CHAPTER 3

EFFECTS OF CITRIC ACID INCORPORATED WITH CHITOSAN-BASED COATING ON PERICARP BROWNING OF FRESH LONGAN FRUIT

3.1 Abstract

The application of citric acid incorporated with chitosan-based coating to control pericarp browning of fresh longan fruit was studied. Fresh longan fruit was dipped in the solutions of 1, 3 and 5% citric acid (CA) mixed with 1% (w/v) chitosan. Results indicated that pericarp browning during storage was related to weight loss percentage and the change of pericarp pH. Dipping in 1% CA mixed with 1% chitosan significantly delayed pericarp browning more than other treatments and storage for 5 days at room temperature and 20 days at 5°C, 85% RH. This treatment not only reduced weight loss, but also delayed an increase in pericarp pH during storage. Whereas higher concentration of CA mixed with 1% chitosan or without chitosan showed damage on outer surface of the fruit and a rapid increase in pericarp browning. Dipping the fruit in CA solution had slight affect on the quality changes of the fruit pulp, such as pH, TA and TSS/TA during the first week of storage at 5°C, whereas no change was observed in the pulp of the fruit treated with CA mixed with 1.2% (w/v) chitosan. Finally, after storage at 5°C for 20 days, treatments with CA mixed with 1.2% chitosan significantly showed higher eating quality and no difference in ethyl alcohol content in the flesh in comparison to those treated with CA solution.

In the second experiment, this research is designed to study the effects of chitosan and CA on pericarp browning and polyphenol oxidase (PPO) activity of longan fruit. The experiment was conducted by dipping longan fruit for 2 min in 1.2% (w/v) chitosan coating solution containing 1.0% CA (pH 3.3), 1.0% CA solution (pH 2.6) or in distilled water (control). The treated fruits in each treatment were then

packaged in foam tray, wrapped with 11 μm PVC film and stored at 5°C, 95% RH for 27 days. Pericarp browning, pericarp color, pericarp pH and titratable acidity (TA), weight loss percentage, PPO activity and total phenolic content were monitored during storage. The results revealed that interaction between chitosan and CA demonstrated the best treatment in delaying pericarp browning which was indicated by the lowest browning index and high L* value, chroma and hue angle. Based on browning index, the fruits treated with citric acid alone and the control fruits were not acceptable after 20 days, which those treated with both CA and chitosan were still acceptable after 27 days of storage. TA had a correlation with the pericarp pH and browning index. 1.2% Chitosan mixed with citric acid exhibited the higher efficacy in preventing TA degradation in pericarp and maintaining low pericarp pH, compared to 1.0% CA treatment. In addition, low PPO activity and high total phenolic content were found when CA was applied in combination with chitosan during storage.

3.2 Introduction

Longan fruit has faced rapid discoloration caused by desiccation during storage at either too low or high temperature. Browning of fresh longan results from phenolic compounds oxidized by endogenous polyphenol oxidase (PPO) and then the pigment forms (Jiang *et al*., 2002; Lin *et al*., 2005). Sulfur dioxide fumigation in commercial use is actually able to resolve the problem (Tongdee, 1994). However, there have been numerous reports on the negative effects of their use such as toxic residues in human beings, asthmatics and reactions in sensitive individuals as well. Therefore, alternative treatments for SO_2 fumigation are required. Citric acid (CA) is an anti-browning agent which prevents polyphenol oxidase by decreasing food pH and binding Cu^{2+} in active site of PPO to form an inactive complex (Martinez and Whitaker, 1995). It has been widely used in the food industry for controlling browning. Although applying CA as a dipping solution has been reported in postharvest fruits with a very satisfactory result for some fruits such as longan (Sardsud *et al*., 2003; Whangchai *et al.*, 2006) and litchi (Terdbaramee *et al*., 2002), the injury of fruit surface and rapid pericarp browning were observed in longkong

(*Aglaia dookoo* Griff.) when high concentrations (2, 4 and 6%) of CA were applied (Lichanporn *et al*., 2002).

Application of an edible coating as a carrier of anti-browning to mitigate browning and water loss has been studied (Cuppett, 1994) and it was found to successfully maintain high quality of fresh produce (Lee *et al*., 2003; Lin *et al*., 2008; RoJas-Grau *et al*., 2007). Chitosan as a high molecular weight cationic polysaccharide from shrimp shells or crab shells can be manufactured in Thailand. It has been widely studied for producing a thin protective coating in many fruits to delay weight loss percentage, as a barrier to O_2 and delay increasing of PPO activity during storage (Jiang and Li, 2001). A control of pericarp browning can be accomplished by mixing CA solvent and chitosan. This solvent has been reported to control pericarp browning in litchi (Joas *et al*., 2005). Results indicated that this mixture efficiently delayed browning by reducing desiccation and pericarp pH during storage. A study on the use of CA and chitosan solution actually demonstrated a higher efficiency in browning control when compared to the fruit dipped in CA alone (Apai *et al*., 2008a). However, the combined effects of CA and chitosan solution on PPO acitivity and total phenolic content have not been reported yet in longan fruit.

The main purpose of this study was to evaluate the effect of CA, chitosan and their combination on pericarp browning and PPO activity of longan fruit during storage at low temperatures.

3.3 Materials and Methods

3.3.1 Effects of citric acid incorporated with chitosan-based coating on pericarp browning

3.3.1.1 Plant material

Mature uniform longan fruits were harvested in May and August, 2006 from a Good Agricultural Practice (GAP) Chiang mai orchard. The fruits were harvested manually and stem were left approximately 5 cm on the fruit. They were then dipped in 0.1% sodium hypochlorite solution (Clorox), dried at room temperature before coating with chitosan.

3.3.1.2 Coating preparation

Chitosan-based coating was prepared by mixing chitosan (high molecular-weighted shrimp flake, Ta Ming Enterprise Co., Ltd., Thailand) and various concentration of citric acid in boiling water with gentle stirring. The pH of coating solutions was measured.

3.3.1.3 Treatments

The longan fruits were dipped for 2 min: in citric acid (CA) plus 1% chitosan at concentration 1, 3 or 5%, or CA solution at 1, 3 or 5% and dipping in distilled water as a control. Treated fruits and control were air dried by electric fan. Fifteen fruits were packed separately foam tray and over-wrapped with 11 μm PVC films. Subsequently, they were stored at room temperature (approximately 28°C) for 5 days. During storage, longan samples were analyzed for weight loss percentage, browning and pericarp pH.

The experiment was repeated and confirmed at temperature 5°C. In creasing of Cts concentration from 1.0% to 1.2% in 1.0% CA on our preliminary experiment slightly decreased weigh loss (data not shown). Thus, this combination of 1% CA and 1.2% chitosan was used in this study. The longan fruits were dipped for 2 min: in CA plus 1.2% chitosan at concentration 1% or acetic acid (AcA) plus 1.2% chitosan at concentration at 1% or CA solution at 1% and dipping in distilled water as a control. They were stored at 5°C with 85% RH for 20 day. During storage, longan sample were analyzed for weight loss percentage, browning, pericarp color, pericarp pH and titratable acidity (TA) in pericarp. During storage at 5°C, fruit quality was analyzed for disease incidence and flesh qualities: total soluble solid (TSS), TA, TSS/TA ratio. Ethanol content and eating quality in the flesh were analyzed before treatment and during 20 day storage. All experiments (treatments) were done in triplicate (three foam trays).

3.3.1.4 Pericarp browning parameter assessments and fruit quality

- **Browning index**

Browning index (BI) was evaluated by following 5-score of pericarp browning. Browning scale was evaluated by measuring total browning areas of the

pericarp on each fruits and fifteen fruits per replication. The following browning scale was used (Figure 3.1). A browning index was calculated using the following formula: Browning index = Σ(browning scale x percentage of corresponding fruits within each class). Fruits having a browning index above 3.0 were rated as unacceptable (Jiang and Li, 2001).

1 = no browning in total area

2 = slight browning in total area

3 = <25% browning in total area

4 = 25-50% browning in total area

5=>50% browning in total area

Figure 3.1 Browning index (scales) of longan pericarp (Jiang and Li, 2001).

- Weight loss percentage and moisture loss

Weight loss percentage was also determined by weighing the whole fruits packed in foam tray before and after storage.

$$\text{Weight loss (\%)} = \frac{[\text{weight before storage} - \text{weight after storage}]}{\text{weight before storage}} \times 100$$

The moisture content was determined by the hot air-oven (Air Oven, Venticell 111, MMM Medcenter Einrichtungen GmbH, Germany) method by Hall (1980) at 105^{o}C for 72 hours. Three grams of longan pericarp were random selected and placed in an individual container (moisture can) and its weight was then recorded.

The containers were consequently placed in a hot-air oven at 105^{o}C for 72 hours. At the end of the heating period the containers were removed to a desiccator until cooled and then weighed. Moisture content (wet weight basis) was determined for each sample as the percentage ratio of the weight loss to the initial wet weight of the sample as following.

$$\text{Moisture content (wet basis) (\%)} = \frac{[\text{initial weight - final weight}]}{\text{initial weight}} \times 100$$

- Pericarp pH

The pericarp pH was determined by using method of Joas *et al*. (2005). The pH of the pericarp homogenate was measured by using a digital pH meter (Titroline easy, Schott, Mainz, Germany) under continuous stirring.

- Total soluble solid (TSS), pH, TA and TSS/TA of longan flesh

The amount of total soluble solids (TSS) of fruit juice was determined by a hand refractometer (ATAGO, Japan). The pH was measured with pH meter (Consort 431, Belgium). Titratable acidity was determined from flesh juice. Sample was titrated with 0.1 N NaOH solutions to pH 8.1 using autotitrator (Titroline easy, Schott, Mainz, Germany) under continuous stirring and expressed as mg acid per 100 g of flesh. TSS/TA ratio was then calculated.

- Eating quality and ethanol content

Eating quality as overall acceptability was assessed by 5 taste panelists, using 9-point hedonic scale, 1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely.

Ethanol contents in juice of the longan flesh were determined by head space gas chromatography (Varian gas chromatograph, model 3400 CX) as described by Bunsoong (2000); Pesis *et al*. (2002). The flesh was blended using Moulinex blender from ten fruit in each replication (30 fruits per treatment) for 2 min and they were stored at 4˚C before analysis. To determine ethanol, 100 µL of each samples of juice and internal standard (isopropanol, AR grade) were transferred to 2.0-ml bottle, which

were sealed with rubber serum caps. Head space auto sampler (Varian, model Genesis) condition was 500 µL sample loop, 50˚C plate temperature, sample equilibration for 4 min, sample mix time for 7 min, 150˚C loop temperature and 200˚C transfer line temperature.

0.5 µL sample of the head space gas within head space was automatically removed by auto sampler and injected into the gas chromatograph equipped with a flame ionization detector (FID) and column RTX volatile 30 m x 0.53 mm 2µm film thickness (Restek). The experimental conditions were: 65˚C oven temperature, 210˚C injector temperature, 220˚C detector temperature. Ethanol was identified individually by comparing retention times against standard (ethanol Clinical standard solution of College of American Pathologist (CAP) (RT = 0.94 min) and internal stand (RT = 1.026 min). Concentration being determined by a regression equation calculated on four sample of standard concentration. Ethanol contents were expressed as µmol/ml juice.

- Disease incidence

Disease incidence percentage was visually observed on a number of the fruit that showed lesions of mycelium or rot on the fruit surface area.

- Statistical analysis

In experiment 3.1.3, the statistical model was a 2 × 3 factorial completely randomized design (CRD) comprising 2 levels of coating material at non-coat and coated fruits and 3 levels of CA concentration at: 1, 3 and 5%. Differences between treatments were analyzed using Duncan's multiple range test (DMRT) at $p \leq 0.05$.

3.3.2 Effects of chitosan-based coating and citric acid on pericarp browning and polyphenol oxidase activity of longan fruit

3.3.2.1 Plant materials and treatments

Mature yellow AA grade longan fruit (100 % of maturity) (*Dimocarus longan* Lour.) cv. Daw were harvested from a commercial GAP orchard in Chiang Mai. Fruit

were selected for uniformity of shape, color and size; any blemished or diseased fruits were then discarded. The average AA grade fruit size was 2.78 cm in width, 2.55 cm in height and 2.52 cm in thickness. The previous experiment showed that 1% (w/v) CA mixed with 1-1.2% (w/v) chitosan demonstrated the most efficiency in reducing pericarp browning of longan fruit during storage for 5 days at 30 °C, 12 days at 10°C and 20 days at 5°C (Apai *et al.*, 2008a). Thus, this combination of 1% CA and 1.2% chitosan was used in this study by dispersing 24 g of chitosan (high molecular-weighted shrimp flake, Ta Ming Enterprise Co., Ltd., Thailand) in 1.5 L of boiling water following the addition of 20 g of CA (food grade, Shandong Ningmeng Biochemistry Co., Ltd., China). The solution was mixed well by magnetic stirrer and cooled down in a hood before addition of distilled water up to 2.0 L and then the pH of the solution was measured. Fruits were dipped for 2 min in 1% CA containing 1.2% chitosan (pH 3.3) or without chitosan (pH 2.6). After dipping, the fruits were air-dried by electric fan, packaged in foam trays wrapped with 11 μm thick PVC film (M Wrap, M.M.P. Packaging Group Co., Ltd., Prakanong, Bangkok, Thailand) (20 fruits per foam tray) and then stored at 5 °C, 95% RH. The fruit dipped in distilled water was used as a control. For each treatment, four replicates were used. Samples were taken initially and then at day 10, 20 and 27 during storage for quality evaluation and the following analyses.

3.3.2.2 Pericarp browning assessment and surface fruit color

Browning was assessed visually by measuring total browning areas of the pericarp on each of twenty 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 (Figure 3.2). The degree of browning was expressed as L*, Chroma ($C^* = [a^{*2} + b^{*2}]^{1/2}$) and hue angle ($h^o = \tan^{-1} b^*/a^*$) values. L* value indicated lightness of color wheel, ranged from black = 0 to white = 100. C* indicated index color saturation or intensity of color wheel, ranged from = 0 at the center and increases according to the distance from the center. Hue angle is expressed in degree from 0˚ to 360˚ of the color wheel; 0˚ = red, 90˚ = yellow, 180˚ = green and 270˚ = blue (McGuire, 1992). Two spots on opposite sides of the fruit were measured and the mean of the two

measurements considered as one reading. The results were expressed as a mean value from four replications of the 20 measured samples.

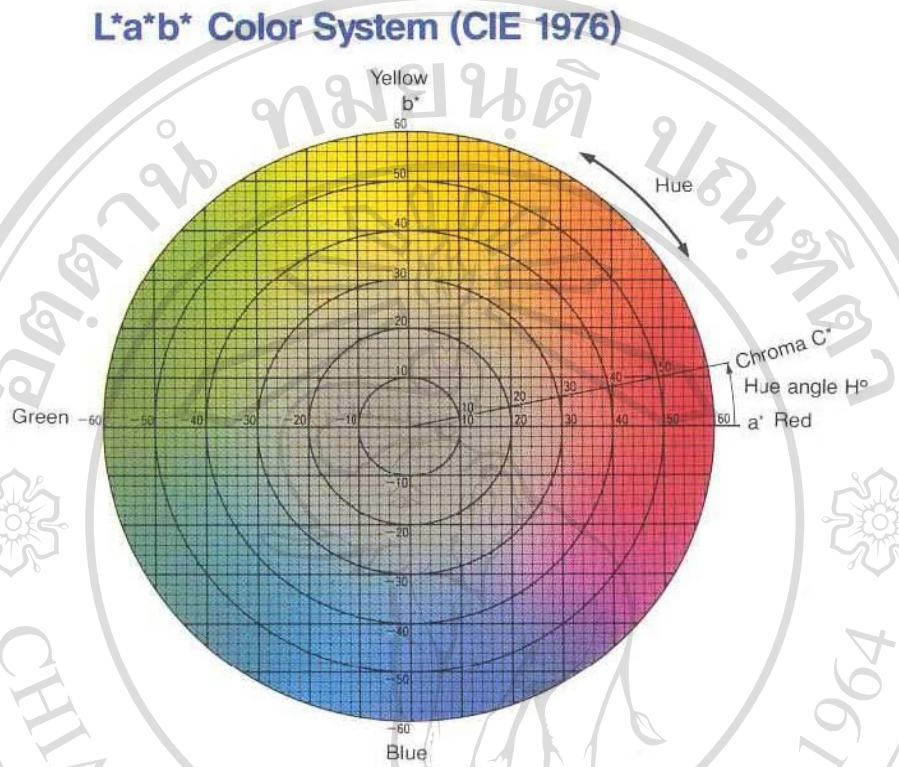

Figure 3.2 Color chart of Minolta model CR-300 (CIE, 1976)

3.3.2.3 Pericarp pH, citric acid content in pericarp, weight loss percentage and disease incidence

Forty fruits, ten in each of the four replications, were used to prepare samples. The pericarp tissues of each sample fruit were finely ground using a blender (Moulinex). Three grams of ground material were then homogenized in 30 ml of distilled water for 60 sec by a homogenizer at a speed of 10,000 rpm (WiggenHauser, Germany). The pH of the pericarp homogenate was measured, using a digital pH meter (Consort C831, Belgium) under continuous stirring. The titratable acidity, expressed as milliequivalents of acids per 100 g of pericarp, was measured by titration with 0.2 N NaOH solution to pH 8.1 using an automated titrimeter (Titroline easy, Schott) (Joas *et al*., 2005). Weight loss percentage was also determined by weighing the whole fruits packed in foam tray before and after storage. Disease incidence

percentage was visually observed on a number of the fruit that showed lesions of mycelium or rot on the fruit surface area.

3.3.2.4 Polyphenol oxidase (PPO) activity assay and protein determination

The pericarps from ten fruits of each treatment were frozen with liquid nitrogen and then powdered using the Moulinex blender. The powdered pericarp (3.0 g) was homogenized in 24 ml of 0.1 M phosphate buffer (pH 6.4) at 4°C. The homogenate was centrifuged at 15,000 x g (Herolab-Unicen 15 DR, Germany) for 20 min and then the supernatant was collected to assay PPO activity according to the modified method of Jiang (1999), by measuring the oxidation of pyrocatechol. The increase in absorbance capacity at 400 nm at 25°C was automatically recorded for 5 min, using a spectrophotometer (SPE Cord M 40, Germany). One unit of enzyme activity was defined as the amount causing a change of 0.001 in absorbance capacity per minute. The protein content was determined according to the dye-binding method of Bradford (1976) using albumin bovine serum as the standard.

3.3.2.5 Determination of total phenolic content

The total phenolic content in longan pericarp tissues was determined by the method of Folin–Ciocalteu reaction (Singleton and Rossi, 1965), using gallic acid as a standard. The phenolic content was expressed as gallic acid equivalents in milligrams on a fresh weight (FW) basis.

3.3.2.6 Statistical Analysis

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. Data were also evaluated using Pearson's correlation analysis of different browning parameters. The SPSS software version 10 for Windows was used as a statistical analysis tool.

3.4 Results and discussion

3.4.1 Effects of citric acid incorporated with chitosan-based coating on pericarp browning

3.4.1.1 Effects of coating on pericarp browning at ambient temperature storage

- Pericarp browning

CA alone at the concentration of 1, 3 and 5% and solution pH at 2.6, 2.4 and 2.2, respectively, showed a high severity of pericarp browning in line with the increasing CA concentration (Table 3.1). Using citric acid (CA) at concentration of 1-3% in chitosan (Cts) significantly delayed pericarp browning for 5 days ($p < 0.01$) (BI < 3.0) (Joas *et al*., 2005). While CA at concentration of 5% in Cts and all CA concentrations without Cts and control (distilled water) delayed pericarp browning less than 5 days (~ 4 days) (BI > 3.0). Interaction between CA and Cts on pericarp browning was found ($p < 0.05$) CA alone at all concentration was the main effects and therefore, severely increased pericarp damage more than those dipped in CA with Cts during day 3 of storage time (Table 3.2). The result was similar to Joas *et al*. (2005) who found that dipping litchi fruit in all CA+Cts at different pH solution: 0.8; 1.0; 1.3 showed good browning control when compared with all CA alone. Lichanporn *et al*. (2002) reported that dip longkong in 2.0, 4.0 and 6.0% CA alone and stored at 20°C, 90-95% RH had tissue damage because of high concentration. The skin of longkong showed browning with weight loss and increasing in respiration rate and ethylene production. Saengnil *et al.* (2006) also reported that dip litchi in 2.5, 5.0, 10 and 15% CA with or without hot water treatment showed the same negative results. Jarinthorn *et al*. 2008 also recently reported in longan fruit and showed the same effects after dipping in many kinds of substances such as 1, 3, 5% of citric acid, ascorbic acid, oxalic acid and tartaric acid, L-cysteins, hexylresocinal and calcium salts. In terms of fresh-cut produces, they are different texture attributes with longan fruits. Cocci *et al*. (2006) reported that the tissue break down in the minimal processed apples (in an aqueous solution of 1% ascorbic acid (AA) and 1% citric acid for 3 min) during

storage had been affected by applying too high CA concentration. The results indicated that CA concentration should be reduced to lower level because the pericarp tissue could not be tolerance. These results suggested that escalating skin damages after CA dipping might also be related to both pH of a solution and natural attribute of each food texture.

As it is possible to see from browning area results (Table 3.1), the dipping treatment adopted in our experimental conditions was effective in slowing down pericarp browning. The synergistic effect of an acidulant (CA) and an edible chitosan coating (Cts) agent to retard browning has been reported in previous investigations (Joas *et al*., 2005; Caro and Joas, 2005) including CA alone (Sardsud *et al*., 2003; Whangchai *et al*., 2006). Unfortunately, this kind of CA dipping treatment alone caused a pericarp tissue breakdown of the fruit skin, with consequent damaging of pericarp longan tissue, also detected by other researchers in similar structural fruits (Lichanporn *et al*., 2002, 2003; Saengnil *et al*., 2006; Jarinthorn *et al*., 2008), in fresh cut apples (Ponting *et al*., 1971) and in our experimental conditions.

This situation could be justified by using the wrap of plastic films which led to a high humidity around the fruits' skin underneath the film and this could prevent the weight loss (Kader, 1994). The accumulation of condensed water under the package within an hour could be observed and was believed to make the fruits squelchy (Figure 3.3). The dry CA residue on the fruits' tissue could then be active again; thus, bleaching, impregnating and damaging fruits' skin which later induced ethylene production which caused a degradation of nutrient and antioxidant (Abeles, 1992). So, pericarp browning could rapidly happen due to a membrane breakdown and O_2 diffusion when phenolic substrate rapidly reacts with PPO. However, when longan fruits were dipped in CA at the same concentration of 1, 3 and 5% but mixed with chitosan, the solution pH were changed to 3.3, 2.5 and 2.4 respectively. The result showed a less severity of damages on outer surfaces of the fruits, and the shelf life was also better prolonged as compared with applying only CA alone. Chitosan, as a thin layer coating substance, has great benefits as a good film forming and biocompatibility.

Table 3.1 Effect of combined treatments on the browning of longan fruits during storage at ambient temperature.

Combined treatments	pH[2]	Storage time (days)			
		0	3	5	6
1.0% CA[4]	2.6	1	1.8±0.12 b	4.5±0.35 a	5.0±0.00 a
3.0% CA	2.4	1	1.9±0.21 b	4.4±0.45 a	5.0±0.00 a
5.0% CA	2.2	1	1.8±0.06 b	4.9±0.06 a	5.0±0.00 a
1.0% CA +1.0% Cts	3.3	1	1.3±0.31 cd	2.0±0.26 d	4.2±0.38 b
3.0% CA +1.0% Cts	2.5	1	1.1±0.10 d	2.8±0.26 c	4.0±0.75 b
5.0% CA +1.0% Cts	2.4	1	1.5±0.06 c	3.6±0.32 b	4.9±0.17 a
DW (control)	5.6	1	2.4±0.06 a	4.6±0.36 a	5.0±0.00 a
F-test		ns	*	*	*
CV (%)			9.24	8.74	7.04

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

[2]pH = pH of solution

[3]browning index: 1 = no browning (excellent quality); 2 = slight browning; 3 =< 25% browning; 4 = 25-50% browning; 5 = > 50% browning (poor quality).

[4]CA = citric acid, Cts = chitosan, DW = distilled water

Adding chitosan into CA can reduce a direct contact between CA and fruits' skin leading to lower pH solution (Table 3.1, 3.2); therefore, fruit damages could be significantly decreased and its shelf life can be better prolonged. This suggested that pericarp browning could also be improved if pH solution is adjusted closest to 3.3. While SO_2 fumigation in commercial practice or sodium metabisulfite and/or combination with oxalic acid at higher concentrations showed similarly positive result to inhibit pericarp browning response (Tongdee, 1994; Boonyong, 2002; Boonin *et al.*, 2006).

Recently, CA dip to prolong shelf life of longan fruit was only investigated by Koslanund *et al*. (2008). This investigated by dipping fresh longan bunches in 6 treatments consisting of water (control), 2% citric + 2% ascorbic acid, 4 % ascorbic acid, 4.0 % citric acid + 2% ascorbic acid, 4.0 % citric acid + 4% ascorbic acid dip and SO_2 fumigation. The result showed that acid dip treatments had more L (lightness) and b (yellow) values of both exterior and interior peels than control but

less than SO_2 fumigation significantly. The 4% ascorbic acid treated fruits had more weight loss percentages than other treatments during week 3 and 4. The sensory evaluation was varied each week but SO_2 fumigated treatment was the least acceptance from the testers because of flesh discoloration.

Table 3.2 Interaction between citric acid and chitosan on browning of longan fruits during storage at ambient temperature.

Treatments	Storage time (days)			
	0	**3**	**5**	**6**
A=chitosan (%)				
0	1	1.84 a	4.61 a	5.00 b
1	1	1.28 b	2.79 b	4.38 a
B=citric acid (%)				
1	1	1.55	3.27 b	4.62
3	1	1.52	3.62 b	4.50
5	1	1.62	4.22 a	4.95
A		*	*	*
B		ns	*	ns
AxB		*	*	ns
C.V. (%)		10.67	8.35	7.5

[1]Same letters in the same column are not significantly different at 0.05. ns = non-significant, $p \leq 0.05$*

A = condensed water under the film wrap B = CA damage on pericarp

Figure 3.3 Scheme of CA damage on pericarp skin (arrow) after dipping in CA alone and wrapping (a) with PVC film at day 3 (b).

A = 1% CA+1% Cts B = 3% CA+1% Cts C = 5% CA+1% Cts

D = 1% CA E = 3% CA F = 5% CA

G = Distilled water dip

Figure 3.4 Effects of chitosan-based coating and citric acid on characteristic of pericarp browning after day 1 of longan fruit during storage at ambient temperature.

A = 1% CA+1% Cts B = 3% CA+1% Cts C = 5% CA+1% Cts

D = 1% CA E = 3% CA F = 5% CA

G = Distilled water dip

Figure 3.5 Effects of chitosan-based coating and citric acid on characteristic of pericarp browning after day 5 of longan fruit during storage at ambient temperature (yellow arrow; browning area).

- Pericarp pH

Pericarp pH during initial of storage showed that 5% CA containing in Cts showed the lowest value reduction from 5.30 (control fruit) to 4.87 (Table 3.3). All CA concentrations in Cts could maintain lower pericarp pH as compared with all CA alone though storage (Table 3.4). Interaction between CA and Cts on pericarp pH was found ($p < 0.01$). Browning index results in this experiment showed a significant positive correlation with pericarp pH (Figure 3.6a). The result indicated that pericarp browning was increased together with pericarp pH. The result was similar to Joas *et al.* (2005) who found that browning index in according to pericarp pH of litchi fruit after dipping in CA+Cts showed the lowest value due to the interaction between CA and Cts (Table 3.4). Chitosan as edible coating has been reported good properties such as biocompatibility and carriers with many food additives, antioxidant, mineral, antagonist etc., (Cuppett, 1994).

Table 3.3 Effect of combined treatments on the pericarp pH of longan fruits during storage at room temperature.

Combined treatments	pH of solution	Storage time (days)			
		1	3	5	6
1.0% CA[2]	2.6	5.27 a	5.60 a	5.67 a	5.67 a
3.0% CA	2.4	5.20 ab	5.47 b	5.57 a	5.67 a
5.0% CA	2.2	5.20 ab	5.60 a	5.63 a	5.63 ab
1.0% CA +1.0% Cts	3.3	5.10 b	5.30 c	5.30 c	5.50 b
3.0% CA +1.0% Cts	2.5	4.97 c	5.23 c	5.40 b	5.50 b
5.0% CA +1.0% Cts	2.4	4.87 c	5.23 c	5.40 b	5.50 b
DW (control)	5.6	5.30 a	5.60 a	5.60 a	5.67 a
F-test		*	*	*	*

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

[2]CA = citric acid, Cts = chitosan, DW = distilled water dip.

Cts along with CA could help to delay CA degradation in pericarp (Joas *et al*., 2005) and therefore pericarp browning was reduced. CA could prevent polyphenol oxidase by suppressing food pH and binding Cu^{2+} in active site of PPO to form an inactive

complex (Martinez and Whitaker, 1995). While all CA alone, pericarp pH rapidly increased within 3-5 days due to surface pericarp damage and then pericarp browning was dramatically increased as well as control fruit. This might be explained by measuring enzymatic browning activity which was needed to investigate to mechanism of CA on pericarp browning with or without Cts.

Table 3.4 Interaction between ciric acid and chitosan on the pericarp pH of longan fruits during storage at room temperature.

Treatments	Storage time (days)			
	0	**3**	**5**	**6**
A=chitosan (%)				
0	5.22 a	5.56 a	5.62 a	5.66 a
1	4.98 b	5.26 b	5.37 b	5.5 b
B=citric acid (%)				
1	5.18 a	5.45 a	5.48	5.58
3	5.08 b	5.35 b	5.48	5.58
5	5.03 b	5.42a	5.52	5.57
A	*	*	*	*
B	*	*	ns	ns
AxB	ns	*	*	ns
C.V. (%)	1.38	0.75	1.05	1.46

[1]Same letters in the same column are not significantly different at 0.05. ns = non-significant, $p \leq 0.05$*

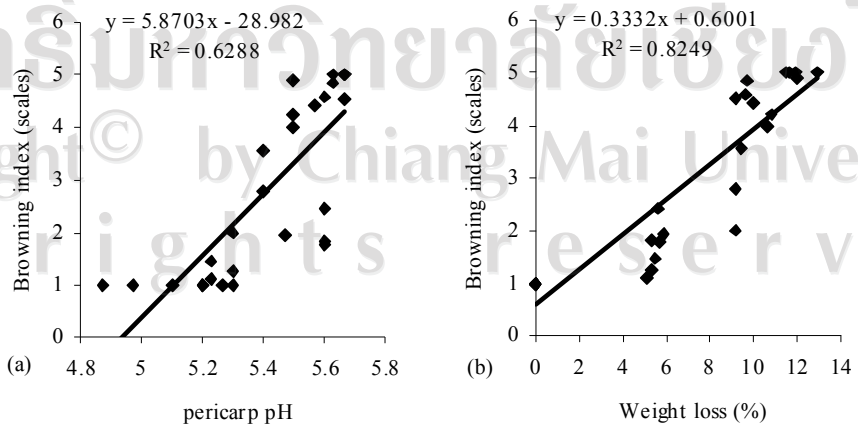

Figure 3.6 Pericarp pH (a) and weight loss percentage (b) of longan fruit in relation to browning index during storage for 6 days (N = 28).

- Weight loss percentage, moisture loss and disease incidence

The browning index (BI) increased progressively and was more closely correlated to weight loss percentage (Figure 3.6b). In the main factor, CA at concentration of 5% increased fruit weight loss percentage during storage compared to 1-3% CA (Table 3.6). This suggested that higher concentration of CA than 3.0% could stimulate rapidly pericarp browning. From Table 3.5, using of CA at concentration of 1-3% in Cts had maintained the lowest weight loss percentage which could observe the lowest glossy or shrivel characteristics of inner pericarp during storage at day 3 as compared with the same CA concentration alone (data not shown). However, the result in weight loss percentage of these treatments (1-3% CA+1% Cts) showed not significant with 1% CA alone and control fruits. This result indicated that Cts had less benefit to protect water loss in pericarp (Table 3.7) because Cts films have relatively high water vapor permeability (Butler *et al*., 1996) due to its hydrophilic property. This result revealed that chitosan might possess a poor water barrier property because of its hydrophilic property, but it had an attribute of a good gas permeability exchange (O_2/CO_2 permeable) which did not create quality-related problems such as off-flavor caused by anaerobic respiration in longan aril (data not shown). The addition of lipid materials to hydrophilic coatings can sometimes improve their moisture barrier properties (Amarante and Banks, 2001). Vargas *et al*. (2006) found that an addition of oleic acid not only enhanced chitosan antimicrobial activity but also improved water vapor resistance of chitosan-coated strawberry. In preliminary study showed that added oleic acid in Cts showed lower weight loss but fruit skin showed glossy characteristics similar to waxed tangerine which consumer might not accept because it did not like the typically fruit skin and also off-flavor (data not shown). In creasing of Cts concentration from 1.0% to 1.2% in 1.0% CA on our preliminary experiment showed the good positive on weigh loss (%) whereas increase Cts to 2.0% in 1.0% CA showed off-flavor, seeing white color on fruit skin and hardness including fruit rot (data not shown). Over wrap with plastic films was possibly the main factor to not significant of weight loss because it permits high relative humidity (RH) and produces condensed water inside the package. However, PVC film as a packaging item is the most optimum packaging for longan because it

permits high water permeability and produces low condensed water when compared with other types of films (Seubrach *et al*., 2006).

Table 3.5 Effect of combined treatments between citric acid and chitosan on the weight loss percentage of longan fruits during storage at room temperature.

Combined treatments	pH	Storage time (days)					
		1	2	3	4	5	6
1.0% CA[2]	2.6	1.13 b	3.09 b	5.31	7.26 bc	9.19 b	10.84 b
3.0% CA	2.4	1.28 a	3.45 a	5.84	7.97 a	9.98 a	11.63 a
5.0% CA	2.2	1.28 a	3.37 a	5.70	7.75 ab	9.69 ab	11.28 ab
1.0% CA +1.0% Cts	3.3	1.14 b	3.08 b	5.30	7.24 c	9.13 b	10.75 b
3.0% CA +1.0% Cts	2.5	1.14 b	3.09 b	5.12	7.23 c	9.12 b	10.70 b
5.0% CA +1.0% Cts	2.4	1.21 ab	3.25 ab	5.53	7.50 abc	9.40 ab	10.78 b
DW (control)	5.6	1.21 ab	3.28 ab	5.57	7.62 abc	9.64 ab	11.18 ab
F-test		*	*	ns	*	*	*

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

[2]CA = citric acid, Cts = chitosan, DW = distilled water dip.

Table 3.6 Interaction between citric acid and chitosan on the weight loss percentage of longan fruits during storage at room temperature.

Treatments	Storage time (days)					
	1	2	3	4	5	6
A=chitosan (%)						
0	1.23 a	3.30 a	5.62	7.66 a	9.62 a	11.25 a
1	1.16 b	3.14 b	5.31	7.32 b	9.22 b	10.75 b
B=citric acid (%)						
1	1.14 b	3.09 b	5.30	7.25 b	9.16	10.80
3	1.21 a	3.27 a	5.48	7.60 a	9.55	11.17
5	1.25 a	3.31 a	5.62	7.63 a	9.56	11.03
A	*	*	**ns**	*	*	*
B	*	*	ns	ns	ns	ns
AxB	*	*	ns	ns	ns	ns
C.V. (%)	3.88	3.56	9.81	3.63	3.60	3.60

[1]Same letters in the same column are not significantly different at 0.05. ns = non-significant, $p \leq 0.05$*

Table 3.7 Effect of combined treatments on the water loss (%) of the pericarp of longan fruits during storage at room temperature.

Combined treatments	pH	Storage time (days)			
		1	3	5	6
1.0% CA[2]	2.6	62.68	52.80	42.71 a	38.53
3.0% CA	2.4	61.40	52.70	40.95 a	35.66
5.0% CA	2.2	60.72	52.19	43.12 a	35.99
1.0% CA +1.0% Cts	3.3	61.85	55.14	42.50 a	35.78
3.0% CA +1.0% Cts	2.5	61.43	52.87	44.09 a	40.58
5.0% CA +1.0% Cts	2.4	61.15	54.11	42.30 a	37.88
DW (control)	5.6	61.39	52.33	35.31 b	28.34
F-test		ns	ns	*	ns

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

[2]CA = citric acid, Cts = chitosan, DW = distilled water dip.

Table 3.8 Effect of combined treatments on the disease incidence (%) of the longan fruits during storage at room temperature.

Combined treatments	pH	0	3	5	6
1.0% CA[2]	2.6	0	90.00 a	100.00 a	100.00 a
3.0% CA	2.4	0	56.67 bc	76.67 ab	100.00 a
5.0% CA	2.2	0	26.67 cd	66.67 ab	93.33 a
1.0% CA +1.0% Cts	3.3	0	86.67 ab	93.33 ab	96.67 a
3.0% CA +1.0% Cts	2.5	0	20.00 e	60.00 b	63.33 b
5.0% CA +1.0% Cts	2.4	0	33.33 cd	70.00 ab	90.00 a
DW (control)	5.6	0	26.67 cd	100.00 a	100.00 a
f-test			*	*	*
CV (%)			25.41	19.25	8.22

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

[2]CA = citric acid, Cts = chitosan, DW = distilled water dip.

3.4.1.2 Effects of coating on pericarp browning and fruit quality at low temperature storage

- Pericarp browning, pericarp pH, weight loss percentage and disease incidence

The results from Table 3.9 showed that at 5°C, 85% RH storage temperature, pericarp pH and weight loss percentage and disease incidence was the lowest from fruits dipped in 1% citric acid in combination with 1.2% chitosan. Joas *et al*. (2005) found that dipping litchi fruits in the combination of CA with 1% chitosan delayed pericarp browning by reducing pericarp pH and weight loss percentage during storage. Dipping solution contains 1.2% chitosan and 1% CA provided the best result as same as the previous experiment at room temperature. There was significant lowest browning index from the fruit stored at 5°C, 85% RH though 20 days from that treatment. Weight loss percentage and pericarp pH of fruits from previous treatment were reduced. Solution contained citric acid and water, without chitosan, including acetic acid containing in chitosan (pH 3.3) did not give a good result, there were more wounds found on outer surface of the fruits compared to those fruits dipped in chitosan containing citric acid solution. Better fruit qualities were found in the fruits treated with a combination of CA and chitosan compared to those treated with CA.

- Total soluble solid (TSS), pH, TA and TSS/TA of longan flesh, eating quality and ethanol content

Duration time in storage showing an affect on the pulp qualities such as pH and TA including TSS/TA (Table. 3.9), which was consistent with decline in eating quality. pH exhibited slightly increased as TA decreased when storage time increased. This suggested possibly due to an increased respiration. Although longan fruit are non-climacteric (Jiang *et al*., 2002) and thus, have no significant changes in total soluble solids and titratable acidity. In our experiment illustrated that fruits treated with CA solution had significant lower pH and TSS/TA ratio but higher TA than other treatments. Ethanol content in longan juice was detected from all treatments during storage which indicated fermentation (Table 3.9). Best eating fruit quality and lowest disease incident (data not shown) after 20 days in storage were found from the fruits dipped in a combination of CA and chitosan solution.

Table 3.9 Changes in the fruit qualities: browning index; weight loss percentage; pericarp pH; disease incidence of longan fruit and the pulp qualities: total soluble solid; juice pH; titratable acidity; acid ratio (TSS/TA); eating quality; ethanol content in the longan flesh and dipped in different treatments stored at 5°C, 85% RH.

		Pericarp				Flesh					
Treats	**pH**	**Browning Index (scale)**	**Weight Loss (%)**	**Pericarp pH**	**Disease Incidence (%)**	**Total soluble Solid (%TSS)**	**Juice pH**	**Titratable Acidity (mg/100g)**	**TSS/TA**	**Eating quality (1-9 score)**	**Ethanol Content µmol/ml juice)**
Day 0		1	0	5.21	0	20	6.75	0.38	54.02	9	37.43
Day 7											
CA+Cts	3.3	1.14c	2.08b	5.10b	6.67	20.16a	6.98a	0.25ab	81.29ab	N/A	N/A
AcA+Cts	3.3	2.73a	2.41a	5.39a	8.89	20.00a	6.98a	0.22b	90.15a	N/A	N/A
CA	2.6	2.44ab	2.28ab	5.14ab	2.22	20.43a	6.88b	0.27a	75.04b	N/A	N/A
DW	5.6	2.20b	2.27b	5.25ab	17.78	18.80b	6.92ab	0.23b	81.11ab	N/A	N/A
Day 20											
CA+Cts	3.3	3.89b	4.50b	5.34b	35.55	19.37b	6.99	0.25	67.7	7.0a	86.46ab
AcA+Cts	3.3	4.53a	4.90a	5.70a	93.33	19.90ab	7.06	0.23	75.72	1.6c	100.62b
CA	2.6	4.27a	4.79ab	5.41ab	73.33	20.37a	7.03	0.22	79.09	5.0b	87.18ab
DW	5.6	4.47a	4.65ab	5.60ab	84.45	20.10ab	7.06	0.20	83.71	5.0b	82.70a

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

[2]CA = 1% citric acid, Cts = 1.2% chitosan, AcA = 1% acetic acid, DW = distilled water, N/A = not analysis

3.4.2 Effects of chitosan-based coating and citric acid on pericarp browning and polyphenol oxidase of longan fruit

3.4.2.1 Pericarp browning, pericarp pH, citric acid content in pericarp and weight loss percentage

Treatment of longan fruits, using a solution of 1% citric acid in combination with 1.2% chitosan (Cts) significantly delayed pericarp browning (browning index; BI). This combination was more efficient than either applying only 1.0% CA alone or using the control (distilled water) ($p<0.01$). The solution of 1.0% CA without chitosan and the control could only postpone pericarp browning for 20 days with the BI $\geq$ 3.0 whereas 1% CA mixed with 1.2% chitosan in solution could delay pericarp browning for 27 days with the BI = 2.53. After day 27 during storage at 5˚C, the solution of 1.0% CA without chitosan; and the control showed pericarp browning development at BI = 4.23 and 4.35, respectively (Figure 3.7a).

This experiment demonstrated that 1.0% CA without chitosan provided the lowest pH (pH = 2.2) as compared to a pH of 2.4 for the solution of 1% CA and 1.2% Cts. However, after applying the solution of 1.2% Cts dissolved in 1% CA, the pericarp pH had the lowest value (pericarp pH = 4.79) from the beginning of the storage until day 27 in comparison to that of CA and the control (Fig. 3.7b). In biochemistry, CA is important as an intermediate in the citric acid cycle and therefore occurs in the metabolism of almost all living things. Kupferman *et al*. (1999) reported that post-storage internal browning of apples was positively related to the amount of water core after storage and negatively related to the level of acidity (TA) and the result was accorded to Vial *et al*. (2005) in grape. In our experiment showed that the accumulation of titratable acidity (TA) in pericarp homogenate was highest in fruits dipped in 1% CA with 1.2% Cts throughout the storage period from 1.22 to 1.06 meq/100 g FW ($p<0.05$) (Figure 3.7D). The result is also consistent with that of Joas *et al*. (2005); Caro and Joas (2005). TA in pericarp homogenate can also be used as an indicator for effectiveness of acid-coating treatment because the TA has a negative correlation with pericarp pH (r = -0.92) (Table 3.11). TA decreased as pericarp pH increased (Figure 3.7c, d). Their studies of dipping litchi(*Litchi chinensis* Sonn.)

fruits in the solvent of CA and 1% chitosan found that pericarp browning effects could be postponed by reducing pericarp pH and weight loss percentage during storage. Chitosan-containing dipping solutions can significantly help delay the rate of CA degradation in fruits' surfaces. As a result, these treated fruits end up with a better and healthier skin look while the single CA component is inferior in its capacity to slow down the rate of CA degradation on fruits' surfaces. The results according to numerous reports also showed a higher capacity in browning control when applying a combination of different edible coatings and anti-browning agents in fresh produces (Baldwin *et al*., 1996; Lee *et al*., 2003; Lin *et al*., 2008; RoJas-Grau *et al*., 2007).

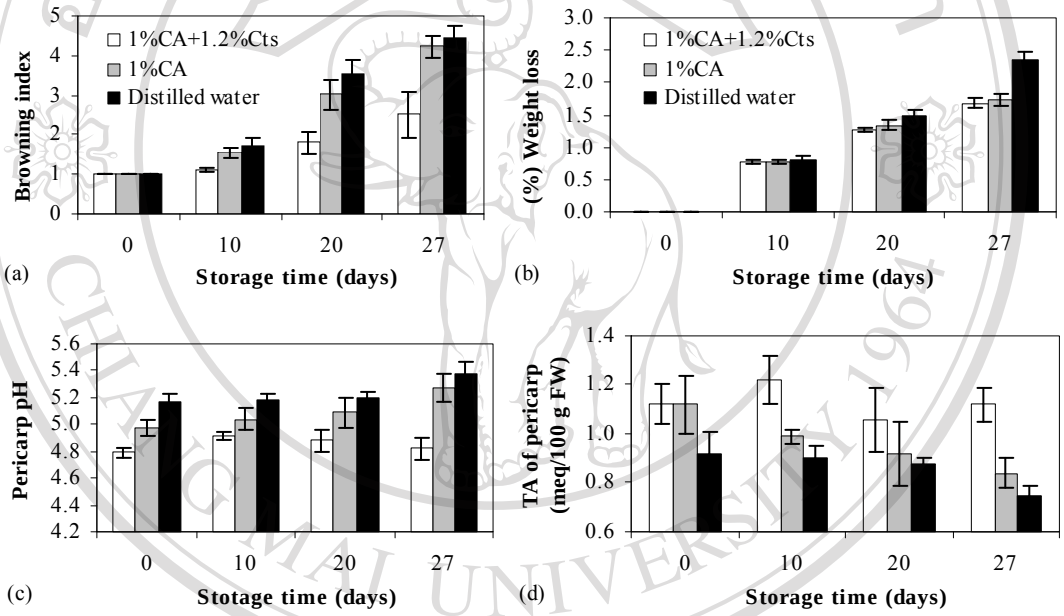

Figure 3.7 Effects of 1% citric acid (CA) solution and 1% CA combined with 1.2% chitosan (Cts) solution on pericarp tissue browning (a), weight loss percentage (b), pericarp pH (c) and titratable acidity of pericarp homogenate (d) of longan fruit during storage at 5°C for 27 days. The distilled water treatd longan fruits were used as the control. Vertical bars indicate standard deviation.

Although, after day 27, the experiment indicated that the longan fruit dipped in chitosan (dissolved in 1.0% CA) showed significant reduction of weight loss percentage compared with the control fruit (Figure 3.7b), but the result was not significantly different in the fruit dipped in CA without chitosan which was in

agreement with the former experiment in longan (Apai *et al*., 2008a). Chitosan films have relatively high water vapor permeability (Butler *et al*., 1996). This result revealed that chitosan might possess a poor water barrier property because of its hydrophilic property, but it has an attribute of a good gas permeability exchange (O_2/CO_2 permeable) which does not create quality-related problems such as off-flavor caused by anaerobic respiration in longan aril. The addition of lipid materials to hydrophilic coatings can sometimes improve their moisture barrier properties (Amarante and Banks, 2001). Vargas *et al*. (2006) found that an addition of oleic acid not only enhanced chitosan antimicrobial activity but also improved water vapor resistance of chitosan-coated strawberry. The use of PVC film as a packaging item is the most optimum for longan because it permits high water permeability and produces low condensed water when compared with other types of films (Seubrach *et al*., 2006).

Table 3.10 Interaction between CA & chitosan on browning control at day 27 during 5˚C.

Factors	(%)	Browning index	Weight loss percentage	Pericarp pH	CA content (meq/100g FW)
A = CA	0	4.43 a	2.34 a	5.37 a	0.75 b
	1.0	3.37 b	1.71 b	5.04 b	0.98 a
B = Cts	0	4.33 a	2.04	5.32 a	0.80 b
	1.2	3.48 b	2.01	5.09 b	0.94 a
A		*	*	*	*
B		*	ns	*	*
A x B		*	ns	*	*

3.4.2.2 Pericarp color and disease incidence

The effectiveness of the 1% CA plus chitosan solution in controlling pericarp browning was confirmed by other research results demonstrating high consistency in pericarp color during storage (Figure 3.8). L* value (lightness), chroma and hue were also well negatively correlated with browning index (Table 3.11). The result was also

in accordance with the recent research of Apai *et al*. (2008a). The high hue in 1.0% CA with chitosan solution indicated that its pericarp color was close to 90° which was expressed as yellowness values due to an increasing surfaces' yellow intensity as the highest C* value, similar to SO_2 fumigation (data not shown).

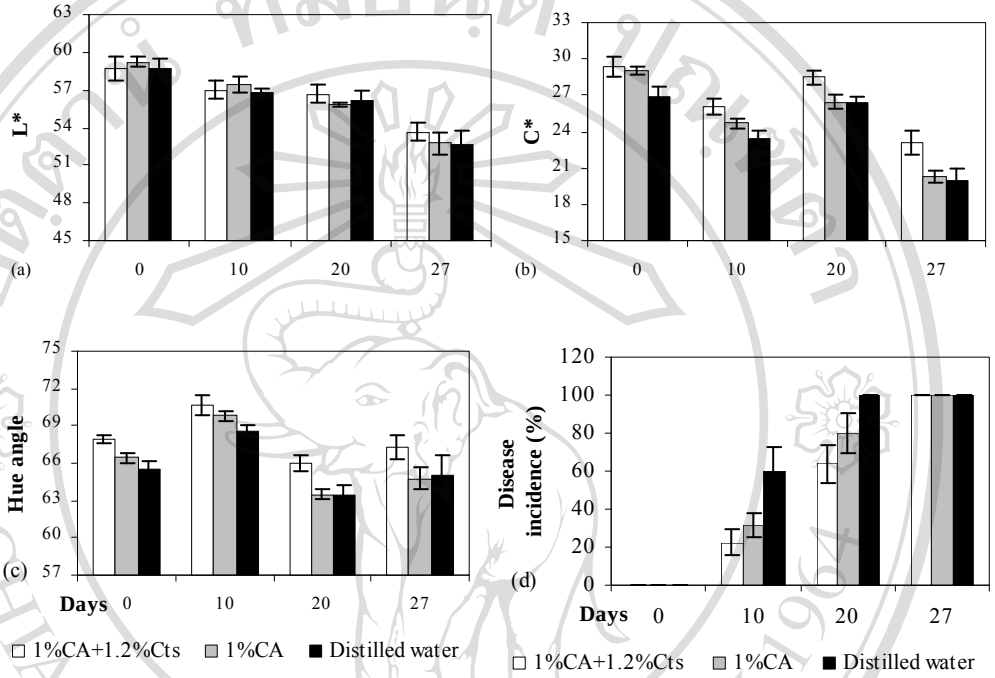

Figure 3.8 Effects of 1% citric acid (CA) solution and 1% CA combined with 1.2% chitosan (Cts) solution on L value (a), chroma (b), hue angle (c) and disease incidence (%) (d) of longan pericarp during the fruit were storage at 5°C for 27 days. The distilled water treatd longan fruits were used as the control. Vertical bars indicate standard deviation.

In Thailand, there have been a number of studies on longan treatment with either a single safe chemical agent or in combination with others as a replacement of SO_2 fumigation. Sardsud *et al*. (2003) found that dipping longan fruits in 5.0% CA could best control the change in pericarp color. Kheuenmanee *et al*. (2005) found that 5.0% CA plus 0.3% potassium sorbate could control pericarp color and disease up to 30 days while Whangchai *et al*. (2006) found that dipping longan fruits in 5.0% CA prior to ozone fumigation for 60 min could control pericarp browning for 20 days at 5°C. However, using CA in high concentration at 2, 4 and 6% was reported to damage the fruits' surface and stimulate pericarp browning in longkong (Lichanporn

et al., 2002). Recently, the same results were also reported in litchi (Saengnil *et al*., 2006) and longan (Apai *et al*., 2008a).

CA alone at the concentration of 1, 3 and 5% with the pH of 2.6, 2.4 and 2.2, respectively showed a high severity of pericarp browning in line with the increasing CA concentration (Apai *et al*., 2008a). This higher level of browning that occurred was a consequence of using the plastic film wrap which maintained a high humidity around the fruit (Kader, 1994). Even though the fruit were dried, the CA residue on the fruit skin would have become active in solution under high humidity conditions, which would have led to bleaching, impregnation and damage of the fruit skin inducing ethylene production and causing a degradation of nutrient and antioxidant content (Abeles *et al*., 1992). These results suggested that escalating skin damage after CA dipping might also be related to both pH of a solution and the natural attribute of each food texture. Adding chitosan to CA can reduce contact between CA and the fruits' skin; therefore, fruit damage could be significantly decreased and its shelf life can be extended. This suggested that pericarp browning could also be improved if pH solution is adjusted closer to 3.3 (CA+Cts). When the same experiment was conducted with the longan fruits of thicker pericarp (greater than 1.0 mm. or 1,000 μm and not dented when pressed), the fruits could better tolerate the acid and control pericarp browning for 27 days as compared to longan with thinner pericarp which could prolong browning for only 14 days at 5°C (Apai *et al*., 2008a). However, the fact that pericarp thickness, pH and relative humidity could have key effects on using CA and chitosan has not yet been previously published elsewhere.

From Table 3.11, disease incidence (DI) had correlation with browning index ($r = 0.89$) and L* ($r = -0.89$). The results revealed that DI was the one factor affecting browning development (Jiang *et al*., 2002). The high humidity inside the packaging rapidly led to increased fungal disease development on the fruit surface within 10 days. CA+Cts and CA treatments could help prolong the DI percentage (Figure 3.8d). The fruit treated with CA+Cts had less DI and the decay development occurred only at the stem end of the fruit or some parts of the fruit surface area. Conversely, CA and the control showed the highest disease development and pulp rot (data not shown) in accordance to the highest browning index (Figure 3.7a; Figure 3.9). This result

suggests that CA+Cts should be added with some additives such as potassium sorbate (Baldwin *et al*., 1996) for delaying DI including pericarp browning.

(A) (B)

Figure 3.9 Effects of chitosan-based coating and citric acid on characteristic of pericarp browning after day 20 (a) and 27 (b) of longan fruit during storage at 5°C.

3.4.2.3 PPO activity and total phenol content

According to the browning index, dipping longan fruits in 1.0% CA with chitosan significantly helped delay PPO activity during storage ($p<0.05$) (Figure

3.10a). This result was in agreement with those reported by Ducamp-Collin *et al*. (2008), which suggested that PPO activity of litchi during storage was inhibited after treatment with CA+Cts. While dipping in 1.0% CA without chitosan, and control (distilled water) only postpones PPO activity at only the beginning of storage. PPO activity in the control treatment continued to increase until day 20, then decreased until the end of the storage. For the fruits treated with CA and CA+Cts, it was found that PPO activities increased during the first 10 days, then continued to decline until the end of the storage. CA inhibited PPO by decreasing pH in food and binding with Cu^{2+} in active site of PPO to form an inactive complex (Martinez and Whitaker, 1995). Jiang (1999) found that PPO of 'Shixia' longan had the optimum pH (pH = 6.5) which was close to 7.0. Tipton and Dixon (1983) found that the active site of PPO was denatured at the low pHs and the substrate was not active due to the reduction in cationic substrate.

Regarding the relationship between PPO activity and browning (Table 3.11), different opinions have been expressed. Mayer and Harel (1979) considered that browning in plant tissue was directly correlated with PPO activity, while Amiot *et al*. (1992) found no correlation between PPO activity and browning potential (BP) in various fruits in accordance with this experiment. It was concluded according to Cheng and Crisosto (1995) that PPO activity was not the unique limiting factor in the enzymatic browning. Both the presence and absence of PPO activity dependency in fruit browning has also been reported among grape cultivars and apples (Cheng and Crisosto, 1995).

Phenolic compounds are the substrates of PPO; most antioxidants are identified in this group. They were found to delay the senescence process in many fresh produce. High total phenolic content was also be detected in longan fruits receiving SO_2 fumigation (Wu *et al*., 1999). This is also consistent with the total phenolic results in this experiment which demonstrated a significant negative correlation with browning index and weight loss percentage (Table 3.11). Application of chitosan combined with CA was found appropriate to retard the loss of total phenolic content in the pericarp as compared with other treatments (Figure 3.10b). Therefore, the browning was delayed till Day 27. Nevertheless, total phenolic content was well negatively correlated with disease incidence (Table 3.11)

which indicated that total phenolic content might have an effect on disease control in pericarp. Since chitosan is able to delay water loss (Jiang and Li, 2001; Lin *et al*., 2005), chitosan coating could well impede total phenolic content loss in many fruits (Zhang and Quantick, 1997; Vangnai *et al*., 2006) which also complied with our study.

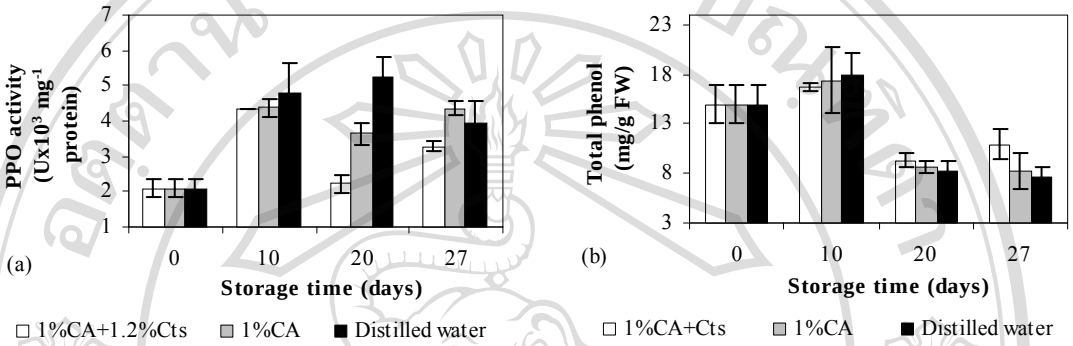

Figure 3.10 Effects of 1% citric acid (CA) solution and 1% CA combined with 1.2% chitosan (Cts) solution on the PPO activity (a) and total phenolic content (b) of longan fruit during storage at 5°C. The distilled water treated longan fruits were used as the control. Vertical bars indicate standard deviation.

3.4.2.4 Pearson's correlation analysis between browning parameters.

Table 3.11 Correlation coefficients between browning parameters results during storage at various temperature.

	BI	p-pH	TA	WL	PPO	TP	L*
BI	1						
p-pH	0.66*	1					
TA	-0.74**	-0.92**	1				
WL	0.90**	NS	NS	1			
PPO	NS	NS	NS	NS	1		
TP	-0.83**	NS	NS	-0.76**	NS	1	
L*	-0.89**	NS	NS	-0.94**	NS	0.70*	1
C*	-0.74**	-0.65*	0.62*	-0.75**	NS	NS	0.87**
h˚	-0.64*	NS	0.62*	NS	NS	0.83**	NS
DI	0.88**	NS	NS	0.94**	NS	0.74**	0.88**

[1]NS = not significant, *p = 0.05, **p = 0.01, N = 12.
[2]BI = browning index, p-pH = pericarp pH, TA = titratable acidity as citric acid content, WL = weight loss, PPO = polyphenol oxidase, TP = total phenol, DI = disease incidence
L* = lightness, C* = chroma, h˚ = hue angle.

3.5 Conclusions

Regardless of the cost, this approach can be considered a very effective alternative method to SO_2 fumigation. Application of CA with chitosan-coated base delayed pericarp browning for 27 days in comparison with CA alone and the control during storage at 5˚C. The treatment delayed the increase of browning parameters: pericarp pH; TA in pericarp homogenate; PPO activity; and total phenolic content loss. This treatment can be used as an alternative treatment in the future for controlling pericarp browning in longan fruits. A similar approach of using an edible coating substance as a carrier of different additives may also be exercised with various produce. Moreover, coating formulation might be improved in order to correct its weakness and add extra benefits, for example, employing fatty acid to prevent water loss, adding other food additives to protect produce from some diseases.