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Control of Enzymatic Browning of Harvested 'Hong Huay' Litchi Fruit with Hot Water and Oxalic Acid Dips

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ABSTRACT: Pericarp browning considerably reduces the shelf life and value of litchi fruits. This research was aimed to evaluate three browning inhibitors for control of litchi browning. Litchi cv. Hong Huay fruits were dipped in hot water (98°C) for 30 s prior to soaking in solutions of oxalic, citric and ascorbic acids at 0, 2.5, 5, 10, and 15% for 15 min. They were then stored at room temperature (25 ± 1°C) and 74 % relative humidity for 5 days. The results showed that oxalic acid at a concentration of 10% was the most effective in controlling browning. Hot water dips enhanced the effectiveness of oxalic acid. Dipping in hot water, followed by treatment with oxalic acid, resulted in the retention of pericarp redness and gave the best browning inhibition during the storage time by reducing the activities of polyphenol oxidase and peroxidase and maintaining a high level of total anthocyanins.

KEYWORDS: browning, oxalic acid, anthocyanins, litchi, PPO, and POD.

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is a subtropical Asian fruit, which is in high demand for its appealing natural red color, sweet taste, and aroma. Exportation through international markets is limited by postharvest storage problems, *i.e.* a rapid loss in the red color of the pericarp. One of the hypothesized mechanisms for this browning is that it results from oxidation and polymerization of phenolic compounds, including the red anthocyanins, caused by polyphenol oxidase (PPO) and peroxidase (POD).[1-4]

Enzymatic browning is a widespread problem in the litchi and other fruit industries, as it leads to undesirable characteristics of fruits, thereby decreasing fruit quality and value. Various physical and chemical methods have been used to control browning and/or inactivate the activities of PPO and POD in litchi fruit.[5-11] Currently, the use of a sulphiting agent plus acid dips is the most efficient chemical approach for control of litchi browning. Sulfur dioxide smoking and an acid dip, used alone or in combination use with cold storage or hot water treatment (HWT), and hydrochloric acid (HCl) treatment reduce pericarp browning in litchi fruits.[9-11] A major limitation of the use of HCl and sulfur dioxide are health concerns since these chemicals are toxic to humans.

Oxalic acid is the most effective antibrowning agent on apple slices,[12] while HWT alone or HWT followed by an HCl dip are also effective in reducing browning and maintaining a distinct red color. These methods seem to be more attractive than the SO_2-treatment of litchi fruit, but after these treatments the aril becomes brown.[6,13] We became interested in examining whether HWT combined with an oxalic acid dip could replace use of HCl and SO_2 to produce high quality and safer litchi fruit. The objectives of this study were to investigate the effects of oxalic acid on browning inhibition and to compare its inhibitory effectiveness with citric and ascorbic acids, combined with hot water treatment. The effects of oxalic acid on the inhibition of PPO and POD activities and total anthocyanin content were also evaluated.

MATERIALS AND METHODS

Litchi (*Litchi chinensis* Sonn. cv. Hong Huay) fruits, at the fully colored and commercially mature stage, were harvested from a commercial orchard in Chiang Mai, Thailand during the 2004 season. Fruits were selected for uniformity of size, shape, color, and lack of physical damage and injury caused by insects, prior to use in the following two experiments.

Experiment 1. Effects of Hot Water and Acids on Pericarp Color and Browning

Fruits were dipped in hot water at 98°C for 30 s, then immersed in a solution of oxalic, citric or ascorbic acids at 0, 2.5, 5, 10, and 15% for 15 min and allowed to air dry. Fruits without hot water and acid treatments

were used as controls. Fruits were then kept under ambient conditions of 25 ± 1°C and 74% relative humidity (RH) in sealed plastic punnets for 5 days, and pericarp color, pH and browning were evaluated. Three replicates per treatment were used, with 15 fruits per replicate.

Experiment 2. Effects of Hot Water and Oxalic Acid on Pericarp Browning, Total Anthocyanin Content, and PPO and POD Activities.

Fruits were dipped in hot water at 98°C for 30 s followed by dips in 5 or 10% oxalic acid for 15 min, or dipped in 15% oxalic acid without HWT. These three treatments gave the best results in terms of maintenance of bright red pericarp color in fresh fruits. These fruits were then kept at 25 ± 1°C and 74% RH for 5 days, and browning was evaluated, while the activities of PPO and POD, and the total anthocyanin content were measured. Three replicates per treatment were used, with 15 fruits per replicate.

Assessments

Fruit Browning

Litchi fruit browning was assessed according to the method of Jiang,[14] by measuring the extent of the total browned area on each fruit pericarp after storage for 0, 6 and 12 h, and 1, 2, 3, 4, and 5 days, on the following scale : 1 = no browning (excellent quality); 2 = slight browning; 3 = <25% browning; 4 = 25 – 50% browning; 5 = >50% browning (poor quality). The browning index was calculated as Σ (browning scale x percentage of corresponding fruit within each class). Fruits evaluated at an index > 3.0 were considered unacceptable for marketing.

Pericarp Color

The Hunter L* and a* pericarp values were measured for each fresh fruit using a Minolta colorimeter (Chromameter CR – 2000, Japan).

Anthocyanin Content

Anthocyanin content was measured according to the method of Ranganna.[15] Litchi pericarp tissue (10 g) from 10 fruits was finely chopped and extracted with 200 ml HCl – ethanol (1 ml HCl in 99 ml 95% ethanol) at 5°C for 24 h. The extract was filtered, diluted, and its absorbance was measured at 530 nm using a spectrophotometer (Thermo Spectronic). Anthocyanin content was expressed as mg per 100 g fresh weight.

Polyphenol Oxidase (PPO) and Peroxidase (POD) Activities

Litchi PPO and POD were extracted by homogenizing the pericarp (2.5 g) in 10 ml of 0.05 M potassium phosphate buffer solution (pH 6.2), 1 M KCl and 2% polyvinylpolypyrroridone (PVPP). The homogenate was centrifuged for 30 min at 13,500 rpm and 4°C, and then the supernatant was collected as the crude enzyme extract. The assay of PPO activity was performed according to the method of Jiang & Fu,[7] using 2.0 ml of 0.05 M potassium phosphate buffer (pH 7.5), 0.2 ml of 0.2 M 4-methyl-catechol and 0.5 ml of enzyme extract. The increase of absorbance at 420 nm was recorded automatically for 5 min. The assay of POD activity was performed according to the method of Nagle & Haard,[17] using 2.5 ml of 0.01 M sodium acetate buffer (pH 6.0), 0.05 ml of 0.1% guaiacol, 0.1 ml of 0.1% H_2O_2, and 0.05 ml of enzyme extract. The absorbance was recorded at 470 nm. One unit of

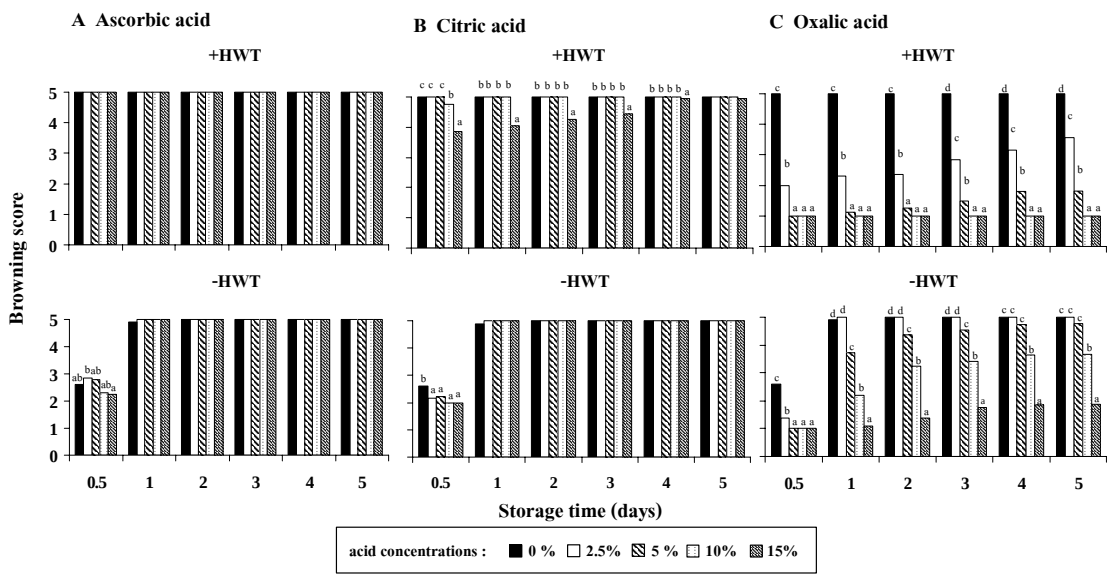

Fig 1. Changes in browning of litchi pericarp after different postharvest treatments during storage at 25 ° C for 5 days. Treatments marked with the same letter are not significantly different ($p \leq 0.05$).

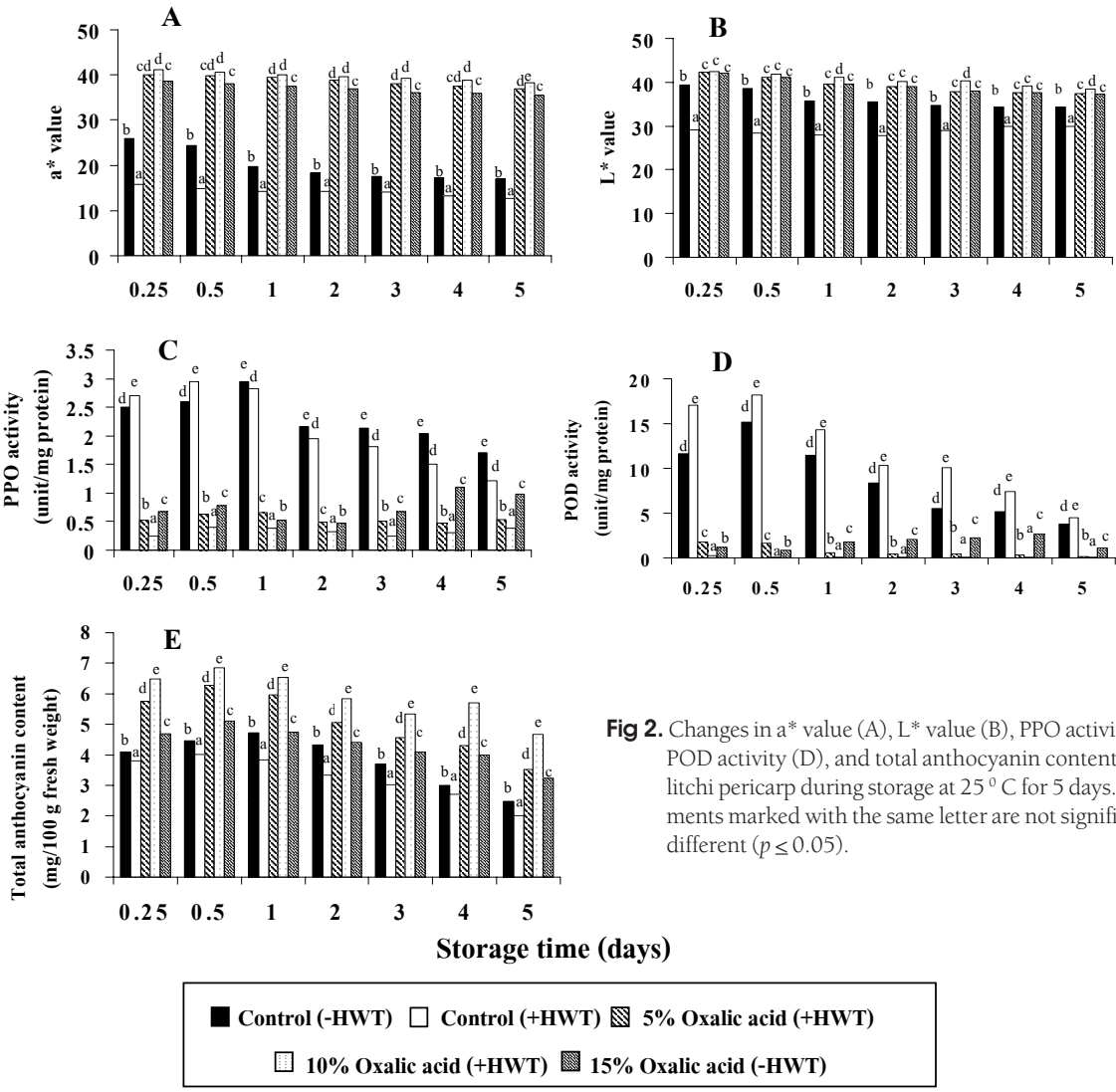

Fig 2. Changes in a* value (A), L* value (B), PPO activity (C), POD activity (D), and total anthocyanin content (E) of litchi pericarp during storage at 25 ^{0} C for 5 days. Treatments marked with the same letter are not significantly different ($p \leq 0.05$).

enzyme activity was defined as the amount that causes an increase of 0.01 in absorbance per min. Protein content was determined according to the method of Lowry *et al.*[18]

pH of Pericarp and Acid Solutions

The pericarp pH was determined according to the method of Underhill & Critchley.[4] Pericarp tissue (4.0 g) was washed in distilled water to remove adhesive aril juice. The pericarp tissue was finely chopped and homogenized in 40 ml of distilled water for 1 min. The pH was measured while stirring the homogenate. The pH of each acid solution was also measured.

Results

Experiment 1. Effects of Hot Water and Acid Treatments on Pericarp Color and Browning

The effects of hot water and acid dip treatments on pericarp browning and red color of litchi fruits are shown in Fig. 1. Pericarp browning increased with storage time. After 1 day of storage, the initial red color of the control fruits had largely disappeared which rendered them commercially unacceptable due to rapid browning, while it took longer to develop this level of browning in fruits treated with oxalic acid (Fig. 1C). The degree of browning inhibition depended on the oxalic acid concentration used. As oxalic acid concentrations increased, browning scores of the treated fruits were lower. Dipping in ascorbic acid or citric acid was not effective in preventing pericarp browning and the treated fruits rapidly turned brown within 1 day (Figs.1B and 1C).

Hot water accelerated pericarp browning (Fig. 1). As expected, fruits that were dipped only in hot water became brown within 12 h of storage. Prior to an oxalic acid dip, HWT treatment significantly reduced pericarp browning, compared to the acid dip alone, whereas

HWT in combination with ascorbic acid or citric acid treatment, resulted in no significant decrease in the pericarp browning index.

Treatment with acid dips resulted in the retention of red pericarp color. Among the acids tested, oxalic acid was the most effective in color retention. Prior to acid dipping, HWT was more effective in the retention of the red color. The pericarp of fruits treated by HWT, followed by acid dipping, had more red than those treated with acid dip alone and control fruits. These results indicated that HWT, followed by acid dips, facilitated reduction of pericarp browning and maintenance of red pericarp color. All treatments resulted in fruit softening after 4 or 5 days of storage (data not shown).

Our study revealed significant inhibition of pericarp browning (browning score < 3) in HWT + 15% oxalic acid, HWT + 10% oxalic acid, HWT + 5% oxalic acid, and 15% oxalic acid without HWT treatments for 5 days of storage. Only fruits treated with HWT + 15% oxalic acid, showed apparent deterioration in fruit aril quality or taste, and caused internal browning in aril (data not shown).

Experiment 2. Effects of Hot Water and Oxalic Acid on Pericarp Browning, Total Anthocyanin Content, and PPO and POD Activities

Results from this experiment (Figs. 2A and 2B) demonstrated that the pericarp of oxalic acid-treated fruits had a brighter red color than those of the control fruits. For the control fruits, the browning scores significantly increased with increased storage time. The browning of oxalic acid-treated fruits was low, but there was no significant difference between the acid treatments (data not shown).

Changes in PPO and POD activities of oxalic acid-treated and control fruits during storage are shown in Figs. 2C and 2D, respectively. PPO and POD activities of acid treated fruits were low and changed slightly during storage, while those of the control fruits were higher and changed significantly. PPO and POD activities of control fruits increased and reached a maximum on the first day and then decreased. During 5 days of storage, there were significant differences in PPO and POD activities between the acid-treated fruits and control fruits. Hot water dipping followed by 10% oxalic acid treatment gave the best inhibition of PPO and POD activities.

The total anthocyanin content of litchi fruit pericarp was associated with browning score and red color. As shown in Fig. 2E, fruits with oxalic acid treatment had higher contents of anthocyanins, less browning, and lower activities of PPO and POD than the control fruits.

To further understand the pH value of each acid treatment in controlling browning and red color of litchi pericarp during storage, evaluations were analyzed. As shown in Table 1, the type and concentration of dipping acid used contributed to the appearance of browning and red color. It was noted that oxalic acid-treated pericarps had the lowest pH. Reduced pH (pH<3; increased acid concentration) led to decreased browning. No marked change in the pH levels of the pericarp homogenates was observed during storage (Table 2).

Discussion

Oxidation of phenolic compounds is the main cause of browning in harvested fruits. PPO and POD are terminal oxidases which catalysed oxidation of phenolics and are involved in the breakdown of anthocyanins, resulting in tissue browning and color changes of litchi fruits.[3-4,19] Dipping fruit in dilute solutions of HCl and some other acids can completely

Table 1. The pH of acid solutions and litchi pericarp after different postharvest treatments.

Acid treatment	pH of acid solution	pH of pericarp homogenate after acid treatment *	
		-HWT	+HWT
control	4.28	4.36±0.01v	4.04±0.01t
2.5 % ascorbic acid	1.99	4.08±0.01u	3.96±0.01r
5 % ascorbic acid	1.94	4.03±0.01t	3.89±0.01q
10 % ascorbic acid	1.92	3.98±0.01s	3.59±0.01n
15 % ascorbic acid	1.89	3.81±0.01p	3.42±0.01k
2.5 % citric acid	1.85	3.77±0.01o	3.52±0.01m
5 % citric acid	1.66	3.58±0.01n	3.44±0.01l
10 % citric acid	1.53	3.35±0.01j	3.35±0.01j
15 % citric acid	1.46	3.29±0.01i	3.29±0.01i
2.5 % oxalic acid	1.25	3.27±0.01h	3.22±0.01g
5 % oxalic acid	1.02	3.17±0.01f	2.64±0.01c
10 % oxalic acid	0.82	2.86±0.01e	2.11±0.02b
15 % oxalic acid	0.76	2.83±0.01d	2.04±0.01a

* Means followed by the same letter are not significantly different ($p \leq 0.05$).

Table 2. The pH of litchi pericarp after different postharvest treatments and storage at 25 ^{0} C for 5 days.

Acid treatment	pH of acid solution	pH of pericarp homogenate after acid treatment			
		-HWT		+HWT	
		day 0	day 5	day 0	day 5
control	5.39	4.51±0.00	4.66±0.01	4.47±0.01	4.51±0.01
5 % oxalic acid	1.08	*ND*	*ND*	3.01±0.01	2.97±0.01
10 % oxalic acid	0.63	*ND*	*ND*	2.34±0.01	2.75±0.01
15 % oxalic acid	0.53	3.10±0.01	2.93±0.01	2.05±0.01	2.50±0.01

ND : not determined.

restore the red color loss and prevent tissue browning in apples and litchi.[12,20-21] The efficacy of acid dipping in preventing browning is dependent on treatment time and acid concentration used.

In our studies, only oxalic acid effectively suppressed color deterioration in litchi fruits at a minimum concentration of 10%. Dipping fruit in oxalic acid prevented pericarp browning and restored the red color, maintained the level of total anthocyanins, and inhibited an increase in PPO and POD activities, which are associated with pericarp browning. This implies that oxalic acid prevents litchi pericarp enzymatic browning by acting as an inhibitor of these enzymes. There are few reports on the inhibition of PPO and POD by oxalic acid.[22-24]

Several studies have been reported on the effect of oxalic acid and the differences in the oxalic acid concentration for an antibrowning in apple[12] and banana slices[25], and litchi pericarp.[26] Oxalic acid at only 0.025% (2 mM) was used as an effective inhibitor to enzymatic browning in litchi cv. Huaizhi. In 'Hong Huay' litchi, the best concentration (10%) used was higher, but showed more effective control on pericarp browning during 5 days of storage. The most effective concentration of oxalic acid may depend on inherent genetic properties (cultivar), pericarp thickness, nature of phenolic substrates, and acid treatment method. The most effective concentration for 'Hong Huay' fruit does not appear to have been reported before.

The mechanism of oxalic acid inhibition on PPO and POD activities may be due to PPO binding with copper and POD to iron to form inactive complexes.[24,27-29] It is also possible that the prevention of browning in litchi pericarp could be due to low pH. Oxalic acid, a chelating agent, is also acidulant, pH reducing, and is a prominent inhibitor of litchi pericarp browning. No PPO activity of litchi pericarp was observed in the enzymatic reaction solution at a pH below 4.2.[30] The oxalic acid treatment resulted in a more acidic pericarp than that of the controls during storage. High concentrations (10 and 15%) of oxalic acid with low pH values were required to prevent browning and effectively maintained the red color. Treatment with oxalic acid at high concentrations may damage litchi pericarp. Internal browning of fruit aril with off-flavor taste, was detected when fruits were dipped in hot water + 15% oxalic acid. This may result in the breakdown of the pericarp membrane system, allowing substrate-enzyme contact, thereby leading to browning.

The accelerated browning may be due to the damage to decompartmentation of enzymes and substrates, resulting in an enhanced enzymatic reaction.[31] After dipping in oxalic acid, fruits exhibited a uniform red color and high levels of anthocyanins. Suitable treatments with acids may form co-pigmentation of anthocyanins in litchi pericarp and stabilize the anthocyanin pigments.[5,19] Anthocyanins can exist in a red stable flavylium ion form at pH 3.0 or below, while there are in less stable anhydro base form at higher pH, resulting in the formation of colorless chromenols.[32] Usually, the physiological pH value in plant vacuoles is about 3.0 with red anthocyanins.[33] The pH of litchi pericarp homogenate treated with oxalic acid, was very low and increased little during storage time. This suggests that prevention of browning of litchi pericarp by oxalic acid may result from the inhibition of PPO and POD activities and stabilization of anthocyanins. Changes in anthocyanins are one of the causes of browning of litchi pericarp. Degradation of anthocyanins is the result of coupled oxidation in the presence of other phenolics.[34]

It was also found that a hot water dip increased the effectiveness of acid dipping, especially with oxalic acid. Dipping in hot water (98^{o}C) for a short time (30 s) may diminish the naturally high surface tention of solutions on the epidermis of litchi pericarp, thus increased permeability of acids into the pericarp in the subsequent dipping stage, which, in turn, may both inhibit the activities of browning-related enzymes and maintain high levels of anthocyanins.[6] Alternatively, short heat treatment may stimulate an increase in the synthesis of wax to fill the cracks.[35]

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