Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.84122

Effects of nitric oxide on postharvest storage quality of *Lycium barbarum* fruit

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Abstract

To investigate the effects of nitric oxide (NO) on the postharvest physiology and storage quality of *Lycium barbarum*, fruits were treated with different concentrations of sodium nitroprusside (SNP), which was used as NO donor. Results showed that SNP soaked at 0.6 mmol/L for 20 min could inhibit the changes during storage of *L. barbarum*. Particularly, the decay rate and weight loss rate were reduced, and the quality parameters of the fruit were increased. By comparing the above indicators, it was found that 0.6 mmol/L soaking for 20 min was more effective in preserving freshness than other concentrations. It was found that total chlorophyll and carotenoid contents were increased, soluble solids and solid acid ratio contents were decreased, ascorbic acid, total phenols and flavonoids contents were increased, and hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), catalase (CAT) and malondialdehyde (MDA) contents were increased. The polyphenol oxidase (PPO) and peroxidase (POD) activities were decreased, and it was concluded that NO treatment improved the freshness of *L. barbarum* fruit and NO could be used as a postharvest preservative for *L. barbarum* fruit.

Keywords: *Lycium barbarum*; nitric oxide (NO); storage quality; freshness preservation.

Practical Application: NO treatment could improve the freshness of *L. barbarum* fruit. NO application could be used as a postharvest preservative for *L. barbarum* fruit.

1 Introduction

Lycium barbarum (goji) is a deciduous perennial shrub of the Solanaceae family. It contains a variety of nutrients which makes it an ideal nutritional resource for both medicinal purpose and functional foods development (Shang et al., 2022; Xing et al., 2022; Zhao et al., 2022). Due to the frail fruit physiology, fruit decay and softening can occur very easily at room temperature after harvesting. The fresh fruit of goji and the original juices can better preserve its active substances, not only as raw materials, and auxiliary materials for food industry, but also as health drinks (Ma et al., 2022a; Ma et al., 2022b). On the one hand, fresh fruit of Chinese goji itself has the advantages of thick flesh and thin skin, and high soluble solids content (Zhang et al., 2022; Muatasim et al., 2018); however, browning, and softening decay after picking greatly limits its industrial development (Huang et al., 2022).

It has been shown that nitric oxide (NO) treatment delays the postharvest senescence of fruits (Dong et al., 2022). Previous reports demonstrated the sodium nitroprusside (SNP) was widely used as NO donor, which acted as a gas signal molecule in plants responsible for the increasing many defense-related enzymes (Filippou et al., 2013). NO may act as a plant growth regulator to regulate plant ripening and senescence (Dong et al., 2022). Previous researchers reported that exogenous NO treatment can palliate adverse damages against abiotic and biotic stresses in plant. In addition, NO application displayed the ability improving crops nutritional quality, alleviating damages during fruit ripening and postharvest period, and regulating ROS and energy metabolism during storage (Dong et al., 2022).

To date, there were no reports about the physiological role of exogenous NO treatment in delaying ripening and senescence of fresh fruits of *L. barbarum* during storage and preservation. So far, there are no reports on the use of NO gas immersion to treat fresh fruit of *L. barbarum* and study the quality changes. In this study, the sodium nitroprusside (SNP) was used as NO donor, and the effect of SNP (20 min soaking treatment) on the postharvest storage quality of *L. barbarum* fruit "Ke qi 608" was investigated to provide theoretical reference for the application of NO in the storage and preservation of *L. barbarum* fruit.

2 Materials and methods

2.1 Experimental materials

The *L. barbarum* fruit "Ke qi 608" was procured from the Institute of Chinese goji, Ningxia Yinchuan Academy of Agriculture and Forestry. For this, disease-free fresh fruit of Chinese goji were selected for processing. Sodium nitroprusside (SNP) reagent was provided by Shanghai Guangnuo Chemical Technology Co. (Shanghai, China)

2.2 Treatment of goji berries

About 2700 freshly ripened goji fruits with uniform size were selected. Three parallel groups of 50 fruits in each group were divided, with no SNP as control (CK). Soak them in 75% alcohol for 2 min to disinfect and air dry naturally. Subsequently, 200 mL SNP solutions of different concentrations (0.0, 0.2, 0.4, 0.6, 0.8,

Received 02 July, 2022
Accepted 21 Aug., 2022
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1.0, 1.2, and 1.4 mM) were prepared as NO donors and placed in polystyrene boxes (PE) for 20 min immersion, followed by placing on filter paper and flattening until dried. The PE were placed in a constant temperature incubation at 25 °C and humidity of 85%-90%, and the optimum concentration of SNP was screened by observing the goji fruits for decay rate and weight loss rate on daily basis.

2.3 Determination of decay rate, weight loss rate, hardness, color, soluble solids, titratable acid, and soluble protein content

The decay rate was determined as the percentage of the number of decayed fruits to the total number of fruits during storage and repeated three times according to the following formula (Equation 1) (Ni et al., 2016; Hu et al., 2019):

$$Fruit\ decay\ rate\ (\%) = number\ of\ decayed\ fruits / total\ fruits \times 100\%. \quad (1)$$

The weight loss rate was determined by the method of weight measurement, expressed as a proportion of the loss of the fruit in the storage process, repeated three times according to the following the formula (Equation 2) (Zhang et al., 2020):

$$Fruit\ weight\ loss\ rate\ (\%):\ W = (mn - m0) / m0 * 100\% \quad (2)$$

The hardness was determined using a mass spectrometer (TAXT plus; SMS) at the equatorial part of the fruit using the puncture method for single point determination with the following parameters: maximum force 8 N, force sensing element range 10 N, trigger force 0.05 N, measurement speed 30 mm/s, puncture distance 7 mm, return speed 60 mm/s, return distance 20 mm.

The fruit color was measured using a precision colorimeter, and the a* and b* values of the fruit peel at the equatorial part of the fruit were determined according to the CIE (International commission on illumination) Lab color space, using a standard white plate and a black cavity as reference objects.

Soluble solids (total soluble solids, TSS) content was determined according to the previously described method (Lv et al., 2022) using a handheld refractometer. For this, fruit juice of *L. barbarum* was squeezed out and dropped on the glass surface of the refractometer.

Titratable acid (TA) of the Ningxia *L. barbarum* fruit was assayed using titration with SNP (0.1 mM) at pH = 8.3 (Lv et al., 2022).

Soluble protein content was determined by the Komas Brilliant Blue G-250 staining method. The samples of *L. barbarum* (1.0 g ± 0.05 g) were taken as homogenate, centrifuged at 4 °C (12000 rpm, 20 min), and then the supernatant (1 mL) was mixed with Kemas Brilliant Blue (5 mL). The OD value of 595 nm was recorded for different samples, and the soluble protein content was calculated and expressed as mgg-1FW.

2.4 Determination of total chlorophyll, carotenoids, total phenols, and flavonoids content

Total chlorophyll and carotenoid content were determined by referring to the spectrophotometric method (Hörtensteiner, 2006). Briefly, 1.0 g of *L. barbarum* fruit sample and 0.5 g of *L. barbarum* fruit stalk sample were mixed with anhydrous ethanol: acetone solution and water volume ratio of 4.5:4.5:1 for static extraction under dark for 24 h. Using extraction solvent as a blank, the absorbance values of the extract solution were determined at the wavelengths of 663, 645, 440 nm. The carotenoid content of the fruits per gram (fresh weight) was expressed as absorbance value at 440 nm, i.e. A440*V/W. The calculation was performed using Amon formula:

$$chlorophyll\ a:\ Ca = 12.7A663 - 2.69A645;$$

$$total\ chlorophyll:\ C(a + b) = Ca + Cb;$$

$$total\ chlorophyll\ content\ (mg / g) = C(a + b) * V / W.$$

Total phenolic content was determined by referring to the method of Lv et al. (2022). The absorbance values of total phenol extracts were measured at 280 nm using 1% hydrochloric acid-methanol solution as a blank control. Meanwhile, the standard curve was prepared with gallic acid as the standard, and the concentration of gallic acid (mg/mL) as the horizontal coordinate and absorbance as the vertical coordinate were used to draw the standard curve, and the regression equation $y = 12.757x + 0.2483$ was obtained, with the correlation coefficient $R^2 = 0.9987$. The concentration of total phenol was calculated from the standard curve, to calculate the total phenol content (per gram) in the sample. The fresh weight of *L. barbarum* was expressed and repeated three times.

The flavonoid content was determined based on the previously described method (Lv et al., 2022), with slight modifications. For plotting the standard curve, 40 mg of rutin was weighed, dissolved in anhydrous methanol, and fixed to 100 mL, and prepared as a standard solution with a concentration of 0.4 mg/mL. The standard solution was prepared to a concentration of 0.4 mg/mL. The standard solution was pipetted into a 25 mL volumetric flask at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL. Then, 1.25 mL of 5% aqueous $NaNO_2$ solution was added, mixed well, and allowed to stand for 6 min. Further, 1.25 mL of 10% aqueous $Al(NO_3)_3$ solution was added and the volume was fixed with distilled water, mixed well and allowed to stand for 15 min. The absorbance of each concentration of standard solution was measured under the wavelength of UV-VIS spectrophotometer at 510 nm. The flavonoid content was determined by making a standard of rutin standard of 0.4 mg/mL with the correlation coefficient $R^2 = 0.9922$. For sample determination, 0.2 g of *L. barbarum* was weighed, grinded to obtain the homogenate with 25 mL of 95% ethanol solution. The obtained solution was sonicated and placed under ice bath conditions, filtered and the volume was fixed to 25 mL. The 7.5 mL of sample was taken in a volumetric flask and 1. 25 mL of 5% $NaNO_2$ aqueous solution was added, shaken well and allowed to stand for 6 min followed by the addition of 1.25 mL of 10% $Al(NO_3)_3$ aqueous solution, shaking and then addition of 10 mL of 4% NaOH aqueous solution. The absorbance of each concentration of standard solution was measured at 510 nm and repeated three times.

2.5 Determination of ascorbic acid (VC), hydrogen peroxide (H_2O_2), malondialdehyde (MDA) content

The ascorbic acid content was determined according to the previously described method (Nath et al., 2011) with slight

modifications. For this, 1 g of *L. barbarum* fruit sample was added with 5 mL of 0.02 g/mL oxalic acid solution, and the homogenate was grinded under ice bath conditions and placed in a 25 mL volumetric flask. The mortar was rinsed with 0.02 g/mL oxalic acid solution and poured into a volumetric flask and the volume was fixed with 0.02 g/mL oxalic acid solution followed by mixing and extraction for 10 min. The extracted solution was poured into a 50 mL centrifuge tube and centrifuged at 12000 r/min for 10 min at 4 °C followed by filtration and collection of the final solution. Then 8 mL of filtrate was absorbed by pipette and placed in a 50 mL triangular flask and titrated with calibrated 2,6-dichlorophenol indophenol solution for 15 s until red color appeared and did not fade. At the same time, 8 mL of 0.02 g/mL oxalic acid solution was used as a blank and titrated and repeated three times. The following formula was used (Equation 3):

$$Ascorbic\ acid\ content\ \left(mg/100g\right)=\left[V\times\left(V_1-V_0\right)\right]\times\rho\times100\div\left(Vs\times m\right) \quad (3)$$

V_1: volume of dye solution consumed for sample titration (mL); V_0: volume of dye solution consumed for blank titration (mL); ρ: mass of 1 mL dye solution equivalent to ascorbic acid (mg/mL); Vs: total volume of sample solution taken for titration (mL); m: mass of sample (g).

The contents of H_2O_2 and MDA were determined using kits according to the manufacturer's instructions (Suzhou Kemin Biotechnology Co., Suzhou, China). The activities of CAT, POD, PPO, and APX were determined using kits according to the manufacturer's instructions (Suzhou Kemin Biotechnology Co., Suzhou, China).

2.7 Data analysis

Three parallel samples were taken for each experiment, and the results were expressed as "mean ± standard deviation (means ± SD)", and analysis of variance (ANOVA) was performed using EXCEL software. The $p < 0.01$ indicates highly significant differences, which were indicated by * and ** in the graphs. The data obtained were statistically analyzed and plotted using origin2021 data analysis software.

3 Results and analysis

3.1 Effect of NO on the phenology of post-harvested L. barbarum during storage period

Different concentrations of SNP solutions (0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mM) were used as SNP donors to treat post-harvested *L. barbarum*, and the apparent results were shown in Figure 1.

It can be seen that SNP could inhibit and delay the decay degree as well as softening of post-harvested goji berries to different

Figure 1. Effect of different concentrations of SNP treatment on the phenology of *L. barbarum* during storage.

degrees, and showed obvious concentration-dependent effect. With the increasing concentration, 0.6 mM of SNP treatment showed better phenological effect. From the 2 d of treatment, softening of control, 0.2 and 0.4, 1.2 mM SNP treated goji fruits was observed, and the softening and senescence of goji treated with different concentrations were slower than the control group. In the following 3-4 days, the fruit became moldy and the fruit pedicels started to fall off. Goji fruits treated with 0.4, 1.2, and 1.4 mM of SNP showed different degrees of fruit moldiness, but the apparent effect was still better than the control. 1.2 and 1.4 mM of SNP showed fruit decay and detachment first. 0.2 mM and 0.4 mM SNP showed significantly better effect than the control and other treatment groups, the fruit pedicels did not appear to fall off. The fruit pedicels did not fall off and the fruit did not show decay. At 5-6 days of storage, compared with the treated groups, the control group showed extensive decay and softening of the fruits, extensive shedding of fruit pedicels, and increased decay rate of the fruits. The fruits of 1.4 mM SNP treated group were obviously softened and extensively rotted. At 7-8 days of storage, the 0.6 mM treated treatment group showed better fruit pedicel abscission, softening, and decay than the control group.

3.2 Determination of decay rate and weight loss rate

The SNP treatment alleviated the increase in fruit decay of *L. barbarum*, and there was a clear upward trend in the 0.6 mmol/L SNP treated *L. barbarum* at the day 3. The weight loss of control *L. barbarum* was always lower than the seven SNP treatments, with the SNP treatment of 1.2 mmol/L always showing the lowest weight loss; whereas the SNP treatment of 0.6 mmol/L showed a consistent upward trend ($p < 0.05$) (Figure 2A). This indicates that SNP can play a role in storage and preservation, and the 0.6 mmol/L of SNP treatment was more effective in preservation.

As seen in Figure 2B, both the control and SNP treated berries showed a gradual increase in weight loss rate with the growth process, and the 0.6 mmol/L treatment significantly increased the weight loss rate ($p < 0.05$) compared to the control and other concentrations of SNP treatment groups.

3.3 Determination of hardness, color, soluble solids, titratable acid, and soluble protein content

As seen in Figure 3A, the overall hardness of the goji fruit showed a gradual decrease throughout the storage period with time, and the hardness of the SNP treatment goji fruit dropped sharply on the first day of storage, all significantly higher ($p < 0.05$) than that of the control group during the same period. The first 0-2 days treatment groups were higher than the control group, however, the middle and late treatment groups were higher than the control group. As seen in Figure 3B, the a* value of flesh color difference can reflect the degree of red and green color of the sample, and the higher a* value indicates the higher redness of the red color. The a* values of post-harvested *L. barbarum* fruits showed an increasing trend in the storage stage at room temperature, and the a* in the SNP treated group was significantly higher than that in the control group from 0-6 days. This indicates that NO treatment could delay the aging of fruits to some extent ($p < 0.05$).

The color difference L* values of the fruit flesh can reflect the degree of color brightness and darkness, and the L values* of post-harvested *Lycium* berries had brightness darkening with the storage time, and the L* of the SNP treatment group was significantly higher than that of the control group during the storage period ($p < 0.05$). It indicates that NO treatment could delay the aging of fruits to some extent (Figure 3C).

During the storage period of post-harvested goji fruit, TSS content declined with the extension of the storage period, and on the first day of storage, both the control and treatment groups first rose slightly and then maintained a decreasing trend, with a small rebound trend (Figure 3D). This rebound of soluble solids as the activation of hydrolytic enzymes during storage and the decomposition of large molecule carbohydrates such as starch into sugars resulted in the accumulation of soluble solids. The soluble solids content of the treated group was consistently higher than that of the control group throughout the storage period and showed significant differences ($p < 0.05$) at the later stages. The treatment group maintained a stable

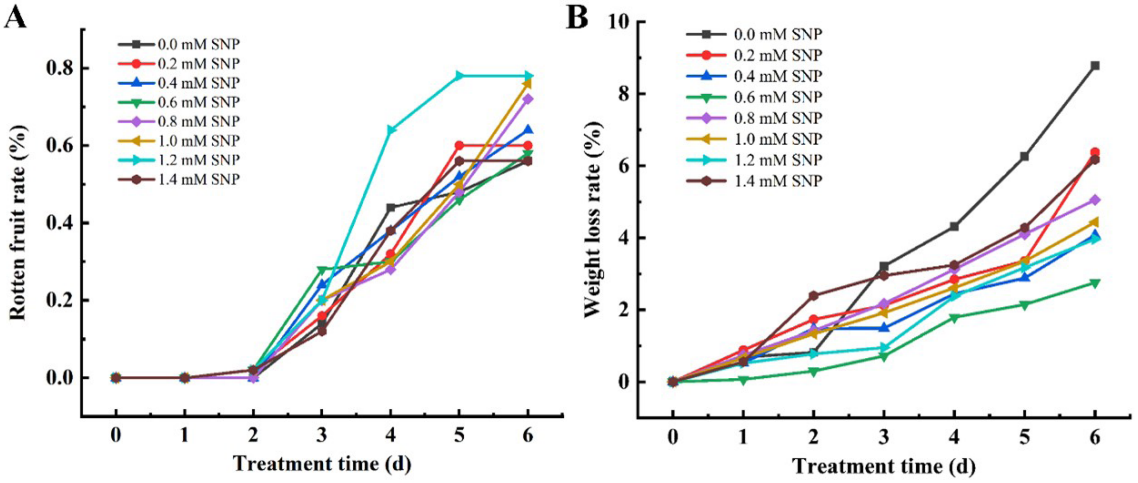

Figure 2. Effect of different concentrations of SNP treatment on the decay rate and weight loss rate of *L. barbarum*. A: The rotten fruit rate (%) of *L. barbarum*. B: The weight loss rate of *L. barbarum*.

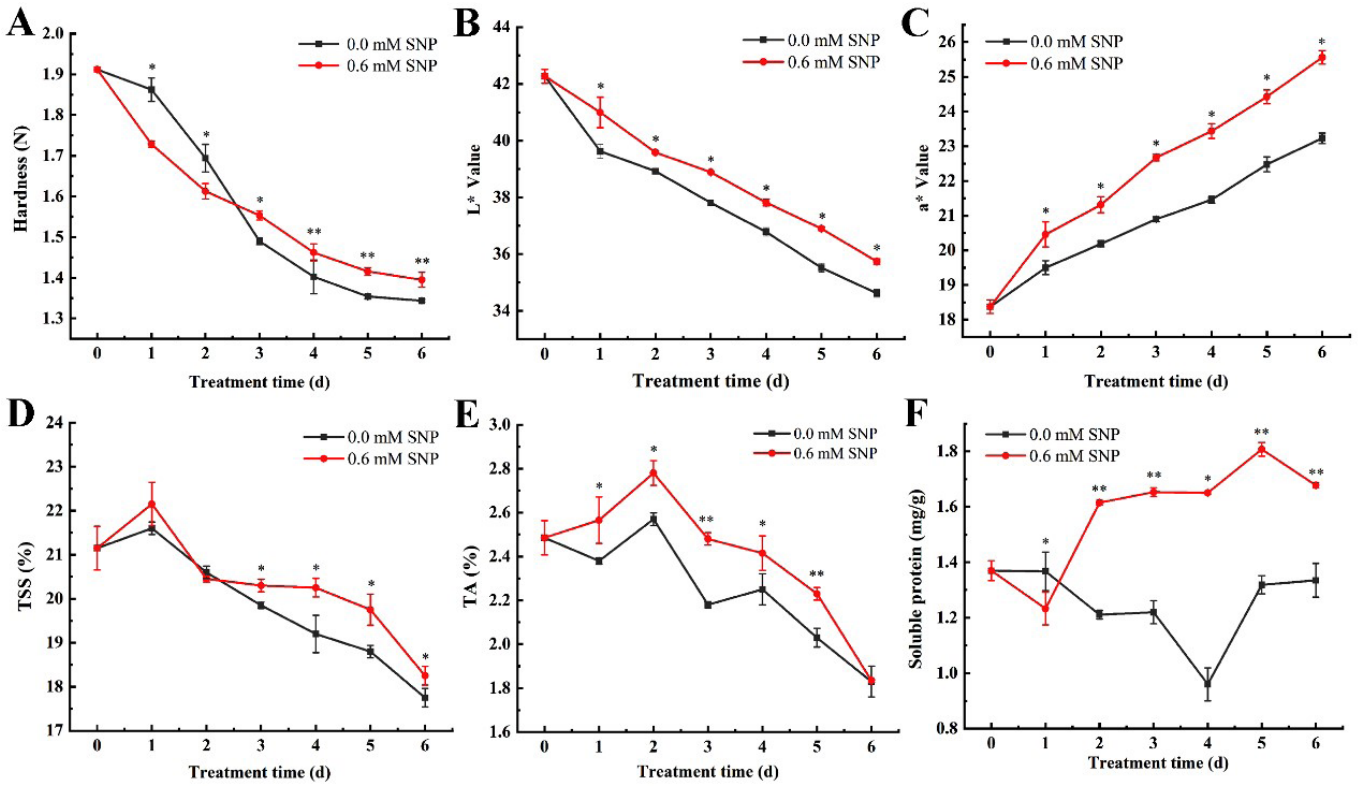

Figure 3. Effect of SNP treatment on the determination of hardness, color, soluble solids, titratable acid, and soluble protein content of *L. barb*arum. A: Hardness of the goji fruit. B: L* values of the fruit. C: a* values of the fruit. D: The total soluble solids (TSS) contents of the fruit. E: Titratable acid (TA) contents of the fruit. F: The soluble protein content of fruit. "*" and "**" represent the significant different at "$P < 0.05$" and "* $P < 0.01$*", respectively.

trend from the second to the fourth day of storage, followed by a small decrease. In the control group, a gradual decrease in soluble solids content occurred in the middle and late stages due to the high consumption of sugars as the main substrate for respiration. The above results indicated that SNP could delay the decomposition of macromolecular carbohydrates while maintaining the TSS content, maintaining the nutrients in the fruit, and delaying the ripening and aging of the fruit.

The substrate associated with respiration during postharvest storage of *L. barbarum* fruit was depleted with the storage time because the amount of external ROS exceeds the amount of ROS that can be inhibited by its own system during storage. Titratable acid is used as one of the indicators of fruit ripening. As shown in the Figure 3E, the content of titratable acid in both the control and treated groups increased slightly and then decreased gradually, while that in the control group increased slightly and then decreased gradually from 0-2 days during storage, and was higher compared to the control group throughout the storage period ($p < 0.05$) (Figure 3E). The above results indicated that SNP could delay the decomposition of macromolecular carbohydrates while maintaining fruit acid content, fruit nutrients, and delaying fruit maturation and senescence.

The soluble protein content of fruits and vegetables is an important physiological and biochemical index, and one of the important evaluation indexes of fruits and vegetables nutrition. In the storage process, post-harvested goji fruit content changed with time; the control group showed the higher content than the treatment group on the first day. Subsequently, the treatment group was higher than the control group and showed a highly significant difference ($p < 0.01$) in the middle and late stages of storage (Figure 3F). SNP treatment significantly inhibited the protein degradation and maintained the soluble protein content of post-harvest *Lycium* fruit at a high level, thus maintaining the nutritional value and storage quality of *Lycium* fruit.

3.4 Determination of total chlorophyll, carotenoids, total phenols, and flavonoids content

The process of fruit ripening and senescence is accompanied by the breakdown of chlorophyll and the accumulation of carotenoids in the fruit (Severo et al., 2015; Balic et al., 2012). Chlorophyll plays an important role in the energy transfer of photosynthesis. Chlorophyll and lutein can determine the degree of ripening and aging of fruits and vegetables (Lv et al., 2022).

The NO treated berries showed a small decrease followed by a small increase and a large decrease trend by the late storage, but the treatment groups showed an increasing trend and a significant difference ($p < 0.05$) in the late storage (Figure 4A). As seen in Figure 4B, NO treatment significantly inhibited the significant decrease in carotenoid pigment substance of the fruit, with a decreasing trend from 1-2 days, followed by a rising and then decreasing trend in the treated group. The control group showed

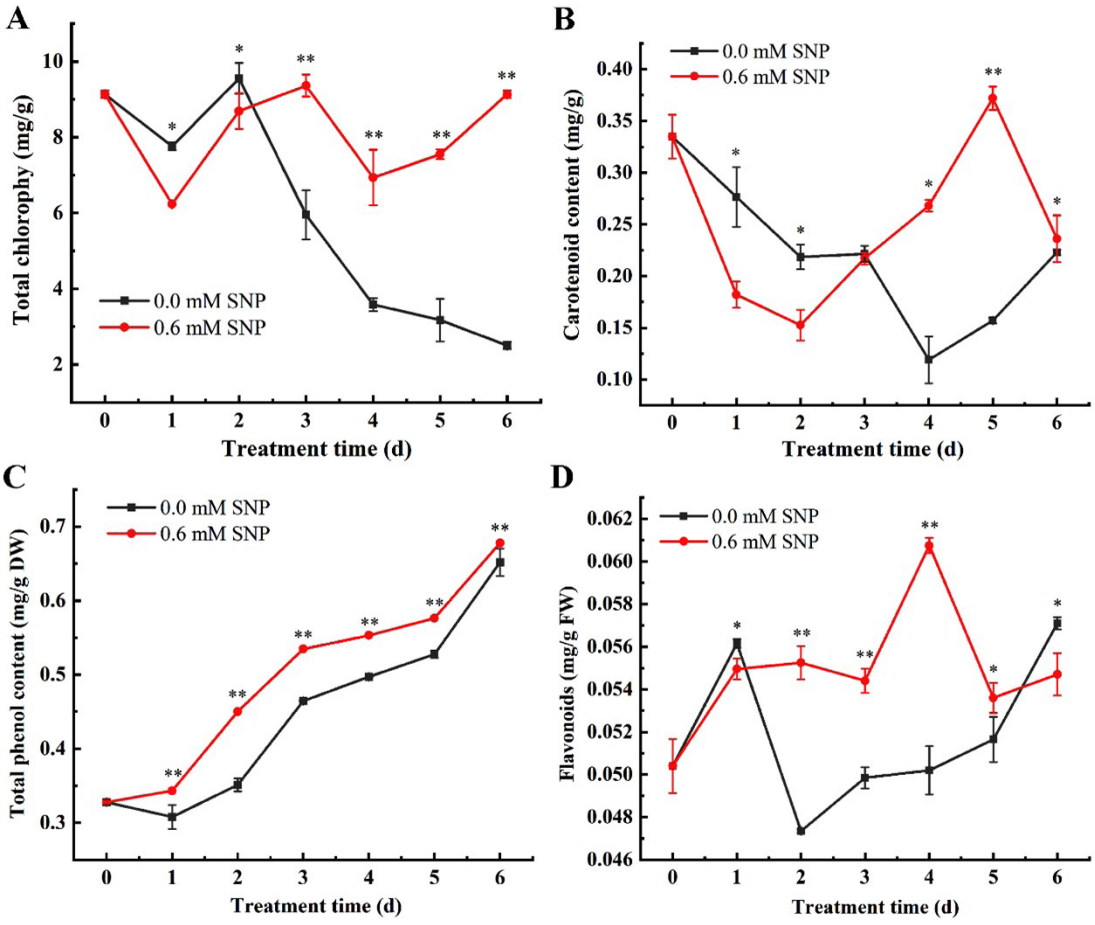

Figure 4. Effect of SNP treatment on the determination of total chlorophyll, carotenoids, total phenols, and flavonoids content of *L. barbarum*. A: The total chlorophyll contents of the fruit. B: The carotenoids contents of the fruit. C: The total phenols contents of the fruit. D: The flavonoids contents of the fruit. "*" and "**" represent the significant different at "$P < 0.05$" and "$* P < 0.01$*", respectively.

higher content than the treated group in the pre-storage period, and by the late storage period, the treated group showed higher content than the control group ($p < 0.05$). Thus, SNP treatment inhibited the reduction of carotenoid substance content and the maintenance of pigmentation may be the main reason for maintaining the color of *L. barbarum*, which in turn maintained the color quality of the fruit.

It was found that phenylalanine aminolytic enzyme activity increased with the tissue maturation and aging thereby catalyzing the synthesis of phenolic compounds from phenylalanine, which explains the rise in total phenolic content that occurred during storage. SNP treatment showed an increasing trend throughout storage and the treated group was always higher than the control group. SNP treatment significantly inhibited the decline in the total phenolic substance content of *L. barbarum* fruit; therefore, SNP treatment significantly delayed the decline in the total phenolic content, thus effectively maintaining the nutrients and good quality of the fruit ($p < 0.05$) (Figure 4C).

Throughout the storage period, the content of flavonoids in the fruit phenotypically showed a decreasing trend, and the decrease rate in the treatment group was significantly lower than that of the control group, so that the flavonoid content was always higher than that of the control group except for day 6 in the treatment group and showed a highly significant difference from the first day of treatment ($p < 0.01$). The treatment group showed a large increase and then a large decrease on day 3, indicating that the flavonoid content increased at day 4 and decreased by day 5. This indicates that the treatment group showed a subtle increase all the time except for day 4. The control group showed a large decrease on day 1 followed by a small gradual increase, indicating that the flavonoid content was not significant on day 2, and a significant increase on day 5, indicating that the control group presented an increase in flavonoid content in the later stages (Figure 4D).

3.5 Analysis of postharvest ascorbic acid (VC), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) activities in SNP treated L. barbarum

As seen in Figure 5A, the VC content in fresh fruit of *L. barbarum* gradually decreased with the extension of storage time, and was significantly higher in the treatment group than in the control group during the whole storage period. The control

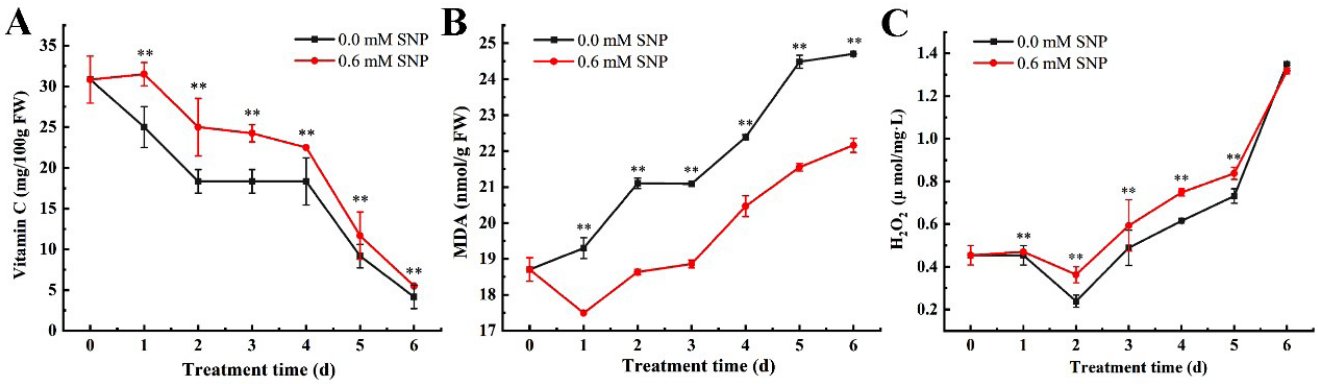

Figure 5. Effect of SNP treatment on the determination of VC, MDA, and H_2O_2 content in *L. barbarum*. A: The ascorbic acid (VC) contents of the fruit. B: The hydrogen peroxide (H2O2) contents of the fruit. C: The malondialdehyde (MDA) contents of the fruit. "*" and "**" represent the significant different at "*P < 0.05*" and "* *P < 0.01**", respectively.

group showed a significant decrease from 0-2 days, remained stable from 2-4 days followed by a gradual decrease from day 5. The treatment group showed a significant decreasing trend from 4d to 6d ($p < 0.05$). This indicates that exogenous NO treatment can significantly delay the decline of VC content in fresh *L. barbarum* after harvest.

MDA is one of the main products of membrane lipid peroxidation, and the magnitude of its content is positively correlated with the degree of membrane lipid peroxidation. Many studies have shown that membrane lipid peroxidation is one of the important causes of fruit senescence (Lv et al., 2022). As seen in Figure 5B, with the prolongation of storage time and the intensification of senescence, the cell membrane structure of *L. barbarum* fruit was damaged and MDA accumulated in its tissues, and the MDA content showed an increasing trend. Throughout the storage process, the MDA content of SNP treated *L. barbarum* fruit maintained an increasing trend, and the trend presented from 0d to 1d was a small decrease, and then maintained a small increasing trend ($p < 0.05$). It was shown that the exogenous SNP treatment could effectively reduce the accumulation of MDA in *L. barbarum* fruits and delay the process of membrane lipid peroxidation during the storage period, thus delaying its maturation and aging, probably because SNP could delay its damaging effect on cell membranes by inhibiting the production of free radical ROS in tissues.

Plant senescence is usually accompanied by the accumulation of ROS, which may cause oxidative damage to cellular components, including lipids, proteins, and nucleic acids (Ni et al., 2016) Excessive production of ROS is an important factor causing disturbance and dysregulation of the dynamic balance of tissue metabolism, which commonly occurs during fruit senescence (Ni et al., 2016). The effect of SNP on the H_2O_2 content in post-harvest *L. barbarum* fruit is shown in Figure 5C. The whole storage period treatment and control groups have been showing a smooth trend from 0-1 days, 1-3 days showed a small decreasing trend and then a small increasing trend, and the treatment group showed a rapid upward trend from 3-6 days. The treatment group showed a decreasing trend followed by increase from 3-6 days. The treatment group showed significantly higher content than the control group from 1-3 days, but the control group was always higher than the treatment group from day 3 and maintained a sharp increasing trend ($p < 0.05$). This indicates that SNP can prevent or delay the burst of ROS, causing a delay in the appearance of the cumulative peak to retard the senescence of post-harvest Lycium berries.

3.6 Effect of SNP on peroxidase (CAT), polyphenol oxidase (PPO), peroxidase (POD), and ascorbate peroxidase (APX) in post-harvested L. barbarum

As seen in Figure 5A, during the whole storage period, the treated group showed the higher CAT activity than the control group at the beginning of the storage period, and the control group showed the higher CAT activity than the treated group toward the end of the storage period, and showed a highly significant difference ($p < 0.01$) at the beginning. SNP treatment led to a rapid increase in CAT activity on the first day until a small decrease on day 3 followed by a gradual increase.

The effect of SNP on the PPO activity of post-harvested *L. barbarum* can be seen in Figure 6B, which was always higher than that of the control group, showing a rising and then falling trend. The control group showed the decreasing trend ($p < 0.05$). It indicates that SNP treatment can inhibit the rapid rise of PPO activity in the fruit, thus alleviating the degree of browning in the fruit during storage.

POD is a variety of metabolic enzymes, whose main function is to participate in the metabolic process of lipids such as fatty acids and oxidative stress regulation (Islinger et al., 2012). As seen in Figure 6C, SNP treated fruits showed an increasing trend in POD activity during storage, and the POD activity of the treated group showed a decreasing trend from 4-6 days after harvest, and was greater than that of the control group throughout the storage period, with significant differences ($p < 0.05$). This indicates that exogenous NO treatment inhibited the POD activity of *L. barbarum* fruit, thus delaying its lignin formation and aging process.

A major hydrogen peroxide detoxifying system in plant chloroplasts and cytosol is called the ascorbate-glutathione

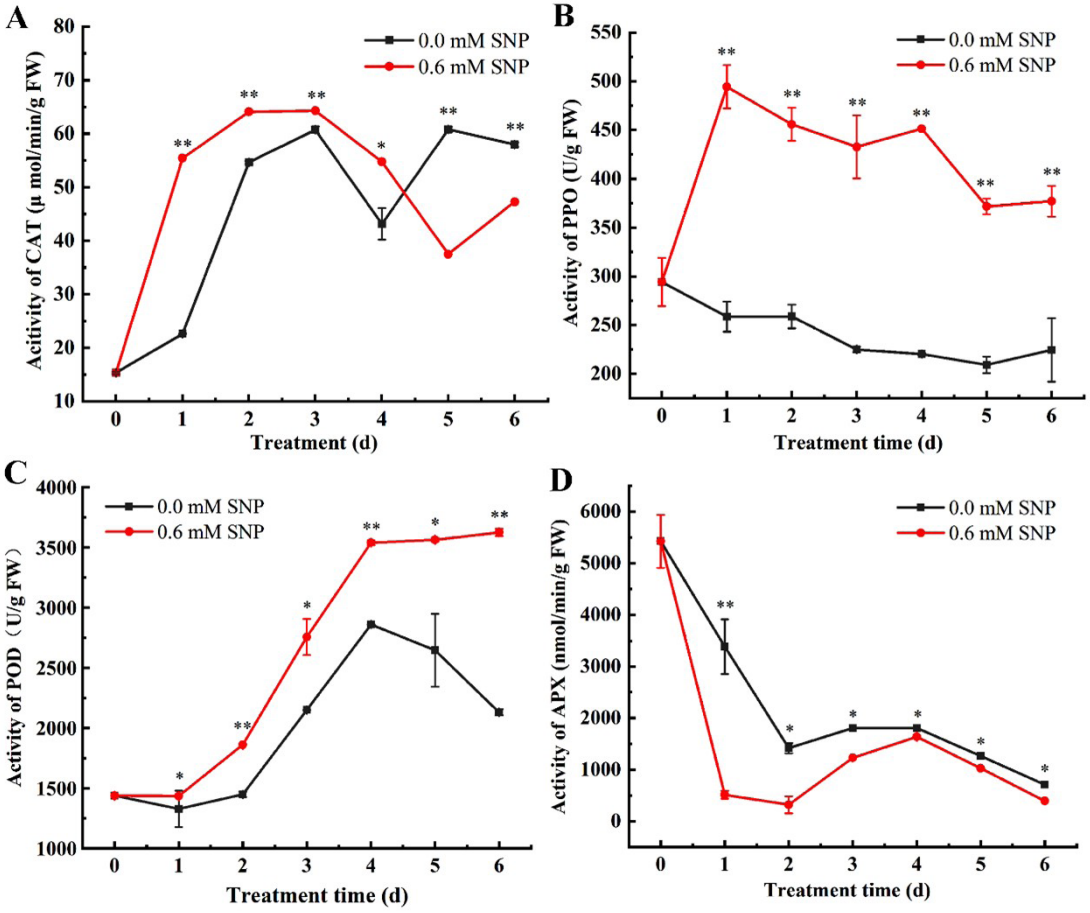

Figure 6. Effect of SNP treatment on CAT, PPO, POD, and APX activities of *L. barbarum*. A: peroxidase (CAT) activity of the fruit. B: The polyphenol oxidase (PPO) of the fruit. C: The peroxidase (POD) activity of the fruit. D: The ascorbate peroxidase (APX) activity of the fruit. "*" and "**" represent the significant different at "$P < 0.05$" and "* $P<0.01$*", respectively.

cycle, in which ascorbate peroxidase (APX) is the key enzyme. As seen in Figure 6D, SNP treatment resulted in a decreasing trend of APX activity in fruits on the first day, followed by a gradual decrease. Throughout the storage process, the APX activity of control fruits was always higher than that of the treated group ($p < 0.05$).

4 Discussion

The decay rate of post-harvested *L. barbarum* after NO treatment was lower than that of untreated SNP. The surface of *L. barbarum* fruit was harder, the content of chlorophyll and carotenoids decreased after NO treatment, and the pigment content in the fruit increased compared to control which was more significant over time. Leshem et al. (1998) concluded that low concentrations of NO promote plant growth, resist stress, and delay senescence, while high concentrations inhibit growth and accelerate decay. inhibits water loss after harvesting, maintains the nutrient content of fruits and vegetables, resulting in delayed aging. Our results showed that exogenous NO treatment inhibited the loss of chlorophyll content of *L. barbarum* after harvesting, and at the same time, delayed the changes in soluble solids and titratable acid content and lowered the decrease in VC, soluble protein content and hardness, and titratable acid, thus better maintaining the nutritional quality of *L. barbarum* after harvesting. In the storage process of *L. barbarum* fruit, POD can catalyze the decomposition of H_2O_2 and cause the polymerization reaction of lignin monomer to generate the degree of lignification. NO could inhibit its activity by acting on the metal cofactor of POD, thus inhibiting the lignification and aging of *L. barbarum* fruit in the postharvest storage process. Previous study showed that the reactive oxygen metabolism of H_2O_2 in fruits and vegetables after harvest was enhanced, causing cellular damage due to membrane lipid peroxidation (Ni et al., 2016).

NO could also increase the content of the antioxidant VC in plant tissues and inhibit membrane lipid peroxidation due to ROS production during storage, thus reducing the MDA accumulation and delaying the aging of *L. barbarum* fruit. NO could inhibit the ethylene synthesis by regulating the activity of ACC synthase to regulate growth hormone and ACC oxidase cofactors (VC and Fe^{2+}) (Leshem et al., 2001), thus indirectly regulating the ROS content in tissues and inhibiting fruit aging. Due to its unique chemical properties, NO can react with H_2O_2 and O_2^- and act on a variety of ion-containing proteins, thus regulating the

activities of enzymes such as SOD, CAT, and APX (Clark et al., 2000). In addition, it can also induce the expression of alternate AOX and inhibit the ROS production (Neill et al., 2003). This indicates that NO delays fruit ripening and leaf senescence (Leshem et al., 1998).

NO is widely present in organisms, not only affecting a series of physiological processes such as growth and development, but also playing a key role in the signal transduction process. To conclude, the exogenous NO treatment maintained the higher activities of MDA, CAT, POD, PPO, and APX *in L. barbarum* fruit compared with the control group, which reduced the degree of membrane lipid peroxidation and could better maintain the stability and integrity of membrane structure. Our study revealed that SNP treatment at appropriate concentration (0.6 mmol/L^{-1}) can exert remarkable preservation effects on the *L. barbarum* fruit. In our research, it was concluded that NO treatment improved the preservation of postharvest *L. barbarum* fruit. However, the mechanism of NO improving the preservation of postharvest *L. barbarum* is yet to be further studied in future, e.g., the regulation respiration and energy metabolism, ethylene biosynthesis and signal transduction, the expression of related genes in mRNA and protein level.

Conflict of interest

There is no declaim.

Acknowledgements

This research was funded by the National Natural Science Foundation of Ningxia Province (2022AAC03280, 2020AAC02030), Innovation Team for Genetic Improvement of Economic Forests of Ningxia Province (2022QCXTD04), the Youth talent cultivation project of North Minzu University (2021KYQD27, FWNX14), Key research and development projects in Ningxia province (2021BEF02013).

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