

## INFLUENCE OF HARVEST LOCATION AND CULTIVAR ON PERICARP BROWNING AND BIOCHEMICAL FRUIT QUALITY OF LITCHI (*Litchi chinensis* Sonn.)

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Litchi is known for its delicious taste and reddish pericarp colour. However, pericarp browning limits the postharvest life of litchi fruit with reduced fruit quality. Therefore, present study was conducted to investigate the influence of harvest location (Lahore and Haripur) as well as cultivar ('Gola' and 'Serai') on pericarp browning, changes in the biochemical quality attributes and enzymatic activities in litchi fruit. Fruit harvested from Lahore exhibited significantly higher pericarp browning index (3.99), weight loss (12.7%), ascorbic acid contents (48.9 mg 100 g<sup>-1</sup>) and activities of enzymes responsible for browning (peroxidase and polyphenol oxidase) as compared to Haripur. Moreover, fruit harvested from Lahore exhibited less SSC: TA ratio (105.3), total phenolic contents (187.35 mg GAE 100 g<sup>-1</sup>), total antioxidants (45.27%) and activity of superoxide dismutase enzyme (40.86 U mg<sup>-1</sup> protein) than that of Haripur. On the other hand, 'Serai' fruit exhibited higher weight loss (12.86%), SSC (19.58°Brix), TA (0.24%), activities of peroxidase (35.81 U mg<sup>-1</sup> protein) and polyphenol oxidase (25.22 U mg<sup>-1</sup> protein) enzymes. Moreover, lower level of TPC (202.9 mg GAE 100 g<sup>-1</sup>), total antioxidants (50.2%), activities of superoxide dismutase (39.85 U mg<sup>-1</sup> protein) and catalase (22.32 U mg<sup>-1</sup> protein) enzymes were observed in 'Serai' than 'Gola' fruit. Results suggested that fruit harvested from Haripur exhibited better fruit quality characteristics and browning index than fruit harvested from Lahore. Cultivar 'Gola' showed better quality with less pericarp browning than 'Serai'.

**Keywords:** Antioxidants, enzymes, fruit quality, litchi, physico-chemical characteristics

### INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) belongs to tropical/subtropical areas that are considered to be originated near Southern China and Northern Vietnam with China being the largest producer in the world (Menzel, 2001). It has high market value due to deliciously flavoured translucent juicy aril. It is one of the most nutritious fruit as it contains 16.83 g carbohydrate, 72 mg vitamin C, 830 mg protein, 171 mg potassium and 10 mg magnesium per 100 g of fruit (Anonymous, 2012). In Pakistan, litchi is grown on an area of about 572 ha with 9250 tons production (Shah, 2003).

However, postharvest pericarp browning is the main issue in litchi fruit which involves rapid browning of fruit skin after harvest (Kumar *et al.*, 2013), thus affecting the fruit quality and hindering its market potential (Rajwana *et al.*, 2010). Different biochemical/physiological changes, enzymatic changes such as, reduction of membrane integrity, degradation of anthocyanins, increase in peroxidase (POD), polyphenol oxidase (PPO) activities and catalyzation of superoxidation reaction have been reported to be associated with pericarp browning in litchi (Jiang, 2000). Several efforts have been made to overcome this issue by using postharvest fruit coatings (Joas *et al.*, 2005), different antioxidants (Kumar *et al.*, 2013), oxalic acid (Zheng and

Tian, 2006), ascorbic acid (Sun *et al.*, 2010), however, with variable and sporadic results.

Due to its unique taste and high nutritional value, the demand of litchi is increasing day by day in Pakistan. At present no information is available about the effect of harvest locations and cultivars on the pericarp browning and fruit quality of litchi. Previously, location influenced many fruit characteristics that vary with cultivars (Knee and Smith, 1989). It is hypothesized that incidence of pericarp browning and quality will be affected by locations of land and cultivars. Therefore, it is the need of the time to further investigate that which area and cultivar is best suited for litchi cultivation with reduced pericarp browning at ambient conditions. Hence, present study was conducted to determine the effect of locations and cultivars on pericarp browning, enzymatic activity and fruit quality of litchi at ambient conditions.

### MATERIALS AND METHODS

**Plant material:** Fruit of litchi (*Litchi chinensis* Sonn.) 'Gola' and 'Serai' was harvested from two locations i.e. Saggian Bridge Lahore (31°35.511'N, 74°15.375'E) and Fruit Farm Nursery Haripur (34°00.114'N, 72°56.779'E). After harvest fruits were transported to Postharvest Research and Training

Center (PRTC) in reefer vein. Fruit of uniform, size, shape, free from physical damage were selected for the study and kept at ambient conditions (25-30°C and 65±1% RH). Various enzymatic and physico-chemical fruit quality attributes were evaluated to test the effects of different harvest locations and cultivars. The study included three factors (i.e. location, cultivar and days) and was conducted under Completely Randomized Design (CRD) with three factor factorial arrangement. The data were recorded on fruit weight loss (%) and pericarp browning index. Fruit quality parameters [soluble solid contents (SSC), titratable acidity (TA), SSC: TA ratio, ascorbic acid] were determined in pulp tissues, whereas, total phenolic contents (TPC), total antioxidants, anthocyanins contents and activities of peroxidase (POD), polyphenol oxidase (PPO), catalase (CAT) and superoxide dismutase (SOD) enzymes were determined in peel tissues of litchi fruit.

**Pericarp browning:** Pericarp browning assessment was done by method given by Jiang *et al.* (2004) based upon measuring the extent of total area under browning of each fruit, using rating scale: 1 = no browning, 2 = slight browning, 3 = <1/4 browning, 4 = 1/4–1/2 browning, 5 = >1/2 browning. The browning index was calculated using the formula:  $\sum (\text{browning scale} \times \text{percentage of corresponding fruit within each class})$ .

**Weight loss:** It was measured in percentage by calculating the decrease in initial weight of the 25 fruit and then average decrease in weight was measured for 1, 3, 5 days in percentage.

**Biochemical analysis:** SSC (°Brix) of fruit juice was determined with a digital refractometer (Atago, ATC-1, Tokyo, Japan). For determination of titratable acidity (TA), fruit juice samples were titrated against 0.1 N NaOH using 2–3 drops of phenolphthalein as an indicator, and the results were expressed in percentage, whereas for the determination of ascorbic acid (AA), 5 mL of aliquot (filtered out of 10 mL of juice and 90 mL of 0.4% oxalic acid solution) was titrated against 2, 6-dichlorophenolindophenol dye solution and results were expressed in “mg 100 g<sup>-1</sup>” as reported by Khalid *et al.* (2012).

**Anthocyanin contents:** Anthocyanin contents were determined by using method of Zheng and Tian (2006). Pericarp tissue sample (1 g) obtained from 25 fruits was extracted in 15 mL HCl with methanol (0.15% HCl: 95% methanol = 15:85) for 4 h. The extract was filtered and its absorbance was determined at 530, 620 and 650 nm, respectively. Determination of anthocyanin contents was done by using the formula:  $\Delta A \text{ g}^{-1} = (A_{530} - A_{620}) - 0.1(A_{650} - A_{620})$ .

**Total phenolic contents and total antioxidants:** Total phenolic contents (TPC) from litchi peel tissues were determined by the method of Ainsworth and Gillespie (2007), using Folin–Ciocalteu (FC) reagent. The concentration of TPC was expressed as the gallic acid equivalent (mg GAE 100 g<sup>-1</sup>) of the lyophilized sample

(Ullah *et al.*, 2013). The DPPH free radical scavenging activity of total antioxidants were measured by bleaching the purple- coloured ethanol solution of the stable DPPH radical, according to the method reported by Ullah *et al.* (2013).

**Enzyme assay:** Pericarp samples from 25 litchi fruits were stored at -80°C. Pooled samples of litchi peel were finely chopped with processor and 1 g of chopped peel was mixed with 10 mL (100 M) potassium citrate buffer (4 pH) containing polyvinylpyrrolidone (PVP) in falcon tubes. Falcon tubes containing sample were centrifuged (5,000 × g) for 5 min at 4°C to get the supernatant. This supernatant was further used for enzyme analysis. The assay mixture for PPO contained freshly prepared 1.45 mL 100 mM potassium citrate buffer (6.8 pH) and 0.50 mL 100 mM 4-methylcatechol (4-MC). The absorption was noted at 412 nm on ELISA plate reader (Model ELX800, Bio-Tek Instruments, Inc, USA). PPO activity was determined as enzyme units (U mg<sup>-1</sup> protein), defined as the quantity of enzyme required to produce 1 µmol min<sup>-1</sup> product (Waite, 1976). SOD activity was measured in terms of its capacity to inhibit photochemical reduction of nitroblue tetrazolium (NBT) according to method described by Stagner and Popovic (2009). CAT was measured as U mg protein<sup>-1</sup>, where one unit was defined as “an absorbance change in 0.01 unit min<sup>-1</sup>”. POD activity was determined by the guaiacol method (Liu *et al.*, 2011).

**Statistical analysis:** The experimental data were subjected to analysis of variance (ANOVA) using Statistix 9 for windows software with three-factor factorial arrangements including cultivars, harvest locations and fruit shelf period. Each experimental unit was consisted of 25 fruits with three replicates. The effects of treatments were determined from the least significant differences test (Fisher’s LSD) at  $P \leq 0.05$ , where the F test was significant (Steel *et al.*, 1997).

## RESULTS

**Weight loss:** Fruit weight loss showed significant increase with the advancement of shelf period regardless of cultivar and harvest location. However, highest fruit weight loss (23.3%) was exhibited by ‘Serai’ fruit harvested from Lahore after 5 days of storage (Table 1). Overall fruit of litchi cultivar ‘Gola’ exhibited 1.05-fold less weight loss than ‘Serai’ (Table 2).

**Pericarp browning, PPO and anthocyanin:** Pericarp browning index was significantly and gradually increased with progress in shelf period (day-1 to day-5), irrespective to location of harvest and cultivar (Fig. 1A and 1D). On day-5 of shelf period fruit harvested from Lahore showed 1.6-fold higher pericarp browning index than fruit harvested from Haripur. However, no significant difference among cultivars was noticed regarding their browning index (Table 2). Increasing trend was observed in the activity of PPO from Day-1 to Day-5 of shelf period (Fig. 1C and 1F). Overall fruit of litchi cv. ‘Gola’ showed significantly lesser PPO activity (10%) than ‘Serai’. Fruit harvested from Lahore exhibited 17% higher activity of PPO than fruit from

**Table 1. Effect of harvest locations and cultivars on weight loss, SSC, TA and SSC: TA ratio of litchi fruit during shelf life**

Location	Cultivar	Weight loss (%)			SSC (°Brix)			TA (%)			SSC: TA		
		Shelf period (Days)			Shelf period (Days)			Shelf period (Days)			Shelf period (Days)		
		1	3	5	1	3	5	1	3	5	1	3	5
Lahore	'Gola'	0.0f	15.0b	20.9c	14.2f	14.4f	17.43e	0.30ab	0.17cd	0.10de	46.2d	88.9d	196.6c
	'Serai'	0.0f	17.2f	23.3a	17.4e	19.7bc	23.3a	0.33a	0.30ab	0.10de	50.0d	66.0d	183.8c
Haripur	'Gola'	0.0f	9.2e	22.3ab	17.3e	18.3cde	17.0e	0.27ab	0.23bc	0.03e	67.6d	81.7d	482.6a
	'Serai'	0.0f	17.6d	22.1ab	17.6de	19.3bcd	20.1b	0.30ab	0.33a	0.10de	57.9d	56.7d	339.1b
LSD value ( $P \leq 0.05$ )		Locations (L)			Cultivars (C)			Shelf period (S)			L x C x S		
weight loss		0.8194			0.8194			1.0036			2.0071		
SSC		0.7405			0.7405			0.9069			1.8139		
TA		0.0281			0.0281			0.0344			0.0688		
SSC:TA		38.687			38.687			47.382			94.763		

The means sharing the same letter are non-significant at  $P \leq 0.05$ , D = number of days after harvest, SSC = soluble solid contents, TA = titratable acidity

**Table 2. Mean effect of harvest locations and cultivars on physiological weight loss, browning index and biochemical quality attributes of litchi fruit**

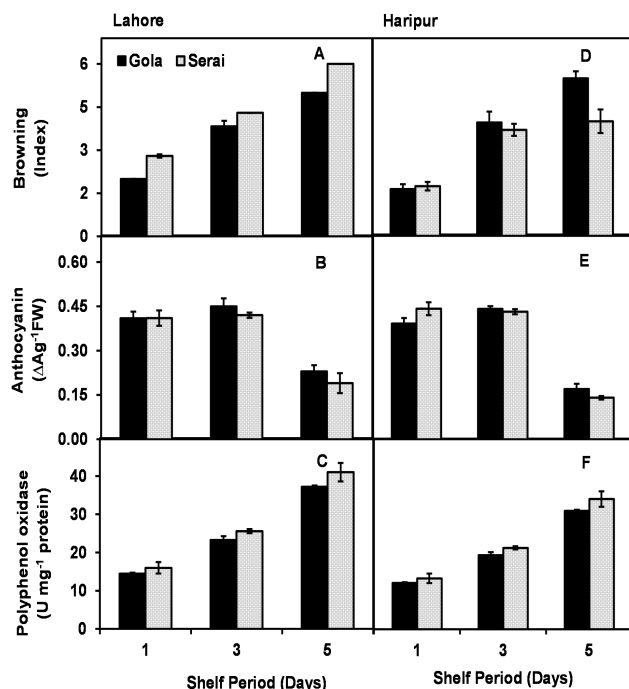
Parameter	Harvest locations		LSD Value $P \leq 0.05$	Cultivars		LSD Value $P \leq 0.05$
	Lahore	Haripur		Serai	Gola	
Weight loss (%)	12.70a	11.30b	0.8194	12.86a	11.23b	0.8194
Pericarp browning	3.99a	3.42b	0.2429	3.76a	3.66a	NS
SSC (Brix°)	17.74a	18.27a	NS	19.58a	16.43b	0.7405
TA (%)	0.22a	0.21a	NS	0.24a	0.18b	0.0281
SSC:TA	105.25b	180.86a	38.687	125.51a	160.60a	NS
Ascorbic acid (mg 100 g <sup>-1</sup> )	48.89a	30.83b	3.5253	41.39a	38.33a	NS
TPC (mg GAE 100 g <sup>-1</sup> )	187.35b	234.18a	3.5224	202.91b	218.62a	3.5224
ASA (%)	45.27b	58.00a	1.8947	50.21b	53.17a	1.8947
Anthocyanin contents ( $\Delta\text{Ag}^{-1}\text{FW}$ )	0.35a	0.334a	NS	0.34a	0.35a	NS
CAT (U mg protein <sup>-1</sup> )	25.87a	27.05a	NS	22.32b	30.60a	3.2340
POD (U mg protein <sup>-1</sup> )	36.74a	30.62b	4.0498	35.81a	31.55b	4.0498
SOD (U mg protein <sup>-1</sup> )	40.86b	52.16a	3.6937	39.85b	53.17a	3.6937
PPO (U mg <sup>-1</sup> Protein <sup>-1</sup> )	26.26a	21.88b	1.3813	25.22a	22.91b	1.3813

Means followed by different letters for a given parameter for harvest location significantly different at  $P \leq 0.05$  (LSD test). NS = non-significant ( $P \leq 0.05$ ). SSC = soluble solid concentration, TA = titratable acidity, AA = ascorbic acid, TPC = total phenolic contents, ASA = antioxidant scavenging activity, CAT = catalase, POD = peroxidase, SOD = superoxide dismutase, PPO = polyphenol oxidase

Haripur (Table 2). Overall decreasing trend in anthocyanin contents was observed with increase in shelf life period. Non-significant increase in anthocyanin contents was observed on day-3 but a significant decline was noticed at day-5 of shelf period (Fig. 1B and 1E). However, non-significant differences were observed between locations of harvest and cultivars (Table 2).

**SSC, TA, SSC: TA and Ascorbic acid:** Significant increase in SSC of fruit juice was observed as number of days at shelf period progressed. SSC showed increasing trend in both cultivars ('Gola' and 'Serai') irrespective of harvest location. It was observed that 'Serai' fruit exhibited higher SSC as compared to 'Gola' in both locations with increase in shelf

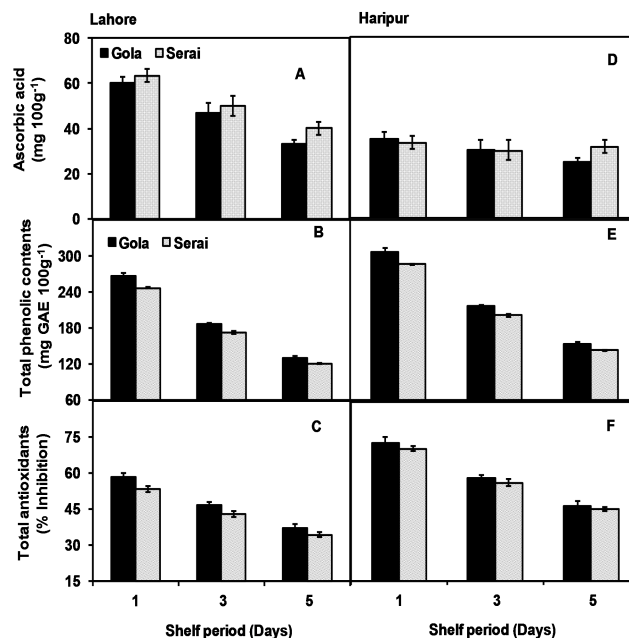
life period (Table 1). There was no significant difference in SSC with respect to location. 'Serai' fruit exhibited 1.2-fold higher SSC as compared to 'Gola' fruit (Table 2). Titratable acidity (TA) percentage showed decreasing trend with increase in number of days at shelf period with maximum TA calculated on day-1 of shelf period (0.33%) and minimum TA was recorded on day-5 (0.10%) (Table 1). Significant increase in SSC: TA ratio was noticed as shelf period increased with minimum SSC: TA being 46.2 on day-1 of shelf period in fruit of 'Gola' cultivar harvested from Lahore and maximum SSC: TA was noted on day-5 of shelf period (482.6) (Table 1).



**Figure 1.** Effect of harvest locations and cultivars on pericarp browning (A, D), anthocyanin contents (B, E) and polyphenol oxidase (C, F) in litchi fruit during shelf life at ambient conditions. Vertical bars represents  $\pm$  SE of means.

Fruit harvested from Haripur exhibited 1.72-fold higher SSC: TA ratio than the fruit harvested from Lahore. However, SSC: TA ratio did not show significant difference among cultivars (Table 2). Ascorbic acid contents were decreased with increase in number of days at shelf period (Fig. 2A and 2D). Maximum ascorbic acid contents were noted in litchi cv. 'Serai' ( $63.3 \text{ mg } 100 \text{ g}^{-1}$ ) harvested from Lahore on day-1 of shelf period (Fig. 2A), whereas, lowest ascorbic acid contents ( $25 \text{ mg } 100 \text{ g}^{-1}$ ) were recorded on day-5 of shelf period in fruit harvested from Haripur (Fig. 2D). Fruit from Lahore showed 1.58-fold higher ascorbic acid contents than collected from Haripur (Table 2).

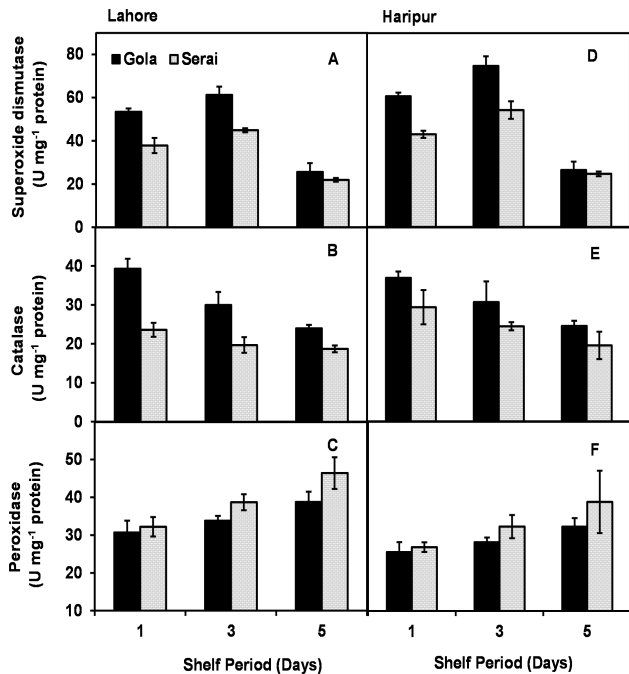
**Total phenolic contents and total antioxidants:** Significant decrease in TPC of both cultivars and locations was observed as number of days at shelf period progressed. On day-1 of shelf period, highest amount of TPC were recorded in both cultivars harvested from both locations while minimum was recorded at day-5 of shelf period (Fig. 2B and 2E). Overall fruit of 'Gola' cultivar exhibited significantly higher TPC as it showed about 1.1-fold more TPC than 'Serai' (Table 2). Fruits harvested from Haripur exhibited 1.25-fold higher TPC than from Lahore (Table 2).



**Figure 2.** Effect of harvest locations and cultivars on ascorbic acid (A, D), total phenolic contents (B, E) and total antioxidants (C, F) in litchi fruit during shelf life at ambient conditions. Vertical bars represents  $\pm$  SE of means.

Similarly total antioxidants decreased significantly in both locations and cultivars with the passage of days at ambient conditions. In both locations highest total antioxidants were recorded on day-1 of shelf period and lowest were recorded on day-5 of shelf period (Fig. 2C and 2F). There was significance difference in concentration of total antioxidants with respect to location as fruit harvested from Haripur showed 1.3-fold higher total antioxidants than fruit harvested from Lahore (Table 2). Fruit of 'Gola' cultivar exhibited significantly higher (1.05-fold) total antioxidants concentration as compared to 'Serai' fruit (Table 2).

**CAT, SOD and POD:** Varying behaviour in SOD activity was noted as it was increased till day-3 and later on decreased significantly on day-5 of shelf period (Fig. 3A and 3D). SOD activity was cumulatively 25% higher in fruit of litchi cv. 'Gola' than 'Serai' (Table 2). Decreasing trend in CAT activity was observed as shelf period progressed (Fig. 3B and 3E). Effect of location on CAT activity did not show any significant difference, whereas, difference among cultivars was significant (Table 2). Overall fruit of litchi cultivar 'Gola' exhibited 27% higher CAT activity than 'Serai' irrespective of location of harvest (Table 2). POD activity was increased significantly from day-1 to day-5 of shelf period (Fig. 3C and 3F). Overall fruit of 'Gola' exhibited 13.7% lower POD activity as compared to 'Serai' (Table 2) and fruit harvested from Haripur showed 19.6% less POD activity as compared to fruit harvested from Lahore (Table 2).



**Figure 3.** Effect of harvest locations and cultivars on superoxide dismutase (A, D), catalase (B, E) and peroxidase (C, F) in litchi fruit during shelf life at ambient conditions. Vertical bars represents  $\pm$  SE of means.

## DISCUSSION

As expected, fruit weight loss was increased significantly with increase in shelf period (Table 1). The increase in weight loss was probably due to moisture loss through transpiration. Tendency of weight loss with increase in time period was also confirmed by Mitra and Kar (2001) in litchi cv. 'Bombai'. Increase in SSC with increase in shelf period might be due to higher water loss (Table 1). These results were also confirmed in grapes berries by Tanada-Palmu and Grosso (2005). Results are also in agreement with Aklimuzzaman *et al.* (2011) who observed increase in SSC with increase in storage duration in litchi cv. 'Bedana'. TA showed continuous decline with passage of shelf period in fruit harvested from both locations (Table 1), due to which SSC: TA ratio was increased with increase in shelf period (Table 1). Decrease in TA might be ascribed to conversion of organic acids into sugars during respiration or due to the metabolic changes in fruit (Gimnez *et al.*, 2003 and Shiri *et al.*, 2011).

Pericarp browning is one of the major postharvest problems in litchi which reduces its commercial value. In the present study, linear increase in pericarp browning index was observed in litchi fruit cv. 'Serai' and 'Gola' from day-1 to day-5 of shelf period at ambient conditions (Fig. 1A and 1D). Whereas, anthocyanin contents decreased regularly at ambient conditions with increase in shelf period (Fig. 1B

and 1E). This increase in pericarp browning and decrease in anthocyanin contents might be ascribed to increase in the activities of POD and PPO enzymes. During shelf period litchi fruit undergo stress with loss of moisture, breakdown of cell membrane and increased activity of PPO. When PPO comes in contact with anthocyanin contents in presence of oxygen, the anthocyanins undergo irreversibly broken down into melanin by-products causing pericarp browning (Lin *et al.*, 1988). Similarly, in this study degradation of anthocyanin contents might be ascribed to higher activities of POD and PPO and these results were also confirmed in litchi fruit by Zauberman *et al.* (1991). Activity of PPO was increased from day-1 to day-5 of shelf period (Fig. 1C and 1F). At ambient conditions humidity was low that increased pH and PPO became active. Jiang (2000) also endorsed these results where maximum activity PPO was observed at pH 6.8 and zero activity below 4.0 pH in litchi cv. 'Huaizhi'. Significant reduction in ascorbic acid contents was noticed with progress in shelf period because ascorbic acid is also one of the organic acids and it decreased continuously in this study due to oxidation (Fig. 2A and 2D) (Gimnez *et al.*, 2003). TPC decreased continuously from day-1 to day-5 of shelf period (Fig. 2B and 2E). This reduction may be attributed to the high rate of oxidation of TPC by PPO with progress in shelf period. These results confirm the findings of Altunkaya and Gokmen (2008) where decline in TPC of lettuce was observed due to oxidation of PPO. Total antioxidants were decreased continuously from day-1 to day-5 of shelf period. The reduction in total antioxidants at ambient conditions with increase in shelf period might be due to oxygen stimulated oxidation of ascorbic acid and phenolic compounds (Stewart *et al.*, 1999). CAT and SOD activities were also decreased as number of days at shelf progressed (Fig. 3A, 3B, 3D and 3E). The decrease in CAT activity may be due to the prolonged oxidative stress at ambient conditions during shelf period. SOD and CAT enzymes are directly related to the senescence of the fruit, as in this study fruit deteriorated rapidly and peel turned brown, activities of SOD and CAT also decreased till day-5 of shelf period, resulting in pericarp browning of the fruit with lowering the quality of aril (Sun *et al.*, 2010). Activity of POD increased from day-1 to day-5 of shelf period at ambient conditions (Fig. 3C and 3F). Moisture loss resulted in increased pH that caused activation of POD. These results were also supported by Mizobutsi *et al.* (2010) who observed that POD activity could be inhibited more effectively at acidic pH rather than at alkaline pH.

**Conclusion:** Cultivars and harvest locations significantly influenced pericarp browning and physico-chemical attributes along with the activities of antioxidative enzymes in litchi fruit. Cultivar 'Gola' exhibited superior fruit quality characteristics than 'Serai', while Haripur location produced fruit with better quality than Lahore.

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