

Full Length Research Paper

Effect of pre-storage salicylic acid, calcium chloride and 2,4-dichlorophenoxyacetic acid dipping on chilling injury and quality of 'Taify' cactus pear fruit during cold storage

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The effects of pre-storage salicylic acid (SA) calcium chloride (CaCl_2) and 2,4-dichlorophenoxyacetic acid (2,4-D) treatments on chilling injury (CI) and quality of cactus pear fruit during storage were investigated. The results showed that SA application at 2.0, 3.0 or 4.0 mM significantly decreased CI index compared to all the other treatments. Increasing SA rate to 3.0 or 4.0 mM did not result in a further reduction in CI index. However, CaCl_2 (at 2, 3, and 4%) and 2,4-D (at 100, 150 and 200 ppm) had no effect on CI index. CI increased during storage and was higher at 30 than at 10 and 20 days of storage. Weight loss was not affected by any of the treatments but was higher at 10 than at 20 and 30 days of storage. Decay was not affected by any of the treatments but was higher at 30 than at 10 and 20 days of storage. Firmness was higher at 200 ppm 2,4-D than all the other treatments. Fruit acidity was not affected by any of the applied treatments but was lower at 20 and 30 days than at 10 days of storage. The pH of fruit juice increased during 30 days of storage and was lower in the SA treatments than the control. Total soluble solids (TSS) concentration was higher in the control than all the other treatments, except for the SA at 4.0 mM treatment. TSS concentration was higher at 10 than at 20 and 30 days of storage. Vitamin C concentration was lower at 20 than at 10 and 30 days of storage and was lower in the CaCl_2 treatments than the control. Total phenols concentration increased during 30 days of storage and was lower in the CaCl_2 and 2,4-D treatments than the control. It was concluded that pre-storage SA dipping at 2.0 mM reduced chilling injury and retained quality of cactus fruit.

Key words: Cactus pear, salicylic acid, CaCl_2 , 2,4-D, chilling injury, storage.

INTRODUCTION

There is a growing demand in international market for excellent quality cactus pear fruit [*Opuntia ficus-indica* (L.) Mill] (Barbera et al., 1995; Mizrahi et al., 1997). The consumption of fruits, in general, and especially cactus pear fruit is important for human health due to their high antioxidants content such as polyphenolics, vitamins, selenium and dietary fibers (Awad and de Jager, 2003; Butera et al., 2002; Tesoriere et al., 2004). Cactus pear fruit are highly perishable having a short shelf life of a few days due to decay and weight loss (Schirra et al., 1999; Rodriguez et al., 2005). However, as most of other tropical fruit species, these fruit are sensitive to chilling

injury (CI) which limits their cold storage. The most symptoms of CI are browning and pitting, dehydration and a decrease in percentage juice when fruit stored below 5°C for longer than few days (Cantwell, 1995). The CI increases susceptibility to decay and negatively affects both external and internal fruit quality. Some postharvest treatments including controlled atmospheres (2% O_2 + 5% CO_2) (Gorini et al., 1993), modified atmosphere packing (Guevara et al., 2003), pre-storage conditioning at 38°C for 24 h (Schirra et al., 1997a), hot water dipping at 52°C for 3 min (Rodriguez et al., 2005) and intermittent warming (Cantwell et al., 1992; Cantwell, 1995) showed

variable success in reducing chilling injury. Postharvest SA dipping at 2.0 mM effectively reduced CI and vitamin C loss in pomegranates (Sayyari et al., 2009). Acetylsalicylic acid (ASA; a derivative of SA) application increased the endogenous SA levels, inhibited ethylene production and maintained firmness of kiwifruit during cold storage (Zhang et al., 2003). Mango fruit treated with SA or oxalic acid (OA) developed lower level of CI than control (Ding et al., 2007). They suggested that the effect of OA or SA on CI probably attributed to more reducing status of ascorbate and glutathione, less O₂ accumulation and more H₂O₂ accumulation. Pre-harvest spray of calcium chloride (CaCl₂) reduced decay but increased the incidence of CI during 5 to 7 weeks of cold storage plus 3 days of shelf life at 20°C (Schirra et al., 1997b; Schirra et al., 1999). Postharvest 2,4-dichlorophenoxyacetic acid (2,4-D) treatment induced healing of injuries, retarded senescence and controlled decay in several fruit and vegetable species (Papadopoulou-Mourkidou, 1991; Kobiler et al., 2001). The postharvest dipping in 2,4-D reduced CI, enhanced the antioxidant enzymes activities and increased the levels of endogenous ABA/GA₃ but decreased the level of IAA in mango fruit during cold storage (Wang et al., 2008). To the best of our knowledge, there is no literature information on the response of cactus pear fruit to postharvest SA, CaCl₂ and 2,4-D treatments. Therefore, the aim of this study was to investigate the effect of pre-storage SA, CaCl₂ and 2,4-D on CI incidence and quality of cactus pear fruit during cold storage at 2°C.

MATERIALS AND METHODS

Plant materials and experimental procedure

Mature cactus pear fruit (green-yellowish color) of a local 'Taify' variety were harvested on 17 July, 2011 from uniform plants growing in a commercial field at Taif region, Kingdom of Saudi Arabia (KSA). The fruits were immediately transported to the laboratory and classified into 81 experimental units, 15 fruits of each (3 SA levels x 3 CaCl₂ levels x 3 2,4-D levels x 3 replicates). Fruit samples were subjected to one of the SA (2, 3 and 4 ppm), CaCl₂ (2, 3, 4%) or 2,4-D (100, 150 and 200 ppm) dipping treatments for 5 min at 20°C ± 1 with the addition of Tween 20 surfactant (1.0 ml/l). A control treatment in which fruit samples were dipped in Tween 20 surfactant (1 ml/L) and water were included. The treated fruit were stored in perforated plastic boxes at 2°C ± 1 and 90 to 95% RH for 30 days. After 10, 20 and 30 days, 5 fruit from each treatment and replicate were sampled at random and stored for a further 3 days at 20°C ± 1 as a shelf life. Additional three replicates (5 fruits of each) for each treatment were stored at the same conditions and were periodically weighed for loss in weight calculation. At harvest, additional three replicates (5 fruits of each) were collected for initial quality measurements as described below.

Chilling injury (CI), weight loss and decay determination

Chilling injury index was individually evaluated in each fruit with a 5 point hedonic scale based on the percentage of fruit surface

affected by CI symptoms (browning and pitting, dehydration): 0 = none; 1 = 1 to 10% damaged area; 2 = 11 to 20% damaged area; 3 = 21 to 30% damaged area; 4 = > 30% damaged area. The CI index was calculated using the following formula: $CI = \sum (\text{value of hedonic scale}) \times (\text{number of fruit with the corresponding scale number}) / \text{total number of fruit in the sample}$ (Sayyari et al., 2009). Fruit was considered unacceptable for the consumer if it had CI indices of 1 or higher. The loss in weight percentage was periodically calculated on initial weight basis. The number of decayed fruit was periodically recorded and expressed as a percentage from the total fruit number.

Fruit pulp firmness, total soluble solids, acidity, vitamin C and pH determinations

Fruit pulp firmness was recorded at two opposite sides independently in each of the 5 fruits per replicate by a digital basic force gauge, model BFG 50N (Mecmesin, Sterling, Virginia, USA) supplemented with a probe of 11 mm diameter and the results expressed as Newton. A homogeneous sample was prepared from these 5 fruits per replicate for total soluble solids (TSS), acidity, vitamin C and pH determinations. TSS was measured as Brix % in fruit juice with a digital refractometer (DR 6000, A. Kruss Optronic GmbH, Hamburg, Germany). Titratable acidity was determined in juice by titrating with 0.1 N sodium hydroxide in the presence of phenolphthalein as indicator and the results were expressed as a percentage of citric acid. Ascorbic acid (vitamin C) was measured by the oxidation of ascorbic acid with 2,6-dichlorophenol endophenol dye and the results were expressed as mg/100 g fresh weight (Ranganna, 1979). The pH was determined in fruit juice samples with a pH meter (WTW 82382, Weilheim, Germany).

Total phenols determination

Total phenols were measured according to Velioglu et al. (1998) using Folin-Ciocalteu reagent. Two hundred milligrams (200 mg) of fruit tissue were extracted with 2 ml of 50% methanol for 2 h at ambient temperature. The mixture was centrifuged for 10 min and the supernatant was decanted into 4 ml vials. A 200 µl of the extract was mixed with 1.5 ml Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand for 5 min before the addition of 1.5 ml of 20% sodium carbonate. After 90 min, absorbance was measured at 750 nm using a UV-Vis spectrophotometer. The blank contains only water and the reagents. Total phenols were quantified from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid.

Statistical analysis of data

The obtained data were statistically analyzed as a factorial experiment in a completely randomized design with three replicates by analysis of variance (ANOVA) using the statistical package software SAS (SAS Institute Inc., 2000, Cary, NC., USA). Comparison between means was made by *F*-test and the least significant differences (LSD) at *P* = 5%.

RESULTS

Salicylic acid application at 2.0, 3.0 or 4.0 mM significantly decreased CI index compared to all the other treatments including the control. Increasing the SA concentration to 3.0 or 4.0 mM did not result in a further

Table 1. Effect of salicylic acid (SA), CaCl₂ and 2,4-D on chilling injury index, weight loss and decay percentages of cactus pear fruit during 30 days of storage at 2°C, season 2011.

Parameter	Chilling injury (index)	Weight loss (%)	Decay (%)
Treatment (T)			
Control	1.78 ^a	1.54	0.33
SA (mM)			
2	1.11 ^b	1.12	0.11
3	1.11 ^b	0.98	0.13
4	1.11 ^b	0.97	0.22
CaCl₂ (%)			
2	1.78 ^a	1.37	0.44
3	1.78 ^a	1.00	0.22
4	1.78 ^a	1.41	0.67
2,4-D (ppm)			
100	1.89 ^a	1.09	0.44
150	1.89 ^a	1.65	0.11
200	1.89 ^a	1.44	0.11
F-test	**	NS	NS
LSD (0.05)	0.43	-	-
Storage period (SP, days)			
10	1.20 ^b	1.58 ^a	0.00 ^b
20	1.43 ^b	0.93 ^c	0.03 ^b
30	2.07 ^a	1.26 ^b	0.77 ^a
F-test	**	**	**
LSD (0.05)	0.23	0.20	0.19
TX SP			
F-test	*	NS	**

Measurements were carried out after each storage period plus 3 days of shelf life. Means within each column followed by the same letter are not significantly different at level $P = 0.05$. (*) and (**), significant at $P = 0.05$ and 0.01 , respectively; (NS), not significant.

reduction in CI index (Table 1). However, the application of CaCl₂ and 2,4-D at the different rates had no significant effect on CI index. The incidence of CI increased during storage and was significantly higher at 30 than at 10 and 20 days of storage (Table 1). The interaction effect between treatment and storage period revealed that in the SA and the control treatments, CI was not significantly increased from 10 to 30 days of storage, in contrast to CaCl₂ and 2,4-D treatments in which CI was significantly increased only after 30 days of storage (Table 2). Also, at 10 days of storage, there were no significant variations in CI index among the treatments, in contrast to the other storage periods (Table 2). Weight loss percentage was not significantly affected by any of the applied treatments but was higher at 10 than at 20 and 30 days of storage (Table 1). Decay percentage was not significantly affected by any of the

applied treatments but was higher at 30 than at 10 and 20 days of storage (Table 1). The interaction effect between treatment and storage period revealed that, in all the treatments, decay was only detected after 30 days of storage (Table 2). Fruit firmness was significantly higher at the highest concentration of 2,4-D (200 ppm) treatment than all the other treatments and was significantly lower at 30 than at 10 and 20 days of storage (Table 3). The interaction effect between treatments and storage period showed that in the control, fruit firmness was not significantly changed during 30 days of storage, in contrast to the other treatments in which fruit firmness was lower at 30 than at 10 and 20 days of storage (Table 4). Fruit acidity was not affected by any of the applied treatments but was lower at 20 and 30 days than at 10 days of storage (Table 3). The pH of fruit juice was lower in the SA treatments than the control and was

Table 2. The interaction effect between treatments and storage period on chilling injury (index), decay (%) and firmness (N) of cactus pear fruit, season 2011.

Treatment (T)	Storage period (day)		
	10	20	30
Chilling injury (index)			
Control	1.67 ^{cde}	1.76 ^{cde}	2.00 ^{bcd}
SA (mM)			
2	1.00 ^e	1.00 ^e	1.33 ^{de}
3	1.00 ^e	1.00 ^e	1.33 ^{de}
4	1.00 ^e	1.33 ^{de}	1.33 ^{de}
CaCl₂ (%)			
2	1.33 ^{de}	2.00 ^{bcd}	2.00 ^{bcd}
3	1.33 ^{de}	1.33 ^{de}	2.67 ^{ab}
4	1.33 ^{de}	1.33 ^{de}	3.00 ^a
2,4-D(ppm)			
100	1.33 ^{de}	1.33 ^{de}	2.67 ^{ab}
150	1.00 ^e	1.76 ^{cde}	2.33 ^{abc}
200	1.00 ^e	1.76 ^{cde}	2.33 ^{abc}
Decay (%)			
Control	0.00 ^e	0.00 ^e	1.00 ^{cb}
SA (mM)			
2	0.00 ^e	0.00 ^e	0.33 ^{ed}
3	0.00 ^e	0.00 ^e	0.00 ^e
4	0.00 ^e	0.00 ^e	0.67 ^{cd}
CaCl₂ (%)			
2	0.00 ^e	0.00 ^e	1.33 ^b
3	0.00 ^e	0.00 ^e	0.67 ^{cd}
4	0.00 ^e	0.00 ^e	2.00 ^a
2,4-D (ppm)			
100	0.00 ^e	0.00 ^e	1.33 ^b
150	0.00 ^e	0.00 ^e	0.33 ^{ed}
200	0.00 ^e	0.33 ^{ed}	0.00 ^e

Measurements were carried out after each storage period plus 3 days of shelf life. For each parameter, means within and between columns followed by the same letter are not significantly different at level P = 0.05.

significantly increased during 30 days of storage (Table 3). The significant interaction effect between treatment and storage period revealed that, during 30 days of storage, the pH increased in the control, CaCl₂ and 2,4-D treatments while it was not significantly changed in the SA treatments (Table 5). The TSS concentration was higher in the control than all the other treatments, except for the SA at 4 mM treatment (Table 3). The TSS

concentration was significantly higher at 10 than at 20 and 30 days of storage (Table 3). Vitamin C concentration was significantly lower in the CaCl₂ treatments than the control and was lower at 20 than at 10 and 30 days of storage (Table 3). There were significant interaction effects between treatments and storage period on vitamin C concentration (Table 5). The concentration of total phenols was significantly lower in the CaCl₂ and

Table 3. Effect of salicylic acid (SA), CaCl₂ and 2,4-D on firmness, and TSS, acidity, pH, vitamin C and total phenols concentration of cactus pear fruit during 30 days of storage at 2°C, season 2011.

Parameter	Firmness (N)	Acidity (%)	pH	TSS (brix %)	Vitamin C (mg/100 g FW)	Phenol (mg/g FW)
Treatment (T)						
Control	32.4 ^{bc}	0.12	5.9 ^a	13.6 ^a	86.8 ^a	2.9 ^a
SA (mM)						
2	30.1 ^{cde}	0.10	5.8 ^{bc}	12.0 ^{bc}	80.8 ^{abc}	2.9 ^{ab}
3	30.0 ^e	0.10	5.7 ^d	12.3 ^b	81.9 ^{ab}	2.2 ^{de}
4	30.4 ^{cde}	0.11	5.8 ^{bc}	13.2 ^a	85.6 ^a	2.2 ^{cd}
CaCl₂ (%)						
2	33.8 ^b	0.10	5.9 ^a	12.1 ^{bc}	74.5 ^c	1.9 ^f
3	32.2 ^{bc}	0.06	5.9 ^a	12.3 ^b	74.9 ^c	1.9 ^{ef}
4	30.6 ^{cde}	0.10	5.9 ^{abc}	12.4 ^b	77.1 ^{bc}	2.3 ^{cd}
2,4-D(ppm)						
100	29.5 ^{de}	0.11	5.8 ^{cd}	12.4 ^b	75.3 ^c	2.9 ^{cd}
150	31.8 ^{bcd}	0.10	5.9 ^{ab}	12.0 ^{bc}	82.1 ^{ab}	2.3 ^{cd}
200	38.0 ^a	0.09	5.9 ^{ab}	11.8 ^c	83.2 ^{ab}	2.7 ^{bc}
F-test	**	NS	**	**	**	**
LSD (0.05)	2.4	-	0.11	0.46	6.5	0.33
Storage period (SP, days)						
10	34.2 ^a	0.12 ^a	5.7 ^c	12.7 ^a	81.8 ^a	0.9 ^c
20	33.5 ^a	0.09 ^b	5.9 ^b	12.3 ^b	76.3 ^b	2.8 ^b
30	27.6 ^b	0.09 ^b	6.0 ^a	12.3 ^b	82.6 ^a	3.3 ^a
F-test	**	**	**	**	**	**
LSD (0.05)	1.3	0.01	0.06	0.25	3.6	0.18
TX SP						
F-test	**	NS	*	NS	**	**

Measurements were carried out after each storage period plus 3 days of shelf life. Means within each column followed by the same letter are not significantly different at level $P = 0.05$. (*) and (**) significant at $P = 0.05$ and 0.01 respectively; (NS), not significant; (-), not calculated. FW = fruit weight.

2,4-D treatments than the control (Table 3). Total phenols concentration significantly increased during 30 days of storage and was lower at 2 and 3% CaCl₂ than all the other treatments (Table 3). There were significant interaction effects between treatments and storage period on the total phenols concentration (Table 5).

DISCUSSION

The results of the current experiment showed that SA application at 2.0, 3.0 or 4.0 mM was effective in the reduction of CI development in cactus pear fruit, as

estimated by CI index, during 30 days of cold storage at 2°C plus 3 days at 20°C. These results are in agreement with those of Wang et al. (2006) in peaches and of Sayyari et al. (2009) in pomegranates where the higher concentration of SA in the range from 0.35 to 2.0 mM was more effective than lower ones. However, in the current experiment increasing the SA concentration to 3.0 or 4.0 mM did not result in a further reduction in CI in cactus pear fruit (Table 1), suggesting a possible physiological active concentration of SA. In confirmation, postharvest treatment with 2.0 mM SA was most effective in reducing ethylene production, fungal decay and retaining quality of strawberry fruit in contrast to 4.0 mM

Table 4. The interaction effect between treatments and storage period on firmness (N) of cactus pear fruit, season 2011.

Treatment (T)	Storage period (day)		
	10	20	30
Firmness (N)			
Control	31.2 ^{fg}	33.6 ^{bcdefgh}	32.8 ^{defghi}
SA (mM)			
2	43.1 ^{bcdef}	31.0 ^{fg}	25.1 ^{kl}
3	34.4 ^{bcdef}	33.7 ^{bcdefgh}	18.9 ^m
4	34.2 ^{bcdef}	33.2 ^{cdefghi}	23.9 ^l
CaCl₂ (%)			
2	35.5 ^{abcde}	36.4 ^{abcd}	29.6 ^{ghij}
3	33.8 ^{bcdef}	33.7 ^{bcdefg}	29.1 ^{ijk}
4	33.2 ^{cdefghi}	31.2 ^{fg}	26.7 ^{kl}
2,4-D (ppm)			
100	34.1 ^{bcdef}	31.6 ^{efghi}	23.0 ^m
150	32.7 ^{efghi}	33.8 ^{bcdef}	29.5 ^{hij}
200	39.3 ^a	37.1 ^{abc}	37.8 ^{ab}

Measurements were carried out after each storage period plus 3 days of shelf life. For each parameter, means within and between columns followed by the same letter are not significantly different at level P = 0.05.

that slightly damaged the fruit and was less effective in retaining fruit quality (Babalar et al., 2007). The oxidative stress caused by the accumulation of reactive oxygen species (ROS) together with a reduction in the antioxidant system were involved in CI development in fruit during storage (Hodges et al., 2004). Ding et al. (2007) attributed the effect of OA or SA on decreasing CI in mango fruit to more reducing status of ascorbate and glutathione, less O₂ accumulation and more H₂O₂ accumulation. Also, the effect of SA on controlling CI of peaches was attributed to its ability to induce antioxidant systems and heat shock protein (HSPs) (Wang et al., 2006). On the other hand, the application of CaCl₂ and 2,4-D at the different rates had no significant effect on CI (Table 1). These results are in agreement with those of Schirra et al. (1997b and 1999) that pre-harvest spray of CaCl₂ reduced decay but increased the incidence of CI during 5 to 7 weeks of cold storage plus 3 days of shelf life at 20°C. However, the current result contradict with those of Wang et al. (2008) that postharvest dipping in 2,4-D reduced CI, enhanced the antioxidant enzymes activities and increased the levels of endogenous ABA/GA₃ but decreased the level of IAA in mango fruit during cold storage. Both weight loss and decay percentages were not significantly affected by any of the applied treatments (Table 1). However, it has been found in other fruits that pre- or postharvest SA treatment

reduced fungal decay in sweet cherries (Yao and Tian, 2005; Xu and Tian, 2008), in peaches (Wang et al., 2006) and in strawberry fruit (Babalar et al., 2007) during cold storage. In this experiment, the maximum weight loss was 1.65% at the 150 ppm 2,4-D treatment (Table 1). For cactus fruit, a weight loss of about 8% was necessary to affect the visual appearance during cold storage (Rodriguez-Felix et al., 1992). In this experiment, along with the alleviated CI, fruit quality characteristics were not obviously affected by SA or CaCl₂ and 2,4-D treatments (Tables 3, 4 and 5). However, SA application maintained firmness and acidity as well as increased TSS during ripening of banana fruit (Srivastava and Dwivedi, 2000). Also, ASA (a derivative of SA), application increased the endogenous SA levels, inhibited ethylene production and maintained firmness of kiwifruit during cold storage (Zhang et al., 2003). Also, in pomegranates SA at 2.0 mM, maintained vitamin C during cold storage compared with lower concentration and the control (Sayyari et al., 2009). In conclusion, the postharvest SA dipping at 2.0 mM of cactus pear fruit reduced the development of CI and maintained quality during cold storage.

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Table 5. The interaction effect between treatments and storage period on pH, vitamin C and total phenols concentration of cactus pear fruit, season 2011.

Treatment (T)	Storage period (day)		
	10	20	30
pH			
Control	5.7 ^{ijklmn}	6.0 ^{abcdef}	6.1 ^{ab}
SA (mM)			
2	5.8 ^{ghijklm}	5.8 ^{fghijkl}	5.8 ^{ghijkl}
3	5.7 ^{klmn}	5.7 ^{ijklmn}	5.6 ⁿ
4	5.8 ^{hijklmn}	5.7 ^{ijklmn}	5.9 ^{defghij}
CaCl₂ (%)			
2	5.8 ^{efghijkl}	5.9 ^{defghijk}	6.1 ^{abc}
3	5.7 ^{ijklmn}	5.9 ^{defghij}	6.2 ^a
4	5.7 ^{lmn}	5.9 ^{efghijk}	6.0 ^{abcde}
2,4-D(ppm)			
100	5.6 ^{mn}	5.7 ^{ijklmn}	6.0 ^{bcdefg}
150	5.7 ^{klmn}	6.0 ^{bcdefgh}	6.0 ^{abcde}
200	5.7 ^{klmn}	5.9 ^{cdefghi}	6.1 ^{abcd}
Vitamin C (mg/100 g FW)			
Control	94.4 ^{ab}	88.0 ^{abcd}	78.0 ^{defgh}
SA (mM)			
2	81.6 ^{cdef}	76.8 ^{defgh}	84.0 ^{bcde}
3	76.8 ^{defgh}	76.8 ^{defgh}	92.0 ^{abc}
4	88.0 ^{abcd}	76.8 ^{defgh}	92.0 ^{abc}
CaCl₂ (%)			
2	78.4 ^{defgh}	72.2 ^{efgh}	70.0 ^{hg}
3	78.4 ^{defgh}	70.0 ^{fgh}	76.0 ^{efgh}
4	83.2 ^{bcde}	80.0 ^{defg}	68.0 ^h
2,4-D (ppm)			
100	75.2 ^{efgh}	76.8 ^{defgh}	74.0 ^{efgh}
150	83.2 ^{bcde}	67.2 ^h	96.0 ^a
200	78.4 ^{defgh}	75.2 ^{efgh}	96.0 ^a

Measurements were carried out after each storage period plus 3 days of shelf life. For each parameter, means within and between columns followed by the same letter are not significantly different at level P = 0.05. FW = fruit weight.

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Table 5. Contd.

Treatment (T)	Storage period (day)		
	10	20	30
Total Phenols (mg/g fw)			
Control	2.02i	3.0def	3.2a
SA (mM)			
2	2.3ghi	3.2cd	3.1ed
3	0.5k	2.8defgh	3.2bcd
4	0.7jk	2.8defg	3.2bcd
CaCl₂ (%)			
2	0.4k	2.5fghi	2.6efgh
3	0.2k	2.3hi	3.2bcd
4	0.8jk	3.0def	3.1ed
2,4-D (ppm)			
100	0.7jk	3.0def	3.1ed
150	0.6k	2.5fghi	3.8ab
200	1.2j	2.8defgh	3.7abc

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