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Research Article

Postharvest Hexanal Application Delays Pericarp Browning Under Cold Storage in Litchi Fruit

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ABSTRACT

Litchi is the fruit of warm climates and has a very short shelf life as the fruit loses its red color within the span of 2-3 days. The current study was planned to evaluate the effect of hexanal fumigation on overall fruit quality and retention of aesthetic appeal of the fruit under low temperature storage conditions. Hence, litchi fruit harvested at the ripening stage was fumigated with 0%, 1%, 2%, 3%, and 4% hexanal concentration followed by cold storage at 5 ± 1°C for 28 days by maintaining 90% relative humidity. During cold storage, fruits were evaluated for physiological weight loss (PLW), browning severity, and anthocyanin depletion in pericarp. Physico-chemical quality characteristics were determined in litchi aril samples. Total phenolics and total antioxidants were also determined from aril samples, whereas antioxidative enzymes such as SOD, CAT, POD PPO were determined from pericarp tissues in litchi pericarp at 7 -day intervals. Postharvest hexanal application significantly controlled pericarp browning and resulted in higher anthocyanin content with reduced weight loss than control fruit. Hexanal preserved a steep rise in SSC and maintained relatively low SSC compared to control fruit throughout the cold storage period; however, titratable acidity, ascorbic acid contents, phenolic components and total antioxidants in litchi fruit aril were significantly augmented in all hexanal formulations, with significantly higher values recorded in 1% hexanal treatment. Likewise, antioxidative enzyme activities, such as SOD (74.54 mg^{-1} protein) and CAT (82.42 mg^{-1} protein), were significantly higher in hexanal-treated fruits, while POD (30.2 mg^{-1} protein) and PPO enzyme (34.42 U mg^{-1} protein) activities were significantly hindered in hexanal-treated litchi fruits which were significantly higher in control treatment. Overall, 1% hexanal formulation showed more promising results in terms of maintaining quality characteristics and color of the fruit during the extended storage period. Conclusively, hexanal application retained the pericarp red color and delayed browning by maintaining fruit quality of 'Gola' litchi longer than control fruits.

Keywords: Antioxidative Enzymes, Pericarp Browning, Physico-chemical Quality.

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INTRODUCTION

Litchi is a sub-tropical fruit, widely grown in the sub-continent and South Asian countries like China, India and Pakistan (Wu et al., 2017). The fruit is known for its appealing red color and juicy, sweet aril with excellent nutritional benefits and medicinal value (Haider et al., 2025; Naik et al., 2018). It is a non-climacteric fruit which is insensitive to ethylene exposure and harvested fully ripe stage (Zhang et al., 2015). Litchi fruit is known for its unpredictable nature and short shelf life, as the fruit starts to deteriorate within three days, resulting in pericarp browning as a major symptom (Bhushan et al., 2015). Postharvest browning of litchi involves rapid loss of skin after harvest (Kumar et al., 2013), which affects the marketability of the fruit (Rajwana et al., 2010). This loss of red color has been attributed to the loss of

anthocyanin compounds and phenolic oxidation simultaneously (Ali et al., 2019), due to enhanced polyphenol oxidase (PPO) and peroxidase (POD) enzyme activities (Sapers and Miller, 1998). PPO enzyme influences chemical reactions like change in monophenol to quinones or o-diphenol, while POD enzyme, associated with phenolic oxidation, has been reported to be the major factor in browning of litchi peel (Jiang et al., 2004; Jiang, 2000). Whereas, PPO-induced oxidation of proanthocyanidin A2 also plays an important role in the loss of red color of peel (Sun et al., 2007); while non-enzymatic processes can be caused by moisture loss, and a change in pH (Jiang et al., 2006).

For a long time, sulphur dioxide fumigation has been employed as a useful tool to overcome browning; nevertheless, its industrial application is banned owing to harmful effects and taste alterations (Sivakumar et al., 2010). Therefore, numerous alternative attempts have been made to delay browning in litchi peel by using diverse postharvest treatments such as application of different coatings, antioxidants, organic acids such as oxalic acid, ascorbic acid, and kojic acid (Joas et al., 2005; Kumar et al., 2013; Zheng and Tian, 2006; Shafique et al., 2016; Sun *et al.,* 2010; Shafique et al., 2022; Shah et al., 2017). While recent studies have reported naturally existing organic acids and gallic acid to maintain the reddish color and delay browning (Zhang et al., 2023), to satisfactory results, Hexanal, being a natural volatile C6 aldehyde compound, has revealed antimicrobial properties against in-vitro microbial deterioration (Song et al., 2007; Utto et al., 2008). Phospholipase D (PLD) has been reported to initiate membrane degradation along with increased ROS activities under oxidative stress (Paliyath and Droillard, 1992) as it inhibits the damage caused by phospholipase D (Paliyath et al., 1999; Paliyath and Subramanian, 2008). Besides, it has also been reported that hexanal treatment inhibited microbial damage and increased the shelf life of apple slices during low temperature storage (Lanciotti et al., 1999). Hexanal also has some other properties in addition to its broad-spectrum antimicrobial activities, and its effectiveness has also been reported in longan fruit (Song et al., 2007). Therefore, current research was focused on the evaluation of hexanal effectiveness on litchi cv. 'Gola' in maintaining the quality characters and red color of the fruit.

MATERIALS AND METHODS

Plant Material

Litchi fruit cv. 'Gola' fruits of uniform size and shape were harvested from well-managed fruit orchard in Haripur, KPK, Pakistan. Fruits were cleared of any inert matter and packed in fiberboard boxes; thereby, transported to Postharvest Research and Training Centre (PRTC), University of Agriculture, Faisalabad, Pakistan. Fruits were offloaded from the reefer van and then immediately subjected to hexanal fumigation treatment for 1 h. A specialized sealed plastic drum installed with a fumigator drum was used for application of hexanal treatment (0,1%, 2%, 3%, 4%). Post-hexanal treatment fruit were shifted to cold storage for 28 days, where the temperature was maintained at 5 ± 1°C. Analysis for physico-chemical characteristics, TPC, and total antioxidants from litchi aril samples. Moreover, activities of CAT, SOD, POD and PPO enzymes, anthocyanin contents in litchi pericarp were also assessed.

Physico-Chemical Parameters and Fruit Weight Loss

Fruit biochemical quality parameters were assessed to evaluate the impact of treatment. Weight loss was measured after each 7 -day interval using an electronic balance (ELB 1200, Japan). SSC (°Brix) of litchi aril was determined by placing a drop of juice on an ATAGO digital refractometer, while TA (%) was determined by the titration method using 0.1 *N* NaOH.

Pericarp Browning and Anthocyanin Contents

Pericarp browning was evaluated by measuring using a scale ranging from 1-5, determining the extent of brown area on litchi peel (Jiang *et al.* 2004). Anthocyanin contents were extracted from 1g of litchi pericarp samples using methanol and HCl (Zheng and Tian, 2006).

TPC, Total Antioxidants and Ascorbic Acid Contents

Litchi fruit TPC was determined from litchi aril and expressed as mg GAE 100 g^{-1}, while total antioxidants were assessed as DPPH scavenging activity (Ainsworth and Gillespie, 2007). Ascorbic acid contents from litchi aril samples were computed titration method 2, 6-dichlorophenolindophenol (Khalid et al., 2012; Haider et al., 2025).

Enzyme Assay

Tissues of litchi pericarp weighing 1 g were mixed with phosphate buffer (2 mL). The obtained mixture was centrifuged for 5 min at 10,000 × *g,* and the supernatant was collected to determine activities of enzymes such as SOD, CAT and POD (Razzaq et al. 2013; Haider et al., 2019; Haider et al., 2024). For PPO enzyme activity, 10 mL of potassium citrate buffer containing polyvinylpyrrolidone was used to obtain supernatant and absorbance was read at 412 nm (Ali et al. 2016).

Statistical Analysis

Data was analyzed using Windows-based software Statistic-10 using the analysis of variance technique. Analysis was based on two-factorial arrangements, which included hexanal concentrations and storage duration. Each experimental unit consisted of twenty-five fruits along with three replications. Significance of means was determined from Fisher's LSD at $P \leq 0.05$ of the *F*-test (Steel et al., 1997).

RESULTS

Fruit Weight Loss, Pericarp Browning, Anthocyanin Contents and PPO Enzyme Activity

Although weight loss was increased steadily; however, postharvest application of hexanal formulations significantly checked the increase in weight loss. Untreated litchi fruits exhibited 13.12% weight loss, which was 2.91-fold higher, as compared to 1% hexanal formulation. Among hexanal-treated fruit, 1% hexanal concentration exhibited 2.91%, which was the lowest yet non-significantly different from other hexanal concentrations (Figure 1A). Significant loss of red color was noted in control fruits during storage with 4.89 browning index. Litchi fruit subjected to different hexanal formulations maintained their red color throughout the cold storage and did not exhibit a significant decline. Among all concentrations, the lowest browning index (2.80) was noted in 1% hexanal formulation, which varied non-significantly with other hexanal treatments (Figure 1B).

Anthocyanin degradation in litchi peel significantly exhibited variations; loss of anthocyanin contents in hexanal-treated fruit was non-significant. Lowest anthocyanin contents (1.82 ΔAg^{-1} FW) were measured in control fruit, whereas hexanal-treated fruits maintained significantly higher anthocyanins than untreated fruit. Evidently, loss of anthocyanins in control fruits was directly related to an increase in pericarp. Among different hexanal treatments highest anthocyanin pigment (2.90 ΔAg^{-1} FW) in litchi pericarp was noted in fruits subjected to 1% hexanal treatment, and 2.45 ΔAg^{-1} FW anthocyanins were recorded 4% hexanal treatment (Figure 1C).

In litchi, PPO enzyme is believed to be one of the main culprits of browning, and its activity determines the extent of browning. Our results for PPO activity can be correlated with the augmented browning index and loss in red color. Activity of the PPO enzyme remained significantly higher in control fruits throughout the cold storage duration compared to hexanal treatments. Control fruit exhibited 43.2 U mg^{-1} protein PPO enzyme in litchi peel tissues, which was about 1.26-fold higher than PPO activity found in 1% hexanal treatment, i.e. 34.2 U mg^{-1} Protein (Figure 1D).

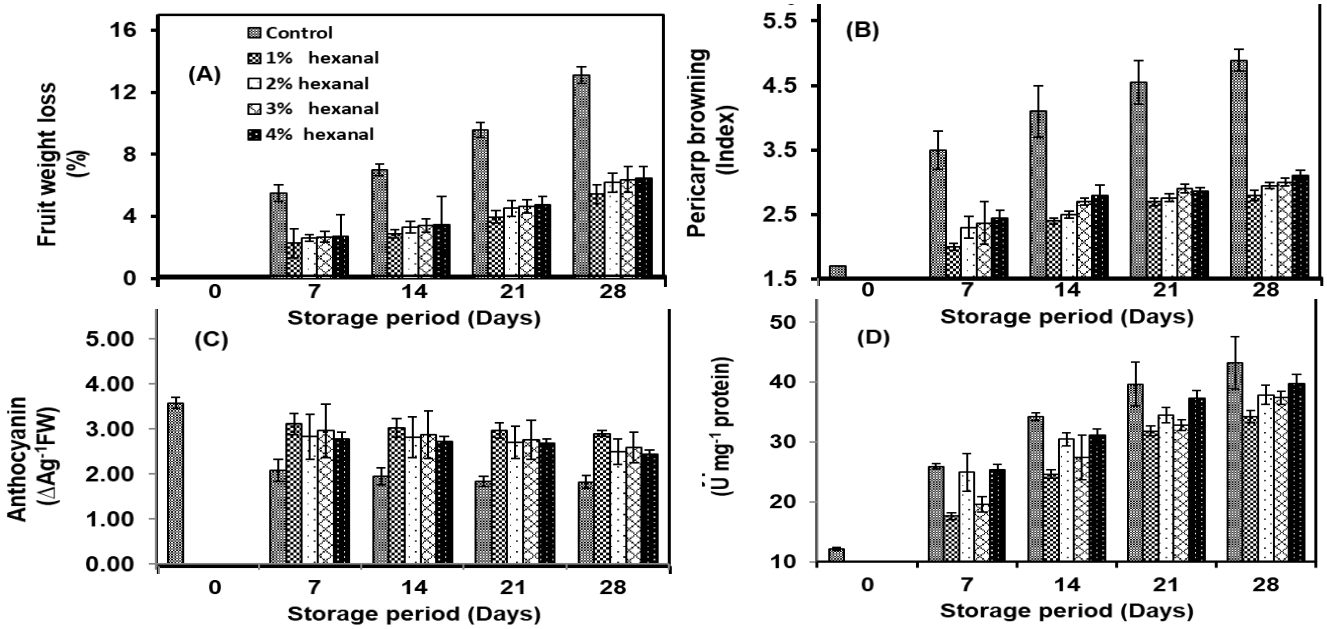

Figure 1. Hexanal fumigation affecting weight loss, pericarp browning, anthocyanin contents, and polyphenol oxidase enzyme in litchi pericarp at 5 ± 1°C.

SSC, TA and SSC: TA

Litchi, being a non-climacteric fruit, exhibited a significant decline in SSC throughout the cold storage period. Decline in SSC of litchi fruit aril was more pronounced than in treated fruits, as hexanal application checked the drastic decline

of SSC and preserved fruit quality. Our results showed that the maximum SSC of untreated litchi aril was 22.2 ˚Brix on the day of harvest, which decreased to 14.9 ˚Brix after 28 days of cold storage, amounting to 33 % loss in SSC. However, hexanal significantly controlled this decline in SSC, and after 28 days of storage, there was a 26.12% decline in 1% hexanal-treated litchi fruits. Other hexanal treatments, i.e. 2%, 3% and 4% exhibited 31%, 29.9%, and 29.14% loss in SSC, respectively (Figure 2A). Besides, titratable acidity was significant reduced and decline in TA was more in untreated litchi fruit, although all hexanal treated fruit retained higher acidity as highest TA (0.27%) was exhibited by 1% hexanal treated fruits (Figure 2B). Similarly, data regarding SSC: TA ratio showed that 4% hexanal treatment had maintained significantly higher SSC: TA ratio (78.4) followed by control fruit (Figure 2C). These results show that hexanal treatments might have reduced respiration, oxidation of organic substrates and enzymatic degradation that resulted in higher SSC and higher TA than control fruits.

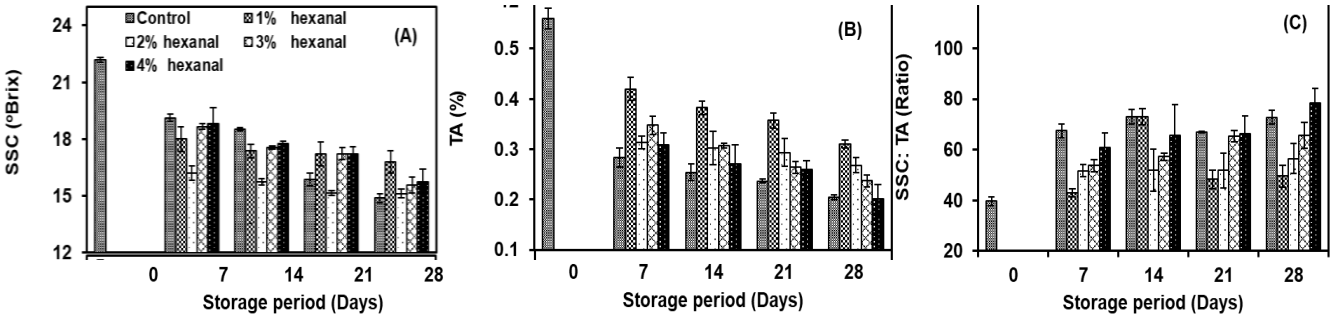

Figure 2. Hexanal fumigation affecting soluble solids (SSC), titratable acidity (TA), and SC: TA ratio in litchi aril at 5 ± 1°C.

Phenolic Compounds, Antioxidants and Ascorbic Acid Contents

Phenolic compounds and antioxidants of litchi fruit aril were decreased during low-temperature extended storage (Figure 3). However, all hexanal treatments were able to maintain significantly higher values of TPC and total antioxidants, as exogenous hexanal application at 1% resulted in 122.3 mg GAE 100g^{-1} TPC, showing about 1.6-fold more phenols than control fruit. Similarly, 2%, 3% and 4% hexanal treatments exhibited 198.4, 192.5 and 180.8 mg GAE 100g^{-1} TPC, respectively, after 28-days of low temperature storage (Figure 3A). Comparable trend was observed regarding total antioxidants of litchi aril as significantly highest total antioxidant value (32.6 % Inhibition) was exhibited by 1% hexanal treatment that was 1.5-times higher than control fruit. Likewise, other hexanal concentrations also resulted in significantly higher values for total antioxidants than control fruit (Figure 3B). Similarly, ascorbic acid, being a key antioxidant, was also significantly higher in hexanal-treated litchi fruit than control fruit. Significantly higher ascorbic acid contents (45.3 mg 100g^{-1}) were measured in 1% hexanal-treated fruits, followed by 2%, 3% and 4% hexanal treatments, whereas the aril of control fruits exhibited 40.7 mg 100g^{-1} ascorbic acid contents (Figure 3C).

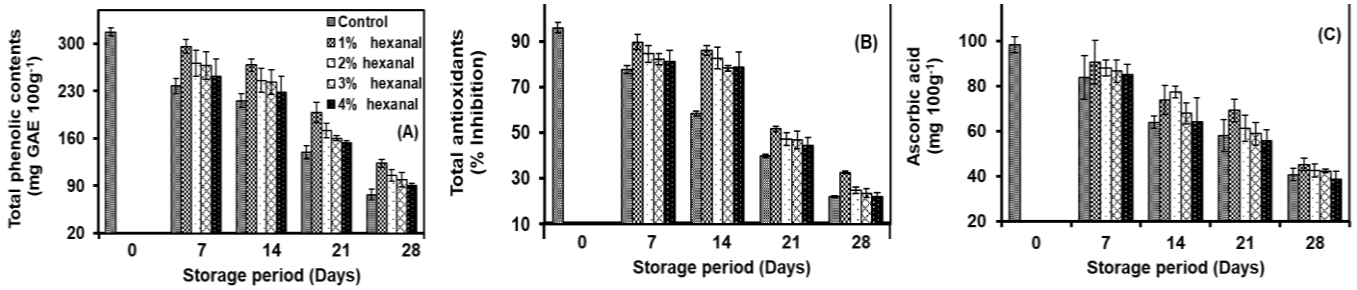

Figure 3. Hexanal fumigation affecting ascorbic acid, total phenolic contents and total antioxidants litchi aril at 5 ± 1°C.

Activities of SOD, CAT and POD Enzymes in Pericarp

Antioxidative enzymes (SOD and CAT) in pericarp tissues of litchi fruit were significantly decreased over time in all treatments, including control fruit (Figure 4A, B). SOD and CAT enzymes, being known as 1st line of defense under stress conditions, were significantly decreased in untreated litchi fruit pericarp. This could be one of the reasons

pericarp browning was more pronounced in control fruits, as hexanal-treated litchi fruits exhibited significantly higher activities. Our result reported that 1% hexanal formulation exhibited 25.4 U mg^{-1} Protein SOD activity which was 1.5-fold more than control fruit (Figure 4A). Comparable observations were recorded for CAT enzyme activity which was significantly higher (36.4 U mg^{-1} Protein) in 1% hexanal treated litchi pericarp followed by gradual decline in other hexanal treatments, respectively (Figure 4B). POD being a key enzyme exhibited significant more value in pericarp tissues during storage. All hexanal treatments exhibited the lowest POD enzyme activities, especially 1% hexanal-treated litchi fruits. Our data indicated that 1% hexanal treatment exhibited about 30.2 U mg^{-1} Protein POD activity compared to 52.2 U mg^{-1} Protein in the control fruit (Figure 4C).

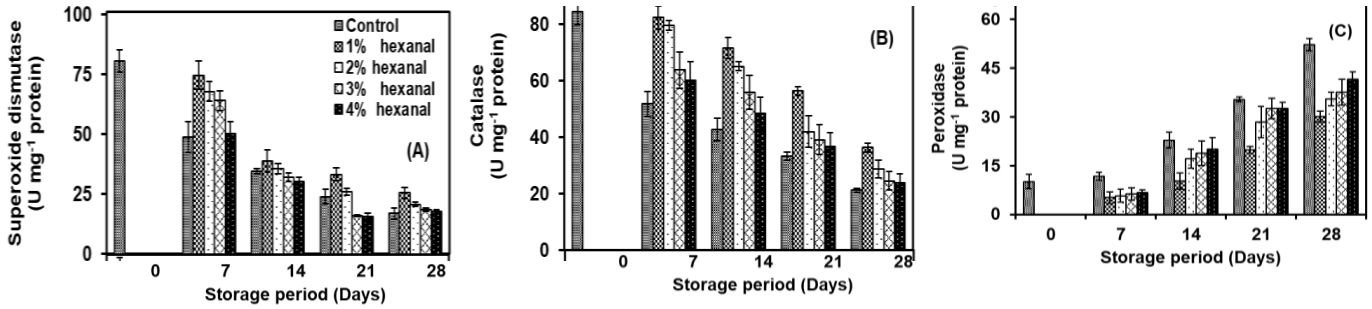

Figure 4. Hexanal fumigation affecting superoxide dismutase, catalase and peroxidase enzymes in litchi aril at 5 ± 1°C.

DISCUSSION

Weight loss was substantially increased by untreated fruit throughout cold storage (Figure 1A), and a similar observation was also reported in 'Bombai' litchi (Mitra and Kar, 2001). Retention of moisture during long-term storage is often linked with overall fruit quality (Lownds et al., 1993). In our study, hexanal fumigation resulted in better moisture retention and substantial weight loss. Preethi et al. (2018) reported similar results where hexanal sprays and dipping treatment reduced weight loss and maintained the freshness of mango fruit. On the other hand, pericarp browning, which is very important aspect of litchi quality, was significantly reduced as hexanal treated litchi fruits retained their pink, red color over 28-days of cold storage, although control fruits exhibited significant browning of pericarp (Figure 1B). Pericarp/peel pH is a key attribute to determine browning, as low pH help in to retain red color of fruit. Increased pericarp browning in control fruits could be attributed to loss of moisture and pH rise that may have resulted in the loss of anthocyanins (Underhill and Critchley, 1995), however, hexanal application inhibited moisture loss and might have resisted the change in pH that resulted in significantly reduced browning over long-term storage. These are consistent with the findings of Bharat et al. (2019), where hexanal application retained the red colour of the fruit and inhibited browning.

Our results showed that anthocyanin content breakdown was in harmony with the development of pericarp browning during cold storage. Evidently, decline in anthocyanin contents was more in control fruits throughout the cold storage, but hexanal application inhibited anthocyanin breakdown and maintained the appealing red color during whole duration (Figure 1C). Hexanal fumigation might have mitigated the oxidative stress under storage conditions due to which litchi pericarp was able to retain higher anthocyanin contents (Sharma et al., 2010) and similar findings were reported by Bharat et al. (2019) where hexanal and inositol application-controlled browning in 'culcuttia' litchi by controlling phospholipase D enzyme activity. Meanwhile, PPO enzyme showed significantly higher activity in controlled that ultimately resulted in pericarp browning (Figure 1D). Hexanal application, as evident from our results, exhibited reduced moisture loss and might have kept pH below 4.5 to inhibit rapid PPO enzyme activity. Jiang (2000) also observed a similar increase in PPO enzyme activity; on the other hand, oxidation of phenolic compounds provides a suitable substrate for PPO and POD enzyme activity, thus inhibiting phenolic degradation, as evident from our results might have inhibited PPO and POD activity (Chen et al., 2001; Bharat et al., 2019).

Among physico-chemical quality parameters, SSC and TA are very important because they describe the acceptability of litchi fruit (Jiang and Fu, 1998). Our results exhibited significantly higher SSC and TA values in hexanal-treated fruit aril compared to control (Figure 2A, B). Comparable findings have also been reported in hexanal-treated tomato fruits (Cheema et al., 2014). On the other hand, our results are also consistent with the findings of Jiang et al. (2004) and Bharat et al. (2019), where both SSC and TA were decreased under storage conditions in litchi fruit. TPC, total antioxidants and ascorbic acid contents of litchi aril were decreased significantly from 0-day to 28-days during cold

storage environment; although decline was more significant control fruits and hexanal fumigated fruits maintained relatively higher TPC, total antioxidants and ascorbic acid (Figure 3). Hydrolytic products of phenolic provide substrates for certain enzyme such as PPO and POD that cause browning (Sun et al., 2007; Baltacioğlu et al., 2011); whereas fruit phenolic compounds also delay senescence (Apai et al., 2007). As for the decline in TPC and antioxidants in the control fruit is concerned, moisture loss due to hexanal treatment could have triggered the PPO enzyme activity, causing a decline in phenolics and antioxidant levels in hexanal-treated fruit. Decline in total antioxidant could also be attributed to the natural role of scavenging of ROS species under uncongenial conditions, while conversion of organic compounds into sugars also decreases ascorbic acid contents (Hounsome et al., 2009; Gimnez et al., 2003). Ascorbic acid, being a critical dietary antioxidant, plays an important role in delaying senescence and scavenging ROS, and its reduction takes place due to oxidative breakdown (Razzaq et al., 2015). Hexanal application might have helped retain significantly higher values of ascorbic by reducing respiration, delaying senescence. Our results for higher ascorbic acid in hexanal-treated fruit are comparable with previous findings reported in mango fruit (Anusuya et al., 2016; Preethi et al., 2018).

SOD and CAT are important antioxidative enzymes and play an important role during long-term uncongenial conditions and help in mitigating oxidative stress (Gill and Tuteja, 2010). Despite a significant decline in control fruit, hexanal formulations maintained significantly higher values of SOD and CAT enzyme activity (Figure 4). Therefore, higher SOD and CAT enzyme activity is often correlated with delaying in senescence of the fruit, and their activities determine the extent of pericarp browning and aril quality was deteriorated (Sun et al., 2010). These enzymes are considered to mitigate stress by converting superoxide anion to hydrogen peroxide (Gill & Tuteja, 2010; Ali et al., 2021).

CONCLUSIONS

Postharvest hexanal application significantly delayed browning in litchi peel browning and effectively maintained edible quality of the fruit during extended storage. Overall, fumigation of fruits with 1% hexanal treatment outperformed all other hexanal concentrations as untreated fruits exhibited rapid browning and quality deterioration.

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Not applicable.

AUTHOR CONTRIBUTIONS

All the authors contributed equally to this research.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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