Research Article

Efficiency of calcium chloride (CaCl₂) treatment on post-harvest performance of pear (*Pyrus communis* L.)

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Abstract

Calcium is present in the primary cell wall and middle lamella in the form of pectic substances of all plant tissues. The gradual penetration of calcium to cell wall results in increasing level of this ion in the cell wall and thus stabilization increases which may protect the fruit from fungal and other microbial attack or contamination. Keeping in view its importance, a post-harvest study was assessed at ARI Tarnab Farm, Peshawar, Khyber Pakhtunkhwa to evaluate the effect of Calcium chloride (CaCl₂) treatment on physio-chemical quality of pear (*Pyrus communis* L.) during storage. Fresh and disease free pear fruits were dipped in CaCl₂ solution for (3, 6 and 9 minutes) and evaluated at 5 days interval for different physio-chemical attributes. The analysis of data showed that pear fruits dipped in CaCl₂ solution for 9 minutes significantly affected the fruit juice pH, ascorbic acid, percent titratable acidity, total soluble solid, reducing sugar, and non-reducing sugar. Similarly, prolonged storage duration resulted a significant increasing trend in fruit juice pH, total soluble solids and non-reducing sugar, while ascorbic acid content, percent titratable acidity and reducing sugar decreased with extension in storage duration from zero to twenty-five days. It was concluded from the results that pear fruits dipped for 9 minutes in CaCl₂ solution was effective in retaining quality attributes during storage at ambient temperature (20°C with 65-70% RH).

Keywords: CaCl₂, Dipping time, Le-Conte, Physiological disorder, Post-harvest losses, Shelf life

Introduction

Pear (*Pyrus communis* L.) belongs to family Rosaceae. It is native to Asia and was first introduced by John Eatton Le-Conte to Georgia in 1856 [1]. In Pakistan, pear are mostly cultivated on the terraces or

in the hills and in the plain areas of Khyber Pakhtunkhwa (Mardan, Peshawar and Hazara). It is usually propagated asexually through whip and cleft grafting but seeds of wild cultivar (Batang) may be used as a rootstock, while for dwarfism quince

rootstocks are used. Fruits and vegetables are a major source of essential dietary nutrients, such as vitamins and minerals [2]. Extending post-harvest life of horticultural products, requires knowledge of all factors, that can cause loss of quality so as to develop affordable technologies, that Post-harvest losses of fresh fruits and vegetables, minimize the rate of deterioration. Length of storage, respiration, transpiration, chemical composition, external appearance, anatomical structures, delay harvesting, taste qualities and other post-harvest behaviors, have significant impact on fruit quality. These controllable and uncontrollable factors, affect the attainment of maximum quality of fruits [3]. Dipping treatments, favor the dispersion of the solution on the surface of the vegetable [4]. Pre and post-harvest Calcium applications have been used, to delay aging or ripening, to reduce post-harvest decay and to control the development of many physiological disorders, in fruits and vegetables [5]. Plums treated with calcium containing compounds extend shelf-life of fruits by showing increased conjugated forms of putrescine (conjugated soluble and cell-wall-bound), thereby, resulting in higher firmness values and minimizing the rate of respiration and thus preventing the disintegration of fruit tissues [6]. Calcium acts to bind cell and maintain the structure stability of cell wall during storage [7, 8]. It conserve fruit qualities by preventing physiological disorders, reduce the rate of respiration, lessens the solubility of pectic substance, maintaining the firmness and slows down the ripening process [9, 10]. Application of calcium on pear fruits significantly increase calcium concentration in fruit peel and cortex which improve fruit quality resulting in overall enhancement of fruit appearance and post-harvest performance [11]. In Pakistan 20-30 % or even up to 40 % losses of fruits occur which worth more than 3 billion rupees due to mishandling, inadequate storage facilities [12]. It is also noted that, an increase in the concentration of calcium

chloride increases the firmness of the fruit [13]. Post-harvest treatment of pineapples, with calcium chloride retards their decay rate [14]. The purpose of this research work was to extend the shelf life of fresh peach fruit and to find an economical and effective control measure to minimize the post-harvest losses so that it can be shipped to distant markets and thus generate larger revenues for all stake holders. The results of this work are not only highly useful for the farmers but also for the fruit processing industries

Materials and methods

An experiment was conducted at Soil Chemistry laboratory, Agriculture Research Institute Tarnab Farm Peshawar, during July 2016 to evaluate the effect of Calcium chloride (CaCl_2) treatment on post-harvest performance of pear. Completely Randomized Design with two factors i.e. Dipping time in CaCl_2 solution (3,6,9minutes) and storage duration (5, 10, 15, 20 and 25 days) having three repetitions were used during experimentation. Fresh and disease free pear fruits cv. Le-Conte were harvested from New Developmental Farm, Horticulture during 2016, at physiological maturity stage.

Procedure for preparation of CaCl_2 solution

Fruits were consequently shifted to Soil Chemistry laboratory and sorted based on size and the absence of physical injuries or infections. Fruits were randomly divided into 4 groups, each group containing 100-120 fruits in three replicates and immersed into solution of (2%(w/v) Ca for 3,6,9 minutes and in distilled water as control. The selected fruits were stored for 25 days at ambient temperature (20°C with 65-70% RH).

Fruit quality evaluation

Fruit juice pH

Fruits were randomly selected and its pH was determined by using pH meter for all treatment in each replication with the help of pH meter [15].

Total soluble solid (°Brix)

Pear fruits were randomly selected from each replication and total soluble solids were determined by hand refractometer and fruit juice drop was placed on clean and dry prism of refractometer and reading was noted.

Ascorbic acid (mg100g⁻¹)

Ascorbic acid (mg100g⁻¹) of randomly selected fruits from each replication was found out by using dye method as described by [10].

Procedure

With help of pipette 10 ml of juice were taken from the extracted fruit and was added to graduated cylinder. With the help of oxalic acid solution the volume was raised up to 100 ml to make 10% solution. 10% solution were titrated from the burette containing dye (50 mg of 2-6 dichlorophenol indo phenol + 42mg baking soda) until pink color was attained. Each sample reading was noted. By using the following formula, Ascorbic acid content were calculated.

$$\text{Ascorbic acid content (mg/100g)} = (F \times T \times 100) / (D \times S) \times 100$$

F = Dye factor

T = ml of dye used for sample titration

D = ml of sample taken for dilution

S = ml of diluted juice taken for titration

Percent titratable acidity

Percent titratable acidity was measured for randomly selected pear fruits in each treatment per replication by the standard method as described in [15].

Calculation

5 ml of Fehling A + 5 ml of Fehling B = Xml of 10% syrup solution = 0.05g of reducing sugar.

$$= 100 \text{ ml of } 10 \% \text{ sample solution will contain } = \frac{0.05 \times 100}{X \text{ ml}} = Y \text{ g of reducing sugar}$$

$$= \% \text{ of reducing sugar in sample } = \% \text{ of reducing sugar} = \frac{Y \times 100}{10}$$

Non reducing sugar

Following procedure was used for determination of non-reducing sugar.

Procedure

Pear juice (10 ml) sample was taken in flask and volume was made up to 100 ml with distilled water. Then 20 ml of diluted sample solution was taken in conical flask along with 10 ml 1N hydrochloric acid. For

Titration of the Sample

In 100ml volumetric flask (10ml) grapefruit juice were taken and diluted up to the mark. In a titration flask 10ml of these diluted samples were taken and as an indicator 2-3 drops of phenolphthalein were added and then titrated against 0.1 N NaOH solutions until the light pink color appeared. Consecutive three readings were taken by the use of following formula:

$$\text{Titratable Acidity (\%)} = (N \times T \times F \times 100) / (D \times S) \times 100$$

N = NaOH Normality

T = in (ml) NaOH used.

F = constant acid factor 0.0064 (citric acid)

D = In ml Citrus Sample taken for dilution

S = Diluted sample taken for titration in ml

Reducing and Non Reducing sugar

[16] method were used for determination of reducing and non-reducing sugar of juice.

Procedure

Pear juice sample of 10 ml was taken and 100 ml volume was made in volumetric flask with distilled water. Diluted juice sample solution was filled in burette. 5 ml of Fehling A + 5 ml of Fehling B + 10 ml distilled water was taken in conical flask. Without disturbing solution was boiled in a conical flask. From burette drop by drop solution was added in conical flask solution till the appearance of red brick color of solution. Methylene blue drop was added in boiling solution without shaking flask for testing the red brick color persistence in solution.

5-10 minutes, solution was heated and then after cooling 10 ml of 1N sodium hydroxide was added in solution and with distilled water 250 ml volume was made. Burette was filled with sample solution. 5 ml Fehling A, 5 ml of Fehling B solution and distilled water of 10 ml was taken in flask for boiling. Flask solution was titrated against the burette sample when it starts

boiling till its color turns to red brick. Methylene blue was used for testing of

sample solution until appearance of red color.

Calculations

X ml of syrup solution contains = 0.05g of reducing sugar

250 ml of syrup solution contain = Y gm of reducing sugars = $\frac{250 \times 0.05}{X \text{ ml}}$

250 ml of syrup solution was prepared from 20 ml of 10 % sample solution contain = $\frac{Y \times 100}{20} =$

P g reducing sugar

10ml of sample solution contain Pg of reducing sugar

=100 ml of sample solution contain = $\frac{P \times 100}{10} = Q \text{ g of total reducing sugar}$

Non reducing sugar = total reducing sugar- free reducing sugar

Experimental design and Statistical analysis

A statistical software package (Statistix 8.1, Inc, Tallahassee FL, USA) was used for calculating ANOVA and LSD value [17]. When F values were significant, the means comparison were done by using Least Significance Difference (LSD) test at 5% level of significance [18].

Results and discussion

Fruit juice pH

Data presented in the (Table 1) showed, that the pH of fruit juice was significantly by CaCl_2 solution and storage duration, while their interaction was found non-significant. Maximum value of fruit juice pH (4.8) was recorded in fruits juice which was dipped for nine minutes in CaCl_2 , while lowest value of fruit juice pH (4.6) was recorded in untreated fruit juice (Figure 1). It has also been observed that increasing storage duration can increase pH of fruit juice of pear. The fresh juice has pH (4.08 to 5.28) in comparison to fruits stored for eighteen to twenty days (Figure 2). In our study, it was observed that, during storage fruit juice pH was increased, because acidity was reduced during storage with the attainment of maturity and ripening [19]. During storage catabolic processes increases due to high rate of respiration that cause breakdown of organic acids and thus results high pH. Percent acidity and pH are inversely related to each other, lower the percent titratable acidity, higher will be the pH and vice versa [20]. Increase in fruit juice pH might be to the breakup of acids, with respiration during storage. The results

are in agreement with the findings of [21].

The biochemical changes in pH of juice occurred along with high rate of respiration and metabolic activity when fruit juice were placed in storage condition. [22] Reported that increasing Calcium chloride prevented decline in the acidity of the fruits. [23] Also reported similar findings that increasing storage duration can increase pH of apple fruit juice.

Total soluble solid ($^{\circ}\text{Brix}$)

CaCl_2 treatment and storage duration significantly affected total soluble solids ($^{\circ}\text{Brix}$) of pear fruit, while their interaction was observed non-significant (Table 1). When the fruits were allowed to dipped for 9 minutes in CaCl_2 , highest value of total soluble solid (16.6 $^{\circ}\text{Brix}$) was observed followed by total soluble solid (16.4 $^{\circ}\text{Brix}$) in fruits dipped for 6 minutes in CaCl_2 . While minimum total soluble was recorded in untreated fruit (Figure 3). It has been observed that total soluble solids show increasing with time duration i.e. increasing storages time from 1 to days 25 showed an increase in total soluble solid from 11.1 to 19.0 $^{\circ}\text{Brix}$ in fruit at zero to twenty-five days of storage (Figure 4). The flavor and marketability of most fruits depend upon on total soluble solids that showed the concentration of sugar and amount of soluble components in the flesh which becomes degraded with prolonged storage duration [24, 25]. The slower increase of TSS of CaCl_2 treated fruits might be due to the fact that more concentration of calcium chloride formed a thin layer on the surface of fruit which delayed degradation process.

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The increase in TSS might be attributed due to the enzymatic conversion of higher polysaccharides such as starches and pectins into simple sugars during ripening [26]. Therefore, the CaCl_2 dip resulted in delaying the increase in TSS in samples subjected to higher concentration of CaCl_2 . Similarly the increase in TSS of cucumbers, treated with Calcium Chloride was less, as the presence of Ca^{2+} ions increases the cohesion of cell-walls and delay fruit ripening [27]. Calcium Chloride delayed fruit ripening, improved resistance to fungal attack and maintained structural integrity of cell walls [28]. [18] Reported that increase in TSS might be due to the changes in pectin and starches in to simple sugars during ripening when action of different enzymes occurred i.e. pectinase, methyl esterase and polygalacturonase. CaCl_2 can delay ripening, senescence and respiration which is responsible for increase as well as decrease of TSS and total sugars. During storage starch present in the fruits converted slowly and gradually into sugar as a result maximum value of total soluble solid was observed in untreated pear fruits. Total dissolved solids and moisture content of the fruit can aggregate and make TSS percentage. It means that the high concentration of calcium chloride application increased the metabolic actions which eventually decreased the TSS of the apple fruits and due to more titratable acidity value [19].

Ascorbic acid (mg/100g)

It is obvious from (Table 1) that ascorbic acid of pear fruit was significantly affected by calcium chloride treatment and storage duration, while their interaction was found non-significant. The lowest (6.66 mg/100 g) ascorbic acid value was noted in untreated fruits, while the highest value (6.91 mg/100 g) of ascorbic acid was recorded in fruit when dipped for 9 minutes in CaCl_2 solution followed by ascorbic acid (6.59 mg/100 g) in fruits dipped for 6 minutes in CaCl_2 solution (Figure 5). The ascorbic acid of pear fruit (when dipped in CaCl_2 solution) decreased along with prolonging

storage period. Similarly, increasing storage duration can decreased the ascorbic acid and it was observed in fruits (7.52 to 5.94 mg/100 g) from zero to twenty days of storage (Figure 6). Ascorbic acid is an important nutrient and is very sensitive to degradation due to its oxidation compared to other nutrients during food processing and storage [29]. The loss of ascorbic acid content might be due to loss of antioxidants activity during post-harvest storage [30]. Ascorbic acid decreased in fruits by increasing storage duration. Our results are in line with the finding of [31] who stated that ascorbic acid of sweet orange decreased with extending storage duration. During extending storage duration of fruit the ascorbic acid reduces due to its volatile nature that evaporates from fruit surface during respiration [32].

Percent titratable acidity (%)

The dipping time of CaCl_2 solution and storage durations significantly influenced the percent titratable acidity of pear fruit except their interaction (Table 2). The highest value of titratable acidity (0.9 %) was observed in the untreated pear fruit which was statistically at par with percent titratable acidity (0.7%) in fruits dipped in CaCl_2 for 3 minutes, while the lowest value of titratable acidity (0.3 %) was recorded in the fruit dipped for 9 minutes (Figure 7). Similarly increasing storage duration from zero to 25 days decreased titratable acid from (0.6 to 0.1%) (Figure 8). During storage reduction in acid contents of juice occur due to use of acid as source of energy which converts organic acid to form sugar [33]. Sugar and acids are related with fruit taste, fruit flavor that should be maintained by having proper amount of titratable acidity. During storage rate of respiration increases which consume organic acid and reduce the fruit acidity that affect the fruit flavor [34]. [35] Reported that with prolonging storage duration decrease in acidity of fruit occurs. The maximum titratable acidity is retained in the application of CaCl_2 solution as compared to untreated apple fruit [36]. The

acidity of fruit juice decrease due to the utilization of organic acids as source of energy and carbon skeleton for the synthesis of new compounds during ripening. Also, sugars accumulation during ripening contributes to decrease of acidity [37]. The ripening process of fruit was delayed due to retention of higher values of acidity in CaCl_2 treatment.

Reducing sugar (%)

The mean value presented in (Table 2) revealed that the reducing sugar (%) of the pear fruit was significantly influenced by the storage duration and calcium chloride treatment, while their interaction had no significant effect on reducing sugar of pear fruit. The highest percent reducing sugar (5.77 %) was recorded in the pear fruit dipped for 9 minute, followed by percent reducing sugar of fruits dipped for 6 minutes (4.56 %). Whereas minimum percent reducing sugar (0.63%) was observed in untreated fruit (Figure 9). Similarly prolonging storage duration, decrease in percent reducing sugar from (5.21 to 4.20%) in freshly harvested fruit to fruit stored for 25 days (Figure 10). Application of calcium chloride solution had a significant effect on reducing the respiration rate of fruit because glucose is the main substrate in respiration [38] which retained the percent reducing sugar. In contrast, CaCl_2 treatment deactivate the activity of hydrolytic enzymes that are responsible for conversion of starch into sugars. These results are in line with findings of [39] in apple.

Non-reducing sugar (%)

It is obvious from data presented in (Table

2) that calcium chloride treatment and storage duration significantly affected percent non-reducing sugar of pear fruit except their interaction. The highest value of non-reducing sugar (4.02%) was recorded in pear fruit dipped for 9 min in CaCl_2 solution followed by non-reducing sugar (2.93) in fruits dipped for 6 min, while lowest value of non-reducing sugar (2.12%) was noted in untreated fruits (Figure 11). An increase in non-reducing sugar (1.22 to 4.84 %) was observed in the pear fruits from day zero to 25 day of storage (Figure 12). The sugar content of apple fruit contributes to the fruit sweetness and thus, is a major fruit quality characteristic. At the early stages of maturation the starch is accumulated which is hydrolyzed to sugars at edible maturity [40] during storage [41], resulted in increased total sugar with increased storage duration [42]. The increase and the subsequent decrease in these biochemical attributes may possibly be attributed to the numerous catabolic processes taking place in the fruits preparing for senescence. [43] stated that in apple, starch, hemicellulose and other polysaccharides acting as a source of sugars get hydrolyzed into mono and disaccharides during ripening which in turn lead to an increase in TSS and sugars during storage. Treated fruits owing to the slow substrate utilization of primary sugars due to decline in respiration rates may have reflected in the increased TSS and sugar contents noted towards the end of storage as calcium, along with other growth substances are known to delay numerous senescence processes [44].

Table 1. Fruit juice pH, total soluble solid and ascorbic acid content of pear fruit as influenced by dipping time and storage duration

Treatments	Fruit juice pH	Total Soluble Solid	Ascorbic acid
	(%)	(°Brix)	(mg.100g ⁻¹)
Dipping time (min)			
0	4.6c	0.63c	6.66b
3	4.6b	4.67b	6.68b
6	4.6ab	4.56c	6.59c
9	4.8a	5.77a	6.91a
LSD≤0.05	0.05	0.07	0.03
Storage duration (days)			
0	4.08f	12.5f	7.52a
5	4.37e	15.4e	7.17b
10	4.58d	16.1d	6.86c
15	4.83d	17.0c	6.58d
20	5.06b	17.9b	6.20e
25	5.28a	18.8a	5.94f
LSD≤0.05	0.04	0.06	0.02

Means value followed by different letter differ from each other at 5% level of significance

Table 2. Titratable acidity, reducing sugar and non-reducing sugar of pear fruit as influenced by dipping time and storage duration

Treatments	Titrateable acidity	Reducing sugar	Non-reducing sugar
	(%)	(%)	(%)
Dipping time (min)			
0	0.09	0.63c	2.12d
3	0.07	4.67b	2.29c
6	0.04	4.56c	2.93b
9	0.03	5.77a	4.02a
LSD≤0.05	NS	0.05	0.03
Storage duration (days)			
0	0.06ab	5.21b	1.22f
5	0.05ab	5.42a	1.81e
10	0.07a	5.17b	2.29d
15	0.03bc	4.86c	3.08c
20	0.02c	4.50d	3.80c
25	0.01c	4.29	4.84a
LSD≤0.05	0.03	0.07	0.04
Interaction			
V.C×T.D	NS	NS	NS

Means value followed by different letter differ from each other at 5% level of significance

Figure 1. Fruit juice pH of pear fruit as affected by calcium chloride treatment

Figure 2. Fruit juice pH of pear fruit as affected by storage duration

Figure 3. Total soluble solid of pear fruit as affected by calcium chloride treatment

Figure 4. Total soluble solid of pear fruit as affected by storage duration

Figure 5. Ascorbic acid content of pear fruit as affected by calcium chloride treatment

Figure 6. Ascorbic acid content of pear fruit as affected by storage duration

Figure 7. Titratable acidity of pear fruit as affected by calcium chloride treatment

Figure 8. Titratable acidity of pear fruit as affected by storage duration

Figure 9. Reducing sugar of pear fruit as affected by calcium chloride treatment

Figure 10. Reducing sugar of pear fruit as affected by storage duration

Figure 11. Non reducing sugar of pear fruit as affected by calcium chloride treatment

Figure 12. Non reducing sugar of pear fruit as affected by storage duration

Conclusion and recommendations

Based on the results, it is concluded that pear fruits dipped in CaCl_2 for 9 minutes maintained quality attributes of pear for 25 days of storage by sustaining acidity, total soluble solid, fruit juice pH, reducing sugar, non-reducing sugar and ascorbic acid and thus recommended for better quality of pear at 20°C with 60-70% RH. Dipping of pear fruit in CaCl_2 solution for 9

minutes retained the quality attributes of pear fruit for 25 days of storage.

Authors' contributions

Conceived and designed the experiments: M Sajid & M Asif, Supervised the experiment: M Sajid, Performed the experiment: M Asif, S Zeb & S Khan, Analyzed the data: M Sajid, M Asif & I Ullah, Contributed materials/ analysis/ tools: A Basit, J Tareen, MK Nawaz & QS Ali,

Wrote the article: I Ullah & A Basit, Review the article: A Basit.

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