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Effect of citric acid and chitosan on maintaining red colouration of litchi fruit pericarp

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Abstract

A postharvest treatment based on citric acid and chitosan was tested on two litchi cultivars of different provenance. Its effects on the activities of polyphenol oxidase (PPO), peroxidase (POD) and anthocyanase and on the anthocyanin content of the pericarp were measured as factors responsible for pericarp browning. The red colour of the pericarp was measured during storage of the fruit. The major anthocyanins present in both cultivars were cyanidin-3-rutinoside and cyanidin-3-glucoside. Although the concentration of cyanidin-3-rutinoside was 64% lower in the cultivar Kwai may (Guiwei) than in Wai chee (Huaizhi), this component represented more than 90% of total anthocyanins in both cultivars. The activity of PPO was six times greater in Kwai may than in Wai chee and the activity of POD was 30-times greater. The activity of POD was greater than that of PPO in both cultivars. The two cultivars, which differ in anthocyanin and oxidative enzyme compositions, responded differently to the acid and chitosan treatment, with the result that the red colour of Kwai may was better preserved during storage than that of Wai chee. This technique could be a future replacement for current sulphur treatments used to treat litchis transported by sea.

Keywords: *Litchi chinensis* Sonn.; Anthocyanins; Polyphenol oxidase (PPO); Peroxidase (POD); Browning

1. Introduction

Of Asian origin, the litchi (*Litchi chinensis* Sonn.) is prone to a number of postharvest problems, among which the most important is browning of the pericarp. This phenomenon can occur in less than 72 h after harvest (Chapman, 1983; Nip, 1988; Holcroft and Mitcham, 1996).

The red colouration of litchis is due to the presence of anthocyanin pigments (Prasad and Jha, 1978; Lee and Wicker, 1991). Cyanidin-3-rutinoside and cyanidin-3-glucoside have been identified as the major anthocyanins present in litchi pericarp (Lee and Wicker, 1991; Le Roux, 1999; Riviera-Lopez et al., 1999; Zhang et al., 2004). The intensity of the colouration of the anthocyanins depends on pH, which influences their structure. The flavylium form is stable at acid pH and is coloured red, but is transformed at basic pH either to its carabinol base (colourless), to chalcone (yellow) or to its quinonic base (blue) (cited by Perret, 2001).

A number of authors have linked browning of the pericarp to the degradation of anthocyanins by the action of polyphenol oxidase (PPO) (Akamine, 1960; Huang et al., 1990; Jiang, 2000) or the action of peroxidase (POD) (Finger et al., 1997; Zhang et al., 2005). Differences in browning between cultivars may be linked to differences in enzyme activity. For example, Chen et al. (2001) reported that PPO activity in Nuomici, a cultivar that browns easily, was higher than in Guiwei, a cultivar that browns more slowly. Enzymes such as PPO and POD are for the most part membrane bound, whereas anthocyanins are vacuolar (cited by Le Roux, 1999). Contact between enzyme and substrate cannot therefore take place unless there is disruption at the cellular level. Thus browning can occur following different types of stress caused by the climatic conditions preceding fruit maturation, diseases, desiccation and thermal shock (Underhill et al., 1997).

The principal postharvest treatment currently used for litchi is fumigation with SO_2. SO_2 is a strong antioxidant that blocks the oxidation reactions that cause browning of the pericarp.

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Furthermore, it acidifies the cellular contents of the pericarp thus stabilising the anthocyanins and acts as an antifungal agent (Zauberman et al., 1991). Nevertheless, the treatment has limitations. The fruit are initially bleached by the treatment and do not completely recover their initial red colouration subsequently (Underhill et al., 1992), and more importantly, sulphur residues can reach the aril. SO_2 represents a health risk for people allergic to sulphur, and in the US it is banned for all food products except grapes for which it can be used as a fungicide (Paull et al., 1995; Holcroft and Mitcham, 1996).

Several alternative treatments to SO_2 fumigation have been proposed, for example, soaking in a solution of N_6-benzyladenine (Jiang and Fu, 1998), the combined use of glutathione and citric acid (Jiang and Fu, 1998) and hot water brushing (Lichter et al., 2000), but until now no method has been established commercially. It has been shown that the use of semi-permeable coatings increases the storage life of perishable crops (Lowings and Cutts, 1982). Chitosan, a high molecular weight polysaccharide, is soluble in organic acids and can be used to conserve fruits. Zhang and Quantick (1997) showed that coating litchis with 1–2 g/100 mL chitosan (in acidic solution) slowed pericarp browning. This treatment limited losses of anthocyanins, flavenoids and total phenolic compounds from the pericarp and, furthermore, retarded the increase in polyphenol oxidase activity and partially inhibited that of peroxidase.

The objective of this work was to study the mode of action of dipping in a solution of citric acid and chitosan to maintain the red colour of litchi pericarp during storage for two different cultivars with different pericarp composition through its effects on the enzymes implicated in browning.

2. Materials and methods

2.1. Plant material and chitosan/acid dip treatment

Two litchi (*L. chinensis* Sonn.) culitvars were used: red Kwai may (Guiwei) from Reunion Island and Wai chee (Huaizhi) from Spain. For both cultivars, the fruit were harvested at maturity and arrived in the laboratory within 72 h (litchis from Réunion were brought by a CIRAD researcher by plane directly to the lab, whilst Spanish litchis were harvested directly and transported to Montpellier by car). The sample lots (20 kg of each variety) were selected in the orchard for uniformity of size and colour, and were free of signs of fungal infection and insect attack. The fruit of both cultivars were harvested from experimental areas and were not chemically treated. They were cooled immediately after harvest to 10 °C and transported in cool boxes.

The dip treatment was to immerse the fruit in a solution of citric acid and chitosan. This was prepared by dissolving citric acid (600 g/L) in boiling water. As the acid dissolved, medium molecular weight (MW 400,000) chitosan was added to a final concentration of 0.75 g/100 mL, after which the solution was allowed to cool to room temperature. The concentration of chitosan was chosen following preliminary experiments with concentrations of 0.25–2 g/100 mL that showed the optimum concentration for maintaining red colouration was 0.75%. Litchis were then dipped in the solution (at room temperature 20 °C), drained, dried with a hair dryer for no more than 30 min and then stored at 4 °C and 90% RH. Samples were evaluated after 21 days storage, this being the average transport time for litchis arriving in Europe from Madagascar by sea. Control fruit were packed in the same way as the treated fruit, but without dipping in the citric acid and chitosan solution, and then also stored at 4 °C and 90% RH.

Five 500 mL polypropylene punnets, each containing 15 fruit, were prepared of both treated and control fruit. At the end of the storage period, the 15 fruit from each punnet were shelled and the pericarps and the arils combined and then frozen at −20 °C until further analysis. Separate punnets of control and treated fruit were prepared and stored in the same way for testing by the taste panel.

2.2. Anthocyanin extraction

Ground frozen pericarp (2 g) was stirred in 20 mL of extraction solution for 20 min with a magnetic stirrer. The extraction medium was 2.5 mL/100 mL HCl in methanol (Le Roux, 1999). After centrifugation, the supernatant was filtered through a sintered glass funnel (porosity no. 1). The sediment was re-extracted in 20 mL of extraction solution using a magnetic stirrer for 5 min and the supernatant again filtered using the sintered glass funnel. The combined filtered extract was then dried under vacuum at 30 °C for 30 min in a rotary vacuum evaporator RE 100 (Bibby Sterilin Ltd., UK). The dry extract was solubilised in 2 mL methanol and filtered through a hydrophilised PTFE membrane (Filtre Millex, 25 mm diameter, 0.45 μm porosity, Millipore, France), hermetically sealed in a tube and stored at −20 °C. The extracts were diluted 20-fold in extraction solution prior to HPLC analysis.

2.3. High pressure liquid chromatography

The extracts were analysed with a SpectraSystem P1000XR chromatograph (Thermo Separation Products, US) equipped with a 20-μL injection loop, an automatic gradient controller and a column oven controlled by PC1000 software. Anthocyanins were separated on a Lichrospher 100RP18E 250 mm × 4.6 mm, 5 μm column (Merk Eurolab S.A., France). The column oven temperature was 30 °C. The mobile phase was a mixture of solution A (98 mL water and 2 mL formic acid) and solution B (18 mL water, 2 mL formic acid and 80 mL acetonitrile), used with the following gradient (A/100 mL:B/100 mL): at 0 min (97:3), at 4 min (97:3), at 52 min (65:35), at 57 min (20:80), at 61 min (20:80) at 62 min (97:3) and at 70 min (97:3). The flow rate was 1 mL/min. The eluted compounds were detected by measuring absorbance at wavelengths of 450–600 nm. Quantification was achieved by using appropriate standards.

2.4. Measurement of PPO and POD

Frozen pericarp (10 g), previously ground in a laboratory blender (Waring Commercial, US), was further homogenised in 50 mL of 0.05 M phosphate buffer (pH 6.8) with an Ultra Turrax blender (T 25 Basic, Ika Labortechnik, Germany). Polyvinyl