CHAPTER 6

EFFECTS OF CHITOSAN COATING ON CHILLING INJURY TOLERANCE AND DISEASE INCIDENCE ON LONGAN FRUIT BY AN ELICITOR ON ACCUMULATION OF PATHOGENESIS-RELATED PROTEIN AT LOW TEMPERATURE

6.1 Abstract

The optimum storage temperatures: 20; 5 and 2˚C of coated longan fruit with 1.2% Chitosan (Cts) dissolved with 3.0% citric acid (CA) and 0.3% potassium sorbate (PS) mixture at a pH of 2.8 in comparison to untreated fruit were investigated. Fresh longan fruits were dipped in solution for 1 min. After being dipped, the fruits were air-dried, packed in 11 μm thick PVC film wrapped foam tray. Results indicated that total phenol (TP) during storage was negatively related to discoloration, polyphenol oxidase (PPO) activity and fruit decay. During all storage temperatures, dipping fruits in Cts + CA + PS at a pH of 2.8 slowed down the total phenol loss and PPO activity in the initial storage but not at the end of storage. Fruit decay was completely controlled during storage at 2˚C but not at 20˚C. Chilling injury symptoms of coated fruits during storage at 2˚C were observed and pericarp browning at 20˚C storage occurred rapidly (within 10 days). However, chilling injury tolerance of this treatment at 5˚C was more improved and pericarp browning was delayed for 20 days as well as weight loss, electrolyte leakage and fruit decay. These results indicated that addition of PS in Cts+CA at a pH of 2.8 clearly showed high efficacy in disease control that prevented a high level of TP degradation rather than browning control. However, TP degradation decreased as PPO activity increased at the last day because pericarp damage of fruit tissue was evident due to lower pH of the acid-coating.

Increasing the pH of 1.2% Cts + 3% CA + 0.3% PS to 3.3 showed a higher efficacy in browning control and retained excellent disease control in comparison to Cts + CA + PS at a

pH of 2.8, CA+PS at pH of 2.4 and untreated fruits. The efficacy of Cts + CA + PS at a pH of 3.3 was indicated by the lowest browning index and the highest pericarp color values (L*, C* and h°) after 32 days. The delay in pericarp browning for fruits treated with Cts + CA + PS at a pH of 2.8 and fruit treated with CA+PS and the control fruit was 24, 20 and 28 days, respectively. Fruits dipped in Cts + CA + PS at a pH of 3.3 exhibited decreased pericarp pH, weight loss, PPO activity, total phenol loss and retained excellent fruit color and eating quality during cold storage and a subsequent shelf life test at ambient conditions. Chitosan along with CA + PS (pH 3.3) prevented sorbic acid degradation in pericarp of fruits when compared with application of CA + PS alone. In this experiment, it was shown that PR-protein was related to chilling injury tolerance (L* value and pericarp pH; $r > 0.70$) more than pathogenic response. SO_2 clearly showed the highest amount of pathogenesis related-protein over other treatments in accordance with the highest efficacies in CI tolerance and disease control more than 32 days. SO_2 produced the lowest pericarp pH, PPO and total phenol loss. Improving pH of Cts + CA + PS at a pH of 3.3 significantly delayed pericarp browning. However, the fruits fumigated with SO_2 showed the poorest eating quality because of off-odors.

6.2 Introduction

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. Joas *et al*. (2005) found that using Cts + CA could control pericarp browning of litchi fruit more than using CA alone. It controlled browning by reducing pericarp pH and weight loss. Ducamp-Collin *et al.* (2008) found that Cts + CA could delay browning for 21 days during simulation of shipping conditions by maintaining anthocyanin and controlling enzymatic browning (PPO and POD activity). 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. Apai *et al*. (2008b) showed that adding the food additive: potassium sorbate, in the coating can improve control of the major important longan pathogen, *Lasiodiplodia theobromae* during in vitro and in vivo trials. Plant responses too many biotic and abiotic stresses are orchestrated locally and systemically by signaling molecules. Bautissa-Banos *et al*. (2006) reviewed that chitosan

induces a series of defense reaction correlated with enzymatic activities. Phenolic compounds and synthesis of specific phytoalexins with antifungal activity were reported in grape and tomato. Although the biological activity of chitosan is well-documented, the mechanisms those underlie its ability to function as both a fungal inhibitor and an elicitor of plant defense reactions in different produces remains unclear. Our experiment suggested that decrease of disease severity and disease incidence did not related to the activity of chitinase and ß-1,3-glucanase (pathogenesis related-protein; PR-protein), but the increase of these enzymatic activities related to wounding and pathogenic infection (data not shown). In nevertheless, Ding *et al*. (2002) reported that the expression of PR-protein genes which was induced by methyl jusmonate enhanced their resistance to chilling tolerance and resistance to pathogens, thereby decreasing the incidence of decay was reported in tomato. In according to heat treatment, could induce PR-protein in tangerine fruit resistance to *P. digitatum* and chilling injury. Interestingly, this stress response has not yet been reported in chitosan coating on longan fruits.

The aims of this combination have been found out the optimum temperature storages during cold storages and to evaluate the treatments with CA+PS and chitosan at various temperature storages on pericarp browning, fruit decay and their relations between enzymatic browning and total phenol. In the last experiment, treatment with CA + PS CTs at two pH conditions and CA + PS on pericarp browning (enzymatic browning, total phenol), fruit decay, active substance degradation and shelf life whether in comparison with SO_2 during optimum cold storage and simulated marketing shelf life at ambient conditions were investigated. The possible mechanism involved elicitation effects of coating material as compared with SO_2 fumigation on chilling injury tolerance and disease incidence by accumulation of pathogenesis-related protein (PR-protein) in pericarp at low temperature was evaluated.

6.3 Materials and methods

6.3.1 Plant materials and experiments

Mature yellow ‘Daw’ longan fruits (*D. longan* Lour.) 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. The fruits were washed in distilled water dried at room temperature before treating.

6.3.1.1 Interactive effects of the storage temperature and chitosan coating on chilling injury and disease incidence of longan fruits

To find the optimum temperature storages after coated fruits with chitosan coating with citric acid and potassium sorbate. Two treatments were used: 1.2% Cts + 3% CA + 0.3% PS (pH 2.8) and non-treated fruit. Fruits were dipped for 1 min in 1.2% Cts + 3% CA + 0.3% PS (pH 2.8). 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 20, 5 and 2ºC with 85% RH. For each treatment, three replicates were used. Samples were taken every d 5 during storage for quality evaluation until d 20. Fruits were evaluated for changes in disease incidence (%), pericarp browning of inner and outer pericarp, fruit surface color both inner and outer pericarp, membrane permeability (%), weight loss percentage, polyphenol oxidase (PPO) assay, protein content and total phenol content determination.

6.3.1.2 Effects of the optimum storage temperature of the coated longan fruits on PR- protein, bio-chemical changes in pericarp and market shelf life

Based on the results of the experiment 6.3.1.1, to more intensively investigate the mechanisms involved in reducing the chilling injury of longan fruit by 1.2% Cts + 3% CA + 0.3% PS, two coatings at different pH values were used. 1.2% Chitosan + 3% CA + 0.3% PS (pH 2.8) was a non-adjusted pH solution and 1.2% Cts + 3% CA + 0.3% PS (pH 3.3) was adjusted to a pH of 3.3 by adding 5 N NaOH. Fruits were dipped for 5 min in 1.2% Cts + 3% CA + 0.3% PS (pH 2.8), 1.2% Cts + 3% CA + 0.3% PS (pH 3.3) or 3% CA + 0.3% PS (pH 2.4). After dipping and the same handling, the fruits were stored at 4 ± 1 ºC at 90% RH. The non-treated fruit (negative control) and SO_2 fumigated fruit (positive control) were used as control. For each treatment, three replicates (foam trays) were used. Samples were taken every 4 d during storage for quality evaluation until day 32. Fruits were evaluated for changes in PR-protein (chitinase and ß-1,3-glucanase) as a defense enzymes which were induced due to pathogenic response and chilling injury tolerance (Ding *et al*., 2002) as well as disease incidence (%) and pericarp browning of outer pericarp. The changes in fruit surface color, weight loss percentage, pericarp pH, sorbic acid degradation in pericarp and flesh and flesh quality as overall acceptability were also evaluated.

6.3.2 Pericarp discolorations and disease incidence

Browning of exterior pericarp was assessed visually by measuring total browning areas of the outer pericarp (Jiang and Li, 2001). Disease incidence (%) was observed. Browning of interior pericarp which was typically observed as chilling injury development (Boonyakiat *et al*., 2002) was assessed visually by measuring total browning or water soaking areas of the inner pericarp on each of ten fruits. The following scale was used for inner pericarp assessment: 1 = no browning or soaking (excellent quality); 2 = browning or water soaking on less than 25% of the total inner surface; 3 = 25–50% browning; 4 = 50–75% browning; 5 ≥ 75% browning (poor quality) (Figure 6.1). The color of outer and inner pericarp of longan was measured.

1 = 0% browning area of inner pericarp

2 = 1 - 25% browning area or water soaking symptoms of inner pericarp

3 = 25 - 50% browning area of inner pericarp

4 = 50 - 75% browning area of inner pericarp

5 = > 75% browning area of inner pericarp

Figure 6.1 Browning index of inner pericarp caused by chilling injury during cold storage.

6.3.3 Membrane permeability and weight loss percentage

Electrolyte leakage was determined by the method of King and Ludford (1983) with some modification. Fifteen pericarp discs (1 cm diameter) weighing about 1 g, were rinsed 3 times in deionized (DI) water, dried with tissue paper and placed in 25 mL of a 0.4 M mannitol solution at room temperature. Electrical conductivity of the solutions (electrolyte leakage from the discs) was determined after 3 hours with conductivity meter (Sartorius Professional Meter PP-20, Sartorius AG, Germany). Afterwards, the samples were autoclaved at 121^{o}C, 15 psi for 30 min, cooled and a final conductivity reading was taken for total tissue electrolytes. Leakage data was expressed as percentage of the total electrolytes. Percentage of leakage was calculated as the ratio of the initial reading to the final reading. Weight loss percentage was also determined.

6.3.4 Total phenol content and activities of PPO

Total phenol assay, the peels from five fruits of each replication were frozen with liquid nitrogen and then powdered using the Moulinex blender. Frozen tissues (3.0 g) were homogenized in 80% ethanol at 4°C. The homogenate was centrifuged at 4,100 x g for 20 min and then supernatant was collected to assay total phenol modified according to the method of Singleton and Rossi (1965), using gallic acid as a standard. The phenol contents were expressed as gallic acid equivalents in milligrams on a fresh weight (FW) basis.

Polyphenol oxidase (PPO) assay, the peels from five fruits of each replications were frozen with liquid nitrogen and then powdered using the Moulinex blender. The powdered pericarp (1.0 g) was homogenized in 8 mL of 0.1 M phosphate buffer (pH 6.4) at 4°C. The homogenate was centrifuged at 4,100 x g for 20 min and then supernatant was collected to assay PPO activity modified according to the method of Jiang (1999), by measuring the oxidation of catechol. 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.

6.3.5 Pathogenesis related- protein as a defense mechanism

In preparing the crude chitinase and ß-1,3-Glucanase, the peels from five fruits of each replication were frozen with liquid nitrogen and then powdered using the Moulinex blender. Frozen tissues (3.0 g) were homogenized in 9 ml of 50 mM sodium acetate buffer, pH 5.0 at 4°C. The homogenate was centrifuged at 4,100 x g for 20 min and then supernatant was collected to assay.

Chitinase activity was assayed using swollen chitin (Monreal and Reese, 1969) following the method of Reissig *et al*. (1955), with slight modifications. Chitinase activity was measured by mixing 1 ml of crude enzyme solution with 1 ml of 1% swollen chitin in 50 mM citrate buffer (pH 6.6). After incubation at 50˚C for 60 min, the reaction was stopped by boiling for 5 min. 0.5 ml supernatant and 0.1 ml of 0.8 M $K_2B_4O_7$ were mixed and boiled for another 3 min, then placed in an ice bath and 3 ml distilled dimethylaminobenzaldehyde (DMAB) was added and incubated for 20 min at 37˚C. The optical absorption was measured at 585 nm using a UV/Visible Spectrophotometer (PerkinElmer lambda 35, USA). The amount of N-acetylglucosamine (NAG) released was calculated from a standard curve prepared with NAG and the chitinase activity was expressed in units (U/mg protein).

ß-1,3-Glucanase activity was determined by a colorimetric method (Burner, 1964). The amount of reducing sugar released from laminarin was measured. The standard assay contained 50 µl of the crude enzyme solution and 50 µl of 5 mg/ml laminarin in 0.1M sodium acetate buffer pH 5.0. After incubation at 35˚C for 30 min, the reaction was stopped by boiling for 5 min and 0.2 ml of 1% dinitrosalicylate (DNS) and 0.2 ml of sodium acetate buffer were added and boiled for another 5 min, then placed in an ice bath and 0.9 ml sterile distilled H_2O was added. The optical absorption was measured at 540 nm. The amount of reducing sugar released was calculated from a standard curve prepared with glucose and the glucanase activity was expressed in units (U/mg protein). The protein content was determined according to the dye-binding method of Bradford (1976) using bovine serum albumin as the standard.

6.3.6 Sorbic acid degradation

Sorbic acid (SA) degradation in the part of fruit pericarp and flesh were analyzed by modifying method of Kamlert (1992). Fifteen fruits, five in each of the three replications, were used to prepare sorbic acid samples. Pericarp and flesh 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 was shaken 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 sorbic acid (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 sorbic acid contents in pericarp or flesh were expressed as mg/kg.

6.3.7 Sensory evaluations

For sensory evaluation, samples were evaluated by 5 trained panel members using 9-point hedonic scale. The following attributes were selected for characterization of the quality of longan fruit: color of pericarp acceptability and eating qualities (appearance, flavor, taste and overall acceptability).

6.3.8 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.

6.4 Results and discussion

6.4.1 Interactive effects of the storage temperature and chitosan coating on chilling injury and disease incidence longan fruits

6.4.1.1 Fruit decay

From Figure 6.2, the results showed that using coating material: 1.2% chitosan (Cts) + 3.0% citric acid (CA) + 0.3% potassium sorbate (PS)-pH 2.8 significantly delayed disease incidence during all storage ($p<0.01$). Coating material could delay disease incidence (DI<25%) during storage at 2, 5 and 20˚C for 20, 20 and 5 days when compared with untreated fruits for 20, 5 and <5 days. This result indicated that using coating material in combination with low temperature storage increased the high efficacy on disease control more than that storage at high temperature.

The important mechanism of Cts+CA+PS-pH 2.8 to control disease development might 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). Cts along with CA + PS could help to delay the rate of sorbic acid degradation in the pericarp that PS may have gradually diffused into the longan surface and consequently interacted with the pathogens on the fruit surface (Apai *et al*., 2008b). 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 + PS in coating materials of a pH 3.0 delayed disease population in strawberry. However, this result was contrasted with Park *et al.* (2005) who found that dipping inoculated strawberry with two pathogens in chitosan coating with or without PS at a pH of 5.5 did not show significant result. This suggests that this coating might cause by too higher pH coating more than pKa of sorbic acid.

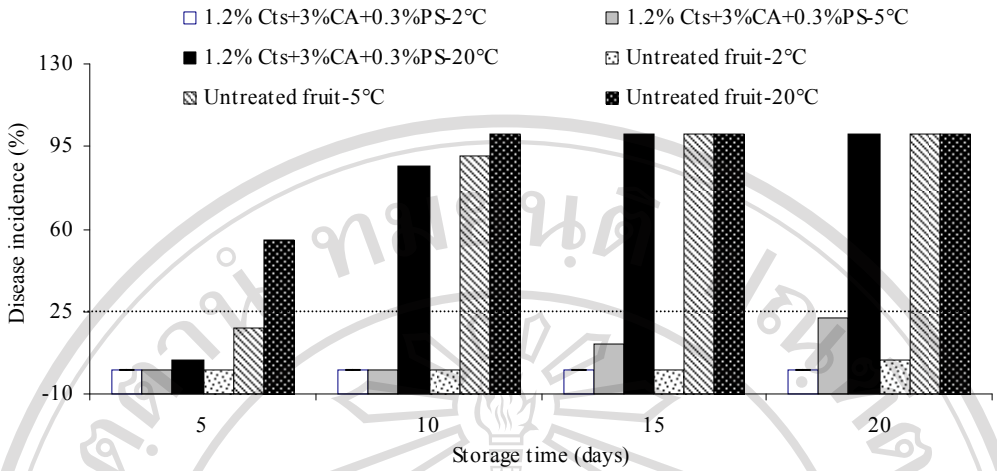

Figure 6.2 Effects of coating on incidence of decay of fresh longan fruits during storage at 20, 5 and 2°C. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

6.4.1.2 Changes in pericarp discolorations

From Table 6.1, high positive correlation between outer and inner pericarp browning was found. Pericarp browning was decreased as temperature storage was reduced from 20 to 5˚C but increased again when stored at 2˚C due to chilling injury induction caused by low temperature within 10 day (Figure 6.3). Using coating material did not delay pericarp browning during 2˚C and was initially induced at 5 days which observed a few water soaking area in inner pericarp and more severely browning damage was observed in both outer and inner pericarp within 10 day. Outer and inner pericarp browning was not acceptable (BI>3.0) at day 15 and 20. This suggested that chilling injury tolerance was related to cultivar, production region and delay time after harvesting. Jaitrong *et al*. (2004) found that microscopic observation of chilling injury was showed damage of epidermal hair and fibrous tissue of exocarp and parenchama damage of mesocarp, however; pulp quality was not affected by this phenomenal and still normal characteristics.

Coating material delayed pericarp browning when stored at 5˚C up to 20 days at a BI of outer pericarp = 2.20 (±0.20) (BI<3.0) as compared with control fruit at a BI = 2.73 (±0.29) (Figure 6.3a) whereas inner pericarp browning showed not significant and BI less than 3.0 (<50%)(Figure 6.3b). Except for decay, chilling injury during low storage is a main problem of longan in term of market shelf life. Coating material could delay only the initial stage of storage compared with control fruit. Chilling damage was slightly occurred after day 5 at 5˚C and after that it slowly increased and finally rapidly increased after day 20 and 25.

This phenomenal indicated that coating material (Cts+CA+PS-pH 2.8) could act as browning delay more than inhibition. Pulp quality was affected in only control fruit due to the highest pathogen infection comparing to coating material (data not shown).

In contrasted during 20˚C, coating material could significantly control pericarp browning (BI<3.0) of both outer and inner peel for 10 and 15 days compared with control fruit. Pericarp browning during high temperature storage showed rapid shrivels and glossy characteristics at inner peel caused by higher water loss in pericarp by 5 day and after that fruit decay was rapidly attraction and then pulp quality was rot and off-flavor after 15 days due to senescence process (data not shown).

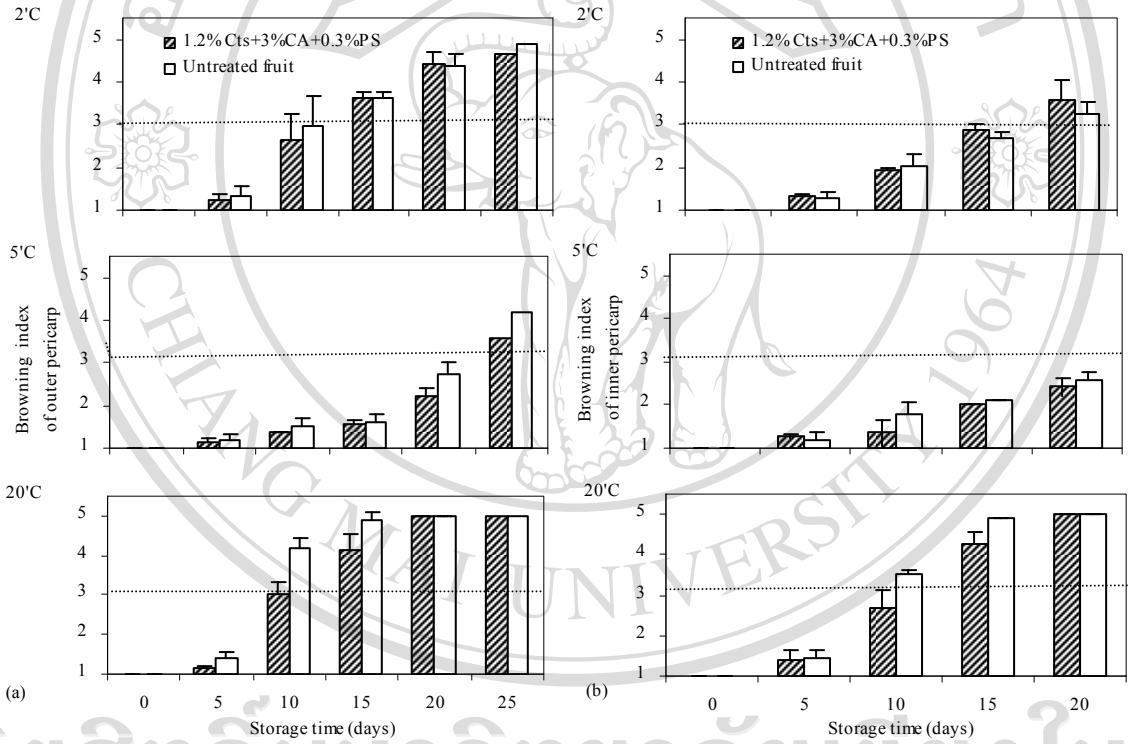

Figure 6.3 The changes in pericarp browning of outer pericarp (a) and inner pericarp (b) during storage at 20, 5 and 2ºC. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

BI of outer pericarp: 1 = no browning (excellent quality); 2 = slight browning; 3 = browning on less than 25% of the total surface; 4 = 25–50% browning; 5 ≥ 50% browning (poor quality).

BI of inner pericarp: 1 = no browning or soaking (excellent quality); 2 = browning or water soaking on less than 25% of the total inner surface; 3 = 25–50% browning; 4 = 50–75% browning; 5 ≥ 75% browning (poor quality).

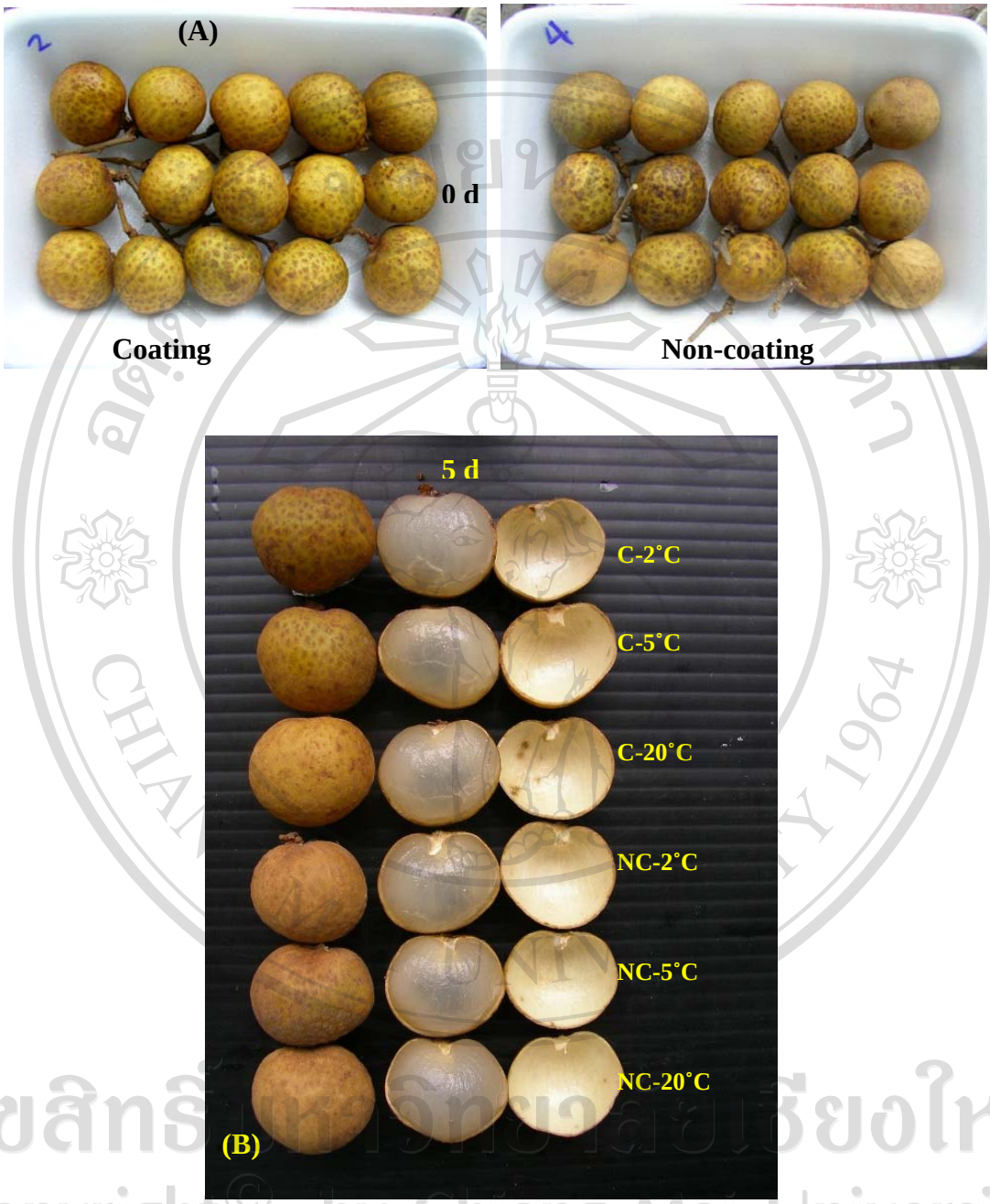

Figure 6.4 Characteristic of pericarp browning development of coated fruit and non-coat (b) during storage for 0 (a) and 5 days (b) at 25 at 5 and 2˚C. (C = coating with PS+Cts+CA-pH 2.8 and NC = non-coating-control fruit).

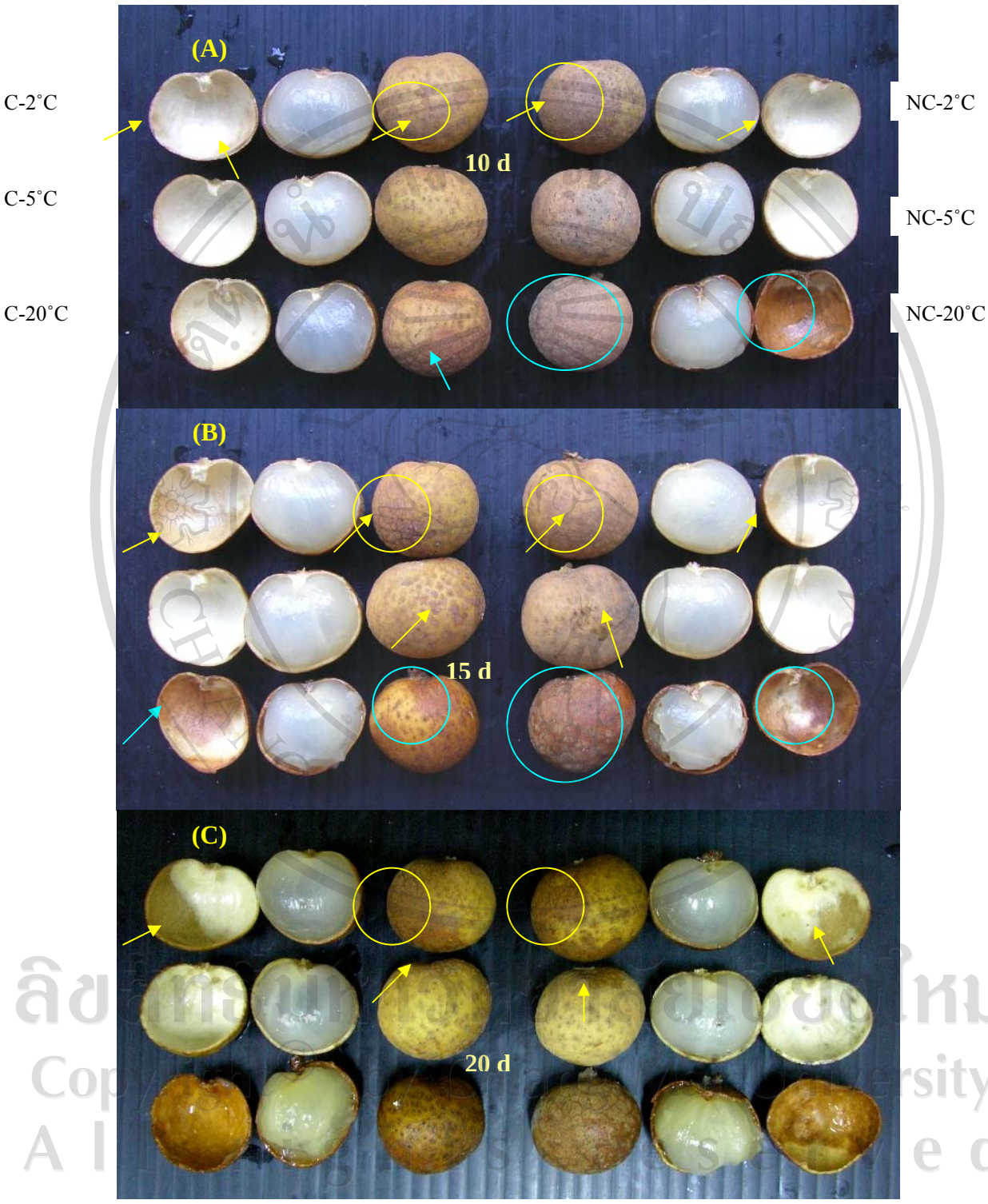

Figure 6.5 Characteristic of pericarp browning development of coated fruit and non-coat during storage for 10 (a), 15 (b) and 20 days (c) at 20, 5 and 2˚C. (C = coating with PS+Cts+CA-pH 2.8 and NC = non-coating-control fruit). (Continue).

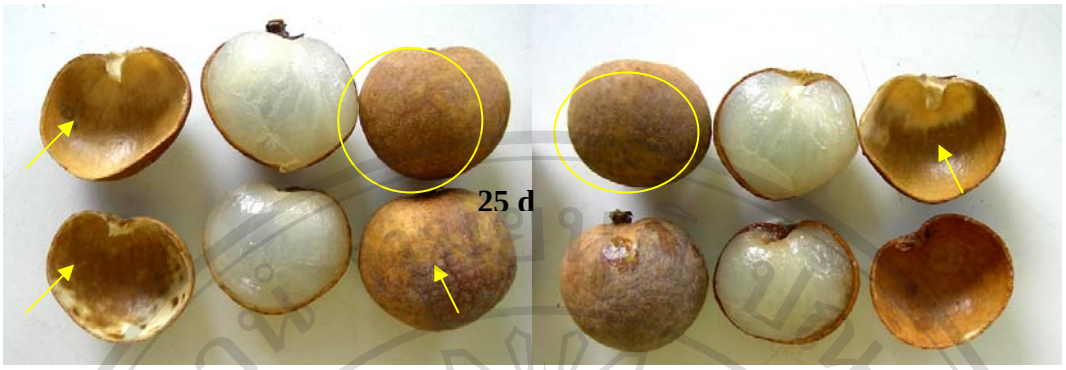

Figure 6.6 Characteristic of pericarp browning development of coated fruit and non-coat during storage after day 25 at 5 and 2˚C. (C = coating with PS+Cts+CA-pH 2.8 and NC = non-coating-control fruit). (Continue).

Note: C and NC-2˚C (upper), inner peel showed dark brown chilling injury area only bottom of fruits and aril showed normal color.

C and NC-5˚C (upper), inner peel of C showed less severe chilling injury area than NC and aril was less soft rotting.

Figure 6.7 and 6.8, pericarp discoloration of colorimeter (objective parameters): L*; C* and h˚ of outer pericarp or L* and h˚ of inner pericarp was declined during storage. From Table 6.1, they were negatively correlated well with inner and outer pericarp browning (subjective parameters). Result indicated that two methods were accordingly high consistence efficacy. In contrasted with C* of inner paricarp, it was not correlated with BI and therefore it could not be used to indicate pericarp discoloration changes.

From Figure 6.7, coating material maintained the highest values of L* and h˚ of both outer and inner pericarp at 5˚C. L* and h˚ values were more declined at 2˚C and non-significant with control according to pericarp browning change. L* and h˚ values was mostly declined rapidly at 20 after 5 days due to senescence from high temperature storage.

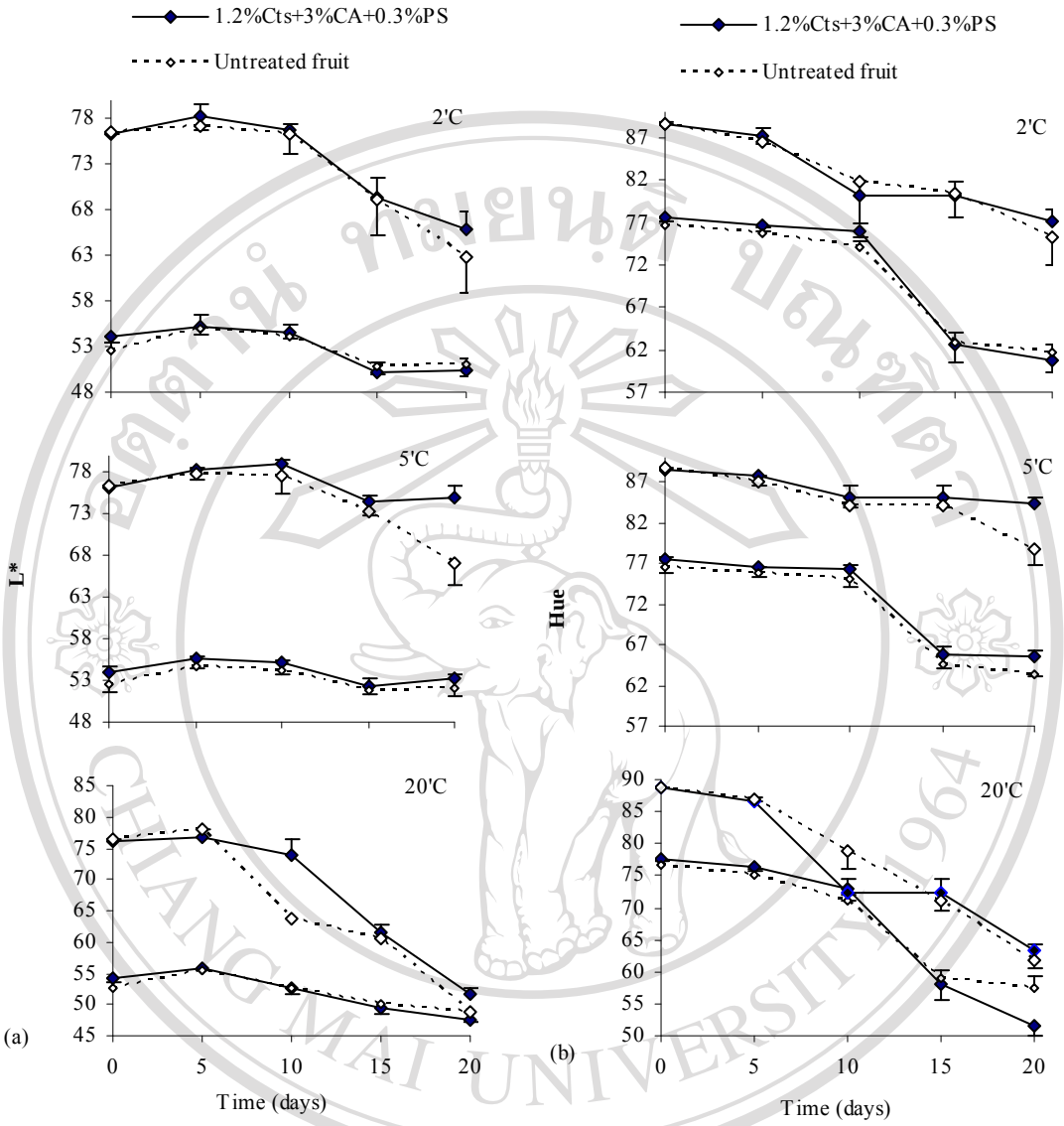

Figure 6.7 The changes in L* (lightness) (a) and h˚ (hue angle) (b) of pericarp longan during storage at 20, 5 and 2ºC. Lower graph = outer pericarp and upper graph = inner pericarp. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

In addition to coating material significantly maintained the highest C* value of outer pericarp at all temperature storages and showed high consistence more than L* and h˚ values especially at 5˚C (Figure 6.8a). While C* value of inner pericarp did not consistence during storage (Figure 6.8b).

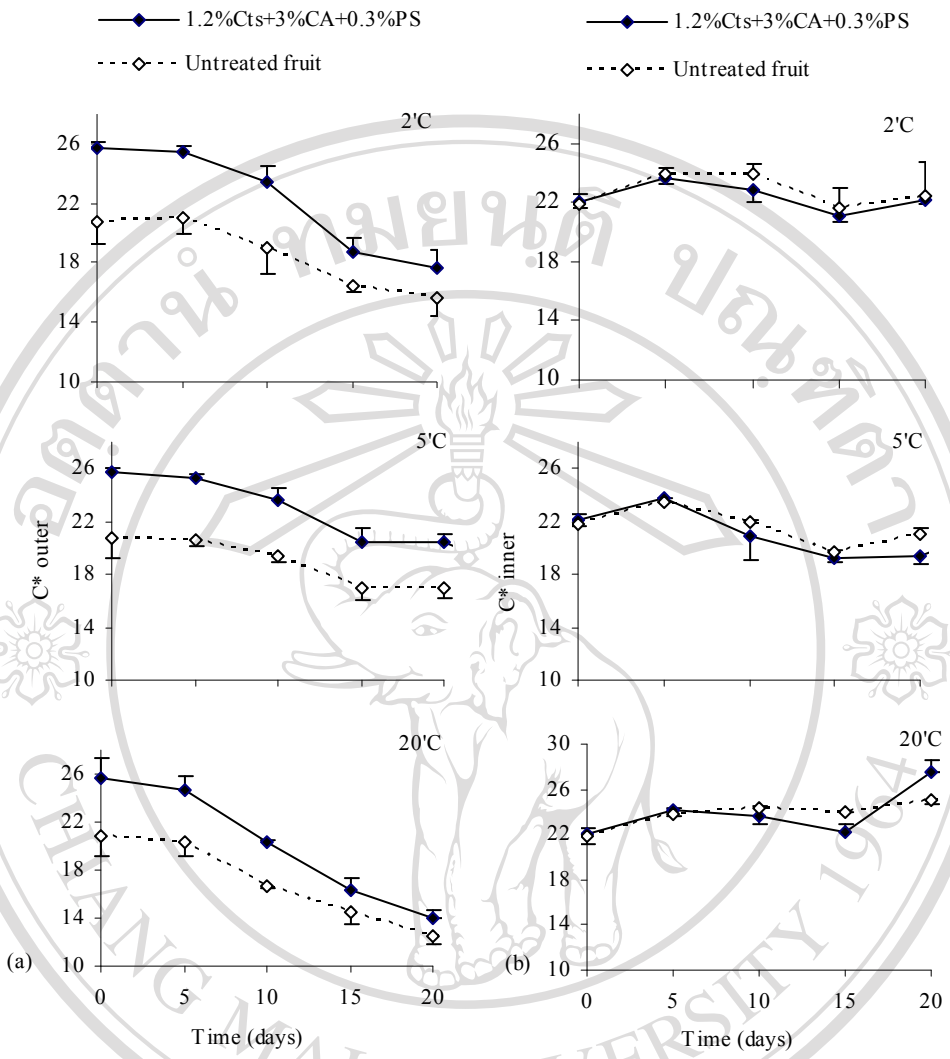

Figure 6.8 Effects of coating on chroma of outer (a) and inner pericarp (b) of longan fruits during storage at 5°C. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

6.4.1.3 Membrane permeability and weight loss percentage

From Table 6.1, high positive correlation of electrolyte leakage percentage (EL express as membrane permeability) and weight loss percentage (WL) with pericarp browning in both outer and inner pericarp were found. They also correlated well with L*, C* and h values. This would indicate that browning is related to at least some structural damage to the plant tissue (Jaitrong *et al*., 2004). In addition, EL and WL were correlated well with disease incidence percentage (r = -0.66 and 0.72). Results concluded that fruit decay was one of important factors as affected on discoloration changes which occurred when cell membrane

integrity is damaged. Thus, enzymatic browning bought into contact and polymeric quinones are rapidly formed by enzymatic oxidation to complete pericarp browning. There were many mycelium or rot cover on fruit surface and pulp quality begin to rot and off-flavor due to fermentation which had many characteristics up to disease species and contents during storage.

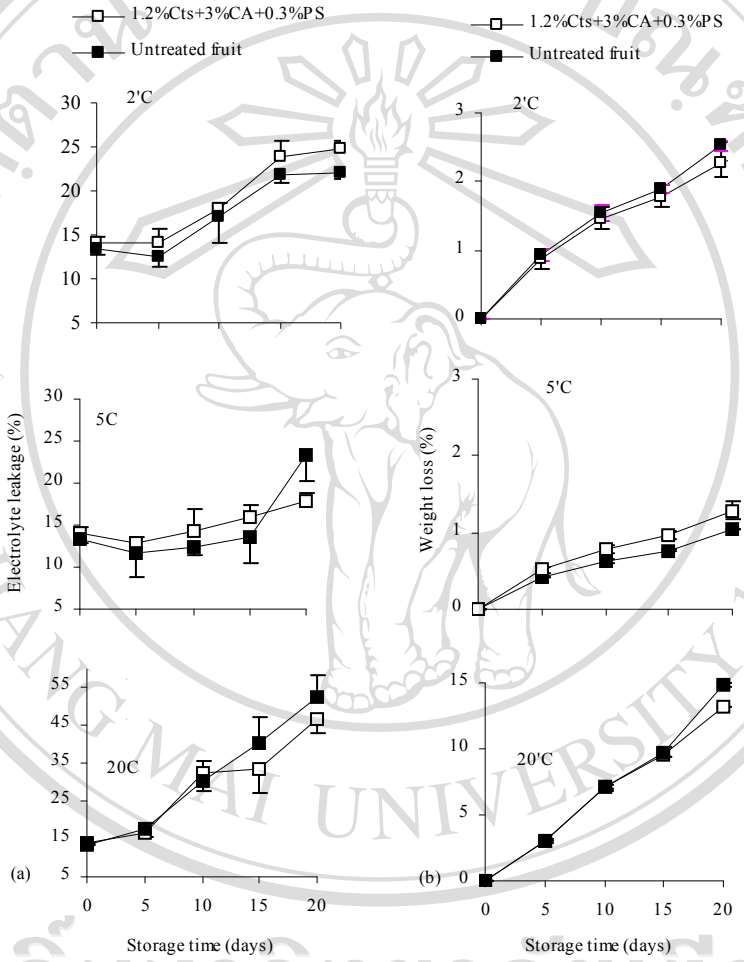

Figure 6.9 Effects of coating on electrolyte leakage (%) (Left) and weight loss (%) (Right) of longan fruits during storage at 5°C. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

From Figure 6.9, EL of pericarp and WL rapidly increased during storage 2˚C more than 5˚C according to discoloration change and rapidly increased after 5 days though storage. Coating material could not reduced EL and WL during storage at 2˚C because the temperature stress was predominantly as a primary event and then induced chilling injury after d 10. Many cell membranes in pericarp were rapidly lost their permeability's which expressed by increasing in electrolyte leakage and weight loss during storage (Murata, 1990).

However, in the best way, the lowest of EL and WL were found under coating material which significantly delayed EL and WL during storage at 5˚C for 20 days, however; they were rapidly increase again when stored at 20˚C.

6.4.1.4 Enzymatic browning and total phenolic content.

From Figure 6.10, coating material significantly delayed increased PPO activity and total phenolic content loss but not the end of storage. The result suggested that this occurrence might be happened from both chilling injury at low storage and water loss in associated disease attraction at high temperature storage. The changes in the PPO pattern were similar under all storages. The highest peak of control during 2 and 5˚C were found at 15 day while 20˚C showed at 10 days and then PPO peak were declined. Total phenolic content decreased at low temperature than at high temperature storage. However, coating material significantly delayed total phenolic content loss during storage at 20˚C followed by 5 and 2˚C, respectively. Result suggest that total phenol of coated fruit was less used as a substrate of PPO because it reduced PPO activity according to the lowest BI and the highest L*, C* and h˚ as compared with control fruit. However, limitation of coating material was found under too lower pH coating which may increase damage on fruit surface and therefore improving pH of coating for shelf life extension was needed.

From Table 6.1, negative correlation between total phenol and PPO activity were found. Total phenolic content and BI of inner and outer pericarp was significantly positively correlated in contrasted with PPO activity with BI which was not correlated. This experiment suggested that PPO did not direct factor on pericarp browning. This suggested that increased protein profiles caused by many diseases in pericarp during fruit senescence near the end of storage might also affect on the pattern of PPO peak (data not shown). However, the predominantly direct factor might be happen from either chilling injury induction during lower temperature storage or disease attraction and water loss during high temperature. This absence of correlation was agreed with the other reports (Amiot *et al*., 1992; Cheng and Crisosto, 1995) whom suggested that the PPO activity was not a limiting factor in the enzymatic browning. This decline is browning tendency may be explained rather by a decline in the concentration of activators such as fatty acids and organic acids (Hutcheson and Buchanan, 1980; Amiot *et al.,* 1992) or by a decrease in phenolic substrate synthesis (Harel *et al*., 1966). In addition to the other factor had affected on pericarp browning such as maturity index, variety, mineral, which remains to be established.

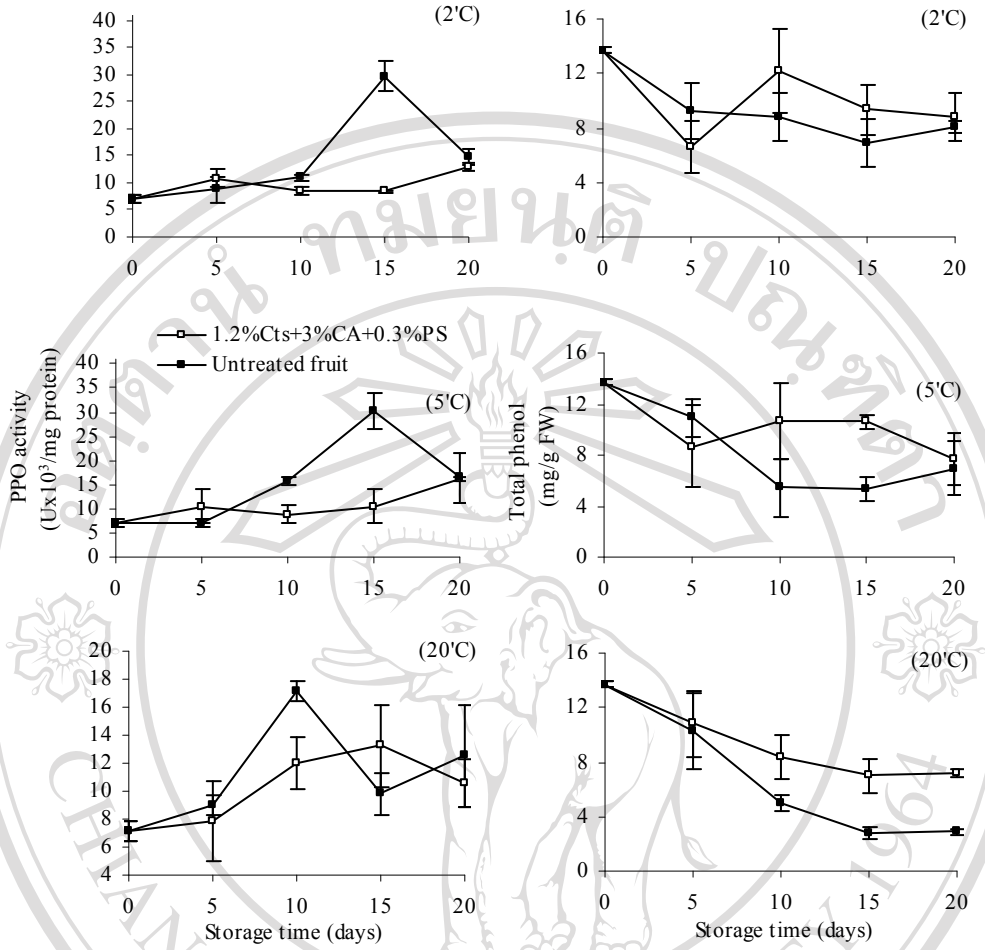

Figure 6.10 Effects of coating on PPO activity (a) and total phenolic content (b) of longan fruit during storage at 20, 5 and 2°C. Cts = chitosan; CA = citric acid; PS = potassium sorbate.

In nevertheless, total phenolic content was well negatively correlated with disease incidence (Table 6.1) which indicated that total phenolic content might affect on disease control in pericarp. Disease incidence had negatively also correlated with weight loss. Coating material could delay total phenol loss during storage according to Zhang and Quantick (1997); Ducamp-Collin *et al*. (2008). Total phenolic content loss rapidly occurred during high temperature storage due to lower weight loss. The increased weight loss during high temperature led to increase of EL and senescence process and latent pathogens in pericarp or saprophytic fungi in the air could be penetrated to decay fruit rapidly. While low storage during 2˚C could delay fungal growth and total phenolic content loss higher than high temperature storage, however; pathogen recovery was rapidly increased after removing to ambient condition.

Table 6.1 Pearson's correlation (r) between peel discoloration, fruit decay and their relations.

	BI outer	L* outer	C* outer	h˚ outer	BI inner	C* inner	h˚ inner	L* inner	PPO	TP	EL	WL	DI
BI outer	1												
L outer	-0.80**	1											
C outer	-0.76**	0.77**	1										
h˚ outer	-0.84**	0.90**	0.73**	1									
BI inner	0.95**	-0.84**	-0.76**	-0.90**	1								
C inner	0.37*	NS	NS	NS	0.36*	1							
h˚ inner	-0.92**	0.80**	0.74**	0.83**	-0.95**	-0.47**	1						
L inner	-0.85**	0.88**	0.79**	0.87**	-0.94**	-0.41*	0.91**	1					
PPO	NS	NS	-0.38**	-0.43*	NS	NS	NS	NS	1				
TP	-0.70**	0.46*	0.60**	0.65**	-0.76**	NS	NS	0.62**	-0.59**	1			
EL	0.86**	-0.79**	-0.69**	-0.78**	0.92**	0.55**	-0.96**	-0.93**	NS	-0.60**	1		
WL	0.79**	-0.68**	-0.66**	-0.69**	0.88**	0.62**	-0.91**	-0.87**	NS	-0.63**	0.97**	1	
DI	0.52**	-0.48**	-0.62**	-0.54**	0.66**	NS	-0.65**	-0.60**	0.39**	-0.72**	-0.66**	0.72**	1

**, Correlation is significant at the 0.01 level.

*, Correlation is significant at the 0.05 level.

BI = browning index, L = lightness, C = chroma, h = hue angle, PPO = polyphenol oxidase, TP = total phemol, EL = electrolyte leakage, WL = weight loss and DI = disease incidence

6.4.2 Effects of the optimum storage temperature of the coated longan fruits on PR-protein, bio-chemical changes in pericarp and market shelf life

6.4.2.1 Changes in browning parameters during cold storage

The results from this experiment showed that, except for SO_2 fumigation, pericarp browning in longan could be considerably controlled when using Cts + CA + PS at a pH level of pH-3.3. This conclusion indicated by the lowest browning index (BI) below 3.0 (Figure 6.11a) after 32 d as compared with Cts + CA + PS (pH-2.8), CA + PS and untreated control fruit which could control pericarp browning for only 24, 20 and 28 d, respectively.

Pericarp browning was reduced if the pH solution is adjusted from 2.8 to 3.3. Adding chitosan into CA + PS can reduce a contact between CA + PS and the fruit skin, therefore, fruit damage in response to these two components could be significantly decreased and thus shelf life prolonged. CA + PS alone showed the highest browning index (Figure 6.11a). This higher level of browning may have been a consequence of using the plastic film wrap which maintained a high humidity around the fruit. Even though the fruit were dried, the CA + PS residue on the fruit skin would have become active in solution under high humidity conditions, which would have led to bleaching and impregnation and damage of the fruit skin inducing ethylene production and causing a degradation of nutrient and antioxidant content (Abeles *et al*., 1992). Application of Cts along with CA + PS (pH-3.3) could be able to reduce CA degradation in the pericarp which is indicated by the higher pH in pericarp homogenate of CA-treated fruit (Figure 6.11c) (Joas *et al*., 2005). Application of Cts along with CA + PS (pH-3.3) showed a reduce in weight loss percentage when compared with non-treated fruit during cold storage (Figure 6.11b) and at shelf life conditions at ambient temperature (Table 6.2) according to Jiang and Li (2001).

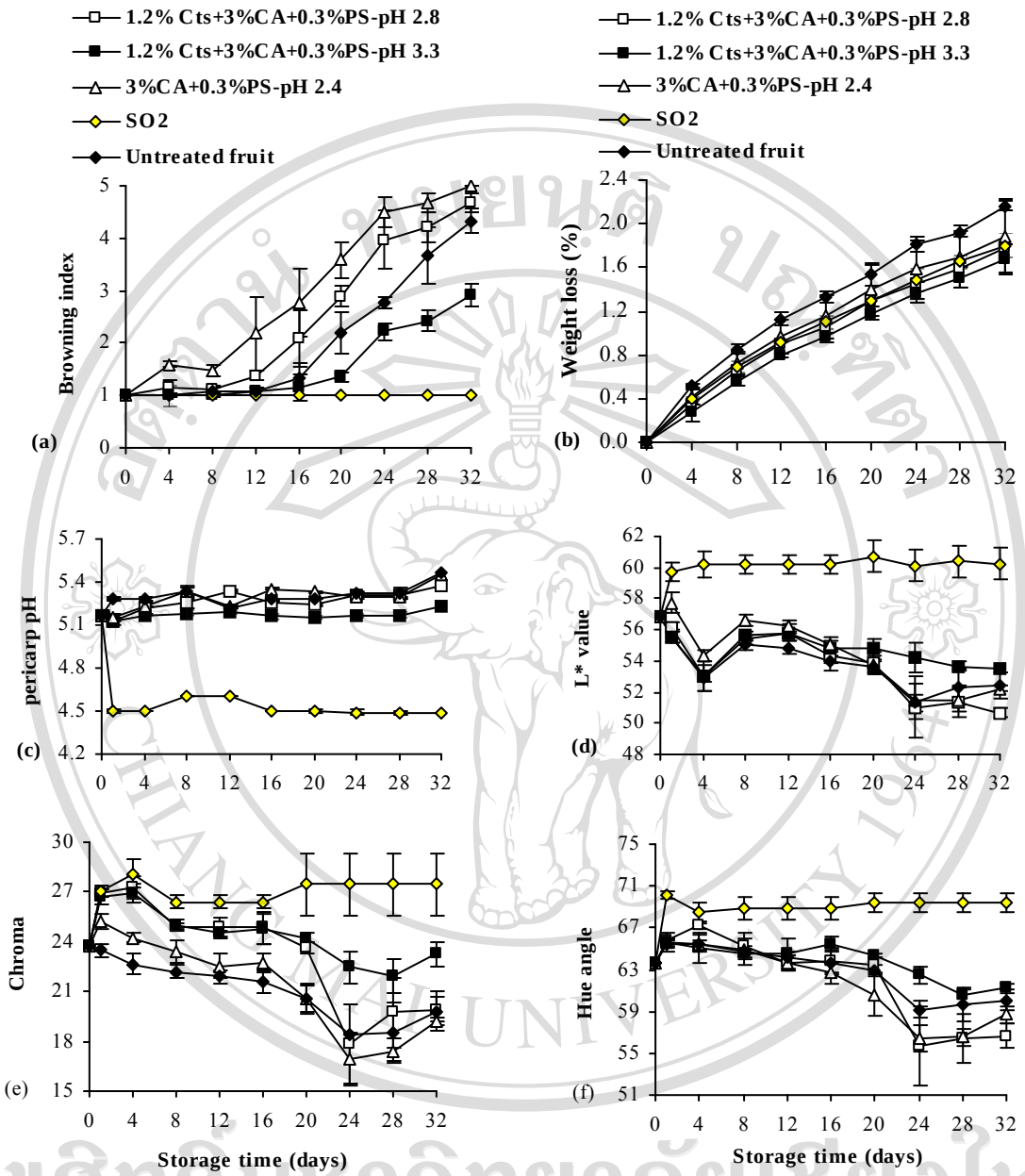

Figure 6.11 Effects of Cts + CA + PS and CA + PS on pericarp browning (a), weight loss percentage (b), pericarp pH (c), lightness (d), chroma (e) and hue angle (f) of longan fruit during storage at 5 °C. Note: Cts = chitosan, CA = citric acid and PS = potassium sorbate.

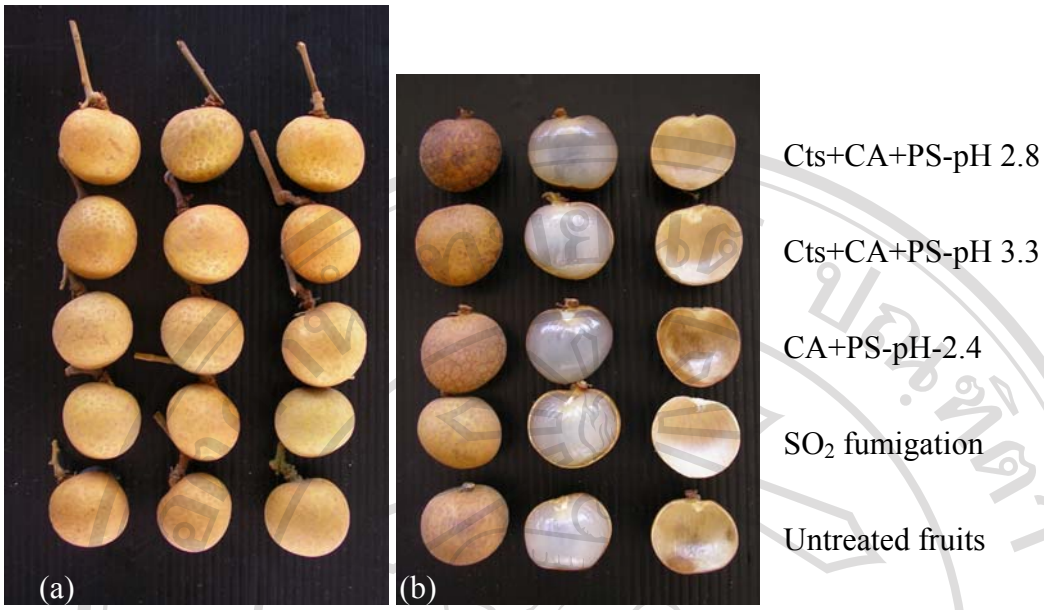

Figure 6.12 Effects of Cts + CA + PS and CA + PS on fruit appearances at day 0 (a) and total day 32 (b) of longan fruit during storage at 5 °C. Cts = chitosan, CA = citric acid and PS = potassium sorbate.

6.4.2.2 PPO activity and total phenolic content

According to highest L*, C* and h˚ values followed by SO_2 (Figure 6.11d, c, f), fruit coating with Cts + CA + PS (pH-3.3) showed lower PPO activity (Figure 6.13a) and total phenolic content loss (Figure 6.13b) due to the effects of the coating components CA and Cts. This conclusion was first made by Ducamp-Collin *et al*. (2008). In the treatments studied, the change in peel PPO activity was also related to the degree of peel browning, which may suggest that CI induced browning is causally related to PPO activity. Significant correlations between BI and PPO (r = 0.76) and between BI and total phenolic content (r = - 0.64) (Table 6.3) were found. This decrease total phenol was therefore correlated with the degree of browning. The phenolic compounds may have been used as substrates for the browning reaction. SO_2 showed the best result in reducing PPO activity and total phenolic content loss which is in accordance with the results of Wu *et al*. (2004) and Whangchai *et al.* (2006).

PPO activity may be a main factor in the browning reaction. PPO, are often found to localize at the chloroplasts, where they are associated with the internal thylakoid membranes. They are also found in the cytoplasm and in vesicles between the plasmalemma and cell wall (Obukowicz and Kennedy, 1981). Free phenolics are present mainly in the vacuole, but are synthesized in the cytoplasm (Vamos-Vigyazo, 1981; Walker and Ferrar, 1998), and perhaps

may also become deposited in the cell walls. If CI induces membrane damage of organelles such as vacuoles, the vacuolar phenolics may become in contact with PPO. Alternatively, the cells may react to chilling injury by depositing phenolic compounds in the cell walls, which would then react with PPO already present in the apoplast.

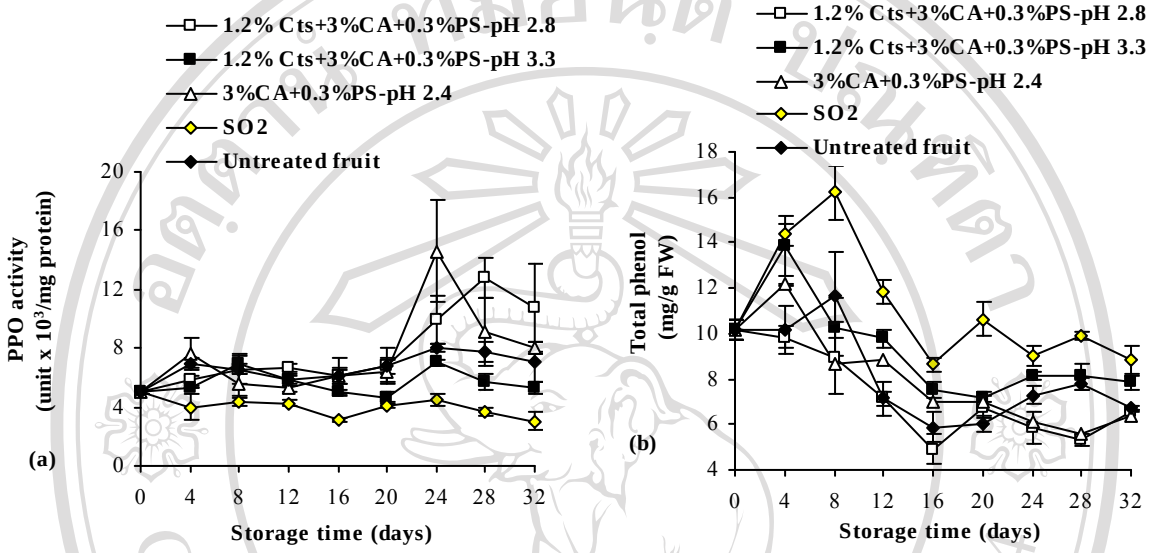

Figure 6.13 Effects of Cts + CA + PS and CA + PS on PPO activity (a) and total phenolic content (b) of longan fruit during storage at 5 °C. Cts = chitosan, CA = citric acid and PS = potassium sorbate.

6.4.2.3 Disease development and sorbic acid distributions

Decay was significantly reduced by coating Cts + CA + PS (pH-3.3) on the fruit, which indicated by lower disease severity (score) of below 2.0 (only the stem-end of the fruit affected) (Figure 6.14a) and lower disease incidence percentage of below 25% (Figure 6.14b) at 32 day as compared with non-treated fruit. However, disease severity (score) and disease incidence percentage did not differ significantly between fruit treated with Cts + CA + PS (pH-3.3), Cts + CA + PS (pH-2.8) and CA + PS. The results revealed that increasing pH coating to 3.3 influence efficacy to control fruit decay. This finding agrees with the work of Sofos and Busta (1981), who found the best activity, was due to the undissociated form of the acid when pH was less than the pKa of 4.75. In addition, the degradation of sorbic acid content in pericarp exhibited negative exponential characteristic (Figure 6.15). Cts along with CA + PS could help to delay the rate of sorbic acid degradation in the pericarp (0.06 mg/ /kg/day) lower than that of CA + PS (0.32 mg/kg/day). While the sorbic acid content in the

aril of two treatments was not detected (data not shown). From Figure 6.5, it appeared that PS may have gradually diffused into the longan surface and consequently interacted with the pathogens on the fruit surface. The highest disease incidence occurred on the control fruit through storage but cold storage below 5ºC reduced disease attack and discoloration effects (Figure 6.11a). However, after a transfer to ambient temperature at day 0 and 20, fungal disease rapidly attacked fruit surface within 5-8 d (Table 6.2).

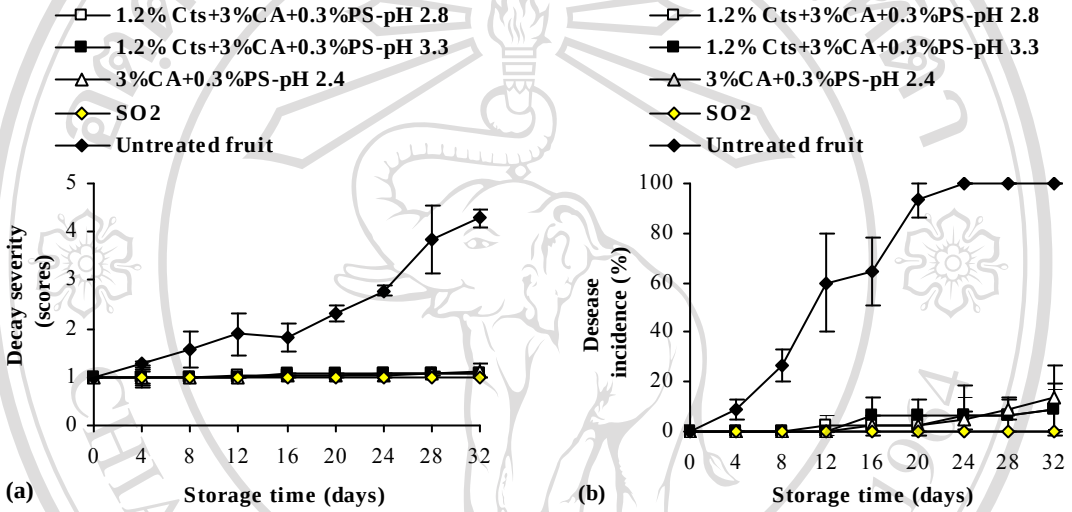

Figure 6.14 Effects of Cts + CA + PS and CA + PS on decay severity (a) and disease incidence (%) (b) of longan fruit during storage at 5°C. Cts = chitosan, CA = citric acid and PS = potassium sorbate.

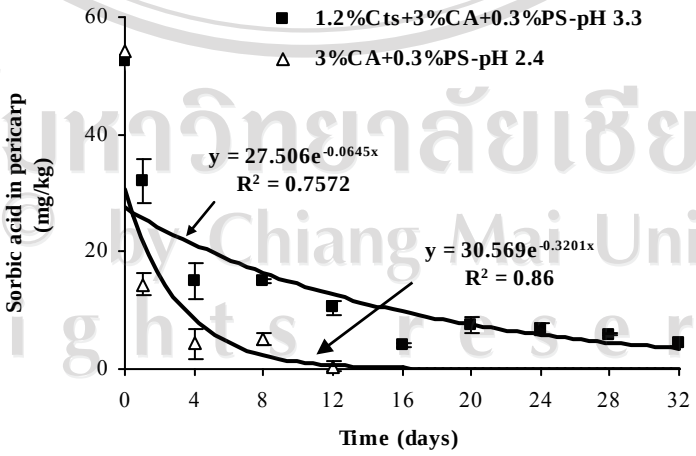

Figure 6.15 Effects of coating with Cts + CA + PS and CA + PS on sorbic acid content in pericarp of longan fruit during storage at 5°C. Cts = chitosan, CA = citric acid and PS = potassium sorbate.

6.4.2.4 PR-protein as a defense mechanism and a relationship with the chilling injury effects

From Figure 6.16, SO_2 fumigation could not only dramatically inhibit pericarp browning during storage but also it induced the highest amount of PR-protein related in plant defense in pericarp which accorded to the firstly reported by Rakwal *et al.* (2003) who found that the response of rice seedlings exposed to the air pollutant sulfur dioxide (SO_2). Most prominent changes in leaves were the induced accumulation of a pathogenesis-related (PR) and a strong production of phytoalexins. SO_2 triggered multiple events linked with defense/stress response in the leaves of rice seedlings. It was similar to this experiment and no report was found in longan. This SO_2 fumigation induced ~ 4 and ~ 10 fold increases in the activities of chitinase and β-1,3-glucanase (Figure 6.16) and reduced the lowest PPO activity and total phenol loss (data not shown). The result was accorded to correlation between chitinase and β-1,3-glucanase with browning parameter such as L* value (r = 0.75** and 0.80**) and pericarp pH (r = 0.83** and 0.88**). In addition to two enzymes also negatively correlated with BI and had medium negative correlation with PPO activity and positively correlation with total phenol but had no correlated with disease incidence. The experiment suggested that PR-protein might relate to chilling injury tolerance more than pathogenic response. This indicator could be used to evaluate the efficacy of each alternative treatment whether in comparison with SO_2 during cold storage. While PR-protein content in other treatments was not significant between treatments and was variation during storage.

Chitosan along with CA+PS was high temporal profiles of PR-protein only day 20 and 24 days and after that was declined with the others. El Ghouth *et al*. (1992) found that mechanisms by which chitosan coating reduced the decay of strawberries appear to be related to its fungistatic property rather than to its ability to induce defense enzymes such as chitinase, chitosanase and β-1,3-glucanase. In this experiment, chitosan in combination with PS+CA or PS+CA alone showed clearly disease control as compared with untreated fruit according to the last experiment.

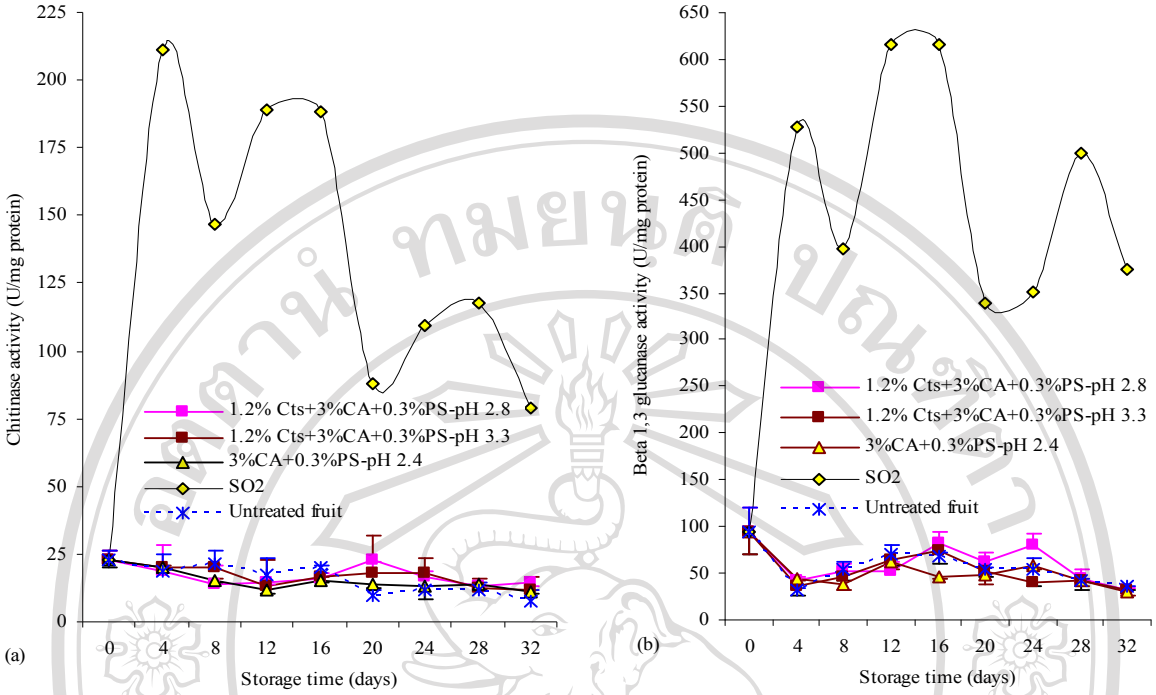

Figure 6.16 Effects of Cts+CA+PS and CA+PS on induced PR-protein in pericarp as defense mechanism; chitinase (a) and glucanase (b) of longan fruit during storage at 5°C. Cts (chitosan), CA (citric acid) and PS (potassium sorbate).

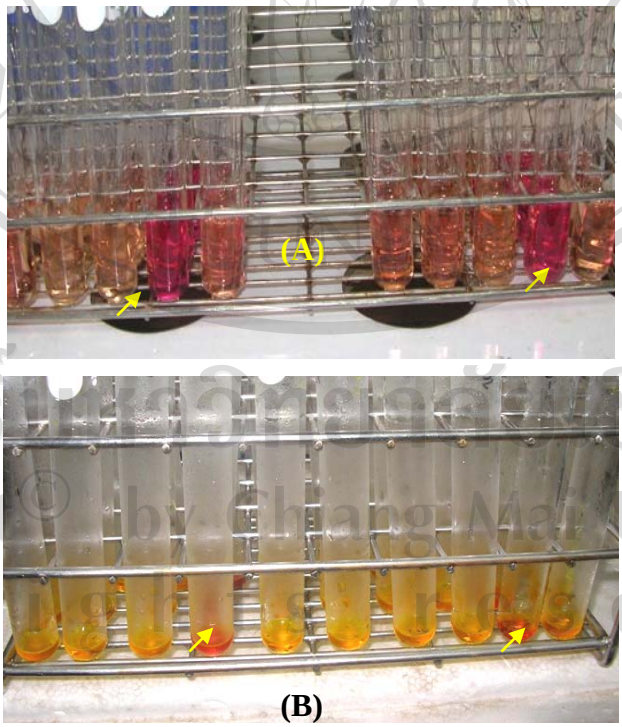

Figure 6.17 Color characteristic of PR-protein in pericarp as defense mechanism; treatment of predominantly SO_2 on chitinase (purple plus) (a) and glucanase (orange plus) (b) of longan fruit during storage at 5°C (yellow arrows).

6.4.2.5 Sensory evaluation during cold storage and taken the samples to simulate marketing shelf life during ambient temperature

Application of Cts + CA + PS (pH-3.3) maintained pericarp color acceptability and eating quality during storage for 32 d (Figure 6.18a-e) and a transfer to simulated marketing shelf life conditions (Table 6.2). Although SO_2 could improve the highest pericarp color acceptability, eating quality showed the lowest score due to off-odor effects caused by film wrap and redden of the aril around stem scar area (Figure 6.18b-e, and Figure 6.19).

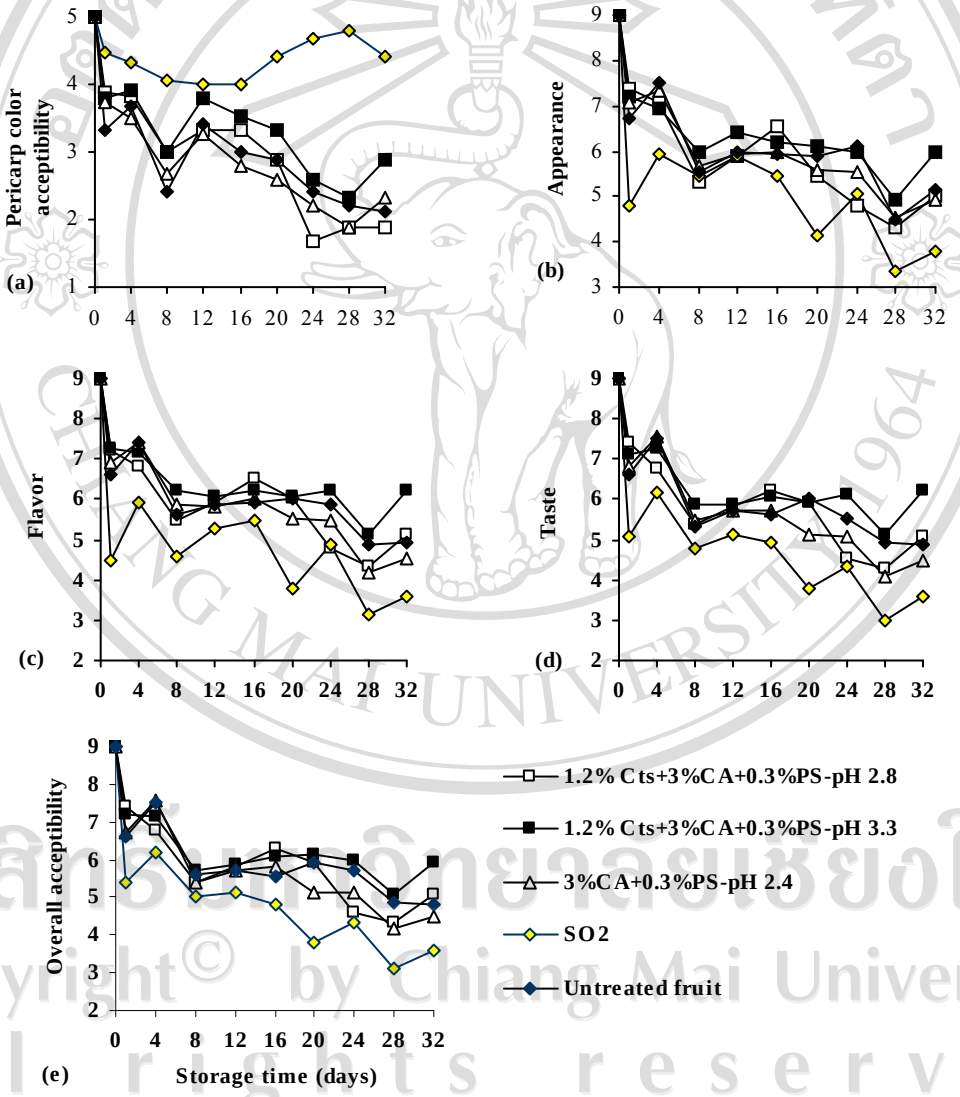

Figure 6.18 Effects of Cts + CA + PS and CA + PS on pericarp color acceptability (a) and eating quality: appearance (b); flavor (c), taste (d) and overall acceptability (e) of longan fruit during storage at 5 °C. Cts = chitosan, CA= citric acid and PS = potassium sorbate.

Figure 6.19 Eating quality: appearances of CA + PS; normal color (a) and SO_2; flesh redden (b) of longan fruit during storage at 5 °C.

Table 6.2 Longan fruit quality after cold storage at 5 ºC, following by holding at ambient simulated shelf conditions.

Treatments	BI	WL	DS	fruit color	Taste
Before storage	1 e	0 d	1 e	5 a	9 a
After 0 days + 8 days at 28 ˚C					
1.2% Cts + 3% CA + 0.3% PS (pH-2.8)	2.3 d	8.5 b	3.5 d	3.8 cd	6.4 b
1.2% Cts + 3% CA + 0.3% PS (pH-3.3)	2.1 d	8.4 b	3.9 bc	3.7 cd	6.4 b
3% CA + 0.3% PS (pH-2.4)	4.5 b	9.9 a	3.7 bcd	3.4 e	6.3 b
SO_2	1.4 e	10.2 a	1.2 e	4.2 b	5.3 c
Untreated fruit	4.9 a	10.8 a	5.00 a	3.5 de	6.3 b
After 20 days at 4˚C + 5 days at 28 ˚C					
1.2% Cts + 3% CA + 0.3% PS (pH-2.8)	2.9 c	5.9 c	3.6 cd	2.3 g	3.9 d
1.2% Cts + 3% CA + 0.3% PS (pH-3.3)	2.0 d	5.8 c	3.9 b	3.0 f	4.9 c
3% CA + 0.3% PS (pH-2.4)	4.1 b	6.9 c	3.6 cd	1.5 h	2.3 e
SO_2	1 e	6.1 c	1 e	4.0 bc	2.6 e
Untreated fruit	3.4 c	6.9 c	5.0 a	2.1 g	3.8 d

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

[2]BI = browning index, WL = weight loss, DS = decay severity (score)

Fruit color acceptability index: 5: excellent; 4: good; 3: fair (acceptable); 2: poor (unacceptable to export); 1: very poor (totally unacceptable).

Score of sensory analysis of eating quality (taste): 1: the lowest (extremely weak) and 9: the highest (extremely strong).

Table 6.3 Pearson's correlation (r) between browning parameters and PR-proteins.

	L*	BI	DS	WL	TP	PPO	P-pH	CTN	GCN	C*	h˚	DI
L*	1											
BI	-0.70**	1										
DS	-0.33*	0.32*	1									
WL	-0.34*	0.69**	0.45**	1								
TP	0.58**	-0.64**	NS	-0.63**	1							
PPO	-0.76**	0.76**	NS	0.37**	-0.51**	1						
P-pH	-0.88**	0.53**	0.30*	NS	-0.53**	0.60**	1					
CTN	-0.75**	-0.38**	NS	NS	0.54**	-0.49**	-0.83**	1				
GCN	-0.80**	-0.40**	NS	NS	0.45**	-0.52**	-0.88**	0.97**	1			
C*	-0.80**	-0.84**	-0.46**	-0.60**	0.66**	-0.79**	-0.70**	0.54**	0.55**	1		
h˚	-0.88**	-0.90**	-0.30*	-0.55**	0.67**	-0.84**	-0.76**	0.59**	0.61**	0.91**	1	
DI	-0.38**	0.29*	0.93**	0.47*	-0.33*	NS	0.32*	NS	NS	-0.52**	-0.33*	1

**, Correlation is significant at the 0.01 level.

*, Correlation is significant at the 0.05 level.

L* = lightness, BI = browning index, DS = decay severity, WL = weight loss, TP = total phenol, PPO = polyphenol oxidase activity, p-pH = pericarp pH, CTN = chitinase activity, GCN = β-1, 3-glucanase activity, C* = chroma, h˚ = hue angle, DI = disease incidence.

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6.5 Conclusion

Our experiment indicated that outer and inner pericarp browning was directly correlated and either could be used to evaluate discoloration change. Correlation between BI (subjective) and L*, C* and h of outer pericarp and L*, h of inner pericarp (objective) were significantly found but C*inner pericarp could be used to be a color index because it did not correlate with BI. BI of inner and outer pericarp was correlated with EL and WL during storage.

Fruit decay was completely controlled during storage at 2˚C but not at 20˚C. Chilling injury symptom (browning index > 25% fruit area) of coated fruits during 2˚C and pericarp browning during 20˚C were rapidly occurred within 10 days which indicated by increasing electrolyte leakage and weight loss. However, chilling injury tolerance of this treatment during 5˚C was more improved and pericarp browning was delayed for 20 days as well as weight loss, electrolyte leakage and fruit decay.

Total phenol (TP) during storage was negatively related to discoloration, polyphenol oxidase (PPO) activity and fruit decay. During all storages, dipping fruits in Cts + CA + PS at a pH of 2.8 slowed down the total phenol loss and PPO activity in the initial storage but not the end of storage. This conclusion showed that addition of PS in Cts+CA at a pH of 2.8 clearly showed high efficacy disease control that prevented a high level of TP degradation rather than browning control. However TP degradation decreased as PPO activity increased at the last day because pericarp damage of fruit tissue was evident due to lower pH of acid-coating.

Improving pH of Cts + CA + PS at a pH of 3.3 showed the higher efficacy in browning control and retained excellent disease control as compared with Cts + CA + PS at a pH of 2.8, CA+PS at pH of 2.4 and untreated fruits. SO_2 fumigation as a commercial practice clearly showed the highest efficacies in browning and disease control in accordance with the highest amount of pathogenesis related-protein: chitinase and β-1,3-glucanase as a defense enzymes which were induced due to pathogenic and chilling stress response during storage at 5˚C over other treatments.