



Pre-storage kojic acid application delays pericarp browning and maintains antioxidant activities of litchi fruit



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ABSTRACT

Pericarp browning is known as one of the leading problems during the supply chains of litchi fruit. The effects of pre-storage kojic acid (KA) application on pericarp browning, activities of antioxidative enzymes in the pericarp tissues, and the quality attributes, soluble solid concentrations, titratable acidity and ascorbic acid concentrations of the flesh of 'Gola' litchis have been investigated. Fruit were treated with 2, 4 or 6 mmol L⁻¹ KA and stored at 5 ± 1 °C with 90 ± 5% relative humidity for 20 d. Fruit treated with 4 mmol L⁻¹ KA had reduced fruit weight loss and fruit decay; while, 6 mmol L⁻¹ KA delayed pericarp browning by maintaining higher total anthocyanin and phenolic concentrations, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Reduced malondialdehyde, and hydrogen peroxide concentrations, and activities of oxidative enzymes such as peroxidase and polyphenol oxidase were also observed in 6 mmol L⁻¹ KA-treated fruit. In contrast, activities of ascorbate peroxidase, catalase and superoxide dismutase enzymes were found to be higher in pericarp tissues of fruit subjected to 6 mmol L⁻¹ KA application. Moreover, soluble solid concentrations, titratable acidity, ascorbic acid concentrations and sensory characteristics were also higher in 6 mmol L⁻¹ KA-treated litchi fruit. In conclusion, pre-storage application of 6 mmol L⁻¹ KA to litchi fruit delayed pericarp browning and maintained activities of antioxidative enzymes.

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is known for its translucent sweet juicy aril, characteristic red colour, delicious flavour and high nutritive value (Holcroft and Mitcham, 1996). It is a non-climacteric fruit and harvested at fully ripe stage, a bright red pericarp being characteristics of its harvest, the red colour becomes brown, and the commercial acceptability of the fruit is reduced (Underhill, 1992; Kumar et al., 2012). Enzymatic degradation of anthocyanin concentrations along with oxidation of phenolic concentrations has been known as the leading cause of litchi pericarp browning (Ali et al., 2016a). Oxidative enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) cause the oxidation of phenolic concentrations due to breakdown in cellular compartmentalization and leads to quinone formation within the cell (Sapers and Miller, 1998). Hydroxylation of monophenol to o-diphenol and o-quinones development by the oxidation of o-diphenol are catalyzed in the presence of O₂ by PPO enzyme; while, POD catalyzes the oxidation of phenolics by using H₂O₂ and cause litchi pericarp browning (Jiang et al., 2004).

Sulfur dioxide (SO₂) fumigation has been found effective to reduce the incidence of pericarp browning in litchi, but alterations of taste and

residual toxicity are its some undesirable effects (Sivakumar et al., 2010). To replace SO₂ fumigation, many techniques have been investigated including pre-cooling (Ketsa and Leelawatana, 1992), acid dip (Zauberman et al., 1991), modified atmosphere storage (Mangaraj et al., 2012), controlled atmosphere storage (Ali et al., 2016a), heat treatment (Saengnil et al., 2006), coatings (Jiang et al., 2005), irradiation (Pandey et al., 2013), polyamines (Jiang and Chen, 1995), 1-methylcyclopropene (Sivakumar and Korsten, 2010), L-cysteine (Ali et al., 2016b), and acidified calcium sulphate (Wang et al., 2010).

KA as a natural antibrowning agent has been reported for the browning management in mushroom (Saruno et al., 1979) and apple slices (Chen et al., 1991a). It showed maximum efficacy among the phenolic acids used for the inhibition of browning in fresh cut slices of 'Liberty' Apple (Son et al., 2001). Combined application of 4-hexylresorcinol, L-cysteine and KA reduced browning in 'Amasya' apple juice (Iyidogan and Bayindirli, 2004). Similarly, KA maintained the quality and colour of potato tuber due to the reduced oxidation of catechol by PPO enzyme (Chen et al., 1991b). However, to the best of our knowledge, effects of KA application on pericarp browning of litchi fruit have not been studied. Hence, there is a need to explore the potential of exogenous application of KA as an alternative of SO₂ fumigation to

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reduce the incidence of pericarp browning in litchi fruit.

The objective of the present study was to investigate the efficacy of different KA concentrations on pericarp browning, activities of anti-oxidative enzymes and biochemical quality of 'Gola' litchi fruit during cold storage.

2. Materials and methods

2.1. Kojic acid and its safety status

Analytical grade kojic acid (5-Hydroxy-2-hydroxymethyl-4H-4-pyr-anone) was procured from Sigma-Aldrich (St Louis, USA). It was extracted from fermentation (exact information about the used micro-organisms was not provided by the manufacture). Kojic acid is known as antioxidant and its use has been reported in several foods with varying concentrations. It is used in syrup (0.05%), flour production (0.1%), meat production (0.2%), flavouring agent (0.2%) and anti-discoloring in vegetables (1%) (SCCP, 2008). Use of kojic acid at the concentrations (up to 1%) generally reported in food/cosmetics does not pose a concern for the safety of consumers (Burdock et al., 2001; Burnett et al., 2010).

2.2. Fruit material

Fruit of litchi (*Litchi chinensis* Sonn.) cv. 'Gola' were harvested from a Government Fruit Farm Nursery (34°00.114'N, 72°56.779'E), Haripur, Khyber Pakhtun Khaw (KPK), Pakistan at commercial maturity (colour = 80-100% red, titratable acidity (TA) = 0.41%, soluble solid concentrations (SSC) = 22.3% and SSC/TA ratio = 55.01). Fruit of uniform size and free from visual defects were pre-cooled at $10 \pm 1^\circ\text{C}$ in refrigerated reefer van and transported to Postharvest Research and Training centre, University of Agriculture, Faisalabad, Pakistan.

2.3. Treatments and storage conditions

Fruit were divided into 16 main groups [treatments (4) \times sampling intervals (4)]. Each main group was further segregated into 3 independent sub-groups having 20 fruit in each replicate. After segregation, fruit were dipped in aqueous solution of different KA concentrations (2, 4 or 6 mmol L⁻¹) along with 'Tween-20' (0.01%) as a surfactant for 5 min. Control fruit were dipped in distilled water along with surfactant only. After treatment, fruit were air dried and kept in plastic crates at $5 \pm 1^\circ\text{C}$ with $90 \pm 5\%$ relative humidity for 20 d. The crates (H \times W \times L = 11 \times 29 \times 39 cm) were completely open from the top with several perforation rows (L \times W = 0.4 \times 3 cm) on the bottom and sides. The level of relative humidity around the fruit was controlled inside the cold room. Fruit were removed after 5 d intervals from cold storage to assess quality characteristics. Fruit weight loss, fruit decay and pericarp browning were determined from whole fruit, and SSC, TA, SSC/TA ratio and ascorbic acid concentrations were evaluated in litchi fruit juice obtained from the pulp tissues. Fruit were manually peeled after assessment of weight loss and pericarp browning. Juice was extracted from the pulp and pericarp samples of the same fruit (used for juice extraction) were pooled, quickly flash frozen with liquid nitrogen and stored at -80°C for biochemical and enzyme analysis. Fruit were sampled cold and 20 fruit per replicate were used for every measurement. Anthocyanin concentrations, malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) concentrations, total phenolic concentrations (TPC), DPPH-radical scavenging activity, activities of ascorbate peroxidase (APX), catalase (CAT), polyphenol oxidase (PPO), peroxidase (POD) and superoxide dismutase (SOD) enzymes were determined from stored tissues samples. This study was conducted under completely randomized design [because conditions (storage temperature and relative humidity) were homogenous for all treatments] with two factors factorial arrangements (KA concentrations and cold storage periods) having 20 fruit as treatment unit replicated three times.

2.4. Fruit weight loss, fruit decay, pericarp browning and anthocyanin concentrations

Fruit samples were assessed before and after cold storage by using digital weight balance (MJ-W176P, Panasonic Japan). Fruit weight loss (%) was calculated according to the formula as given below:

$$\text{Fruit weight loss(\%)} = \frac{\text{Weight before storage} - \text{Weight after storage}}{\text{Weight before storage}} \times 100$$

Fruit decay percentage was estimated by ratio of the number of decayed fruit with total number of fruit per replicate. Pericarp browning (score) was assessed according to the scale described by Jiang and Chen (1995) with little modification. The used scale was 1 = no browning; 2 = slight browning; 3 = < 1/4 browning; 4 = 1/4- 1/2 browning and 5 = > 1/2 browning. The browning grade was calculated as Σ (browning scale \times percentage of corresponding fruit within each class). Anthocyanin concentrations in pericarp tissues were determined by the method of Zheng and Tian (2006) and expressed as $\Delta\text{A g}^{-1}$ on a fresh weight basis by using the following formula:

$$\Delta\text{A g}^{-1} = (\text{A}_{530} - \text{A}_{620}) - 0.1 (\text{A}_{650} - \text{A}_{620})$$

2.5. MDA, H₂O₂ radical, TPC and DPPH-radical scavenging activity

MDA concentrations were determined according to the method of Zheng and Tian (2006). Pericarp tissues were homogenized in 10% trichloroacetic acid (TCA, 15 mL) and centrifuged at $10,000 \times g$ (20 min). The supernatant (2 mL) was reacted with 2-thiobarbituric acid (0.6%, 2 mL) and absorbance was noted at 450, 532 and 600 nm. MDA concentrations were expressed as $\mu\text{mol kg}^{-1}$ on a fresh weight basis. H₂O₂ radical concentrations were determined according to the detailed method of Velikova and Loreto (2005). Briefly, samples (1 g) were extracted with 0.1% (1 mL) TCA and resultant homogenate was centrifuged at $12000 \times g$ (15 min). The 0.5 mL supernatant was mixed with 0.5 mL phosphate buffer (10, mmol L⁻¹, pH 7.0) and 1 mL of 1 molar potassium iodide. The absorbance was monitored at 390 nm and H₂O₂ concentrations were expressed in $\mu\text{mol kg}^{-1}$ on a fresh weight basis. TPC of pericarp tissues was assessed by the method of Ainsworth and Gillespie (2007) using Folin-Ciocalteu reagent and expressed as mg kg^{-1} on a fresh weight basis by using gallic acid standard. Bleaching of methanol solution by DPPH-radical was used to estimate the DPPH-radical scavenging activity as outlined by Ullah et al. (2013). The results were expressed as DPPH scavenging activity and were calculated according to the formula as described by Zhang and Hamauzu (2004).

$$\text{DPPH - radical scavenging activity (\%)} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

2.6. Enzyme assays

The pulverized peel tissues (1 g from 20 fruit) of the fruit were homogenized in 2 mL of the citrate buffer (pH 4) containing polyvinylpyrrolidone in the chilled mortar and pestle. After homogenization, the samples were centrifuged (235-A, Pegasus Scientific Inc., USA) at $10,000 \times g$ for 10 min at 4°C and the resultant supernatant was used for assays of the following enzymes.

PPO enzyme (EC 1.14.18.1) activity was assayed as reported by Ali et al. (2016a). The 50 μL enzyme extract was mixed with 1.45 mL of 100 mmol L⁻¹ citrate buffer (pH 6.8) and 0.50 mL of 100 mmol L⁻¹ 4-methylcatechol. The absorbance was noted at 412 nm and PPO activity was expressed as $\mu\text{mol s}^{-1} \text{kg}^{-1}$.

The activity of POD enzyme (EC 1.11.1.7) was determined as described by Ali et al. (2016a). Briefly, a reaction mixture was prepared comprising 800 μL of 50 mmol L⁻¹ phosphate buffer (pH 5), 100 μL 40 mmol L⁻¹ H₂O₂ and 100 μL of 20 mmol L⁻¹ guaiacol. Finally,

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