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Effects of Calcium Infiltration and Chitosan Coating on Storage Life and Quality Characteristics During Storage of Papaya (*Carica papaya* L.)

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Abstract: Mature green stage (Index 2) papaya cv Eksotika II fruits were treated either with 2.5% calcium chloride infiltration, 0.75% chitosan coating, calcium infiltration at 2.5% then subsequently with chitosan coating at 0.75% or untreated fruits as the control. The fruits were then stored at 13±1°C for 35 days. Calcium infiltration was observed to be effective in maintaining the firmness with a slight effect on the weight loss by 2.9 folds and 2.8%, respectively compared to the control. Chitosan coating had less effect on maintaining firmness (2 folds) but gave better effect in preventing weight loss (5.3%). Chitosan coating treatment markedly slowed the ripening of papaya as shown by their retention of weight loss, delayed changes in their external color and other quality aspects. Calcium infiltration and subsequently chitosan coating as a combined treatment further extended the storage life up to 35 days with better retention of fruits firmness and water loss control compared to the treatments mentioned.

Key words: Papaya, postharvest, calcium chloride infiltration, chitosan, quality

INTRODUCTION

Papayas are a good source of vitamin C and A and ranks first among 13-17 fresh fruits for vitamin C content per 100 g edible tissue (Vinci *et al*., 1995; Gebhardt and Thomas, 2002). Papaya (*Carica papaya* L.) fruit harvested at the color break stage can be held for 2-3 weeks at 8-10°C and could as well have 1 week retail period at 22°C. This short postharvest life and chilling susceptibility limit the time available to ship and market fruits from the tropics (Chen and Paull, 1985; An and Paull, 1990).

The susceptibility of papaya fruits to several diseases is a major reason for extensive postharvest losses during handling and storage. The most important of these is the fungal disease, Anthracnose, caused by *Colletotrichum gloeosporoides* (Paull *et al*., 1997). Papaya diseases greatly increase incidence and severity following refrigerated storage. The postharvest life of papaya can be extended by several techniques combined with refrigeration. Control of decay is usually achieved by Hot Water Treatment (HWT), heat or chemical fungicides (Couey and Farias, 1979; Couey *et al*., 1984). However, in papaya, hot water dip treatment affects the ripening process and the use of fungicides for extended periods may cause the emergence of strains of fungus resistant to these fungicides. Further, the residues of fungicides present on the fruits may be harmful to consumers (Paull, 1990; Gamagae *et al*., 2003).

It is well known that calcium plays a major role in maintaining the quality of fruit and vegetables. Increasing the calcium content in the cell wall of fruit tissue can help to delay softening and mold growth and decrease the incidence of physiological disorders (Poovaiah, 1986). Thus, calcium is applied before and after harvest to prevent physiological disorders, delay ripening and to improve

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quality of various fruits crops (Asrey *et al*., 2004; Hernandez-Munoz *et al*., 2006). However, little is known on the efficiency of postharvest application of calcium in improving storage life and fruit quality of papaya.

Edible coatings have long been known to protect perishable food products from deterioration by retarding dehydration, suppressing respiration, improving textural quality, helping retain volatile flavor compounds and reducing microbial growth (Debeaufort *et al*., 1998). In postharvest studies, chitosan has been reported to maintain the quality of fruits and vegetables, reducing respiration rates, ethylene production and transpiration. Another important attribute of this natural compound is associated with its fungistatic or fungicidal properties against pathogens of various fruits and vegetables (El-Ghaouth *et al*., 1992a, b; Li and Yu, 2000).

Useful information could be obtained by conducting research using calcium infiltration and chitosan coating and their combination to study their effects on storage life and quality during storage period intervals. Thus, the objectives of this research were to evaluate the effect of calcium and chitosan and their combination on prolonging storage life and maintenance of the quality of papaya during storage period at low temperature condition 13±1°C.

MATERIALS AND METHODS

Plant Materials and Treatments

This research was conducted in September, 2007 using mature green stage of papaya fruits of color index 2 cv. Eksotika II were obtained from Exotic Star (M) Sdn Bhd, Sg. Chua, Kajang, Selongor, Malaysia within same day of harvesting. The fruits were selected for uniformity, shape, colour and size and any blemished or diseased fruits were discarded. The fruits were randomly distributed into groups of 25, five replicates for each treatment was used. Papaya fruits were treated either with 2.5% calcium chloride infiltration, 0.75% chitosan coating, calcium infiltration 2.5% then subsequently chitosan coating at 0.75% combined treatment or with untreated fruits as a control.

Calcium solution was prepared using calcium chloride commercial grade of 74% made up as 2.5% solution with distilled water and added 0.03% of Tween-80 as surfactant agent. Calcium chloride treatment was applied by using vacuum infiltration method according to the method of Wills and Tirmazi, (1979) with some modifications. The fruits were vacuum infiltrated by placing in calcium solutions for 10 min under 33 kPa vacuum and then held in solution for 5 min after vacuum release. After words, fruits were rinsed with distilled water and air dried at ambient temperature for 30 min in an attempt to reduce possible chemical injury.

A locally prepared of shrimp shell chitosan (88% deacetylated) was obtained from chitin chitosan research centre of Universiti Kebangsaan Malaysia (UKM), Malaysia used in this study. The solution of chitosan was prepared according to El Ghaouth *et al*. (1991) by dissolving the purified chitosan 88% of deacetylated degree in 2% (v/v) acetic acid under continuous stirring. The pH of the chitosan solution was adjusted to 5.6 using 1 N NaOH and 0.1% (v/v) Tween-80 added as a surfactant to improve the wetting properties of the solution.

Fruits were dipped in the chitosan solution for 5 min at concentration 0.75% and allowed to dry for 1 h at ambient temperature. The combined treatment of calcium and chitosan was applied initially by using the vacuum infiltration method with calcium chloride solution and allowed to dry in air for 1 h at ambient temperature. The fruits were then dipped in the chitosan solution for 5 min and then allowed to dry for 1 h at ambient temperature. Control treatment was used the untreated fruit for comparison. After applying the treatments and drying the fruits, each papaya fruit was sleeved with white styrofoam nettings and packed in single layer with stem end facing down in a commercial export packaging carton (40×30×15 cm^3). The fruits were stored at 13±1°C and 80-90% relative humidity for 35 days of storage period.

Fruit Quality Evaluation

Weight loss was determined. Five fruits in each replication for each treatment were marked before storage and weighed using electronic balance (EK-600H, Japan). At each storage interval (7 days) fruits were taken out from cold storage and weighed. Immediately after weighing, they were kept at cold storage refrigerator for subsequent weighings. Percentage of weight loss at particular storage interval was determined.

Fruit firmness was measured by the Instron Universal Testing Machine (Model 5540, USA) using the compression mode. The test was done for five fruits in each replication by using probe diameter size of 50 mm, speed 50 mm min^{-1} and load range 100 load cells. Compression force was measured at the maximum peak of recorded force on the chart and expressed as Newton (N).

Peel colour of the fruits was determined using a Minolta CR-300 Chroma Meter (Minolta Corp., Japan). The peel color determination was expressed in chromaticity values of L*, C* and h°. The L* coordinate indicated the lightness, C* indicated the chroma and h° indicated the hue angle of color.

Soluble Solids Concentration, pH, Titratable Acidity and Ascorbic Acid

Initially after applying the treatments and every 7 days of storage, Soluble Solid Concentration (SSC), pH, titratable acidity and ascorbic acid of fruit pulp were analysed according to the method of Ranganna (1977). SSC (Brix°) was determined with a Baush Lomb Abbe 3 L digital refractometer (Rochester, NY).

Fruit juice pH determination was determined by using the remainder of the filtrate from SSC determination using pH meter model Crison Micro pH 2000, Crison Instruments, Spain. pH meter was calibrated by using buffer solutions of pH 4 and 7. Titratable acidity was analyzed using the titration method (Ranganna, 1977). Five milliliter of the filtrate with one to two drops of phenolphthalein (1%) as indicator was titrated using 0.1 N NaOH to an endpoint pink (pH 8.2). The results were expressed as percentage of citric acid per 100 g fresh weight. Ascorbic acid was determined using the Dye method (Ranganna, 1977). The ascorbic acid content (Vitamin C) was expressed as (mg 100 g^{-1}) of fresh fruits.

Statistical Analysis

The experimental design was Complete Randomized Design (CRD) with five replicates. Analysis of Variance (ANOVA) was defected treatment effect. Mean separations were performed by using Least Significance Difference (LSD) at the $p \leq 0.05$ level. The data were analyzed using SAS 8.2 satatistical data analysis.

RESULTS AND DISCUSSION

Weight Loss

All fruit showed a progressive loss of weight during storage (Fig. 1). The control (untreated) had higher loss of fruit weight over all the storage period compared with other treatments. The maximum weight loss was observed for control treatment was about 13% compared to calcium infiltration treatment which reduced by 10.8% at 28 days of storage. Chitosan coating treatment was observed to prevent weight loss more than that of calcium infiltration treatment throughout the storage period but was less than that of combined treatment. However, it only had significance difference ($p<0.05$) with calcium treatment where reduction in weight loss was 8.2% of 28 days of storage. Weight loss under the combined treatment was consistently the lowest throughout the storage period (28 days). The combined treatment had the ability to reduce the weight loss significantly by 6.1% at 28 days compared to other treatments. After 28 days of storage, all the treatments had significant effect on weight loss. The effect of all the treatments on weight loss lasted for 28 days except for the combined treatment. The combined treatment was clearly effective in conferring a physical barrier to moisture loss and therefore retarding dehydration and fruit shriveling. Loss of weight in fruit was mainly due to the loss of water caused by transpiration and respiration processes.

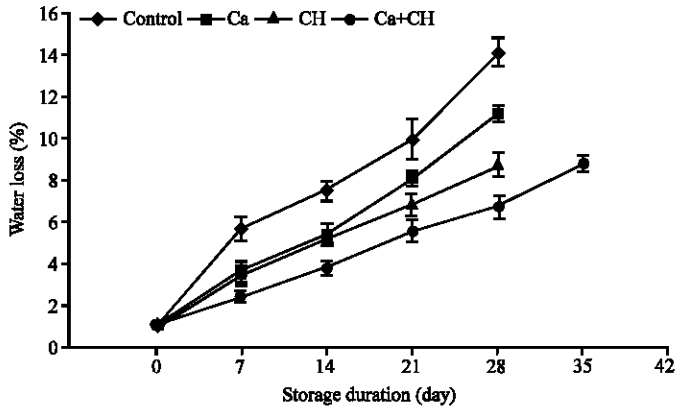

Fig. 1: Effect of calcium infiltration and chitosan coating on the weight loss of papaya fruits during storage at 13±1°C. Vertical bar indicates standard errors

This result confirms the findings of Garcia *et al*. (1998) who reported that the chitosan film formed on the surface of the fruit delayed migration of moisture from the fruit into the environment, thus reducing weight loss during storage. Postharvest water retention prevents rapid deterioration by shriveling. However, before shriveling becomes apparent, postharvest water loss may also alter metabolism and, in some instances, accelerate fruit ripening (Burdon *et al*., 1994). Therefore, reducing water loss from fruit during storage or ripening helps to maintain the quality of fruit. It was reported by Woods (1990) that weight loss is a consequence of fruit dehydration due to changes in surface transfer resistance to water vapour, in respiration rate and the occurrence of small fissures connecting the internal and external atmospheres. In this sense, Lester and Grusak (1999) showed that calcium application was effective in terms of membrane functionality and integrity maintenance, with lower losses of phospholipids and proteins and reduced ion leakage which could be responsible for the lower weight loss found in calcium treated plums.

Firmness

The initial flesh firmness values were similar to those of control and treated samples ($p>0.05$). Seven days after storage, the fruits with control treatment began to show a gradual loss of firmness followed by chitosan treatment and they only showed significant difference at 28 days of storage (Fig. 2). After 28 days of storage, the loss of fruit firmness for the control treatment was 88% whilst fruit firmness for coating treatment decreased by 76%. Calcium treatment showed greater effect on maintaining fruit firmness during storage than that obtained for chitosan treatment, which was more pronounced at 28 days of storage where it was reduced by 64%. The samples treated with combined treatment showed higher effect in maintaining firmness during storage and was obvious after the 28 days of storage during which it reduced to 51% compared with other treatments. Loss of texture is one of the main factors limiting quality and the postharvest shelf life of fruits and vegetables. Fruit softening considerably occurs during ripening which mainly occurs as a result of degradation of the middle lamella of the cell wall of cortical parenchyma cells (Perkins-Veazie, 1995).

Other characteristics influencing fruit firmness are cell wall strength, cell to cell contact and cellular turgor (Harker *et al*., 1997). It has been shown that chitosan coatings and other edible biopolymers are selective barriers to O_2 and CO_2, modifying internal atmospheres and slowing down the respiration rate of fresh fruit and vegetables (Debeaufort *et al*., 1998). As has been noted above, the high percentage of water lost by uncoated samples could contribute to firmness differences. This result proves that edible coating controlled the migration of moisture from the fruits, thus controlling

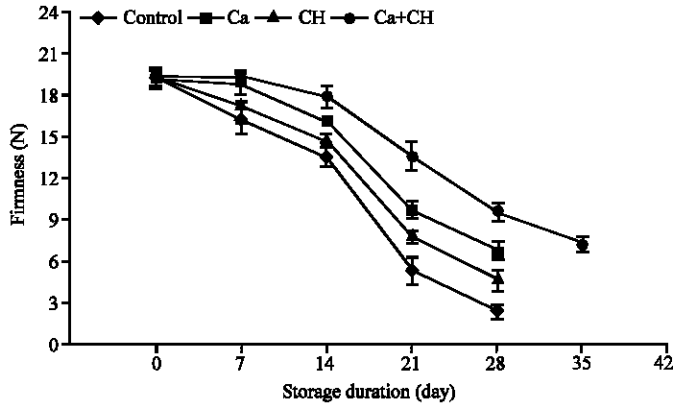

Fig. 2: Effect of calcium infiltration and chitosan coating on the firmness of papaya fruits during storage at 13±1°C. Vertical bar indicates standard errors

the integrity and texture of papaya. Present findings were consistent with that of Rolle and Chism (1987) who reported that chitosan containing calcium demonstrated the best result, probably because calcium may interact with pectic acid in cell walls to form calcium pectate, a compound helpful for maintaining structure of the fruit.

Peel Colour

Changes in the peel colour of papaya were monitored by measuring lightness (L*), chroma (C*) and hue angle (h°) during 35 days of storage are shown in Fig. 3-5. Calcium infiltration and chitosan coating combined treatment caused darkening as evidenced by decreasing values of L* which became significantly different ($p<0.05$) after the 14 days of storage until the end of storage period compared with the control treatment. The chitosan coating had significant effect in delaying darkening of papaya ($p>0.05$) at 28 days of storage compared with calcium treatment (Fig. 3). Both of the control (untreated samples) and calcium infiltrated papaya initially showed a decrease in L*. Upon subsequent storage, the control had a higher L* compared with the calcium infiltrated fruits, which was significantly different ($p>0.05$) at 21 and 28 days of storage, respectively. The untreated (control) and calcium infiltrated samples showed a significant decrease in hue angle and significant increase of chroma after 28 days of storage.

After 28 days of storage, the calcium infiltrated samples developed a more yellow and less vivid color as noted by significantly higher values in the lightness and chroma compared with coated samples. Calcium infiltrated and chitosan coated samples combined treatment had greatest effect in delaying color surface changes of fruit as noted from the lower values of lightness and chroma and higher values of hue angle. This may be that the chitosan coating may have caused control of fruit color during storage, suggesting a delay in the maturation/ripening of the fruits.

Calcium has been shown to be effective in reducing chlorophyll and inhibiting plant tissue senescence (Poovaiah *et al*., 1988). Saftner *et al*. (2003) found treatment with calcium inhibited color changes and development of tissue translucency in honeydew chunks. Thus, these findings are consistent with ours.

Soluble Solids Concentration

The SSC of the coated fruit was lower than that observed with calcium and control treatments, respectively (Fig. 6).

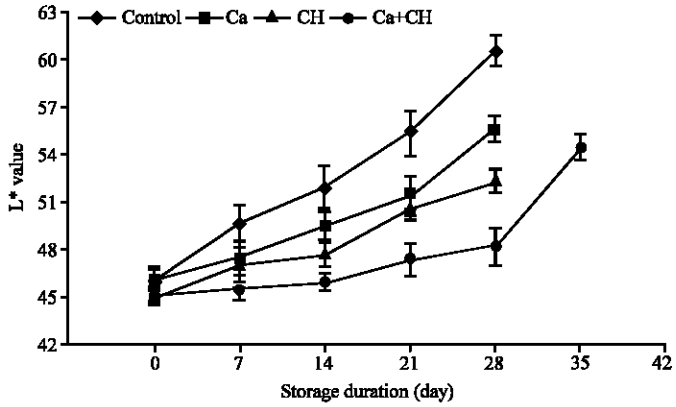

Fig. 3: Effect of calcium infiltration and chitosan coating on the development of peel color (L* Value) of papaya fruits during storage at 13±1°C. Vertical bar indicates standard errors

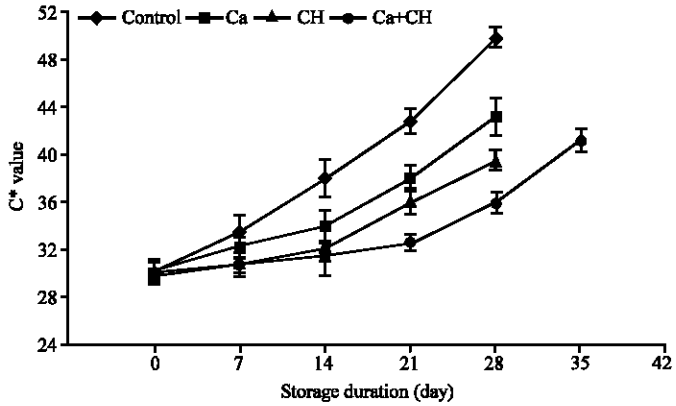

Fig. 4: Effect of calcium infiltration and chitosan coating on the development of peel color C* Values of papaya fruits during storage at 13±1°C. Vertical bar indicates standard errors

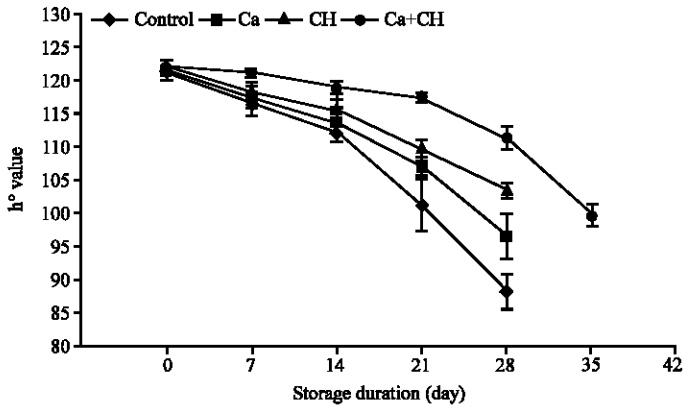

Fig. 5: Effect of calcium infiltration and chitosan coating on the development of peel color C* and h° Values of papaya fruits during storage at 13±1°C. Vertical bar indicates standard errors

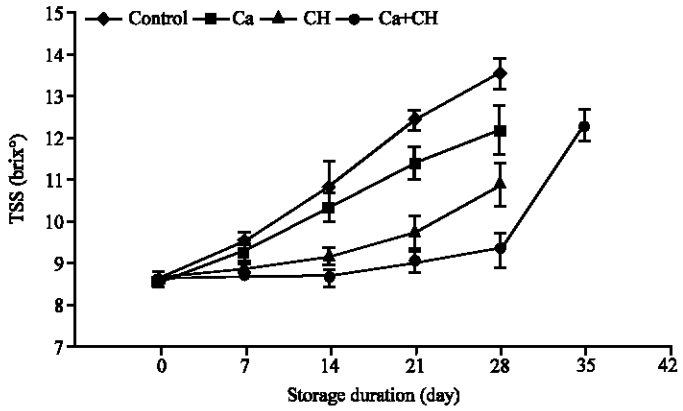

Fig. 6: Effect of calcium infiltration and chitosan coating on the soluble solids concentrations of papaya fruits during storage at 13±1°C. Vertical bar indicates standard errors

Whilst the SSC of control and calcium infiltrated papaya the greatest increment progressively with time. The combined treatment had higher effect in reducing the SSC during storage compared with other treatments, but had significant effect ($p>0.05$) on the SSC of fruits at 28 days of storage. This was probably due to the semipermeable chitosan film may have formed on the surface of the fruits and modify the internal atmosphere and the endogenous CO_2 and O_2 concentration of the fruit, thus retarding ripening as shown by Lowings and Cutts (1982) and Bai *et al*. (1988). The results obtained in this study can be explained by the considerable loss of water experienced by papaya fruits during storage at 13±1°C, especially by the uncoated fruit (control treatment).

By contrast, the SSC of mature strawberries has been reported to decrease under cold storage as a result of respiration (Garcia *et al*., 1998). The effect of calcium on reducing SSC of fruits probably due to slowing down metabolism activity hence, retarding the ripening process. This results is consistent with the findings of Singh *et al*. (1993) who reported that calcium treated mango fruits had lower respiration than control though large differences occurred among calcium treatments. Calcium treated fruits had a relatively slow rate of ripening as could be perceived from lower SSC and titratable acidity.

pH

The change in pH was lower over storage time for both combined treatment followed by coated fruit compared with other treatments. In general, the pH of papaya increased after the first days of storage, yielding significant differences ($p>0.05$) between chitosan coating, combined and control treatments by 28 days of storage (Fig. 7). The combined treatment slowed the changes in pH effectively, delaying fruit ripening and senescence. After 28 days of storage, the pH values continued to increase until 35 days of storage whereas, the other treatments lasted only at 28 days of storage. This was probably because the semipermeable chitosan coating on the surface of the fruit may have modified the internal atmosphere and the endogenous CO_2 and O_2 concentration of the fruits, thus retarding ripening (Lowings and Cutts, 1982; Bai *et al*., 1988).

Titratable Acidity

TA of control and calcium infiltration treatments decreased gradually until the end of the storage period. They had only significant changes ($p<0.05$) at 21 days of storage (Fig. 8). The combined and chitosan treatments had higher ability in maintaining the decrease in the TA content of fruits, respectively. The combined treatment was significantly ($p<0.05$) greater than that with chitosan alone

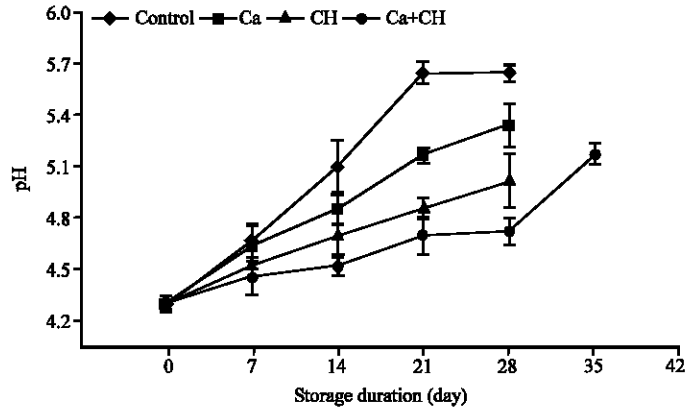

Fig. 7: Effect of calcium infiltration and chitosan coating on the pH of papaya fruits during storage at 13±1°C. Vertical bar indicates standard errors

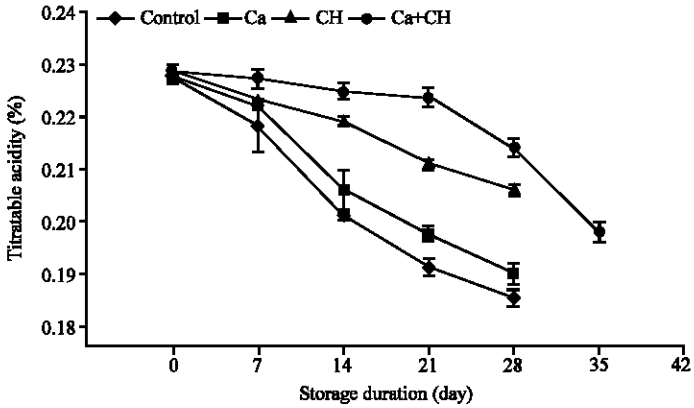

Fig. 8: Effect of calcium infiltration and chitosan coating on the titratable acidity of papaya fruits during storage at 13±1°C. Vertical bar indicates standard errors

at 21 days of storage. Slowing down the papaya ripening by means of a polymer coating and calcium causing inhibition enzyme activity could explain the delay in the use of organic acids in the enzymatic reactions of respiration.

This result was consistent with reports of Garcia *et al*. (1998) and Zhang and Quantick (1998) who reported that TA of chitosan or starch based coated strawberries kept under cold storage decreased with time but to a lesser extent than that of uncoated fruit. It also agreed with those reported by El-Ghaouth *et al*. (1991) and Garcia *et al*. (1998) that the decrease of TA during storage demonstrated fruit senescence. Similar results was obtained by Cheour *et al*. (1990) who reported that the quantity of organic acids expressed as citric acid decreased in apple fruits during storage, calcium treatment delayed the decrease in the TA observed after 14 days of storage.

Ascorbic Acid

As shown in Fig. 9, the ascorbic acid tended to increase during storage after 7 days until 21 days with control, calcium and chitosan treatments, respectively and then declined after words except for the combined treatment. The ascorbic acid content of calcium treatment increased after 7 days until 21 days then declined, followed by chitosan treatment. They did not have significance difference

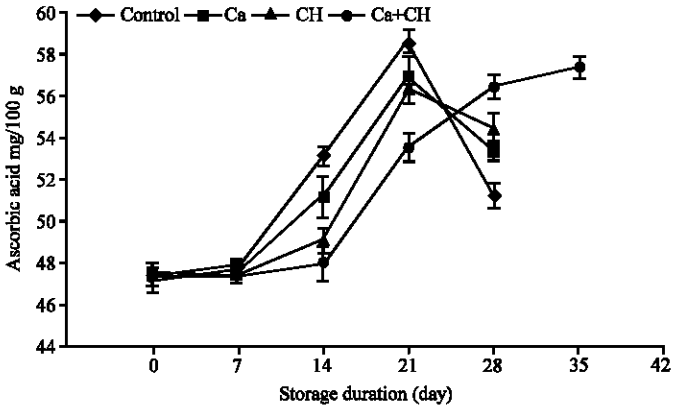

Fig. 9: Effect of calcium infiltration and chitosan coating on the ascorbic acid content of papaya fruits during storage at 13± 1°C. Vertical bar indicates standard errors

($p<0.05$) among each other during storage compared with control treatment. This study has demonstrated that the combined treatment, delayed the increase of ascorbic acid and the loss at ripening stage of papaya. This may be due to the combined effect of calcium infiltration and chitosan coating on retarding of ripening and slowing down the metabolism activity, which causing oxidation of ascorbic acid. These results are supported by the research of Burton (1982) and Chen and Paull (1985) who reported that tissues entering senescence or ripening have low biosynthesis of ascorbic acid. Losses in vitamin C were associated with senescence and quality deterioration of the fruits (Watada *et al*., 1984).

Slowing down of vitamin loss was attributed to the low oxygen permeability of the coating (Ayranci and Tunc, 2003). Keeping oxygen away from the food delays the deteriorative oxidation reaction of vitamin C (Ayranci and Tunc, 2004). Postharvest calcium treatment has been reported to reduce respiration rate of apples, delay onset of ripening of tomatoes and avocados (Bangerth *et al*., 1972; Wills *et al*., 1977; Wills and Sirivatanapa, 1988).

CONCLUSION

The use of calcium infiltration at 2.5% and subsequently chitosan coating at 0.75% has positive effect on storage life and preservation of postharvest quality of papaya. Hence, it provides good handling procedures to enhance papaya export to long distances with minimal losses as well as being environmental friendly.

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