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Food Chemistry 88 (2004) 443–446
Food Chemistry
www.elsevier.com/locate/foodchem

Advances in understanding of enzymatic browning in harvested litchi fruit

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Abstract

Litchi (*Litchi chinensis* Sonn.) is a subtropical to tropical fruit of high commercial value in international trade. However, harvested litchi fruit rapidly lose their bright red skin colour. Peel browning of harvested litchi fruit has largely been attributed to rapid degradation of red anthocyanin pigments. This process is associated with enzymatic oxidation of phenolics by polyphenol oxidase (PPO) and/or peroxidase (POD). PPO and POD from litchi pericarp cannot directly oxidize anthocyanins. Moreover, PPO substrates in the pericarp are not well characterised. Consequently, the roles of PPO and POD in litchi browning require further investigation. Recently, an anthocyanase catalysing the hydrolysis of sugar moieties from anthocyanin to anthocyanidin has been identified in litchi peel for the first time. Thus, litchi enzymatic browning may involve an anthocyanase–anthocyanin–phenolic–PPO reaction. Current research focus is on characterising the properties of the anthocyanase involved in anthocyanin degradation. Associated emphasis is on maintenance of membrane functions in relation to loss of compartmentation between litchi peel oxidase enzymes and their substrates.

Keywords: Anthocyanase; Anthocyanin; Browning; Litchi; *Litchi chinensis*; Lychee; Oxidase; Peroxidase; Phenolic; Polyphenol oxidase

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a subtropical to tropical fruit of high commercial value in international trade. The fruit typically has a bright red peel colour and is sweet, acidic, juicy and soft but with crisp pulp (Nakasone & Paull, 1998). Harvested litchi fruit are highly perishable. They can rapidly lose their bright red skin colour and turn brown within 1–2 days at ambient temperatures (Huang & Scott, 1985; Jiang & Fu, 1998a; Zhang & Quantick, 1997). Post-harvest browning of litchi fruit has been attributed mainly to degradation of red pigments in association with oxidation of phenolics by polyphenol oxidase (PPO) and/or peroxidase (POD) enzymes (Huang, Hart, Lee, & Wicker, 1990; Zauberman, Ronen, Akerman, Weksler, Rot, & Fuchs, 1991; Zhang & Quantick, 1997). Li and Yan (1963) first discerned the relationship between PPO activity and litchi peel browning. Significant progress in purification and characterisation of PPO and its substrates in litchi pericarp tissue has since been made. Nonetheless, enzymatic browning is still the major practical limitation to litchi fruit storage (Jiang, Yao, Lichter, & Li, 2003). This paper reviews enzymatic browning of litchi fruit after harvest, with an emphasis on recent advances.

2. Enzymes

2.1. Polyphenol oxidase

Litchi pericarp tissue browning is mainly due to the oxidation of phenolics and degradation of red pigments by polyphenol oxidase. This oxidase is also referred to as catechol oxidase, tyrosinase, catecholase or *o*-diphenol oxygen oxidoreductase. PPO has been isolated and

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0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.02.004

purified from litchi fruit peel. Its pH and temperature optima are 6.5 and 70 °C, respectively (Jiang, Zauberman, & Fuchs, 1997b; Jiang, Zauberman, Fuchs, & Fu, 1999). The enzyme can be inhibited by antioxidants, such as glutathione and L-cysteine, and activated by divalent cations, such as Mn^{2+} and Ca^{2+} (Jiang & Fu, 1998b; Jiang, Zauberman, Fuchs, & Fu, 1998). However, PPO activity during litchi fruit storage is evidently inconsistent. Lin et al. (1988a) demonstrated a rapid increase in PPO activity during the first 48 h of storage. However, Zauberman et al. (1991) found no significant change in the PPO activity during the same period. In contrast, Underhill and Critchley (1994) reported a progressive reduction in PPO activity. Apparently contradictory findings for PPO activity may be due to differences in methods and/or cultivars, and this merits investigation.

2.2. Peroxidase

The relative significance of PPO activity is further obscured by the presence of POD, a similar oxidative enzyme, in litchi pericarp. Lin et al. (1988b), Chen and Wang (1989) and Underhill and Critchley (1995) have recorded an increase in POD activity during litchi pericarp browning. Gong and Tian (2002) have recently partially purified POD from litchi fruit peel. They found that the enzyme can rapidly oxidize 4-methylcatechol in the presence of H_2O_2 and, thereby, form brown polymeric pigments. This finding further supports the case for involvement of POD in enzymatic browning of litchi fruit.

The success of commercial sulphite treatment in controlling litchi pericarp browning is an evidence for the hypothesis that the browning is due to some types of oxidative enzymes (Zauberman et al., 1991; Jiang et al., 1997a). Involvement of both, PPO and POD, is consistent with the author's research results which showed that inhibition of activities of the PPO and POD delayed litchi pericarp browning. (Jiang & Fu, 1999c, 1999d; Jiang & Li, 2003). Moreover Underhill and Critchley (1995) demonstrated that there was a correlation between POD activity and cellular browning, such that there was higher POD activity in the browned pericarp.

2.3. Anthocyanase

An involvement of PPO in litchi pericarp browning has become generally accepted. However, PPO cannot directly oxidize anthocyanins. The oxidative product 4-methylcatechol, yielded by PPO, can accelerate anthocyanin degradation (Jiang, 2000). Furthermore, PPO can oxidize products of the anthocyanin degradation, resulting in the formation of brown-coloured substances (Jiang, unpublished data). Recently, Zhang, Pang, Ji, and Jiang (2001) identified an anthocyanase catalysing the hydrolysis of sugar moieties from anthocyanin to yield anthocyanidin. It was suggested that anthocyanase may contribute to litchi pericarp browning by rendering major phenolic constituents (anthocyanins) accessible to POD or PPO. Properties of the anthocyanase involved in anthocyanin degradation require detailed characterisation.

3. Pigments and browning substrates

Compared with the literature on PPO and POD enzymes, there are very few publications relating to the role of anthocyanins in litchi pericarp browning. Prasad and Jha (1978) and Rivera-Lopez, Ordorica-Falomir, and Wesche-Ebeling (1999) identified anthocyanins as the red pigments present in litchi pericarp. Lee and Wicker (1991) subsequently reported that litchi pericarp contains seven types of anthocyanins (cyanidin-3-rutinoside, cyanidin-3-glucoside, cyanidin-3-galactoside, malvidin-3-acetylglucoside, pelargonidin-3-glycoside, and quercetin-3-rutinosde). Zhang, Grigor, and Quantick (2000) and Sarni-Manchado, Le Roux, Le Guerneve, Lozano, and Cheynier (2000) identified (by HPLC) the anthocyanins as cyanidin-3-rutinoside, cyanidin-3-glucoside, quercetin-3-rutinoside and quercetin-3-glucoside. Recently, Zhang, Pang, Yang, Ji, and Jiang (2004), using HPLC-MS, showed that the major anthocyanin is the cyanidin-3-rutinoside. Thus, anthocyanins, together with various phenolic compounds, were progressively degraded or oxidized in association with formation of polymeric brown pigments. Although anthocyanin degradation has been observed (Prasad & Jha, 1978), anthocyanins may be decolourised, to some degree, prior to the degradation as a consequence of increased vacuolar pH, which results in an increase in the rate of visual browning (Zhang et al., 2001).

As implied above, PPO has very low affinity for litchi peel anthocyanins (Jiang, 2000). Thus, there may be non-anthocyanin substrates for the enzyme. Potential substrates for PPO were extracted from litchi pericarp with methanol–acetone–water, separated by DEAE–cellulose column chromatography and indentified primarily as phenolics similar in structure to catechol-based compounds (Tan & Zhou, 1987). This characterization was by analysis of the infrared absorption and UV absorption spectrums. However, the exact structure of the substrate was not determined, due to current technology limitations. Further identification of non-anthocyanin PPO substrates in litchi skin tissue is needed in conjunction with improved analytical technology.

4. Peroxidative activity and membrane lipids

Oxidative enzymes and their substrates are in different subcellular compartments in red intact litchi fruit

pericarp (Liu, Jiang, Chen, Zhang, & Li, 1991). Accordingly, compartmentation limits mixing that results in enzymatic browning (Liu et al., 1991). Peroxide content and malondialdehyde (i.e. a product from peroxidated membrane lipids) concentrations increase in aging litchi fruit. Conversely, superoxide dismutase activity, associated with the anti-oxidant capacity of litchi pericarp tissue, decreased with increasing storage time at ambient temperature (Jiang & Chen, 1995a; Jiang & Fu, 1998b; Lin et al., 1988b). Membrane permeability, assessed as electrolyte leakage, and the ratio of saturated: unsaturated fatty acids, increased (Jiang & Chen, 1995a, 1995b). Conversely, membrane fluidity, as determined by the fluorescent probe 1,6-diphenylhexatriene (DPH), decreased with increasing storage period (Jiang & Chen, 1995b). Collectively, these changes indicate a decreased ability of harvested litchi fruit to eliminate active oxygen. Thus, membranes become more affected by oxidative activity. Consequently, loss of compartmentation between enzymes and substrates leads to enzymatic browning.

5. Concluding remarks

Impetus for research on litchi fruit deterioration in China and elsewhere has come in conjunction with increased production and demand around the world. The major producer, China, seeks to identify domestic and international markets for this unique and popular fruit. Post-harvest browning of litchi fruit skin is the main limitation to market acceptance. The biochemistry of enzymatic browning has not yet been fully elaborated (Jiang et al., 2003; Peng, 1998). However, it is proposed that anthocyanins may first be hydrolysed by anthocyanase, forming an anthocyanidin. In turn, this compound may be oxidized by PPO and/or POD. Oxidative products of phenolics, such as 4-methylcatechol, resulting from PPO activity, then accelerate anthocyanidin degradation, resulting in enzymatic browning (Fig. 1). With an increasing research effort on litchi, our understanding of the enzymatic browning mechanism in the fruit pericarp is likely to be much more complete in the near future.

For red intact litchi fruit pericarp, compartmentation of enzymes and substrates in different organelles limits enzymatic browning. However, litchi fruit pericarp cells rapidly senesce after harvest in association with the enhanced lipid peroxidation, reduced membrane fluidity and increased membrane permeability (Jiang & Chen, 1995a, 1995b; Lin et al., 1988b). Deterioration in membrane function may result in loss of compartmentation between enzymes and their substrates and, thereby, may aid enzymatic browning (Fig. 1).

cDNA sequences for PPO from avocado and mango have recently been reported (Kahn, 1977; Robinson, Loveys, & Chacko, 1993). Studies along these lines are in progress in litchi (Wang, Personal communication). The potential for genetic manipulation, using anti-sense or cosuppression of PPO RNA, should be explored to prevent litchi browning. However, in view of consumer concerns, products of such technology are not likely to be practical in the short-term. In the meantime, researchers supporting the litchi industry require a better understanding of enzymatic browning. Based on improved understanding, reliable technological approaches may be developed to control the browning of the fruit during storage, transport and marketing.

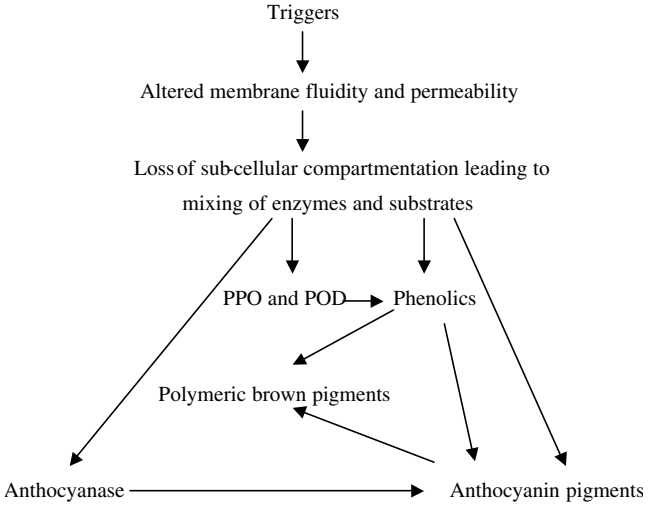

Fig. 1. A proposed scheme for enzymatic browning in the pericarp of harvested litchi fruit.

Acknowledgements

This work was supported by the International Foundation for Science (Grant No. E2265/3F) and the National Science Foundation of China (Grant No. 39900102).

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