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Pre-storage methionine treatment inhibits postharvest enzymatic browning of cold stored 'Gola' litchi fruit

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ABSTRACT

Antibrowning effect of methionine was investigated on litchi fruit. Fruit were treated with 0.25% methionine and kept under cold-storage at 5 ± 1 °C for 28 d. Methionine treatment reduced weight loss, and inhibited fruit decay. Methionine application to litchi fruit significantly reduced pericarp browning, malondialdehyde content, hydrogen peroxide, electrolyte leakage and peroxidase, and polyphenol oxidase activities with conserved pericarp anthocyanins. The treated fruit also had higher ascorbic acid contents, total phenolics, 2, 2–diphenyl–1–picrylhydrazyl–radical (DPPH) scavenging activity, and activities of ascorbate peroxidase, catalase, and superoxide dismutase enzymes. Methionine treated fruit also showed higher soluble solid contents, titratable acidity, and sensory attributes with reduced sugar: acid ratio. In conclusion, postharvest methionine application to litchi fruit showed significantly reduced browning, higher antioxidative activities, and maintained quality.

1. Introduction

Litchi is an imperative subtropical fruit. Due to appealing red colour, litchi fruit has high demand in the international markets. However, due to high perishability, its fruit exhibit relatively shorter postharvest shelf-life of only 2–3 d at ambient conditions (Zhang and Quantick, 1997). Numerous factors such as desiccation, rapid cellular de-compartmentalization, increased peroxidation of the membrane lipids, over production of the reactive oxygen species (ROS), and decreased pericarp antioxidants have been found correlated with the litchi pericarp browning (Zhang et al., 2015; Ali et al., 2016a). Moreover, increased oxidation of phenolics and anthocyanins catalyzed by anthocyanase, peroxidase (POD), and polyphenol oxidase (PPO) enzymes has also been reported to be the major cause of litchi pericarp browning (Zhang et al., 2015).

Different exogenous treatments *i.e.* ascorbic acid (Sun et al., 2010), 1-methylcyclopropene (Sivakumar and Korsten, 2010), hydrochloric acid (Kumar et al., 2012), oxalic acid (Tran et al., 2016), irradiation (Kumar et al., 2012), salicylic acid (Kumar et al., 2013; Kumari et al., 2015), pyrogallol (Jing et al., 2013), potassium metabisulfite (Kumar et al., 2012), apple polyphenols (Zhang et al., 2015), kojic acid (Shah et al., 2017), tea seed oil (Zhang et al., 2017), biocontrol bacteria (Wu et al., 2017), and novel chitosan formulation (Jiang et al., 2018) have been used to delay litchi pericarp browning. The pericarp browning still needs to be addressed efficiently to reduce its quality loss. Furthermore, to safeguard the fruit safety, some premium and eco-friendly treatments need to be found and instantly communicated to the stakeholders/growers. It is also indispensible to ensure provision of the safe fruit (by excluding use of sulphur dioxide) during its postharvest supply chain in the world (Tran et al., 2016). Moreover, beside postharvest handling treatments, some most suitable and effective antibrowning agent/browning inhibitor is also required to inhibit its enzymatic browning (Ali et al., 2016b).

Based on mode of action, the enzymatic browning inhibitors have been classified into six groups *viz*; complexing agents, chelating agents, acidulants, reducing agents, enzyme treatments, and enzyme inhibitors. Among various reducing agents, chemical compounds with sulfhydryl groups *i.e.* methionine, glutathione, thiourea, cysteine, and mercaptoethanol have been found as highly effective for reducing enzymatic browning in numerous fruits (apple, litchi, loquat and grapes) (Son et al., 2001; Ding et al., 2002; Wu, 2014; Ali et al., 2016b), and vegetables (artichoke, potato and eggplant) (Ghidelli et al., 2014; Cabezas-Serrano et al., 2013; M-Ali et al., 2016). The methionine application has also been used for inhibition of enzymatic browning of fresh-cut 'Liberty' apple fruit (Son et al., 2001), and 'Spunta' potatoes (M-Ali et al., 2016). Nevertheless, no comprehensive work is available regarding application of methionine in inhibiting browning of the litchi fruit. Therefore, the present research aimed to study the impact of exogenous

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methionine treatment on pericarp browning, oxidative stress, overall quality and activities of pro-oxidant, and antioxidant enzymes of litchi fruit under cold-stored conditions.

2. Materials and methods

2.1. Fruit material, treatments and storage conditions

'Gola' litchi (*Litchi chinensis* Sonn.) fruit were harvested at commercially mature stage from Government Fruit Farm Nursery orchard (34°00′N; 72°56′E), Haripur, Pakistan. Fruit were selected based on uniform colour, free from any mechanical damage, and pre-harvest cracks. Fruit were kept in reefer van at 10 ± 1 °C, and transferred to Postharvest Laboratory, University of Agriculture Faisalabad, Pakistan. Litchi fruit were dipped in 0.25% aqueous solution of methionine for 5 min (as L-methionine, Sigma-Aldrich, St Louis, USA). Tween-20 (0.01%) was used as a surfactant. The said methionine treatment was selected on the basis of preliminary trial by using 0, 0.25, 0.50, 0.75, and 1.0% concentrations. The control fruit were dipped in distilled water having only Tween-20. After air drying, fruit were kept under cold storage [in open top plastic crates (L × W × H = 39 × 29 × 11 cm)] at 5 ± 1 °C with 90 ± 5% relative humidity level for 28 d. Sampling of the fruit was done at 0, 7, 14, 21 and 28th d of storage to assess various quality attributes. The research was conducted under completely randomized factorial design. Each treatment was repeated three times having 25 litchi fruit as a single replicate. At each sampling period, 150 fruit [treatments (2) × replicates (3) × fruit per replicate (25)] were removed, and used for various quality attributes. Overall, the study comprised of 675 fruit.

2.2. Studied parameters

Weight loss, fruit decay, and pericarp browning were determined from the whole fruit. Total phenolic contents (TPC), DPPH scavenging activity, ascorbate peroxidase [APX (EC 1.11.1.11)], peroxidase [POD (EC 1.11.1.7)], catalase [CAT (EC 1.11.1.6)], and superoxide dismutase [SOD (EC 1.15.1.1)] enzymes activities were assessed in pericarp/peel, and pulp (data have been reported only in supplementary figures) tissues. Activities of polyphenol oxidase enzyme [PPO (EC 1.14.18.1)], total anthocyanins, hydrogen peroxide (H_2O_2) content, membrane electrolyte leakage, and malondialdehyde (MDA) were studied in pericarp tissues. Soluble solid contents (SSC), titratable acidity (TA), and ascorbic acid were investigated only from litchi pulp samples. Sensory attributes such as taste, aroma, flavour, and overall acceptability were also evaluated.

2.3. Weight loss, fruit decay, pericarp browning and total anthocyanins

Fruit weight loss was assessed as described previously by Ali et al. (2016a), and expressed as percent. Fruit decay incidence was calculated by dividing the number of decayed fruit over the total number of fruit, and reported as percentage. Pericarp browning was evaluated by assessing extent of the browned portion/spots on surface of the fruit, and expressed in score (Zhang and Quantick, 1997). Total anthocyanins of the litchi pericarp samples were assessed as reported by Zheng and Tian (2006), and expressed as $\Delta A\ g^{-1}$ fresh weight.

2.4. Membrane electrolyte leakage, MDA contents, DPPH scavenging activity and TPC

Membrane electrolyte leakage was determined as reported by Jiang and Chen (1995), and expressed as percent. MDA contents were quantified by method of Zheng and Tian (2006), and expressed in nmol kg^{-1} fresh weight. The DPPH scavenging activity was evaluated by using protocol of Brand-Williams et al. (1995) with some modifications. The 50 µL methanolic pericarp extract was mixed with 5 mL of DPPH radical (0.1 mmol L^{-1}) in methanol. The reaction mixture was incubated at 25 ± 1 °C for 30 min under dark conditions. The absorbance of samples was measured at 517 nm. The control reaction mixture only contained methanol; whereas, blank had methanol instead of DPPH radical solution. Finally, DPPH scavenging activity was calculated with the equation: DPPH scavenging activity = [1 – (sample absorbance – blank absorbance)/control absorbance] × 100, and reported in percent. Folin–Ciocalteu reagent was used to determine TPC (Ainsworth and Gillespie, 2007). A standard curve was prepared with analytical grade gallic acid (GAE), and the concentration of TPC was expressed as mg GAE kg^{-1} fresh weight.

2.5. H_2O_2 content and enzyme assays

H_2O_2 content was assayed according to detailed procedure of Velikova and Loreto (2005), and expressed in µmol kg^{-1} fresh weight.

Pericarp/pulp samples were extracted either with phosphate [pulp (pH 7.2)] or citrate [peel (pH 4.0)] buffer, and centrifuged at 10,000 × *g* for 5 min (Ali et al., 2016a). The obtained supernatant was utilized for the assays of PPO, APX, POD, CAT, and SOD enzymes.

For PPO activity determination, 50 µL crude enzyme extract was reacted with 1.45 mL of the 100 mmol L^{-1} citrate buffer, and 0.50 mL of 100 mmol L^{-1} 4-methylcatechol. Finally, the enzyme activity was assessed by monitoring the change in absorbance at 412 nm (Ali et al., 2016a).

For the assay of APX activity, the 100 µL enzyme extract was reacted with 0.5 mmol L^{-1} L-ascorbate (100 µL), 0.1 mmol L^{-1} H_2O_2 (100 µL), and 50 mmol L^{-1} phophate buffer (pH 5, 200 µL). Finally, the activity of APX enzyme was determined by monitoring the oxidation of L-ascorbate at 290 nm (Nakano and Asada, 1987).

CAT enzyme activity was studied as reported by Ali et al. (2016a). The 100 µL extract of the enzyme was mixed with 5.9 mmol L^{-1} of 100 µL H_2O_2 solution, and the decomposition of H_2O_2 was monitored at 240 nm.

POD enzyme activity was assayed according to the modified methodology of Ali et al. (2016a). Briefly, 100 µL crude enzyme extract was mixed with 100 µL reaction mixture [100 µL guaiacol (20 mmol L^{-1}), 100 µL H_2O_2 (40 mmol L^{-1}), and 800 µL phosphate buffer with pH 4.0 (50 mmol L^{-1})], and the increase in the absorbance was noted at 470 nm.

SOD enzyme activity was assayed with nitro-blue-tetrazolium (NBT) method. In short, 50 mmol L^{-1} phosphate buffer [pH 5, (500 µL)], distilled water (800 µL), 20 µmol L^{-1} NBT (100 µL), 22 µmol L^{-1} methionine (200 µL), 0.1 µmol L^{-1} triton-X (200 µL), and 0.6 µmol L^{-1} riboflavin (100 µL) were reacted with the 100 µL extract of enzyme. After assay mixture illumination under UV-light (15 min), the change in absorbance was observed at 560 nm (Ali et al., 2016a).

All enzymes activities were expressed as µmol s^{-1} kg^{-1} protein, and protein contents were assayed according to the detailed method of Bradford (1976).

2.6. SSC, TA, SSC: TA ratio and ascorbic acid contents

SSC, TA, SSC: TA ratio and ascorbic acid contents were assessed according to detailed methodology of Ali et al. (2016a), and expressed in percent (%), % malic acid, ratio and mg kg^{-1} fresh weight, respectively.

2.7. Sensory evaluation

Sensory attributes such as taste, flavour, aroma, and overall acceptability were assessed according to our previous study (Ali et al., 2016a). The hedonic scale consisted of 1 = extremely dislike, 5 = neither dislike nor like, and 9 = extremely like.