CHAPTER 4

EFFECTS OF POTASSIUM SORBATE INCORPORATED WITH CHITOSAN COATING ON LONGAN FRUIT DECAY

4.1 Abstract

This work investigated the effectiveness of the incorporation of potassium sorbate (PS) as an antimicrobial additive in the chitosan (Cts) coating when dissolved with citric acid (CA) on the longan fruit decay. The fruits were dipped in edible coating after washing in 0.10% (pH~7.0) sodium hypochlorite for 1 min. The coating solutions were mixtures of 1.2% Cts and 3.0% CA that contained 0, 0.3, 0.6 and 1.0% (w/v) PS respectively. The fruits were air dried, packed in a foam tray and wrapped with 11 µm PVC film. The samples were stored at 10˚C for 15 days. During storage, samples including a control (dipping fruit in distilled water) were collected every three days for fruit decay analysis of yeast and mold population (log colony forming unit/ml) on the fruit surfaces and disease incidence percentage, sorbic acid as active substance degradation in pericarp and aril together with the effect on the discoloration parameters: browning index, Hunter's L* C* h˚ scales, pericarp pH and titratable acidity (TA) of pericarp homogenate were analyzed. Application of 0.3 – 1.0% (w/v) PS in Cts coating produced a better fruit decay control in the fruit by reducing in mold population including disease incidence percentage in comparison with control fruit and no additive of PS in the coating. Addition of 0.3% PS in coating showed the optimum concentration to control fruit decay because its efficacy on disease control was not significant with the higher PS in the coating. Moreover, sorbic acid residue of this treatment was not detected on the aril whereas sorbic acid degradation in pericarp was increased as application of PS concentration in coating was increased. Its degradation during storage was a negative exponentials curve. However, application of PS in coating more than 0.6% showed more darkening of fruit pericarp due to phytotoxicity of this compound which indicated by higher pericarp pH and

lower TA in pericarp homogenate but not weight loss percentage. Addition of PS at higher concentrations could disturb some coating properties by reducing viscosity and increasing the pH of the solution.

4.2 Introduction

Chitosan coating could prolong shelf life of fresh longan (Jiang and Li, 2001) and litchi fruit (Zhang and Quantick, 1997). Chitosan is a good film forming property, biocompatibility with carrier compounds and therefore it can be added with the other substances (fungicides, food additives, antioxidants and coloring agents) for improving its efficacy (No *et al*., 2007). Apai *et al*. (2008a) found that dipped longan fruit in Cts + CA showed the best browning control but its efficacy in disease control was not as good according to Joas *et al*. (2005) reported that litchi after coating with CA+Cts should store at low storage more than ambient storage for protecting fungal attraction (personal communication).

The growth of pathogenic microorganisms may be prevented by incorporating antimicrobial agents into edible films or coatings (Debeaufort *et al*., 1998). The antimicrobial agents most commonly used in edible coatings are: sorbic acid, proprionic acid, potassium sorbate, benzoic acid, sodium benzoate and citric acid (Quintavalla and Vicini, 2002). Potassium sorbate (PS) as well as sodium benzoate is one of most popular antimicrobial agents which is added in edible coatings and films (Pranoto *et al*., 2004) and edible coating (Park *et al.,* 2005). It improved fruit decay control in many fresh produces such as banana (Al Zaemey *et al*., 1994), strawberry (Garcia *et al*., 1998; Park *et al*., 2005) and fresh-cut produces (Baldwin *et al*., 1996) PS was also used for controlling fruit decay in longan (Kaewsorn *et al*., 2005; Kheuenmanee *et al*., 2005; Chartupos and Kongbangkerd, 2002). In a preliminary showed that addition of PS in CA at concentraton of 1.0% in 1.2% Cts showed less viscosity than that added in 3.0% CA+ 1.2% Cts. Therefore, 1.2% Cts+3.0% CA in combination with PS at various concentrations were used.

The objectives of the present work were: (1) to investigate the effectiveness of the incorporation of potassium sorbate (PS) as an antimicrobial additive in the chitosan (Cts) coating dissolved with citric acid (CA) on the longan fruit decay; (2) to

study the interactive effects between PS and Cts+CA on fruit decay and discoloration parameters; and (3) to find the optimum PS concentration incorporating Cts+CA on the microbial growth and sorbic acid degradation in edible portion and peel during storage at 10°C.

4.3 Materials and Methods

4.3.1 Plant material and coating preparation

Mature longan fruits (*D. longan* Lour.) cv Daw were harvested from a commercial GAP orchard in Chiang Mai. Fruits were selected in uniformity of shape, color and size; then any blemished or diseased fruits were discarded. Stem of the fruits were left approximately 5 cm. They were washed using sodium hypochlorite (0.1%) at pH 7.0 for 1 min and air dried.

To prepare 2,000 ml of 1.2% chitosan (Cts) solution, 24 grams of chitosan (high molecular-weighted shrimp flake, Ta Ming Enterprise Co., Ltd., Thailand) were dispersed in 1,500 ml of boiling water under magnetic stirring to which 60 grams of 3% citric acid (CA; food grade) was added to dissolve the chitosan. 0, 0.3, 0.6 and 1.0% w/v Potassium sorbate (PS; food grade) as an antimicrobial agent were added in coating solution and stirring until completely soluble. The solution was cooled down in a hood. After that made up the solution to 2,000 ml, measured pH solution using pH meter (Consort C321) and viscosity using Brookfield viscometer (model LVDV-III) with spindle no 1 at 200 rpm and 25°C.

4.3.2 Experiments

4.3.2.1 The interaction effects between potassium sorbate and coating material on fruit decay

The interaction effects between PS and Cts+CA on discoloration and fruit decay was studied. PS concentration at 0, 0.3 and 1.0% was used for addition together with or without Cts+CA. Fruits were dipped for 5 min in different solutions. After dipping, the fruits were air-dried by electric fan, packed in foam tray wrapped with 11 μm thick PVC film (10 fruits per foam tray) and then stored at 10ºC with 85% RH. For each treatment, three replicates were used. Samples were taken every day 5

during storage for quality evaluation until day 15. Fruits were evaluated for changes in disease incidence, browning index, fruit surface color both inner and outer pericarp and weight loss percentage.

4.3.2.2 Effects of potassium sorbate incorporated with chitosan coating on fruit decay and sorbic acid degradation in longan

Based on the results of the recent study, to more intensively investigate the optimum PS concentration optimum. PS concentration incorporating Cts+CA on the microbial population and sorbic acid degradation in edible portion and peel was investigated. Fruits were dipped for 5 min in 0, 0.3, 0.6 and 1.0% (w/v) potassium sorbate (PS; food grade) were added in coating solution in above (3.1). After dipping, the fruits were air-dried by electric fan, packed in foam tray wrapped with 11 μm thick PVC film (15 fruits per foam tray) and then stored at 10ºC. Dipping in distilled water was used as control. For each treatment, three replicates were used. Samples were taken every day 3 during storage for quality evaluation until day 15. Fruits were evaluated for changes in microbial population including disease incidence and sorbic acid degradation in edible portion and peel, browning index and fruit surface color of outer pericarp, TA, pericarp pH and weight loss.

4.3.3 Fruit decay and sorbic acid degradation in longan fruit.

Yeasts and molds on the fruit surface was analyzed modifying method of Whangchai *et al*. (2006). Five longan fruits per replication were placed in 50 ml sterile distilled water in Erlenmeyer flask and shaken at 250 rpm for 30 min. For each replication, a sample (0.1 ml) of the suspension was spread over acid potato dextrose agar (PDA) medium. The PDA plates were incubated at 25 °C for 5 day and the survival of microorganisms was expressed as the mean number of colony forming units (log cfu ml^{-1}). Disease incidence (%) was observed using magnified lens or visual on a number of the fruit that showed lesion of mycelium or rot on the fruit surface.

Sorbic acid (SA) degradation in pericarp and aril were analyzed by modifying method of Kamlert (1992). Thirty fruits, ten of each of the three replications, were used to prepare sorbic acid samples. Pericarp and aril were removed from the fruit at

the time of decaying analysis, finely ground using the Moulinex blender and weighing about ~5.00 g in 100 ml volumetric flask. Adding 80 mL of extract solution (HPLC grade methanol : 0.01 M Ammonium acetate buffer (pH 4.5-6) (60:40) and each of 1 mL of Carrez I (15% potassium ferro cyanate) and Carrez II (23% zinc acetate) respectively, shaking by hand thoroughly, adjusted volume to 100 mL and stored at 25 °C for 15 min. The solutions were filtered through no. 42 paper filters and then through a 0.45-µm nylon membrane filter before injection. The sorbic acid concentration in each solution was determined using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) with a diode array detector, auto sampler, Hypersil BDS C18 column (inner dia, 150 × 5 mm) with guard column and computer with chemstation software. The mobile phase was methanol: 0.01 M Ammonium acetate buffer (pH 4.5-4.6) (60:40), the injection volume was 20 µL and the flow rate was 1.0 mL/min. Absorbance was read at 235 nm and run time was 10 min. Calibration curve was created by diluting PS calculation as sorbic acid in the concentration ranges from 0.01 to 100 mg/L. The method recovery test as the accuracy of sorbic acid analysis was done by spiking 20 mg/L SA (middle range) in the blank samples and it was 93-96%. The determination of linearity (R^2) of the standard curve was 0.9995. The SA contents in pericarp or aril were expressed as mg/kg.

4.3.4 Effect of PS in coating material on pericarp browning, pericarp color, pericarp pH, titratable acidity, weight loss

Browning was assessed visually by measuring total browning areas of the pericarp on each of fifteen fruit (Jiang and Li, 2001). The color of outer pericarp of longan was measured with a colorimeter (Color Quest XE) according to the CIELAB scale (L*, C* and h˚). The pH of the pericarp homogenate and the titratable acidity, expressed as milliequivalents of acids per 100 g of pericarp, was measured (Joas *et al*., 2005). Weight loss percentage was determined.

4.3.5 Statistical Analysis

In experiment 3.2.1, the statistical model was a 2 × 3 factorial completely randomized design (CRD) comprising 2 levels of coating material at non-coat

(distilled water) and coated fruit (Cts+CA) and 3 levels of PS concentration at: 0, 0.3 and 1.0%.

In next experiment, they were arranged as CRD Analysis of variance (ANOVA) and the test of mean comparison according to least significant difference (LSD) were applied with a significance level of 0.05. The SPSS software version 10 for Windows was used as a statistical analysis tool.

4.4 Results and discussion

4.4.1 The interaction effects between potassium sorbate and coating material on fruit decay

4.4.1.1 Disease incidence

One reason for the experiment was to compare the efficacy of PS and acid coating in a coating matrix to an aqueous solution of PS at low pH for controlling fruit decays. Figure 4.1displayed the results of decay incidence in longan fruit. It was found that an addition of all PS concentration in acid coating (0, 0.3 and 1.0% PS in Cts+CA and pH 2.3, 2.6 and 3.1) could delay fruit decay. The efficacy of all PS and PS in Cts were better than PS alone in alkaline (0, 0.3 and 1.0% PS and pH 6.0, 7.4 and 8.0). Result indicated that using PS in combination with acid coating dip helped control fruit decay compared to using PS alone. Higher PS concentration showed higher efficacy in fruit decay (Table 4.1). McGuire and Baldwin (1998) found that Nature seal® (0.15% PS+CA containing in cellulose coating, pH 2.5) was be able to delay disease incidence in litchi fruit by maintaining acidic environments in food (Torres, 1985). The main antimicrobial effect of sorbic acid has been attributed to the undissociated acid penetrating the microbial cell wall and then disassociating in higher pH cytoplasm. (Stratford and Anslow, 1996). Chitosan is a hydrophilic polymer obtained industrially by hydrolyzing the aminoacetyl group of chitin using an alkaline treatment. It has been widely used in antimicrobial films and coatings because of its antimicrobial effect. Chitosan reacts with the strongly electronegative microbial surface leading to changes in membrane permeability, metabolic disturbances and eventually death (Romanazzi *et al*., 2002). Difference in pH of

solution and the types of organic acid solvents had affected on the charge density of cationic in chitosan molecule which observed by FTIR spectra (Kim *et al*., 2006) and response to fruit quality.

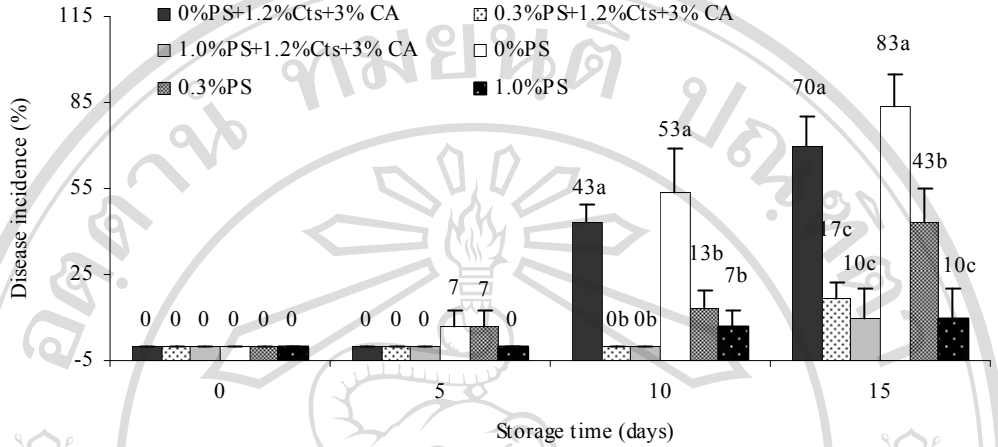

Figure 4.1 Chitosan coating and PS on disease incidence during storage at 10ºC for 15 days. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

Table 4.1 The interaction between chitosan coating and PS on disease incidence during storage at 10ºC for 15 days.

	Disease incidence (%)			
	0	**5**	**10**	**15**
Factor A				
1.2% Cts+3% CA[2]	0	0b[1]	14.44b	32.22b
DW	0	4.44a	24.44a	45.56a
Factor B				
0%PS	0	3.33	48.33a	76.67a
0.3%PS	0	3.33	6.67b	30.00b
1.0%PS	0	0	3.33b	10.00c
A		*	*	*
B		ns	*	*
A x B		ns	ns	*
CV. (%)		150	38.33	25.71

[1]Same letters in the same column are not significantly different at 0.05.

[2]Cts (chitosan), CA (citric acid), PS (potassium sorbate) and DW = distilled water.

4.4.1.2 Pericarp browning and surface fruit color

Browning index of pericarp was shown in Table 4.2, acid coating (1.2% Cts+3% CA) was the main effect to reduce the change in pericarp browning. Addition of all PS concentration in acid coating (0, 0.3 and 1.0% PS in 1.2% Cts+3% CA and pH 2.3, 2.6 and 3.1) delayed pericarp browning more effective than all alkaline PS concentrations alone (0, 0.3 and 1.0% PS solution and pH 6.0, 7.4 and 8.0) (Figure 4.2). Results indicated that using PS in combination with acid coating dip could control pericarp browning compared to using PS alone. Higher PS concentration showed higher severity on fruit browning. Higher PS concentration at 1.0% showed higher severity in pericarp browning more than the lower PS concentration (0 and 0.3% PS).

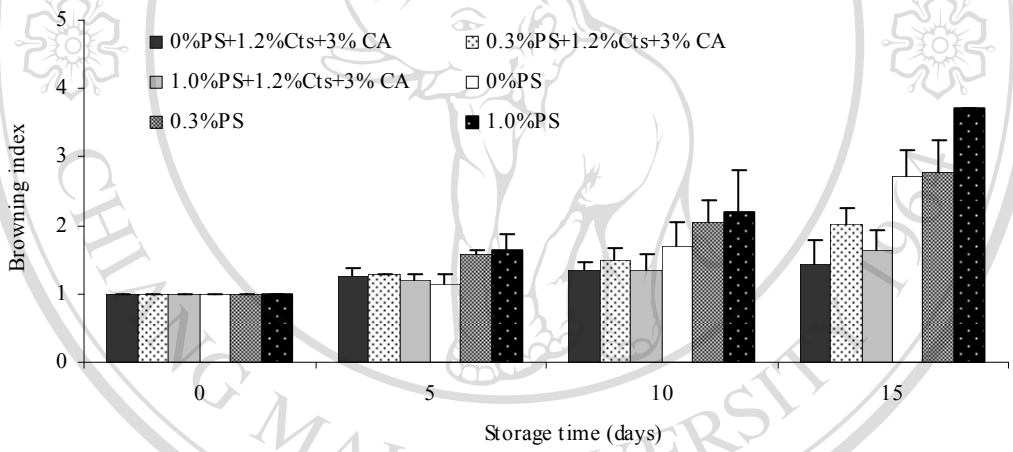

Figure 4.2 Effects of chitosan coating and PS on browning index during storage at 10ºC for 15 days. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

Figure 4.3 Effect of chitosan coating + 0.3% PS (a) and 0.3% PS without chitosan (b) on browning appearance during storage at 10˚C for 15 days.

Table 4.2 The interaction between chitosan coating and PS on browning index during storage at 10ºC for 15 days.

		Browning index			
	Phytotoxic[3]	**0**	**5**	**10**	**15**
Factor A					
1.2% Cts+3% CA[2]	1.53[1]a	1	1.26b	1.39b	1.70b
DW	1.28b	1	1.44a	1.98a	3.07a
Factor B					
0%PS	1.47	1	1.20b	1.52	2.08b
0.3%PS	1.47	1	1.43a	1.77	2.40ab
1.0%PS	1.28	1	1.42a	1.77	2.67a
A	*		*	*	*
B	ns		*	ns	*
A x B	ns		*	ns	*
CV. (%)	9.63		10.18	14.95	13.67

[1]Same letters in the same column are not significantly different at 0.05.

[2]Cts (chitosan), CA (citric acid), PS (potassium sorbate) and DW = distilled water.

[3]Phytoxic of substances on fruit surface = visual evaluation after treating at day 1 in 3 scores: 1 = Normal, 2 = moderate, 3 = virulent.

Effect of acid coating (Cts+CA) was highly significant reduce color intensity (C*). Higher PS concentration significantly decreased in L* value including C* and h values in fruit pericarp which indicated darkening during storage. Interaction between the effects of % PS levels and coating levels significantly influenced in L* value including C* and hue during storage (Table 4.4). Using only PS at concentration of 1.0% alone increased darkening of outer and inner pericarp skin which had the lowest L* value as well as C* and h values during storage at 15 day (Table 4.3). This suggested that all alkalizes pH influenced on pericarp skin damage in comparison with all acidic coating containing in PS due to its good moisture absorption. According to appearance of the longan fruits, as assessed visually during the 15-day storage period, is presented in Figure 4.3.

Table 4.3 Effects of chitosan coating and PS concentrations on the changes in L* C* and h during storage at 10ºC for 15 days.

	L*					C*				h			
	0	5	10	15	15 L* inner	0	5	10	15	0	5	10	15
0%PS+1.2%Cts+3%CA[2]	52.75a	53.15b	52.86c	52.03a	74.33a	26.23a	27.25a	24.19a	24.92a	67.80a	67.54a	64.66a	63.69a
0.3%PS+1.2%Cts+3%CA	53.27a	53.72ab	52.42c	51.76a	73.47a	25.94a	27.12a	24.09a	23.43a	68.08a	68.09a	64.17a	63.24a
1.0%PS+1.2%Cts+3%CA	53.00a	53.18b	53.47b	52.54a	74.03a	25.49a	26.61a	24.40a	24.00a	67.26a	67.23a	64.77a	63.37a
0%PS	53.50a	53.91a	54.14a	52.59a	72.34ab	21.11b	21.95b	20.88b	20.10b	65.63b	65.01b	63.04b	61.79ab
0.3%PS	52.63a	53.17b	52.28c	51.88a	72.24ab	20.12bc	21.16b	19.45c	19.84b	64.88b	64.42b	61.44c	60.75b
1.0%PS	50.82b	51.47c	51.38d	50.08b	71.07b	18.65c	19.70c	18.98c	17.36c	62.96c	63.05c	59.56d	57.49c
CV (%)	1.01	0.64	0.63	0.86	1.69	2.61	2.17	2.08	3.08	1.35	0.99	0.65	1.65
F-test	*	*	*	*	ns	*	*	*	*	*	*	*	*
LSD0.05	0.95	0.61	0.59	0.79	2.2	1.06	0.93	0.81	1.81	1.58	1.66	0.72	1.82

[1]Same letters in the same column are not significantly different at 0.05.

Ns = non significant; P< 0.05*

[2]Cts (chitosan), CA (citric acid), PS (potassium sorbate) and DW = distilled water.

Table 4.4 Interaction between chitosan coating and PS concentrations on the changes in L* C* and h during storage at 10ºC for 15 days.

	L* value of outer peel					Chroma				Hue			
	0	5	10	15	15 L* inner	0	5	10	15	0	5	10	15
Factor A													
1.2%Cts+3%CA[2]	53.01a	53.35a	52.92	52.1a	73.95a	25.89a	27.00a	24.23a	24.12a	67.72a	67.62a	64.54a	63.44a
DW	52.32b	52.86b	52.60	51.52b	71.89b	19.96b	20.94b	19.77b	19.11b	64.49b	64.16b	59.56b	60.01b
Factor B													
0%PS	53.13a	53.53a	53.50a	52.3a	73.34	23.67a	24.60a	22.54a	22.52a	66.72a	66.28a	63.86a	62.74a
0.3%PS	52.96a	53.45a	52.35b	51.82ab	72.86	23.03ab	24.14a	21.77b	21.64ab	66.48a	66.26a	62.81b	62.00ab
1.0%PS	51.91c	52.33b	52.43b	51.31b	72.56	22.08b	23.16b	21.69b	20.68b	65.11b	65.89b	62.16b	60.44c
A	*	*	ns	*	*	*	*	*	*	*	*	*	*
B	*	*	*	*	ns	*	*	*	*	*	*	*	*
A x B	*	*	*	*	ns	ns	ns	*	*	ns	ns	*	*
CV(%)	1.01	0.64	0.63	0.86	1.69	2.61	2.17	2.08	3.13	1.35	0.99	0.65	1.65

Ns = non significant; P< 0.05*

[2]Cts (chitosan), CA (citric acid), PS (potassium sorbate) and DW = distilled water

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4.4.1.3 Weight loss percentage

The weight loss percentage of longan increased with storage time increased (Figure 4.4). Hydrophilic polymers, especially chitosan, had medium moisture barrier properties but good O_2 barrier (Wong *et al*, 1992). However, addition of Cts in CA (Table 4.5) appeared to further improved weight loss control in comparison with non-added treatment ($p < 0.01$). Addition of all PS treatments increased weight loss percentage at day 10 ($p < 0.05$), but it showed no difference in day 5 and 15. Application only PS at 1% in water showed the highest weight loss percentage in contrast with all PS added in Cts which showed the lowest weight loss percentage (Figure 4.4). Thus, addition of 0 or 0.3% PS in Cts might be adequately effective against weight loss percentage control.

Table 4.5 The interaction between chitosan coating and PS on the changes in weight loss percentage during storage at 10ºC for 15 days.

	Weight loss percentage			
	0	**5**	**10**	**15**
Factor A				
1.2% Cts+3% CA	0	1.83	3.16b	4.39b
DW	0	1.92	3.57a	4.69a
Factor B				
0%PS	0	1.82	3.23b	4.52
0.3%PS	0	1.87	3.41ab	4.51
1.0%PS	0	1.92	3.47a	4.59
A		ns	*	*
B		ns	*	ns
A x B		ns	ns	ns
CV. (%)		4.73	4.11	4.47

[1]Cts (chitosan), CA (citric acid) and PS (potassium sorbate).

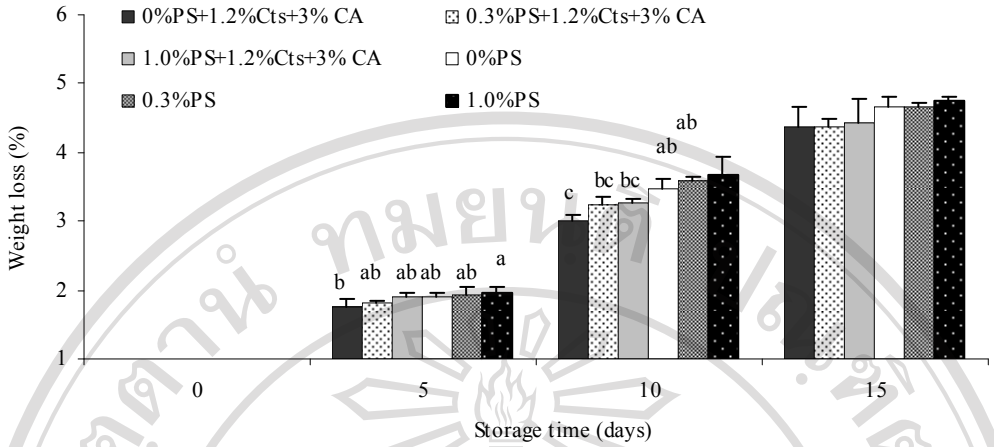

Figure 4.4 Effects of chitosan coating and PS on the changes in weight loss percentage during storage at 10ºC for 15 days. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

4.4.2 Effects of potassium sorbate incorporated with chitosan coating on fruit decay and sorbic acid degradation in longan

4.4.2.1 Coating property changes: viscosity and pH

The viscosity and pH of the treatment solution was shown in Table 4.6. The results showed that CA is another way was used for dissolving chitosan film and coating (Begin and Calsteren, 1999) which might be prepared in boiling water. The different response of the types of acid coating was found by Caro and Joas (2005) who found that dipping litchi fruit in Cts+CA at low pH dip showed the best consistency of dispersion of chitosan in surfaces comparing to Cts+tartaric acid.

Adding of PS at higher concentration up to 1.0% in Cts+CA disturbed the change in coating properties which showed higher pH and lower viscosity. It is important to know that the addition of PS to a food product will raise the pH by approximately 0.1-0.5 pH unit depend on the amount, pH, and type of product. If pH of the product is below 4.50, it could provide greater protection against a wider variety of microorganisms (Branen and Davidon, 1983). The result found the negative correlation between pH solution and viscosity (Figure 4.5). The pH solution increased as viscosity decreased due to PS addition in Cts+CA (Table 4.6). The disturbance of PS incorporating Cts+CA on film structure did not show at any report.

In Cts+acetic acid, Chen *et al*. (1996); Pranoto *et al*. (2004) similarly reported that the FT-IR spectrum showed the ionic interaction between -COO^- of preservatives and -NH^{3+} of chitosan existed in the film. However, the incorporation of preservatives did not affect the tensile strength and elongation property of the methylcellulose/chitosan film.

Table 4.6 The changes in coating properties (viscosities and pH) after PS addition in coating materials.

Coating formulations	pH solution[2]	Viscosity[2] (Cps)
0.0% PS+1.2% Cts+3.0% CA	2.42±0.03[1] a	4.43±0.02d
0.3% PS+1.2% Cts+3.0% CA	2.71±0.02 b	4.25±0.05 c
0.6% PS+1.2% Cts+3.0% CA	2.98±0.02 c	3.99±0.01 b
1.0% PS+1.2% Cts+3.0% CA	3.30±0.01 d	3.86±0.03 a
$LSD_{0.05}$	5.51	0.73
CV.	0.7	3.95

[1]Same letters in the same column are not significantly different at 0.05.

[2]Consort C831 as pH meter and Brookfield viscometer LVDV-spindle no.1-220 rpm were conducted at 25±0.4°C (Cps = centipoises)

[3]Cts (chitosan), CA (citric acid) and PS (potassium sorbate).

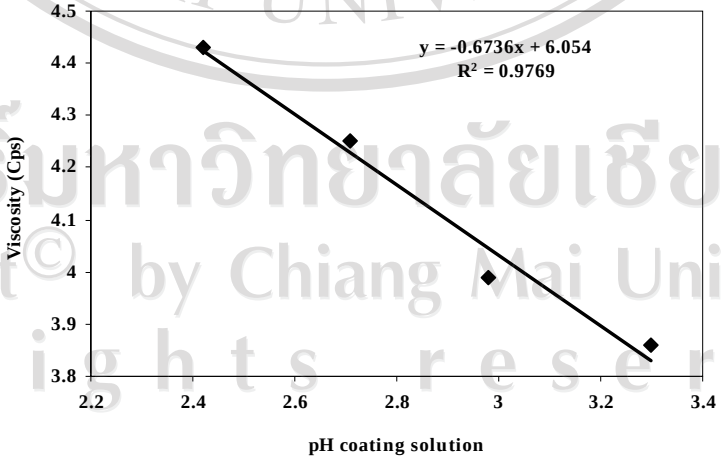

Figure 4.5 Positive relations between pH coating and viscosity measuring at 25˚C.

4.4.2.2 The effects on disease incidence (%) and yeasts and molds counts on fungal occurrence surface of fruit

From Figure 4.6, the results showed that addition of all PS in coating material: 1.2% chitosan (Cts) + 3.0% citric acid (CA) significantly delayed disease occurrence (disease incidence; DI) and molds and yeasts population during storage as compared with control and non-added PS in coating material ($p<0.01$). It was recognized that higher concentration of PS in the edible coating material, promoted high effectiveness of the antimicrobial properties. The presence of PS in the edible coating material at 0.3 up to 1.0% similarly showed greater effectiveness on the growth inhibition of molds and yeasts growth than washing with 0.1% chlorine alone and non-added PS in coating material. Sorbic acid action on dehydrogenase enzymes makes molds and yeasts unable to metabolize carbon double bonds in the alpha position with the carboxyl group (Eklund, 1983). It controlled disease incidence (DI < 25%) during storage at 10˚C for 15 days when compared with untreated fruits and non-added PS in coating material for 5 and 9 days.

The important mechanism PS incorporating Cts+CA to control disease development might therefore be involved with pH of coating. The main antimicrobial effect of sorbic acid has been attributed to the undissociated acid penetrating the microbial cell wall and then disassociating in higher pH cytoplasm. The H^+ released was believed to inhibit glycolysis and growth (Stratford and Anslow, 1996). The antimicrobial activity was therefore very dependent on the pH as reported by Sofos and Busta (1981), who found the best activity was due to the undissociated form of the acid when pH was less than pKa (pKa-4.75). These results were in accordance with those of Baldwin *et al*. (1996) who reported that Nature Seal (an edible coating, pH-2.5) containing PS significantly reduced mould count. Garcia *et al.* (1998) also reported that addition of CA in 0.2 g/L PS in coating materials enhanced antimicrobial action of PS which showed the lowest microbial counts in strawberry. Difference of pH solution and the types of organic acid solvents had been affected on chitosan properties (Kim *et al.,* 2006). They found that lower pH of Cts film showed a lot of charge density of cationic in chitosan molecule which observed by FTIR spectra.

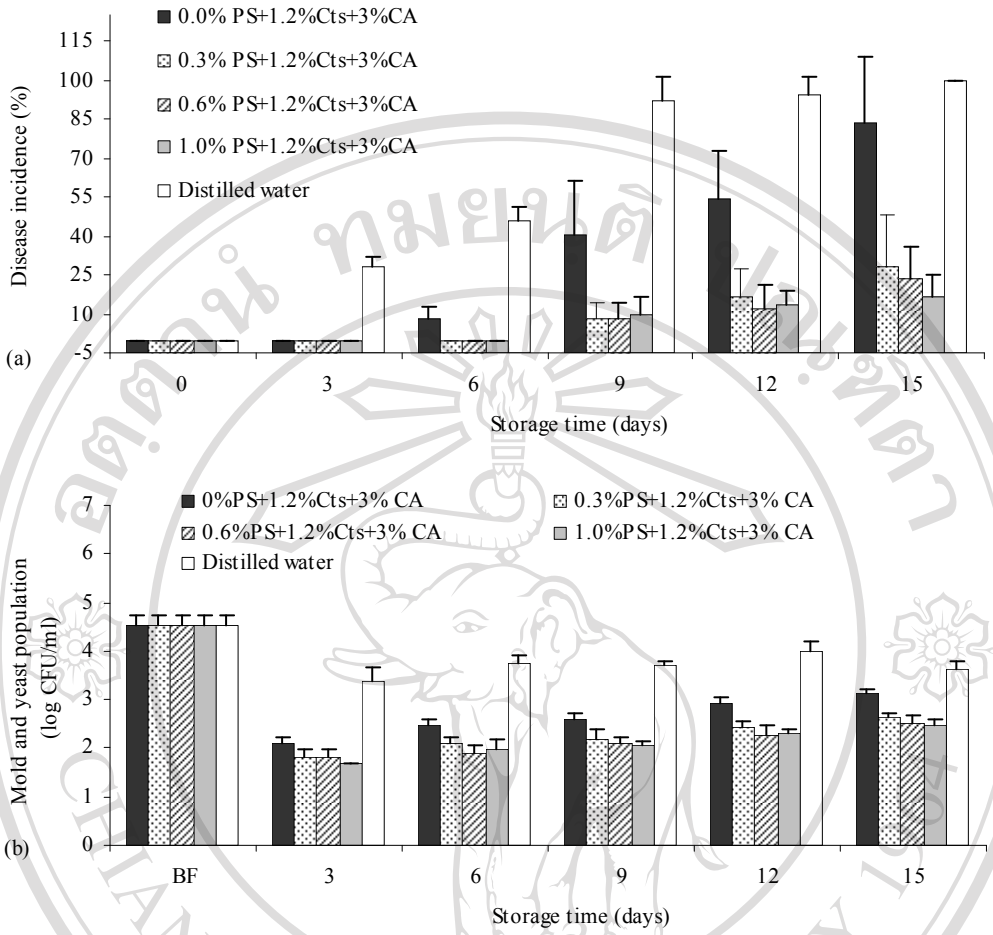

Figure 4.6 Effects of PS+Cts on disease incidence percentage (a) and yeast and mold population expressed as colony forming unit (log CFU/ml) of longan fruit during storage at 10°C. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

It was suggested that the cationic amine group (NH_2) increased ionization as pH decreased and could react with anionic ion in the cell membrane causing leakage (Leuba and St¨ossel, 1986; Liu *et al*., 2007). Generally, PS is effective against yeasts, molds, and select bacteria and is widely used at 0.025 to 0.10% levels in cheeses, dips, yogurt, sour cream, bread, cakes, pies and fillings, baking mixes, dough's, icings, fudge, toppings, beverages, margarine, salads, fermented and acidified vegetables, olives, fruit products, dressings, smoked and salted fish, confections and mayonnaise. Sorbate at the levels used in food product at will not control the growth

of high levels of microorganisms. Therefore, always use good quality ingredients and follow good manufacturing practices to keep the microbial load to a minimum.

4.4.2.3 Sorbic acid degradation on pericarp and aril

Sorbic acid degradation in pericarp and aril as a function of time stored at 10˚C. The result showed that sorbic acid content positively increased following the concentration (Figure 4.7). High positive correlation between sorbic acid in pericarp and aril were found (Figure 4.8). At the beginning storage, coated fruit with 1.0% PS+Cts+CA showed the highest amount of sorbic acid content (835.0 mg/kg) followed by 0.6 and 0.3% PS+Cts+CA (259.2 and 90.13 mg/kg), respectively. The degradation of sorbic acid in pericarp of all PS concentration in Cts+CA was different ($p<0.01$) and relation between active substance degradation and storage time. The degradation of PS in longan pericarp was higher during the first 6 days, as seen in Figure 4.7a and at the end of 15 days there was 71.0, 16.18 and 2.89 mg/kg of 1.0, 0.6 and 0.3% PS+Cts+CA. Coated fruit with the highest PS concentration of 1.0% PS Cts+CA showed the highest amount of sorbic acid in the pericarp.

At the beginning of the storage, coated fruit with 1.0% PS+Cts+CA showed the highest amount of sorbic acid content (5.48 mg/kg) in the aril compared to 0.6% PS (2.18 mg/kg) and 0.3% Cts+CA was not detectable. The degradation of PS in longan aril was higher during the first 6 days, as seen in Figure 4.7b and at the end of 15 days there was 0.35 mg/kg of 1.0 % PS+Cts+CA. Whereas 0.6% PS+Cts+CA was 0.17 mg/kg at day 9 and after that it was not detected. Coated fruit with the highest PS concentration of 1.0% PS+Cts+CA showed the highest sorbic acid residue in the aril greater than lower PS concentration of 0.6% PS Cts+CA. While coated fruit with 0.3% PS+Cts+CA was not detected as well as control fruit.

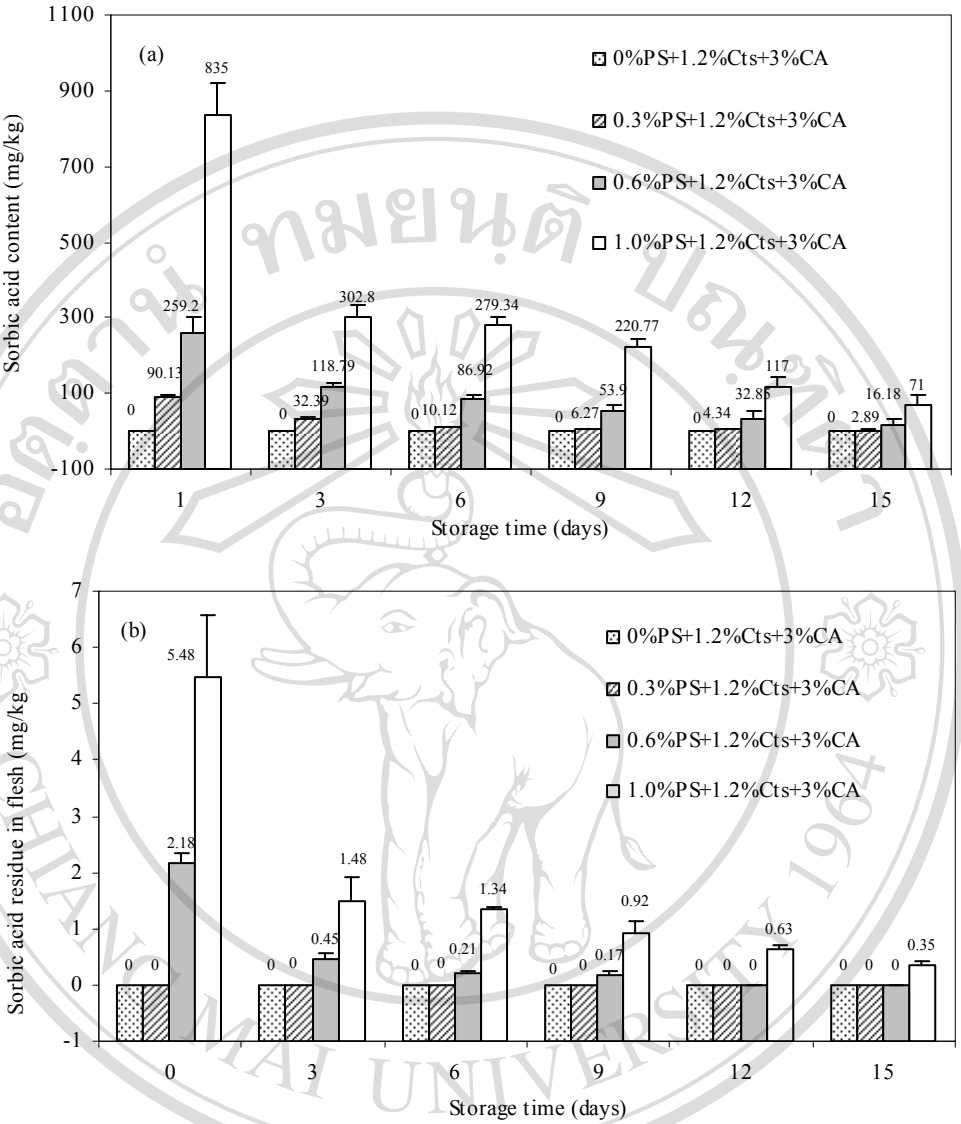

Figure 4.7 Effects of PS+Cts+CA on sorbic acid profiles in pericarp (a) and aril (b) of longan fruit during storage at 10°C. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

PS is considered one of the least harmful preservatives in use; the World Health Organization (WHO) has set its acceptable daily intake (ADI) at 25 mg/kg of body weight (Sofos and Busta, 1983). Sorbic acid is not toxic for human being, because it is metabolized similarly as a fatty acid. To prolong storage life of fruits and vegetables, generally the product is dipped in 50 – 100 g/L (Restaino *et al*., 1981). In our coatings, 3 g/L plus CA provided a sufficient high and effective concentration of preservative that allowed a reduction of its microbial counts and fruit

decay percentage on longan. Thus, edible coatings and films act as surface retention agents, particularly when additives are included in the formulation and limit preservative diffusion in the food core (Vodjani and Torres, 1989).

Limjaroen *et al*. (2005) reported that using sorbic acid–containing films, common spoilage organisms were also inhibited on meat product and cheese. After 28 days of contact with bologna and Cheddar cheese, these films retained 7% and 60% of their original sorbic acid content, respectively, with the control film retaining 85% of its original sorbic acid content. Longan pericarp did not like chitosan film which was made in the same structural and chemical composition. Longan pericarp was many variations in morphologies such as different in non-consistency cuticle, structural pore, thickness, water content, and chemical substances in tissue (Jaitrong, 2006; Suwanakood, 2007) and these parameters could affect on diffusion process of active substance of surface coating in pericarp skin including food industry (Franssen and Krochta, 2003). Therefore, diffusion process of edible coating on fruit skin is difficult to study and no report was studied in diffusion process of edible coating in fruits. Min and Krochta (2008) supported hypothesized of Chen *et al.* (1999) who suggested that edible coating could serve as food additive carriers by accumulation of benzoic acid of both fruit preserves contained 50–100 μg of benzoic acid (per gram) and yeast growth was inhibited and no effect on sensory evaluation.

Ou *et al*. (2002) found that estimation of amount of benzoic acid content in filled tilapia surface coated with gelatin containing benzoic acid on microbial load were between 16.3 mg and 17.3 mg per g of fillets. Film casting incorporating food additives was directly measured by releasing in buffer as representative diffusion of edible coating (Torres *et al*., 1985; Vodjani and Torres, 1990). In this study on sorbic acid distribution on edible coating on pericarp skin, although coated fruit with the highest PS concentration of 1.0% PS+Cts+CA could help to slow down the rate of sorbic acid degradation in the pericarp greater than lower PS concentration of 0.6 and 0.3% PS+Cts+CA, respectively (Figure 4.7a). In nevertheless, pericarp tissue damage after coated fruit at above 0.6% was occurred during storage because of phytotoxic from sorbic acid substance due to interaction between PS and CA (Table 4.2). At above PS at concentration of 0.6% showed not compatible it together which indicated by higher viscosity and lower pH after adding of PS in coating materials (Table 4.6).

In conclusion, 0.3% PS+Cts+CA could be used for the optimum treatment for protecting fruit decay because it showed less phytotoxicity on pericarp tissue and low disease incidence comparing to control fruit.

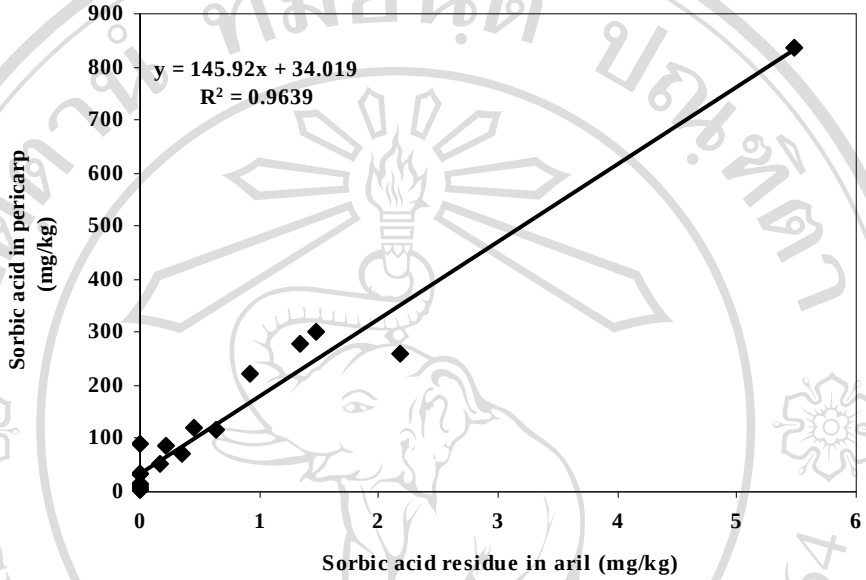

Figure 4.8 Positive relation between sorbic acid in pericarp and aril of longan fruit during storage at 10°C.

4.4.2.4 Pericarp browning

Addition of all PS concentrations in Cts+CA did not affect on pericarp browning in comparison with control fruit during the first 12 days (Figure 4.9). However, PS at concentration of 0-0.6% in Cts+CA was quite low browning index compared to 1.0% PS+Cts+CA and control fruit at day 15. These results related to (%) PS addition in Cts+CA which was much lower browning index in Cts+CA alone than that PS added in Cts+CA as their concentrations increased. Higher PS at concentration up to 1.0% might induce pericarp damage due to high relative humidity inside the packaging. Ethylene and respiration production were rapidly increased during tissue damage and become browning (Abeles *et al*., 1992). This experiment suggested that 0.3% PS in Cts+CA might be used to protect pericarp browning and fruit decay.

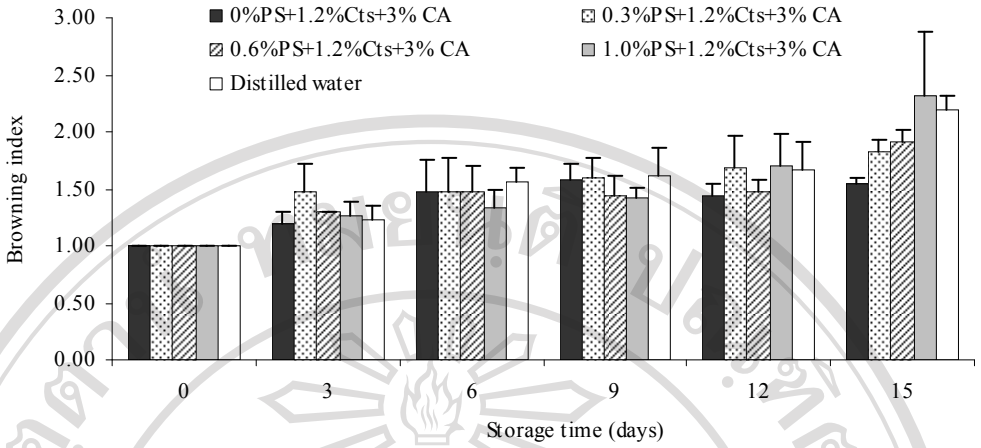

Figure 4.9 Effects of PS+Cts on browning index of longan fruit during storage at 5°C. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

4.4.2.5 Weight loss percentage

Addition of all PS concentrations in Cts+CA did not influence on weight loss percentage in comparison with control fruit during the first 12 days (Figure 4.10). However, PS at concentration of 0-1.0% in Cts+CA was slightly low weight loss percentage compared to control fruit at day 15. It was showed that storage condition with high humidity inside packaging during storage minimized direct water losses and preserved fruit color of pericarp at least three weeks. After storage for four weeks, fruits were infected by fungi on all treatments (data not shown).

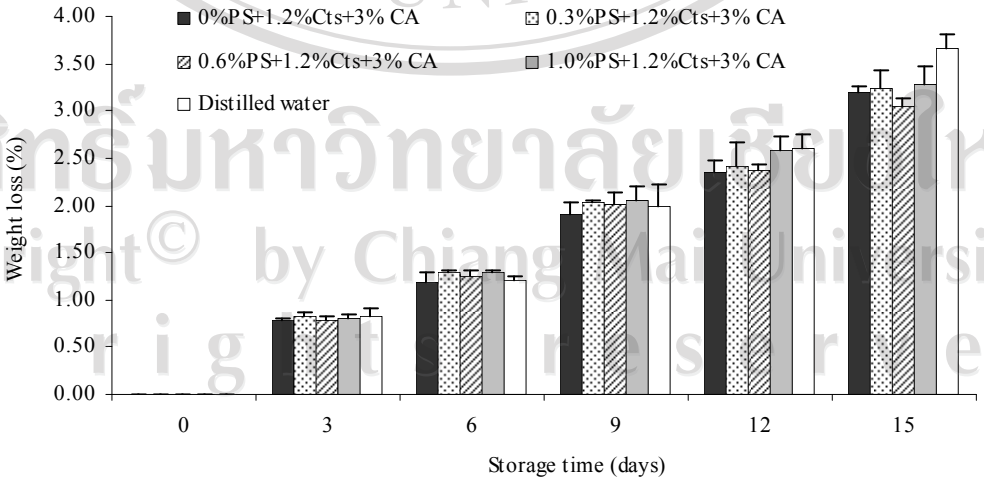

Figure 4.10 Effects of PS+Cts on weight loss percentage of longan fruit during storage at 10°C. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

4.4.2.6 Pericarp pH and citric acid content in pericarp homogenate

Results on pH and titratable acidity (TA) were presented in Figure 4.11. The increasing in pericarp pH of all PS concentration in Cts+CA was increased with PS concentration increased. They were related to citric acid degradation (TA) and storage time. Pericarp pH was lower and titratable acidity higher in 0-0.6% PS in Cts+CA than in 1.0% PS in Cts+CA and control fruit (distilled water) after 15 days of storage. Adding of PS at higher concentration up to 1.0% in Cts+CA disturbed the change in coating properties which showed higher pH and lower viscosity due to interaction either PS and CA or Cts, this may have an impact on acid impregnation in pericarp skin during storage. Higher PS at concentration of 1.0% might induce pericarp damage caused by a large amount of sorbic acid content in pericarp skin (Figure 4.9) and reduction of TA after this treatment was occurred during storage as same as control fruit at day 12 and 15. Lower pericarp pH and higher acidity resulted in a lower browning index in coated fruit at low PS concentration at 0-0.3% in edible coating (Figure 4.9; 4.11).

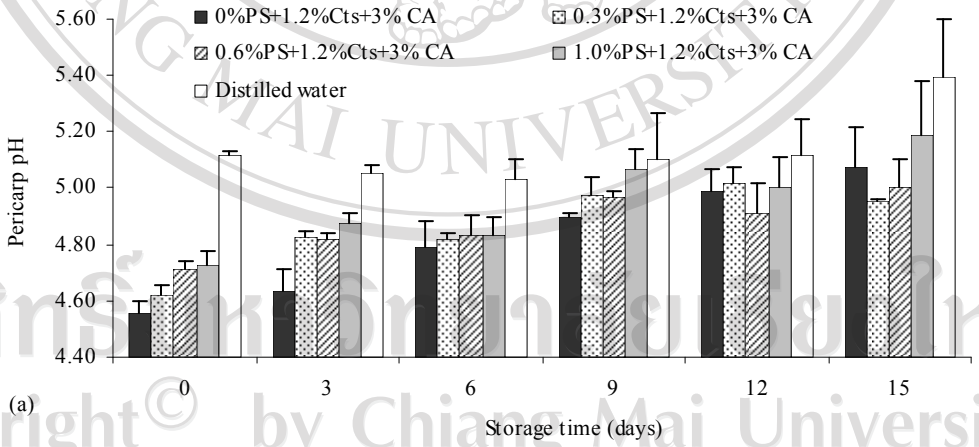

Figure 4.11 Effects of PS+Cts on pericarp pH (a) and citric acid content as TA in pericarp homogenate (b) of longan fruit during storage at 10°C.
Cts = chitosan; CA = citric acid; PS = potassium sorbate.

Table 4.11 (Continue)

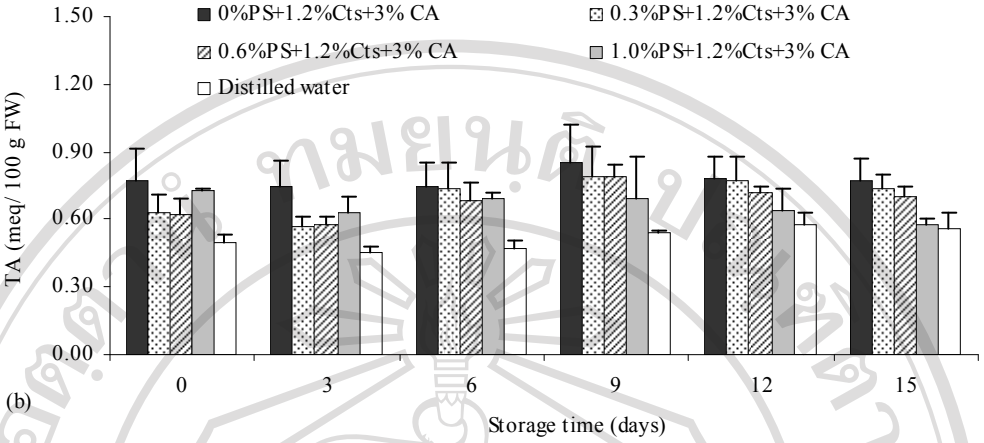

Figure 4.11 Effects of PS+Cts on pericarp pH (a) and citric acid content as TA in pericarp homogenate (b) of longan fruit during storage at 10°C. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

4.4.2.7 Surface fruit color

Surface fruit color was displayed in Figure 4.12, which decreased as the storage time increased. Addition of all PS concentrations in Cts+CA caused decreasing in L* and h˚ values during the first 12 days compared to the control fruit. However, after 15 days, the result was not significant. L* value decreased as their PS concentrations in coating material increased. The reason was the damage of the fruit pericarp during coating (Figure 4.12a). However, addition of all PS concentrations in Cts+CA significantly increased C* values during storage in comparison with control fruit during the first 15 days.

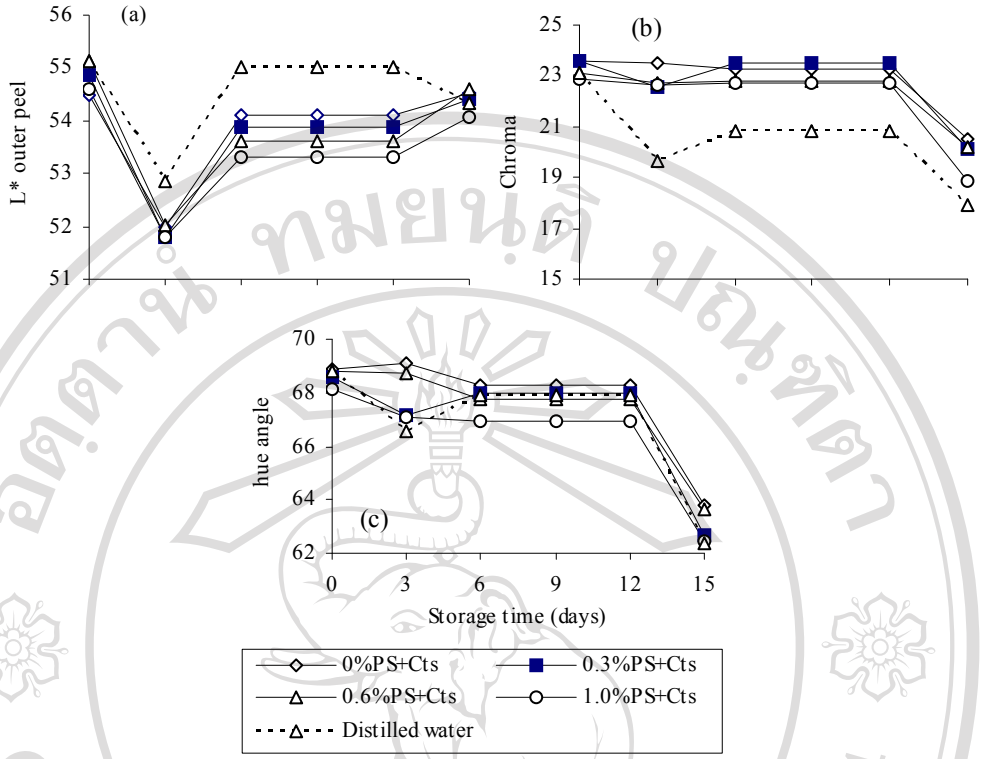

Figure 4.12 Effects of PS+Cts L* (a), chroma (b) and hue angle (c) in outer pericarp of longan fruit during storage at 10°C. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

4.5 Conclusion

Dipping is a direct surface application clearly showed some limitations on disease control in comparison to incorporating coating material. The limitations were caused by neutralization of the solution (alkaline pH) and inadequately, diffusion into the bulk food (Torres *et al*., 1985; Siragusa and Dickson, 1992). The efficacy of edible coatings (Cts+CA) in disease control can be improved by incorporating antimicrobial agents (chemical preservatives like PS) in the solution which could help delay fruit decay in comparison with no PS in coating. Application of 0.3 % (w/v) PS in Cts coating sufficiently produced a better fruit decay control in the fruit by reducing in mold population including disease incidence percentage in comparison with control fruit and no additive of PS in coating. Sorbic acid residue in the aril of this treatment was not detected in comparison with the added PS in coating at high

concentration. Cts along with CA and PS could help delay the loss of active substance in fruit surface; maintaining of sorbic acid and citric acid content (TA) in pericarp. However, application of PS in coating more than 0.6% showed darker of fruit pericarp due to phytotoxicity of this compound which indicated by higher pericarp pH and lower TA in pericarp homogenate but not weight loss percentage. Addition of PS at higher concentration could disturb some coating properties by reducing viscosity and increasing pH of solution.