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PRE-STORAGE APPLICATION OF CALCIUM CHLORIDE AND SALICYLIC ACID MAINTAIN THE QUALITY AND EXTEND THE SHELF LIFE OF STRAWBERRY

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Strawberry is highly nutritious and economically important fruit crop having short shelf life. Fruit has sensitivity to fungal decay and due to fast metabolic activity heavy losses occur before reach to the consumers. Pre storage application of calcium chloride ($CaCl_2$) and salicylic acid (SA) is used as an alternative to synthetic fungicides for controlling decay problem and to extend the shelf life of harvested fruits. The present study was conducted to standardize the doses of calcium chloride and salicylic acid to extend the shelf life of strawberry fruit. Marketable strawberries were dipped in different concentrations of $CaCl_2$ (2, 4, 6 mM) and SA (3, 5, 7 mM) solutions for 10 minutes along with control (simple water) and stored at 4°C with 80-85% RH for 15 days. All fruit quality parameters were analyzed during 0, 3, 6, 9, 12 and 15 days after storage. It was observed that decay (%) significantly reduced in all treated fruits than fruits in control treatment. Maximum decay (39%) was noted in control treatment while minimum decay percentages (1.5 and 2.3) were in medium doses of SA and $CaCl_2$ (5mM and 4mM), respectively. The physiochemical analysis showed that $CaCl_2$ (6 mM) proved better for reducing weight loss of strawberries where it was 3.3% while in control it was 9.3%. Maximum firmness (0.42 kg. cm^{-2}), vitamin C contents (43.90 mg 100 g^{-1}) and TPC (132.75 mg 100 g^{-1}) were also found from strawberries those were treated with 6 mM $CaCl_2$ as compared to control when analyzed after 15 days. However, minimum TSS (7.85ºBrix), maximum retaining of acid contents (0.62%), total antioxidants (39.0% DPPH) and enzymatic activities were found with SA (5 mM) dipping application as compared to other treatments. Consequently, it is concluded that pre storage treatment of SA @ 5 mM is recommended for extending shelf life and for retaining maximum quality attributes of strawberry during 15 days of storage. Moreover $CaCl_2$ (6 mM) also performed better to maintain the quality parameters up to 15 days but decay (%) was somewhat increased.
Keywords: Strawberry, fungal decay, firmness, non-climacteric, antioxidants, total phenolic contents.

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.) is delicious, sweet flavored small fruit belongs to family Rosaceae (Sharma, 2002). Amongst all small fruits strawberry is most popular and economically important fruit crop all over the world (Santos and Chandler, 2009). Strawberry is newly emerging small fruit crop in Pakistan; therefore yield is very low as compared to major strawberry producing countries (Mabood, 1994). In Pakistan strawberry is cultivated on 179 hectares with 609 'tons' annual production (GOP, 2015).

Strawberries are delicious low calorie fruits with rich source of vitamins, minerals and antioxidants (Adda-Bjarnadottir, 2012). Strawberries lower the level of cholesterol, blood pressure, reduce inflammation and decrease oxidative stress. Important characteristic of strawberry fruit include aroma, taste and flavor (Giampieri *et al*., 2012). The nature of strawberry fruit is non-climacteric and has short shelf life due to that fruit is consumed as fresh and in processed form (Mabood, 1994). Major storage issues are rapid metabolic activity, sensitive to fungal decay and grey mold disease (Hernandez-Munoz *et al*., 2006). Postharvest losses (25-40%) of strawberries are more than pre harvest due to inappropriate management practices, poor handling techniques, low quality packaging, poor transportation system and complex marketing channels (Aday and Caner, 2014). Strawberries are highly perishable due to extreme tenderness, high level of respiration and their susceptibility to fungal spoilage (Almenar *et al*., 2006). After harvest quality of strawberry fruit deteriorates rapidly due to water loss and soft texture. Strawberries should be kept at 0-4°C after harvest to retain fruit quality (Khreba *et al*., 2014). Postharvest application of $CaCl_2$ employed directly on fruit surface is best method for increasing internal calcium content and extending shelf life (Conway *et al*., 1994). Calcium chloride as an inorganic salt plays significant role for retaining fruit quality and firmness. It is involved in maintaining fruit firmness because major component of pectin which improved the inflexibility of cells, membrane rigidity and maintained cell structure (Maas, 1998; Sams, 1999).

Salicylic acid reported as signaling hormone provides resistance against oxidative stresses inhibits ethylene production and also delay senescence process in fruits during storage (Asghari and Aghdam, 2010). Salicylic acid and its derivatives including acetyl salicylic acid have capability for delaying ripening process, maintaining quality and controlling postharvest diseases of fruits (Zhang *et al*., 2010). Exogenous application of SA induces systemic acquired resistance at the site of pathogen attack and also produces pathogenesis related proteins in plants (Beckers and Spoel, 2006). Pre and postharvest application of SA on strawberry cultivar 'Camarosa' delayed the ripening of strawberry fruit, increased vitamin C contents due to slow down of metabolic activities and carbohydrate depletion rate (Lolaei *et al*., 2012). Application of SA during storage slowed down the ripening process of kiwi fruit due to reduction in ethylene production (Zhang *et al*., 2003). In literature it was reported that SA prevented fungal decay in peaches and grapes (Wang *et al*., 2006; Ranjbaran *et al*., 2011). During storage, application of SA enhanced defense system by increasing activities of antioxidant enzymes which improved resistance against fungal attack (Xu and Tian, 2008). Salicylic acid delays fruit ripening and softening process in banana fruit by delaying the activity of cell wall degrading enzymes (Srivastava and Dwivedi, 2000). In Pakistan synthetic fungicides are used to control postharvest decay of fruits which contained harmful chemicals that showed harmful effects on human health (Babalar*et al*., 2007). Salicylic acid is used as an alternative to chemical fungicides for extending shelf life of fruits (Khademi and Ershadi, 2013). Calcium chloride ($CaCl_2$) and Salicylic acid (SA) have potential for commercial control of qualitative properties and extend the shelf life of harvested fruits. Therefore, the aim of present research was to optimize the best concentration of $CaCl_2$ and SA which extend the shelf life and maintain the quality of strawberry fruit during 15 days of storage at 4°C.

MATERIALS AND METHODS

***Experimental Material*:** Marketable strawberries were collected from strawberry farm (Sharaqpur Sharif Lahore, Pakistan) during March, 2017. After harvesting fruit was transported to the lab of Post-Harvest Research Centre, AARI, Faisalabad (30°31 N and 73°74 E). Strawberries were pre cooled at 0°C to remove the field heat. This experiment was consisted of seven treatments with 4 replications in each treatment and there were 60 strawberries per replication. For this experiment 1680 marketable strawberries were used. Different treatments were used including T_1 (Control), T_2 (2 mM $CaCl_2$), T_3 (4 mM $CaCl_2$), T_4 (6 mM$CaCl_2$), T_5 (3 mM SA), T_6 (5 mM SA), T_7 (7 mM SA). Each concentration was used for one liter of water and strawberries were dipped in different concentrations of solutions for 10 minutes. After dipping strawberries were dried at room temperature (25°C) and then packed in plastic punnets and stored at 4°C, 80-85% RH for 15 days. All fruit quality parameters were analyzed during 0, 3, 6, 9, 12 and 15 days of interval.

***Physical parameters*:**

***Fruit weight loss (%)*:** Fruit weight loss was recorded before and during each storage interval by using formula:

Weight loss (%) = [Initial weight of strawberries - Final weight of strawberries × 100/Initial weight of strawberries]

***Fungal decay (%)*:**

Decay (%) was recorded during 3, 6, 9, 12 and 15 days of storage interval by using following formula.

Fungal decay (%) = [Number of diseased and decayed strawberries per treatment× 100/Total number of strawberries per treatment]

***Firmness (kg. cm^{-2})*:** Fruit firmness was measured with digital penetrometer (Humboldt H-1240D) by using 3 mm diameter probe which measure the penetration force.

Biochemical parameters

***TSS (^{o}Brix), Titratable acidity (%) and TSS: TA ratio*:** Total soluble solids were measured with digital refractrometer (Atago, Japan). Titratable acidity of strawberry juice was determined by method given by Hortwitz (1960). 10 ml of strawberry juice was taken in 100 ml conical flask, dilution was made up to 50 ml with distilled water and titrated against 0.1 N NaOH, using 2- 3 drops of phenolphthalein as an indicator till pink color end point was obtained. Calculations were made according to the formula:

TA (%) = [0.1 N NaOH× 0.0064 ×100/ml juice used]. The ratio was measured by dividing TSS/TA.

***Vitamin C (mg 100 g^{-1})*:** For the measurement of vitamin C contents in strawberry extract, first of all strawberry extract was filtered then (10 ml) aliquot was taken in (100 ml) volumetric flask and volume was made up to mark after addition of (0.4%) oxalic acid. For titration purpose (5 ml) aliquot was taken in beaker and titration was done with 2, 6-dichlorophenol indophenol when pink color appeared it was indication of end point. Calculations were done with the method described by Ruck (1969).

***Total phenolic contents (mg GAE 100 g^{-1})*:** For the determination of TPC of strawberry Folin Ciocalteu (FC) method was used. Extracted sample (100 µL) and FC reagent (200 µL) was taken in centrifuge tube and vortexed only for one minute. After it sodium carbonate (800 µL) was added in it and it was vortexed again only for one minute. Incubation was done for 2 hours at room temperature and then it was added into a 96-well plate and read at 765 nm using spectrophotometer against the standard curve of Gallic acid (R^2 = 0.7884). Calculations were performed according to method as described by Ainsworth and Gillespie (2007).

***Total antioxidants (% DPPH)*:** For TA determinations 50 µL supernatant and methanolic solution 0.004 % (5 ml) was taken in test tube. Methanolic solution expressed in (2, 2-

diphenyl-1 picrylhydrazyl radical) % DPPH. Samples were tested with the interval of 30 minutes. Changes in absorbance were measured at 517 nm by microplate reader using spectrophotometer. Calculations were performed by using method described by Brand-William *et al*. (1995).

Activities of anti-oxidative enzymes

***Catalase (U mg^{-1} protein)*:** For CAT activity hydrogen peroxide was used to initiate the reaction mixture. Enzyme extract (100 µL) was mixed in H_2O_2 (100 µL) and then activity was observed at 240 nm by microplate reader. Calculations were performed by using method described by Jimenez *et al*. (2003) and Liu *et al*. (2009).

***Peroxidase (U mg^{-1} protein*):** For POD activity reaction mixture was prepared by using potassium phosphate buffer (0.1 M), hydrogen peroxide (40 mM) and guaiacol (20 mM) with different ratios (8: 1: 1). Enzyme extract (100 µL) was added in reaction mixture (100 µL) and then absorbance was noted at 470 nm by using microplate reader. Calculations were performed by using method described by Liu *et al*., 2009.

***Superoxide dismutase (U mg^{-1} protein*):** For estimation of SOD activity nitro blue tetrazolium was used for 50% inhibition of photochemical reduction. Reaction mixture was prepared by adding phosphate buffer (500 µL), methionine (200 µL), nitro blue tetrazolium (100 µL), Triton X, (200 µL), riboflavin (100 µL), distilled water (800 µL) and enzyme extract (100 µL). Illumination of this mixture was done with fluorescent lamp. Absorbance was noted at 560 nm by using microplate reader. Calculations were performed by using method described by Jimenez *et al*. (2003) and Stanger and Popovic (2009).

***Statistical Analysis*:** Results were statistically analyzed by using (MINITAB®18.0 and SPSS 21 Software). Completely randomized design (CRD) with two factors factorial arrangement was used. To test the overall significance of data ANOVA techniques were employed. To compare the differences among treatment means ($P \leq 0.05$) least significant difference (LSD) test was used (Steel *et al*., 1997).

RESULTS

Physical Parameters

***Fruit weight loss (%)*:** Fruit weight was significantly reduced in stored strawberry fruit of all treatments with continuation of storage. Maximum weight loss (15.08%) was found in control treatment while minimum (6.08%) was noticed in fruits treated with 6 mM $CaCl_2$ after 15 days of storage (Table 1). Interaction effects also revealed that maximum weight loss increased in all treated fruits after 9 days but in control treatment it was started after 3 days of storage. In all treatments $CaCl_2$ (6 mM) acted as barrier to reduce weight loss during storage.

***Fungal decay (%)*:** Fruit decay (%) was enhanced with

Table 1. Effect of $CaCl_2$ and SA treatments on fruit weight loss (%) of strawberry stored for 15 days at 4ºC.

Treatments	Days						Mean
	0	3	6	9	12	15	
Control	0.00 x	8.05 e	9.05 d	11.08 c	13.08 b	15.08 a	**9.39 A**
2 mM $CaCl_2$	0.00 x	3.80 pq	4.23 no	5.64 k	6.63 h	7.92 f	**4.70 B**
4 mM $CaCl_2$	0.00 x	3.53 qrs	3.34 st	4.50 mn	5.74 jk	6.89 h	**4.00 D**
6 mM $CaCl_2$	0.00 x	2.04 w	3.01 uv	4.02 op	5.08 l	6.08 i	**3.37 E**
3 mM SA	0.00 x	3.64 qr	4.29 no	5.14 l	6.76 h	7.95 f	**4.63 B**
5 mM SA	0.00 x	2.75 v	3.12 tu	4.13 o	5.15 l	6.13 hi	**3.55 D**
7 mM SA	0.00 x	3.46 rs	3.45 rs	4.77 m	5.93 ij	7.44 g	**4.17 C**
Mean	**0.00 F**	**3.89 E**	**4.35 D**	**5.61 C**	**6.91 B**	**8.21 A**	
Note:	LSD (P=0.05) treatments = 0.11; LSD days = 0.10; LSD interaction = 0.28 CV =3.51%						

Treatments mean which represent same letter are statistically non-significant (P>0.05). Small letters indicate interaction effects and capital letters indicate overall mean.

Table 2. Effect of $CaCl_2$ and SA treatments on fungal decay (%) of strawberry stored for 15 days at 4ºC.

Treatments	Days						Mean
	0	3	6	9	12	15	
Control	0.00 r	21.00 d	47.83 c	53.50 b	53.33 b	56.80 a	**38.74 A**
2 mM $CaCl_2$	0.00 r	1.50 pq	3.50 m	7.50 g	8.50 f	10.50 e	**5.25 B**
4 mM $CaCl_2$	0.00 r	1.13 q	1.50 pq	2.50 no	3.50 lm	5.50 i	**2.35 F**
6 mM $CaCl_2$	0.00 r	1.23 pq	2.00 op	5.00 ij	5.50 i	7.50 g	**3.54 D**
3 mM SA	0.00 r	1.35 pq	2.50 no	4.00 kl	6.50 h	8.50 f	**3.81 C**
5 mM SA	0.00 r	1.00 q	1.00 q	1.50 pq	2.50 no	3.50 lm	**1.58 G**
7 mM SA	0.00 r	1.10 q	1.50 pq	3.00 mn	4.50 jk	6.50 h	**2.77 E**
Mean	**0.00 F**	**4.04 E**	**8.55 D**	**11.00 C**	**12.05 B**	**14.11 A**	
Note:	LSD (P=0.05) treatments = 0.33; LSD days = 0.31; LSD interaction = 0.82						

expansion in storage days however this increase was more in control treatment. In control treatment maximum decay problem was started during initial 6 days of storage while in treated fruits decay was minimum during entire storage. After 15 days of storage interval, control treatment showed maximum fungal decay (56%) while those fruits treated with 5 mM SA showed minimum decay (3.5%). Overall, mean values of treatments showed that different concentrations of $CaCl_2$ and SA were proved better to minimize the decay incidence during entire storage as compared to control treatment (Table 2).

***Firmness (kg. cm^{-2})*:** Strawberry firmness was progressively declined during storage but fruits treated with different concentrations of $CaCl_2$ and SA maintained fruit firmness during entire storage. After 15 days maximum strawberry firmness (0.42 kg. cm^{-2}) was retained with 6 mM $CaCl_2$ as compared to other treatments. The response of control treatment and lower doses of $CaCl_2$ and SA was non-significant after 15 days. Interaction effects also exhibited that maximum decreasing trend in firmness was noticed in all treated fruits after 12 days of storage while in control fruits it was noted after 6 days of storage (Table 3).

Fruit quality parameters

***TSS (ºBrix)*:** Mean values of treatments demonstrated that maximum TSS contents (8.14 ºBrix) were noted in control treatment while minimum (6.52 ºBrix) were observed with 5 mM SA at the end of storage. Strawberries which were treated with different concentrations of $CaCl_2$ and SA retained TSS contents during storage (Table 4). Interaction effects also indicated that retention of TSS contents was noted in treated fruits while gradual increase in TSS contents was noticed in control treatment during overall storage.

***Titratable acidity (%)*:** After 15 days of storage higher TA (0.62%) was exhibited from strawberry fruits treated with 5 mM SA while minimum TA (0.35%) was observed with control treatment (Table 5). During storage acid contents were rapidly decreased in control fruits due to maximum increment in TSS contents. In treated fruits, due to retention of TSS contents minimum loss of acid contents was observed. Overall, the response of SA (5 mM) was highly effective for retaining maximum acid contents of strawberry fruit as compared to other treatments.

***TSS: TA ratio*:** TSS: TA ratio is an important indicator of strawberry quality which depicts the shelf life of fruits.

Table 3. Effect of $CaCl_2$ and SA treatments on firmness (kg. cm^{-2}) of strawberry stored for 15 days at 4ºC.

Treatments	Days						Mean
	0	3	6	9	12	15	
Control	0.90 a	0.58 m	0.32 z	0.29 z	0.27 z	0.23 z	**0.43 F**
2 mM $CaCl_2$	0.91 a	0.72 f	0.67 i	0.55 o	0.43 u	0.34 z	**0.60 E**
4 mM $CaCl_2$	0.92 a	0.74 de	0.68 h	0.56 n	0.48 s	0.37 x	**0.62 C**
6 mM $CaCl_2$	0.89 a	0.81 b	0.74 e	0.62 l	0.52 q	0.42 v	**0.67 A**
3 mM SA	0.91 a	0.64 k	0.61 k	0.53 p	0.44 u	0.32 z	**0.58 E**
5 mM SA	0.92 a	0.78 c	0.70 g	0.58 m	0.49 r	0.39 w	**0.64 B**
7 mM SA	0.91 a	0.75 d	0.65 j	0.55 o	0.45 u	0.36 y	**0.61 D**
Mean	**0.91 A**	**0.72 B**	**0.63 C**	**0.53 D**	**0.44 E**	**0.34 F**	
Note:	LSD (P=0.05) treatments = 3.46; LSD days = 3.20; LSD interaction = 8.47 CV = 1.02%						

Treatments mean which represent same letter are statistically non-significant (P> 0.05). Small letters indicate interaction effects and capital letters indicate overall mean.

Table 4. Effect of $CaCl_2$ and SA treatments on TSS (ºBrix) of strawberry stored for 15 days at 4ºC.

Treatments	Days						Mean
	0	3	6	9	12	15	
Control	6.01 x	7.88 i	8.20 g	8.73 d	8.95 b	9.30 a	**8.14 A**
2 mM $CaCl_2$	6.04 x	6.65 p	7.15 m	7.65 j	7.85 i	8.85 c	**7.33 B**
4 mM $CaCl_2$	6.00 x	5.83 x	6.10 v	6.43 rs	7.10 m	8.10 h	**6.56 E**
6 mM $CaCl_2$	6.02 x	6.33 t	6.50 qr	6.75 o	7.43 l	8.45 e	**6.88 D**
3 mM SA	6.04 x	6.45 r	6.90 n	7.13 m	7.58 jk	8.65 d	**7.08 C**
5 mM SA	6.04 x	6.00 w	6.28 tu	6.35 st	6.83 no	7.85 i	**6.52 F**
7 mM SA	6.01 x	6.23 u	6.55 q	6.88 n	7.55 k	8.35 f	**6.89 D**
Mean	**6.02 F**	**6.48 E**	**6.81 D**	**7.13 C**	**7.61 B**	**8.51 A**	
Note:	LSD (P=0.05) treatments = 0.03; LSD days = 0.03; LSD interaction = 0.09 CV = 0.93%						

Treatments mean which represent same letter are statistically non-significant (P> 0.05). Small letters indicate interaction effects and capital letters indicate overall mean.

Balance between TSS: TA ratio is essential for maximum shelf life of fruits. After 15 days maximum ratio (26.96) was noticed in control treatment as compared to other treatments. Increment in TSS: TA ratio was more in control treatment due to maximum increase in TSS contents and reduction in acid contents during storage while in treated fruits this ratio was intermediate. Irrespective to treatments, increasing trend was noticed regarding TSS: TA ratio during each storage interval (Table 6).

***Vitamin C (mg 100g^{-1})*:** Significant differences were observed among treatments, storage days and their interaction effects regarding vitamin C contents of strawberry fruit. Interaction effects revealed that as the storage period prolonged vitamin C contents decreased. Increment in vitamin C contents was observed in treated fruits during 3-9 days and then gradually decreased while in control fruits increasing trend was noticed during 3-6 days of storage then decreased. Maximum retention of vitamin C contents (43.90 mg 100 g^{-1}) was noted in fruits treated with 6 mM $CaCl_2$ as compared with control (24.90 mg 100 g^{-1}) after 15 days of storage (Table 7).

***Total phenolic contents (mg GAE 100g^{-1})*:** Total phenolic contents (59 mg GAE 100 g^{-1}) rapidly decreased in control treatment while maximum values for total phenolic contents (132.75mg GAE 100 g^{-1}) were noted in fruits treated with 6 mM $CaCl_2$ (Table 8). Treatments including 4 mM $CaCl_2$ and 7 mM SA were statistically at par with each other. Interaction effects also revealed that increasing trend was observed in treated fruits regarding TPC during 3-9 days of storage while in control treatment it was noticed during 3-6 days of storage then gradually decreased. Results demonstrated that the response of $CaCl_2$ (6 mM) was highly effective for retaining maximum TPC during storage.

***Total antioxidants (% DPPH)*:** Maximum antioxidant activities (39.0% DPPH) after 15 days were noted in strawberry extract treated with 5 mM SA compared to control (28.75% DPPH) (Table 9). The response of control treatment and lower doses of SA and $CaCl_2$ was similar when analyzed after 15 days of storage and these treatments were statistically at par with each other. Interaction between treatments and storage days exhibited that antioxidant activity of all treated strawberry fruits increased during 3-9

Table 5. Effect of $CaCl_2$ and SA treatments on Titratable acidity (%) of strawberry stored for 15 days at 4ºC.

Treatments	Days						Mean
	0	3	6	9	12	15	
Control	1.00 a	0.55 u	0.42 x	0.39 y	0.38 z	0.35 a	**0.51 G**
2 mM $CaCl_2$	1.01 a	0.81 g	0.75 j	0.63 p	0.58 s	0.50 w	**0.71 F**
4 mM $CaCl_2$	1.02 a	1.00 b	0.84 e	0.79 h	0.68 n	0.59 rs	**0.82 B**
6 mM $CaCl_2$	1.01 a	0.88 d	0.79 h	0.68 n	0.62 q	0.55 u	**0.75 D**
3 mM SA	1.01 a	0.83 f	0.73 k	0.64 o	0.60 r	0.52 v	**0.72 E**
5 mM SA	1.00 a	1.01 ab	0.91 c	0.82 f	0.71 l	0.62 q	**0.85 A**
7 mM SA	1.00 a	0.88 d	0.77 i	0.69 m	0.63 op	0.57 t	**0.76 C**
Mean	**1.01 A**	**0.85 B**	**0.74 C**	**0.66 D**	**0.60 E**	**0.53 F**	
Note:	LSD (P=0.05) treatments = 3.24; LSD days = 3.0; LSD interaction = 7.95 CV = 4.78%						

Treatments mean which represent same letter are statistically non-significant (P> 0.05). Small letters indicate interaction effects and capital letters indicate overall mean.

Table 6. Effect of $CaCl_2$ and SA treatments on TSS: TA ratio of strawberry stored for 15 days at 4ºC.

Treatments	Days						Mean
	0	3	6	9	12	15	
Control	5.71 v	14.45 h	19.76 d	22.23 c	23.87 b	26.96 a	**18.84 A**
2 mM $CaCl_2$	5.70 v	8.26 qr	9.54 p	12.14 l	13.48 j	17.62 e	**11.13 B**
4 mM $CaCl_2$	5.72 v	5.84 v	7.31 t	8.19 r	10.52 n	13.79 i	**8.56 F**
6 mM $CaCl_2$	5.74 v	7.23 t	8.28 qr	10.00 o	11.98 l	15.51 g	**9.79 D**
3 mM SA	5.74 v	7.82 s	9.52 p	11.13 m	12.73 k	16.64 f	**10.60 C**
5 mM SA	5.73 v	5.97 v	6.88 u	7.75 s	9.58 p	12.77 k	**8.11 G**
7 mM SA	5.76 v	7.08 tu	8.51 q	10.04 o	11.94 l	14.72 h	**9.67 E**
Mean	**5.74 F**	**8.09 E**	**9.97 D**	**11.64 C**	**13.44 B**	**16.86 A**	
Note:	LSD (P=0.05) treatments = 0.12; LSD days = 0.11; LSD interaction = 0.29 CV = 1.92%						

Treatments mean which represent same letter are statistically non-significant (P> 0.05). Small letters indicate interaction effects and capital letters indicate overall mean.

Table 7. Effect of $CaCl_2$ and SA treatments on vitamin C (mg $100g^{-1}$) contents of strawberry stored for 15 days at 4ºC.

Treatments	Days						Mean
	0	3	6	9	12	15	
Control	41.50 rs	45.85 v	49.15 z	33.65 z	28.08 z	24.90 z	**32.52 G**
2 mM $CaCl_2$	43.50 rs	49.00 p	50.68 ln	55.25 g	42.98 s	33.13 y	**45.75 E**
4 mM $CaCl_2$	42.50 rs	51.50 lm	53.03 ij	60.98 e	46.05 q	36.45 w	**48.58 C**
6 mM $CaCl_2$	44.50 rs	53.75 hi	69.03 c	74.28 a	52.73 jk	43.90 r	**56.20 A**
3 mM SA	43.50 rs	49.58 op	50.58 n	53.78 h	41.90 t	31.03 z	**45.06 F**
5 mM SA	40.50 rs	53.28 hij	63.28 d	70.00 b	50.18 no	39.95 u	**53.36 B**
7 mM SA	43.50 rs	50.80 mn	52.08 kl	58.85 f	43.95 r	34.98 x	**47.36 D**
Mean	**43.50 D**	**49.39 C**	**52.83 B**	**57.54 A**	**43.69 D**	**34.90 E**	
Note:	LSD (*P*=0.05) treatments = 0.30; LSD days = 0.28; LSD interaction = 0.74 CV = 1.13%						

Treatments mean which represent same letter are statistically non-significant (*P*> 0.05). Small letters indicate interaction effects and capital letters indicate overall mean.

Table 8. Effect of $CaCl_2$ and SA treatments on total phenolic contents (mg GAE $100g^{-1}$) of strawberry stored for 15 days at 4ºC.

Treatments	Days						Mean
	0	3	6	9	12	15	
Control	151.00 p	160.75 r	162.50 y	101.00 z	69.75 z	59.00 z	**101.33 F**
2 mM $CaCl_2$	152.00 p	165.50 l	177.50 hi	184.50 f	157.50 o	109.75 w	**158.29 D**
4 mM $CaCl_2$	155.00 p	172.50 j	180.50 g	195.50 cd	160.50 n	123.75 u	**164.63 C**
6 mM $CaCl_2$	155.00 p	184.75 f	207.50 b	218.50 a	177.00 hi	132.75 s	**179.25 A**
3 mM SA	154.00 p	163.50 m	178.75 gh	192.00 e	151.00 q	107.50 x	**157.96 E**
5 mM SA	155.00 p	176.50 i	195.00 d	207.50 b	167.00 l	126.50 t	**171.25 B**
7 mM SA	157.00 p	170.50 k	183.25 f	197.00 c	162.75 m	120.25 uv	**164.79 C**
Mean	**155.00 D**	**168.14 C**	**173.86 B**	**183.00 A**	**149.36 E**	**111.36 F**	
Note:	LSD (*P*=0.05) treatments = 0.78; LSD days = 0.73; LSD interaction = 1.93 CV = 0.88%						

Treatments mean which represent same letter are statistically non-significant (*P*> 0.05). Small letters indicate interaction effects and capital letters indicate overall mean.

Table 9. Effect of $CaCl_2$ and SA treatments on total antioxidant (% DPPH) activities of strawberry stored for 15 days at 4ºC.

Treatments	Days						Mean
	0	3	6	9	12	15	
Control	42.00 r	44.50 t	48.25 w	34.50 z	31.25 z	28.75 z	**35.88 F**
2 mM $CaCl_2$	41.00 r	45.25 o	51.50 k	58.75 f	38.75 v	31.25 z	**44.75 E**
4 mM $CaCl_2$	44.00 r	51.75 k	57.50 g	71.50 b	44.50 p	35.50 y	**50.63 B**
6 mM $CaCl_2$	45.00 r	48.50 m	53.25 j	60.75 e	41.50 s	33.25 z	**46.71 D**
3 mM SA	43.00 r	45.50 o	52.00 k	57.25 g	39.50 u	32.50 z	**44.96 E**
5 mM SA	43.00 r	55.50 h	63.50 d	76.50 a	47.00 n	39.00 uv	**54.08 A**
7 mM SA	41.00 r	50.25 l	54.50 i	64.50 c	43.75 q	36.50 x	**48.75 C**
Mean	**43.00 D**	**48.18 C**	**52.79 B**	**60.54 A**	**40.89 E**	**33.82 F**	
Note:	LSD (*P*=0.05) treatments = 0.28; LSD days = 0.26; LSD interaction = 0.68 CV = 1.06%						

Treatments mean which represent same letter are statistically non-significant (*P* > 0.05). Small letters indicate interaction effects and capital letters indicate overall mean.

days and then gradually decreased while in fruits of control treatment increasing trend was observed during 3-6 days then decreased. Salicylic acid treatments showed superiority for retaining maximum total antioxidants during storage.

Activities of anti-oxidative enzymes

***Catalase (U mg^{-1} protein)*:** Catalase activity was significantly increased with different concentrations of $CaCl_2$ and SA; and decreased with the advancement of storage days. Maximum CAT activity (12.8 U mg^{-1} protein) was noted in the strawberry fruits treated with 5 mM SA while minimum activity (10.2 U mg^{-1} protein) was observed

Table 10. Effect of $CaCl_2$ and SA treatments on catalase (U mg^{-1} protein) activity of strawberry stored for 15 days at 4ºC.

Treatments	Days						Mean
	0	3	6	9	12	15	
Control	10.6 o	11.7 l	11.8 k	10.4 r	10.2 s	10.2 s	**10.8 G**
2 mM $CaCl_2$	10.1 o	11.8 k	12.9 h	11.7 l	10.5 q	10.4 r	**11.3 F**
4 mM $CaCl_2$	10.0 o	12.9 h	13.7 e	12.9 h	11.7 l	10.6 p	**12.1 C**
6 mM $CaCl_2$	10.8 o	12.8 i	13.5 f	12.8 hi	11.6 m	10.5 q	**12.0 D**
3 mM SA	10.8 o	11.8 k	12.9 h	11.7 l	11.3 n	11.3 n	**11.6 E**
5 mM SA	10.5 o	13.0 g	14.2 a	14.0 b	13.8 d	12.8 i	**13.1 A**
7 mM SA	10.8 o	12.9 h	13.9 c	13.9 c	12.7 j	11.6 m	**12.6 B**
Mean	**10.8 E**	**12.4 B**	**13.2 A**	**12.5 B**	**11.6 C**	**11.1 D**	
Note:	LSD (P=0.05) treatments = 4.84; LSD days = 4.48; LSD interaction = 9.31 CV = 3.17%						

Treatments mean which represent same letter are statistically non-significant (P > 0.05). Small letters indicate interaction effects and capital letters indicate overall mean.

Table 11. Effect of $CaCl_2$ and SA treatments on superoxide dismutase (U mg^{-1} protein) activity of strawberry stored for 15 days at 4ºC.

Treatments	Days						Mean
	0	3	6	9	12	15	
Control	16.2 s	18.4 pq	20.2 o	18.2 q	17.1 r	16.1 t	**17.7 G**
2 mM $CaCl_2$	16.3 s	19.3 op	23.4 l	19.3 op	18.2 q	17.2 r	**19.0 F**
4 mM $CaCl_2$	16.3 s	23.5 l	32.5 c	30.5 e	26.4 i	21.3 n	**25.0 C**
6 mM $CaCl_2$	16.1 s	22.4 m	29.5 f	28.3 g	24.3 k	19.2 p	**23.3 D**
3 mM SA	16.0 s	19.3 op	25.5 j	24.4 k	22.2 lm	18.2 q	**21.0 E**
5 mM SA	16.3 s	25.5 j	34.7 a	31.5 cd	28.4 fg	24.3 k	**27.0 A**
7 mM SA	16.7 s	24.4 k	33.5 b	31.4 d	27.3 h	23.2 l	**26.0 B**
Mean	**16.3 F**	**21.8 D**	**28.5 A**	**26.2 B**	**23.4 C**	**20.0 E**	
Note:	LSD (P=0.05) treatments = 3.45; LSD days = 3.19; LSD interaction = 8.45 CV = 1.71%						

Treatments mean which represent same letter are statistically non-significant (P > 0.05). Small letters indicate interaction effects and capital letters indicate overall mean.

Table 12. Effect of $CaCl_2$ and SA treatments on peroxidase (U mg^{-1} protein) activity of strawberry stored for 15 days at 4ºC.

Treatments	Days						Mean
	0	3	6	9	12	15	
Control	0.41 q	0.46 p	0.35 u	0.24 x	0.21 y	0.15 z	**0.31 G**
2 mM $CaCl_2$	0.45 q	0.48 o	0.58 h	0.41 r	0.31 uv	0.26 wx	**0.41 F**
4 mM $CaCl_2$	0.44 q	0.57 i	0.70 c	0.59 g	0.49 n	0.35 u	**0.53 B**
6 mM $CaCl_2$	0.45 q	0.51 m	0.62 e	0.52 l	0.41 r	0.28 w	**0.46 D**
3 mM SA	0.43 q	0.49 n	0.59 g	0.48 o	0.38 t	0.26 wx	**0.44 E**
5 mM SA	0.45 q	0.60 f	1.06 a	0.82 b	0.56 j	0.39 s	**0.64 A**
7 mM SA	0.42 q	0.53 k	0.63 d	0.53 k	0.45 q	0.29 v	**0.48 C**
Mean	**0.45 C**	**0.52 B**	**0.65 A**	**0.51 B**	**0.40 D**	**0.28 E**	
Note:	LSD (P=0.05) treatments = 3.48; LSD days = 3.23; LSD interaction = 8.54 CV = 1.32%						

Treatments mean which represent same letter are statistically non-significant (P > 0.05). Small letters indicate interaction effects and capital letters indicate overall mean.

in control treatment after 15 days (Table 10). The response of different doses of SA was highly effective for retaining maximum CAT activity during storage. Catalase activity was increased from 3-6 days of storage then decreased in all treatments.

***Superoxide dismutase (U mg^{-1} protein)*:** Increasing trend was observed regarding SOD activity during storage. Fruits treated with 5 mM SA exhibited maximum SOD activity (24.3 U mg^{-1} protein) after 15 days of storage (Table 11). Control treatment showed maximum decline in SOD activity

(16.1 U mg^{-1} protein) during entire storage. Superoxide dismutase activity was increased from 3-6 days of storage then decreased. From results it was concluded that SA (5 mM) dipping application showed superiority over all treatments for retaining maximum SOD activity during storage.

***Peroxidase (U mg^{-1} protein)*:** Maximum POD activity (0.39 U mg^{-1} protein) was exhibited when strawberry fruits treated with 5 mM SA after 15 days as compared to other treatments (Table 12). Peroxidase activity was increased in treated fruits during 3-6 days while in control fruits increasing trend was observed from 0-3 days. Fruits treated with different concentrations of $CaCl_2$ and SA retained POD activity during storage while in control treatment minimum activity (0.15 U mg^{-1} protein) was observed. From results it was observed that medium concentration of SA (5 mM) retained maximum POD activity in strawberry fruits during storage.

DISCUSSION

Strawberry is very sensitive fruit crop which requires careful handling and appropriate management practices to retain fruit quality after harvest (Picha, 2006).

In present study increasing trend in fruit weight loss was noted during storage. It could be due to higher metabolic activity of fruit which occurred due to depletion of moisture from surface of fruit because of higher transpiration process which continued during whole storage (Boynton *et al*., 2005; Cordenunsi *et al*., 2005; Hussain *et al*., 2012). Maximum weight loss was found in control treatment after 15 days while minimum was noticed in fruits treated with $CaCl_2$ (6 mM) followed by other treated fruits (Table 1). The lower weight reduction in $CaCl_2$ treated strawberries showed the superiority of $CaCl_2$ to reduce the weight loss during entire storage compared to control. Our results regarding weight loss (%) of strawberry are also in association with those of Pila *et al*. (2010) who reported that $CaCl_2$ on fruit surface acted as physical barrier reduced the rapid metabolic activity due to its anti-senescent properties, so water loss from fruit surface reduced and shelf life of tomato increased during storage. Bagheri *et al*. (2015) also evaluated that weight loss in persimmon fruit decreased with $CaCl_2$ treatments (0.5, 1, and 2%) as compared to control.

Strawberry decay is still serious problem during storage which is caused by fungal pathogens. At the end of storage, fruits from control treatment showed maximum decay problem while fruits treated with SA (5 mM) showed minimum decay (Table 2). Salicylic acid reduced decay incidence during storage because it increased the activity of pathogenesis related proteins (chitinase and β-1, 3-glucanase) which played significant role in enhancing defense mechanism in fruits against oxidative damage caused by ROS species (Meena *et al*., 2001; Hussain *et al*., 2015). Postharvest application of SA also delays fruit softening, fruit ripening and senescence process due to inhibition of ethylene process and also beneficial for reduction of fungal decay of strawberries (Zhang *et al*., 2010). Our findings regarding SA minimized decay during storage are similar with those of Baninaiem *et al*. (2016) who exhibited that different concentrations of SA (1, 2 and 4 mM) reduced the decay incidence of tomato fruit when stored at 10°C for 40 days. Therefore, it was noted from results that different concentrations of $CaCl_2$ and SA effectively minimized the decay incidence and also acted as barrier for nutrients availability to pathogens on the fruit surface (Moline, 1994).

Strawberry firmness is a major fruit quality index during storage which was positively affected by different concentrations of $CaCl_2$ and SA as compared to control treatment. After 15 days of storage maximum strawberry firmness was achieved with $CaCl_2$ (6 mM) as compared to other treatments (Table 3). Firmness decreased during storage due to higher metabolic activity of strawberries after harvest which caused more water loss from fruit surface and cells lose their rigidity (Conway *et al*., 1994; Maas, 1998). Our results regarding firmness suggested that higher concentration of $CaCl_2$ (6 mM) was more effective for maintaining strawberry firmness during storage. It is because of dipping application of $CaCl_2$ improves the fruit cell wall pectin due to that membrane rigidity and cell structure maintained during storage (Sams, 1999; Franco *et al*., 2008). In some previous studies it was reported that dipping treatment of $CaCl_2$ (2%) solution maintained firmness of peaches when stored at 4°C for 2 weeks (Lysiak *et al*., 2008). In some other studies it was also observed that salt solution of $CaCl_2$ (3%) maintained the firmness of nectarines during storage (Manganaris *et al*., 2005).

Application of different concentrations of $CaCl_2$ and SA effectively maintained the TSS contents of strawberry during storage (Table 4). Increase in TSS contents during storage was due to higher respiration process of fruit which converted starch contents into sugars (Avina *et al*., 2014). After 15 days of storage maximum TSS contents were recorded in control fruits while minimum were observed in fruits treated with SA (5 mM). Our results are in accordance with previous findings where SA application leads to reduction in invertase enzymatic activity and reduced maximum increment in TSS contents of banana fruit during storage (Srivastava and Dwivedi, 2000). Decreasing trend was observed regarding acid contents of strawberry fruit during storage because of conversion of acids into sugars but the response of SA (5 mM) was highly effective for retaining strawberry acid contents as compared to other treatments (Table 5). Our findings regarding acid contents of strawberry are in association with previous study who reported that titratable acidity was more in SA treated tomatoes as compared to control (Baninaiem *et al*., 2016). The level of TSS: TA ratio was found higher among all

treatments during storage period however; this increment was more in control treatment because of maximum reduction in acidity and rapidly increasing TSS and sugar contents (Table 6).

According to our findings vitamin C contents reduced during storage. Degradation process of vitamin C contents during storage due to fluctuation in temperature and higher metabolic activity of fruit (Silva *et al*., 2013). Low temperature during storage cause oxidation of vitamin C contents of fruit (Rapisarda *et al*., 2008). Maximum vitamin C contents were observed in fruits treated with $CaCl_2$ (6 mM) as compared to other treatments after 15 days of storage (Table 7). The reason of decreasing vitamin C contents during storage due to reduction in ascorbate peroxidase enzymatic activity but $CaCl_2$ application enhanced the activity of several catalytic enzymes which played major role in biosynthesis of vitamin C contents (Kadir, 2004). Calcium chloride application is helpful for decreasing oxidation process and retains the vitamin C contents (Davey *et al*., 2000; Ruoyi *et al*., 2005). Our results are also in accordance with those of Akhtar *et al*. (2010) who observed the effects of $CaCl_2$ on storage behavior of loquat cultivar 'Surkh' which were dipped in calcium solutions (1%, 2% and 3%) for 2 minutes and then stored at 4°C for 10 weeks. Minimum loss was observed regarding vitamin C contents when treated with $CaCl_2$ (1% and 2%).

Maximum TPC were noted in strawberry fruits treated with $CaCl_2$ (6 mM) after 15 days of storage (Table 8). Increase in total phenolic contents during storage was due to exposure to low temperature while decrease in TPC was due to reduction in internal resistance of fruits (Silva *et al*., 2013). Our results confirmed the previous findings where storage life and quality of persimmon fruit increased with different concentrations of $CaCl_2$ (0.5, 1 and 2%) and total phenolic contents increased with 2% $CaCl_2$ treatment. Phenolic contents have maximum antioxidant activity for protecting fruit cells against oxidative stress caused by scavenging free radicals (Bagheri *et al*., 2015). In previous studies it was also observed that $CaCl_2$ application increased the phenolic contents in apricot by reduction in softening process, acidity and respiration rate (Ali *et al*., 2013).

Antioxidants (enzymatic and non-enzymatic) plays major role in decreasing oxidative stress in fruits caused by ROS species and also helpful for reducing chronic diseases in humans (Yamaguchi *et al*., 1998). Oxidative damage in fruits recovered with antioxidants like total phenolic contents, flavonoids, anthocyanin's and ascorbic acid contents (Kinsella *et al*., 1993). Maximum total antioxidants were noted in fruits treated with SA (5 mM) followed by other treated fruits while minimum were noticed in control treatment during entire storage (Table 9). These results are supported by previous studies where SA (1 mM) enhanced the antioxidant capacity of cornelian cherry fruit during storage (Dokhanieh *et al*., 2013). Increasing concentration of SA enhanced antioxidant activity in peach fruit (Tareen *et al*., 2012).

During stress condition enzymatic activities (CAT, SOD and POD) protect the cell structure by directly scavenging ROS species (superoxide radicals and hydrogen peroxide) and converted them into less reactive species (Racchi, 2013). In present study higher activities of antioxidant enzymes (CAT, SOD and POD) were noticed in strawberry fruits treated with SA (5 mM) while, lower activities were noticed in control (Table 10, 11, 12). Salicylic acid is an important phenylpropanoid compound which enhanced the fruit resistance against stress causing factors by activating ascorbate peroxidase enzymatic activity which prevents oxidative damage of cells (Stolfa *et al*., 2014). In literature it was reported that postharvest dipping of tomatoes in SA before storage and kept at low temperature induced heat shock proteins (HSPs) biosynthesis which create resistance against low temperature by activating enzymatic activities (Baninaiem *et al*., 2016). During storage peaches treated with SA increased the CAT and SOD activity by reduction in superoxide radical level (Cao *et al*., 2010). Superoxide dismutase is ubiquitous defensive enzyme which has ability to protect the plant cell from damage during stress conditions. Catalase and peroxidase enzymatic activities are also responsible for scavenging ROS species which damage the cells under stress conditions (Hertwig *et al*., 1992; Racchi, 2013). However, decreasing trend regarding enzymatic activities during storage could be due to interaction with low temperature and higher metabolic activities of strawberries.

***Conclusion*:** It is concluded that pre storage dipping of strawberry fruit in $CaCl_2$ (6 mM) and SA (5 mM) for 10 minutes can be used successfully to minimize the decay %, weight loss % and to maintain the quality related parameters of strawberry fruit during 15 days of cold storage (4°C). Compassion of both showed that SA (5 mM) is more effective than $CaCl_2$ to reduce decay % and to maintain TSS, acid contents, total antioxidants and enzymatic activities.

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