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Postharvest L-cysteine application delayed pericarp browning, suppressed lipid peroxidation and maintained antioxidative activities of litchi fruit

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ABSTRACT

Efficacy of L-cysteine as an anti-browning agent was investigated on 'Gola' litchi fruit. Fruit were treated with its different concentrations (0.0, 0.25, 0.50, 0.75 and 1.0%) and stored at 5 ± 1 °C with 90 ± 5% relative humidity (RH) for 28 d. Among the used concentrations, 0.25% treatment was most effective. L-cysteine (0.25%) treated-fruit showed significantly reduced weight loss, disease incidence, disease severity, browning index, membrane leakage and malondialdehyde (MDA) contents. Application of L-cysteine (0.25%) to litchi fruit maintained acidic pericarp pH, exhibited higher total anthocyanins, 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) radical-scavenging-activity and total phenolic contents (TPC) along with reduced activities of peroxidase (POD) and polyphenol oxidase (PPO) enzymes. L-cysteine (0.25%) treatment also maintained substantially higher soluble solid contents (SSC), titratable acidity (TA), ascorbic acid contents and activities of catalase (CAT) and superoxide dismutase (SOD) enzymes. In conclusion, pre-storage L-cysteine (0.25%) application to litchi fruit exhibited reduced pericarp browning index and maintained antioxidative system for 28 d.

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a well-known subtropical sapindaceae fruit. Being non-climacteric, it is generally harvested at the fully ripe stage. Its harvest maturity is usually judged by development of pink or reddish colour on surface of fruit (Kumar et al., 2012; Barman et al., 2014). However, once harvested, the bright reddish pericarp colour quickly turns brown within 1–2 d at room temperature. The development of undesirable brown colour technically known as pericarp browning severely decreases its market potential (Holcroft and Mitcham, 1996). Pericarp browning of litchi fruit is primarily attributed to the desiccation of pericarp tissues and anthocyanin degradation accompanied by phenolic oxidation in the presence of some oxidative enzymes such as peroxidase and polyphenol oxidase (Zauberman et al., 1991; Jiang et al., 2004).

A large number of approaches such as sulphur dioxide (SO_2) fumigation (Ray et al., 2005); hydrochloric acid dips (Jiang et al., 1997); heat treatments (Olesen et al., 2004); pre-cooling (Pornchaloempong et al., 1997); edible coatings (Sun et al., 2010); cold storage (Khan et al., 2012); modified atmosphere packaging (Somboonkaew and Terry, 2010); controlled atmosphere storage (Sivakumar and Korsten, 2010; Ali et al., 2016); and irradiation (Kumar et al., 2012) have been used to manage enzymatic browning, and shelf-life extension of the litchi fruit. In general, SO_2 fumigation is used commercially to preclude pericarp browning of litchi fruit. Nevertheless, due to undesirable chemical residues, SO_2 fumigation is now in question because it usually alters the taste of fruit and possesses some serious health threats for consumers and the packinghouse workers (Kremer-Kohne, 1993). Moreover, when litchi fruit is treated with SO_2 fumigation, these may absorb around 30–65% of the applied sulphur contents, which is considered well beyond the permitted SO_2 residual limit (10 ppm) in various countries such as Australia, Europe, and Japan (Kumar et al., 2012). Hence, some alternative anti-browning agent is needed for the reduction in litchi pericarp browning and overall quality management which could be considered safe for packinghouse workers and consumers all over the world.

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L-cysteine being an anti-browning agent is known as very effective inhibitor of PPO and POD enzymes (Eidhin et al., 2005; Sharma and Rao, 2013). During the oxidation of phenolic compounds, cysteine usually traps o-quinones by developing the colourless products technically known as 'cysteinyl-adducts' which has been found the competitive inhibitor of PPO enzyme (Richard-Forget et al., 1992). Numerous studies have reported L-cysteine as one of the most effective anti-browning agent on different fresh cut fruits and vegetables such as avocados (Dorantes-Alvarez et al., 1998); pear (Gorny et al., 2002); loquat (Ding et al., 2002); mango (Guerrero-Beltran et al., 2005); cherimoya (Campos-Vargas et al., 2008); apple (Rojas-Grau et al., 2006); banana (Bico et al., 2009); artichokes (Amodio et al., 2011; Cabezas-Serrano et al., 2013); carambola (Sharma and Rao, 2013); eggplant (Ghidelli et al., 2014); and peach (Colantuono et al., 2015) either alone or in combination with different organic acids/edible coatings. Similarly, combine application of 2 m*M* L-cysteine and 2 m*M* citric acid reduced browning index, inhibited PPO enzyme activity and reduced the loss of SSC and TA of litchi fruit stored at 25 °C conditions (Jiang et al., 2008). However, as per our information, no work has been reported regarding the use of different concentrations of L-cysteine for reduction of postharvest pericarp browning and quality maintenance of litchi fruit under extended cold stored conditions. Moreover, L-cysteine has been recognized as GRAS (Generally Recognized as Safe) (Food and Drug Administration, 2013); hence, its use in litchi industry of the world would be considered safe without any potential health menaces. Hence, present research was conducted to investigate the effects of postharvest application of L-cysteine on pericarp browning, membrane permeability, lipid peroxidation, changes in phenolic contents, antioxidant capacity, antioxidant enzymes activities and biochemical characteristics of clod stored 'Gola' litchi fruit.

2. Materials and methods

2.1. Fruit source

Fruits of litchi cv. 'Gola' were harvested at commercial maturity (colour = 85–100% red) from Government Fruit Farm Nursery Haripur (34°00.114′N, 72°56.779′E), Pakistan. After harvest, fruits free from pre-harvest cracks, injury and bruise were packed in plastic crates, pre-cooled in forced air refrigerated reefer van (10 ± 1 °C) and transported to Postharvest Research and Training Center (PRTC), University of Agriculture Faisalabad, Pakistan.

2.2. Treatments, storage conditions and studied parameters

Fruits were randomly divided into five groups and dipped in the aqueous solutions of different concentrations of L-cysteine (0, 0.25, 0.50, 0.75 and 1.0%) with 0.01% Tween-20 as a surfactant for 5 min. After treatment application, samples were segregated into groups of 25 fruit per replication; air dried and stored at 5 ± 1 °C with 90 ± 5% RH conditions for 28 d. Fruit were removed from the cold storage and manually peeled. Pulp of each fruit (total 25 fruit per replication) was divided into two equal parts. Juice was extracted from one part and used for determination of SSC, TA and ascorbic acid; whereas, remaining part of pulp was treated with liquid nitrogen and stored at −80 °C for further analysis of TPC, DPPH radical-scavenging-activity and enzymes. On the other hand, peel was taken from all 25 fruit and a composite sample was prepared; while, remaining procedure was same as for pulp TPC, DPPH radical-scavenging-activity and enzymes. Sampling was done at 0-, 7-, 14-, 21- and 28 d after cold storage. Weight loss, disease incidence, disease severity and pericarp browning index were assessed from whole fruit. SSC, TA, SSC: TA ratio and ascorbic acid contents were determined only from the pulp tissues. TPC, DPPH radical-scavenging-activity, activities of POD, CAT, and SOD enzymes were determined from both peel and pulp tissues. On the other hand, PPO enzyme activities, pericarp pH, MDA contents, membrane leakage, and total anthocyanins were determined only from the peel tissues. Experiment was conducted under completely randomized design with two-factor factorial arrangement (chemical concentrations and storage periods). Each treatment was replicated thrice with 25 fruit as a single replication.

2.3. Fruit weight loss, disease incidence and disease severity

Fruit were weighed on the digital weight balance (ELB-1200, Shimadzu, Kyoto Inc., Japan) and loss of weight was calculated with the following equation and expressed in terms of percentage.

$$\text{Fruit weight loss}\,(\%) = \frac{\text{Initial fruit weight} - \text{Final fruit weight}}{\text{Initial fruit weight}} \times 100$$

Disease incidence was assessed at each sampling period. Fruit showing symptoms of fungal and bacterial growth were considered as diseased. Finally, disease incidence was calculated with the following equation and expressed as percent.

$$\text{Disease incidenece}\,(\%) = \frac{\text{Number of diseased fruits}}{\text{Total number of fruits}} \times 100$$

Disease severity was recorded following the scale as described by Sivakumar et al. (2002) and expressed as score.

2.4. Pericarp pH, pericarp browning and total anthocyanins

Pericarp pH of litchi fruit was determined with digital pH meter (Model HI-98107, Hanna Instruments, Mauritius) as reported previously by Joas et al. (2005). Pericarp browning was assessed by evaluating the extent of browned portion on surface of the fruit at each removal as reported by Zhang and Quantick (1997) and expressed as score. For total anthocyanins, one g litchi pericarp was finely ground and extracted in 15 mL freshly prepared solution of HCl with methanol (15:85 v/v) for 4 h. Finally, total anthocyanins were calculated as $\Delta A\ g^{-1} = (A_{530} - A_{620}) - 0.1(A_{650} - A_{620})$ and expressed in terms of $\Delta A\ g^{-1}$ on the basis of fresh weight (Zheng and Tian, 2006).

2.5. Membrane leakage, MDA contents, DPPH-radical scavenging activity and TPC

For MDA determination, one g sample was homogenized in 15 mL trichloroacetic acid [(TCA) (10%)] and centrifuged at $10{,}000 \times g$ for 20 min. The 2 mL supernatant was reacted with 2 mL of 2-thiobarbituric acid (0.6%), heated at 100 °C for 20 min in water bath and solution was centrifuged at $10{,}000 \times g$ for 20 min. Finally, absorbance of supernatant was noted at 450, 532, and 600 nm, respectively and MDA contents were expressed as nmole kg^{-1} on the basis of fresh weight (Zheng and Tian, 2006). Membrane leakage of pericarp tissues was determined as described by Jiang and Chen (1995). Pericarp discs (10 mm) were incubated in 0.3 *M* mannitol solution for 30 min at 25 °C and initial electrolyte leakage was noted directly from the incubated solution with conductivity meter (HI-98304, Hanna Instruments Inc., Mauritius). After initial reading, discs were heated at 98 °C for 15 min and cooled at 25 °C to note total electrolytes and membrane leakage was expressed as percent. DPPH radical-scavenging-activity was assessed by bleaching purple–coloured–solution of the 2, 2–diphenyl–1–picrylhydrazyl–radical in methanol and expressed in terms of percent (Brand-Williams et al., 1995). TPC were determined with Folin–Ciocalteu reagent. Gallic acid was