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Effects of Chitosan-Plant Extract Coatings on the Postharvest Quality of Mango Fruits (*Mangifera indica*) with Anthracnose Disease

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Abstract

Anthracnose is the most prevalent and devastating fungal disease both before and after harvesting of mango worldwide. The anthracnose pathogen infects fruit in the field and remains quiescent until the fruit is harvested and starts to ripen. This is the major constraint in the commercialization of fresh mango. Currently, this is the first study that has evaluated the impact of a chitosan-based coating with *Aloe vera* gel and papaya leaf extracts on the postharvest quality of mango fruit infected with anthracnose under ambient storage conditions. After coating and drying, mangoes were artificially inoculated with *Colletotrichum gloeosporioides* (10^5 conidia per mL) and then air-dry, placed in perforated open-top plastic baskets, and stored at room conditions (average temperature 34.3 ± 2°C and relative humidity 71.8 ± 10%). The respiration rate, weight loss, color, firmness, vitamin C content, and spoilage rate were measured during storage. The results showed that the coating formula made with 0.5% chitosan combined with 0.3 % *Aloe vera* gel and 0.2% papaya leaf extracts was the most effective formula for maintaining postharvest quality and minimizing the spoilage of mango fruit infected with anthracnose under ambient conditions. Further in-depth studies are needed to determine both the mode of action in the successful commercialization of this new edible coating in the fresh mango fruit industry.

Keywords

Aloe vera gel, anthracnose, chitosan, mango, papaya leaf

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Introduction

Mango (*Mangifera indica*) is produced in more than 100 countries and is the fifth most consumed fruit in the world (Javier, 2014). Most mangoes are consumed fresh, but trading has been limited because of their short shelf-life and lack of postharvest management. Due to its climacteric nature, mango fruit ripening is

very fast, and hence, is susceptible to anthracnose pathogens, mainly *C. gloeosporioides*, that initiate infection in the field and remain dormant until the beginning of ripening. The decay due to *C. gloeosporioides* can reach almost 100 percent in fruits produced under humid conditions (Arauz, 2000). For years, synthetic fungicides have been used as the main approach to control *C. gloeosporioides* to extend the life of mango fruit after harvesting. However, the need for food without chemicals has initiated the demand for safe alternatives.

Edible coatings with antifungal activity against *C. gloeosporioides* have been considered one of the most promising means to improve safety and increase the shelf life of mango fruits. Studies have indicated that anthracnose disease caused by *C. gloeosporioides* in mango was reduced by a postharvest treatment with papaya leaf extract (Bautista-Banos *et al.*, 2002) or coating the fruit with chitosan (Le Nguyen Doan Duy *et al.*, 2014). In addition, *A.vera* gel has also been reported as a coating to extend the shelf life and maintain the quality of mango fruit (Ochiki *et al.*, 2015). Sai *et al.* (2011) reported a combined antifungal effect when papaya leaf extract was incorporated in an *A.vera* gel coating on papaya fruit. Similarly, Vieira *et al.* (2016) indicated that the antimicrobial activity of a chitosan coating was greatly enhanced by adding *A. vera* extract. However, a report on the postharvest treatment of chitosan combined with the extracts of both *A.vera* gel and papaya leaf to control anthracnose in mango was unavailable. Therefore, the objective of this work was to evaluate the efficacy of a chitosan-based coating with *A.vera* gel and papaya leaf extract in maintaining the quality of mango infected with anthracnose under ambient storage conditions.

Materials and Methods

Materials

Chitosan (deacetylated ≥ 95%) was obtained from the Vietnam Chitosan Limited company, Rach Gia, Kien Giang, Vietnam. Lactic acid 90%, glycerol 99%, and Tween 80 (analytical grade) were obtained from the Xilong Chemical Industry Incorporated Co. Ltd., China.

Fresh *A.vera* leaves were purchased from Big C supermarket located in Long Bien district, Hanoi, Vietnam, with homogenous leaves according to size and maturity. Fresh papaya leaves with uniform size and maturity were collected from Hai Duong province, Vietnam.

Fresh mango var. Keo cultivated in Gia Lam, Hanoi, were harvested at the mature green stage when they were just turning olive green, and the fruits were hard without visible blemishes and transported to the lab within 2h after harvesting.

Methods

Preparation of coating solution

The method to prepare the coating solutions was developed from methods described by Satish *et al.* (1999) and Vieira *et al.* (2016).

Fresh leaves of *A.vera* and papaya free from the disease were washed under running tap water 2-3 times, then washed with chlorinated water (150 mL L^{-1}), and finally air-dried. The colorless hydroparenchyma of the *A.vera* leaves and papaya leaves were mixed separately with distilled water (ratio 1:3 kg L^{-1}), ground in a blender (HR2115, Philip) for 10 minutes, and then the mixtures were filtered through double-layered muslin cloth and Whatman no. 1 filter paper. The extracts were preserved separately in brown bottles at 5°C until used.

Chitosan (0.5 % w/v) was dissolved in a 1% (v/v) lactic acid solution under agitation at room temperature (35°C) to obtain a homogeneous solution. Glycerol was added at the concentration of 0.5% mL plasticizer/g of chitosan. Tween 80 was added at 0.1% (w/v). The *A.vera* gel and papaya extracts of leaves as antimicrobial agents were added under agitation at room temperature to reach complete dissolution (**Table 1**).

Application of chitosan-plant extracts coating

Mangoes selected based on size, color, and absence of physical injuries or disease infection were washed under running tap water, then surface-disinfected with 150ppm of chlorine, and air-dried. Fruits were dipped into one of the coating solutions for 3min and thereafter dried with ventilation to ensure coating dryness. Uncoated fruits were the control (code DC)

Table 1. Formulas of the chitosan-plant extracts coating solutions

Code	Chitosan (% w/v)	*A.vera* extract (% v/v)	Papaya leaf extract (% v/v)
CT1	0.5	0.3	0.2
CT2	0.5	0.4	0.1
CT3	0.5	0.5	0

After treatment, mangoes were sprayed with a spore suspension of *C. gloeosporioides* ($1x10^5$ spore mL^{-1}). The fruits were weighed and then stored for 12 days in perforated open-top plastic baskets under room conditions (average temperature 34.3 ± 2°C and relative humidity 71.8 ± 10%). There were 30 fruits in each treatment. The parameters analyzed included respiration rate, weight loss, color, firmness, vitamin C content, and spoilage rate.

Respiration rate

Respiration rate was determined by the amount of CO_2 generated from the mangoes per unit of weight and time (mL $CO_2kg^{-1}h^{-1}$) and measured by placing the fruits in a hermetically sealed container. After 1h, the concentration of CO_2 in the container was measured by an ICA250 dual gas analyzer (International Control Analyzer Ltd.).

Weight loss

Weight loss was determined by weighing the fruits before and during storage by an Ohaus PA214 (accuracy of 0.0001g) analytical balance. Weight loss was calculated by the equation (A-B)*100/A, where, A is the initial weight of the fruit (day 0) and B is the weight of fruit after the storage period.

Color change

The color change of the mangoes was measured by a portable colorimeter CR400, Konica Minolta, Japan. The color parameters included L* (lightness, value from 0 to 100), a* (color change from green to red, value from -60 to 60), and b* (color change from blue to yellow, value from -60 to 60).

The color change of the fruit was calculated by the equation: $\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$, $\Delta L = L^*_s - L^*$, $\Delta a = a^*_s - a^*$, $\Delta b = b^*_s - b^*$, where, L*, a*, and b* are the initial color parameters of the fruit (day 0) and L^*_s, a^*_s, and b^*_s are color parameters at the time of analysis.

Firmness

Firmness was measured by a portable penetrometer FT321, USA indicating the force (kg) required to puncture a whole 5mm depth into the fruit flesh randomly (usually in 3 parts of the fruit) with 5-mm and 11-mm stainless steel plunger tips. Measured values were expressed in kg/cm^2.

Vitamin C content

Vitamin C content was analyzed by the titration method with I_2 0.01 N. The results were expressed as mg% of vitamin C.

Fruit spoilage rate

The fruits were visually observed for fungal spoilage and fruit rots. The number of spoiled fruits was recorded and the disease percentage was calculated as follows: % Disease = A/B x 100, where, A is the number of spoiled fruits and B is the total number of fruits in the sample.

Statistical analysis

The data obtained from the experiments were expressed as mean ± standard errors (SE) of five measurements (n = 5). Significant differences ($P< 0.05$) among means were subjected to one-way analysis of variance (ANOVA) with Tukey's test.

Results and Discussion

Respiration rate

After harvest, fruits still respire to maintain their life. During respiration, stored substrates are used up resulting in a decrease in the quality and shelf life of fruits. The changes in the respiratory behavior of edible coated and non-coated mango fruit during 12 days of storage at

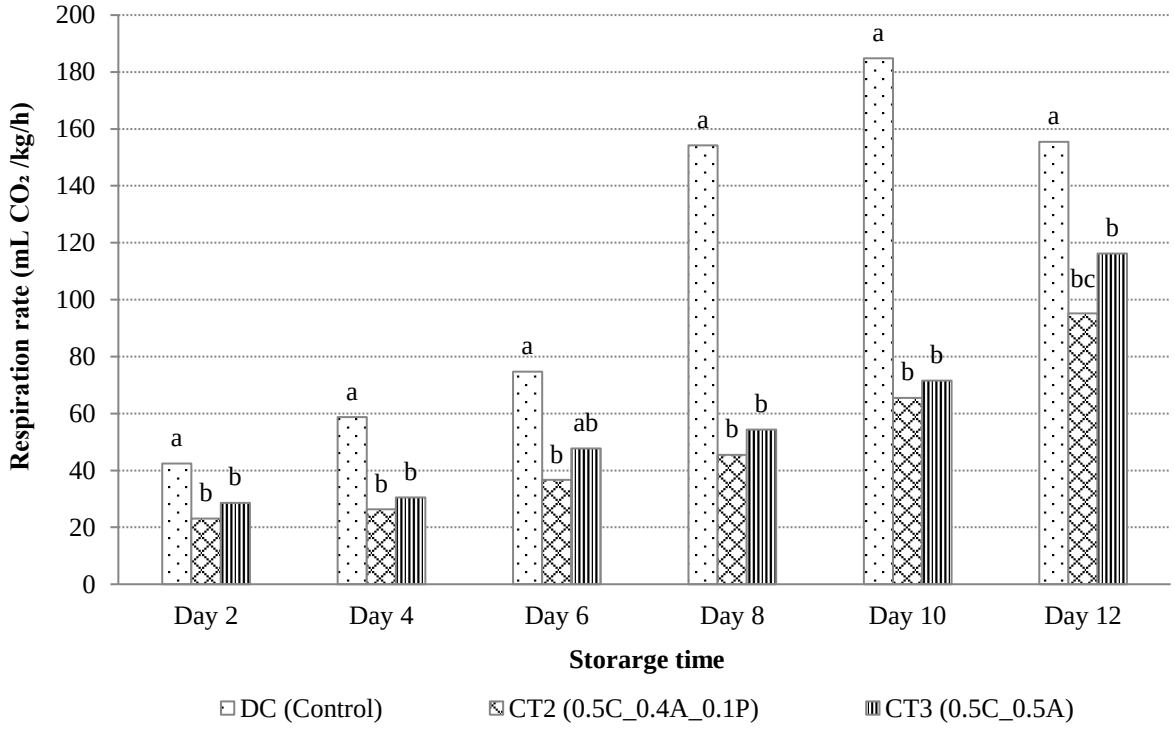
34.3 ± 2°C and relative humidity 71.8 ± 10% were determined (**Figure 1**).

The respiration rate of the control fruits gradually increased in the first 6 days, showed a sharp increase, peaked on day 10, and then decreased at the end of storage. The changes in the respiration activity of mango fruit during storage can be explained by the fact that mango is a climacteric fruit with a postharvest ripening process; the mangoes were at the mature green stage when harvested and the respiration rate increased for the ripening stage then decreased when transitioning into the senescence stage. The rates of CO_2 production in the coated mango fruits had an upward trend until the last day of the storage period and were significantly lower than the control at all time points ($P< 0.05$). These results indicated that the coating reduced the respiration rate of the mangoes and thus, delayed the ripening process.

In this study, mangoes coated with formula CT1 (0.5% chitosan – 0.3% *A.vera* – 0.2% papaya) had the lowest respiration rate and were significantly different from those coated with CT3 (0.5% chitosan – 0.5% *A.vera*) and the control fruits ($P< 0.05$). This could not only be due to the complex polymer created by combining chitosan and *A. vera*, which completely covered the stomata on the mango peel that prevented oxygen from contacting the fruit while carbonic accumulated inside the coating (Chauhan *et al.*, 2013; Jongsri *et al.*, 2016), but also due to a synergistic antifungal effect when papaya leaf extract was incorporated in the chitosan-*A.vera* coating, which might be in charge of decreasing the respiration rate (Sai *et al.*, 2011). This suggests that the composite coating containing chitosan, *A.vera*, and papaya leaf extract reduced the respiration rate of mango fruit better than the combination of only chitosan and *A.vera* extract.

Weight loss

The effect of the coating on the weight loss of mango fruit stored at 34.3 ± 2°C and relative humidity 71.8 ± 10% was observed. Weight loss of the fruits increased during storage, but the increase rate in control was much higher than the



Note: Bars not sharing a common superscript differ significantly at P< 0.05.

Figure 1. Effects of chitosan-plant extract coatings on the respiration rate of mango fruits cv. 'Keo'

treated fruits (**Figure 2).** The reduction in weight loss for the coated fruits was potentially due to the effects of their coatings as barriers against gas and moisture movement, resulting in the reduction of respiration and water loss (Park, 1999). These results are in agreement with the findings of Vieira *et al.* (2016) who reported that the weight loss of blueberries coated with an *A.vera* gel incorporated into a chitosan coating was 1.67 times lower than in uncoated fruits.

After 12 storage days, the final weight loss value of the CT1 (0.5% chitosan – 0.3% *A.vera* – 0.2% papaya) treatments was smallest and significantly lower than the other coated fruits and uncoated fruits ($P< 0.05$). The minimal weight loss in the fruits treated with CT1 was possibly due to the increased water holding capacity when *A.vera* was mixed with chitosan (Chauhan *et al.*, 2013; Vieira *et al.*, 2016) and decreased transpiration through a lack of cuticle fractures, which are generally caused by fungal attacks because the *A.vera*-papaya leaf extract had antifungal activity (Sai *et al.*, 2011). This result was in harmony with the respiration rates of mango fruits presented above (**Figure 1**).

Color

Color is prime quality and greatly influences the purchasing decision of customers. During storage, the color of mango fruits constantly changes from green to yellow due to the fruit's metabolic activity as well as the impact of environmental factors such as temperature, relative humidity, and microbial infection, etc. Data on color change revealed significant differences between uncoated and coated fruit (**Figure 3**).

The ripening of mango fruit is characterized by color change due to the transformation of chlorophyll into other pigments and the synthesis of carotenoids and anthocyanins in fruit, and at the end of storage, the control fruit had the highest color change. This might have been due to the high contact surface of gas, moisture and pathogens, and the fact that the control fruits had the highest respiration rate, thereby hasten the ripening process, oxidation reaction, transpiration, and diseases that trigger color changes.

Increasing the papaya leaf extract concentration in the chitosan - *A.vera* coating

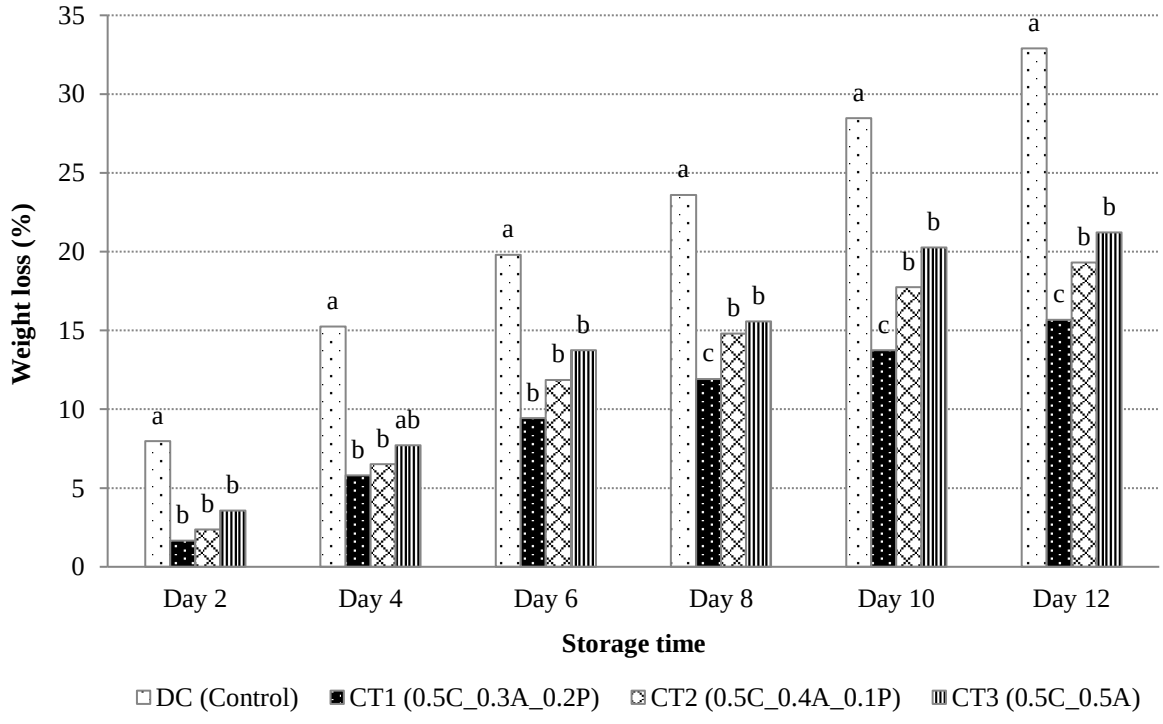


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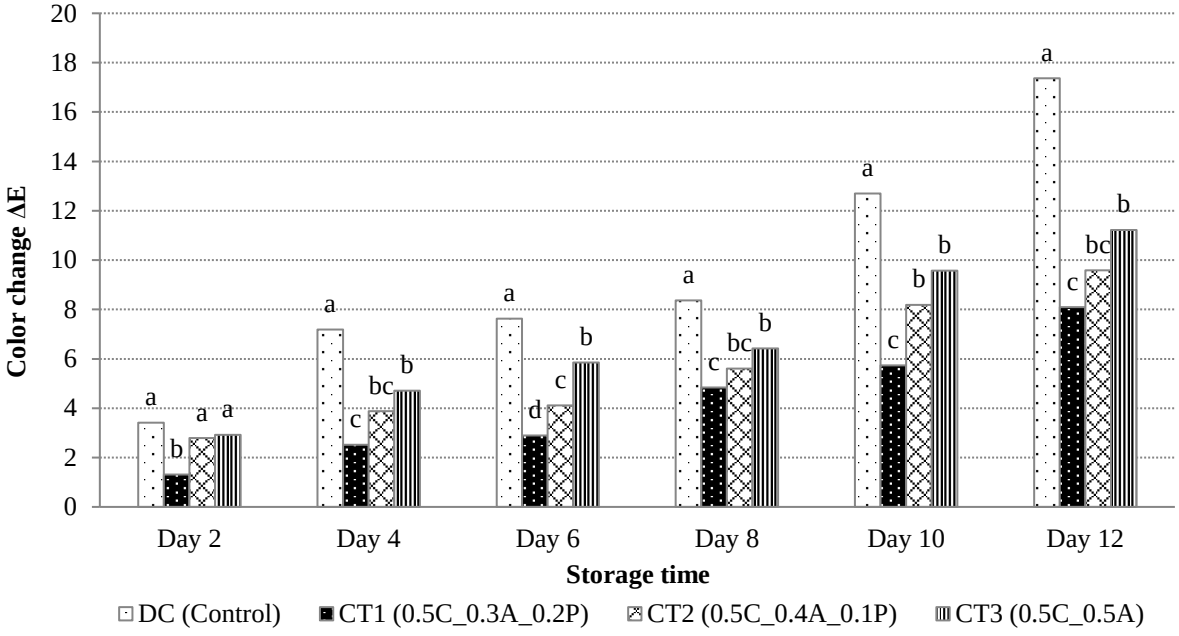
Figure 2. Percent weight loss of mango fruits cv. 'Keo' treated with chitosan-plant extract coatings .



Note: Bars not sharing a common superscript differ significantly at P< 0.05.

Figure 3. Color changes in mango fruits cv. 'Keo' as affected by chitosan-plant extract coatings

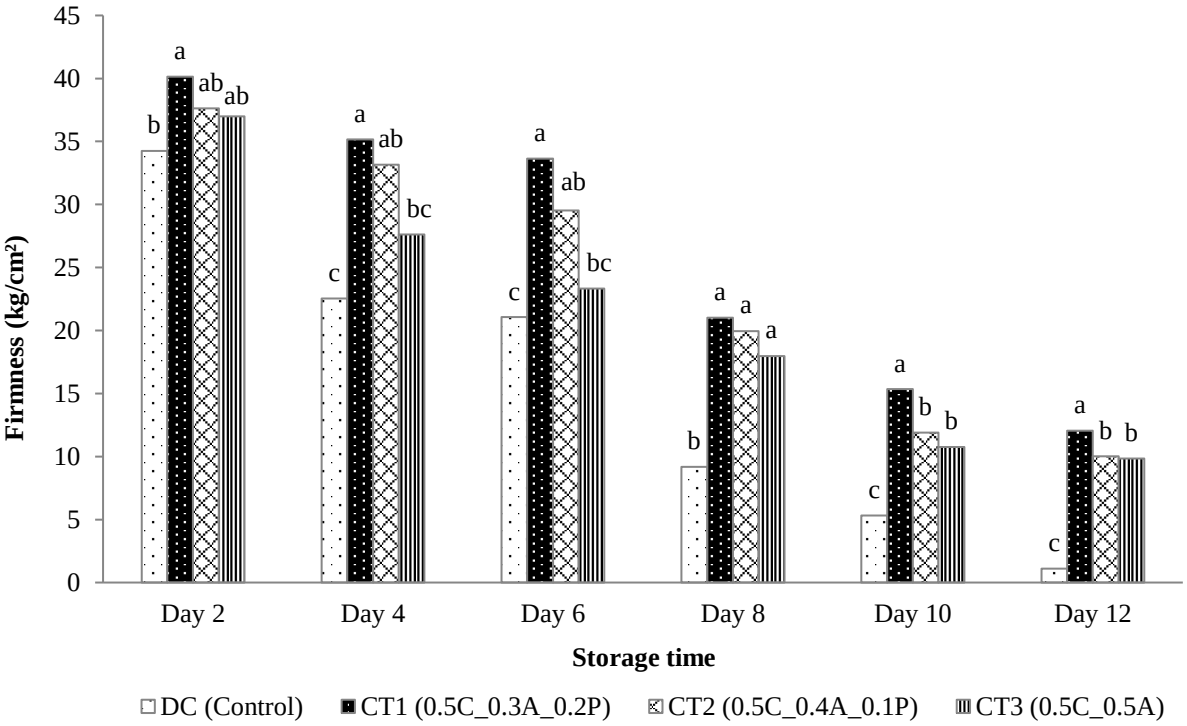
solutions increased the color retention of mango fruit and the CT1 coating formula with the highest papaya leaf extract concentration (0.2%) was the best in maintaining the color of mango fruit. This result was in accordance with the lowest respiration rate of this treatment and in agreement with Sai *et al.* (2011) that papaya leaf extract incorporated in *A.vera* gel coating controlled color development even at the end of 15^{th}-day storage at 30 ± 3°C.

Apart from the cooperative effect of chitosan and *A.vera* in slowing down the chlorophyll degradation process (Ochiki *et al.*, 2015; Jongsri *et al.*, 2016), papaya leaf extract incorporated in chitosan-*A.vera* coating acted as a barrier that reduced anthracnose infection of mango (Bautista-Banos *et al.*, 2002) while the chitosan and *A.vera* coating controlled gas exchange and limited the penetration of *C. gloeosporioides* germs, making it more effective in preserving the color during storage of mango fruit.

Firmness

Firmness is the physiological parameter thatindicated fruit quality and storage life. Mango fruits softened for both the treated and control fruits during storage (**Figure 4**). This phenomenon was due to the disruption of cell walls and loss of cellular turgidity during fruit ripening with increases in the enzyme activities of pectinmethylesterase and polygalacturonase that shorten the pectin substances' chain lengths, thus, converting insoluble protopectin to soluble pectic acid and pectin (Ali *et al.*, 2004). Besides, fruit wilting due to weight loss also led to a decrease of firmness (**Figure 2**), therefore, the final firmness value of uncoated fruits was the lowest and significantly lower than those of the coated fruits ($P< 0.05$). Prolonged firmness in the coated fruits as compared to the control can be explained by modifications in internal gas levels created by the chitosan-plant extract coating, which reduced respiration and transpiration (**Figure 1** and **Figure 2**), delayed fruit ripening, and hence, resulted in firmer fruit. The results of this study are consistent with the findings by Jongsri *et al.* (2016) and Silva *et al.* (2017) who who reported that untreated mango cv. Nam Dok Mai and mango cv. Palmer exhibited higher losses in firmness than mango coated with chitosan.

Furthermore, the treated fruits with the 0.5% chitosan – 0.3% *Aloe vera* – 0.2% papaya coating exhibited significantly firmer fruits than the other treatments, which may be related to the lower



Note: Bars not sharing a common superscript differ significantly at P< 0.05.

The initial firmness of the mangoes was 43.91 kg cm^{-2}.

Figure 4. Firmness (kg cm^{-2}) of mango fruits cv. 'Keo' treated with chitosan-plant extract coatings

respiration rate and lower weight loss as reported earlier in papaya treated with papaya leaf extract incorporated into *A. vera* gel coating (Sai *et al.*, 2011) and chitosan coated mango fruits (Silva *et al.*, 2017).

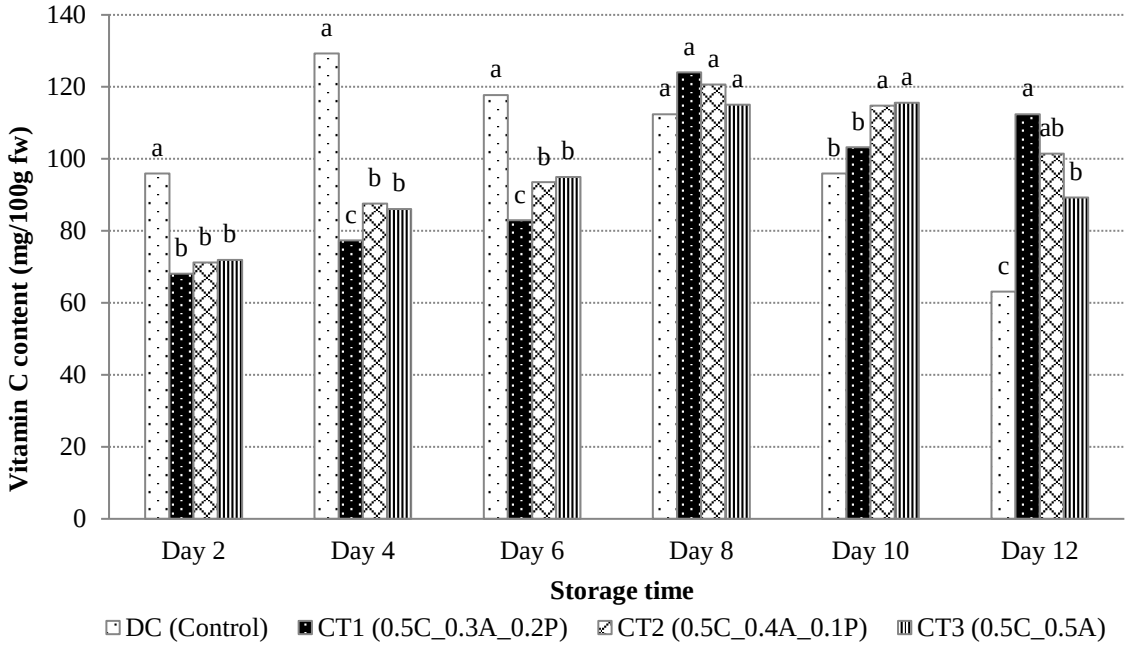
Vitamin C content

Mango is a good source of vitamin C, the most important vitamin for human nutrition in fruits and vegetables. However, it is very sensitive and easily degraded in high temperatures, low relative humidity, O_2-rich atmospheres, or high CO_2 atmospheres of more than 10% (Lee & Kader, 2000). The vitamin C content of uncoated fruits increased on the 4th day and then decreased thereafter. This result is in agreement with the reports of Aina (1990) and Hossain *et al.* (2014), who reported that vitamin C content peaked on the 4th day of storage and decreased afterwards. The vitamin C content seems to have been synthesized during early storage and then decreased by being used as substrates for respiration (Hossain *et al.*, 2014) and through oxidative devastation at high temperatures (Thomas & Oke, 1980; Vazques-Salinas & Lakshiminarayana, 1985).

It is interesting to note that coated fruits exhibited a continued increase in the vitamin C level till the 8th day of storage then gradually decreased starting the following day. The vitamin C contents of the coated mangoes were significantly higher than those of the uncoated fruits after 12 storage days (**Figure 5**). The coatings were effective in reducing vitamin C loss attributed to the low oxygen permeability of the coating, which reduced the respiration rate (**Figure 1**) and delayed the ripening process (**Figure 3** and **Figure 4**) leading to an extended vitamin C synthesis phase from days 4 to 8 in the coated fruit. The lowest respiration rate (**Figure 1**) was under the synergistic effect of the coating with 0.5% chitosan – 0.3% *Aloe vera* – 0.2% papaya, which led to the best retention of vitamin C content.

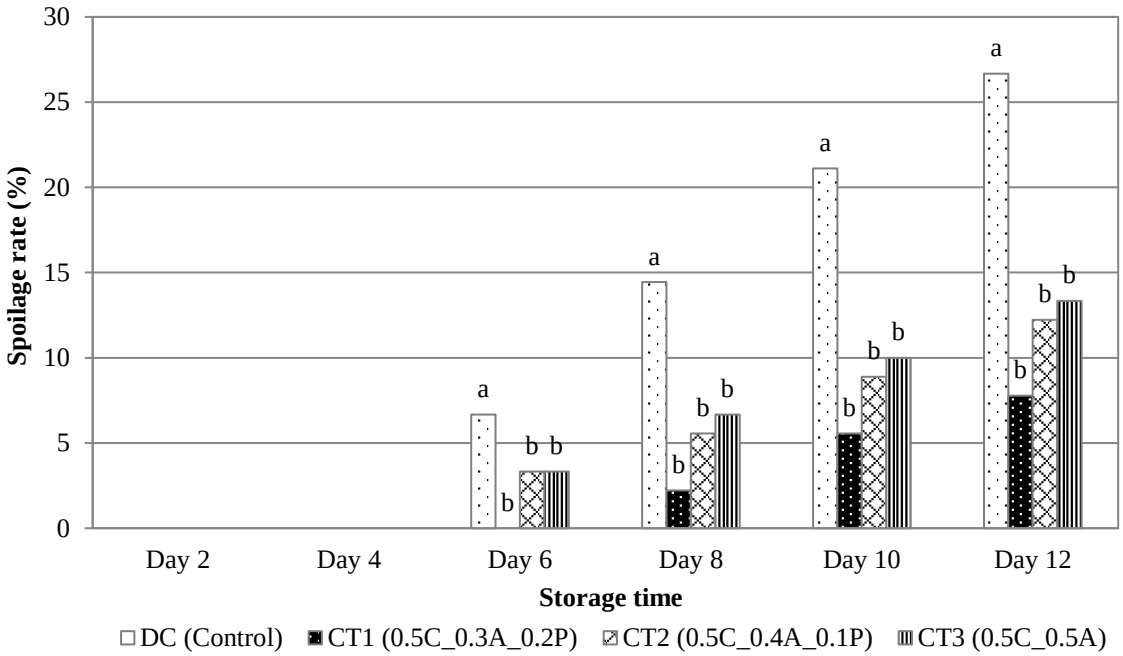
Spoilage rate

The rate of fruit spoilage indicated the effects of the coating on the microbial growth of



Note: Bars not sharing a common superscript differ significantly at P< 0.05.
The initial vitamin C content of the mangoes was 37.4 mg%.

Figure 5. Vitamin C content (mg/100 g fw) in mango fruits cv. 'Keo' treated with chitosan-plant extract coatings



Note: Bars not sharing a common superscript differ significantly at P< 0.05.

Figure 6. Spoilage rate (%) in mango fruits cv. 'Keo' treated with chitosan-plant extract coatings

the fruits. Spoilage due to anthracnose fungi *C. gloeospoirioides* by artificial inoculation application was higher in non-coated fruits than in coated ones during the 12 storage days (**Figure 6**). After the first 6 days of storage, fruit deterioration did not appear in the CT1 coated

fruits, while anthracnose began to grow on the control fruits on this day and drastically increased for the rest of the storage period. After 12 days of storage, the spoilage rate of fruits was found to be significantly higher in the uncoated fruits at 26.67% compared to the CT1, CT2, and CT3 treatments at the same time point ($P< 0.05$).

It was observed that the uncoated fruits ripened faster than the coated fruits due to higher oxygen levels and higher respiration rates (**Figure 1**). The ripening hastened the softening of the fruits, which provided good conditions for microbial spoilage. The coating treatments delayed the ripening of fruits and prevented penetration of the germ tubes of *C. gloeospoirioides*, resulting in reductions of spoilage in the coated fruits. In addition, it can be explained that the antifungal and antimicrobial activities of both the chitosan and plant extracts, which included *A.vera* gel and papaya leaf, protected the coated fruits against the attacks of *C. gloeospoirioides* during storage.

The positive charge of chitosan interacted with the negatively charged cell surface of the fungi, which affected the fungal cells' permeability. This resulted in the leakage of cellular components and hence, the death of cells (Kong *et al.*, 2010). Therefore, chitosan alone affected *C. gloeospoirioides* both *in vitro*, including mycelial growth, sporulation, and conidial morphology and *in situ* in the experiment (Bautista-Banos *et al.*, 2003).

Anthraquinone derivatives, which are aloin and aloe-emodin in the *Aloe vera* gel extract, had antifungal activity against *C. gloeospoirioides* (Nidiry *et al.*, 2011). Similarly, papaya leaf extract affected sporulation of *C. gloeospoirioides* (*in vitro*) (Bautista-Banos *et al.*, 2003) and reduced anthracnose infection of mango as well (*in vivo*) (Bautista-Banos *et al.*, 2002). Thus, a synergistic effect of damaging the normal growth of fungi might have contributed to the low decay incidence in chitosan plant extract coated fruits. There were no significant differences in the spoilage rates of coated fruits after 12 days of storage at 34.3 ± 2°C and relative humidity 71.8 ± 10%. Hence, in-depth studies are needed to define both the action mode and application of this new edible coating for use in the fresh mango fruit industry.

Conclusions

A coating of 0.5 % chitosan with 0.3% *A. vera* and 0.2% papaya leaf extracts applied on mango fruits gave the best results in maintaining postharvest quality and protecting the fruits against anthracnose. This is the first study to prove that *A.vera* gel and papaya leaf extracts mixed with a chitosan coating can reduce the respiration rate, reduce weight loss, delay color change, retain firmness, and sustain high vitamin C content while delaying and reducing the decay incidence during the storage of mango fruit. These findings suggest that chitosan combined with *A.vera* and papaya leaf extracts can be considered as a natural product to prolong the postharvest storage life and control postharvest anthracnose diseases of mango (cv. Keo). For the commercial application of this new edible coating in the fresh mango fruit industry, it is recommended that more in-depth studies be done.

Acknowledgments

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