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*Full Length Article*

# Cultivar and Harvest Location Influence Fruit Softening and Antioxidative Activities of Peach during Ripening

**Sami Ullah[1], Ahmad Sattar Khan[1*], Aman Ullah Malik[1] and Muhammad Shahid[2]**
[1]*Postharvest Research and Training Centre, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, 38040, Pakistan*
[2]*Protein Molecular Biology Lab, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, 38040, Pakistan*
[*]For correspondence: ahmad_khan157@yahoo.com

## Abstract

Fruit softening and quality management is very important to reduce postharvest losses in peach. Present study was conducted to observe the effect of harvest location and cultivars on peach fruit softening and quality during ripening at ambient conditions (30±1°C and 60-65% RH). Fruit harvested from Sillanwali exhibited significantly higher ethylene production (24.27 µL $C_2H_4$ $kg^{-1}$ $h^{-1}$), respiration rate (1.04 mL $CO_2$ $kg^{-1}$ $h^{-1}$), ascorbic acid contents (11.11 mg 100 $g^{-1}$), activities of catalase (CAT; 47.53 U mg $protein^{-1}$), peroxidase (POD; 32.48 U mg $protein^{-1}$), *endo*-polygalacturonase [(*endo*-PG; 11.61 Δ viscosity (mg $protein^{-1}h^{-1}$)], *exo*-polygalacturonase (*exo*-PG; 138.9 µg gal acid mg $protein^{-1}$ $h^{-1}$) enzymes, and significantly lower fruit firmness (25.83 N), ground colour (1.65 score), soluble solid contents (SSC; 11.15 °Brix), total phenolic contents (TPC; 72.28 mg GAE $100g^{-1}$), antioxidant scavenging activity (ASA 47.72%), activities of pectin esterase (PE; 1.19 m*M* NaOH mg $protein^{-1}h^{-1}$) and endo-1,4-*β*-D-Glucanase [(EGase; 2.57 Δ viscosity (mg $protein^{-1}$ $h^{-1}$)] enzymes as compared to fruit harvested from Soan Valley. Peach cv. 'Early Grand' showed significantly higher ethylene production (22.35 µL $C_2H_4$ $kg^{-1}$ $h^{-1}$), respiration rate (0.85 mL $CO_2$ $kg^{-1}$ $h^{-1}$), ASA (59.5%), activities of CAT (49.35 U mg $protein^{-1}$), *endo*-PG [(11.43 Δ viscosity (mg $protein^{-1}$ $h^{-1}$)] and *exo*-PG (147.22 µg gal acid mg $protein^{-1}$ $h^{-1}$) enzymes, whereas lower fruit firmness (30 N), TPC (108.33 mg GAE 100 $g^{-1}$), activities of superoxide dismutase (SOD; 27.75 U mg $protein^{-1}$), PE (1.17 m*M* NaOH mg $protein^{-1}$ $h^{-1}$) and EGase [(2.54 Δ viscosity (mg $protein^{-1}$ $h^{-1}$)] enzymes than 'Flordaking'. In conclusion, harvest locations and cultivars have significant influence on the various physico-chemical attributes including activities of various fruit softening and antioxidative enzymes in peach fruit. 

**Keywords:** Antioxidant; Cultivars; Fruit quality; Harvest location; Peach; Softening

## Introduction

Cultivation of *Prunus* fruit is common in temperate regions throughout the world. Peaches and nectarines are the most prominent fruits within the group. The annual world production of peach and nectarine is about 20.27 million tonnes with an area of 1.53 million ha (FAOSTAT, 2011). In Pakistan, peach is an emerging fruit crop cultivated on an area of 15.3 thousand ha with 54 thousand tonnes production (GOP, 2010). During the last decade its production and area had been doubled in the country, which is highly appreciated due to its unique aesthetic, organoleptic characteristics, and high neutraceautical values.

During ripening fruit exhibit various pattern and mechanism of softening. Some fruit soften rapidly during ripening after harvesting such as peach, apricot and strawberry, whereas others soften slowly like apple and pear (Bourne, 1979). Therefore, individual fruit must be cared for changes during ripening, even in some circumstances fruit ripening pattern in one cultivar may not be applicable to other cultivars within the same species (Goulao and Oliveira, 2008). Previously, fruit appearance, sugars and organic acids are the traditional attributes which had been studied in relation to fruit quality during ripening in different fruit like apricot (Drogoudi *et al.*, 2008). However, myriad of phytochemicals present in relatively low concentration have a key role in overall fruit softening and quality (Terry, 2011). Among the phytochemicals, total antioxidants are the key bioactive compounds present in peach fruit. Various factors have been reported to affect the levels of enzymatic and non-enzymatic antioxidants in *Prunus* species, such as genotype (Hegedus *et al.*, 2010) and geographic region of cultivation (Dragovic-Uzelac *et al.*, 2007). It has been reported that the concentration of these compounds showed dramatic differences during fruit ripening in different varieties of the same fruit. For instance in apricot, differences of about ten folds in carotenoid contents have been observed among the 37 varieties (Ruiz *et*

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*al.,* 2005), and total phenolic contents have also showed a great variation in 29 varieties of apricot including some hybrids (Drogoudi *et al.,* 2008). Variation among these phytochemicals has been found with respect to different geographic conditions like an increased level of bioactive compounds including ascorbic acid has been related with sunlight exposure in fruit (Lee and Kader, 2000). Similarly, fruit development period also influences quality of peach fruit as Baron *et al.* (1995) reported that short fruit development period can reduces fruit quality in early ripening peach cultivars.

On the other hand dynamics of fruit softening and antioxidative enzymes during fruit ripening is very important as fruit texture, internal quality and the senescence rate during ripening and storage have been associated with oxidative stress. Fruit softening is a very important aspect of ripening process, which depends on activity of fruit softening enzymes (Payasi *et al.,* 2009). The antioxidants delay lipid peroxidation and concomitant decrease in membrane leakage associated with senescence (Lurie *et al.,* 2003). Ascorbic acid level has been found to first drop and then increase progressively to reach maximum with advancement of fruit ripening in sweet cherry (Serrano *et al.,* 2005). To the best of our knowledge, presently no information is available on effect of harvest locations and cultivars on changes in the fruit antioxidative enzymes along with fruit softening in peach during ripening. We hypothesized that peach cultivars harvested from different growing locations will exhibit differences in their eating quality during ripening. Keeping in view the above factors two peach cultivars harvested from two locations having different fruit development period were studied for changes in fruit quality during ripening at ambient conditions. The aim of this experiment was to investigate the effect of cultivars and harvest locations on fruit quality and changes in fruit softening and antioxidative enzymes in peach during ripening.

## Materials and Methods

### Experimental Treatments

Four and five years old healthy peach (*Prunus persica* L. Batsch) trees of uniform size, grafted on peach seedling rootstock, planted in a north–south direction (7 m between rows and 7 m within rows), trained on a central open leader system were selected for the experiment at Horticulture Research Station, Noshehra, Soan Valley Distt. Khushab (72°12′ 27.02″E; 32°1′12.62″N) and a private Farm from Sillanwali (72°40′16″E; 32°5′1″N), District Sargodha, Punjab, Pakistan, respectively. Fruit of uniform size and maturity, free from diseases and visual symptoms of blemishes of peach cultivars 'Early Grand' and 'Flordaking' were harvested at physiological mature stage [('Early Grand': fruit firmness = 65 N, SSC = 8.5 °Brix); ('Flordaking': fruit firmness = 73 N, SSC = 7 °Brix)] from Horticulture Research Station, Noshehra, Soan Valley Distt. Khushab and a private Farm [('Early Grand': fruit firmness = 57 N, SSC = 7.5 °Brix); ('Flordaking': fruit firmness = 70 N, SSC = 6.5 °Brix)] from Sillanwali, District Sargodha, Punjab, Pakistan. Both the orchards were following the recommended standard cultural operations along with plant protection measures. These fruit were transported to Postharvest Lab, IHS, UAF, Pakistan in a forced air temperature controlled reefer van (7±2°C Temp. and 85-90% RH). Uniform size fruit, apparently free from any defect were selected and ripened at room temperature under ambient conditions at 30±1°C and 60-65% RH. Data regarding different fruit quality parameters (respiration rate, ethylene production, fruit weight loss, firmness, colour, SSC, TA, TPC, AS activity) and activities of various enzymes (fruit softening and antioxidative) were recorded on daily basis until fruit ripening (eating soft). Eight fruit were used as an experimental unit. The experiment was conducted using CRD under factorial arrangement replicated three times.

### Determination of Fruit Physiological Quality

For determination of respiration rate ($CO_2$) and ethylene production, two peach fruit were put in an air tight plastic jar of known volume (2200 mL). Respiration rate was determined with a $CO_2$ gas analyzer (Model MI-70, Vaisala, Finland), while ethylene production was determined with help of hand held ethylene analyser (Model -56, ICA Storage Limited, UK). Respiration rate and ethylene production were expressed as mL $CO_2$ $kg^{-1}$ $h^{-1}$ and µL $C_2H_4$ $kg^{-1}$ $h^{-1}$ of fruit weight, respectively.

### Determination of Fruit Physical Quality

An electronic balance (Model ELB 1200 Shimadzu, Japan) was used to determine fruit weight. Fruit weight loss was calculated as percentage of fruit weight as outlined by Tareen *et al.* (2012). Penetrometer (Model DFM50, Ametek Inc., USA) fitted with 8 mm plunger was used to determine firmness of peach fruit. The fruit peel was removed with a peeler on both cheeks and was calculated in Newton (N). For ground colour determination a scale was developed based on visual observations as described earlier by Hussain (2010) by using the rating scale of 1-4 (4 = 25% yellow and 75% green; 3 = 50% yellow and 50% green; 2 = 75% yellow and 25% green; 1 = 100% yellow and 0% green).

### Determination of Fruit Biochemical Quality

A handheld digital refractometer (Model RX 5000 Atago, Japan) was used to determine SCC of peach fruit juice. Method described by Khan *et al.* (2012) was used to determine TA of fruit juice and was expressed as percentage of malic acid. Ascorbic acid contents were determined by the method outlined by Ullah *et al.* (2012) and were expressed as mg 100 $g^{-1}$. Bradford reagent was used to determine fruit protein content (Bradford, 1976) and was expressed as mg $g^{-1}$ of fruit weight.

**Determination of Total Phenolic Contents and Antioxidant Scavenging Activity**

For determination of TPC and ASA, liquid nitrogen treated 13 g frozen stored (-80°C) fruit pulp samples were homogenized in 5 mL extraction buffer (methanol, acetone and HCl; 90:8:1) using pestle and mortar, followed by centrifugation at 10,000 × *g* for 5 min at 4°C. Supernatant was used for further determination of TPC and ASA. ASA was determined by using the method reported by Mimica-Dukic *et al.* (2003). Supernatant (50 µL) mixed with 5 mL 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.004% solution made in methanol) was kept in dark conditions at room temperature for 30 min. Absorbance was noted at 517 nm and ASA was determined as percentage inhibition described earlier by Zhang *et al.* (2011). TPC from peach fruit pulp were determined by the method of Ainsworth and Gillespie (2007) using Folin–Ciocalteu (FC) reagent. Extracted supernatant (100 µL) was mixed with freshly prepared 200 µL FC reagent followed by 1-2 min thorough vortex. Then 800 µL $Na_2CO_3$ (700 m*M*) was added in the reaction mixture and vortexed for further 1-2 min and incubated at room temperature for 1 h before taking OD at 765 nm. TPC was express as mg GAE 100 $g^{-1}$ (gallic acid equivalent) by using gallic acid as standard.

**Determination of Activities of Fruit Softening and Antioxidative Enzymes**

Activities of fruit softening enzymes including PE (EC 3.1.1.11), EGase (EC 3.1.1.4), *endo*-PG (EC 3.2.1.67) and *exo*-PG **(**EC 3.2.1.15) from peach fruit pulp tissues were determined by using the method reported by Khan and Singh (2007). The activities of EGase and *endo*-PG enzymes were expressed as Δ viscosity mg protein$^{-1}$ h$^{-1}$, while of PE and *exo*-PG as m*M* NaOH mg protein$^{-1}$ h$^{-1}$ and µg galacturonic acid mg protein$^{-1}$ h$^{-1}$, respectively.

For determination of antioxidative enzymes, liquid nitrogen treated 13 g fruit pulp tissue samples were stored at -80°C till further analysis. Then these samples were homogenized in pestle and mortar with 5 mL $K_3PO_4$ buffer (pH 7.2) followed by centrifugation at 10,000 × *g* for 5 min at 4°C. The supernatant was used to determine the activities of CAT (EC 1.11.1.6) and POD (EC 1.11.1.7) enzymes using method described by Liu *et al.* (2009) with some modifications. Supernatant (100 µL) was mixed with 100 µL hydrogen peroxide (5.9 m*M*) and absorbance was recorded at 240 nm by using ELISA plate reader (Model ELX800, Bio-Tek Instruments, Inc, USA). It was expressed as U mg protein$^{-1}$, where one unit was defined as "an absorbance change in 0.01 unit min$^{-1}$". The POD reaction mixture was prepared by mixing 800 µL 50 m*M* phosphate buffer (pH 5), 100 µL hydrogen peoxide (40 m*M*) and 100 µL guaiacol (20 m*M*). Reaction was initiated by mixing an equal amount of reaction mixture and supernatant (100 µL). The activity was interpreted as U mg protein$^{-1}$. SOD (EC 1.15.1.1) was determined by using the method described by Stagner and Popovic (2009). The photochemical reduction of nitro blue tetrazolium (NBT) was measured by ELISA plate reader at a wavelength of 560 nm. Reaction mixture contained phosphate buffer (50 m*M*, pH 5), 22 µ*M* methionine, 20 µ*M* NBT, 0.1 µ*M* Triton X, 0.6 µ*M* riboflavin, distilled water and enzyme extract in a ratio of 5:2:1:2:1:8:1, respectively taken in test tube was exposed to UV light for 15 min to initiate the reaction. It was expressed as U mg protein$^{-1}$. One unit of SOD activity was defined as "the quantity of enzyme used to inhibit 50% photoreduction of NBT".

**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 ripening periods. 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). Pearson's correlations were also performed to estimate relationship between fruit firmness and fruit softening enzymes using Statistix 9 for windows software.

## Results

**Changes in Fruit Weight Loss, Ground Colour and Firmness**

Fruit weight loss showed a non-significant increasing trend with ripening period regardless of cultivars and harvest locations. However, the highest fruit weight loss was exhibited by the peach cv. 'Flordaking' harvested from Sillanwali about 65% more than at day-1 of fruit ripening (Fig. 1A). The lowest weight loss was recorded in 'Early Grand' fruit harvested from Soan Valley, about 42% more than at day-1 of fruit ripening (Fig. 1D). Both the locations and cultivars showed non-significant decreasing trend for changes in fruit ground colour. The least ground colour was noted in 'Flordaking' harvested from Soan Valley at day-4 of fruit ripening about 22% less than day-1 (Fig. 1B and 1E). If we compare the mean effect of harvest locations and cultivars then fruit harvested from Soan Valley revealed more ground colour than Sillanwali and 'Early Grand' showed more colour than 'Flordaking'(Table 1 and 2).

Fruit firmness showed a significant decreasing trend as fruit ripening period progressed, irrespective of locations of harvest and cultivars (Fig. 1C and 1F). Lowest fruit firmness was recorded in 'Flordaking' fruit harvested from Soan Valley on day-4 of ripening, almost 8-fold less than at day-1 of fruit ripening. Fruit harvested from Soan Valley exhibited about 33% more fruit firmness than from Sillanwali, while peach cv 'Flordaking' were about 13% more firmer than 'Early Grand' (Table 2). Peach fruit firmness showed a

**Table 1:** Mean changes in fruit softening and quality of peach fruit as influenced by harvest locations

| Parameters | Harvest locations | | LSD |
|---|---|---|---|
| | Sillanwali | Soan Valley | ($P \leq 0.05$) |
| Weight loss (%) | 6.78 | 6.73 | NS |
| Ground colour (score) | 2.61a | 1.66b | 0.186 |
| Firmness (N) | 25.83b | 38.75a | 2.182 |
| SSC (Brix°) | 11.15b | 11.97a | 0.256 |
| TA (%) | 0.64 | 0.67 | NS |
| SSC:TA | 17.79 | 18.23 | NS |
| Ascorbic acid (mg 100 $g^{-1}$) | 11.11a | 9.03b | 1.063 |
| TPC (mg GAE 100 $g^{-1}$) | 72.28b | 174.36a | 3.147 |
| ASA (%) | 47.72b | 66.85a | 1.008 |
| CAT (U mg $protein^{-1}$) | 47.53a | 44.73b | 2.387 |
| POD (U mg $protein^{-1}$) | 32.48a | 25.34b | 4.219 |
| SOD (U mg $protein^{-1}$) | 28.93 | 33.24 | NS |
| PE (m*M* NaOH mg $protein^{-1}$ $h^{-1}$) | 1.19b | 1.48a | 0.043 |
| EGase [Δ viscosity (mg $protein^{-1}$ $h^{-1}$)] | 2.57b | 2.74b | 0.421 |
| *endo*-PG [Δ viscosity (mg $protein^{-1}$ $h^{-1}$)] | 11.61a | 10.07b | 0.267 |
| *exo*-PG (µg gal acid mg $protein^{-1}$ $h^{-1}$) | 138.79a | 132.92b | 1.163 |
| Ethylene (µL $C_2H_4$ $kg^{-1}$ $h^{-1}$) | 24.27a | 18.46b | 0.768 |
| Respiration (mL $CO_2$ $kg^{-1}$ $h^{-1}$) | 1.04a | 0.61b | 0.047 |

Means followed by different letters for a given parameter for harvest locations and cultivars significantly different at $P \leq 0.05$ (LSD test), NS = non-significant, SSC = soluble solid concentration, TA = titratable acidity, AA = ascorbic acid, TPC = total phenolics contents, ASA = antioxidant scavenging, CAT = catalase, POD = peroxidase, SOD = superoxide dismutase, PE = pectin esterase, EGase = endo-1,4-*β*-D-Glucanase, *endo*-PG = endo- polygalacturonase, *exo*-PG = exo-polygalacturonase

**Table 2:** Mean changes in fruit softening and quality of peach fruit influenced by cultivars

| Parameters | Peach cultivars | | LSD |
|---|---|---|---|
| | Early Grand | Flordaking | ($P \leq 0.05$) |
| Weight loss (%) | 6.94 | 6.57 | NS |
| Ground colour (score) | 2.17 | 2.09 | NS |
| Fruit firmness (N) | 30b | 34.58a | 2.182 |
| SSC (Brix°) | 11.46 | 11.66 | NS |
| TA (%) | 0.65 | 0.66 | NS |
| SSC:TA | 17.94 | 18.08 | NS |
| Ascorbic acid (mg $100g^{-1}$) | 9.89 | 10.24 | NS |
| TPC (mg GAE $100g^{-1}$) | 108.33b | 138.30a | 3.147 |
| ASA (%) | 59.50a | 55.07b | 1.008 |
| CAT (U mg $protein^{-1}$) | 49.30a | 42.97b | 2.387 |
| POD (U mg $protein^{-1}$) | 27.76 | 30.06 | NS |
| SOD (U mg $protein^{-1}$) | 27.75b | 34.41a | 4.413 |
| PE (m*M* NaOH mg $protein^{-1}$ $h^{-1}$) | 1.17b | 1.50b | 0.043 |
| EGase [Δ viscosity (mg $protein^{-1}$ $h^{-1}$)] | 2.54b | 2.77a | 0.421 |
| Endo PG [Δ viscosity (mg $protein^{-1}$ $h^{-1}$)] | 11.43a | 10.34b | 0.264 |
| Exp PG (µg gal acid mg $protein^{-1}$ $h^{-1}$) | 147.22a | 124.48b | 1.163 |
| Ethylene production (µL $C_2H_4$ $kg^{-1}$ $h^{-1}$) | 22.35a | 20.38b | 0.768 |
| Respiration rate (mL $CO_2$ $kg^{-1}$ $h^{-1}$) | 0.85a | 0.80b | 0.047 |

Means followed by different letters for a given parameter for harvest location and cultivars significantly different at $P \leq 0.05$ (LSD test). NS = non-significant, SSC = soluble solid concentration, TA = titratable acidity, AA = ascorbic acid, TPC = total phenolics contents, ASA = antioxidant scavenging, CAT = catalase, POD = peroxidase, SOD = superoxide dismutase, PE = pectin esterase, EGase = endo-1,4-*β*-D-Glucanase, *endo*-PG = endo- polygalacturonase, *exo*-PG = exo-polygalacturonase

significant positive ($P \leq 0.01$) correlations with PE ($r$ = 0.846) and EGase ($r$ = 0.6388) activities in fruit pulp tissues, while significant negative ($P \leq 0.01$) correlations with *endo*-PG ($r$= -0.781) and *exo*-PG ($r$ = -0.6706) activities during fruit ripening at ambient condition (Table 3).

**Changes in Respiration Rate and Ethylene Production**

Respiration rate and ethylene production increased as the ripening period progressed (Fig. 2). Fruit exhibited their ethylene production and respiratory peaks on day-3 of fruit ripening irrespective of harvest locations and cultivars. Fruit harvested from Sillanwali showed about 31% and 70% higher ethylene production and respiration rate as compared to Soan Valley (Table 1). Peach cv 'Early Grand' exhibited about 10% and 6% more ethylene production and respiration rate, respectively as compared to 'Flordaking' (Table 2).

**Changes in SSC, TA and SSC: TA Ratio**

Harvest locations, cultivars and ripening periods did not show significant affect on SSC, TA and SSC:TA of peach fruit during ripening at room temperature (Fig. 3). However, mean effect of harvest locations remained significant and higher level of mean SSC was observed in peach fruit harvested from Soan Valley than Sillanwali (Table 1).

**Changes in Ascorbic Acid, Total Phenolic Contents and Antioxidant Scavenging Activities**

Significant increasing trend was observed in TPC (Fig. 4B and 4E) and ASA (Fig. 4C and 4F), but significant decreasing trend was observed in ascorbic acid contents (Fig. 4A and 4D). However, regarding mean effect of harvest location, Sillanwali showed about 19% more ascorbic acid, while about 59% and 29% less TPC and ASA compared with Soan Valley, respectively (Table 1). Peach cv 'Early Grand' showed about 22% less mean TPC and 8% more ASA as compared with 'Flordaking' (Table 2).

**Changes in CAT, POD and SOD Enzymes**

Both harvest locations and cultivars showed significantly increased activities of CAT (Fig. 5A and 5D) and SOD (Fig. 5C and 5F), as fruit ripening period progressed. However, a non-significant change was observed in activities of POD enzyme (Fig. 5B and 5E). Fruit harvested from Sillanwali showed about 6% and 28% significantly higher activities of CAT and POD enzymes as compared to Soan Valley (Table 1). A significant difference in CAT and SOD activities was observed in peach cultivars (Table 2). About 15% more and 19% less activities of CAT and SOD, respectively were observed in cv 'Early Grand' as compared to 'Flordaking' fruit.

**Table 3:** Relationship of fruit firmness with fruit softening enzymes in pulp tissue of two peach cultivars harvested from two different locations during ripening at ambient conditions

| Compared variable | Pearson's correlation |
|---|---|
| Firmness vs. PE | 0.8463** |
| Firmness vs. EGase | 0.6388** |
| Firmness vs. *endo*-PG | -0.7810** |
| Firmness vs. *exo*-PG | -0.6706** |

** = significant at $P \leq 0.01$, PE = pectin esterase, EGase = endo-1,4-$\beta$-D-Glucanase, *endo*-PG = endo- polygalacturonase, *exo*-PG = exo-polygalacturonase

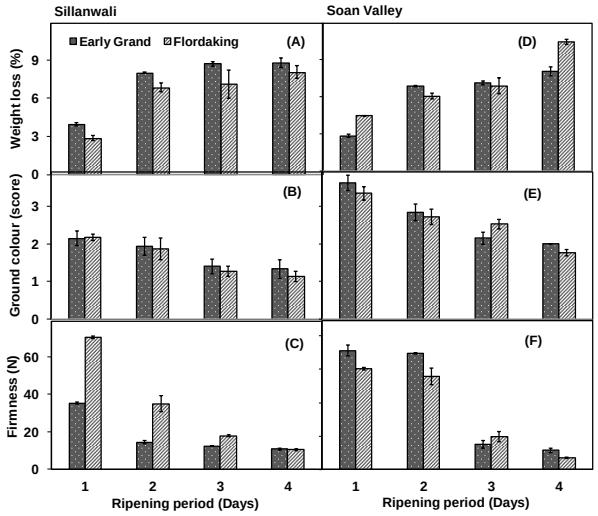


**Fig. 1:** Effect of cultivars, harvest locations and ripening period on fruit weight loss (A, D), ground colour (B, E) and firmness (C, F) of peach fruit. Vertical bars represent ± SE of means. n = 18 (6 fruit x 3 replicates)

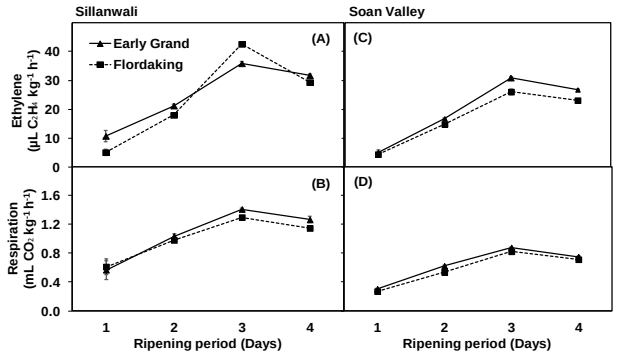


**Fig. 2:** Effect of cultivars, harvest locations and ripening period on ethylene production (A, C) and respiration rate (B, D) of peach fruit. Vertical bars represent ± SE of means. n = 6 (2 fruit x 3 replicates)

### Changes in PE, EGase, *endo*-PG and *exo*-PG Enzymes

Activities of all softening enzymes under study showed significant differences as affected by harvest locations.

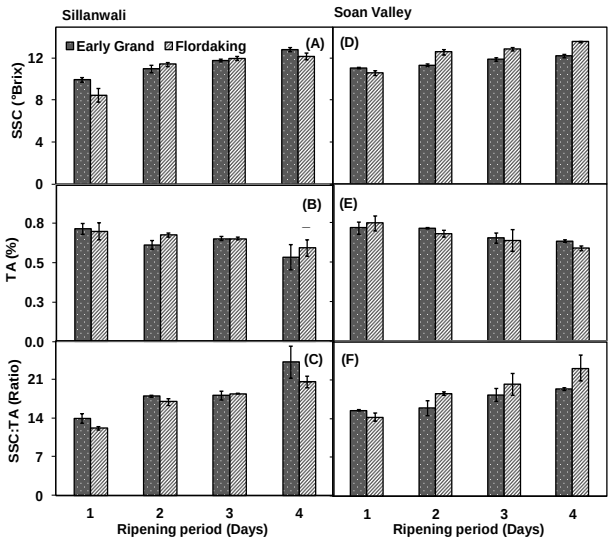


**Fig. 3:** Effect of cultivars, harvest locations and ripening period on SSC (A, D), TA (B, E), and SSC: TA ratio (C, F) of peach fruit. Vertical bars represent ± SE of means. n = 3 replicates. SSC = soluble solid concentration, TA = titratable acidity

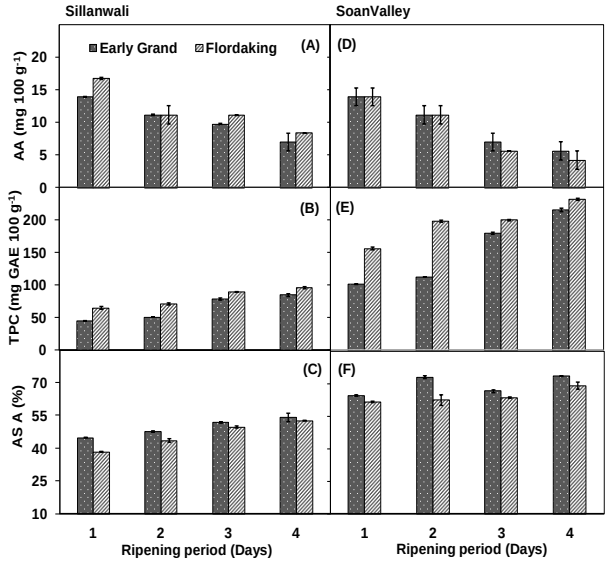


**Fig. 4:** Effect of cultivars, harvest locations and ripening period on AA contents (A, D), TPC (B, E) and ASA (C, F) activity of peach fruit. Vertical bars represent ± SE of means. n = 3 replicates. AA = ascorbic acid, TPC = total phenolic contents, ASA = antioxidant scavenging activity

Fruit harvested from Sillanwali exhibited about 20% and 6% less activities of PE (Fig. 6A and 6E) and EGase (Fig. 6B and 6F) enzymes, while about 15% and 5% more activities of *endo*-PG (Fig. 6C and 6G) and *exo*-PG (Fig. 6D and 6H) enzymes respectively than fruit harvested from Soan Valley (Table 1). A significant increasing trend was

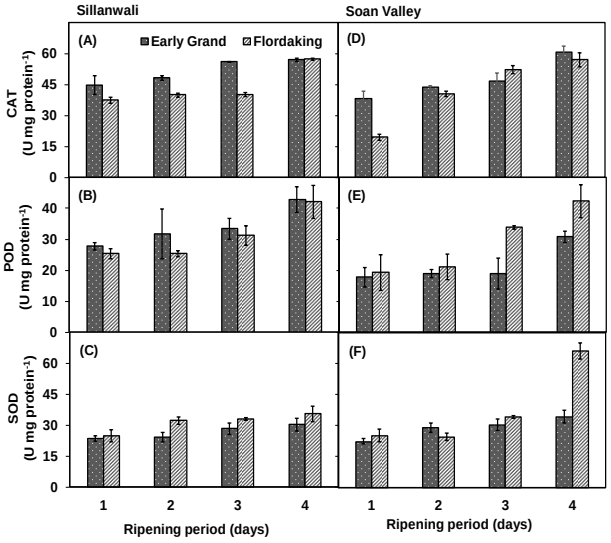


**Fig. 5:** Effect of cultivars, harvest locations and ripening period on the activities of catalase (A, D), peroxidase (B, E) and superoxide dismutase (C, F) enzymes in pulp tissues of peach fruit. Vertical bars represent ± SE of means. n = 3 replicates. CAT = catalase, POD = peroxidase, SOD = superoxide dismutase

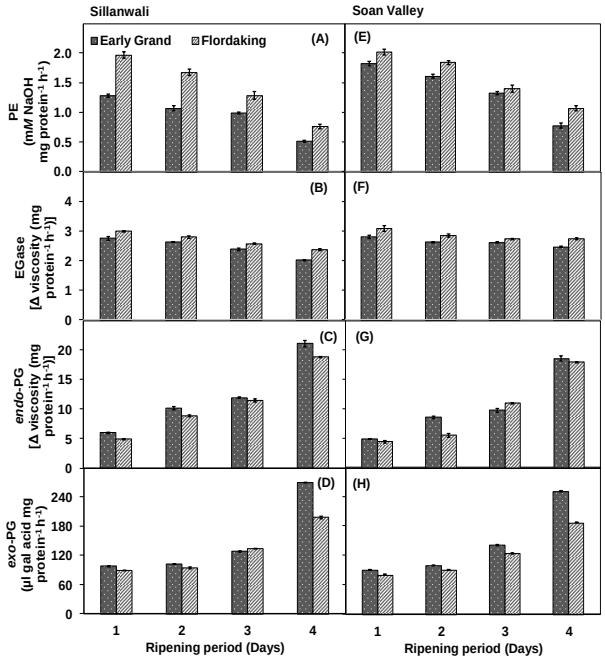


**Fig. 6:** Effect of cultivars, harvest locations and ripening period on activities of PE (A, E), Egase (B, F) *endo*-PG (C, G) and *exo*-PG (D, H) enzymes in pulp tissue of peach fruit. Vertical bars represent ± SE of means. n = 3 replicates. PE = pectin esterase, EGase = endo-1,4-*β*-D-Glucanase, *endo*-PG = endo- polygalacturonase, *exo*-PG = exo-polygalacturonase

noted during ripening in peach fruit for *endo*-PG and *exo*-PG activities, whereas a significant decrease was observed in PE activity during fruit ripening. During ripening fruit exhibited significant decrease in the activity of EGase enzymes. Peach cv. 'Early Grand' showed significantly about 22% and 8% less mean activities of PE and EGase enzymes respectively, while about 10% and 18% more mean activities of *endo*-PG and *exo*-PG enzymes, respectively than 'Flordaking' (Table 2).

## Discussion

Softening plays a key role in the ripening of climacteric fruit. Modifications in cell wall during ripening are considered to be involved in the coordination and interdependence of a range of hydrolytic enzymes including polygalacturonase (PG), EGase and PE. Activities of these enzymes determine the texture (juiciness, crispness) of fruit during ripening (Payasi *et al.,* 2009). Fruit firmness progressively declined throughout fruit ripening period (Fig. 1). Our results have shown that peach fruit softening during ripening is positively correlated with activities of PE and EGase enzymes (Table 3). Reduction in fruit firmness during ripening maybe ascribed to the pectin degradation (Brady, 1987) augmented by hydrolysis of cell walls (Fischer and Bennett, 1991) by the action of fruit softening enzymes. In the present study fruits of both cultivars harvested from both locations showed an increased respiration rate and ethylene production with advancement of fruit ripening (Fig. 2). Fruit exhibited the climacteric peak on day-3 of fruit ripening with autocatalytic rise in ethylene production. This increase in ethylene production and respiration rate is ascribed to rapid conversion of sugars, organic acid to $CO_2$ during the ripening period. As respiratory metabolism of climacteric fruit have been found to involve dramatic rise in respiration rate including peach (Saltveit, 2004).

In both peach cultivars harvested from Sillanwali as well as Soan Valley, the SSC and SSC:TA ratio of the fruit progressively increased, whilst TA decreased during fruit ripening (Fig. 3). Increased SSC could be attributed to oxidative breakdown of starch to sugars, and organic acids (Akhtar *et al.,* 2010). A declined TA during ripening might be due to decarboxylation of malate and the consequent decarboxylation of pyruvate (Hawker, 1969). The increase of phenolic compounds observed in this study for both the cultivars at both locations might be attributed to increase in the production of ethylene during ripening process. Higher level of ethylene production during ripening at ambient temperature stimulated the biosynthetic pathway of phenol compounds. In fact, ethylene motivates phenylalanine ammonialyase activity, an important enzyme involved in biosynthesis of phenolic compounds followed by the resultant phenolics accumulation (Ritenour *et al.,* 1995) coupled with accumulation of oxygen species as reported in peach (Camejo *et al.,* 2010). Fruits contain different types of

antioxidant components. Most of the antioxidant capacity of a fruit or vegetable may be from compounds other than enzymatic antioxidants. Phenolic contents also had been demonstrated to exhibit strong antioxidant activities in fruits (Hanasaki *et al.,* 1994). Many factors are involved in alteration of fruit antioxidant activities including cultivars, storage techniques, geographical location and duration between fruit harvesting and consumption. It is evident that postharvest life of peaches has been reported to influence deeply their antioxidants capability (Di Vaio *et al.,* 2008). Our results revealed significant higher activities of antioxidant enzymes irrespective of harvest locations and cultivars during fruit ripening. It might be attributed to lowered lipoxygenase activity, which is partly responsible for superoxide radical formation during ripening. As earlier reduction in lipoxygenase level has been reported during ripening of tomato fruit regulated by increased level of ethylene production (Griffiths *et al.,* 1999). These superoxide radicals are converted into hydrogen peroxide in result of SOD activity and the same is disintegrated into $H_2O$ and $O_2$ by CAT and POD activities. This leads to oxidative stress during ripening. The antioxidative mechanism of fruit include activities of CAT, POD and SOD as front line defense (Tareen *et al.,* 2012).

As evidenced from the results that fruit softening is highly correlated to activities of PE, EGase, *endo*-PG and *exo*-PG enzymes (Table 3). Activities of PE decreased while that of *endo*-PG and *exo*-PG increased at both locations and in cultivars during ripening (Fig. 6). This might be due to variation in the prevailing climatic conditions such as light and temperature at both locations as they determine rate of photosynthesis and supply of carbohydrates to fruit which are indispensable for all biochemical reactions in fruit after harvest (Tromp, 2005). It occurs owing to the breakdown of cell walls as well as conversion and dissociation of cell wall polymers during ripening (Singh and Singh, 2011). On the other side, loss of neutral sugar like galactose from pectin associated compound is proposed to coincide with the beginning of fruit softening. Subsequent solubilization of pectins are subjected to depolymerise in the later stages of ripening through the action of *endo*- or *exo*-PG (Dawson *et al.,* 1992). Different fruits had been reported to show a marked difference in their softening rates during ripening like mango (Lazan and Ali, 1993), banana (Kojima *et al.,* 1994) and in carambola (Chin *et al.,* 1999). It was reported that pectin solubilisation occurs prior to depolymerisation in peach fruit (Brummell *et al.,* 2004). Breakdown of polyuronides has been found to be started at mid-softening stages in fruits such as avocado and tomato, or during later stages of fruit softening in melon and peach (Brummell, 2006). During fruit ripening both cultivars exhibited similar trend in the changes of fruit quality characteristics. However, significant variations occurred with respect to levels of various physical, biochemical fruit quality traits as well as activities of antioxidative and fruit softening enzymes.

In conclusion, cultivars and harvest locations significantly influenced the various physico-chemical attributes along with the activities of fruit softening and antioxidative enzymes in peach fruit. Cultivar 'Flordaking' exhibited superior fruit quality characteristics than 'Early Grand', while Soan Valley location produced fruit with better quality than Sillanwali.

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