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Purification and structural analysis of anthocyanins from litchi pericarp

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Abstract

Litchi fruit peel anthocyanins were extracted with 0.5 M HCl, and then purified by an Amberlite XAD-7 column and Sephadex LH-20 column chromatography. The major part of the anthocyanins of litchi pericarp, which (94.3% of total), was obtained. Furthermore, the partially purified anthocyanin was identified as cyanindin-3-rutinoside, with a molecular weight of 595, using HPLC equipped with mass spectrometry. The results corroborate the previous observation of Zhang, Pang, Ji, and Jiang (2001 *Food Chemistry*, *75*, 217–221), who suggested that nonenzymatic degradation of anthocyanins could participate in postharvest browning of litchi pericarp.

Keywords: Anthocyanin; HPLC-MS; Litchi; Peel; Structure

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a tropical and subtropical fruit of high commercial value because of its white and translucent aril and attractive red colour. However, the fruit rapidly lose their bright colour and turn brown once harvested (Holcroft & Mitcham, 1996; Jaiswal, Sah, & Prasad, 1986; Nip, 1988). Postharvest browning of litchi pericarp may be attributed to a degradation of the red pigment (Akamine, 1960; Huang & Wang, 1990), and the red pigments were identified as anthocyanins (Lee & Wicker, 1991; Prasad & Jha, 1978). Furthermore, the content of anthocyanins decreased quickly as the fruit browned, with a significant negative relationship (Zhang, Pang, Ji, & Jiang, 2001). However, Jiang (2000) reported that litchi polyphenol oxidase (PPO) did not oxidise the anthocyanins. Zhang et al. (2001) suggested that the presence of the sugar moiety of anthocyanins may cause steric hindrance of PPO accessibility to the anthocyanins and, thus, the hydrolysis of the anthocyanins, leading to the formation of anthocyanidin, accelerated the enzymatic degradation of the anthocyanins. Further understanding of the anthocyanin molecular structure could therefore aid in explaining the mechanism of browning of litchi pericarp.

Prasad and Jha (1978) first reported that red colour of litchi fruit pericarp was probably due to a mixture of cyanidin and pelargonidin, using a thin-layer chromatograph (TLC) analysis. The major anthocyanin of the red pigments was identified as cyanidin-3-rutinoside by TLC and high-performance liquid chromatography (HPLC) (Lee & Wicker, 1991). Sarni-Manchado, Roux, Guerneve, Lozano, and Cheynier (2000) further identified the anthocyanins as cyanidin-3-rutinoside, cyanidin glucoside, quercetin-3-rutinoside, and quercetin glucoside, using low-pressure chromatography, HPLC, UV-nisible spectral analysis, mass spectrometry (MS), and nuclear magnetic resonance. However, different sugar groups may exist in these cyanindin-containing anthocyanins (Zhang, Quantick, & Grigor, 2000). Recent research showed that the electrospray mass spectrum was a powerful tool for identification of molecular weight and groups of the chemicals (Giusti, Rodriguez-Saona, Griffin, & Wrolstad, 1999). Thus, the litchi

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anthocyanin structure can be characterized by mass spectrometry in combination with HPLC.

The objective of this study was to extract and purify litchi pericarp anthocyanins, and then analyze the molecular structure of the major anthocyanins by high-performance liquid chromatography-mass spectrometry (HPLC-MS) in relation to the degradation involved in the anthocyanin degradation.

2. Materials and methods

2.1. Materials

Mature red fruit of litchi (*Litchi chinensis* Sonn.) cv. Huaizhi, a major cultivar with bright red colour skin, were obtained from a commercial orchard in Guangzhou, China. Fruit were peeled and the pericarp was stored at −20 °C before extraction and purification of anthocyanins.

2.2. Extraction of anthocyanins

Freozen litchi pericarp was extracted overnight at 25 °C in an extraction medium consisting of 0.5 M HCl (in water) by the method of Zhang et al. (2001). Extract was filtered through Whatman No. 1 paper, and the filter residue was re-extracted until the absorbance at 510 nm of the extract was $\leqslant 0.01$ using a spectrophotometer (Shimadu 300). Filtrates were combined and concentrated to a small volume at 40 °C by a rotary evaporator for further purification of anthocyanins.

2.3. Anthocyanin purification

According to the procedures described by Baublis, Spomer, and Berber-Jimenez (1994). The filtrates were loaded onto an Amberlite XAD-7 resin column (1.5×40 cm) (Sigma), and then washed with distilled water, followed by an elution with 0.1% HCl in methanol. The fractions with the highest absorbance at 510 nm were pooled and concentrated to a small volume to remove methanol at 40 °C by a rotary evaporator (Heidolph, Germany). The partially purified concentrated anthocyanins were then loaded onto a Sephadex LH-20 column (1.0×60 cm) and eluted with 1% formic acid at 24 ml/h. Formic acid has been shown to give a good separation of the red pigments (Lee & Wicker, 1991; Jiang, 2000). Four fractions, based on the absorbance values ($\geqslant 0.1$) at 510 nm were collected using a fraction collector (2 ml/tube). The fraction with the highest absorbance value was concentrated by a rotary evaporator at 40 °C. Anthocyanin content of these fractions was determined by the method of Zhang et al. (2001) and expressed as absorbance values at 510 nm using a Shimadu 300 UV spectrophotometer.

2.4. Anthocyanin identification and molecular weight estimation by HPLC-MS

A 20 μl solution of the major fraction of the purified litchi anthocyanins was injected directly into the HPLC-MS system (LCQ^{DECA}, Finigan Company, USA). The system was equipped with a Hewlett-Packard 1040A photodiode array detector and Hewlett-Packard 9000 computer system which detected at 280 nm. HPLC analysis was carried out on a PolyLC ODS C-18 column (5×150 mm), with a mobile phase of acetonitrile: 5% acetic acid (70:30, v/v) and a flow rate of 1 ml/min, while mass spectroscopy was recorded with a heat capillary voltage of 5 kV, a heat capillary temperature of 275 °C, sheath gas flow rate of 80 units, and auxiliary gas flow rate of 20 units. The scan range of molecular weight was 50–2000 D.

3. Results and discussion

3.1. Anthocyanin purification

The Amberlite XAD-7 resin column exhibited a high affinity for litchi anthocyanins. Salts, carbohydrates and other soluble compounds can be well separated from the anthocyanins. One major fraction of the anthocyanins, based on the highest absorbance at 510 nm, was achieved by the column (Fig. 1). Further purification by a Sephadex LH-20 column, based on the various molecular sizes of the anthocyanins, showed that there were four fractions, whose relative absorbances at 510 nm were 1.67%, 94.3, 1.2 and 2.87%, respectively (Fig. 2). The results also suggested that the procedure used in this study was validated for anthocyanin purification. Lee and Wicker (1991) reported that the major anthocyanin in litchi pericarp comprised 67.7% of the total quantity by Sephadex LH-20 chromatography, but it appeared earlier in elution. Discrepancies in elution time might be attributed to various extract solutions or various cultivars.

In the study, the major anthocyanin obtained from the Sephadex LH-20 column was chosen for further

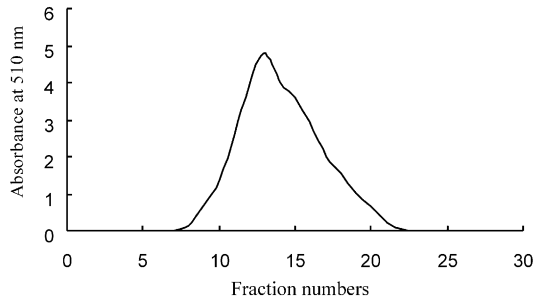

Fig. 1. Elution profiles of litchi fruit pericarp anthocyanins partially purified by Amberlite XAD-7.

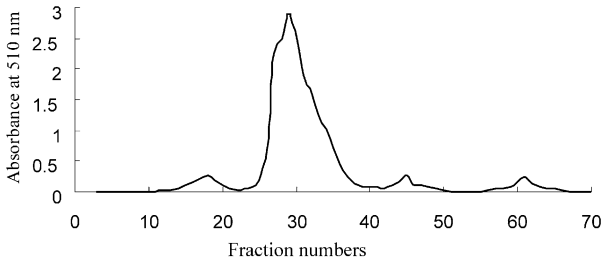

Fig. 2. Elution profiles of the major anthocyanin fraction separated by Amberlite XAD-7, followed by a further purification on Sephadex LH-20.

analysis by HPLC-MS. The major fraction showed a big peak with a high purity (Fig. 3). Underhill and Critchley (1993) reported that cyanidin-3-rutinoside was the major anthocyanin present in fresh skin of litchi fruit (97% of total anthocyanin). However, other anthocyanins (e.g. cyaniding-3-glucoside, cyaniding-3-galactoside, pelargonidin-3,7-diglucoside, quercetin-3-rutinoside, quercetin glucoside) may vary with various cultivars (Sarni-Manchado et al., 2000). In comparison to the anthocyanins present in various litchi cultivars (Jaisweal et al., 1986; Lee & Wicker, 1991; Prasad & Jha, 1978; Sarni-Manchado et al., 2000; Underhill & Critchley, 1994; Zhang et al., 2000), the other three fractions of 'Huaizhi' fruit preicarp in this study could be very low amounts of cyaniding-3-glucoside, cyaniding-3,5-diglucoside and malvidin-3-glucoside, but they need to be further studied. Generally, HPLC and TLC have been used to determine the purity and variety of anthocyanins (Robards & Antolovich, 1997), while ion-exchange resin column or gel-filtrate chromatography

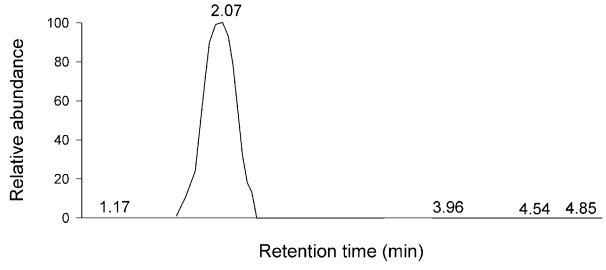

Fig. 3. Total ion chromatogram of the major anthocyanin fraction by HPLC-MS.

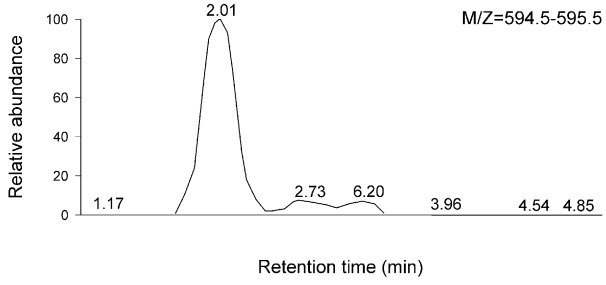

Fig. 4. Molecular weight estimation of the major anthocyanin fraction by HPLC-MS.

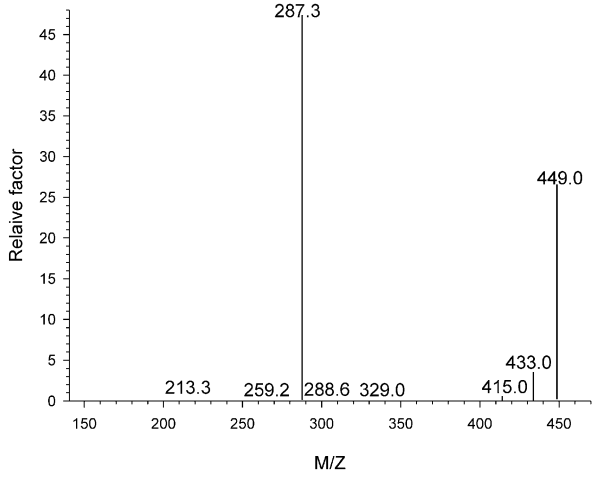

Fig. 5. Electrospray mass spectrum of the major anthocyanin fraction.

were effective for preparing the anthocyanins on a large scale (Anderse-Oyvind, Wiksund & Pederse, 1995; Choung et al., 2001; Matsumoto, Hanamura, Kawakami, Sato, & Hirayama, 2001).

3.2. Molecular weight and structure of the major anthocyanin

The mass chromatography profile of $m/z = 594.5$–595.5 showed that the molecular weight of the major anthocyanin in litchi pericarp was 595 (Fig. 4), which could be cyanidin-3-rutinoside, as supposed by Lee and Wicker (1991). Two components from the $m/z = 595$ constituent were further confirmed by electrospray mass spectrometry (Fig. 5). The component with the molecular weight of ion peak 449 coincided with cyanidin-3-glucoside ($C_{21}H_{21}O_{11}$), while ion peak 287 coincided with cyanidin. This conclusion corroborates that of Zhang et al. (2001), who suggested, based upon experiments to investigate anthocyanin degradation, that nonenzymatic degradation of anthocyanins could participate in postharvest browning of litchi pericarp. The pathway of cyanidin-3-rutinoside (595) degradation was proposed to be as follows.

cyanindin-3-rutinoside (595) → cyanindin-3-glucoside (449) → cyanindin (287)

↓ ↓

rhammosyl (146) glucosyl (162)

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