Application of Calcium Chloride at Different Phenological Stages Alleviates Chilling Injury and Delays Climacteric Ripening in Peach Fruit during Low-Temperature Storage

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ABSTRACT
Owing to the high degree of perishability of the fruit, the peach industry faces serious challenges tackling physiological disorders, microbial/chemical decay, and the suboptimal quality of the fruit during and following cold storage. The present study was aimed to investigate the effects of spray applications of calcium chloride (CaCl₂) at various physiological stages of fruit growth and development, on ripening, flesh softening, chilling injury (CI) and nutritional quality of peach fruit cv “Flordaking” during low-temperature storage. The aqueous solutions of CaCl₂ [0, 1, 2, and 3% (w/v)] were sprayed on peach trees during cell division, pit hardening and cell expansion stages in 2011 and 2012. At commercial harvest, the fruit were hand-picked and were subjected to cold storage at 1 ± 1°C (RH: 90 ± 5%) for 6 weeks. The data on CI index and other quality characteristics of the fruit were recorded at harvest and then weekly for up to 6 weeks. The results showed that the pre-harvest spray application of CaCl₂ substantially reduced the incidence of chilling injury (CI) and delayed climacteric ripening seemingly by lowering the rates of ethylene production and fruit softening during storage as compared to control, regardless the doses of CaCl₂ and its application time, however higher doses of calcium chloride proved to be toxic causing blemishes on the surface of “Flordaking” peaches. In conclusion, pre-harvest spray application of CaCl₂ @ 1% may be a successful production management tool to maintain the quality and to extend storability of peach fruit during cold storage.

KEYWORDS
Ethylene; firmness; fruit quality; ascorbic acid; cold storage

INTRODUCTION
The good blend of volatile compounds, organic acids, phenolic pigments, dietary antioxidants, flavonoids, traces of lipids and proteins contained in the fruit makes peaches (Prunus persica L.) attractive to the consumers. However, it is characteristically a soft-fleshed and highly perishable stone-fruit with a very limited potential of postharvest life, specifically when it is stored at ambient conditions. To slow down the ripening process and to delay the deterioration of quality, peaches are usually stored at low (however, above the freezing) temperature. But improper storage conditions can cause decay and internal breakdown of the flesh which further results in deterioration of fruit quality during storage (Wang et al., 2019).

The internal breakdown of the fruit flesh known as chilling injury (CI) is a genetically controlled phenomenon usually triggered by improper combination of storage duration and conditions (Lurie and Crisosto, 2005). It expresses itself as mealy, woolly, dry, and hard-textured (usually juiceless) fruit along with browning and development of off flavor. It has been reported that the symptoms of CI
development in the fruit are more intense and appear faster when the susceptible fruits are stored at the temperature range of 2.2–7.6°C (generally called ‘killing zone’ temperature), compared to those stored at 0°C or below (Cao et al., 2018).

A variety of remedial measures have been tested to alleviate or limit the incidence of CI in peach fruit. Application of calcium-containing compounds or plant growth regulators, providing controlled atmospheric environment, interrupting storage conditions, delaying the pre-storage cooling operation and using modified atmosphere packaging (MAP) reduces the problem to some extent (Lurie and Crisosto, 2005; Mendes et al., 2019; Tanou et al., 2017). However, the results have been erratic and inconsistent while mostly remained inconclusive when tested under diverse agro-climatic conditions or on different genotypes (Wang et al., 2019).

Chilling injury results in loss of flavor and the fruit fails to ripen uniformly (Lurie and Crisosto, 2005). Flesh browning is considered as main chilling injury (CI) symptom caused by oxidation of phenolics, which leads to discoloration and decay of fruits, limiting the storage life and causing appreciable economic losses (Jin et al., 2011). Long-term storage is only possible in certain late cultivars. Therefore, interest has been renewed in alternative treatment to reduce post-harvest losses in fruits. Techniques leading to improved post-harvest storage are fundamental for guaranteeing its quality from the initial production to the final stage of consumption. Although enzymatic browning was successfully controlled by sulfites, a replacement has been urgently sought due to consumer’s increasing awareness of health but governments have banned the use of the sulfites for fresh fruits and vegetables (Manganaris et al., 2007). Thus, alternative means of browning control are needed. Since, peach fruit in Pakistan and most other parts of the world is produced in the areas far from markets of interest within the country, which means a longer time of transport to reach to the consumer. It is necessary to search for new techniques to extend the post-harvest life of peach.

Calcium is a nutritional element that differs from others by being imported into fleshy fruit only in small amounts, much less than into leaves. Although Ca is sufficiently available in the soil of the most orchards, due to its immobility within plant cells. Ca deficiency may become a problem in several fruits causing large economic losses (Gayed et al., 2017). Some authors postulated a competition for Ca between low-transpiring fruit, vigorously growing and highly transpiring leafy shoots (Montanaro et al., 2006). Exogenous application of calcium markedly increases the calcium content in the flesh and affects some of the changes associated with ripening and senescence (Ferguson, 2006).

In some fruit, calcium treatments have been useful to maintain firmness and to reduce decay (Lara et al., 2004). In strawberry fruit, foliar treatments with CaCl₂ delayed ripening and reduced the incidence of postharvest rots (Wojcik and Lewandowski, 2003). Positive results were also found by immersion in 1% CaCl₂ in raspberries (Montealegre and Valdes, 1993) and blueberries (Hanson et al., 1993). However, in other work, no beneficial effects have been observed in strawberries (Erincik et al., 1998). Several factors could contribute to explain these differences, such as differences in calcium uptake, and/or the reduced capacity of pectins to bind calcium due to a high degree of esterification (Swietlik and Faust, 2011). In addition, the reduced mobility and translocation of calcium might be a factor in the lack of positive results.

Foliar applications of calcium imply the absorption by the fruit. To be effective, foliar applications need to be followed several times during the phenological stages of fruit growth and development (Yuri et al., 2002). Exogenous calcium application during fruit development can be a safer mode to improve endogenous calcium levels in various fruits (Raese and Drake, 2000). Although the process of calcium diffusion into fruits is not well defined and contradictory results have been found regarding the effect in improving calcium content in peaches (Serrano et al., 2004). The lenticels (both in size and number) seem to have a significant positive effect on calcium penetration. Additionally, cracks and surface discontinuities, which are more apparent during the late phase of fruit growth, seem to offer sites for calcium penetration (Glenn et al., 1985).
The quality of the fruit can only be improved when the fruit is still attached to the plant while it can be maintained for reasonable duration in proper storage. To our information, there is very little work done on the role of pre-harvest use of calcium chloride application in peach during critical developmental stages, i.e., cell division, pit hardening and cell enlargement stage of fruit growth to improve quality of peach fruit at harvest and its effects during storage at low temperatures. Thus, the objective of the work was to probe the role of foliar calcium chloride applications to improve quality and storability of peach cv. ‘Flordaking’ at low temperature.

Material and Methods

Thirty-six, 8-year-old peach trees cv. “Flordaking” with visually uniform architecture of plant canopy, grafted on ‘Peshawar Local’ rootstock were selected from a commercial orchard at Madrota village (33° 52’ 24” N, 72° 21’ 44” E), in Attock district of the Punjab province in Pakistan. Under the vertical row planting pattern, the trees were planted following the square system setting the planting distance of 7 m each way and the rows were oriented in the north–south direction.

The aqueous solution of CaCl₂ [0, 1, 2, or 3% (w/v)] was sprayed onto the selected trees till run-off at three (3) stages of fruit growth and development (i.e., cell-division, pit-hardening, and cell-expansion). To lower the interfacial tension, Tween-20 (polyoxyethylene esobitanmonolaurate) was used as a surface-active agent. The trial was laid out following the randomized complete block design (RCBD) with three (3) replications (80 fruits per replication).

At the physiological maturity (break in ground color of peach fruit from green toward yellow), 80 peach fruit free from the symptoms of decay and mechanical damages, visibly uniform in shape and size were carefully hand-picked from replication and were transported to the laboratory for analysis. The fruit were divided into four equal lots. Each lot contained 20 fruit. The first lot was immediately analyzed for color perception, rate of ethylene production, flesh firmness, soluble solids content (SSC), titratable acidity (TA), and ascorbic acid (AsA) content. The other three lots were packed in corrugated boxes, which were subjected to low-temperature (1 ± 1°C) storage with 90 ± 5% RH, for 2, 4 and 6 weeks at regular atmosphere. Data for the incidence of CI and disease/decay incidence were recorded following 4 and 6 weeks of storage, respectively. The soluble solids content (SSC), titratable acidity (TA), flesh firmness, the objective color of the fruit in terms of Commission Internationale de l’Eclairage (CIE) units, loss in fruit weight, rate of ethylene production and integrity of cell-membrane of peach fruit, were evaluated fortnightly during cold storage.

Analysis of Peach Fruit

Fruit Yield, Yield Components

Samples of 10 mature fruit were taken for determination of the physical fruit quality characteristics like fruit weight (g.), fruit diameter (cm), pulp-to-stone ratio. Harvest dates and marketable yield (total weight of marketable fruit in Kg/tree) were recorded at the time of harvest. Initial harvest dates were recorded at the day of first harvest. The fruit was harvested at three different occasions. Three fruit slices from harvested fruit were taken for fruit tissue analysis followed by dry ashing procedure (Chapman and Pratt, 1961) and grinding subsequently. Calcium contents were determined as described by Berry and Johnson (1966).

Flesh Firmness

The flesh firmness of individual fruit in each replication was measured on the two opposite sides along the equatorial region with the portable penetrometer (BKD020; WEL, Willow bank Electronics Ltd., Napier, New Zealand) equipped with an 8 mm diameter tip. The average flesh firmness of the fruit was expressed as newton (N) force.
**Soluble Solids Content (SSC)**

Using the hand-held refractometer (FG-103; Chincan brand, Hangzhou Weiku Co. Ltd., Zhejiang, China), the SSC of peach juice (from a composite sample of eight fruits) was measured at room temperature and the results were reported in °Brix.

**Titratble Acidity (TA)**

To estimate titratable acidity (TA), 10 mL of peach juice (from a composite sample of 8 fruits) was diluted with distilled water (40 mL). The aliquot (10 mL) was titrated against 0.1 N NaOH to the endpoint (pH: 8.2), using phenolphthalein as the indicator. The percent acidity was calculated by the following formula:

\[
TA(\%) = \frac{\text{Volume of 0.1 NNaOH(used)} \times 0.0064}{\text{Volume of the sample used}} \times 100
\]

**Ascorbic Acid (Asa) Content**

To estimate the level of ascorbic acid in peach fruit, the method of Malik and Zora (2005) with some modifications (Petriccione et al., 2015). Briefly, the fruit pulp (2.5 g) was homogenized with 10 mL of 16% (v/v) metaphosphoric acid that contained 0.18% (w/v) of disodium salt (ethylene diamine tetraacetic acid; EDTA). The homogenate was centrifuged at 5000 × g for 10 min. Following incubation at 23 ± 1°C for 10 min, the optical density (OD) of the supernatant was recorded at 760 nm. Using L-ascorbic acid as the reference standard, a calibration curve was plotted against the series of its known concentrations to quantify the AsA content in the fruit sample. The results were expressed as microgram of ascorbic acid (AsA) per gram of fresh weight (µg AsA g⁻¹fm).

**Physiological Loss in Fruit Weight (PWL)**

The initial weight (W₀) of the corrugated box in each replication was noted at harvest and then subsequently at the end of each storage period. The physiological loss in fruit weight (PWL), expressed as percent weight loss relative to its initial (W₀) value, was calculated by the following equation:

\[
\text{PWL} (\%) = \frac{(W₀-Wₙ)}{W₀} \times 100
\]

Where \( W₀ \) = Initial weight, \( Wₙ \) = Weight of the fruit following the subsequent storage period.

**Objective Color of Fruit**

The objective color of the fruit’s exterior was recorded in terms of the Commission Internationale de L’Eclairage (CIE) units (L*, a*, and b*) using the chroma meter (CR-400; Konica Minolta 7 Sensing, Inc., Osaka, Japan).

**Fruit Decay (%)**

Peach fruit visibly showing the symptoms of bacterial lesion, brown rot (common), or fungal growth were considered as fruit decay. During storage, the affected fruit were counted fortnightly and the percent decay incidence relative to the total number of fruit were calculated.
Rate of Ethylene Production

To estimate the rate of ethylene production, a group of three peaches randomly selected from each replication were weighed and incubated for 1 h in an airtight jar equipped with a rubber septum. Using a syringe, 1 mL air was drawn out from the jar following the incubation period. The air sample was immediately injected onto the gas-chromatograph (Agilent Technologies, 6890 N Network GC system, Palo Alto, CA, USA) fitted with the flame ionization detector (FID). The temperatures of the column, injector, and the detector were maintained at 110°C, 100°C, and 250°C, respectively (Whale and Singh, 2007). The rate of ethylene production of peach fruit was estimated by the method described by Beyer and Morgan (1970) and was reported as μmol kg⁻¹ h⁻¹.

Permeability of Cell-Membrane

The permeability of cell-membrane of peach fruit was estimated as a function of electrolyte leakage (EL). The percent EL of the fruit skin was measured by the method described by Guo et al. (2017) with minor modifications. In short, 15 skin discs (8-mm diameter) of peach fruit were submerged in 15 mL of double deionized water (DDW), vacuum infiltrated for 30 min and then continuously shook for 2 h. Using the electrical conductivity meter (HI98309; Hanna Instruments, Ann Arbor MI, USA), the initial electric conductance (EC₀) of the sample was noted. The final electric conductance (ECₙ) was recorded after digesting the sample at 95°C for 30 min. The percent EL was calculated by the following expression:

\[ \text{TA(\%)} = \frac{\text{Volume of 0.1 NNaOH(used) x 0.0064Volume of the sample used X100}}{\text{Volume of 0.1 NNaOH}} \]  

(3)

Chilling Injury (Internal Browning)

The degree of chilling injury (CI) was assessed by visual observation of the extent of flesh browning on the flat surface of mesocarp in peach fruit by cutting five fruits from the middle along the axial diameter and observing the symptoms visually as described by Wang et al. (2006).

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) using Statistix 8.1 software (Eberhart and Russell, 1966). The multiple comparisons among the means were executed by the Duncan’s multiple range test (DMRT) at \( P \leq 0.05 \).

Results

Fruit Analysis at Harvest

Toxicity of CaCl₂

The pre-harvest spray applications of higher doses (2 and 3%) of CaCl₂ resulted in noticeable leaf and fruit burn which caused excessive pre-harvest fruit drop (data not shown). Following the spray application, the leaf burn appeared as brown necrotic tissue progressing to encompass the entire leaf with the passage of time. In the present study, the young developing leaves and the fruit appeared to be more affected with CaCl₂ toxicity compared to the older ones.

Fruit Yield, Yield Components, and Flesh Firmness

In both seasons (2011 and 2012), the pre-harvest spray application of 1% CaCl₂ significantly increased the weight, diameter, pulp-to-stone ratio, and flesh firmness of peach fruit at harvest as compared to control and other treatments regardless the time of application in both seasons (Tables 1 and 2).
Consequently, the yield of peach fruit from the tree treated with 1% CaCl₂ was also increased. However, the spray application of 2 and 3% CaCl₂ did not result in making significant changes in the physical traits of peach fruit, at harvest in any season. Instead, these spray applications resulted in fruit burn and heavy fruit drop prior to the physiological maturity which caused the yield to significantly decline as compared to control.

**Soluble Solids Content (SSC), Titratable Acidity (TA), and Ascorbic Acid (Asa) Content**

No significant change in SSC of peach fruit occurred in response to pre-harvest spray application of any level of CaCl₂ as compared to control in both seasons. However, the pre-harvest spray application of 1% CaCl₂ significantly increased the levels of AsA content and TA of the fruit at harvest as compared to control and other treatments both in 2011 and 2012 (Table 2). Peach fruit harvested from the control (untreated) trees had the lowest levels (4.3 and 4.9 mg Kg⁻¹, respectively), whereas those harvested from the trees treated with 1% CaCl₂ solution contained the highest levels (5.6 and 6.5 mg Kg⁻¹, respectively) of AsA in their pulp in both seasons.

The levels of Ca in both kinds of the fruit tissue were proportional to the level of CaCl₂ applied at the pit-hardening stage of fruit development. Pre-harvest spray application of CaCl₂ significantly increased the level of calcium content in the peel and pulp of peach fruit at harvest as compared to control regardless of the season (Table 3). All the treatments were at par in increasing the peel calcium contents as compared to control. While for pulp calcium contents higher doses of calcium were found significantly better as compared to the rest of the treatments yet statistically being at par.

**Fruit Analysis during Storage**

**Flesh Firmness**

The pre-harvest spray application of 1, 2, and 3% CaCl₂ resulted in significantly firmer peaches compared to control, during storage, in both of the seasons (Table 4). Peach fruit from the trees treated with CaCl₂ solution maintained the higher levels of their flesh firmness during storage,

| Table 1. Effects of different treatments of calcium chloride on peach fruit physical characteristics at harvest in the year 2011 and 2012. |
|----------------|----------------|----------------|----------------|----------------|----------------|
|                | Fruit Weight (g) | Diameter (mm)  | Pulp:stone     | Harvesting dates | Yield (Kg)     |
|                | 2011  2012       | 2011  2012     | 2011  2012     | 2011  2012      | 2011  2012     |
| Control        | 123.3 B 106.7 B  | 57.1 B 54.4 B  | 9.3 B 9.9 B    | 0 0             | 49.1 B 43.2 B |
| CaCl₂ 1%       | 148.3 A 120.6 A | 65.9 A 59.2 A  | 13.9 A 13.1 A  | 0 0             | 61.4 A 48.2 A |
| CaCl₂ 2%       | 123.9 B 108.9 B | 57.1 B 55.1 B  | 9.8 B 11.0 B   | 0 0             | 32.0 C 30.5 C |
| CaCl₂ 3%       | 125.0 B 107.2 B | 56.4 B 55.2 B  | 9.8 B 10.7 B   | 0 0             | 27.2 C 28.6 C |
| LSD            | 8.10  3.94       | 4.44  2.64     | 1.36  1.38     |                | 7.70  4.74     |

(+, days after, −, days before the control harvest date). Average of 3 replicates.

| Table 2. Effects of different treatments of calcium chloride on peach fruit physico-chemical characteristics at harvest in the year 2011 and 2012. |
|-----------------|----------------|----------------|----------------|----------------|
|                 | Fruit firmness (N) | TSS (°Brix) | TA (%)         | Ascorbic Acid (mg) |
|                 | 2011  2012       | 2011  2012   | 2011  2012     | 2011  2012     |
| Control         | 75.3 C 97.4 B    | 9.32 A 9.17 A | 0.93 C 1.09 B  | 4.33 C 4.90 D  |
| CaCl₂ 1%        | 96.5 A 105.1 A   | 9.32 A 9.30 A | 1.14 A 1.16 A  | 5.60 A 6.45 A  |
| CaCl₂ 2%        | 85.1 B 98.2 B    | 9.37 A 9.33 A | 1.03 B 1.10 B  | 4.83 B 5.40 C  |
| CaCl₂ 3%        | 93.6 A 98.2 B    | 9.71 A 9.27 A | 1.03 B 1.11 B  | 4.83 B 6.03 B  |
| LSD             | 3.6472 4.4262    | 0.6420 0.4380 | 0.0438         | 0.3693 0.3224  |

Average of 3 replicates. Means within a column having same letters are statistically non significant using Least Significant Difference Test.
comparing to those sprayed with water only (control). At the conclusion of the cold storage, the fruit from the trees treated with 1% CaCl$_2$ solution were significantly firmer than those harvested from the trees treated with 2 and 3% CaCl$_2$ solution or from the control trees. The fruit harvested from the water-treated (control) trees had the least flesh firmness at the end of 6 weeks of low-temperature storage in both seasons. A rapid loss in flesh firmness of peaches was recorded following the 2$^{nd}$ week of cold storage irrespective of the season and the treatments applied.

**Fruit Weight Loss**

Though the loss in fruit weight continued to increase along the time span during low-temperature storage irrespective of the treatments applied. However, a significant reduction in the rate of weight loss was recorded for the fruit harvested from the trees treated with pre-harvest spray application of 1, 2 and 3% CaCl$_2$ solution as compared to control both in 2011 and 2012 (Table 4). The fruit harvested from the peach trees treated with 1% CaCl$_2$ solution showed the least rate of weight loss compared (21.16 and 26.96%) to other treatments including control (35.16 and 34.66%). At the conclusion of the storage, the fruit harvested from the control (untreated) trees exhibited 1.6- and 1.3-fold higher losses in their weight in 2011 and 2012, respectively, compared to those harvested from the trees treated with 1% CaCl$_2$ solution.

**Fruit Skin Lightness (L*) and Fruit Skin Color Index (A*/b*)**

A linear, steady, and gradual, decrease in the lightness (L*) of skin color of peach fruit was observed during the course of low-temperature storage in this study irrespective of the application of CaCl$_2$ and the season of investigation (Table 4). Although the pre-harvest spray applications of 1–3% CaCl$_2$ solution significantly decreased the rate of change in the lightness of skin color. However, the peach fruit harvested from the trees treated with 1% CaCl$_2$ solution had significantly (P < 0.05) higher levels of L*, compared to all other treatments including control, at the conclusion of the six-week cold storage in both of the seasons.

Contrarily the color index (a*/b*) of peach fruit increased along the passage of time during cold storage regardless of the season and the treatments applied (Table 4). At the end of the low-temperature storage, the least increase in the color index of the fruit was observed in peaches harvested from the trees treated with 1% calcium chloride solution, whereas those harvested from the untreated (control) trees exhibited the highest level of increase in their color index.

**SSC, TA, SSC: TA (Ratio), and AsA Content**

After harvesting, the SSC of peach fruit continued to increase linearly throughout the low-temperature storage (Table 5). The pre-harvest spray applications of 1, 2, and 3% CaCl$_2$ solution onto the trees significantly reduced the rate of increase in SSC of peach fruit during storage. However, the fruit from the trees treated with 1% CaCl$_2$ solution had the least level of SSC at the conclusion (6 weeks) of the cold storage.

**Table 3. Effects of different treatments of calcium chloride on peach fruit physico-chemical characteristics at harvest in the year 2011 and 2012.**

|                     | Fruit Peel Calcium Contents (%)  | Fruit Pulp Calcium contents (%)  |
|---------------------|----------------------------------|----------------------------------|
|                     | 2011  | 2012  | 2011  | 2012  |
| Control             | 0.057 B | 0.058 B | 0.022 C | 0.023 C |
| CaCl$_2$ 1%         | 0.069 A | 0.069 A | 0.025 B | 0.028 B |
| CaCl$_2$ 2%         | 0.070 A | 0.072 A | 0.027 AB | 0.029 AB |
| CaCl$_2$ 3%         | 0.070 A | 0.071 A | 0.029 A | 0.031 A |
| LSD                 | 2.256E-03 | 3.311E-03 | 2.909E-03 | 2.596E-03 |

Average of 3 replicates. Means within a column having same letters are statistically non significant using Least Significant Difference Test.
Table 4. Soluble Solid Contents (SSC) Titratable acidity (TA) and Ascorbic acid (AA) in control and calcium chloride (CaCl₂) treated peach fruits during storage.

| CaCl₂% (Conc.) | Storage period (Weeks) | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 |
|---------------|------------------------|------|------|------|------|------|------|------|------|
| Control (A)   | 0                      | 0.00±L | 0.00±L | 68.45±AB | 72.47±A-D | 1.0±H | 0.0±I | 0.0±I | 0.0±I |
| Day 1         | 1                      | 90.30±A | 90.30±A | 69.38±A | 73.60±A | 1.0±H | 0.0±I | 0.0±I | 0.0±I |
| Day 2         | 2                      | 85.10±AB | 85.10±AB | 68.77±AB | 72.99±AB | 1.0±H | 0.0±I | 0.0±I | 0.0±I |
| Day 3         | 3                      | 93.60±A | 93.60±A | 68.23±AB | 72.64±ABC | 1.0±H | 0.0±I | 0.0±I | 0.0±I |
| Day 4         | 4                      | 78.20±F | 78.20±F | 67.14±AB | 72.93±A | 1.0±H | 0.0±I | 0.0±I | 0.0±I |
| Week 1        | 5                      | 36.35±F-L | 36.35±F-L | 60.04±A-F | 70.58±B-G | 0.35±FG | 0.14±H | 0.14±H | 0.14±H |
| Week 2        | 6                      | 36.35±F-L | 36.35±F-L | 60.04±A-F | 70.58±B-G | 0.35±FG | 0.14±H | 0.14±H | 0.14±H |
| Week 3        | 7                      | 36.35±F-L | 36.35±F-L | 60.04±A-F | 70.58±B-G | 0.35±FG | 0.14±H | 0.14±H | 0.14±H |
| Week 4        | 8                      | 36.35±F-L | 36.35±F-L | 60.04±A-F | 70.58±B-G | 0.35±FG | 0.14±H | 0.14±H | 0.14±H |
| Week 5        | 9                      | 36.35±F-L | 36.35±F-L | 60.04±A-F | 70.58±B-G | 0.35±FG | 0.14±H | 0.14±H | 0.14±H |
| Week 6        | 10                     | 36.35±F-L | 36.35±F-L | 60.04±A-F | 70.58±B-G | 0.35±FG | 0.14±H | 0.14±H | 0.14±H |

LSD (P < .05)

** Concentration **
** Storage period **
** Interaction **
With the passage of time during storage, the TA of peach fruit showed the trend of gradual decline irrespective of the CaCl₂ treatments or the season of study (Table 5). After the 6 weeks of cold storage, the peaches harvested from the trees treated with 1% CaCl₂ solution exhibited higher level of TA compared to those harvested from the trees sprayed with water (control) or 2–3% CaCl₂ solution in both seasons in the present study.

However, we observed the trend of decline in SSC: TA ratio of peach fruit during low-temperature storage regardless of season and CaCl₂ treatments. However, at the conclusion of the cold storage, the fruit from the untreated (control) trees had the highest SSC: TA ratio. Whereas those harvested from the trees treated with 1% CaCl₂ solution had the lowest SSC: TA ratio (Table 5).

The duration of the storage of peach fruit at 1 ± 1°C resulted in a linear decrease in the level of AsA content in the fruit regardless of the season and the treatments with CaCl₂ solution (Table 5). However, the pre-harvest spray application of 1% CaCl₂ solution onto the trees resulted in 1.3- and 1.6-fold higher levels of AsA content in the fruit, in 2011 and 2012 respectively, as compared to control at the conclusion of the 6 weeks of cold storage.

**Biosynthesis of Ethylene**

The treatment of peach trees with 1–3% CaCl₂ solutions did not significantly delay the climacteric rise, yet it caused significantly lower rate of ethylene production in the fruit, compared to control, during cold storage (Table 6). It was observed that the decrease in the rate of ethylene production was inversely proportional to the level of CaCl₂ solution applied. The pre-harvest spray application of 1% CaCl₂ solution resulted in 1.4 and 1.3-fold lower levels of climacteric peak in peach fruit, compared to control, at the end of 6 weeks of cold storage. The interaction between CaCl₂ treatments and the storage period for the rate of ethylene production was highly significant (Table 6).

**Chilling Injury (Internal Browning) and Electrolyte Leakage**

The spray application of 1, 2, and 3% CaCl₂ significantly delayed the onset of CI in peach fruit by four weeks during low-temperature storage regardless of seasons (Table 7). The highest level of CI index was recorded in the fruit harvested from the control trees. During storage, the changes in CI index of peach fruit were parallel to the EL of their skin discs (Table 6). While the least levels of EL were observed in the fruit collected from the trees treated with 1% CaCl₂, in both of the seasons.

**Disease and Decay Incidence**

Pre-harvest spray application of 1, 2, and 3% CaCl₂ onto the peach trees significantly reduced the rate of disease/decay occurrence in the fruit during low-temperature storage in both seasons (Table 7). At the conclusion of the cold storage, the highest degree of disease/decay incidence (51 and 52%, for 2011, and 2012, respectively) was recorded in peach fruit harvested from the untreated (control) trees. The fruit from the trees treated with 1% CaCl₂ solution exhibited the least disease/decay incidence, at the conclusion of cold storage in the present study (Table 7).

**Discussion**

The results from the present investigation showed that pre-harvest spray application of CaCl₂ significantly affected the physico-chemical traits (fruit weight, diameter, SSC, and TA) of peach fruit at harvest. Additionally, the application of CaCl₂ significantly reduced the rates of decay incidence, physiological loss in weight, quality deterioration in terms of TA, pH, SSC, AsA, and ethylene production in the fruit as compared to control during low-temperature storage. It suggests that the
treatment of peach fruit with CaCl₂ at pit-hardening stage of fruit development may effectively slow down the ripening phenomenon in peaches without compromising the nutritional quality of the fruit, during cold storage.

**Effects of Pre-Harvest Treatments of Calcium Chloride on the Quality of Peach Fruit at Harvest**

In the present study, the higher concentrations (2 and 3%) of CaCl₂ proved to be toxic for the leaves and fruits. In the aqueous solution, CaCl₂ is dissociated into free ions (Ca²⁺ and Cl⁻). The sodium ions (Na⁺) present in irrigation water, soil residues and in the tissues of peach fruit and/or leaves form sodium salt by combining with the Cl⁻ ions. This buildup of the salt dries out the leaf and fruit tissues causing sunburn and tissue corrosion. The change in the chemical structure of CaCl₂ when dissolved in water or combined with other toxic ions of the fruit and leaves produced during the normal physiological phenomena such as respiration, condensation, and transpiration, may also have toxic effects on the fruit and leaves. Free hydrogen ion (H⁺) forms hydrochloric acid
**Table 6.** Ethylene biosynthesis and Electrolyte leakage in control and calcium chloride (CaCl₂) treated peach fruits during storage.

| CaCl₂% (Conc.) | Storage period (Weeks) | Electrolyte Leakage | Electrolyte Leakage |
|----------------|-----------------------|---------------------|---------------------|
|                | 2011                  | 2012                | 2011                | 2012                |
| 0              | 0                     | 9.60 ± L            | 7.34 ± M            | 44.06 ± E-J         | 34.92± GH         |
| 1              | 0                     | 8.92 ± L            | 7.47 ± M            | 35.32 ± J           | 26.37 ± I         |
| 2              | 0                     | 9.10 ± L            | 7.09 ± M            | 40.27± HJ           | 32.67± HI         |
| 3              | 0                     | 9.53 ± L            | 7.13 ± M            | 40.76± HJ           | 36.41± GH         |
| Day 0          | 1                     | 9.29E               | 7.26 G              | 40.10 F             | 32.59 C           |
|                | 2                     | 25.77 ± G-J         | 26.59± UK           | 49.53 ± C-H         | 41.88 ± D-G       |
|                | 1                     | 21.85 ± K           | 22.95 ± L           | 37.64 ± J           | 39.57 ± FGH       |
|                | 1                     | 23.55± JK           | 24.85 ± JKL         | 41.84 ± F-J         | 42.83 ± C-G       |
|                | 1                     | 24.48± UK           | 24.83 ± JKL         | 41.76 ± F-J         | 44.65 ± B-F       |
| Week 1         | 1                     | 23.91D              | 24.80 F             | 42.69EF             | 42.23B            |
|                | 2                     | 30.17± DE           | 30.39± FGH          | 57.45 ± A-D         | 46.33 ± A-F       |
|                | 1                     | 24.25± UK           | 26.42± UK           | 41.41 ± F-J         | 41.83 ± D-G       |
|                | 2                     | 25.56± HJ           | 28.49± GHI          | 41.62 ± F-J         | 46.41 ± A-F       |
|                | 2                     | 28.09 ± E-H         | 31.70± D-G          | 41.64 ± F-J         | 45.01 ± B-F       |
| Week 2         | 2                     | 27.02 C             | 29.25D              | 45.53DE             | 44.89B            |
|                | 3                     | 31.95± BCD          | 44.00 ± A           | 59.54± ABC          | 48.00 ± A-E       |
|                | 3                     | 28.02 ± E-H         | 32.67 ± DEF         | 40.95 ± G-J         | 41.22± EFG        |
|                | 3                     | 30.18± CDE          | 34.06± DE           | 45.58 ± E-I         | 44.98 ± B-F       |
|                | 3                     | 31.98± BCD          | 38.33 ± B           | 46.03 ± E-I         | 45.58 ± B-F       |
| Week 3         | 3                     | 30.53B              | 37.27A              | 48.03 CD            | 44.95B            |
|                | 4                     | 39.07 ± A           | 39.36 ± B           | 62.08 ± AB          | 51.19± AB         |
|                | 4                     | 28.64± EFG          | 29.21± GHI          | 47.09 ± E-I         | 46.04 ± B-F       |
|                | 4                     | 33.73 ± B           | 34.91± CD           | 46.78 ± E-I         | 47.58 ± A-F       |
|                | 4                     | 36.91 ± A           | 37.64± BC           | 47.98 ± D-H         | 50.03± A-D        |
| Week 4         | 4                     | 34.59A              | 35.28B              | 50.98BC             | 48.71A            |
|                | 5                     | 36.78± A            | 39.26 ± B           | 64.42 ± A           | 50.02 ± A-D       |
|                | 5                     | 25.28± HJ           | 26.76± UK           | 47.19 ± E-I         | 47.53 ± A-F       |
|                | 5                     | 26.76± FHGL         | 28.59± GHI          | 52.78 ± B-E         | 51.84± AB         |
|                | 5                     | 32.19± BCD          | 31.40± EFG          | 51.38 ± C-F         | 51.29± AB         |
| Week 5         | 5                     | 30.25B              | 31.50 C             | 53.94B              | 50.17A            |
|                | 6                     | 33.09± BC           | 31.03± EFG          | 66.43A              | 50.61± ABC        |
|                | 6                     | 21.76 ± K           | 23.59± KL           | 50.91 ± C-G         | 48.43 ± A-E       |
|                | 6                     | 24.14± UK           | 24.38± JKL          | 58.29± ABC          | 54.43 ± A         |
|                | 6                     | 29.32± DEF          | 27.44± HJ           | 58.50± ABC          | 50.85± ABC        |
| Week 6         | 6                     | 27.08 C             | 26.61E              | 58.53A              | 51.08A            |
|                |                       | 2.91                | 3.22                | 10.02               | 2.10              |
| LSD (p < .05)  |                       | 0.04                | 0.06                | 9.05                | 11.93             |

| Storage period | Concentration | Interaction | LSD (p < .05) |
|----------------|---------------|-------------|---------------|
|                | **            | **          | NS            |
|                | **            | **          | NS            |

**Table 7.** Chilling injury (browning index) after four weeks at low temperature +3 days shelf life and disease and decay incidence in control and calcium chloride (CaCl₂) treated peach fruits after six weeks of storage.

| SA mM (concentration) | Chilling Injury | Disease and Decay (%) |
|-----------------------|-----------------|-----------------------|
|                       | 2011            | 2012                  | 2011            | 2012                  |
| **Control**           |                 |                       |                 |                       |
| CaCl₂ 1%              | 0.23 A          | 0.45 A                | 50.7 A          | 51.7 A                |
| CaCl₂ 2%              | 0.11 B          | 0.13 D                | 32.0 C          | 28.3 C                |
| CaCl₂ 3%              | 0.14 B          | 0.21 C                | 34.7 BC         | 31.7 BC               |
| LSD (p < .05)         | 0.04            | 0.06                  | 9.05            | 11.93                 |

after reacting with chloride (Cl⁻) ions, which may reduce fruit skin brightness and burn lenticels (Ali et al., 2014). The symptoms of toxicity similar that we observed in peach fruit and leaves in response to spray application of CaCl₂ had also been reported in ‘Boskoop’ and ‘Elstar’ apples (Mayr and Schröder, 2002), kiwi fruit (Cooper et al., 2007) and strawberry (Hernandez-Munoz et al., 2008).
The foliar application of 1% CaCl₂ significantly improved the physical characteristics such as fruit weight, fruit size, and pulp: stone ratio, thereby resulting in greater economic return as the large-sized fruits are appreciated in the fresh market. The similar results of improving physical traits of various horticultural commodities by the use of CaCl₂, have been reported in bell pepper (Toivonen and Bowen, 1999), tomato (Hao and Papadopoulos, 2003), nectarines (Serrano et al., 2004), apple (Khalifa et al., 2009), pomegranate (Ramezanian et al., 2009) and dates (Marzouk and Kasem, 2011). Khalaj et al. (2017) showed that foliar application of calcium results in increasing the levels of pectin substances in the cell wall that in turn causes the increase in thickness of the pericarp tissues and ultimately the bio-weight of the fruit. Previously, we suggested that calcium plays a vital role in fruit growth and development, especially at the cell division stage, which consequently affects the size and weight of peach fruit, and finally the productivity of the crop (Ali et al., 2014). The influence of calcium on the levels of endogenous growth substances especially on the activities of cytokinins has been reported (Bangerth, 1979). The increased levels of plant growth substances in response to calcium application may also be involved in improving the physical characteristics of the fruit (Lownds et al., 1993). The toxic effects of higher doses (2 and 3%) of CaCl₂ led to excessive fruit drop prior to harvest that ultimately resulted in the lowest yield of peaches at commercial harvest.

The poor development of xylem may cause the leaves and the fruit deficient in calcium content. The exogenous application of calcium may solve the problems associated with calcium deficiency (Al Eryani-Raqeeb et al., 2009). The pre-harvest spray application of CaCl₂, especially its higher doses (2 and 3%), in the present investigation substantially increased the levels of calcium content both in the leaves and the fruit tissues. The similar results were reported in tomato fruit by Tuna et al. (2007).

The peach fruit treated with CaCl₂ solution in the present investigation were firmer than those treated with water only. Increase in the levels of pectin in the cell wall, in response to exogenous application of calcium compounds in a variety of fresh production, is well documented (Toivonen and Bowen, 1999; Wang et al., 2018). Calcium may also inhibit the activities of polygaracturonase (Uhlm et al., 2003) the enzyme that is involved in fruit softening.

The application of calcium did not significantly affect the SSC of peach fruit in the present study. These results are in line with those reported by Lester and Grusak (2004) in melons, and Peyvast et al. (2009) in tomato. Contrarily, pre-harvest calcium application increased the level of AsA in the peach juice, which may be attributed to lower permeability of the cell-membrane or reduced rate of fruit respiration (Godara et al., 2002). These results were in line with those reported by Wills et al. (1988) and Singh et al. (1998) in mango, Samaan et al. (2001) in citrus, and Ramezanian et al. (2009) in pomegranate.

**Effects of Pre-Harvest Treatments of Calcium Chloride on Fruit Quality Parameters during Storage**

During storage, the rate of physiological loss in peach weight was substantially reduced in response to pre-harvest spray application of CaCl₂ onto the intact fruit, compared to control, in the present investigation. The results from the present study were in agreement with those reported by in plums (Kirmani et al., 2013), in peaches (Belge et al., 2019), in mangosteens (Sripong et al., 2019) and in cucumbers (Nasef, 2018). The major reason of the postharvest physiological loss in fruit weight is the loss of moisture content from the fruit by evaporation and respiration through cuticle, stomata and stem scar (Paul and Chen, 1989). The role of calcium in maintaining and improving the turgidity of cell wall is well documented (Hocking et al., 2016; Ogden et al., 2018). In the present investigation, the reduced rate of physiological loss in fruit weight may be ascribed to the improved tissue turgidity or firmness of the fruit flesh in response to the application of CaCl₂ probably by decreasing the rate of fruit respiration (Faust and Timon, 2010) or through the decreased activities of the enzymes involved in disintegration of cellular structure of the fruit (Chea et al., 2019).
Fruit firmness declined during storage. However, ca application resulted in a higher fruit firmness compared to control both at harvest as well as after 6 weeks of low-temperature storage. CaCl₂ (1%) treated peaches exhibited a significantly (p < .05) higher firmness during the entire storage period than none treated ones. Softening of fruits is one of the common physical parameters to assess the progress of ripening (Brummel, 2006) and softening is a major problem of peach that limits the quality. Fruit softening is caused either by breakdown of insoluble proto-pectins into soluble pectin or by hydrolysis of starch (Pantastico et al., 1975), or by increased membrane permeability caused by cellular disintegration (Wara-aswapati et al., 1990). The loss of pectic substances in the middle lamellae of the cell wall is perhaps the key step in ripening process that leads to the loss of cell integrity or firmness (Kirmani et al., 2013). The desired effect of calcium in maintaining fruit firmness may be due to the calcium binding to free carboxyl groups of polygalacturonate polymer, stabilizing, and strengthening the cell wall, which in turn may strengthen the tissue thus becoming more resistant to hydrolytic enzyme activity, where calcium inhibits the polygalacturonase activity in cell walls (Buescher and Hobson, 1982). Results here regarding the role of CaCl₂ in the reduction of fruit softening are in correlation with those obtained on plums (Kirmani et al., 2013) and strawberry (Hernandez-Munoz et al., 2008). All these reports evidenced a similar reduction in the firmness loss following the pre-harvest application of CaCl₂. These results are in agreement with those of Conway et al. (2001) in apple, Selvan and Bal (2005) in guava, Prasad et al. (2015) in pears.

During the experimental period fruit color L* (brightness) and a*/b* (fruit skin color index) values changed as compared to the harvest date, L* value decreased while a*/b* value increased with the passage of time. Among the treatments 1% CaCl₂ recorded the minimum changes in fruit color as compared to the other treatments. These findings are in agreement with Ganai et al. (2015) who observed minimum changes in fruit color during storage as a result of calcium chloride treatment. During normal ripening process of fruit, rapid degradation of chlorophyll occurs in the tissues with an increased level of carotenoids and colored pigments. Similarly loss of water through the membrane also caused the darker appearance of the fruit. The reduction in color development of the fruit with foliar application of calcium chloride in the present experiment might be attributed to lower chlorophyll degradation and delay in the senescence process during the storage.

Calcium chloride has been shown to have an effect in ethylene production, where it suppresses its synthesis Ishaq et al. (2009), thereby delaying its role in unmasking the yellow and red carotenoids in climacteric fruits (Pinzón-Gómez et al., 2014). This could possibly explain the cause of reduced color change (a*/b*) in peaches, as ethylene is considered the key factor for changing the color of the horticultural commodities (Freitas and Nassur, 2017).

CaCl₂ treatments significantly delayed the increase in SSC of the fruit till the end of the low-temperature storage as compared to control. Results of calcium as a pre-harvest treatment in maintaining the SSC are in harmony with those mentioned by Montanaro et al. (2006) on kiwifruit, Bhat et al. (2012) on pear and El-Badawy (2012) on peach. Increased TSS percentages throughout the storage period are presumably due to increased activity of enzymes responsible for starch hydrolysis to soluble sugars and can be caused by the decline in the amount of carbohydrates, pectines, partial hydrolysis of protein and decomposition of glycosides into subunits during respiration (Ali et al., 2014). The effect of calcium treatment in slowing the increase of SSC content of the fruits was probably due to retarding the ripening process. Thus, lower SSC due to the slower change from carbohydrates to sugars (Rohani et al., 1997) and reduction in water loss from the fruit surface as observed in the experiment.

Titratable acidity decreases with increasing storage time, thus, the decrease of acidity during storage demonstrated fruit ripening. As for treatments, in both seasons highest means of TA contents were obtained with all the combinations of 1% CaCl₂. The calcium treated fruits could maintain a higher acidity during the storage might be due to reduced respiration rate. Similar findings have been reported on aonla (Singh et al., 2005), guava (Goutam et al., 2010), and peach (Sajid et al., 2017).

Ascorbic acid content of peach fruits decreased during storage period. During storage, oxidizing enzymes like ascorbic acid oxidase, peroxidase, catalase, and polyphenol oxidase might be causing decrease in ascorbic acid content of fruits (Singh et al., 2005). Activities of oxidizing enzymes might be
reduced. This effect could also be explained due to delayed ripening; hence, this process might have delayed the oxidation of ascorbic acid, which can occur rapidly during the normal ripening. This finding is in agreement with those of Goutam et al. (2010), Islam et al. (2013) and Samant et al. (2008) in guava, mango, and ber fruits, respectively.

In peach fruits, chilling injury manifested as internal browning was affected by the applied treatment. The highest chilling injury was observed for the control fruits after fourth week of low-temperature storage, whereas the lowest value was for 1% CaCl2. Similarly, calcium treatments were effective in controlling the chilling injury in pear (Rosen and Kader, 1989), loquat (Akhtar et al., 2010) and pomegranate (Ramezanian et al., 2009). It has been shown in many studies that Ca2+ could improve the integrity of plasma membrane (De Souza et al., 1999), consequently, lower chilling damage, as observed in the present study. Ramezanian et al. (2009) found higher antioxidant defense system in Ca treated fruit, so it can also be concluded that higher level of antioxidant enzymes as a result of the Ca treatment, might also be responsible for membrane integrity thus reducing chilling injury in peach fruits.

The results in Table 1 show that the storage period has a significant effect on ethylene of fruits (p ≤ 0.05). The results indicate that maximum ethylene was observed in control treatment, while, the lowest ethylene was recorded in 4% (w/v) Ca. Oxidative membrane injury allows the mixing of the normally separated enzyme (PPO) and oxidizable substrates (polyphenols), which lead to browning (Hodges, 2003). High calcium concentrations result in decreased ethylene production, electrolyte leakage and flesh browning symptoms which are directly associated with calcium content in fruits (Hewajulige et al., 2003). Decreased electrolyte leakage by calcium application increases the cell wall integrity and stability (Mortazavi et al., 2007). Ethylene possesses an important role in integrating developmental signals and responses to abiotic stresses, like cold storage, and it has been suggested that calcium delays the onset of the ethylene climacteric period and climacteric peak (Ben-Arie et al., 1995). The results in Table 1 show that the storage period has a significant effect on ethylene of fruits (p ≤ 0.05). The results indicate that maximum ethylene was observed in control treatment, while, the lowest ethylene was recorded in 4% (w/v) Ca. Oxidative membrane injury allows the mixing of the normally separated enzyme (PPO) and oxidizable substrates (polyphenols), which lead to browning (Hodges, 2003). High calcium concentrations result in decreased ethylene production, electrolyte leakage, and flesh browning symptoms which are directly associated with calcium content in fruits (Hewajulige et al., 2003). Decreased electrolyte leakage by calcium application increases the cell wall integrity and stability (Mortazavi et al., 2007). Ethylene possesses an important role in integrating developmental signals and responses to abiotic stresses, like cold storage, and it has been suggested that calcium delays the onset of the ethylene climacteric period and climacteric peak (Ben-Arie et al., 1995).

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The results showed that the treatments significantly lowered ethylene peak of the stored fruit as compared to control. The results are in accordance with the findings of Shirzadeh et al. (2011), Shirzadeh et al. (2011) who observed similar pattern of ethylene in calcium treated apples as compared to control. It has been shown that once the intracellular calcium concentration increased to at least 1 μM (Saunders and Helper, 1983), calcium is bound to calmodulin, which is one of the most common
intracellular calcium receptors and the accruing calcium-calmodulin complex modulates many physiological processes. Calcium may be inactivating the ethylene forming enzyme in the ethylene biosynthetic pathway via calcium-calmodulin mediated reactions (Senevirathna and Daundasekera, 2010). Produce spoilage and deterioration is a function of degradation of the cell wall (Franco-Mora et al., 2005). Application of a substance that delays any form of cell disintegration has been found to extend the postharvest life of produce. Calcium chloride has previously been reported to delay onset of deterioration in pear fruits (Sugar and Basile, 2011). This is attributed to calcium’s interaction with the cell walls components resulting in maintenance of the cell for a long period of time (Babu et al., 2011) thus resulting in improved postharvest life. Calcium ions have been observed to form bridges with peptic molecules of the middle lamella. As a result, better cell cohesion is maintained leading to better pH quality of produce (Franco-Mora et al., 2005). The results here may also be attributed to the role of calcium ions in reducing fruit softening by strengthening the cell walls. Jawandha et al. (2012) reported that calcium compounds significantly thickened the middle lamella of fruit cells owing to increased deposition of calcium pectate and thereby maintained the cell wall, which inhibits the penetration and spread of pathogens in fruits ultimately reducing the spoilage percentage of fruits. Similar observations were made by Selvan and Bal (2005) in guava fruits and Singh et al. (2013) in ber fruits. Results of this study are in agreement with previous reports which indicated that calcium chloride spray reduces physiological disorders of fruits and increases their resistance to infection than untreated ones (Kirimani et al., 2013).

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No potential conflict of interest was reported by the author(s).

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Literature Cited

Akhtar, M.J., M. Ahamed, S. Kumar, H. Siddiqui, G. Patil, M. Ashquin, and I. Ahmad. 2010. Nanotoxicity of pure silica mediated through oxidant generation rather than glutathione depletion in human lung epithelial cells. Toxicology 276(2):95–102. doi: 10.1016/j.tox.2010.07.010.

Al Eryani-Raqeeb, A., T.M.M. Mahmud, S.R. Syed-Omar, A.R.M. Zaki, and A.R. Al-Eryani. 2009. Effects of calcium and chitosan treatments on controlling anthracnose and postharvest quality of papaya (Carica papaya L.). Int. J. Agric. Res. 4:53–68. doi: 10.3923/ijar.2009.53.68.

Ali, I., N.A. Abbasi, and I.A. Hafiz. 2014. Physiological response and quality attributes of peach fruit cv. Flordaking as affected by different treatments of calcium chloride, putrescine and salicylic acid. Pak. J. Agric. Sci. 51:33–39.

Babu, M.M., R. Van Der Lee, N.S. de Groot, and J. Gsponer. 2011. Intrinsically disordered proteins: Regulation and disease. Curr. Opin. Struct. Biol. 21(3):432–440. doi: 10.1016/j.sbi.2011.03.011.

Bangerth, F. 1979. Calcium-related physiological disorders of plants. Annu Rev Phytopathol 17(1):97–122. doi: 10.1146/annurev.phy.17.090179.000525.

Belge, B., L.F. Goulao, E. Comabella, J. Graell, and I. Lara. 2019. Postharvest heat and CO2 shocks induce changes in cuticle composition and cuticle-related gene expression in ‘October Sun’ peach fruit. Postharvest Biol. Technol. 148:200–207. doi: 10.1016/j.postharvbio.2018.11.005.

Berry, W., and C. Johnson. 1966. Determination of calcium and magnesium in plant material and culture solutions, using atomic absorption spectroscopy. Appl. Spectrosc. 20(4):209–211. doi: 10.1366/000370266774386029.

Beyer, E.M., and P.W. Morgan. 1970. A method for determining the concentration of ethylene in the gas phase of vegetative plant tissues. Plant Physiol. 46(2):352–354. doi: 10.1104/pp.46.2.352.
Bhat, M.Y., H. Ahsan, F.A. Banday, M.A. Dar, A.J. Wani, and G.I. Hassan. 2012. Effect of harvest dates, pre harvest calcium sprays and storage period on physico-chemical characteristics of pear cv. Bartlett. J. Agric. Res. Dev. 2 (4):101–106.

Brummell, D.A. 2006. Cell wall disassembly in ripening fruit. Funct. Plant Biol. 33(2):103–119. doi: 10.1071/FP05234.

Buescher, R.W., and G.E. Hobson. 1982. Role of calcium and chelating agents in regulating the degradation of tomato fruit tissue by polygalacturonase. J. Food Biochem. 6(3):147–160. doi: 10.1111/j.1745-4519.1982.tb00682.x.

Cao, S., J. Shao, L. Shi, L. Xu, Z. Shen, W. Chen, and Z. Yang. 2018. Melatonin increases chilling tolerance in postharvest peach fruit by alleviating oxidative damage. Sci Rep 8(1):806. doi: 10.1038/s41598-018-19363-5.

Chapman, H.D., and P.F. Pratt 1961. Methods of analysis for soils, plants, and waters. Univ. of Calif., Div. Agr. Sci, Berkeley, Calif. 309.

Chea, S., D.J. Yu, J. Park, H.D. Oh, S.W. Chung, and H.J. Lee. 2019. Fruit softening correlates with enzymatic and compositional changes in fruit cell wall during ripening in ‘Blucrop’ highbush blueberries. Sci. Horticult. 245:163–170. doi: 10.1016/j.scientia.2018.10.019.

Conway, W.S., C.E. Sams, and K.D. Hickey. 2001. Pre-and postharvest calcium treatment of apple fruit and its effect on quality. Int Symp Foliar Nutr Perennial Fruit Plants. 594:413–419.

Cooper, T., A. Gargiulo, and J. Retamales. 2007. Kiwifruit softening: Comprehensive research approach in chile and relevant results, p. 289–296. In: A.R. Ferguson, E.W. Hewett, F.A. Gunson, and C.N. Hale (eds.), Vol. 753. VI International Symposium on Kiwifruit. International Society for Horticultural Science (ISHS), Rotorua, New Zealand. Acta Horticulturae.

De Souza, A.B., Q.S. De Paula, M.F. Chittarar, and A.B. Chittarar. 1999. Post-harvest application of CaCl2 in strawberry fruits: Evaluation of fruit quality and post-harvest life. Ciência E Agrotec. Lavras 23:841–848.

Eberhart, S.A., and W.A. Russell. 1966. Stability Parameters for Comparing Varieties1. Crop Sci. 6(1):3640. doi: 10.2135/cropsci1966.0011183X000600010011x.

El-Badawy, H.E.M. 2012. Effect of chitosan and calcium chloride spraying on fruits quality of Florida Prince peach under cold storage. Res. J. Agric. Sci. 8(2):272–281.

Erincik, O., L.V. Madden, J.C. Scheerens, and M.A. Ellis. 1998. Evaluation of foliar applications of calcium chloride for control of Botrytis bunch rot on strawberry and effects on strawberry fruit quality. Adv. Strawberry Res. 17:7–17.

Faust, M., and B. Timon. 2010. Origin and dissemination of peach. Hortic Rev. 17:331–379.

Ferguson, I.B. 2006. Ca2+ in plant senescence and fruit ripening. Plant Cell Environ. 7(6):477–489. doi: 10.1111/j.1365-3040.1984.tb01438.x.

Franco-Mora, O., K. Tanabe, F. Tamura, and A. Itai. 2005. Effects of putrescine application on fruit set in ‘Housui’ Japanese pear (Pyrus pyrifolia Nakai). Sci. Horticult. 104(3):265–273. doi: 10.1016/j.scienta.2004.10.005.

Freitas, S.T., and R.D.C.M.R. Nassur 2017. Calcium Treatments. In Novel Postharvest Treatments of Fresh Produce, pp. 51–78. CRC Press.

Ganai, S.A., H. Ahsan, I.A. Wani, A.A. Lone, S.A. Mir, and S.M. Wani. 2015. Colour changes during storage of apple cv. Red delicious-influence of harvest dates, precooling, calcium chloride and waxing. Int. Food Res. J. 22(1):196.

Gayed, A.A.N.A., S.A.M.A. Shaarawi, M.A. Elkhishen, and N.R.M. Elsherbini. 2017. Pre-harvest application of calcium chloride and chitosan on fruit quality and storability of “Early Swelling” peach during cold storage. Ciência e Agrotecnologia 41(2):220–231. doi: 10.1590/1413-70542017412005917.

Glenn, G., B. Poovalah, and H. Rasmussen. 1985. Pathways of calcium penetration through isolated cuticles of ‘Golden Delicious’ apple fruit. J. Am. Soc. Hortic. Sci. 110:166–171.

Godara, A.K., K.S. Chauhan, and K. Ashwani. 2002. Effect of various pre-harvest treatments on the quality of Thompson Seedless grapes. Haryana J. Hort. Sci. 31:164–167.

Goutam, T., H.S. Dhaliwal, and B.V.C. Mahajan. 2010. Effect of pre-harvest calcium sprays on post-harvest life of winter guava (Psidium guajava L.). J. Food Sci. Technol. 47(5):501–506. doi: 10.1007/s13197-010-0085-2.

Guo, Z.W., J.J. Hu, S.L. Chen, Y.C. Li, Q.P. Yang, and H.J. Cai. 2017. Nitrogen addition and clonal integration alleviate water stress of dependent ramets of Indocalamus decorus under heterogeneous soil water environment. Sci Rep 7 (1):44524. doi: 10.1038/srep44524.

Hanson, E.J., J.L. Beggs, and R. Beaudry. 1993. Applying calcium chloride postharvest to improve highbush blueberry firmness. HortScience 28(10):1033. doi: 10.21273/HORTSCl.28.10.1033.

Hao, X., and A.P. Papadopoulos. 2003. Effects of calcium and magnesium on growth, fruit yield and quality in a fall greenhouse tomato crop grown on rockwool. Can. J. Plant. Sci. 83(4):903–912. doi: 10.4141/P02-140.

Hernandez-Munoz, P., E. Almenar, V. Del Valle, D. Velez, and R. Gavara. 2008. Effect of chitosan coating combined with postharvest calcium treatment on strawberry (Fragariax ananassa) quality during refrigerated storage. Food Chem. 110(2):428–435. doi: 10.1016/j.foodchem.2008.02.020.

Hocking, B., S.D. Tyerman, R.A. Burton, and M. Gillham. 2016. Fruit calcium: Transport and physiology. Front Plant Sci. 7:569.

Ishaq, S., H.A. Rathore, T. Masud, and S. Ali. 2009. Influence of post harvest calcium chloride application, ethylene absorbent and modified atmosphere on quality characteristics and shelf life of apricot (Prunus armeniaca L.) fruit during storage. Pak. J. Nutr. 8(6):861–865. doi: 10.3923/pjn.2009.861.865.
Islam, M., M.Z.H. Khan, M.A.R. Sarkar, N. Absar, and S.K. Sarkar. 2013. Changes in acidity, TSS, and sugar content at different storage periods of the postharvest Mango (Mangifera indica L.) Influenced by Bavistin DF. Int. J. Food Sci. 2013:1–8. doi: 10.1155/2013/939385.

Jawandha, S.K., N. Gupta, and J.S. Randhawa. 2012. Effect of post-harvest treatments on enzyme activity and quality of cold stored ber fruit. Notulae Scientiae Biologicae 4(4):86–89. doi: 10.15835/nsb448181.

Jin, P., H. Shang, J. Chen, H. Zhu, Y. Zhao, and Y. Zheng. 2011. Effect of 1-methylcyclopropene on chilling injury and quality of peach fruit during cold storage. J. Food Sci. 76(8):485–491. doi: 10.1111/j.1750-3841.2011.02349.x.

Khalaj, K., N. Ahmadi, and M.K. Souri. 2017. Improvement of postharvest quality of asian pear fruits by foliar application of boron and calcare. Horticulturae 3:3–15.

Khalifa, R.K.M., O.M. Hafez, and H. Abd-El-Khair. 2009. Influence of foliar spraying with boron and calcium on productivity, fruit quality, nutritional status and controlling of blossom end rot disease of anna apple trees. World J. Agric. Res. 5:237–249.

Kirmapi, S.N., G.M. Wani, M.S. Wani, M.Y. Ghani, M. Abid, S. Muzamil, H. Raja, and A.R. Malik. 2013. Effect of preharvest application of calcium chloride (CaCl₂), gibberellic acid (GA₃) and naphthenic acetic acid (NAA) on storage of plum (Prunus salicina L.), cv. Santa Rosa, under ambient storage conditions. Afr. J. Agric. Res. 8(9):812–818.

Lara, I., P. Garcia, and M. Vendrell. 2004. Modifications in cell wall composition after cold storage of calcium-treated strawberry (Gragaria x ananassa Duch.) fruit. Postharvest Biol. Technol. 34(3):331–339. doi: 10.1016/j.postharvbio.2004.05.018.

Lester, G.E., and M.A. Grusak. 2004. Field application of chelated calcium: Postharvest effects on cantaloupe and honeydew fruit quality. HortTechnology 14(1):29–38. doi: 10.21273/HORTTECH.14.1.0029.

Lownds, N.K., M. Banaras, and P.W. Bosland. 1993. Relationships between Postharvest water loss and physical properties of Pepper Fruit (Capsicum annuum L.). HortScience 28(12):1182–1184. doi: 10.21273/HORTSCI.28.12.1182.

Lurie, S., and C.H. Crisostos. 2005. Chilling injury in peach and nectarine. Postharvest Biol. Technol. 37(3):195–208. doi: 10.1016/j.postharvbio.2005.04.012.

Malik, A.U., and S. Zora. 2004. Pre-storage application of polyamines improves shelf-life and fruit quality of mango. J. Hortic. Sci. Biotechnol. 80(3):363–369. doi: 10.1080/14620316.2005.11511945.

Manganaris, G.A., A.R. Vicente, C.H. Crisostos, and J.M. Labavitch. 2007. Effect of dips in a 1-methyl-cyclopropene-generating liquid formulation on “Harrow Sun” plums stored under different temperature regimes. J. Agric. Food Chem. 55(17):7015–7020. doi: 10.1021/jf071065p.

Marzouk, H.A., and H.A. Kassem. 2011. Improving fruit quality, nutritional value and yield of Zaghloul dates by the application of organic and/or mineral fertilizers. Sci. Hortic. 127(3):249–254. doi: 10.1016/j.scienta.2010.10.005.

Mayr, U., and M. Schröder. 2002. Influence of calcium sprays with different concentrations: spray timing and combinations with prohexadione-ca on the mineral content in “boskoop” and “elstar” apples, p. 553–556. In: M. Tagliavini, M. Toselli, L. Bertschinger, P. Brown, D. Neilsen, and M. Thalheimer (eds.), International symposium on foliar nutrition of perennial fruit plants. 594 ed. International Society for Horticultural Science (ISHS), Merano, Italy.

Mendes, L.D.S., E. Aguayo, C.D.O. Pessoa, B.T. Nastaro, and R.A. Kluge. 2019. Enhancement of the antioxidant capacity and reduction of chilling injury in Durarrado peppers refrigerated under pre-storage and modified atmosphere. Acta Sci. Agron. 41(1):39641. doi: 10.4025/actasciagron.v41i1.39641.

Montanaro, G., B. Dichio, C. Xiloynnis, and G. Celano. 2006. Light influences transpiration and calcium accumulation in fruit of kiwifruit plants (Actinidia deliciosa var. delicosa). Plant Sci. 170(3):520–527. doi: 10.1016/j.plantsci.2005.10.004.

Monteagle, J.R., and J.M. Valades. 1993. The effect of calcium applied before harvest on the susceptibility of raspberry fruits to Botrytis cinerea. Fitopatologia 28(93):96.

Nasef, I.N. 2018. Short hot water as safe treatment induces chilling tolerance and antioxidant enzymes, prevents decay and maintains quality of cold-stored cucumbers. Postharvest Biol. Technol. 138:1–10. doi: 10.1016/j.postharvbio.2017.12.005.

Ogden, M., R. Hoefgen, U. Roessner, S. Persson, and G.A. Khan. 2018. Feeding the walls: How does nutrient availability regulate cell wall composition. Int J Mol Sci. 19(9).

Pantastico, A.B., A.K. Matto, T. Murata, and K. Ogata. 1975. Physiological disorders and diseases: Chilling injury, p. 339–362. In: Postharvest physiology, handling and utilization of tropical and subtropical fruits and vegetables. The AVI Publ. CO. Inc., Connecticut.

Paul, R.E., and N.J. Chen. 1989. Waxing and plastic wraps influence water loss from papaya during storage and ripening. J. Am. Soc. Hortic. 114:937–942.

Petriccione, M., F. Mastrobuoni, M. Pasquariello, L. Zampella, E. Nobis, G. Capriolo, and M. Scortichini. 2015. Effect of chitosan coating on the postharvest quality and antioxidant enzyme system response of strawberry fruit during cold storage. Foods 4(4):501–523. doi: 10.3390/foods4040501.

Peyvast, G., J.A. Olfti, P. Ramezani, and S. Kamari-Shahmaleki. 2009. Uptake of calcium nitrate and potassium phosphate from foliar fertilization by tomato. J. Hortic. For. 7:7–13.
Pinzón-Gómez, L.P., Y.A. Deaquiz, and J.G. Álvarez-Herrera. 2014. Postharvest behavior of tamarillo (Solanum betaceum Cav.) treated with CaCl₂ under different storage temperatures. Agronomía Colombiana 32(2):238–245. doi: 10.15446/agron.colomb.v32n2.42764.

Prasad, B., D.C. Dimiri, and L. Bora. 2015. Effect of pre-harvest foliar spray of calcium and potassium on fruit quality of Pear cv. PATTERNAKH. Sci. Res. Essays 10(11):392–396.

Raese, J.T., and S.R. Drake. 2000. Effect of calcium spray material, rate, time of spray application, and rootstocks on fruit quality of ‘Red’ and ‘Golden Delicious’ apples. J Plant Nutr 23(10):1435–1447. doi: 10.1080/0190416009382113.

Ramezanian, A., M. Rahemi, and M.R. Vazifehshenas. 2009. Effects of foliar application of calcium chloride and urea on qualitative and quantitative characteristics of pomegranate fruits. Sci. Hort. 121(2):171–175. doi: 10.1016/j.scienta.2009.01.039.

Rohani, M.Y., M.Z. Zaipun, and M. Norhayati. 1997. Effect of modified atmosphere on the storage life and quality of Eksotika papaya. J. Trop. Agric. Food Sci. 25:103–113.

Rosen, J.C., and A.A. Kader. 1989. Postharvest physiology and quality maintenance of sliced pear and strawberry fruits. J. Food Sci. 54(3):656–659.

Sajid, M., M.A. Khan, W. Bilal, A. Rab, and Z. Iqbal. 2017. Anti-oxidant activities, chemical attributes and fruit yield of peach cultivars as influenced by foliar application of ascorbic acid. Gesunde Pflanzen 69(3):113–121. doi: 10.1007/s10343-017-0395-7.

Samaan, L.G., M.S.S. El-Boray, F.G. Guirguis, and M.E. Helal. 2001. Post-harvest calcium treatments to improve keeping quality and marketing season of citrus fruits. J. Agric. Sci. Mansoura University 20:1619–1631.

Samant, D., N.K. Mishra, A.K. Singh, and R.L. Lal. 2008. Effect of micronutrient sprays on fruit yield and quality during storage in ber cv. Umran under ambient conditions. Indian J. Hort. 65(4):399–404.

Saunders, M.J., and P.K. Helper. 1983. Calcium antagonists and calmodulin inhibitors block cytokinin-induced bud formation in Funaria Hygrometrica. Dev. Biol. 99(1):41–49. doi: 10.1016/0012-1606(83)90252-X.

Selvan, M.T., and J.S. Bal. 2005. Effect of post-harvest chemical treatments on shelf life of guava during ambient storage. Haryana J. Hort. Sci. 34(1/2):33.

Senevirathna, P.A.W.A.N.K., and W.A.M. Daundasekera. 2010. Effect of postharvest calcium chloride vacuum infiltration on shelf life and quality of tomato (cv. ‘Thilina’). Ceylon J. Sci. (Biological Sciences) 39(1):35. doi: 10.4038/cjsbs.v39i1.2351.

Serrano, M.A., D. Martinez-Romero, S. Castillo, F. Guillén, and D. Valero. 2004. Role of calcium and heat treatments in alleviating physiological changes induced by mechanical damage in plum. Postharvest Biol. Technol. 34(2):155–167. doi: 10.1016/j.postharvbio.2004.05.004.

Shirzadeh, E., V. Rabiei, and Y. Sharafi. 2011. Effect of calcium chloride (CaCl₂) on postharvest quality of apple fruits. Afr. J. Agric. Res. 6(22):5139–5143.

Singh, B.P., G. Pandey, D.K. Sarolia, M.K. Pandey, and R.K. Pathak. 2005. Shelf-life evaluation of aonla cultivars. Indian J. Hortic. 62(2):137–140.

Singh, S., V.S. Brahmacari, and K.K. Jha. 1998. Effect of calcium and polythene wrapping on storage life of mango. Indian J. Hortic. 55:22–28.

Singh, S.K., R.S. Singh, and O.P. Awasthi. 2013. Influence of pre-and post-harvest treatments on shelf-life and quality attributes of ber fruits. Indian J. Hortic. 70(4):610–613.

Sriong, K., P. Jitareerat, and A. Uthairatanakij. 2019. UV irradiation induces resistance against fruit rot disease and improves the quality of harvested mangosteen. Postharvest Biol. Technol. 149:187–194. doi: 10.1016/j.postharvbio.2018.12.001.

Sugar, D., and S.R. Basile. 2011. Orchard calcium and fungicide treatments mitigate effects of delayed postharvest fungicide applications for control of postharvest decay of pear fruit. Postharvest Biol. Technol. 60(1):52–56. doi: 10.1016/j.postharvbio.2010.11.007.

Swietlik, D., and M. Faust. 2011. Foliar nutrition of fruit crops. Hortic Rev (Am Soc Hortic Sci) 6:287–355.

Tanou, G., I.S. Minas, F. Scossa, M. Belghazi, A. Xanthopoulou, I. Ganopoulos, P. Madesis, A. Fernie, and A. Molassiotis. 2017. Exploring priming responses involved in peach fruit acclimation to cold stress. Sci Rep 7(1):11358. doi: 10.1038/s41598-017-11933-3.

Toivonen, P.M.A., and P.A. Bowen. 1999. The effect of preharvest foliar sprays of calcium on quality and shelf life of two cultivars of sweet bell peppers (Capsicum annum L.) grown in plastic culture. Can. J. Plant. Sci. 79(3):411–416. doi: 10.4141/P98-092.

Tuna, A.L., C. Kaya, M. Ashraf, H. Altunlu, I. Yokas, and B. Yagmur. 2007. The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. Environ. Exp. Bot. 59(2):173–178. doi: 10.1016/j.envexpbot.2005.12.007.

Uh, K.H., I.P. Ahn, S. Kim, and Y.H. Lee. 2003. Calcium/Calmodulin-Dependent signaling for prepenetration development in colletotrichum gloeosporioides. Phytopathology 93(1):82–87. doi: 10.1094/PHYTO.2003.93.1.82.

Wang, D., T.H. Yeats, S. Uluisik, J.K.C. Rose, and G.B. Seymour. 2018. Fruit softening: Revisiting the role of pectin. Trends Plant Sci. 23(4):302–310. doi: 10.1016/j.tplants.2018.01.006.
Wang, L., S. Chen, W. Kong, S. Li, and D.D. Archbold. 2006. Salicylic acid pretreatment alleviates chilling injury and affects the antioxidant system and heat shock proteins of peaches during cold storage. Postharvest Biol. Technol. 41 (3):244–251. doi: 10.1016/j.postharvbio.2006.04.010.

Wang, L., T. Shan, B. Xie, C. Ling, S. Shao, P. Jin, and Y. Zheng. 2019. Glycine betaine reduces chilling injury in peach fruit by enhancing phenolic and sugar metabolisms. Food Chem. 272:530–538. doi: 10.1016/j.foodchem.2018.08.085.

Wara-aswapati, O., J. Sornsrivichai, J. Uthaibutra, and C. Oogaki. 1990. Effect of seal packaging by different plastic films on storage life and quality of litchi (Litchi chinensis Sonn.) fruits stored at three different temperatures. Jpn. J. Trop. Agric. 34(2):68–77.

Whale, S.K., and Z. Singh. 2007. Endogenous ethylene and colour development in the skin of ‘Pink Lady’ apple. J. Am. Soc. Hortic. 132(1):20–28. doi: 10.21273/JASHS.132.1.20.

Wills, R.B.H., C.M.C. Yuen, S.D.L. Sabri, and S. Suyanti. 1988. Effect of calcium infiltration on delayed ripening of three mango cultivars in Indonesia. ASEAN Food J. 4:67–68.

Wojcik, P., and M. Lewandowski. 2003. Effect of calcium and boron sprays on yield and quality of “Elsanata” strawberry. J Plant Nutr 26(3):671–682. doi: 10.1081/PLN-120017674.

Yuri, J., J. Retamales, C. Moggia, and J. Vasquez. 2002. Bitter pit control in aples cv. Braeburn through foliar sprays of different calcium sources. Acta. Hort. (594):453–460. doi: 10.17660/ActaHortic.2002.594.58.