

INFLUENCE OF CALCIUM CHLORIDE ON STORABILITY AND QUALITY OF APPLE FRUITS

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The influence of calcium chloride treatment on storability of three apple cultivars (Royal Gala, Mondial Gala and Golden Delicious) harvested at commercial maturity stage, from the orchards at Matta, Swat was examined. The fruits were treated with 0 and 9% CaCl₂ solution for the period of 12 minutes and stored for 150 days at 5±1°C with 60-70% relative humidity. After storage, apple cultivar Royal Gala had the highest ascorbic acid (12.88 mg/100g) and the least bitter pit (2.92%). Mondial Gala had the maximum titratable acidity (0.54%). Total soluble solids (TSS), total sugar, TSS/acid ratio and bitter pit increased while, starch, titratable acidity and ascorbic acid declined during storage. The application of CaCl₂ significantly decreased TSS (10.92%) total sugar (10.50%), TSS/acid ratio (21.69) and bitter pit (1.03 %), while a starch score (5.49), titratable acidity (0.53%) and ascorbic acid (12.97 mg/100g) increased with CaCl₂ treatment.

Keywords: Acidity, apple, bitter pit, calcium, storage, sugars

INTRODUCTION

Apple is prone to qualitative and quantitative losses after harvest, therefore it is stored in cold storage to minimize the losses and ensure its availability in the market throughout the year (Shah *et al.*, 2002). The storage life is reduced by loss of firmness (Kov *et al.*, 2005), loss of chemical attributes (Golias *et al.*, 2008), physiological disorders like bitter pit (Juan *et al.*, 1999) and disease incidence or decay (Hribar *et al.*, 1996; Ingle *et al.*, 2000). Thus, attempts have been made to explore different methods to reduce the postharvest losses during storage (Conway *et al.*, 2002; Mahmud *et al.*, 2008; Gupta and Jawandha, 2010).

Calcium plays an important role in regulating the metabolism in apple fruit, and adequate concentration maintains fruit firmness, delay fruit ripening, lower the incidence of physiological disorders such as water core, bitter pit, and internal breakdown (Mason, *et al.*, 1975; Reid and Padfield, 1975) and suppress *Erwinia carotovora* (Jones) incidence on apple fruits (Sharples and Johnson, 1977; Conway, 1982). The apple fruit grown on soil having optimum calcium level experienced Ca deficiency symptoms (Petersen, 1980) which may lead to several physiological disorders. The apple fruits having less than 50 mg kg⁻¹ Ca content of fresh weight, are sensitive to physiological disorders like bitter pit and internal breakdown (Petersen, 1980). By contrast optimum level of calcium in apple fruit maintain fruit firmness and reduces the incidence of physiological disorders such as water core, bitter pit and internal breakdown and postharvest decay (Conway *et al.*, 2002).

Soil treatments with calcium to increase fruit calcium content have often met with very little success but direct application of calcium to the fruit is the most effective method for increasing fruit calcium content, accomplished by pre-harvest sprays or postharvest dips or vacuum or pressure infiltration (Conway *et al.*, 2002). According to Martin *et al.* (1960), magnesium nitrate spray increased the incidence of bitter pit but calcium nitrate decreased it. Kader (2005) reported that five to eight CaCl₂ applications to 'Jonathan' apple at fruit sizes of 0.9 and 1.6 cm average diameters increased the fruit firmness by 26% and the SSC/TA by 35% as compared to control but fewer CaCl₂ applications were required to sustain fruit skin color during storage. The optimum level of Ca content may be different for various cultivars (Dris and Niskanen, 1999). The present experiment was, therefore, conducted to evaluate the influence of CaCl₂ treatment on physico-chemical changes in fruit of important apple cultivars during storage.

MATERIALS AND METHODS

The fruits were harvested at commercial maturity stage from apple cultivar Royal Gala, Mondial Gala and Golden Delicious at Matta, Swat. The healthy and uniform size fruits were selected and were divided into two groups each containing 150 fruits. One group of fruits were treated with 0 and the other with 9% CaCl₂ solution for 12 minutes. After dipping solution, the fruits were spread over clean transparent plastic sheet and the surface moisture was removed with a gentle air blow from an electric fan. The fruit were then packed in cardboard packages and shifted to

cold storage and stored at $5\pm1^{\circ}\text{C}$ with 60-70% relative humidity for the period of 150 days.

Starch content: Starch content was calculated at 30 days intervals of 150 days storage with the help of starch-iodine test. Iodine solution was prepared by dissolving 6 g of KI in 400 ml of water, and then added 1 g of I_2 . Slices of fruit dipped into iodine solution for 1 minute. Then the slices removed from the solution and let stand for 2 minutes. Each slice washed quickly in water and estimated the percentage of starch. Starch showed up as dark blue area and white areas represented sugar. The starch content was calculated according to Generic chart scores (1-8), where 1 represents the least and 8 the highest starch scores (Jan *et al.*, 2012).

Total sugars: Reducing and non-reducing sugars was determined by the method as described in AOAC (1990).

Total soluble solids: Total Soluble Solids of the fruit was determined at 30 days intervals of 150 days storage accordingly. Total soluble solids (TSS) were measured with a hand refractometer (Kernco, Instruments Co. Texas). The juice from sample fruits were thoroughly mixed and drop of juice was placed on the slab of brix refractometer and covered with a transparent led. The rotation was observed through the eye piece of the equipment.

Titrateable acidity: Acidity was determined as suggested by Khan *et al.* (2011).

TSS/Acid Ratio: The total soluble solids and acid ratio was calculated with the help of following formula.

$$\text{TSS/Acid} = \frac{\text{Total soluble solids}}{\text{Titrateable acidity}}$$

Ascorbic acid (mg/ml): Ascorbic acid was determined by the standard method as reported in AOAC (1990).

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 150 days of cold storage.

Statistical analysis: The data were analyzed by using a completely randomized design (CRD) with a factorial arrangement having twenty four treatment combinations repeated three times and 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 (Steel *et al.*, 1997).

RESULTS

Loss of starch content (score): The starch content score decreased significantly during storage from 6.95 for fresh harvested fruit to 3.23 for fruit stored for 150 days. The application of CaCl_2 resulted in significantly high starch score 5.49 as compared to 4.69 in control. The interaction effect of storage and CaCl_2 application was also significant. The maximum starch score (7.00) recorded in untreated

fruits 0 day storage, followed by apple fruits with a starch score (6.90) treated with 9% CaCl_2 application after 150 days storage, however these two starch scores were at par with each other. The minimum starch score (2.38) observed in untreated fruits after 150 days storage (Fig.1).

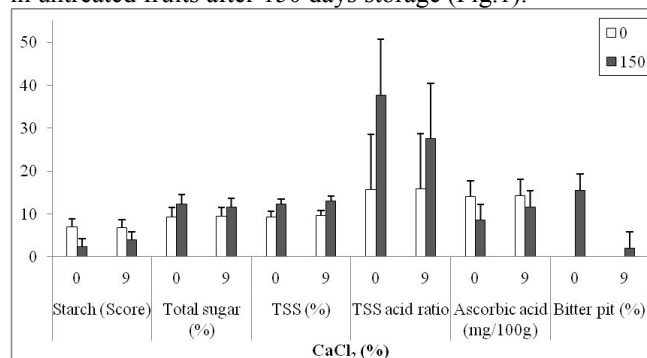


Figure 1. Interaction effect of CaCl_2 and storage on starch, total sugar, TSS, TSS/acid ratio, ascorbic acid and bitter pit of apple.

Total sugars (%): The total sugars significantly increased during storage from 9.41% for fresh harvested fruit to 11.96% with 150 days storage, but were significantly higher (10.87%) in non treated apple fruits as compared to 10.50% with a CaCl_2 application. The interaction effect of storage and CaCl_2 application was also significant. The maximum total sugars (12.36%) were recorded in untreated fruits (11.56%) at 150 days storage, followed by apple fruits treated with CaCl_2 solution after 150 days storage. The minimum total sugars (9.39%) were observed in untreated fruits at 0 day storage (Fig. 1).

Total soluble solids: The total soluble solids contents of the fruit increased significantly during storage from 9.91 in fresh harvested fruit to 12.46 % in fruit stored for 150 days but CaCl_2 application decreased the total soluble solids to 10.92% in contrast to 11.44% in control. The interaction effect of storage and CaCl_2 application was also significant. The maximum total soluble solids (13.0%) recorded in untreated fruits after 150 days storage, followed by apple fruits with total soluble solids (11.91%) in fruits treated with CaCl_2 solution, however these two values was at par with each other. The minimum total soluble solids (9.88%) observed in untreated fruits at 0 day storage (Fig. 1).

Titrateable acidity (%): The apple cultivars showed significant variations in percent titrateable acidity with the maximum titrateable acidity (0.54%) recorded in Mondial Gala and followed by Royal Gala with 0.52% while, the minimum titrateable acidity (0.48%) recorded in Golden Delicious. The percent acidity significantly decreased from 0.64%, recorded in fresh harvested fruits to 0.39% in apple fruit stored for 150 days. The titrateable acidity increased significantly from 0.49% for non treated apple fruits to 0.53% with CaCl_2 solution (Table 1). Titrateable acidity of

Table 1. The effect of storage and CaCl₂ application on starch, total sugar, TSS, titratable acidity TSS/acid ratio, ascorbic acid and bitter pit of apple fruit

Cultivars	Starch (Score)	Total sugar (%)	TSS (%)	Titratable acidity (%)	TSS/Acid ratio	Ascorbic acid (mg/100g)	Bitter pit (%)
Royal Gala	5.12	10.59	11.08	0.52 ab	23.45	12.88 a	2.92 b
Mondial Gala	4.97	10.63	11.02	0.54 a	23.42	12.73 a	3.34 b
Golden Delicious	5.18	10.84	11.45	0.48 b	25.71	10.79 b	6.96 a
LSD value	NS	NS	NS	0.06	NS	1.10	1.13
Storage (days)							
0	6.95 a	9.41 b	9.91 b	0.64 a	15.75 b	14.16 a	0.00 b
150	3.23 b	11.96 a	12.46 a	0.39 b	32.63 a	10.11 b	8.81 a
Significance level	*	*	*	*	*	*	*
CaCl ₂ (%)							
0	4.69 b	10.87 a	11.44 a	0.49 b	26.69 a	11.30 b	7.78 a
9	5.49 a	10.50 b	10.92 b	0.53 a	21.69 b	12.97 a	1.03 b
Significance level	*	*	*	*	*	*	*
Interactions				Significance Level			
C × S	NS	NS	NS	*	NS	*	*
C × Ca	NS	NS	NS	NS	NS	NS	*
S × Ca	*	*	*	*	*	*	*
C × S × Ca	NS	NS	NS	NS	NS	NS	*

Mean followed by similar letter(s) in column do not differ significantly from one another; NS = Non Significant and * = Significant at 5% level of probability; C × S = Interaction of cultivar and storage; C × Ca = Interaction of cultivar and CaCl₂; S × Ca = Interaction of storage and CaCl₂; C × S × Ca = Interaction of cultivar, storage and CaCl₂.

apple juice was significantly affected by the interaction of apple cultivars and storage. The maximum titratable acidity (0.69%) was recorded in cultivar Mondial Gala at 0 day storage, followed by Royal Gala with acidity of 0.65%, with the difference being non significant. The minimum titratable acidity (0.38%) was observed in cultivars Mondial Gala after 150 days storage, followed by Golden Delicious and Royal Gala with titratable acidity of 0.39 and 0.40% respectively, however, the variation was non-significant among these three cultivars (Fig. 2). The interaction effect of storage and CaCl₂ application was also significant. The maximum titratable acidity (0.64%) recorded in untreated fruits at 0 day storage, followed by apple fruits with 0.63% in fruits treated with CaCl₂ solution, however these two values was at par with each other. The minimum titratable acidity (0.35%) observed in untreated fruits at 0 day storage (Fig. 3).

TSS/Acid ratio: Storage had a significant effect on TSS/Acid ratio of apple juice. The TSS/Acid ratio increased from 15.75 in fresh fruit (S0) to 32.63 with storage for 150 days. The apple fruits dipped in CaCl₂ solution also had lower TSS/acid ratio of 21.69 as compared to 26.69 recorded for control treatment. The interaction effect of storage and CaCl₂ application was also significant. The maximum TSS/Acid ratio (37.75) recorded in untreated fruits after 150 days storage, followed by apple fruits with 27.51 in fruits treated with CaCl₂ solution, however these two values was at par with each other. The minimum TSS/Acid ratio (15.63) observed in untreated fruits at 0 day storage (Fig. 1).

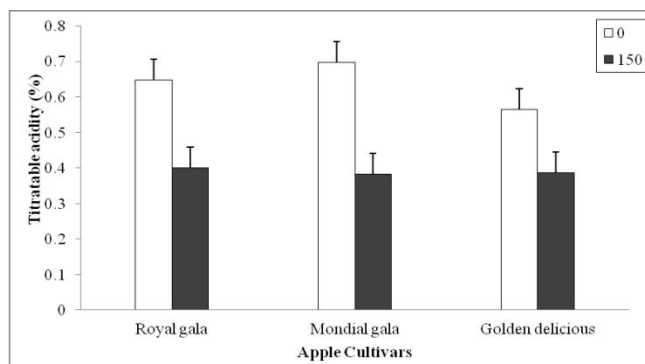


Figure 2. Interaction effect of cultivars and storage on titratable acidity of apple.

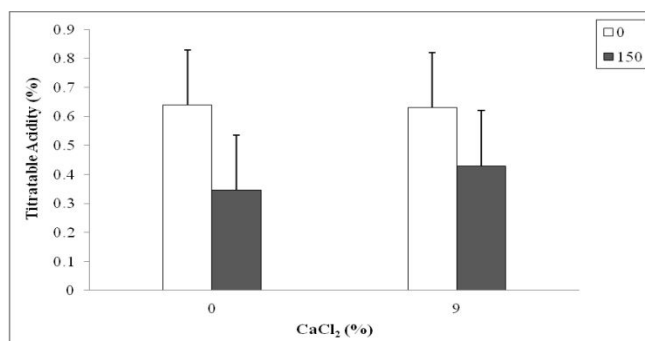


Figure 3. Interaction effect of CaCl₂ and storage on titratable acidity of apple.

Ascorbic acid (mg/100g): The maximum ascorbic acid content was recorded in cultivar Royal Gala (12.88 mg/100g) followed by Mondial Gala with 12.73 mg/100g, however, these two cultivars were at par with each other. The least ascorbic acid was observed in cultivar Golden Delicious (10.79 mg/100g) (Table 1). The ascorbic acid decreased significantly from 14.16 mg/100g observed in fresh fruits to 10.11 mg/100g after 150 days storage at $5\pm1^{\circ}\text{C}$. The ascorbic acid was significantly high (12.97 mg/100g) in non treated apple fruits to 11.30 mg/100g in fruits dipped CaCl_2 solution. The interaction effect of cultivars and storage on ascorbic acid was also significant. The maximum ascorbic acid (15.12 mg/100g) was recorded in cultivar Mondial Gala, followed by Royal Gala with 15.03 mg/100g after 0 day storage, however, these two cultivars was at par with each other. The minimum ascorbic acid (9.26 mg/100g) was observed with 150 days storage in cultivar Golden Delicious, followed by Mondial Gala and Royal Gala with ascorbic acid content of 1.35 and 10.73 mg/100g, respectively. However, the differences among all the three cultivars was statistically non significant after 150 days storage (Fig. 4). The interaction effect of storage and CaCl_2 application was also significant. The maximum ascorbic acid (14.26 mg/100g) recorded in fruits dipped in 9% CaCl_2 solution at 0 day storage while, the minimum ascorbic acid (8.55 mg/100g) observed in untreated fruits at 0 day storage (Fig. 1).

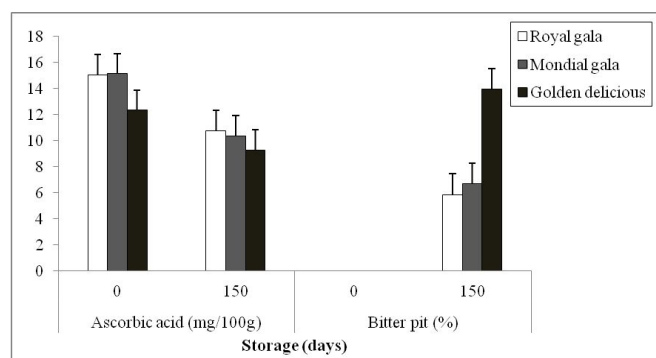


Figure 4. Interaction effect of cultivars and storage on ascorbic acid and bitter pit of apple.

Bitter pit (%): The apple cultivars varied significantly in bitter pit incidence in apple fruits. The lowest bitter pit incidence (2.92%) was measured in Royal Gala, followed by the Mondial Gala with 3.34% of bitter pit incidence, with the difference being non significant. The highest incidence of bitter pit (6.96%) was observed in cultivar Golden Delicious. The bitter bit incidence was 0% in freshly harvested fruits, but increased to 8.81% in fruit stored for 150. The bitter pit incidence in non treated apple fruits (7.78%) significantly decreased to 1.03% in fruits dipped in CaCl_2 solution. The bitter pit incidence increased significantly with increasing

storage in all the cultivars under study. After 150 days storage, the bitter pit incidence was significantly lower in Royal Gala and Mondial Gala with 5.84 and 6.67% respectively. The maximum bitter pit incidence of 13.93% was recorded in cultivar Golden Delicious (Fig. 4). The interaction of cultivar and CaCl_2 application also significantly affected the bitter pit incidence. The maximum bitter pit incidence (12.10%) observed in untreated fruits of cultivar Golden Delicious. The minimum bitter pit incidence of 0.60% recorded in cultivar Royal Gala treated with CaCl_2 solution (Fig. 5). The interaction of storage and CaCl_2 application significantly affected the incidence of bitter pit. The maximum incidence of bitter pit (15.56%) observed in fresh fruits treated with 9% CaCl_2 solution, followed by 2.06% in fruits treated with the same application after 150 days storage (Fig. 1). The interaction of cultivar, storage and CaCl_2 solution also significantly affected the incidence of bitter pit incidence. The maximum bitter pit incidence (24.20%) recorded in untreated fruits of cultivar Golden Delicious after 150 days storage which was significantly decreased to 3.65% after treated with 9% CaCl_2 solution (Fig. 6).

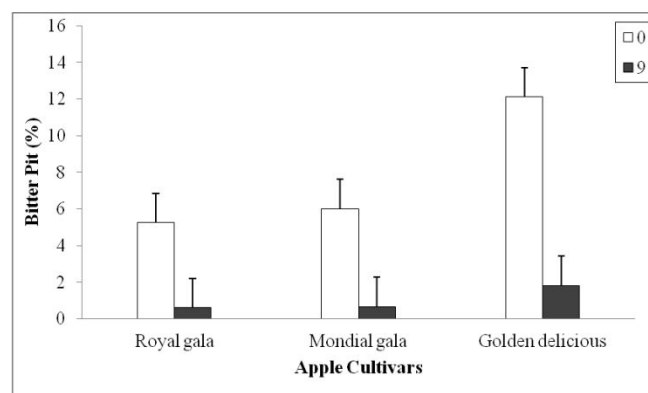


Figure 5. Interaction effect of cultivars and CaCl_2 on bitter pit of apple.

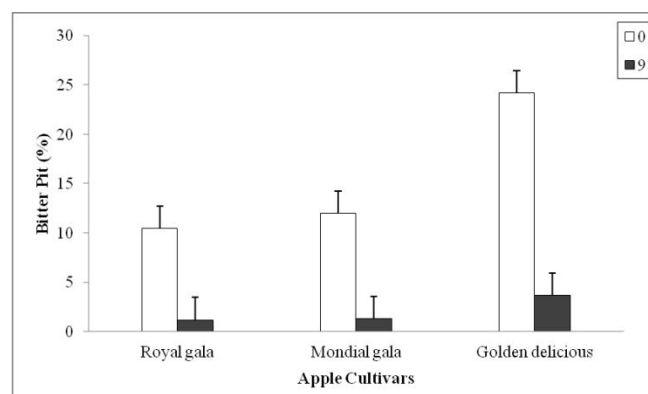


Figure 6. Interaction effect of cultivar, storage and CaCl_2 on bitter pit of apple.

DISCUSSION

While the starch content score was non-significant among different apple cultivars, it has been decreased by 53.53% during storage for 150 days. The decline in starch content was retarded by the application of CaCl_2 which resulted in 14.57% lower loss of starch content than the non treated control fruits (Table 1). The starch is the major storage carbohydrates in apple fruit (Beaudry *et al.*, 1989), which is converted to sugars at the onset of ripening and during storage to meet the respiratory demand of the fruit (Crouch, 2003), thus it is likely to observe the decline in starch content during storage of apple fruit. Since calcium application delay the ripening process of fruit, therefore the conversion of starch to sugar is also less with the CaCl_2 application (Kader, 2005). The decline in starch score was accompanied by an increase in sugar content, indicating that the stored starch to free sugars to meet the respiratory demands and contribute to the fruit sweetness. The total sugars were non significantly different among different apple cultivars, but apple fruit stored for 150 days had 21.32 % higher total sugars than freshly harvested fruits (Table 1). The apple fruit accumulates starch at the early stages of maturation, which is hydrolyzed to sugars at edible maturity (Magein and Leurquin, 2000) and during storage (Beaudry *et al.*, 1989), resulting in increased total sugar with maturation (Wani *et al.*, 2008) and during storage (Crouch, 2003). The calcium treatment of the apple fruit retarded the trend of increasing total sugars so that there was 3.4% less total sugars as compared to the untreated fruits. The apple cultivars, under study, had no significant difference in TSS, which increased significantly during storage so that it was 20.46% higher in fruit stored for 150 days as compared to the fresh harvested fruits stored for 0 days (Table 1). The increase in TSS was decreased by 4.55% when the fruits were treated with CaCl_2 as compared to non treated control. The increase in TSS may be due to the enzymatic conversion of higher polysaccharides such as starches to simple sugars during ripening (Hussain *et al.*, 2008). The less increase in TSS with CaCl_2 may be due to the delay in natural physiological processes like ripening and senescence, due to the inhibition or retardation of conversion to simple sugars from complex polysaccharides (Kader, 1986; Rosen and Kader, 1989; Agar *et al.*, 1999;). It is, however, also important that the loss of moisture may concentrate the cell sap, resulting in low juice content but high TSS percentage. The apple cultivars differed significantly in percent titratable acidity, which was 7.69 and 11.11% higher than Golden Delicious in Royal Gala and Mondial Gala, respectively. Generally, the percent acidity decreases with the increase in storage duration (Khan *et al.*, 2012). The percent acidity decreased by 39.06% with storage for 150 days in contrast to freshly harvested fruits. The decrease in titratable acidity was significantly retarded by CaCl_2 treatment, and was

7.54% higher than the non treated apple fruits (Table 1). The decline in titratable acidity depends on the rate of metabolism (Murata and Minamide, 1970) especially respiration which consumed organic acid and, thus, decline acidity (Rivera, 2005; Ghafir *et al.*, 2009). Since, the organic acids are consumed in respiration, resulting in lower acidity with increasing storage duration (Rivera, 2005; Ghafir *et al.*, 2009). The TSS/Acid ratio is a function of TSS and acidity of different apple cultivars. Hence, it followed a comparable pattern. The three apple cultivars, under study, were statistically at par in a TSS/Acid ratio, which increased by 107.17% during 150 days storage. The CaCl_2 treatment of apple fruit decreased the trend of increasing TSS/Acid ratio. Thus, the TSS/Acid ratio in control fruit was 18.73% higher than the fruits treated with a CaCl_2 solution (Table 1). Since CaCl_2 application caused significant decrease in TSS and the increase in titratable acidity, it is reasonable to observe lower TSS/acid ratio with CaCl_2 application. The TSS/ Acid ratio of apple and other fruits is a major quality parameter (Weibel *et al.*, 2004; Peck *et al.*, 2006). The TSS/acid ratio generally increases with increasing storage. The increase in TSS/acid ratio can be attributed to starch breakdown resulting in free sugars (Beaudry *et al.*, 1989) and decline in organic acids due to its consumption in respiration (Rivera, 2005; Ghafir *et al.*, 2009). Ascorbic acid is usually considered as an index of nutritional quality in apple fruit. Ascorbic acid is a bioactive compound with antioxidant properties (Lata, 2007). The ascorbic acid content of apple is generally 12.8 mg/100 g fruit (Lee *et al.*, 2003). The apple cultivars differ significantly in their ascorbic acid content (Davey *et al.*, 2007; Nour *et al.*, 2010). There was no significant difference in ascorbic acid content of Royal Gala and Mondial Gala but the maximum ascorbic acid recorded in cultivar Royal Gala was 16.23% higher than cultivar Golden Delicious (Table 1). The ascorbic acid decreased significantly (28.60%) during storage for 150 days as compared to fresh fruits (Table 1). The decrease in ascorbic acid was 12.97% less in CaCl_2 treated fruits as compared to non treated control (Table 1). The ascorbic acid is a high labile vitamin which tends to decline during storage (Hayat *et al.*, 2003; Ali *et al.*, 2004). The ascorbic acid loss during storage is known to be due to its antioxidant activity, especially under postharvest storage conditions (Davey *et al.*, 2000). The ascorbic acid can be irreversibly oxidized (Parviainen and Nyyssonen, 1992; Pardio-Sedas *et al.*, 1994), thus causing a decrease during storage (Jung and Watkins, 2008). The retention of relatively high ascorbic acid with CaCl_2 may due to the regulation of oxidative processes in the cytosol, leading to ascorbic acid degradation (Faust and Shear, 1972). Bitter pit is a physiological disorder, characterized by depressed brown lesions in the skin of the fruit (Ferguson and Watkins, 1989). The apple cultivars varied significantly in bitter pit incidence on fruits. Cultivar Royal Gala has the lowest bitter pit incidence (2.92%) which

was statistically at par with Mondial Gala but significantly lower than cultivar Golden Delicious, which had 58.05% higher bitter pit incidence than Royal Gala (Table 1). Crouch (2003) reported that the incidence of bitter pit depends on genetic factors and apple cultivar Red Delicious is more susceptible to bitter pit as compared to Golden Delicious. The bitter bit incidence increased by 8.81% in fruit stored for 150 days, but decreased significantly (11.32%) with CaCl_2 treatment. The incidence of bitter pit generally increased with increasing storage duration (Pesis *et al.*, 2009). The lower bitter pit incidence with CaCl_2 treatment is in accordance with earlier reports that Ca treatments can decrease bitter pit incidence. The incidence of bitter pit is related to plant nutrition (Fallahi *et al.*, 1997) especially Ca concentration (Crouch, 2003; Pesis *et al.*, 2009) and can be decreased by pre- (Peryea *et al.*, 2003) and post-harvest Ca application (Reid and Padfield, 1975; Jones and Higgs, 1982).

Conclusions: Apple cultivars Royal Gala and Mondial Gala had higher acidity and ascorbic acid content while the least bitter pit incidence as compared to Golden Delicious. Thus, these two cultivars can be stored for longer duration than Golden Delicious. Application of CaCl_2 significantly reduced the decline in starch, acidity and ascorbic acid content as well as the increase in sugars, TSS and bitter pit incidence during storage, thus, retained the nutritional quality during storage.

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