International Journal of Horticultural Science and Technology
Journal homepage: https://ijhst.ut.ac.ir

Impact of Postharvest Sodium Nitroprusside Application on Physicochemical Quality of Apple Fruit cv. 'Golden Delicious' during Cold Storage

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ARTICLE INFO

Article history:

Received: 4 January 2025,
Received in revised form: 2 June 2025,
Accepted: 3 June 2025,

Article type:

Research paper

Keywords:

Ethylene
Fruit quality
Storage life
Vitamin C

ABSTRACT

This study examines the effects of sodium nitroprusside (SNP) on 'Golden Delicious' apples, focusing on biochemical and quality changes during cold storage (0 ± 1 °C; 90–95% relative humidity). Apples were treated with SNP at concentrations of 0, 5, and 7 mM and assessed at 90, 150, and 210 d post-storage. The results demonstrated that SNP significantly influenced peroxidase activity, total phenolic content, vitamin C levels, fruit firmness, and ethylene production. Over extended storage, activities of catalase and peroxidase, soluble protein content, reducing sugars, and ethylene production increased, whereas total phenolic compounds, vitamin C, titratable acidity, and fruit firmness decreased. Higher SNP concentrations were associated with enhanced fruit firmness between 90 and 150 d of storage. Notably, the 5 mM SNP treatment increased fruit firmness by 24.5% after 210 d, whereas the 7 mM treatment showed no significant difference compared to the control. SNP-treated apples exhibited improved visual quality, with 5 and 7 mM SNP treatments mitigating vitamin C loss and reducing titratable acidity. These findings indicated that SNP treatment may reduce ethylene production, delay senescence, and enhance fruit quality.

Abbreviation: Sodium Nitroprusside (SNP)

Introduction

The apple (*Malus domestica* Borkh.), a member of the Rosaceae family, is a cornerstone of temperate fruit production, valued for both fresh consumption and processed products, and plays a pivotal role in global trade (Yuri et al., 2012). Its high polyphenol content contributes significantly to human health and nutrition, with regular consumption associated with reduced risks of cardiovascular diseases and atherosclerosis (Yuri et al., 2012). However, maintaining postharvest quality remains a challenge due to physiological and biochemical changes during storage, which lead to fruit softening, nutrient loss, and diminished marketability (Hasan et al., 2024). Key indicators of apple quality and marketability include fruit freshness, appearance, and qualitative parameters such as texture and nutrient composition, which are critical for determining the storage duration of horticultural crops (Han et al., 2015; Liu et al., 2023).

Understanding the metabolic dynamics of volatile compounds in apples provides insights into fruit maturity and ripening, processes influenced by cold storage duration. Effective management of ripening can mitigate losses of titratable acids due to malic acid metabolism (Defilippi et al., 2004) and enhance the stability of soluble acid content (Larrigaudière et al., 2008), thereby extending the shelf life of aromatic compounds. Postharvest treatments that regulate oxidative stress, delay senescence, and

maintain fruit firmness are essential for prolonging apple storage life (Li et al., 2022).

Nitric oxide (NO), a bioactive growth regulator, has gained attention in postharvest horticulture for its potential to extend shelf life when applied at low concentrations (Jayarajan and Sharma, 2018). NO influences ethylene synthesis by suppressing respiration and inhibiting 1-aminocyclopropane-1-carboxylic acid (ACC) activity (Nolpradubphan and Lichanporn, 2016). Sodium nitroprusside (SNP), an NO-releasing compound, shows promise in extending fruit shelf life by reducing pericarp browning, preserving antioxidant activity (Barman et al., 2014), and mitigating fruit softening (Li et al., 2014; Madebo et al., 2023).

Ren et al. (2017) reported that SNP treatment of mango fruit (Mangifera indica L. cv. 'Tainong') reduced respiration rates, rotting indices, and fruit weight loss while enhancing tissue firmness during storage. Additionally, SNP treatment increased the activity of antioxidant enzymes, including superoxide dismutase, catalase, and peroxidase, throughout the storage period. Similarly, Wang et al. (2015) observed enhanced antioxidant enzyme activity in banana fruit following SNP treatment. Although these studies demonstrate SNP's efficacy in improving postharvest storage attributes in various fruits, its application in apples remains underexplored. Given the economic importance of apples and their susceptibility to postharvest deterioration, investigating SNP treatment to enhance storability is of considerable interest.

This study aims to elucidate the potential of SNP in horticulture by examining its effects on extending the shelf life and preserving the quality of apples during postharvest storage. By exploring the biochemical mechanisms influenced by SNP treatment, this research seeks to provide valuable insights into improving fruit quality and extending shelf life in the horticultural industry.

Materials and Methods

Experimental procedure

Apples were hand-picked in 2023 from 21-year-old apple trees (*Malus* × *domestica* Borkh. cv. 'Golden Delicious') in a commercial orchard located in Oshnavieh, West Azarbaijan Province, Iran (45°5′ E, 37°2′ N, at an elevation of 1400 m). For each treatment, 60 apples were selected from one side of each of the 18 trees at a harvest index of 150 d after full bloom, with 20 apples per replicate harvested early in the morning (temperature 14 °C, relative humidity 55%) and transported to the laboratory for treatment with sodium nitroprusside (SNP). SNP (Merck, Germany) was dissolved in distilled water at the specified concentrations for each treatment, and the apples were immersed in the SNP solutions for 10 min. Distilled water was used for the control group. After a 2 h drying period, the treated and control apples were placed in separate polyethylene boxes (45 × 28 × 12 cm) and stored under controlled conditions (0 ± 1 °C, 90–95% relative humidity). The experiment was conducted as a factorial experiment based on a completely randomized design with three replications. Each replication consisted of 20 apples. The first factor was the different concentrations of sodium nitroprusside (0 (control), 5, and 7 mM), and the second factor was the different storage durations (90, 150, and 210 d).

Monitoring the enzymatic antioxidant capacity

The modified method of Kang and Saltveit (2002) was used to prepare the plant extracts. Initially, 0.5 g of fruit tissue was ground in a cold mortar with 3 mL of buffer (50 mM Tris-HCl, pH 7.0) containing 3 mM $MgCl_2$ and 1 mM disodium EDTA. The resulting homogenate was centrifuged for 20 min at 4 °C at 10,000 rpm (Hermle Z216 MK; Germany). The supernatant was then stored in a freezer at −26 °C for subsequent measurement of the enzymatic activity of peroxidase and catalase enzymes.

Catalase activity assay

Catalase activity was measured using the Beers and Sizer (1952) method, which monitors the rate of hydrogen peroxide (H_2O_2) degradation. Initially, 0.5 mL of fruit extract was mixed with 1.5 mL of phosphate buffer (50 mM, pH 7.0) and centrifuged (10,000 rpm, 15 min, 4 °C). Then, 50 µL of the supernatant was combined with 2.5 mL of phosphate buffer. This solution was transferred to a spectrophotometer, followed by the addition of 20 µL of H_2O_2. Absorbance was recorded at 240 nm for 3 min. Catalase activity, expressed as units per mg of protein, was calculated based on the change in absorbance (Equation 1).

$$\mathrm{CAT} = \frac{\Delta E \times \text{Dilution factor}}{(\text{time} \times \text{EC} \times \text{FW})} \qquad \text{Equation 1}$$

Where ΔE is the change in absorbance, EC is the extinction coefficient (39.4 $mM^{-1}cm^{-1}$), FW is the fresh weight of the fruit tissue, and Time is the duration of enzyme activity in the reaction mixture.

Peroxidase activity assay

The activity of peroxidase was determined using the method described by Upadhyaya et al. (1985). The reaction mixture consisted of 2.5 mL of 50 mM phosphate buffer (pH 7.0), 1 mL of 1% guaiacol, 1 mL of 1% hydrogen peroxide, and 0.1 mL of fruit extract. Peroxidase activity was quantified by measuring the increase in absorbance at 420 nm over 1 min using a spectrophotometer (Perkin Elmer UV/VIS Lambda 25; USA). The extinction coefficient (26.6 $mM^{-1}cm^{-1}$) was used in Equation (2) to calculate enzyme activity.

$$Units\ (mM\ min^{-1}) = \frac{\frac{\text{doD}}{\text{min(slop)}} * \text{Vol.of assay}(0.0001)}{\text{Extinction coefficient}(26.6)} \quad \text{Equation 2}$$

Where ΔA = change in absorbance, min = total minutes, Volume of Assay = total volume of the assay, and Extinction Coefficient = 26.6 $mM^{-1}cm^{-1}$.

Soluble protein measurement

To measure soluble protein, 1 g of fresh fruit tissue was centrifuged in 3 mL of Tris-HCl buffer (pH 6.8) for 15 min at 13,000 rpm in a refrigerated centrifuge. Then, 0.1 mL of the supernatant was mixed with 0.9 mL of Bradford reagent. After 5 min at room temperature, the absorbance was measured at 595 nm. Bovine serum albumin (BSA) was used as a standard to construct the standard curve. The protein content was expressed in mg g^{-1} of fresh tissue (Bradford, 1976).

Fruit quality indices

Fruit firmness, was assessed using a Lutron (FG-5020) Fruit Hardness Tester. Each apple was tested to determine its firmness. To measure reducing sugars, 2 mL of the prepared fruit extract was transferred to test tubes, followed by 2 mL of copper sulfate solution. The tubes were sealed with cotton and heated in a water bath at 100 °C for 8 min. After cooling, 2 mL of phosphomolybdic acid solution was added, producing a blue color. Absorbance was measured at 600 nm, and concentrations were calculated using a D-glucose standard curve (Somogyi, 1952). Total phenolics were quantified using the Folin-Ciocalteu method (Slinkard and Singleton, 1977), with results expressed in mg gallic acid equivalent per g of fruit extract. This method forms a blue complex, measured at 760 nm. Vitamin C content was determined via iodometric titration (Kashyap and Gautam, 2012), expressed as mg ascorbic acid per 100 mL of extract.

Total soluble solids (TSS) were measured using a manual refractometer (ATAGO Model, Japan) at 20 °C and expressed as °Brix (AOAC, 1980). Titratable acidity was determined by diluting 20 mL of each extract with 80 mL of distilled water. The diluted solution was titrated against 0.1 N NaOH using phenolphthalein as an indicator. The titration endpoint was determined by the first stable color change to pale pink, typically corresponding to pH 8.2. The volume of NaOH required to reach the endpoint was recorded to calculate the titratable acidity. Given that malic acid is the predominant organic acid in apple extracts, Equation (3) was used to determine the titratable acidity for each replicate (Nadeem et al., 2010).

$$A = \frac{N \times V \times E}{M} \times 100 \quad \text{Equation 3}$$

Where A is the amount of organic acids in the extract (mg per 100 mL of fruit extract), N is the normality of NaOH (0.1 N), V is the volume of NaOH used, E is the equivalent weight of malic acid, and M is the volume of fruit extract (mL).

Ethylene measurement

Ethylene production was measured using a Shimadzu Gas Chromatograph with the injection and detector temperatures set at 90 and 120 °C, respectively. Helium was used as the carrier gas. Three similarly sized apples per replicate were placed in 2.5 L sealed glass containers with 25 mL of 1 M potassium hydroxide solution. After 16 h, 1 mL of headspace gas was sampled through a rubber septum and analyzed by gas chromatography. Ethylene production was calculated using Equation (4).

$$\text{Ep}(\mu\text{l}h^{-1}g^{-1}) = \frac{(\text{E} \times \text{V} \times 60)}{(\text{T} \times \text{W})} \quad \text{Equation 4}$$

Where Ep = ethylene production, E = concentration of ethylene in the headspace (µL), V = volume of the container, T = time the sample was kept in the container, and W = wet weight of the sample (g).

Data analysis

Initially, all recorded data were checked for outliers. The normality of the data was then assessed using the Kolmogorov–Smirnov test in SPSS 21.0 software. Two-way analysis of variance was conducted considering fixed effects for the factors studied (fixed-effects model) in SAS 9.2 software. Duncan's Multiple Range Test (DMRT) was used for comparing treatment means at a 5% probability level.

Results

Catalase

The results of the analysis of variance indicated a significant effect ($P < 0.01$) on catalase enzyme activity with respect to storage time. However, neither SNP treatment nor the interaction between SNP and storage time had a significant effect on this parameter (Table 1). During fruit storage, catalase enzyme activity increased by 23.3 and 10.22% when storage time was extended from 90 to 150 d and from 150 to 210 d, respectively (Fig. 1).

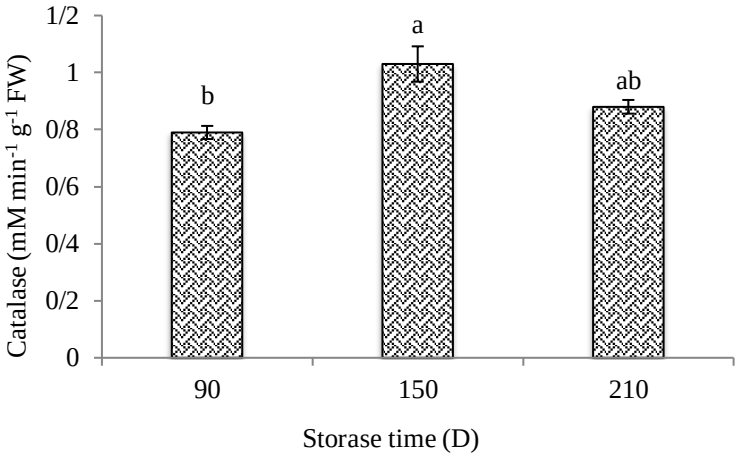

Fig. 1. Effect of storage duration on catalase enzyme activity in the 'Golden Delicious' apple cultivar. Columns sharing the same superscript letter(s) indicate no significant difference based on Duncan's Multiple Range Test ($P \leq 0.05$). Error bars represent standard errors (mean ± SE, n = 3).

Table 1. Two-way ANOVA effects of sodium nitroprusside (SNP) on antioxidant activity and quality attributes of the 'Golden Delicious' apple cultivar during storage.

Source of variation	df	Mean Square									
		Catalase	Proxidase	Soluble Protein	Reducing Sugars	Total Phenol	Vitamin C	Titratable Acidity	Total Soluble Solid	Firmness	Ethylene
SNP†	2	0.001^{ns}††	2.94**	0.00003^{ns}	0.001^{ns}	44.33*	0.52**	0.045**	0.27^{ns}	13.6**	1.04**
ST††	2	0.125**	10.72**	0.0049**	0.004**	678.54**	7.21**	1.10**	0.79**	21.7**	11.97**
SNP × ST	4	0.013^{ns}	0.07^{ns}	0.0002^{ns}	0.003**	92.12**	0.004^{ns}	0.024**	0.004^{ns}	2.3**	0.33^{ns}
Total Error	18	0.016	0.19	0.0002	0.00	18.6	0.08	0.004	0.11	0.3	0.08
CV (%)		14.39	7.71	7.77	9.56	4.41	5.26	5.94	2.38	2.38	15.00

† SNP: Sodium Nitroprusside; †† ST: Storage Time; ††† ^{ns}: Means no significant, ** and * show being significant (α = 0.01 and 0.05, respectively).

Peroxidase

The activity of the peroxidase enzyme was significantly influenced by both SNP treatment and storage time ($P < 0.01$). However, the interaction between these two factors did not result in a statistically significant effect on enzyme activity (Table 1). The application of SNP at concentrations of 5 and 7 mM resulted in a notable decrease in peroxidase enzyme activity by 17.52 and 12.36%, respectively (Fig. 2A). Furthermore, the activity of the peroxidase enzyme showed a gradual increase with extended storage time of the apples. Specifically, after 210 d of storage, the enzyme activity was 32.06% higher compared to d 90 of the storage period (Fig. 2B).

Soluble protein

Soluble protein content was significantly affected by storage time; however, no significant effects were observed on soluble protein content as a result of SNP application or the interaction of SNP × storage time (Table 1). According to the findings, the maximum value of soluble protein was observed at 150 d of storage time (Fig. 3).

Reducing sugars

The analysis of variance indicated that SNP had no significant effect on reducing sugars. However, storage time and its interaction with SNP showed significant effects ($P < 0.01$) (Table 1). The highest reducing sugar content (0.28 mg g^{-1} fresh weight) was observed in the control (without SNP) after 150 d of storage, significantly different from other treatments. The lowest content was recorded with SNP treatment at 210 d. Mean comparisons revealed that 0 and 5 mM SNP resulted in initial increases followed by decreases in reducing sugars over time. In contrast, 7 mM SNP consistently increased reducing sugar content, with a significant 17.39% increase on d 210 compared to d 90 (Fig. 4).

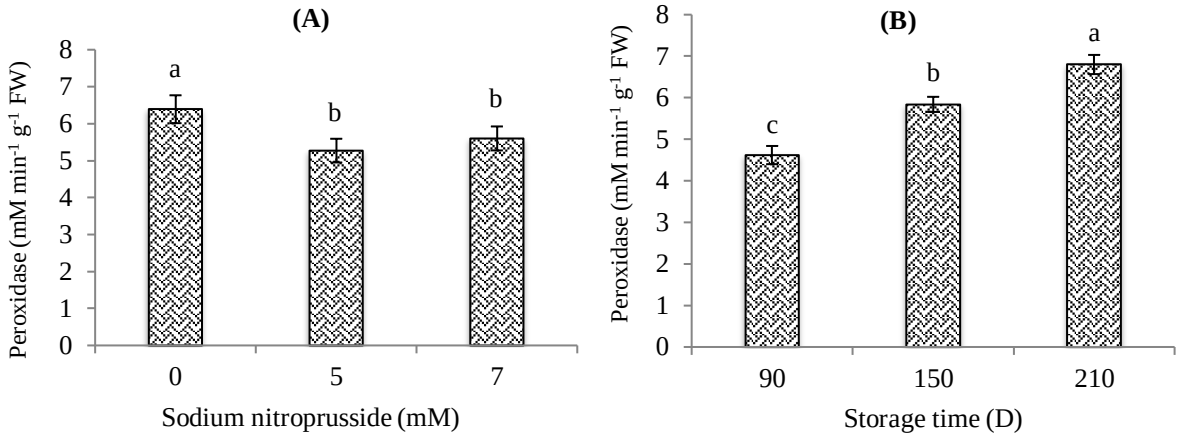

Fig. 2. Peroxidase enzyme activity in the 'Golden Delicious' apple cultivar under (**A**) sodium nitroprusside (SNP) treatment and (**B**) different storage durations. Columns sharing the same superscript letter indicate no significant difference based on Duncan's Multiple Range Test ($P \leq 0.05$). Error bars represent standard errors (mean ± SE, n = 3).

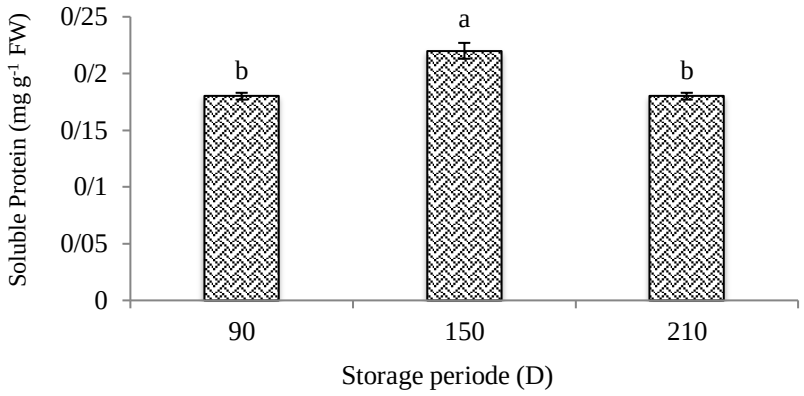

Fig. 3. Effect of storage duration on soluble protein content in the 'Golden Delicious' apple cultivar. Columns sharing the same superscript letter indicate no significant difference based on Duncan's Multiple Range Test ($P \leq 0.05$). Error bars represent standard errors (mean ± SE, n = 3).

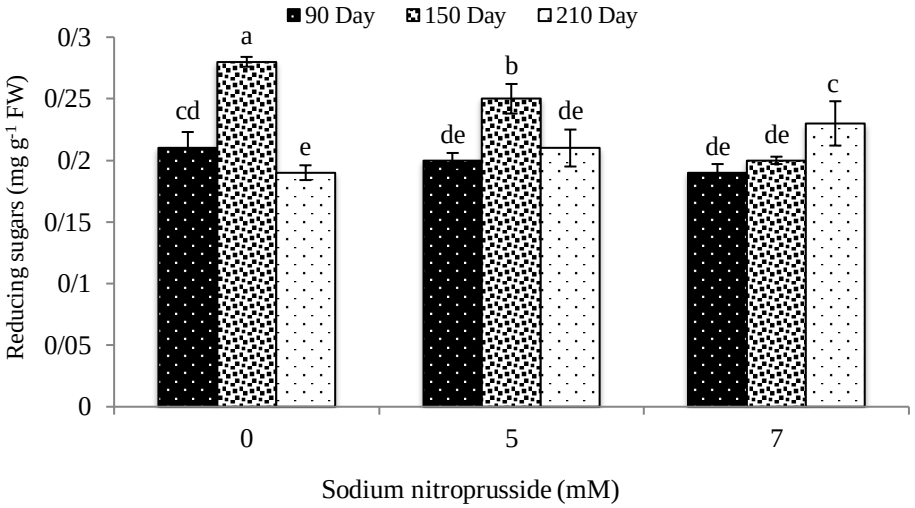

Fig. 4. Interaction effects of sodium nitroprusside (SNP) and storage time on reducing sugar content in the 'Golden Delicious' apple cultivar. Columns sharing the same superscript letter indicate no significant difference based on Duncan's Multiple Range Test ($P \leq 0.05$). Error bars represent standard errors (mean ± SE, n = 3).

Total phenolic compounds

The analysis of variance showed a significant effect of total phenolic compounds in response to SNP ($P < 0.05$), storage time, and the interaction of SNP and storage time ($P < 0.01$) (Table 1). By comparing the mean interaction of SNP and storage time, the apples had the lowest phenolic content after 210 d of storage at all levels of SNP. This indicated a significant decrease compared to d 90 of storage. The highest phenolic content (110.4 mg g^{-1} fresh weight) was observed in the control treatment after 150 d of storage. However, at the same storage time, with 5 mM SNP, the total phenolic content decreased significantly (10.75%) (Fig. 5).

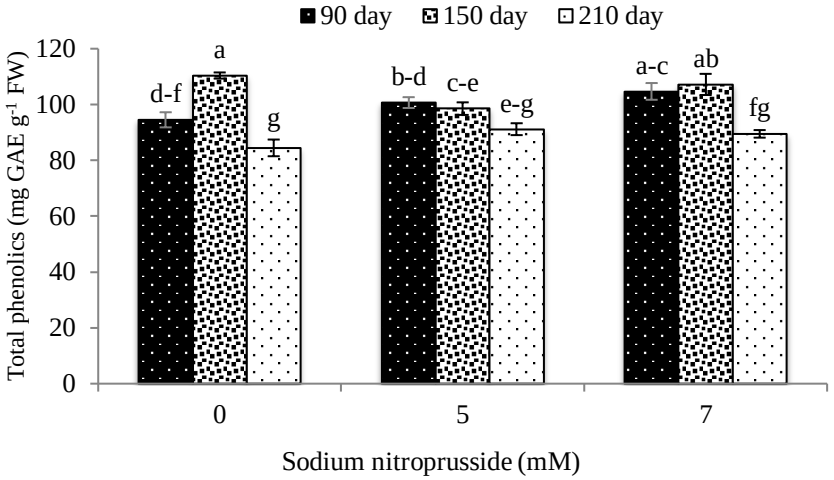

Fig. 5. Effects of storage duration and sodium nitroprusside (SNP) concentrations on total phenolic content in the 'Golden Delicious' apple cultivar. Columns sharing the same superscript letter indicate no significant difference based on Duncan's Multiple Range Test ($P \leq 0.05$). Error bars represent standard errors (mean ± SE, n = 3).

Vitamin C

The vitamin C content of the apples was significantly affected by SNP treatment and storage time ($P < 0.01$), whereas no significant effects were observed for the interaction between these two factors (Table 1). Application of 5 and 7 mM SNP concentrations resulted in an increase in the vitamin C content of the apples by 14.52 and 27.51%, respectively, compared to the control (Fig. 6A). Based on the results, prolonged storage time of apples resulted in a significant decrease in vitamin C content. Consequently, apples exhibited the lowest vitamin C content (5.7 mg g^{-1} fresh weight) on d 210 of the storage period (Fig. 6B).

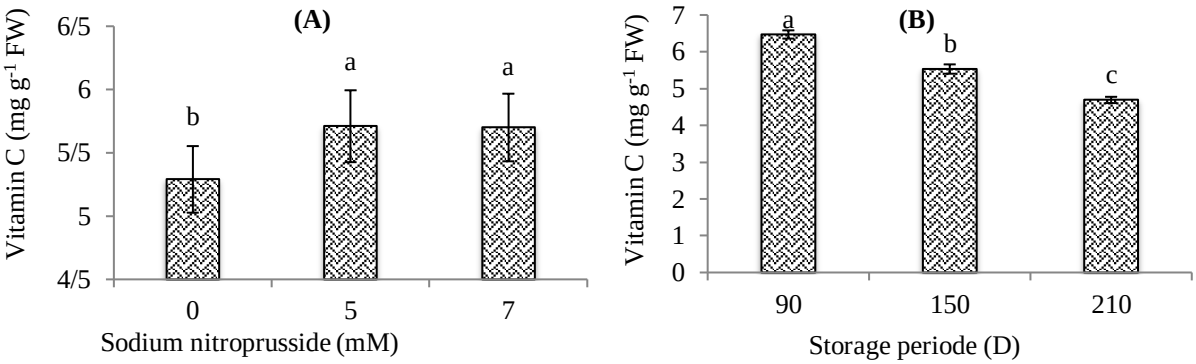

Fig. 6. Effects of (**A**) sodium nitroprusside (SNP) treatment and (**B**) storage duration on vitamin C content in the 'Golden Delicious' apple cultivar. Columns sharing the same superscript letter indicate no significant difference based on Duncan's Multiple Range Test ($P \leq 0.05$). Error bars represent standard errors (mean ± SE, n = 3).

Titratable acidity

Titratable acidity was significantly influenced by storage time and the interaction between SNP and storage time ($P < 0.01$), but it did not show a significant response to SNP alone (Table 1). By comparing the mean interaction, the highest titratable acidity value (1.71 mg g^{-1} fresh weight) was observed with 7 mM SNP on d 90 of storage, representing a significant increase of 14.62% compared to the control (absence of SNP) over the

same storage period (Fig. 7). Titratable acidity showed a decreasing trend at all levels of SNP over the storage duration. While the application of 5 mM SNP did not result in significant changes in titratable acidity on d 90 and 210 of storage, the application of 7 mM SNP significantly increased titratable acidity. Notably, SNP did not cause significant changes in titratable acidity during the initial 150 d of storage (Fig. 7).

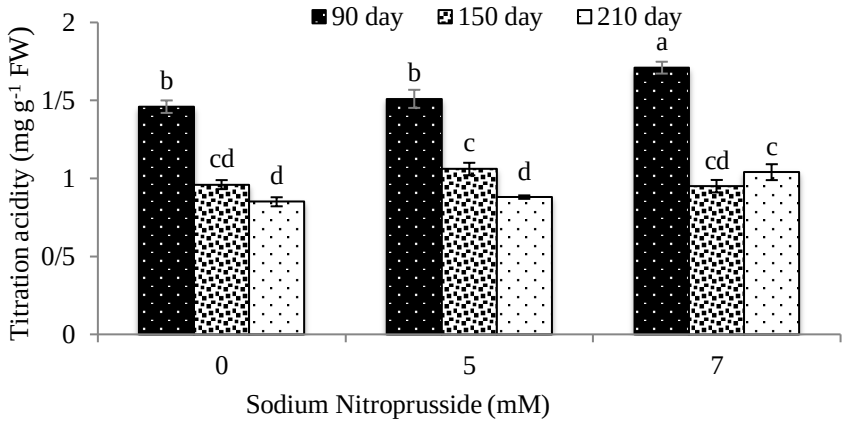

Fig. 7. Effects of storage duration and sodium nitroprusside (SNP) treatment on titratable acidity in the 'Golden Delicious' apple cultivar. Columns sharing the same superscript letter indicate no significant difference based on Duncan's Multiple Range Test ($P \leq 0.05$). Error bars represent standard errors (mean ± SE, n = 3).

Total soluble solids (TSS)

The total soluble solids (TSS) content demonstrated a statistically significant effect ($P < 0.05$), primarily driven by storage time. However, neither SNP nor its interaction with storage time had a significant effect on TSS content, as presented in Table 1. When comparing the mean values of treatments, an increase was recorded in the TSS content over storage time. The TSS value increased by 3.98% from d 90 to d 210 of the storage period (Fig. 8).

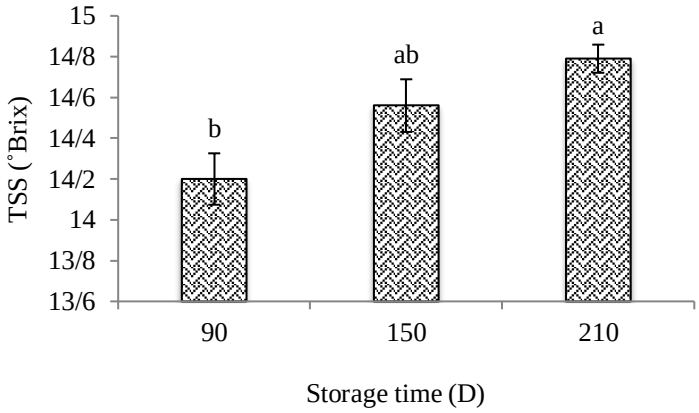

Fig. 8. Effect of storage duration on total soluble solids content in the 'Golden Delicious' apple cultivar. Columns sharing the same superscript letter indicate no significant difference based on Duncan's Multiple Range Test ($P \leq 0.05$). Error bars represent standard errors (mean ± SE, n = 3).

Fruit firmness

Fruit firmness was significantly influenced by SNP treatment, storage time, and the interaction between these two factors ($P \leq 0.01$, Table 1). When comparing the mean values of the treatments, it was observed that firmness gradually decreased over the storage duration across all concentrations of SNP. Notably, this decrease was more pronounced with the application of 7 mM SNP. On d 90 of storage, the effects of SNP were significant in enhancing fruit firmness. Specifically, treatment with 7 mM SNP resulted in apples maintaining significantly higher firmness, reaching 12.17 N. Subsequently, after 150 d of storage, apples treated with SNP showed significant retention of firmness compared to the control treatment. After 210 d of storage, 5 mM SNP resulted in a significant increase (24.51%) in fruit firmness compared to the control treatment without SNP. However, increasing the concentration from 5 to 7 mM led to a significant decrease in fruit

firmness, although it was not significantly different from the control treatment (Fig. 9).

Ethylene

A significant effect on ethylene production was observed in response to SNP ($P < 0.05$) and storage time ($P < 0.01$). However, the interaction between SNP and storage time had no significant effect on ethylene production in the apples (Table 1). SNP resulted in a significant decrease in ethylene production in apples, compared to the control (zero SNP concentration). The decrease was similar in apples treated with 5 mM SNP and those treated with 7 mM (Fig. 10A). The mean ethylene production in apples showed an increasing trend over storage time. An increase of 60% was observed by d 210 of storage, compared to d 90 (Fig. 10B).

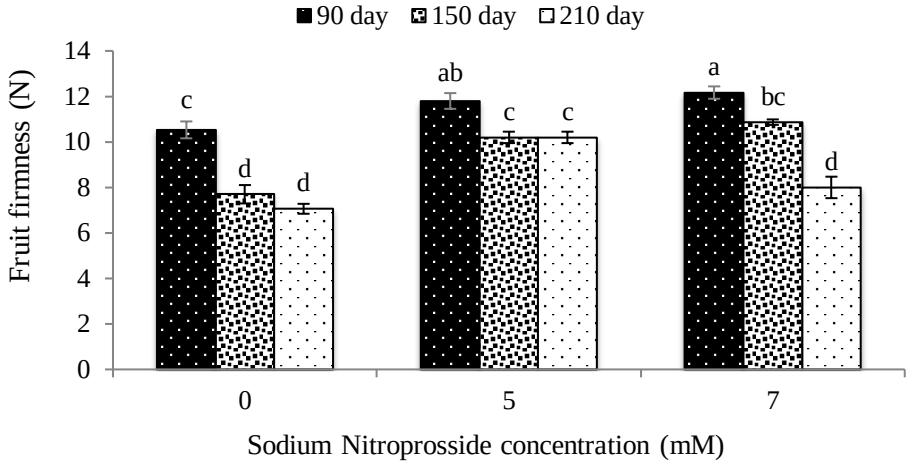

Fig. 9. Effects of storage duration and sodium nitroprusside (SNP) treatment on fruit firmness in the 'Golden Delicious' apple cultivar. Columns sharing the same superscript letter indicate no significant difference based on Duncan's Multiple Range Test ($P \leq 0.05$). Error bars represent standard errors (mean ± SE, n = 3).

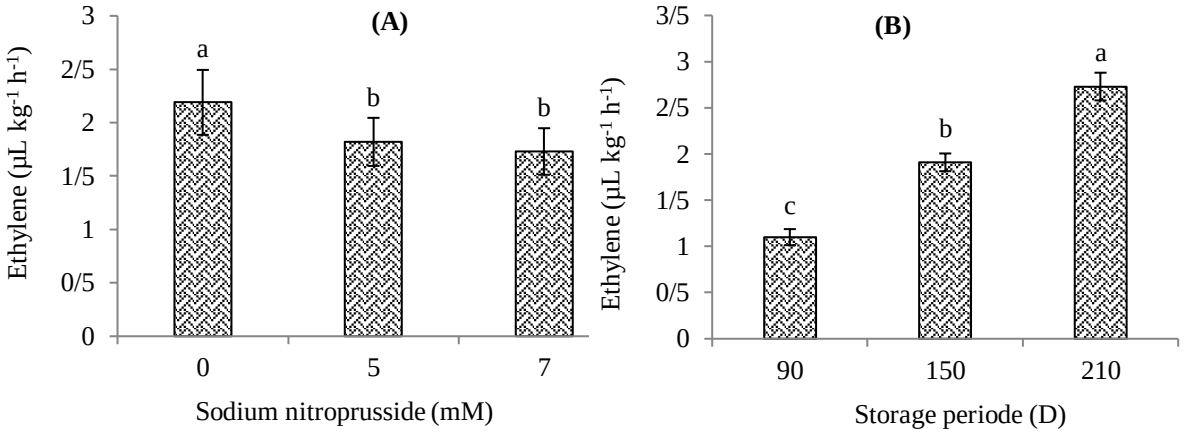

Fig. 10. Effects of (**A**) sodium nitroprusside (SNP) treatment and (**B**) storage duration on ethylene production in the 'Golden Delicious' apple cultivar. Columns sharing the same superscript letter indicate no significant difference based on Duncan's Multiple Range Test ($P \leq 0.05$). Error bars represent standard errors (mean ± SE, n = 3).

Discussion

The metabolism of volatile compounds in apples indicates maturity and ripening, varying with storage duration and environmental conditions. Maturation and senescence are oxidative processes that produce reactive oxygen species (ROS), such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2). Overproduction of ROS leads to oxidative stress, lipid peroxidation, protein denaturation, and cell death (Dong et al., 2015). Plants counteract ROS through an endogenous defense system, including non-enzymatic antioxidants (ascorbate, glutathione, tocopherol, carotenoids) and antioxidant enzymes (superoxide dismutase, catalase, peroxidase) (Ezhilmathi et al., 2007). This study found that increased antioxidant enzyme activity, such as catalase (Fig. 1) and peroxidase (Fig. 2B), during storage suppresses ROS, thereby controlling fruit ripening and senescence. Similar findings were reported by Wang et al. (2009) in mangoes. Catalase

activity (Fig. 1) correlates with phenolic content (Fig. 5), as phenolic compounds enhance antioxidant activity in apples (Gorinstein et al., 2004). Sodium nitroprusside (SNP) reduces oxidative damage in plants by acting as an antioxidant (Ruan et al., 2015). In this study, SNP reduced peroxidase activity, likely through direct scavenging of reactive oxygen species (ROS) and suppression of oxidative enzyme activity. An increase in protein content during storage is associated with the increase in antioxidant enzymes involved in fruit ripening, contributing to ethylene production, sugar metabolism, and oxidative stress responses (Zheng et al., 2013). Soluble protein content initially increased (90 to 150 d) and then decreased (at 210 d) (Fig. 3). These findings are consistent with those reported for apples (Jan et al., 2012) and cherries (Wei et al., 2011).

Apples are rich in carbohydrates, accumulating starch during development and converting it to reducing sugars as they mature. This study observed variations in reducing sugar content. At 0 and 5 mM SNP, reducing sugars initially increased (up to 90 d) before decreasing. However, 7 mM SNP consistently increased reducing sugar content during storage. Wright and Whiteman (1955) attributed the increase in reducing sugars to metabolic processes and the conversion of sorbitol to simple sugars. Similarly, Liu et al. (2011) found that soluble sugar content in pear fruit increased during storage. The decrease in reducing sugars from 150 to 210 d in 0 and 5 mM SNP treatments is likely due to respiratory consumption. In contrast, 7 mM SNP may have prevented the depletion of reducing sugars, thereby reducing the respiration rate.

Based on the results of this study, the total phenolic content during storage did not follow a consistent trend and varied in response to concentrations of 0 and 5 mM SNP. The total phenolic content in apples increased as the storage time extended from 90 to 150 d, and then exhibited a decreasing trend from d 150 to d 210. This variation during the storage period may be attributed to metabolic processes such as respiration, ethylene production (Fig. 10B), and the activity of antioxidant enzymes (Figs. 1 and 2B) following fruit harvest. Duma et al. (2017) reported that phenolic content varied during storage, supporting the results of this study. The current findings indicate that the total phenolic content exhibited varying increasing and decreasing trends under different SNP concentrations at various storage times. In another study by Shahkoomahally et al. (2015), pre-storage treatment of persimmon fruit (*Diospyros kaki* L.) with nitric oxide resulted in a reduction in phenolic content during storage. The current study revealed an increasing trend in the total phenolic content on d 90 of the storage period with higher SNP concentrations. These results are consistent with previous findings by Ren et al. (2017), which indicated an increase in phenolic content following SNP treatment. The treatment of apples with SNP may inhibit the activity of polyphenol oxidase (PPO) and peroxidase (POD) enzymes and maintain phenolic compounds at elevated levels (Duan et al., 2007). The current study showed that the vitamin C content decreased as the apple storage time increased. Studies by other researchers also confirm the reduction in ascorbic acid during storage (Wang and Gao, 2013). The decrease in vitamin C content during storage may be attributed to the role of this vitamin as an electron donor to oxidants that neutralize free radicals (Smirnoff, 1995). The results also indicated that vitamin C content in apples treated with SNP showed a significant increase compared to the control. By inhibiting ethylene production (Fig. 10A) and delaying the senescence process, nitric oxide likely prevented cell wall degradation and reduced the softening of fruit tissue (Fig. 9). Through this mechanism, the depletion of ascorbic acid was minimized, and vitamin C was preserved by reducing the production of free radicals (Fig. 6A). The findings of Sharma and Sharma (2015) on Japanese plum (*Prunus salicina* Lindell) also support the positive role of nitric oxide in preserving and enhancing vitamin C content, which is consistent with the results of this study. Elevated levels of titratable acidity (TA) under SNP treatment (Fig. 7) may also contribute to preserving vitamin C content during storage. Ren et al. (2017) found that an increase in TA levels can effectively contribute to maintaining vitamin C content in fruit during storage. Controlling the fruit ripening process and slowing it down can reduce the loss of titratable acids resulting from malic acid metabolism (Defilippi et al., 2004), stabilize soluble acids (Larrigaudière et al., 2008), and enhance the shelf life of aromatic compounds. Several studies have shown a decreasing trend in postharvest titratable acidity during storage (Jan et al., 2012; Duma et al., 2017), which supports the results of this study. The reduction in titratable acidity during storage may be associated with a higher respiration rate during fruit ripening, whereby organic acids are used as substrates in the respiratory process (Tigist et al., 2013). Treating apples with an SNP solution can significantly reduce ethylene production and respiration. It also prevents the depletion of organic acids and increases the content of soluble solids by reducing the rate of cell wall degradation and polysaccharide breakdown (Zhu et al., 2008). In a study by Ren et al. (2017), the treatment of mango (‘Tainong’ cultivar) with nitric oxide significantly delayed the decrease in titratable acidity during ripening, handling, and storage. This is consistent with the results of this study. The researchers suggested that there is a correlation between titratable acidity and phenolic content (Ren et al., 2017). The current findings indicate that total soluble

solids (TSS) followed an increasing trend during storage. Other researchers have also reported that the content of total soluble solids increased during storage (Jan et al., 2012), supporting the results of this study. An increase in TSS during storage likely occurred due to ongoing metabolic activities in the apples and the further production of simple sugars in the fruit extract (Akhtar et al., 2010).

Fruit firmness is considered one of the most critical quality attributes during the postharvest stage. In part, the duration of fruit storage is determined by the stability and firmness of the fruit texture. The decrease in fruit firmness following the storage period is well-documented (Sis et al., 2012; Liu et al., 2014), which aligns with the results of this study. Given that ethylene promotes fruit ripening and softening in apples, thereby reducing firmness, the removal of ethylene or use of an anti-ethylene compound can help maintain the firmness of the fruit texture during storage time. SNP has been recognized as an anti-ethylene compound in recent years, although variations exist in its application during storage and its positive role in reducing ethylene production and maintaining fruit firmness (Li et al., 2014). Previous studies on peach fruit demonstrated that the application of 1 mM SNP helped maintain and enhance fruit firmness by reducing ethylene production (Sis et al., 2012). An increase in ethylene production during storage has been confirmed in various studies (Tran et al., 2015; Brackmann et al., 2017), which aligns with the findings of this study. Nitric oxide, recognized as an anti-ethylene compound, can function as an antagonist in the senescence process of plant tissues (Duan et al., 2007). Several studies have confirmed that nitric oxide and ethylene act antagonistically in higher plants. In this context, studies by Tran et al. (2013) on mango fruit showed that the application of 0.5 and 1 mM SNP reduced ethylene content and respiration rate. Sis et al. (2012) reported in their studies that the application of 1 mM SNP can prolong the postharvest shelf life of peaches by reducing ethylene production, delaying softening, and increasing the activities of superoxide dismutase and catalase enzymes. In another study on papaya, treatment with nitric oxide also effectively reduced ethylene production and respiration rate in fruit during 20 d of storage (Li et al., 2014). This decrease in ethylene production is attributed to the binding of nitric oxide with 1-aminocyclopropane-1-carboxylic acid (ACC) and ACC oxidase, thereby forming a stable compound, which limits the production of ethylene (Tierney et al., 2005).

Conclusion

In summary, the storage duration of apples induced time-dependent biochemical changes, significantly affecting fruit quality. Notably, catalase activity, soluble protein content, and phenolic content showed an increase from d 90 to d 150 of the storage period. Furthermore, extended storage and ripening durations resulted in increased ethylene production, leading to fruit softening and reduced firmness during the storage period. Moreover, sodium nitroprusside (SNP) treatment at both concentrations of 5 and 7 mM reduced peroxidase activity and mitigated the loss of vitamin C throughout the storage duration. Ultimately, SNP played a pivotal role in preserving fruit quality and maintaining firmness by reducing ethylene production, thereby delaying the fruit senescence process.

Acknowledgments

We express our sincere gratitude to the esteemed authorities of the Islamic Azad University, Mahabad Branch, for their generous support in facilitating the execution of this research project.

Author Contributions

Conceptualization, MA and MJN; methodology, MJN; software, MJN; validation, MA; investigation, MJN; data curation, MA and MJN; writing-original draft preparation, MA; writing-review and editing, MA and MJN; visualization, supervision, project administration, MA. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Conflict of Interest

The authors indicate no conflict of interest in this work.

References

AOAC, 1980. Official methods of analysis. 13th Ed Washington D.C.

Barman K, Siddiqui MW, Patel VB, Prasad M. 2014. Nitric oxide reduces pericarp browning and preserves bioactive antioxidants in litchi. Scientia Horticulturae 171, 71–77. https://doi.org/10.1016/j.scienta.2014.03.036

Beers RF, Sizer IW. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. Journal of Biological Chemistry 195(1), 133-140. https://doi.org/10.1016/s0021-9258(19)50881-x

Brackmann A, Ludwig V, Thewes FR, Anese RO, Schultz EE, Bergetti MRP. 2017. Ethanol and nitric oxide in quality maintenance of 'Galaxy' apples stored under controlled atmosphere. Revista Brasileira de Fruticultura 39(5), 313–322. https://doi.org/10.1590/0100-29452017322

Bradford MM. 1976. A rapid and sensitive method

for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248-254. https://doi.org/10.1006/abio.1976.9999

Defilippi BG, Dandekar AM, Kader AA. 2004. Impact of suppression of ethylene action or biosynthesis on flavor metabolites in apple (*Malus domestica* Borkh) fruits. Journal of agricultural and food chemistry 52(18), 5694-5701. https://doi.org/10.1021/jf049504x

Dong Y, Liu L, Zhao Z, Zhi H, Guan J. 2015. Effects of 1-MCP on reactive oxygen species, polyphenol oxidase activity, and cellular ultra-structure of core tissue in 'Yali' pear (*Pyrus bretschneideri* Rehd.) during storage. Horticulture, Environment, and Biotechnology 56(2), 207-215. https://doi.org/10.1007/s13580-015-0094-1

Duan X, Su X, You Y, Qu H, Li Y, Jiang Y. 2007. Effect of nitric oxide on pericarp browning of harvested longan fruit in relation to phenolic metabolism. Food Chemistry 104(2), 571-576. https://doi.org/10.1016/j.foodchem.2006.12.007

Duma M, Alsina I, Dubova L, Erdberga I. 2017. Quality of tomatoes during storage. Conference: 11th Baltic Conference on Food Science and Technology "Food science and technology in a changing world". 130-133. https://doi.org/10.22616/foodbalt.2017

Ezhilmathi K, Singh VP, Arora A, Sairam RK. 2007. Effect of 5-sulfosalicylic acid on antioxidant activity in relation to vase life of gladiolus cut flowers. Plant Growth Regulation 51(2), 99-108. https://doi.org/10.1007/s10725-006-9142-2

Gorinstein S, Zachwieja Z, Katrich E, Pawelzik E, Haruenkit R, Trakhtenberg S, Martin-Belloso O. 2004. Comparison of the contents of the main antioxidant compounds and the antioxidant activity of white grapefruit and his new hybrid. LWT-Food Science and Technology 37(3), 337-343. https://doi.org/10.1016/j.lwt.2003.10.005

Han C, Zuo J, Wang Q, Xu L, Wang Z, Dong H, Gao L. 2015. Effects of 1-MCP on postharvest physiology and quality of bitter melon (*Momordica charantia* L.). Scientia Horticulturae 182, 86-91. https://doi.org/10.1016/j.scienta.2014.07.024

Hasan MU, Singh Z, Shah, HM S, Kaur J, Woodward A. 2024. Water Loss: A Postharvest Quality Marker in Apple Storage. Food and Bioprocess Technology 1-26. https://doi.org/10.1007/s11947-023-03305-9

Jan I, Rab A, Sajid M, Ali A, Shah ST. 2012. Response of apple cultivars to different storage durations. Sarhad Journal of Agriculture 28(2), 219-225.

Jayarajan S, Sharma RR. 2018 Impact of nitric oxide on shelf life and quality of nectarine (*Prunus persica* var. nucipersica). Acta physiologiae plantarum 40(12), 207. https://doi.org/10.1007/s11738-018-2779-4

Kang HM, Saltveit ME. 2002. Chilling tolerance of maize, cucumber and rice seedling leaves and roots are differentially affected by salicylic acid. Physiologia Plantarum 115(4): 571-576. https://doi.org/10.1034/j.1399-3054.2002.1150411.x

Kashyap G, Gautam MD. 2012. Analysis of Vitamin C in Commercial and Naturals substances by Iodometric Titration found in Nimar and Malwa regeion. Journal of Scientific Research in Pharmacy 1(2): 77-78.

Larrigaudière C, Vilaplana R, Soria Y, Recasens I. 2008. Comparative study of the effects of 1-MCP treatment on apple quality by instrumental and multivariate analysis. Journal of the Science of Food and Agriculture 88(9): 1614-1621. https://doi.org/10.1002/jsfa.3258

Li C, Yu W, Liao W. 2022. Role of nitric oxide in postharvest senescence of fruits. International Journal of Molecular Sciences 2:23(17):10046. https://doi.org/10.3390/ijms231710046

Li XP, Wu B, Guo Q, Wang JD, Zhang P, Chen WX. 2014. Effects of nitric oxide on postharvest quality and soluble sugar content in papaya fruit during ripening. Journal of food processing and preservation 38(1): 591-599. https://doi.org/10.1111/jfpp.12007

Liu H, Lin S, Zhang M, Lv Y, Ma Y, Rao J, Zhu Q. 2023. Changes in cell wall sugar neutral composition contribute to apple texture loss during storage among cultivars. Horticulturae 9(3): 292. https://doi.org/10.3390/horticulturae9030292

Liu K, Wang X, Young M. 2014. Effect of bentonite/potassium sorbate coatings on the quality of mangos in storage at ambient temperature. Journal of Food Engineering 137, 16-22. https://doi.org/10.1016/j.jfoodeng.2014.03.024

Liu LQ, Yu DONG, Guan JF. 2011. Effects of nitric oxide on the quality and pectin metabolism of Yali pears during cold storage. Agricultural sciences in China 10(7): 1125-1133. https://doi.org/10.1016/s1671-2927(11)60102-8

Madebo MP, Ayalew Y, Zheng Y and Jin P (2023) Nitric oxide and its donor sodium-nitroprusside regulation of the postharvest quality and oxidative stress on fruits: a systematic review meta-analysis. Food Reviews International 39(9): 6648-6676. https://doi.org/10.1080/87559129.2022.2122995

Nadeem SM, Zahir ZA, Naveed M, Asghar HN, Arshad M. 2010. Rhizobacteria capable of producing ACC-deaminase may mitigate salt stress in wheat. Soil Science Society of America Journal 74(2): 533-542. https://doi.org/10.2136/sssaj2008.0240

Nolpradubphan A, Lichanporn I. 2016. Effect of nitric oxide on postharvest quality of lime fruit (*Citrus aurantifolia* Swingle). Asia-Pacific Journal of Science and Technology 21(1): 86-96.

Ren Y, He J, Liu H, Liu G, Ren X. 2017. Nitric oxide alleviates deterioration and preserves antioxidant properties in 'Tainong'mango fruit during ripening. Horticulture, Environment, and Biotechnology 58(1): 27-37. https://doi.org/10.1007/s13580-017-0001-z

Ruan J, Li M, Jin H, Sun L, Zhu Y, Xu M, Dong J. 2015. UV-B irradiation alleviates the deterioration of cold-stored mangoes by enhancing endogenous nitric oxide levels. Food Chemistry 169, 417-423. https://doi.org/10.1016/j.foodchem.2014.08.014

Shahkoomahally S, Ramezanian A, Farahnaky A. 2015. Postharvest nitric oxide treatment of persimmon (*Diospyros kaki* l.) improves fruit quality during storage. Fruits 70(2): 63-68. https://doi.org/10.1051/fruits/2014045

Sharma S, Sharma RR. 2015. Nitric oxide inhibits activities of PAL and PME enzymes and reduces chilling injury in 'Santa Rosa' Japanese plum (*Prunus salicina* Lindell). Journal Of Plant Biochemistry and Biotechnology 24(3): 292-297. https://doi.org/10.1007/s13562-014-0271-9

Sis SA, Mostofi Y, Boojar MM, Khalighi A. 2012. Effect of nitric oxide on ethylene biosynthesis and antioxidant enzymes on Iranian peach (*Prunus persica* cv. Anjiri). Journal of Food, Agriculture and Environment 10(2): 125-129.

Slinkard K, Singleton VL. 1977. Total phenol analysis: automation and comparison with manual methods. American journal of enology and viticulture 28(1): 49-55. https://doi.org/10.5344/ajev.1977.28.1.49

Smirnoff H. 1995. Antioxidant systems and plant response to the environment. Environment and plant metabolism 217– 43.

Somogy M. 1952. Notes on sugar determination. Journal of Biochemistry 195, 19-29.

Tierney DL, Rocklin AM, Lipscomb JD, Que L, Hoffman BM. 2005. ENDOR studies of the ligation and structure of the non-heme iron site in ACC oxidase. Journal of the American Chemical Society 127(19): 7005-7013. https://doi.org/10.1021/ja0500862

Tigist M, Workneh S, Woldetsadik K. 2013. Effects of variety on the quality of tomato stored under ambient conditions. Journal of Food Science and Technology 50(3): 477-486. https://doi.org/10.1007/s13197-011-0378-0

Tran TTL, Aiamla-or S, Srilaong V, Jitareerat P, Wongs-Aree C, Uthairatanakij A. 2013. Application of nitric oxide to extend the shelf life of mango fruit. In II Southeast Asia Symposium on Quality Management in Postharvest Systems. 1088, 97-102.

Tran TTL, Jitareera P, Aimla-or S, Srilaong V, Boonyaritthongchai P, Uthairatanakij A. 2015. Applying of sodium nitroprusside (SNP) on postharvest Nam Dok Mai No.4 mango fruits delay ripening and maintain quality. African Journal of Agricultural Research 10(31): 3067-3072. https://doi.org/10.5897/ajar2015.9530

Upadhyaya A, Sankhla D, Davis TD, Sankhla N, Smith BN. 1985. Effect of paclobutrazol on the activities of some enzymes of activated oxygen metabolism and lipid peroxidation in senescing soybean leaves. Journal of plant physiology 121(5): 453-461. https://doi.org/10.1016/s0176-1617(85)80081-x

Wang B, Wang J, Feng X, Lin L, Zhao Y, Jiang W. 2009. Effects of 1-MCP and exogenous ethylene on fruit ripening and antioxidants in stored mango. Plant Growth Regulation 57(2): 185. https://doi.org/10.1007/s10725-008-9335-y

Wang SY, Gao H. 2013. Effect of chitosan-based edible coating on antioxidants, antioxidant enzyme system, and postharvest fruit quality of strawberries (*Fragaria aranassa* Duch.). LWT-Food Science and Technology 52(2): 71-79. https://doi.org/10.1016/j.lwt.2012.05.003

Wang Y, Luo Z, Du R. 2015. Nitric oxide delays chlorophyll degradation and enhances antioxidant activity in banana fruits after cold storage. Acta physiologiae plantarum 37(4): 74. https://doi.org/10.1007/s11738-015-1821-z

Wei J, Qi X, Guan J, Zhu X. 2011. Effect of cold storage and 1-MCP treatment on postharvest changes of fruit quality and cell wall metabolism in sweet cherry. Journal of Food, Agriculture and Environment 9(3/4 part 1): 118-122.

Wright RC, Whiteman TM. 1955. Some changes in Eastern apples during storage (No. 1120). US Department of Agriculture.

Yuri JA, Maldonado FJ, Razmilic I, Neira A, Quilodran Á, Palomo I. 2012. Concentrations of total phenols and antioxidant activity in apple do not differ between conventional and organic orchard management. Journal of Food Agriculture and Environment 10(2): 207-216.

Zheng Q, Song J, Campbell-Palmer L, Thompson K, Li L, Walker B, Cui Y, Li X. 2013. A proteomic investigation of apple fruit during ripening and in response to ethylene treatment. Journal of Proteomics 93: 276–294. https://doi.org/10.1016/j.jprot.2013.02.006

Zhu S, Sun L, Liu M, Zhou J. 2008. Effect of nitric oxide on reactive oxygen species and antioxidant enzymes in kiwifruit during storage. Journal of the Science of Food and Agriculture 88(13): 2324-2331. https://doi.org/10.1002/jsfa.3353