Postharvest treatment with nitric oxide influences the physiological and quality attributes of 'Santa Rosa' plums during cold storage

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ABSTRACT

Studies were conducted to observe the effect of nitric oxide (NO) on 'Santa Rosa' plum, a Japanese plum grown extensively in India. 'Santa Rosa' plums were dipped in solution of sodium nitroprusside (SNP @ 0.25, 0.5, 1.0 and 1.5 mM) and distilled water (control) for 5 min. After treatment, fruits were air-dried under fan and stored at 2°C temperature and 90 ± 5% RH for 36 days. Results revealed that SNP treatments significantly delayed the weight loss, fruit softening, and fruit decay in plums. However, minimum weight loss (8.3%), maximum firmness (3.463 N) and lowest fruit decay (0.0%) were recorded in SNP (0.5 mM) treated plums, whereas untreated fruits showed maximum weight loss (13.8%), lowest fruit firmness (1.595 N) and highest decay loss (18%). All SNP treatments significantly suppressed and delayed the rates of respiration and ethylene production by the fruits. Maximum phenolics content (106 mg/ 100 g pulp) and titratable acidity (1.1%) was observed in SNP @ 0.5 mM treated fruits, while it was lowest (65.3 mg/ 100 g pulp, 0.8% respectively) in untreated plums. Untreated fruits reached the highest SSC content on 16th day of storage (16.7°Brix) followed by a decline, while SNP (0.5 mM) treated fruits showed slower increase in SSC content. Hence, SNP 0.5 mM treatment can be effectively used for maintenance of desired postharvest quality and extending the market life of 'Santa Rosa' plums up to 36 days when stored at 2°C.

Key words: Fruit firmness, nitric oxide, plum, quality attributes, respiration rate.

INTRODUCTION

The Japanese plum probably originated in China and has been in cultivation in Japan before being introduced to America and Europe. Plum is a juicy and nutritious fruit. There are good number of plum species, which are economically important, major among them being European plum (*Prunus domestica*), Damson plum (*Prunus insititia*), Cherry plum (*Prunus cerasifera*), Japanese plum (*Prunus salicina*) and American plum (*Prunus americana*). However, Japanese plums perform excellent under Indian climatic conditions mainly due to its hardy nature and prolific bearing habit. Japanese plum cv. Santa Rosa belongs to the climacteric group (Sharma *et al*., 10; Sharma *et al*., 11) with high metabolic and respiration rates, which makes it a very perishable product. There are huge post harvest losses (25-30%) due to compression injury even during transportation (Sharma *et al*., 8; Sharma *et al*., 9). Thus, it demands special attention on the postharvest practices used in order to sustain quality and prolong its shelf-life.

Nitric oxide (NO), a highly reactive free radical gas, acts as a multifunctional signaling molecule in various physiological processes in plants (Wendehenne *et al*., 15). In horticultural crops, NO levels decrease with maturation and senescence (Leshem *et al*., 4), thereby offering an opportunity for modulation of their levels with exogenous application to exert the opposite effect. Short-term exposure of intact and fresh-cut horticultural commodities to very low concentrations of NO is known to retard their postharvest senescence (Wills *et al*., 16). Postharvest exogenous application of NO has been reported to delay fruit ripening in a range of climacteric or non-climacteric fruits such as banana, plum, strawberry and kiwifruit through different mechanisms such as suppressed respiration rate, reduced ethylene biosynthesis, disease incidence, flesh softening and reduced activity of softening enzymes (Ku *et al*., 3; Manjunatha *et al*., 5). Postharvest NO fumigation has also been reported to alleviate chilling injury (CI) in Japanese plum (Singh *et al*., 12) and mango (Zaharah and Singh, 17).

The previous studies conducted on the effect of NO treatment in extending shelf-life; NO was administered through fumigation method (Zaharah and Singh, 17), which seems costly and difficult. Therefore, we attempted to apply nitric oxide as aqueous dip treatment of sodium nitroprusside (SNP, a NO donor) on plums, as no information is yet available. This prompted us to address above gap(s) and investigate the effect of SNP (a NO donor) enhancing shelf-life and preserving plum cv. Santa Rosa fruit quality during storage at 2ºC.

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MATERIALS AND METHODS

The studies were conducted in the Division of Food Science & Post Harvest Technology, IARI, New Delhi in July-August' 2012. 'Santa Rosa' plums were harvested in the month of June from a private orchard at Katrain, Kullu (Himachal Pradesh). The fruits were packaged in corrugated fibre board boxes and transported by road to Delhi from the orchard. These plums were given sodium nitroprusside (a nitric oxide donor) aqueous dip treatments (control, SNP @ 0.25, 0.5, 1.0 & 1.5 mM) for 5 min. at 20°C in the laboratory. For this, the desired quantity of SNP was dissolved in small quantity of distilled water and then the solution poured in the container containing 10 l of tap water. The fruits in control were also dipped in plain water. The fruits were then air-dried for 30 min. by spreading on blotting paper under fan and packed in plastic punnets followed by storage at 2° C and $90 \pm 2\%$ RH (500 g capacity) having holes for ventilation and observations on different parameters were recorded at 4 days interval during the 36 days storage period.

The observations on physiological loss in weight (PLW), firmness, fruit rot, rate of respiration and ethylene evolution, and fruit quality attributes such as total phenols, soluble solids content (SSC), titratable acidity (TA) were recorded at 4 day interval. Physiological loss in weight (PLW) was determined by weighing the fruits at the initial day and then at regular intervals. Fruit firmness in plums was determined using a texture analyzer (model: TA+Di, Stable micro systems, UK) using compression test and expressed as Newton (N). The fruits showing visible fungal or bacterial invasion, abnormal softening or oozing out of fluids were considered to be rotten and accordingly the fruits showing rotting were expressed as percentage. Ethylene production and respiration rates were measured using the static headspace technique (Sharma *et al*., 10; Sharma *et al*., 11). The respiration rate was expressed as ml CO_2 kg 1 h 1 using Model: Checkmate 9900 O_2 /CO₂, PBI Dan sensor, Denmark. For ethylene, 1 ml of the headspace atmosphere of the container was withdrawn with a gas-tight syringe and injected into a gas chromatograph (Model HP 5890, Hewlett Packard, USA), which was calibrated using pure ethylene gas. The gas chromatograph was equipped with Porapak-N (80-100 mesh) column and a flame ionization detector (FID) and the rate of ethylene evolution was expressed as μ l kg⁻¹ h⁻¹.

Total phenolics of the fruit extracts were determined by the mehod described by Singleton and Rossi (13) with some modifications, and expressed in mg of gallic acid equivalents (GAE)/100 g of extract. soluble solids content (SSC) of samples was estimated using Fisher hand refractometer (0-50).The results were expressed as degree Brix (ºBrix) at 20ºC. Titratable acidity in the plums was determined by titrating a known amount of fruit sample against 0.1 N NaOH using a few drops of 1% phenolphthalein solution as an indicator (Ranganna, 7). The experiments were laid out in factorial CRD design with each treatment consisting of 120 fruits in three replications. The data obtained were analysed by following standard procedures and the results were compared from ANOVA by calculating the CD (Panse and Sukhatme, 6).

RESULTS AND DISCUSSON

Physiological loss in weight is an important parameter, which is mainly responsible for quantitative as well as qualitative loss of produce. It is evident from the Fig. 1A that PLW irrespective of treatments increased with the advancement of the storage period, however, untreated fruits (control) showed significantly higher loss in weight (1.8%) from $4th$ day of storage, which gradually increased with the advancement of storage period. Shriveling was noticed on fruits, which have lost 5-7% or more moisture. However, fruits treated with SNP have significantly influenced to reduce the PLW during the entire storage period up to 36 days. Among the treatments, SNP (0.5 mM) gave better result in terms of minimum PLW followed by other concentrations of SNP. After 36 days of storage, minimum PLW (8.3%) was recorded in fruits treated with SNP 0.5 mM, followed by SNP 1 mM (8.7%), while the untreated plums showed the highest weight loss (13.8%) at the termination time of the experiment. Higher PLW in untreated fruits might be due to fact that these were metabolically more active than SNP treated fruits in respect to respiration and ethylene evolution and thus they deteriorated at a faster rate. The reduced PLW in SNP treated plums may also be influenced by consolidation of both cell integrity and permeability of tissues resulting in lower moisture loss (Barman *et al*., 1; Ku *et al*., 3). The tissue disruption allows the transference of water vapours from the fruit. Our results are in line with Barman *et al*. (1) and Ku *et al*. (3), who reported reduced weight loss in NO treated mango fruits.

Fruit firmness of 'Santa Rosa' plums during storage at 2ºC was significantly influenced by the SNP treatments (Fig. 1B). Fruit firmness showed a gradual decline with the progressive increase in storage period under all the treatments. The firmness loss was found to be significantly higher in untreated fruits than SNP treated fruits. Plums treated with SNP (0.5 & 0.25 mM) showed much slower reduction in fruit firmness than other treatments. Among the treatments, highest firmness (3.463 N) was retained with SNP (0.25 mM), while it was lowest (1.595 N) in control fruits. After

Nitric Oxide Influences on 'Santa Rosa' Plum during Cold Storage

Fig. 1. Effect of nitric oxide on PLW (%) (A), firmness (N) (B) and fruit rot (%) (C) of 'Santa Rosa' plums during cold storage at 2° C & 90 ± 5% R.H. Data are the mean ± S.E. of three replicate (n = 3). (NO1 = 0.25 mM; NO2 = 0.5 mM; NO3 = 1.0 mM; NO4 = 1.5 mM).

36 days of storage, SNP (0.25 mM) treated plums maintained \approx 117% higher fruit firmness than untreated fruits. The higher firmness in SNP treated fruits may be due to reduced enzyme activities and decreased rates of respiration and ethylene production. Earlier study on Japanese plum has also indicated the involvement of ethylene directly in increasing the activities of fruit softening enzymes activity such as pectin methyl esterase, polygalacturonase (Sharma *et al*., 10; Sharma *et al*., 11). Reduced softening in NO-fumigated fruit has also been observed in mango (Zaharah and Singh, 17), kiwifruit (Zhu *et al*., 19), litchi (Barman *et al*., 1), plum (Zhang *et al*., 18; Singh *et al*., 12) and peaches (Flores *et al*., 2) during post harvest storage.

Fruit rotting in 'Santa Rosa' plums during storage has been presented in the Fig. 1C. No symptoms of disease were observed in any of the treatments up to

5 days of storage. However, in untreated fruits, rotting started from $4th$ day of storage. Remarkably, SNP (0.25 and 0.5 mM) treated fruits showed zero rotting throughout the storage period, and it was delayed up to 20 days in SNP (1.0 and 1.5 mM) treated fruits. At the end of storage period, highest fruit rot (18%) was observed in untreated fruits, while it was lowest (0.0 %) in 0.25 and 0.5 mM SNP treated fruits. The lower fruit rot in SNP treated fruits may be because of induction of disease resistance and reducing effects on chilling injury by NO (Singh *et al*., 12).

SNP treatments were highly effective in reducing the rate of respiration (Fig. 2A). The rate of $CO₂$ production in untreated plums increased rapidly and a typical climacteric peak was observed after 12 days of storage. However, the SNP treatments suppressed the respiration rate and also delayed the onset of the

respiratory climacteric, where the peak was observed after 16 & 20 days of storage in 0.25 and 0.5 mM SNP treatments, respectively. Finally after 36 days of storage, highest respiration rate (50 ml CO₂ kg⁻¹ h^{-1}) was observed in untreated fruits, while it was minimum (30 ml $CO₂$ kg⁻¹ h⁻¹) in SNP (0.5 mM) treated fruits. Further, the impact of lower doses of SNP (0.5 & 1.0 mM) was found better over highest (1.5 mM) and lowest dose of SNP (0.25 mM). The reduction in respiration rate in SNP treated plums may be ascribed to delaying the ripening process. Delayed ripening in SNP treated fruits might be attributed to anti-senescence property of NO, which was liberated from SNP (Manjunatha *et al*., 5). Suppression of respiration rate during fruit ripening in NO fumigated peaches and plums has also been reported by Flores *et al*. (2) and Singh *et al*. (12), respectively.

Different SNP treatments significantly influence the ethylene production rates of 'Santa Rosa' plums during low temperature storage (Fig. 2B). Ethylene evolution increased rapidly in untreated fruits, which achieved a climacteric peak (56 μl kg -1 h -1) on 12th day of storage. Further, the fruits treated with SNP showed climacteric peak of ethylene on 16, 20, 16 & 12 days of storage, respectively in fruits treated with 0.25, 0.5, 1.0 & 1.5 mM SNP aqueous dip. The suppression of climacteric peak of ethylene with SNP (0.25, 0.5, 1.0 & 1.5 mM) treatments was found to be 39.0, 29.5, 33.5 and 47.5 μ I C₂H₄ kg⁻¹ h⁻¹, respectively. At the end of the storage, lowest ethylene evolution rate (6.5 μl C_2H_4 kg⁻¹ h⁻¹) was detected in SNP (1.5 mM) treated fruits, followed by other treatments of SNP (0.5 & 1.0 mM). Lower production of ethylene by SNP treated fruits may be due to formation of a ACC oxidase-NO complex, which decreases the ethylene production (Tierney *et al*., 14).

There was a gradual decrease in total phenolics content with the progress in storage period (Fig. 3A.). However, the decline was at a slower rate in 'Santa Rosa' fruits treated with SNP than untreated fruits. Maximum phenolics content (106 mg/ 100 g pulp) was recorded in 0.5 mM SNP treated fruits while it was lowest (65.3 mg/ 100 g pulp) in untreated plums. After 36 days of storage, SNP (0.25, 0.5 and 1 mM) treated fruits retained ≈ 35%, ≈47% and ≈62% higher phenolics content, respectively compared to control. The decrease in phenolic content might be attributed to lower activity of polyphenol oxidase (PPO) and higher activity of phenylalanine ammonia lyase (PAL) enzymes.

Sugar content of plums during storage increased in all the treatments. However, untreated fruits showed

Fig. 2. Effect of nitric oxide on respiration rate (A) and ethylene evolution rates (B) of 'Santa Rosa' plums during cold storage at 2° C & 90 ± 5% R.H. Data are the mean ± S.E. of three replicate determination (n = 3). (NO1 = 0.25 mM; NO2 = 0.5 mM; NO3 = 1.0 mM; NO4 = 1.5 mM).

rapid increase in SSC content with highest value on $16th$ day of storage (16.7 \textdegree B) followed by a decline with progressive increase in storage period (Fig. 3B.). While SNP (0.5 mM) treated fruits showed much slower increase in SSC content. At the end of the experiment, highest SSC content (15.3ºB) was recorded in SNP (0.5 mM) treated fruits while, it was lowest (14.0ºB) in 1.5 mM SNP treated fruits. Higher SSC in untreated plum fruits in beginning might be due to quicker ripening though decline in SSC content at later stages could be due to higher respiration rate (Sharma *et al*., 8; Sharma *et al*., 9) as also reported by Barman *et al*. (1) in litchi.

A decreasing trend in titratable acidity (TA) was observed in SNP treated as well as untreated plums with the increase in storage period. However, untreated fruits showed faster decrease in titratable acidity, while SNP treatments significantly delayed the decrease in titratable acidity during storage up to 36 days (Fig. 3C.). On 36th day of storage, highest TA (1.1%) was recorded in SNP (0.5 mM) treated 'Santa Rosa' plums, while it was lowest (0.8%) in control. Higher acidity in SNP treated plums may be because of slower ripening rate (Barman *et al*., 1).

This study revealed that postharvest aqueous dip treatment with sodium nitroprusside (SNP 0.25, 0.5, 1.0 and 1.5 mM) was effective in reducing PLW, decay rot and influenced respiration rate and ethylene production rate, which helped in improving shelf-life and quality of plums during storage at \approx 2°C. The SNP treatment is easy to adopt by farmers since it is simple as well as cheap. It will also help in reducing the postharvest losses, if included in the integrated postharvest chain of 'Santa Rosa' plums, thus increasing the availability of fruit for an extended period.

Fig. 3. Effect of nitric oxide on total phenol content (mg 100 g⁻¹ pulp) (A), SSC (°B) (B), titratable acidity (%) (C) of 'Santa Rosa' plum during cold storage at 2°C & 90 ± 5% R.H. Data are the mean ± S.E. of three replicate determination $(n = 3)$. (NO1 = 0.25 mM; NO2 = 0.5 mM; NO3 = 1.0 mM; NO4 = 1.5 mM)

REFERENCES

- 1. Barman, K., Siddiqui, W.M., Patel, V.B. and Prasad, M. 2014. Nitric oxide reduces pericarp browning and preserves bioactive antioxidants in litchi. *Scientia Hort*. **171**: 71-77.
- 2. Flores, F., Sanchez-Bel, P., Valdenegro, M., Romojaro, F., Martinez-Madrid, M. and Egea, M. 2008. Effects of a pretreatment with nitric oxide on peach (*Prunus persica* L.) storage at room temperature. *European Food Res. Tech*. **227**: 1599-1611.
- 3. Ku, V.V.V., Wills, R.B.H. and Leshem, Y.Y. 2000. Use of nitric oxide to reduce postharvest water loss from horticultural produce. *J. Hort. Sci. Biotech*. **75**: 268-70.
- 4. Leshem, Y.Y., Wills, R.B.H. and Ku, V.V.V. 1998. Evidence for the function of the free radical gas nitric oxide (NO) as an endogenous maturation and senescence regulating factor in higher plants. *Plant Physiol. Biochem*. **36**: 825-33.
- 5. Manjunatha, G., Lokesh, V. and Neelwarne, B. 2010. Nitric oxide in fruit ripening: trends and opportunities. *Biotech. Adv*. **28**: 489-99.
- 6. Panse, V.G. and Sukhatme, P.V. 1984. *Statistical Methods for Agricultural Workers* (3rd edn.), Indian Council of Agricultural Research, New Delhi.
- 7. Ranganna, S. 1999. *Handbook of Analysis and Quality Control for Fruits and Vegetable Products* (3rd edn), Tata McGraw-Hill Publishing Company Ltd., New Delhi, India.
- 8. Sharma, S., Sharma, R.R. and Pal, R.K. 2012a. Effect of ethylene absorbents on compression injury and quality of 'Santa Rosa' Japanese plum (*Prunus salicina*) during transportation. *Indian J. Agri. Sci.* **82**: 223-26.
- 9. Sharma, S., Sharma, R.R., Pal, R.K. and Singh, S.K. 2012b. Influence of 1-MCP on compression injury of Japanese plums during transportation. *Indian J. Hort*. **69**: 101-06.
- 10. Sharma, S., Sharma, R.R., Pal, R.K., Jhalegar, J., Singh, J., Srivastav, M. and Dhiman, M.R. 2012c. Ethylene absorbents influence fruit firmness and activity of enzymes involved in fruit softening

of Japanese plum (*Prunus salicina* Lindell) cv. 'Santa Rosa'. *Fruits*, **67**: 257-66.

- 11. Sharma, S., Sharma, R.R., Pal, R.K., Paul, V. and Dahuja, A. 2012d. 1-Methylcyclopropene influences biochemical attributes and fruit softening enzymes of 'Santa Rosa' Japanese plum (*Prunus salicina* Lindl.). *J. Plant Biochem. Biotech*. **21**: 295-99.
- 12. Singh, S.P., Singh, Z. and Swinny, E.E. 2009. Post harvest nitric oxide fumigation delays fruit ripening and alleviates chilling injury during cold storage of Japanese plums (*Prunus salicina* Lindell). *Postharvest Biol. Tech*. **53**: 101-8.
- 13. Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. *American J. Enol. Vitic*. **16**: 144-58.
- 14. Tierney, D.L., Rocklin, A.M., Lipscomb, J.D., Que, J.L. and Hoffman, B.M. 2005. ENDOR studies of the ligation and structure of the non-heme iron site in ACC oxidase. *J. American Chem. Soc*. **127**: 7005-13.
- 15. Wendehenne, D., Durner, J. and Klessig, D.F. 2001. Nitric oxide: a new player in plant signaling and defense responses. *Curr. Opinion Plant Biol*. **7**: 449-55.
- 16. Wills, R.B.H., Ku, V.V.V. and Leshem, Y.Y. 2000. Fumigation with nitric oxide to extend the postharvest life of strawberries. *Postharvest Biol. Tech*. **18**: 75-9.
- 17. Zaharah, S.S. and Singh, Z. 2011. Postharvest nitric oxide fumigation alleviates chilling injury, delays fruit ripening and maintains quality in cold stored 'Kensington Pride' mango. *Postharvest Biol. Tech*. **60**: 202-10.
- 18. Zhang, D.D., Cheng, G.P., Li, J., Yi, C., Yang, E., Qu, H.X., Jiang, Y.M. and Duan, X.W. 2008. Effect of nitric oxide on disorder development and quality maintenance of plum fruit stored at low temperature. *Acta Hort*. **804**: 549-54.
- 19. Zhu, S.H., Sun, L. and Zhou, J. 2010. Effects of different nitric oxide application on quality of kiwifruit during 20ºC storage. *Intl. J. Fd. Sci. Tech*. **45**: 245-51.

Received : November, 2013; Revised : May, 2015; Accepted : August, 2015