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Effects of Calcium and Chitosan Treatments on Controlling Anthracnose and Postharvest Quality of Papaya (*Carica papaya* L.)

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Abstract: This study was conducted to evaluate the *in vitro* fungicidal effects of calcium and chitosan on *Colletotrichum gloeosporioides* and to as well determine their effects on storage life and quality of papaya. Potato Dextrose Agar (PDA) incorporated with calcium at different concentrations (1.5, 2.5 or 3.5%) or in combination with chitosan at 0.75% or chitosan alone were used as treatments for *in vitro* tests. Uncorporated treatments with PDA and untreated fruits as control used on papaya fruits for storage life and quality evolutions. Chitosan had the greatest effect against *Colletotrichum gloeosporioides* in both *in vitro* and in disease incidence (%) on papaya fruits compared to calcium treatment and as well as control. Calcium reduced spores germination significantly as calcium concentrations increased from 2.5 to 3.5%, compared to the 1.5% and control treatments. However, it did not show any fungicidal effects on mycelial growth. The combination of 2.5% calcium with chitosan 0.75% completely inhibited spore germinations and significantly inhibited mycelia growth compared to calcium individual treatments and as well as control. Anthracnose disease incidence (%) was significantly controlled (5.6%) using calcium at 2.5% combined with chitosan compared with the other treatments. This demonstrated the best effect on controlling anthracnose disease incidence for papaya fruits. Moreover, this treatment proved able to extend the storage life of papaya fruits up to 33 days of storage life while maintaining valuable attributes of quality.

Key words: Papaya, *Colletotrichum gloeosporioides*, chitosan, calcium, papaya quality

INTRODUCTION

Over the last decade, papaya fruit handling and ripening research has been considered as importance. This importance is due to the increase of worldwide consumer's awareness for this fruit and subsequently expansion in production and exports. The major research issues are quality retention and postharvest storage life that place constraints on marketing. The frequent problems are harvesting, postharvest latent infections and postharvest diseases (Chen *et al*., 2007).

The susceptibility of papaya fruit 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). Fungicides still provide a primary means of control (Zhang, 2007). However, pathogen resistance to fungicides and concern for public safety, as well as deregistration of some effective fungicides, making biocontrol an alternative method to fungicidal treatment with the aim of controlling fruit diseases (Holmes and Eckertm, 1999).

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In this regard, chitosan has been reported to have important attribute as a natural compound associated with its fungistatic or fungicidal properties against pathogens of various fruits and vegetables. Growth of important postharvest fungi such as *Alternaria alternata*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Penicillium* sp. are inhibited on nutrient media amended with various concentrations of chitosan (Bhaskara-Reddy *et al*., 1998; Bautista-Banos *et al*., 2003). Luna *et al*. (2001) also reported less postharvest rots when papaya fruits were dipped in chitosan solutions compared with other postharvest treatments such as heat and thiabendazole applications.

Calcium chloride has been widely used as preservative and firming agent in fruits and vegetables industry for whole and fresh cut commodities. There are positive effects of calcium chloride in terms of increasing fruits firmness and reducing decay after harvesting apples (Chardonnet *et al*., 2003). Saftner *et al*. (2003) work also focused on the firming effect of calcium chloride treatment on fresh cut honeydew. They found that calcium can reduce pathogen spore germination, sporulation and growth that are common in root vegetables such as potato. This ultimately reduces the storage decay of fruits or vegetables (Conway *et al*., 1994).

Garcia *et al*. (1996) reported that calcium is involved in maintaining the textural quality of produce since calcium ions form cross links or bridges between free carboxyl groups of the pectin chains, hence resulting in strengthening of the cell wall. Calcium complexes with cell wall and middle lamella polygalacturonic acid residues, improving structural integrity (Bakshi *et al*., 2005). This makes the cell wall less accessible to the enzymes that cause softening. This practice controls ripening, softening and decay at the same time (Sams *et al*., 1993). Lara *et al*. (2004) have indicated that calcium enhanced tissue resistance to fungal attack by stabilizing or strengthening cell walls, thereby making them more resistant to harmful enzymes produced by fungi and also delays aging of fruits.

Therefore, application of calcium together with chitosan coating might have positive effects on controlling anthracnose, prolonging storage life and retaining fruit quality of papaya. Hence, the objectives of this study are to evaluate the effect of calcium and chitosan on controlling anthracnose caused by *C. gloeosporioides* and to study their effects on storage life and fruit quality of papaya stored at 13±1°C.

MATERIALS AND METHODS

Materials

This research was conducted in June, 2007 using papaya fruits cv. Eksotika II of color index 2. Fruits were obtained from Exotic Star (M) Sdn Bhd, Sg. Chua, Kajang, Selangor, Malaysia within same day of harvesting. Calcium chloride commercial grade (74%) was obtained from a wholesaler shop. Shrimp shell chitosan (88% deacetylated) was obtained from the Chemistry Department, Universiti Putra Malaysia, Serdang, Selangor.

Preparations of Calcium and Chitosan

Calcium with concentrations of 1.5, 2.5 or 3.5% were prepared by dissolving 1.5, 2.5 or 3.5 g in distilled water (w/v) amended with 0.03% Tween 80 as surfactant agent. Chitosan solution (0.75%) was prepared by dissolving 0.75 g of chitosan in 75 mL of distilled water added with 2 mL of glacial acetic acid. The mixture was heated with continuous stirring for proper dissolution of chitosan. The final pH of the solution was adjusted to 5.6 with 2 N NaOH and made up to 100 mL with sterilized distilled water. To improve the wettability, 0.1 mL of Tween 80 was added to the solution (El Ghaouth *et al*., 1991a).

Colletotrichum gloeosporioides Isolate and Culture Conditions

Small portions of symptomatic tissue were surface sterilized in 5% sodium hypochlorite solution and washed in three changes of sterile distilled water. The tissues were then dried on sterilized filter

paper and then placed on petri plates containing Potato Dextrose Agar (PDA) and incubated at 28±2°C temperature for 7 days. Mycelia growth was observed with an optical microscopy and identification was done based on published description of Barnett and Hunter (1972). The fungus isolate was transferred to fresh PDA plates and incubated at room temperature for 2-4 days to obtain pure cultures. Pure culture of fungal isolate was maintained on PDA slants for further use.

***In vitro* Evaluation of the Fungicidal Activity of Calcium and Chitosan**

The effects of three concentrations of calcium (1.5, 2.5 and 3.5%) and chitosan (0.75%) or combinations of both on the growth of *C. gloeosporioides* were studied *in vitro*. Agar disks (5 mm diameter) taken from the margin of 4 days old pure culture of *C. gloeosporioides* were placed in the center of the respective PDA plates containing calcium at 1.5, 2.5 or 3.5% and with 0.75% chitosan and in combination of both. Control plates contained PDA only. The plates were then incubated at 28±2°C for 7 days. Daily radial of growth were measured until the fungus reached the edge of the plates. For spore germination test, spores were harvested by scrapping them off the agar with the aid of a glass rod and distilled water. The mycelium and the spore mixtures were double filtered through cheesecloth and resulting filtrate was adjusted to 20 mL. The number of spores in mL^{-1} of filtrate was determined using a haemocytometer (5×10^5 spores mL^{-1}) according to (Obagwu and Korsten, 2003).

For spore germination test, 100 µL of the spore suspension (5×10^5 spore mL^{-1}) were pipetted onto each PDA plate amended with calcium (1.5, 2.5 and 3.5%), chitosan (0.75%) or with their combinations and spread with a sterile bent glass rod. Inoculated plates were incubated at 28±2°C for 7 h before spore germination were recorded. Germination of 100 spores per plate was determined microscopically. A spore was considered to have germinated when the germ tube length equaled or exceeded the length of the spore (El Ghaouth *et al*., 1992a). The experiment was arranged in a CRD with five replications.

Postharvest Treatments

Mature green stage papaya fruits were treated either with 1.5, 2.5 and 3.5% calcium chloride infiltration, 0.75% chitosan coating, calcium infiltration at 1.5, 2.5 and 3.5% then subsequently chitosan coating or untreated fruits as control. Calcium chloride treatment was done using vacuum infiltration method according to Wills and Tirmazi (1979). Fruits were placed in each calcium concentrations for 9.5 min then fruits were maintained submerged in the solution for another 5 min after release the pressure. Finally, the fruits were removed, rinsed with distilled water to remove the calcium from the fruit surfaces. Chitosan coating application was done according to the method of El Ghaouth *et al*. (1991a). Fruits were dipped in chitosan solution for 5 min, then air dried at ambient temperature for 1 h. After treatments, papaya fruits were packed using commercial export packaging boxes. The boxes were randomly stored in cold room set at 13±1°C and 80-90% relative humidity for 33 days of storage period.

Determination of Physical Quality

Disease incidence (DI) was monitored by determined the number of fruits showing the visible symptoms of anthracnose lesions on the surface and recorded as percentage. Five fruits in each replication for each treatment were used. Storage life was determined based on reaching fruits to the full ripe index of fruits and initial symptoms of anthracnose lesions. Five fruits in each replication for each treatment were used. Storage life was monitored and records were taken until the end of storage and expressed by day of storage. Weight loss was determined using the same set of fruits at intervals of storage period. Five replications for each treatment were used.

Peel colour changes 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. The determination was done on five fruits for each replication.

Fruit firmness was measured using the Instron Universal Testing Machine (Model 5540, USA) with the compression mode and expressed as Newton (N). The determination was done on five fruits for each replication for each treatment.

Determination of Chemical Quality

After 21 days of storage, soluble solid content (SSC), pH, titratable acidity and ascorbic acid of fruit pulp were analyzed 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 pH meter model Crison Micro pH 2000, Crison Instruments, Spain. Titratable acidity was analyzed using the titration method. The results were expressed as percentage of citric acid per 100 g fresh weight. Ascorbic acid was determined using the Dye method. The ascorbic acid content (Vitamin C) was expressed as (mg/100 g) of fresh fruits. These determinations were done using five replications for each treatment.

Calcium analysis determination was made using the single dry ashing method (Cottenie, 1980). Calcium was determined by atomic absorption spectrophotometory. Results were expressed as mg Ca g^{-1} dry weight of sample.

Statistical Analysis

Analysis of variance (ANOVA) was used to detect treatment effect. Mean separation were performed using least significance difference (LSD) at the $p \leq 0.05$ level. The data were analyzed using Statistical Analysis System (SAS) version 8.2.

RESULTS AND DISCUSSION

Mycelial Growth and Spore Germination of *C. gloeosporioides*

In vitro test, calcium at different concentrations of 1.5, 2.5 or 3.5% amended with PDA did not affect the mycelial growth of *Colletotrichum gloeosporioides* but had stimulation effect. The stimulation effect on mycelial growth decreased as calcium concentration increased among these three concentrations (Table 1). Calcium concentration of 1.5% amended with PDA stimulated the mycelial growth of *Colletotrichum gloeosporioides* significantly compared with the control (PDA only) during the period of incubation. However, it did not showed any significant difference with calcium

Table 1: Effects of calcium and chitosan treatments on mycilial growth of *Colletotrichum gloeosporioides in vitro* during 5 days of incubation at 28±2°C

	Fungal growth (cm)				
	Days				
Treatments	1	2	3	4	5
Control					
0%	0.86b	2.24b	3.92b	4.88b	6.22b
Calcium (Ca)					
1.5%	1.20a	2.66a	4.54a	6.42a	8.06a
2.5%	1.08ab	2.40ab	4.38ab	6.22a	7.58a
3.5%	0.98ab	2.30b	4.24ab	6.04a	6.62b
Chitosan (CH) 0.75%	0.24d	1.00e	1.66e	2.68d	3.30e
(CH) 0.75%+ Ca 1.5%	0.58c	1.76c	2.88c	3.86c	4.94c
(CH) 0.75%+ Ca 2.5%	0.42cd	1.58cd	2.68cd	3.74c	4.56cd
(CH) 0.75%+ Ca 3.5%	0.36cd	1.34de	2.32d	3.24cd	4.08d
LSD	0.22	0.35	0.47	0.71	0.68

Means with the same letter(s) within column are not significantly different at $p \leq 0.05$ using LSD. Each value is the mean for five replicates

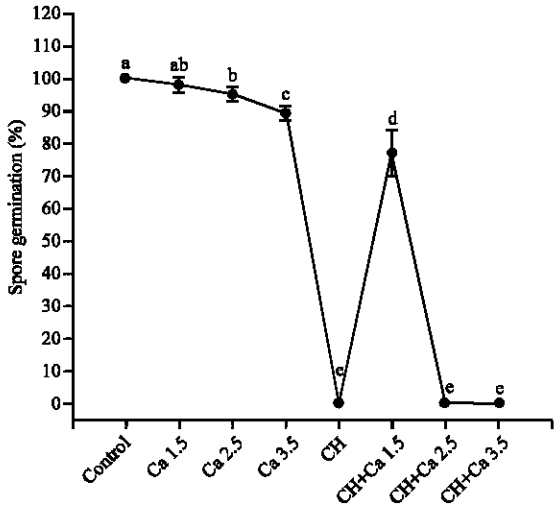

Fig. 1: Effects of calcium and chitosan treatments on spore germination of *Colletotrichum gloeosporioides in vitro* after 7 h of incubation at 28±2°C

concentration of 2.5% amended with PDA during the period of incubation. Similar growth of mycelia of *Colletotrichum gloeosporioides* was observed for calcium concentration of 2.5% amended with PDA compared with the control during the incubation period except for the fourth and fifth days of incubation which grown higher significantly. Calcium at 3.5% amended with PDA showed similar mycelial growth compared with the control during the incubation period with no significant different except on the fourth day of incubation which was grown higher. However, chitosan (0.75%) amended with PDA showed the highest fungicidal effect on mycelial growth of *Colletotrichum gloeosporioides* during the period of incubation and growth suppression became evident at the end of the incubation period demonstrating its significant effect (3.3 cm diameter) compared with the other treatments.

The combined treatments of calcium at different concentrations (1.5, 2.5 and 3.5%) with chitosan amended with PDA was observed to decrease mycelial growth gradually as calcium concentration increased with no significant differences among these treatments on the first and fourth days of incubation period. There were no significant differences between 1.5 and 2.5% and between 2.5 and 3.5%, respectively during the 5 days of incubation period. In general, the combined treatments of calcium at different concentrations (1.5, 2.5 and 3.5%) with chitosan amended with PDA showed better effects on mycilial growth than that obtained for calcium treatments alone amended with PDA. Whereas chitosan alone amended with PDA concern the best treatment, demonstrated the greatest effect on mycilial growth than the other treatments.

Spore germination of *C. gloeosporioides* was tested after 7 h of incubation period (Fig. 1). Spore germination was completely inhibited by chitosan amended with PDA and all calcium concentrations combined with chitosan amended with PDA except for 1.5%. Spore germination was reduced significantly for calcium concentrations of 2.5 and 3.5% amended with PDA, respectively, compared with the control. Whereas, calcium concentration of 1.5% amended with PDA had less effect on spore germination but it did not showed any significant different compared with 2.5% or with the control (PDA alone).

In vitro test, the decrease of stimulation effect on mycelial growth by increasing calcium concentrations (2.5 and 3.5%) amended with PDA was observed. Similarly, significant reduction of spores germination of *C. gloeosporioides* were found with increasing calcium concentrations from 2.5 to 3.5% amended with PDA compared with the control.

These effects were probably due to the toxicity of higher concentration of calcium on *C. gloeosporioides* by affecting the osmotic balance in the fungal cells and inhibition of pectinolytic enzymes (Arras *et al*., 1998). In addition, calcium did not reduced mycelial growth of *C. gloeosporioides* in the *in vitro* study but reduced germination of spores exposed to calcium has been observed in this study, indicating that the higher sensitivity of the pathogen to calcium at the spores stage more that of mycilaial growth stage of development. Biggs (2004) obtained similar results that fungal isolates grew similarly or were stimulated in the presence of calcium salts compared to the control.

The mechanisms by which fungi are sensitive to or tolerant to calcium salts are not known. One hypothesis is that high external concentrations of calcium may lead to increased concentration of calcium in the cytosol, which may be toxic to the fungus. Since maintenance of low basal concentrations of internal calcium is essential for normal cell functions, organisms with the inability to regulate intracellular calcium may exhibit compromised growth and development. Those that can regulate intracellular calcium may grow normally (Droby *et al*., 1997).

The control of decay by chitosan is believed to originate, in part, from its antifungal property. *In vitro* studies showed that chitosan not only inhibited the radical growth of major postharvest pathogens, but also induced severe morphological alterations in *Rhizopus stolonifer* and *Botrytis cinerea*, as well as increased cellular leakage in both fungi, presumably by interfering with fungal plasma membranes (El Ghaouth *et al*., 1992c).

In the present study, chitosan amended with PDA showed the highest fungicidal effect on mycelial growth of *Colletotrichum gloeosporioides* during the period of incubation. Similarly, spore germination was significantly inhibited by chitosan amended with PDA and with all calcium concentrations combined with chitosan amended with PDA except for 1.5%. The mechanism by which chitosan affects the growth of several phytopathogenic fungi has not been fully elucidated, but several hypotheses have been proposed in the explanation of antimicrobial activity.

It is believed that the polycationic nature of this compound is the key to its antifungal properties and that the length of the polymer chain enhances its antifungal activity (Hirano and Nagao, 1989). Due to its polycationic nature, chitosan interferes with negatively charged residues of macromolecules exposed on the fungal cell surface. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents (Leuba and Stossel, 1986). Similar findings was obtained by El Ghaouth *et al*. (1992a) who reported that chitosan inhibited spore germination and radial growth of *Botrytis cinerea* and *Rhizopus stolonifer in vitro*. This prove, is strong evidence that mycelial growth can be inhibited or retarded when the growth media of fungi are amended with chitosan.

Disease Incidence Percentage of Anthracnose

The effects of treatments on the number of fruits infected with anthracnose were significantly different (Table 2). The higher sign of lesions was observed in untreated papaya fruits. Disease incidence was reduced significantly with 2.5% calcium treatment compared with the other concentrations 1.5 and 3.5%. However, chitosan treatment controlled the disease incidence better than that obtained with calcium treatments. Further enhancement of controlling disease incidence was obtained using chitosan in combination with calcium treatments. Chitosan in combination with calcium at 2.5% had significantly reduced the disease incidence compared with the other combinations with calcium at 1.5 and 3.5%. Moreover, this treatment showed the highest fungicidal effect observed in papaya fruits, demonstrated the lowest disease incidence compared to other treatments.

The positive effect of reducing disease incidence due to the fungicidal effect of chitosan on the disease incidence of anthracnose caused by *Colletotrichum gloeosporioides* confirmed and this is consistent with reports that chitosan has antifungal ability against several postharvest pathogens (El Ghaouth *et al*., 1992a; Zhang and Quantick, 1998; Jiang and Li, 2001). It was possible that chitosan

Table 2: Effects of calcium and chitosan treatments on disease incidence and storage life of papaya fruits after storage at 13±1°C

Treatments	Disease incidence (%)	Storage life (day)
Control		
0%	88.13a	18.40g
Calcium (Ca)		
1.5%	70.38b	21.20f
2.5%	38.07d	24.20d
3.5%	49.30c	22.70e
Chitosan (CH) 0.75%	27.92e	26.30c
(CH) 0.75% + Ca 1.5%	20.67ef	27.70bc
(CH) 0.75% + Ca 2.5%	5.60g	33.40a
(CH) 0.75% + Ca 3.5%	17.76f	29.10b
LSD	8.20	1.48

Means with the same letter(s) within column are not significantly different at $p \leq 0.05$ using LSD. Each value is the mean for five replicates

coating on papaya fruit acted as a barrier limiting penetration of the germ tube of *C. gloeosporioides*. However, other physiological events might be involved in controlling anthracnose during papaya storage since chitosan has also been noticed to induce several host defense mechanisms such as the induction of physical barriers and production of phytoalexins (El Ghaouth *et al*., 1994). El Ghaouth *et al*. (1992b) suggested that chitosan induces chitinase, a defense enzyme, catalyzes the hydrolysis of chitin, a common component of fungal cell walls, thus preventing the growth of fungi on the fruits.

Other possibility explained that the maintaining of fruit firmness resulted by calcium application thus, making the fruits more difficult to access by the pathogen. Firming and resistance to softening resulting from addition of calcium have been attributed to the stabilization of membrane systems and the formation of calcium pectates, which increase rigidity of the middle lamella and cell wall to increased resistance to polygalacturonase enzyme attack and to improve turgor pressure (Poovaiah, 1986; Mignani *et al*., 1995).

These results indicated that adding calcium into fruits would increase the antifungal activity of chitosan. These findings confirmed that reported by Chaiprasart *et al*. (2006) that treating strawberries with chitosan and calcium chloride can reduce fruit decay of all strawberry cultivars during low storage temperature.

Storage Life

Storage life was extended significantly ($p<0.05$) with treatments (Table 2). Storage life was significantly prolonged to some extent using calcium infiltration treatments at 1.5, 2.5 and 3.5% by 21.2, 24.2 and 22.7 days, respectively compared to the untreated fruits (control). Chitosan coating (0.75%) prolonged the storage life significantly up to 26.3 days compared to calcium treatments or untreated fruits which lasted for 18.4 days. The positive effect of chitosan on storage life improved further when combined with calcium (2.5%) where the fruits lasted for about 33 days followed by 3.5% (29 days). There were no significant differences between chitosan treatment alone with chitosan plus 1.5% and between the later with chitosan plus 3.5% calcium. The calcium could probably was incorporated into the cell wall of the fruits, conferring high resistance infection (Wang *et al*., 1993; Souza *et al*., 1999). On the other hand, chitosan coatings to improved quality and storability have been reported for fruits such strawberries and raspberries (Zhang and Quantick, 1998). The positive effect of chitosan coating on storage life could probably due to forms an excellent film over the fruits, modifying the atmosphere within as in modified atmosphere (MA) storage. The depolymerization process of metabolism substrates is possibly influenced by the modified atmosphere created inside fruits. The MA created can therefore delay ripening by delaying ethylene production and by reducing the level of internal oxygen and consequently prolong the storage life of fruits (El Ghaouth *et al*., 1991b, 1992d).

Table 3: Effects of calcium and chitosan treatments on weight loss of papaya fruits every 7 days intervals of storage at 13±1°C

	Weight loss (%)		
Treatments	7 days	14 days	21 days
Control			
0%	4.47a	6.72a	9.10a
Calcium (Ca)			
1.5%	3.92b	5.85b	8.26b
2.5%	2.24c	4.61c	7.48c
3.5%	3.85b	5.66b	8.00bc
Chitosan (CH) 0.75%	2.20c	4.43c	6.20d
(CH) 0.75%+ Ca 1.5%	2.56c	4.81c	5.79de
(CH) 0.75%+ Ca 2.5%	1.63d	2.82d	4.30f
(CH) 0.75%+ Ca 3.5%	1.71d	3.19d	5.34e
LSD	0.34	0.56	0.73

Means with the same letter(s) within column are not significantly different at $p \leq 0.05$ using LSD. Each value is the mean for five replicates

Calcium chloride proved to have significant impact on the shelf life of various fruits and vegetables such as strawberries, tomatoes, pear and apples (Rosen and Kader, 1989; Floros *et al*., 1992; Sams *et al*., 1993). The benefits of applying calcium chloride probably due to delaying of aging or ripening, reduces postharvest decay, controls the development of many physiological disorders and increases the calcium content, thus improving their nutritional value (Rosen and Kader, 1989; Floros *et al*., 1992; Sams *et al*., 1993). Wills and Mahendra (1989) reported that the use of 1% calcium chloride as postharvest treatment significantly extended the time for peach fruits to ripen by about 30%, which is of significant commercial value.

Weight Loss

Treatments significantly ($p<0.05$) reduced weight loss of papaya fruits during storage period of 21 days compared to the control (Table 3). Calcium treatment at 2.5% significantly reduced weight loss when stored for 7 and 14 days compared to other concentrations and control. However, after 21 days of storage, this treatment was significant in ration to the control and 1.5% but not with 3.5% calcium treatment. In fact, results on the weight loss of fruits treated with calcium infiltrations at 2.5 and 3.5% combined with chitosan coating demonstrated that the moisture barrier properties of chitosan coating was significantly ($p<0.05$) improved compared with the other treatments during 7 and 14 days intervals. After 21 days of storage, the calcium infiltration at 2.5% combined with chitosan coating showed the best effect in reducing weight loss (4.3%) compared to chitosan alone and the other treatments. Whereas, control treatment had the highest loss about 9%.

This observation may be that addition of 2.5% calcium into fruits may have increased the resistant of water permeability of the cell walls, thus resulting in decreased diffusivity of water vapor through the film matrix and a decrease weight loss. The influence of calcium additives on the weight loss is generally expected to improve the water vapor barrier properties by providing hydrophobicity and increasing film resistance to water transmission (Han *et al*., 2004). Similar results on weight loss reduction trend were observed using calcium caseinate combined with chitosan based film coating (Mei and Zhao, 2003). These results also confirmed by the findings of Garcia *et al*. (1998) 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.

Peel Color Changes

Peel color changes were significantly ($p<0.05$) by the treatments (Table 4). After 21 days of storage, the effects of treatments on lightness, chroma and hue angle of peel color of papaya were

Table 4: Effects of calcium and chitosan treatments on peel color lightness (L*), chroma (C*) and hue angle (h°) of papaya fruits after 21 days storage at 13±1°C

	Peel color changes		
Treatments	Lightness (L*)	Chroma (C*)	Hue (h°)
Control			
0%	65.22a	54.47a	102.34f
Calcium (Ca)			
1.5%	62.50ab	52.30b	107.56f
2.5%	58.86c	49.06c	120.08d
3.5%	60.01bc	51.38b	113.38e
Chitosan (CH) 0.75%	55.37d	47.37cd	126.12c
(CH) 0.75%+ Ca 1.5%	53.29de	45.77d	133.40b
(CH) 0.75%+ Ca 2.5%	47.39f	43.06e	143.12a
(CH) 0.75%+ Ca 3.5%	50.96e	45.53d	136.46b
LSD	3.49	2.05	5.73

Means with the same letter(s) within column are not significantly different at $p \leq 0.05$ using LSD. Each value is the mean for five replicates

measured. In general, while lightness and chroma decreased the hue angle increased with applying treatments. Higher values of lightness and chroma and the lowest values of hue angle were obtained with untreated fruits compared with the other treatments. Calcium infiltration treatments were found to reduce lightness values gradually with increasing calcium concentrations up to 2.5% then declined at 3.5% with no significant differences between 1.5 and 3.5% and between 2.5 and 3.5%. Chitosan coating reduced the lightness more than the calcium infiltration treatments significantly ($p<0.05$). Whereas it was greater when combined with calcium infiltration up to 2.5% then declined with 3.5%. There was no significant difference between chitosan coating alone and when combined with 1.5% but there were significant differences ($p<0.05$) with other combinations at 2.5 and 3.5%. The best effect was observed for the treatment with 2.5% calcium with chitosan coating.

Chroma values were observed to have similar trend as that of lightness where the lightness decreased gradually with increasing calcium concentrations up to 2.5% after which it declined at 3.5% for calcium infiltration treatments alone or combined with chitosan coating. The calcium combined treatments with chitosan had greater effects than the calcium treatments alone significantly ($p<0.05$) and it demonstrated the best effect with calcium at 2.5% combined with chitosan compared with 1.5 and 3.5%.

Hue angle value was observed to be maintained higher with calcium infiltration treatments by increasing calcium concentration to 2.5% then declined at 3.5% with significant differences ($p<0.05$) for 2.5 and 3.5%, respectively compared with untreated fruits. Hue angle maintained in its higher value greater with the treatment of chitosan coating alone compared with calcium infiltrations as individual treatments. Whereas, this ability of maintaining the changes of hue angle was improved with the combined treatments of calcium at 1.5, 2.5 and 3.5% with chitosan coating with no significant difference between 1.5 and 3.5%. The combined treatment of calcium infiltration at 2.5% with chitosan coating demonstrated the highest effect in maintaining the changes of hue angle significantly ($p<0.05$) compared with other treatments.

The retardation of color development in the papaya skin treated with calcium and chitosan in this investigation can be attributed to the modified internal fruit atmosphere, which caused slowing down the ripening process indicating by lower changes of peel color of fruits. The coating reduced the fruit respiration and ethylene production. The slow respiration and reduced ethylene synthesis, in turn, delayed ripening and senescence, resulting in less change in the greenish yellow color of fruits (Kader *et al*., 1989). The results from this study agree with Lazan *et al*. (1990) who observed that modify atmosphere (MA) with sucrose polyester was effective in retarding the development of

Table 5: Effects of calcium and chitosan treatments on firmness (N), soluble solids concentrations (SSC) and pH of papaya fruits after 21 days of storage at 13±1°C

Treatments	Firmness (N)	SSC (°Brix)	pH
Control			
0%	1.66f	13.62a	5.49a
Calcium (Ca)			
1.5%	2.11ef	13.22ab	5.02b
2.5%	4.84c	12.50c	4.80bcd
3.5%	2.81e	12.88bc	4.93bc
Chitosan (CH) 0.75%	3.93d	11.82d	4.57cd
(CH) 0.75%+ Ca 1.5%	5.90b	11.66d	4.53d
(CH) 0.75%+ Ca 2.5%	8.21a	10.32f	4.08e
(CH) 0.75%+ Ca 3.5%	6.64b	11.00e	4.47d
LSD	0.75	0.64	0.38

Means with the same letter(s) within column are not significantly different at $p \leq 0.05$ using LSD. Each value is the mean for five replicates

skin color in Eksotika papaya. Postharvest application of calcium chloride delayed color changes of treated sapodilla, compared to distilled water treatment (0% calcium chloride) as a control (Poonsawat *et al*., 2007).

Fruit Firmness

There were significant effects of treatments on the firmness of papaya fruits after 21 days of storage (Table 5). Calcium infiltration at 1.5% did not significantly control the loss of firmness compared with untreated fruits. Calcium infiltration of 2.5% resulted with much firmer fruits compared to other concentrations. However, when combined with chitosan (0.75%) the fruits treated with 2.5% calcium (8.2, 3.9 N) almost do were the former (4.8, 3.9 N). Chitosan coating alone showed better effect in controlling firmness loss compared with calcium infiltration treatments or untreated fruits. The three treatments with chitosan showed the highest ability in controlling firmness loss of fruits with no significant difference between 1.5 and 3.5%. Among the other treatments, the combination treatment with calcium at 2.5% and chitosan coating showed the best effect in maintaining the loss of firmness.

These results proved that edible coating and calcium as firming agent can controlled the migration of moisture from the fruits, thus controlling the integrity and texture of papaya fruits. This maybe due to calcium interaction with pectic acid in cell walls to form calcium pectate, a compound helpful for maintaining structure of the fruits (Rolle and Chism, 1987).

These findings are consistent with the previous studies, which reported greater firmness of fruit such as papaya, tomatoes and peaches when coated with chitosan solutions (El Ghaouth *et al*., 1992d; Li and Yu, 2001; Luna *et al*., 2001). The improvement of firmness in fruits treated with calcium and chitosan could be also due to the higher antifungal activity of chitosan, covering of the cuticle and lenticels, thereby reducing infection, respiration and other ripening processes during storage, hence maintaining firmness of fruits (Martinez-Romero *et al*., 2006).

Soluble Solids Concentrations (SSC)

Untreated fruits resulted with highest content of SSC but was not significantly different with 1.5% calcium treatment (Table 5). Significant ($p<0.05$) increase in SSC was observed for calcium infiltration treatment at 1.5% than calcium treatment at 2.5% compared with control treatment. However, it was not significantly different with 3.5% calcium treatment. Chitosan coating treatment significantly lower contents of SSC compared to calcium treatments. Chitosan effect significantly enhanced with the incorporation of calcium treatments where the SSC further reduced with addition of 2.5 and 3.5% of calcium. The lowest SSC content found when chitosan was in combination with 2.5% calcium infiltration treatment compared with the other treatments.

The effect of calcium at 2.5% and chitosan combine 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). These findings are consistent with that obtained by Cheour *et al*. (1991) who reported that the concentration of free sugars progressively increased with storage, this increase was quite markedly delayed by calcium treatment for Glooscap variety of strawberry. Cheour *et al*. (1990) reported that the application of calcium on strawberry plants increased fruit calcium content and influenced several postharvest senescence changes involving free sugars content of fruits. Other reports reported a slow rise in SSC of mango and banana treated with chitosan and a higher rise in treated peaches (Kittur *et al*., 2001; Li and Yu, 2001). This might be due to chitosan effect on slowing down the ripening, respiration rise and metabolism processes of the fruits.

pH Determination

The pH value was significantly ($p<0.05$) higher in the untreated fruits compared with other treatments (Table 5). This would be due to higher concentrations of organic acids which normally occurred when the fruits ripened. pH values were not affected by calcium treatments. However, calcium combinations with chitosan coating showed better control on pH (lower pH) than that of calcium infiltrations as individual treatments or chitosan coating alone. Calcium at 2.5% in combination with chitosan had a significantly lower pH compared to the other treatments. This might be attributed to the relatively delaying of fruits ripening probably because of the semipermeable chitosan film formed on the surface of the fruit modified the internal atmosphere, such as CO_2 and O_2 concentrations of the fruits, thus retarding breakdown of organic acids (Bai *et al*., 1988). Present results are consistent with the findings of Han *et al*. (2004) who reported that chitosan alone and chitosan coatings containing calcium showed better control on pH (lower pH) than that of chitosan containing Vitamin E on both fresh and frozen strawberries.

Titratable Acidity (TA)

Titratable acidity (TA) content was significantly ($p<0.05$) lower in the untreated fruits compared with the other treatments (Table 6). The calcium infiltration treatment at 1.5% slowed the reduction of TA significantly compared to the untreated fruits. Whereas calcium infiltration at 2.5 and 3.5% had better effects but they did not showed any significant differences between each other. Chitosan slowed the reduction of TA effectively compared with calcium infiltration treatments. However, chitosan in combination with calcium treatments had a significantly effect than the chitosan alone. Calcium at 2.5% in combination with chitosan showed significant effect in slowing TA reduction compared with the

Table 6: Effects of calcium and chitosan treatments on titratable acidity and ascorbic acid contents of papaya fruits after 21 days of storage at 13±1°C

Treatments	Titratable acidity (%)	Ascorbic acid (mg 100 g^{-1})
Control		
0%	0.140g	40.00e
Calcium (Ca)		
1.5%	0.149f	41.67e
2.5%	0.167e	45.17cde
3.5%	0.161e	43.12de
Chitosan (CH) 0.75%	0.180d	47.83bcd
(CH) 0.75% + Ca 1.5%	0.193c	49.17bc
(CH) 0.75% + Ca 2.5%	0.228a	57.83a
(CH) 0.75% + Ca 3.5%	0.202b	52.67ab
LSD	0.007	5.72

Means with the same letter(s) within column are not significantly different at $p \leq 0.05$ using LSD. Each value is the mean for five replicates

other concentrations 1.5 and 3.5%, demonstrated the best effect on slowing down changes of TA compared with the other treatments. Slowing the reduction of TA in papaya fruits was probably due to slowing down of acid oxidation for the fruits delayed its ripening caused by the treatments during ripening of fruits.

These results agreed with those reported by El Ghaouth *et al*. (1991b) and Garcia *et al*. (1998) that the titratable acidity decreases with increasing storage time in both uncoated and coated fruit. Thus, the decrease of acidity during storage demonstrated fruit ripening. Present results confirmed the findings obtained by Cheour *et al*. (1991) who reported that the quantity of organic acids expressed as citric acid decreased in strawberry fruits during storage. Calcium treatment delayed the decrease in the citric acid, which was observed after 14 days of storage. This effect might be due to slowing ripening process of the fruits, hence slowing the breakdown of organic acid occurred during ripening.

Ascorbic Acid (AA)

Ascorbic acid content (Vitamin C) was observed to be lower in the untreated fruit (Table 6). Ascorbic acid was not affected by calcium treatments. However, chitosan treatment showed better effect in slowing the decrease of AA significantly compared with untreated fruits. The combined treatments of calcium and chitosan coating improved the maintenance of ascorbic acid as calcium concentrations increased for 1.5 and 2.5% then declined with 3.5%. The combined treatment with calcium at 2.5% and chitosan coating had the best effect in maintaining ascorbic acid changes significantly compared to the other treatments except for 3.5% calcium in combination with chitosan. However, other combinations with calcium at 1.5 or 3.5% did not show any significant difference among each other or with chitosan alone.

This effect could due to the combined effect of calcium and chitosan which caused the best result of delaying ripening of fruits hence, this process might have delayed the oxidation of ascorbic acid which can occur during the normal ripening. Slowing down of vitamin loss was attributed to the low oxygen permeability of the coating film formed on the fruit surfaces (Ayranci and Tunc, 2003). Keeping oxygen away from the fruits delays the deteriorative oxidation reaction of vitamin C (Ayranci and Tunc, 2004). An increase in vitamin C content of pineapples and guava treated with calcium chloride has been observed (Mohamed *et al*., 1993). In line with this finding, it was reported that Kiwifruit slices stored in ethylene free air contained 3 fold AA than controls. When dipped in 1% calcium chloride after cutting and kept in an ethylene free atmosphere, slices had a slightly higher AA content than those treated with 1% calcium chloride only (Agar *et al*., 1999). It is also reported that the application of calcium chloride increased ascorbic acid content of apples (Poovaiah, 1986). This effect probably due to slowing ripening process, hence keeping vitamin C out of the oxidation occurred at normal ripening.

In papaya, ascorbic acid (Vitamin C) initially increases during ripening then decreasing during senescence (Selvaraj *et al*., 1982; Lazan *et al*., 1990). It has been indicated that once fruits reach ripe stage, ascorbic acid contents start to decline (Selvaraj *et al*., 1982). It seems that modifying atmosphere (MA) by chitosan coating suppresses the synthesis of ascorbic acid but does not impair the fruit capability to synthesize the vitamin. Mathooko (2003) reported that the ascorbic acid content in tomato increased with maturity and ripening. However, once fruit became fully ripe, the vitamin content started to decline.

Calcium Content

Pell and flesh calcium contents were observed to increase significantly ($p<0.05$) by increasing calcium concentrations (Table 7). Calcium treatments increased calcium content of peel fruits significantly from 1.47% for calcium treatment at 1.5 to 2.65% with calcium treatment at 3.5%

Table 7: Effects of calcium treatment on calcium contents of peel and flesh of papaya fruits after 21 days of storage at 13±1°C

	Calcium content of fruits (%)	
Treatments	Peel	Flesh
Control		
0%	0.96d	0.73d
Calcium (Ca)		
1.5%	1.47c	1.09c
2.5%	2.05b	1.57b
3.5%	2.65a	1.86a
LSD	0.28	0.22

Means with the same letter(s) within column are not significantly different at $p \leq 0.05$ using LSD. Each value is the mean for three replicates

compared with the untreated fruits (0.96%). Calcium contents of flesh fruits also significantly increased, whereby it increased from 1.09 to 1.86% with calcium treatment at 1.5 to 3.5% compared with the untreated fruits (0.73%). The increment was higher in the peel than that of the flesh of fruits.

The high calcium content in the peel fruits of papaya is attributed to its large surface area to volume ratio, thus carrying more nutrients than that with flesh fruits. Calcium infiltration of 2.5% combined with chitosan enhanced the nutritional value of fruits without causing any negative effects on their quality attributes. This study demonstrates that calcium infiltration could be used effectively as a method of applying calcium to develop papaya fruits nutritionally. These results are consistent with Garcia *et al*. (1996) who reported that strawberries fruits treated with 1% calcium chloride had significantly higher concentration of calcium content about 37% than the control. This increase in the calcium content could be considered nutritional value that has been added to the fruit.

CONCLUSION

Anthracnose disease was effectively controlled *in vitro* study using chitosan treatment compared to the other treatments. Whereas calcium at 2.5% combined with chitosan demonstrated the best effect in controlling anthracnose disease incidence in papaya fruits. This was due to maintenance of fruit firmness and chitosan antifungal property. The combined treatment of calcium at 2.5% with chitosan coating proved to extend the storage life of papaya fruits by indirect maintenance of firmness hence, decreasing the disease incidence, weight loss and control of changes in color and chemical characteristics. In addition, calcium infiltration demonstrated their capabilities to increase calcium content of papaya fruits significantly, thus increasing the content of these nutrients in the peel and flesh of papaya fruits. The combination treatment of calcium at 2.5% with chitosan coating significantly improved its storability, moisture barrier functionality and maintaining quality of fruits. Therefore, future study to fully understand the changes of weight loss and other quality characteristics for this treatment during storage at 13±1°C should be investigated.

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