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Pre-harvest application of salicylic acid and n-propyl gallate for enhancing shelf-life of guava

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ABSTRACT

Present investigation was conducted to evaluate the efficacy of pre-harvest application of salicylic acid (SA) and N-propyl gallate (n-PG) for enhancing the postharvest shelf-life and quality of winter guava fruit cv. Allahabad Safeda during two seasons. Pre-harvest application of aqueous solutions of salicylic acid (150, 300, 450 ppm) or N-propyl gallate (100, 200, 300 ppm) was done at 2 and 4 weeks before harvest. Fruits were harvested during third week of December at the firm mature stage, packed in corrugated fibre board boxes with newspaper lining and stored in a walk-in-cold room maintained at 6-8°C and 90-95% RH. Fruits were analyzed at weekly intervals to evaluate various quality attributes. In control fruits harvested from untreated trees, weight loss and decay incidence increased, whereas, firmness, ascorbic acid, acidity, total phenols and pectin content decreased during the storage. Fruits treated with n-PG (300 ppm) showed minimum decay incidence (16.55%), reduced pectin methylesterase activity (2.96 µg/ g FW), enhanced firmness (66.1 N), total soluble solids (9.96°Brix), acidity (0.35%), pectin (1.16% calcium pectate) and phenol contents (145.58 mg GAC/ 100 g pulp), and also maintained highly acceptable organoleptic rating up to 3 weeks of storage. Fruits subjected to SA treatments were intermediate in quality attributes between that of the control and n-PG treated fruits.

Key words: Cold storage, *guava*, n-propyl gallate, pre-harvest spray, salicylic acid, shelf-life.

INTRODUCTION

Guava (*Psidium guajava* L.) is considered one of the most exquisite, nutritionally valuable and remunerative fruit crops of tropical and sub-tropical regions of the world. Guava contains high amounts of vitamins - A, B_1 (Thiamine), B_2 (Riboflavin) and vitamin C content of guava fruit is 2-5 times more than that of citrus fruits (Singh, 14). India is the world's largest producer of guava followed by China, Kenya, Thailand, Indonesia, Pakistan, Mexico, Brazil, Bangladesh, Nigeria, Philippines, Vietnam and Egypt (FAO, 9). In India, it ranks fifth in area and production after mango, banana, citrus and apple. At present, it occupies an area of 268.2 thousand ha with an annual production of 3,667 thousand MT and has productivity of 13.7 MT per hectare. The important guava growing states in India are Maharashtra, Madhya Pradesh, Uttar Pradesh, Bihar, West Bengal, Punjab, Gujarat and Karnataka (Anon, 3).

In spite of its high nutritive value, world trade of guava fruit is limited due to its delicate nature, short post-harvest life and susceptibility to chilling injury and diseases. It is a climacteric fruit exhibiting respiratory and ethylene peaks during ripening (Bashir and Abu-Goukh, 6). Under ambient conditions guava fruits become overripe and mealy within a week, whereas, in cold storage the shelf-life can be extended up to two weeks at 6-8°C and 90-95% RH (Mahajan *et al*., 11). This necessitates the search for new technologies to improve its shelf-life and marketability. At present, there are no satisfactory procedures for enhancing the storage life and quality of guava fruits, and need immediate marketing and utilization after harvest. Most of the techniques meant for enhancing the post-harvest life of fruits aims at reducing the respiration rate and thereby the catabolism. Other approaches to achieve this goal may be disruption of ethylene production, application of antioxidants and by inhibiting cell wall hydrolysing enzymes like lipoxygenases *etc*. Therefore, an attempt was made in present study to prolong the post-harvest life and maintain the quality of winter guava cv. Allahabad Safeda by pre-harvest application of salicylic acid and n-propyl gallate.

MATERIALS AND METHODS

Randomly distributed 21 trees of guava cv. Allahabad Safeda of 10 years age and uniform vigour were selected during two consecutive cropping seasons of 2012 and 2013 at Punjab Agricultural University-Regional Fruit Research Station, Bahadurgarh, Patiala. The plants were maintained under uniform schedule of fertilizer, irrigation and phytosanitory treatments for insect pest control recommended under Punjab conditions. The selected plants were subjected to two sprays of either salicylic

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acid (150, 300, 450 ppm) or n-propyl gallate (100, 200, 300 ppm) at 4 and 2 weeks prior to harvest, whereas, the control trees were sprayed with water only. Experimental unit consisted of three trees for each treatment with one plant per replication. During third week of December, fruits from experimental plants were harvested at firm green stage. Fruits (10 kg/ replicate, a total of 30 kg per treatment) were harvested and packed in corrugated fibre board (CFB) boxes of 2 kg capacity (5% ventilation) and lined with paper clippings. The boxes were kept in a walk-in-cold room at 6-8°C and 90-95% RH. Fruits for decay analysis (100 fruits/ replicate) and weight loss study (25 fruits/ replicate) were kept separate and the same lot of fruits were assessed at different storage interval to obtain comparative data during the period. For physico-chemical analysis 25 fruits/ replicate were taken out from the cold storage at 7, 14, 21, 28 and 35 days after storage and analyzed for various quality parameters.

Weight loss of fruits was determined on the basis of initial fresh weight of the fruits and subsequent loss in weight that occurred during storage and expressed as percentage loss. Fruit firmness was measured with the help of a penetrometer (Model FT-327, QA Supplies, Norfolk, VA, USA). The pressure required to force a stainless steel probe of 8 mm in diameter, into peeled guava flesh through an automatic lever device to obtain uniform application of force was recorded in terms of Newton (N). Palatability rating was determined on the basis of colour and taste of fruits by a panel of 10 expert judges as per Hedonic scale (1 to 9 points) as described by Amerine *et al*. (2). Decay incidence was assessed by counting the number of fruits spoiled, and/or displaying fungal mycelia or sporulation from a total of 100 fruits per replication. TSS was measured by a temperature compensated digital refractometer (Atago PAL-1, model 3810, Japan) and expressed as percent soluble solids. Ascorbic acid and titratable acidity were estimated by the method described in AOAC (1). Total phenolic content was determined by the Folin-Ciocalteu method (Jacob *et al*., 10). Phenol extraction was carried out with 80% ethanol and the absorbance was measured at 765 nm against a blank using a spectrophotometer (Spectronic 200+, Thermo Scientific). The results were expressed as mg of gallic acid eqv./100 g FW using a gallic acid standard curve. Pectin content as per cent calcium pectate per 100 g pulp was estimated using gravimetric method (Ruck, 13). The enzyme activity of pectin methyl esterase was measured with 5 g of tissue sample as per the AOAC (1). The volume of 0.02 N NaOH consumed to adjust the pH to 7.5 was recorded and the results were expressed as µg/g FW.

The experiment was laid out in a randomized block design with factorial arrangements. Comparisons were made between treatments (salicylic acid, n-propyl gallate and control), and observed changes in parameters during storage period. Data were analyzed for variance by using the SAS (V 9.3, SAS Institute Inc., USA) package. The effect of each treatment and storage interval was determined by pairwise mean comparison using LSD ($p \leq 0.05$), where interactions between factors under study were found significant ($P \leq 0.05$).

RESULTS AND DISCUSSION

In general, physiological weight loss of the stored guava fruits increased during the storage period irrespective of treatment applied (Table 1). n-propyl gallate (n-PG) treated fruits registered the lowest average fresh weight loss (4.20%) as compared to other treatments, whereas, control fruit recorded the highest weight loss (7.25%). The fruits treated with salicylic acid (SA) also maintained lower weight loss than the control. In the case of fruits, a weight loss of 5% during storage is considered as the maximum acceptable limit that a fresh produce can have under storage, above which the fruits show shrivelling and become unmarketable (Mahajan *et al*., 11). Using this as a standard, it can be assumed that n-PG and SA treatments helped in maintaining the marketability of fruits up to 3 weeks, as against 2 weeks in the untreated fruits. However, n-PG treated fruits maintained significantly lower weight loss of ~6% up to 4 weeks so that they can be stored for a few days more than 3 weeks. The lower weight loss in fruits treated with n-PG and salicylic acid may be due to their role in delaying respiration rate of fruits (Asgharia and Aghdam, 5).

Fruit firmness registered a linear decline during storage (Fig. 1). Initial fruit firmness at the beginning of storage ranged from 94 N for control to nearly 115 N for n-PG treated fruits. Fruits treated with n-PG in general, maintained significantly higher average fruit firmness during storage, followed by those treated with SA (450 ppm). n-PG (300 ppm) proved to be the best treatment, and recorded the highest mean fruit firmness (66.11 N) over the storage period. Among the salicylic acid treatment the fruit treated with 450 ppm SA recorded the second highest firmness value of 63.31 N. On day 21, the treated fruits showed a firmness of ~50-60 N, while the control fruits showed 38.19 N. Control fruits suffered a quick loss of firmness during storage that caused excessive softening and shrivelling of fruits. Softening of fruits is caused either by breakdown of insoluble protopectin into soluble pectin in most fruits, or by hydrolysis of starch as in banana (Mattoo *et al*., 12). The loss of

Table 1. Effect of pre-harvest spray and storage interval on the physical fruit quality attributes of guava cv. Allahabad Safeda.

Treatment	Storage period (days)						
	0	7	14	21	28	35	Mean ± SE
Weight loss (%)							
Salicylic acid 150 ppm	0.00	1.17	2.62	5.18	9.60	12.70	5.21 ± 1.11^{b}
Salicylic acid 300 ppm	0.00	1.32	2.91	4.57	9.20	12.82	5.14 ± 1.09^{b}
Salicylic acid 450 ppm	0.00	1.27	2.80	4.33	9.25	11.85	4.92 ± 1.04^{c}
n-Propyl gallate 100 ppm	0.00	1.40	2.87	3.85	6.70	11.45	4.38 ± 0.92^{d}
n-Propyl gallate 200 ppm	0.00	1.32	2.67	3.67	6.60	11.65	4.32 ± 0.94^{de}
n-Propyl gallate 300 ppm	0.00	1.19	2.78	3.88	6.20	11.15	4.20 ± 0.89^{e}
Control	0.00	2.56	4.19	8.70	12.25	15.81	7.25 ± 1.34^{a}
Mean ± SE	0.00 ± 0.00^{f}	1.46 ± 0.10^{e}	2.97 ± 0.11^{d}	4.88 ± 0.36^{c}	8.54 ± 0.45^{b}	12.35 ± 0.34^{a}	
LSD (p≤0.05)	Treatment (T) = 0.16		Storage interval (D) = 0.15			Interaction (T × D) = 0.39	
Decay Incidence (%)							
Salicylic acid 150 ppm	0.00 (0.00)	0.00 (0.00)	1.78 (0.02)	22.59 (0.23)	39.60 (0.41)	69.37 (0.77)	0.24 ± 0.07^{b}
Salicylic acid 300 ppm	0.00 (0.00)	0.00 (0.00)	3.00 (0.03)	18.68 (0.19)	35.53 (0.36)	72.33 (0.81)	0.23 ± 0.07^{b}
Salicylic acid 450 ppm	0.00 (0.00)	0.00 (0.00)	1.40 (0.01)	15.94 (0.16)	25.54 (0.26)	67.63 (0.74)	0.20 ± 0.06^{c}
n-Propyl gallate 100 ppm	0.00 (0.00)	0.00 (0.00)	1.42 (0.01)	19.62 (0.20)	32.90 (0.34)	65.07 (0.71)	0.21 ± 0.06^{c}
n-Propyl gallate 200 ppm	0.00 (0.00)	0.00 (0.00)	0.12 (0.00)	18.54 (0.19)	31.52 (0.32)	64.17 (0.70)	0.20 ± 0.06^{c}
n-Propyl gallate 300 ppm	0.00 (0.00)	0.00 (0.00)	0.41 (0.00)	12.02 (0.12)	25.39 (0.26)	61.50 (0.66)	0.17 ± 0.06^{d}
Control	0.00 (0.00)	0.00 (0.00)	11.03 (0.11)	35.50 (0.36)	57.61 (0.61)	100.00 (1.57)	0.44 ± 0.13^{a}
Mean ± SE	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.03 ± 0.01^{d}	0.21 ± 0.02^{c}	0.37 ± 0.02^{b}	0.85 ± 0.07^{a}	
LSD (p ≤ 0.05)	Treatment (T) = 0.01		Storage interval (D) = 0.01			Interaction (T × D) = 0.03	
Organoleptic Rating (Out of 9)							
Salicylic acid 150 ppm	6.46	7.31	7.25	6.50	5.65	4.71	6.31 ± 0.22^{e}
Salicylic acid 300 ppm	6.59	7.38	7.25	6.65	5.78	4.97	6.43 ± 0.21^{d}
Salicylic acid 450 ppm	6.30	7.22	7.25	7.00	6.84	5.57	6.70 ± 0.15^{bc}
n-Propyl gallate 100 ppm	6.45	7.25	7.90	6.99	6.11	5.20	6.65 ± 0.21^{c}
n-Propyl gallate 200 ppm	6.80	7.55	7.75	7.22	6.30	5.65	6.88 ± 0.18^{a}
n-Propyl gallate 300 ppm	6.48	7.35	8.03	7.09	6.30	5.50	6.79 ± 0.20^{ab}
Control	6.45	7.45	7.00	5.18	4.65	0.00	5.12 ± 0.29^{f}
Mean ± SE	6.50 ± 0.03^{d}	7.36 ± 0.04^{b}	7.49 ± 0.09^{a}	6.66 ± 0.15^{c}	5.95 ± 0.15^{e}	4.51 ± 0.11^{f}	
LSD (p ≤ 0.05)	Treatment (T) = 0.12		Storage interval (D) = 0.11			Interaction (T × D) = 0.29	

Footnote: Means with the same letter are not significantly different (LSD, p≤0.05). Figures in parenthesis are Arc Sine transformation values. Each value represents pooled mean of two years (2012 and 2013)

pectic substances in the middle lamella of the cell wall is a key step in the ripening process that leads to the loss of cell wall integrity resulting in fruit softening (Solomos and Laties, 17).

Fruits treated with n-PG (300 ppm) recorded a minimum mean decay incidence score (16.55%), whereas the control fruits recorded maximum mean decay (34.02%) (Table 1). Salicylic acid and n-PG are known for their antioxidant properties that help to maintain the membrane structure, as well as provide protection from the oxidative damage (Yao and Tian, 19; Apelbaum *et al*., 4). Irrespective of the treatment the organoleptic rating of all the treated fruits increased up to 14 days of storage, and

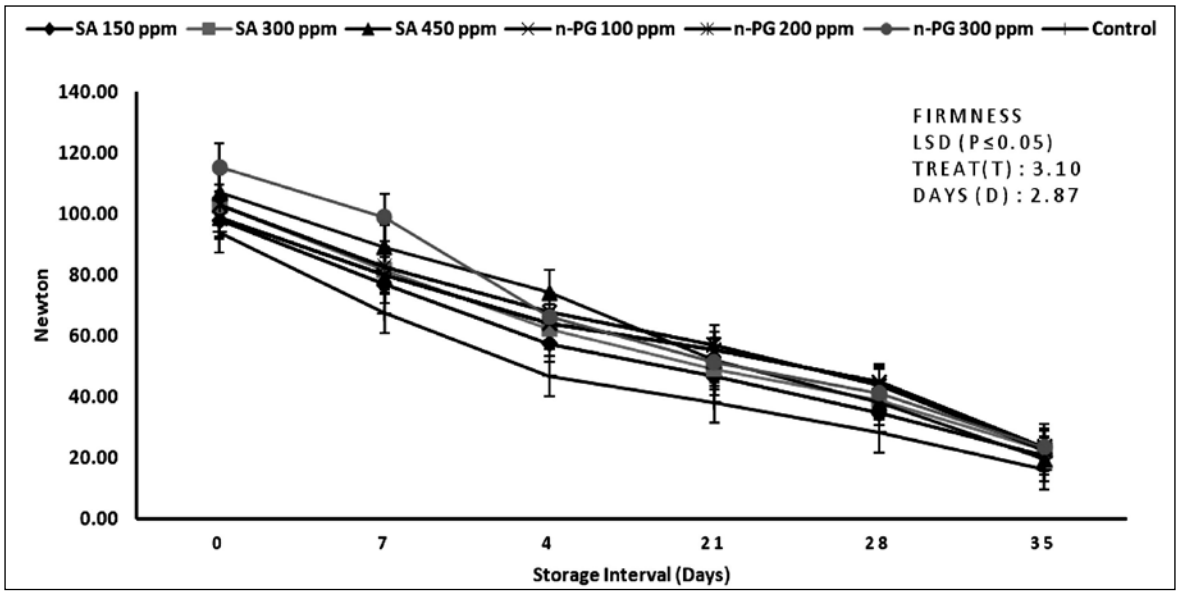

Fig. 1. Changes in fruit firmness (N) as a result of pre-harvest application of the salicylic acid (SA) and n-Propyl gallate (n-PG). Vertical bars represent ± SE of three replicates of two years pooled data.

thereafter, a gradual decline in sensory score was noted (Table 1). However in control fruit, maximum acceptability rating (7.45) was recorded after 7 days of storage and rated as 'highly desirable' up to 15 days, followed by a rapid decline, and were declared unfit for consumption after 21 days of storage. Fruits treated with all treatments of n-PG and SA (450 ppm) recorded significantly higher palatability rating of 7 and rated as 'highly desirable' for 21 days of storage. By contrast, untreated fruits were rated 'highly desirable' till 14 days of cold storage. The improvement of palatability rating in fruits during storage might be due to the build-up of sugars and acids as a result of hydrolysis of starch and other complex molecules leading to the development of flavour in fruits. Delay in the rate of catabolic processes in treated fruits may also affect the overall sensory qualities, thus prolonging a higher organoleptic rating during extended periods of storage.

TSS content in the fruits increased gradually up to 21 days in the treated fruits, and thereafter declined until 35 days of storage (Table 2). The Highest mean TSS (9.96 and 9.85%) was recorded in n-PG (300 ppm) and n-PG (200 ppm), respectively. On the other hand, control fruits recorded an increase in TSS up to 14 days (10.90%) followed by a sharp decline during storage. The increase in TSS content during storage might result from hydrolysis of starch into sugars. On completion of starch hydrolysis, no further increase in sugar content occurred and subsequently the TSS content declined as the sugars were metabolized during respiration (Wills *et al.*, 18).

Acid content in the fruits followed a declining trend throughout the storage period (Table 2). Both n-PG and SA treated fruits maintained significantly higher acidity content than the untreated fruits, which recorded a minimum mean acidity (0.28%). However, most of the n-PG and SA treatments except control were observed to be statistically at par with each other. A decline in the acid content was also more rapid in the untreated fruits, whereas the treated fruits registered a gradual fall in acidity level. The decrease in acid content of fruits during storage could be attributed to the use of organic acids in respiratory process by the fruit at a level higher than the treated fruits (Echeverria and Valich, 8). Fruits treated with n-PG and SA maintained a higher acidity value during storage, possibly due to reduction in the respiration rate, and delayed ripening.

Ascorbic acid (vitamin C) content declined irrespective of the treatment during storage period (Table 2). N-PG (300 ppm) treated fruits registered the maximum mean ascorbic acid (212.69 mg/100 g pulp) content, followed by n-PG (100 and 200 ppm) treatments. Salicylic acid treatments also helped in retention of ascorbic acid at significantly higher levels than those in the control fruit. Ascorbic acid level (175.93 mg per 100 g fruit pulp) was considerably lower in untreated fruits. The decrease in ascorbic acid during storage is due to conversion of ascorbic acid to dehydroascorbic acid by the action of ascorbic acid oxidase (Singh *et al*., 15). The gradual decline in ascorbic acid in n-PG treated fruits might be due to increased biosynthesis, or decreased oxidation during storage.

Table 2. Effect of pre-harvest sprays and storage intervals on the bio-chemical quality attributes of guava cv. Allahabad Safeda.

Treatment	Storage period (days)						
	0	7	14	21	28	35	Mean ± SE
TSS (°Brix)							
Salicylic acid 150 ppm	7.65	9.10	11.20	10.20	8.80	7.75	9.12 ± 0.31^{c}
Salicylic acid 300 ppm	7.75	9.60	10.45	11.10	8.90	7.70	9.25 ± 0.34^{c}
Salicylic acid 450 ppm	7.90	9.20	10.45	10.65	9.30	7.50	9.17 ± 0.29^{c}
n-Propyl gallate 100 ppm	8.50	9.50	10.30	11.80	9.55	7.80	9.58 ± 0.32^{b}
n-Propyl gallate 200 ppm	8.05	10.15	9.65	11.60	10.50	9.15	9.85 ± 0.27^{ab}
n-Propyl gallate 300 ppm	9.05	10.15	10.95	11.30	10.05	8.25	9.96 ± 0.29^{a}
Control	8.50	10.55	10.90	8.50	7.45	6.20	8.68 ± 0.40
Mean ± SE	8.20 ± 0.11	9.75 ± 0.12	10.56±0.12	10.74±0.27	9.22 ± 0.23	7.76 ± 0.19	
LSD (p ≤ 0.05)	Treatment (T) = 0.30		Storage interval (D) = 0.28			Interaction (T × D) = 0.73	
Acidity (%)							
Salicylic acid 150 ppm	0.44	0.41	0.36	0.28	0.23	0.20	0.32 ± 0.02^{d}
Salicylic acid 300 ppm	0.49	0.42	0.39	0.32	0.22	0.16	0.33 ± 0.03^{cd}
Salicylic acid 450 ppm	0.46	0.43	0.37	0.33	0.23	0.18	0.33 ± 0.02^{bc}
n-Propyl gallate 100 ppm	0.48	0.42	0.37	0.33	0.25	0.20	0.34 ± 0.02^{abc}
n-Propyl gallate 200 ppm	0.46	0.44	0.39	0.30	0.26	0.20	0.34 ± 0.02^{ab}
n-Propyl gallate 300 ppm	0.49	0.43	0.38	0.34	0.26	0.18	0.35 ± 0.03^{a}
Control	0.52	0.43	0.29	0.17	0.14	0.11	0.28 ± 0.04^{e}
Mean ± SE	0.48 ±0.01^{a}	0.43±0.00^{b}	0.36±0.01^{c}	0.29±0.01^{d}	0.23±0.01^{e}	0.18 ± 0.01^{f}	
LSD (p ≤ 0.05)	Treatment (T) = 0.01		Storage interval (D) = 0.01			Interaction (T × D) = 0.02	
Vitamin C (mg/100 g)							
Salicylic acid 150 ppm	247.25	223.71	202.22	185.90	169.37	144.81	195.54±8.23^{c}
Salicylic acid 300 ppm	243.97	224.20	207.23	192.11	171.80	150.09	198.23±7.68^{c}
Salicylic acid 450 ppm	245.33	234.24	209.04	190.02	167.79	152.39	199.80±8.30^{c}
n-Propyl gallate 100 ppm	247.77	232.51	209.84	194.46	187.04	160.04	205.28±7.12^{b}
n-Propyl gallate 200 ppm	254.54	240.02	218.09	202.59	180.01	145.47	206.79±8.97^{b}
n-Propyl gallate 300 ppm	259.60	242.58	222.19	203.61	179.84	168.36	212.69±8.03^{a}
Control	243.56	214.80	186.01	152.68	134.03	124.40	175.91±10.63^{d}
Mean ± SE	248.86 ± 1.82^{a}	230.29 ± 2.25^{b}	207.80 ± 2.89^{c}	188.77 ± 3.77^{d}	169.98 ± 3.73^{e}	149.36 ± 3.36^{f}	
LSD (p ≤ 0.05)	Treatment (T) = 4.77		Storage interval (D) = 4.42			Interaction (T × D) = 11.69	

Footnote: Means with the same letter are not significantly different (p≤0.05) according to LSD. Each value represents pooled mean of 2 years

The phenolic content of guava fruit registered a linear decline during cold storage in all the treatments as well as untreated fruits, and was found to be maximum (187.07 mg GAC/100 g pulp) at harvest (Fig. 2). Treated fruits maintained significantly higher levels of phenolics content as compared to the control. A sudden decline in the phenolics content was observed in untreated fruits after 7 days of cold storage synchronizing with the ripening and fruit softening. SA (450 ppm) and n-PG (300 ppm) treated fruits possessed higher phenolics content (146.64 and 145.58 mg GAC/100 g pulp) and maintained this level during storage. Quick ripening in untreated fruits during storage led to a decline in total phenols content earlier

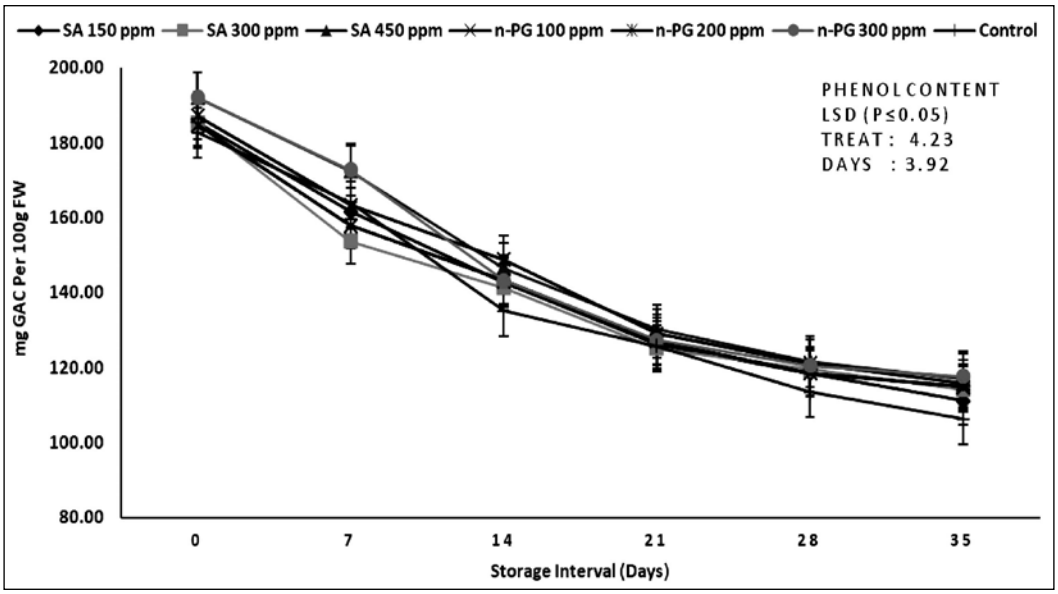

Fig. 2. Changes in phenol content (mg GAC / 100 g FW) as a result of pre-harvest application of the salicylic acid (SA) and n-propyl gallate (n-PG). Vertical bars represent ± SE of three replicates of two years pooled data.

than the treated ones, which is consistent with the observations of Bashir and Abu-Goukh (6) in guava. Singh and Pal (16) also reported similar declining trend of phenolic content during storage in guava.

Pectin content was found to follow a decreasing trend during storage irrespective of treatment (Fig. 3). The decrease in pectin can be correlated with a decrease in molecular size and esterification of pectin during storage. Fruits treated with n-PG recorded a lower decline as compared to those treated with SA and untreated fruits. In n-PG treated fruits, pectin content ranged from 1.09-1.18% and was significantly higher over the other salicylic acid treatments throughout the storage period. However, salicylic acid treated fruits also registered significantly higher pectin content as compared to the untreated fruits. The minimum pectin content was recorded in control fruits (0.65%), which was statistically lower than that observed in fruits subjected to n-PG and SA treatments.

Both n-PG and SA treatments significantly influenced the PME activity during storage (Fig. 4). PME activity peaked between 7 and 14 days of storage

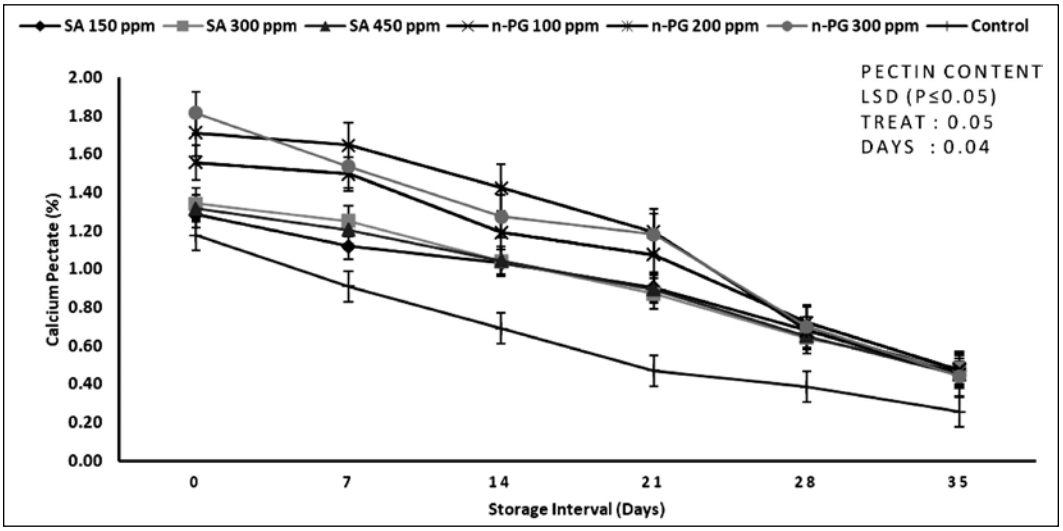

Fig. 3. Changes in pectin content (per cent calcium pectate) as a result of pre-harvest application of the salicylic acid (SA) and n-propyl gallate (n-PG). Vertical bars represent ± SE of three replicates of two years pooled data.

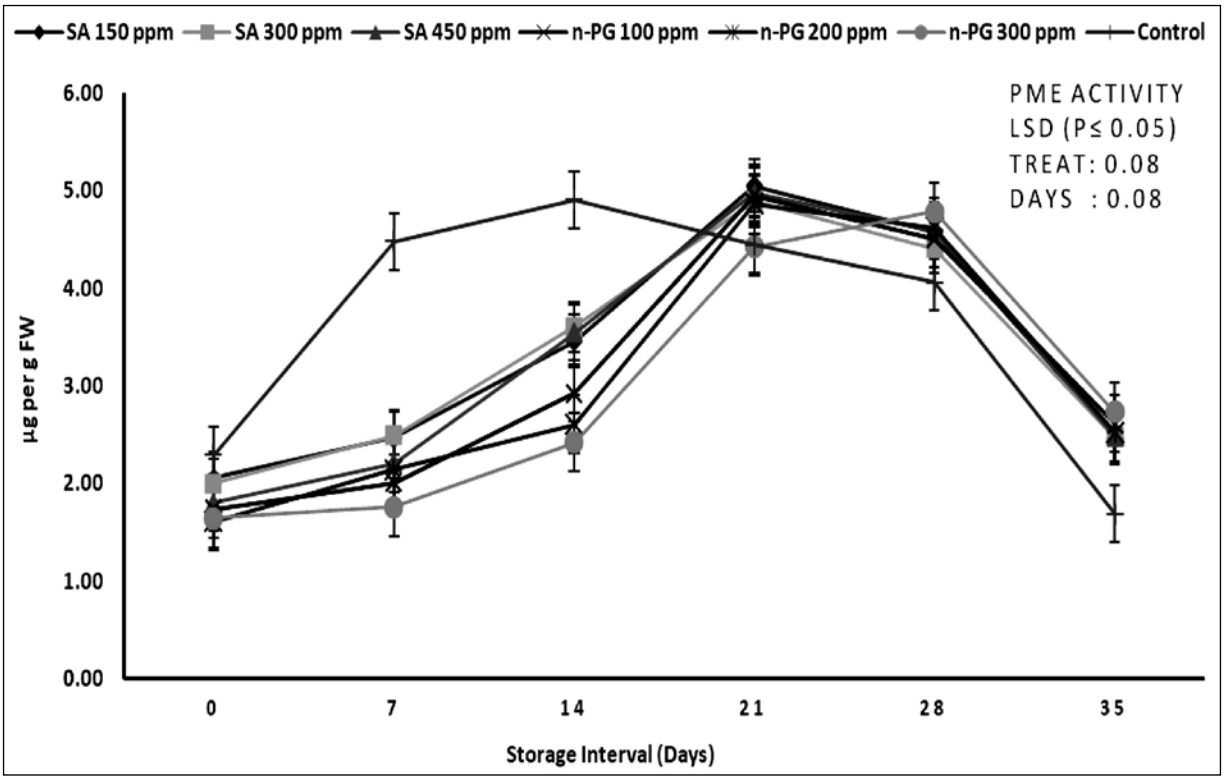

Fig. 4. Changes in PME activity (µg/g FW) as a result of pre-harvest application of the salicylic acid (SA) and n-Propyl gallate (n-PG). Vertical bars represent ± S.E. of three replicates of two years pooled data.

in control fruits and thereafter showed a sharp and abrupt decline in parallel with the loss in fruit quality. n-PG (300 ppm) treated fruits showed a gradual increase in PME activity, which peaked at 28 days of storage, and then declined. Salicylic acid treated fruits also registered a similar pattern of increase with the peak temporally appearing earlier at 14 days. PME removes the methyl group of galacturonic acid polymers of pectin. De-esterification of pectin chain by PME may make the chain more susceptible to polygalacturonase mediated degradation (Carpita and Gibeaut, 7) facilitating a rapid loss of cell wall structure. Thus, PME activity appears to show a significant influence in determining the postharvest shelf-life and quality in guava. The present investigation has shown that guava fruits treated with pre-harvest sprays of n-PG (300 ppm) at 4 and 2 weeks before harvest and packed in CFB boxes (with 5% ventilation) most effectively retained the fruit quality attributes up to 21 days during cold storage at 6-8°C and 90-95% RH.

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