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Browning Control and Quality Maintenance of Litchi Fruit Treated with Combination of *N*-acetyl Cysteine and Isoascorbic Acid

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Abstract: Experiments were conducted to test effects of anti-browning agents on inhibition of browning and decay of litchi fruit. Litchi fruit were soaked for 2 min in a solution of 0.1% *N*-Acetyl Cysteine (AC) +1% Isoascorbic Acid (IAA) and held for 7 days at 25°C and 80-90% relative humidity for quality assessments. Use of AC and IAA combination significantly reduced fruit decay and browning, markedly inhibited polyphenol oxidase activity and maintained higher level of anthocyanin in pericarp tissues. Fruit treated with 0.1% AC +1% IAA had good visual appearance. The use of the combination of 0.1% AC +1% IAA could be considered for commercial application in inhibiting pericarp browning and maintaining flesh quality of litchi fruit during storage.

Key words: Acetyl cysteine, browning, fruit, isoascorbic acid, *Litchi chinensis*, storage

INTRODUCTION

Litchi (*Litchi chinensis sonn.*) is a tropical to subtropical fruit with high commercial value in the international market. However, the fruit after harvest are very perishable and rapidly lose their bright skin colour and turn brown, resulting in reduced market value[1-3]. Browning was thought to be due to a rapid degradation of the red pigments and oxidation of phenolics by Polyphenol Oxidase (PPO), producing brown-colored byproducts[4-6].

Enzymatic browning can be prevented effectively by use of bisuphite[7-9]. SO_2 fumigation has been used commercially to control pericarp browning but SO_2-fumigation leaves undesirable residues, alters the fruit taste and results in health hazards for consumers and packed house workers[10]. Furthermore, the strict standards enforced by the European Community on fruit, allowing maximum sulphur residue levels of only 10 μg g^{-1} in the edible portion of the fruit, has necessitated the development of an alternative postharvest technology to maintain overall fruit quality of litchis during storage and transportation.

Use of some anti-browning agents and their derivatives, such as 4-hexylresorcinol, *N*-Acetyl Cysteine (AC), ascorbic acid, Isoascorbic Acid (IAA) and calcium chloride, alone or in combination at different concentrations, have been effective in reducing browning and inhibiting decay of different fruits and vegetables[11-16]. Furthermore, use of AC and IAA exhibited a better inhibitory effect of PPO activity, compared with cysteine or ascorbic acid[17]. Jiang and Fu[18] reported that the combination of glutathione and citric acid could give good control of browning of litchi fruit. Recently, we find that the combination of AC and IAA is the most effective in inhibiting litchi PPO activity among these chemicals. Unfortunately, there are no published data on effects of use of *N*-acetyl cysteine and isoascorbic acid combination on pericarp browning of harvested litchi fruit. The objective of this study was to investigate the effect of the combination of AC and IAA in inhibiting browning and deterioration of litchi fruit during storage.

MATERIALS AND METHODS

Plant materials and treatments: Mature fruit of litchi (*Litchi chinensis* sonn.) cv. Huaizhi were obtained from a commercial orchard in Guangzhou, China. Fruit were selected for uniformity of shape, color and size and any blemished or diseased fruit discarded. Fruit were dipped for 2 min in a solution of 0.1% AC +1% IAA for within

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3 h of harvest. In this study, the treatment was the mostx effective in maintaining visual appearance of the fruit. Fruit treated with distilled water for 2 min were used as control. After these treatments, the fruit were air-dried for 2 h at 25°C before being packed in units of 20 fruit into plastic punnets, overwrapped with plastic films and held at 25°C and 80-90% relative humidity for quality assessments. There were three replicates for each treatment.

Fruit quality evaluation: Appearance was assessed by measuring the extent of the total browned area on each fruit pericarp, using 300 fruit during shelf life evaluation, on the following scale: 1 = no browning (excellent quality); 2 = slight browning; 3 = <1/4 browning; 4 = 1/4-1/2 browning; 5 = >1/2 browning (poor quality). The browning index was calculated as Σ (browning scale × percentage of corresponding fruit within each class). Fruit at higher than 2.0 (browning index) was considered unacceptable for marketing. Disease incidence was monitored by randomly collecting 300 fruit and then recording the percentage of fungal growth or bacterial lesions on the fruit surface.

Relative leakage rate: Membrane permeability, expressed by relative electrolyte leakage rate, was determined by the method of Jiang and Chen[19]. Peel discs were removed with a 5 mm cork borer from the equatorial region of 30 fruit. Fifty discs were rinsed twice in distilled water and then incubated in 30 mL of 0.3 M mannitol solution at 25°C with shaking for 30 min. Electrolyte leakage was determined with a conductivity meter (Model DDS-11A, Shanghai Scientific Instruments, China). Total electrolyte leakage was determined after boiling another batch of 50 discs for 30 min and cooling to 25°C (total electrolytes). Relative leakage rate was expressed as a proportion of total electrolyte leakage.

PPO activity: PPO activity was assayed with 4-methylcatechol as a substrate according to the method of Zauberman *et al.*[20]. Peel (6.0 g) from 15 fruit was homogenized in 30 ml of 0.02 M phosphate buffer (pH 6.8) containing 0.6 g of polyvinylpyrrolidone (insoluble). The homogenate was centrifuged for 20 min at 19 000 g (Beckman J20-2) and 4°C and the supernatant was then collected as crude enzyme extract. Assay of PPO activity was performed using 1.0 mL of 0.1 M phosphate buffer (pH 6.8), 0.5 mL of 0.1 M 4-methylcatechol and 0.5 mL enzyme solution. The increase in absorbance at 410 nm at 25°C was automatically recorded for 3 min (Beckman DU-7). One unit of enzyme activity was defined as the amount which caused a change of 0.01 in absorbance per minute. Protein contents were determined by the method of Bradford[21] with albumin bovine serum as the standard. Anthocyanin concentration: Litchi pericarp (10 g) from 15 fruit was finely sliced and extracted with 200 mL of 0.1% HCl-methanol (1 mL HCl in 99 mL methanol) for 2 h, according to the method of Pirie and Mullins[22]. After the extract was filtered and diluted, its absorbance was measured at 530 and 600 nm using a spectrophotometer (Beckman DU-7). A unit of anthocyanin was expressed as a difference of 0.1 in the absorbance between 530 and 600 nm and anthocyanin concentrations were presented as unit/g fresh weight.

Total soluble solids and titratable acidity concentrations: Total soluble solids and titratable acidity concentrations in control and treated litchi fruits were analysed at harvest and after 3, 5 and 7 days of storage. Pulp (20 g) from 15 fruit was homogenized in a grinder and then centrifuged or 20 min at 15 000 g (Beckman J20-2). The supernatant was collected for analyses of total soluble solids using a hand refractometer (J1-3A, Guangdong Scientific Instruments) and titratable acidity as % citric acid determined by titration with 0.1 M NaOH[23].

Statical analysis: The experiments were arranged in completely randomized designed and each treatment comprised of three replicates. Data were tested by analysis of variance using SPSS version 7.5. Least Significant Differences (LSD) were calculated to compare significant effects at the 5% level.

RESULTS AND DISCUSSION

Effects of AC and IAA combination on browning and decay: As shown in Fig. 1A, peel browning index of litchi fruit rapidly increased during storage. The browning indexes of the control fruit were 2.6 and 4.0 after 5 days and 7 days of storage at 25°C, respectively. Treatment with AC and IAA was effective in reducing peel browning of litchi fruit. The treated fruit retained red color after 7 days of storage. Similar results were reported on browning control of fresh-cut pineapple and banana slices by application of AC or IAA[24-25]. In addition, an additive effect on inhibition of browning was achieved when AC and IAA were combined in apple, pear and mango slices[11,26].

Spoilage pathogens of litchi fruit were significantly inhibited by the use of AC and IAA combination (Fig. 1B) during storage. No diseases development was observed within 3 days. However, about 12.6% of control fruit begun to rot after 5 days of storage. After 7 days of storage, treatment with 0.1% AC +1% IAA

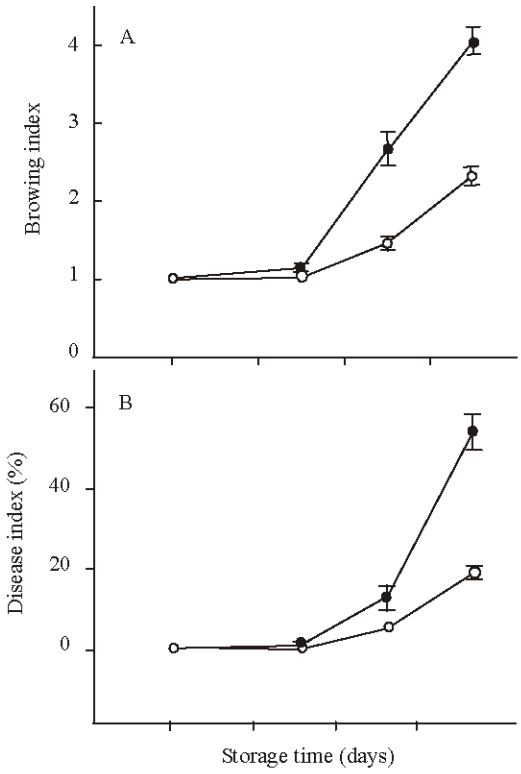

Fig. 1: Effects of the combination of acetyl cysteine and isoascorbic acid on browning index (A) and disease rate (B). Litchi fruit were treated with 0% AC + 0% IAA (●) or 0.1% AC + 1% IAA (○) and then stored for up to 7 days at 25°C. Each value is the mean for three replicates and vertical bars indicate the standard errors where they exceed the symbol size

significantly suppressed disease development. González-Aguilar *et al.*[25] reported that individual use of AC or IAA could significantly reduced deterioration of fresh-cut pineapple, while Buta *et al.*[27] found that the combination of AC and IAA was more effective in preventing deterioration of apple slices.

Effects of AC and IAA combination on anthocyanin concentration, PPO activity and relevant leakage rate: Fruit discoloration correlated well with the PPO activity and the concentration of phenolics[28-30]. The bright red colour of litchi fruit has been attributed to anthocyanin in pericarp tissues[4]. In this investigation, anthocyanin concentration decreased with storage time (Fig. 2A), while PPO activity rapidly increased within 5 days and then remained relatively a high level (Fig. 2B. Treatment with AC and IAA combination reduced the decrease in anthocyanin concentration and delayed increase in PPO

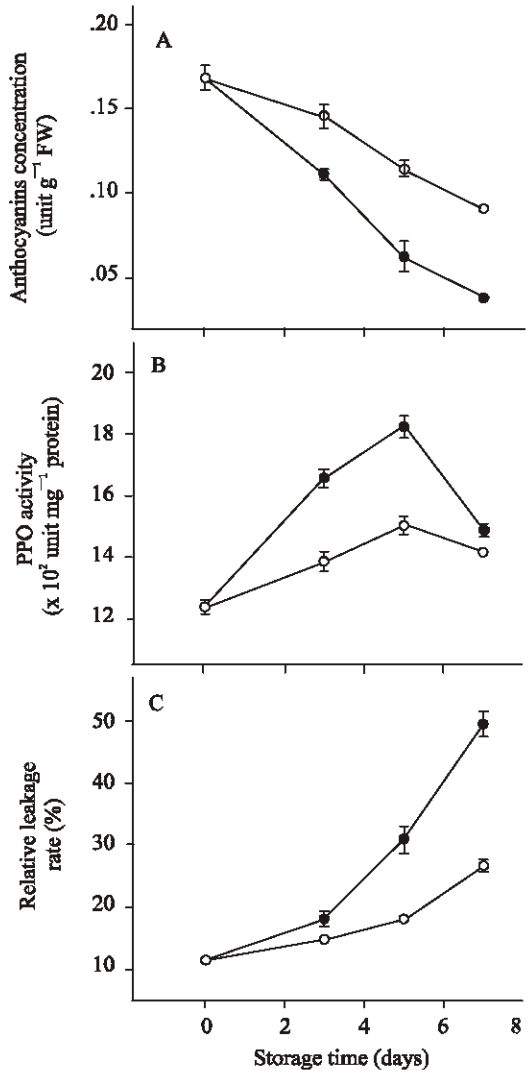

Fig. 2: Effects of combination of acetyl cysteine and isoascorbic acid on anthoncyanin content (A), PPO activity (B) and relative leakage rate (C). Litchi fruit were treated with 0% AC + 0% IAA (●) or 0.1% AC + 1% IAA (○) and then stored for up to 7 days at 25°C. Each value is the mean for three replicates and vertical bars indicate the standard errors where they exceed the symbol size

activity. Monsalve-Gonzalez *et al.*[11] and Buta *et al.*[27] reported that application of AC and IAA, as reducing agents, were effective in reducing browning of apple and pear slices, while Gonzalez-Aguilar *et al.*[25] found that IAA was more effective than ascorbic acid in preventing the browning. Apparently, IAA had a mode of action different from AC and a combination of AC and IAA was more effective in inhibiting browning of fresh-cut

Table 1: Effects of the combination of 0.1% AC and 1% IAA on concentrations of Total Soluble Solids (TSS) and Titratable Acidity (TA) of aril of litchi fruit after 6 days of storage at 25°C

	Storage time (days)					
	3		5		7	
Treatment	TSS (%)	TA (%)	TSS (%)	TA (%)	TSS (%)	TA (%)
Control	14.9b	0.16b	14.7b	0.15a	14.5b	0.13b
Treatment	15.4a	0.19a	15.3a	0.18a	15.1a	0.17a

For each measurement, corresponding means within a column between control and treatment followed by the same letter are not significantly different at the 5% level. Concentrations of total soluble solids and titratable acidity in aril of litchi fruit at harvest were 16.8 and 0.21%, respectively.

radishes[31]. Moreover, the efficacy of these compounds may be altered by concentrations used and characteristics of fruits and vegetables.

Postharvest browning of litchi fruit was also thought to be due to the cellular decompartmentation, which resulted in oxidation of phenolics by PPO, forming browning-colour by-products[32-33]. To further understand the role of AC and IAA in browning inhibition of litchi fruit, relative leakage rate was analyzed. Membrane permeability can be expressed by relative leakage rate, which was correlated negatively with membrane integrity[34]. As shown in Fig. 2C, relative leakage rate increased and treatment with AC and IAA combination delayed the increase in the relative leakage rate. AC or IAA can protect membrane damage by eliminating the reactive oxygen species[35]. Therefore, application of AC and IAA maintained relatively membrane integrity and, thus, inhibited peel browning of harvested litchi fruit.

Effects of AC and IAA combination on concentrations of total soluble solids and titratable acidity: Titratable acidity and total soluble solids are important factors in assessing flavor and nutritive quality of litchi fruit[18]. Table 1 showed the concentrations of titratable acidity and total soluble solids of litchi flesh during storage. The fruit treated with AC and IAA combination had higher concentrations of total soluble solids and titratable acidity, which could be due to respiration inhibition[25].

In inclusion, in terms of good browning control and quality maintenance, combination of 0.1% AC + 1% IAA could be considered for commercial application to harvested litchi fruit during storage and marketing.

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