

## Nitric Oxide, Oxalic Acid and Hydrogen Peroxide Treatments to Reduce Decay and Maintain Postharvest Quality of 'Valencia' Orange Fruits During Cold Storage

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**T**HE EFFECTIVENESS of postharvest treatments of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) treatments and their combinations on postharvest quality and enzyme activity of 'Valencia' orange fruits were examined after harvest in season 2014 and 2015. The experiment were included the following treatments: distilled water (control), 1 mM SNP, 10 mM OA, 2% H<sub>2</sub>O<sub>2</sub>, 1 mM SNP + 10 mM OA, 1 mM SNP + 2% H<sub>2</sub>O<sub>2</sub> and 10 mM OA + 2% H<sub>2</sub>O<sub>2</sub> for 5 minutes. All treatments were stored at 8±1°C and 85-90% relative humidity (RH) followed by one week as a shelf life at ambient temperature 18-23°C and 55-65% RH for 15 weeks. All postharvest studied maintained postharvest quality of 'Valencia' oranges as compared to untreated fruits (control) during storage. Moreover, combined treatments were more effective than individual treatments. Treated 'Valencia' oranges with 1 mM SNP plus 10 mM OA or 2% H<sub>2</sub>O<sub>2</sub> alleviated decay incidence and reduced weight loss percentage with an increase of marketable fruit percentage. In addition, these applications reduced the activities of polyphenol oxidase (PPO) and pectinase (PE) enzymes, while increased the activity of peroxidase (POX) enzyme. These treatments also decreased loss of firmness, hue angle, lightness values and juice content of fruits. In addition, these applications slowed the increase of soluble solids content (SSC) and SSC/TA ratio as well as decreased loss of titratable acidity (TA) and ascorbic acid contents during cold storage period followed by shelf life. Therefore, the use of postharvest treatments with 1 mM SNP in combination with 10 mM OA or 2% H<sub>2</sub>O<sub>2</sub> have good potential strategy to improve the storability and reduce decay incidence as well as maintain postharvest quality of 'Valencia' oranges during cold storage.

**Keywords:** Oxalic acid, Sodium nitroprusside, Hydrogen peroxide, 'Valencia' orange, Fruit quality, Enzyme activity.

Although citrus is considered the greatest planted area among all grown fruits in Egypt, the exported quantity of fresh citrus fruits to foreign markets is restricted.

Therefore, any effort directed towards keeping fruit quality and decreasing postharvest losses is important to increase the national proceeds. During the last two decades, many investigations were done to try identifying an effective natural disinfecting substance, which may be acceptable to consumers. However, until now there is no alternate applied method.

Postharvest decay is considered the most important factor that limit the shelf life of oranges. Orange fruits are susceptible to a wide variety of fungal diseases. These days, there is a great concern about human health and environmental contamination hazards associated with fungicide residues (Wisniewski & Wilson, 1992). Thus, there is a stringent need for the development of reliable and environmental friendly methods for protecting perishable crops, particularly fresh fruits, against losses after harvest. Nitric Oxide (NO) is a highly reactive free radical gas engaged in fighting vegetative stress and deterioration of horticultural products. Short term exposure to low doses of NO or its donors can prolong the postharvest life of several fresh fruits and vegetables (Wills *et al.*, 2007 and Zhu & Zhou, 2007). External postharvest application of NO delayed disease incidence, peel colour changes and reduced activity of softening enzymes (Manjunatha *et al.*, 2010). In addition, application of NO reduced postharvest water loss from horticultural product (Ku *et al.*, 2000). No fumigation reduced the activity of polygalacturonase (PG) enzyme of kiwifruit and peach fruits (Zhu *et al.*, 2010 a, b), banana fruits (Cheng *et al.*, 2009) and Yang *et al.*, 2010). Also, No fumigation reduced the activities of fruits softening enzymes such as exo and endo PG in 'Kensington Pride' mango fruits during cold storage at 13°C (Zaharah & Singh, 2011a, b).

Oxalic acid (OA) is a natural organic acid and playing an important function in systemic resistance and response to environment (Zheng *et al.*, 2012 and Jin *et al.*, 2014). OA application is a secure and hopeful postharvest handling technology for keeping quality and prolonging storage life of fruit (Zheng & Tian, 2006). OA has shown some antioxidant activities and play a serious function in systemic strength, programmed cell death, redo homeostasis in plants and an anti-senescence effectiveness in harvested fruits (Ding *et al.*, 2007, Zheng *et al.*, 2007a and Wu *et al.*, 2011). In addition, postharvest treatment of OA reduced the activity of PPO enzyme (Yoruk *et al.*, 2002).

Pre-storage application with OA enhanced the antioxidant capacities of banana and pomegranate fruits (Sayyari *et al.*, 2010 and Huang *et al.*, 2013a, b). Moreover, OA and oxalate treatments induced systemic resistance against diseases caused by fungi, bacteria and viruses in plants (Mucharromah & Kuc, 1991 and Toal & Jones, 1999). Pre-storage application of OA can suppress postharvest disorders and prolong the storage life of mangoes because of delaying the ripening process (Zheng *et al.*, 2007b, c and Zheng *et al.*, 2012). Moreover, postharvest treatment of OA decreased loss of fruit firmness and reduced the activity of exo-PG enzyme beside enhanced the activities of antioxidative enzymes (superoxide dismutase, catalase and peroxidase) (Razzaq

et al., 2015). Furthermore, who added that, SSC and SSC/TA ratio were lowered, while, TA and ascorbic acid contents were higher in treated fruits as compared to untreated fruits. In addition, postharvest application of bananas with OA maintained peel appearance, prolonged the shelf life of fruits and displayed the potential suitable for commercial application to store the bananas at ambient temperature (Huang et al., 2013 a, b).

Postharvest treatment with H<sub>2</sub>O<sub>2</sub> has been proposed as alternative method to replace currently used chemicals to control postharvest diseases and increasing the storability of fruits and vegetables as well as it pliable as water, surface antiseptic and postharvest aides for organic crops (Suslow, 1997). H<sub>2</sub>O<sub>2</sub> is an environment friendly compound whose activity is based on oxidation of microbes and it was successfully used to inhibit pathogens of vegetable during storage (Afeke et al., 1999). It is now obvious that H<sub>2</sub>O<sub>2</sub> function as signaling molecules in plants, it is a form of reactive oxygen species (ROS) created because of oxidative stress. Oxidative strain arises from an imbalance in the generation and metabolism of ROS. H<sub>2</sub>O<sub>2</sub> is one of ROS and used as alternative to chemical materials for disinfecting fruits and vegetables to reduce microbial populations and prolong the shelf life without leaving significant residues or causing loss of postharvest quality (Sapers & Simmons, 1998 and Sapers et al., 2001). Postharvest treatment with H<sub>2</sub>O<sub>2</sub> controlled postharvest green mold of lemons (Smilanick et al., 1995). In addition, postharvest applications of H<sub>2</sub>O<sub>2</sub> of pepper fruits reduced weight loss and rot rate as well as enhanced antioxidants content and improved general appearance during cold storage at 10°C for four weeks or during shelf life at 20°C for two weeks as compared to untreated fruits (Bayoumi, 2008).

Therefore, the aims of this work were to study the effects of postharvest treatments of 'Valencia' oranges with SNP, OA and H<sub>2</sub>O<sub>2</sub> alone or in combination on postharvest decay and fruit quality during cold storage.

### Materials and Methods

The present study has been done during two successive seasons 2014 and 2015 on 'Valencia' orange (*Citrus sinensis* (L.) Osbeck). At maturity stage, 'Valencia' orange fruits were picked randomly from a private orchard at El-Behera Governorate, Egypt. 'Valencia' orange trees were about 23 years old, grafted on 'Volkamariana' rootstock and planted in a sandy soil at 5x5 meters, irrigated by drip system and subjected to all ideal agriculture practices. Fruits were picked directly from similar trees, apparently uniform in size. Fruit were directly transported to postharvest laboratory at Horticulture Research Institute, Agriculture Research Center, Giza governorate. Once arrival to the laboratory, oranges sorted to eliminate defected fruits. Uniform size and appearance orange fruits were washed by tap water then dried by air and held for 24 hours at room temperature.

Orange fruits were selected and randomly divided into seven groups. Each group included nine boxes consist of 12 individual fruits (108 fruits per treatment, 756 fruits for the whole experiment). Each treatment was immersed for five minutes into one of the following treatments: a- distilled water and used as control, b- 1 mM sodium nitroprusside (SNP), a nitric oxide donor that can release nitric oxide upon in aqueous solvents, c- 10 mM oxalic acid (OX), d- 2% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), e - 1 mM SNP + 10 mM OX, f- 1 mM SNP + 2% H<sub>2</sub>O<sub>2</sub> and g- 10 mM OX + 2% H<sub>2</sub>O<sub>2</sub>. Tween-80 at 0.05% (v/v) was added in each solution to improve wettability and adherence to oranges surface. After immersing treatments, all fruits were dried for one hour at room temperature by electric fan and then packaged in perforated polyethylene bags (five holes of 7 mm diameter). After that, all treatments were stored at 8±1°C and 85-90% relative humidity (RH) for 15 weeks. Fruit physical and chemical characteristics were measured at three weeks intervals of cold storage followed by one week at ambient temperature 18-23°C and 55-65% RH as a shelf life period. The activities of peroxidase (POX), polyphenol oxidase (PPO) and pectinase (PE) enzymes were determined at 0, 3, 9 and 15 weeks of storage period followed by one week shelf life at ambient temperature.

#### *Measurements of fruit physical and chemical properties*

Weight loss percentage was calculated by the following equation [(initial fruit weight - fruit weight at examination date) / (initial fruit weight)] × 100.

Decayed fruit percentage was determined by the following equation [(number of decayed fruits at examination date) / (initial number of fruits)] × 100.

Marketable fruit percentage was calculated by the following formula [(sound fruits at examination date) / (initial fruit weight)] × 100.

Fruit colour was measured using a Minolta CR-400 Chroma Meter (Minolta Co. Ltd. Osaka, Japan). The measurements of skin colour and gloss were expressed in chromaticity values of hue angle (h°) and lightness (L), respectively. Three readings were taken at different locations of each orange fruit during each data observation (McGuire, 1992 and Voss, 1992).

Fruit firmness of the peel was assessed by using Ifra texture analyzer instrument. The force required to penetrate 1 cm inside the fruit using a needle probe diameter of 5 mm was measured. The machine was set with peak mode and speed of 0.3 mm/sec. Readings were recorded on the two opposite sides of the orange fruit and the results were expressed as the resistance force to the penetrating tester in units of pressure g/cm<sup>2</sup> (Watkins & Harman, 1981).

Fruit juice content was measured by squeezing six fruits for each treatment represent three replicates and then juice percentage was calculated (w/w).

Fruit juice content of ascorbic acid (AsA) was determined according to method of adopting the procedure described by AOAC (1990) and was calculated as mg/100 ml juice.

Fruit juice soluble solids content (SSC) was determined by hand refractometer, 0-32 scale (ATAGO N-1E, Japan) and expressed in °Brix after making the temperature correction at 20°C according to AOAC (1990).

Fruit juice content of titratable acidity (TA) was measured by titration as mentioned by AOAC (1990) and was calculated as grams of citric acid/100 ml juice.

Fruit juice SSC/TA ratio was calculated from the values recorded for fruit juice SSC and TA percentages determined.

#### *Enzyme activities*

0.5 gram of fresh orange peel was homogenized by using a mortar and pestle with 0.1 M buffer of phosphate at 4°C (pH=6.5) and stirred for 20 min. The suspension obtained was filtered through one piece of muslin cloth and afterwards centrifuged at 18,000×g for 15 minutes, 4°C. Polyphenol oxidase (PPO) enzyme was measured as mentioned by Fernandez et al. (2011), while peroxidase (POX) and pectinase (PE) enzymes were determined according to Horwitz et al. (1975). The activities of these enzymes were expressed as units per gram fresh weight (U g<sup>-1</sup> fW).

#### *Statistical analysis*

This experiment was arranged in a completely randomized design having three replications (Steel et al., 1997) and consisting of two factors (postharvest treatments and storage periods). This experiment was analysis as factorial. Data calculated as percentage were transformed to arcsine of square root before statistical analysis and non-transformed means are shown. The effects of postharvest treatments and cold storage periods on different attributes were analyzed statistically by analysis of variance (ANOVA) using the MSTAT-C statistical package (M-STAT, 1993). Comparisons between means were done by Duncan's multiple range tests (DMRT) at probability ≤ 0.05.

### **Results and Discussion**

*Influence of postharvest treatments of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) solutions on 'Valencia' orange fruit quality during cold storage*

*Fruit weight loss, decay and marketable percentages*

The present study clearly indicated that, weight loss and decay percentages of 'Valencia' orange fruits gradually and significantly increased with prolonging of cold storage period at 8°C plus one week as a shelf life at 18-23°C in the two

seasons in this work (Tables 1 and 2). While the percentage of marketable 'Valencia' orange fruits gradually and significantly decreased with prolonging of cold storage period in the two seasons in this study (Table 3).

Data also cleared that, all postharvest treatments significantly decreased the deterioration rate of all these characteristics during cold storage in the two seasons in this experiment as compared with untreated fruits (control). Moreover, it is clear that, postharvest treatment of 1 mM SNP in combination with 10 mM OA followed by 1 mM SNP in combination with 2% H<sub>2</sub>O<sub>2</sub> had the lowest fruit weight loss and decay percentages with the highest marketable fruit percentage as compared to the other postharvest treatments during cold storage in the two seasons in this study.

NO and OA treatments decreased fruit weight loss because of reduced the respiration rate, transpiration through skin, various metabolic activities and inhibited the ethylene production in plums (Singh *et al.*, 2009 and Wu *et al.*, 2011), mangoes (Zheng *et al.*, 2007b, c and Zaharah & Singh, 2011b) and banana fruits (Huang *et al.*, 2013a).

**TABLE 1. Influence of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on weight loss percentage of 'Valencia' oranges during storage at 8°C followed by one week shelf life at 18-23°C**

Postharvest treatments	Storage period (weeks)						Means
	0	3	6	9	12	15	
<b>First Season (2014)</b>							
Distilled water (control)	2.44 opq	5.74 i-l	7.72 fgh	12.17 bc	14.72 a	16.19 a	9.83 A
1 mM SNP	1.78 pq	4.53 k-n	6.96 g-j	7.87 fgh	9.26 def	12.94 b	7.22 B
10 mM OX	1.63 pq	4.29 lmn	6.32 h-k	8.23 efg	10.02 de	12.35 bc	7.14 B
2% H <sub>2</sub> O <sub>2</sub>	1.95 pq	4.65 k-n	7.28 ghi	8.78 efg	9.40 def	13.05 b	7.52 B
1 mM SNP + 10 mM OX	1.20 q	3.34 nop	5.08 k-n	7.17 ghi	8.11 fgh	9.18 def	5.68 C
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	1.28 q	3.79 mno	5.21 j-m	7.21 ghi	8.30 efg	9.46 def	5.87 C
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	2.00 pq	4.95 k-n	6.36 h-k	8.23 efg	9.47 def	10.87 cd	6.98 B
<b>Means</b>	1.75 F	4.47 E	6.42 D	8.52 C	9.90 B	12.01 A	
<b>Second Season (2015)</b>							
Distilled water (control)	2.27 n	5.22 lm	9.05 e-i	12.95 b	15.59 a	16.60 a	10.28 A
1 mM SNP	2.13 n	4.85 lm	6.62 jkl	6.26 kl	10.86 cde	12.85 b	7.26 C
10 mM OX	2.07 n	5.81 lm	6.38 kl	10.03 ef	11.88 bcd	13.29 b	8.24 B
2% H <sub>2</sub> O <sub>2</sub>	2.17 n	5.38 lm	7.65 ijk	10.06 ef	10.41 de	12.96 b	8.10 B
1 mM SNP + 10 mM OX	1.52 n	4.29 m	5.79 lm	7.83 h-k	8.13 g-j	9.83 efg	6.23 D
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	1.58 n	4.39 m	6.10 klm	8.30 f-j	8.30 f-j	10.02 ef	6.45 D
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	1.86 n	5.04 lm	6.06 klm	9.57 e-h	9.22 e-i	12.40 bc	7.36 C
<b>Means</b>	1.94 F	5.00 E	6.81 D	9.29 C	10.63 B	12.56 A	

Means followed by the same letters within postharvest treatments, storage periods and their interactions in each season are not significantly different at level  $P \leq 0.05$  according to DMRT.

**TABLE 2. Influence of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on decay percentage of 'Valencia' oranges during storage at 8°C followed by one week shelf life at 18-23°C**

Postharvest treatments	Storage period (weeks)							Means
	0	3	6	9	12	15		
<b>First Season (2014)</b>								
Distilled water (control)	0.00 d	6.13 cd	9.88 bcd	19.13 abc	26.08 ab	34.08 a	15.88 A	
1 mM SNP	0.00 d	0.00 d	3.92 cd	8.88 bcd	10.62 bcd	12.75 bcd	6.03 BC	
10 mM OX	0.00 d	0.00 d	4.70 cd	9.68 bcd	11.84 bcd	14.43 bcd	6.78 BC	
2% H <sub>2</sub> O <sub>2</sub>	0.00 d	2.86 cd	6.76 cd	11.66 bcd	13.22 bcd	15.27 bcd	8.29 B	
1 mM SNP + 10 mM OX	0.00 d	0.00 d	0.00 d	0.00 d	2.40 cd	4.27 cd	1.11 C	
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	0.00 d	0.00 d	0.00 d	2.55 cd	4.08 cd	9.50 bcd	2.69 BC	
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	0.00 d	0.00 d	3.14 cd	5.69 cd	8.45 bcd	11.70 bcd	4.83 BC	
<b>Means</b>	0.00 D	1.28 D	4.06 CD	8.23 BC	10.96 AB	14.57 A		
<b>Second Season (2015)</b>								
Distilled water (control)	0.00 d	6.66 cd	11.24 bcd	19.61 abc	25.36 ab	32.90 a	15.96 A	
1 mM SNP	0.00 d	3.70 cd	5.10 cd	7.53 cd	9.19 cd	11.40 bcd	6.15 BC	
10 mM OX	0.00 d	4.15 cd	6.37 cd	8.84 cd	10.94 bcd	12.06 bcd	7.06 BC	
2% H <sub>2</sub> O <sub>2</sub>	0.00 d	4.85 cd	7.60 cd	10.59 bcd	11.96 bcd	13.35 bcd	8.06 B	
1 mM SNP + 10 mM OX	0.00 d	0.00 d	0.00 d	0.00 d	3.06 cd	4.52 cd	1.26 C	
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	0.00 d	0.00 d	0.00 d	0.00 d	4.77 cd	6.34 cd	1.85 C	
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	0.00 d	0.00 d	3.21 cd	5.17 cd	7.52 cd	9.75 bcd	4.28 BC	
<b>Means</b>	0.00 D	2.77 CD	4.79 CD	7.39 BC	10.40 AB	12.90 A		

Means followed by the same letters within postharvest treatments, storage periods and their interactions in each season are not significantly different at level  $P \leq 0.05$  according to DMRT.

The safeguard of fruit from infestation of fungal pathogens is largely due to activation of a highly coordinated biochemical and constitutional protection system that helps ward off the dispersal of pathogens (Lawton et al., 1996). Chitinase and  $\beta$ -1,3-glucanase hydrolyze polymers of fungal dikes walls are thinking to be involved in plant defense mechanisms against fungal infection (Schlumbaum et al., 1986 and Collinge et al., 1993).

NO enhanced fruit resistance to fungal pathogens by inducing the abundance of pathogen related proteins (Kang et al., 2016). OA treatments are contributes to induced systemic resistance in plants by increasing the activity of POX enzyme (Toal and Jones, 1999).

In this study, reduced the decay incidence of 'Valencia' oranges by 1 mM SNP in combination with 10 mM OA treatment may be due to an increase in the activity of POX enzyme and an reduce in the activities of PPO and PE enzymes during storage as mentioned by Tian et al. (2006) and Zheng et al. (2012).

The reduction of rot rate by using H<sub>2</sub>O<sub>2</sub> attributed to H<sub>2</sub>O<sub>2</sub> as a reactive oxygen species (ROS), which play an important role in resistance of plant diseases to infection with pathogens (Bayoumi, 2008).

These results are in harmony with those mentioned by Bayoumi (2008) on pepper fruits, Sayyari *et al.* (2010) on pomegranates and Razzaq *et al.* (2015) on mangoes. They mentioned that, postharvest treatments with NO, OA and H<sub>2</sub>O<sub>2</sub> treatments reduced the weight loss percentage during storage.

Moreover, These results are in agreement with the findings of Zheng & Tian (2006) on litchi fruits, Zheng *et al.* (2007a) on peach fruits and Zheng *et al.* (2007b, c), Bayoumi (2008) on pepper fruits and Zheng *et al.* (2012) on mangoes. They mentioned that, the application of postharvest treatments with NO, OA and H<sub>2</sub>O<sub>2</sub> reduced weight loss and decay incidence of stored fruits.

**TABLE 3. Influence of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on marketable percentage of ‘Valencia’ oranges during storage at 8°C followed by one week shelf life at 18-23°C**

Postharvest treatments	Storage period (weeks)						Means
	0	3	6	9	12	15	
<b>First Season (2014)</b>							
Distilled water (control)	97.56 ab	88.13 a-j	82.41 c-l	68.70 i-m	59.21 klm	49.74 m	74.29 D
1 mM SNP	98.22 a	95.47 a-e	89.12 a-i	83.25 b-l	80.12 f-l	74.32 j-m	86.75 BC
10 mM OX	98.37 a	95.71 a-d	88.98 a-i	82.09 d-l	78.14 g-l	73.22 klm	86.09 C
2% H <sub>2</sub> O <sub>2</sub>	98.05 a	92.50 a-g	85.96 a-l	79.57 f-l	77.38 h-l	71.68 lm	84.19 CD
1 mM SNP + 10 mM OX	98.80 a	96.66 abc	94.92 a-e	92.83 a-f	89.49 a-i	86.55 a-k	93.21 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	98.72 a	96.21 a-d	94.79 a-e	90.25 a-h	87.62 a-j	81.05 e-l	91.44 AB
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	98.00 a	95.05 a-e	90.50 a-h	86.07 a-k	82.07 d-l	77.43 h-l	88.19 ABC
<b>Means</b>	98.25 A	94.25 A	89.52 B	83.25 C	79.15 C	73.43 D	
<b>Second Season (2015)</b>							
Distilled water (control)	97.73 ab	88.12 a-g	79.71 c-h	67.44 fgh	59.05 hi	50.50 i	73.76 C
1 mM SNP	97.87 a	91.44 a-e	88.28 a-g	86.21 a-g	79.95 c-h	75.74 e-h	86.58 AB
10 mM OX	97.93 a	90.05 a-g	87.25 a-g	81.13 b-h	77.18 e-h	74.65 fgh	84.70 B
2% H <sub>2</sub> O <sub>2</sub>	97.83 a	89.77 a-g	84.76 a-g	79.35 c-h	77.63 d-h	73.69 gh	83.84 B
1 mM SNP + 10 mM OX	98.48 a	95.71 abc	94.21 a-d	92.18 a-e	88.82 a-g	85.65 a-g	92.51 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	98.42 a	95.61 abc	93.90 a-d	91.70 a-e	86.93 a-g	83.64 a-g	91.70 A
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	98.14 a	94.96 abc	90.73 a-f	85.27 a-g	83.25 a-g	77.86 d-h	88.37 AB
<b>Means</b>	98.06 A	92.24 B	88.41 BC	83.32 CD	78.97 DE	74.53 E	

Means followed by the same letters within postharvest treatments, storage periods and their interactions in each season are not significantly different at level  $P \leq 0.05$  according to DMRT.

*Fruit firmness, gloss (lightness), colour (hue angle) and juice content*

Skin colour development represented as hue angle value (greenish yellow, around 75 and yellow, around 60) and gloss represented as lightness. Our results in the present study shown that, fruit firmness, lightness and juice percentage gradually and significantly decreased with prolonging of cold storage period during the two seasons in this investigation. Data also cleared that, fruit peel colour changed directly from greenish yellow to yellow with prolonging of cold storage period at 8°C followed by one week as a shelf life at ambient temperature during the two seasons in this study (Tables 4, 5, 6 and 7).

Moreover, the results of our study displayed that, postharvest treatments of 1 mM SNP, 10 mM OA and 2% H<sub>2</sub>O<sub>2</sub> individually or in combination significantly decreased these changes rate in comparison to untreated fruits (control) during the two seasons in this work. Moreover, it is obvious that, postharvest treatment of 1 mM SNP in combination with 10 mM OA had the most effective influence in this aspect followed by 1 mM SNP in combination with 2% H<sub>2</sub>O<sub>2</sub>.

**TABLE 4. Influence of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on fruit firmness (g/cm<sup>2</sup>) of ‘Valencia’ oranges during storage at 8°C followed by one week shelf life at 18-23°C**

Postharvest treatments	Storage period (weeks)						
	0	3	6	9	12	15	Means
<b>First Season (2014)</b>							
Distilled water (control)	205.00 ab	187.00 e	164.33 k	152.67 l	136.67 m	127.33 n	162.17 E
1 mM SNP	206.67 ab	203.67 ab	194.00 d	184.33 efg	174.33 i	166.00 k	188.17 BCD
10 mM OX	206.33 ab	203.33 ab	194.00 d	181.33 e-h	173.67 ij	164.67 k	187.22 CD
2% H <sub>2</sub> O <sub>2</sub>	205.67 ab	202.67 bc	193.67 d	180.33 fgh	173.33 ij	163.67 k	186.56 D
1 mM SNP + 10 mM OX	209.00 a	206.00 ab	197.67 cd	186.00 ef	178.67 ghi	168.33 jk	190.94 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	208.67 ab	205.67 ab	196.67 d	185.67 ef	177.67 hi	168.00 jk	190.39 AB
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	207.67 ab	204.67 ab	196.00 d	184.33 efg	177.00 hi	166.67 k	189.39 ABC
<b>Means</b>	207.00 A	201.86 B	190.90 C	179.24 D	170.19 E	160.67 F	
<b>Second Season (2015)</b>							
Distilled water (control)	204.67 ab	193.00 fgh	179.67 k-n	166.00 pq	135.00 r	126.00 s	167.39 D
1 mM SNP	206.67 a	203.67 abc	196.67 c-f	184.33 i-l	179.00 k-n	166.67 pq	189.50 BC
10 mM OX	205.33 ab	202.33 a-e	195.67 d-g	184.00 i-l	177.00 l-o	165.00 q	188.22 C
2% H <sub>2</sub> O <sub>2</sub>	205.33 ab	202.33 a-e	195.33 efg	182.33 i-m	176.33 mno	164.33 q	187.67 C
1 mM SNP + 10 mM OX	209.67 a	206.67 a	199.00 b-f	189.00 ghi	182.67 i-m	173.00 nop	193.33 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	208.67 a	205.67 ab	196.67 c-f	187.67 hij	180.33 j-n	171.33 opq	191.72 AB
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	206.00 ab	203.00 a-d	195.33 efg	185.33 ijk	179.33 k-n	167.67 pq	189.44 BC
<b>Means</b>	206.62 A	202.38 B	194.05 C	182.67 D	172.81 E	162.00 F	

Means followed by the same letters within postharvest treatments, storage periods and their interactions in each season are not significantly different at level  $P \leq 0.05$  according to DMRT.

**TABLE 5. Influence of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on fruit lightness of 'Valencia' oranges during storage at 8°C followed by one week shelf life at 18-23°C**

Postharvest treatments	Storage period (weeks)						Means
	0	3	6	9	12	15	
<b>First Season (2014)</b>							
Distilled water (control)	69.51 a-e	57.01 l-o	53.77 opq	51.41 pqr	48.38 r	46.61 r	54.45 E
1 mM SNP	71.55 abc	68.55 a-f	66.27 c-h	62.91 g-k	59.49 j-n	56.39 m-p	64.19 BCD
10 mM OX	71.11 a-d	68.11 a-g	65.79 d-i	62.52 g-l	57.91 k-o	54.47 n-q	63.32 CD
2% H <sub>2</sub> O <sub>2</sub>	70.56 a-d	67.56 a-h	65.51 d-i	62.51 g-l	56.61 mno	51.17 qr	62.32 D
1 mM SNP + 10 mM OX	72.75 a	69.75 a-e	67.99 a-g	64.72 e-j	62.32 h-l	60.42 i-m	66.33 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	72.75 a	69.75 a-e	66.65 b-h	64.10 e-j	60.24 i-m	59.75 j-n	65.54 AB
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	72.22 ab	69.22 a-e	66.38 c-h	63.53 f-j	59.75 j-n	59.65 j-n	65.13 ABC
<b>Means</b>	71.49 A	67.14 B	64.62 C	61.67 D	57.81 E	55.49 F	
<b>Second Season (2015)</b>							
Distilled water (control)	68.72 a-e	60.75 j-o	56.32 pq	54.05 q	49.99 r	45.75 s	55.93 C
1 mM SNP	69.75 abc	66.42 c-h	63.99 f-k	61.39 i-o	59.65 k-p	57.08 opq	63.05 B
10 mM OX	69.75 abc	65.42 c-i	63.32 g-l	61.05 j-o	58.99 l-p	55.75 pq	62.38 B
2% H <sub>2</sub> O <sub>2</sub>	69.09 a-d	65.09 d-j	62.65 h-m	60.72 j-o	58.65 m-p	55.42 pq	61.94 B
1 mM SNP + 10 mM OX	72.09 a	69.09 a-d	67.99 a-f	65.05 d-j	62.32 h-n	59.08 l-p	65.94 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	71.42 a	68.42 a-e	67.99 a-f	64.39 e-j	61.32 i-o	58.42 m-p	65.33 A
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	71.09 ab	68.09 a-f	66.99 b-g	64.05 f-j	60.99 j-o	58.08 n-q	64.88 A
<b>Means</b>	70.27 A	66.18 B	64.18 C	61.53 D	58.84 E	55.65 F	

Means followed by the same letters within postharvest treatments, storage periods and their interactions in each season are not significantly different at level  $P \leq 0.05$  according to DMRT.

Fruit softening is associated with changes in cell wall mechanical strength (Valero & Serrano, 2010). These results are in conformity with those mentioned by Wu *et al.* (2011) on plums, Zheng *et al.* (2012) and Razzaq *et al.* (2015) on mangoes and Li *et al.* (2014) on papaya fruits. They reported that, NO and OA reduced cell wall hydrolytic enzymes and consequently decreased loss of firmness of fruits during storage.

Our results are supported by the findings of Zhang *et al.* (2008) and Singh *et al.* (2009) in plums, Zaharah & Singh (2011a, b) in mangoes, Cheng *et al.* (2009), Yang *et al.* (2010) and Huang *et al.* (2013a) in bananas and Zhu *et al.* (2010b) and Kang *et al.* (2016) in peaches. They mentioned that, NO treatment reduced the activities of hydrolytic enzymes and consequently reduced the deterioration rate of fruit firmness.

**TABLE 6. Influence of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on fruit colour represented as hue angle of 'Valencia' oranges during storage at 8°C followed by one week shelf life at 18-23°C**

Postharvest treatments	Storage period (weeks)						Means
	0	3	6	9	12	15	
<b>First Season (2014)</b>							
Distilled water (control)	69.76 a-e	65.33 e-i	60.47 i-l	56.07 lm	52.88 mn	50.59 n	59.18 C
1 mM SNP	71.15 abc	70.15 a-e	67.95 a-f	63.94 f-k	61.01 i-l	60.38 i-l	65.76 B
10 mM OX	71.33 abc	70.33 a-e	67.61 a-g	63.81 f-k	60.80 i-l	59.84 jkl	65.62 B
2% H <sub>2</sub> O <sub>2</sub>	70.84 a-d	69.84 a-e	67.25 b-h	62.23 g-k	60.25 i-l	59.62 kl	65.01 B
1 mM SNP + 10 mM OX	72.95 a	71.95 abc	69.53 a-e	67.66 a-f	65.57 d-i	63.48 f-k	68.52 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	72.29 abc	71.29 abc	68.87 a-f	67.00 b-h	65.24 e-j	62.15 h-k	67.81 A
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	72.49 ab	71.49 abc	68.50 a-f	66.80 c-h	65.15 e-j	61.86 h-k	67.72 A
<b>Means</b>	71.54 A	70.05 A	67.17 B	63.93 C	61.56 D	59.70 E	
<b>Second Season (2015)</b>							
Distilled water (control)	68.74 abc	64.43 d-i	59.34 n-q	54.13 r	50.04 s	49.62 s	57.72 E
1 mM SNP	68.96 abc	67.96 a-d	64.20 e-k	63.00 g-n	60.24 l-q	59.15 opq	63.92 BCD
10 mM OX	69.01 ab	67.72 a-e	63.96 f-m	61.43 h-o	60.00 n-q	57.58 pq	63.28 CD
2% H <sub>2</sub> O <sub>2</sub>	68.87 abc	67.54 a-f	63.12 g-n	60.58 j-p	59.49 n-q	56.73 qr	62.72 D
1 mM SNP + 10 mM OX	71.29 a	70.95 a	67.53 a-f	64.33 d-j	62.24 g-o	61.48 h-o	66.30 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	69.96 a	68.96 abc	65.87 b-g	64.00 f-l	61.91 h-o	60.48 k-p	65.20 AB
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	69.64 ab	68.64 abc	65.21 c-h	63.01 g-n	60.92 i-p	60.16 m-q	64.60 BC
<b>Means</b>	69.50 A	68.03 B	64.18 C	61.50 D	59.26 E	57.89 F	

Means followed by the same letters within postharvest treatments, storage periods and their interactions in each season are not significantly different at level  $P \leq 0.05$  according to DMRT.

Moreover, these results are in agreement with the outcome of Cheng et al. (2009) and Huang et al. (2013a) in banana fruits and Zaharah & Singh (2011a, b) in mangoes. They illustrated that, NO and OA delayed colour development by maintained lightness and higher hue angle of the skin during storage. Moreover, they added that, the influence of NO and OA in delaying colour development of fruits may due to suppression of ethylene production and consequently reduced biosynthesis of carotenoids in the skin.

In addition, OA treatment delayed ripening process, reduced the activities of cell wall hydrolytic enzymes in fruits, thus reduced the decline in fruits firmness during storage as reported by Zheng & Tian (2006) on litchi fruits and Zheng et al. (2007c) and Zheng et al. (2012) on mangoes.

**TABLE 7. Influence of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on juice percentage of 'Valencia' oranges during storage at 8°C followed by one week shelf life at 18-23°C**

Postharvest treatments	Storage period (weeks)						Means
	0	3	6	9	12	15	
<b>First Season (2014)</b>							
Distilled water (control)	50.77 a-h	48.46 c-k	44.14 k-p	41.42 n-q	38.23 qr	35.66 r	43.11 D
1 mM SNP	52.75 a-d	50.75 a-h	48.32 c-k	45.77 h-o	42.97 l-q	41.18 opq	46.96 BC
10 mM OX	51.58 a-f	49.58 b-j	47.10 f-m	45.56 i-o	42.67 l-q	40.29 pq	46.13 C
2% H <sub>2</sub> O <sub>2</sub>	51.56 a-f	49.56 b-j	46.43 f-n	44.66 j-p	42.41 m-q	40.30 pq	45.82 C
1 mM SNP + 10 mM OX	54.79 a	52.79 a-d	51.10 a-g	47.71 d-l	47.72 d-l	44.14 k-p	49.71 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	54.25 ab	52.25 a-e	50.43 a-i	46.94 f-m	47.27 e-m	42.98 l-q	49.02 A
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	53.41 abc	51.41 a-f	49.62 b-j	46.64 f-m	46.06 g-o	42.72 l-q	48.31 AB
<b>Means</b>	52.73 A	50.69 B	48.16 C	45.53 D	43.90 E	41.04 F	
<b>Second Season (2015)</b>							
Distilled water (control)	51.54 a-f	48.46 f-i	43.18 l-q	42.77 l-q	38.10 st	35.43 t	43.25 E
1 mM SNP	52.00 a-d	50.00 b-g	47.41 g-j	44.98 j-n	42.19 n-r	40.34 p-s	46.15 CD
10 mM OX	52.28 a-d	49.14 d-g	47.28 g-j	44.29 j-o	41.32 o-r	40.05 qrs	45.73 D
2% H <sub>2</sub> O <sub>2</sub>	51.73 a-e	48.70 e-h	47.12 g-j	43.91 k-o	40.20 qrs	39.54 rs	45.20 D
1 mM SNP + 10 mM OX	53.79 a	51.79 a-e	49.69 c-g	47.41 g-j	45.49 i-m	43.46 l-p	48.61 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	53.14 ab	51.14 a-f	48.37 f-i	46.94 g-k	43.65 l-o	42.85 l-q	47.68 AB
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	52.84 abc	50.84 a-f	48.74 e-h	45.90 h-l	42.53 m-r	41.60 o-r	47.08 BC
<b>Means</b>	52.47 A	50.01 B	47.40 C	45.17 D	41.93 E	40.47 F	

Means followed by the same letters within postharvest treatments, storage periods and their interactions in each season are not significantly different at level  $P \leq 0.05$  according to DMRT.

#### *Fruit activities of pectinase (PE), polyphenol oxidase (PPO) and peroxidase (POX) enzymes*

The present study cleared that, the activities of POX, PPO and PE enzymes gradually and significantly increased with prolonging of cold storage period in the two seasons in this investigation (Tables 8, 9 and 10).

Moreover, it is obvious that, all postharvest treatments significantly reduced the activities rate of PPO and PE enzymes of 'Valencia' oranges as compared to untreated fruits (control) during cold storage at 8°C followed by one week as a shelf life at ambient temperature. On contrary, all postharvest applications significantly increased the activity rate of POX enzyme. In addition, postharvest treatment of 1 mM SNP in combination with 10 mM OA followed by the other two combinations treatments were more effective in reducing the activities of PPO and PE enzymes as well as increasing the activity POX enzyme than individual treatments during storage in the two seasons in this work.

**TABLE 8. Influence of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on activity of POX enzyme (U g<sup>-1</sup> fW) of 'Valencia' oranges during storage at 8°C followed by one week shelf life at 18-23°C**

Postharvest treatments	Storage period (weeks)				
	0	3	9	15	Means
<b>First Season (2014)</b>					
Distilled water (control)	0.127 jk	0.225 h-k	0.239 h-k	0.246 hij	0.209 D
1 mM SNP	0.178 ijk	0.276 hi	0.292 ghi	0.535 cde	0.320 C
10 mM OX	0.136 jk	0.234 h-k	0.287 ghi	0.521 cde	0.294 C
2% H <sub>2</sub> O <sub>2</sub>	0.107 k	0.205 h-k	0.207 h-k	0.412 efg	0.233 D
1 mM SNP + 10 mM OX	0.262 hij	0.627 c	0.767 b	1.084 a	0.685 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	0.178 ijk	0.277 hi	0.419 ef	0.819 b	0.423 B
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	0.220 h-k	0.318 fgh	0.490 de	0.608 cd	0.409 B
<b>Means</b>	0.173 D	0.309 C	0.386 B	0.603 A	
<b>Second Season (2015)</b>					
Distilled water (control)	0.134 mn	0.232 ijk	0.234 ijk	0.236 ijk	0.209 E
1 mM SNP	0.161 lmn	0.259 hij	0.313 gh	0.542 e	0.319 C
10 mM OX	0.137 mn	0.235 ijk	0.309 gh	0.544 e	0.306 C
2% H <sub>2</sub> O <sub>2</sub>	0.106 n	0.204 jkl	0.250 ij	0.454 f	0.254 D
1 mM SNP + 10 mM OX	0.257 hij	0.422 f	0.935 b	1.162 a	0.694 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	0.179 klm	0.277 ghi	0.449 f	0.858 c	0.441 B
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	0.221 ijk	0.319 g	0.540 e	0.610 d	0.423 B
<b>Means</b>	0.171 D	0.278 C	0.433 B	0.629 A	

Means followed by the same letters within postharvest treatments, storage periods and their interactions in each season are not significantly different at level  $P \leq 0.05$  according to DMRT.

NO positively maintains the balance between the formation and detoxification of ROS (Wu et al., 2012). Low doses of NO encouraged the expression or activation of antioxidant enzymes in plants (Leshem et al., 1998). NO also could detoxify ROS by reacting with O<sub>2</sub> and generating peroxynitrite (Crawford, 2006).

Moreover, OA applications are contributes to induced systemic resistance in plants by increasing the activity of POX enzyme (Toal and Jones, 1999). In this experiment, treated fruits by 1 mM SNP in combination with 10 mM OA increased the activity of POX enzyme and reduced the activities of PPO and PE enzymes of 'Valencia' oranges during storage, thus reduced the decay incidence of fruits.

POX activity produces the oxidative power for transit connecting of proteins and phenylpropanoid radicals generating an encouragement of cell walls against attempted fungal penetration (Yao & Tian, 2005)

These results were supported by the findings of Tian et al. (2006) on pears, Bayoumi (2008) on peppers, Zheng et al. (2012) on mangoes, Li et al. (2014) on

papaya fruits and Wang *et al.* (2016) on apricots. They reported that, postharvest applications of NO, OA and H<sub>2</sub>O<sub>2</sub> increased the activities of antioxidant enzymes in fruits as compared to control.

These results are supported by the findings of Ueda *et al.* (2001) and Zaharah & Singh (2011a, b) on mangoes, Cheng *et al.* (2009) and Yang *et al.* (2010) on banana fruits, Zhu *et al.* (2010a) on kiwifruit fruits and Zhu *et al.* (2006), Zhu *et al.* (2010b) and Kang *et al.* (2016) on peaches. They postulated that, NO reduced the activities of fruits softening enzymes.

In addition, postharvest applications of OA and oxalate treatments delaying ripening process of mango fruits (Zheng *et al.*, 2007c and Zheng *et al.*, 2012) and bananas (Huang *et al.*, 2013a) by reducing the activities of softening enzymes during storage.

**TABLE 9. Influence of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on activity of PPO enzyme (U g<sup>-1</sup> fW) of 'Valencia' oranges during storage at 8°C followed by one week shelf life at 18-23°C**

Postharvest treatments	Storage period (weeks)				Means
	0	3	9	15	
<b>First Season (2014)</b>					
Distilled water (control)	0.238 e	0.296 d	0.468 b	0.764 a	0.441 A
1 mM SNP	0.091 ghi	0.149 fg	0.166 f	0.363 c	0.192 BC
10 mM OX	0.091 ghi	0.149 fg	0.169 f	0.367 c	0.194 BC
2% H <sub>2</sub> O <sub>2</sub>	0.092 ghi	0.151 fg	0.221 e	0.367 c	0.208 B
1 mM SNP + 10 mM OX	0.091 ghi	0.149 fg	0.120 f-i	0.225 e	0.146 D
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	0.088 hi	0.146 fgh	0.150 fg	0.323 cd	0.177 C
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	0.084 i	0.143 f-i	0.162 f	0.369 c	0.190 BC
<b>Means</b>	0.111 D	0.169 C	0.208 B	0.397 A	
<b>Second Season (2015)</b>					
Distilled water (control)	0.245 h	0.303 g	0.441 b	0.709 a	0.424 A
1 mM SNP	0.091 n	0.149 l	0.174 k	0.405 c	0.205 D
10 mM OX	0.092 n	0.150 l	0.205 j	0.416 c	0.216 C
2% H <sub>2</sub> O <sub>2</sub>	0.111 m	0.170 k	0.237 hi	0.385 d	0.226 B
1 mM SNP + 10 mM OX	0.099 mn	0.157 kl	0.149 l	0.223 i	0.157 F
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	0.092 n	0.150 l	0.150 l	0.366 e	0.190 E
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	0.088 n	0.146 l	0.169 k	0.349 f	0.188 E
<b>Means</b>	0.117 D	0.175 C	0.218 B	0.407 A	

Means followed by the same letters within postharvest treatments, storage periods and their interactions in each season are not significantly different at level  $P \leq 0.05$  according to DMRT.

**TABLE 10. Influence of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on activity of PE enzyme (U g<sup>-1</sup> fW) of 'Valencia' oranges during storage at 8°C followed by one week shelf life at 18-23°C**

Postharvest treatments	Storage period (weeks)				
	0	3	9	15	Means
<b>First Season (2014)</b>					
Distilled water (control)	0.161 gh	0.179 ef	0.286 b	0.420 a	0.262 A
1 mM SNP	0.106 l-o	0.124 jkl	0.143 hi	0.213 d	0.147 C
10 mM OX	0.105 l-o	0.124 jkl	0.123 jkl	0.242 c	0.149 C
2% H <sub>2</sub> O <sub>2</sub>	0.130 ijk	0.148 ghi	0.187 e	0.277 b	0.185 B
1 mM SNP + 10 mM OX	0.096 no	0.114 k-n	0.137 ij	0.133 ijk	0.120 E
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	0.102 mno	0.120 j-m	0.119 j-m	0.191 e	0.133 D
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	0.092 o	0.110 l-o	0.145 hi	0.164 fg	0.128 DE
<b>Means</b>	0.113 D	0.131 C	0.163 B	0.234 A	
<b>Second Season (2015)</b>					
Distilled water (control)	0.167 f	0.185 e	0.282 b	0.400 a	0.259 A
1 mM SNP	0.104 k-n	0.122 h-k	0.136 gh	0.212 d	0.144 CD
10 mM OX	0.105 j-n	0.123 hij	0.126 hi	0.252 c	0.152 C
2% H <sub>2</sub> O <sub>2</sub>	0.127 hi	0.145 g	0.184 ef	0.251 c	0.177 B
1 mM SNP + 10 mM OX	0.094 mn	0.112 i-m	0.120 h-l	0.135 gh	0.115 F
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	0.092 n	0.110 i-n	0.145 g	0.185 e	0.133 E
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	0.103 lmn	0.121 h-l	0.148 g	0.189 e	0.140 DE
<b>Means</b>	0.113 D	0.131 C	0.163 B	0.232 A	

Means followed by the same letters within postharvest treatments, storage periods and their interactions in each season are not significantly different at level  $P \leq 0.05$  according to DMRT.

*Fruit juice contents of soluble solids content (SSC), titratable acidity (TA), SSC/TA ratio and ascorbic acid (AsA)*

Juice contents of TA and AsA in 'Valencia' orange fruits declined during cold storage at 8°C followed by one week as a shelf life at ambient temperature, while an increasing trend in SSC and SSC/TA ratio were recorded. This trend was cleared during the two seasons in this work (Tables 11, 12, 13 and 14).

Data also cleared that, all postharvest treatments especially in combinations significantly reduced these deterioration in 'Valencia' orange fruits during the two seasons in this work in comparison to untreated fruits, except in the case of SSC, which was insignificant during the first season.

**TABLE 11. Influence of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on SSC (°Brix) of 'Valencia' oranges during storage at 8°C followed by one week shelf life at 18-23°C**

Postharvest treatments	Storage period (weeks)						Means
	0	3	6	9	12	15	
<b>First Season (2014)</b>							
Distilled water (control)	9.87 ef	9.93 def	10.03 b-f	10.13 a-f	10.47 ab	10.50 ab	10.16 A
1 mM SNP	9.70 f	9.90 ef	10.03 b-f	10.20 a-e	10.40 abc	10.43 ab	10.11 A
10 mM OX	9.77 ef	9.97 c-f	10.13 a-f	10.20 a-e	10.43 ab	10.50 ab	10.17 A
2% H <sub>2</sub> O <sub>2</sub>	9.77 ef	9.97 c-f	10.20 a-e	10.13 a-f	10.47 ab	10.50 ab	10.17 A
1 mM SNP + 10 mM OX	9.70 f	9.90 ef	10.13 a-f	10.20 a-e	10.43 ab	10.57 a	10.16 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	9.77 ef	9.97 c-f	10.10 a-f	10.20 a-e	10.37 a-d	10.50 ab	10.15 A
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	9.77 ef	9.97 c-f	10.17 a-f	10.17 a-f	10.47 ab	10.57 a	10.19 A
<b>Means</b>	9.76 D	9.94 C	10.11 B	10.18 B	10.43 A	10.51 A	
<b>Second Season (2015)</b>							
Distilled water (control)	9.70 f-k	9.80 c-k	10.00 a-g	10.03 a-f	10.17 ab	10.20 a	9.98 A
1 mM SNP	9.60 ijk	9.87 a-i	9.77 d-k	9.87 a-i	10.00 a-g	9.97 a-h	9.84 B
10 mM OX	9.57 ijk	9.87 a-i	9.80 c-k	9.83 b-j	10.03 a-f	10.00 a-g	9.85 B
2% H <sub>2</sub> O <sub>2</sub>	9.60 ijk	9.70 f-k	9.83 b-j	9.90 a-i	10.07 a-e	10.07 a-e	9.86 B
1 mM SNP + 10 mM OX	9.47 k	9.67 g-k	9.77 d-k	9.83 b-j	10.03 a-f	10.10 a-d	9.81 B
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	9.50 jk	9.63 h-k	9.73 e-k	9.77 d-k	10.03 a-f	10.07 a-e	9.79 B
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	9.57 ijk	9.77 d-k	9.87 a-i	9.87 a-i	10.00 a-g	10.13 abc	9.87 B
<b>Means</b>	9.57 D	9.76 C	9.82 BC	9.87 B	10.05 A	10.08 A	

Means followed by the same letters within postharvest treatments, storage periods and their interactions in each season are not significantly different at level  $P \leq 0.05$  according to DMRT.

Moreover, it is clear that, the postharvest treatment with the 1 mM SNP individual or in combination with 10 mM OA or 2% H<sub>2</sub>O<sub>2</sub> gave the most effective influence on fruit content of SSC. While in case of titratable acidity and SSC/TA ratio it is clear that, the treatments of 1 mM SNP in combination with 2% H<sub>2</sub>O<sub>2</sub> followed by the other two combinations had the most effective in this aspect.

In case of ascorbic acid content changes, it is clear that, all combinations postharvest treatments had the most effective in this aspect and reduced the deterioration rate of 'Valencia' orange fruits content in AsA as compared to individual and control treatments during the first season. While during the second season, postharvest application of 1 mM SNP in combination with 10mM OA were superior in this aspect followed by postharvest treatments by the other two combinations.

**TABLE 12. Influence of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on TA (g citric acid/100 ml juice) of 'Valencia' oranges during storage at 8°C followed by one week shelf life at 18-23°C**

Postharvest treatments	Storage period (weeks)							Means						
	0	3	6	9	12	15								
<b>First Season (2014)</b>														
Distilled water (control)	1.27	a-e	1.20	a-h	1.04	e-j	0.96	hij	0.92	ij	0.90	j	1.05	D
1 mM SNP	1.34	ab	1.24	a-f	1.15	b-i	1.07	d-j	1.04	e-j	1.00	f-j	1.14	ABC
10 mM OX	1.31	abc	1.21	a-g	1.20	a-h	1.09	c-j	0.98	g-j	0.98	g-j	1.13	BCD
2% H <sub>2</sub> O <sub>2</sub>	1.29	a-d	1.19	a-h	1.15	b-i	1.07	d-j	0.98	g-j	0.96	hij	1.11	CD
1 mM SNP + 10 mM OX	1.40	a	1.30	a-d	1.20	a-h	1.13	b-j	1.09	c-j	1.02	f-j	1.19	ABC
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	1.42	a	1.32	abc	1.28	a-e	1.15	b-i	1.11	b-j	1.04	e-j	1.22	A
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	1.40	a	1.30	a-d	1.24	a-f	1.13	b-j	1.11	b-j	1.03	f-j	1.20	AB
<b>Means</b>	1.35	A	1.25	B	1.18	B	1.09	C	1.03	CD	0.99	D		
<b>Second Season (2015)</b>														
Distilled water (control)	1.24	a-e	1.11	d-k	1.02	g-m	0.96	j-m	0.92	lm	0.85	m	1.02	D
1 mM SNP	1.32	abc	1.22	a-f	1.11	d-k	1.04	f-l	1.00	h-m	0.96	j-m	1.11	ABC
10 mM OX	1.30	abc	1.20	a-g	1.09	d-l	1.02	g-m	0.98	i-m	0.94	klm	1.09	BC
2% H <sub>2</sub> O <sub>2</sub>	1.27	a-d	1.17	a-h	1.07	e-l	0.96	j-m	0.96	j-m	0.92	lm	1.06	CD
1 mM SNP + 10 mM OX	1.34	ab	1.24	a-e	1.15	b-i	1.09	d-l	1.04	f-l	0.96	j-m	1.14	AB
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	1.36	a	1.26	a-d	1.18	a-h	1.11	d-k	1.06	e-l	1.00	h-m	1.16	A
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	1.32	abc	1.22	a-f	1.13	c-j	1.07	e-l	1.02	g-m	0.98	i-m	1.12	ABC
<b>Means</b>	1.31	A	1.20	B	1.11	C	1.04	D	1.00	DE	0.94	E		

Means followed by the same letters within postharvest treatments, storage periods and their interactions in each season are not significantly different at level  $P \leq 0.05$  according to DMRT.

OA treatment could be suppressed the lipid peroxidation and ultimately decreased the ascorbic acid oxidation (Kayashima & Katayama, 2002). H<sub>2</sub>O<sub>2</sub> treatment have beneficial effects on fruit physiology such as delaying ripening of oranges by the increasing antioxidants content in fruits (Saltveit and Sharaf, 1992).

The stability of ascorbic acid directly increased in the presence of H<sub>2</sub>O<sub>2</sub> during storage of orange, grape and pomegranate fruit juices (Özkan et al. 2004 and Bayoumi, 2008).

In addition, the highest value of SSC in untreated fruits may be due to the increasing of water loss (Troncoso et al., 2005 and Bayoumi, 2008). Moreover, the decreasing juice content of TA with prolonging of cold storage period might be due to the degradation of citric acid during storage or their conversion into sugars and further utilization in metabolic process in the fruit (Rathore et al., 2007).

**TABLE 13. Influence of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on SSC/TA ratio of 'Valencia' oranges during storage at 8°C followed by one week shelf life at 18-23°C**

Postharvest treatments	Storage period (weeks)							Means						
	0	3	6	9	12	15								
<b>First Season (2014)</b>														
Distilled water (control)	7.78	i-l	8.34	f-l	9.61	b-i	10.58	a-d	11.52	ab	11.75	a	9.93	A
1 mM SNP	7.28	kl	8.03	h-l	8.73	d-l	9.57	b-i	10.39	a-f	10.81	a-d	9.14	BC
10 mM OX	7.45	jkl	8.23	g-l	8.49	e-l	9.46	b-j	10.78	a-d	10.88	abc	9.22	ABC
2% H <sub>2</sub> O <sub>2</sub>	7.56	i-l	8.36	f-l	8.91	c-l	9.64	b-i	10.67	a-d	10.98	abc	9.35	AB
1 mM SNP + 10 mM OX	6.95	l	7.64	i-l	8.49	e-l	9.06	c-k	9.61	b-i	10.32	a-g	8.68	BC
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	6.87	l	7.54	i-l	7.89	i-l	8.88	c-l	9.40	c-j	10.12	a-h	8.45	C
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	6.97	kl	7.66	i-l	8.25	g-l	9.57	b-i	9.48	b-j	10.49	a-e	8.74	BC
<b>Means</b>	7.27	E	7.97	D	8.62	C	9.54	B	10.26	A	10.76	A		
<b>Second Season (2015)</b>														
Distilled water (control)	7.85	m-r	8.87	f-p	9.79	b-k	10.48	a-g	11.17	ab	11.96	a	10.02	A
1 mM SNP	7.31	pqr	8.13	k-r	8.85	f-p	9.44	c-m	10.03	b-j	10.61	a-e	9.06	BCD
10 mM OX	7.39	o-r	8.26	k-r	9.03	e-o	9.62	b-l	10.29	b-h	10.85	a-d	9.24	BC
2% H <sub>2</sub> O <sub>2</sub>	7.77	m-r	8.55	i-r	9.23	d-n	10.35	a-h	10.52	a-g	11.03	abc	9.58	AB
1 mM SNP + 10 mM OX	7.11	qr	7.85	m-r	8.49	i-r	9.07	e-o	9.61	b-l	10.55	a-f	8.78	CD
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	7.02	r	7.69	n-r	8.32	j-r	8.81	g-p	9.44	c-m	10.07	b-i	8.56	D
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	7.28	pqr	8.05	l-r	8.75	h-q	9.31	d-n	9.82	b-k	10.34	a-h	8.92	CD
<b>Means</b>	7.39	F	8.20	E	8.92	D	9.58	C	10.13	B	10.77	A		

Means followed by the same letters within postharvest treatments, storage periods and their interactions in each season are not significantly different at level  $P \leq 0.05$  according to DMRT.

Based on these explanation could be suggested that, postharvest treatments especially 1 mM SNP in combination with 10 mM OA or 2% H<sub>2</sub>O<sub>2</sub> applications increased fruit juice contents of AsA and TA and decrease of SSC as compared to untreated fruits during cold storage period at 8°C followed by one week at ambient temperature.

Our results are in accordance the findings of Zheng *et al.* (2007b, c), Zaharah & Singh (2011a, b) and Razzaq *et al.* (2015) on mangoes, Sayyari *et al.* (2010) on pomegranates and Li *et al.* (2014) on papaya fruits. They claimed that, postharvest application of NO and OA reduced fruit content of SSC and SSC/TA ratio as well as increased TA and AsA as compared to untreated fruits during cold storage.

**TABLE 14. Influence of sodium nitroprusside (SNP), oxalic acid (OA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on AsA (mg/100 ml juice) of 'Valencia' oranges during storage at 8°C followed by one week shelf life at 18-23°C**

Postharvest treatments	Storage period (weeks)						
	0	3	6	9	12	15	Means
<b>First Season (2014)</b>							
Distilled water (control)	63.61 a-f	58.18 h-m	51.52 qrs	47.57 t	42.12 u	37.27 v	50.05 D
1 mM SNP	65.52 abc	61.52 c-h	58.79 g-l	56.67 j-p	54.24 m-r	51.21 q-t	57.99 C
10 mM OX	65.21 a-d	61.21 d-h	57.57 h-o	56.06 k-p	53.94 n-r	50.91 rst	57.48 C
2% H <sub>2</sub> O <sub>2</sub>	64.91 a-e	60.91 e-i	56.67 j-p	55.15 l-q	52.73 p-s	49.09 st	56.58 C
1 mM SNP + 10 mM OX	66.85 a	64.85 a-e	62.73 a-g	61.52 c-h	59.09 g-l	56.66 j-p	61.95 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	66.55 ab	64.55 a-e	62.42 b-g	60.31 f-j	57.88 h-n	54.55 m-r	61.04 AB
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	65.94 ab	63.94 a-f	62.43 b-g	59.39 g-k	56.97 i-o	53.64 o-r	60.39 B
<b>Means</b>	65.51 A	62.17 B	58.88 C	56.67 D	53.85 E	50.48 F	
<b>Second Season (2015)</b>							
Distilled water (control)	62.81 a-d	57.58 g-l	52.42 o-s	46.36 t	40.30 u	36.06 v	49.26 E
1 mM SNP	63.94 abc	60.61 b-h	57.88 g-k	55.46 j-p	53.94 l-q	50.91 qrs	57.12 C
10 mM OX	64.03 abc	59.70 d-i	56.36 i-n	54.24 k-q	53.03 n-r	49.39 rst	56.13 CD
2% H <sub>2</sub> O <sub>2</sub>	63.74 abc	59.09 d-j	56.06 i-o	53.94 l-q	52.12 p-s	48.79 st	55.62 D
1 mM SNP + 10 mM OX	65.94 a	63.94 abc	62.12 a-f	60.30 c-h	58.48 f-j	56.37 i-n	61.19 A
1 mM SNP + 2% H <sub>2</sub> O <sub>2</sub>	64.43 ab	62.43 a-e	61.21 b-g	58.79 e-j	56.97 h-m	53.34 m-q	59.53 B
10 mM OA + 2% H <sub>2</sub> O <sub>2</sub>	64.12 abc	62.12 a-f	60.30 c-h	57.88 g-k	55.45 j-p	52.43 o-s	58.72 B
<b>Means</b>	64.14 A	60.78 B	58.05 C	55.28 D	52.90 E	49.61 F	

Means followed by the same letters within postharvest treatments, storage periods and their interactions in each season are not significantly different at level  $P \leq 0.05$  according to DMRT.

In conclusion, postharvest application of 'Valencia' orange fruits with 1 mM SNP, 10 mM OA and 2% H<sub>2</sub>O<sub>2</sub> especially 1 mM SNP in combination with 10 mM OA or 2% H<sub>2</sub>O<sub>2</sub> treatments controlled decay incidence and reduced fruit weight loss percentage with increased marketable percentage as well as maintained fruit firmness. Moreover, these applications reduced the activities of PPO and PE enzymes besides enhanced the activity of POX enzyme with maintained the inner fruit quality during cold storage at 8°C and 85-95%RH for up to 15 weeks.

*Author contributions:* A.F. Abd El-khalek conceived of study, designed the experiment and purchased the chemicals. A.F. Abd El-khalek and H.G. Elmehrat performed the experiment. Gehan. A. Mahmoud determined enzyme activities only. A.F. Abd El-khalek analyzed the data and wrote the manuscript. M.A.A Mohamed revised the manuscript.

*Conflicts of interest:* the authors declare that there are no conflicts of interest related to the publication of this study.

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## المعاملة بأكسيد النيتريك وحامض الأوكساليك وفوق أكسيد الهيدروجين لتقليل التلف والمحافظة علي جودة ثمار البرتقال الفالانشيا أثناء التخزين المبرد

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تم دراسة تأثير معاملات ما بعد الحصاد بأكسيد النيتريك وحامض الأوكساليك وفوق أكسيد الهيدروجين والخلط فيما بينهم في المحافظة علي جودة ثمار البرتقال الفالانشيا والفترة التسويقية و مضادات الأكسدة بعد الحصاد في موسمي ٢٠١٤ و ٢٠١٥. تم معاملة الثمار عن طريق نقعها لمدة خمس دقائق في الماء المقطر (كنترول) ، محاليل نيتروبروسيد الصوديوم ١ مللي مولار، حامض الأوكساليك ١٠ مللي مولار، فوق أكسيد الهيدروجين ٢٪ ، نيتروبروسيد الصوديوم ١ مللي مولار + حامض الأوكساليك ١٠ مللي مولار ، نيتروبروسيد الصوديوم ١ مللي مولار + فوق أكسيد الهيدروجين ٢٪ ، حامض الأوكساليك ١٠ مللي مولار + فوق أكسيد الهيدروجين ٢٪ وتم تخزين جميع العينات علي درجة ٨±١ مئوية ورطوبة نسبية ٨٥-٩٠٪ متبعة بأسبوع فترة محاكاة تسويقية علي درجة حرارة الغرفة ١٨-٢٣ درجة مئوية ورطوبة نسبية ٥٥-٦٥٪ لمدة ١٥ أسبوع. معاملات ما بعد الحصاد للثمار حافظت علي جودة الثمار بعد الحصاد بالمقارنة بالثمار غير المعاملة. علاوة علي ذلك، معاملة الثمار عن طريق الخلط كانت أكثر كفاءة عن تلك غير المعاملة أو المعاملة بصورة منفردة. المعاملة التطبيقية بواسطة خلط محاليل نيتروبروسيد الصوديوم بتركيز ١ مللي مولار مع حامض الأوكساليك بتركيز ١٠ مللي مولار أو فوق أكسيد الهيدروجين بتركيز ٢٪ قللت حدوث التلف مع زيادة النسبة المئوية لثمار البرتقال الفالانشيا القابلة للتسويق وتقليل النسبة المئوية لفقد الثمار في الوزن. إضافة إلي ذلك، خفضت هذه المعاملات النشاط الإنزيمي للبولي فينول أكسيديز والبكتينيز بينما عملت علي زيادة النشاط الإنزيمي للبيروكسيديز. هذه المعاملات قللت أيضاً من الفقد في قيم الصلابة، اللعنان، زاوية اللون ومحتوي الثمار من العصير. إضافة إلي ذلك، فإن هذه المعاملات أخرجت محتوى الثمار من المواد الصلبة الذائبة الكلية ، النسبة بين المواد الصلبة الذائبة الكلية/الحموضة المقدره وأيضاً عملت علي تقليل الفقد في الحموضة المقدره وحامض الأسكوربيك بالمقارنة بالثمار غير المعاملة أثناء التخزين المبرد. لذا فإن استخدام معاملات ما بعد الحصاد عن طريق خلط نيتروبروسيد الصوديوم بتركيز ١ مللي مولار مع حامض الأوكساليك بتركيز ١٠ مللي مولار أو فوق أكسيد الهيدروجين بتركيز ٢٪ ممكن أن تكون إستراتيجية جيدة لتحسين المقدره التخزينية وتقليل التلف الحادث وأيضاً المحافظة علي جودة ثمار البرتقال الفالانشيا أثناء التخزين المبرد.