

Effect of Nitric Oxide and Film Wrapping on Quality Maintenance and Alleviation of Chilling Injury on Pomegranate Fruit

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ABSTRACT

The aim of this study was to assess the effectiveness of the individual application of nitric oxide or cellophane wrapping, and combination effects of these treatments on reducing chilling injury and quality improvement of pomegranate fruit cv. 'Shishe-Kab' during storage. Fruits were dipped in nitric oxide (0 or 300 μM) solution for 2 minutes, followed by cellophane wraps (wrapped or unwrapped) as treatments and then stored at two different storage temperatures (1 or 5°C) for 90 days. Application of 300 μM nitric oxide significantly increased the antioxidant activity, total anthocyanin content, and the a^* value of aril color, and also led to the lowest chilling injury and electrolyte leakage in fruit compared with the control. The lowest weight loss, chilling injury, and total soluble solids and the highest total anthocyanin content and the a^* value of aril color was observed in cellophane wrapped fruits, compared with unwrapped fruits. The combination of nitric oxide and cellophane wrapping had a greater effect on reducing the electrolyte leakage of fruits as it decreased about 72 and 63% compared to the control in stored fruit for 45 and 90 days, respectively.

Keywords: Anthocyanin, Antioxidant, Electrolytes leakage, *Punica granatum*, Sodium nitroprusside.

INTRODUCTION

Pomegranate (*Punica granatum*) arils were demonstrated to be high in antioxidant activity, vitamins, sugars, acids, polysaccharides, polyphenols, and some important minerals (Gil *et al.*, 2000) that are reported having various medicinal properties and health benefits. However, improper postharvest management and subsequent deterioration are a serious concern in pomegranate industry.

Storage of pomegranate fruit at lower than 5 °C temperature resulted in chilling injury with symptoms of brown discoloration of the skin, internal discoloration, aril browning, and susceptibility to decay organisms, which reduce consumer acceptance and market life of fruits as reported for different cultivars (Caleb *et al.*,

2012; Fawole and Opara, 2013). 'Shishe-Kab' pomegranate, which has a high quality fruit with excellent color and flavor, is the main commercial cultivar in South Khorasan province, Iran. However, its postharvest life is short with a maximum 10 weeks at cold room (5°C), primarily because of water loss and chilling injury as reported in our previous study (Moradinezhad *et al.*, 2013) and also significant losses in product quantity and quality through physical damage (poor postharvest handling) and fungal pathogens, particularly on damaged tissues (Moradinezhad and Khayyat, 2014; Teksur, 2015).

Packing and packaging methods can greatly influence air flow rates around the commodity, thereby affecting temperature and relative humidity management of the produce while in storage or in transit (Kader

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and Rolle, 2004). Sealed polyethylene films are reported to retain the grapefruit weight and quality better than wax paper (Purvis, 1985). In addition, Ladaniya and Singh (1999) indicated that polyethylene uni-packing dramatically decreases weight loss of mandarin fruits as compared to unwrapped fruits. Previous research on Modified Atmosphere (MA) has also demonstrated the beneficial effect of plastic films in improving storability of pomegranates. Sealing 'Mollar de Elche' pomegranates in either perforated or non-perforated polypropylene bags highly permeable to gases prevented the fruit from transpiration and any physiological disorders both during cold storage at 2 or 5°C (Artés *et al.*, 2000). D'Aquino *et al.* (2010) also reported that individual film wrapping of 'Primosole' pomegranates almost completely inhibited weight loss and husk scald and preserved fruit freshness after 12 weeks of cold storage at 8°C.

Nitric Oxide (NO) is a small, lipophilic, and bioactive gas molecule that plays an important role in different physiological processes (Lamotte *et al.*, 2005). It has been reported that NO could delay the senescence of fruits by inhibiting ethylene biosynthesis (Zhu *et al.*, 2006). Several reports have shown that NO can effectively extend postharvest life of various fruits, such as strawberry (Soegiarto and Wills, 2006), peach (Zhu *et al.*, 2006) and plum (Singh *et al.*, 2009). Recently, we compared the effect of NO postharvest application at different concentrations on the quality of pomegranate fruit (Ranjbari *et al.*, 2017) and reported the benefit of NO postharvest treatment on pomegranate. However, no study has been conducted yet on the combined effect of pre-storage nitric oxide application with film wrapping on pomegranate fruit held at different storage temperatures, particularly for 'Shishe-Kab' cultivar. Therefore, the aims of this study were to determine the influence of nitric oxide pre-storage treatment and cellophane wrapping on quality attributes and chemical changes as well as the chilling resistance of

pomegranate fruit held at cold and chilling storage temperatures (5 and 1°C).

MATERIALS AND METHODS

Preparing the Plant Material

Pomegranate fruits (cv. 'Shishe-Kab') were harvested from a commercial orchard in Ferdows, South Khorasan province, Iran, in the 2015 growing season. Fruits were harvested at full maturity stage early in November, and immediately transported to the Postharvest Laboratory at University of Birjand, Birjand. Diseased, sunburn, bruised and injured fruits were discarded. About 640 fruits were then selected for uniformity in size (300-350 g), shape and color, and prepared for the following treatments in four replications. Ten fruits were used for each replication; therefore, 320 fruits for each sampling date have been used.

Treatments and Storage Conditions

Fruits were washed with distilled water and dipped in Tiabendazole 1% solution for 1 minute prior to treatments. The donor of nitric oxide, sodium nitroprusside (SNP), was purchased from Merck Company and was dissolved in distilled water. Half of the fruits at the ambient temperature were dipped in 300 µM of SNP solution for 2 minutes and then allowed to air-dry. The remaining fruits were dipped in distilled water for 2 minutes. Thereafter, half of the treated fruits from each treatment were wrapped with common cellophane (0.012 mm thickness) individually, while the rest of fruits remained unwrapped. Both treated and control fruits were then placed in open plastic crates and stored at 1 or 5±0.5°C and 85±5% RH. Physical and biochemical properties and electrolytes leakage of samples were assessed after 45 and 90 days of storage.

Physicochemical Properties

To determine weight loss, the individual fruit was weighted at harvest time (day 0) and after each storage period (at days 45 and 90). The weight loss was calculated as follows [Equation (1)]:

$$WL (\%) = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

Where, WL = Weight Loss (%) of fruit; W_1 = Initial Weight (g) of the fruit at the beginning of storage, and W_2 = Final Weight (g) of the fruit at the end of storage period. Weight loss was calculated for each storage temperature. The chilling injury occurrence and its intensity symptoms were recorded on a 4-point hedonic scale based on the percentage of husk surface affected by CI symptoms (dehydration, browning, and pitting): 1= (No CI symptoms), 2= (1–25% of surface damaged), 3= (26–50% of surface damaged), and 4= (> 51% of the surface damaged) (Moradinezhad and Khayyat, 2014). Titratable acidity was determined by titration of 2 mL of juice with 0.1M NaOH to an end point of pH 8.2 which was recorded with pH meter and results were calculated as a percentage of citric acid. The pH was measured at room temperature using a pH meter. Total soluble solids were determined with a hand-held refractometer (RF 10, °Brix 0-32, Extech Co., USA) at 25°C, and expressed as percentage.

The DPPH method was used to measure the antioxidant activities of pomegranate juices based on the evaluation of the free radical scavenging capacities of the juices (Blois, 1958). The absorbance was measured at 517 nm using spectrophotometer (Unico 2100, China). Antioxidant activity was expressed as the percentage decline in absorbance relative to the control, corresponding to the percentage of DPPH scavenged, which was calculated as Equation (2):

$$DPPHHsc (\%) = 1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (2)$$

Where, A is absorbance.

Total anthocyanin content was determined spectrophotometrically by the pH differential method (Lako *et al.*, 2007). Absorbance was measured at 510 and 700 nm in buffers at pH 1.0 and 4.5, using a spectrophotometer, and then calculated according to the following equation [Equation (3)]:

$$A = \frac{[(A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5}]}{\epsilon} \quad (3)$$

Results were expressed as mg of cyanidin-3-glucoside per 100 mL of juice, using a molar absorptive coefficient (ϵ) of 26,900 and a molecular weight of 449.2, and then total anthocyanin content was calculated as follows (Eq. 4):

$$\text{Total anthocyanin (mg/l)} = \frac{A \times MW \times DF \times 1000}{\epsilon} \quad (4)$$

Where, A = Absorbance; MW = Molecular Weight of cyanidin-3-glucoside; DF = The Degree of Dilution; ϵ = Molar absorptive coefficient.

Ion leakage of fruit husk (disks of rind) was measured according to the method by Li *et al.* (2014) with some modifications. Disks (2.5 cm diameter) of fruit skin per replication/treatment were taken from fruits and placed in 20 mL of deionized water at ambient temperature for 24 hours and electrical Conductivity was measured with an electrical conductivity meter (C1). The same disks were kept in a boiling water bath (100°C) for one hour to release all electrolytes, cooled at the ambient temperature, and again Conductivity was recorded (C2). The Electrolyte Leakage (EL) was expressed in percentage and calculated using Eq. 5 (Beckerson *et al.*, 1980):

$$EL (\%) = \frac{C_1}{C_2} \times 100 \quad (5)$$

Aril color of pomegranate fruits was measured by using colormeter (TES-135A, Taiwan) and recorded as L^* (lightness), a^* (-greenness to +redness), and b^* (-blueness to +yellowness).

The experiment was conducted as a factorial in a completely randomized design with eight treatments and four replications, using the SAS program version 6.1 to



analyze the data. The factors were nitric oxide (0 or 300 μM), cellophane (wrapped or unwrapped) and storage temperatures (1 or 5°C). Data were analyzed using GLM (Generalized linear model) procedure and means were compared using LSD at $P < 0.05$.

RESULTS AND DISCUSSION

Results showed that the interaction effects of treatments had no significant differences in pomegranate fruit parameters, except the electrolyte leakage of fruits peel.

Fruit Weight Loss

Results indicated that Weight Loss (WL) was not affected by nitric oxide treatment (Table 1). Wrapping significantly decreased WL in both storage time and the lowest (1.97%) WL was observed in wrapped fruits with cellophane at day 45 of storage (Table 1), which was in agreement with findings of Rub *et al.* (2010) in sweet orange when cellophane and uni-packing were used. They stated that loss of moisture leads to reduced visual quality and physiological

dysfunctions in orange fruit. One way to reduce evaporation through the skin is film coatings (Hernandez-Munoz *et al.*, 2008) including cellophane, polyethylene, and polyethylene green, which are impermeable to water and raise the humidity around the commodity and decrease moisture loss. Storage temperature affected fruit WL, which significantly decreased in fruit stored at 1 compared to 5°C, and the lowest WL was obtained at day 45 of storage (Table 1). Similarly, it was reported by Cohen *et al.* (1994) in citrus, lemon, and grapefruit. The reducing weight loss (water loss) in fruits stored at a lower temperature is probably due to the lower transpiration rate by the fruit as well as the prevailing higher relative humidity at lower temperatures (Fawole *et al.*, 2013). According to Paull and Chen (1989), loss of weight and development of symptoms resulting from water loss, i.e. loss of glossy appearance, softness, shriveling, and dryness of the peel, in papaya fruits are greatly influenced by the relative humidity and temperature of the storage area (Nunes *et al.*, 2006) which was in agreement with the present results. High storage temperature leads to accelerated water loss and, subsequently, to shriveling and softening of the fruit (Emond *et al.*, 2005). This might

Table 1. Main effects of pre-storage nitric oxide treatment, film wrapping and storage temperature on Weight Loss (WL), Chilling Injury (CI) and Electrolyte Leakage (EL) of 'Shishe-Kab' pomegranate at days 45 and 90 of storage.^a

Parameter		WL (%)		CI index		EL (%)	
Storage time (Days)		45	90	45	90	45	90
Treatment							
NO (μM)	0	3.30 ^a	8.07 ^a	0.75 ^a	2.25 ^a	61.57 ^a	77.62 ^a
	300	2.67 ^a	8.26 ^a	0.12 ^b	1.37 ^b	54.84 ^b	70.45 ^b
Film wrapping	Unwrapped	4.01 ^a	10.6 ^a	0.62 ^a	2.25 ^a	59.93 ^a	77.91 ^a
	Wrapped	1.97 ^b	5.71 ^b	0.25 ^a	1.37 ^b	56.48 ^a	70.16 ^b
Storage temperature (°C)	1	1.90 ^b	5.72 ^b	0.56 ^a	2.12 ^a	60.68 ^a	81.09 ^a
	5	4.08 ^a	10.61 ^a	0.31 ^a	1.50 ^b	55.72 ^b	66.98 ^b

^a Mean values in each column, for each treatment in evaluated parameter, followed by the same letter are not significantly different by the LSD ($P < 0.05$, $n = 15$).

associate with a faster metabolism and ripen at a higher temperature, increased cell wall degradation, and higher membrane permeability leading to exposure of cell water to easy evaporation (Lee *et al.*, 1995).

Chilling Injury Index

Data showed that application of nitric oxide significantly decreased the incidence of chilling injury in pomegranate fruits compared to the control during storage periods, as the highest amount of chilling injury index was obtained in untreated fruits after 45 days of storage (Table 1). The NO was also effective in alleviating chilling injury symptoms or improving chilling tolerance in Japanese plum (Singh *et al.*, 2009) and banana fruit (Wu *et al.*, 2014) during cold storage. They reported that the chilling injury index in NO-treated fruit was significantly lower than non-treated control. It has been reported that low temperatures cause the generation of Reactive Oxygen Species (ROS), which induce oxidative stress and CI in fruit (Ding *et al.*, 2007). NO has antioxidant properties and also plays an important role in ROS metabolism and in plant signaling networks under normal and stress conditions (Manjunatha *et al.*, 2010). SNP, a NO-donor, may have down-regulated the generation of ROS and, consequently, reduced oxidative stress in fruits during cold storage, which then alleviated the incidence of CI (Zaharah and Singh, 2011). This result was in accordance with Ranjbari *et al.* (2017) reports on pomegranate fruit. The cellophane wrapped pomegranates at day 90 of storage showed significantly lower symptoms of chilling injury than unwrapped fruits, but there was no significant difference at day 45 of storage (Table 1). Probably, it is because of reducing the desiccation of the skin cells by cellophane, which leads to retention of cell stability and, therefore, a lower rate of phenol oxidation that is the main cause of the discoloration associated with chilling injury (Eksteen and Truter, 1985). Storage of pomegranate at 1°C

significantly increased the chilling injury index compared to 5°C at day 90 of storage; however, there was no significant difference between storage temperatures at day 45 of storage (Table 1). Cold storage of harvested fruit is commonly used for extending postharvest life. However, many tropical and subtropical fruits are extremely sensitive to chilling injury, and this reduces the overall quality and marketability of many fruits (Wu *et al.*, 2014). Development of chilling injury symptoms by fruits stored at lower critical temperatures possibly indicates the beginning of disruption of cell structures and membranes (Concellon *et al.*, 2007).

Titrateable Acidity, pH, and Total Soluble Solids

The Titrateable Acidity (TA) was significantly affected by storage temperature (Table 2). The highest TA was found in fruits stored at 1°C in both storage times (Table 2) as reported by Marcilla *et al.* (2006) in citrus. It is also reported that higher storage temperature decreased the amount of TA more than low temperature (Znidarcic *et al.*, 2006). Storage temperature also significantly affected pH at day 90 of storage as the lowest pH was found in stored fruit at 1°C. Decreased acidity might be due to acidic hydrolysis of polysaccharides, where acid is utilized for converting non-RS into RS (Bhardwaj and Pandey, 2011). Temperature has no significant effect on Total Soluble Solids (TSS). Packaging significantly decreased TSS of the fruits after 90 days of storage, but at day 45 of storage, it had no significant effect on TSS (Table 2). It has been reported that unpacking material significantly reduced TSS content in sweet orange fruits (Rub *et al.*, 2010). Since TSS percentage is a function of total dissolved solids and moisture content of the fruit, the increase in TSS may be due to low fruit moisture content. This also could be due to free access of the non-packaged fruits to O₂, which increases respiration rates, resulting in the faster

**Table 2.** Main effect of pre-storage nitric oxide treatment, film wrapping and storage temperature on Total Acidity (TA), Total Soluble Solids (TSS) and pH of 'Shishe-Kab' pomegranate at days 45 and 90 of storage.^a

Parameter		TA (%)		TSS (%)		pH	
Storage time (Days)		45	90	45	90	45	90
Treatment							
NO (μM)	0	1.15 ^a	0.99 ^a	17.18 ^a	17.87 ^a	5.84 ^a	6.00 ^a
	300	1.04 ^a	0.95 ^a	17.04 ^a	17.78 ^a	5.82 ^a	5.97 ^a
Film wrapping	Unwrapped	1.09 ^a	0.94 ^a	17.15 ^a	18.46 ^a	5.83 ^a	5.99 ^a
	Wrapped	1.09 ^a	1.00 ^a	17.07 ^a	17.18 ^b	5.84 ^a	5.99 ^a
Storage Temperature (°C)	1	1.22 ^a	1.08 ^a	16.96 ^a	17.59 ^a	5.84 ^a	5.95 ^b
	5	0.90 ^b	0.82 ^b	17.26 ^a	18.06 ^a	5.83 ^a	6.02 ^a

^a Mean values in each column, for each treatment in evaluated parameter, followed by the same letter are not significantly different by the LSD (P < 0.05, n = 15).

conversion of starch to soluble sugars (Lam, 1989).

Antioxidant Activity

Application of 300 μM of nitric oxide significantly maintained antioxidant activity in treated fruit compared to the control for 90 days of storage; however, there was no

significant effect on day 45 of storage (Table 3). Similar results were found in kiwifruit (Saadatian *et al.*, 2012) and pomegranate fruit (Ranjbari *et al.*, 2017) where nitric oxide had significant effects on the enhancement of antioxidant capacity. Fan *et al.* (2008) reported that NO maintained high levels of antioxidant compounds and inhibited the ROS production in tomatoes. Dhindsa *et al.* (1981) stated that the NO may

Table 3. Main effect of pre-storage nitric oxide treatment, film wrapping and storage temperature on antioxidant activities and total anthocyanin of 'Shishe-Kab' pomegranate at dayS 45 and 90 of storage.^a

Parameter		Antioxidant (%)		Anthocyanin (mg l ⁻¹)	
Storage time (Days)		45	90	45	90
Treatment					
NO (μM)	0	26.73 ^a	19.20 ^b	17.93 ^b	16.7 ^b
	300	23.86 ^a	25.51 ^a	22.17 ^a	21.32 ^a
Film wrapping	Unwrapped	24.46 ^a	20.91 ^a	17.51 ^b	16.14 ^b
	Wrapped	26.13 ^a	23.80 ^a	22.59 ^a	21.93 ^a
Storage temperature (°C)	1	18.7 ^b	14.17 ^b	19.63 ^a	18.51 ^a
	5	31.88 ^a	30.53 ^a	20.47 ^a	19.56 ^a

^a Mean values in each column, for each treatment in evaluated parameter, followed by the same letter are not significantly different by the LSD (P < 0.05, n = 15).

act as an antioxidant, and the protective role of that may be attributed to its mediating the expression of genes encoding these ROS-scavenging antioxidants under different conditions. Wrapping fruit had no significant effect on antioxidant activity during storage period as shown in Table 5. Antioxidant activity of fruits also maintained significantly higher in both times of storage at 5°C compared to 1°C (Table 3), in agreement with Ayala-Zavala *et al.* (2004) data, which noted that enhancement of temperature increases the amount of antioxidant activity of strawberry. The results presented here suggest that the adaptation to the cold environment is a challenging condition to the redox state of the tissue (Cordenunsi *et al.*, 2005).

Total Anthocyanin Content

Total anthocyanin content of treated fruits with NO at 300 µM was higher than the control during storage (Table 3), as stated in Zhu *et al.* (2009) study in Chinese winter jujube. Villarreal *et al.* (2009) also have detected an enhancement of anthocyanin synthesis of strawberry fruit by NO application. Seemingly, nitric oxide with an increment of antioxidant systems protects the plant against destructive elements such as PPO, POD, and H₂O₂ that have an important role in anthocyanin degradation (Khodaei *et al.*, 2015). Administration of NO donors to tobacco plants induced the expression of *Phenylalanine AmmoniaLyase* (PAL) gene (Durner *et al.*, 1998), which is a key enzyme in the synthesis of anthocyanin and different compounds related to plant defense. It is suggested that the increase in anthocyanin amount after NO treatment could be due to the induction of PAL gene expression in the fruit (Villarreal *et al.*, 2009). The packaging also showed a significant effect on anthocyanin as wrapped fruits with cellophane showed higher anthocyanin compared to unwrapped fruits during storage periods (Table 3). The storage temperature (Table 3) had no significant effect on total anthocyanin at both storage times.

This result was in agreement with Ranjbari *et al.* (2017) findings on pomegranate fruit.

Aril Color Properties

Differences in aril color property measured as CIE were significant among nitric oxide treatment or cellophane wrapping of fruits during cold storage. Results showed that the *a** component of aril color was significantly affected by the application of 300 µM nitric oxide up to day 90 of storage and the lower amount of *a** value was found in the control, however, there was no significant difference between treatments at day 45 of storage. Wrapping fruits in cellophane significantly increased the amount of *a** value of aril color compared to the unwrapped fruits after 90 days of storage, but at day 45 of storage, there was no difference between treatments (Table 4). One of the most important quality characteristics of the pomegranate is the red pigmentation of its arils and juice. This red color depends on anthocyanin concentration and on the chemical structure of the individual anthocyanin (Holcroft *et al.*, 1998). Since *a** value shows the redness of the fruit, probably, redder fruits shows a higher amount of *a** component due to the higher level of anthocyanin. Therefore, by the preservation of anthocyanin, NO likely caused the higher level of *a** index. It has been reported that coating and shrink wrapped tray packing was effective for reduction in losses of anthocyanin pigment in apple, and this may possibly be due to the delayed senescence of tissues which involves the degradation of these pigments (Wijewardane and Guleria, 2013). So, fruits which were wrapped with cellophane were redder than unwrapped fruits and showed higher amount of *a** index.

Electrolyte Leakage

The NO treatment significantly reduced the EL of pomegranate fruits during storage period (Table 1), which was in agreement with findings of Wu *et al.* (2014) on banana

**Table 4.** Main effect of pre-storage nitric oxide treatment, film wrapping and storage temperature on aril color properties of 'Shishe-Kab' pomegranate at days 45 and 90 of storage.^a

Parameter		L*		a*		b*	
Storage time (Days)		45	90	45	90	45	90
Treatment							
NO (μM)	0	30.25 ^a	24.27 ^a	23.44 ^a	20.97 ^b	5.3 ^a	4.57 ^a
	300	29.23 ^a	24.49 ^a	24.15 ^a	22.98 ^a	4.96 ^a	5.26 ^a
Film wrapping	Unwrapped	31.48 ^a	25.73 ^a	23.24 ^a	20.67 ^b	5 ^a	5.33 ^a
	Wrapped	28 ^a	23.23 ^a	24.36 ^a	23.28 ^a	5.27 ^a	4.50 ^a
Storage temperature (°C)	1	31.92 ^a	26.33 ^a	24.33 ^a	22.28 ^a	5.58 ^a	4.29 ^a
	5	27.56 ^a	22.63 ^a	23.27 ^a	21.67 ^a	4.68 ^a	5.54 ^a

^a Mean values in each column, for each treatment in evaluated parameter, followed by the same letter are not significantly different by the LSD ($P < 0.05$, $n = 15$).

Table 5. Interactive effect of nitric oxide treatment×cellophane wrapping×temperature on electrolyte leakage of 'Shishe-Kab' pomegranate at days 45 and 90 of storage.^a

Storage time (Days)		45		90	
Treatments		Storage temperature (°C)		Storage temperature (°C)	
NO (μM)	Film wrapping	1	5	1	5
0	Unwrapped	70.38 ^a	65.27 ^{ab}	87.64 ^a	74.50 ^c
	Wrapped	66.71 ^b	57.87 ^b	78.98 ^b	69.36 ^d
300	Unwrapped	63.82 ^{ab}	50.98 ^c	80.82 ^b	68.69 ^d
	Wrapped	62.82 ^{ab}	49.88 ^c	76.91 ^{bc}	55.38 ^e

^a Mean values in each column followed by the same letter are not significantly different by the LSD ($P < 0.05$, $n = 15$).

fruit. However, unwrapped fruits had significantly higher EL compared to wrapped fruits after 90 days of storage, but there was no difference between wrapped and unwrapped fruits at day 45 of storage (Table 1). Storage temperature also had significant effects on fruits EL and the highest level was obtained at 1°C in both storage times (Table 1). The interactive effects of treatments significantly affected EL in both storage periods (Table 5). Unwrapped fruits which were not treated with NO and stored at 1°C showed the highest EL, however, wrapped fruits in cellophane that were treated by nitric oxide and stored at 5°C had the lowest amount of EL. Generally, CI occurs primarily at the cell membrane with changes in the fatty acid phospholipids composition (Mirdehghan *et*

al., 2007) and the membrane damages initiate a cascade of secondary reactions leading to disruption of cell structures. This membrane damage can be measured by the electrolyte leakage and often used to indicate chilling tolerance of plant tissues (Wu *et al.*, 2014).

CONCLUSIONS

This experiment showed the beneficial effects of pre-storage treatments of nitric oxide and cellophane on pomegranate fruit quality. Cellophane vastly improved moisture retention around the fruit, resulting in lower weight loss and also enhanced the amount of anthocyanin and, consequently, the a^* value of the aril color. Application of

NO maintained antioxidant activity and the total anthocyanin content resulting in the higher amount of a^* value of aril color. The combination of NO treatment and cellophane wrapping have more beneficial effects on fruit chilling injury resistance at 1°C, which can lead to extending postharvest life of pomegranate fruit cv. 'Shishe-Kab' in prolonged cold storage.

REFERENCES

1. Artés, F., Villaescusa, R. and Tudela, J. A. 2000. Modified Atmosphere Packaging of Pomegranate. *J. Food Sci.*, **65**: 1112-1116.
2. Ayala-Zavala, J. F., Wang, S. Y., Wang, C. Y. and Gonzalez-Aguilar, G. A. 2004. Effect of Storage Temperatures on Antioxidant Capacity and Aroma Compounds in Strawberry Fruit. *LWT-Food Sci. Technol.*, **37**: 687-695.
3. Beckerson, D. and Hofstra, G. 1980. Effects of Sulphur Dioxide and Ozone, Singly or in Combination, on Membrane Permeability. *Can. J. Bot.*, **58**: 451-457.
4. Bhardwaj, R. L. and Pandey, S. 2011. Juice Blends: A Way of Utilization of Under-Utilized Fruits, Vegetables, and Spices: A Review. *Crit. Rev. Food Sci.*, **51**: 563-570.
5. Blois, M. S. 1958. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature*, **181**: 1199-1200.
6. Caleb, O. J., Opara, U. L. and Witthuhn, C. R. 2012. Modified Atmosphere Packaging of Pomegranate Fruit and Arils: A Review. *Food Bioprocess Tech.*, **5**: 15-30.
7. Cohen, E., Shapiro, B., Shalom, Y. and Klein, J. D. 1994. Water Loss: A Nondestructive Indicator of Enhanced Cell Membrane Permeability of Chilling-Injured Citrus Fruit. *J. Am. Soc. Hortic. Sci.*, **119**: 983-986.
8. Concellon, A., Anon, M. C. and Chaves, A. R. 2007. Effect of Low Temperature Storage on Physical and Physiological Characteristics of Eggplant Fruit (*Solanum melongena* L.). *LWT-Food Sci. Technol.*, **40**: 389-396.
9. Cordenunsi, B. R., Genovese, M. I., Nascimento, J. R. O., Hassimotto, N. M. A., Dos santos, R. J. and Lajolo, F. M. 2005. Effects of Temperature on the Chemical Composition and Antioxidant Activity of Three Strawberry Cultivars. *Food Chem.*, **91**: 113-121.
10. D'Aquino, S., Palma, A., Schirra, M., Continella, A., Tribulato, E. and La Malfa, S. 2010. Influence of Film Wrapping and Fludioxonil Application on Quality of Pomegranate Fruit. *Postharvest Biol. Technol.*, **55**: 121-128.
11. Dhindsa, R. S., Plumb-Dhindsa, P. and Thorpe, T. A. 1981. Leaf Senescence: Correlated with Increased Levels of Membrane Permeability and Lipid Peroxidation, and Decreased Levels of Superoxide Dismutase and Catalase. *J. Exp. Bot.*, **32**: 93-101.
12. Ding, Z. S., Tian, S. P., Zheng, X. L., Zhou, Z. W. and Xu, Y. 2007. Responses of Reactive Oxygen Metabolism and Quality in Mango Fruit to Exogenous Oxalic Acid or Salicylic Acid under Chilling Temperature Stress. *Physiol. Plantarum.*, **130**: 112-121.
13. Durner, J., Wendehenne, D. and Klessig, D. F. 1998. Defense Gene Induction in Tobacco by Nitric Oxide, Cyclic GMP, and Cyclic ADP-Ribose. *P. Nat. Acad. Sci.*, **95**: 10328-10333.
14. Eksteen, G. J. and Truter, A. B. 1985. Effects of Controlled and Modified Atmosphere Storage on Quality of Eating Ripe Avocados. *SAAGA*, **8**: 78-80.
15. Emond, J. and Brecht, J. K. 2005. Quality Attributes Limiting Papaya Postharvest Life at Chilling and Non-Chilling Temperatures. *Proc. Fla. State Hortic. Soc.*, **118**: 389-395.
16. Fan, B., Shen, L., Liu, K., Zhao, D., Yu, M. and Sheng, J. 2008. Interaction between Nitric Oxide and Hydrogen Peroxide in Postharvest Tomato Resistance Response to *Rhizopus nigricans*. *J. Sci. Food Agr.*, **88**: 1238-1244.



17. Fawole, O. A. and Opara, U. L. 2013. Effects of Storage Temperature and Duration on Physiological Responses of Pomegranate Fruit. *Ind. Crop. Prod.*, **47**: 300-309.
18. Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B., Holcroft, D. M. and Kader, A. A. 2000. Antioxidant Activity of Pomegranate Juice and Its Relationship with Phenolic Composition and Processing. *J. Agr. Food Chem.*, **48**: 4581-4589.
19. Hernandez-Munoz, P., Almenar, E., Del Valle, V., Velez, D. and Gavara, R. 2008. Effect of Chitosan Coating Combined with Postharvest Calcium Treatment on Strawberry (*Fragaria ananassa*) Quality during Refrigerated Storage. *Food Chem.*, **110**: 428-435.
20. Holcroft, D. M., Gil, M. I. and Kader, A. A. 1998. Effect of Carbon Dioxide on Anthocyanins, Phenylammonialyase and Glucosyltransferase in the Arils of Stored Pomegranates. *J. Am. Soc. Hortic. Sci.*, **123**: 136-140.
21. Kader, A. A. and Rolle, R. S. 2004. *The Role of Post-Harvest Management in Assuring the Quality and Safety of Horticultural Produce*. Food and Agriculture Organization, FAO, United Nations Rome, 152 PP.
22. Khodaei, M., Nahandi, F. Z., Motallebi-Azar, A. and Dadpour, M. 2015. Effect of Salicylic Acid and Sodium nNitro Proside on the Pomegranate Aril Browning Disorder. *Biol. Forum.*, **7**: 1014-1020.
23. Ladaniya, M. and Singh, S. 1999. *Post-Harvest Technology and Processing of Citrus Fruits in India*. National Research Centre for Citrus, Nagpur, India.
24. Lako, J., Trenerry, V. C., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S. and Premier, R. 2007. Phytochemical Flavonols, Carotenoids and the Antioxidant Properties of a Wide Selection of Fijian Fruit, Vegetables and Other Readily Available Foods. *Food Chem.*, **101**: 1727-1741.
25. Lam, P. 1989. Respiration Rate, Ethylene Production and Skin Color Change of Papaya at Different Temperature. *Acta Hortic.*, **269**: 257-266.
26. Lamotte, O., Courtois, C., Barnavon, L., Pugin, A. and Wendehenne, D. 2005. Nitric Oxide in Plants: The Biosynthesis and Cell Signalling Properties of a Fascinating Molecule. *Planta*, **221**: 1-4.
27. Lee, L., Arul, J., Lencki, R. and Castaigne, F. 1995. A Review on Modified Atmosphere Packaging and Preservation of Fresh Fruits and Vegetables: Physiological Basis and Practical Aspects: Part I. *Packag. Technol. Sci.*, **8**: 315-331.
28. Li, X. P., Wu, B., Guo, Q., Wang, J. D., Zhang, P. and Chen, W. X. 2014. Effects of Nitric Oxide on Postharvest Quality and Soluble Sugar Content in Papaya Fruit during Ripening. *J. Food Process. Pres.*, **38**: 591-599.
29. Manjunatha, G., Lokesh, V. and Neelwarne, B. 2010. Nitric Oxide in Fruit Ripening: Trends and Opportunities. *Biotechnol. Adv.*, **28**: 489-499.
30. Marcilla, A., Zarzo, M. and Del Río, M. A. 2006. Effect of Storage Temperature on the Flavour of Citrus Fruit. *Span. J. Agric. Res.*, **4**: 336-344.
31. Mirdehghan, S. H., Rahemi, M., Martinez-Romero, D., Guillen, F., Valverde, J. M., Zapata, P. J., Serrano, M. and Valero, D. 2007. Reduction of Pomegranate Chilling Injury during Storage after Heat Treatment: Role of Polyamines. *Postharvest Biol. Technol.*, **44**: 19-25.
32. Moradinezhad, F. and Khayyat, M. 2014. Effects of Intermittent Warming and Prestorage Treatments (Hot Water, Salicylic Acid, Calcium Chloride) on Postharvest Life of Pomegranate Fruit cv. 'Shishe-Kab' during Long-Term Cold Storage. *Int. J. Hort. Sci. Technol.*, **1**: 43-51.
33. Moradinezhad, F., Khayyat, M. and Saeb, H. 2013. Combination Effects of Postharvest Treatments and Modified Atmosphere Packaging on Shelf Life and

- Quality of Iranian Pomegranate Fruit cv. 'Sheshi-Kab'. *Int. J. Postharvest Technol. Innov.*, **3**: 244-256.
34. Nunes, M., Emond, J. and Brecht, J. 2006. Brief Deviations from Set Point Temperatures during Normal Airport Handling Operations Negatively Affect the Quality of Papaya (*Carica papaya*) Fruit. *Postharvest Biol. Technol.*, **41**: 328-340.
35. Paull, R. and Chen, N. 1989. Waxing and Plastic Wraps Influence Water-Loss from Papaya Fruit during Storage and Ripening. *J. Am. Soc. Hortic. Sci.*, **114**: 937-942.
36. Purvis, A. C. 1985. Relationship between Chilling Injury of Grapefruit and Moisture Loss during Storage: Amelioration by Polyethylene Shrink Film. *J. Am. Soc. Hortic. Sci.*, **11**: 385-388.
37. Ranjbari, F., Moradinezhad, F. and Khayyat, M. 2017. Effect of Nitric Oxide on Biochemical and Antioxidant Properties of Pomegranate Fruit cv. Shishe-Kab during Cold Storage. *Int. J. Hort. Sci. Technol.*, **3**: 211-219.
38. Rub, A., Haq, S., Khalil, S. A. and Ali, S. G. 2010. Fruit Quality and Senescence Related Changes in Sweet Orange Cultivar Blood Red Unpacked in Different Packing Materials. *Sarhad J. Agric.*, **26**: 221-227.
39. Saadatian, M., Ahmadiyan, S., Akbari, M. and Balouchi, Z. 2012. Effects of Pretreatment with Nitric Oxide on Kiwifruit Storage at Low Temperature. *Adv. Environ. Biol.*, 1902-1909.
40. Singh, S. P., Singh, Z. and Swinny, E. E. 2009. Postharvest Nitric Oxide Fumigation Delays Fruit Ripening and Alleviates Chilling Injury during Cold Storage of Japanese Plums (*Prunus salicina* Lindell). *Postharvest Biol. Technol.*, **53**: 101-108.
41. Soegiarto, L. and Wills, R. B. H. 2006. Effect of Nitric Oxide, Reduced Oxygen and Elevated Carbon Dioxide Levels on the Postharvest Life of Strawberries and Lettuce. *Anim. Prod. Sci.*, **46**: 1097-1100.
42. Teksur, P. K. 2015. Alternative Technologies to Control Postharvest Diseases of Pomegranate. *Stewart Postharvest Rev.*, **11**: 1-7.
43. Villarreal, N. M., Martinez, G. A. and Civello, P. M. 2009. Influence of Plant Growth Regulators on Oolygalacturonase Expression in Strawberry Fruit. *Plant Sci.*, **176**: 749-757.
44. Wijewardane, R. M. N. A. and Guleria, S. P. S. 2013. Effect of Pre-Cooling, Fruit Coating and Packaging on Postharvest Quality of Apple. *J. Food Sci. Tech.*, **50**: 325-331.
45. Wu, B., Guo, Q., Li, Q., Ha, Y., Li, X. and Chen, W. 2014. Impact of Postharvest Nitric Oxide Treatment on Antioxidant Enzymes and Related Genes in Banana Fruit in Response to Chilling Tolerance. *Postharvest Biol. Technol.*, **92**: 157-163.
46. Zaharah, 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. Technol.*, **60**: 202-210.
47. Zhu, S., Liu, M. and Zhou, J. 2006. Inhibition by Nitric Oxide of Ethylene Biosynthesis and Lipoyxygenase Activity in Peach Fruit during Storage. *Postharvest Biol. Technol.*, **42**: 41-48.
48. Zhu, S., Sun, L. and Zhou, J. 2009. Effects of Nitric Oxide Fumigation on Phenolic Metabolism of Postharvest Chinese Winter Jujube (*Zizyphus jujuba* Mill. cv. Dongzao) in Relation to Fruit Quality. *LWT-Food Sci. Technol.*, **42**: 1009-1014.
49. Znidarcic, D. and Pozrl, T. 2006. Comparative Study of Quality Changes in Tomato cv. Malike (*Lycopersicon esculentum* Mill.) whilst Stored at Different Temperatures. *Acta Agric. Slov.*, **87**: 235-243.



تأثیر نیتریک اکسید و لاف پلاستیکی بر حفظ کیفیت و کاهش سرمازدگی میوه انار

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چکیده

هدف از این مطالعه ارزیابی تأثیر کاربرد جداگانه نیتریک اکسید و پوشش سلوفان و ترکیب این تیمارها در کاهش سرمازدگی و بهبود کیفیت میوه انار رقم شیشه کب در انبار سرد و سرمازدگی بود. تیمارها شامل غوطه‌وری میوه در محلول نیتریک اسید (صفر یا ۳۰۰ میکرومول) به مدت ۲ دقیقه و به دنبال آن پوشش سلوفان (با و یا بدون بسته بندی) بود، و سپس در انبار با دو دمای مختلف (۱ و ۵ درجه سانتی گراد) به مدت ۹۰ روز قرار داده شدند. کاربرد ۳۰۰ میکرومول نیتریک اکسید به طور معنی داری فعالیت آنتی‌اکسیدانی، محتوی فنول کل، مقدار a رنگ آریل را افزایش داد، در نتیجه کمترین سرمازدگی و نشت یونی میوه در مقایسه با شاهد حاصل شد. کمترین افت وزن، سرمازدگی و مواد جامد محلول و بیشترین محتوی آنتوسیانین و مولفه a رنگ آریل در میوه‌های پیچیده شده با پوشش، در مقایسه با بدون پوشش مشاهده شد. ترکیب نیتریک اکسید و پوشش سلوفان اثر بیشتری در کاهش نشت یونی میوه‌ها داشت، به طوری که موجب کاهش ۷۲ و ۶۳ درصدی آن در مقایسه با میوه‌های شاهد که بترتیب برای مدت ۴۵ و ۹۰ روز انبار شده بودند شد.