



## GELATIN BASED EDIBLE COATING COMBINED WITH CALCIUM CHLORIDE ENHANCES THE SHELF LIFE AND NUTRITIONAL QUALITY OF SAPOTA (*MANILKARA ZAPOTA L.*) CV. KALIPATTI

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### ABSTRACT

Sapota (*Manilkara zapota L.*) is a climacteric fruit that ripens shortly after harvest. Harvest maturity plays a crucial role in deciding the marketability of climacteric fruits in general. Attempt has been made to evaluate the influence of gelatin and calcium chloride on the physicochemical and biochemical changes that occur during ripening of harvested sapota cv. Kalipatti. Thus the present study has objective of delaying the ripening process of sapota during transit and extend its marketability during storage at room temperature ( $22 \pm 5^\circ\text{C}$  and 65–75% R.H.). The treatments of gelatin in its different concentrations delayed the changes of weight loss, total soluble solids, decaying percentage, and sugar content in sapota fruit of the experimental set than that of control set. The significant impact of the coating solution is found causing least decay percentage of fruits treated with gelatin 1%. The lower activity of cell wall softening related enzymes was noticed in fruit treated with gelatin 1% followed by gelatin 2% treated sapota fruit. From the present study, it can be concluded that gelatin (1%) has significant effect in extending the shelf life of sapota fruits while retaining its nutritional quality. Among all the treatments, tested under the present study, gelatin 1% was found to be the most effective in delaying the ripening of sapota fruit and enhanced the shelf life by 6 days more than that of the control fruit.

**Key Words:** Calcium chloride, Edible coating, Fruit, Gelatin, *Manilkara zapota L.*

### INTRODUCTION

Sapota (*Manilkara zapota L.*) is a tropical fruit with unique taste and medicinal properties [1]. Sapota ranks fifth both in production and consumption next to mango, banana, citrus and grapes in India and also the Kallipatti cultivar represents a major proportion of export of sapota from India [2]. The shelf life of sapota fruit is short at ambient temperature and it is sensitive to cold storage. The production of sapodilla fruit has increased so much. As it ripens and decays rapidly within 7–9 days at ambient temperature, the fruit is only intended for local markets, due to the lack of effective postharvest technologies [3]. Thus, development of postharvest technology related to quality maintenance is an essential issue for market expansion of sapota fruit. Their post harvest losses are high in tropical countries particularly in India and it ranges between 25-30%.

Edible coatings are traditionally used to improve food appearance and conservation. They act as barriers during processing, handling and storage, and retard food deterioration. These edible coatings are also safe due to their natural biocide activity, or because of the incorporation of antimicrobial compounds [4]. Proteins are widely used to form edible films [5]. Protein-based edible films show satisfactory gas barrier or mechanical properties. Park [6] indicated that proteins (*e.g.* zein, wheat gluten, albumin, keratin, and gelatin) are better gas barriers than polysaccharides (*e.g.* pectin, methylcellulose, hydroxyl propyl methylcellulose, and starch) due to their unique structure and high intermolecular binding potentiality.

Calcium chloride has been effective in quality retention of fruit. Studies on fruit ripening processes showed that tissue calcium content usually affects senescence aspects *e.g.* respiration rate. Calcium chloride has been reported to reduce onset of ripening in sugar apple, avocado, and strawberry [7]. The higher

concentration of calcium chloride is reported to delay softening and decrease the incidence of physiological disorders. In recent years, significant advances have been made in fruit storage by the use of calcium chloride dipping alone or combined with other treatments. Pre and postharvest application of calcium prevented postharvest disorders, retarded fruit ripening and decreased postharvest fruit weight loss and decay [8]. According to Torres *et al.* [9] the highest concentration of calcium chloride can help to delay softening and decrease the incidence of physiological disorders.

In view of foregone account, the present study has been undertaken to examine the effects of postharvest dipping of sapota in aqueous calcium chloride and gelatin on its storage at room temperature ( $22 \pm 5^\circ\text{C}$  and 65–75% R.H.).

### MATERIALS AND METHODS

Sapota fruit were procured from an agricultural farm in Balasinor village, Khaira district, Gujarat, India. The fruits were brought to the laboratory and washed with the water and then with sodium hypochlorite solution (2%) and allowed to dry. All fruit were grouped into four sets having 15 fruits in each set were of uniform size, shape, age (according to stage of maturity) and without any physiological disorder. All fruit were treated with 5% solution of calcium chloride ( $\text{CaCl}_2$ ) for 30 sec except control set, followed by air-drying of them at room temperature. On drying, these treated fruits were put into four homogeneous groups with fifteen fruits in each group and they were given the following treatments (T): T1= gelatin 1%, T2= gelatin 2%, T3= gelatin 3%, and T4= control, and all the sets were stored at room temperature  $22 \pm 5^\circ\text{C}$  and 60-70% R.H. Stored fruits were subjected for the physico-chemical and biochemical analysis at 0, 4, 8, and 12 days of storage period. For control fruit and T3, the data were recorded only up to 8 days of storage period, as thereafter, they began to decompose.

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### Preparation of Coating Solutions

The gelatin (final concentration in the film forming solution of 4 g/100 ml) was firstly dissolved in distilled water to a ratio of 4 g/50 ml. Based on the study reported by Thomazine *et al.* [10], tween80 (0.15 g/g gelatin) as a surfactant and glycerol (0.15 g/g gelatin) as a plasticizer were added.

### Determination of Physical Quality

Decay or rotting of the stored sapota fruit was determined by their visual observations. Decay percentage of sapota fruit was calculated as the number of decayed fruit divided by initial number of all fruit multiplied by 100. Shelf life of stored sapota fruit was calculated by counting the days required them to attain the last stage of ripening, but up to the stage when they remained acceptable for marketing [11].

### Determination of Biochemical Quality

The pH of the fruit samples was determined as per the method described in AOAC [12]. The TSS content of the fruit was determined by using refractrometer (Atago Co., Tokyo, Japan). A homogenous sample of fruit was prepared by crushing it in the mortar and pestle and a few drops of juicy fruit pulp were put on the prism of refractrometer and direct reading was taken by reading the scale in meter as described in AOAC [12]. Reducing sugars, non-reducing sugars and total phenols were estimated by using the methods cited by Thimmaiah [13]. The quantitative analysis of ascorbic acid was carried out as per the method of Roe [14].

### Total Proteins, Enzyme Extraction and Assay

The concentration of protein was determined as per the Lowry *et al.*, method [15], with bovine serum albumin as a standard. Polygalacturonase (PG), Pectin methyl esterase (PME) and pectate lyase (PL) were extracted and assayed according to the procedures described by Lohani *et al.* [16].

### Statistical Analysis

The data presented in this paper was statistically analyzed by SPSS 17 software. All the performed analyses were carried out in triplicate and the mean and standard deviation (SD) were calculated. The statistical significance of the data was assessed by one way Analysis of variance and LSD test. Mean comparisons were performed using HSD of Tukey's test to examine if differences between treatments and storage time were significant at  $P < 0.05$ . The overall least significance difference (LSD;  $p \leq 0.05$ ) was calculated and used it to detect significant differences among all the treatments and control set [17].

## RESULTS AND DISCUSSION

### Effect on Decaying Percentage and Shelf Life

The data summarized in Table 1 shows the steadily increasing decay percentage of sapota fruit, from 0 days to the end of storage period. On 4<sup>th</sup> day of storage period, the lowest decay percentage was noticed in fruit

treated with gelatin 1%-T1 (13.33%), while control fruit had significantly more decay (33.33%). Furthermore, at the end of the storage period, the percentage of decay was found to be 33.33% and 13.33% in fruits of T1 and T2 sets respectively. Baldwin [18] suggested that the coatings are a simple, environmentally friendly, and relatively inexpensive technology that can be used to extend the shelf life of tropical fruits and vegetables provided that there is good storage and temperature control. The shelf life of sapota fruit has been extended significantly ( $P \leq 0.05$ ) with some of the treatments tested in the current study. The shelf life of T1 (gelatin 1%) treated sapota fruit was highest among all the treatments up to 14 days followed by gelatin 2% (T2) with 12 days and gelatin 3% (T3) with 8 days of shelf life. Similar results were reported by Cheour *et al.* [19] and Pila *et al.* [20] who stated that the application of calcium prolonged the storage life of strawberries and tomato as measured by a delay in accumulation of sugars, decrease in organic acids, increase of color saturation index and mold development.

### Effect on pH and TSS

The pH of freshly harvested (i.e. 0 days storage) sapota fruit was 5.93 and it increased continuously up to 12 days of storage (Table 2). On 4<sup>th</sup> day of the storage, the pH of control fruit (untreated set) was found to have significantly ( $P \leq 0.05$ ) higher (6.54) as compared to that of the treated fruits. Coating reduces respiratory and metabolic rates, and thereby the utilization of organic acids, reported by Baraiya *et al.* [21]. In the present study, the changes in pH values of fruits treated with gelatin (1%) were found to be lesser than that of all other treatments. On 4<sup>th</sup> day, the pH was 5.95 followed by 6 and 6.08 on 8<sup>th</sup> and 12<sup>th</sup> days of storage respectively. In this regard, Beaulieu and Gorny [22] stated that the reduced respiration rate may be reflected in lesser changes in pH, titrable acidity and total soluble sugars. In the present study, the TSS of freshly harvested sapota fruit on 0 days of storage period was 1.9% (Table 2) but it had increased gradually as the storage period increased. Biale [23] attributed the increase in TSS during fruit ripening to the increased activity of enzymes responsible for the hydrolysis of starch to soluble sugars. Qiuping *et al.* [24] also noted little increase TSS concentration initially of sapodilla fruit but decreased during storage at ambient temperature. All the currently tested treatments showed better effect i.e. on 8 days of the storage the amount of TSS in control fruit was found to be (4.5 Brix<sup>o</sup>), whereas gelatin 1% (T1) treated fruits showed lowest amount of TSS content (4.1 Brix<sup>o</sup>), which was also significantly ( $P \leq 0.05$ ) lower than the other treated fruits. Similar results were reported by Carrillo-Lopez *et al.* [25] who found that the "Semperfresh" coating was effective in maintaining a lesser TSS in coated mangoes than observed in non coated mangoes.

### Effect on Total Sugars

In general, the rate of conversion of insoluble polysaccharide into organic acids that leads to an increase in sugar content was faster in the initial stages of post harvest storage than that of mono and disaccharides. In the current study, the lesser concentration of reducing sugar noticed in the gelatin 1% treated fruit which was 62.31 mg/g significant ( $P \leq 0.05$ ), whereas the highest amount

**Table 1:** Effect of treatments on Decay percentage and shelf life of sapota fruit stored at room temperature ( $22 \pm 5$  °C). T1 – Gelatin 1%, T2 – Gelatin 2%, T3 – Gelatin 3% and T4 – Control.

| Decay percentage |                          |       |       |       |
|------------------|--------------------------|-------|-------|-------|
| Treatments       | Storage period (in days) |       |       |       |
| (T)              | 0                        | 4     | 8     | 12    |
| T1               | 0                        | 13.33 | 20    | 33.33 |
| T2               | 0                        | 26.67 | 33.33 | 13.33 |
| T3               | 0                        | 40    | 33.33 | ----  |
| T4               | 0                        | 33.33 | 40    | ----  |

| Shelf life |    |    |    |    |
|------------|----|----|----|----|
| Treatments | T1 | T2 | T3 | T4 |
| Days       | 14 | 12 | 8  | 8  |

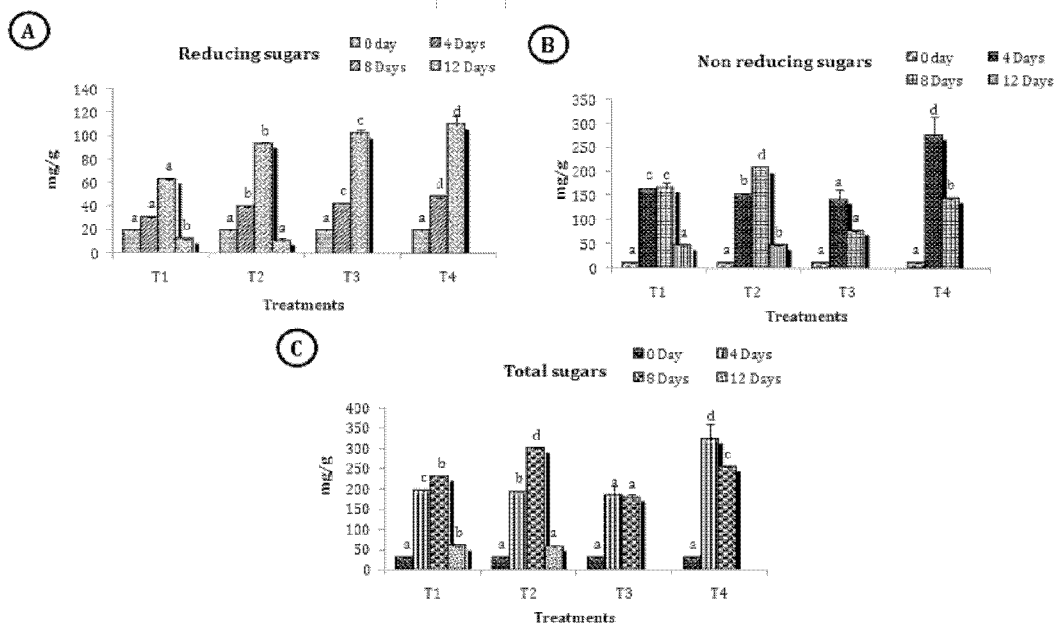
**Table 2:** Effect of treatments on pH and TSS of sapota fruit stored at room temperature ( $22 \pm 5$  °C). Each value is the mean for three ( $n = 3$ ) replicates. T1 – Gelatin 1%, T2 – Gelatin 2%, T3 – Gelatin 3% and T4 – Control. (Different letters in the same column means significantly different at  $p \leq 0.05$ ).

| pH         |                          |                   |                   |                   |
|------------|--------------------------|-------------------|-------------------|-------------------|
| Treatments | Storage period (in days) |                   |                   |                   |
| (T)        | 0                        | 4                 | 8                 | 12                |
| T1         | $5.93 \pm 0.061a$        | $5.95 \pm 0.036d$ | $6.00 \pm 0.000a$ | $6.08 \pm 0.006a$ |
| T2         | $5.93 \pm 0.061a$        | $6.14 \pm 0.059a$ | $6.24 \pm 0.041b$ | $6.43 \pm 0.020b$ |
| T3         | $5.93 \pm 0.061a$        | $6.18 \pm 0.006b$ | $6.44 \pm 0.026c$ | ----              |
| T4         | $5.93 \pm 0.061a$        | $6.47 \pm 0.006c$ | $6.54 \pm 0.004d$ | ----              |

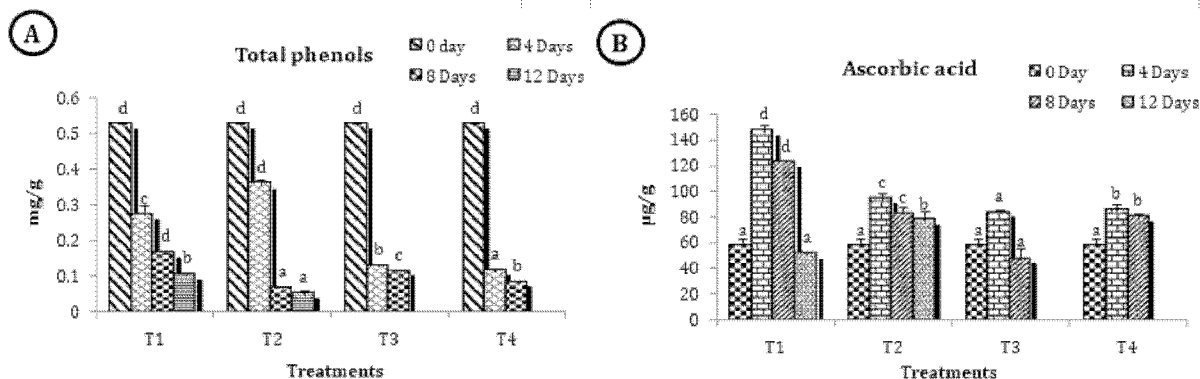
  

| TSS (Brix°) |                          |                  |                  |                  |
|-------------|--------------------------|------------------|------------------|------------------|
| Treatments  | Storage period (in days) |                  |                  |                  |
| (T)         | 0                        | 4                | 8                | 12               |
| T1          | $1.90 \pm 0.00a$         | $2.80 \pm 0.00a$ | $4.14 \pm 0.06c$ | $4.80 \pm 0.00a$ |
| T2          | $1.90 \pm 0.00a$         | $4.74 \pm 0.05d$ | $4.29 \pm 0.06d$ | $5.04 \pm 0.06b$ |
| T3          | $1.90 \pm 0.00a$         | $4.00 \pm 0.00b$ | $4.21 \pm 0.00b$ | ----             |
| T4          | $1.90 \pm 0.00a$         | $4.50 \pm 0.00c$ | $4.40 \pm 0.00a$ | ----             |

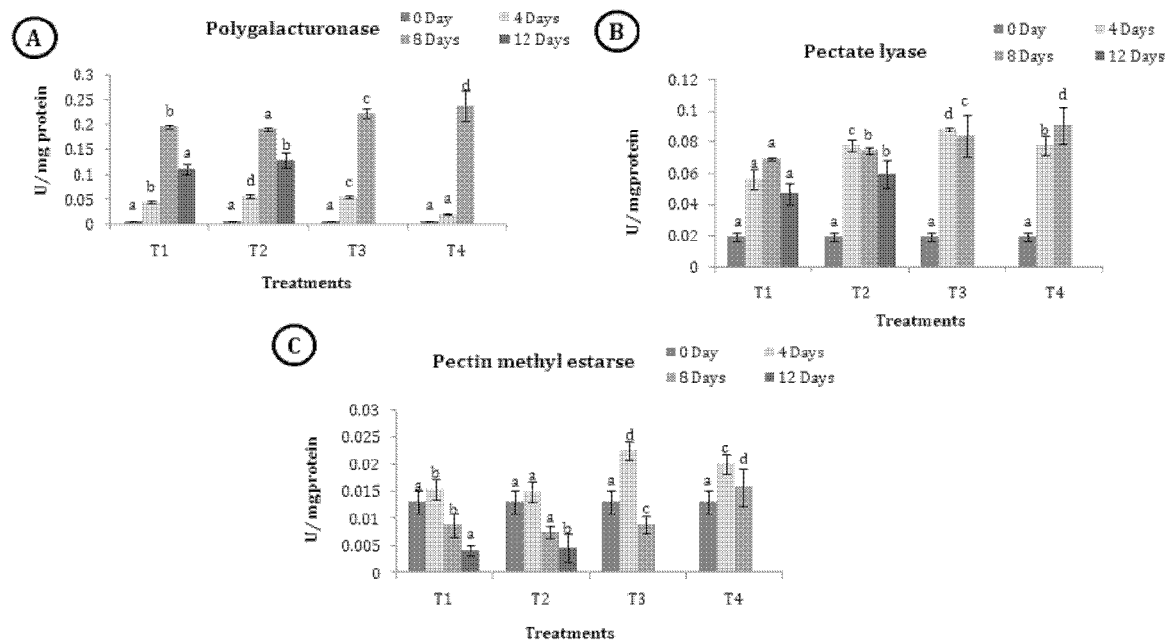
**Figure 1:** Effect of treatments on, (A) Reducing sugars of sapota fruit stored at room temperature ( $22 \pm 5^\circ\text{C}$ ). (B) Non-reducing sugars of sapota fruit stored at room temperature ( $22 \pm 5^\circ\text{C}$ ). (C) Total sugars of sapota fruit stored at room temperature ( $22 \pm 5^\circ\text{C}$ ). (Vertical bars represent  $\pm$  SD of means for three replicates T1 – Gelatin 1% T2 – Gelatin 2%, T3 – Gelatin 3% and T4 – Control. Different letters on the bars means significantly different at  $p \leq 0.05$ ).



**Figure 2:** Effect of treatments on, (A) Total phenols of sapota fruit stored at room temperature ( $22 \pm 5^\circ\text{C}$ ). (B) Ascorbic acid of sapota fruit stored at room temperature ( $22 \pm 5^\circ\text{C}$ ). (Vertical bars represent  $\pm$  SD of means for three replicates T1 – Gelatin 1% T2 – Gelatin 2%, T3 – Gelatin 3% and T4 – Control. Different letters on the bars means significantly different at  $p \leq 0.05$ ).



**Figure 3:** Effect of treatments on activity of (A) PG of sapota fruit stored at room temperature ( $22 \pm 5^\circ\text{C}$ ). (B) Pectate lyase of sapota fruit stored at room temperature ( $22 \pm 5^\circ\text{C}$ ). (C) PME of sapota fruit stored at room temperature ( $22 \pm 5^\circ\text{C}$ ). (Vertical bars represent  $\pm$  SD of means for three replicates T1 – Gelatin 1% T2 – Gelatin 2%, T3 – Gelatin 3% and T4 – Control. Different letters on the bars means significantly different at  $p \leq 0.05$ ).



was found to be in control fruits (108.6 mg/g) after 8 days of storage (Figure 1 A). This might be due to the fact that higher concentration of calcium chloride delayed the hydrolysis of polysaccharide and other physiological changes in fruits. In the current study, the non-reducing sugar levels increased progressively from 0 days to the 8 days of storage period and then it decreased. According to Preiss and Levi [26], as the fruit develops, the non-reducing sugar, sucrose, accumulates in the cytoplasm but as the ripening progresses the non-reducing sugar level decreases because the sucrose present in the cytoplasm is hydrolyzed into the glucose and fructose. The concentration of total sugars in fresh sapota fruit was 8.90 mg/g at 0 day of storage period (Figure 1 B). After 4 days of storage period, the amount of non reducing sugar was found to be lower in gelatin 1%- T1 treated fruits (163.0 mg/g) as compared to that of the control fruits (273.1 mg/g). It is clear from the results of present study that at the time of harvest of sapota fruit the amount of total sugars was very low i.e. 27.34 mg/g (Figure 1 C) but as the ripening enhances, the amount of total sugars increases and only at the end of storage it was slightly declined. Vyas *et al.* [27] reported that the amount of total sugar in freshly harvested papaya fruit was lower but it increased gradually with the period of storage at room temperature and all the treatments of CMC and carrageenan were found to cause a lowering of the total sugars, reducing sugars, and non-reducing sugars compared to the control fruit. Gelatin 1% (T1) treated sapota fruits had lesser amount of the total sugars than that of the control fruits

after 8 days of the storage. These results are in accordance with the findings of Hussein *et al.* [28] who also found that reducing and total sugar increased in Golden delicious apple during storage.

#### Effect on Total Phenols and Ascorbic Acid Content

The phenolics are widely distributed in plants and have the ability to scavenge free radicals, superoxide and hydroxyl radical by a single electron transfer. In the present study, the decrease in the total phenols was found in gelatin 1% treated fruit 0.268 mg/g (on 4<sup>th</sup> day) and 0.161 mg/g (on 8<sup>th</sup> day) significant ( $P \leq 0.05$ ), whereas in control fruit it was 0.112 mg/g on 4<sup>th</sup> day and 0.080 mg/g on 8<sup>th</sup> day (Figure 2 A). Among all the treatments, gelatin 1% -T1 and gelatin 2% -T2 showed higher levels of total phenols up to the end of the storage period indicating the retention of phenols in treated sapota fruits. These results are in agreement with the results reported by Meng *et al.* [29] regarding the decrease of total phenolic compounds with the increase of storage time and that postharvest chitosan treatment significantly inhibited the decrease of phenols in the table grape fruit stored at 20 °C. This indicates that decline in the total phenol content can be slowed down using edible coating treatments. This difference in flavor retention might be due to different nature of treatment and modified atmospheric conditions created by different levels of coatings. It was observed earlier that coatings improve the flavor of fruit that depends upon the type and permeability [30].

The ascorbic acid content in freshly harvested sapota fruit used for the present study (i.e. at 0 days of storage) was 56.04 µg/g (Figure 2 B). The significantly ( $P \leq 0.05$ ) higher amount of ascorbic acid was noticed in fruits treated with gelatin 1%- T1 (146.0 µg/g) on 4<sup>th</sup> day followed by gelatin 2%- T2 (93.33 µg/g), while in the control fruit the amount of ascorbic acid was only 84.38 µg/g after 4 days and 79.38 µg/g after 8 days of the storage respectively. The reason for high ascorbic acid in calcium treated fruits might be that, metabolic activities not fast as in untreated fruits. Therefore in untreated fruits the respiration rate and ethylene production were at higher rate due to which ascorbic acid constantly decreased rapidly as compared to CaCl<sub>2</sub> treated fruits. [31]. Qiuping *et al.* [24] reported that ascorbic acid content in sapodilla fruit declined gradually during storage, the fruit exposed to 1-MCP had higher ascorbic acid content. This decrease in the level of ascorbic acid in the coated fruit may be due to the general effect of the treatment on the ripening delay as treatment has also been found to reduce the ethylene production and respiration rate [32]. Zapata *et al.* [33] found the positive effects of protein based zein coating in retention of the ascorbic acid in tomatoes.

#### Effect on PG, PME, and Pectate Lyase

PG is important softening related enzyme which is responsible for cell wall degradation and ripening of the fruits. All the presently tested treatments were effective in delaying the activity of PG of the sapota fruit at the end of the storage period (Figure 3 A). Control fruit, without any treatment, had the highest level of PG activity i.e. 0.237 U/mg protein ( $P \leq 0.05$ ) after 8 days of the storage period, whereas all the treated fruit showed lower activity of PG enzyme as is evident from the values recorded: 0.196 U/mg protein of T1, 0.191 U/mg protein of T2 and 0.222 U/mg protein of T3 treated fruits. Among all the treatments, gelatin 1% (T1) and gelatin 2% (T2) showed better effect on the sapota fruits and maintained lower activity of PG till the end of the storage. Qiuping *et al.* [24] reported that Sapodilla fruit had a very low PG activity at harvest, but the enzyme activity increased later and then reached a peak before climacteric respiration rate appeared.

Pectate lyase activity increased in both the sets of sapota fruits control as well as treated samples at room temperature. At the beginning of the storage period, the sapota fruits exhibited less pectate lysase enzyme activity i.e. 0.02 U/mg protein ( $P \leq 0.05$ ) (Figure 3 B). The specific activity of untreated sapota fruits after 8 days of storage was 0.091 U/mg protein which was higher than that of the activity of pectate lyase found in treated fruit. The lower activity was observed in fruits treated with T1 (0.069 U/mg protein) after 8 days of the storage period. During 12 days of storage period, the activity declined little and in the fruit treated with T1 and T2, have shown less enzyme activity i.e. 0.047 U/mg protein and 0.059 U/mg protein respectively, compared to other treatments.

Pectinmethylesterase catalyzes the demethylesterification of galacturonic acid of pectin chains and since PG depolymerizes these galacturonic acid chains, PG activity is dependent on PME for making substrate available [34]. In sapodilla, it is reported that the

high pectin demethylesterification activity catalyzed by PME is probably required not only for subsequent PG activity, which was very low, but also to modify pH and cation exchange properties of the cell wall, which might impact on other cell wall enzymes [35]. In the present study the activity of PME enzyme was very less at 0 days i.e. 0.013 U/mg protein and then increment in it occurred at T4 days of storage period in control as well as treated sapota fruits (Figure 3 C). The control fruits showed highest activity of PME (0.020 U/mg protein) ( $P \leq 0.05$ ) after 4 days of storage, whereas T1 and T2 treated sapota fruits showed lesser activity (0.015 U/mg protein). On 8<sup>th</sup> days of the storage the activity of PME enzyme declined in all the treated as well as control sapota fruits. At the end of the storage, the PME activity of gelatin 1% (T1) treated fruits was 0.004 U/mg protein, while the fruit treated by gelatin 2% (T2) was having the activity (0.005 U/mg protein). During the present study, the lower activity was maintained by gelatin 1% and gelatin 2% treated sapota fruits.

#### CONCLUSION

Based on the results obtained from the present study, it may be concluded that the Gelatin as an edible coating could enhance the shelf life of sapota fruit without any quality deterioration. In fact, this nondestructive property of gelatin coating helped in retention of nutritionally important quality components in sapota fruit. The role of CaCl<sub>2</sub> in retarding fruit ripening and decreasing postharvest decay was confirmed. The delay in decay incidence, increased pH, retained TSS and total sugar content indicating the delayed ripening while the antioxidants like ascorbic acid and phenols were maintained higher in coated fruit than that of the control set. Thus, the treatments applied during the present investigation may be useful as one of the viable postharvest technologies to reduce the decay and extend the shelf life of perishable fruit like sapota and several other fruits as well.

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