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Original Article

Effects of chitosan and citric acid on pericarp browning and polyphenol oxidase activity of longan fruit

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Abstract

This research was designed to study the effects of chitosan and citric acid (CA) on pericarp browning and polyphenol oxidase (PPO) activity of longan fruit. The experiment was conducted by dipping longan fruit in 1.2% (w/v) chitosan coating solution containing 1.0% CA (pH 3.3) for 2 min, in 1.0% CA solution (pH 2.6) for 2 min, and in distilled water for 2 min as a control. The treated fruits in each treatment were then packaged in a foam tray, wrapped with 11 μm PVC film and stored at 5°C, 95% RH for 27 days. Pericarp browning, pericarp color, pericarp pH, titratable acidity (TA), weight loss, polyphenol oxidase (PPO) activity, and total phenol were monitored during storage. The results revealed that interaction between chitosan and CA demonstrated the best treatment in postponement of 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. Chitosan mixed with citric acid exhibited a 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 phenol content were found when CA was applied in combination with chitosan during storage.

Key words: Chitosan, citric acid, longan, pericarp browning, PPO

1. Introduction

Fresh longan is one of the leading export products of Thailand. Postharvest longan has faced rapid discoloration caused by desiccation during storage at either too low or too high temperatures. Browning of fresh longan resulted from phenolic compounds oxidized by endogenous polyphenol oxidase (PPO) and then the pigment was formed (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 its use such as toxic residues in humans and asthmatics and other reactions with sensitive individuals. Therefore, alternative treatments for SO_2 fumigation are required. Citric acid (CA) is an anti-browning agent, which prevents polyphenol oxidase by suppressing the food pH and binding the Cu^{2+} in an active site of PPO to form an inactive complex (Martinez and Whitaker, 1995). It has also been widely used in the food industries for controlling the browning. Although applying CA as a dipping solution has been reported in postharvest fruits with very satisfactory results for fruits such as longan (Sardsud *et al*.,

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2003; Whangchai *et al.*, 2006) and litchi (Terdbaramee *et al*., 2002), injury of fruit surface and rapid pericarp browning were observed in longkong when high concentrations (2, 4 and 6%) of CA were applied (Lichanporn *et al*., 2002).

The application of an edible coating as a carrier of anti-browning to mitigate the browning and water loss has been studied (Cuppett, 1994) and it was found to successfully maintain a high quality of the fresh products (Lee *et al*., 2003; RoJas-Grau *et al*., 2007; Lin *et al., 2008*). 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, as a barrier to O_2 and delay the increase 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*., 2008). However, the combined effects of CA and chitosan solution on PPO activity and total phenols have not been reported yet in longan fruit. The main purpose of this study was to evaluate the effects of CA, chitosan, and their combination on pericarp browning and PPO activity of longan fruit during storage at low temperature.

2. Materials and Methods

2.1 Plant materials and treatments

Mature yellow AA grade longan fruit (100 % of maturity) (*Dimocarpus longan* Lour.) cv. Daw were harvested from a commercial Good Agricultural Practice (GAP) orchard in Chiang Mai. The fruits were selected for uniformity of shape, color, and size, so that any blemished or diseased fruits were subsequently discarded. The average AA grade fruit size was 2.78 cm in width, 2.55 cm in height and 2.52 cm in thickness. A preliminary study showed that 1% (w/v) CA mixed with 1-1.2% (w/v) chitosan showed the highest efficiency to reduce 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*., 2008). 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 followed by the addition of 20 g of CA (food grade, Shandong Ningmeng Biochemistry Co., Ltd., China). The solution was mixed well by a magnetic stirrer and cooled down in a hood before the addition of distilled water up to 2.0 L, and then the pH of the solution was measured. Fruit 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 an electric fan, packed in foam trays and wrapped with 11 μm thick PVC film (M Wrap, M.M.P. Packaging Group Co., Ltd., Prakanong, Bangkok, Thailand) (20 fruits per a foam tray), and then stored at 5°C, 95% RH. Fruits dipped in distilled water were used as a control. For each treatment, four replicates were used. Samples were taken directly after treatment and then at day 10, 20, and 27 after storage for quality evaluation and analyses.

2.2 Pericarp browning assessment and color changes

Browning was assessed visually by measuring the total browning areas of the pericarp on each of the twenty fruits. The following scale was used: 1 = no browning (excellent quality), 2 = slightly browning, 3 = browning on less than 25% of the total surface, 4 = 25-50% browning, and 5 $\geq$ 50% browning (poor quality) (Jiang and Li, 2001). A browning index was calculated using the following formula, Σ(browning scale $\times$ percentage of fruit in each class). Fruits having a browning index above 3.0 were rated as unacceptable. The color of the outer pericarp of longan was measured with a colorimeter (Color Quest XE) according to the CIELAB scale. 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. The results were expressed as a mean value from four replications of 20 measured samples.

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

Forty fruits, ten in each of the four replications, were used to prepare the 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 s 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 fruits that showed lesions of mycelium or rot on the fruit surface area.

2.4 Polyphenol oxidase (PPO) assay and protein determination

The peels 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.

2.5 Determination of total phenols

The total phenol contents in longan pericarp tissues were determined by the method of Folin-Ciocalteu reaction (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.

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. Results and Discussion

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

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 distilled water (the control) ($p<0.01$). 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, the solution of 1.0% CA without chitosan; and the control showed pericarp browning development at BI = 4.23 and 4.35, respectively (Figure 1a).

This experiment demonstrated that 1.0% CA without chitosan provided the lowest pH (pH 2.2) as compared to a higher 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 (Figure 1b). 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 1). The result is also consistent with that of Caro and Joas (2005) and Joas *et al*. (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 1). TA decreased as pericarp pH increased (Figure 1c, d). Caro and Joas (2005) and Joas *et al*. (2005) studies of dipping litchi fruits in the solvent of CA and 1% chitosan found that pericarp browning effects could be postponed by reducing pericarp pH and weight loss during storage. Chitosan-contained dipping solution can significantly help delay the

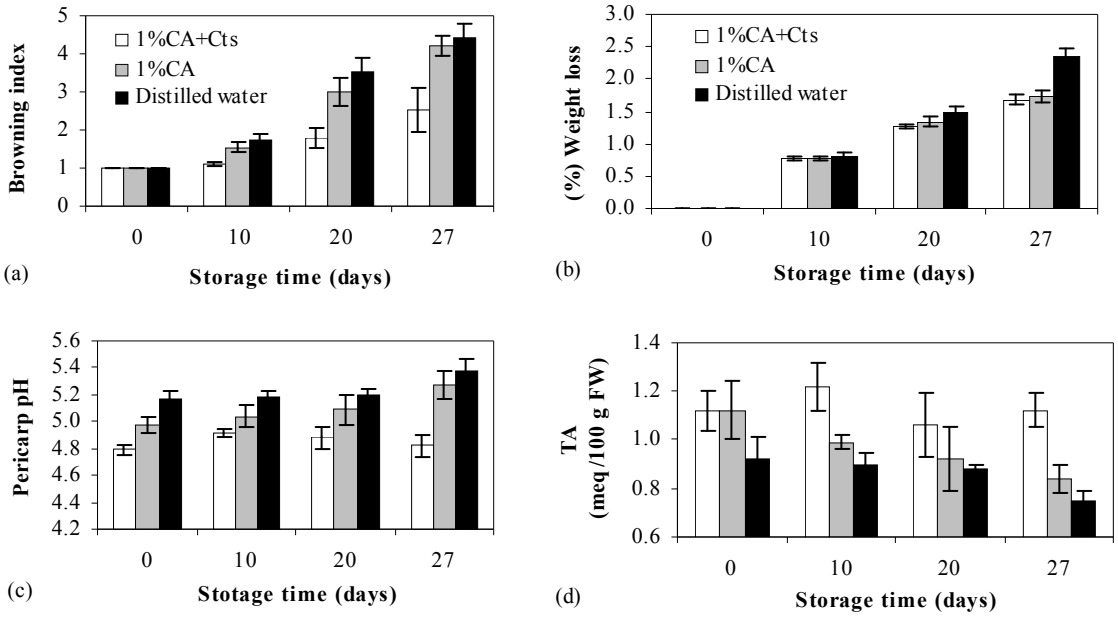

Figure 1. Effects of 1% citric acid (CA) solution and 1% CA combined with 1.2% chitosan (Cts) solution on pericarp tissue browning (a), weight loss (b), pericarp pH (c), and titratable acidity (d) of longan fruit during storage at 5°C for 0, 10, 20, and 27 days. The distilled water treated longan fruits were used as the control. Vertical bars indicate standard deviation (n = 4).

rate of CA degradation on the 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 the fruits' surfaces. The results according to numerous reports also showed a higher capacity in browning control when applying combination of different edible coating and anti-browning agents in fresh products (Baldwin *et al*., 1996; Lee *et al*., 2003; RoJas-Grau *et al*., 2007; Lin *et al., 2008*).

Although, after day 27, the experiment indicated that the longan fruit dipped in chitosan (dissolved in 1.0% CA) showed a significant reduction of weight loss (%) compared with the control fruit (Figure 1b), 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*., 2008). 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 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. The addition of lipid materials to hydrophilic coatings can sometimes improve the moisture barrier properties (Amarante and Banks, 2001). Vargas *et al*. (2006) found that an addition of oleic acid not only enhanced the chitosan antimicrobial activity but also improved water vapor resistance of chitosan-coated strawberry. The use of PVC film as a packaging item is the 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).

3.2 Pericarp color

The effectiveness of the 1% CA plus chitosan solution in controlling pericarp browning was confirmed by other research results demonstrating the high consistency in pericarp color during storage (Figure 2). L* value (lightness), chroma, and hue were also well correlated with the browning index (r = -0.89), (r = -0.74) and (r = -0.64) (Table 1). The result was also in accordance with the recent research of Apai *et al*. (2008). 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).

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 concentrations at 2, 4 and 6% was reported to damage the fruits' surface and stimulate pericarp browning in longkong (Lichanporn *et al*., 2002).

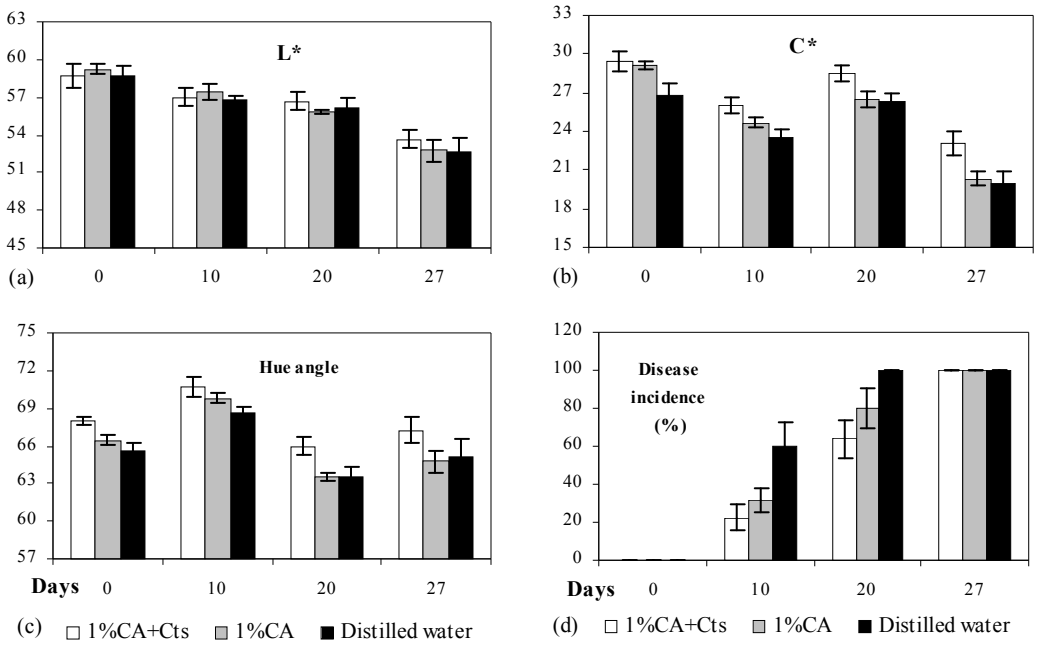

Figure 2. Effects of 1% citric acid (CA) solution and 1% CA combined with 1.2% chitosan (Cts) solution on L* value (a), chroma (b), hue angle (c), and disease incidence (%) (d) of longan pericarp during the fruit storage at 5°C for 0, 10, 20, and 27 days. The distilled water treated longan fruits were used as the control. Vertical bars indicate standard deviation (n = 4).

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

CA alone at concentrations of 1, 3, and 5% with pH of 2.6, 2.4, and 2.2, respectively, showed a high severity of pericarp browning inline with increasing CA concentration (Apai *et al*., 2008). 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 nutrients and antioxidant contents (Abeles *et al*., 1992). These results suggested that escalating skin damage after CA dipping might also be related to both pH of the solution and the natural attribute of each food texture. Adding chitosan to CA can reduce the 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 better control pericarp browning for 27 days compared to longan with thinner pericarp, which could prolong browning for only 14 days at 5°C (Apai *et al*., 2008). However, the fact that pericarp thickness, pH, and relative humidity could have key effects on using CA and chitosan has not yet been published elsewhere.

From Table 1, disease incidence (DI) shows a correlation with the browning index (r = 0.89) and L* (r = -0.89). The results revealed that DI was one factor affecting the browning development (Jiang *et al*., 2002). The high humidity inside the packaging rapidly led to an increased fungal disease development on the fruit surface within 10 days. CA+Cts and CA treatments could help prolong the DI percentage (Figure 2d). 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 1a). This result suggested that CA+Cts should be added with some additives for delaying DI including pericarp browning.

3.3 Polyphenol oxidase (PPO) activity and total phenol

According to the browning index, dipping longan fruits in 1.0% CA with chitosan significantly helped to delay PPO activity during storage ($p<0.05$) (Figure 3a). 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. Dipping in 1.0% CA without chitosan and control (distilled water) only postpones PPO activity at the beginning of the 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 an 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 substrate was not active due to the reduction in cationic substrate.

Regarding the relationship between PPO activity and browning (Table 1), 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 been reported among grape cultivars and apples (Cheng and Crisosto, 1995).

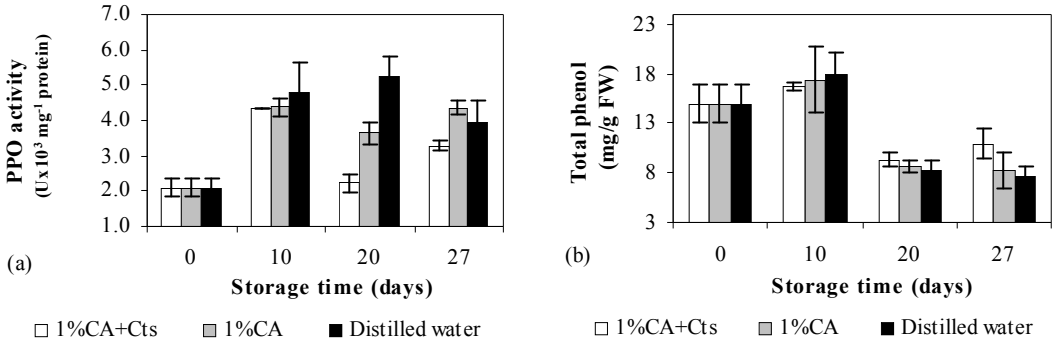

Figure 3. Effects of of 1% citric acid (CA) solution and 1% CA combined with 1.2% chitosan (Cts) solution on the PPO activity (a) and total phenols (b) of longan fruit during storage at 5°C for 0, 10, 20, and 27 days. The distilled water treated longan fruits were used as the control. Vertical bars indicate standard deviation (n = 4).

Table 1. Correlation coefficients (r) between browning and physio-chemical changes of longan during storage at 5°C for 27 days.

	BI	p-pH	TA	WL	PPO	TP	L*
BI	1						
p-pH	0.6641*	1					
TA	-0.7424**	-0.9211**	1				
WL	0.8976**	NS	NS	1			
PPO	NS	NS	NS	NS	1		
TP	-0.8314**	NS	NS	-0.7641**	NS	1	
L*	-0.8881**	NS	NS	-0.9441**	NS	0.6963*	1
C*	-0.7395**	-0.6468*	0.6200*	-0.7489**	NS	NS	0.8660**
h°	-0.6411*	NS	0.6159*	NS	NS	0.8320**	NS
DI	0.8950**	NS	NS	0.9460**	NS	-0.7710**	-0.8960**

[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, L* = lightness, C* = chroma, h° = hue angle.

Phenolic compounds were the substrates of PPO; most antioxidants were identified in this group. They were found to delay the senescence process in many fresh products. High phenol content was also be detected in longan fruits receiving SO_2 fumigation (Wu *et al*., 1999). This is also consistent with the phenol results in this experiment, which demonstrated a significant correlation with browning index (r = -0.83) and weight loss (r = -0.76). Application of chitosan combined with CA was found appropriate to retard the loss of phenol in the pericarp as compared with other treatments (Figure 3b). Therefore, the browning was delayed till day 27. Nevertheless, total phenol was well correlated with disease incidence (r = -0.77) (Table 1), which indicated that total phenol content might have an effect on disease control in pericarp. Due to the fact that chitosan was able to delay water loss (Jiang and Li, 2001; Lin *et al*., 2005), chitosan coating could well impede phenol loss in many fruits (Zhang and Quantick, 1997; Vangnai *et al*., 2006), which also complied with our study.

4. Conclusion

Regardless of the costs, 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 phenol 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 products. 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, and adding other food additives to protect products from some diseases.

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