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ORIGINAL ARTICLE

Postharvest nitric oxide treatment of persimmon (*Diospyros kaki* l.) improves fruit quality during storage

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Abstract – Introduction. The effects of nitric oxide (NO) on postharvest ripening of persimmon were investigated. **Materials and methods.** Fruit were dipped for 30 min in 1.0 and 1.5 mM sodium nitroprusside (SNP), a nitric oxide donor, and stored at 1 °C and 90% relative humidity for 56 days. Changes in total antioxidant activity, total phenol compounds, color, firmness, soluble tannins and weight loss were evaluated. **Results and discussion.** The results showed that fruit ripening was significantly delayed by SNP. Application of 1.0 and 1.5 mM SNP delayed weight loss and retained greater total antioxidant activity, total phenolic compounds and firmness compared to the control treatments. No significant differences were observed between the two concentrations of SNP. **Conclusion.** These results demonstrated that postharvest NO application has potential to delay ripening and maintain quality of harvested persimmon fruit.

Keywords: Iran / *Diospyros kaki* / phenolic compounds / antioxidant activity / refrigeration / postharvest management

Résumé – Le traitement post-récolte du kaki (*Diospyros kaki* l.) à l'oxyde nitrique améliore la qualité des fruits au cours du stockage. Introduction. Les effets de l'oxyde nitrique (NO) sur la maturation post-récolte du kaki ont été étudiés. **Matériel et méthodes.** Les fruits ont été plongés pendant 30 minutes dans une solution à 1,0 ou 1,5 mM de nitroprussiate de sodium (SNP), un donneur d'oxyde nitrique, puis stockés à 1 °C et 90% d'humidité relative pendant 56 jours. Les changements observés dans l'activité anti-oxydante totale, les composés phénoliques totaux, la couleur, la fermeté, les tanins solubles et la perte de poids ont été étudiés. **Résultats et discussion.** La maturation des fruits a été considérablement retardée par les traitement au SNP. Les solutions de 1,0 ou 1,5 mM SNP ont retardé la perte de poids des fruits, ont maintenu leur fermeté et leur ont conservé une plus grande activité anti-oxydante et une plus forte teneur en composés phénoliques totaux, par rapport au contrôle. Aucune différence significative n'a été observée entre les deux concentrations de SNP. **Conclusion.** Ces résultats ont démontré que l'application post-récolte de NO pourrait être une méthode pratique permettant de retarder la maturation et de maintenir la qualité post-récolte des fruits du kaki.

Mots clés : Iran / *Diospyros kaki* / composés phénoliques / réfrigération / technologie post-récolte / activité / anti-oxydante

1 Introduction

Persimmon (*Diospyros kaki* L.), which belongs to the Ebenaceae family, is cultivated in a wide area including Eastern Asia, Spain and Israel. In addition to its fresh market value, persimmon has been traditionally used for many medicinal purposes such as reducing blood pressure, coughs, as a diuretic and is touted to reduce degenerative diseases [1].

Control of ripening is very important for the development of the persimmon industry. The delicate nature of fruit, poor handling and inadequate transportation and storage all limit its marketing. Persimmon is a climacteric fruit where ripening is regulated by ethylene. Therefore, inhibiting ethylene biosynthesis or action may play an important role in slowing down the ripening process and prolonging storage life [2]. Considering these facts, one strategy to delay the senescence of persimmon after harvest could be through pretreatments with an antagonist of ethylene biosynthesis or action.

Nitric oxide (NO) is a relatively stable free radical gas. It can be a signaling molecule in plants that mediates various pathophysiological and developmental processes, including expression of defense-related genes and programmed cell death, stomatal closure, seed generation and root development [3, 4]. Additional evidence indicates that NO may have anti-senescence and ripening properties [5]. For example, Leshem and Wills [6] found that exogenous NO could considerably prolong the shelf life of some leaf vegetables, flowers and fruits by inhibiting the emission of ethylene, implying that NO may have important roles in regulating aging processes.

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Leshem and Haramaty [7] found that application of a NO donor to pea leaves under senescence-promoting conditions inhibited ethylene production. In recent years, some research reports have shown that NO at low concentrations can effectively extend the postharvest life of various fruits, such as strawberry [8], peach [9], longan [10, 11], plum [12] and winter jujube [13], and a number of other horticultural crops [14, 15].

Our studies were carried out to observe the effects of SNP applied through fruit immersion during the entire period of cold storage [16–18]. We determined its effects on fruit softening and other functional properties and quality attributes during storage at 1 °C over two months. No previous research work has been reported on the effects of NO on fruit ripening and storage life of persimmon.

2 Materials and methods

2.1 Plant material

'Shiraz' persimmon at the commercial maturity stage (orange color and rigid) were harvested from a commercial orchard in Shiraz, Iran (Fars province 29°06′N longitude, 54°01′E latitude, 1,860 m above sea level). Fruit were selected according to their maturity and color with the aim of having higher homogeneity within the samples to reduce the variability of the results. Fruit without any physical injuries or decay were washed in a sodium hypochlorite solution at 0.5% (v/v) for 2 min, then rinsed with tap water and air-dried prior to use.

2.2 Treatment with SNP

Sodium nitroprusside (SNP) was purchased from Sigma–Aldrich. Fruit were immersed in 1.0 mM and 1.5 mM SNP aqueous solution, or water as a control, for 30 min, and dried in air at 25 °C for 2 h. Fruit were put into closed plastic trays and stored at 1 °C and 90 ± 5% relative humidity (RH) for up to 56 days. Fruit samples were taken after 0, 14, 28, 42 and 56 days for quality evaluation and further analyses. The experiment was designed in a completely randomized factorial arrangement with 12 fruits per replicate and three replicates for each treatment.

2.3 Physical and physicochemical assays

2.3.1 ABTS radical scavenging activity

Each sample (0.1 mL), potassium phosphate buffer (0.1 mL, 0.1 M, pH 5.0), and hydrogen peroxide (20 mL, 10 mM) were mixed and pre-incubated at 37 °C for 5 min [19]. After pre-incubation, ABTS (2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) (30 mL, 1.25 mM, in 0.05 M phosphate-citrate buffer, pH 5.0) and peroxidase (30 mL, 1 unit mL^{-1}) were added to the mixture and then it was incubated at 37 °C for 10 min. The absorbance level was obtained with a microplate reader (Bio-Tek ELx808, USA) at 405 nm.

2.3.2 Total phenolic contents (TPC)

To measuring TPC, 5 mg of dried samples was extracted with 5 mL of methanol. Sample were centrifuged at 3500 rpm for 10 min. TPC of each extract was determined according to the method of Gutfinger [20]. Each extract (1 mL) at 1 mg mL^{-1} was mixed with 1 mL of 2% Na_2CO_3. After standing for 3 min, 0.2 mL of 50% Folin-Ciocalteu reagent was added to the mixture for 30 min and centrifuged at 13,400 × *g* for 5 min. The absorbance was read at 750 nm and TPC was expressed as gallic acid equivalent (GAE).

2.3.3 Soluble tannins

Soluble tannins were measured using the Folin-Denis method as described by Arnal and Del Río [21]. This colorimetric method is based on the reduction of Folin-Denis reagent by soluble tannins in alkaline solution. The calibration curve was made with gallic acid. Soluble tannins were assayed four times from frozen replicates. Five grams of the fruit sample was placed directly into a solution of 25 mL of 80% methanol, and was homogenized. Thereafter, samples were filtered and centrifuged at 14,000 rpm for 20 min at 4 °C and the supernatant was reserved. More supernatant was extracted from the precipitant with 80% methanol and added to the first supernatant. The total supernatant was brought to 100 mL with distilled water. One mL of this sample solution and 6 mL of distilled water were mixed and vortexed. Thereafter, 0.5 mL of 1N phenol reagent (Folin Ciocalteu reagent) was added. After 3 min, 1mL of saturated Na_2CO_3 was added, vortexed, and 1.5 mL of distilled water was added. Absorbance was measured after 1 h with a WVP spectrophotometer (WVP, UK) at 725 nm. Soluble tannins were expressed as GAE (mg GAE 100 g^{-1}).

2.3.4 Weight loss

The percentage of weight loss was determined according to the following equation:

$$\%WL(t) = \frac{W_0 - W_t}{W_0} \times 100$$

where W_0 is the initial weight and W_t is the weight of fruit after various storage times.

2.3.5 Color

Color was determined using digital imaging [22] performed in a photograph chamber. Angle light with the horizontal surface of the images was 45°. After transferring the images to a computer, Photoshop image processing software was employed. Following the recording of individual L^*, a^* and b^* parameters. L^* is lightness, a^* and b^* are chromaticity coordinates. The a^* and b^* values were converted to chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and *hue* angle ($h^\circ = \tan^{-1}(b*/a*)$).

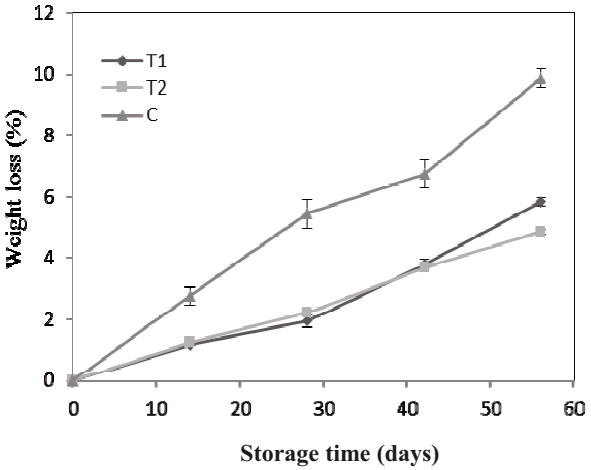

Figure 1. Effects of 1.0 mM (T1) and 1.5 mM (T2) nitric oxide (SNP) on weight loss of persimmon fruit during 56 days storage at 1 °C. LSD for treatments, storage time and interaction of treatments, storage time are 0.09, 0.12 and 0.2 at level of 5% of significance, respectively. Vertical bars represent standard deviations of the means.

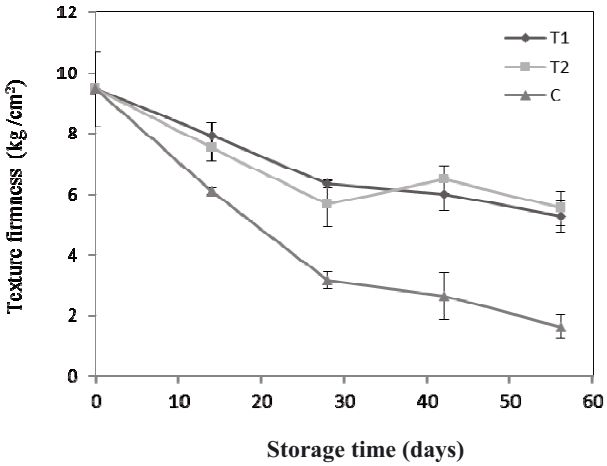

Figure 2. Effects of 1.0 mM (T1) and 1.5 mM (T2) nitric oxide (SNP) on texture firmness of persimmon fruit during 56 days storage at 1 °C. LSD for treatments, storage time and interaction of treatments, storage time are 0.51, 0.67 and 1.16 at level of 5% of significance, respectively. Vertical bars represent standard deviations of the means.

2.3.6 Texture analysis

Texture analysis was performed using a texture analyzer (StevensLfra, UK). Flesh firmness was measured as the maximum penetration force (kg cm^{-2}) reached during tissue breakage and determined with an 8 mm diameter cylindrical probe. The penetration depth was 3 mm and the cross-head speed was 1 mm s^{-1}. Ten fruits for each treatment were measured.

2.4 Statistical analysis

Data were subjected to analysis of variance (GLM). Sources of variation were treatment, time of storage and the interaction of treatment × storage time. Following normalization, data were smoothed to improve the validity of statistical inferences and to reduce inter-individual variation. Mean comparisons were performed using the Duncan's test to examine if differences between treatments and storage time were significant at $P \leqslant 0.05$. P values were calculated according to Student's t test analysis. All analyses were performed with SAS software package v. 9.1 for Windows. Correlation coefficient was obtained using simple regression analysis (Excel software).

3 Results

3.1 Weight loss

Figure 1 shows weight loss during storage of persimmon fruit treated with 1.0 and 1.5 mM SNP as compared with the non-treated fruit. All samples demonstrated a gradual loss of weight during storage. During storage, the weight loss of non-treated fruit was significantly greater than fruit treated with SNP. SNP treatment significantly decreased weight loss but there was no significant difference between SNP concentrations ($P \leqslant 0.05$). The control fruit showed an increase in weight loss reaching up to 9.85% after 56 days, whereas the weight losses of samples treated with 1.0 and 1.5 mM SNP were 5.82% and 4.83%, respectively.

3.2 Texture analysis

SNP treatment at both concentrations resulted in greater fruit firmness during storage (*figure 2*). Firmness of non-treated fruit decreased rapidly during ripening. After 14 days of storage, the firmness of control fruit was less than 0.6 kg cm^2, while it was more than 0.7 kg cm^{-2} in the SNP-treated fruit. The firmness of SNP-treated fruit dropped to less than 0.6 kg cm^{-2} after 42 days of storage. No significant differences were observed between the two SNP levels.

3.3 Soluble tannins

Immediately after harvest fruit showed a high level of soluble tannins (ST). Soluble tannins concentrations in persimmon fruit treated with SNP decreased similar to that of the untreated fruits (*figure 3*), but the rate of soluble tannins loss was lower in fruit treated with SNP. No differences were observed between SNP concentrations.

3.4 Total phenolic compounds (TPC)

Control fruit exhibited a significant reduction in TPC during cold storage from the initial values (50.3 mg GAE 100 g^{-1}). A sharp decrease occurred during the first 14 days of storage (35.3 mg GAE 100 g^{-1}), and continued slowly thereafter. On the contrary, TPC in SNP-treated persimmons remained significantly unchanged during 14 days at 1°C, with a slight reduction observed at day 56 (*figure 4*) The TPC of fruit treated with SNP decreased more slowly than the control fruits.

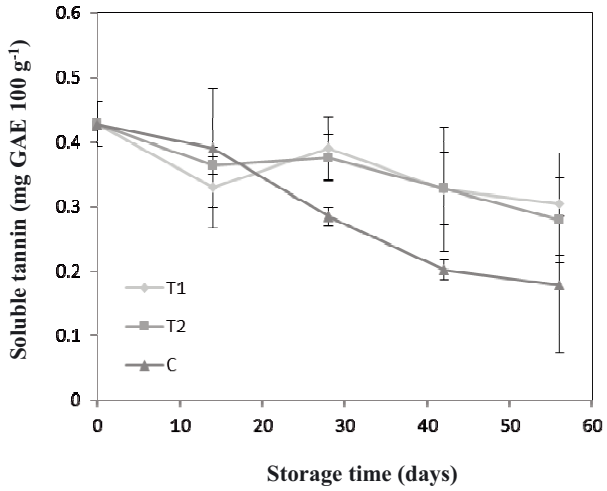

Figure 3. Effects of 1.0 mM (T1) and 1.5 mM (T2) nitric oxide (SNP) on soluble tannins of persimmon fruit during 56 days storage at 1 °C. LSD for treatments, storage time and interaction of treatments, storage time are 0.04, 0.6 and 0.1 at level of 5% of significance, respectively. Vertical bars represent standard deviations of the means.

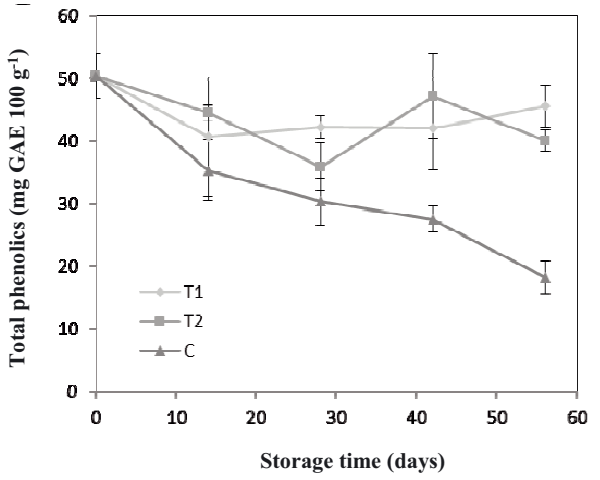

Figure 4. Effects of 1.0 mM (T1) and 1.5 mM (T2) nitric oxide (SNP) on total phenol compounds in persimmon fruit during 56 days storage at 1 °C. LSD for treatments, storage time and interaction of treatments, storage time are 3.2, 4.24 and 7.3 at level of 5% of significance, respectively. Vertical bars represent standard deviations of the means.

3.5 Total antioxidant activity (TAA)

During cold storage, a slight reduction in TAA was detected at day 56 in treated fruit (*figure 5*) SNP treatments significantly delayed TAA, but this was similar after 56 d with only minor differences between the 1.0 and 1.5 mM SNP treatments. During storage at 1 °C and subsequent ripening, all treated fruit exhibited significantly ($P \leqslant 0.05$) higher TAA than the control fruit (*figure 5*). The positive effects of SNP on the retention of TAA during storage declined significantly in week 2, but fruit treated with SNP had higher TAA than the non-treated fruit during storage.

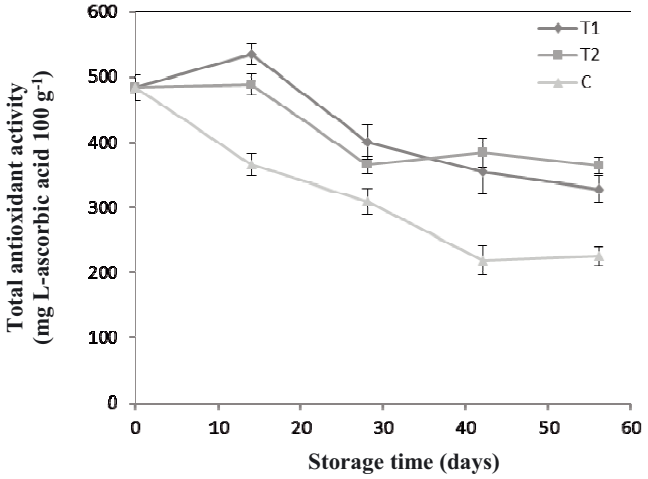

Figure 5. Effects of 1.0 mM (T1) and 1.5 mM (T2) nitric oxide (SNP) on TAA in persimmon fruit during 56 days storage at 1 °C. LSD for treatments, storage time and interaction of treatments, storage time are 14.87, 19.19 and 33.25 at level of 5% of significance, respectively. Vertical bars represent standard deviations of the means.

Table I. Effects of nitric oxide (SNP treatment) on chromatic parameters of persimmon fruit after 14, 28, 42 and 56 days storage at 1 °C.

Days at 1 °C	Treatment	Chromatic parameters		
		Hue	Chroma	*L**
0		58.5	71.0	51.8
14	Control	44.3	76.1	47.4
	SNP (1 Mm)	45.9	71.9	49.1
	SNP (1.5 Mm)	45.3	73.6	48.0
28	Control	41.8	73.7	43.7
	SNP (1 Mm)	47.7	76.3	47.6
	SNP (1.5 Mm)	44.6	76.4	46.1
42	Control	40.8	70.8	42.5
	SNP (1 Mm)	45.2	72.4	47.8
	SNP (1.5 Mm)	45.8	72.5	48.1
56	Control	40.4	69	42.4
	SNP (1 Mm)	45.3	72	46.7
	SNP (1.5 Mm)	47.3	70	47.9
Time		$(1.14)^c$	NS	$(1.15)^c$
Treatment		$(0.88)^b$	$(1.65)^c$	$(0.89)^b$
Time× Treatment		$(1.97)^c$	NS	$(2)^c$

NS: not significant. LSD values are in brackets. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$.

3.6 Color

Hue angle values were higher in fruit treated with 1.0 and 1.5 mM SNP than the control fruit after 14, 28, 42 and 56 days storage. The highest degree in color set off was seen at SNP treated fruit 1.0 (45.39) and 1.5 mM (47.38) while the lowest degree occurred for control fruit (40.41) (*table I*). Objective evaluation showed that the control fruit lost luminosity more rapidly (decrease in *L**), while significant differences

in comparison with the other treatments were found to occur from 14 days storage onwards. This parameter remained constant throughout storage time when fruits were treated with SNP (*table I*). Chroma values showed a slight decrease for all treatments (*table I*). This pattern is characteristic of more advanced stages of ripening or in other words orange-red color in contrast to yellow-orange, which is indicative of less ripe fruit.

4 Discussion

Some evidence has shown an antagonistic effect between NO and ethylene [6, 23], while ethylene plays an important role in regulating ripening and senescence of fruit [24]. Application of exogenous NO has been found to delay ripening and senescence of some horticultural crops by inhibiting ethylene production [5]. For example, NO extended the postharvest life of strawberry fruit [25], pear [26], and mango [27]. In this study, SNP treatment markedly extended the postharvest life of persimmon fruit, and maintained high levels of TPC and ST (*figures 3, 4*), indicating that NO treatment delayed the senescence of persimmon fruit during storage. The difference between treated and control fruit was highly significant. Bibi *et al.* [28] reported that some astringent fruit show reduction in tannins during ripening due to decrease in extractability/ polymerization accompanied by loss in fluidity and decrease in astringency. The TPC of fruit treated with SNP decreased more slowly than the control fruit. At the late storage duration, the treatment with SNP might have inhibited activities of polyphenol oxidase (PPO) and peroxidase (POD) that catalyze the oxidation of phenolic compounds associated with high TPC [10].

SNP-treated fruit had lower weight loss compared to the control fruit. Fruit weight loss is mainly associated with respiration and transpiration [29]. Not only respiration and transpiration, but also the suppression of ethylene production has a close relationship with the suppression of softening by SNP.

SNP treatment retarded fruit softening during storage This delay of softening probably resulted from the retardation of senescence processes due to the inhibition of the respiration rate caused by SNP. It is known that fruit softening is accelerated by ethylene in persimmon [30].

TAA has been correlated to phenolic content in nectarine, peach, and plum [31] and in small fruits such as blackberry, raspberry, and strawberry [32]. Some of these polyphenols are natural antioxidants in foods [33, 34].

In this study it was observed that NO could effectively retard color changes (reddening) associated with ripening, thus extending the postharvest life of persimmon (*table I*). The reason for the lowest decrease in *hue* angle values in control fruit may be the fact that SNP inhibits surface color change Similar results were also reported in analogous experiment performed on tomato fruit [35] It may be said that brightness loss is generally caused by physiological deteriorations, especially peel browning. Thus, the reason for the lowest decrease in chromaticity value of treated fruit is the fact that SNP inhibits color change. Lai *et al.* [35] observed that NO could effectively retard pericarp color reddening and suppress ethylene production in tomato fruit at the mature green stage.

5 Conclusion

As a whole, this study showed positive effects of postharvest application of sodium nitroprusside (SNP) to delay ripening of persimmon at 1 °C. Based on the results obtained SNP is suggested to be used for maintaining favorable quality and extending storability and postharvest life of persimmon fruit. Further research is needed to explore the mechanism of action of SNP in delaying persimmon ripening.

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