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Effects of Different Molecular Weights of Chitosan Coatings on Postharvest Qualities of 'Nam Dok Mai' Mango

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Abstract. The effects of varying molecular weights of chitosan and storage time of chitosan solutions on postharvest quality of 'Nam Dok Mai' mango fruit were investigated. Chitosan coating solutions were prepared from low Mw (65,000 Da = LM-CTS), medium Mw (240,000 Da = MM-CTS) and high Mw (410,000 Da = HM-CTS). Coating solutions were separated into 2 groups: freshly prepared and 14-days stored solutions. Mango fruits coated with 14-days stored chitosan coating solutions had higher disease incidences and lower postharvest qualities compared to those coated with freshly prepared solutions. Among the freshly prepared solutions, freshly prepared MM-CTS showed the best results, in term of longer shelf life, fewer disease incidences and delayed ripening characteristics. In conclusion, our results suggest that freshly prepared MM-CTS solution can be used as an effective coating agent for the extension of 'Nam Dok Mai' mango's shelf life.

Keywords: 'Nam Dok Mai' mango, chitosan coating, postharvest disease, storage, ripening

1. Introduction

'Nam Dok Mai' mango (*Mangifera indica* cv. 'Nam Dok Mai no. 4') is a very popular tropical fruit. The golden pulp of this ripened fruit has a great flavor. However, mango is a climacteric fruit in that the ripening process and senescence continue after fruit has been harvested [1] resulting in some physiological changes, e.g., fresh weight and fruit firmness losses, peel color change, depletion of total acid, increasing of total soluble solids, and increased fruit softening [2]. All of these factors contribute to unacceptable commercial qualities for customers. Thus, a proper postharvest management is needed to prolong shelf life and maintain an ideal mango fruit quality.

Chitosan is a biopolymer prepared from shells of shrimp, crab or squid. Furthermore, chitosan is a natural elicitor that can induce defensive mechanism in plants and can reduce growth of plant pathogens [3]. Previously, numerous researches have applied chitosan on varieties of fruits. However, molecular weight of chitosan varies due to sources of materials. Differences in molecular weights of chitosan may affect postharvest physiological characters of mango fruit. In addition, chitosan solution is a non-Newtonian fluid, i.e., viscosity of chitosan solution drops sharply after preparation and then gradually becomes stable [4]. Additionally, chitosan is a carbon source for microbes that have chitosanase activity [5]. Thus, these properties of chitosan may affect the shelf life of mango fruit. The aims of this study were to investigate the effects of different molecular weights of chitosan coatings on postharvest qualities of 'Nam Dok Mai' mango and to compare physiological changes between mango fruits coated with freshly prepared and 14-days stored chitosan coating solutions.

2. Materials and Methods

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2.1. Plant materials and chitosan coating

Mature green ‘Nam Dok Mai’ mango fruits were harvested at a commercial maturity stage from a local orchard in Chaiyaphum, Thailand. Mango fruits of uniform size and lack of disease were selected, rinsed with tap water, and then allowed to dry. Three different molecular weights of chitosan flakes prepared from shrimp shell: 65 kDa (low molecular weight chitosan: LM-CTS), 240 kDa (medium molecular weight chitosan: MM-CTS) and 410 kDa (high molecular weight chitosan: HM-CTS) (A.N. Lab, Thailand) were dissolved in a 0.5% acetic acid solution and stirred for 4 hr. Tween-80® (0.1%) was added as a surfactant. Before application, all coating solutions were stored for 24 hr to eliminate microbubbles caused by agitation. The solutions were separated into 2 groups: freshly prepared and 14-days stored solutions. After both solutions were ready for application, fruits were dipped in distilled water (control), 0.5% acetic acid, freshly prepared LM-CTS (fLM-CTS), 14-days stored LM-CTS (14dLM-CTS), freshly prepared MM-CTS (fMM-CTS), 14-days stored MM-CTS (14dMM-CTS), freshly prepared HM-CTS (fHM-CTS) and 14-days stored HM-CTS (14dHM-CTS) for 1 min and allowed to dry before storing at 25 °C for 15 days.

2.2. Physiological analysis

Fresh weigh, fruit firmness, peel color, total soluble solids (TSS) content and titratable acidity (TA) were analyzed. Firmness of mango fruit was measured using a FHR-1 fruit firmness tester (Nippon optical work, Japan) at the top, middle and bottom of the fruit. Data was reported in Newton (N). Peel color of mango fruit was measured using a CR-10 color reader (Konica Minolta, Japan). Ten grams of fruit pulp was homogenized with 10 ml deionized water and centrifuged at 8,000 rpm for 5 min. The supernatant was used to determine TSS with an N-1E hand refractometer (Atago, Japan). Raw data was multiplied by 2 (as dilution factor) and reported in °Brix. The TA method was modified from Amador [6] by homogenizing 20 g of fruit pulp with 20 ml deionized water. Samples were titrated with 0.1 M NaOH and used phenolphthalein as an indicator. Percentage of acidity was calculated following the equation (ml of NaOH used x NaOH molar x 0.64)/ g pulp weight. The experiment applied a completely randomized design (CRD) with 3 replications and 3 fruits per each replication. All data were analyzed using analysis of variance (ANOVA) with SPSS software. Means were separated by test of least significant difference (LSD) at $p < 0.05$.

3. Results and Discussion

The result showed that control, 0.5% acetic acid, fLM-CTS and 14dLM-CTS had 12 days of shelf life while fMM-CTS, 14dMM-CTS, fHM-CTS and 14dHM-CTS could be stored for 15 days (Fig. 1). Chitosan coatings affected weight loss, fruit firmness, peel color changes, TSS and TA (Table 1). Weight loss was the highest in 0.5% acetic acid and fLM-CTS treatments but not significantly different among other treatments. Firmness was the lowest in 14dLM-CTS and the highest in freshly prepared HM-CTS treatments. It has been shown that mango fruits lose their cell wall strength because of ripening and senescence [2]. Our results suggested that chitosan application could retain fruit firmness. ‘Ataulfo’ mango fruit treated with chitosan and hydrothermal process exhibited low polygalacturonase activities (PG) and pectin methylesterase (PME), key enzymes that reduced plant cell wall strength during fruit ripening [7]. However, most experiments used freshly prepared chitosan solution. It could be implied that stored chitosan might lose its properties, e.g., reduction of viscosity and degradation caused by some microbes that had chitosanase activity and could use chitosan as their carbon source [8]-[10].

Peel color changes were prominently different. Lightness of mango peel increased when fruit ripened. However, the peel lightness value of both fHM-CTS and 14dHM-CTS was lower than other treatments. Another peel color parameter is hue angle which determines peel color changing from green to yellow. The result showed that hue angle of both fHM-CTS and 14dHM-CTS treatments was higher than others which indicated that peel colors of these treatments were still green while other treatments turned yellow. Also, peels of fHM-CTS and 14dHM-CTS treatments were still green until the end of the experiment. Thus, using high MW of chitosan might interrupt the peel color changing process of ‘Nam Dok Mai’ mango [11].

Fig. 1: ‘Nam Dok Mai’ mango fruits on day 12. Fruits were dipped in distilled water (control), 0.5% acetic acid, freshly prepared LM-CTS (fLM-CTS), 14-days stored LM-CTS (14dLM-CTS), freshly prepared MM-CTS (fMM-CTS), 14-days stored MM-CTS (14dMM-CTS), freshly prepared HM-CTS (fHM-CTS) and 14-days stored HM-CTS (14dHM-CTS) for 1 min and stored at 25 ℃ for 15 days.

TSS can be referred to the sugar content in fruit pulp. TSS contents in all treatments were not significantly different on day 12, but on day 15, fHM-CTS showed the highest TSS content while fMM-CTS had the lowest. This finding showed that the ripening process of mango fruit in fHM-CTS was interrupted. On the other hand, TSS of fMM-CTS dropped because fruits became senescence, i.e., most of sugar was used for fruit metabolism [2], [11].

TA was determined as an additional indicator of the degree of fruit ripeness. The result showed that all treatments had low acid content during fruit ripening since citric and malic acids were used as respiratory substrates which was similar to Ali et al. [11]. However, fHM-CTS maintained very high acid content compared with other treatments. This suggested that high MW chitosan interfered with acid metabolism in fruit.

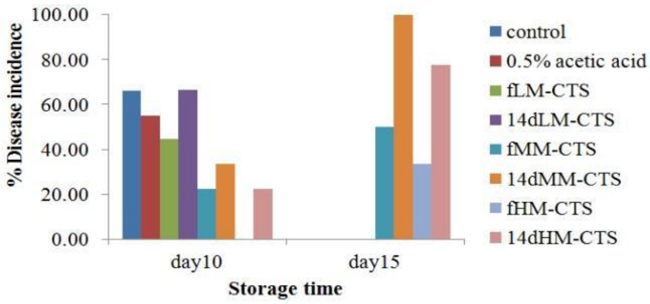

Fig. 2: Effect of chitosan coating solution on postharvest anthracnose disease incidence. Fruits were dipped in distilled water (control), 0.5% acetic acid, freshly prepared LM-CTS (fLM-CTS), 14-days stored LM-CTS (14dLM-CTS), freshly prepared MM-CTS (fMM-CTS), 14-days stored MM-CTS (14dMM-CTS), freshly prepared HM-CTS (fHM-CTS) and 14-days stored HM-CTS (14dHM-CTS) for 1 min and stored at 25 ℃ for 15 days. Each value is the mean of 3 replicates (*n*=9).

Fruits which had black spots of anthracnose disease larger than 0.5 cm diameter were determined as infected and counted toward disease incidence. Fruits that had more than 1.0 cm diameter of black spots were defined as defect fruits. Treatments were no longer stored when defect fruits were found to comprise of more than 30% of all samples. The result showed that fHM-CTS had no disease incidence on day 12 and then increased to 30% on day 15 (Fig. 2). Postharvest disease resistance of mango fruit was greatly improved when MW of chitosan increased. According to Hernández-Lauzardo et al. [12], higher MW of chitosan could reduce germination rate of *Rhizopus stolonifer* more effectively. A major postharvest disease of ‘Nam Dok Mai’ mango fruits is anthracnose which is caused by *Colletotrichum gloeosporioides*. According to Bautista-Baños et al. [13], different MW chitosan was added into a fungal medium and found that the proper MW should be around 200,000 Da. Moreover, this study showed that fMM-CTS could prolong shelf life and allow normal ripening of mango fruit to which both HM-CTS treatments failed. In addition, application of high levels of MW chitosan not only interrupted the ripening process but also reduced disease resistant capacity in fruits [14]. Furthermore, all 14-days stored chitosan solutions had obviously higher disease incidence than those of freshly prepared coating solution. The result of No et al. [15] reported that long storage time reduced stability of chitosan in both viscosity and antibacterial properties. Also, Ren et al. [16] reported that MW of chitosan in solution form reduced simultaneously when it was stored for an extended time and was degraded by microbes [9].

Table 1: Effect of chitosan coating solutions on postharvest physiological characteristics of 'Nam Dok Mai' mango fruits. Fruits were dipped in distilled water (control), 0.5% acetic acid, freshly prepared LM-CTS (fLM-CTS), 14-days stored LM-CTS (14dLM-CTS), freshly prepared MM-CTS (fMM-CTS), 14-days stored MM-CTS (14dMM-CTS), freshly prepared HM-CTS (fHM-CTS) and 14-days stored HM-CTS (14dHM-CTS) for 1 min and stored at 25 ℃ for 15 days.

Treatments	Weight loss (%)			Fruit firmness (N)		
	day0	day12	day15	day0	day12	day15
control	0	3.32 ±0.91 b		4.52 ±0.31	3.30 ±0.56 ab	
0.5% acetic acid	0	4.22 ±0.66 a		4.52 ±0.31	3.42 ±0.41 a	
fLM-CTS	0	3.56 ±0.47 b		4.52 ±0.31	3.00 ±0.25 b	
14dLM-CTS	0	4.17 ±0.38 a		4.52 ±0.31	2.53 ±0.20 c	
fMM-CTS	0	3.59 ±0.24 b	7.55 ±0.89 ns	4.52 ±0.31	3.29 ±0.25 ab	2.64 ±0.29 ns
14dMM-CTS	0	3.77 ±0.48 ab	7.06 ±0.64 ns	4.52 ±0.31	3.45 ±0.33 a	2.51 ±0.30 ns
fHM-CTS	0	3.62 ±0.64 b	7.81 ±1.24 ns	4.52 ±0.31	3.57 ±0.12 a	2.50 ±0.21 ns
14dHM-CTS	0	3.15 ±0.46 b	7.19 ±0.87 ns	4.52 ±0.31	2.72 ±0.30 bc	2.38 ±0.30 ns
Treatments	Peel lightness (L value)			Peel color (Hue angle)		
	day0	day12	day15	day0	day12	day15
control	66.63 ±0.68	70.41 ±1.90 a		89.92 ±1.71	76.35 ±6.53 c	
0.5% acetic acid	66.63 ±0.68	69.97 ±1.24 ab		89.92 ±1.71	73.22 ±3.58 cd	
fLM-CTS	66.63 ±0.68	70.33 ±1.66 ab		89.92 ±1.71	74.05 ±1.73 cd	
14dLM-CTS	66.63 ±0.68	69.01 ±1.63 b		89.92 ±1.71	72.50 ±2.70 d	
fMM-CTS	66.63 ±0.68	69.83 ±0.63 ab	68.70 ±1.22 a	89.92 ±1.71	72.79 ±1.69 cd	73.13 ±2.04 b
14dMM-CTS	66.63 ±0.68	68.93 ±0.98 b	68.51 ±0.83 a	89.92 ±1.71	75.04 ±4.91 cd	73.02 ±3.08 b
fHM-CTS	66.63 ±0.68	65.80 ±1.45 c	65.96 ±1.57 b	89.92 ±1.71	88.84 ±1.54 a	85.93 ±4.05 a
14dHM-CTS	66.63 ±0.68	66.02 ±1.40 c	65.20 ±2.23 b	89.92 ±1.71	84.89 ±3.41 b	86.02 ±2.17 a
Treatments	Total soluble solid (°Brix)			Titratable acidity (%)		
	day0	day12	day15	day0	day12	day15
control	8.07 ±0.22	13.33 ±1.62 ns		1.18 ±0.09	0.11 ±0.03 b	
0.5% acetic acid	8.07 ±0.22	13.61 ±1.22 ns		1.18 ±0.09	0.07 ±0.02 b	
fLM-CTS	8.07 ±0.22	14.07 ±0.80 ns		1.18 ±0.09	0.08 ±0.02 b	
14dLM-CTS	8.07 ±0.22	13.16 ±2.06 ns		1.18 ±0.09	0.05 ±0.01 b	
fMM-CTS	8.07 ±0.22	13.44 ±1.36 ns	12.75 ±1.20 b	1.18 ±0.09	0.03 ±0.01 b	0.09 ±0.02 b
14dMM-CTS	8.07 ±0.22	13.30 ±0.94 ns	13.12 ±1.91 ab	1.18 ±0.09	0.07 ±0.01 b	0.04 ±0.01 b
fHM-CTS	8.07 ±0.22	13.63 ±1.37 ns	14.44 ±1.12 a	1.18 ±0.09	0.78 ±0.16 a	0.15 ±0.07 a
14dHM-CTS	8.07 ±0.22	13.86 ±1.09 ns	13.81 ±2.09 ab	1.18 ±0.09	0.11 ±0.03 b	0.15 ±0.10 ab

Each value is the mean of 3 replicates (n=9). Means with different letters are significantly different at p = 0.05 + standard error. All values with the same letters/or ns are not significantly different.

In conclusion, the most appropriate MW of chitosan for prolonging the shelf life of 'Nam Dok Mai' mango fruit was 240 kDa, and the coating solution should be freshly prepared in order to minimize the susceptibility to postharvest disease. Mango fruits coated with fMM-CTS had 15 days of shelf life and low anthracnose incidence. This finding would be an advantage for improvement of ‘Nam Dok Mai’ mango quality.

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