Journal of Pure and Applied Agriculture (2022) 7(4): 73-89
ISSN (Print) 2617-8672, ISSN (Online) 2617-8680
http://jpaa.aiou.edu.pk/

Pre-storage application of ascorbic acid and salicylic acid to preserve quality of peach fruits during cold storage

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Abstract

Peach fruit has a very short postharvest life due to its high perishability which makes it more crucial to preserve the quality of fruits during transportation and storage. The current study was designed to examine the storage behavior of peach cultivar 8-A treated with salicylic acid (SA) and ascorbic acid (AA) at Horticulture Laboratory, The University of Haripur, Pakistan in September-November 2020. Freshly harvested peach fruits were treated with four different levels of SA and AA (T_0 = Control, T_1 = SA 10 mM, T_2 = AA 5 mM, T_3 = SA + AA (10 Mm + 5 mM) and T_4 = SA + AA (20 mM + 10 mM)). Treated fruits were then kept at cold storage (2-4 ˚C and 60% RH) for 20 days. Various attributes including fruit weight loss, fruit decay, fruit firmness, pH of juice, total soluble solid and sugar contents (TS: Total sugar, RS: Reducing sugar, NS: Non-reducing sugar) along with total phenolic contents, total antioxidant, enzymatic activities (CAT, POX, SOD) and phytochemical screening were measured during the study. Data on the above-mentioned parameters were recorded regularly after five days intervals. The results showed a significant decrease in fruit firmness (maximum fruit firmness (70.93 Nm in fresh fruits and least fruit firmness (30.66 Nm) in fruits stored for 20 days) while an increase in rest of all parameters were observed, in contrast, the reducing sugars showed no significant variation in storage duration. In addition, results regarding SA and AA interaction showed the highest fruit firmness (75.19 Nm), maximum weight loss (52.53%), fruit decay (1.80%), total sugar (21.55%), reducing sugar (6.84%) and non-reducing sugar (14.70%) were observed in control. In conclusion, the application of SA + AA (10 mM + 5 mM) performed best to prolong the shelf life of peach fruit under cold storage and will benefit the farmers and consumers by having good quality fruits.

Keywords: Antioxidants, Cold storage, Enzymes, Peach, Phenolic content, Post-harvest, Sugar contents, TSS

To cite this article: Ayub, M. U., Ayub, Q., Khan, S, M., Khan, M, A., Ahmad, S., Hussain, I., Ahmed, W., Mehmood, A., Hassaan, M., & Shaukat, M, F., (2022). Pre-storage application of ascorbic acid and salicylic acid to preserve quality of peach fruits during cold storage. *Journal of Pure and Applied Agriculture, 7*(4), 73-89.

Introduction

Peach (*Prunus persica* L.) belong to the family Rosacea (Shaukat et al., 2022). Peach is originated from China and was domesticated in Persia. In Khyber Pakhtunkhwa province of Pakistan peach occupy approximately 5000 ha of total area with total production of 30,000 tons (Shah et al., 2013). Like other fruits and vegetables, the peaches have high perishability and shorter post-harvest life. Many undesirable changes occur inside the fruit which reduces the quality and economic values of the product, like high respiration rate, increased cellular respiration and ethylene production (Shalan, 2020). This growing behavior of the peach fruit natural product characterizes some genuine limitations for proficient administration practices, for example, postharvest dealing with, capacity and transportation. Hence in order to prevent these unwanted degradation scientists have been continuously working to develop such methods and techniques which can help to maintain post-harvest quality of fruits (Mehmood et al., 2022). These strategies include, use of semi-permeable/biodegradable coatings (Kamal et al., 2022), pre-conditioning (Shah et al., 2021), intermittent warming, controlled atmosphere storage (Bakshi et al., 2003). These practices are related with unwanted attributes of natural product and its quality, for example, chilling and additionally CO_2 injury (Shahzad et al., 2020), creation of ethanol and off-flavor taste because of organic product breathe under anaerobic conditions (Khan et al., 2008; Mehmood et al., 2018). With the progression of storage duration, internal browning can cause softening of fruit which make them more prone to decay and rotting (González-Agüero et al., 2008).

Hence in order to control natural post-harvest stresses of fruits and vegetables, its freshness and quality and also its nutritional and physical appearance the use of environmentallyfriendly techniques is of most importance (Mehmood et al., 2021). Like for example, use of salicylic acid (SA) has a characteristic and safe flagging atom has been suggested (Ayuba et al., 2020). SA have been appeared to display a high prospective in postponing the aging process, improving quality, and also prevent fruits and vegetables from post-harvest damages (Asghari & Aghdam 2010). Besides,

nutritive salicylates from fruits and vegetables are depicted as bioactive atoms with healthiness, abilities and are considered as generally recognized as safe (Khan et al., 2020). To maintained the shelf life and retaining the quality of harvested fruits and vegetables by application of pre-harvest and post-harvest treatment with SA for commercial uses to reduce the risk of damage (Turan et al., 2022). The technique was used to delay ripening in various fruits by post-harvest application of SA including strawberry (El-Mogy et al., 2019), banana (Arif et al., 2020), peach (Shaukat et al., 2022), and sweet pepper (Rao et al., 2011). Post-harvest dipping of banana and pear in SA was reported to have an inhibitory effect on its ethylene biosynthesis (Ali et al., 2021).

Application of ascorbic acid (AA) results in reduction of browning in fruits and vegetables (Singh et al., 2018). It was found that ascorbic acid is most effective in discoloration deterioration and helpful in reduction of browning in mushroom (Hsu et al., 2010); Rehman et al. (2022) discovered that AA is mostly used to prevent enzyme discoloration of fruits by reduction of colorless o-quinines to biphenyl's compound (Ayub et al., 2021 Mehmood et al., 2021). Various researches have reported that controlling the browning of fruits and vegetables is very effective measure by application of AA (Ali et al., 2021). Different sort of molds (Fungi) in various nuts can also be eliminated by application of AA solution along with water (Acero et al., 2015). AA has a good antioxidant nature that used to prevent fruit from darkening and also help to reduce the bacterial attacks during drying process. Application of AAalso resulted in reduction of browning in fruits and vegetables (Singh et al., 2018).

Although, postharvest application of SA and AA on peach fruit has been reported earlier by many scientists, but the application and effects of these chemicals in combination with each other have not been documented. As mentioned earlier that both chemicals in individual have prominent effects in increasing the shelf life and post-harvest quality of the peach so it can be hypothesized that the combined application of SA and AA might have suitable effects on different post-harvest attributes of the fruit. Hence, this study was planned to evaluate the effects of SA and AA on quality and shelf life of peach fruits stored under cold storage and to study the effect of storage interval on shelf life and post-harvest quality of peach fruit.

Materials and Methods

Present was conducted at Department of Horticulture, The University of Haripur, Pakistan during April-June 2020. Healthy, mature and uniformly sized fruits of Peach cultivars 8A were harvested from orchards of Swat Valley, Pakistan. The fruits were then transported to the Horticulture lab and were washed thoroughly by tap water to remove any foreign materials and were left to dry at room conditions. Peach fruits were then treated with ascorbic acid and Salicylic acid. Peach fruits were treated with Ascorbic acid and Salicylic acid. The treatments include; T0 = Control, T1 = Salicylic acid (10 mM); T2 = Ascorbic acid (5 mM); T3 = Salicylic acid + Ascorbic acid (10 mM + 5 mM); T4 = Salicylic acid + Ascorbic acid (20 mM + 10 mM). All the treatments were prepared by the methods as described by (AOAC, 2001).

The washed and air-dried peach fruits were dipped for 15 minutes in above mentioned treatments for 15 minutes. Treatment was repeated three times and every repetition comprised of a total 15 fruits. Fruits treated with SA and AA were kept under cold storage having temperature 2-4°C for 20 days. Observations regarding different parameters were noted on regular intervals of 5 days.

Storage duration 1 = 0 day
Storage duration 2 = 5 days
Storage duration 3 = 10 days
Storage duration 4 = 15 days
Storage duration 5 = 20 days

Phytochemical screening

For phytochemical screening peach juice from each replication was put in marked plastic bottles then tests for Saponins, Phytosterol, Tannins and Flavonoids was held, Phytochemical analysis carried out by using the process defined by Puerta-Gomez et al. (2011); Oliveira et al. (2012).

Fruit weight loss and firmness

Gravimetrical method was used for the fruit weight loss measurement by using standard digital weight balance (MJ-W176P, Panasonic Japan) and the discrepancy between the initial weight (before storage) and final weight was measured and divided by initial weight and calculated in the percentage value with the following equation:

$$Weight\ loss\ (\%) \frac{Initial\ weight - Final\ weight}{Initial\ Weight} \times 100$$

The penetrometer was used to calculate the firmness of the fruits. For this purpose, its needle was inserted into the peel and reading from each replication was noted.

Fruit decay (%)

Fruit decay was noted as per storage day after watching the physical appearances of the fruits. Total number of decayed fruits from each treatment was counted and following formula was implemented to calculate total fruit decay (%):

$$Fruit\ decay\ (\%) \frac{Decayed\ Fruits}{Total\ number\ of\ fruits} \times 100$$

pH and total soluble solids

Fruit juice pH was measured with a digital pH meter (HI 98107, Hanna, Mauritius at 18 ºC±2 ºC). Hand-held Refractometer (KROSS HRN-16), was utilized for measurement of Total soluble solids (TSS) by following standard procedure.

Sugars (Total sugars, reducing and non-reducing sugars)

In peach fruit sugar content was measured by using the method defined by Horowitz, and Gentili, 1960.

Total sugars

In order to measure the total sugar, 25 mL of Aliquot already formulated for sugar reduction have been taken into a 100 mL volumetric flask in which 20 mL of purified water and the condensed HCl of 5 mL have been added and a stored for overnight. It was then neutralized and rendered with 100 mL of purified water and 50% condensed NaOH. A 10 mL solution for the brick red end point using methylene blue as the symbol was taken in and titrated Fehling. The following formula was used to measure the total sugars:

Total sugar (%) = 25 × (X/Z)

Where,

25 = correction factor

X = mL of standard sugar solution used against 10 mL Fehling's solution

Z = mL of sample aliquot used against 10 mL Fehling's solution

Reducing sugars

Applying 2-3 drops of Methylene blue to the red brick end stage, a desk filtrate was taken in desk and titrated against the 10 ml Fehling-solution. Sugar reduction (RS) was decided by:

Reducing sugar (%) = 6.25 (X/Y)

Where,

6.25 = correction factor

X = mL of standard sugar solution used against 10 mL of Fehling's solution

Y = mL of sample aliquot used against 10 mL of Fehling's solution

Non reducing sugars

Non-reducing sugars (NRS) for juice samples were estimated with the formula:

Non reducing sugars (%) = 0.95 × (% Total sugars - % Reducing sugars)

Where 0.95 = Correction factor

Total phenolic contents and antioxidant activity

Total phenolic contents (TPC) of juice were calculated by using the Folin-Ciocalteu reagent as outlined by Ainsworth and Gillespie (2007) with some modifications. In pure water, the FC-reagent 10 ml was dissolved in 100ml solution. FC (200 µL) reagent and vortex have been extensively applied to each sample (100 ml). Each sample was fitted with 700 mM Na_2C0_3 (800 µL), which is incubated for 2 hours at room temperature. The sample (200 µL) was moved to the transparent platform of 96-pit, with absorption at 765 nm. TPC levels are measured by using a Gallic acid reference curve. Antioxidant activity was measured by using redical scavenging potential with 2, 2-diphenyl-1-picrylhydrazyl. Using an ELISA micro-plate reader (Bio Tek, USA), the absorbance was read against a 517 nm blank. In the existing (percent) inhibition of DPPH free radicals was determined by the formula below.

1% = (A blank-A sample /A blank) × 100

Where the samples are excluded by an absorbent blank, the control response mixture is absorbed by the test compounds. Due to the plot of the inhibition percentage toward amounts, the IC50 value was computed, reflecting the concentration of date fruit extracts inducing 50% neutralization of the DPPH radicals.

Enzymes activities

Catalase determination (Umg^{-1}protein)

The CAT behavior in peach peel has been calculated with some modifications by a process outlined by Awad et al. (2011). Enzyme extract (100 µL) has been combined to initiate the enzyme reaction by freshly prepared 5.9 mM 100 µL H_2O. ELX800 Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) was specified for CAT operation at 240 nm and was expressed as U mg^{-1} protein.

Peroxidase determination (Umg^{-1}protein)

The activities of POX were examined with modification of El-Hilali et al. (2003). The new phosphate buffer (pH 5) 50 mM was added to 40 mM 100 µL hydrogen peroxide and about 20 mM 100 µL of guaiacol. Fresh reaction mixture was formed. Enzyme extract (100 µL) absorption has been combined with reaction mixture, reported as a protein, U mg^{-1} with the use of ELX800 Microplate Reader, Inc. (Winooski, VT, USA). An absorption shift in 0.01 units per minute has been described as a unit of POX operation.

Superoxide dismutase determination (Umg^{-1}protein)

SOD was carried out using the process defined by Kaynar et al. (2005), to calculate a 50 percent photochemical reduction of nitro-blue tetrazolium (NBT). There were 500 µL phosphate buffer (50 mM, pH 5), two hydrocarbons (22 µM) of methionine (200 µL NBT), 200 µL Triton X (0.1 µM), and 1

hundred µL Ribophlavine (0.6 µM). In a box lit with fluorescent lamps for 15 min, test tubes were kept. The absorbance of ELX800 Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) at 560 nm and is expressed in U mg^{-1} protein. The amount of enzyme that inhibited 50 percent of the NBT reduction was described as one unit of SOD action.

Statistical analysis

Data was analyzed by application of standard error and analysis of variance (ANOVA) using two factorial design under Complete Randomized Design (CRD) using Least significant difference (LSD) at $p<0.05$ by using latest version of Statistix 8.1.

Results and Discussion

Phytochemical screening

Phytochemical analysis of Juice of peach fruit stored for different durations (0, 5, 10, 15 and 20) and treated with different concentrations of SA and AA has been shown in Table 1. Saponins shows their high presence in fruits treated with salicylic acid and ascorbic acid (10 mM and 5 mM) stored for 10, 15 and 20 days. Whereas Phytosterol showed their high presence in fruits treated with Salicylic Acid and ascorbic acid (10 mM and 5mM). Regarding Tannins, it indicated its presence regardless of the storage duration and chemical application. Flavonoids are moderately present in all treatments stored for 5 and 10 days' fruits. After increasing the storage duration of the fruit, flavonoids presence slowly increases and peaked when stored for more than 10 days. In recent years many spirostane, furostane, saponins, sapogenins, and glycosides have been isolated (Alamgir et al., 2018). However, Studies on secondary metabolites of peach is marginal. Nevertheless, there are studies of qualitative phytochemical analyses in peach (Shehzad et al., 2020). It comprises a large variety of phytochemicals, including saponins, alkaloids, flavonoids and phenolic acids (Visavadiya et al., 2010).

Table 1 Qualitative tests of phytochemical in Peach fruits

Storage days	Treatments	Saponins	Phytosterol	Tannins	Flavonoids
5	T0	++	++	++	++
5	T1	+++	+++	++	++
5	T2	++	+++	++	++
5	T3	+++	+++	++	++
5	T4	+++	++	++	+++
10	T0	++	+++	++	++
10	T1	+++	++	++	++
10	T2	++	+++	++	++
10	T3	++	+++	+++	+++
10	T4	++	++	+++	+++
15	T0	+++	++	++	+++
15	T1	++	+++	++	+++
15	T2	++	+++	+++	+++
15	T3	++	++	+++	+++
15	T4	++	++	+++	++
20	T0	+++	++	_	++
20	T1	++	++	+++	+++
20	T2	+++	++	+++	++
20	T3	++	++	+++	++
20	T4	++	++	++	+++

+++ = Highly present; ++ = Moderate presence

Table 2 Effects of salicylic acid and ascorbic acid on post-harvest quality and sugar content of peaches

Storage duration	WL	FF	FD	pH	TSS	TS	RS	NRS
0 days	0.00 e	59.62 a	0.00 c	3.99 e	8.74 e	12.78 d	3.90 e	8.09 b
5 days	47.68 d	57.39 b	0.00 c	4.34 d	8.88 d	13.41 d	4.27 d	23.38 a
10 days	48.96 c	54.96 c	0.00 c	4.54 c	9.20 c	16.80 c	6.46 c	23.38 a
15 days	50.02 b	52.91 d	1.00 b	4.70 b	9.36 b	22.54 b	7.24 b	23.38 a
20 days	51.40 a	50.98 e	1.60 a	4.82 a	9.50 a	32.55 a	9.16 a	23.38a

LSD (0.05)	**0.33**	**0.56**	**0.18**	**0.06**	**0.08**	**0.64**	**0.26**	**0.97**
Treatments								
T0	52.53a	34.91e	1.8a	3.46e	6.6e	21.55a	6.84a	21.3a
T1	42.96b	45.10d	0.73b	3.9d	7.51d	20.03b	6.25b	21.2ab
T2	37.8c	65.04b	0.06c	4.54c	8.52c	19.22c	6.14b	20c
T3	30.32e	75.19a	0.00c	4.97b	10.52b	18.1d	5.72c	18.7d
T4	34.46d	55.62c	0.00c	5.53a	12.55a	19.16c	6.08b	20.3bc
LSD (0.05)	**0.33**	**0.56**	**0.18**	**0.06**	**0.08**	**0.64**	**0.26**	**0.97**
LSD (SD × T*)	**0.73**	**1.26**	**0.42**	**0.15**	**0.19**	**1.43**	**0.59**	**2.18**
CV (%)	**1.14**	**1.4**	**49.65**	**2.04**	**1.29**	**4.45**	**5.81**	**6.57**

FF = Fruit firmness (Nm); FD = Fruit decay (%), WL = Weight loss (%); TSS = Total soluble solids (Brix^{o});
TS = Total sugar; RS = Reducing sugar; NRS; Non-reducing sugar

Table 3 Analysis of variance results (Mean square values) showing significance of pre-storage application of salicylic acid and ascorbic acid on Peach fruits during cold storage

Source	DF	WL	FF	FD	pH	TSS	TS	RS	NRS
Storage duration	4	7384.33*	177.67*	8.280*	1.597*	1.513*	1008.7*	70.964*	701.65*
Treatments	4	1103.77*	3789.1*	9.1133*	10.220*	86.125*	24.62*	2.465*	16.52*
Storage durations × Treatments	16	69.92*	0.16NS	3.8633*	0.013*	0.007*	2.84*	0.722*	0.704NS
Error	50	0.20	0.59	0.06667	0.0084NS	0.0139	0.76	0.1300	1.783
Total	74								

* Shows significance of results at p≤0.05; whereas NS represents non-significant variations p≥0.05; FF = Fruit firmness (Nm); FD = Fruit decay (%); WL = Weight loss (%); TSS = Total soluble solids (Brix^{o}); TS = Total sugar; RS = Reducing sugar; NRS = Non-reducing sugar

Fruit weight loss

Analysis of variance regarding weight loss (%) showed that highly significant difference (*p≤0.05*) exists between storage duration applied treatment and their interaction (Table 3 and 5). It was noted that the weight of peach fruits was decreased from 0 to 51.4% at the end of storage period. Similarly, the SA and AA have affected the peach fruits weight loss and it was noted that peach fruits which were treated with T3 and T4 exhibited the lowest reduction in weight i.e., 30.32% and 34.46%, respectively while untreated fruits shoed highest reduction in peach fruits (52.53%) as shown in Table 2. The combine effects of experimental treatments on stored peach fruits showed that the peach fruits which were treated with T4 showed least weight loss (39.46%) whereas untreated fruits showed highest weight loss (69%) (Fig. 1). The loss of weight in stored fruits can be attributed to respiration rate, transpiration and metabolic activities occurring inside the fruits. It was noted that application of combined dose of SA and AA has reduced the weight loss during the storage of peach fruit (Manthe et al., 1992; Zheng & Zhang, 2004). SA has been reported to responsible for closure of stomata which results in suppression of respiration rate and hence reduces the transpiration of moisture which results in minimization of weight loss (Shafiee et al., 2010). Similarly, peach fruits cv. 'Delicia' treated with SA exhibited less weight loss than control (Abbasi et al., 2019). Thus, the results of this study suggested that SA and AA might have reduced respiration and transpiration which concomitantly delayed senescence

Fruit firmness (Nm)

Fruit firmness is one of the most important physical parameters to monitor the ripening progress. Thus, the effect of SA and AA on flesh firmness of peach fruits, to assess the storage life, has been examined. Analysis of variance for fruit firmness (Nm) showed statistically significant difference (*p≤0.01*) for SA and AA treatments and for storage duration whereas the interaction of storage duration and treatmentswas found non-significant (*p≥0.05*) (Table 5). Results pertaining fruit firmness exhibits a decreasing trend during the storage period. Maximum fruit firmness (59.62 Nm) was recorded at 1st day, while minimum Fruit firmness (50.98 Nm) was noted at 20th day. The application of AA and SA showed that fruits treated with T3 exhibited highest fruit firmness (75.19 Nm) and those fruits which were kept untreated showed least fruit firmness (34.91 Nm) (Table 2). The combine effects of SA and AA treatments on peach fruits under cold storage conditions reveled that on 20th day of storage peach fruits treated with T3 showed maximum fruit firmness (70.93 Nm) fallowed by fruits treated with T2 (60.9 Nm) whereas untreated peach fruits showed least fruit firmness (30.66Nm) as evident from Fig. 2. Our results related to fruit firmness are in agreement with those of Khan et al. (2016) and Gupta et al. (2011) who reported the

firmness of peach cultivars decrease during post-harvest storage.

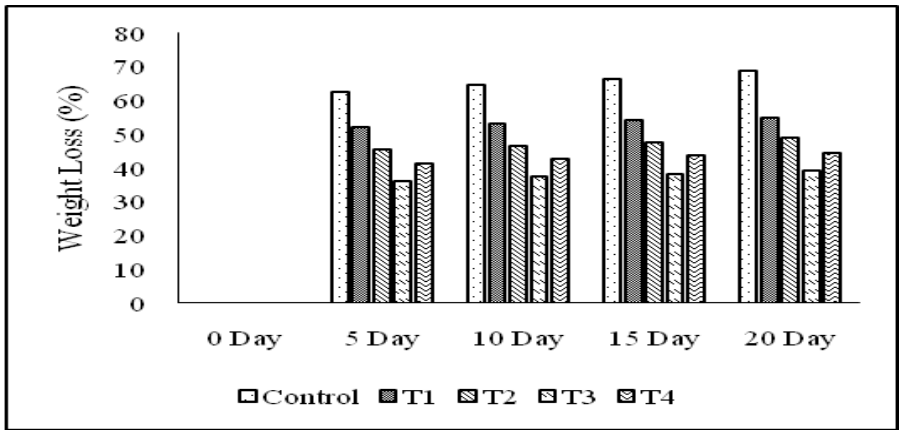

Fig. 1 Fruit weight Loss as affected by SA and AA treatments

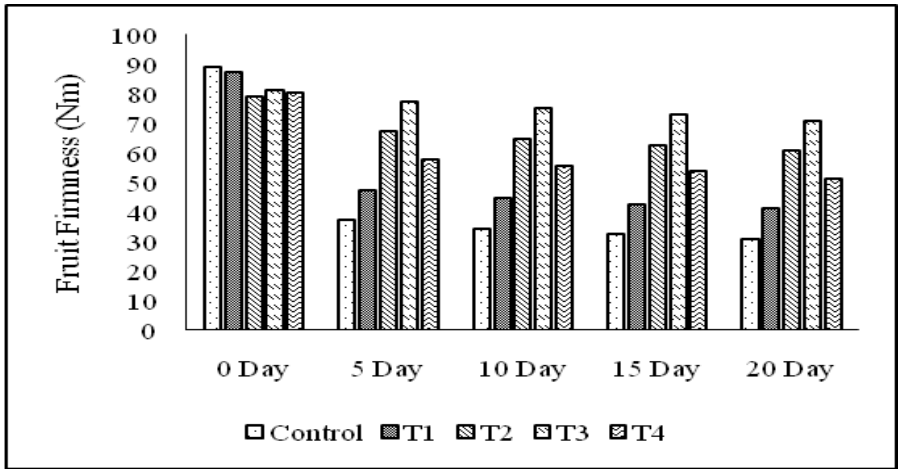

Fig. 2 Fruit Firmness as affected by SA and AA treatments

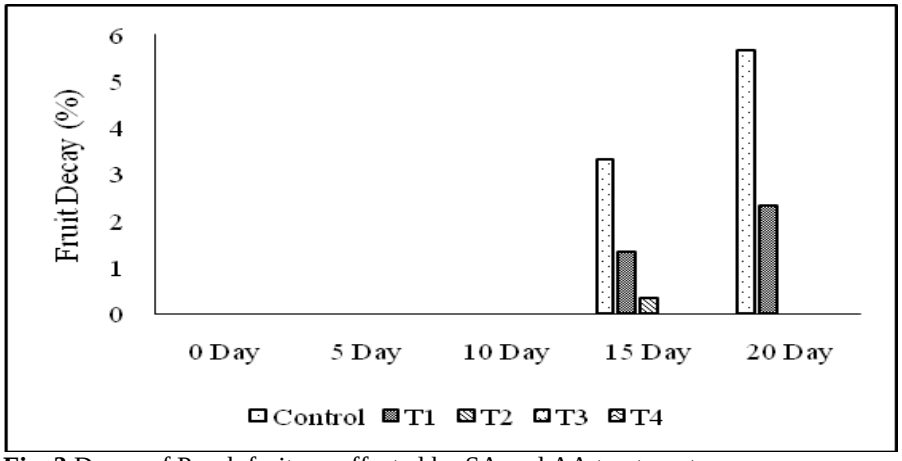

Fig. 3 Decay of Peach fruits as affected by SA and AA treatments

The ripening stage directly influences the fruit firmness. Stored fruits start to loss their firmness as their ripening stage progress which is evident from the findings of this experiment. The decrease in firmness might be due to several physiological activities such as that turgor pressure, starch degradation and cell wall breakage during ripening which depends upon the conditions of storage facility the result in loss of textural firmness (Lurie et al., 2005). It was also noted that application of SA and AA helps in maintains fruit firmness which can be associated to the fact that SA and AA reduce the respiration rate of stored fruits and slows down the ripening process hence sustained the fruit firmness and quality of stored fruits (Wang et al., 2006). These compounds also act as a protective agent for cell wall and prevent the breakdown of cell wall and maintain its structural integrity hence caused an increased fruit firmness during the storage (Aghdam et al., 2009).

Fruit decay (%)

The results regarding fruit decay suggested statistically significant variations for applied SA and AA treatments, storage intervals and their interactions (Table 5). An increasing pattern was noted in Fruit decay (%) over the progression of storage intervals. Highest Fruit decay (1.60%) was recorded on 20th storage day followed by (1.00%) at 15th day, while nofruit decay was recorded during 0, 5th and 15th day respectively. The effects of SA and AA showed that maximum fruit decay (1.80%) was observed in controlled (untreated) fruits, whereas nofruit decay (0.00%) was noted for T3 and T4 respectively (Table 2). The interactive results of SA and AA treatments on stored peach fruits revealed that on last day of storagehighest fruit decay (5.66%) was observed in peach fruits treated with neither SA nor AA, followed by fruit decay of 2.33% in fruits treated with T1. On the other hand, fruits treated with T3 and T4 have showed no signs of fruits decay till the end of experiment (Fig. 3). The increased fruit decay during the storage time can be attribute to ethylene production, insect pest and pathogen attack, breakdown of cell wall, enzymatic activity of cells and rate of ripening all these factors increase the chances of fruit decay (Asghari & Aghdam, 2010). But it was noted exogenous application of SA and AA to the peach fruit with efficiently controlled fruit decay caused by decreasing postharvest physiological process and pathogens. Khademi et al. (2013) mentioned that SA and AA treatments decreases the activity of cell wall degrading enzymes which enhances the postharvest life and delayed fruit ripening. Ethylene production could be effectively decreased by SA and AA treatments accompanied with cell swelling (Serrano et al., 2012) and inducing systemic resistances against postharvest pathogen which extend storability of fruits with higher antioxidant activity that activates natural defense mechanism of fruits (Khan et al., 2020).

Total sugar contents

Analysis of variance regarding Total Sugar (%) showed a significant difference ($p<0.05$) between applied treatment, storage durations and their interaction (Table 5). An overall increase in total sugar content was recorded for total sugar throughout the storage duration. It was noted that during the 20th day of storage peach fruits exhibited maximum total sugar content (32.55) while minimum total sugar (12.78) was recorded on 1st day. The application of SA and AA showed that untreated peach fruits produced highest total sugar content (21.55) whereas lowest total sugar values (18.10) were noted in peach fruits treated with T3 (Table 2). The effects of storage duration and SA and AA treatments on peach fruitsshowed that SA and AA treatments kept a lowered sugar content throughout the storage intervals and during 20th day of storage untreated peach fruits showed maximum total sugar (35.14) and least sugar content (29.38) was recorded for peach fruits treated with T3 (Fig. 4). The rise in total sugars is due to accumulation and biosynthesis of sucrose in peach fruit during ripening period. During the ripening of peach fruit chlorophyll degradation and increased in lycopene synthesis the results in the representative color development in peach fruit (Singh et al., 2013). The fruit stored at improved conditions and pack significantly overdue the sugar biosynthesis. Reducing the respiration rate and also delayed in ripening period of packed fruits, the same results have been reported by (Nor et al., 2020).

Reducing and non-reducing sugars (%)

Analysis of variance regarding reducing sugars (%) showed that highly significant difference ($p<0.01$) exists between applied treatment, storage durations and their interaction (Table 5). Results regarding reducing sugars (%) showed an increasing trend over the storage duration. Maximum reducing sugars (9.16) were recorded on 20th day, while lower reducing sugars (3.90) was recorded 1st day of storage. T0 produced highest reducing sugars (6.84) whereas lowest reducing sugars (5.72) were noted in T3 (Table 2). The combine effect of storage duration and treatment indicated that greatest reducing sugars (10.60) was obtained in those peach fruit which were left untreated and stored up to 20th day, while least amount of reducing sugars (3.42) were recorded in T1 on 1st day (Fig. 5). For Non-reducing sugar analysis of variance regarding non-reducing sugars (%) showed that highly significant difference ($p<0.01$) exists between applied treatment, storage durations and their interaction show non-significant results ($P\geq0.05$) (Table 5). Results regarding non-reducing sugars (%) showed an increasing trend over the storage duration. Maximum non-reducing sugars (23.38) were recorded on 20th day while lower non-reducing sugars (8.09) were recorded at 1st day. Results concerning treatments of SA and AA showed that T0 produced highest non-reducing sugars (21.33) whereas lowest non-

reducing sugars (18.74) were noted in T3. The interaction results of storage and treatments revealed that maximum non-reducing sugars (24.54) were observed in peach fruit kept at control and stored for 20 days, while lowest non-reducing sugars (8.53) were recorded in control fruits during the 1st day (Fig. 6).

The activity of sucrose-phosphate synthase is the cause of increased sugar content (Srivastava 2002; Shahzad et al., 2020). As stated by Ghasemnezhad et al., (2010) chemical analysis that fruits are between 4.6% and 9.6% sugar. The predominance of glucose and fructose has been identified (Han et al., 2018). Peach fruit is a major sugar-free component of sucrose and fructose is a primary sugar-free component (Mangku et al., 2018). Unripe fruits store high-quantity starch and turn them into sugars after maturing starch. Other evidence of changes in fruit during maturation includes color, loss of firmness and production of flavor. Satisfaction of consumers affects fruit and vegetable consumption as a significant factor (Bovi et al., 2018). The attributes of fruit taste are primarily dependent on the ratio of sugars and acids in the fruit (Raffo et al., 2007). Our results showed that total sugar and sugar reduction generally increased in both SA and AA concentrations and control as the storage period progressed. However, during the whole storage period, the sum of T3 treated peach fruit was higher and reduced in sugar compared to control. Complete losses in control fruits were increased and the content of sugar was decreased. This may be because of SA treatments that dramatically reduce losses by minimizing both complete deterioration and the reduction fruit sugar content. Increased content of these sugars was also shown for the continuous storage of fruits treated with calcium chloride (Mehmood et al., 2021; Chardonnet et al., 2003). Complete sugars and sugar reduction have been strengthened and achieved during the 20th storage day to the best of all therapies. However, although the non-reduction of the sugar content was also significantly influenced by SA and AA treatments T3 concentrations had maximum amounts of non-reduction in their sugar content than control fruits (Castellanos et al., 2015). In short, in the course of storage, the sugar content in fruits collectively increased, while in the concentration-dependent way, SA altered the content.

Total phenolic contents

SA and AA treatments and storage duration have statistically significant effect ($p<0.05$) on total phenolic content of stored peach fruits but their interaction was non-significant ($p>0.05$) (Table 5). It was noted that highesttotal phenolic content (74.36) was recorded on 20th day while lower total phenolic content (65.66) was recorded at 1st day of storage. The application of SA and AA treatments reveled that peach fruits treated with T3 showed maximumtotal phenolic content (90.40) while lowest TPC (48.40) were observed in control (untreated) fruits (Table 4). The interactive results of storage duration and SA and AA treatments revealed that on 20th day of storage maximum total phenolic content (94.7) was observed in fruits treated with T3 and least values of total phenolic content (53.3) were recorded in untreated fruits (Fig. 7).

All plants synthesize phenolic as secondary plant metabolites in their different parts fruits. They are responsible for the fruit products' taste and color (Jeong et al., 2008). Robbins et al., (2003) suggested those phenolics are involved in a variety of functions, such as nutrient absorption, protein synthesis, enzymatic and photosynthesis activities. Many phenolic compounds serve as antioxidants, being browning substrates in larger concentrations, and PPO and reactive oxygen species behave as key oxidants, as substrates and antioxidants during phenolic behave (Balasundram et al., 2006). Generally, they occur as flavonoids in fruit peel (Hamauzu et al., 2006). This study showed that, compared with regulation, the application of SA and AA greatly increased the overall phenolic content of peach fruits during the storage time. Huang et al., (2008) have mentioned related observations that SA treated oranges had an improved overall phenolic content. SA-treated peach fruits preserved fruit content by inhibiting browning and encouraging the loop of ascorbate-glutathione (Wang et al., 2009).

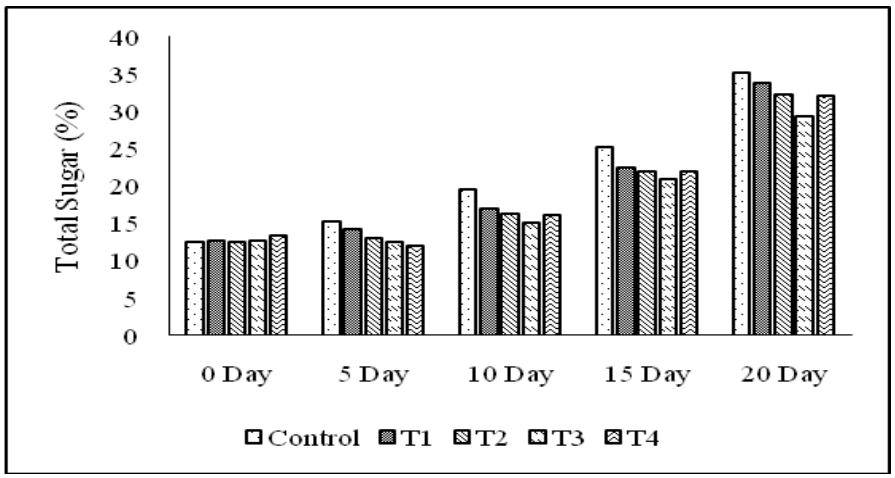

Fig. 4 Total Sugar (%) of Peach fruit as affected by SA and AA treatments

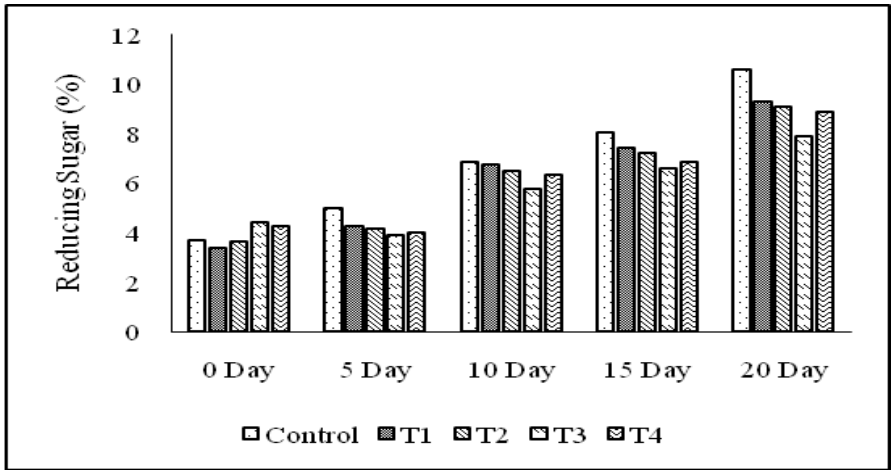

Fig. 5 Reducing sugar (%) of Peach fruits as affected by SA and AA treatments

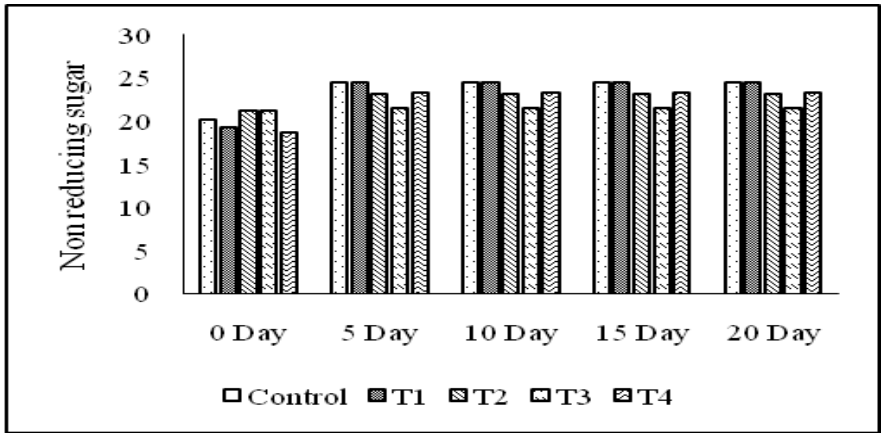

Fig. 6 Non reducing sugar (%) of Peach fruits as affected by SA and AA treatments

Table 4 Effects of post-harvest application of salicylic acid and ascorbic acid treatments on enzymes activity of Peaches

Storage duration	Total phenolic content	Total antioxidant	Super oxidase determination	Catalyze determination	Peroxidase determination
0 days	65.66 e	3.13 e	362.65 e	43.98 a	35.59 e
5 days	67.15 d	3.28 d	365.58 d	43.64 ab	36.70 d
10 days	69.41 c	3.49 c	369.08 c	43.28 b	37.74 c
15 days	71.78 b	3.69 b	373.40 b	42.64 c	38.40 b
20 days	74.36 a	3.91 a	378.03 a	41.48 d	39.73 a
LSD (0.05)	**0.735**	**0.555**	**1.661**	**0.452**	**0.463**
Treatments					
T0	48.40 e	1.54 e	329.37 e	32.97 e	27.37 e
T1	59.64 d	2.61 d	351.51 d	38.18 d	32.96 d
T2	70.08 c	4.45 b	389.03 b	43.38 c	43.32 b
T3	90.40 a	5.42 a	409.69 a	52.94 a	47.31 a
T4	79.84 b	3.39 c	369.15 c	47.54 b	37.20 c
LSD	**0.735**	**0.555**	**1.661**	**0.452**	**0.463**
LSD (SD×T*)	**1.644**	**0.124**	**3.714**	**1.011**	**1.036**
CV (%)	**1.44**	**2.16**	**0.61**	**1.43**	**1.68**

Similar letters in same column shows statistically non-significant variation at $p<0.05$

Table 5 Analysis of variance results (Mean square values) showing significance of pre-storage application of salicylic acid and ascorbic acid on Peach fruits during cold storage

Source	DF	Total phenolic content	Total antioxidant	Super oxidase determination	Catalyze determination	Peroxidase determination
Storage duration	4	183.38*	1.466*	563.0*	37.662*	14.581*
Treatments	4	4073.8*	35.328*	14739.5*	950.294*	912.492*
SD × T	16	1.12NS	0.008NS	4.5NS	0.398NS	7.280*
Error	50	1.01	0.0057	5.1	0.400	0.380
Total	74					

***** Shows significance of results at $p\leq0.05$; whereas NS represents non-significant variations $p\geq0.05$

Antioxidants activity

As suggested by the results of ANOVA (mg 100 mL^{-1}) statistically significant difference (*p<0.05*) existedfor SA and AA treatments and for storage durations and but their interaction was not significant (*p≥0.05*) (Table 5). It was noted that antioxidants activityshowed an increasing trend over the storage duration. Maximum antioxidantsactivity (3.91) was recorded on 20th day of storage while lowestantioxidantsactivity (3.13) was recorded on 1st day. The application of SA and AA treatments showed that T3 showed highest antioxidants activity (5.42) whereas the leastantioxidants (1.54) were noted in control fruits Table (Table 4). The interaction of Storage and SA and AA treatments on peach fruits revealed that on the 20th day highest antioxidants (5.90) were observed in peach fruit treated with T3 and lowest antioxidants (1.93) were recorded in untreated (control) fruitsduring the same storage day (Fig. 8).

DPPH is a very high fruit activity due to its antioxidant properties, including vitamins and other polyphenolic compounds that have a vital role in free radicals of scavenger (Akhtar et al., 2010). Post-harvest storage times related to certain physical and chemical parameters alterations such as flesh firmness and peel color, which can be determined by using DPPH (Dalla et al., 2007). Di Vaio et al., (2008) claimed that fruit picking times, storage conditions and duration between harvesting and consummation of fruits could change the antioxidant contents. It is also important to estimate the antioxidant activity of fruits in order to determine their dietary value (Awad et al., 2001). Also, it is worth noting that SA or AA has been reported to constitute a strong antioxidant potential and have ability to scavenge the action of DPPH radical (Campanella et al., 2003). The DPPH assessment was carried out with different concentrations of SA and AA for the peach fruits in this report. The DPPH started to develop, and this has also been recorded in melons from the beginning of the storage period (Oms-Oliu et al., 2008). The DPPH concentration of the combined application of SA and AA had

increased by the same chemicals and control applications. These combination SA and AA therapies may have replaced increased free radicals with increased DPPA. SA is a phenolic compound that influences biosynthesis, including antioxidants, in certain fruit protection systems and nutritional components (Huang et al., 2008). The consistency and speed of the internal fruits when they have been processed corresponds to the state of antioxidants (Hodges et al., 2004).

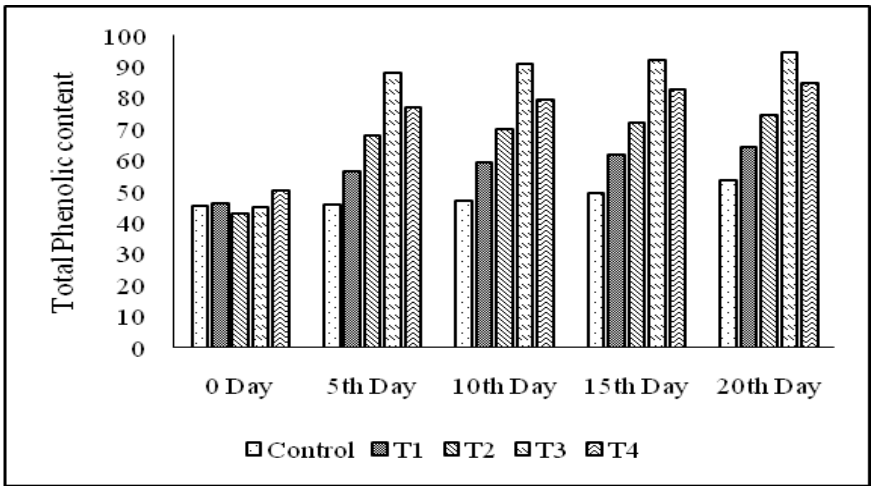

Fig. 7 Total Phenolic content of Peach fruits as affected by SA and AA treatments

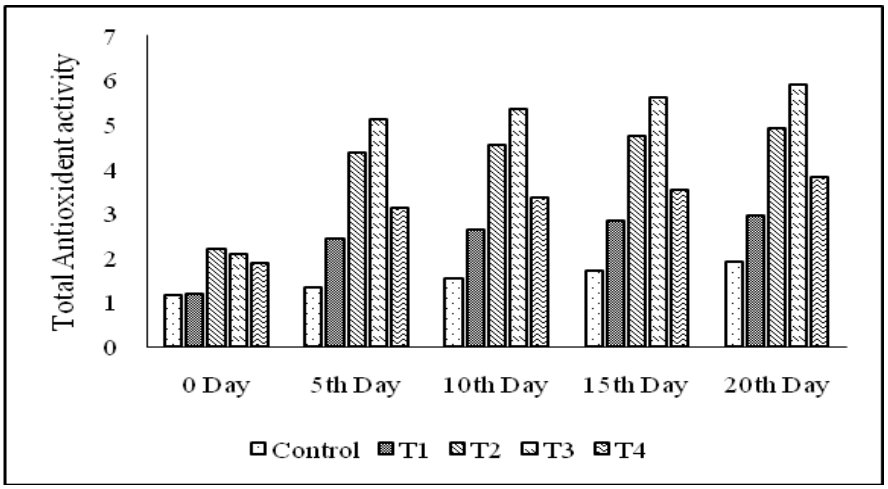

Fig. 8 Antioxident activity of Peach fruits as affected by SA and AA treatments

Superoxide dismutase showed significant differences ($p \leq 0.05$) between SA and AA treatments and for storage durations whereas the interaction of storage duration and treatment were non-significant ($p \geq 0.05$) (Table 5). It was noted that SOD exhibitedan increasing trend during the storage duration. Maximum SOD (378.03) was recorded on 20th day, while lower SOD (362.65) was recorded on 1st day of storage. SA and AA treatments reveledthat peach fruits treated with T3 highest SOD activities (409.69), whereas lowest SOD activities (329.37) were noted for untreated (control) fruits (Table 4). As far as the results of combine effects of SA and AA treatments are concerned it was noted that maximum SOD activities (418.73) were showed by peach fruitswhich were treated with T3 and stored till 20thday, while during the same day those fruits which were untreated (control) have lowest SOD activities (336.9) was recorded in control fruits during the first day of storage (Fig. 9). The first enzyme in antioxidants is the superoxide dismutase that plays a major part in the fruit's postharvest existence. The controversy over free superoxide (O_2^-) to hydrogen peroxide (H_2O_2) is SOD-catalyzed. Hydrogen peroxide serves as a chemical messenger under stress conditions before being metabolized by catalase and peroxidases (Tian et al., 2007). The use of exogenous salicylic acids in sweet cherry fruits, the post-harvest life has been shown to alter H_2O_2 metabolize enzymes (Chan et al., 2006). Increased SOD activity in loquat fruits were also noted when treated with SA by Tian et al. (2007). In our studies higher

SOD activity was maintained by T3. The findings of the study were in line with Mittler et al. (2002).These elevated SOD activities of peach fruits when treated with SA and AA can be attributed to fact that these chemicals have the potential to minimize respiratory intensity, free superoxide radicalproductionand MDA/LOX activity (Mo et al., 2008).

Catalase determination (Umg^{-1}protein)

Analysis of variance regarding Catalase determination (CATactivity) showedsignificant variations ($p<0.05$) forSA and AA treatments, for storage durations and for their interaction (Table 5). It was noted thatCATactivities were reduced during the storage duration. During the first day of storage maximum CAT (43.98) was recorded, while lower CAT activities (41.48) were recorded during the 20th day. Application of SA and AA treatments reveled that T3 had maintained the CAT activity and showed highest activity (52.94) whereas lowest CAT activity (32.97) was noted in control fruits (Table 4). The interaction of storage duration and treatments indicated that during the last day of storage maximum CAT activity (55.1) wasobserved in peach fruit which were treated with T3, while lowest CAT activity (30.63) was recorded in control fruits during the 20th day (Fig. 10). CAT is one of the most important enzymes for antioxidant regulation (ROS, in particular H_2O_2 (hydrogen peroxide) of the reactive oxygen species. Increased CAT activity is a key to oxidative damage protection (Najiet al., 2017) while lower CAT activity proposes that the cells' ability to scavenge H_2O_2 be decreased (Ng et al., 2005). According to the findings of Mo et al. (2008), salicylic acid prevented decline of CAT activity and also stated that a higher concentration of SA and AA had a greater impact than lower concentrations and control on sustaining increased CAT activity in fruitsof apple.

Peroxidase determination (U mg^{-1} protein)

Analysis of variance for peroxidase showed that statistically significant differences ($p<0.05$) for storage duration applied SA and AA treatments, but the interaction of these were found non-significant ($p>0.05$) (Table 5). Peroxidase showed an increasing patternthroughout the storage period. Maximum peroxidase (39.73) was recorded on 20th day while lower peroxidase (35.59) was recorded on 1st day of storage. Application of SA and AA treatments showed that peach fruits treated with T3 showed highest peroxidase (47.31) whereas lowest peroxidase (27.37) was noted in control (untreated) fruits (Table 4). The combine results of storage duration and treatment revealed that maximum peroxidase (49.26) was observed in those peach fruitswhich were treated with T3 and stored for 20 days, while during the same storage day lowest peroxidase (29.56) was recorded in control fruits (Fig. 11). Fruit kept in cold storage for longer periods is subjected to chilling, starvation or internal browning causing poor-quality fruit. Different parameters are used as stick yard to measure and track these physiological problems. The results of our study is in accordance with El-Hilali et al. (2003) who noted that during the storage of Mandarin fruit and papaya fruits for thirty days (at 5°C and 15°C) the POX activity continuous to increase.The several researches have reported POX activity in peach fruits during the storage period (Sultan et al., 2013) and (Ding et al., 2009). Peroxidases typically occur in fruit cells. It is also found in the chloroplast of life cells, cytoplasm, peroxisomes, vacuoles or apophasis (Zhang et al., 2008). Catalytic reactions of catalase and peroxidase clear the development of hydrogen peroxide as a result of superoxide free radicals disfigured by SOD (Valero et al., 2011). Growing POX activity in peach fruit in comparison with untreated (control) fruits over the entire duration of storage has been a superior effect according to our findings for SA and AA application. T3 increased POX activity compared to single SA or AA treatment.

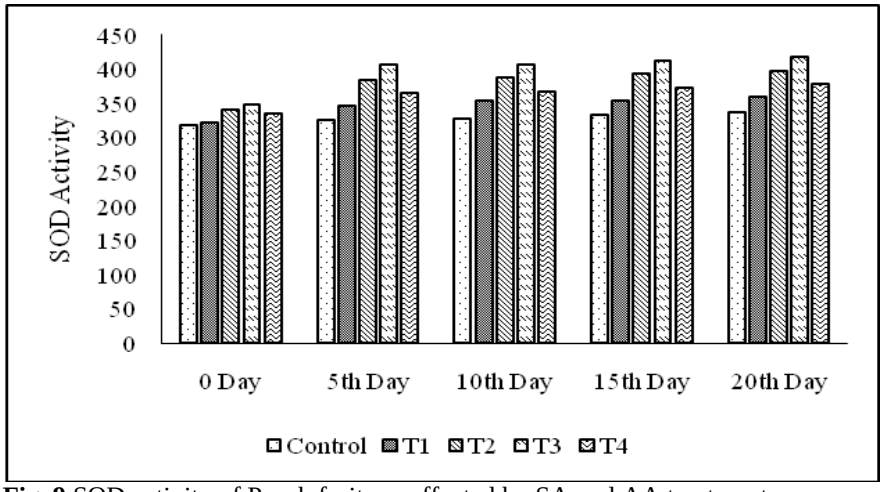

Fig. 9 SOD activity of Peach fruits as affected by SA and AA treatments

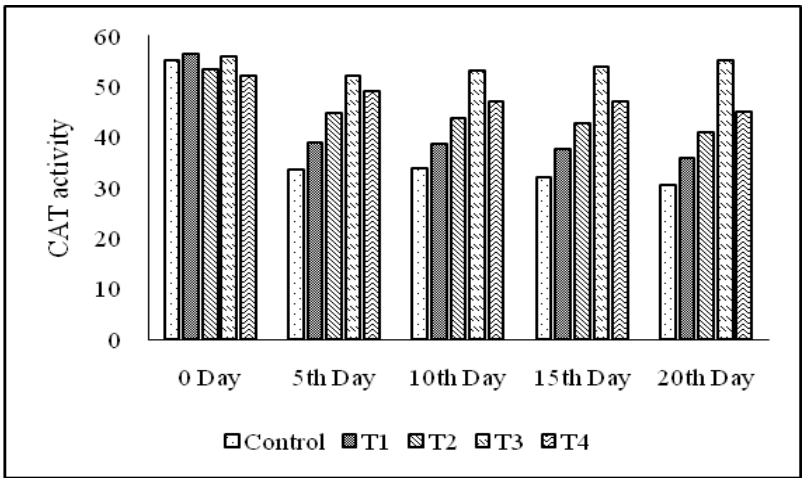

Fig. 10 CAT activity of Peach fruits as affected by SA and AA treatments

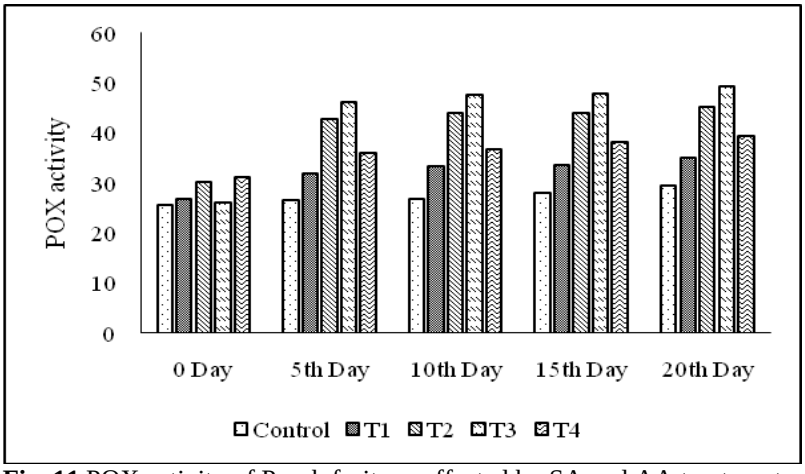

Fig. 11 POX activity of Peach fruits as affected by SA and AA treatments

Conclusion

The experimental trail concluded that peach showed significant variation towards storage intervals and applied treatments. Storage day have reduced most of the studied parameter and at 20th day maximum reduction in quality and enzymatic attributes were observed. Whereas application of AA and SA have enhanced the overall quality and shelf life of peach. It was also concluded that post-harvest treatment of T3 (Salicylic acid 10 mM and ascorbic acid 5mM) and T4 had significantly increased quality parameters of peach fruit CV. 8-A during 20 days of storage period. While other treatments T1 (salicylic acid (10 mM), T2 (ascorbic acid 5 mM) did not perform significantly as compared to that of control. Since this study was conducted on postharvest quality of peaches during cold storage to check its quality and freshness, so the results shows that peaches stored in cold storage are highly suitable for commercial purpose and can give high revenue to the farmers. It has been concluded that the combined application of salicylic acid & ascorbic acid on peaches to attain the best quality parameters.

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