Research Article

Temporal Effect of Calcium Foliar Application on Fruit Quality and Postharvest Physiological Disorders During Storage

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Abstract | Field and laboratory experiments on the impact of foliar calcium application on fruit quality and the incidence of postharvest physiological disorders were conducted at Swat regions of Kalam and Matta and Postharvest Laboratory of Horticulture, University of Agriculture, Khyber Pakhtunkhwa, Pakistan. During the 1st phase, apple plants were applied with calcium chloride (1%) at time intervals of 0 (Berry size fruit of 9mm), 30 (1st spray), 60 (2nd spray), 90 (3rd spray), 120 (4th spray) and 150 (5th spray) days during the fruit growing season (RCB design replicated three times). During the 2nd phase, the harvested fruits were brought to the postharvest laboratory of horticulture to determine the influence of calcium chloride application biochemical attributes of apple using CRD repeated three times. The results showed that the time of calcium spray significantly affected all the studied parameters. Regarding the means for foliar application of calcium, the highest total soluble solids (9.19 °brix), Reducing sugars (7.77%), ascorbic acid content (2.72 mg $100g^{-1}$), calcium content (0.19%), specific gravity (0.906), fruit juice pH (4.83), fruit firmness (7.45 kg cm^{-2}) with minimum bitter pit incidence (4.69%), internal breakdown (3.12%) was obtained when calcium was applied after 150 days as a foliar spray. However, highest non reducing sugars (3.60%) and acidity (2.11%) was recorded when calcium as foliar spray was applied at 60 and 120 days respectively. Regarding means for storage durations, the total soluble solids (13.59 °brix), acidity (1.43%), Reducing sugars (5.53%), non-reducing sugars (2.97%),_ascorbic acid content (2.17 mg $100g^{-1}$), fruit firmness (6.40 kg cm^{-2}), bitter pit incidence (2.0%), internal breakdown (3.4%) fruit taste (6.30) and fruit aroma (4.56) was significantly retained for 90 days during storage. However, calcium content (0.19%), specific gravity (0.791), fruit juice pH (5.05) and weight loss (2.08%) was significantly retained up to 120 days of storage at storeroom. Fruit color (10.0) and aroma (9.5) was retained for 75 days while fruit taste (8.0) up to 105 days of storage at storeroom. Based on the results it is concluded that foliar application of calcium up to five months prior to harvest can improve the shelf life of apple fruits stored for longer time.

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Introduction

Postharvest losses occur in most horticultural products going through various stages, including harvesting, handling, storage, marketing, and delivery to consumers (Baliyan and Kgathi, 2009). Several factors led to postharvest deterioration of horticultural products. These include lack of expertise in harvesting crops at the appropriate stage, inadequate pre-harvest cultural practices, inadequate hygiene management, suboptimal production techniques, excessive or improper fertilizer application, inefficient transportation, unsuitable rootstock selection, Postharvest issues, poor pest and disease management, failure to remove field heat, the use of substandard packaging and grading materials, poor storage and marketing strategies which further exaggerate the problem (Kader, 2002). Due to its year-round demand, apple fruit is commonly preserved in cold storage. However, its perishability makes it susceptible to both qualitative and quantitative postharvest losses. Insufficient attention to the production and postharvest management of apples contributes to their decay and limited storage life (Khan *et al*., 2016). Prolonged storage can lead to enzymatic browning, which results in undesirable changes in color, flavor, and nutritional quality. On average, postharvest losses in apples amount to approximately 17% (Argenta *et al*., 2021). Pre-harvest calcium spray is one of the most important practices in horticultural crop production systems which increase fruit firmness and decrease the use of fungicide sprays with increasing resistance to bitter pit and internal browning (Lester and Grusak, 2004).

Calcium is an essential macronutrient that plays a vital role in apple metabolic processes. Optimum calcium levels contribute to lower physiological disorders occurrence such as internal breakdown, bitter pit, and water core, as less than 50 mg kg^{-1} of calcium in their fresh weight make it more susceptible to internal breakdown and bitter pit development (Conway *et al*., 2002). Additionally, calcium delaying ripening, and maintaining fruit tissue firmness. Despite the abundance of calcium in orchard soils and its uptake by apple trees, various physiological disorders occur in fruits due to calcium deficiency. Consequently, extensive research has been conducted to identify strategies for minimizing postharvest losses during storage (Uikey *et al*., 2023).

Calcium, as a constituent of cell wall, plays a vital role in forming cross-linked polymers that enhanced cell wall strength and considered final layer of defense before cell wall breakdown (Fry, 2004). Calcium sprays during fruit growth ensures calcium availablit which is needed for better growth and development of fresh fruits. It also defends the cell wall from cell wall-deteriorating enzymes to strengthens it (White and Broadley, 2003). Similarly, in fig fruits, a 4% foliar calcium spray has been shown to be effective in extending the shelf life by maintaining texture and preserving fruit color (Irfan *et al*., 2013).

Considering the significance of apple fruits and the role of calcium in improving and maintaining their shelf life, this study was conducted to determine the optimal timing for calcium spray application to enhance fruit quality and storage performance. Additionally, the experiment aimed to maximize the apples shelf life during storage by identify the most suitable storage duration.

Materials and Methods

Experimental plan

This experiment was conducted in two phases to analyze the impact of calcium foliar application on fruit quality and the incidence of postharvest physiological disorders. The study was carried out at an apple orchard in Swat and the Postharvest Laboratory, Department of Horticulture, The University of Agriculture Peshawar. In the first phase, calcium chloride was applied to apple plants for 150 days of highly susceptible apple cultivar (Golden Smoothee) to bitterpit and internal breakdown (Begum and Khattak, 2023) during the first growing season. The experiment followed a Randomized Complete Block Design (RCBD) with three replications. 1% calcium chloride (foliar spray) were applied at 30-day intervals during the fruit growth stage (starting from the 9 mm fruit size until the physiological maturity stage). The data were recorded at 30 days' interval at the time of each spray.

Three trees were assigned for each treatment, so a total of 15 trees were used for the whole experiment during the 1st phase. In the second stage, using Completely Randomized Design (CRD) with three repetitions, a total of 150 apple fruits (50 fruits from each tree) were harvested for each storage duration, which were brought to Postharvest Laboratory, Department of Horticulture to determine the effect of calcium chloride application for 240 days stored at 5°C with 80-90% relative humidity and 15 days' interval during storage. With 15 days of interval, various qualitative features including acidity, ascorbic acid, total soluble solids, calcium content, pH, firmness, reducing sugars, non-reducing sugars, specific gravity, weight loss, bitter pit incidence, internal browning, color, taste and aroma in apple fruits were recorded.

Experimental details

Foliar spray: Calcium chloride application after 30 days interval: 0, 30 (1st spray), 60 (2nd spray), 90 (3rd spray), 120 (4th spray) and 150 (4th spray) days.

Storage duration: 17 (0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225 and 240 days)

Data were recorded on chemical and physical attributes of apple fruits at 30- and 15-days interval, respectively.

Chemical attributes

The total soluble solids (TSS using a hand refractometer), total titratable acidity (TTA) ascorbic acid, reducing, and non-reducing sugars were measured following the method described by AOAC (1990) at room temperature. Calcium content was recorded by atomic absorption spectrophotometry. To determine the pH of the samples, Inolab Digital pH meter was used. The extent of fruit firmness was evaluated by penetrometer (fruit pressure tester) and articulated in kg cm^{-2}.

Physical attributes

The specific gravity of fruits was calculated using the following formula:

$$Specific\ gravity = \frac{Mass\ of\ the\ fruit}{Volume\ of\ the\ fruit}$$

The weight loss index was expressed as a percentage and determined using the formula below for each cultivar:

$$Weightloss\ (\%) = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100$$

The observance of flesh cells folding below the peel linked with dark depressions on the fruit surface led to Bitter pit incidence in apple to record at that time (Amarante *et al*., 2013). The average of pitted fruits and inner brown fruits were analyzed by finding percentage for each treatment. The inflated fruits by means of brown pits were counted, discarded and washed.

Organoleptic attributes

The panel of 20 judges of Horticulture Department (expert in sensory evaluation) evaluated fruit samples organoleptically using 9-point hedonic scale described by Larmond (1977) based on aroma, color, and taste. Upon samples presentation, each panelist recorded their observations by assigning scores on a scale of 1 to 9, where 1 indicated "extremely disliked," scores from 2 to 5 represented varying levels of "liked," and scores from 6 to 9 denoted "extremely liked".

Statistical analysis

The data were organized following a Randomized Complete Block Design (RCBD) and analyzed using analysis of variance (ANOVA) in the first stage. While Completely Randomized Design (CRD) was used for second phase data. Statistix 8.1 software was utilized for statistical analysis (Statistix, 2003). Least Significant Difference (LSD) test was applied to compare the means when significant differences were observed (Jan *et al*., 2009).

Results and Discussion

Total soluble solids (°brix)

The data regarding total soluble solids of apple fruits as affected by storage duration after foliar application of calcium chloride are presented in Figure 1. Total soluble solids were significantly affected by calcium foliar application and storage durations. The extent of total soluble solids gradually increased from 5.55 °brix recorded earlier than spray application to 9.19°brix recorded for fruits after 150 days foliar spray. The total soluble solids (6.96°brix) recorded with calcium sprayed after 30 days, followed by 7.27°brix and 8.01°brix recorded with calcium sprayed after 60 and 90 foliar spray of calcium chloride, respectively. The total soluble solids of 8.50°brix recorded for fruits after 120 days foliar spray. Concerning the means for storage duration, total soluble solids of 11.30°brix were noted at the time of storing apple fruits in storeroom, which gradually increased to 15.70°brix during seven months storage period, while further decrease 14.84°brix in total soluble solids was noted for 240 days of storage. The highest total soluble solids (15.70°brix) content was recorded in apple fruits at 210 days storage, followed by total soluble solids (15.39°brix) content in apple fruits at 195 days storage. The lowest total soluble (11.30°brix) was recorded in fruits of control treatment.

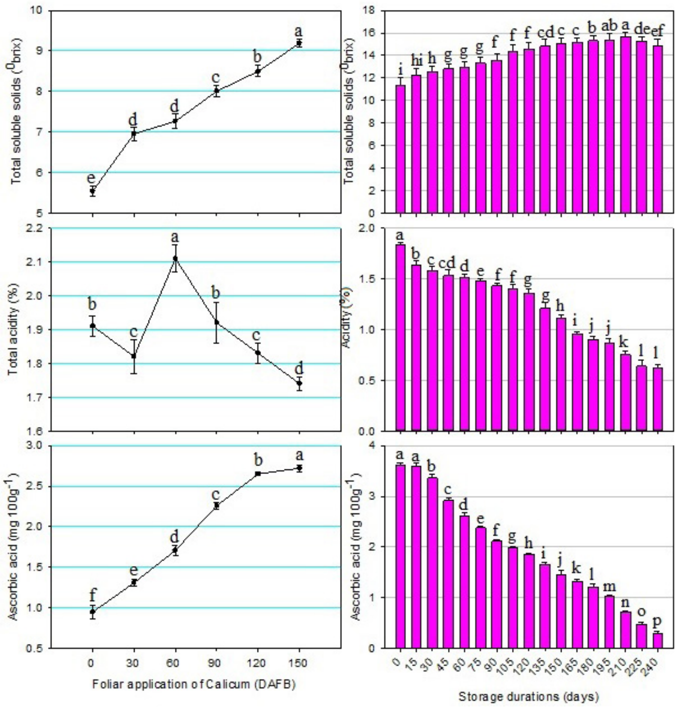

Figure 1: *Total soluble solids, acidity and ascorbic acids of apple fruits as affected by time of foliar application of calcium and storage duration.*

The total soluble solid is a significant quality parameter as well as narrates to soluble sugars in the juice content (Ganai *et al*., 2018). The moisture of fruits is an important factor and the loss of weight and subsequent softening greatly depends on moisture loss (Lee *et al*., 2003). Total soluble solids were significantly affected by calcium spray application. Total soluble solids in apple fruits sprayed for 150 days resulted in 76% increase and this increase was due to calcium spray application. During ripening process, the breakdown of complex polysaccharides into simpler sugars led to increase in total soluble solids (Hussain *et al*., 2008). Foliar application of calcium resulted in improvement of postharvest life of fruits (Khalaj *et al*., 2017). The combined application of calcium chloride and boric acid was found to be helpful in increasing total soluble solids compared to other nutrient spray applications (Ganai *et al*., 2018). Storage duration also has a significant influence on apple TSS as 210 days stored apples have shown the highest TSS. The present results in line with previous studies that early season calcium spray results in increased total soluble solids during three to six months cold storage (Ghorbani *et al*., 2017). Moreover, Storage resulted in an increase in total soluble solids from 12 to 14°brix (Rehman *et al*., 2017).

Total acidity (%)

The data concerning total acidity of apple fruits as affected by storage duration after foliar application of calcium chloride is shown in Figure 1. Significant variation in total acidity was observed both in storage and with foliar application of calcium. The total acidity of 1.82% recorded with calcium foliar sprayed after 30 days, followed by 2.11% recorded with 2nd foliar calcium spray. The total acidity of 1.92% and 1.83% was recorded for fruits after 90- and 120-days foliar spray. In relation to the means for storage period, storage duration significantly affected total acidity in apple fruits. The total acidity of 1.83% was noted at the time of storing apple fruits in storeroom which steadily decreased to 0.62% during eight months storage period. A slow decrease in total acidity was observed during the first three months of storage. The highest total acidity 1.83% recorded in apple fruits earlier than foliar spray application and the lowest total acidity (0.62%) was seen in apple fruits stored for 240 days. The results indicated that foliar spray application of calcium chloride earlier than pre-harvest stages and storage duration significantly reduced the level of total acidity in stored apple fruits.

Total acidity in apple fruits was significantly affected by calcium spray application and increased due to preharvest calcium spray application at 60 days. Foliar spray application of calcium chloride earlier than pre-harvest stages significantly reduced the level of total acidity in stored apple fruits. Total acidity decreased from the maximum of 1.83% to the minimum of 0.62%. The decrease in total acidity depends on the rate of respiration, which consumes organic acid and thus decline acidity. Similar decline rate from 0.5% to 0.094 % has also been recorded in 15 days stored apples (Rathore *et al*., 2007). The decrease in total acidity may be due to the consumption of organic acids during growing period, because the organic acids are inspired in respiration, which tend to decrease the acidity with increasing storage duration (Riveria, 2005). The present results are confirmed from previous results that combined application of calcium and boron increased fruit firmness, total soluble solids, while decreased total acidity (Omaina and Karima, 2007; Ganai *et al*., 2018).

Ascorbic acid content (mg $100g^{-1}$)

The data regarding ascorbic acid content in apple fruits as affected by storage duration after foliar application of calcium chloride are presented in Figure 1. Foliar application of calcium and storage durations significantly affected ascorbic acid of apple fruits. The amount of ascorbic acid content was increased due to calcium spray application, while decrease in ascorbic acid content was noted in apple fruits during an eight-month storage period. The extent of ascorbic acid content ranged from 0.95 to 2.72 mg 100 g^{-1} during five months of calcium spray. The highest ascorbic acid content (2.72 and 2.65 mg 100 g^{-1}) was recorded after 150 and 120 days of calcium sprayed on apple fruits and lowest ascorbic acid content (0.95 mg 100 g^{-1}) was recorded in fruits of control treatment. The amount of ascorbic acid content was 1.31 and 1.71 mg 100 g^{-1} during the 30 and 60 spray application. Regarding the means for storage period, significant differences were observed for ascorbic acid content in apple fruits stored for eight months. The amount of ascorbic acid content ranged from 3.62 to 0.30 mg 100 g^{-1} in storeroom. The highest ascorbic acid content of 3.62 mg 100 g^{-1} observed at 0-day storage which steadily decreased during storage. The lowest ascorbic acid content of 0.30 mg 100 g^{-1} was recorded in fruits stored for eight months. The stage of ascorbic acid content in apple fruits was found to be in adequate level up to three months storage period but further decreased. The results pointed out that calcium spray application increased ascorbic acid content, but storage time decreased its amount in stored apple fruits.

Foliar application of calcium chloride increased the ascorbic acid content, while storage duration decreased ascorbic acid content. Ascorbic acid is usually considered as important characteristics to judge the quality in apple fruit specially desired for its antioxidant properties (Lata, 2007). Highest ascorbic acid content (2.72 and 2.65 mg 100 g^{-1}) was recorded after 150 and 120 days of calcium sprayed on apple fruits, respectively. Significant differences were observed for ascorbic acid content in apple fruits stored for eight months. Ascorbic acid content in apple fruits found in adequate level up to three months' storage period but further decreased. Various scientists also reported similar result and concluded that during postharvest storage, the ascorbic acid levels decreased significantly (Hayat *et al*., 2003; Feszterova *et al*., 2023), which is due to the antioxidant activity (oxidative process) of ascorbic acid (Abeysuriya *et al*., 2024).

Calcium content (%)

The data in Figure 2 reveal that calcium content in apple fruits was significantly affected by foliar application of calcium and storage duration. It is evident from the data that calcium content in apple fruits significantly increased due to various calcium sprays. Calcium content in apple fruits ranged from 0.03% observed in control treatment to 0.19% in fruits treated for 150 days of calcium spray, followed by calcium content 0.16% treated with 4th spray (120 days). Concerning the means of calcium content for storage period, considerable variations were observed for calcium content in apple fruits stored for eight months. The amount of calcium content in apple fruits decreased ranged from 0.19 to 0.04% in storeroom. The highest calcium content of 0.19% observed at 0-day storage which gradually decreased during storage. The lowest calcium content of 0.04% was recorded in fruits stored for eight months. The degree of calcium content in apple fruits found in enough level up to four months storage period but further decreased. The results revealed that calcium spray application increased the level of calcium content however storage period decreased its amount in stored apple fruits. Considerable variations were observed for calcium content in apple fruits significantly increased due to various calcium sprays. Calcium content increased from the minimum of 0.03% to the maximum of 0.19% in fruits treated with 5th spray (150 days). Torres *et al*. (2024) stated that there is relation among calcium level, protein levels in the fruit and bitter pit incidence. Beiparysa *et al*. (2023) stated that pre-harvest calcium treatments used to increase the calcium content of the cell walls of fruit tissue after harvest and more effective in delay senescence, resulting in firmness and higher quality fruits. Koutinas *et al*. (2010) reported that the calcium sprays were effective on increasing the calcium content in the fruit and decreases with length of storage of apple cv. Golden smoothee. Significant differences were observed for calcium content in apple fruits stored for eight months. The amount of calcium content in apple fruits decreased during storage. The highest calcium content (0.19%) observed at 0-day storage, gradually decreased during storage. Baneh *et al*. (2003) also reported similar results and reported that calcium chloride at the rate of 1% concentration significantly affected fruit firmness and calcium content.

Fruit juice pH

The data concerning pH in apple fruits as affected by storage duration after foliar application of calcium chloride are presented in Figure 2. The data reveal that pH of apple fruits is significantly affected by calcium foliar application and storage duration indicating significant differences observed for pH in apple fruits. Mean values indicated that pH in apple fruits increased due to various calcium sprays. The array of pH values in apple fruits ranged from 3.19 observed in control treatment to 4.83 in fruits treated with 5th foliar spray (150 days), followed by pH 3.21 treated with 4th foliar spray (120 days). The amount of pH was 3.63 with 2nd foliar spray (60 days), afterward increased to 3.87 observed with 3rd foliar spray (90 days) of calcium. Concerning the means of pH for storage period, considerable variations were observed for pH in apple fruits stored for eight months. The amount of pH in apple fruits increased ranged from 3.17 to 7.01 in storeroom. The lowest pH values of 3.17 observed at 0-day storage which gradually increased during storage. The highest pH of 7.01 was recorded in fruits stored for 240 days. The level of pH in apple fruits found in deficit stage up to three months storage period but further increased. The results revealed that pre-harvest calcium spray application of 150 days increased the level of pH in apple fruits stored for 240 days.

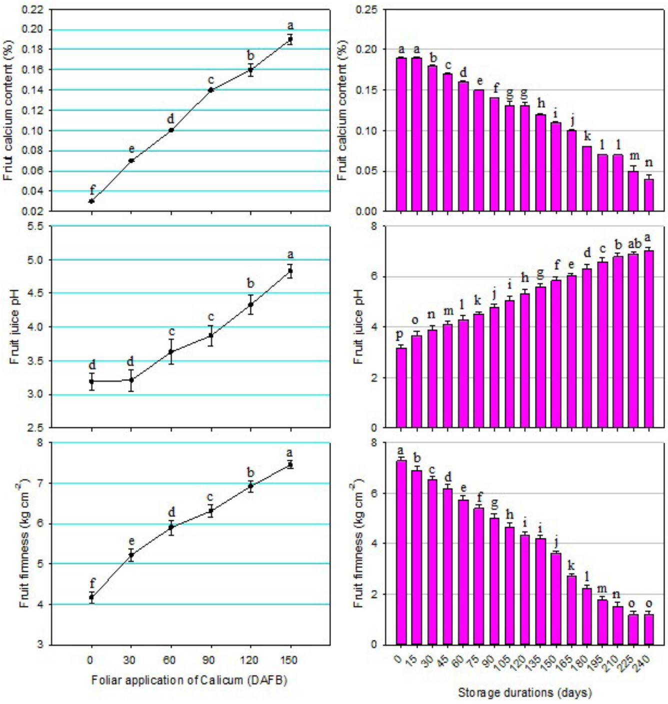

Figure 2: *Fruit calcium content, fruit juice pH and fruit firmness of apple fruits as affected by time of foliar application of calcium and storage duration.*

The pH in apple fruits comparatively increased due to various calcium sprays. The pH values increased from the minimum of 3.19% to the maximum of 4.83% in fruits treated with 5th spray (150 days). Calcium lactate concentration on postharvest life of apple fruit showed an increase in pH from 4.0 to 5.2% for three months storage (Rehman *et al*., 2017). Pre-harvest calcium spray application of 150 days increased the level of pH in apple fruits stored for 240 days. Considerable variations were observed for pH in apple fruits stored for eight months. The amount of pH in apple fruits increased ranged from 3.17% to 7.01% during storage. The level of pH in apple fruits was slight up to three months storage period but further increased. pH and acidity are linked factors and decrease in acidity leads to increase in pH, when acidity decreases the acids, decline resulting in decrease in acidity and increase in pH. Vatanparast *et al*. (2012) observed an increase in pH of fruit juice due to potassium sulfate (1.5%) application during storage. The results of Rehman *et al*. (2017) are in line with the present study who reported that calcium lactate 2% concentration showed significant increase in pH of stored apple fruits.

Fruit firmness (kg cm^{-2})

The data regarding fruit firmness in apple fruits as affected by storage duration after calcium spray application are presented in Figure 2. The data showed that firmness in apple fruits was significantly affected by foliar application of calcium and storage durations. It is clear from the data that firmness in apple fruits rather increased due to various calcium sprays. The highest firmness in apple fruits ranged from 4.17 Kg cm^{-2} observed in control treatment to 7.45 Kg cm^{-2} in fruits treated with 5th foliar spray (150 days), followed by fruit firmness 6.92 Kg cm^{-2} treated with 4th foliar spray (120 days). The extent of fruit firmness was Kg cm^{-2} with 2nd foliar spray (60 days), afterward increased to 6.31 Kg cm^{-2} observed with 3rd foliar spray (90 days) of calcium. In relation to the means of fruit firmness for storage period, significant differences were observed for inflexibility in apple fruits stored for eight months. The degree of firmness in apple fruits decreased during storage ranged from 7.28 to 1.19 Kg cm^{-2} in storehouse. The highest fruit firmness 7.28 Kg cm^{-2} observed at 0-day storage which progressively decreased during storage. The lowest fruit firmness Kg cm^{-2} was recorded in fruits stored for 225 days later than increased to Kg cm^{-2} stored for further 15 days. The scope of firmness in apple fruits was boundless up to three months storage period but further decreased. The observation revealed that pre-harvest calcium spray application of 150 days increased the level of firmness in stored apple fruits for 225 days.

Fruit firmness is a decisive factor for edible quality and market value of apples. The loss of fruit firmness is a serious concern resulting in quality losses (Kov *et al*., 2005). Foliar calcium chloride spray resulted in higher fruit firmness than other treatments (Ghorbani *et al*. 2017). Significant differences were observed for firmness apple fruits stored for eight months. The degree of firmness in apple fruits decreased ranged from 7.28 to 1.19 kg/cm^2 during storage. The highest fruit firmness was recorded in control treatment which progressively decreased during storage. The degradation of the cell wall led to firmness reduction which is primarily due to changes in the texture of the flesh (Fuller, 2008). Calcium combines with pectic acid, thus promoting cell adhesion, and helps to maintain fruit firmness by strengthening the cell wall structure and forming Ca pectate (Zhang *et al*., 2019). Furthermore, fruit firmness improves through the number of pectin network enhancements under exogenous calcium application, leading to the accumulation of homogalacturonans (Huang *et al*., 2023). The present results are in line with Suljević and Drkenda (2011) who reported significant variation in biochemical attributes especially fruit firmness, TSS and acidity of Idared and Granny Smith apples.

Reducing sugars (%)

The data concerning reducing sugars in apple fruits as affected by storage duration after foliar application of calcium chloride are shown in Figure 3. The data reveal that reducing sugars in apple fruits considerably affected by foliar application of calcium and storage duration. Reducing sugars in apple fruits ranged from 4.84% observed in control treatment to 7.77% in fruits treated with 5th foliar spray (150 days), followed by reducing sugars 6.56% treated with 4th foliar spray (120 days). The amount of reducing sugars was 5.44% with 2nd foliar spray (60 days), afterward increased to 5.93% observed with 3rd foliar spray (90 days) of calcium. Concerning the means of reducing sugars for storage period, considerable variations were observed for reducing sugars in apple fruits stored for eight months. The amount of reducing sugars in apple fruits increased ranged from 7.54 to 10.68% in storeroom. The lowest reducing sugars 7.54% observed at 0-day storage which gradually increased during storage. The highest reducing sugars 10.68% were recorded in fruits stored for 240 days. The level of reducing sugars in apple fruits found in restricted level up to four months storage period but auxiliary increase was noted in further storage. The observation revealed that pre-harvest calcium spray application of 150 days increased the level of reducing sugars in stored apple fruits for 240 days.

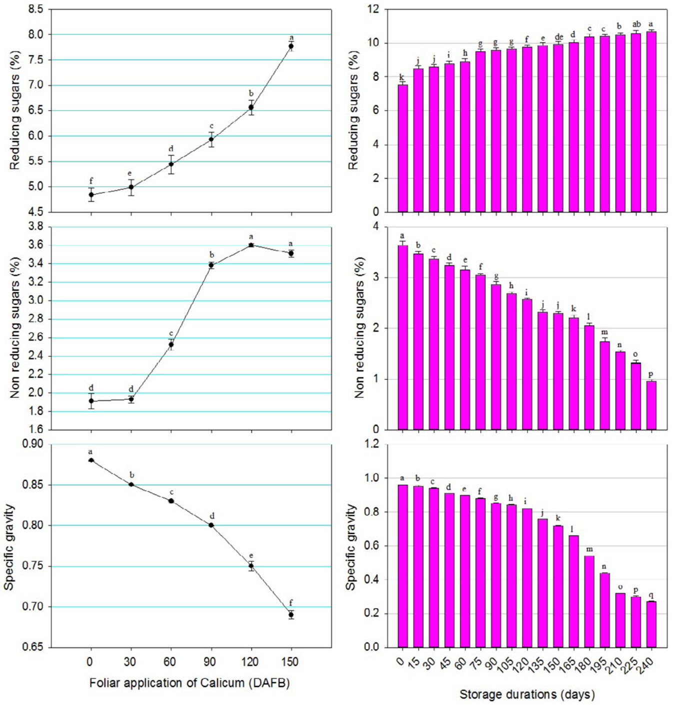

Figure 3: *Reducing sugars, non-reducing sugars and specific gravity of apple fruits as affected by time of foliar application of calcium and storage duration.*

Reducing sugars in apple fruits considerably affected by foliar spray application representing significant differences observed for reducing sugars in apple fruits. Sindha *et al*. (2018) stated that the applications of chemicals increased reducing sugars in custard apple fruits. The application of calcium was found to be very helpful in enhancing the shelf life of apple fruits (Hayat *et al*., 2003). Considerable variations were observed for reducing sugars in apple fruits stored for eight months. The amount of reducing sugars in apple fruits increased ranged from 7.54 to 10.68% during storage. Pre-harvest calcium spray application of 150 days increased the level of reducing sugars in apple fruits stored for 240 days. The storage temperature and acids are major factors during storage to enhance reducing sugar by converting sucrose into reducing sugars such as glucose and fructose. The present results are in line with previous which clearly concluded that foliar application of calcium improved fruit quality and shelf life of apple (Chandel *et al*., 2019). Moreover, Haleema *et al*. (2018) also reported similar results in tomato fruits.

Non-reducing sugars (%)

The data pertaining to non-reducing sugars in apple fruits as influenced by storage duration after foliar application of calcium chloride are presented in Figure 3. Storage durations and foliar application of calcium significantly affected non reducing sugars of apple. The level of non-reducing sugars in apple fruits ranged from 1.91% observed in control treatment to 3.60% in fruits treated with 4th foliar spray (120 days), followed by non-reducing sugars 3.51% treated with 5th foliar spray (150 days). The amount of non-reducing sugars was 2.52% with 2nd foliar spray (60 days), subsequently increased to 3.38% observed with 3rd foliar spray (90 days) of calcium. Concerning the means of non-reducing sugars for storage period, significant differences were observed for non-reducing sugars in apple fruits stored for eight months. The amount of non-reducing sugars in apple fruits decreased ranged from 3.63 to 0.95% in storehouse. The highest non-reducing sugars 3.63% observed at 0-day storage which gradually decreased during storage. The lowest non-reducing sugars 0.95% were recorded in fruits stored for 240 days. The ideal level of non-reducing sugars in apple fruits was noted up to a three-month storage period but further decrease was noted in additional storage. The data showed that pre-harvest calcium spray application of 120 days increased the level of non-reducing sugars, while storage period up to 240 days decreased the extent of non-reducing sugars.

Calcium spray applications influence non-reducing sugars in apple fruits. Non-reducing sugars in apple fruits moderately increased because of various calcium sprays. The level of non-reducing sugars in apple fruits ranged from 1.91% observed in control treatment to 3.60% in fruits treated with 4th foliar spray. The amount of non-reducing sugars was ideal up to three months but further decreased. Sindha *et al*. (2018) stated that the applications of chemicals increased non-reducing sugars in custard apple fruits. The application of calcium chloride was found to be very helpful in enhancing the shelf life of apple fruits (Hayat *et al*., 2003). Significant differences were observed for non-reducing sugars in apple fruits stored for eight months. The amount of non-reducing sugars in apple fruits decreased ranged from 3.63 to 0.95% during storage. The highest non-reducing sugars 3.63% observed at 0-day storage which gradually decreased. The ultimate level of non-reducing sugars in apple fruits was noted up to three months storage period but further decrease. Pre-harvest calcium spray application of 120 days increased the level of non-reducing sugars, while storage period decreased its amount. The results are in close conformity with Haleema *et al*. (2024) who reported significant enhancement in yield and quality of tomato by combine application of calcium, boron and zinc.

Specific gravity (w/v)

The data regarding specific gravity in apple fruits as affected by storage duration after calcium spray application are presented in Figure 3. The results indicated that specific gravity of apple fruits was significantly influenced by calcium as a foliar spray and storage durations. The extent of specific gravity in apple fruits ranged from 0.88 w/v observed in control treatment to 0.69 w/v in fruits treated with 5th foliar spray (150 days), followed by specific gravity 0.75 w/v treated with 4th foliar spray (120 days). The level of specific gravity was 0.83w/v due to 2nd foliar spray (60 days) and then decreased to 0.80 w/v observed with 3rd foliar spray (90 days) of calcium. Concerning the means of specific gravity for storage period, significant differences were observed for specific gravity in apple fruits stored for eight months. The level of specific gravity in apple fruits decreased during storage ranged from 0.96 to 0.27w/v in storehouse. The highest specific gravity 0.96 w/v observed at 0-day storage which gradually decreased during storage. The lowest specific gravity 0.27 w/v was recorded in fruits stored for 240 days. The extent of specific gravity in apple fruits was unlimited up to five months storage period but further decreased. The results indicated that pre-harvest calcium spray application of 150 days decreased the level of specific gravity in stored apple fruits for 240 days.

Specific gravity in apple fruits influenced by foliar spray application representing considerable divergence observed for specific gravity in apple fruits. Specific gravity of apple fruits slightly decreased due to various calcium sprays. Specific gravity in apple fruits ranged from 0.88 w/v observed in control treatment to 0.69 w/v in fruits treated with 5th foliar spray. Ghafir *et al*. (2009) stated that calcium may well decrease the growth of air spaces linked with ripening accountable for the loss of specific gravity. Significant differences were observed for specific gravity in apple fruits stored for eight months. The level of specific gravity in apple fruits decreased during eight months storage. The highest specific gravity 0.96w/v observed at 0-day storage which gradually decreased during storage. Prior to harvest, calcium spray applied at 150 days decreased the level of specific gravity in stored apple fruits for eight months. The specific gravity is high in fresh fruits and declines during storage due to fall of intercellular spaces with loss of moisture (Ozturk and Polat, 2016). Various research has shown significant effect of calcium on the specific gravity of apple fruits (Jan *et al*. 2013) and date palm (Sarrwy *et al*., 2012).

Weight loss (%)

The data concerning weight loss in apple fruits as affected by storage duration is shown in Figure 4. Mean values of the data reveal that weight loss in apple fruits is significantly influenced by storage duration. Significant differences were observed for weight loss in apple fruits stored for eight months. Weight loss in apple fruits increased ranged from 0 to 6.69% in storeroom. The fruits stored for 240 days resulted in significant weight loss of apple fruits. The maximum weight loss (6.69%) observed after 240 days storage, followed by weight loss of 6.01% in apple fruits stored for 225 days. Weight loss significantly increased with the increase in storage time. The slight increase in weight loss observed up to three months storage which considerably increased because of ongoing storage time up to eight months. The results revealed that storage period after three months (90 days) increased the stage of weight loss in stored apple fruits up to 240 days.

Weight loss depends on water present in the fruit, the shape and thickness of the skin and nature of waxes on fruit surface (Veravrbeke *et al*., 2003). Significant differences were observed for weight loss in apple fruits stored for eight months. Weight loss in apple fruits increased ranged from 0 to 6.69% in storehouse. The fruits stored for 240 days resulted in significant weight loss (6.69%) of apple fruits. Weight loss considerably decreases with increase in calcium spray application. Hayat *et al*. (2003) stated that increased calcium concentration decreases weight loss in apple fruits. Apple fruits sprayed with calcium concentrations at ripen stage reduce weight loss. Post-harvest calcium treatments inhibited fruit softening and decreased weight losses (Shirzadeh *et al*., 2011). Foliar application of calcium significantly affected weight loss during storage (Baneh *et al*., 2003). Foliar spray application of calcium chloride resulted best fruit quality and decreased weight loss (Omaina and Karima, 2007). Weight loss significantly increased with the increase in storage time. The slight increase in weight loss observed up to three months storage which considerably increased because of ongoing storage time up to eight months. Lower respiration rate and water content during storage led to weight loss in apple fruits (Ghafir *et al*., 2009). Hayat *et al*. (2003) and Jan *et al*. (2013) also observed similar results in decreased weight loss from apple fruits with calcium treatment.

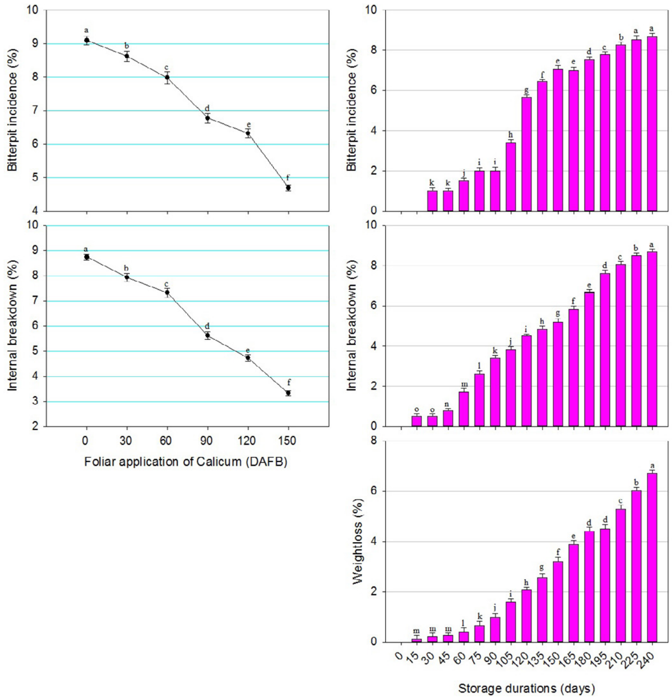

Figure 4: *Bitterpit incidence, internal breakdown and weight loss of apple fruits as affected by time of foliar application of calcium and storage duration.*

Bitter pit incidence (%)

Bitter pit incidence of apple fruits was significantly influenced by foliar application of calcium and storage durations (Figure 4). It is clear from the data that bitter pit incidence in apple fruits slightly decreased due to time of calcium as a foliar spray. Bitter pit incidence in apple fruits ranged from 9.09% observed in control treatment to 4.69% in fruits treated with 5th foliar spray (150 days), followed by bitter pit incidence 6.32% treated with 4th foliar spray (120 days). The level of bitter pit incidence was 7.98% due to 2nd foliar spray (60 days) and then decreased to 6.77% observed with 3rd foliar spray (90 days) of calcium. Concerning the means of bitter pit incidence for storage period, significant differences were observed for bitter pit incidence in apple fruits stored for eight months. The incidence of bitter pit on apple fruits increased during storage ranged from 0 to 8.68% in storehouse. The lowest bitter pit incidence 1% observed at 30- and 45-days storage which gradually increased during storage. The highest bitter pit incidence 8.68% was recorded in fruits stored for 240 days. The incidence of bitter pit was 8.53% in apple fruits stored for 225 days, followed by bitter pit (8.26%) incidence in fruits stored for 210 days. The results indicated that pre-harvest calcium spray application of 150 days decreased the level of bitter pit incidence, while storage period up to 240 days increased the extent of bitter pit incidence in stored apple fruits.

As a physiological disorder, bitter pit is described by brown scratches in the skin of the fruit. The incidence of bitter pit is possibly associated to inherited characters, however develops at the onset of ripening and at harvest (Crouch, 2003). Bitter pit incidence in apple fruits is influenced by foliar spray application. The incidence of bitter pit in apple fruits was slightly decreased due to various calcium sprays. Calcium helps in changing the metabolism in apple fruit, as well as sufficient concentration keeps fruit flesh firmness, delay fruit ripening and reduce the incidence of bitter pit, water core and internal breakdown (Frank *et al.*, 2007). The frequency of bitter pit in apple fruits ranged from the maximum of 9.09% observed in control treatment to the minimum of 4.69% in fruits treated with 5th foliar spray. The application of calcium spray of 150 days decreased the level of bitter pit before fruit harvesting. Calcium is absorbed during fruit development and frequent calcium application in low concentration throughout the fruit growth stage to increase calcium levels and decrease bitter pit incidence in apple (Lee *et al.*, 2017). Significant differences were observed for bitter pit incidence in apple fruits stored for eight months. The occurrence of bitter pit on apple fruits increased during storage ranged from 0 to 8.68% in storehouse. Long-lasting storage causes quick decay and maximum rotting resulting fruit spoilage (Sempere and Santamarina, 2007). The main reasons of the occurrence of superficial scald are the reactive oxygen species, long storage and oxidative stress to the fruits. The results of the present study are in line with Dris and Niskanen (1999) who observed significant reduction in physiological disorders especially bitter pit of apple fruits.

Internal browning (%)

Calcium application as a foliar spray and storage duration significantly influenced the internal browning in apple fruits (Figure 4). Mean values of the data confirmed internal browning in apple fruits significantly decreased as a result of various calcium sprays. Internal browning of apple fruits ranged from 8.74% observed in control treatment to 3.32% in fruits treated with 5th foliar spray (150 days), followed by internal browning 4.72% treated with 4th foliar spray (120 days). The level of internal browning was 7.32% due to 2nd foliar spray (60 days) and then decreased to 5.62% observed with 3rd foliar spray (90 days) of calcium. In relation to the means of internal browning for storage period, significant differences were observed for internal injuries in apple fruits stored for eight months. The occurrence of internal browning in apple fruits increased during storage ranged from 0 to 8.70% in storehouse. The lowest internal browning 0.50% observed at 15- and 30-days storage which gradually increased during storage. The highest internal browning 8.70% was recorded in fruits stored for 240 days. Internal browning in apple fruits was 8.50% stored for 225 days, followed by inner bruising (8.08%) in fruits stored for 210 days. The results indicated that pre-harvest calcium spray application of 150 days decreased internal browning, while storage period up to 240 days increased internal browning in stored apple fruits.

The internal browning in apple fruits is a serious problem in apple cultivation. The internal browning in apple fruits influenced by foliar spray application Calcium sprays and significantly decreased the occurrence of internal browning. The occurrence of internal browning ranged from the maximum of 8.74% observed in control treatment to the minimum of 3.32% in fruits treated with 5th foliar spray. The physiological disorders (such as internal breakdown, bitter pit, and water core) associated with apples are controlled under calcium treatment along with firmness maintenance (Conway *et al.*, 2002). Frank *et al.* (2007) stated that pre-harvest sprays of calcium chloride are more effective than postharvest sprays for the control of disease incidence in apple. Calcium sprays are commonly used to control Ca-related disorders such as bitter pit and internal browning in apples (Lee *et al.*, 2017). Significant differences were observed for internal browning in apple fruits stored for eight months. The occurrence of internal browning in apple fruits increased during storage ranged from 0 to 8.70% in storehouse. Internal browning in apple fruits was 8.50% stored for 225 days. Continuous storage causes speedy decay and highest rot resulted in fruit spoilage (Sempere and Santamarina, 2007). Pre-harvest calcium sprays are effective for extending the shelf life of stored apple fruits. The eight applications of liquid calcium fertilizers significantly reduced the occurrence of disease incidence during storage (Lanauskas *et al.*, 2012).

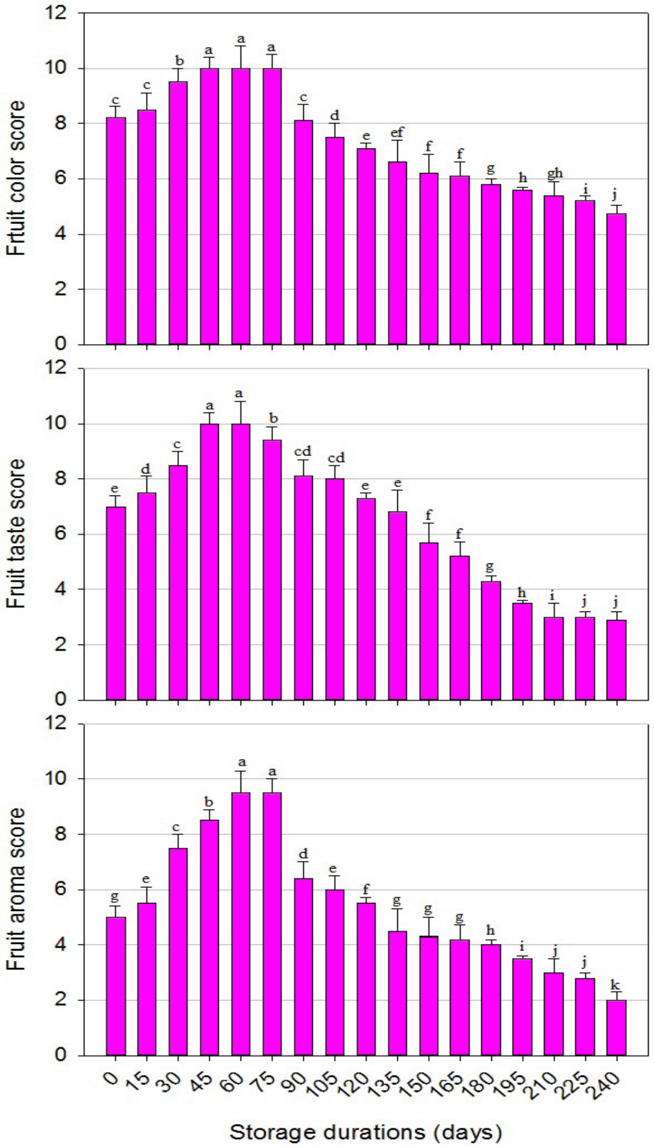

Figure 5: *Fruit color, taste and aroma score of apple fruits as affected by time of foliar application of calcium and storage duration.*

Fruit color

Significant differences for fruit color score of stored apple fruits were observed with storage durations (Figure 5). The color score of fruit was initially increased from 8.22 to 10.00 during 75 days of storage because of pre-harvest calcium spray application, afterward steadily decrease had occurred during eight months storage period. Least color score (4.73) was noted in fruits stored for 240 days, followed by color color of 5.20, 5.40 and 5.60 recorded in fruits during 225, 210 and 195 days of storage. Maximum fruit color grade (10.00) was recorded at 45, 60 and 75 days of storage. The estimated increase (21.65%) for 75 days storage and then decrease (52.7%) in fruit color grade up to eight months of storage happened in apple fruits. The results indicated that color grade of stored fruits was satisfactory up to four months of storage afterward declining in color grade was started up to eight months.

Colour is one of the top qualitative features for buyers to get high prices, as it not only affects the flavor but also gains customers' attention. Significant differences were observed in the color grade of stored apple fruits. The color grade of fruit was initially increased from 8.22 to 10.00 during 75 days of storage but afterward steadily decreased. During storage, the chemical reaction may be considered as discoloration factor. The color score of stored fruits was satisfactory up to four months of storage afterward declined. Red color is based on the amount of anthocyanin that depends on the breakdown of chlorophyll. Furthermore, under 5 days of storage similar discoloration in mango has also been observed (Suwapanich and Haewsungcharoen, 2007). The results are in line with Solhjoo *et al.* (2017) who observes significant improvement in skin color of apple fruits with foliar application of calcium and potassium.

Fruit taste

Significant differences were observed in taste of stored apple fruits with storage durations (Figure 5). Taste score was statistically parallel during the 1st two months of storage. The taste of fruit was first increased from 7.00 to 10.00 during 60 days of storage because of pre-harvest calcium spray application later a steady decrease occurred during eight months of storage period. Minimum taste (2.90) was noted in fruits stored for 210 days, followed by slight taste (3.00) recorded in fruits during 225 and 240 days of storage. Maximum fruit taste (10.00) was recorded at 45 and 60 days of storage. The estimated increase (42.85%) for 60 days storage and then decrease (70%) in fruit taste up to eight months of storage occurred in apple fruits. The results indicated that taste of stored fruits was satisfactory up to three months of storage and then declining in taste of apple fruits was started up to eight months.

The most favorable temperature is a key factor for sensory evaluation in stored products, as anthocyanin is negatively influenced under higher temperatures during storage (Marti *et al*., 2002). Similarly, colour, TSS, and flavor are maintained under a low temperature of 6 °C (Fardin *et al*., 2011). Higher temperature affects the anthocyanin, coloring accountable for red color of apple during. Significant differences were observed in the taste of stored apple fruits. The taste of fruit was first increased from 7.00 to 10.00 during 60 days of storage because of pre-harvest calcium spray application and later decreased during storage period. The taste of stored fruits was satisfactory up to three months of storage and then decline in taste of apple fruits was started up to eight months storage. The results of the present study are in line with Netravati and Jagadeesh (2018) who reported maximum score for taste and flavor (4.69) in the custard fruits treated with combined use of calcium chloride and wax (1:10).

Fruit aroma

Significant variations were observed in aroma of stored apple fruits with storage durations (Figure 5) however, aroma was statistically parallel during 2nd month of storage. The aroma of fruit was initially increased from 8.00 to 9.50 during 60 days of storage because of pre-harvest calcium spray application afterward gradual decrease occurred during eight months storage period. Minimum fruit aroma (2.50) was noted in fruits stored for 240 days, followed by least fruit aroma 2.80, 3.00 and 3.50 recorded in fruits during 225, 210 and 195 days of storage. Maximum fruit aroma (9.50) was recorded at 60 and 75 days of storage. The estimated increase (90%) during 60- and 75-days storage and then decreased (73.68%) in fruit aroma up to eight months of storage happened in apple fruits. The results indicated that aroma of stored fruits was satisfactory up to three months of storage afterward declining in aroma was started up to eight months.

Considerable variations were observed in aroma of stored apple fruits. The aroma of fruit was initially increased from 8.00 to 9.50 during 60 days of storage. Minimum smell (2.50) was noted in fruits stored for 240 days and maximum fruit aroma (9.50) was recorded at 60 and 75 days of storage. The aroma of stored fruits was better after three months of storage; afterwards, it declined up to eight months due to higher temperature and prolonged storage duration, which caused fruit softening to negatively influence its aroma. Similar results were reported and are in line with the present study in which maximum scores for appearance (4.50), texture (4.69), taste and aroma (4.69) were observed in the fruits treated with calcium chloride and wax (Netravati and Jagadeesh, 2018).

Conclusions and Recommendations

Calcium application up to five months has enhanced the quality of apple fruits by increasing total soluble solids, ascorbic acids, calcium content, pH, fruit firmness and decreasing bitter pit incidence and internal browning thus can be recommended for comprehensive storage period of apple fruits. Storage period increased pH, reducing sugars, weight loss, bitter pit incidence and internal browning hence measures need to be taken to decrease weight loss, bitter pit incidence and internal browning during storage. However, apple fruits should be stored up to 3 months keeping quality up to a safe level for consumer preferences.

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Novelty Statement

This study is very useful for the apple growers of Swat District. This study also key stage of apple growth where calcium deficiency can cause most prone physiological disorders of apple i.e. bitter bit incidence and internal browning. Moreover, the role of calcium in overcoming these physiological disorders with maximum retained quality for 5 months storage.

Author's Contribution

Nasra Begum and Abdul Mateen Khattak: Planned and executed the experiment. Analyzed the data and wrote the draft of the manuscript

Conflict of interest

The authors have declared no conflict of interest

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