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Effect of oxalic acid on control of postharvest browning of litchi fruit

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Abstract

Litchi (*Litchi chinensis* Sonn.) fruit, cv. Huaizhi, was treated with 2 and 4 mM oxalic acid and stored at room temperature to investigate the effect of oxalic acid on pericarp browning. The results showed that the pericarp browning indices of the fruit, treated with both oxalic acid concentrations, were significantly lower than that of the control, due to increase of membrane integrity, inhibition of anthocyanin degradation, decline of oxidation, and maintanance of relatively low peroxidase activity in the fruit during storage. It appears that application of oxalic acid can effectively control the pericarp browning of litchi fruit during postharvest storage.

Keywords: Litchi; Oxalic acid; Postharvest browning

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a tropical and subtropical fruit with a high market value. However, rapid pericarp browning and decay of litchi fruit during storage are the main problems that result in drop of its market value. Much research work has indicated that many biochemical and physiological changes, such as decrease of membrane integrity, degradation of anthocyanin, increase of peroxidase (POD) and polyphenol oxidase (PPO) activities, and enhancing of superoxidation reaction, are involved in these problems (Jiang, 2000; Jiang & Fu, 1999a, 1999b; Lin et al., 1988; Underhill & Critchley, 1995).

Oxalic acid, as a final metabolic product of plants, was identified as having many functions in plants by recent research work. For instance, oxalic acid was shown to be an anti-browning agent for apple and banana slices by decreasing PPO activity (Yoruk, Balaban, Marshall, & Yoruk, 2002). Many researchers (Zhang, Peng, Jiang, Xu, & Li, 1998; Zheng, Zhao, & Peng, 1999) have considered that oxalic acid should induce systemic resistance of plants, related to both increase of POD activity and synthesis of new POD isoforms. In addition, oxalic acid was found to enhance intrinsic heat-tolerance of pepper leaves by increasing the membrane stability (Zhang, Li, & Wang, 2001). However, there are no reports about the efficiency of oxalic acid for litchi fruit during storage. The objective of this study was to investigate the effect of oxalic acid on the browning of litchi fruit in order to improve the texture of litchi using exogenous oxalic acid.

2. Materials and methods

2.1. Fruit treatment and storage condition

Litchi fruit, cv. Huaizhi, (about 80%-matured stage), were harvested from an orchard of Zhangjiang City,

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Guangdong Province, China. Fruits were selected for uniformity of size and colour, and blemished and diseased fruit were discarded. The selected fruit were dipped in different oxalic acid solutions ((A) 2 mM oxalic acid + 0.05% fungicide, Bavistin; (B) 4 mM oxalic acid + % fungicide, Bavistin, and (C) 0.05% fungicide, Bavistin, as the control) for 10 min within 4 h of harvest, and then air-dried. Each 500 g of the treated fruit was packed into a plastic punnet and wrapped with a 0.2-mm polyethylene bag, and then stored in a room held at 25(±1 °C) with 80–90% relative humidity (RH).

2.2. Browning assessment

The browning was assessed by measuring the extent of the total browned area on each fruit pericarp, on the following scale (Jiang & Fu, 1999a): 1 = no browning; 2 = slight browning; 3 = <1/4 browning; 4 = 1/4–1/2 browning; 5 = >1/2 browning. The browning index was calculated using the formula: $\sum$(browning scale × percentage of corresponding fruit within each class). Three replicates (50 fruit per replicate) of each treatment were carried out.

2.3. Measurement of relative leakage rate

Relative leakage rate was determined by the method of Li (2000). Litchi pericarp discs, (1 g), from 10 fruits were rinsed and incubated in 60 ml of distilled water for 4 h, and then the initial electrolyte leakage was monitored with a conductivity meter (DDS-11A, China). Each sample was continually rinsed for 2 h after being boiled for 5 min, and the final electrolyte leakage (total electrolyte) was again monitored. Relative leakage rate was defined as percent of initial electrolyte.

2.4. pH measurement of pulp juice

Pulp tissues (20 g) from 10 fruits were homogenized and filtered, and then pH values of both solutions were monitored with a pH meter (PHS – 3B, China) at 27 °C.

2.5. Anthocyanin assay

Pericarp (1 g) from 10 fruits was quickly sliced and extracted with 15 mlHCl–methanol (0.15% HCl:95%methanol = 15:85) for 4 h. The extract was filtered and its absorbance was determined at 530, 620 and 650 nm, respectively. The anthocyanin content measurement was based on the formula: $\Delta A/\text{ml} = (A_{530} - A_{620}) - 0.1\,(A_{650} - A_{620})$ (Proctor, 1974).

2.6. Enzyme assay

Litchi pericarp (2 g) from 10 fruits was homogenized with 25 ml of 50 mM sodium phosphate buffer (pH 7.0) containing 0.25 g of polyvinylpyrrolidone (PPVP, Sigma). After centrifugation at 18,000*g* for 20 min, the supernatant was used as enzyme extract. POD (EC 1.11.1.7) activity was based on the determination of guaiacol oxidation at 470 nm by H_2O_2. The change in absorbance at 470 nm was followed every 30 s by spectrophotometer (GBC Centra-10, Australia) (Lacan & Baccou, 1999). POD activity was defined as ΔA_{470}/min/g FW (Li, 2000). Total SOD (SOD, EC 1.15.1.1) activity was assayed according to the method of Oberley and Spitz (1985), based on the ability of SOD to inhibit the reduction of nitroblue tetrazolium (NBT, Sigma). The absorbance was monitored at 550 nm. One unit of SOD is the amount of extract that gives 50% inhibition of the reduction rate of NBT.

2.7. Malondialdehyde (MDA) determination

MDA determination was followed the method described by Li (2000). Litchi pericarp, (1 g), from 10 fruits was homogenized in 15 ml of 10%TCA. The homogenate was centrifuged at 10,000*g* for 20 min, and then 2 ml supernatant of sample was reacted with 2 ml of 0.6% 2-thiobarbituric acid. The absorbance was monitored at 600, 532 and 450 nm, respectively. Calculation of MDA was based on the following formula: $C(\mu\text{m/l}) = 6.45(A_{532} - A_{600}) - 0.56\,A_{450}$.

3. Results and discussion

3.1. The changes in pericarp browning index and anthocyanin content in pericarp of the fruit treated with oxalic acid

The browning index increased with storage time. However, the indices of the fruit treated with 2 and 4 mM oxalic acid were significantly lower than that of the control after 5 d of harvest (Fig. 1(a)). Comparing the inhibitory efficiencies of different oxalic acid concentration on browning, showed that 2 mM oxalic acid was the most effective concentration for litchi, cv. Huaizhi. pH value of pulp was initially low and had potential to increase with storage time but, in fact, no significant change in pH (initially 4.43) occurred as a result of oxalic acid treatment during four days of storage. This suggests the experimental treatment did not cause a leaking of oxalic acid into the mesocarp of fruit (Table 1).

The anthocyanin content in the pericarp increased at 2 d after harvest with respect to the day of harvest and then markedly decreased with storage time. No difference in anthocyanin content, between treated fruit and control, was presented at 2 d after harvest, but its content in oxalic acid-treated fruit was significantly higher than in the control after 4 d of harvest (Fig. 1(b)). Postharvest browning of litchi was thought to be due to

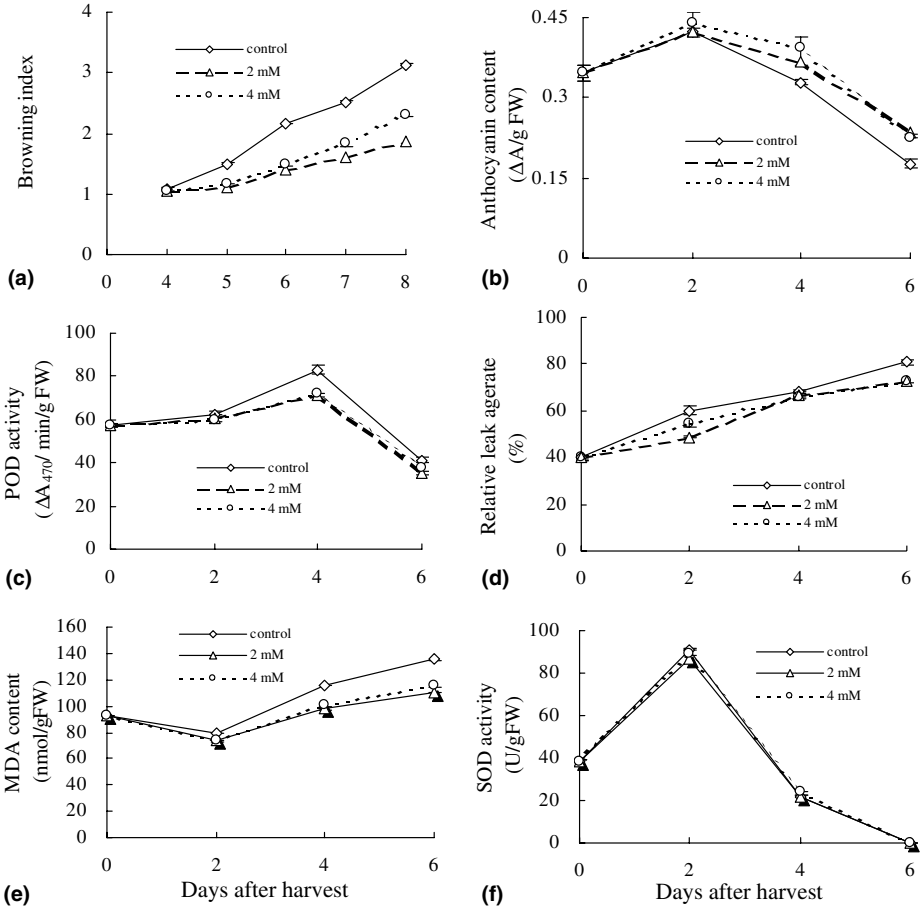

Fig. 1. The effect of oxalic acid on browning index (a), anthocyanin content (b), POD activity (c), relative leakage rate (d), MDA content (e) and SOD activity (f) in pericarp of litchi fruit. Data are means ± SE of three independent replicates.

Table 1
The pH value of pulp of the fruit treated with oxalic acid during storage

Days after harvest	Control	2 mM	4 mM
0	4.43 ± 0.02a	4.43 ± 0.02a	4.43 ± 0.02a
2	4.54 ± 0.02a	4.53 ± 0.01a	4.54 ± 0.01a
4	4.73 ± 0.01a	4.86 ± 0.02a	4.73 ± 0.03a

Variance analysis by new multiple range test.
Means within a line among different oxalic acid concentration treatment followed by the same letter are not significantly different at 5% level.

anthocyanin degradation (Zauberman et al., 1991). However, no evidence of browning was observed in the anthocyanin-containing mesocarp and application of 0.1 M HCl to already brown tissue significantly improved pericarp redness, independently of anthocyanin synthesis (Underhill & Critchley, 1994). Jiang (2000) suggested that anthocyanins, PPO and phenols contribute to litchi pericarp browning involved in the anthocyanin–PPO–phenol reaction, because litchi PPO cannot cause the degradation of anthocyanins, but anthocyanin degradation was accelerated upon mixing of anthocyanins, phenolics and PPO. Moreover, anthocyanin decolourization involved structural changes (Markakis, 1982), and anthocyanin structure and colour were directly dependent on pH (Nakayama & Powers, 1982). Date here indicate that the effect of oxalic acid on pericarp browning of litchi during the storage was associated with inhibition of the anthocyanin degradation rather than direct maintenance of the reddish colour of anthocyanin by pH change. The increase of anthocyanin content at the beginning of the storage time might result from anthocyanin synthesis after harvest.

3.2. Physiological responses in litchi pericarp to oxalic acid treatment during storage

POD activity in the litchi fruit increased at the beginning of storage time and reached maximum activity at 4 d of harvest and then markedly declined. The fruit treated with oxalic acid presented relatively lower POD activity during storage in comparison to the control (Fig. 1(c)). In litchi, POD has been considered as an enzyme responsible for pericarp browning (Jaiswal, Sah, & Prasad, 1987; Lin et al., 1988; Underhill &

Critchley, 1995). Recently, Gong and Tian (2002) found that, like PPO, the activity of POD in litchi pericarp was markedly inhibited by $NaHSO_4$ and Na_2SO_4, and they suggested that soluble POD should play an important role in litchi postharvest browning. So the effect of oxalic acid on pericarp browning of litchi during storage was partly due to causing a relative decrease of POD activity in the fruit.

The relative leakage rate of litchi fruit treated with oxalic acid did not increase markedly as did the control (Fig. 1(d)). MDA content, the oxidative enzymic product, in the treated fruit, was significantly lower than that in the control after 4 d of storage (Fig. 1(e)). Furthermore, SOD activity in the fruit increased at the beginning of storage time and markedly decreased 2 d after harvest and, at each storage time, and the levels of SOD activity in oxalic acid-treated fruit were similar to those in control (Fig. 1(f)). SOD is believed to play a crucial role in antioxidant defence, and a high level of SOD in non-netted muskmelon, combined with changes in the three different classes of SOD during storage, contribute to delay the senescence process of fruit (Lacan & Baccou, 1999). Conversely, SOD activity in rice leaves increased under oxidative stress when rice was pretreated with oxalic acid, which might account for part of the protective effect of oxalic acid (Peng, Liu, & Li, 2001). Pepper leaves were sprayed with oxalic acid for 3 d before heat stress, the damage to the membrane was alleviated, and the production of MDA and hydrogen peroxide was decreased (Zhang et al., 2001). Our results showed that oxalic acid could not effect SOD activity in litchi pericarp, but might play a protective role in membrane integrity in litchi because of the declining oxidation during storage and, thereby, might contribute to inhibition of enzymatic browning.

3.3. The potential use of oxalic acid for controlling litchi postharvest browning

It is well known the reddish skin of litchi fruit is one of the most important aspects of the fruit's quality. Unfortunately, litchi fruit rapidly deteriorates once harvested, which results in skin browning, the first visual symptom of deterioration (Jaiswal et al., 1987). Currently, sulphur dioxide fumigation and acid-dipping were shown to be effective for controlling skin browning and are commercially available in many countries (Swarts, 1985; Tongdee, 1986; Zauberman et al., 1991). Zauberman, Roren, Akerman, and Fuchs (1990) reported that application of 1 M HCl, after sulphur dioxide treatment, could restore fruit to the initial skin colour. Jiang, Liu, Chen, Li, and Zhang (1997) also found litchi that fruit presented the best red colour after soaking in 1% $NaHSO_3$ containing 0.5% HCl for 8 min and proposed $NaHSO_3$ with HCl be an alternative method for preserving litchi red colour. However, with respect to the side-effects, such as the residue problems and tainting that can be harmful to human health (Kremer-Kohne & Lonsdale, 1990; Taylor & Bush, 1986), alternative chemicals without toxic effects are still urgently needed for commercial use. Oxalic acid is a metabolic product that is distributed among different organs of plants (Davies & Asker, 1983; Libert & Franceschi, 1987). Kayashima and Katayama (2002) considered that oxalic acid was available as a natural antioxidant and might play an important role in the natural and artificial preservation of oxidized materials. This fact, together with our present study, suggests that treatment with controlled oxalic acid is a safe and promising method for controlling litchi pericarp browning during postharvest storage.

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