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Efficacy of postharvest sodium nitroprusside application to suppress the fruit softening and extend storability by regulating physicochemical quality of peach (*Prunus persica* L. Batsch) fruit under low temperature conditions

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ABSTRACT

The climacteric nature of peach fruit entails its ongoing biological activity, including respiration, transpiration, and other biochemical processes even after harvest, leading to rapid quality deterioration. In an effort to mitigate softening and maintain peach fruit quality, the effectiveness of postharvest dip treatment with sodium nitroprusside (SNP) at concentrations of 0.5mM, 1.0mM, and 2.0mM was explored on 'Shan-i-Punjab' peaches during storage at 0-1°C and 90-95% relative humidity for 40 days. The application of sodium nitroprusside to 'Shan-i-Punjab' peach fruits exhibited notable benefits, including reduced mass loss, retained firmness, suppressed polyphenol oxidase (PPO) activity, lowered malondialdehyde (MDA) content and relative electrical conductivity (REC), and sustained levels of soluble solids, titratable acidity, total phenolics, ascorbic acid, total carotenoids, and antioxidant activities. This treatment effectively conserved the fruit quality for an extended period during storage. Furthermore, sodium nitroprusside treatment demonstrated a softening reduction by inhibiting the activities of cell wall wall-degrading enzymes such as pectin methylesterase, polygalacturonase and cellulase. Among the various concentrations tested, the SNP1.0 mM concentration proved to be the most effective in reducing fruit softening and extending the storage life of peaches up to 30 days under cold storage conditions.

Keywords: Antioxidant activities, Cold storage, Enzymatic activity, Peach, *Prunus persica* L. Batsch, Shan-i-Punjab, Sodium nitroprusside, Storage quality.

Introduction

Peaches (*Prunus persica* L. Batsch) are very famous all over the world because of their delicious flavour, high nutritional content, eye-catching colour and therapeutic qualities. Peaches are growing well in sub-tropical to semi-tropical conditions in northwest India due to comparatively less hours of chilling hours requirement in these regions. Peach fruits are rich in phenolic compounds, ascorbic acid, carotenoids (provitamin A) as well as an excellent source of antioxidants [13]. Numerous physiological and biochemical processes such as respiration, transpiration and cell wall disintegration affect the quality of harvested peaches. After the fruit harvest, the 'Shan-i-Punjab' variety of peach achieves early maturity and deteriorates quickly due to the high temperature and humidity of the season. Being a climacteric fruit, peach fruit lose a lot of useful chemicals through quick biochemical changes in storage, which shortens its shelf life and affects marketability. Low quality fruit is produced as a result of inadequate postharvest management, which reduces consumer health benefits and jeopardizes nutritional security. It is noted in the FAO study that postharvest losses account for 14% of global food loss [33].

Peach postharvest losses are made worse by India's inadequate cold chain infrastructure, which further speeds up the decline in peach fruit quality after harvest. Due to an excess of peaches during the harvest season, growers in northwest India are difficult to get a profit at local market places and hence sell their produce at a very low rate. For a continuous supply of fresh peaches in the consumer market, using food-grade phytochemicals or phenolic compounds is one of the way that have been investigated to preserve postharvest quality. Additionally, certain methods have been tried to extend the postharvest life of peach fruit and maintain its quality for a longer period of time, including cold storage [20], modified atmosphere storage [5], controlled atmosphere storage [26], hot water dips [35], intermittent warming [22], gamma irradiation [42], 1-methylcyclopropene (1-MCP) [21], polyamines (PA) [49], aminoethoxyvinylglycine (AVG) [30] and nitric oxide [89]. Fruit storage life is significantly extended by postharvest chemical treatments. Sodium nitroprusside (SNP) is used in fruit preservation to extend shelf life by releasing nitric oxide (NO), a signalling molecule that delays senescence [62]. Nitric oxide with its high reactivity, serves the dual purposes of being a biological messenger and an antioxidant in the plant system. It functions in plants as a multipurpose signalling molecule by taking part in many physiological processes, such as the ripening of climacteric and non-climacteric fruits [68]. Nitric oxide fumigated fruits have lower respiration rate and produce less ethylene.

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This happens when NO attaches to the enzymes ACC synthase and ACC oxidase's active sites, reducing the production of ethylene gas. When it comes to contact with climacteric fruits, the effects of NO are more noticeable than in non-climacteric fruits, because NO has the ability to maintain fruit quality after harvest and delay the ripening processes [90]. Furthermore, by increasing the antioxidant enzyme potential in the plant system, nitric oxide is essential in decreasing reactive oxygen species [61]. Nitric oxide can play a role in postharvest storage procedures as a senescence-retarding agent. Research has indicated that nitric oxide postpones the deterioration of fruit quality in a number of fruits such as peaches [62], pears [2], apples [16] and plums [65]. But using gas treatment is difficult and requires unique closed chamber setups, which makes it less feasible, particularly in areas with inadequate cold chain infrastructure. Sodium nitroprusside (SNP) application, on the other hand, is thought to be more user-friendly. When SNP is utilized in an aqueous solution, it produces nitric oxide gas, which is similar to NO gas treatment [16,27]. As far as we are aware, not much research has been done on the use of sodium nitroprusside (SNP) to extend the shelf life of subtropical peach fruit. So the present study was planned to determine the efficacy of SNP in extending the storage life and maintaining the quality of subtropical peaches during cold storage.

2. Materials and Methods

2.1 Materials

Fruits of peach variety cultivar 'Shan-i-Punjab' randomly harvested at the physiological maturity stage from Fruit Research Farm, Punjab Agricultural University, Ludhiana, during the years 2019-2020 and 2020-2021. The harvested fruits were immediately shifted to the Postharvest Laboratory of the Department of Fruit Science, Punjab Agricultural University, Ludhiana Ludhiana. Visual sorting to ensure uniformity in size and colour of the fruits was carried out. Blemished, diseased and physically defective fruits were removed. After sorting, the fruits were thoroughly washed under running tap water and were left for surface drying in the shade.

2.2. Treatments and storage

A total of 2000 uniform and defect free fruits were divided into four groups. These fruits were then subjected to dipping in aqueous solutions of sodium nitroprusside (SNP) at concentrations of 0.5mM, 1.0mM, and 2.0 mM for a duration of 5 minutes by following the methodology outlined by [16]. Control fruits were immersed in distilled water. After the treatments, fruits were air-dried in shade. Each treatment was replicated four times, with each replication comprising 125 fruits. Packaging was done using three-ply corrugated fibreboard boxes (5% perforation) with paper lining. Subsequently after packaging, all fibreboard boxes were kept in a cold storage at 0-1°C and 90-95% relative humidity for a period of 40 days. The physicochemical and enzymatic alterations in the peach fruits were assessed at intervals of 0, 10, 20, 30, and 40 days during the storage period.

2.3 Determination of mass loss (ML)

The initial mass (M_1) and subsequent masses (final mass) (M_2) of peach fruits at each storage interval were measured in order to calculate the mass loss (ML) by using the formula as given by [69] and mass loss was expressed as a percentage.

$$\text{Mass Loss (\%)} = \frac{M_1 - M_2}{M_1} \times 100$$

2.4 Determination of fruit firmness

Fruit firmness was measured using a stand-mounted penetrometer (Model FT-327, USA) with a stainless-steel probe with an 8 mm plunger. After peeling, the fruit firmness was measured at two opposite positions on its equator [36] and expressed in Newton(N) force.

2.5 Measurement of the soluble solid content (SSC) and titratable acidity (TA)

The soluble solids content was determined using a digital hand refractometer, (PAL-1 model from Atago Co., Ltd. in Tokyo, Japan). The titratable acidity was estimated using maleic acid on reference following the methodology described by [57].

2.6 Determination of Total phenolics content (TPC)

Total phenolic content was measured by homogenizing one gram of peach fruit tissue in three milliliters of 80% methanol. The homogenate was then centrifuged for twenty minutes at 4°C at 10,000 x g. Subsequently, 500 µL of the methanolic extract was mixed with 10 mL distilled water. After this, a test tube was loaded with four milliliters of 20% sodium carbonate solution, 1.5 millilitres of freshly prepared Folin Ciocalteu reagent and 100 microliters of the divided aliquot. To each tube, 4.4 mL of distilled water was added to get a final combined volume of 10 mL. The reaction mixture was incubated in the dark for 30 minutes. A spectrophotometer (Spectronic 20D+, Thermo Scientific, USA) was used to detect absorbance at 738 nm. The method described by [72] was followed to express the results as milligrams of gallic acid equivalent per kilogram of fresh weight.

2.7 Measurement of Ascorbic acid content

The ascorbic acid content in the fruit pulp sample was determined by oxidizing ascorbic acid with 2,6-dichloroindophenol dye, following the method outlined by [57]. The ascorbic acid content in the fruit juice was then quantified as mg 100mL⁻¹ (milligram per 100 millilitres). To initiate the extraction procedure, 10mL of fruit pulp juice was macerated in 70 mL of freshly prepared 3% metaphosphoric acid. The mixture was then diluted to a final volume of 100mL. Following the extraction, the product was filtered, and a 10 mL aliquot was titrated against 2,6-dichloroindophenol dye until a distinct rose-pink colour was retained for approximately 15 to 20 seconds.

2.8 Determination of total antioxidant activity

The total antioxidant activity of peach pulp tissue extracted with methanol was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following the protocol given by [14]. To extract the material, a homogenized mixture comprising 3 g of fruit tissue and 30 mL of methanol was centrifuged at 10,000 x g for 15 minutes at 4°C. An aliquot of 100 µL of the clear supernatant was combined with 3mL of a 0.1 mM DPPH methanol solution. All reagents, except for the sample, were included as controls. The reaction mixture was then incubated for 20 minutes at 25°C in the dark, and the absorbance (Abs) was measured at 517 nm. An equation was used to determine the total antioxidant activity:

$$\text{DPPH radical scavenging (\%)} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100.$$

2.9 Determination of total carotenoids

The estimation of total carotenoid content with the method proposed by [8].

A fresh sample of peach fruit (100 mg) was taken and thoroughly crushed in a mortar and pestle with 30 mL of 80% acetone. The crushed sample was then transferred to centrifuge tubes and centrifuged at 3000 x g for 10 minutes. The resulting supernatant was carefully transferred to test tubes. The pellet was subjected to another extraction with 2.0 mL of 80% acetone, followed by re-centrifugation. The two supernatants were combined in the same test tubes, and the final volume was adjusted to 10 mL with 80% acetone. The absorbance of the extract was measured at 645, 665 and 480 nm using 80% acetone as a blank. The total carotenoids content was determined using the following formula and expressed as micrograms per gram of fresh weight ($\mu\text{g g}^{-1}$ FW). To convert it to micrograms per 100 grams ($\mu\text{g 100g}^{-1}$), the result was multiplied by a common factor of 100.

$$\text{Total carotenoids } (\mu\text{g g}^{-1}\text{FW}) = A_{480} + 0.114A_{665} - 0.638A_{645} \times \frac{V}{1000 \times W}$$

Where, A_{480} = Absorbance at 480nm

A_{665} = Absorbance at 665nm

A_{645} = Absorbance at 645nm

V = Total volume of the extract (mL)

W = Weight of sample (g)

2.10. Malondialdehyde content and Membrane permeability (relative electrolyte leakage)

Using the techniques outlined by [47], the concentration of malondialdehyde (MDA) content was estimated and expressed as micromoles per kilogram of fresh weight ($\mu\text{mol kg}^{-1}$ FW). Fruit pulp tissue was homogenized in 10% trichloroacetic acid and the resulting mixture was centrifuged at 12,000 x g for 30 minutes at 4°C. Following this process, two milliliters of thiobarbituric acid were mixed with a three-milliliter aliquot, heated to boiling for twenty minutes and then quickly cooled. The absorbance at 532, 600, and 450 nm was measured after the mixture was centrifuged at 12,000 x g for 15 minutes.

The membrane permeability was measured in terms of relative electrolyte loss using a conductivity meter [48]. After thoroughly cleaning the fruit in double-distilled water, peel discs of 1 cm in diameter and 1 mm in thickness were cut. These disc fragments were soaked in 40 mL of double-distilled water before their initial conductivity (P_0) was determined. After incubating for 10 minutes at 25°C, the conductivity (P_1) was measured. After boiling the samples for ten minutes, they were allowed to cool to room temperature before the final conductivity (P_2) was determined. The relative electrolyte leakage was calculated using a specific equation:

$$\text{Relative electrolyte leakage } (\%) = [(P_1 - P_0) / (P_2 - P_0)] \times 100\%$$

2.11. Extraction and assay of pectin methylesterase (PME), cellulase and polygalacturonase (PG) activity

Ten grams of fruit pulp obtained from five different fruits were homogenized with 20 mL of sodium citrate buffer (pH 4.6) containing 1M NaCl, 13 mM EDTA, 10 mM β -mercaptoethanol, and 1% w/v polyvinyl (PVPP). Throughout the process, the mixture was kept completely cool. After filtering the homogenized mixture with Whatman's filter paper, a Sigma3-18K centrifuge (Germany) was used to centrifuge it for 30 minutes at 4°C and 15,000 x g. Cellulase, polygalacturonase, and pectin methylesterase (PME) activities were measured using the crude enzyme extract that was produced from the resulting supernatant, following the procedure described by [7].

The reaction combination used to assess the pectin methylesterase (PME) activity was 1 mL of 0.01% pectin solution (pH 7.5), 0.1 mL of 0.01% bromothymol blue solution, 0.2 mL of NaCl (0.15 mol L⁻¹), 0.2 mL of distilled water, and 0.1 mL of the enzyme extract. After three minutes, the absorbance of the reaction mixture was immediately measured at 620 nm using a spectrophotometer (Spectronic 20D+, Thermo Scientific, USA), and again measured after an additional three minutes. The absorbance difference between 0 and 3 minutes was used to calculate the PME activity. The enzyme activity was quantified as millimoles per kilogram per minute (mmol kg⁻¹ min⁻¹) methyl ester and assessed using galacturonic acid as a reference, in accordance with the approach outlined by [29]. Cellulase activity was measured using the following assay mixture containing 0.1 mL of the enzyme extract, 0.15 mL of 1% w/w carboxymethylcellulose, and 0.5 mL of 0.01M sodium citrate buffer (pH 6.0). The reaction mixture was incubated for thirty minutes at 37°C. The mixture was then immersed in a boiling water bath for five minutes after the addition of 1 mL of 3,5-dinitrosalicylic acid (DNS) reagent. By comparing the absorbance spectra at 540 nm to a standard of glucose, the activity of the cellulase enzyme was ascertained. Based on the methodology outlined by [19], the outcomes were expressed as millimoles of D-glucose per kilogram per minute (mmol kg⁻¹ min⁻¹).

A reaction mixture containing 0.1 mL of sodium chloride (2 mM), 0.4 mL of polygalacturonic acid (1%), 0.1 mL of crude enzyme extract and 2 mM sodium acetate buffer (pH 4.5) was used to evaluate the activity of polygalacturonase (PG). Following an hour of incubation at 37°C, the mixture was heated for a further five minutes in a boiling water bath after 0.1 mL of 3,5-dinitrosalicylic acid (DNS) reagent was added. The polygalacturonase activity was then ascertained using absorbance spectra at 450 nm. The calculation was performed against a standard curve for galacturonic acid. The polygalacturonase activity was measured as millimoles per kilogram per minute (mmol kg⁻¹ min⁻¹) D-galacturonic acid using the method described by [51].

2.12 Extraction and assay of polyphenol oxidase (PPO) activity

The activity of polyphenol oxidase (PPO) enzyme was measured using the procedure outlined by [2]. The enzyme was extracted by homogenizing 0.2 g of fruit pulp tissue in 2 mL of ice-cooled phosphate buffer (0.1 mM, pH 6.8). The homogenized samples' supernatants were used to make PPO extracts after they were centrifuged at 10,000 x g for 20 minutes at 4°C. The polyphenol oxidase enzyme's activity was measured by mixing 500 μ L of the generated enzyme extracts with 1 mL of phosphate buffer (0.1 mol L⁻¹, pH 6.8) and 0.5 mL of 4-methyl catechol (0.1 mol L⁻¹). At intervals of one minute, the rise in absorbance was monitored every three minutes at 410 nm. The unit of measurement for enzyme activity was units per minute per kilogram of fresh weight ($\text{U min}^{-1} \text{Kg}^{-1}$) and was defined as an increase of 0.01 in absorbance per minute per kilogram.

2.13 Statistical analysis

The present investigation employed a factorial completely randomized design and was conducted over the course of two consecutive years (2019-2020 and 2020-2021). There were four replications in each treatment group. Using the procedures of the Statistical Analysis System 9.3 (S.A.S. Institute Inc., Cary, NC, USA), a two-way analysis of variance (ANOVA) was

performed on the combined data for various physico-chemical characteristics and enzymatic activities collected from both years. The Least Significant Difference (LSD) test was utilized to ascertain the significance of various treatment means, with all comparisons being carried out at a 5% probability level. The Pearson correlation coefficient was used to demonstrate the correlation between the several measured attributes ($p < 0.05$ and $p < 0.01$).

3. Results

3.1 Weight loss

Significant weight increase in weight loss occurred in peach fruit during cold storage for 40 days, irrespective of the treatments (Fig. 1A). However, the application of SNP (sodium nitroprusside) solution demonstrated a significant reduction in weight loss compared to control throughout the storage period. The lowest loss (5.37%) was noted in fruits treated with SNP 1.0mM, while the highest weight loss (8.92%) was observed in the SNP 2.0mM treatment at the end of storage when compared to other treatments. The highest weight loss indicated a negative impact likely attributed to the toxicity of the higher SNP dose on the fruits.

3.2 Fruit firmness

Firmness is directly correlated with fruit texture, consumer preference concerning crispiness and the overall storage life of the fruit (Fig. 1B). Despite a general decrease in fruit firmness was observed throughout the cold storage period, irrespective to the treatments SNP treated fruits exhibited at comparatively lower rate of softening. The minimum reduction in fruit firmness (28.38%) was recorded SNP 1.0mM treatment, while the highest firmness reduction (56.76%) was observed in with SNP 2.0mM treated fruits.

3.3 Total soluble solids (TSS) content and titratable acidity (TA)

The total soluble solids (TSS) content increased during storage for all the treatments, but the rate of increase was faster in control compared to the SNP treated fruits until the 20th day of storage, except for SNP 2.0mM treatment. After the 20th day of storage, TSS decreased in all treatments, except for SNP 1.0mM treated fruits. At end of the storage, the maximum retention of TSS corresponding to 11.55% was observed in SNP 1.0mM treated fruits. The titratable acidity decreased with the progression of storage as the storage. A rapid reduction in TA was observed in SNP 2mM treatment in comparison to SNP 1.0 mM at the end of storage.

3.4 Total phenolic content (TPC)

A consistent decrease in total phenolic content (TPC) was observed as the storage period advanced for all treatments (Table 1). At the end of storage period, fruits treated with SNP1.0mM exhibited the highest TPC, while those treated with SNP 2.0mM had the lowest TPC, corresponding to 11.35% and 29.59% reduction respectively.

3.5 Ascorbic acid

The storage period had a decreasing effect on ascorbic acid in all treatments (Table 1). However, SNP treatments resulted in better retention of ascorbic acid compared to the control throughout the storage. At the end of storage, SNP1.0 mM treated fruits exhibited the highest ascorbic acid content, while those treated with SNP2.0 mM had the lowest ascorbic acid corresponding to 75.15% and 54.05% retention, respectively.

3.6 Total antioxidant activity (TAA)

A gradual decrease in total antioxidant activity (TAA) was noticed in all treated peach fruits over the storage period, regardless of the treatment as depicted in Figure 1D. At the end of storage, the least reduction (18%) in total antioxidant activity was measured in fruits treated with SNP 1.0mM. While the highest reduction (62.30%) was observed in fruits treated with SNP 2.0mM.

3.7 Total carotenoids

The total carotenoid content of peach fruit consistently increased during storage across all treatments, as indicated in Table 1. However, sodium nitroprusside treatments led to a notable reduction in total carotenoids throughout the storage period. When compared to control at the end of 40th day of storage, fruits treated with SNP1.0mM exhibited the lowest total carotenoids content (375.06 μ g/100g) compared to other treatments.

3.8 Malondialdehyde (MDA) content and membrane permeability (Relative electrolyte leakage)

Lipid peroxidation was assessed through MDA content of peaches which was examined over the storage period. The alterations in MDA content exhibited similar patterns to electrolyte leakage with both parameters rapidly increasing during storage. However, at the end of storage, SNP 1.0mM treated fruits retained the lowest levels of MDA content (67.68 μ mol/kg⁻¹ FW) and membrane permeability (108.90%), whereas the highest levels of both were observed in the SNP 2.0mM treatment (Fig 1 G & H).

3.9 Polyphenol oxidase (PPO) enzyme activity

Polyphenol oxidase (PPO) enzyme activities increased with the prolonged storage period, and notable statistically significant variations were observed among different treatments (Fig. 1C). The application of sodium nitroprusside (SNP) to the fruits resulted in a reduction in PPO activity during storage. At the end of the storage period, the lowest recorded PPO activity was associated with the SNP 1.0mM treatment (12.75 U/min/g), while the highest was noted in fruits treated with SNP 2.0mM (22.83 U/min/g).

3.10 Pectin methylesterase (PME), polygalacturonase (PG) and cellulase enzymes activity

The pectin methyl esterase (PME) activity exhibited an increase up to the 20th day of storage in all treatments, except for SNP 2.0mM (Fig 1D). Following the 20th day, a decline in PME activity was observed across all treatments, except for those treated with SNP 1.0mM. However, at the end of storage period, a decrease in PME activity was noted in all treatments, with the highest PME activity recorded in SNP 1mM. A similar trend was observed during storage for cellulase and polygalacturonase (PG) enzyme activities in peach fruits (Fig 1E and F).

4. Discussion

Peach fruits have climacteric ripening behaviour, which frequently leads to a short postharvest life due to physiological and biochemical changes that cause an economic loss to the peach industry. During the storage, a steady increase in weight loss was observed in peach fruits. In the present investigation, SNP 1.0mM concentration exhibited superior efficacy in preserving most quality characteristics during low temperature storage, highlighting the importance of finding an optimal SNP concentration.

It is suggested that nitric oxide (NO) has dual characteristics, as the higher concentration of SNP (2.0mM) may adversely affect peaches, leading to deterioration in fruit quality. This negative impact could be attributed to the generation of reactive nitrogen oxide species in conjunction with superoxide ions. These reactive nitrogen species (RNOS) have the potential to affect the fruit through processes such as oxidation, nitrosation and nitration [62]. Similar findings of deterioration of fruit quality have been with higher SNP concentrations in Patharnakh pear fruits stored at 0-1°C and 90-95% relative humidity reported by [2]. Cellular breakdown accelerates the transpiration process, facilitating the exchange of water between the internal and external atmosphere, resulting in weight loss during storage and subsequent shrinkage of the fruit [80]. The loss of carbon atoms in each respiration cycle also contributes to the reduction in weight [76]. In this investigation, the treatment with SNP 1.0mM exhibited a significant inhibitory effect on the weight loss of peach fruit during storage, suggesting that optimal SNP treatment could effectively reduce weight loss. The reduction in weight can be attributed to water transpiration through the cell cuticle, leads to the loss of turgor pressure and the degradation of the cell wall, this is facilitated by enzymes such as pectin methyl esterase and cellulase, contributing to the decline in firmness during storage [17]. [17] reported banana weight loss during storage is attributed to the changes in metabolic activities, such as water transpiration, respiration rate and the activity of softening enzymes. Similar findings were reported in plum [68], kiwi fruit [86], navel orange [88], and guava [63] during storage.

Sodium nitroprusside (SNP) has the potential to act as a triggering molecule, leading to an increase in salicylic acid levels, which play a crucial role in imparting tolerance against stress conditions [53]. Notably, the exogenous application of nitric oxide was found to significantly extend fruit storage life by inhibiting the respiration rate and consequently retarding the ripening process [62]. The softening of fruit, resulting in reduced firmness, has adverse effect on consumer acceptability and can impact the economic value of the fruit. The decrease in fruit firmness during storage primarily results from the softening of the cell wall, either through the breakdown of insoluble pectin into soluble pectin or through cellular disintegration [54]. The ripening process involves the degradation of the cell wall and pectic substances, contributing to the loss in fruit firmness [59]. Firmness serves as the most dependable indicator of quality and shelf life for pear fruit during storage. Despite a significant reduction in fruit firmness observed throughout the storage period independent of treatments (Fig1B). Application of SNP treatment resulted in a decrease in the activity of pectin esterase (PE), pectin methyl esterase (PME) and poly-galactouronase (PG), enzymes responsible for cell wall degradation [85], leading to the preservation of firmness. In the present study, the treatment with SNP 1.0 mM maintained higher fruit firmness throughout the entire storage period compared to all other samples. Consistent with these findings, similar positive effects of SNP treatment were observed in fruits of pear [2], peach [24], plum [68] and kiwifruit [91].

An initial increase in soluble solids content during the early stages of ripening is attributed to the hydrolytic conversion of insoluble polysaccharides and pectic substances into simpler mono and disaccharide sugars [39]. Higher soluble solids in fruits may also result from moisture loss, leading to dehydration and concentrated juice [3].

Conversely, the gradual decline in soluble solids content during later phases of storage is linked to carbohydrate consumption and the metabolic breakdown of glycosides during respiration [75]. The increase in total sugars during ripening is associated with enhanced sugar permeability across the tonoplast, leading to higher sugar accumulation in the free cell space [75]. Additionally, the hydrolytic conversion of starch into sugars occurs more rapidly at higher temperatures due to an accelerated respiration process [11]. The increase in soluble solids content is likely due to sugar formation from organic acids with an increase in the activity of glycolytic enzymes during storage [58]. However, in the present work the SNP 1.0mM treatment significantly delayed the increase in SSC compared to other treatments and retained higher SSC at the end of storage. After 30 days of storage, a decline in soluble solids content occurred as stored carbohydrates might have consumed in metabolic activities during storage, aligning with the findings of [15] in grapes. Similar delays in SSC increase during storage were observed in SNP treated papaya fruit [46] and 'Fuji' apple [16]. This effect of SNP can be attributed to the lower rate of respiration, reduced ethylene production and delayed ripening process [62]. Organic acids, namely malic acid, serve as major substrates for respiration, and a higher rate of postharvest respiration leads to a decrease in the acid content of fruits[52,78].

Titratable acidity (TA) is a crucial quality indicator that reflects fruit flavour, with malic acid being the predominant contributor to TA in pear fruit. The gradual decline in TA during storage results from the utilization of these organic acids as respiratory substrates and their subsequent conversion into sugars. In the current study, TA in peach fruit decreased during storage. However, fruit treated with SNP 1.0mM showed a minimal reduction and retained higher TA throughout the storage period. Similar findings were observed by [16] in apple fruit that SNP treated fruit retained higher TA compared to the control. [12] also demonstrated the effectiveness of postharvest nitric oxide treatment in litchi fruit to retard ripening by reducing the loss of soluble solids, acidity, ascorbic acid, total phenolic content and lowering decay, thereby extending the storage life. Phenolics play a crucial role as antioxidant compounds, and there is a growing recognition of their functional properties. These compounds, being principal secondary metabolites, act as potent antioxidants. The current study observed a notable reduction in total phenolic content (TPC) during storage, likely attributed to the increased activities of polyphenol oxidase (PPO) enzymes, leading to the utilization of polyphenols as substrates, consequently decreasing total phenolic content [28]. However, peaches treated with SNP, the rate of decline in TPC was slower compared to untreated fruits under low temperature storage conditions. Previous studies have also shown a restricted loss in TPC as a result of SNP treatment in fruits of persimmons[64], pears [2] and apples[40]. Regardless of treatments, a decrease in TPC during storage was also reported in table grapes, and this reduction might be attributed to oxidation by the PPO enzyme [67]. In our study, a significantly negative correlation was identified between TPC and PPO activity (Tables 2 and 3, Fig. 2 F). A similar and highly significant negative correlation between total phenolic content (TPC) and polyphenol oxidase (PPO) was identified in pears under low temperature storage conditions[2]. In pears, PPO consists of a diverse structure belonging to different classes such as flavonoids and phenolic acids.

The degradation of phenolic compounds in pears may occur through direct oxidation by polyphenol oxidases, followed by coupled oxidation [18]. The observed slower degradation of TPC in SNP treated fruits is attributed to the delayed activity of the PPO enzyme [77].

Ascorbic acid serves as a major antioxidant in fruit, playing a crucial role in scavenging reactive oxygen species and preventing browning. It is considered an integral component of the fruit's defence system and is also regarded as a quality parameter concerning the nutritional aspect of the fruit. Ascorbic acid is an important nutritional compound in fruit but often undergoes oxidative losses during storage. However, in fruits treated with SNP 1.0mM, this loss was controlled to the maximum compared to the other treatments. Sodium nitroprusside appears to form a protective layer that prevents ascorbic acid loss in fruit during storage, potentially due to the formation of a protective barrier on the fruit surface against gas permeability [43]. This barrier may inhibit the auto-oxidation of ascorbic acid and regulate the enzymatic activity of ascorbic acid oxidase and phenoloxidase, which is highly influenced by oxygen uptake in storage conditions [81]. Similarly, SNP treatment has been shown to maintain ascorbic acid content in mangoes [31], blueberries [27] and apples [16] during storage. The decline in antioxidant activities in fruit during storage often indicates the oxidation of phenolics and ascorbic acid compounds. The reduction in antioxidant activity observed in stored fruit is often indicative of the oxidation of phenolic and ascorbic acid compounds [43]. A rapid decline in total antioxidant activity during storage may result from increased metabolic activities leading to cell disruption, allowing oxidizing enzymes like polyphenol oxidase etc., access to their bioactive substrates [9]. The swift degradation of ascorbic acid and total phenolics content during storage contributes to the decline in antioxidant activity. Consequently, a significant negative correlation was identified between ascorbic acid, total phenolic content and total antioxidant activity of peach fruits during storage (Table 2, Fig 2E, I). The effectiveness of SNP in preserving antioxidant activity has been reported in pistachios [28], navel oranges [88] and lemons [37] during storage. Similarly SNP was found to delay the senescence of apples by inhibiting the respiration rate and maintaining a higher antioxidant capacity, aligning with the observed effects of SNP treatment on persimmons [31], mangos [64], plums [65], peaches [83] and bananas [62]. As the ripening occurs, chlorophyll pigment degradation occurs, revealing previously hidden pigments such as carotenoids. The transformation of chloroplasts in the peel fruits into coloured plastids further contributes to changes in fruit color [6]. The degradation of chlorophyll, coupled with carotenoid synthesis during storage, influences the overall fruit colour [32]. Peach fruits treated with SNP maintained the lowest total carotenoid content throughout storage, attributed to the slower degradation of chlorophyll pigments. Nitric oxide treatment has been shown to down-regulate the transcriptional levels of chlorophyllase and Mg dechelatase, leading to reduced enzyme activities and consequently slowing down chlorophyll degradation in bananas during the ripening process [79]. Similarly, [63] reported delayed ripening in guava fruits treated with sodium nitroprusside (1.0 mM), stored at ambient conditions ($20 \pm 3^\circ\text{C}$) resulting in minimal chlorophyll loss and a slower increase in the synthesis of carotenoid pigments. Electrolyte leakage and the accumulation of MDA serve as indicators of cellular damage, typically increasing during fruit ripening [34].

Prolonged storage of fruits and vegetables induces the production of reactive oxygen species like H_2O_2 , damaging membrane fatty acids and leading to increased Malondialdehyde (MDA) content. Malondialdehyde content and relative electrolyte leakage (REC) primarily contribute to membrane lipid peroxidation, reflecting cellular membrane integrity [66]. Therefore, higher MDA content and REC were noted in the SNP (2.0mM) and control treatments, causing cellular damage and resulting in a loss of fruit firmness during storage. The MDA content and REC of peach fruits demonstrated a negative correlation with firmness (Tables 2 and 3, Fig G & H). The elevated MDA content and REC also played a role in chlorophyll degradation, indicating that damaged membranes influence chlorophyll breakdown [84]. In our study, the increased MDA content and REC were positively correlated with carotenoid contents, contributing to rapid chlorophyll degradation with storage. Similarly, low levels of MDA content and electrolyte leakage were maintained, and cell membranes remained more intact in nitric oxide (NO) treated mushrooms. Exogenous NO treatment reduced reactive oxygen species accumulation, as reported by [25]. This finding aligns with research on *Zingiber officinale* Roscoe and cucumber by [45,82] respectively.

Polyphenol oxidase identified as a copper-containing metalloprotein, plays a crucial role in catalyzing the oxidation of phenols to quinones within fruits, leading to the formation of brown substances [50,41]. Consequently, the study emphasizes the strong correlation between PPO activity and the total phenolic content (TPC) in fruits, and both are closely linked to the occurrence of fruit browning [44]. During storage, decline in TPC was observed, potentially attributable to the oxidative conversion of phenolic to quinones facilitated by the presence of the PPO enzyme [28]. The present study also identified a comparable inverse relationship between PPO activity and total phenolics content, as detailed in Table 2 and Fig. 2F. The slower degradation of TPC in SNP treated fruits indicates a delay in the activity of the PPO enzyme, consistent with the findings of [77]. Similarly, treatments involving sodium nitroprusside applied to 'Patharnakh' pear fruits demonstrated suppression of polyphenol oxidase activity when stored under conditions of $0-1^\circ\text{C}$ and 90-95% relative humidity, as reported by [2]. The application of sodium nitroprusside (SNP) resulted in a reduction of cell wall degrading enzyme activity during ambient storage of peach fruits.

Polygalacturonase (PG) catalysed PME activity, contributing to the depolymerization of pectin and consequent fruit softening [60]. A group of enzymes, including PG, PME, pectin esterase (PE), and cellulase, collectively contribute to the degradation of pectic substances and impact fruit quality during storage [23]. Pectin methyl esterase, identified as a cell wall degrading enzyme, plays a role in the hydrolysis of pectic components in the cell wall, influencing cell-to-cell adhesion [55]. Lower activity of fruit softening enzymes corresponds to maintained fruit quality as observed in pear by [36]. Sodium nitroprusside was found to delay the rise in PME activity. Since pectin is a primary component of the fruit's cell wall and middle lamella, its hydrolysis by PME generates demethylated pectin, making it more susceptible to degradation by PG. This process aids in depolymerizing pectin and retaining fruit crumbliness and firmness during storage [87]. Similarly, the use of SNP 0.5mM in the plum cultivar 'Santa Rosa' was associated with maintaining fruit quality and extending shelf life.

This was achieved by inhibiting fruit softening through interference with phenylalanine ammonia lyase (PAL) and PME activities during cold storage, as reported by [65]. In a study conducted by [46], it was observed that the fumigation of winter jujube (*Ziziphus jujuba* Mill. cultivar Dongzao) fruits with 20 μ L/L of nitric oxide (NO) for 3 hours led to the suppression of activities of pectin methylesterase, polygalacturonase, β -galactosidase and cellulase during a 75 days storage period at 0 \pm 1°C and 90-95% relative humidity. The softening process in fruits is believed to result from the de-esterification of pectin catalysed by PME, followed by pectin depolymerisation catalysed by PG, with PG activity dependent on PME for substrate availability [1].

Postharvest immersion treatment of sodium nitroprusside (SNP) at a concentration of 0.002 mol L⁻¹ on 'Patharnakh' pear fruits resulted in decreased activity of the fruit softening enzyme cellulase under cold storage conditions (0-1°C and 90-95% relative humidity), as observed in the study by [2]. Cellulase is pivotal in breaking down hemicellulosic polysaccharides and degrading cellulosic and β -1, 4-glucan linkages within the cell wall, as explained by [87]. Enzymes like polygalacturonase, pectin methylesterase, pectin esterase, and cellulase contribute to the reduction in firmness and quality during storage, as noted by [23]. [4] reported that cellulase activity in Kinnow fruits leads to firmness and quality loss by breaking down cellulose into simple sugars. In the research conducted by [2], the relationship between enzymatic activity and firmness during storage demonstrated a statistically significant negative correlation ($p \leq 0.05$) between firmness and the activities of pectin methylesterase (PME), polygalacturonase (PG) and cellulase. Firmness is a crucial postharvest quality parameter influenced significantly by pectic substances. High levels of pectin dimethyl esterification, catalysed by PME, not only serve as substrates for PG but also alter the pH level and cation exchange mechanism of the cell wall, affecting the activity of other cell wall degrading enzymes such as cellulase [56]. In the study, cellulase activity exhibited a steady increase over time, at a lower level in SNP treated fruits. Control fruits showed the lowest activities of PG, PME, and cellulase at later stages of storage, likely due to the rapid ripening related changes occurring earlier in these fruits. Consequently, during the initial storage period, the utilization of enzyme substrates was greater in control fruits, leading to a reduction in enzyme activity at later stages. Conversely, SNP treated fruits exhibited higher enzyme activities towards the end of storage, possibly due to a slower ripening process during the initial stages of storage. Similar observations regarding the inhibitory effect of nitric oxide on fruit softening have been reported in plum [68], kiwi fruit [90] and pear[70,36].

5.1 Correlation and regression analysis

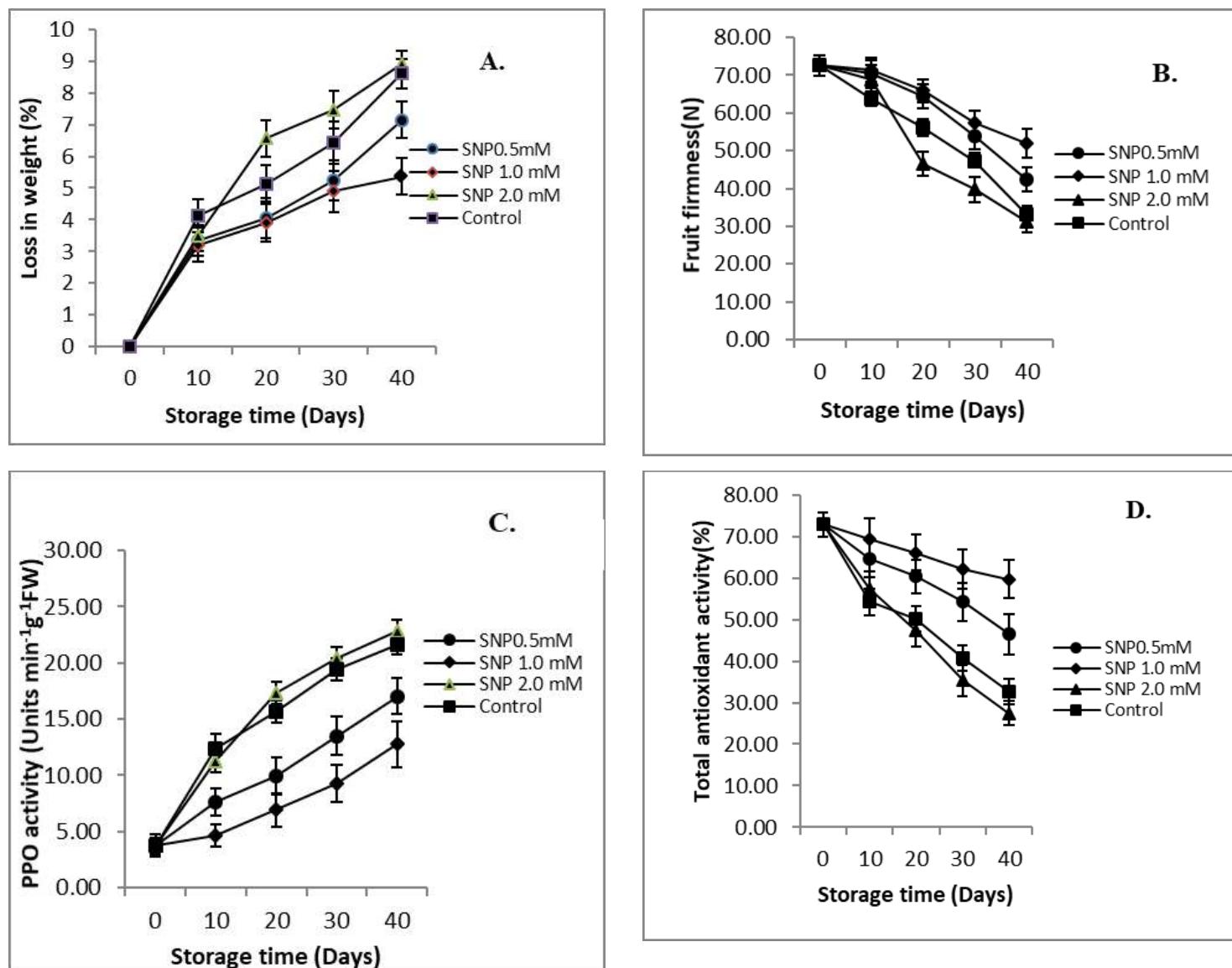
Table 2 illustrates the association between several attributes as determined by the Pearson correlation coefficient. Fruit firmness was negatively correlated with softening enzyme activities such as pectin methylesterase (PME), polygalacturonase (PG), and cellulase, as well as parameters like mass loss, total soluble solids (TSS), total carotenoids, malondialdehyde (MDA) content, and relative electrical conductivity (REC). Soluble solid content (SSC), on the other hand, increased as fruit weight decreased but had the opposite effect on fruit firmness. Interestingly, titratable acidity showed a strong positive link with fruit firmness but an inverse relationship with fruit mass loss. Furthermore, ascorbic acid concentration and antioxidant activity were positively correlated, although total phenolic content (TPC) and polyphenol oxidase (PPO) activity were negatively correlated. Subsequently, regression analysis was performed on correlated to establish the relationship between two attributes, as presented in Table 3 and Fig. 2.

Correlation and regression analyses revealed a substantial negative correlation (-0.924, $R^2 = 0.855$) between weight loss and firmness (Tables 2 and 3, Fig. 2A). Fruit firmness also demonstrated significant negative relationships with the activities of pectin methylesterase, polygalacturonase, and cellulase. Specifically, the correlation analysis indicated negative relationships between fruit firmness and pectin methylesterase activity (-0.076, $R^2=0.006$), cellulase activity (-0.335, $R^2=0.112$) and polygalacturonase activity (-0.248, $R^2=0.062$) (Tables 2 and 3, Fig. B, C, and D, respectively). Moreover, ascorbic acid exhibited a positive relationship with antioxidant activity (0.940, $R^2=0.883$) (Tables 2 and 3, Fig. 2E) and total phenolic content showed a positive relationship with antioxidant activity (0.987, $R^2=0.974$) (Table 2 and 3, Fig 2 I). Conversely, there was an inverse relationship between total phenolic content and polyphenol oxidase activity (-0.985, $R^2=0.969$) (Tables 2 and 3, Fig. 2F). Similar correlations and regression relationships between firmness and weight loss, firmness and pectin methylesterase activity, and firmness and cellulase activity were reported by [69,70,71] in postharvest treatments of putrescine-treated pear fruits of 'Punjab Beauty' stored in cold storage conditions (0-1 °C and 90-95% RH). Similarly, [2] reported correlations and regression relationships between polyphenol oxidase and total phenolic content, firmness and pectin methylesterase, cellulase, and polygalacturonase in sodium nitroprusside treated pear fruits of 'Patharnakh' stored under similar conditions. [73,74] also found analogous correlations between total phenolic content (TPC) and polyphenol oxidase (PPO) activity, as well as TPC and antioxidant activity, in 'Punjab Beauty' pear fruits (*Pyrus pyrifolia* x *Pyrus communis*) treated with different coatings such as chitosan enriched salicylic acid and beeswax plus salicylic acid under cold storage conditions (0-1°C and 90-95% RH).

Table 1. Effect of postharvest application of sodium nitroprusside (SNP) at different concentrations on ascorbic acid (AsA), total phenolic content (TPC), Soluble solids content (SSC), Titratable acidity and total carotenoids of peach fruits under cold storage (0-1 °C, 90-95% RH) for 40 days.

Parameter	Treatment	Storage time (days)				
		0	10	20	30	40
Ascorbic acid (mg100mL ⁻¹ juice)	SNP 0.5mM	27±0.55 ^a	22.07±1.13 ^{bcd}	21.01±1.07 ^{cde}	19.25±0.93 ^{efg}	17.33±0.80 ^{hi}
	SNP 1.0mM		23.37±1.20 ^b	22.66±0.96 ^{bc}	21.83±1.02 ^{bcd}	19.84±1.07 ^{ef}
	SNP 2.0mM		20.80±1.28 ^{de}	17.62±1.07 ^{ghi}	15.75±0.74 ^{jk}	14.66±1.17 ^k
	Control		19.67±0.95 ^{fghi}	18.47±0.89 ^{fgh}	16.56±0.48 ^{ll}	15.58±0.51 ^{jk}
Total Phenolic content (mg100g ⁻¹ FW)	SNP 0.5mM	256.77±4.38 ^a	244±7.25 ^{bc}	233.39±7.31 ^{de}	224.89±5.99 ^{ef}	209.94±4.69 ^{gh}
	SNP 1.0mM		251.20±5.36 ^{ab}	245.79±6.42 ^{abc}	236.49±6.47 ^{cd}	227.62±5.18 ^{def}
	SNP 2.0mM		231.81±6.32 ^{de}	212.34±6.05 ^{gh}	194.92±6.78 ^{ji}	180.78±4.88 ^k
	Control		224.30±6.73 ^{ef}	217.71±4.43 ^g	202.33±4.72 ^{hi}	188.37±4.13 ^{jk}
Soluble solids content (%)	SNP 0.5mM	9.80±0.21 ^{hi}	10.74±0.44 ^{efg}	11.96±0.36 ^{abc}	11.73±0.54 ^{bcd}	10.75±0.59 ^{efg}
	SNP 1.0mM		10.57±0.39 ^{fgi}	11.70±0.58 ^{bcd}	12.24±0.20 ^{ab}	11.55±0.47 ^{bcd}
	SNP 2.0mM		11.14±0.39 ^{def}	10.49±0.56 ^{fgi}	10.31±0.53 ^{ghi}	9.54±0.45 ⁱ
	Control		11.88±0.28 ^{abcd}	12.63±0.04 ^a	11.28±0.24 ^{cde}	10.36±0.26 ^{gh}
Titratable acidity (%)	SNP 0.5mM	0.73±0.03 ^a	0.67±0.05 ^{ab}	0.62±0.03 ^{bcd}	0.58±0.05 ^{def}	0.52±0.03 ^{fg}
	SNP 1.0mM		0.71±0.04 ^a	0.68±0.04 ^{ab}	0.64±0.05 ^{bc}	0.60±0.03 ^{cde}
	SNP 2.0mM		0.60±0.04 ^{cde}	0.44±0.03 ^{hi}	0.40±0.03 ^{ji}	0.34±0.02 ^l
	Control		0.58±0.02 ^{def}	0.54±0.02 ^{ef}	0.46±0.02 ^{gh}	0.41±0.02 ^{hi}
Total carotenoids (μ g 100g ⁻¹)	SNP 0.5mM	307.86±3.16 ⁱ	333.55±7.46 ^h	355.65±6.75 ^g	373.56±5.84 ^{de}	384.85±5.98 ^e
	SNP 1.0mM		325.53±7.19 ^h	348.81±7.63 ^g	365.78±6.20 ^{ef}	375.06±5.18 ^{de}
	SNP 2.0mM		345.90±7.32 ^g	383.85±5.75 ^g	403.80±5.14 ^b	417.48±5.76 ^a
	Control		354.89±3.09 ^g	371.25±2.70 ^e	382.41±2.82 ^{cd}	407.97±3.12 ^{ab}

Fig. 1. Variation in loss in weight (A), fruit firmness (B), sensory score (C), PPO activity (D), total antioxidant activity (E), PG activity (F) and cellulase activity (G) of peach fruit during cold storage in relation to different treatments of sodium nitroprusside (SNP). Vertical bars represent \pm S.E. of means for 4 replicates ($p \leq 0.05$)



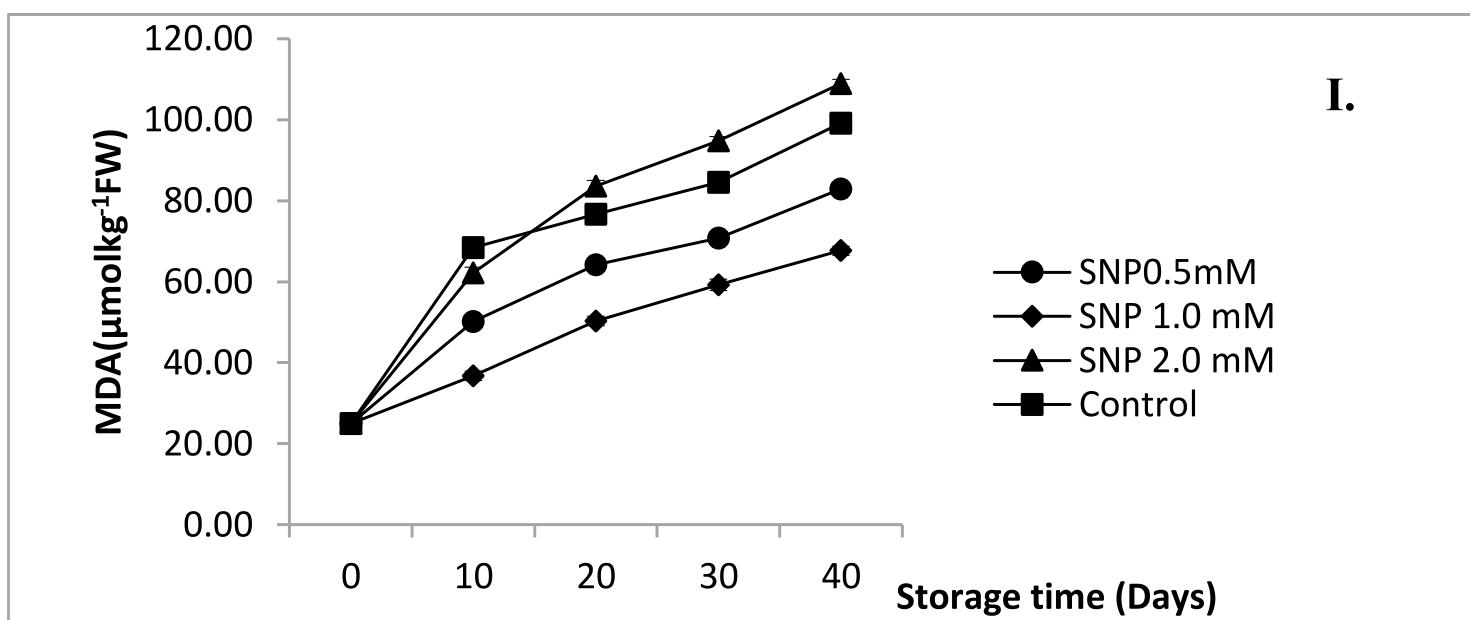
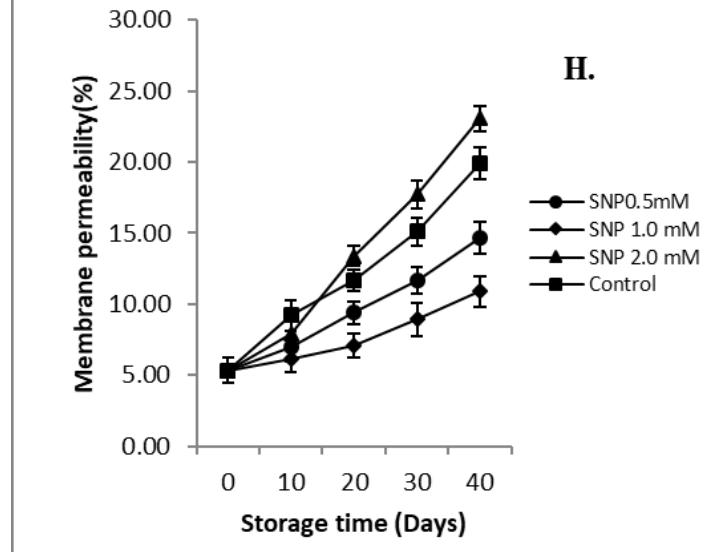
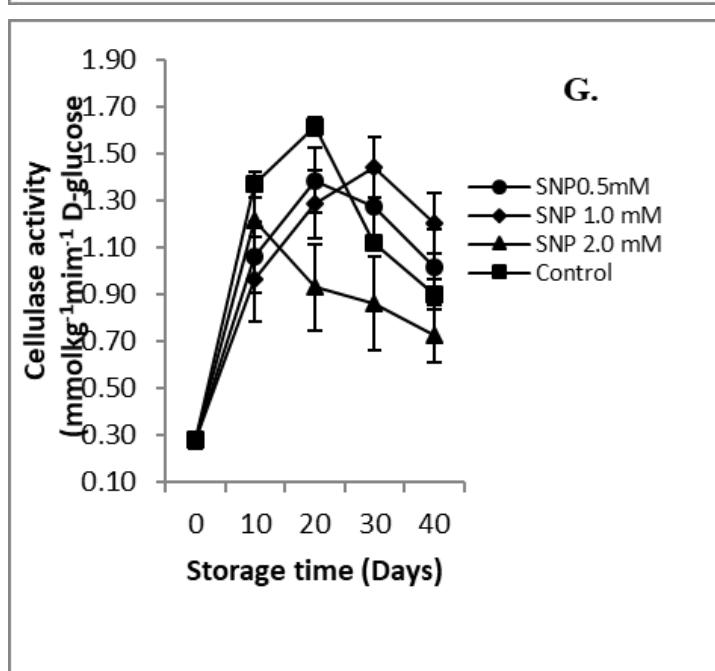
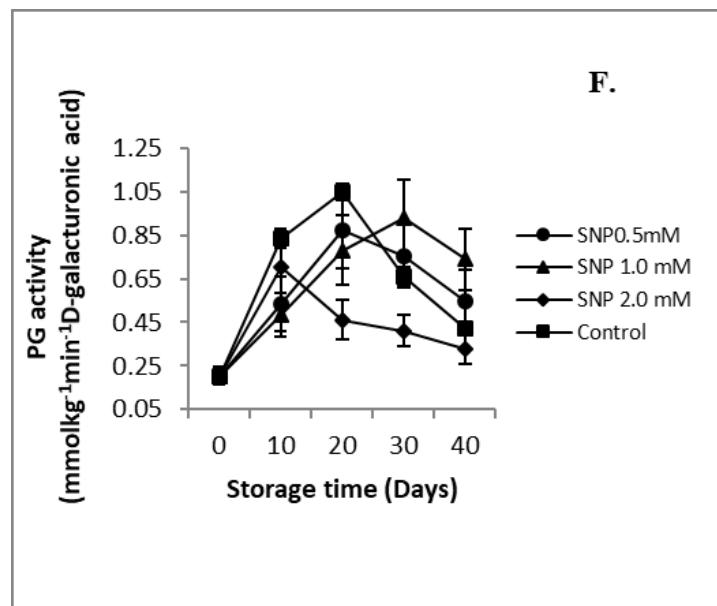
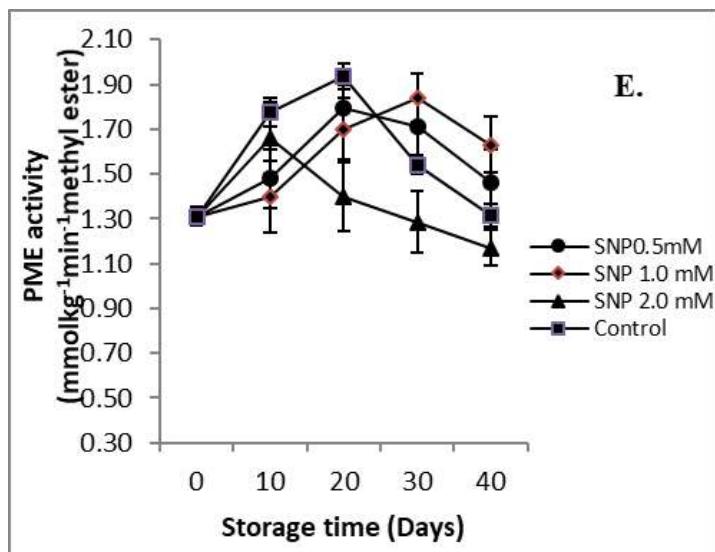
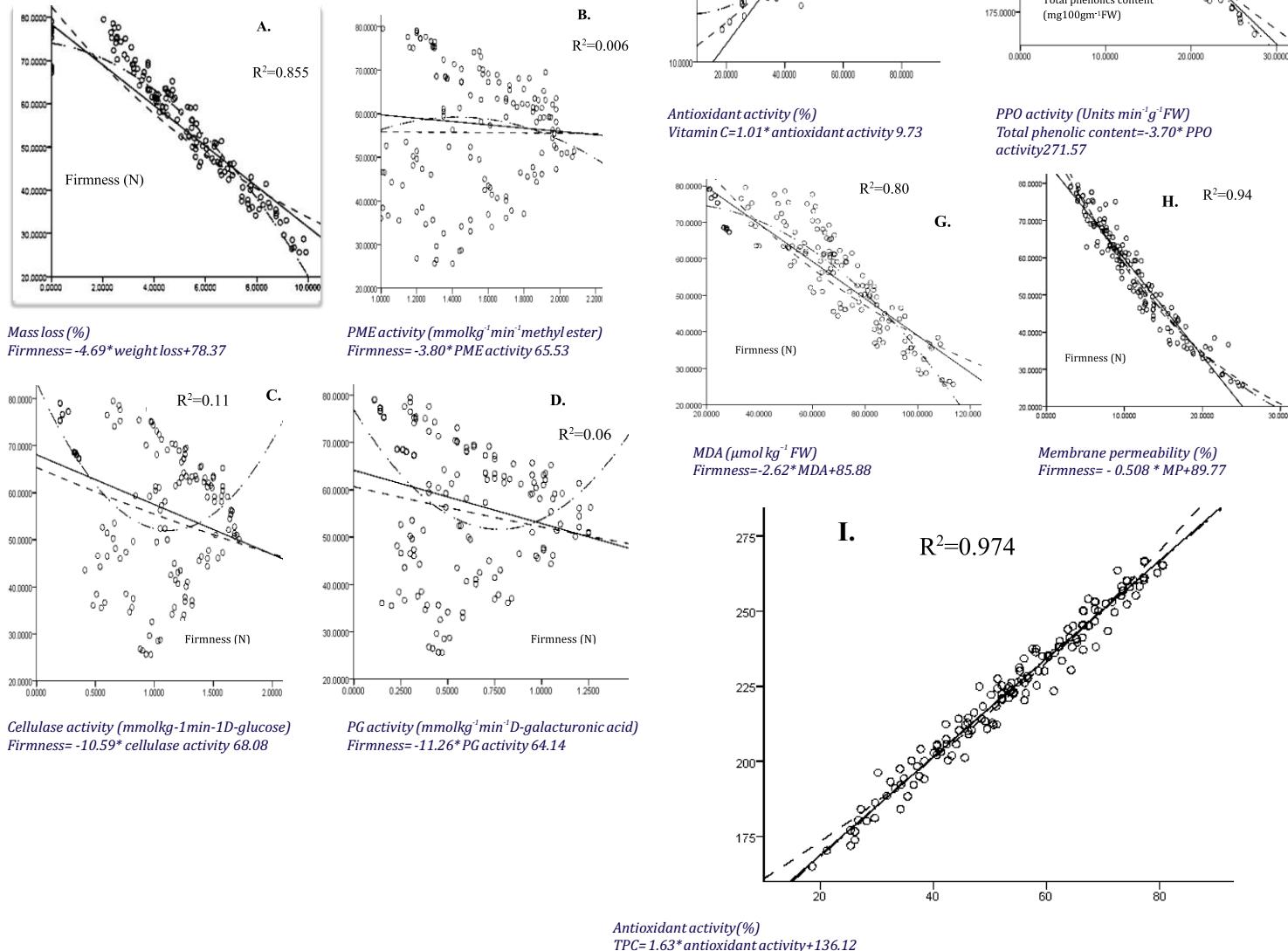


Table 2. Pearson's correlation coefficients between various attributes of peach fruit

	Mass loss	Firmness	SSC	TA	TPC	PPO activity	Ascorbic acid	Antioxidant activity	Total carotenoids	PME activity	PG activity	Cellulase activity	MDA	Membrane permeability
Mass loss	1													
Firmness	-0.924**	1												
SSC	0.320**	-0.184*	1											
TA	-0.894**	0.926**	-0.225**	1										
TPC	-0.927**	0.950**	-0.257**	0.966**	1									
PPO activity	0.933**	-0.946**	0.275**	-0.958**	-0.985**	1								
Ascorbic acid	-0.944**	0.886**	-0.386**	0.920**	0.949**	-0.955**	1							
Antioxidant activity	-0.912**	0.940**	-0.247**	0.967**	0.987**	-0.974**	0.940	1						
Total carotenoids	0.973**	-0.949**	0.315**	-0.914**	-0.945**	0.952**	-0.948	-0.926**	1					
PME activity	0.209**	-0.076	0.934**	-0.127	-0.154	0.165*	-0.283	-0.162*	0.191*	1				
PG activity	0.413**	-0.248**	0.921**	-0.303**	-0.327**	0.350**	-0.464	-0.332**	0.396**	0.922**	1			
Cellulase activity	0.552**	-0.335**	0.876**	-0.376**	-0.418**	0.448**	-0.581	-0.415**	0.516**	0.849**	0.938**	1		
MDA	0.944**	-0.893**	0.213*	-0.889**	-0.918**	0.933*	-0.927**	-0.889**	0.961**	0.081	0.305**	0.460**	1	
Membrane permeability	0.910**	-0.967**	0.114	-0.934**	-0.964**	0.948**	-0.889**	-0.958**	0.933**	0.006	0.186*	0.286**	0.909**	1

Significant at $p \leq 0.01$ *Significant at $p \leq 0.05$ **Table 3. Linear regression relationship between various attributes

Combination	Equation	R ²
Weight loss	Firmness= -4.69 *weight loss+78.38	0.855
PME activity	Firmness= -3.80 *PME activity +65.53	0.006
Cellulase activity	Firmness= -10.59 * cellulase activity+ 68.08	0.112
PG activity	Firmness= -11.26 * PG activity +64.14	0.062
Antioxidant activity	Ascorbic acid=0.26 *antioxidant activity+ 5.96	0.883
Antioxidant activity	Total phenolic content=1.63* antioxidant activity+136.12	0.974
PPO activity	Total phenolic content= -3.70 PPO * activity+271.57	0.969
MDA	Firmness= - 0.508 * MDA+89.77	0.798
MP	Firmness= -2.62* MP+85.88	0.935

**Fig. 2.** Linear regression relationship between various attributes.

Conclusion

The current study investigated the efficacy of sodium nitroprusside (SNP) as a safe antioxidant in preserving bioactive compounds and modulating fruit ripening in peaches stored at low temperatures. Various SNP treatments were applied to assess their impact on delaying fruit softening and maintaining peach fruit quality, thereby extending postharvest storage life under cold storage conditions. The findings revealed that SNP treatments, acting as nitric oxide (NO) donors, effectively delayed ripening changes in peach fruit during cold storage. Specifically, treatment with 1.0 mM sodium nitroprusside showed significant inhibition of ripening related changes, including the retention of fruit firmness, soluble solids content, titratable acidity, ascorbic acid content, total phenolic content and antioxidant activity. Moreover, this treatment led to a slower degradation of chlorophyll pigments and reduced synthesis of carotenoids. Additionally, SNP treated peach fruits exhibited decreased mass loss, MDA content, REC, and activities of PME, PG, cellulase and PPO enzymes compared to other treatments. In summary, postharvest application of SNP proved to be an effective method for extending the storability and maintaining the quality of peach fruit, particularly in the 'Shan-i-Punjab' cultivar. However, further investigation into the biotechnological intervention regarding gene expression induced by SNP application is warranted to gain a better understanding of the relationship between SNP treatment and peach storability.

Future Thrust

Future research should focus on investigating the optimal concentrations of sodium nitroprusside (SNP) for various peach varieties, its combined effects with other postharvest technologies, and a deeper understanding of its metabolic mechanisms to ensure food safety and human health. The goal is to move beyond its efficacy in lab settings to develop practical, scalable, and safe industrial applications for commercial use in extending peach storability.

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Conflict of Interest Statement

The author declare that they have no competing interests

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