Pak. J. Bot., 48(4): 1415-1420, 2016.

EFFECT OF CALCIUM SALT ON SOFT ROT, BITTER PIT AND PHYSICOCHEMICAL PROPERTIES OF STORED APPLES

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Abstract

Postharvest losses are one of the main problems in leading fruit producing countries. Therefore, an attempt has been made to decrease the postharvest losses during apple storage. To achieve this objective, fruits were dipped in 0, 3, 6 and 9% $CaCl_2$ solution for the period of 3, 6, 9 and 12 minutes and were stored for 150 days at 5 ± 1°C with 60–70% relative humidity. After the storage time, fruits were analysed for percent weight loss, total sugar, bitter pit incidence, soft rot, ascorbic acid and firmness. The ascorbic acid contents and firmness of the fruits significantly increased, when the fruits were dipped in 9% $CaCl_2$ solution as compared to control (dipped in water). Whereas, weight loss, total sugar, bitter pit and soft rot incidences were reduced with increased concentration of $CaCl_2$. The firmness of the fruits dipped for 3 minutes in a $CaCl_2$ solution was significantly lower in comparison to the fruits dipped for 12 minutes in a $CaCl_2$ solution. Likewise, the weight loss, bitter pit and the soft rot incidence reduced with increase in dipping duration in $CaCl_2$ solution from 3 minutes to 12 minutes.

Key words: Calcium salts, Apple firmness, Bitter pit, Soft rot, Shelf life.

Introduction

Apple fruit is generally stored in cold storage because of consumer demand throughout the year. Apple is prone to postharvest losses due to high perishability (Hussain *et al.*, 2012). The storage life is limited because of various reasons i.e. loss of firmness (Jan *et al.*, 2015), loss of chemical quality (Golias *et al.*, 2008), physiological disorders (Hayat *et al.*, 2005) and disease incidence or decay (Jan *et al.*, 2013). These losses along with loss in flavour can be minimized by foliar application of Ca and amino acids (Gou *et al.*, 2015). Various attempts have been made to explore different methods to decrease the postharvest losses during storage (Gupta & Jawandha, 2010; Tengku Muda Mohamed *et al.*, 2008).

Calcium (Ca) is an important macronutrient, which play an important role in regulating the metabolism in fruits. In orchards fruit may experience Ca deficiency despite of high Ca content, which may lead to several physiological disorders like loss in firmness, bitter pit, soft pit etc. Generally the apple fruits containing less than 50 mg kg^{-1} Ca on fresh weight basis are sensitive to rot and internal breakdown (Jan*, et al.*, 2015; Ullah *et al.*, 2007). But adequate Ca helps to maintain apple fruit firmness and decreases the incidence of postharvest decay (Jan*, et al.*, 2015).

Soil treatments to regulate Ca uptake by plant is not very successful as compare to direct application of Ca like NPK (nitrogen, phosphorus and potassium) through pre-harvest sprays (Shabbir *et al.*, 2015), postharvest dips, vacuum or pressure infiltration (Beirao-da-Costa *et al.*, 2008; Nigro *et al.*, 2006; Senevirathna & Daundasekera, 2010). According to Lanauskas and Kvikliene (2006), spraying trees of apples with calcium chloride decreases the incidence of bitter pit in fruits. Kadir (2005) reported that five to eight $CaCl_2$ applications to 'Jonathan' apple at fruit size of 0.9 and 1.6 cm in diameters retained fruit firmness. Omania and Karima (2007) observed that pre-harvest application of Ca sprays, vacuum infiltration or post-harvest dipping of fruit in Ca solution could decrease the rate of fruit softening during storage.

Materials and Methods

The experiment was conducted at Horticulture Postharvest Laboratory, The University of Agriculture, Peshawar-Pakistan during 2009-10. The fruits of apple cv. 'Red Delicious' were harvested from three different plants (in triplicate) at commercial maturity stage. Healthy fruits of uniform size were selected and dipped in 0, 3, 6 and 9% $CaCl_2$ solution for a period of 3, 6, 9 and 12 minutes. Ca solution was prepared from analytical grade $CaCl_2$. The fruits were then placed carefully in plastic buckets covered with wooden top. The surface moisture was removed with a gentle air blower. The fruits were then shifted to cold storage at 5 ± 1°C and 60–70% relative humidity for a period of 150 days.

Calcium content of fruit (mg 100 g^{-1} dry weight): Each fruit was washed thoroughly with distilled water and cut into four equal pieces. The weight of each piece was recorded. These pieces were then dried in an oven at 70°C till constant weight. The fruit pieces were grounded by using Tema mill, which was washed thoroughly with a brush and acetone between each treatment. The mineralization was achieved by the addition of 4 ml of 65% nitric acid solution and heating. The concentration of Ca in the fruit was determined by Atomic Absorption Spectrophotometer (GBC AA 932).

Weight loss (%): Five fruits in each treatment were separated for weight loss test. The initial weight of each fruit was noted with the help of electronic balance (Seed buro, Model: 8860). The weight loss (%) was calculated as under:

$$\text{Fruit weight loss (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Total sugars: Reducing and non-reducing sugars were determined by Fehling tests. The juice extracted for determination of sugar was filtered through Whattman No. 4 filter paper. The filtered juice (25 gm) was transferred to 250 ml volumetric flask and 100 ml of water was added to it. The solution was neutralized with 1 N NaOH. Lead acetate (2 ml) was added to the solution and was shaken vigorously. The content of the flask was allowed to stand for 10 minutes. Potassium oxalate was then added to remove the excess lead and the final volume was made up till the mark with water. Sugars were calculated as:

a. $$\text{\% Reducing sugar} = \frac{\text{Factor x dilution x 100}}{\text{Titer x wt. or vol. of sample}}$$

a. Non-reducing sugar = (% Total sugar - % Reducing sugar) x 0.95

Ascorbic acid (mg 100 g^{-1}): Juice sample of 10 ml was transferred to 100 ml volumetric flask and added 0.4% oxalic acid and the volume was made up to the mark. From this solution, an aliquot (10 ml) was taken into the flask and titrated against the dye (2,6-dichlorophenolindophenol) till the color turned light pink and remain stable for 15 seconds. The ascorbic acid content of the juice was determined by following formula:

$$\text{Ascorbic acid} = \frac{\text{F x T x 10}}{\text{D x S}} \times 100$$

where F = factor for standardization = $\frac{\text{(ml of ascorbic acid)}}{\text{ml of dye}}$, T = ml of dye used for sample – ml of dye used for blank, D = ml of sample taken for dilution, S = ml of dilute sample taken for titration.

Fruit firmness (kg cm^{-2}): Data pertaining to fruit firmness was recorded with the help of Penetrometer (Effigi, 11 mm prob.) for five fruits per treatment.

Bitter pit (%): Percent bitter pit incidence was observed visually in each treatment by calculating the surface area of each fruit covered with the symptoms of bitter pit at time 0 and 150 days interval of cold storage.

Soft rot (%): Percent soft rot in each replication of treatments was examined visually and counted during 150 days storage and the disease percentage of fruits was calculated by formula:

$$\text{Percent disease incidence (\%)} = \frac{\text{Number of disease fruits}}{\text{Total number of fruit}} \times 100$$

Statistical analysis: The data were analysed by using Completely Randomized Design (CRD) having thirty-two treatment combinations replicated three times. In case where the differences were significant, the means were further assessed for differences through least significant difference (LSD) test. Statistical computer software *MSTATC* (Michigan State University, USA), was applied for computing both the ANOVA and LSD.

Results and Discussion

Calcium content of the fruit: Ca content of apple cv. 'Red Delicious' was significantly affected by $CaCl_2$ concentration and dipping time. The Ca content was significantly higher in fruits dipped in increased $CaCl_2$ concentration for longer time (Table 1). Dipping of apple fruit in 9% $CaCl_2$ solution for 12 minutes resulted in 2.44 fold increase in the calcium content. The calcium might enters the fruits through apoplasm that bound to both cell wall and outer surface of the cell membrane in an exchangeable form (Valero & Serrano, 2010). The results indicate that dipping of fruits in $CaCl_2$ solution is an effective way of increasing the calcium content of the fruit.

Percent weight loss: Storage of apple fruits for 150 days had resulted in a significant weight loss. However the weight loss were controlled when the fruits were treated with increased concentration of $CaCl_2$ (Fig. 1a). Also the dipping durations of fruits in $CaCl_2$ solution had affected the weight loss; the weight loss was decreased, when dipping duration was extended from 3 to 12 minutes (Fig. 1b). The interaction effect between dipping duration × $CaCl_2$ concentration was also significant (Fig. 1c). The loss of turgor pressure and subsequent softening of the fruits leads to the destruction of the structure, which mainly depends on moisture loss (Sagar & Kumar, 2010). The data revealed that storage of apples for 150 days resulted in significant weight loss, which is due to loss in water and respiration (Ghafir *et al*., 2009). The decrease in weight of the fruits depends on water losses, which in turns rely on structure and nature of the cell wall (Toivonen & Brummell, 2008) and surface polymers .

Table 1. Effect of $CaCl_2$ concentration and dipping duration on the calcium content (mg kg^{-1}) of apple fruit cv. 'Red Delicious'.

Dipping duration (minutes)	CaCl2 concentration (%)				% Change	Mean
	0	3	6	9		
3	39.3 ± 1.45	49.0 ± 2.48	57.6 ± 1.52	62.2 ± 1.67	58.3	52.0d
6	38.4 ± 1.39	55.1 ± 2.30	64.7 ± 1.03	74.1 ± 3.49	93.0	58.1c
9	39.8 ± 1.33	58.8 ± 2.07	71.1 ± 1.88	81.5 ± 3.10	104.8	62.8b
12	39.2 ± 2.27	69.2 ± 2.24	78.2 ± 1.69	94.3 ± 3.09	140.6	70.2a
Mean	39.18d	58.03c	67.90b	78.03a		

Means in columns or rows with different letters are significantly different from one another ($p<0.05$). Each column consists of means of triplicated data and ± S.E of means

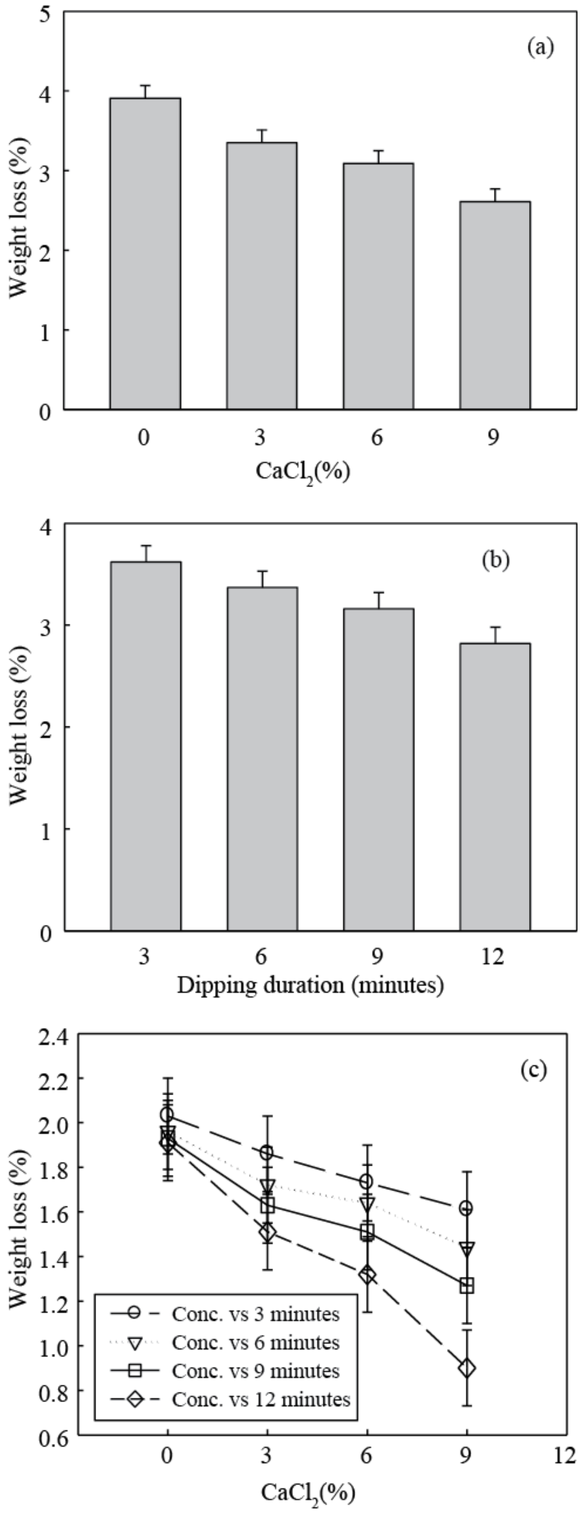

Fig. 1. Effect of $CaCl_2$ on percent weight loss of apple fruit during storage

a) Influence of $CaCl_2$ concentrations on percent weight loss in apple fruits stored for 150 days; b) Influence of dipping durations in $CaCl_2$ solution on percent weight loss in apple fruits stored for 150 days; c) Interaction effect of $CaCl_2$ concentrations x dipping durations on percent weight loss. The error bars represents LSD values at α= 0.05%.

Total sugar (%): The total sugar increased significantly during storage of fruits for 150 days. The sugar contents of apple fruits were significantly decreased with increase in $CaCl_2$ concentration as compared to untreated/control fruits. The lowest sugars were observed in apple fruits that were dipped in 9% $CaCl_2$ solution (Fig. 2a). Generally in fruits the sugars tend to increase with maturation (Wani *et al.*, 2008), which might be due to the breakdown/hydrolysis of starch. The application of Ca delays the changes associated with ripening and senescence in fruits (Gupta *et al.*, 2011; Hayat, *et al.*, 2005). Thus decrease in sugars with increasing $CaCl_2$ concentration was probably due to slow senescence and decreasing the water loss.

Ascorbic acid (mg 100 g^{-1}): The ascorbic acid in apple fruits was decreased significantly during storage (i.e. from 0 day to 150 days storage). Whereas the fruits treated with $CaCl_2$ had retained the ascorbic acid compared to control fruits or untreated fruits. The maximum ascorbic acid was recorded in fruits treated with 9% $CaCl_2$ solution, followed by 6% and 3%, respectively (Fig. 2(b)). Ascorbic acid is usually considered as an index of nutrient quality in apple fruits (Lata, 2007) but it is highly labile, which tends to decline during storage (Hayat, *et al.*, 2005). The losses in ascorbic acid during storage are known to be due to its antioxidant activity especially under postharvest storage conditions (Davey *et al.*, 2007; Jung & Watkins, 2008). The retention of relatively high ascorbic acid with increasing $CaCl_2$ concentrations or dipping duration is might be due to the regulation of oxidative processes in the cytosol.

Firmness (kg cm^{-2}): The firmness of apple fruits were decreased significantly during storage from 0 day to 150 days storage but it was retained with $CaCl_2$ treatment, the higher the $CaCl_2$ concentration the more firm the fruits were (Fig 3a). Also dipping of the fruits in 9% $CaCl_2$ solution for various time intervals had significantly affected fruit firmness (Fig. 3(b)). Fruit firmness is actually an important criterion that determines market value of the fruits (Peck *et al.*, 2006). The firmness of the apple fruit is due to texture of the flesh and cell wall assembly. Pectin solubilisation (Jin *et al.*, 2006) also reduces the mechanical strength of cell walls and decrease the firmness in apple fruits (Toivonen & Brummell, 2008). The retention of firmness with increasing calcium concentration or dipping duration can be attributed to the formation of calcium pectate which leads to increased rigidity of the cell wall and improved turgor pressure.

Bitter pit (%): The incidence of bitter pit on apple fruit significantly increased during storage but $CaCl_2$ concentration significantly affected the incidence of bitter pit (Fig. 4b). Dipping duration in $CaCl_2$ solution also affected the incidence of bitter pit that decreased with increase in dipping duration (Fig. 4d). The interaction of $CaCl_2$ concentration × dipping duration were also significant, the incidence of bitter pit was high in control/untreated fruits compared to fruit dipped in 9% $CaCl_2$ solution for 12 minutes (Fig. 4a). Bitter pit is a physiological disorder, characterized by necrotic spot due to calcium deficiency in apple fruits (Zupan *et al.*, 2013). The incidence of bitter pit is related to genetic factors but other factors that are also responsible includes maturity at harvest and Ca concentration in fruit (Pesis *et al.*, 2009). The bitter pit incidence is generally high in fruits stored for long time (Pesis, *et al.*, 2009) that can be controlled by postharvest Ca application (Zupan, *et al.*, 2013).

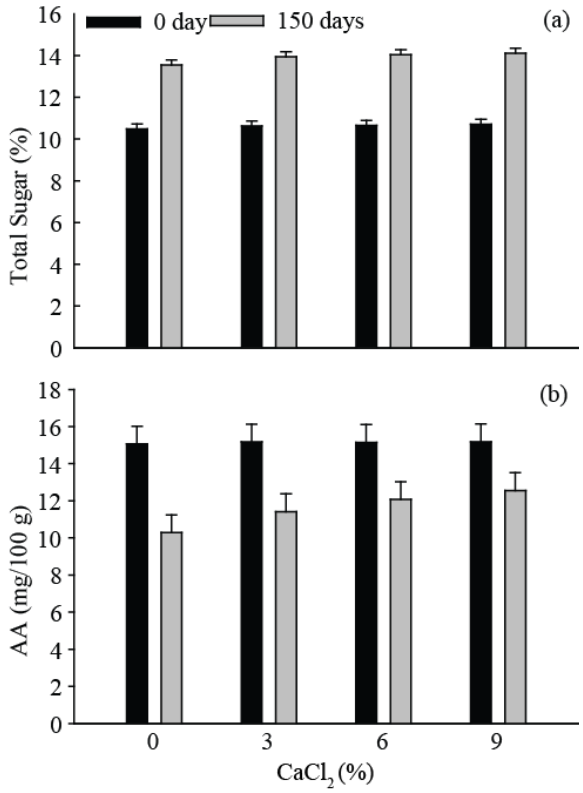

Fig. 2. Effect of $CaCl_2$ concentrations on TS and AA
a) Represents influence of $CaCl_2$ concentration on percent total sugar after 150 days of storage; b) Represents Influence of $CaCl_2$ concentration on Ascorbic acid (mg/100g). The error bars represents LSD values at α= 0.05%.

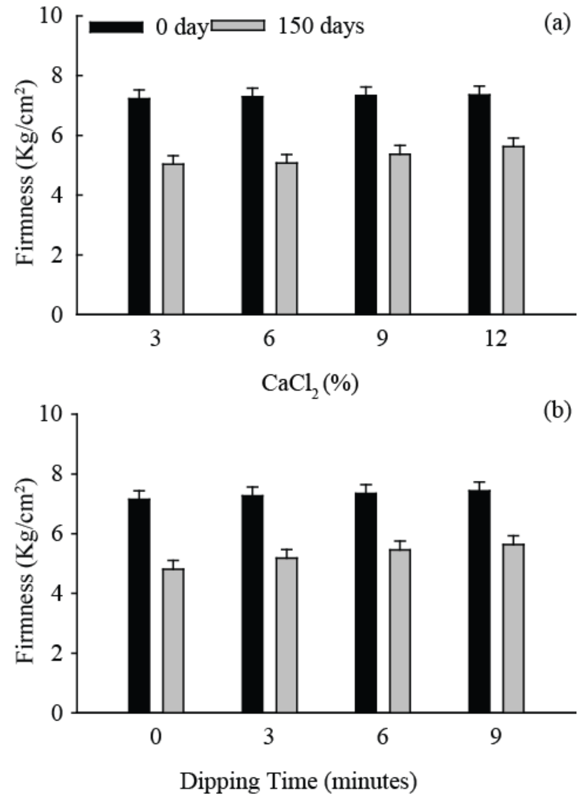

Fig. 3. Effect of $CaCl_2$ on apple fruit firmness
a) Influence of $CaCl_2$ concentration on fruit firmness after 150 days of storage; b) Influence of dipping time in $CaCl_2$ solution on fruit firmness after 150 days of storage. The error bars represents the LSD values at α= 0.05%.

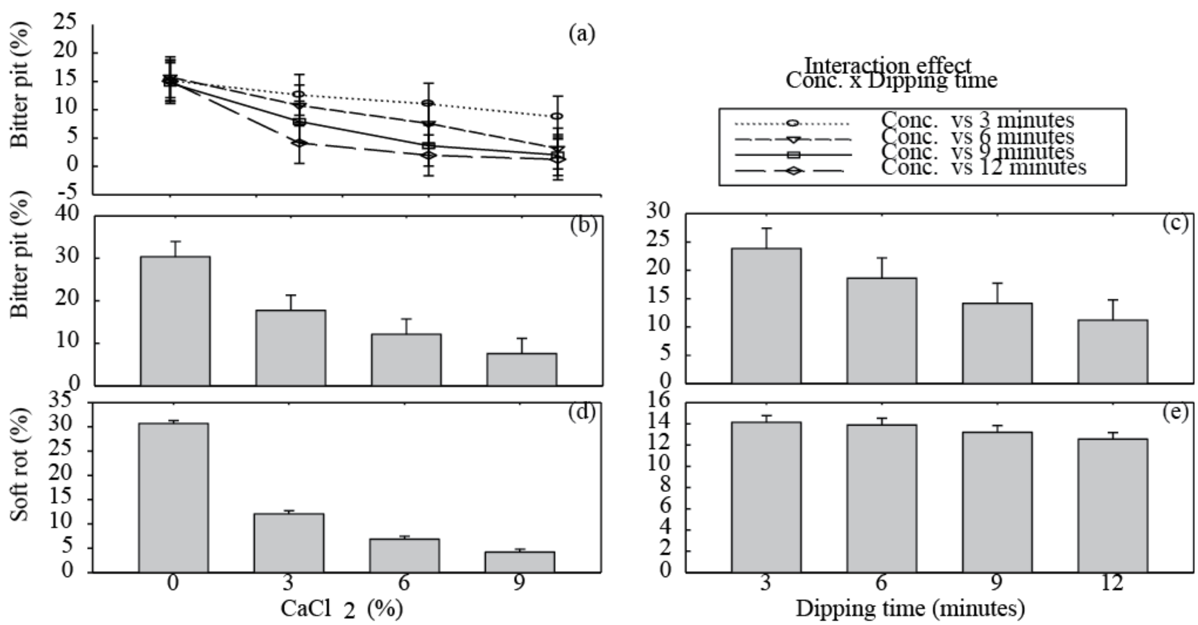

Fig. 4. Influence of $CaCl_2$ on bitter pit and soft rot
a) Interaction effect of $CaCl_2$ concentrations x dipping durations on % bitter pit; b) Influence of $CaCl_2$ concentrations on % bitter pit in apple fruits stored for 150 days; c) Influence of $CaCl_2$ concentrations on % soft rot in apple fruits stored for 150 days; d) Influence of dipping durations in $CaCl_2$ solution on % bitter pit in apple fruits stored for 150 days; e) Influence of dipping durations in $CaCl_2$ solution on % soft rot in apple fruits stored for 150 days. The error bars represents LSD values at α= 0.05%.

Soft rot (%): Results indicated that incidence of soft rot in apple fruit was decreased with increasing $CaCl_2$ concentration (Fig. 4c) and prolonged dipping duration in $CaCl_2$ solution (Fig. 4e). The lowest soft rot incidence was observed in apple fruit treated with 9% $CaCl_2$ concentration compared to control. The decreased in soft rot incidence with increased calcium concentration is might be due to delay in natural ripening and senescence induced by calcium. Whereas, Davey *et al.* (2007) suggested that during storage the increased susceptibility of fruits to *Botrytis cinerea* is because of low ascorbic acid contents and hence low antioxidant activity. However, no evidence were found latter to support that low ascorbic acid is responsible for pathogenic activity in fruits (Mattheis & Rudell, 2008).

Conclusion

As postharvest losses are the leading problems in the major fruit producing countries. But from our findings we concluded that these lose can be decreased by dipping of fruits in $CaCl_2$ solution of higher concentration for longer time. Thus, the results indicated that not only the dipping time is important but also the concentration of $CaCl_2$ in solution is necessary to retard any changes in the physiochemical characteristics of the fruits. Beside this, the time of dipping fruits in $CaCl_2$ solution has significantly reduced the incidence of soft rot and bitter pit. Though the combination effect (dipping time × concentration) have played a vital role in the retention of the quality parameters of the tested fruits, but sensory evaluation of the fruits is necessary before further recommendation.

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(Received for publication 15 June 2015)