Pertanika J. Trop. Agric. Sci. 35 (3): 439 - 458 (2012)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

# Potential Co-application of *Burkholderia cepacia*, Calcium and Chitosan on Enhancement of Storage Life and Quality of Papaya Fruits

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## ABSTRACT

The fruit of harvested papayas (cv. Sekaki), at colour stage two (mature-green with trace yellow), were treated with fungicide benocide® (0.33 $gL^{-1}$) or with a combination of *Burkholderia cepacia* B23 ($10^9$ CFU $mL^{-1}$) and 0.75% chitosan solution, amended with 3% calcium chloride and stored at 14 ± 0.5ºC and 90-95% RH for 28 d. The effectiveness of the treatments was assessed by evaluating their impacts on storability and changes in the quality attributes of fruits. Results indicated that fruit treated with the combination of *B. cepacia* B23-chitosan-$CaCl_2$ showed delayed climacteric ethylene evolution and reduced respiration rate. The combined treatment reduced weight loss by more than 25% to the control. It also markedly slowed down the ripening of fruits, as shown by their retention of firmness 4.17 N after storage. Moreover, a delayed change in external colour and pH without compromising fruit quality was observed in the fruit receiving the combined treatment. The storage life was thus extended up to 15 d compared to the control. In addition, the incorporation of 3% $CaCl_2$ into the combined treatment significantly increased the calcium content (81%) in the fruit compared to the control, resulting in the improved nutritional value of the papaya. This study provided an alternative method for fungicides treatment of papaya at post-harvest.

ARTICLE INFO

*Article history:*
Received: 5 February 2010
Accepted: 12 April 2011

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ISSN: 1511-3701 

*Keywords*: Papaya, bioactive coating, natural compounds, storage life, quality maintenance

## INTRODUCTION

Being a climacteric fruit, papaya (*Carica papaya* L.) is characterized by increased respiration and ethylene evolution during ripening. Generally the fruit ripens in a rather short period between seven to nine days from harvest (Ali *et al.*, 1994). Proper storage practices are required for papaya fruits to avoid quality deterioration which occurs primarily due to post-harvest diseases and accelerated softening. For the fruits to be competitive in the market, it is important to control the disease and to delay the onset of the ripening processes while maintaining the quality.

It is important to note that synthetic fungicides is the primary means used to control post-harvest diseases of fruits; however, environmental and health risks are high (Janisiewicz & Korsten, 2002). Controlled atmosphere (CA) techniques are expensive, while modified atmosphere packaging (MAP) has been shown to ameliorate chilling injury and fungal decay in several crops (Yahia & Paull, 1997). Thus, there is a need to have alternative technique to reduce disease incidence and improve storability of papaya without undesirable physico-chemical changes taking place during the storage. In this sense, post-harvest application of biocontrol agent, in combination with chitosan and calcium chloride, is considered to be an alternative tool.

In our previous study, the antifungal activities of *Burkholderia cepacia* strain B23 were demonstrated in petri plate assays (Kadir *et al.*, 2008; Rahman *et al.*, 2007). The principal mode of disease control of this particular strain is antibiosis. *B. cepacia* has also been shown to protect against or decrease the severity of various post-harvest diseases of fruits, including apples and pears, which are caused by *Penicillium expansum* and *Botrytis cinerea* (Janisiewicz & Roitman, 1988) and banana infection caused by *Colletotrichum musae* (De Costa & Subasinghe, 1999).

Chitosan, which is a high molecular weight cationic polysaccharide, can theoretically be used as a coating material for fruit (Jiang & Li, 2001). Due to its ability to form a semi-permeable film, chitosan coating may be expected to modify the internal atmosphere of fruit and decrease transpiration losses (Zhang & Quantick, 1998). Results of some previous studies have shown that chitosan coating has the potential to prolong storage life and to control decay of many fruit such as strawberries (Hernandez-Munoz *et al.*, 2006), apples (Du *et al.*, 1998) and papaya (Sivakumar *et al.*, 2005).

Calcium has been identified as an important nutraceutical that plays significant roles in the human body to prevent certain diseases (Pszczola, 1998). Many authors have reported that calcium dip increases nutritional value, maintains firmness and extends the storage life of a wide range of fruit, including strawberries and raspberries

(Han *et al.*, 2004), pears (Mahajan & Dhatt, 2004) and peaches (Mahajan & Sharma, 2000).

One of the unique characteristics of chitosan-based coating is that it can be used as a carrier for incorporating functional ingredients, such as antimicrobial agents and nutraceuticals (Park & Zhao, 2004). It can not be denied that works on *B. cepacia*, chitosan and calcium chloride, as post-harvest treatments, are readily available but the literature is still scarce for the local strain of *B. cepacia* and a variety of papayas. Thus, the objective of the study was to determine the potential of postharvest application of *B. cepacia* B23 in combination with chitosan and calcium chloride on the post-harvest storage and quality of papaya fruits under low temperature conditions.

## MATERIALS AND METHODS

### *Preparation of Aqueous Suspension of B. cepacia B23*

A local strain of *B. cepacia* B23, isolated from the surface of a papaya fruit, was used as a biocontrol agent in this study. In a previous study, *B. cepacia* B23 was isolated following standard methods and identified using BIOLOG identification system (Rahman *et al*., 2007). To prepare the aqueous antagonist suspension, isolate B23 was grown on nutrient agar (NA) at 28 ± 2ºC for 24 h. A loop of the bacterial culture was then transferred into a 250 mL Erlenmeyer flask containing 50 mL of sterilized nutrient broth (NB) and incubated on a rotary shaker at 150 rpm for 48 h at 28 ± 2ºC. The isolate was re-cultured in fresh NB and incubated for another 72 h before use. At the time of use, the cell concentration of *B. cepacia* B23 in the suspension was adjusted to approximately $1x10^9$ CFU $mL^{-1}$ with sterilized distilled water using spectrophotometer at 600 nm.

### *Preparation of Chitosan Solutions*

To prepare 100 mL of 0.75% chitosan solution, 0.75 g of chitosan (Shrimp shell chitosan, Chito-Chem (M) Sdn. Bhd., Malaysia) was dissolved in 75mL of distilled water added with 2mL 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 volume made up to 100mL with sterilized distilled water. To improve wettability, 0.1mL of Tween 80 was added into the solution (Jiang & Li, 2001).

### *Fruits and Treatments*

Fully matured papayas cv. 'Sekaki' with colour stage two (mature-green with trace yellow) were obtained from an exporter Seng Chew Hup Kee (M) Sdn. Bhd., Kajang, Selongor, Malaysia, on the same day of harvest. Surface sterilization with 75% ethanol was followed by rinsing in sterilized distilled water and air-drying for 10 min for a total of 132 fruit. For one treatment, each of the 44 fruit was dipped for 15 min in (i) sterilized distilled water (control) or (ii) commercial fungicide, benocide® (benomyl 50% WP) of 0.33 g $L^{-1}$. For the combined treatment, 44 fruit were initially immersed in aqueous suspension of *B. cepacia* B23 ($10^9$ CFU $mL^{-1}$) for 15 min and allowed to

air dry for 5 min. Once again, the fruit were immersed in 0.75% chitosan solution which was amended with 3% $CaCl_2$ for 15 min and allowed to surface-dry for 5 min. Each fruit was sleeved with Styrofoam netting, packed in a commercial packaging, and held at 14 ± 0.5 ºC and 90-95% RH for 28 d. Every week, eight fruit (representing four replications for each treatment) were used for the determination of physico-chemical characteristics. A different set of four fruit from each treatment was used to determine the respiration rate and ethylene production, and the same set of fruits was also used throughout the whole storage period. Data were recorded every week, and this was started immediately after the treatment.

*Determination of Respiration Rate and Ethylene Production*

Respiration rate and ethylene evolution were assayed on a weekly basis. Individual fruit was sealed in a 2.5 L airtight plastic container and incubated for 3 h at 14 ± 0.5ºC. After incubation, one mL of gas sample was withdrawn from headspace by a gas-light hypodermic syringe and analyzed using gas chromatography (Clarus 500, Perkin Elmer, Shelton, USA), equipped with a thermal conductivity detector (TCD), a flame ionization detector (FID) and a Porapack Q, 50/80 stainless steel column. Standard $CO_2$ and $C_2H_4$ gasses (Air Products Pte. Ltd., Singapore) were used for calibrating the chromatography. The respiration rate was expressed as mL $kg^{-1}$ $h^{-1}$ of $CO_2$ evolved, whereas, ethylene production was expressed as µL $kg^{-1}$ $h^{-1}$.

*Measurements of Weight loss, Surface Colour and Flesh Firmness*

To determine weight loss, an individual fruit was weighed with a top pan electronic balance (BP2100, Sartorius, Germany) at the beginning of the experiment just after the treatment and then air-dried, and thereafter, this was done each week during the storage period. Eight fruit per treatment (representing four replications) were marked for the measurements of weight loss and surface colour. The same set of fruits was used until the end of the experimental period. Weight loss was expressed as the percentage loss of the initial total weight.

The colour of the surface of papaya was determined using a Chroma Meter (Model CR-300, Minolta Corp., Japan) and expressed in the chromaticity values of lightness (L*), chroma (C*) and hue angle (hº). Before measurement, the equipment was calibrated against a standard white tile, with standard values of L* = 97.30, C* = 1.88, hº = 85.8 using the Illuminate C (CIE 1976). The measurements were taken at stem end, mid region and blossom end on each fruit so as to obtain a mean value.

Meanwhile, pulp firmness of the fruit was measured using an Instron Universal Testing Machine (Model 5543, Instron Corp, USA), which was supported by an Instron Merlin Software Version M12-13664-EN. The instrument was equipped with a 6 mm diameter flat probe that was programmed to penetrate in a normal direction at a cross-head speed of 20 mm $min^{-1}$. Round slices of 25 mm thick, containing both peel and pulp, were cut horizontally from the stem

end, equatorial and blossom end of each fruit with a razor blade. The measurements were taken at three different places of each slice and the readings were recorded in Newton (N), while the mean was also calculated.

*Measurements of Total Soluble Solids, pH, Titratable Acidity and Ascorbic Acid*

After the firmness analysis, the pulp tissues of papaya were cut into small pieces. Ten grams of pulp tissues was homogenized in 50 mL of distilled water for 2 min using a kitchen blender and filtered through a Whatman filter paper No. 2. The supernatant was collected in order to measure the total soluble solids using a digital refractometer (Model N-1 α, Atago, Japan), pH using a glass electrode pH meter (GLP 21, Crison, Bercelona, EEC), whereas titratable acidity expressed as citric acid (%) was determined by titration with 0.1 mol $L^{-1}$ NaOH to pH 8.1 according to the method by Ranganna (1977). For ascorbic acid measurement, 10 g pulp tissue was immediately homogenized in 50 mL of 3% cold metaphosphoric acid ($HPO_3$) using a blender for 2 min, and filtered through Whatman filter paper No. 2. The clear supernatant was collected for assaying ascorbic acid by 2,6-dichlorophenolindophenol titration, following the method of Ranganna (1977). Ten millilitres of aliquot was titrated with 0.1% 2,6-dichlorophenolindophenol solution until the filtrate changed to pink, persisting for at least 15 s and the titration volume of 2,6-dichlorophenolindophenol was recorded. Prior to titration, 2,6-dichlorophenolindophenol solution was calibrated by ascorbic acid standard solution. Ascorbic acid content was calculated according to the titration volume of 2,6-dichlorophenolindophenol and the results were expressed as mg 100 $g^{-1}$ fresh weight.

*Calcium Determination*

For skin calcium determination, peel with outer flesh of the treated fruits was removed to a depth of 2 mm with a mechanical peeler, and cut into small pieces with a sharp knife. The next 2 mm of the pulp tissue was used for flesh calcium analysis. Each sample was a pooled of peel or flesh from two papayas; four replicates from each treatment were analyzed. The samples were dried in a mechanical convection oven (Memmert, Germany) at 80ºC for two days and ground into powder. Dry ashing procedure was used to digest the powder. The calcium content was analyzed by atomic absorption spectrophotometer (AAnalyst 400, Perkin-Elmer). Calcium measurement was done only at day 0 after the treatment as $Ca^{+2}$ is very stable during storage (Mei *et al.*, 2002); the calcium content is reported in mg $kg^{-1}$.

*Scanning Electron Microscopic Observation of Papaya Fruit Pericarp*

Water treated control and *B. cepacia* B23-chitosan-$CaCl_2$ treated papaya fruit were used in this study. The peel samples of ~2 $mm^3$ were taken from the mid region of the fruit and fixed separately in 2.5% buffered glutaraldehyde for 24 h at 4ºC. The samples were prepared following the standard procedure, as described by Benhamau and

Chet (1996). The samples were dried in a Baltec 030 Critical Point Drying apparatus. The dried samples were stuck on aluminium stubs and coated with gold in a Polaron Sputter Coater and viewed under SEM (JOEL JSM 6400).

*Experimental Design and Statistical Analysis*

All the experiments were carried out in a completely randomized design (CRD) with three treatments replicated four times. The data were subjected to the analysis of variance (ANOVA) using the SAS statistical software version 8.2. The results showing significant differences were then subjected to mean separation using Tukey's Studentized Range (HSD) Test at $P \leq 0.05$.

## RESULTS

*Respiration and Ethylene Production*

The rate of $CO_2$ production showed a characteristic of climacteric respiratory pattern occurring during storage at 14 ± 0.5ºC (Fig.1). Immediately after the treatment, the production of $CO_2$ was found to be higher (7.75 mL $kg^{-1}$ $h^{-1}$) in the fruit dipped into the combination of *B. cepacia* B23-chitosan-$CaCl_2$, indicating a higher respiration rate than the control (6.69 mL $kg^{-1}$ $h^{-1}$) or benocide® (6.41 mL $kg^{-1}$ $h^{-1}$) treated fruits. However, the respiration rate in all the treatments decreased up to 7 d of storage following the initial storage period and then sharply increased in the control and benocide® treated fruit up to 21 d of storage. In the control and benocide® treated fruit, the production of $CO_2$ reached the maximum levels of 8.23 and 8.42 mL $kg^{-1}$ $h^{-1}$, respectively on day 21, which were identical to each other. On the other hand, the combination of *B. cepacia* B23-chitosan-$CaCl_2$ suppressed the respiratory production and delayed the onset of the respiratory climacteric. This was markedly lower to the control or benocide® treated fruit. Thus, the

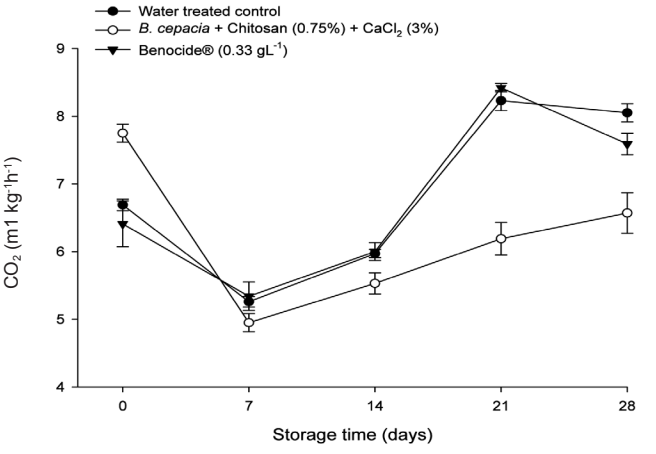


Fig.1: The effect of different treatments on the respiration rate of papayas during storage at 14ºC and 95% RH for 28 d. Each value is the mean of four replications. Means are separated by Tukey's Studentized Range Test at $P \leq 0.05$. Vertical bar represents standard error

combined treatment delayed the respiratory climacteric pattern by almost seven days, as compared to the control or benocide® treated fruit.

As with respiration, ethylene production followed the same climacteric pattern during the storage of fruits for all the treatments (Fig.2). However, the peak was suppressed in the fruit receiving the combination of *B. cepacia*-chitosan-$CaCl_2$. Meanwhile, the fruit under the combined treatment did not produce ethylene up to 14 d of storage. The onset of ethylene production was evidenced after this period, with a substantial increase until the end of storage period with significantly ($P \leq 0.05$) lower rate (0.19 µL $kg^{-1}$ $h^{-1}$) than the control fruit. Water treated fruit, on the other hand, showed a higher rate of ethylene production after 7 d of storage, and it peaked at 0.59 µL $kg^{-1}$ $h^{-1}$ after 21 d of storage. There were no significant differences in the ethylene production rate throughout the storage period between the control and benocide® treated fruit.

### *Weight Loss, Surface Colour and Flesh Firmness*

Under all the treatments, the papaya fruit showed a progressive loss of weight during four weeks of storage at 14 ± 0.5 ºC and 90-95% RH (Fig.3). However, significantly ($P \leq 0.05$) lower weight loss was consistently recorded with the combination of *B. cepacia* B23-chitosan-$CaCl_2$ dipped fruit as compared to the control or benocide®-dipped fruit. The values ranged between 1.26 to 4.05% for the combined treatment after 7 to 28 d of storage. The control and benocide® treated fruit, on the other hand, exhibited the maximum weight loss at each storage interval with the values 5.46% and 4.81%, respectively after end of storage. No significant differences were observed in the weight loss between the control and benocide®-dipped fruit up to 21 d of storage;

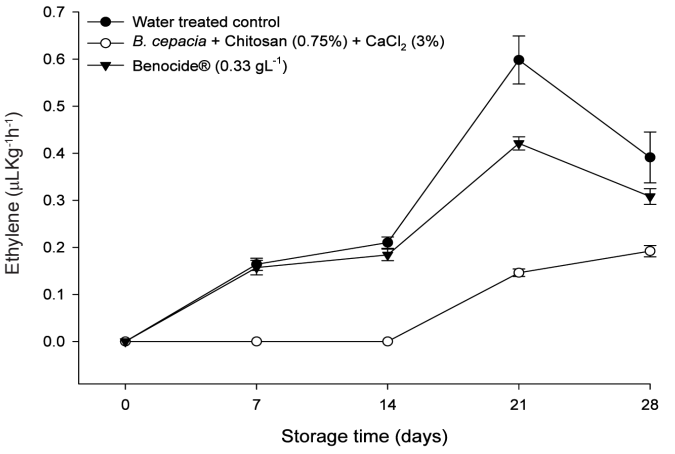


Fig.2: Effects of different treatments on the ethylene production from papayas during storage at 14ºC and 95% RH for 28 d. Each value is the mean of four replications. Means are separated by Tukey's Studentized Range Test at $P \leq 0.05$. Vertical bar represents standard error

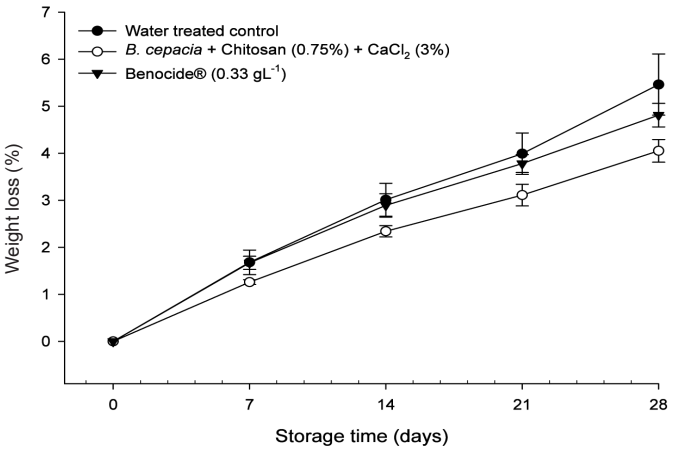


Fig.3: Effect of different treatments on the weight loss of papaya fruits during storage at 14ºC and 95% RH for 28 d. Each value is the mean of four replications. Means are separated by Tukey's Studentized Range Test at $P \leq 0.05$. Vertical bar represents standard error

however, the control fruit showed a higher weight loss than those of other treatments by the end of the storage period.

Scanning Electron Microscopic (SEM) observations showed that *B. cepacia* B23-chitosan-$CaCl_2$ treatment created a film over the fruit surface (Fig.4). The cuticles of the fruit surfaces treated with a combined application were found well arranged with no visible cracks observed (Figure 4A) in relation to the control, whereas, many deep cracks were visible on the epidermal cells of the fruit skin, and cleavages were also apparent (Fig.4B). These cracking on the waxy cuticle and epidermal cells might facilitate water loss from the surface.

The colour changes on the surface of the papaya fruit were monitored by measuring lightness (L*), chroma (C*) and hue angle (h°) during the storage period (Fig.5A-C). The intensity of the green colour of the fruit skin gradually decreased with advancing storage period and this turned to orange-yellow as evidenced by the increasing values of L* and C* of ~48 and ~34, respectively. The fruit under combined treatment consistently exhibited a slower change in the skin colour, as indicated by a more gradual increase in the L* and C* values, ranging from 48.9 to 56.0 and 35.0 to 47.5 respectively after 7 to 28 d of storage. The control fruit, on the other hand, demonstrated the maximum colour changes at each storage interval, as shown by the rapid increases in the L* and C* values, ranging from 54.9 to 63.6 and 46.5 to 59.3, respectively. There were no significant differences between the changes in the L* and C* values in the control and benocide®-treated fruit throughout the evolved storage period.

The initial value of hue angle for all the treated fruit was ~123. Generally, all the fruit showed a significant decrease in their

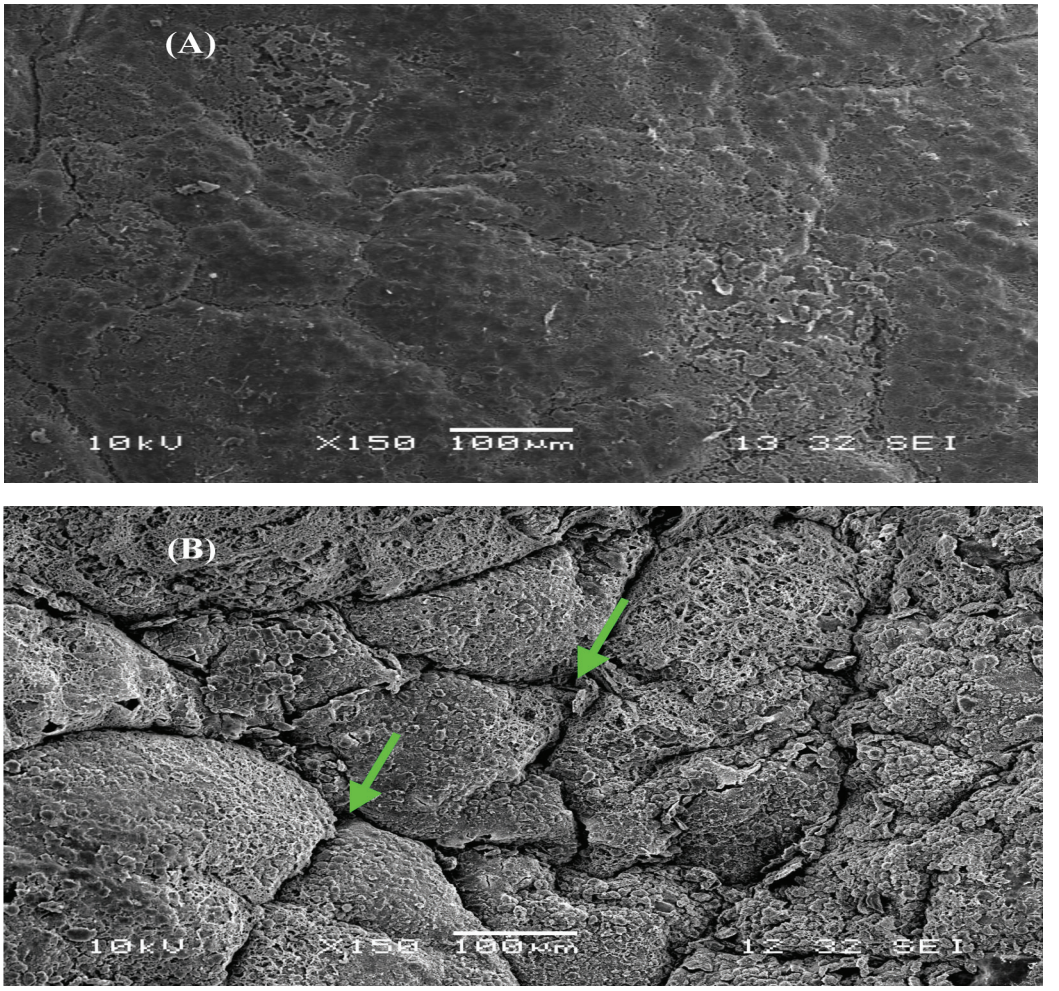


Fig.4: The Scanning Electronic Microscopic (SEM) observations of the fruit pericarp of papaya from *B. cepacia* B23-chitosan-$CaCl_2$ dipped fruit (A) and water dipped fruit (B). Arrow shows deep cracks on the fruit surface

TABLE 1
Calcium content of papaya fruits dipped in benocide® solution or in suspension of *Burkholderia cepacia* B23 incorporated with chitosan and calcium chloride

| Treatments | Calcium content (mg $kg^{-1}$) | |
|---|---|---|
| | Peel calcium | Flesh calcium |
| Water treated control | 2614.3 ± 31.1 b* | 1334 ± 60.35 b* |
| Benocide® (0.33 g $L^{-1}$) | 2575.0 ± 65.5 b | 1312 ± 87.5 b |
| *B. cepacia* + chitosan (0.75%) + $CaCl_2$ (3%) | 6087.5 ± 68 a (132.8%)[1] | 2415 ± 76 a (81%)[1] |

*Values in each column, followed by the same letter, are not significantly different at $P<0.05$, based on Tukey's Studentized Range Test (HSD).
[1]Values in the parenthesis are the percentage increase in the calcium content over control.

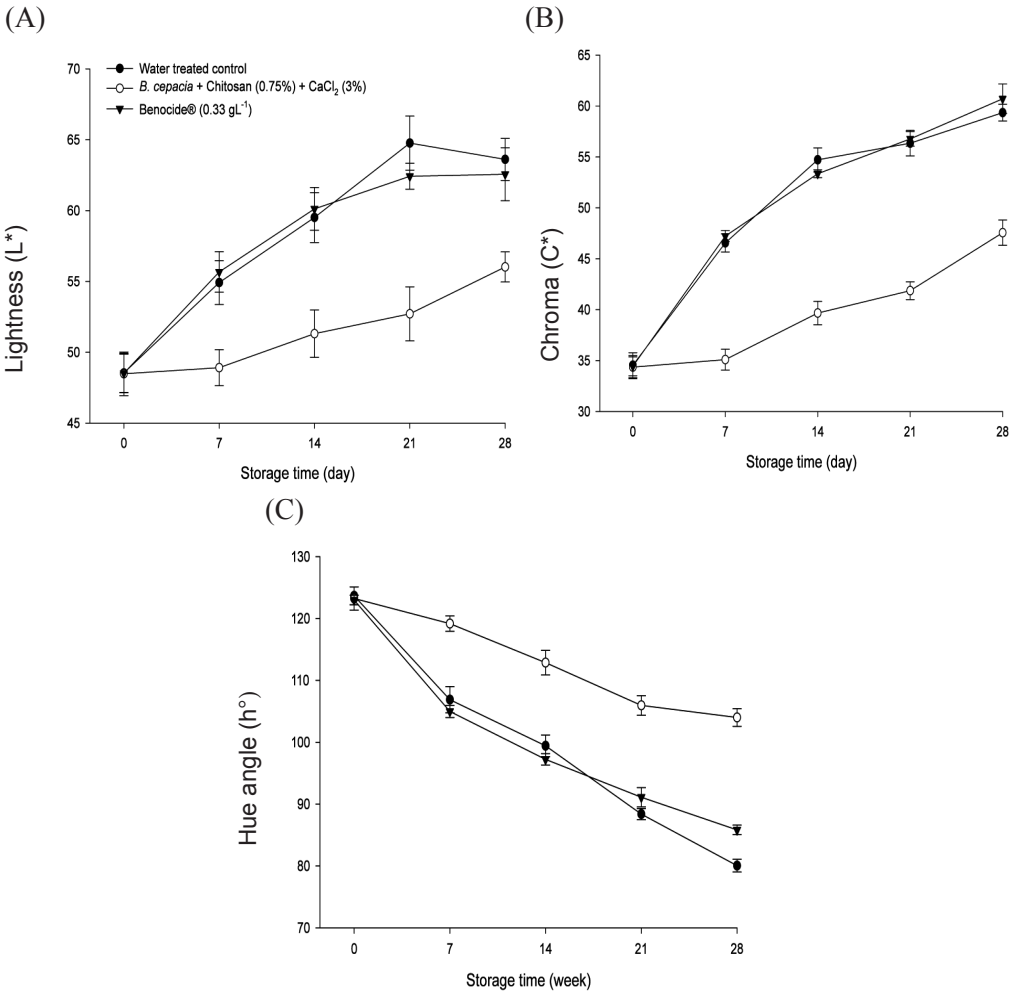


Fig.5: Effects of different treatments on skin colour, lightness (A); chroma (B); hue angle (C) of the papayas during storage at 14ºC and 95% RH for 28 d. Each value is the mean of four replications. Means are separated by Tukey's Studentized Range Test at $P \leq 0.05$. Vertical bar represents standard error

hue angle up to 28 d of storage. At the end of each week of storage, the papaya fruit under the combined treatment exhibited significantly ($P \leq 0.05$) higher h° values, ranging from 119.1 to 104 after 7 and 28 d of storage respectively compared to the control fruit. This indicated a lower rate of colour changes of the skin. In the control fruit, on the contrary, hue angle sharply decreased with storage advanced for which the values were 106.8 to 80 after 7 to 28 d of storage, respectively. A similar trend was also shown by the benocide® treated fruit.

Initially, the firmness of papaya flesh was the maximum (18.7-19.0 N) in all the treatments (fig. 6). The firmness gradually declined for all the fruit, with extended storage period. The rate of the decrease

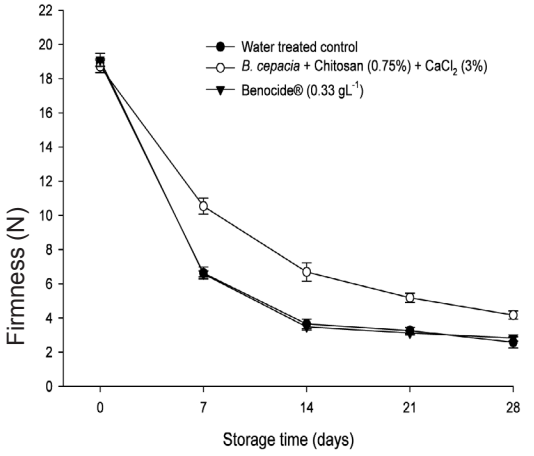


Fig.6: Effects of different treatments on the flesh firmness of papayas during storage at 14ºC and 95% RH for 28 d. Each value is the mean of four replications. Means are separated by Tukey's Studentized Range Test at $P \leq 0.05$. Vertical bar represents standard error

was significantly ($P \leq 0.05$) lower in the fruit subjected to the combined treatment *B. cepacia* B23-chitosan-$CaCl_2$ than those of the control and benocide® treatments. The flesh firmness under the combined treatment was consistently higher than the control or the benocide®-treated fruit during the entire storage period remaining, with the firmness of 4.17 N after 28 d of storage. On the contrary, the control and benocide®-treated fruit manifested sharp decreases in their firmness up to 14 d of storage and thereafter exhibited more or less constant firmness until the end of the storage period. Based on the data on firmness, there was a gain of at least 15 d of extra storage life with the application of the combined treatment. Both the control and benocide®-treated fruit did not show significant differences in term of their firmness throughout the storage period when compared with each other.

*Total Soluble Solids, pH, Titratable Acidity and Ascorbic Acid*

Changes in the total soluble solids (TSS) content of the papaya fruit during storage are presented in Figure 7. The initial TSS of all the fruit samples was fairly low (~8.2), and this generally increased with ripening. In the control and benocide® treated fruit, the TSS contents reached the maximum level with the values of 12.1 and 11.9, respectively, after 21 d of storage, and these were significantly ($P \leq 0.05$) higher than that of the fruits treated with the combination of *B. cepacia* B23-chitosan-$CaCl_2$. After this period, noticeable decrease in the TSS was recorded in the control and benocide® treated fruit. In contrast, the fruit under the combined treatment showed a gradual improvement in TSS content registering the maximum value of 10.88 at the end of storage period. This showed that the fruit

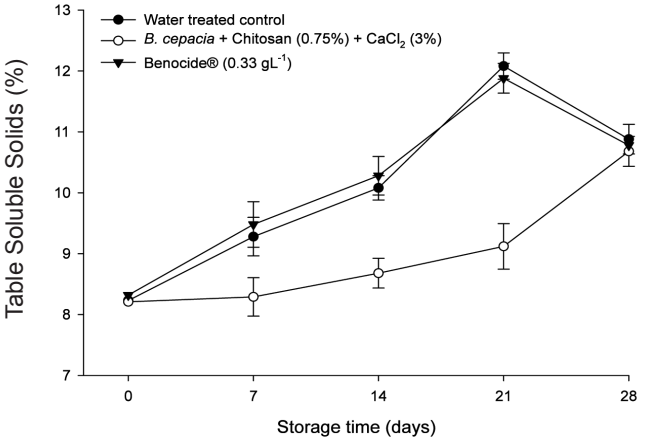


Fig.7: Effects of different treatments on total soluble solids of papayas during storage at 14ºC and 95% RH for 28 d. Each value is the mean of four replications. Means are separated by Tukey's Studentized Range Test at $P \leq 0.05$. Vertical bar represents standard error

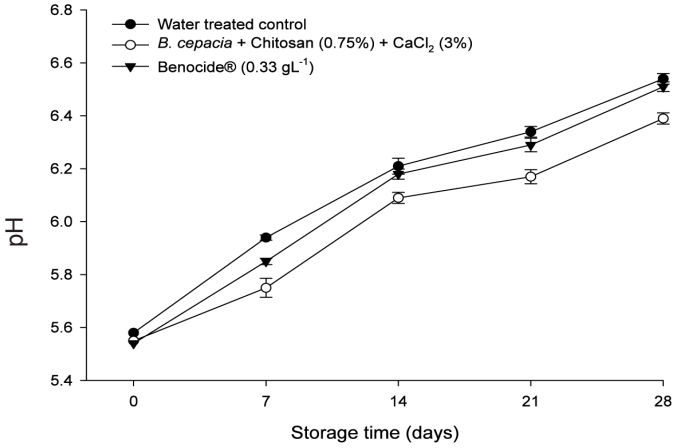


Fig.8: Effects of different treatments on the pH of papayas during storage at 14ºC and 95% RH for 28 d. Each value is the mean of four replications. Means are separated by Tukey's Studentized Range Test at $P \leq 0.05$. Vertical bar represents standard error

had not reached the full ripening stage for them to be immediately marketable.

The changes in the pH value of papaya, as a function of different treatments and storage time, are shown in Fig.8. The pH value of fruit gradually increased as storage progressed with significant differences ($P \leq 0.05$) between the treatments. At the end of a storage period of 28 d, the pH value was significantly lower (6.3) in the fruit that were subjected to the combination of *B. cepacia* B23-chitosan-$CaCl_2$ to the control fruit (6.5). Nonetheless, no significant variation was observed in the pH values of the control

and the benocide®-treated fruit throughout the storage period.

It is evident that the combination of *B. cepacia* B23-chitosan-$CaCl_2$ induced a significant variation in the ascorbic acid content of the fruits during storage (Fig.9). Initially, the ascorbic acid content was ~52 mg 100 $g^{-1}$ for all the treatments. With the control fruit, however, the content sharply increased over time and reached the maximum value of 72.5 mg 100 $g^{-1}$ on day 14 but it declined until the end of storage thereafter. A similar trend was observed for the benocide®-treated fruit. On the contrarily, the fruit subjected to the combined treatment showed a more gradual decline with time and exhibited the maximum value of 64.7 mg 100 $g^{-1}$ after 21 d of storage, but slightly declined thereafter.

*Fruit Calcium Content*

As expected, fruit treated with the combination of *B. cepacia* B23-chitosan-$CaCl_2$ resulted in significantly ($P \leq 0.05$) higher calcium contents of 6087.5 and 2415 mg $kg^{-1}$ in the peel and flesh tissues, respectively, as compared to that those found in the control or Benocide®-treated fruit (Table 1). The addition of 3% $CaCl_2$ into the chitosan solution increased the content of $Ca^{+2}$ by 132.8 and 81% in the peel and flesh tissues, respectively as compared to the control.

## DISCUSSION

Generally, climacteric fruit exhibits a rapid rise in respiration rate at the onset of ripening, which subsequently slows down as the fruit ripens (Sirivatanapa, 2006). Thus, the storage life of climacteric fruit is usually shorter than that of non-climacteric fruit. Likewise, in this study, the papaya fruit exhibited a respiratory climacteric, which appeared simultaneously with an increase in the ethylene synthesis. The application of *B. cepacia* B23, in combination with

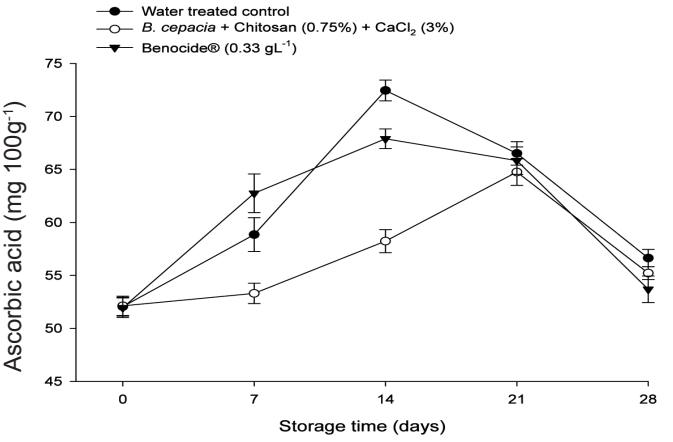


Fig.9: Effects of different treatments on the ascorbic acid content of papayas during storage at 14ºC and 95% RH for 28 d. Each value is the mean of four replications. Means are separated by Tukey's Studentized Range Test at P≤0.05. Vertical bar represents standard error

calcium incorporated-chitosan coating on the papaya fruit as a post-harvest treatment, showed beneficial effects on the respiration rate, ethylene production, weight loss and loss of firmness. Moreover, in the previous study, this combined treatment exhibited a good control of anthracnose on artificially inoculated and naturally infected papaya fruit (Rahman *et al.*, 2009).

The chitosan-based coating can form a protective barrier on the surface of fresh fruit, reduce water loss, inhibit gas exchange, decrease nutrient loss, and prevent micro-organism growth that causes fruit rotting (Qiuping & Wenshui, 2007). In this study, the combined treatment was found to significantly reduce the respiration rate, ethylene production and weight loss. The effectiveness of this particular treatment might be due to the biological activity of *B. cepacia* B23 and the filmogenic properties of chitosan-$CaCl_2$. In this case, chitosan acted as a carrier of *B. cepacia* B23 and $CaCl_2$, together with its coating capability, which modified the atmospheric compositions inside the fruits. Since an inhibition of $CO_2$ evolution was the consequence of bioactive coating, ethylene production of the fruits would also be reduced (Bautista-Banos *et al.*, 2006). Such inhibitory effects on both the respiration and ethylene productions were reported in tomatoes and peaches coated with chitosan (El Ghaouth *et al.*, 1992; Li & Yu, 2000). Immediately after the treatment, the papayas that were subjected to the combination of *B. cepacia* B23-chitosan-$CaCl_2$ exhibited an increased respiration rate, probably because of an induced stress of the acetic acid solution (Devlieghere *et al.*, 2004). In an earlier study, El Ghaouth *et al.* (1991) observed an immediate stimulation of the respiration in the chitosan coated strawberry, and it disappeared gradually.

The role of *B. cepacia* B23 in reducing respiration rate and ethylene production was not very clear; however, this bacterium might directly assist in the removal of ethylene from the fruit surroundings by using it as a biochemical substrate (Reid, 1992). Moreover, in our previous study, it was found that *B. cepacia* significantly reduced the anthracnose disease in papaya fruit (Rahman *et al.*, 2009), and this might be associated with reduced respiration and ethylene production rate through by controlling the infection. This contention is in agreement with some previous researchers, who have reported that the degree of microbial spoilage of fresh-cut honeydew and cantaloupes is correlated to the increase in the respiration rate (Luna-Guzman & Barrett, 2000; Saftner *et al.*, 2003). Thus, any reduction of disease infection will eventually lead to lower rates of respiration and ethylene synthesis.

Surface coating with chitosan-based matrix was reported to reduce weight loss of various fruit types, such as strawberries and raspberries (Hernandez-Munoz *et al.*, 2006), longan (Jiang & Li, 2001) and papayas (Sivakumar *et al.*, 2005). In this study, the combined treatment of *B. cepacia* B23-chitosan-$CaCl_2$ significantly reduced the weight loss of fresh papaya fruit during storage at 14ºC for 28 d. Due to its ability

to form a semi-permeable coating around the fruit, chitosan reduces the weight loss by controlling the migration of water vapour through the surface of fruit. The anti-fungal and moisture barrier functions of chitosan-based coating were not altered by the incorporation of 3% $CaCl_2$ into the treatment (Han *et al.*, 2004). Meanwhile, the beneficial effect of $CaCl_2$ in reducing post-harvest weight loss has been reported in Asian pear (Mahajan & Dhatt, 2004). The authors explained that the reduction in weight loss was attributed to the influence of calcium in maintaining the firmness of the fruit and tissue rigidity, thereby checking moisture loss from fruit.

Loss of firmness is one of the major factors limiting the post-harvest quality and storage life of fruit and vegetables. In the present study, better firmness was attributed to the papaya fruit subjected to the combination of *B. cepacia* B23-chitosan-$CaCl_2$. In this combined treatment, chitosan coating reduced weight loss, while slowing down the migration of water vapour from the fruit surface and thus, controlling the integrity and texture of cells, resulting in the maintenance of firmness (Hernandez-Munoz *et al.*, 2006), which was further enhanced by the incorporation of calcium. The firming effect with the incorporation of 3% $CaCl_2$ was expected as calcium plays an important role in stabilizing cell membrane through the formation of calcium pectates, which might increase the rigidity of cell wall and the middle lamella of the fruit (Picchioni *et al.*, 1996) and therefore, maintaining cell turgor potentials (Mignani *et al.*, 1995). Hence, the application of *B. cepacia* B23 with chitosan-$CaCl_2$ probably has a synergistic or additive effect in maintaining the firmness of papaya. This result is in agreement with that of Han *et al.* (2004) who found the highest firmness in strawberries and raspberries treated with chitosan containing higher concentration of calcium.

Generally, the external colour of fruit is retained when coated with chitosan solution (Bautista-Banos *et al.*, 2006). In this study, the extent of skin colour development of the papaya fruit was significantly slowed down when treated with the combination of *B. cepacia* B23-chitosan-$CaCl_2$ compared to the control. Meanwhile, the application of the combined treatment formed a semi-permeable film, which caused modification of gaseous compositions around the interior of fruit surface and consequently reduced respiration rate and ethylene production and action (Kader *et al.*, 1989). These conditions delayed ripening and senescence process, resulting in retention of green colour and firmness of fruit. The results of this study support the findings by Sivakumar *et al.* (2005) who found that chitosan coating amended with ammonium chloride retarded colour development of skin and the flesh of papaya during storage. Since attack by pathogens is a major factor causing discoloration of harvested fruit (Jiang *et al.*, 2005), the delay in the skin colour development by the combination of *B. cepacia* B23-chitosan-$CaCl_2$ could be partially beneficial due to the control of decay in this study. This result is in concordant with the work of Jiang and Li

(2001) who noted that inhibiting decay by chitosan coating resulted in the delay in skin colour changes of longan fruit.

TSS, ascorbic acid, titratable acidity and pH are important quality parameters of papaya. The results of the current study showed that the treatment with the combination of *B. cepacia* B23-chitosan-$CaCl_2$ exhibited a beneficial effect on the changes in the quality of papayas during storage. This combined treatment slowed down the accumulation of TSS and ascorbic acid, and reduced the change in pH of fruit during storage. This could be due to the reduction of oxygen supply on the fruit surface which resulted in a lower respiration rate and the growth inhibition of spoilage organisms (Yonemoto *et al.*, 2002). The results of this study are in agreement with the findings of the previous works on various fruit coated with chitosan-based coatings, such as Indian jujube (Qiuping & Wenshui, 2007) and mangoes (Srinivasa *et al.*, 2002).

The addition of 3% $CaCl_2$ into the combined treatment enriched $Ca^{+2}$ content in the papayas, where flesh $Ca^{+2}$ content was increased by 81% as compared to the control fruits, and thus, resulting in an increased nutritional value of the fruit. The results of the current work further strengthened the findings by Han *et al.* (2004) who reported that chitosan-based coatings containing calcium or vitamin E significantly increased the content of these nutrients in both fresh and frozen strawberries and raspberries during storage.

## CONCLUSION

The combination of *B. cepacia* B23-chitosan-$CaCl_2$ extended the storage life of papaya by inhibiting its respiration rate and ethylene production. It reduced weight loss and delayed changes in colour and pH during storage without impairing the quality of the fruit. In addition, chitosan-based coating demonstrated its potentiality to carry microbial antagonist and high concentration of $CaCl_2$, which thus significantly increased the content of calcium in papayas. It is therefore obvious that the combination of *B. cepacia* B23-chitosan-$CaCl_2$ has the potential to improve storability and enhance the nutritional value of fresh papayas and can be commercially used as a post-harvest treatment.

## ACKNOWLEDGEMENTS

The authors are grateful to the Ministry of Science, Technology and Innovation, Malaysia, for the research funding through e-Science Fund (Grant No. 05-01-04-SF0104).

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