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Exogenous salicylic acid inhibits browning of fresh-cut Chinese water chestnut

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Abstract

The potential usage of salicylic acid (SA) as a powerful anti-browning agent in fresh-cut Chinese water chestnut (CWC) was investigated. The fresh-cut CWC were dipped for 1 min in solutions of 0, 1, 2 or 4 mM SA, then placed in trays over-wrapped with plastic films, and finally stored at 4 °C. Changes in color, eating quality, and disease incidence were evaluated, while activities of phenol-associated enzymes, polyphenol oxidase (PPO), peroxidase (POD) and phenylalanine ammonia lyase (PAL), and concentrations of total soluble solid, titratable acidity and ascorbic acid were measured. SA treatment delayed discoloration, maintained eating quality with higher content of the quality attributers, and reduced activities of or delayed the increases in activities of PPO, POD and PAL in fresh-cut CWC. However, SA had no significant inhibition of the activities of PPO and POD in an in vitro test, indicating that the beneficial effect of SA was indirect. Further research is needed to elucidate the inhibition of the surface browning of the fresh-cut CWC by SA.

Keywords: Chinese water chestnut; Browning; *Eleocharis tuberosa*; Fresh-cut; Inhibition; Salicylic acid

1. Introduction

Fresh-cut Chinese water chestnut (CWC) can meet the ever-increasing demands of consumers for high quality, fresh, nutritive, and conveniently prepared fruits and vegetables (Peng & Jiang, 2003). However, the produce is very perishable compared to intact fruit, similar to other minimally processed produce (Peng & Jiang, 2003). It is, thereby, necessary to develop a suitable technology to extend the shelf life for commercial use.

Cut-surface browning, considered to be mediated by polyphenol oxidase (PPO) and peroxidase (POD), is a major concern with regard to quality deterioration and short shelf life of fresh-cut fruits and vegetables (Loaiza-Velarde & Saltviet, 2001; Luo & Barbosa-Canovas, 1996). Various approaches are now applied to prevent browning of fresh-cut fruits and vegetables, one of which is the use of different modified atmosphere conditions during low temperature storage (Annese, Manzano, & Nicoli, 1997). Application of low O_2 atmosphere below 1 kPa is effective in inhibiting browning of many fresh-cut fruits and vegetables mediated by PPO, but off-flavors often occur due to anaerobic respiration under low O_2 atmosphere conditions (Annese et al., 1997). Other approaches are to use chemical inhibitors of enzymatic browning (Friedman, 1996). Although, enzymatic browning was successfully controlled by sulfites, a replacement has been urgently sought due to consumer's increasing awareness of health and government bans on the use of sulfites for fresh fruits and vegetables. Unfortunately, the food-approved anti-browning additives, such as ascorbic acid,

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in combination with citric acid, have not been very effective in preventing discoloration of fresh-cut fruits and vegetables (Buta, Molie, Spaulding, & Wang, 1999; Sapers & Miller, 1995; Son, Moon, & Lee, 2001). Thus, alternative means of browning control are needed.

Salicylic acid (SA), a ubiquitous phenolic in plants, is the active part for the anti-inflammatory properties of aspirin, and has shown lots of benefits for human health (Baxber, Graham, Lawrence, Wiles, & Paterson, 2001; Deng, Ruan, Du, Saunders, & Wu, 2001; Scheier, 2001). In plants, SA was shown to induce flowering in some angiosperm species (Raskin, Ehmann, Melander, & Meeuse, 1987), mediate hypersensitive and systemic acquired resistance against pathogen attack (Raskin, 1992a, 1992b), activate the expression of several defense related genes (Lu, Rate, Song, & Greenberg, 2003), and interfere with biosynthesis and action of ethylene, abscisic acid and cytokinins, and improve responses concerning drought, chilling, heat, salt stress and UV-irradiation (Borsani, Valpuesta, & Botella, 2001; Janda, Szalai, Tari, & Paldi, 1999; Larkindale & Knight, 2002; Vahala, Keinänen, Schützendübel, Polle, & Kangasjärvi, 2003). It is also reported that SA treatment can delay fruit ripening and/or reduce decay of banana, nectarine, peach, apple, pear, strawberry, kiwifruit and citrus (Li & Han, 2000; Srivastava & Dwivedi, 2000; Yan & Shen, 1998; Zhang, Chen, Zhang, & Ferguson, 2003), and alleviate chilling injury of tomato and cucumber stored at low temperature (Han, Li, & Feng, 2002). In addition, SA is effective in the prevention of cardiovascular disease (Janssen, Katan, van Staveren, Hollman, & Venema, 1997) and shows many benefits for human health (Baxber et al., 2001; Deng et al., 2001). Scheier (2001) suggested that people should eat fruits and vegetables to intake more SA. Thus, SA can be used for postharvest handling of fruits and vegetables as a food additive (Fan, Mattheis, and Fellman, 1998; Li & Han, 2000; Srivastava & Dwivedi, 2000; Yan & Shen, 1998; Zhang et al., 2003).

In search for powerful anti-browning agents, our previous investigation exhibited that treatment with SA can effectively prevent discoloration of fresh-cut CWC. The objective of this study is to further investigate effects of exogenous SA treatment on inhibition of activities of phenolic-related enzymes phenylalanine ammonia lyase (PAL), PPO and POD, and quality maintenance of fresh-cut CWC stored at low temperature.

2. Materials and methods

2.1. Plant materials

Chinese water chestnut (*Eleocharis tuberosa*) cv. Guilin was obtained from a commercial market in Guangzhou, selected for uniformity and size, and any bruised or diseased fruit were discarded. Fruit were washed, peeled and cut into 4-mm thick slices (6 pieces/fruit) with a sharp stainless knife. The prepared slices were surface-sterilized by immersion in a 0.1% (w/v) NaClO solution for 1 min, air-dried for 30 min, and then dipped for 1 min in the solution containing 1, 2 or 4 mM SA. The slices treated with 0 mM SA solution was used as a control. After air-drying for another 30 min, the slices were placed into trays overwrapped with plastic films (0.02-mm thick polyethylene films, Xingxin plastic Corporation, Panyu, China) and then stored at 4 °C for progressive assessments. For each treatment, six replicates were used.

2.2. Quality evaluation

Appearance was estimated by measuring the extent of the total browned area on each slice surface on the scale: 0 = no browning, 1 = browning spots, 2 = slight browning (<1/5), 3 = moderate browning (1/5–1/3), 4 = moderate–serious browning (1/3–1/2), 5 = serious browning (> 1/2). The browning index was calculated using the following formula: $\sum$(browning scale × proportion of corresponding slices within each class). Slices evaluated at a higher index than 2.0 were considered to be unacceptable for marketing. Overgrowth of spoilage organisms was measured observing visible fungal growth or bacterial lesions on the surface. Eating quality of slices was assessed using a six-member panel. At each withdrawal, 12 slices were randomly selected and scored on a scale of 9 = excellent to 1 = poor in terms of appearance and flavor.

2.3. Extractions and assays of PAL, PPO and POD activities

PAL was extracted by the method of Lister, Lancaster, and Walker (1996). Tissue (10 g) from six slices was homogenized in 30 ml of 0.05 M phosphate buffer (pH 7.0) containing 5% polyvinylpyrolidone (Mr 44,000), 0.05 M sodium ascorbate, 0.018 M mercaptoethanol and 0.1% (v/v) Triton X-100. The homogenate was filtered through four layers of cotton cloth and then centrifuged for 20 min at 20,000*g* (Beckman J20-2, Pato, Alto, USA) and 4 °C. The supernatant was collected as the enzyme extract. PAL activity was assayed by a slight modification based on the method of McCallum and Walker (1990) using a reaction mixture of 0.06 M sodium borate buffer (pH 8.8) containing 11 mM L-phenylalanine and 0.4 ml of crude enzyme, with a final volume of 2.4 ml. Tubes were incubated for 2 h at 30 °C, and the reaction was stopped after addition of 0.6 ml of 35% (w/v) trichloroacetic acid. After the tubes were centrifuged for 5 min at 5000*g* to pellet the denatured proteins, the absorbance was measured at 290 nm by a Beckman DU-7 spectrophotometer. One unit of the

enzyme activity was defined as the amount of enzyme that caused a change of 0.001 in absorbance per hour.

To measure PPO and POD activities, tissue (5 g) from six slices was homogenized in 20 ml of 0.05 M phosphate buffer (pH 6.8) and the homogenate was filtered through two layers of cotton cloth to remove cell debris. The clear supernatant after centrifugation at 19,000*g* (Beckman J20-2) for 20 min at 4 °C was collected as enzyme extracts. PPO activity was assayed according to the method of Jiang (1999), by measuring the oxidation of 4-methylcatechol. The increase in absorbance at 410 nm was automatically recorded for 3 min, using a spectrophotometer (Beckman, DU-7). One unit of enzyme activity was defined as the amount that caused a change of 0.001 in absorbance per minute. POD activity was measured by the procedure of MacAdam, Nelson, and Sharp (1992). The assay mixture consisted of 0.05 M sodium phosphate buffer (pH 7.0), 0.012 M H_2O_2, 0.007 M guaiacol and 0.1 ml of enzyme solution in a final volume of 3.0 ml. The increase in absorbance at 470 nm was recorded for 3 min using a Beckman DU-7 spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme that caused a change of 0.001 in absorbance per minute.

The protein content was determined according to the dye-binding method of Bradford (1976) with bovine serum albumin as the standard.

2.4. In vitro experiments with SA effects on PPO and POD activities

To analyse the effects of SA on PPO and POD activities, 0.1 ml of SA solutions at the final concentrations of 0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 and 10 mM, as given in Fig. 3, was respectively, added to the reaction solution. PPO and POD activities were determined using the methods described previously and the relative enzymatic activities were expressed considering the activity without citric acid as 100. Each measurement was conducted three times.

2.5. Measurements of total soluble solids, titratable acid, and ascorbic acid

No disease development was observed on the fresh-cut CWC treated with 2 and 4 mM SA after 12 days of storage, and concentrations of total soluble solids, titratable acid and ascorbic acid of the CWC were analysed. Tissue (20 g) from six slices was homogenized in a grinder and then centrifuged for 20 min at 15,000*g* (Beckman J20-2). The supernatant phase was collected to analyse for: total soluble solids, using a hand refractometer (J1-3a, Guangdong Scientific Instruments); titratable acid was determined with 0.1 M NaOH, and ascorbic acid by 2,6-dichlorophenolindophenol titration.

2.6. Statistical analysis

All results were tested by analysis of variance (ANOVA procedure), and the means were separated using Fisher's LSD at $p \leqslant 0.05$.

3. Results

3.1. Effect of SA on sensory quality of fresh-cut CWC

As shown in Fig. 1, discoloration of fresh-cut CWC appeared after 3 days of storage at 4 °C and became serious after 6 days, while eating quality decreased markedly. SA treatment delayed the development of discoloration. Generally, increasing SA concentration

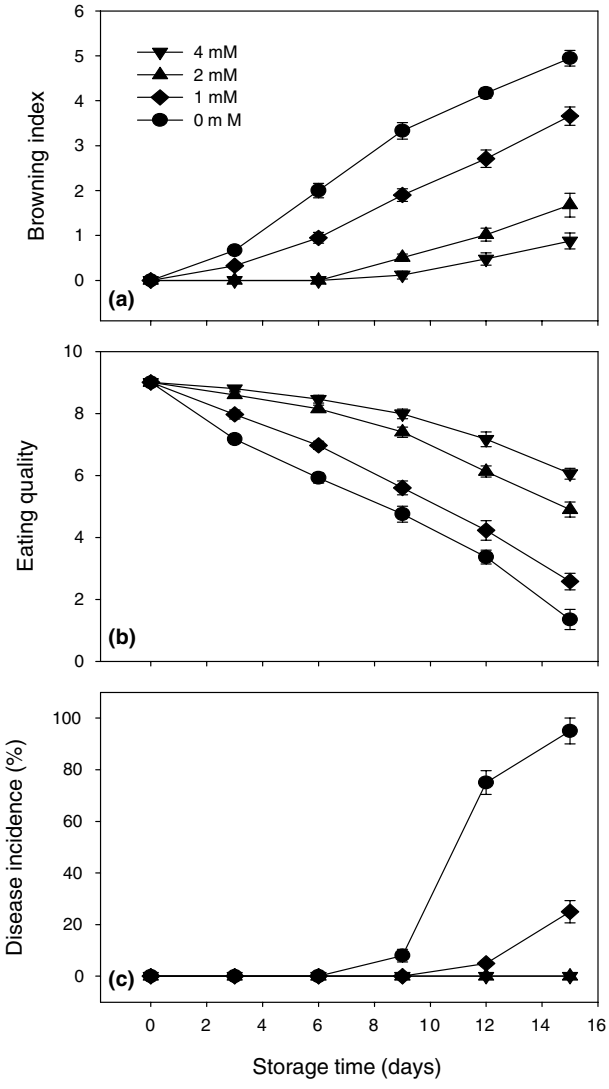

Fig. 1. Effects of SA at various concentrations on browning index (a), eating quality (b) and disease incidence (c) of fresh-cut CWC during storage at 4 °C. The vertical bars indicate the standard errors of six replicates.

(from 0 to 4 mM) enhanced browning inhibition and maintained eating quality. Meanwhile, SA treatment effectively inhibited disease development on fresh-cut CWC during storage at 4 °C (Fig. 1(c)). Thus, the surface browning of the sliced CWC appears to be the major factor limiting storage life.

3.2. Effect of SA on PAL, PPO, and POD activities

As mentioned above, the major problem that limits the shelf life of fresh-cut CWC is surface discoloration, which is associated with the phenol metabolic enzymes, such as POD, PPO and PAL (Jiang, 1999; Loaiza-Velarde & Saltviet, 2001; Lopez-Serrano & Ros Barcelo, 1999; Zhang et al., 2003). The activity in PPO increased at the early stage of storage, then reached a peak at day 9, and finally decreased (Fig. 2(a)). SA treatment inhibited markedly the increase in PPO activity of fresh-cut CWC, and increasing SA concentrations enhanced the inhibition of the enzyme.

POD activity of fresh-cut CWC decreased markedly when stored at low temperature within 3 days, and then increased (Fig. 2(b)). Treatments with SA delayed the increase in POD activity. At day 12, the slices treated with 1, 2 and 4 mM SA had a lower POD activity compared with the control.

Like PPO, PAL activity exhibited a peak at day 6 or 9 (Fig. 2(c)). SA treatment markedly inhibited the increase in the PAL activity. At day 6, the PAL activities of the CWC slices treated with 1, 2 and 4 mM SA, respectively, were 77.7%, 41.3% and 24.8% of the control in which the enzyme activity reached a peak.

3.3. In vitro experiments with SA effects on PPO and POD activities

To determine whether SA had direct inhibition on activities of PPO and POD, changes in the activities of the PPO and POD by SA were analysed in vitro (Fig. 3). SA stimulated POD activity within a concentration range of 0.05, 0.1, 0.2, 0.5, 1, 2, 5 and 10 mM (Fig. 3(b)), with the highest activity at 10 mM. Compared to POD, SA at low concentration has little effect on PPO

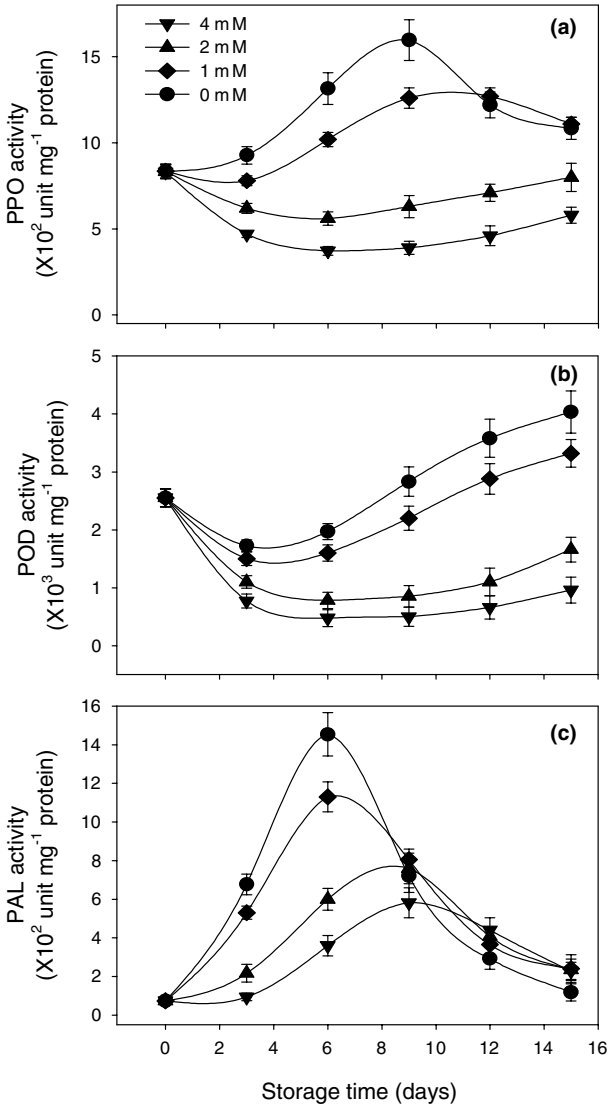

Fig. 2. Effects of SA at various concentrations on activities of PPO (a), POD (b) and PAL (c) of fresh-cut CWC during storage at 4 °C. The vertical bars indicate the standard errors of three replicates.

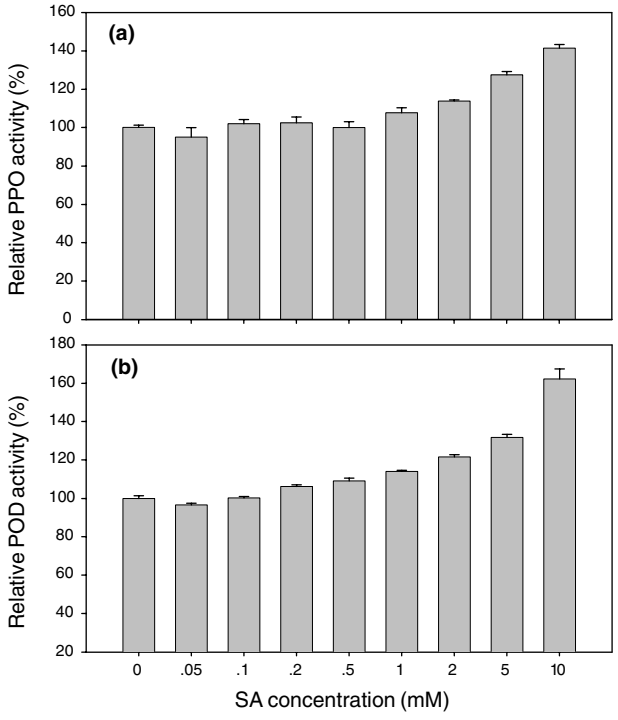

Fig. 3. Direct inhibitory effects of SA at various concentrations on activities of PPO (a) and POD (b) of fresh-cut CWC. The vertical bars indicate the standard errors of three replicates.

Table 1
Analyses of fresh-cut CWC after 12 days of storage at 4 °C

SA (mM)	Total soluble solids (%)	Titratable acidity (mmol/g)	Ascorbic acid (mg/100 g pulp)
Before treatment	16.0	0.165	8.175
After storage			
0	11.2c	0.109b	5.8d
1	12.5b	0.117b	6.42c
2	14.2a	0.145a	7.38b
4	14.8a	0.156a	7.80a

Means within a column followed by the same letter are not significantly different at the 5% level.

activity, but the presence of SA at higher than 2 mM significantly increased the activity (Fig. 3(a)).

3.4. Effects of SA on concentrations of total soluble solids, titratable acidity and ascorbic acid

The concentrations of total soluble solids, titratable acidity and ascorbic acid decreased after 12 days of storage at 4 °C (Table 1), which was consistent with the decline in eating quality (Fig. 1). SA treatment maintained higher concentrations of total soluble solids, titratable acidity and ascorbic acid than the control.

4. Discussion

SA treatment effectively prevented surface browning (Fig. 1(a)), which limits the shelf life of fresh-cut CWC (Peng & Jiang, 2003). It is generally accepted that surface browning is due to the oxidation of phenolics caused by PPO and POD, resulting in the formation of brown colored substances (Laurila, Kervinen, & Ahvenainen, 1998; Martinez & Whitaker, 1995). The increase in PPO activity of fresh-cut CWC at the early stage of storage accelerated the oxidation of polyphenols and thus led to rapid lose of surface white color of the CWC (Figs. 1(a) and 2(a)). Similar results were obtained on fresh-cut apples, pears, and lettuce (Buta & Abbott, 2000; Buta et al., 1999; Heimdal, Kuhn, Poll, & Larsen, 1995; Luo & Barbosa-Canovas, 1996; Rolle & Chism, 1989). In this study, SA at lower concentration had little inhibition on PPO activity, but it at higher concentration increased significantly the PPO activity in vitro (Fig. 3(a)). Thus, the inhibition of the PPO activity by SA could be indirect.

POD is also related to surface browning of fruit and vegetables (Laurila et al., 1998; Saltveit, 2000). POD and PPO have a synergistic effect on the formation of the brown polymers (Lopez-Serrano & Ros Barcelo, 1999; Subramanian, Venkatesh, Ganguli, & Sinkar, 1999). In this study, high POD activity correlated with browning index (Figs. 1(a) and 2(b)). The in vitro experiments indicated that inhibition of POD activity by SA is an indirect effect (Fig. 3(c)).

PAL is the first key enzyme involved in the biosynthesis of phenols in fruits, and it is induced by wounding in minimal processing (Dixon & Paira, 1995; Saltveit, 2000). Inhibition of PAL activity reduced biosynthesis of precursors for the formation of the brown substances (Loaiza-Velarde & Saltviet, 2001; Loaiza-Velarde, Tomas-Barbera, & Saltveit, 1997). SA treatment inhibited PAL activity of fresh-cut CWC, which was associated with reduced browning index (Fig. 1(a)).

SA has shown benefits for human health (Baxber et al., 2001; Deng et al., 2001; Janssen et al., 1997) and had a high LD_{50} of 891 mg/kg oral rat (Mallinckrodt Baker Inc, 2003). In this study, SA at 1, 2 and 4 mM was used for handling of fresh-cut CWC. A very low SA residue was detected in the treated fresh CWC after 12 days of storage at 4 °C. Thus, it is safe to use SA in fresh-cut CWC.

In conclusion, as SA treatment effectively prevented browning and maintained eating quality with higher concentrations of quality attributers of fresh-cut CWC, SA could be considered for commercial application in extending shelf-life and maintaining quality of fresh-cut CWC, but the inhibition of PPO and POD activities in the CWC slices by SA is unclear and thus requires further investigation.

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