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Role of anthocyanin degradation in litchi pericarp browning

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Abstract

Pericarp browning is the main problem of post-harvest litchi fruit, resulting in an accelerated shelf life and reduced commercial value of the fruit. Underhill and Critchley (1994). Anthocyanin decolorisation and its role in lychee pericarp browning. *Australian Journal of Experimental Agriculture*, *34*, 115-122 found that there was not an obvious change in the content of anthocyanins when the fruit browned. This work was conducted with a view to explaining this unexpected observation. Litchi pericarp browning index increased while the content of anthocyanins decreased with storage time when 0.1 M HCl was used as the extract solution instead of acidic methanol. The visible spectum of the anthocyanin extract, at a range of 400–600 nm and pH values of 1.0, 3.0 and 5.0, were recorded, with an absorbance peak of about 510 nm. The colour of the extract depended on the pH values and the half-degradation constants for anthocyanins at pHs 1.0, 3.0 and 5.0 were, respectively, 29, 15.3 and 10.5 days, as calculated from the kinetics of the degradation. Compared with the anthocyanin extract, anthocyanidin is more vulnerable, with a half-degradation of about 5.3 min at pH 5.0. Furthermore, the product from the anthocyanidin degradation had a similar structure to catechol (a good substrate for polyphenol oxidase), which, in turn, could accelerate enzymatic browning reaction by the enzyme polyphenol oxidase. In addition, an anthocyanase, catalyzing anthocyanin hydrolysis and producing anthocyanidin was extracted from litchi fruit pericarp. High activity of the enzyme was observed in the pericarp. Thus, it is suggested that anthocyanase might contribute to the browning of litchi pericarp involved in the anthocyanase-anthocyanin-PPO reaction.

Keywords: Anthocyanin; Anthocynase; Browning; Degradation; Litchi

1. Introduction

Litchi (*Litchi chinensis* Sonn.*)* is a tropical and subtropical fruit of high commercial value for its white, transulent aril and attractive red colour. However, the fruit rapidly lose their redness and turn brown once harvested (Holcroft & Mitcham, 1996; Nip, 1988). Post-harvest browning of litchi was thought to be caused by a rapid degradation of the red pigment by polyphenol oxidase (PPO), producing brown-coloured by-products (Akamine, 1960; Huang, Hart, Lee, & Wicker, 1990). The red pigment of litchi pericarp was identified as cyanidin-3-rutinoside and cyanidin-3-glucoside (major) and malvidin-3-acetylglucoside (minor; Lee & Wicker, 1991). However, Underhill and Critchley (1994) found that there was no obvious change in the content of anthocyanins when the fruit browned. Recently, Jiang (2000) reported that litchi PPO cannot oxidize anthocyanin, but the anthocyanin might be degraded rapidly in an anthocyanin-PPO-phenol system and, thus, suggested that it may be the presence of the sugar moiety which caused steric hindrance. Since anthocyanins are unstable, they could be degraded nonenzymatically or enzymatically. Simpson, Lee, Rodrigue, and Chichester (1976) proposed two possible mechanisms for the nonenzymatic degradation of anthocyanin: 1, the hydrolysis of the 3-glycosidic linkages to produce the more labile aglucone, and 2, hydrolytic opening of the pyrylium ring to form a substituted chalcone. In addition, anthocyanase (anthocyanin-β-glucosidase) could play a role in removing the sugar groups, leading to the anthocyanin decolorization (Huang, 1955). However, the enzyme has been found only in fungi and has been used in making white wine from red grapes(Huang, 1955; Sanchez-Torres, Gonzalez-Candelas, & Ramon, 1998). The objective of this current study was to investigate the kinetics of nonenzymatic anthocyanin degradation of

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litchi pericarp at various pH values, to determine whether there was an anthocyanase in the litchi pericarp, and then to understand the role of both nonenzymatic and enzymatic degradation of the anthocyanin in the browning of litchi pericarp.

2. Materials and Methods

2.1. Plant materials

Fruit of litchi (*Litchi chinensis* Sonn.) cv. Huaizhi at the commercially mature stage were obtained from a commercial orchard in Guangzhou, China. Fruit were selected for uniformity of shape and colour and then divided into two groups. One group of the fruit was peeled and the pericarps were then stored at −20°C while the other was held in a controlled environment room at 30°C and 70% relative humidity (RH).

2.2. Fruit browning assessment

According to the method of Jiang (2000), litchi browning was assessed by measuring the extent of the total browned area on each fruit pericarp, using 18 fruit after storage for 0, 1, 2, 4 and 6 days, 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).

2.3. Measurement of anthocyanin content

Litchi pericarp (10 g) from 10 fruit with various browning indices, described above, during storage, was blanched with 200 ml of 0.1 M HCl. Anthocyanin content of the extraction solution was determined using a pH-differential method (Wrolstad, Culbertson, Cornwell, & Mattick, 1982). The extract (5 ml) was diluted in 25 ml of pH 1.0 KCl-HCl buffer solution and 25ml of pH 4.5 citric acid-Na_2HPO_4 buffer solution, respectively. A spectrophotometer (Shimadu 300 UV), with 1 cm pathlength cells, was used for optical measurements at 510 nm. Anthocyanin content was calculated as cyanidin-3-glucoside by the method of Wrolstad et al. (1982).

2.4. Anthocyanin extraction

According to the method of Lee and Wicker (1991), an ethanolic solution of litchi pericarp extract was prepared by blenching the pericarp (50 g) from fully red fruit with 100 ml of acidified ethanol (1.5 M HCl /ethanol, 15:85, v/v), refrigerating overnight and filtering through Whatman No. 41 paper. The extract was concentrated to remove ethanol with a rotary evaporator at 40°C, and was then used for the following experiments.

2.5. Acidic hydrolysis of litchi anthocyanin

The acidic hydrolysis procedure previously described by Hong and Wroslstad (1986), was used for preparation of anthocyanidin. The extract (10 ml) was obtained with 50 ml of 2 M HCl for 60 min in a boiling water bath. To remove HCl and concentrate anthocyanindin, the hydrolysate was purified using an Amberlite XAD-7 resin column, as suggested by Baublis, Spomer, and Berber-Jimenez (1994).

2.6. Analysis of anthocyanin and anthocyanidin degradation

The spontaneous degradation of anthocyanin and anthocyanidin was conducted by the method of Baublis et al. (1994). To determine the pH effect on the kinetics of the degradation, the anthocyanin and anthocyanidin extracts (1 ml) were, respectively, dissolved in 50 ml of 0.4 M KCl–HCl buffer solution (pH 1.0) or 0.1 M citric acid-Na_2HPO_4 buffer solutions with a pH of 3.0 or 5.0. The final concentration of anthocyanin or anthocyanidin in these buffer solutions was 0.1 mg/l. Then the extract solutions were diluted with respective buffer solutions and the absorbance of each solution was automatically scanned at a range of 400–600 nm and recorded, using a spectrophotometer (Shimadu 300 UV). After the scanning, the solutions were transferred to screw-top test tubes at 30°C. These tubes were degassed with nitrogen to eliminate oxygen transfer. Samples were taken out of the tubes at various times, according to the respective degradation rate, and the amount of anthocyanin or anthocyanidin in these solutions was measured. For the kinetics, lg (Co/Ct) was made (Co: the initial content of the pigment; Ct: the content of the pigment at various time). Also, the half-degradation constants were calculated from the kinetic curves (Masterton, Slowinski, & Stanitski, 1985).

2.7. Determination of anthocyanase activity

Litchi anthocyanase was extracted by homogenising the pericarp (4 g) with 10 ml of 0.1 M sodium lactate buffer solution (pH 4.0) and Polyclar AT (insoluble polyvinylpyrrolidone; 10% of peel by weight). The homogenate was centrifuged for 20 min at 16 000 g and 4°C and then the supernatant was collected as the crude enzyme extract. According to the method of Martino, Pifferi, and Spagna (1994), 1 ml of enzyme extract was added to 4 ml of 0.05 mM cyanidin-3-glucoside (Extrasynthese, France), which was dissolved in 0.1 M sodium lactate buffer (pH 4.0). After the mixture was incubated for 10 min at 40°C, 5 ml of 0.1 M HCl was added to the

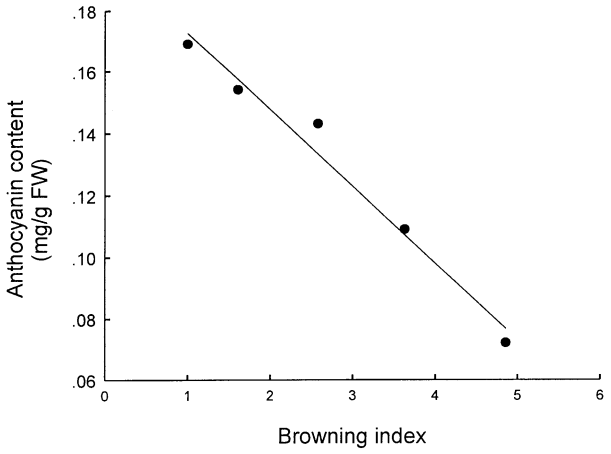

Fig. 1. Relationship between anthocyanin content and browning index of litchi pericarp.

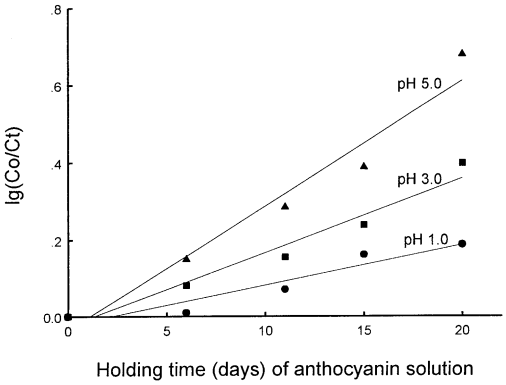

Fig. 3. The kinetics of the anthocyanin degradation of litchi pericarp extract at pH values of 1.0, 3.0 and 5.0.

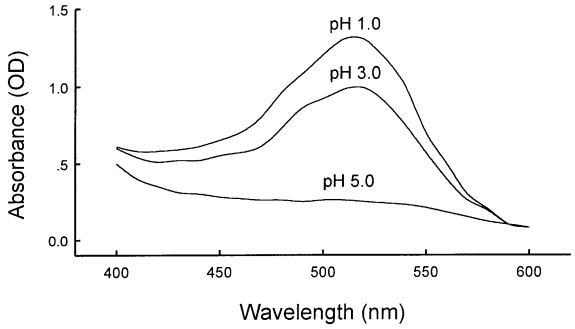

Fig. 2. Visible spectra of the anthocyanin extract of litchi pericarp at pH values of 1.0, 3.0 and 5.0.

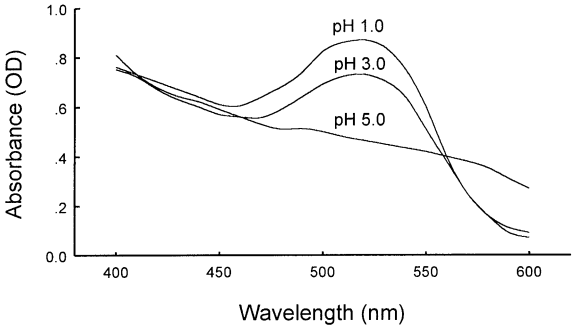

Fig. 4. Visible spectra of the acidic hydrolysis of the anthocyanin extract of litchi pericarp at pH values of 1.0, 3.0 and 5.0.

reaction and the absorbance recorded at 510 nm. One unit of enzyme activity was defined as the degradation of 1 nmol of cyanidin-3-glucoside per minute at 40°C.

3. Results and discussion

3.1. Relationship between pericarp browning and anthocyanin degradation

Litchi pericarp browning index increased with storage time while the content of anthocyanins decreased (Fig. 1). Furthermore, there was an obviously inverse relationship ($R^2 = 0.976$, $P < 0.05$) between anthocyanin content and peel browning index. Lin et al. (1988) reported that the anthocyanin content did not markedly change when the fruit browned. Underhill and Critchley (1994) suggested that the colour change of litchi peel was independent of the content of anthocyanin. The difference might be attributed to the procedure of anthocyanin extraction. In this current study, we used 0.1 M HCl as the extract solution instead of acidic methanol (Lin et al., 1988; Underhill & Critchley, 1994) because the authors found that the browned substances extracted by acidic methanol were insoluble in 0.1 M HCl. Thus, there was a higher absorbance at 530 nm in the acidic methanol extract (data not shown), which resulted in an increased content of the anthocyanins of the browned pericarp extract.

3.2. Degradation of anthocyanins at various pH values

The visible spectrum of the anthocyanin extract at a range of 400–600 nm and various pH values of 1.0, 3.0 and 5.0 was recorded with an absorbance peak of about 510 nm (Fig. 2). Obviously, the colour of the extract depended on the pH values, i.e. red at pHs 1.0 and 3.0 and colourless at pH 5.0. The intensity of characteristic absorption at 510 nm decreased as the pH values increased. With the decrease of the pH values, the anthocyanins were more stable but still showed a loss during storage, which can be confirmed by the dependence of lg Co/Ct (Fig. 3). Furthermore, the half-degradation constants for anthocyanins at pHs 1.0, 3.0 and 5.0 were, respectively, 29, 15.3 and 10.5 days, as calculated from the lg Co/Ct kinetics of the degradation.

Anthocyanins can exist in a red stable flavylium ion form at pH 3.0 or below, while they are in a less stable anhydro base form at higher pH, resulting in the formation of colourless chromenols(Jurd, 1972). Usually,

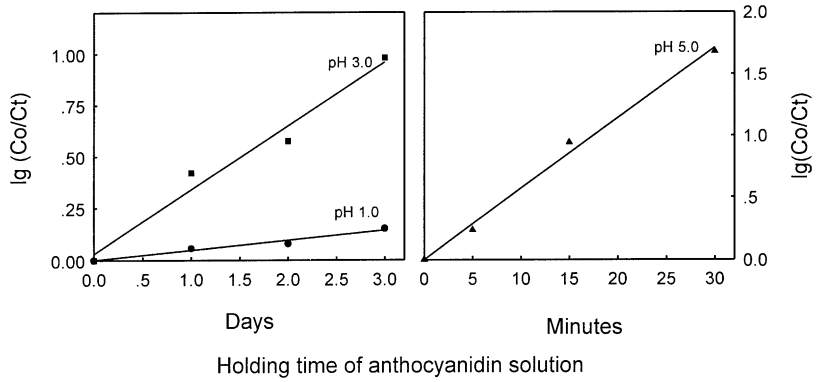

Fig. 5. The kinetics of the anthocyanidin degradation of litchi pericarp extract at pH values of 1.0, 3.0 and 5.0.

the physiological pH value in the plant vacuole is about 3.0 with red anthocyanins(Brouillard, Figueiredo, Elhabiri, & Dangles, 1997). Underhill and Critchley (1994) reported that the pH values of the litchi pericarp increased from 4.1 to 4.7 with fruit desiccation. However, compared to the half-degradation constant (10.5 days, Fig. 3) for anthocyanins at pH 5.0 in vitro, the anthocyanin degradation in litchi fruit was more rapid, and the fruit lost more than 50% of their anthocyanin content when they were stored for 3 days at 30°C and 70% RH. Thus, the spontaneous degradation of anthocyanins could not account for the rapid browning of the litchi pericarp and suggested that the enzymatic degradation of the anthocyanins may play a key role in the browning.

3.3. Degradation of anthocyanidin at various pH values

Anthocyanidin had similar absorbance spectra to anthocyanins (Fig. 4). Compared with the anthocyanins, the degradation of the anthocyanidin was more pronounced, especially at pH 5.0, and the half-degradation constants at pHs 1.0, 3.0 and 5.0 were, respectively, 6.4 days, 7.5 h and 5.3 min (Figs. 3 and 5). Richardson and Finley (1985) reported that anthocyanidins can exist as colourless pseudobases at pH 3.0 or higher, and then irreversibly split to form phenolic benzoic acids. Actually, the anthocyanidin spontaneously breaks down once it is produced at pH 5.0. Because the major anthocyanin is cyanidin-3-rutinside, the opening of cyanidin would produce an *o*-phenol, a similar structure to catechol (a good substrate for PPO), which, in turn, could accelerate the enzymatic browning reaction by PPO(Jiang, 2000).

3.4. Anthocyaninase in litchi pericarp

There was a high activity of anthocyanase (2.19×10^3 Units/g FW) in litchi pericarp. So far, the anthocyanase has been found only in fungi. For example, certain *Aspergillus niger* strains exhibited high anthocyanase activity and are used to reduce the redness of juice and wine (Blom, 1983, Huang, 1955; Yang & Steel, 1958). In addition, mold-contaminated fruit, with the enzyme, is also used to degrade anthocyanins in strawberry wine (Pilando, Wrolstad, & Heatherbell, 1985), strawberry juice (Rwabahizi & Wrolstad, 1988) and red raspberry juice (Cassignard, Ducasse, Barrere, & Artigau, 1977). Thus, anthocyanin degradation could be attributed to the action of the enzyme.

Recently, Jiang (2000) suggested that litchi anthocyanins can be rapidly degraded in an anthocyanin-PPO-phenol reaction. Similar results were reported in blueberry, strawberry, plum and grape fruits(Kader, Rovel, Girardin, & Metche, 1997; Raynal & Moutounet, 1989; Sarni, Fulcrand, Souquet, & Cheynier, 1995; Wesche-Ebeling & Montgomery, 1990). Since there was a high activity of anthocyanase in litchi pericarp, it was suggested that the litchi browning may involve: 1, co-oxidation of phenols and anthocyanins by PPO with the formation of *o*-quinones, and 2, the hydrolysis of anthocyanins by anthocyanase, leading to the formation of anthocyanidin, which, in turn, accelerates the enzymatic degradation of the anthocyanins.

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