \leq 0.05$) loss noted in control fruits (13.9 ± 0.29 %) as compared to treated fruits. However, higher concentration of salicylic acid provided better control of weight loss during storage. Treatment with SSA at 2 mM concentration (6.21 ± 0.37 %) resulted in least changes in fruit weight till the end of storage and the fruits remained marketable. Reduction in transpiration losses and control of the respiration process as a consequence of salicylic acid application maybe the reason behind such a trend. Previously, Madhav *et al.* (14) have also reported reduced weight loss in salicylic acid treated guava fruits. Also, as per earlier reports, chilling injury is one of the causes of weight loss (Razavi *et al.*, 15). In our experiments, the results, as discussed later, show that there was lower incidence of chilling injury in treated guava fruits that further contributes to reduced weight loss.

Firmness, one of the most crucial of all quality attributes of a fruit, directly affects their shelf life and acceptability. In the present study, firmness of guava fruits reduced gradually with the progress in storage period for all the treatments (Table 1). A significant ($p \leq 0.05$) reduction in firmness was recorded for untreated fruits at the end of the storage (1.33±0.67 N). Fruits treated with SSA or SA at higher concentration (2 mM) retained 40.84% and 39.89% higher firmness, respectively, when compared to the control fruits after 12+3 days of storage. This may be as a result of better efficacy of salicylic acid at higher concentration towards decreasing the ethylene production and also retarding the activity of polygalactouronases (PG), pectin methyl esterases (PME), lipoxygenases that are enzymes responsible for cell degradation. Earlier study conducted by Madhav *et al.* (14) have also shown similar results in guava fruits.

CI in guava fruits is mainly manifested by surface pitting of the affected area that increases with the progression of storage (Singh and Pal., 16). Postharvest application of salicylic acid to Allahabad Safeda fruits given before storage significantly reduced the incidence of chilling injury. The untreated fruits showed CI symptoms just after 3+3 days of storage. In contrast, fruits treated with SA or SSA at 2 mM concentration showed CI symptoms after 12+3 days of storage while fruits treated with SA or SSA at 1 mM concentration showed CI symptoms after 9+3 days of the storage (Table 1). Exposure of fruits to chilling temperature has been reported to induce the generation of reactive oxygen species that causes oxidative stress and eventually CI to the fruits (Barman and Asrey, 5**).** The exogenous application of SA causes an induction of reactive oxygen species (ROS) avoidance genes and ROS scavenging genes (Asghari and Aghdam, 4) thereby suppressing the chilling injury. Further, the action of defence enzymes also increases with a reduction in lipid peroxidation upon SA application thus, preventing chilling injury. Our results are in agreement with previous work done by Barman and Asrey. (5).

Hue angle represents the predominant colour of the fruits that represents green at 120° and yellow at 90°. Guava developed yellow hue as the fruit ripen. The change in the hue angle of the skin of guava fruits during our experiment is shown in Table 1. Fruits treated with 2 mM concentration either of SA or SSA did not turn completely yellow till 12+3 days of storage as observed by the minimal change in the hue angle whereas, control fruits turned completely yellow after 9+3 days of storage. Thus, application of salicylic acid to harvested guava fruits delayed their

Table 1. Effect of salicylic acid treatments on weight loss, firmness, chilling injury index and hue in guava fruits stored at 5°C followed by supermarket conditions for 3 days.

Treatment	Storage period (days)				
	0	3+3	6+3	9+3	12+3
Weight loss (%)					
SA @ 1 mM	0±0.00^{l}	4.53±0.61^{i}	6.86±0.45^{hgf}	8.74±0.64^{d}	10.22±0.58^{c}
SA @ 2 mM	0±0.00 ^{l}	2.19±0.38^{k}	4.23±0.36^{ji}	7.43±0.45^{egf}	8.18±0.65^{ed}
SSA @ 1 mM	0±0.00 ^{l}	3.48±0.29^{j}	4.59±0.59^{i}	6.66±0.95^{hg}	7.78±0.49^{def}
SSA @ 2 mM	0±0.00 ^{l}	2.35±0.32^{k}	3.48±0.45^{j}	4.16±0.59^{ji}	6.21±0.37^{h}
Control	0±0.00 ^{l}	7.86±0.71^{ed}	9.98±0.55^{c}	11.95±0.49^{b}	13.9±0.29^{a}
Firmness (N)					
SA @ 1 mM	10.12±0.58^{a}	7.16±0.49^{bc}	5.89±0.47^{dce}	2.78±0.45^{gf}	1.873±0.56^{g}
SA @ 2 mM	10.12±0.58^{a}	9.12±0.36^{a}	8.36±0.34^{ba}	6.28±0.62^{dc}	4.16±0.27^{fe}
SSA @ 1 mM	10.12±0.58^{a}	7.18±0.53^{bc}	5.91±0.29^{dce}	2.82±0.38^{gf}	1.89±0.75^{g}
SSA @ 2 mM	10.12±0.58^{a}	9.14±0.71^{a}	8.39±0.37^{ba}	6.32±0.71^{dc}	4.22±0.59^{fe}
Control	10.12±0.58^{a}	6.49±0.65^{dc}	5.08±0.35^{de}	2.43±0.39^{gf}	1.33±0.67^{g}
Chilling injury index					
SA @ 1 mM	0±0.00 ^{g}	0±0.00 ^{g}	0±0.00 ^{g}	2±0.00 ^{dc}	2.5±0.16^{c}
SA @ 2 mM	0±0.00 ^{g}	0±0.00 ^{g}	0±0.00 ^{g}	0±0.00 ^{g}	1±0.00^{fe}
SSA @ 1 mM	0±0.00 ^{g}	0±0.00 ^{g}	0±0.00 ^{g}	1.5±0.16^{fe}	2±0.16^{c}
SSA @ 2 mM	0±0.00 ^{g}	0±0.00 ^{g}	0±0.00 ^{g}	0±0.00 ^{g}	1±0.00 ^{f}
Control	0±0.00 ^{g}	1.5±0.16^{dfe}	2±0.33 ^{dc}	3±0.16 ^{b}	4±0.16^{a}
Hue					
SA @ 1 mM	115±1.15^{a}	103±1.15^{becd}	99±1.15^{fegd}	92±1.7^{hgi}	90±1.73^{i}
SA @ 2 mM	115±1.15 ^{a}	107±1.73^{bc}	103±1.73^{becd}	98±1.15^{fheg}	93±1.15^{hgi}
SSA @ 1 mM	115±1.15 ^{a}	104±1.73^{becd}	102±1.15^{fbecd}	91±2.31^{hi}	88±1.15^{i}
SSA @ 2 mM	115±1.15 ^{a}	109±0.57^{ba}	106±0.57^{bd}	101±1.15^{fecd}	91±1.73^{hi}
Control	115±1.15 ^{a}	102±1.15^{fbecd}	95±1.15^{fhgi}	91±1.73^{hgi}	88±1.15^{i}

SA- Salicylic acid, SSA- Sulfosalicylic acid. Each value is the mean of three replicates ±SE. $^{a-l}$Means with different alphabet superscripts in the same column differ significantly (P ≤ 0.05) in that particular interval.

ripening process. The results are in tune with the earlier observations of Madhav *et al.* (14) in guava fruits.

Respiratory processes in the fruits majorly contribute to postharvest losses, converting stored carbohydrates to energy thus advancing senescence. Therefore, it is important to maintain the respiration rate at low level to prolong the storage life of fruit. During the course of our experiment, we observed a gradual increase in the rate of respiration followed by declining trend with the advancement in storage period. As shown in Fig 1, the onset of respiratory peak in the untreated guava fruits was observed on the 3+3 days of storage while the climacteric peak was suppressed and postponed up to 6+3 days in fruits treated with 1 mM concentration of SA or SSA. Moreover, higher concentration of SA (2 mM) further delayed the respiratory peak to 9+3 days of low temperature

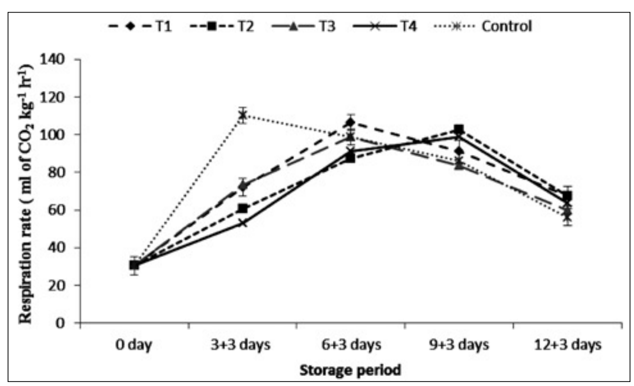

Fig. 1. Effect of salicylic acid treatments on respiration rate of guava during storage (T_1-SA @ 1mM, T_2-SA @ 2mM, T_3-SSA @ 1mM, T_4-SA @ 2mM and control). Bars in lines represent standard error of three replicates for each determination.

storage in Allahabad Safeda fruits. SA has negative effect on PME, PG, cellulose and antioxidant enzymes leading to decreased ethylene production and action. Moreover, SA retards the ripening process and also lowers the incidence of chilling injury as previously reported by Barman and Asrey (5) in mango fruits.

AOX capacity in guava fruits is mainly because of presence of phenolic compounds and ascorbic acid. Total antioxidant activity of the Allahabad Safeda fruits was observed to reduce during storage in all the treatments (Table 2), with the loss being more pronounced in control fruits. Retention of AOX activity is significantly higher ($p \leq 0.05$) upto 9+3 days of storage in fruits treated with 2 mM SSA (11.01±0.65 µmol TE/g) or SA (10.89±0.17 µmol TE/g). SSA (2 mM) was able to maintain maximum antioxidant activity (10.01±0.65 µmol TE/g) till the termination of the experiment. Since, SA induces expression of antioxidants and increases the antioxidant capacity of the cells, its postharvest application helped to retain higher phenolics and ascorbic acid content that ultimately resulted in higher AOX capacity. Dokhanieh *et al.* (7) have also reported higher antioxidant capacity of salicylic acid treated cherry fruits.

PAL activity is one of the enzymes involved in phenol synthesis. In our study, PAL activity in all samples of guava fruits increased initially followed by a decline with the progress in storage period (Table 2). In control fruits, the maximum PAL activity was observed on 3+3 days of storage in contrast to the treated guava fruits that showed a slow increase upto 6+3 days of storage. SA treated guava fruits showed greater PAL activity as it stimulates the synthesis of these antioxidant enzymes. Although PAL activity was higher in fruits treated with SSA @ 2 mM, it varied non-significantly with the fruits treated with SA @ 2 mM. The higher PAL activity of treated fruits helped in retention of phenolic compounds in the fruits during the storage which ultimately led to reduced incidence of chilling injury of these fruits. Our results are in agreement with previous studies done by Cao *et al.* (6) in cucumber.

MDA levels in tissues is indicative of the membrane integrity of cells subjected to low temperature with higher values indicating greater lipid peroxidation of cell membrane. In our study, MDA content was found to increase irrespective of the treatment applied (Table 2). Maximum MDA content was observed in untreated

Table 2. Effect of salicylic acid treatments on total phenolic content, total AOX activity, PAL activity and MDA content in guava fruits stored at 5°C followed by ambient conditions for 3 days.

Treatment	Storage period (days)				
	0	3+3	6+3	9+3	12+3
Total antioxidant activity (µmol Trolox g^{-1})					
SA @ 1 mM	14.13 ± 1.13^{a}	11.06 ± 0.86^{d}	9.68 ± 1.02^{g}	8.91 ± 0.75^{h}	7.42 ± 0.63^{j}
SA @ 2 mM	14.13 ± 1.13^{a}	13.12 ± 0.54^{b}	11.58 ± 0.86^{c}	10.89 ± 0.17^{d}	9.71 ± 0.89^{fg}
SSA @ 1 mM	14.13 ± 1.13^{a}	11.41 ± 0.66^{c}	9.71 ± 0.64^{fg}	9.12 ± 0.89^{h}	8.03 ± 0.63^{i}
SSA @ 2 mM	14.13 ± 1.13^{a}	13.25 ± 0.91^{b}	11.45 ± 0.35^{c}	11.01 ± 0.65^{d}	10.01 ± 0.65^{fe}
Control	14.13 ± 1.13^{a}	10.06 ± 0.78^{e}	7.98 ± 0.43^{i}	6.86 ± 0.63^{k}	6.01 ± 0.57^{l}
PAL activity (µmol cinnamic acid h^{-1} g^{-1} FW)					
SA @ 1 mM	0.136 ± 0.005^{ih}	0.145 ± 0.003^{egdf}	0.154 ± 0.002^{ebac}	0.148 ± 0.003^{egdfc}	0.141 ± 0.002^{gh}
SA @ 2 mM	0.136 ± 0.005^{ih}	0.152 ± 0.002^{ebdc}	0.161 ± 0.005^{ba}	0.155 ± 0.003^{bdac}	0.151 ± 0.005^{edfc}
SSA @ 1 mM	0.136 ± 0.005^{ih}	0.147 ± 0.003^{egdf}	0.155 ± 0.003^{bdac}	0.145 ± 0.005^{egdf}	0.140 ± 0.005^{gjh}
SSA @ 2 mM	0.136 ± 0.005^{ih}	0.155 ± 0.002^{bdac}	0.163 ± 0.002^{a}	0.157 ± 0.003^{bac}	0.152 ± 0.002^{edbc}
Control	0.136 ± 0.005^{ih}	0.142 ± 0.005^{ghf}	0.137 ± 0.005^{ih}	0.131 ± 0.002^{ij}	0.125 ± 0.005^{j}
MDA content (µmol g^{-1})					
SA @ 1 mM	22 ± 1.15^{i}	32 ± 0.94^{hfg}	37 ± 0.85^{efgd}	46 ± 1.31^{bc}	53 ± 0.87^{ba}
SA @ 2 mM	22 ± 1.15^{i}	25 ± 1.41^{hi}	29 ± 1.53^{hgi}	38 ± 1.25^{efcd}	44 ± 1.35^{ecd}
SSA @ 1 mM	22 ± 1.15^{i}	27 ± 1.71^{hi}	31 ± 1.10^{hfg}	39 ± 0.89^{efcd}	45 ± 1.15^{bcd}
SSA @ 2 mM	22 ± 1.15^{i}	22 ± 1.15^{i}	26 ± 1.25^{hi}	33 ± 1.73^{hfg}	39 ± 1.41^{efcd}
Control	22 ± 1.15^{i}	36 ± 1.56^{efg}	42 ± 1.63^{ecd}	53 ± 1.52^{ba}	59 ± 1.65^{a}

SA- Salicylic acid, SSA- Sulfosalicylic acid. Each value is the mean of three replicates ±SE.
$^{a-n}$Means with different alphabet superscripts in the same column differ significantly ($P \leq 0.05$) in that particular interval.

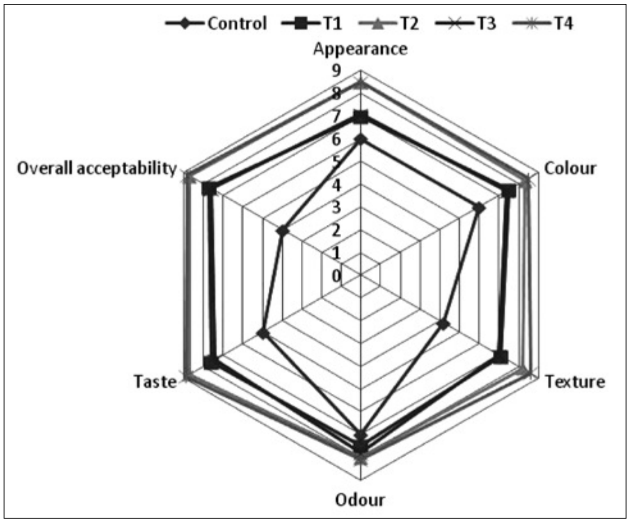

Fig. 2. Effect of salicylic acid treatments on sensory scores of guava after 9+3 days of storage. (T_1-SA @ 1mM, T_2-SA @ 2mM, T_3-SSA @ 1mM, T_4-SA @ 2mM and control).

guava fruits. Fruits of Allahabad Safeda treated with 2 mM concentration of SSA (39±1.41 µmol g^{-1} FW) or SA (44±1.35 µmol g^{-1} FW) showed significantly lower MDA content till the end of the storage compared to other treatments. This clearly indicates that damage caused by chilling stress could be alleviated by SA treatment. The reduced level of MDA by exogenous application of salicylic acid has also been previously reported in orange (Huang *et al.*, 10).

Sensory evaluation studies revealed that the salicylic acid treated fruits were better acceptable over control fruits after 9+3 days of storage (Fig. 2).The untreated fruits became organoleptically unacceptable at 3+3 days of storage due to enhanced softening and chilling injury (Table 1). The treatments retarded the softening, chilling injury and maintained overall acceptability which resulted in higher sensory scores. Earlier, Faheem *et al*. (8) have also reported maintenance of sensory attributes in sweet orange cv Blood Red.

Postharvest application of Allahabad Safeda fruits with salicylic acid could effectively control the senescence processes of the fruits. Higher concentration of SA and SSA (2 mM) could successfully impart chilling tolerance and maintain postharvest quality of guava fruits cv. Allahabad Safeda. The treatments helped to delay the ripening process indicated by reduced weight loss, respiration rate, colour change and PME activity and maintained the overall quality of the fruits as manifested by higher retention of firmness, total phenols and total antioxidant activity.

AUTHORS' CONTRIBUTION

Conceptualization and designing of research (JVM, SS), Preparation of manuscript (JVM, SS, RRS), Experimental material (AN), Data analysis (EV).

DECLARATION

The authors declare no conflict of interest.

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