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ASCORBIC ACID INFLUENCES PERICARP COLOR, BIOCHEMICAL QUALITY AND ANTIOXIDATIVE CAPACITY OF LITCHI FRUIT UNDER COLD STORAGE

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Abstract
Litchi being suited for tropical and sub-topical agro-climatic conditions is very perishable and loses it bright red color in few hours after harvest. Current experiment was planned to evaluate potential of ascorbic acid concentrations (0, 15, 30, 45 or 60 m*M*) to delay pericarp browning and preserve fruit quality for longer period of time under storage conditions (5 ± 1^{o}C). Results of ascorbic acid dipping treatments revealed higher anthocyanin contents, besides significantly inhibited weight loss and occurrence of pericarp browning; along with better physico-chemical quality attributes together as well as higher antioxidative capacity in 45 m*M* ascorbic acid treatment. Meanwhile, augmented antioxidative enzyme activities like superoxide dismutase (SOD) and catalase (CAT) activities coupled with reduced polyphenol oxidase (PPO) and peroxidase (POD) activities were recorded with 45 m*M* ascorbic acid treated litchi pericarp as well as aril tissues. Irrefutably, postharvest dipping treatment of litchi fruit in 45 m*M* ascorbic acid inhibited manifestation of pericarp browning and maintained higher quality under long term storage conditions.
Keywords: Ascorbic Acid; Fruit Quality; Litchi; Oxidative Enzymes; Pericarp Browning

Introduction

Litchi is considered the most remunerative fruit crops of tropical and subtropical regions that has very high demand among consumer (Jiang et al. 2004). Its fruit is popular for its bright redcolour and a luminous aril with crucial dietary importance (Salomao et al. 2006); but loses its visual appeal as pericarp of freshly harvested fruit turns brown within 2-3 days (Del-Aguila et al. 2009). Pericarp browning is favored by multiple factors that include moisture loss, high temperature or chilling stress, senescence, and diseases incidence. Several changes take place in litchi pericarp with the development of browning like anthocyanin breakdown due to activation of peroxidase, ascorbic acid oxidase (PO) and polyphenol oxidase (PPO) enzymes (Mizobutsi et al. 2010) or by activation of superoxide,

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hydrogen peroxide (H_2O_2) and hydroxyl radicals called as reactive oxygen species (ROS) (Wang et al. 2007).

Usually production of ROS could be the outcome of suppressed antioxidative enzyme defense mechanism (Kanazawa et al. 2000) that may result in protein damage and membrane dysfunctionality (Yang et al. 2008). There are number of chemical treatments like hydrochloric acid application alone or in combination (Jiang et al. 2004), application of oxalic acid (Saengnil et al. 2006; Shafique et al. 2016), SO_2 fumigation (Ducamp-Collin et al. 2008) and edible coating of chitosan plus ascorbic acid delayed or prevented rind browning by boosting up the antioxidative enzyme defense mechanism (Sun et al. 2010) consisting of catalase (CAT) or superoxide dismutase (SOD) enzymes (Razzaq et al. 2013).

Ascorbic acid being known for antioxidative properties that help inhibiting browning development and decay in freshly harvested fruits (Suttirak and Manurakchinakorn 2010). Ascorbyl formed from ascorbic acid not only inhibited ROS (Yamaguchi et al. 1999) but also inhibit o-quinones formation by suppressing PPO enzyme activity (Robert et al. 2003). Previously, ascorbic acid treatment induced increased membrane integrity, reduced membrane leakage and suppressed POD and PPO enzymes that ultimately delayed browning (Sun et al. 2010). However, there are instances where ascorbic application could not delay browning as reported in 'Bengal' litchi (Silva et al. 2010); although, antioxidative potential of aril tissues was amplified under low temperature storage in litchi cv. 'Feizixiao' (Sun et al. 2010).

Above literature clearly shows that pericarp browning as well as quality of quality was affected by ascorbic acid treatments; although, with inconsistent outcomes. However, comprehensive information about activities of antioxidative enzymes in pericarp and aril tissue as influenced by ascorbic acid is not available in literature. Furthermore, its role in deferring pericarp browning and maintaining quality of litchi fruit under prolonged low temperatures necessitates detailed investigations. Therefore, hypothesis of current investigation was based on that application of ascorbic acid application would maintain fruit quality and reduce browning of cv. 'Gola' peel under cold storage.

Material and Methods

Plant Material

Litchi fruits at commercially maturity (TA = 0.56%, SSC = 15^{o}Brix) were harvested from uniform healthy trees (30-35 years old) located at Haripur (34^{o}00.114'N, 72^{o}56.779'E) Khyber Pakhtunkhwa Province of Pakistan. Fruits were transferred for evaluation of pericarp brown and biochemical fruit quality analysis to Postharvest Laboratory, University of Agriculture, Faisalabad. After initial sorting and grading fruit were washed with 0.01% Tween 20 and dipped in 0, 15, 30, 45 and 60 *mM* ascorbic acid solutions for 5 min followed by drying at room temperature. After drying fruits were kept at 5 ± 1^{o}C with

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90 ± 5% RH in a cold storage room for 28 days. Cold stored fruits were evaluated at weekly (7 days) interval for physico-chemical quality attributes from aril tissues; while antioxidative capacity [total antioxidants and phenolic contents (TPC)] along with antioxidative enzymes (SOD, POD and CAT) were determined from pericarp as well as aril tissues at 7 days interval. On the other hand, pericarp browning along with anthocyanin pigments and PPO enzyme were determined from pericarp tissues. Each treatment was comprised as 25 fruits replicated three times and obtained data were subjected to completely randomized design (CRD) where concentrations of ascorbic acid and storage period served as factors.

Estimation of weight loss, pericarp browning and biochemical quality attributes

Weight loss of whole litchi fruit was determined in percentage with the help of ELB 1200, Shimadzu, Japan, electronic balance. Browning of litchi fruit under cold storage intervals was evaluated as by the method described by Jiang et al. (2004).SSC (^{o}Brix) was assessed by placing a drop of juice on lens of ATAGO-Japan digital refractometer. Meanwhile, litchi juice extracted from litchi pulp was titrated using 0.1 N NaOH for the determination of TA and calculated in percentage (%). Ascorbic acid contents were calculated specifically from litchi pulp (Shafique et al. 2015).

Estimation of anthocyanin contents, TPC and total antioxidants

Litchi pericarp tissues were used to determine anthocyanin contents as peel tissues (1 g) were extracted using methanol and HCL at 85:15 ratio which was centrifuged for 5 min at 4000 x *g*. Measurement of anthocyanin content was done by reading absorbance at 530, 620, 650 nm (Zheng and Tian 2006).

TPC in litchi pericarp and aril tissues was estimated as mg GAE 100 g^{-1} using the process elaborated by Ainsworth and Gillespie (2007). Total antioxidants from both tissues were calculated by the procedure presented by Mimica-Dukic et al. (2003).

Estimation of CAT, SOD, POD and PPO Enzymes Activities

Litchi tissues (pericarp and aril) were grinded simultaneously, homogenized and centrifuged separately to prepare the extract that was further used for the quantification of CAT, SOD, POD and PPO enzymes by adopting the assay elaborated by Shafique et al. (2015).

Statistical analysis

The data after collection was analyzed using windows based analytical software, Statistix 10 (Tallahassee, FL 32317, USA). Ascorbic acid concentrations and cold storage period were considered as factors under CRD; whereas least significant differences test (Fisher's LSD) was applied at $P \leq 0.05$ to calculate treatment effects (Steel et al. 1997). Correlation of pericarp browning with anthocyanin contents and antioxidative enzymes was drawn by applying Pearson's correlation using Statistix 10.

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RESULTS

Influence of ascorbic acid on weight loss, pericarp browning, anthocyanin contents and activity of PPO enzyme

Litchi fruit lost its weight continuously under cold storage; although, ascorbic acid treatments maintained reduced weight loss with 45m*M* ascorbic acid treatment prominently surpassed all other treatment (Fig. 1A). Persistent weight loss resulted in gradually resulted in development of brown pericarp, however, ascorbic acid treatment with 45 m*M* presented relatively 1.48-fold less browning as related with control fruit (Fig. 1B). Pericarp tissues of litchi fruit showed rapid degradation of anthocyanin contents; while correlation observed between peel browning and anthocyanin pigments was found negative ((R^2 = -0.51) (Table). Degradation of anthocyanin contents in untreated litchi pericarp was 2.71-fold more than 45 m*M* ascorbic acid treatment (Fig. 1C).

Meanwhile, litchi pericarp tissues showed increasing tendency in PPO enzyme activity as storage period proceeded. Litchi fruits dipped in various ascorbic acid concentrations maintained reduced PPO enzyme activity. Among different concentrations of ascorbic acid, litchi fruit dipped in 45 m*M* ascorbic acid maintained significantly reduced action of PPO enzyme which was 1.22-fold lower than 30 m*M* treatment (Fig. 1D). Expectedly, browning exhibited positive (+) correlation (R^2 = 0.76) with PPO enzyme (Table 1).

Influence of ascorbic acid on SSC, TA and SSC: TA ratio

As litchi aril is known for its sweet taste and flavor therefore SSC along TA and SSC: TA are of foremost importance. SSC of litchi fruit showed a general increasing trend during cold storage; however, ascorbic acid dipping treatment at 15 m*M* concentration showed 1.07-fold more SSC than untreated fruit (Fig. 2A). Meanwhile, persistent drop in acidity of litchi aril was noted throughout cold storage. TA drop in aril of untreated fruit was more rapid as much as 1.37-fold more than other treatments. Conversely, application of ascorbic acid (30 m*M*) resulted in resulted in 1.15-fold augmented TA than other ascorbic acid treatments (Fig. 2B). Litchi fruit under cold storage showed constant rise in SSC: TA ratio regardless of treatments applied. Dipping treatment with different ascorbic acid concentration inhibited the rapid increase SSC: TA ratio; while, 45 m*M* ascorbic acid maintained significantly more SSC: TA ratio when compared with 30 m*M* treatment that resulted in unarguably lowest SSC: TA ratio (Fig. 2C).

Influence of on TPC, total antioxidants and ascorbic acid contents

Litchi pericarp exhibited steady drop in TPC and antioxidative potential under progressing low temperature storage (Fig. 3). Litchi fruits dipped in ascorbic acid decelerated the decline in antioxidative potential with as much as 1.56-fold augmented TPC and 1.30-fold higher total antioxidants with 45 m*M* ascorbic acid treatment than control fruit (Fig. 3A, B). Browning in litchi pericarp was negatively correlated with TPC and total antioxidants in pericarp (R^2 = -0.83; R^2 = -0.71) as well as aril tissues (R^2 = -0.81; R^2 = -0.68), respectively (Table 1).

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Meanwhile, aril tissues of litchi fruit exhibited comparable results to pericarp tissues with declining trend in TPC and total antioxidant count (Fig. 4). Litchi fruit dipping in ascorbic acid inhibited rapid fall in TPC and total antioxidants; however, treating litchi fruit with 45 m*M* ascorbic acid was proved to be most effective concentration and maintained 2.04-fold elevated TPC together with 1.60-fold more total antioxidants as compared to control (Fig. 4A, B). Correspondingly, aril tissues of litchi fruit faced continuous decline in vitamin C/ascorbic acid contents during cold storage; yet dipping in 45m*M* ascorbic acid resulted in 1.40-fold more vitamin C or ascorbic acid contents in an aril of litchi fruit than undipped fruits (Fig. 4C).

Influence of ascorbic acid on SOD, CAT and POD activities

Generally, antioxidative enzymes are involved in protection against oxidative stress. In present study litchi fruits underwent considerable decline in SOD and CAT enzymes in litchi fruit aril and pericarp tissues; nevertheless, ascorbic acid dipping treatment inhibited the prompt reduction and maintained significantly higher enzymatic activities of SOD as well as CAT than unprocessed litchi fruits throughout the cold storage (Fig. 5A and B). All ascorbic acid concentrations exhibited higher SOD and CAT enzymes activities than control fruits; whereas, litchi fruits dipped in 45 m*M* showed maximum SOD and CAT activities in litchi pericarp and aril tissues compared with other ascorbic acid concentrations (Fig. 5B). Meanwhile, POD enzyme activity in litchi fruit kept on increasing in all treatments during cold storage. Ascorbic dipping treatment suppressed rapid increase and maintained significantly reduced POD enzyme activity; as untreated fruits exhibited about 1.84-fold and 1.48-fold higher activities in litchi pericarp as well as aril tissues, respectively.

Adding further pericarp browning was negatively associated with CAT (R^2 = -0.65) and SOD (R^2 = -0.81) enzyme activities in litchi pericarp tissues; likewise, similar correlation was observed in litchi aril tissues for CAT (R^2 = -0.79) as well as SOD (-0.64) enzymes activities. While, development of browning in ascorbic acid-treated fruit was positively correlated with POD enzyme activity in pericarp (R^2= 0.67) as well as aril tissues (R^2 = 0.77) (Table 1).

DISCUSSION

Persistent weight loss caused steady loss of red colour of litchi pericarp under low temperature storage (Fig. 1A). Augmented weight loss in litchi fruits could possibly be ascribed to constant loss of moisture during low temperature storage duration. Application of ascorbic acid prevented rapid weight loss by protecting cellular integrity (Shalata and Neumann 2000); while, our results were also testified in ‘Bombai’ litchi (Mitra and Kar 2001). Ascorbic acid dipping might have enhanced membrane integrity with reduced oxidation of anthocyanins as well as phenolics that ultimately hindered browning of litchi pericarp (Fig. 1B). Likewise, litchi cv. ‘Feizixiao’ subjected to ascorbic acid and chitosan

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coating exhibited reduced pericarp browning with enhanced antioxidative capacity (Sun et al. 2010).

On the other hand, breakdown of anthocyanin pigments was rather obvious under low temperature storage (Fig. 1C); as degradation of anthocyanin are often related with advanced PPO and POD enzyme activities (Jiang 2000; Zhang et al. 2005). Our results are in harmony with previous findings that anthocyanin depletion was low in ascorbic acid and chitosan treated litchi of fruits (Silva et al. 2010; Sun et al. 2010). While, current results for lesser increase in PPO activity as shown in Fig. 1D are in agreement with former results where 'Feizixiao' litchi treated with ascorbic acid and chitosan exhibited reduced PPO activity **coupled with less pericarp browning** (Sun et al. 2010). Rise in PPO enzyme activity could be associated with loss of moisture that indirectly raised pH of the pericarp tissues; previously, comparable observation was reported by Jiang in 'Huaizhi' litchi (2000).

Since taste as well as flavor are based on sweetness or sourness of fruit; therefore, SSC and TA carry premium importance in determining edible quality of litchi fruit. Results of current study indicated that SSC of litchi juice was increased continuously under cold storage (Fig. 2A). Breakdown of organic acid into sugars as testified by Echeverria and Valich (1989) or constant depletion of moisture might have caused in higher SSC (Tanada-Palmu and Grosso 2005). However, SSC of ascorbic acid treated fruit was increased at much slower rate owing to delayed breakdown of carbohydrates and comparable results were described in HCl-treated 'Bengal' litchi (Hojo et al. 2011). Besides, acidity (TA) of the litchi fruit was declined with the advancement of storage time but untreated litchi fruits showed steep drop in TA than litchi fruits subjected to ascorbic acid treatment (Fig. 2B); whereas SSC: TA ratio showed consistent increase throughout storage intervals (Fig. 2C). Organic acids depletion during respiration process might have played a role in decrease of TA; our outcomes are in conformity with the observations of Hojoet al. (2011) where TA of the 'Bengal' litchi was decreased during low temperature storage.

Meanwhile, results revealed that antioxidative capacity were decreased continuously in litchi pericarp/aril but postharvest dipping in ascorbic acid prevented reduction in phenolic and antioxidants under cold storage (Figs. 3 and 4). Phenolic compounds scavenge ROS; although, PPO enzyme associate the degradation of phenolics (Baltacig et al. 2011); likewise, antioxidants reduction in this study could be ascribed to depletion while scavenging ROS at low temperature (Hounsome et al. 2009). Ascorbic acid itself being an antioxidant protected phenolic compounds of cantaloupe by deterring the action of polyphenols and reconverting organic compounds to phenolic substrates (Lamikanra and Watson 2001). Likewise, ascorbic acid had a synergistic effect on membrane integrity and phenolic compounds of berries (Kahkonen et al. 2001) and correspondingly ascorbic acid increased antioxidants in lettuce (Altunkaya and Gokmen 2008). Conversely, litchi fruit dipped in ascorbic acid exhibited more ascorbic acid contents (Fig. 4C). Formerly, parallel trend was observed for ascorbic acid percentage in 'Bengal' litchi (Silva et al. 2010).

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These observations are comparable with the observations of Hojo et al. (2011) where comparable behavior of ascorbic acid contents was noticed in 'Bengal' litchi. Decline of ascorbic acid/vitamin C could be related to the breakdown of complex organic acids into simple sugars under extended storage period (Gimnez et al. 2003).

Fruits exposed to ROS develop a protect mechanism in which they activate CAT and SOD enzymes (Niranjana et al. 2009). These enzymes keep ROS production below threshold levels due to their antioxidative mechanism (Rao et al. 1996). Decrease in SOD and CAT enzyme in present study could be ascribed to their consumption in scavenging of ROS;while relatively higher SOD and CAT activities in treated fruit determine the protective function of ascorbic acid (Figs. 5). Ascorbic acid reduced oxidation of SOD and CAT substrates and comparable observation was reported in fruit of 'Feizixiao' litchi (Sun et al. 2010). While, enzymatic activity of POD kept increasing with the advancement of storage intervals (Fig. 5); therefore, POD enzyme is assumed to accelerate the degradation of phenolic and anthocyanin pigments in litchi fruit (Zhang et al. 2005). Nevertheless, results of current study suggested that ascorbic acid repressed the POD enzyme activity and maintained reduced pericarp browning as formerly observed by Sun et al. (2010). Meanwhile, Mizobutsi et al. (2010) correlated inhibition of POD (H_2O_2) enzyme with acidic pH in litchi pericarp.

CONCLUSIONS

Postharvest dipping of litchi in ascorbic acid solution prohibited litchi pericarp browning for longer period of time by sustaining higher antioxidative enzymes activities (CAT and SOD) along with improved physico-chemical qualities in 'Gola' litchi under low temperature storage.

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Vol: 65 Issue 02 | 2022
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FIGURE LEGENDS

Fig. 1 Effect of postharvest application of ascorbic acid (T) on fruit weight loss (A), pericarp browning (B), anthocyanin contents (C) and polyphenol oxidase (D) in pericarp tissues of litchi fruit during storage period (SP). Vertical bars represent ± SE of means and are invisible when the values are smaller than the symbol. n = 25, LSD ($P \leq 0.05$) for fruit weight loss: T = 0.64, SP = 0.57, T × SP = 1.28; pericarp browning: T = 0.22, SP = 0.20, T × SP = 0.45; anthocyanin contents: T = 0.09, SP = 0.08, T × SP = NS and polyphenol oxidase enzyme: T = 5.98, SP = 5.35, T × SP = NS. NS = not significant.

Fig. 2 Effect of postharvest application of ascorbic acid (T) SSC (A), TA (B) and SSC: TA (C) in aril tissues of litchi fruit during storage period (SP). Vertical bars represent ± SE of means and are invisible when the values are smaller than the symbol. n = 25, LSD ($P \leq 0.05$) for SSC: T = 0.50, SP = 0.45, T × SP = 1.01; TA: T = 0.05, SP = 0.04, T × SP = NS and SSC: TA: T = 11.69, SP = 10.45, T × SP = 23.38. NS = not significant.

Fig. 3 Effect of postharvest application of ascorbic acid (T) on total phenolic contents (A) and total antioxidants (B) in pericarp tissues of litchi fruit during storage period (SP). Vertical bars represent ± SE of means and are invisible when the values are smaller than the symbol. n = 25, LSD ($P \leq 0.05$) for total phenolic contents: T = 16.87 SP = 15.08, T × SP = 33.73 and total antioxidants: T = 3.84, SP = 3.44, T × SP = NS. NS = not significant.

Fig. 4 Effect of postharvest application of ascorbic acid (T) on total phenolic contents (A), total antioxidants (B) and ascorbic acid contents (C) in aril tissue of litchi fruit during

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Vol: 65 Issue 02 | 2022
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storage period (SP). Vertical bars represent ± SE of means and are invisible when the values are smaller than the symbol. n = 25, LSD ($P \leq 0.05$) for total phenolic contents: T = 20.86, SP = 18.66, T × SP = 41.73; total antioxidants: T = 5.31, SP = 4.75, T × SP = NS and ascorbic acid contents: T = 5.91, SP = 5.28, T × SP = NS. NS = not significant.

Fig. 5 Effect of postharvest application of ascorbic acid (T) on the activities of superoxide dismutase (A), catalase (B) and peroxidase (C) enzymes on pericarp and aril tissues of litchi fruit during storage period (SP). Vertical bars represent ± SE of means and are invisible when the values are smaller than the symbol. n = 25, LSD ($P \leq 0.05$) for superoxide dismutase: T = 3.31, SP = 2.96, T × SP = 6.61; catalase: T = 7.89, SP = 7.06, T × SP = NS and peroxidase enzymes: T = 4.09, SP = 3.66, T × SP = NS. NS = not significant; whereas, for aril tissues for superoxide dismutase: T = 3.64, SP = 3.26, T × SP = NS; catalase: T = 3.76, SP = 3.36, T × SP = NS and peroxidase enzymes: T = 3.10, SP = 2.77, T × SP = 6.20. NS = not significant.

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Fig. 1.

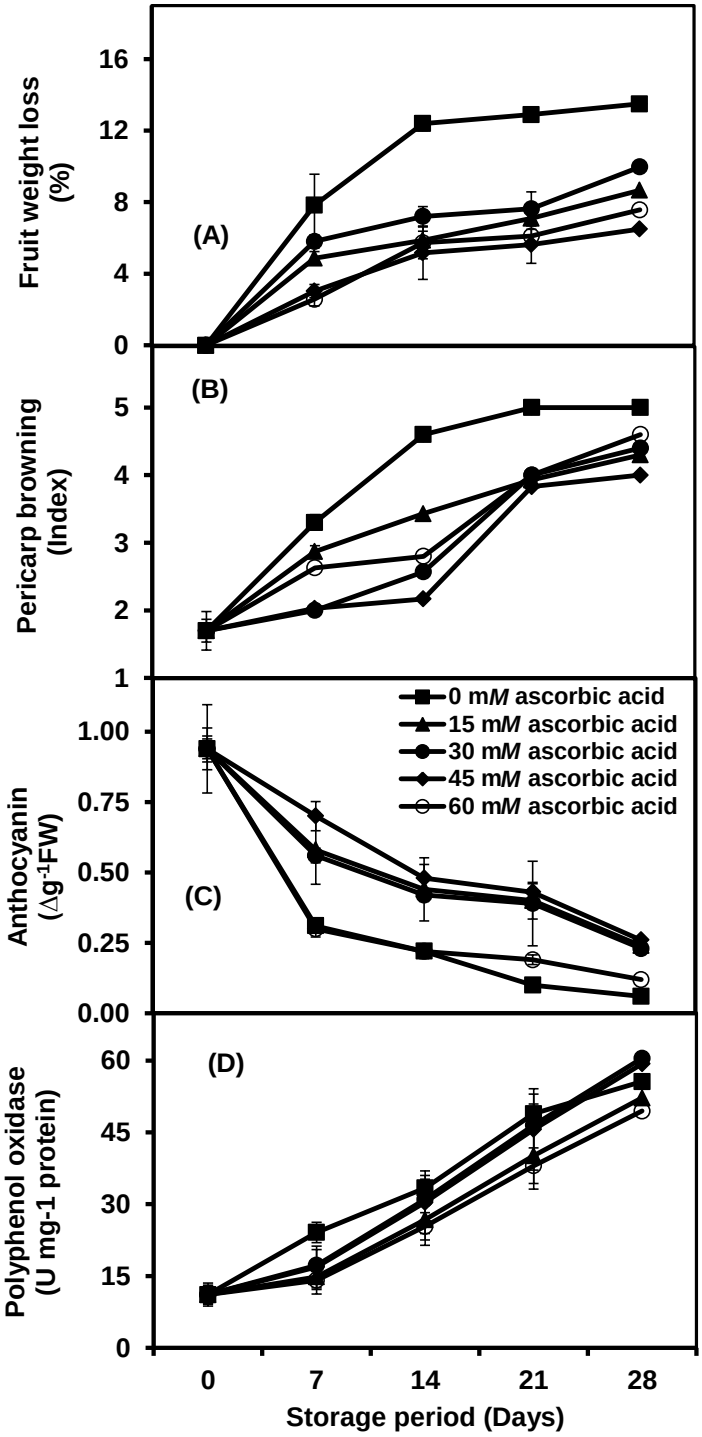

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Fig. 2.

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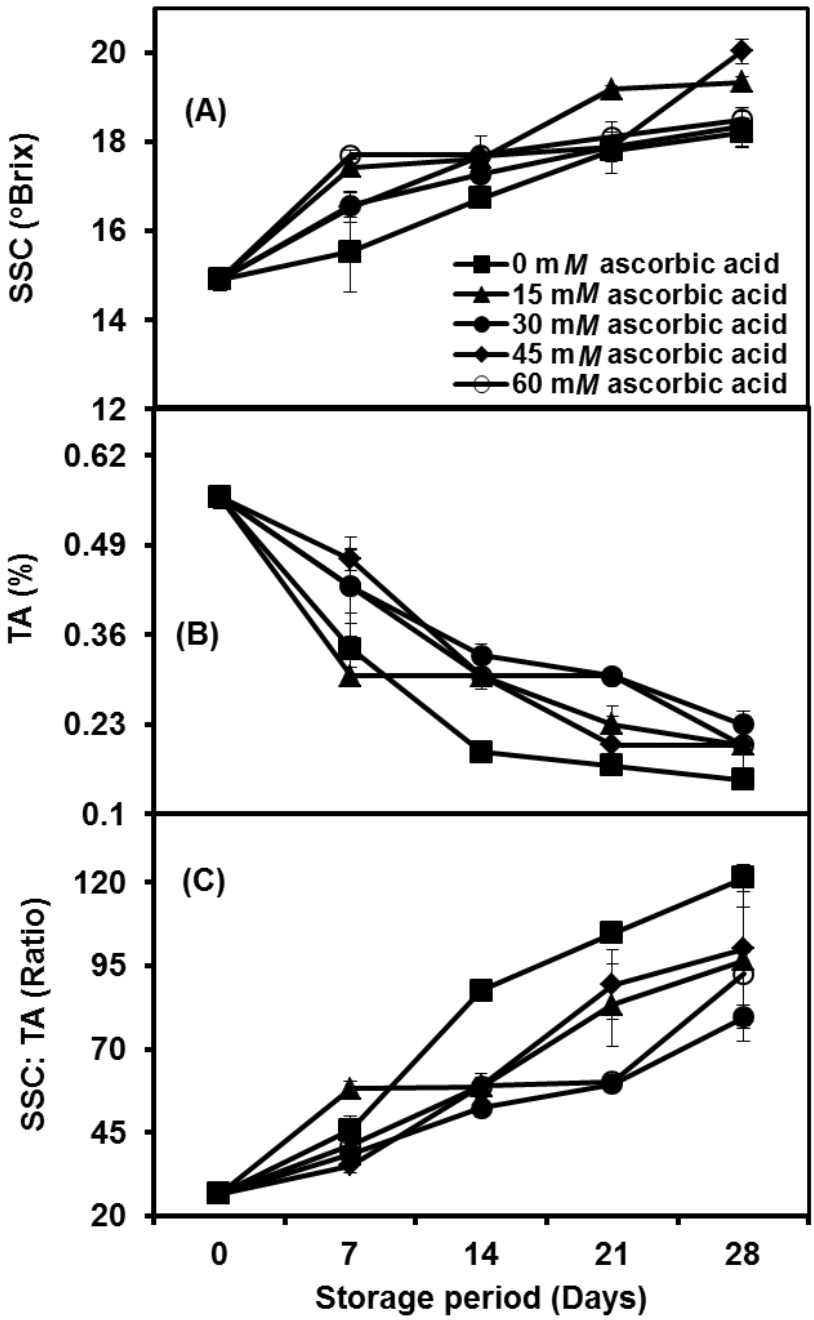

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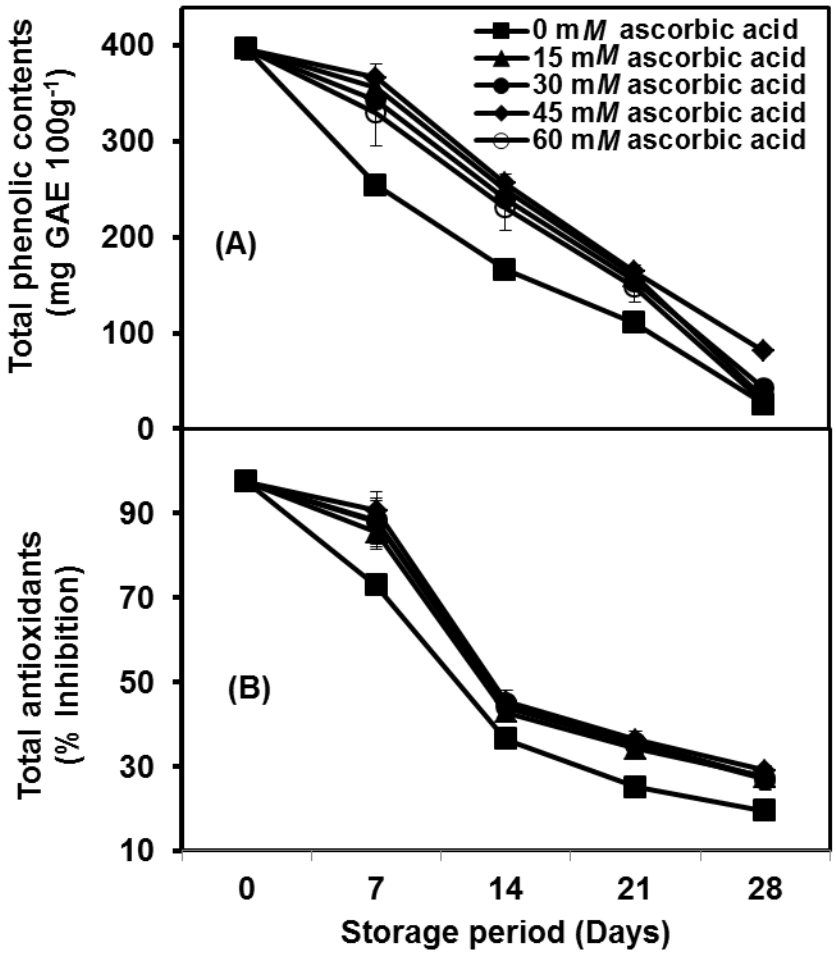
Fig. 3.



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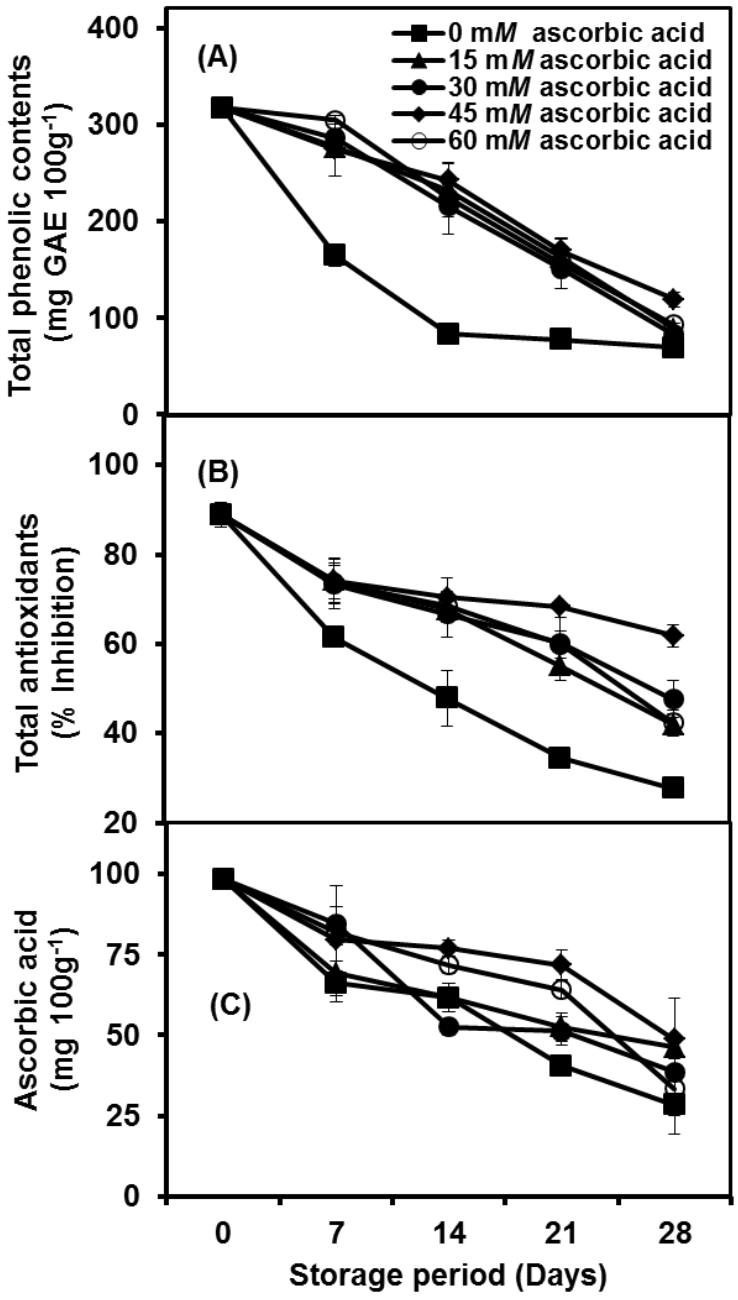

Fig. 5.

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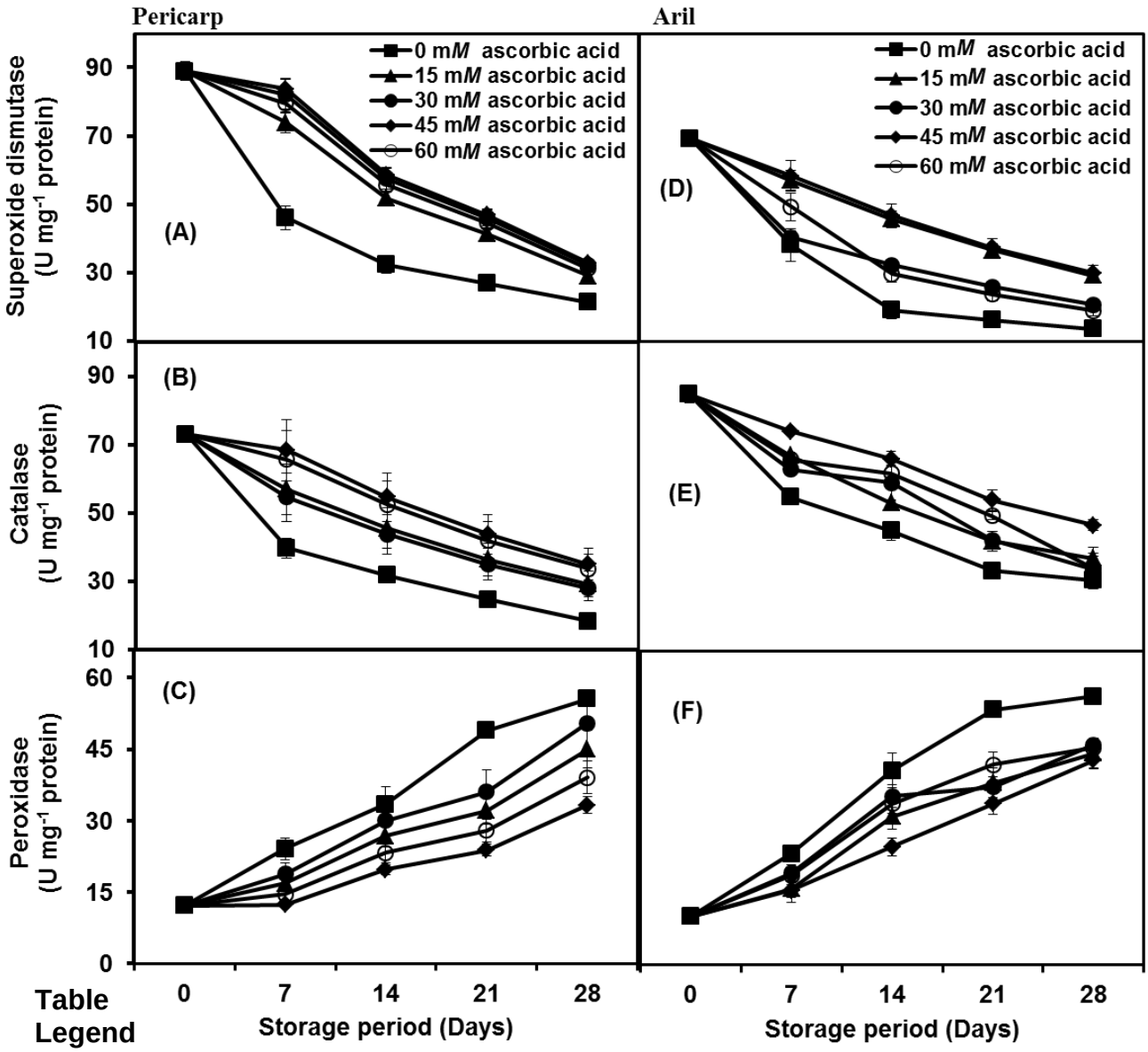

Table 1 Relationship of pericarp browning index with activities of PPO, POD, SOD, CAT enzymes, TPC and total antioxidants in ascorbic acid-treated litchi peel/pulp tissues

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PBI = pericarp browning index, PPO = polyphenol oxidase, POD = peroxidase, TPC =

Parameter	Peel	Pulp
PBI *vs* anthocyanin Contents	-0.511**	---
PBI *vs* PPO	0.762**	---
PBI *vs* POD	0.678**	0.772**
PBI *vs* SOD	-0.810**	-0.646**
PBI *vs* CAT	-0.658**	-0.793**
PBI *vs* TPC	-0.831**	-0.815**
PBI *vs* total antioxidants	-0.714**	-0.6872**

total phenolic contents, SOD = superoxide dismutase, CAT = catalase, ** = significant at $P \leq 0.01$.