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# Enzymatic browning and antioxidant activities in harvested litchi fruit as influenced by apple polyphenols

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ABSTRACT

'Guiwei' litchi fruit were treated with 5 g a.i. $L^{-1}$ apple polyphenols (APP) and then stored at 25 °C to investigate the effects on pericarp browning. APP treatment effectively reduced pericarp browning and retarded the loss of red colour. APP-treated fruit exhibited higher levels of anthocyanins and cyanidin-3-rutinoside, which correlated with suppressed anthocyanase activity. APP treatment also maintained membrane integrity and reduced oxidative damage, as indicated by a lower relative leakage rate, malondialdehyde content, and reactive oxygen species (ROS) generation. The data suggest that decompartmentalisation of peroxidase and polyphenoloxidase and respective browning substrates was reduced. In addition, APP treatment enhanced the activities of antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase), as well as non-enzymatic antioxidant capacity (DPPH radical-scavenging activity and reducing power), which might be beneficial in scavenging ROS. We propose that APP treatment is a promising safe strategy for controlling postharvest browning of litchi fruit.



## 1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a popular tropical fruit due to its bright red pericarp, translucent white aril, exotic flavour and particular nutritional qualities (Holcroft & Mitcham, 1996). The fruit after harvest, however, is highly perishable and susceptible to pericarp browning, resulting in compromised quality and reduced commercial value (Jiang, Duan, Joyce, Zhang, & Li, 2004). Previous studies have shown that pericarp browning of litchi fruit is related to the oxidation of phenolic compounds by peroxidase (POD) and polyphenol oxidase (PPO), membrane lipid peroxidation, degradation of anthocyanins, and redox imbalance resulting from overproduction of reactive oxygen species (ROS) and decreased antioxidant capacity (Jiang et al., 2004; Neog & Saikia, 2010). Therefore, inhibiting these physiological processes could be important for controlling browning and extending the shelf life of harvested litchi fruit. Traditional postharvest techniques including sulphur dioxide ($SO_2$) fumigation and acid treatment can effectively retain the red colour of litchi fruit pericarp through blocking oxidative reactions (Ramma, 2003). However, alternative strategies for colour retention have been sought due to public concerns for food safety and increasing restriction in use of chemicals (Jiang, Yao, Lichter, & Li, 2003).

Plant polyphenols have received much attention over the past decades for their diverse roles in human health issues (Pandey & Rizvi, 2009). Polyphenols display strong antioxidant activity and various beneficial physiological functions *in vivo* and *in vitro* (Pandey & Rizvi, 2009). Apples are one of the most commonly consumed sources of phenols with a phenol content ranging from 50 to 380 mg per 100 g fresh weight depending on cultivar (Ceymann, Arrigoni, Schärer, Nising, & Hurrell, 2012). Apple polyphenols (APP) are composed of flavonols, anthocyanins, dihydrochalcones, as well as other phenolic acids (Bao et al., 2013). APP intake by means of fresh apple consumption or oral administration of APP may exert immunomodulatory and antiinflammatory effects in humans and animals. For example, APP reduced cardiovascular disease risks by decreasing oxidised low-density lipoprotein (Zhao, Bomser, Joseph, & DiSilvestro, 2013), ameliorated dextran sulphate sodium-induced colitis (Skyberg et al., 2011), prevented damage to gastric epithelial cells (Graziani et al., 2005), attenuated food allergy (Zuercher, Holvoet, Weiss, & Mercenier, 2010), and provided protection against cigarette smoke-induced acute lung injury (Bao et al., 2013). In addition to

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drug function, APP have also been incorporated into chewing gum for removing ozostomia, used as a fish-odour removing agent in aquatic products-processing industries, and employed as a clarificant in the beverage industry (Zhao, 2010).

In spite of the strong antioxidant properties of APP, there is surprisingly little information regarding the efficacy of APP as a postharvest anti-browning/senescence treatment. The objective of the present study was to investigate the effects of APP on the postharvest pericarp browning of litchi fruit. Included were analyses of browning severity and marketability, prooxidant and antioxidant systems, and membrane effects. The results provide a new strategy for maintaining postharvest quality and extending the storage life of harvested litchi fruit.

## 2. Materials and methods

### 2.1. Plant material and treatments

Litchi (*L. chinensis* Sonn.) fruit cv. Guiwei were harvested at commercial maturity from an orchard in Gaozhou city, Guangdong province of China. Fruit were packed in polyethylene bags and transported to postharvest laboratories within 3 h. Fruit of uniform shape and colour without visible blemish and disease were selected for the experiments. Fruit were disinfected with 0.1% Sportak fungicide solution for 3 min, rinsed with tap water, air-dried for 2 h and then divided randomly into 2 treatment groups, with 20 kg for each treatment. The two groups of fruit were immersed in distiled water (control) and 5 g a.i $L^{-1}$ (w/v) aqueous solution of APP [food grade, 80% purity; comprised of procyanidins (≈40%), chlorogenic acid (≈15%), phlorizin and phloretin (≈5%), anthocyanins (≈5%), *p*-coumaric acid (≈5%), hydrochalcone (≈5%), and [−]-epicatechin, [+]-catechin and gallic acid (≈5%); Changyue Phytochemistry Co., Ltd., Xi'an, China] at 25 ± 1 °C for 3 min. This APP concentration was determined to be the optimum based on preliminary experiments using 0, 2.5, 5 and 10 g a.i. $L^{-1}$ APP. After air-drying, fruit were packed into polyethylene bags (200 × 150 mm, 0.03 mm thickness, and 15 fruit per bag) with small holes and stored at 25 °C and 85–90% relative humidity. Browning index, colour and membrane permeability were evaluated at 0, 1, 3, 5, 7 and 9 days during storage. For the assay of enzymes and metabolites, the pericarp tissue samples at the same time interval as above were collected and frozen in liquid nitrogen and stored at −80 °C until analysed. Each treatment replicate contained 15 fruit at each sampling time, with 3 replicates for each treatment.

### 2.2. Assessment of pericarp browning and marketable fruit

Litchi pericarp browning was visually assessed by evaluating the extent of the browned area on each fruit surface using the following scale: 0 = no browning; 1 = slight browning (<5%); 2 = <1/4 browning; 3 = 1/4–1/2 browning; 4 = >1/2 browning. The browning index was calculated as $\Sigma$ (browning scale × number of corresponding fruit within each class)/(4 × total number of fruit). Each treatment contained three replicates, and 45 fruit were included in each replicate.

Marketable fruit (%) was determined by calculating the proportion of fruit without browning (browning scale = 0) relative to total fruit number. Each treatment had three replicates, and 45 fruit were included in each replicate.

### 2.3. Pericarp colour measurement

Pericarp colour was measured using a chroma meter (Minolta CR 400, Konica Minolta Sensing, Inc., Osaka, Japan), which provided the $L^*$, $a^*$ and $b^*$ values according to the CIE system. $L^*$ values represent the lightness. Positive $a^*$ values indicate red and negative values indicate a green colour. Positive $b^*$ values indicate yellow and negative $b^*$ values indicate blue colour. Two measurements were conducted at 2 equidistant points on the equatorial axis. Three replicates for each treatment were performed, and each replicate contained 15 fruit.

### 2.4. Measurement of total anthocyanins

Total anthocyanins content in pericarp was measured by a pH-differential method as described in Zhang, Pang, Ji, and Jiang (2001). Briefly, 3 g of thawed pericarp tissues were bleached with 45 ml of 0.1 M HCl in a shaking water bath at 25 °C for 24 h. Aliquots of 0.5 ml extract were diluted by adding 2.5 ml of 0.4 M KCl–HCl buffer solution (pH 1.0) and 2.5 ml of 0.4 M citric acid–$Na_2HPO_4$ buffer solution (pH 5.0), respectively. The absorbance of each mixture was measured at 510 nm, and the difference between absorbance of mixture under pH 1.0 and 5.0 was calculated. One unit (U) of anthocyanins content is defined as a change in absorbance of 0.1, and the data are expressed U $g^{-1}$ fresh weight (FW).

### 2.5. Measurement of cyanidin-3-rutinoside

Cyanidin-3-rutinoside content was determined by high performance liquid chromatography (HPLC). One gram of thawed pericarp was extracted with 5 ml of 1% HCl–methanol in a shaking water bath at 25 °C for 24 h. Two millilitres of extract were passed through a 0.45 μm-Millipore membrane and 20 μl aliquots were injected into the HPLC (SPD-20A series, Shimadzu, Kyoto, Japan) equipped with an ultraviolet (UV) detector using a Shim-pack-VP-ODS RP C18 (4.6 × 250 mm, 5 μm) column. A gradient HPLC system was eluted with solvent A (0.1% trifluoroacetic acid in ultrapure water) and B (100% acetonitrile). The gradient procedure initiated with solvent B from 5% to 40% at 20 min, 100% at 25 min, from 100% to 5% at 35 min and 10% B at 45 min, with a flow rate of 1.0 ml $min^{-1}$. Monitoring was carried out by a UV detector at 520 nm. Cyanidin-3-rutinoside was indentified and quantified by comparison with retention time and peak area from an authentic compound (HPLC purity). Cyanidin-3-rutinoside content is expressed as mg $kg^{-1}$ FW.

### 2.6. Measurement of membrane permeability

Membrane permeability was expressed by relative leakage rate. Pericarp discs were removed from the equatorial region with a cork borer (10 mm in diameter). Fifteen discs derived from 15 fruits, with 3 replicates for each treatment, were washed twice and then incubated in 20 ml of distiled water at 25 °C for 30 min. Initial electrolyte content of the bathing solution was measured using a conductivity meter (Model DDS-11A, Shanghai Scientific Instruments, Shanghai, China). After the 30 min incubation period, the solution with discs was placed in a boiling water bath for 15 min, quickly cooled, and then the total electrolyte leakage of the solution was again measured. Leakage rate is expressed as a percentage (%) of the electrolyte values obtained after the 30 min incubation period relative to the total electrolyte content after boiling.

### 2.7. Malondialdehyde (MDA) determination

MDA content was measured using the method of Duan et al. (2011) with minor modifications. Frozen pericarp tissues were ground using a FW-100 grinder (Yongguangming Medical Equipment Co., Ltd., Beijing, China). The grinder chamber was pre-chilled with liquid nitrogen prior to processing of each tissue. Three grams of powders were homogenised in 15 ml of 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 8000*g* for 10 min at 4 °C. One ml