

Effects of Multilayer Coating of Chitosan and Polystyrene Sulfonate on Quality of ‘Nam Dok Mai No.4’ Mango

N. Hadthamard, P. Chaumpluk, M. Buanong, P. Boonyariththongchai, C. Wongs-Aree

Abstract—Ripe ‘Nam Dok Mai’ mango (*Mangifera indica* L.) is an important exported fruit of Thailand, but rapidly declined in the quality attributes mainly by infection of anthracnose and stem end rot diseases. Multilayer coating is considered as a developed technique to maintain the postharvest quality of mangoes. The utilization of alternated coating by matching oppositely electrostatic charges between 0.1% chitosan and 0.1% polystyrene sulfonate (PSS) was studied. A number of the coating layers (layer by layer) were applied on mature green ‘Nam Dok Mai No.4’ mangoes prior to storage at 25 °C, 65-70% relative humidity (RH). There were significant differences in some quality attributes of mangoes coated by 3½ layers, 4½ layers and 5½ layers. In comparison to coated mangoes, uncoated fruits were higher in weight loss, total soluble solids, respiration rate, ethylene production and disease incidence except the titratable acidity. Coating fruit at 3½ layers exhibited the ripening delay and reducing disease infection without off flavour. On the other hand, fruit coated with 5½ layers comprised the lowest acceptable score, caused by exhibiting disorders from fermentation at the end of storage. As a result, multilayer coating between chitosan and PSS could effectively maintain the postharvest quality of mango, but number of coating layers should be thoroughly considered.

Keywords—Multilayer, chitosan, polystyrene sulfonate, Nam Dok Mai No.4.

I. INTRODUCTION

MANGO (*Mangifera indica* L.) cv. ‘Nam Dok Mai’ is a popularly exported fruit of Thailand [1]. It is highly perishable and has a short shelf life by only a week at room temperature (25 °C). Although ripening stage is suitable for consumer preference of attractive yellow peel and pulp, sweet taste and good flavour, fast softening and fungal infection are major problems of the ripe fruit during the transportation and marketing chains.

Anthracnose caused by *Collectotrichum gloeosporioides*

N. Hadthamard is a Ph.D. student of the Postharvest Technology Program, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi (Bang Khun Thian), Bangkok, 10150 Thailand and works at the Vegetable Integration Technology Program, Faculty of Science and Technology, Kanchanaburi Rajabhat University, Kanchanaburi, 71190, Thailand (phone: ++66-5596-3339; fax: ++66-5596-3301; e-mail: nanthawan.aor04@gmail.com).

P. Chaumpluk works at the Botany Program, Faculty of Science, Chulalongkorn University, Bangkok, 10330 Thailand (phone: ++66-2218-5485; fax: ++66-2252-8979; e-mail: piyasak.cha@gmail.com).

M. Buanong, P. Boonyariththongchai, and C. Wongs-Aree work at the Postharvest Technology Program, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand, and the Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok 10400, Thailand (phone: ++66-2470-7725; fax: ++66-2470-7728; *e-mail: chalermchai.won@kmutt.ac.th).

(Penz.) [2] and stem end rot caused by *Lasiodiplodia theobromae* (Pat.) are the major postharvest diseases of ‘Nam Dok Mai’ mango. The fungi can infect since the flower blooming and are latent in fruit during development on tree [3], [4]. Under high humid condition, anthracnose symptoms first appear as small brown spots on mango during ripening and are quickly developed to the bigger lesions [5]. On the other hand, brown spots are initially generated adjacent the fruit pedicle for stem end rot and turn to the black lesions within 2-3 days [6]. Postharvest diseases cause economic valuable loss of the ‘Nam Dok Mai’ mango logistics.

Several postharvest technologies including low temperature storage [7], irradiation [8], and modified atmosphere packaging [9] can extend the storage life of mangoes by delaying the fruit ripening and reducing the disease infection. Coating, one of practical techniques in modified atmosphere aspects, has been studied for maintaining mango fruit quality after harvest. Typical coating techniques may not properly cover whole fruit, causing improper permeability of gases and water vapour between the coated fruit and atmosphere. In the present study, multilayer coating by 2 different materials was developed for ‘Nam Dok Mai’ mango after harvest. Multicoating film can retard the permeability of oxygen, carbon dioxide, moisture, and volatiles between fruit and micro-atmosphere that can reduce the respiration rate, ripening, weight loss, and fungal growth [10], [11]. Layer by layer film with cohesion and adhesion produces ultrathin film by combining opposite electrostatic charges of two coating materials [2], [12]-[15]. Increasing number of layers to multilayer is connected by covalently opposite charges of each layer [16]. Unsuitable thickness and material concentrations of coating may create disorder from fermentation in anaerobic respiration [17].

Coating materials are based on polysaccharides, lipids, and proteins [13]. Multilayer coating between chitosan and PSS was applied on mango fruit at postharvest in this study. Chitosan, natural cationic polysaccharide, obtained from *N*-deacetylation of chitin is nontoxic, biodegradable, antimicrobial and is a stable barrier to gas and water vapour transfer [18]. Thereby, chitosan has been used to be combined with opposite charge biodegradable materials such as calcium caseinate [14], sodium alginate [14], carboxymethyl cellulose (CMC) [15], and pectin [19] to improve film properties for controlling physicochemical characteristics of some fruits. On the other hand, PSS, an ionic polymer, made by sulfonation of polystyrene. PSS layer raises large number of ion-exchange

sites and high binding capacity [20]. Accordingly, PSS was considered as an anionic material for multilayer coating.

The success of multilayers coated on mango totally depends on factors of film such as type of materials, number of layers, and coating techniques. Therefore, the aim of this research was to optimize the number of multilayers of chitosan and PSS to maintain the quality of 'Nam Dok Mai No.4' mango.

II. MATERIALS AND METHODS

A. Preparation of Fruit and Coating Materials

'Nam Dok Mai No.4' Mango (*Mangifera indica*) fruits at 80% maturation (95-100 days after full bloom) were collected from a commercial farm in Phitsanulok province, the Northern of Thailand between 2017 and 2018 and were transported to the Postharvest Technology Laboratory at King Mongkut's University of Technology Thonburi, Bangkok, Thailand. 300 fruits were sorted for uniformity of size and free from defects. Fruits were rinsed with tap water, dipped into 200 ppm sodium hypochlorite solution for 3 minutes, and then rinsed with distilled water for 1 minute to remove the residual chlorine. Fruits were then air dried.

Chitosan (CTS) powder (310-375 kDa) and PSS (70 kDa) (Better Syndicate Co., Ltd., Thailand) were separately prepared at a concentration of 0.1% (w/v). CTS solution was dissolved in 0.5% acetic acid by magnetic stirring for 3 hours and adjusted the pH to 3.0. PSS solution was dissolved in deionized water and adjusted the pH to 7.0.

B. Process of Multilayer Coating on Mango

Mango fruit were coated in 5 different treatments of coating procedures. Each treatment comprised 60 fruits. Fruits were dipped in deionized water for 20 seconds as a control, and in 0.1% CTS as another control treatment. For multilayer coating, fruits were first dipped in 0.1% CTS and then alternately dipped in 0.1% PSS, layer by layer to 3½, 4½, and 5½ layers.

Layer by layer coating was processed by firstly dipping mangoes into CTS solution for 20 seconds and then rinsing fruits with deionized water. Fruits were air dried for 5 minutes. For an additional layer, mangoes were dipped in PSS for 20 seconds and then air dried. Fruits were alternately coated with CTS and PSS until reaching to 3½, 4½, and 5½ layers. All treatments were started and ended up with a CTS layer. Fruit were stored at 25±3 °C, 65-70% RH and measured for the quality every 3 days.

C. Evaluation of Multilayer Coating on Mango

Contact angle of water was used to confirm an alternated deposition of each layer by making a 5 µl water drop on the surface of coated mango skin at room temperature in the dark. Light was horizontally shined to the fruit and the angles of water drop shadow were measured on the left and right sides and averaged [21].

D. Analysis of Mango Quality

For nondestructive parameters of respiration rate, ethylene production, weight loss, peel colour, disease incidence and

severity, ten fruits in each treatment were used for continuous determination.

1. Respiration and Ethylene Production Rates

Mango was placed in a closed jar for 2 hours at the storage condition. 1 ml of the head space was withdrawn and injected into a Li-700 CO₂/H₂O Analyzer (Li-700, LI-COR, Inc., U.S.A.) for detecting the concentration of carbon dioxide. For ethylene, the head space was detected by the gas chromatograph (GC-8A, Shimadzu, Japan) connected with a flame ionization detector, using nitrogen as the carrier gas.

2. Weight Loss

Weight loss of fruit was calculated as percentage of fresh weight lost from initial. Each fruit was weighted at the beginning (W_0) and during interval days (W_n). Weight loss was calculated from:

$$\text{Weight loss}_n (\%) = ((W_0 - W_n) / W_0) \times 100$$

3. Pulp Firmness

Pulp firmness was analysed by a Texture Analyzer (TA.XT Express, Stable Micro Systems Ltd., United Kingdom) in the middle of fruit. Pulp was penetrated by a 6 mm probe with a compression speed of 20 mm/min for 5 mm in distance [22]. The result was reported in Newton.

4. Acetaldehyde and Ethanol Contents

Acetaldehyde and ethanol contents in fruit were measured on day 3 and day 9. 10 ml of mango juice were put into a vial, top sealed, and incubated in a water bath at 50 °C for 15 minutes. 1 ml of the headspace gas was immediately injected into a GC-FID (GC-2014, Shimadzu, Japan) equipped with a 3 mm Porapak-Q 80-100 G-8016. Ultrapure helium as the carrier gas was set for a flowed rate of 30 ml/min and the final temperature of the column was 240 °C for 4.5 minutes [15].

5. Peel and Pulp Colour Changes

Peel and pulp colours at the blossom, the middle, and the stem end region of fruit were detected using a colorimeter (CR-10, Konica Minolta, Japan) and reported in L values (lightness) and hue angles.

6. Total Soluble Solids, Titratable Acidity, and pH Value

Total soluble solids (TSS), titratable acidity (TA), and pH were measured from pulp juice.

TSS content was detected by a digital hand held pocket refractometer (PAL-1 3810, Atago, Japan) and reported in degree brix.

TA content was analysed using a titration method. 10 ml of mango juice were mixed with 90 ml of distilled water and 1-2 drops of 1% phenolphthalein indicator. The mixed sample was titrated with 0.1 N NaOH until the end point (pink colour) [2], [23].

$$\text{TA} (\%) = (\text{volume of NaOH} \times 0.1 \times 0.07 \times 100) / 10$$

pH was measured by a pH meter (FE20-ATC Kit, Mettler Toledo, Switzerland) connected to LE438 electrode probe.

7. Disease Incidence and Severity

Incidence of mango fruit showing disease symptoms was calculated by counting number of infected fruit out of total. The result was reported as mean percentage of disease incidence [2].

Disease severity shown as mean percentage lesion area on the fruit surface was evaluated from visible lesions and decided by scoring; 0 = no disease, 1 = 1-2% disease, 2 = 5% disease, 3 = 10% disease, 4 = 20% disease, 5 = 40% disease, 6 = 60% disease, and 7 = more than 80% disease [2].

8. Degree of Anthracnose and Stem End Rot

Appearance of anthracnose and stem end rot on mangoes was observed from the disease severity scale using visible and decided by using score; 0 = non-development, 1 = less than 0.1 cm², 2 = 2-3 cm², 3 = 3-12 cm², 4 = 12-25 cm², and 5 = more than 25 cm² [6].

9. Total Counts of Yeast and Mold

On the last day of uncoated control fruit, every treatment was analysed for yeast and mold growth. Fruit at 100 g was crushed in a sterile plastic bag containing 500 ml of peptone water. Sample was diluted for 10⁻², 10⁻³, and 10⁻⁴. The total yeast and mold were carried out on potato dextrose agar at 25 °C for 5 days and reported in CFU/g [6].

10. Acceptance Scores and Shelf life

The mean of acceptance scores below 3 was used to indicate the end of storage. Acceptance scores from 5 panelists were observed from visual quality rating as followed; 1 = inedible, 2 = edible but not marketable, 3 = poor, 4 = fair, 5 = excellent.

11. Statistical Analysis

All data were statistically analysed by one-way analysis of variance (ANOVA) using SPSS statistical software. Significant differences were regarded when $p < 0.05$. Mean separations were performed by employing Duncan's Multiple Range Test comparison procedure.

III. RESULTS AND DISCUSSION

A. Evaluation of Contact Angles of Coating Layers on Mango Fruit

Fig. 1 shows the angles of water drop on skin of fruit coated with CTS and PSS. The angles were represented as the alternate deposition between CTS and PSS. Water contact angle on CTS hydrophobic surface was higher than PSS hydrophilic surface. The degree of contact angle of CTS coated mango was similar to the multi-coating of CTS combined with pectin on 'Tommy Atkins' mango [23]. PSS layer was reported to raise a large number of ion-exchange sites and high binding capacity [20], [24]. Decreased contact angles of hydrophobic fruit skin were changed to hydrophilic surface after the deposition of only few polyelectrolyte layers [25].

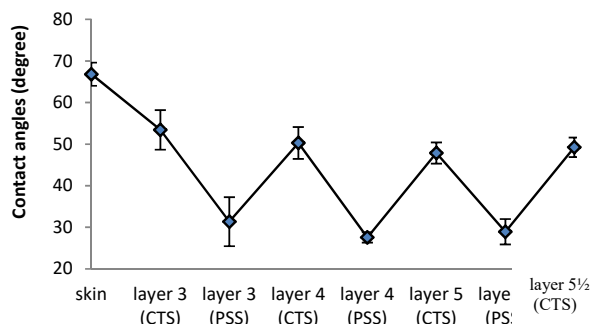


Fig. 1 Contact angles of mango fruit coated by 0.1% CTS and 0.1% PSS at different layers. Vertical bars represent the SD of means (n = 10)

B. Effects of Multilayer Coating on Physiological Changes of Mango Fruit

Respiration and ethylene production rates are relevant index for postharvest metabolism and indicate signals of the ripening patterns for climacteric fruits. The respiration and ethylene production rates between coated and uncoated fruit revealed the same patterns (Figs. 2 (a) and (b)). The respiratory climacteric peaks of fruits in all treatments were exhibited on day 6 (Fig. 2 (a)). There were 2 groups of ethylene production, comprising of the lower rates from multilayer coated fruits and the higher rates from uncoated and single layer coated fruits (Fig. 2 (b)). The respiration and particularly ethylene production rates of multilayer coated mango were significantly lower than uncoated and single layer coated fruit. There were no differences in both physiological changes between multilayer coated fruits. Multilayers of CTS and PSS coating affect the permeability of CO₂, C₂H₄, and O₂ inside and outside of fruit, lead to a reduced rate of respiration, transpiration and production of ethylene [26].

C. Effects of Multilayer Coating on Quality and Physicochemical Characteristics of Mango Fruit

1. Weight Loss and Firmness

Weight loss of mangoes increased successively (Fig. 3 (a)) whereas firmness decreased during storage (Fig. 3 (b)). The weight loss of multicoated fruit was significantly lower than uncoated one after 3 days of storage. The more coating layer was responsible for the lower weight loss. Coating fruit by 3½ and 5½ layers were best to retain the fruit softness, whereas the firmness of uncoated fruit decreased rapidly.

Increasing number of coating layers effectively maintained weight loss and fruit firmness. Higher levels of multilayers could reduce the transpiration process and respiration rate by creating modified atmospheric conditions in the fruit, which reduces water transfer and delays ripening [27]. Coating seals scar pedicel/lenticels of fruit, and adds more cuticle on the surface [28], [29]. Furthermore, previous research revealed that CTS film as a barrier prevented the passage of heat from high temperature surrounding into coated litchi fruit [30]. Therefore fruit coated with CTS could produce less bio-heat leading to less water loss to atmosphere.

There are some reports associated with the utilization of layer by layer coating on fruit softening. Coating with 2% sodium alginate and 0.2% olive oil delayed biochemical changes in cell turgidity and cell wall structure of Ber fruit [31]. Coating 'Choke Anan' mangoes with 10% gum Arabic combined with 1% CTS significantly reduced the weight loss and retained the high firmness during storage [22].

2. Acetaldehyde and Ethanol Contents

Acetaldehyde contents in all fruits on day 3 were lower than in ripe fruit on day 9 (Table I). This may be due to the fruit producing energy toward the ripening physiology. On the other hand, an accumulation of ethanol content in uncoated and 5½ layers fruit on day 9 occurred by a conversion of acetaldehyde to ethanol in anaerobic respiration pathway. Uncoated fruit produced high ethanol which could be caused by over ripening and disease infection [27]. Even coating at 5½ layers effectively delayed ripening, but high levels of coating layers produced poor gaseous permeability and started an accumulation of anaerobic metabolites since day 9. Ethanol content was undetectable in fruit coated by 3½ layers. High contents of ethanol in fruit coated by 5½ layers were related with low consumer acceptance scores.

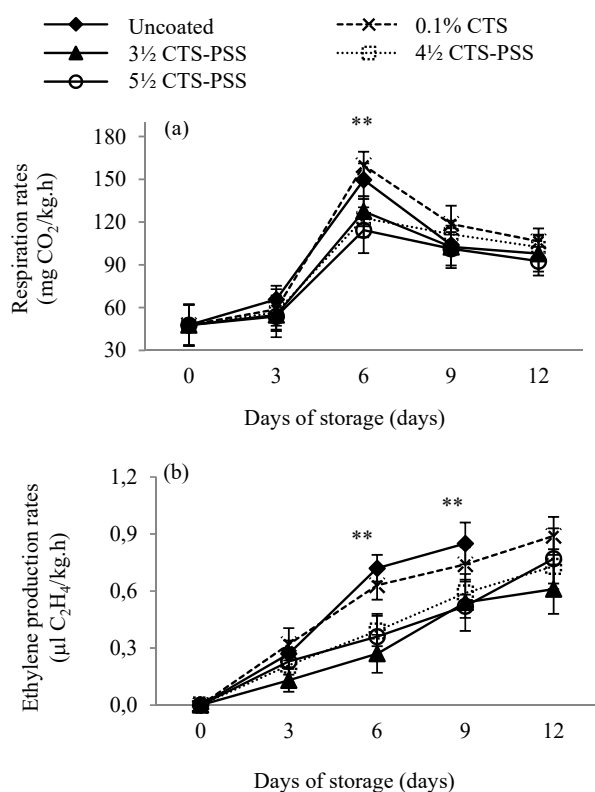


Fig. 2 Respiration (a) and ethylene production (b) rates of mango fruit coated by 0.1% CTS and 0.1% PSS at different layers and then stored at 25±3°C for 12 days. Vertical bars represent the SD of means (n = 10)

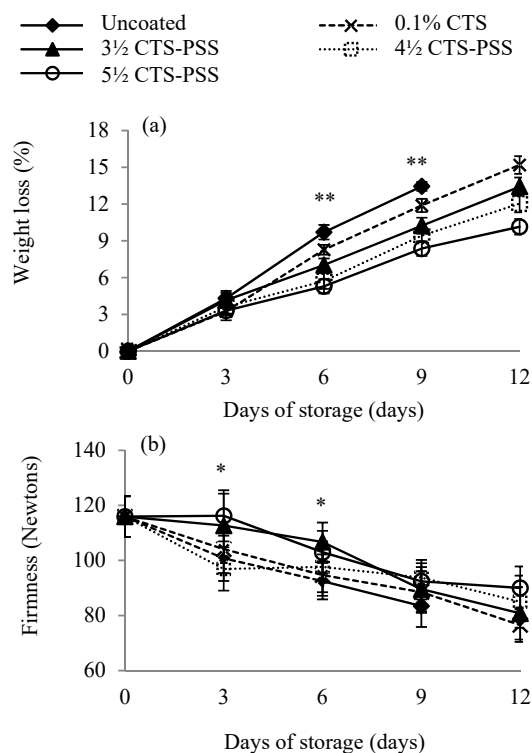


Fig. 3 Weight loss (a) and firmness (b) of mango fruit coated by 0.1% CTS and 0.1% PSS at different layers and then stored at 25±3°C for 12 days. Vertical bars represent the SD of means (n = 10)

TABLE I
ACETALDEHYDE AND ETHANOL CONTENTS IN PULP OF MANGO COATED BY 0.1% CTS AND 0.1% PSS AT DIFFERENT LAYERS AND THEN STORED AT 25±3 °C

Time (days)	Treatment	Acetaldehyde (mg/l)	Ethanol (mg/l)
3	Uncoated	8.99 ^a	0
	3½ CTS-PSS	6.54 ^b	0
	5½ CTS-PSS	0.87 ^c	0
9	Uncoated	15.56 ^a	1.1 ^a
	3½ CTS-PSS	12.05 ^b	0
	5½ CTS-PSS	8.00 ^c	2.8 ^a

^a The same letters in column of the mean values were not significantly different at $p \leq 0.05$ (n=3).

3. Quality Attributes

Changes in fruit colour and taste were used as the index for the ripe mango. When compared with uncoated mangoes, multilayer coating positively retarded the changes in colour and taste of mangoes. A single layer CTS coating showed less effect on delaying the mango ripening. Ripe mango fruit showed increasing L values and decreasing hue angles that present colour changes from green to yellow. Furthermore, the sweetness increased continuously.

The degradation of green colour occurred continuously in every treatment. Loss of green colour of peel was dramatically quick in uncoated fruit. Multilayer coating significantly delayed colour changes of the peel and the pulp during the first 6 days of storage (Table II). The previous research presented the utilization of CTS coating to decrease the

oxidation of chlorophyll degradation [32] and the ripening process by modification of the internal atmosphere in mango [33] and papaya [34]. Moreover, ratio TSS:TA indicating the sweetness level of ripe mangoes increased rapidly after day 6 and was highest at the end of storage (Table II). Multicoating at 4½ and 5½ layers significantly delayed the sweetness, followed by 3½ layers, and a single CTS coating. The increasing of TSS during the ripening was probably due to a conversion of polysaccharides in tissues to soluble sugars in respiration process and the water loss leading to an increase in sugar concentrations [23]. The relationship between TA content and pH, the decline in TA content and the increase in pH during the ripening may be associated with a reversion of organic acids to such substrates in the respiration process [35]. Thus, the decline of gas and water permeability by fruit multiple coating affected the eating quality of mango.

4. Disease Incidence and Severity

Small black spots on the peel were the starting point of anthracnose and stem end rot. The symptoms of uncoated mangoes started on day 3, whereas coating process retarded anthracnose and stem end (Fig. 4 (a)). When fruit showed the disease symptoms, disease infection was spread out on the fruit and led to rapid decay. There was no significant difference in disease incidence and severity between 4½ or 5½ layers of coating (Fig. 4 (b)).

The efficiency of multilayer coating to decrease the disease severity may depend on delayed ripening. CTS and PSS multilayers prevent the diseases by creating modified atmosphere in the fruit which affects microbial metabolisms [19], [36], [37]. Furthermore CTS has been reported to induce plant defensive mechanism [18], while the ionic strength of PSS could obstruct the compatibility of diseases [24]. As a result, the multilayer coating prolonged the disease timing and slowed down the fungal growth about 2 folds.

TABLE II
CHANGES IN COLOUR AND TASTE OF MANGO FRUIT COATED WITH 0.1% CTS AND 0.1% PSS AT DIFFERENT LAYERS AND THEN STORED AT 25±3°C FOR 12 DAYS

Time (days)	Treatment	Peel		Pulp		Taste			
		L values	Hue angles	L values	Hue angles	TSS (°brix)	TA (%)	TSS:TA	pH
0	Uncoated	71.97	106.24 ^{ab}	64.60	99.86	9.34	2.66	3.51	3.05
	0.1% CTS	71.98	108.75 ^a	64.60	99.86	9.34	2.66	3.51	3.05
	3½ CTS-PSS	70.51	104.89 ^b	64.60	99.86	9.34	2.66	3.51	3.05
	4½ CTS-PSS	71.94	105.13 ^b	64.60	99.86	9.34	2.66	3.51	3.05
	5½ CTS-PSS	70.21	107.03 ^a	64.60	99.86	9.34	2.66	3.51	3.05
3	Uncoated	75.27 ^a	104.51 ^b	68.53 ^a	95.74 ^{ab}	12.10 ^a	1.75 ^c	6.91 ^a	4.09 ^a
	0.1% CTS	73.85 ^{ab}	106.06 ^{ab}	67.73 ^a	94.75 ^b	11.00 ^{ab}	2.53 ^b	4.35 ^b	3.33 ^b
	3½ CTS-PSS	72.53 ^b	104.88 ^b	66.58 ^a	96.09 ^{ab}	10.24 ^b	2.44 ^b	4.20 ^b	3.16 ^{bc}
	4½ CTS-PSS	73.15 ^{ab}	107.11 ^a	65.53 ^{ab}	97.86 ^a	10.26 ^b	2.73 ^a	3.76 ^c	3.43 ^b
	5½ CTS-PSS	74.04 ^a	104.94 ^b	63.90 ^b	97.43 ^a	10.24 ^b	2.56 ^b	4.00 ^b	3.56 ^b
6	Uncoated	77.49 ^a	93.12 ^b	68.59 ^a	93.68 ^b	17.72 ^a	0.76 ^b	23.32 ^a	6.51 ^a
	0.1% CTS	75.51 ^{ab}	95.21 ^{ab}	67.51 ^{ab}	94.56 ^b	15.44 ^b	1.04 ^a	12.92 ^c	4.53 ^b
	3½ CTS-PSS	74.84 ^b	95.13 ^{ab}	69.48 ^a	96.68 ^a	16.14 ^b	1.15 ^a	14.03 ^b	4.36 ^b
	4½ CTS-PSS	74.22 ^b	97.75 ^a	67.61 ^{ab}	95.65 ^{ab}	13.82 ^c	1.03 ^a	13.42 ^{bc}	4.18 ^b
	5½ CTS-PSS	73.26 ^b	98.86 ^a	66.15 ^b	97.23 ^a	13.62 ^c	1.14 ^a	11.95 ^c	4.27 ^b
9	Uncoated	81.38 ^a	85.67 ^b	72.35 ^a	85.34 ^b	20.52 ^a	0.41 ^c	50.05 ^a	6.39 ^a
	0.1% CTS	77.92 ^b	88.78 ^a	71.79 ^a	87.26 ^a	18.32 ^b	0.66 ^b	27.76 ^b	5.91 ^b
	3½ CTS-PSS	75.34 ^c	91.94 ^a	68.05 ^b	87.98 ^a	17.72 ^{bc}	0.96 ^a	18.45 ^c	5.55 ^{bc}
	4½ CTS-PSS	74.48 ^c	90.45 ^a	69.48 ^b	88.35 ^a	16.66 ^c	1.01 ^a	16.50 ^c	4.88 ^c
	5½ CTS-PSS	75.09 ^c	89.33 ^a	69.63 ^b	87.22 ^a	16.85 ^c	0.98 ^a	17.19 ^c	5.27 ^c
12	Uncoated	-	-	-	-	-	-	-	-
	0.1% CTS	80.61 ^a	84.61 ^b	72.51 ^a	86.61	21.67 ^a	0.36 ^b	60.19 ^a	6.69 ^a
	3½ CTS-PSS	77.20 ^b	88.16 ^a	69.43 ^b	86.49	19.72 ^b	0.41 ^a	48.10 ^b	6.36 ^b
	4½ CTS-PSS	76.25 ^b	86.93 ^a	69.05 ^b	86.11	18.48 ^c	0.50 ^a	39.96 ^c	6.28 ^b
	5½ CTS-PSS	76.34 ^b	87.08 ^a	67.89 ^b	87.08	18.17 ^c	0.45 ^a	40.38 ^c	6.13 ^b

^a The same letters in each column of the mean values were not significantly different at $p \leq 0.05$ ($n = 10$)

D. Effects of Multilayer Coating on Qualities' Acceptance

Table III presents the total yeast and mold counts of all treatments on day 9. The multilayer coating creating antimicrobial films were shown by a dramatic reduction of yeast and mold colony growth, when obviously, the highest counts of yeast and mold were exhibited in uncoated fruit. On day 9, uncoated mango had acceptance scores of 2.2 with high disease appearance. More layers of coating showed better inhibition of disease growth on coated 'Nam Dok Mai No.4'

mango, especially to stem end rot (Fig. 5). Although 5½ CTS-PSS was shown in the highest scores of visual acceptance (Table III), coated fruit produced high fermented volatiles (Table I).

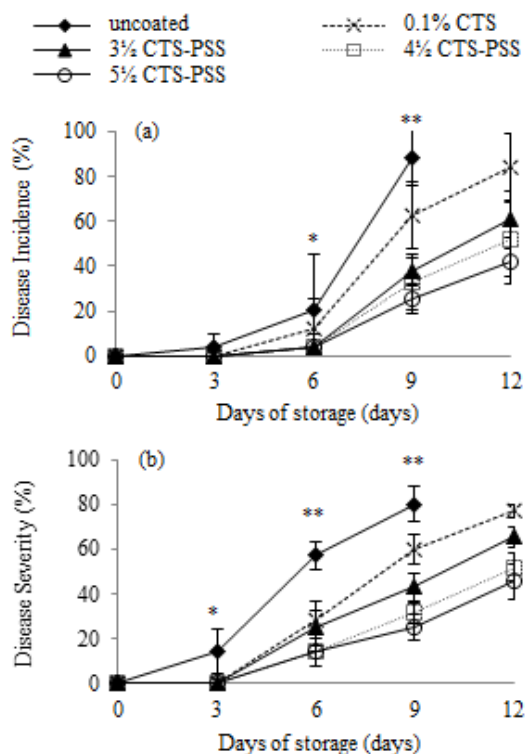


Fig. 4 Disease incidence (a) and disease severity (b) of mango fruit coated by 0.1% CTS and 0.1% PSS at different layers and then stored at $25\pm 3^\circ\text{C}$ for 12 days. Vertical bars represent the SD of means ($n = 10$)

TABLE III

YEAST AND MOLD GROWTH AND QUALITY ACCEPTANCES OF MANGO FRUIT COATED 0.1% CTS AND 0.1% PSS AT DIFFERENT LAYERS AND THEN STORED AT $25\pm 3^\circ\text{C}$ ON DAY 9

Treatment	Yeast & Mold (CFU/g)	Appearance (scores)		Visual Acceptance score
		Anthraco	Stem End Rot	
Uncoated	8.49×10^4 ^a	3.88 ^a	4.75 ^a	2.38 ^c
0.1% CTS	3.27×10^4 ^b	2.88 ^b	3.50 ^b	3.13 ^b
3½ CTS-PSS	5.54×10^3 ^c	2.63 ^b	3.63 ^b	3.25 ^b
4½ CTS-PSS	4.18×10^3 ^c	2.63 ^b	3.38 ^c	3.38 ^{ab}
5½ CTS-PSS	4.36×10^3 ^c	2.38 ^c	3.25 ^c	3.50 ^a

^a The same letters in each column of the mean values were not significantly different at $p \leq 0.05$ ($n = 5$).

IV. CONCLUSION

Among coating procedures of multilayers between CTS and PSS (0, 1, 3½, 4½, and 5½ layers) on 'Nam Dok Mai No.4' mangoes on, coating at 3½ layers was appropriate to maintain the storage quality and decreased anthracnose and stem end rot diseases without off-flavour generation. Although coating at 5½ layers was given in high overall quality and low disease incidence, coated fruit released fermented flavour. These findings suggested that the 3½ layer of CTS combined with PSS was best to prolong the shelf life of mango stores at $25\pm 3^\circ\text{C}$. Thus, multilayer coating for extending 'Nam Dok Mai' mango in retail markets is needed to be further developed.

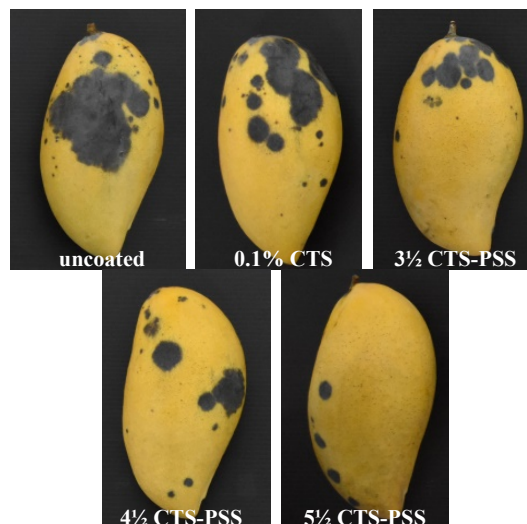


Fig. 5 Disease appearance of mango fruit coated by 0.1% CTS and 0.1% PSS at different layers and then stored at $25\pm 3^\circ\text{C}$ on day 9

ACKNOWLEDGMENT

The authors would like to thank the Postharvest Technology Innovation Center, Commission on Higher Education, Thailand, for financial support (PL. P. 3/2559).

REFERENCES

- [1] P. Jongsri, P. Rojsitthisak, T. Wangsomboondee, and K. Seraypheap, "Influence of chitosan coating combined with spermidine on anthracnose disease and qualities of 'Nam Dok Mai' mango after harvest," *Sci Hortic.*, vol. 224, pp. 180-187, Oct. 2017.
- [2] P. Jongsri, T. Wangsomboondee, P. Rojsitthisak, and K. Seraypheap, "Effect of molecular weights of chitosan coating on postharvest quality and physicochemical characteristics of mango fruit," *JFST.*, vol. 73, pp. 28-36, May 2016.
- [3] A. Malik, S. Iram, A. Rasool, "Current status of mango pre and post-harvest diseases with respect to environmental factors," *Int. J. Agri. Agri. R.*, vol. 8, no. 4, pp. 101-110, Apr. 2016.
- [4] S. F. Ullah, Y. Hussain, and S. Iram, "Pathogenic characterization of *Lasiodiplodia* causing stem end rot of mango and its control using botanicals," *Pak. J. Bot.*, vol. 49, no. 4, pp. 1605-1613, Jan. 2017.
- [5] P. Singh, "Integrated Management of Storage Anthracnose of Mango," *J. Mycol Pl Pathol*, vol. 41, no. 1, Jan. 2011.
- [6] M. T. Malik, T. Tariq, A. H. Khan, H. Ullah, M. Imran, J. Iqbal, and A. Zainab, "Outbreak of anthracnose and stem end rot diseases of mango in changing climate and their management through hot water treatment," *Pak. J. Phytopathol.*, vol. 30, no.1, pp. 91-98, June. 2018.
- [7] L. Ayele, and A. Bayleyegn, "Effect of Low Temperature Storage on Ripening and Quality of Mango (*Mangifera indica* L.)," *Acad. Res. J. Agri. Sci. Res.*, vol. 5, no. 7, pp. 570-576, Nov. 2017.
- [8] A. Safitri, T. Theppakorn, M. Naradison, and S. Setha, "Effects of UV-C irradiation on ripening quality and antioxidant capacity of mango fruit cv. Nam Dok Mai Si Thong," *JFAT.*, vol. 1, no. 1, pp. 164-170, Feb. 2015.
- [9] G. Ruth, A. Jane, M. Hutchison, and W. Owino, "Effect of Activebag® Modified Atmosphere Packaging on the Postharvest Characteristics of Mango Fruits, *Mangifera indica* L, Cultivar Tommy Atkins," *J. Appl. Biosci.*, vol. 83, pp. 7535-7544, Nov. 2014.
- [10] R. K. Dhall, "Advances in edible coatings for fresh fruits and vegetables: A review," *J. Food Sci Nutr.*, vol. 53, no. 5, pp. 435-450, Feb. 2013.
- [11] H. Arnon-Rips and E. Poverenov, "Improving food products quality and storability by using Layer by Layer edible coatings," *JFST.*, vol. 75, pp. 81-92, May 2018.
- [12] P. Appendini and J. H. Hotchkiss, "Review of antimicrobial food

- packaging," *IFSET*, vol. 3, no. 2, pp. 113-126, June 2002.
- [13] S. Mangaraj, T. K. Goswami, and P. V. Mahajan, "Applications of Plastic Films for Modified Atmosphere Packaging of Fruits and Vegetables: A Review," *Food Eng Rev*, vol. 1, pp. 133-158, Dec. 2009.
 - [14] J. Duan, R. Wu, B. C. Strik, and Y. Zhao, "Effect of edible coatings on the quality of fresh blueberries (Duke and Elliott) under commercial storage conditions," *Postharvest Biol. Technol.*, vol. 59, pp. 71-79, Jan. 2011.
 - [15] J. Yan, Z. Luo, Z. Ban, H. Lu, D. Li, D. Yang, M. S. Aghdam, and L. Li, "The effect of the layer-by-layer (LBL) edible coating on strawberry quality and metabolites during storage," *Postharvest Biol. Technol.*, vol. 147, pp. 29-38, Jan. 2019.
 - [16] S. Soh, X. Chen, S. J. Vella, W. Choi, J. Gong, and G. M. Whitesides, "Layer-By-Layer Films for Tunable and Rewritable Control of Contact Electrification," *soft matter*, vol. 9, pp. 10233-10238, Sep. 2013.
 - [17] P. K. Raghav, N. Agarwal, and M. Saini, "Herbal Edible Coatings of Fruits and Vegetables: A Newer Concept," *IJSR*, vol. 4, no.6, pp. 1452-1458, June 2016.
 - [18] P. K. Dutta, S. Tripathi, G. K. Mehrotra, and J. Dutta, "Review: Perspectives for chitosan based antimicrobial films in food applications," *Food. Chem.*, vol. 114, pp. 1173-1182, June 2009.
 - [19] M. Z. Elsabee, E. S. Abdou, K. S. A. Nagy, and M. Eweis, "Surface modification of polypropylene films by chitosan and chitosan/pectin multilayer," *Food Chem.*, vol. 71, pp. 187-195, Jan. 2008.
 - [20] G. Liu, D. M. Dotzauer, and M. L. Bruening, "Ion-Exchange Membranes Prepared Using Layer-by-Layer Polyelectrolyte Deposition," *J. Memb Sci.*, vol. 354, no. 1-2, pp. 198-205, May 2010.
 - [21] A. Ghanem and M. Katalinich, "Characterization of chitosan films for tissue engineering application," *ABBI*, vol. 2, no. 1, pp. 9-16, Jan. 2005.
 - [22] G. Khaliq, M. T. M. Mohamed, P. Ding, H. M. Ghazali, and A. Ali, "Storage behavior and quality responses of mango (*Mangifera indica* L.) fruit treated with chitosan and gum arabic coating during cold storage conditions," *IFRJ*, vol. 23(suppl.), pp. S141-S148, Dec. 2016.
 - [23] B. G. de S. Medeiros, A. C. Pinheiro, M. G. Carneiro-da-Cunha, and A. A. Vicente, "Development and characterization of a nanomultilayer coating of pectin and chitosan-Evaluation of its gas barrier properties and application on 'tommy Atkins' mangoes," *J. Food Eng.*, vol. 110, no. 3, pp. 457-464, June 2012.
 - [24] K. Tand and N. A. M. Besseling, "Formation of polyelectrolyte multilayers: ionic strengths and growth regimes," *Soft Matter*, vol. 12, pp. 1032-1040, Jan. 2016.
 - [25] P. Kittitheeranun, S. T. Dubas, and L. Dubas, "Layer-by-Layer Surface Modification of Fruits with Edible Nano-Coatings," *Appl Mech Mater.*, vol. 229-231, pp.2745-2748, Nov. 2012.
 - [26] A. Ali, M.T.M. Muhammad, K. Sijam, and Y. Siddiqui, "Effect of chitosan coatings on the physicochemical characteristics of Eksotika II papaya (*Carica papaya* L.) fruit during cold storage," *Food Chem.*, vol. 124, no. 2, pp. 620-626, Jan. 2011.
 - [27] F. A. Baldwin, J. K. Burns, W. Kazokas, J. K. Brecht, R. D. Hagenmaier, R. J. Bender, and E. Pesis, "Effect of two edible coatings with different permeability characteristic on mango (*Mangifera indica* L.) ripening during storage," *Postharvest Biol. Technol.*, vol. 17, no.3, pp. 215-226, Nov. 1999.
 - [28] K. Prasad, R. R. Sharma, and M. Srivastav, "Postharvest treatment of antioxidant reduces lenticel browning and improves cosmetic appeal of mango (*Mangifera indica* L.) fruits without impairing quality," *J. Food Sci Technol.*, vol. 53, no. 7, pp. 2995-3001, July 2016.
 - [29] H. Rymbai, M. Srivastav, R.R. Sharma, and S.K. Singh, "Lenticels on mango fruit: origin, development, discoloration and prevention of their discoloration," *Sci Hortic.*, vol. 135, pp. 164-170, Feb. 2012.
 - [30] B. Lin, Y. Du, X. Liang, X. Wang, and J. Yang, "Effect of chitosan coating on respiratory behavior and quality of stored litchi under ambient temperature," *J. Food Eng.*, vol. 102, pp. 94-99, Jan. 2011.
 - [31] T. V. R. Rao, N. S. Baraiya, P. B. Vyas, and D. M. Patel, "Composite coating of alginate-olive oil enriched with antioxidants enhances postharvest quality and shelf life of Ber fruit (*Ziziphus mauritiana* Lamk. Var. Gola)," *J Food Sci Technol.*, vol. 53, no. 1, pp. 748-756, Jan. 2016.
 - [32] Z. Fang, J. C. Bouwkamp and T. Solomos, "Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of *Phaseolus vulgaris* L.," *JXB.*, vol.49, no. 320, pp. 503-510, March 1998.
 - [33] P. C. Srinivasa, R. Baskaran, M. N. Ramesh, K. V. Harish Prashanth, and R.N. Tharanathan, "Storage studies of mango packed using biodegradable chitosan film," *Eur Food Res Technol*, vol. 215, pp. 504-508, Oct. 2002.
 - [34] L.S. Bhanushree, K.R. Vasudeva, G.J. Suresha, G.K. Sadananda, H.M. Tayeebulla, and G.K. Halesh, "Influence of chitosan on postharvest behavior of papaya (*Carica papaya* L.) Fruits under different storage conditions," *RJPP*, vol. 7, no. 2, pp. 2010-2014, Feb. 2018.
 - [35] O. H. Malik, A. U. Malik, M. S. Khalid, M. Amin, S. Khalid, and M. Umar, "Effect of Modified Atmosphere Packaging on Postharvest Quality of Mango cvs. Sindhri and Sufaid Chaunsa During Storage," *TURJAF*, vol. 4, no. 12, pp. 1104-1111, Oct. 2016.
 - [36] O. Feygenbery, V. Hershkovitz, R. Ben-Arie, S. Jacob, and E. Pesis, "Postharvest Use of Organic Coating for Maintaining Bio-Organic Avocado and Mango Quality," *Acta Hort.*, vol. 682, no. 1, pp. 507-512, June 2005.
 - [37] R. A. Shiekh, M. A. Malik, S. A. Al-Thabalti, and M. A. Shiekh, "Chitosan as a Novel Edible Coating for Fresh Fruits," *Food Sci. Technol. Res.*, vol. 19, no. 2, pp. 139-155, Jan. 2013.