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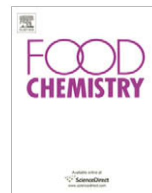

Role of endogenous and exogenous phenolics in litchi anthocyanin degradation caused by polyphenol oxidase

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ABSTRACT

The degradation of anthocyanins and/or the oxidation of phenolics caused by polyphenol oxidase (PPO) results in an enzymatic browning reaction of fruits and vegetables. This work was conducted with a view to explaining the unexpected observation that litchi (*Litchi chinensis* Sonn.) PPO did not directly oxidise litchi anthocyanins. PPO and anthocyanin from litchi fruit pericarp were extracted and purified, respectively, and then the anthocyanin degradation by PPO in the presence of (−)-epicatechin (endogenous PPO substrate), and catechol and gallic acid (exogenous PPO substrates) were analysed comparatively. The results showed that catechol was the most effective in litchi anthocyanin degradation, followed by (−)-epicatechin and gallic acid, but no significant differences existed between catechol and (−)-epicatechin. The study suggested that litchi PPO directly oxidised (−)-epicatechin; then oxidative products of (−)-epicatechin in turn catalysed litchi anthocyanin degradation, and finally resulted in the browning reaction, which can account for pericarp browning of postharvest litchi fruit.

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1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a subtropical to tropical tree in the Sapindaceae family. The fruit has high commercial value due to its white translucent aril and attractive red skin colour (Jiang, Duan, Joyce, Zhang, & Li, 2004). The red colour of litchi fruit peel is mainly attributed to anthocyanins (Jiang et al., 2006; Prasad & Jha, 1978). Cyanidin was identified as the major anthocyanin present in pericarp tissues of fresh litchi fruit, followed by delphinidin and pelargonidin (Jiang et al., 2004; Oomah & Mazza, 1999), and malvidin (Lee & Wicker, 1991). Pericarp browning is a common and important defect of harvested litchi fruit, which results in reduced market value (Huang & Scott, 1985). Postharvest browning of litchi fruit has mainly been attributed to the degradation of anthocyanins and/or the oxidation of phenolics by polyphenol oxidase (PPO) (Jiang et al., 2004). Thus, postharvest technologies have been developed to reduce the anthocyanin degradation and then maintain red colour of harvested litchi fruit (Jiang et al., 2006). Some studies indicate that PPO plays an important role in the anthocyanin degradation in postharvest fruits and vegetables (Cheynier, Osse, & Rigaud, 1988; Cheynier, Souquet, Kontek, & Moutounet, 1994). Recently Sun et al. (2006), have identified (−)-epicatechin as an endogenous PPO substrate present in pericarp tissues of litchi fruit. Unfortunately, PPO did not directly oxidise litchi anthocyanins but an obvious degradation of litchi anthocyanins was observed in the presence of catechol (Jiang, 2000). It is suggested that the litchi anthocyanin degradation may involve a co-oxidation of *o*-quinones formed enzymatically but this needs to be investigated further. In the present study, PPO and anthocyanins were extracted and purified from pericarp tissues of harvested litchi fruit, respectively. The anthocyanin degradation extent, caused by PPO in the presence of (−)-epicatechin (endogenous PPO substrate), and catechol and gallic acid (exogenous PPO substrates), were analysed comparatively. Elucidation of the role of endogenous PPO substrate in the anthocyanin degradation in pericarp tissues of litchi fruit caused by PPO can help to better understand the pericarp browning and then improve postharvest handling of litchi fruit during storage and transport.

2. Materials and methods

2.1. Plant materials

Fresh fruits of litchi (*L. chinensis* Sonn.) cv. Huaizhi, at a commercially mature stage (a fully red colour), were obtained from an orchard in Guangzhou, China. The fruits were selected and then the uniform size and colour fruits were peeled. The pericarp tissues were collected, then frozen and finally stored at −20 °C.

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2.2. PPO extraction and purification

PPO extraction and purification were carried out at 4 °C by the method of Jiang (1999), with some modification. Litchi pericarp tissues (7.5 g) were homogenised with 50 ml of 0.1 M sodium phosphate buffer (pH 6.8) containing 1% (w/v) Polyclar AT (insoluble polyvinylpyrrolidone) and 1% (v/v) Triton X-100. The homogenate was filtered through cheesecloth and then centrifuged for 20 min at 8500*g*. The supernatant was collected and then fractionated with solid ammonium sulphate at 50–80% saturation. The precipitated phase was collected after centrifugation at 10,000*g* for 10 min, then redissolved in a small volume of 0.1 M sodium phosphate buffer (pH 6.8), and finally dialysed overnight in a cellulose dialysis tubing (Yuanju Co., Shanghai, China). The dialysed solution was loaded onto a Sephadex G-100 (Superfine, Sweden) column (1.5×50 cm) by eluting with 0.1 M sodium phosphate buffer (pH 6.8) containing 10% glycerol, and then the elute was collected at 1.5 ml per tube, using a fraction collector. The fraction with the highest PPO activity was collected and then used to examine the effects of (−)-epicatechin, catechol and gallic acid on litchi anthocyanin degradation by PPO. The enzymatic activity of each fraction, using 50 mM catechol as a substrate, was determined by measuring the change in absorbance at 420 nm for 2 min, using a spectrophotometer (Unic 2305, Shanghai, China), by the method of Jiang, Zauberman, and Fuchs (1997). One unit of PPO activity was defined as the amount of enzyme which caused an increase in absorbance of 0.001 per minute at 25 °C. The protein content was determined according to the method of Bradford (1976) with bovine albumin.

2.3. Extraction and purification of litchi anthocyanins

Anthocyanin extraction was conducted according to the method of Ranganna (1997). Litchi pericarp tissues (50 g) were homogenised and then extracted with 500 ml of acidic ethanol (1.5 M HCl in 95% ethanol, 15:85, v/v) at 4 °C overnight. The extract was filtered through Whatman #1 paper and then concentrated by a rotary evaporator under vacuum at 40 °C. The concentrated extract was purified with an Amberite XAD-7 resin (Sigma, St. Louis, MO, USA) column (2.0×50 cm) by the method of Zhang, Pang, Yang, Ji, and Jiang (2004). The eluate was collected at 1.5 ml per tube, using a fraction collector. The anthocyanin content of each fraction was measured at 530 and 657 nm by the method of Padmavati, Sakhivel, Thara, and Reddy (1997) and then expressed as μmol/ml from the expression, $[(A530 - 0.33 \times A657)/31.6] \times$ [volume (ml)], where *A* indicated the absorbance and the extinction coefficient of 31.6 was used to convert the absorbance value into anthocyanin concentration. The fraction with the highest anthocyanin content was collected and then used to examine the effects of (−)-epicatechin, catechol and gallic acid on litchi anthocyanin degradation by PPO.

2.4. Effects of (−)-epicatechin, catechol and gallic acid on litchi anthocyanin degradation by PPO

Two exogenous PPO substrates, catechol (Peng & Markakis, 1963), and gallic acid (Prabha & Patwardhan, 1986), and an endogenous litchi PPO substrate (−)-epicatechin (Sun et al., 2006) were used to examine their effects on litchi anthocyanin degradation caused by PPO in this study. The reaction mixture consisted of 0.1 ml of 0.2 mM litchi anthocyanin solution and 1.8 ml of 10 mM catechol, gallic acid or (−)-epicatechin solution. They were mixed immediately prior to the addition of 0.1 ml of PPO solution (1 unit/ml). The absorbances of the mixture solutions after 1, 3 and 5 min of incubation at 25 °C were automatically scanned at 350–700 nm. The relative anthocyanin degradation rate, expressed as a percentage, was calculated from the following expression: $(A/A_{max}) \times 100$, where *A* indicated the absorbance.

2.5. Statistical analysis

Data were analysed using the SAS Version 6.12 (SAS Institute Inc., Cary, NC) according to Duncan's multiple range test. Differences between the means at the 5% level were considered to be significant.

3. Results and discussion

3.1. Extraction and purification of litchi PPO

In the study, the addition of insoluble polyvinylpyrrolidone was used to bind phenolics, to prevent the phenol–protein interaction (Ziyan & Pekyardimci, 2004) whilst Triton X-100 was used to increase the extraction effectiveness (Galeazzi & Sgarbieri, 1981). Fang, Zhang, Sun, and Sun (2007) reported that application of polyvinylpyrrolidone, combined with Triton X-100, slightly increased PPO activity. The purification of PPO from pericarp tissues of litchi fruit is summarised in Table 1. The profile of PPO activity of these fractions eluted from Sephadex G-100 was well associated with the absorbance value at 280 nm (Fig. 1), which was in agreement with the report of Jiang et al. (1997). Thus, the obtained PPO in this study can be used to examine the effects of (−)-epicatechin, catechol or gallic acid on the litchi anthocyanin degradation by PPO.

3.2. Extraction and purification of litchi anthocyanins

Amberlite XAD-7 column chromatography or Sephadex LH-20 column chromatography are commonly used for anthocyanin purification. Zhang et al. (2004) reported that litchi anthocyanins can be easily purified using an Amberlite XAD-7, and a major anthocyanin (about 94% of a total quantity) from pericarp tissues was obtained. In this study, one major fraction, with the highest absorbance at 510 nm, was also obtained (Fig. 2). The fraction had an anthocyanin concentration of 5.6 μmol/ml.

3.3. Effects of endogenous and exogenous phenolics on the anthocyanin degradation by PPO

The litchi PPO showed no affinity for anthocyanins from pericarp tissues of litchi fruit (Jiang, 2000), probably due to the presence of a sugar moiety, causing a steric hindrance effect (Jiang et al., 2004; Kader, Haluk, Nicolas, & Metche, 1998; Sarni, Fulcrand,

Table 1
PPO purification from pericarp tissues of litchi fruit.

Purification step	Volume (ml)	Activity[a] (unit/ml)	Total activity[a] (unit)	Protein (mg/ml)	Total protein (mg)	Specific activity[a] (units/mg)	Recovery (%)	Purification
Crude extract	50	58	2900	0.70	32.08	82.7	100	1
$(NH_4)_2SO_4$	24	94	2256	0.61	14.61	154	77.8	1.87
Sephadex G-100	10	103	1025	0.167	1.67	615	35.3	7.44

[a] PPO activity was measured using 50 mM catechol as a substrate.

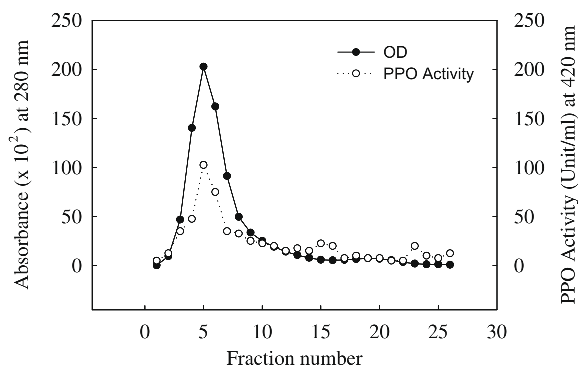

Fig. 1. Elution profile of absorbance value (OD) at 280 nm and PPO activity by Sephadex G-100.

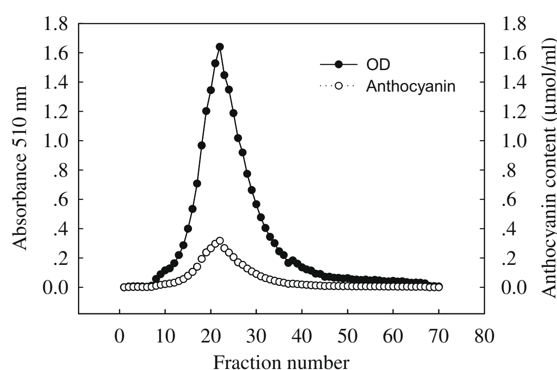

Fig. 2. Elution profile of absorbance value (OD) at 510 nm and anthocyanin content purified by Amberlite XAD-7.

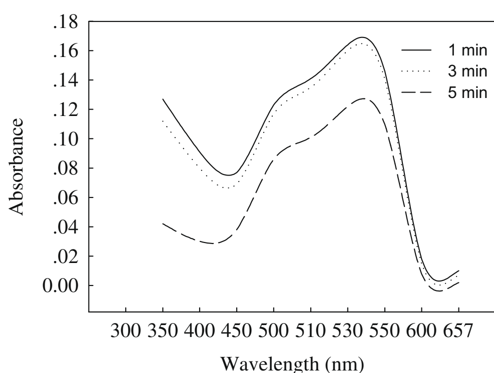

Fig. 3. Profile of absorbance at 350–700 nm of litchi anthocyanin incubated with litchi PPO for 1, 3 or 5 min.

Souquet, & Cheynier, 1995). In this study, the degradation rate of litchi anthocyanin incubated with litchi PPO solution for 1, 3 or 5 min slightly increased, based on the absorbance values at 350−700 nm (Fig. 3) but the litchi anthocyanin degradation caused by PPO was markedly enhanced in the presence of (−)-epicatechin, catechol or gallic acid (Fig. 4), which indicated a decreased anthocyanin content. Furthermore, the highest degradation rate of the litchi anthocyanin caused by PPO was observed in the presence of catechol, followed by (−)-epicatechin and gallic acid (Table 2), but no significant differences in the anthocyanin degradation between catechol and (−)-epicatechin were obtained. The study indicated that *o*-diphenols, such as catechol and (−)-epicatechin, exhibited a better effect on litchi anthocyanin degradation, by PPO, than did tri-hydroxy phenolics, such as gallic acid. Thus, it is proposed that litchi PPO directly oxidised (−)-epicatechin; then oxidative products of (−)-epicatechin in turn catalysed litchi anthocyanin degradation, and finally resulted in the browning reaction, which can account for pericarp browning of litchi fruit during storage. Further identification of oxidative products of (−)-epicatechin is needed to better elucidate the browning reaction of litchi anthocyanins.

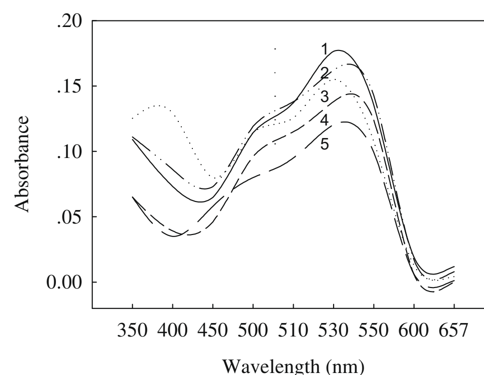

Fig. 4. Profile of absorbance at 350–700 nm of litchi anthocyanin after 3 min of incubation. 1, litchi anthocyanin; 2, litchi anthocyanin + litchi PPO; 3, litchi anthocyanin + gallic acid + litchi PPO; 4, litchi anthocyanin + (−)-epicatechin + litchi PPO; 5, litchi anthocyanin + catechol + litchi PPO.

Table 2
Effects of various PPO substrates on litchi anthocyanin degradation by PPO after 1 and 3 min of enzymatic reaction.

Substrate	Relative anthocyanin degradation rate (%)	
	1 min	3 min
(−)-Epicatechin	97.5 ± 0.150[a]	99.2 ± 0.334[a]
Gallic acid	94.2 ± 0.314[b]	97.0 ± 0.757[b]
Catechol	97.2 ± 0.163[a]	1000 ± 0.000[a]
F-test	0.0001	0.0048

The means ± standard errors ($n = 3$) within a column followed by different letters were significantly different at the 5% level.

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