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Effect of controlled atmosphere storage on pericarp browning, bioactive compounds and antioxidant enzymes of litchi fruits

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ABSTRACT

'Gola' litchi fruits were stored under ten different CA-combinations at 5 ± 1 °C to investigate its effects on pericarp browning, biochemical quality and antioxidative activities. Control fruit turned completely brown after 28 days of storage and were excluded from the study. Fruit-stored under CA_7-combination (1% O_2 + 5% CO_2) showed reduced weight loss, pericarp browning, membrane leakage and malondialdehyde contents. Soluble solid contents, titratable acidity and ascorbic acid contents were higher in CA_7-stored fruit. Activities of catalase and superoxide dismutase enzymes, levels of total anthocyanins, DPPH radical-scavenging-activity and phenolic contents were significantly higher in CA_7-stored litchi fruit. In contrast, activities of polyphenol oxidase and peroxidase enzymes were substantially lower in fruit kept under CA_7-combination. Fruit subjected to CA_7-conditions also maintained higher organoleptic quality. In conclusion, 1% O_2 + 5% CO_2 CA-conditions delayed pericarp browning, maintained antioxidative activities and biochemical characteristics along with better organoleptic quality of litchi fruit for 35 days.

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a tropical to subtropical fruit, highly admired for its characteristic appealing bright red color, delicious taste, and attractive aroma (Holcroft & Mitcham, 1996; Underhill & Simons, 1993). Export of this premium quality fruit in the international markets is restricted due to different postharvest problems such as pericarp browning and fruit decay. Besides different other issues, pericarp browning of litchi fruit is one of the major postharvest constraints which greatly reduces its market value around the globe (Jiang, Zauberman, & Fuchs, 1997; Underhill & Critchley, 1995). Different factors have been found associated with litchi pericarp browning; however, water loss/desiccation is considered one of the leading causes of this problem. Desiccation of litchi pericarp tissues leads to different physiological and biochemical changes that ultimately results in the direct contact of peel phenolics with polyphenol oxidase (PPO) and peroxidase (POD) enzymes (Zauberman et al., 1991). Oxidation of peel phenolics by PPO and POD enzymes finally leads to pericarp browning of litchi fruit (Zauberman et al., 1991; Zhang, Pang, Zuoliang, & Jiang, 2001).

Several approaches such as sulfur dioxide fumigation (Swarts, 1985); modified atmosphere packaging (Chen, Wu, Ji, & Su, 2001); hot-water dips (Olesen, Nacey, Wiltshire, & O'Brian, 2004); CA-storage (Mahajan & Goswami, 2004); cold-storage (Khan, Ahmad, Malik, & Amjad, 2012); postharvest exogenous application of oxalic acid (Zheng & Tian, 2006); ascorbic acid (Sun, Liang, Xie, Lei, & Mo, 2010) and antioxidants (Kumar, Mishra, Chakraborty, & Kumar, 2013) have been used for the management of pericarp browning, overall quality retention and storage-life extension of litchi fruit. However, among these approaches, CA-storage has been found more suitable to slow down the rapid rate of skin color loss of litchi fruit (Jiang & Fu, 1999). CA-storage with 3.5% O_2 and 3.5% CO_2 gaseous conditions maintained sensory and biochemical characteristics of 'Bombay' litchi fruit (Mahajan & Goswami, 2004). Duan et al. (2011) also reported reduced pericarp browning, lipid peroxidation and reactive oxygen species along with higher activities of antioxidative enzymes under pure O_2 conditions in 'Huaizhi' litchi fruit for 6 days. Similarly, fruit of litchi cv. 'Heiye' kept under CA-storage (CA-I = 5% O_2 + 5% CO_2; CA-II = initially at 70% O_2 + 0% CO_2 for 7 days and later on 5% O_2 + 5% CO_2 combination) showed reduced pericarp browning (Tian, Bo-Qiang, & Xu, 2005). Similarly, reduced pericarp browning has also been reported with application of

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1-methylcyclopropene under CA-storage (17% O_2 + 6% CO_2) in fruit of litchi cv. 'McLean's Red' for 21 days (Sivakumar & Korsten, 2010). Although, they reported reduced pericarp browning and maintained quality; but, these studies did not describe detailed changes in postharvest physiology of litchi fruit. Moreover, information about changes in different enzymatic (catalase, peroxidase and superoxide dismutase) and non-enzymatic (DPPH radical scavenging activity and phenolic contents) antioxidants in both peel and pulp tissues in relation to development of litchi pericarp browning under CA-storage is also lacking. The knowledge about occurrence of pericarp browning under prolonged CA storage could further enhance our understanding to devise most appropriate strategies for its management and quality control during the supply chain operations of litchi fruit. Hence, objectives of the current research were to investigate the effects of different concentrations of O_2 and CO_2 on pericarp browning, membrane integrity, lipid peroxidation, bioactive compounds, antioxidative activities, biochemical attributes and organoleptic quality of 'Gola' litchi fruit under extended storage conditions.

2. Materials and methods

2.1. Fruit source

Fruits of litchi cv. 'Gola' were harvested at anticipated commercial maturity [color = 85–100% red, SSC = 18.5 °Brix, TA = 0.70% and SSC/TA = 26.42 (as per local maturity standards)] from the "Government Fruit Farm Nursery (34°00.114′N, 72°56.779′E), Haripur, Khaybar Patkhtun Khaw (KPK), Pakistan. After harvest, uniform sized fruits free from visual defects were pre-cooled in refrigerated reefer van (10 ± 1 °C) and shifted to Postharvest Research and Training Center, University of Agriculture Faisalabad, Pakistan.

2.2. Storage conditions and treatments

The experiment comprised of ten treatments viz; control (air), CA_1 = 1% O_2 + 3% CO_2, CA_2 = 2% O_2 + 3% CO_2, CA_3 = 3% O_2 + 3% CO_2, CA_4 = 1% O_2 + 4% CO_2, CA_5 = 2% O_2 + 4% CO_2, CA_6 = 3% O_2 + 4% CO_2, CA_7 = 1% O_2 + 5% CO_2, CA_8 = 2% O_2 + 5% CO_2 and CA_9 = 3% O_2 + 5% CO_2. Air, N_2 and CO_2 were mixed via pressure regulator to establish initial O_2 and CO_2 gases levels in CA-pallistores by a continuous flow through system (VPSA-6 Van CA Technology, Amerongen, the Netherlands) automatically regulated, and controlled by the analyzer (S-904 Van CA Technology, Amerongen, the Netherlands). Fruits were stored in CA-pallistores ($L \times W \times H$ = 43 × 33 × 1 50 cm) at 5 ± 1 °C with 90 ± 5% RH for 35 days. Gas mixtures of all CA-pallistores were monitored thrice daily to ensure the anticipated gaseous compositions. Fruits were removed from CA-pallistores at 7 days interval, and evaluated for different quality characteristics. Fruit weight loss, decay incidence, and pericarp browning index were assessed from whole fruit; while, soluble solid contents, titratable acidity, sugar: acid ratio, and ascorbic acid contents were determined only from the pulp tissues. DPPH radical scavenging activity, total phenolic contents, activities of catalase, peroxidase and superoxide dismutase enzymes were determined from both peel, and pulp tissues. Membrane leakage, malondialdehyde, total anthocyanins, and activities of polyphenol oxidase enzyme were determined only from the peel tissues. The experiment was conducted under completely randomized design with factorial arrangement. Fruits were placed in plastic crates ($L \times W \times H$ = 39 × 29 × 11 cm) and stored under CA-pallistores. At each sampling interval, every treatment contained three independent crates and each crate contained 25 fruits as a single replication. Overall the experiment comprised of 174 crates having total 3675 fruits.

2.3. Fruit weight loss, decay incidence, pericarp browning and browning rate

Fruits 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.

$$\text{Fruit weight loss} = \frac{\text{Initial fruit weight before storage} - \text{Final fruit weight after storage}}{\text{Initial fruit weight before storage}} \times 100$$

Decay incidence was assessed by dividing the number of decayed/rotten fruits over total number of fruits in each replicate, and expressed as percentage. Pericarp browning was assessed by evaluating the extent of browned area on the surface of fruit as reported by Sivakumar and Korsten (2010) and calculated according to the formula of Zhang and Quantick (1997). Browning rate was calculated by dividing the number of brown fruits over total number of fruits in each replicate, and expressed in percentage.

2.4. Pericarp pH, membrane leakage and malondialdehyde (MDA) contents

Pericarp pH of litchi fruit was determined by the method as reported previously by Joas, Caro, Ducamp, and Reynes (2005). Membrane leakage of the pericarp tissues was determined as described by Jiang and Chen (1995). Membrane leakage was finally expressed in terms of percent of the initial electrolytes to the total electrolytes. MDA contents of litchi pericarp tissues were determined as described by Zheng and Tian (2006), and expressed as nmol g^{-1} FW.

2.5. Total anthocyanins, DPPH radical scavenging activity and total phenolic contents

Total anthocyanin contents of litchi pericarp tissues were extracted according to the method as described by Zheng and Tian (2006), and calculated as: $\Delta A\ g^{-1}$ FW = $(A_{530}–A_{620})–0.1(A_{650}–A_{620})$. Total antioxidant activity of 2,2-diphenyl-1-picrylhydrazyl-radical (DPPH) was assessed by bleaching purple-colored-solution (stable-DPPH-radical) of methanol, and expressed in terms of percent (Brand-Williams, Cuvelier, & Berset, 1995). Total phenolic contents were determined with Folin–Ciocalteu reagent (Ainsworth & Gillespie, 2007), and their concentration was expressed as gallic acid equivalent (mg GAE 100 g^{-1} FW).

2.6. Soluble solid contents (SSC), titratable acidity (TA), SSC: TA ratio and ascorbic acid contents

SSC of litchi fruit juice were determined by a digital refractometer (RX-5000 Atago, Japan), and expressed in terms of °Brix. TA was determined by juice titration against 0.1 N NaOH and expressed as percent (%) malic acid. SSC: TA ratio was determined by dividing the SSC with their corresponding TA values. Ascorbic acid contents were determined by titrating 5 mL of litchi juice aliquot (obtained from 10 mL juice and 90 mL of 0.4% oxalic acid) against 2,6-dichlorophenolindophenol and expressed as mg 100 mL^{-1} of fruit juice.

2.7. Enzymes assays

Litchi peel and pulp tissues (1 g each) were homogenized in 2 mL phosphate buffer [for pulp (pH 7.2)] and citrate buffer [for peel (pH 4) in pre-chilled mortar, and pestle. After thorough