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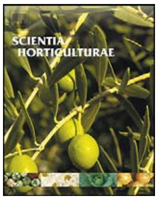

Short communication

Variations in contents of browning substrates and activities of some related enzymes during litchi fruit development

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ABSTRACT

Pericarp browning is a major factor to limit storage and transportation of postharvest litchi fruit. Experiments were conducted firstly to determine variations in contents of browning substrate (−)-epicatechin in pericarp tissues of litchi fruit during development. Changes in the activities of polyphenoloxidase (PPO), peroxidase (POD) and phenylalanine ammonialyase (PAL) in association with the synthesis or oxidation of (−)-epicatechin were also examined. (−)-Epicatechin content tended to decrease during fruit development. PAL activity had a high level at the initial stage of fruit development, then decreased rapidly, and finally increased slightly, which was in agreement with the variation in (−)-epicatechin content. The higher contents of total phenolics and (−)-epicatechin of fruit of 'Nuomici' than 'Feizixiao' were also related to the higher PAL activity. PPO had a similar change to PAL in activity, but POD activity slowly decreased at the early fruit development and then increased markedly. It is suggested that (−)-epicatechin content might be largely regulated by PAL activity. This study could help to understand the variations in the content of the browning substrate and the activities of some substrate-related enzymes of litchi fruit during development and then control better pericarp browning of harvested litchi fruit by the application of appropriate preharvest treatments.

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a subtropical fruit with a high commercial value. The fruit has a white, translucent, sweet and juicy aril, but it turns easily brown once harvested (Zheng and Tian, 2006). Thus, pericarp browning of harvested litchi fruit is a major factor to affect fruit quality and storage life (Zhang et al., 2001). The pericarp browning of harvested litchi fruit has been attributed to the oxidation of endogenous phenolics caused by polyphenoloxidase (PPO) and/or peroxidase (POD) (Jiang et al., 2004). Phenylalanine ammonialyase (PAL, EC 4.3.1.5) catalyzes the first step in the biosynthesis of phenylpropanoid-derived secondary products such as phenolic compounds (Boudet, 2007) while the major browning substrates present in pericarp tissues of litchi fruit belong to phenolic compounds (Sun et al., 2006). Therefore, the level of browning substrate may be regulated by a dynamic equilibrium of PPO, POD and PAL activities.

Litchi has a sigmoidal growth curve (Holcroft and Mitcham, 1996). Initially, the seed and pericarp develop simultaneously, followed by aril growth (Wang et al., 2006). During litchi fruit development, various phenolic compounds especially a number of anthocyanins involving PPO, POD and PAL activities were reported (Rivera-López et al., 1999; Hu et al., 2000; Wang et al., 2005). In our previous study (Sun et al., 2006), the major PPO substrate for enzymatic browning from pericarp tissues of litchi fruit was firstly identified as (−)-epicatechin, and the oxidation of (−)-epicatechin could account for pericarp browning of litchi fruit. It is suggested that understanding of the variation in the level of browning substrate involved in the activities of some substrate-related enzymes during litchi fruit development could be helpful to control the pericarp browning of harvested litchi fruit by applying appropriate preharvest treatments. Unfortunately, little information of the endogenous browning substrates present in litchi pericarp has been available during fruit development. The objective of this present study was to examine the variations in the levels of the major browning substrate and activities of PPO, POD and PAL during litchi fruit development. This study can provide an important basis to prevent pericarp browning of harvested litchi fruit by the inhibition of the formation of browning substrates.

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2. Materials and methods

2.1. Plant materials

Two common litchi cultivars ('Nuomici' and 'Feizixiao') in China were used in this study. Litchi fruits at various stages of development were collected from Guangdong Academy of Agricultural Sciences every 10 days from April 30 (20 and 30 days after full bloom for 'Nuomici' and 'Feizixiao', respectively) to June 10 (61 and 71 days after full bloom for 'Nuomici' and 'Feizixiao', respectively) in 2007. The uniform fruits (50 fruits per stage) from a 12-year-old tree were selected and then peeled. The fruit pericarp tissues were collected and then used for extraction and analysis.

2.2. Measurements of contents of total phenolics and PPO substrates

Pericarp tissues (3 g) of litchi fruit from various developmental stages were ground into powder in liquid nitrogen, and then extracted for 20 min at 4 °C with 15 mL of methanol containing 0.2% (w/v) ascorbic acid. The extract was centrifuged for 10 min at 7500 × g, and then the supernatant was collected and used to determine contents of total phenolics and the major PPO substrates.

Total phenolic content was measured by the method of Price and Butler (1977). A 50-μL aliquot of the supernatant was diluted with 6 mL of distilled water. Then, 0.1 mL of 50 mM $FeCl_3$ (prepared in 0.1 M HCl solution) and 0.1 mL of 8 mM $K_3Fe(CN)_6$ were added and allowed to stand for 20 min at 25 °C. Absorbance was recorded at 720 nm using a UV-2802 spectrophotometer (Unic Inc., Shanghai, China). Total phenolic content was calculated against gallic acid and then expressed as micrograms of gallic acid equivalents (GAE) on fresh weight (FW) basis.

The contents of the major PPO substrates for enzymatic browning were analyzed using a Waters 2695 high-performance liquid chromatograph (Waters Co., Milford, MA, USA) coupled with a Waters 2487 dual wavelength absorbance detector (Waters Co.). The above-mentioned extract was filtered through a 0.45 μm membrane filter (Millex-HV, Millipore Co., Billerica, MA, USA) before injection. The separation was performed on a 250 mm × 4.6 mm (i.d.) and 5 μm Symmetry Shield RP18 column (Waters Co.) maintained at 30 °C. The mobile phase was water (A), acetonitrile (B) and acetic acid (C), with a flow rate of 1 mL/min. The elution was allowed to run for 4 min with 97% A, 1% B and 2% C, and then solvent A decreased from 97 to 57.8% while solvent B increased from 1 to 40.2% within 24 min. Solvent A further decreased from 57.8 to 18% while solvent B increased from 40.2 to 80% within 2 min. Finally, the elution system returned to 97% A, 1% B and 2% C within 2 min. The injection volume was 10 μL. The monitoring wavelength of 280 nm was used. The identification of the major PPO substrates was based on a combination of retention time and spectral matching with standard (−)-epicatechin (Sigma–Aldrich Canada Chemical Ltd., Oakville, ON, Canada). The contents of the major PPO substrates were calculated by a calibration against the standard compound using the external standard method. Results were expressed as mg/100 g pericarp on fresh weight (FW) basis. Three determinations were carried out.

2.3. Extraction and assays of PPO and POD

PPO and POD were extracted according to the modified methods of Jiang (2000) and Dong et al. (2004). All steps were performed at 4 °C. Litchi pericarp tissues (2 g) were ground in liquid nitrogen with a mortar and pestle, and then extracted for 20 min with 10 mL of 0.1 M sodium phosphate buffer (pH 6.8) containing 0.1% (w/v) polyvinyl pyrrolidone (PVP) and 0.1% (v/v) Triton X-100. The extract solution was centrifuged for 15 min at 10,000 × g, and the supernatant was collected for the determinations of PPO and POD activities. PPO activity was assayed with catechol as a substrate according to the spectrophotometric procedure (Jiang et al., 1997). The enzyme solution (0.2 mL) was rapidly added to 2.8 mL of 10 mM catechol prepared in 0.01 M sodium phosphate buffer (pH 6.8). The increase in absorbance at 400 nm was recorded for 3 min at 25 °C using the UV-2802 spectrophotometer. One unit of enzymatic activity was defined as the amount of the enzyme that causes a change of 0.001 in absorbance per minute. POD activity was spectrophotometrically measured by the procedure of Macadam et al. (1992). The assay mixture consisted of 2.7 mL of 0.1 M sodium phosphate buffer (pH 6.8), 0.1 mL of 0.46% (v/v) H_2O_2, 0.1 mL of 4% (w/v) guaiacol and 0.1 mL of enzyme solution in a final volume of 3 mL. The increase in absorbance at 470 nm was recorded for 3 min at 25 °C. One unit of enzymatic activity was defined as the amount of the enzyme that causes a change of 0.01 in absorbance per minute.

2.4. Extraction and assay of PAL

PAL was extracted according to the methods of Lister et al. (1996). All steps were performed at 4 °C. Litchi pericarp tissues (1 g) were ground in liquid nitrogen with a mortar and pestle, and then extracted for 20 min with 5 mL of 0.05 M sodium borate

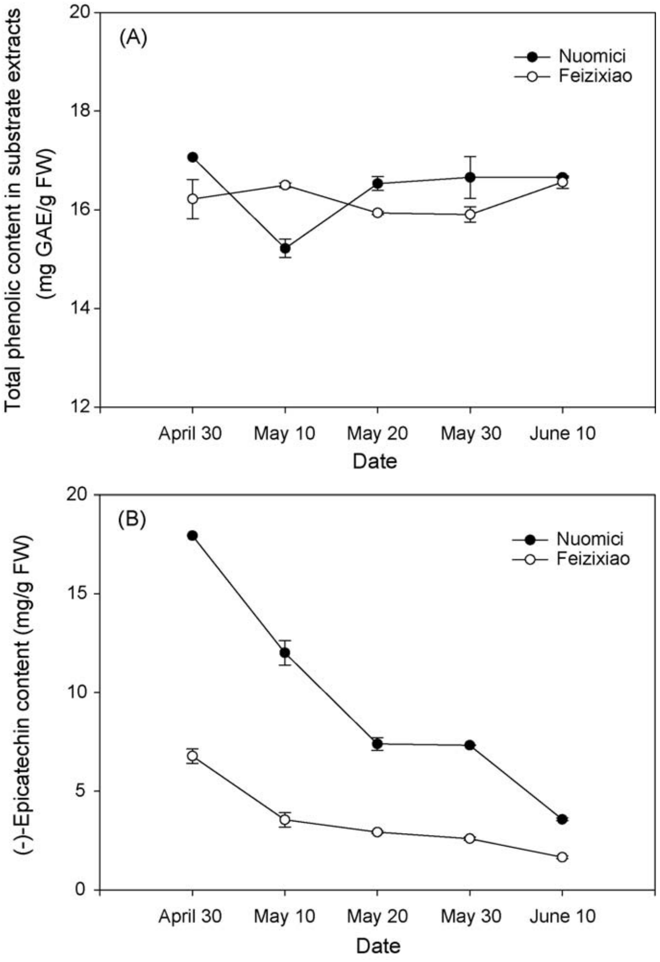

Fig. 1. Contents of total phenolics (A) and (−)-epicatechin (B) in the phenolic extracts from pericarp tissues of 'Nuomici' and 'Feizixiao' fruits at different developmentat stages. April 30, May 10, 20 and 30, and June 10 corresponded to 20, 30, 40, 50 and 61 days for 'Nuomici' fruit and 30, 40, 50, 61, and 71 days after full bloom for 'Feizixiao'fruit, respectively.