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Reduction of enzymatic browning of harvested 'Daw' longan exocarp by sodium chlorite

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ABSTRACT: Post-harvest exocarp browning is a major problem resulting in reduced shelf-life of longan fruits. The objective of this study was to evaluate the possibility of using sodium chlorite (SC) as an anti-browning agent for controlling enzymatic browning of harvested longan fruits during storage at ambient conditions. Longan fruits cv. Daw were dipped in 0.001%, 0.005%, 0.01%, and 0.05% SC (W/V) for 10 min. The fruits were packed in cardboard boxes and stored at 25 ± 1 °C with a relative humidity of 82 ± 5% for 72 h. Changes in browning index, colour parameter (L* and b* values), polyphenol oxidase (PPO) activity, peroxidase (POD) activity, and total phenolic content were measured. The results showed that the fruits treated with SC had lower browning index, but higher L* (lightness) and b*(yellowness) values than those of the control group during storage for 48 h. SC at a concentration of 0.01% was the most effective in reducing exocarp browning. The application of SC reduced PPO and POD activities and delayed a decrease in the total phenolic content. The treatment with 0.01% and 0.05% SC had the lowest PPO and POD activities, and maintained the highest total phenolic content. It was concluded that an application of SC is an alternative method for reducing exocarp browning and maintaining quality of harvested longan fruits.

KEYWORDS: *Dimocarpus longan*, peroxidase (POD), polyphenol oxidase (PPO)

INTRODUCTION

Longan is a commercial subtropical fruit, widely grown in China, Thailand, India, and Vietnam[1–3]. Unfortunately, the fruit has a very short post-harvest life and the visual appeal of longan can deteriorate within 3 days under ambient conditions[4–6] due to pericarp browning and breakdown, resulting in reduced market value[7–9]. Colour is one of the most important visual characteristics for marketing longan fruits[10]. Browning of longan has mainly been attributed to oxidation of phenolic compounds by polyphenol oxidase (PPO) and peroxidase (POD), producing brown-coloured by-products[7,11]. Reducing enzymatic browning is important to extend storage life and maintain quality of longan fruits[12].

Several methods have been used to prevent enzymatic browning of fruits and vegetables[13]. One of these is the use of anti-browning agents such as chitosan[11], citric acid, ascorbic acid, oxalic acid[14], and nitric oxide[12]. Using these agents is constrained by their high cost and harmfulness. Consequently, research and development studies to find effective substitutes are still ongoing[15,16].

Sodium chlorite (SC) is an oxidizing and sanitizing agent which is able to generate chlorine dioxide (ClO_2) in an acidic environment[17]. The American Food and Drug Administration has approved its use on raw fruits and vegetables in the concentration range of 0.05% to 0.12%[18]. It has been reported that SC and ClO_2 have been used to reduce enzymatic browning of fruits and vegetables[19–24]. For example, Lu et al[19] demonstrated that SC at a concentration of 0.09% (w/v) significantly inhibited PPO activity extracted from fresh-cut apples (*Malus domestica* Borkh. cv. Red Delicious). Similarly, Lu et al[20] found that dipping in 0.05% (w/v) SC for 1 min inhibited enzymatic browning of fresh-cut Red Delicious apples. Fu et al[21] reported that an aqueous solution of ClO_2 at 0.005% (w/v) inhibited the activity of PPO extracted from Golden Delicious apples by 63%. In the same way, Guan and Fan[22] reported that dipping in SC at 0.05% (w/v) for 5 min reduced enzymatic browning and microbial population of fresh-cut Granny Smith apples. Du et al[23] found that dipping fresh-cut lotus (*Nelumbo nucifera* Gaertn cv. Bai Hua) roots in 0.01%

(w/v) ClO_2 solution for 10 min significantly decreased the activity of PPO and delayed browning. Chen et al[24] reported dipping in 0.01% (w/v) ClO_2 for 20 min inhibited enzymatic browning and extended shelf-life of fresh-cut asparagus lettuce (*Lactuca sativa* L. var. *angustana* Irish).

Although SC has been reported to reduce browning in many fruits and vegetables, there is no report on the effect of SC on enzymatic browning in longan. The objective of this study was to evaluate the possibility of using SC as an anti-browning agent for longan fruits during storage at ambient conditions.

MATERIALS AND METHODS

Plant materials

Longan (*Dimocarpus longan* Lour. cv. Daw) fruits at commercial maturity were harvested from a commercial orchard in Lamphun province, Thailand, in July and August 2010 and February and March 2011 for repetition. Fruits were delivered to a laboratory room in the Department of Biology, Chiang Mai University, within 1.5 h. The fruits were individually selected from a bunch for uniformity of shape, colour, size, and lack of defects. The fruits were randomly distributed into five groups of 120 fruits. The fruits were then dipped in sodium chlorite (SC) solutions at concentrations of 0.001, 0.005, 0.01, and 0.05% (W/V) (pH 5.5) for 10 min at room temperature (25 ± 1 °C). Fruits dipped in distilled water (pH 5.5) were used as a control. After dipping, the fruits were air-dried for 10 min, packed in cardboard boxes, and then stored for 72 h at room temperature with a relative humidity of 82 ± 5%. Fruits from each treatment and control were randomly sampled at 12, 24, 48, and 72 h after storage to measure browning index, colour of exocarp, PPO and POD activities, and total phenolic content.

Browning index

Exocarp browning was estimated visually by measuring the extent of the total brown area on each fruit surface using the following scale[11]: 1=no browning (excellent quality), 2=slight browning, 3=less than 25% browning of the total surface, 4=25–50% browning, and 5=> 50% browning (poor quality). A browning index was calculated using the following formula: $\sum$ (browning scale $\times$ percentage of fruit in each class)[11]. Fruits having a browning index above 3.0 were considered as unacceptable for visual marketing quality[11].

Colour measurement

The colour of the exocarp was measured using a chromameter (Model Miniscan XE plus, Germany) and the degree of browning was expressed as L* and b* values (CIE 1976). L* values indicated lightness of the exocarp, ranging from black=0 to white=100[10], whereas b* values indicated classification of yellow to blue ranging from yellow (> 0) to blue (< 0)[23].

Extraction of enzymes and assay for PPO and POD

Enzymes were extracted by the modified method of Huang et al[25]. Longan exocarp (2 g) from 20 fruits was homogenized in 20 ml of 0.05 M potassium phosphate buffer (pH 6.2) containing 1 M KCl and 2% polyvinylpyroritidone for 5 min by using a mortar and pestle, and centrifuged for 5 min at 20 000g (Hermel model Z383K, Germany) and 4 °C. The supernatant was then collected for PPO and POD activity assays as a crude enzyme extract.

PPO activity, using catechol as a substrate, was assayed based on the method of Jiang and Fu[26] using the reaction mixture (2 ml) containing 1.3 ml of 0.05 M potassium phosphate buffer (pH 7.5), 0.2 ml of 0.2 M catechol, and 0.5 ml of crude enzyme. Tubes were incubated for 5 min at 30 °C. The absorbance was measured at 420 nm in a visible spectrophotometer (Model Thermo Spectronic, USA). The unit of enzyme activity was defined as the amount of enzyme that caused a change of 0.01 in absorbance per minute[14].

POD activity, using guaiacol as a substrate, was assayed based on the method of Nagle and Harrd[27] using a reaction mixture (2.5 ml) containing 2.3 ml of 0.01 M sodium acetate buffer (pH 6.0), 0.05 ml of 0.1% guaiacol (V/V), 0.1 ml of 0.1% H_2O_2 (V/V), and 0.05 ml of crude enzyme. Tubes were incubated for 5 min at 30 °C and the absorbance was measured at 470 nm in a visible spectrophotometer (Model Thermo Spectronic, USA). The unit of enzyme activity was defined as explained for PPO activity.

Protein levels were assayed from crude enzyme extracts according to Lowry et al[28] with Folin-Ciocalteu reagent as a standard.

Determination of total phenolic content

The total phenolic content was determined by the method of Singleton and Rossi[29]. Longan exocarp (2 g) from 20 fruits was homogenized in 20 ml of 80% ethanol for 5 min by using a mortar and pestle, and then centrifuged for 20 min at 16 000g (Hermel model Z383K, Germany) and 4 °C. Two hundred microlitres of clear supernatant were mixed with 10 ml of 10% Folin-Ciocalteu reagent (V/V) for 8 min. Then, 8.0 ml of 7.5% sodium carbonate (W/V) was added. Tubes were incubated for 2 h at 30 °C and

the absorbance was measured at 765 nm in a visible spectrophotometer (model Thermo Spectronic, USA). A standard curve of gallic acid 0–0.01% (W/V) was used to quantify the total phenolic content.

Statistical analysis

The experiments were designed as a completely randomized design. Statistical analysis was carried out using SPSS version 16 (SPSS incorporation Chicago, IL, USA). Duncan's Multiple Range Tests ($P = 0.05$) were performed to determine significant differences among the treatments.

RESULTS AND DISCUSSION

Exocarp browning is the main factor influencing postharvest quality and storage life of longan fruits[7–9]. It has been reported that the visual appeal of longan could deteriorate within 3 days under ambient conditions following harvest[4–6]. In our study, the inhibitory effect of SC on exocarp browning and activities of PPO and POD in longan fruits was investigated. As shown in Fig. 1, exocarp browning, represented by a browning index, increased with increasing storage time. The browning symptom was significantly reduced ($p < 0.05$) when fruits were dipped in SC at concentrations of 0.001–0.05% (w/v) for 10 min and stored at room temperature (25 ± 1 °C) for 48 h (Fig. 1). SC at a concentration of 0.01% was the most effective treatment in reducing exocarp browning, reducing by 73.8% and 36.7% at 24 and 48 h, respectively, (Fig. 1). Our results are consistent with previous studies by Lu et al[19,20] and Guan and Fan[22] who found that SC prevented browning of Red Delicious and Granny Smith apples. In addition, ClO_2, a derivative of SC, has also been reported to reduce browning of Golden Delicious apples, fresh-cut lotus roots and fresh-cut asparagus lettuce[21,23,24]. Although 0.05% SC was the highest concentration used in this experiment, the results showed that it was less effective than 0.01% SC (Fig. 1). A possible explanation is that the high concentration of 0.05% SC might cause tissue damage[20]. Therefore, phenolic compounds may easily be oxidized by PPO and POD, resulting in exocarp browning of longan fruits.

As shown in Fig. 2, L* and b* values gradually decreased with increasing storage time, but dipping in 0.001–0.05% SC significantly delayed the decrease in these values, indicating that SC could maintain lightness and yellowness of longan exocarp. The exocarp browning of longan fruits is primarily attributed to the oxidation of phenolic compounds by PPO and POD[7,11], leading to rapid browning after storage at ambient conditions. In our study, changes

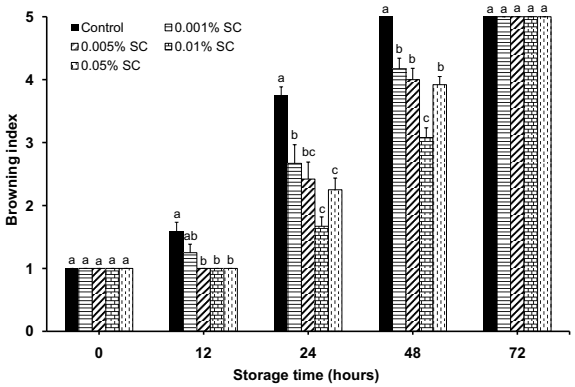

Fig. 1 The effects of SC on browning index of longan fruits during storage at 25 ± 1 °C. Bars with the same letters (in each storage time) are not significantly different at $P < 0.05$ using LSD. Means and standard errors ($n = 20$).

in PPO and POD activity during storage of longan are shown in Fig. 3. PPO activity of the control group dramatically increased and reached the highest value at 48 h, and this activity gradually decreased at 72 h (Fig. 3a). The result agrees with that of Duan et al[12] who also reported in longan cv. Shixia. All SC treatments significantly decreased the activity of PPO as compared to the control group ($p < 0.05$; Fig. 3a). The results show that 0.01% and 0.05% SC significantly reduced PPO activity more than 0.001% and 0.005% SC treatments at 12–48 h ($p < 0.05$; Fig. 3a). The results are consistent with the work of Lu et al[19], Fu et al[21], and Du et al[23] who reported that SC and ClO_2 inhibited PPO activity in fresh-cut apples and lotus roots. It has been reported that PPO contains copper in its active site, which is essential for enzyme activity[30,31]. Consequently, SC might affect the oxidation level of copper and alter the catalysing activity of PPO[32].

In addition to PPO, POD can catalyse the oxidation of many kinds of phenolic compounds in the presence of oxygen, which results in enzymatic browning of harvested fruits such as peach[33], litchi[34], pear[35], and pineapple[36]. Consequently, the control of POD activity is important in the preservation of fruits[37]. An increase in POD activity is commonly associated with injury, flavour loss, or biodegradation[38]. In our study, the activity of POD in exocarp of longan fruits dramatically increased and reached the highest value at 48 h, and then the activity gradually decreased at 72 h (Fig. 3b). When the fruits were dipped in 0.001–0.05% SC, the POD activity decreased (Fig. 3b). Our results show that 0.01% and 0.05% SC significantly reduced POD activity, with 0.001% and 0.005% being

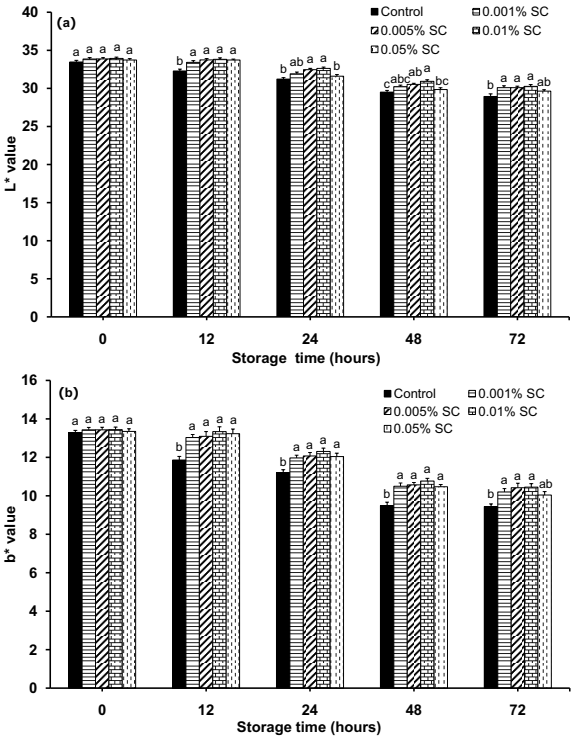

Fig. 2 The effects of SC on (a) L* value and (b) b* value of longan fruits during storage at 25 ± 1 °C. Bars with the same letters (in each storage time) are not significantly different at $p < 0.05$ using LSD. Means and standard errors ($n = 20$).

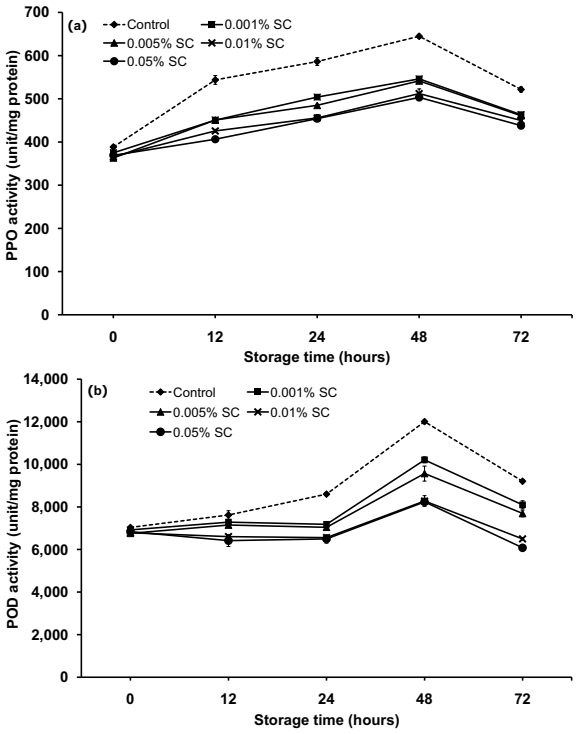

Fig. 3 The effects of SC on (a) PPO activity and (b) POD activity of longan fruits during storage at 25 ± 1 °C. Means and standard errors ($n = 6$).

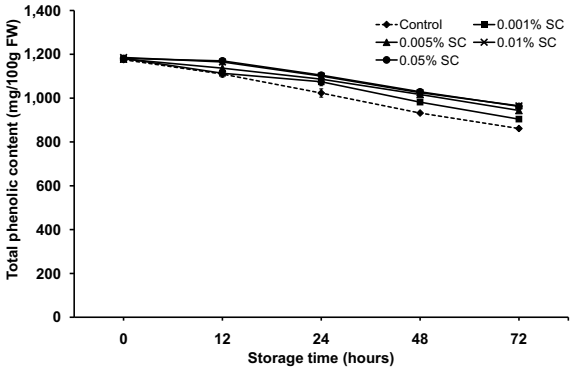

Fig. 4 The effects of SC on total phenolic content of longan fruits during storage at 25 ± 1 °C. Means and standard errors ($n = 6$).

more effective SC treatments ($p < 0.05$; Fig. 3b). The underlying mechanism of SC and ClO_2 treatments on the inhibitory effect of POD has not been clearly elucidated[24]. It is possible that SC might affect the oxidation level of iron at the active site of POD and alter the catalysing activity of POD[39,40].

Phenolic compounds are plant secondary metabolites synthesized mostly through the phenylpropanoid pathway and are involved in the defence of plants against invading pathogens[41]. Various classes of phenolic compounds such as catechins, catechol hydroxycinnamic acid derivatives, and anthocyanins have been found to contribute to non-enzymatic and enzymatic browning of foods[41]. Usually phenolic compounds in plant organs or tissues are oxidized into quinones under enzymatic catalysis and then the quinone is polymerized into brown polymeric pigments by PPO and POD[42]. In our study, the total phenolic content in longan exocarp decreased dramatically during storage, suggesting that phenolic compounds were oxidized during the browning process (Fig. 4). It was found that dipping in 0.001%-0.05% SC could significantly delay the decrease in total phenolic content during storage of longan fruits and the result is compatible with a decrease in the activity of PPO and POD (Fig. 3).

CONCLUSIONS

The results showed that dipping in 0.001–0.05% SC for 10 min has the potential to reduce exocarp browning in longan fruits cv. Daw by reducing the activity

of PPO and POD as well as maintaining total phenolic content during storage at ambient conditions for 48 h. It was recommended that an application of SC may be an alternative method for reducing exocarp browning and maintaining quality of harvested longan fruits.

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