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Effect of Salicylic acid and Polyethylene Glycol Application on Physiological and Chemical Changes of Valencia' Oranges During Cold Storage

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Abstract: The effect of salicylic acid at 0.5, 1.0 and 1.5 mM and polyethylene glycol at 0.2, 0.4 and 0.6 % on physiological and chemical changes of Valencia Oranges during cold storage were evaluated during 2014 and 2015 seasons. Fruit treated with high concentrations of both substances exhibited trace to light chilling injury which recorded 1.75 and 2.25 scale, respectively. For that, it could be considered that polyethylene glycol at 0.2 & 0.4 % and salicylic acid at 0.5mM treatments are a good pre – storage treatments to minimize chilling injury, reduction in the weight loss %, attaining the highest SSC content and the highest values of L- Ascorbic of oranges storied at low temperature (5°C ± 1).Whereas the high concentrations of both PEG and SA exhibited the highest values of weight loss, the lowest SSC values, low titratable acidity content and L- Ascorbic. Additionally, total phenol and total flavonoid components increased with high SA and PEG concentrations which could be considered the main reason for minimizing chilling injury on Valencia fruits during cold storage. No signs of decay on control or the other used treatments during the two months of cold storage.

Key wards: Salicylic acid (SA) · Polyethylene Glycol (PEG) · Cold storage · Valencia Orange Chilling injury · Phenol components · Flavonid components

INTRODUCTION

Citrus are tropical fruits which known to be susceptible to chilling injury (CI) development during cold storage [1, 2]. Occurrence of postharvest chilling injury and decay has been found as the main reason for losses of about 40% of the total production of sweet oranges [3, 4]. The most symptoms of CI are pitting, water soaked areas, browning of the flavedo (the outer pigmented layer of the rind) and a decrease in percentage juice after the fruit is removed from cold storages. Several post-harvest treatments have been used to alleviate chilling injury and decay of citrus fruit [5]. The application of wax coatings to fresh fruit in order to replace the natural waxes that have been removed by handling washing and brushing procedures is a usual practice by the postharvest fresh citrus industry [6].

Salicylic acid (SA), which belongs to a group of phenolic compounds, is widely distributed in plants and it is now considered as a hormonal substance, playing an important role in regulating stress responses as well as many processes regarding plant growth and development, inhibiting ethylene biosynthesis and delaying the fruit senescence [7-10]. The involvement of SA in systemic acquired resistance, associated with the production of pathogenesis-related proteins, has been extensively reported [11]. Moreover, dietary salicylates from fruit and vegetables are described as bioactive compounds with health care potential and considered as generally recognized as safe [12]. There are several studies indicating beneficial influences of SA treatment on storability of fruits as supplementary refrigeration and coated treatments, SA significantly increased resistance to chilling injury in plants such as peach and pomegranate [13 -15]. Application of SA prevented the increase in the concentration of H_2O_2, reduced the peroxidase and the increase in the activity of catalase, superoxide dismutase and ascorbate peroxidase [13]. Also prevented chilling-induced increase in lipid peroxidation in membranes [16, 14].

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Polyethylene Glycol (PEG) is a compound of high molecular weight is a non–penetrating inert osmoticum lowering the water potential of the nutrient solution without being taken up or being phytotoxic [17]. Ion and osmotic homeostasis are necessary for plant to be salt and drought tolerant. Osmotic homeostasis is accomplished by accumulation of compatible osmolytes in the cytosol for intracellular osmotic homeostasis [18, 19]. These include the accumulation of endogenous free proline which contributes in preventing dehydration and cellular damage via balancing the osmotic potential of the cytoplasm with surrounding environment [20].

PEG treatments enhanced the biosynthesis of soluble protein contents, increase in antioxidant enzyme activity and enhance membrane fusion [21, 22]. PEG stimulates water stress in cultured plant cells in the same way it does in the cells of intact plants. The phenomenon of cross tolerance has also gained the attention of researchers in recent years. Cross tolerance refers to the exposure of tissue to a moderate stress that induces resistance to another stress [23].Polyethylene glycol (PEG) treatment can improve chilling tolerance of soybean seeds and barley cultivars [24, 25]. Also used as a coatings reduced ethane permeance of orange and apple peels [26].

The goal of this study was to determine the effect of SA and PEG application on key components of the phenolic content and flavonoid content associated with chilling injury as well the effect on physiological and chemical changes of Valencia' oranges during cold storage.

MATERIALS AND METHODS

Valencia oranges were harvested randomly from a private orchard in El- Behera Governorate. Fruit were harvested in late March of the a commercial maturity during two seasons of 2014 and 2015. Average fruit weight at harvested were 195.9 ± 11.5 g., total soluble solids 8.7± 0.22 % and total acidity 1.12± 0.11%.

Fruit were delivered to the laboratory on the day of harvest, sorted for uniform size and lack of defects, washed with tap water containing Clorox solution at 1 % (0.05% Sodium hypochlorite) and air dried, then divided into 3 groups and treated with the following treatments:

Salicylic Acid Treatments: The first groups were dipped in different concentrations of salicylic acid aqueous solutions (0.5, 1.0 and 1.5 mM) for 5 min at 20°C ± 1 with the addition of Tween 20 surfactant (1.0 ml/l).

Polyethylene Glycol -6000 Treatments: The second groups were dipped in solution at PEG 6000 was used 0.2, 0. 4 and 0.6 % (2, 4 and 6 g PEG-6000 in soluble water until 1000 ml) for 5 min at 20°C ± 1 with the addition of Tween 20 surfactant (1.0 ml/l).

Control Treatment: Fruits of the third groups were dipped in distilled water containing Tween 20 surfactant (1 ml/L), then the fruits were coated with 5% commercial wax.

The treatments used were: Control, 0.5 mM salicylic acid, 1.0 mM salicylic acid, 1.5 mM salicylic acid, 0.2 % PEG 6000, 0. 4% PEG 600 and 0.6 % PEG 6000.

Each treatment consisted of 36 fruits and was replicated thrice (12 fruits of each). Fruits of all treatments were placed in Carton boxes (60×40×15 cm). at 5°C (chilling temperature) and 80-85% RH for up to 60 days five fruits from each treatment and replicate were sampled and stored at random and stored for a further 5 days at 20°C ± 2 as a market conditions.

A sample of randomly selected fruits at the beginning of cold storage duration (0 day) and bimonthly (15 days) intervals was collected from each replication for all treatments during the cold storage 5+1°C and the end of market conditions at 20± 2°C.

Effect of the tested treatments on "Valencia" oranges fruits were evaluated through the following determinations.

Fruit Physical Properties: Estimation of chilling injury (CI) index: Fruit were evaluated for chilling injury severity according to the following scale: 0 = normal (nil rind damage), 1 = trace (a few scattered pits), 2 = slight (pitting covering up to 10% of the fruit surface), 3 = moderate(pitting covering up to 30 % of the fruit surface) and 4= severe (extensive pitting covering >30% of the fruit surface). The CI index was calculated using the following formula:

CI = Σ (value of hedonic scale) x (number of fruit with the corresponding scale number)/ total number of fruit in the sample [27]. Fruit was considered unacceptable for the consumer if it had CI indices of 1 or higher.

Fruit Weight Loss Percentage: The initial weight of " Valencia" oranges fruits was recorded in each treatment and at weekly intervals, then fruit weight loss% was calculated by weighing the same fruits at each interval and at the end of cold storage duration using the following formula:

$$WL\% = \frac{\text{Fruit initial weight - Fruit weight at each sampling date}}{\text{Fruit initial weight}} \text{X } 100$$

Fruit Decay Percentage: The decayed fruits of each treatment were discarded and weighed. The weight of such discarded fruits related to the initial weight of fruits per each treatment was estimated and decay percentage was calculated.

Overall Quality: The end of market conditions at 20± 2 °C overall visual and eating quality was rated subjectively into one of five categories: 4 (excellent), 3 (good), 2 (fair), 1 (poor) and 0 (very poor), by an informal panel of five people familiar with oranges [2].

Fruit Chemical Properties: Soluble solids content, total acidity and L- ascorbic acid: Soluble solids content (S.S.C) of fruit juice were measured using a hand refractometer and expressed as a percent. Moreover, fruit titratable acidity (TA) grams of citric acid per 100ml of juice and L- ascorbic acid (LA) content (milligrams ascorbic acid per 100ml fruit juice) according to A.O.A.C. [28].

Determination of Total Phenolic Content: Total phenolic contents of the fruit extracts was measured using a modified colorimetric Folin-Ciocalteu method with further slight modifications [29]. Fruit extracts (0.5 mL) were placed in a test tube. Folin-Ciocalteu reagent (2.5 mL) was added to the solution and allowed to react for 3 min. The reaction was neutralized with 2 mL of sodium carbonate (7.5 %). Absorbance at 765 nm was read after 30 min. Gallic acid was used as standard and data were expressed as mg Gallic acid equivalents (GA)/ g FW.

Determination of Total Flavonoid Content: The total flavonoid content of the samples was measured using a colorimetric method [30, 31]. The methanolic extract (1 mL containing 0.1 g/mL FW) was mixed respectively with 4 mL DI water in a 10 mL volumetric flask and 3 mL of 5% $NaNO_2$ solution, then allowed to mix for 6 min. After addition of 0.3 mL of 10% $AlCl_3$ solution and mixing for 5 min, the reaction was initiated by adding 2 mL of 1.0 M NaOH and the total volume was made up to 10 mL with DI water. Sample absorbance was read at 510 nm using a spectrophotometer. Catechin standard was used as standard and total flavonoid content was expressed as ìg. Catechin equivalents (CA)/g FW

Statistical Analysis: Data obtained in the two studied seasons were conducted in a Completely Randomized Design (CRD) with three replications. Data obtained were subjected to Analysis of variance (ANOVA) using MSTAT-C software (MSTAT Michigan University East Lansing). Duncan multiple rang test (LSR) was performed to determine any significant difference among various treatments. $p<0.05$ was selected as decision for significant differences according to Steel *et al.* [32].

RESULTS AND DISCUSSION

Fruit Physical Analysis

Visual Chilling Injury Symptoms (CI): Data tabulated in Table (1) showed that, chilling injury symptoms greatly increased with advanced in days of cold storage at 5 ± 1°C regardless the used pre storage treatments. Fruits treated with high concentrations of both substances under study (SA and PEG) exhibited chilling injury recorded (1.75 and 2.25 scale)respectively during the 1st season, whereas SA at 0.5 mM and 0.2 % PEG treatments achieved (0.48 & 0.57 and 0.39 &0.61 scale) at the end of cold storage, during the first and second season respectively. However, the other treatments recorded scale between (slight) with the use of salicylic acid at 1.0 mM & 0.4%PEG. This finding is logic due to that same citrus fruits are sensitive to chilling injury and suggested treatments in this work are a trial to minimize this sensitivity. Generally, it could be noticed that PEG at 0.2& 0.4 % and SA at 0.5 mM treatments could be considered a good pre-storage treatments to minimize chilling injury in orange fruits during transportation at low temperature (5°C ±1). Various secondary metabolites are produced through the phenylpropanoid pathway, including lignins, flavonoids and coumarins, most of which are important metabolites to plants [33]. This pathway starts with cinnamic acid synthesis from phenylalanine by phenylalanine ammonia lyase (PAL) in the cytosol and the pathway is modulated by PAL, which is the rate-limiting enzyme [34]. PAL activity may be regulated by feedback inhibition by the pathway product, cinnamic acid, which may modify the expression of the PAL gene [35, 36]. Phenylpropanoid compounds have important roles in plant defense mechanisms, reproduction and development [33]. Activities of PAL were induced by various stimuli, such as chilling [37, 38, 27, 39, 40]. PAL, may have distinct metabolic functions, such as flavonoids, lignin biosynthesis, salicylic acid (SA). Low temperature increased the activity of PAL that leads to reduced accumulation of SA, making the plants more susceptible to Pseudomonas syringae [27, 41].

Table 1: Effect of salicylic acid and polyethylene glycol postharvest treatments on the visual chilling injury symptoms of " Valencia" oranges under cold storage at 5±1° C, during 2014 and 2015 seasons

	Days in cold storage at 5±1° C			
	15	30	45	60
Treatments	Season 2014			
Control	0.0 b	0.47 b	0.79 b	1.17 c
0.5 mM SA	0.0 b	0.0 c	0.13 c	0.48 d
1.0 mM SA	0.0 b	0.77 a	1.31 a	1.54 bc
1.5 mM SA	0.0 b	0.81 a	1.35 a	1.75 ab
0.2 % PEG	0.0 b	0.23 bc	0.32 bc	0.57 d
0. 4% PEG	0.0 b	0.32 b	0.41 bc	0.66 d
0.6 % PEG	0.33 a	0.97 a	1.65 a	2.25 a
	Season 2015			
Control	0.0 a	0.32 b	0.68 cd	0.97 cd
0.5 mM SA	0.0 a	0.0 c	0.24 e	0.39 e
1.0 mM SA	0.0 a	0.81 a	1.18 ab	1.68 ab
1.5 mM SA	0.0 a	0.64 a	0.92 bc	1.35 bc
0.2 % PEG	0.0 a	0.15 bc	0.52 de	0.61 de
0. 4% PEG	0.0 a	0.22 bc	0.45 de	0.75 de
0.6 % PEG	0.18 a	0.88 a	1.35 a	2.19 a

Values followed by the same letter (s) of each column are not significantly different at 5% level.

PEG: polyethylene glycol, SA: salicylic acid.

Table 2: Effect of salicylic acid and polyethylene glycol postharvest treatments on weight loss % of " Valencia" oranges under cold storage at 5±1° C, during 2014 and 2015 seasons

	Days in cold storage at 5±1°C			
	15	30	45	60
Treatments	Season 2014			
Control	0.84 bc	1.54 bc	2.11 bc	2.76 d
0.5 mM SA	0.50 c	0.87 d	1.05 d	1.43e
1.0 mM SA	1.05 ab	1.83 ab	2.31 bc	3.59 b
1.5 mM SA	1.32 ab	2.11 ab	2.71 ab	4.10 bc
0.2 % PEG	0.66 c	0.95 cd	1. 47 cd	1.92 cd
0. 4% PEG	0.78 bc	1.21 cd	1.83 cd	2.16 cd
0.6 % PEG	1.69 a	2.58 a	3.75 a	4.88 a
	Season 2015			
Control	0.92 bc	1.33 bc	1.96 bc	2.25 d
0.5 mM SA	0.76 c	1.12 c	1.50 c	1.79 d
1.0 mM SA	1.31 ab	1.84 b	2.69 b	3.92 b
1.5 mM SA	1.53 a	1.96 b	2.55 b	3.48 bc
0.2 % PEG	1.01 bc	1.31 bc	2.03 bc	2.63 cd
0. 4% PEG	0.82 c	1.14 c	1.76 c	2.33 cd
0.6 % PEG	1.88 a	2.77 a	4.05 a	5.19 a

Values followed by the same letter (s) of each column are not significantly different at 5% level.

PEG: polyethylene glycol, SA: salicylic acid

The effect of SA on CI probably attributed to more reducing status of ascorbate and glutathione, less O_2 accumulation and more H_2O_2 accumulation. The oxidative stress caused by the accumulation of reactive oxygen species (ROS) together with a reduction in the antioxidant system were involved in CI development in fruit during storage [42]. Also, the effect of SA on controlling chilling injury of peaches was attributed to its ability to induce antioxidant systems and heat shock protein (HSPs) [13].

Decay Fruits %: There were no decay % on control or other treatments until the end of storage period.

Weight Loss %: Data in Table (2) revealed that the low concentrations of salicylic acid at 0.5 mM concentration showed better results during the storage period and PEG at 0.2% significantly decreased fruit weight loss as compared with control and other treatment. The highest values of fruit losses were achieved with increasing storage period to 60 days and increase SA & PEG concentrations. Data of weight loss showed a slight differences among treatments at the first sample (15 days) where (ranged from 0.50 to 1.69 in the first season and 0.76 to 1.88 % in the second season). With advanced in storage durations and after 30 days, PEG 0.6% treated fruits lost higher weight (2.58 and 2.77 %) than the other treatments during both seasons respectively. When chilling injury occurs, the associated abnormal metabolism may be reflected in an increase the damaged tissues causing microscopic cracks in the peel and pits. Such cracks enable a greater loss water from the fruit [43, 44]. The end of cold storage, the high concentration of PEG and SA exhibited the highest values of weight loss than others. Whereas, low level of salicylic acid at 0.5 mM and PEG at 0.2% and 0.4 % led to a reduction in the weight loss % without significant different between them.

SA, gradually returned to control level. Treatment with elicitor molecules, such as SA resulted in the activation of defense mechanisms, including induction of cell wall strengthening and defense enzymes [45]. PAL transcripts from Lycoris radiata (LrPAL) were significantly induced by MeJA and moderately increased by SA [46]. The addition of exogenous SA also induced PAL activity in pear and *Saussurea medusa* cell cultures [14, 47]. Addition of SA induced expression of enzymes that were important to the PAL pathway, including the PAL gene [48-50].

Table 3: Effect of salicylic acid and polyethylene glycol postharvest treatments on Soluble solids content (SSC) of " Valencia" oranges under cold storage at 5±1° C, during 2014 and 2015 seasons

	Days in cold storage at 5±1° C			
	15	30	45	60
Treatments	Season 2014			
Control	10.5 a	10.5 a	10.5 ab	10.8 ab
0.5 mM SA	10.6 a	10.7 a	10.9 a	11.2 a
1.0 mM SA	10.0 a	10.0 a	9.8 bc	9.6 c
1.5 mM SA	10.3 a	10.2 ab	10.0 bc	9.2 c
0.2 % PEG	10.4 a	10.5 a	10.7 a	10.9 ab
0. 4% PEG	10.5 a	10.6 a	10.6 ab	10.8 ab
0.6 % PEG	9.7 a	9.5 b	9.1c	9.0 c
	Season 2015			
Control	11.0 a	11.0 a	11.2 ab	11.4 ab
0.5 mM SA	11.2 a	11.3 a	11.7 a	11.8 a
1.0 mM SA	10.8 a	10.7 a	10.3 c	10.5 bc
1.5 mM SA	11.0 a	10.8 a	10.6 bc	10.0 c
0.2 % PEG	11.0 a	11.3 a	11.5 ab	11.5 a
0. 4% PEG	11.0 a	11.2 a	11.3 ab	11.5 a
0.6 % PEG	10.3 a	10.5 a	10.3 a	9.8 c

Values followed by the same letter (s) of each column are not significantly different at 5% level.
PEG: polyethylene glycol, SA: salicylic acid

Fruit Chemical

Soluble Solids Content: The results illustrated in Table (3) showed that SSC of fruits increased slightly during storage, the highest levels of salicylic acid and PEG maintained significantly lower S.S.C than the control and other treatments in both seasons. The end of storage period at 5± 1° C, the highest SSC content was detected in SA at 0.5 mM treatment which give (11.2 and 11.8%) during 2013 and 2014 seasons respectively.

On the other hand, the lowest SSC content were determined in SA at 1.5 mM and PEG at 0.6 % treatments which recorded (9.2 & 9.0 and 10.0 & 9.8 %) during both seasons under study at the end of cold storage period (60 days). The increased amounts of soluble solids over the storage period could due to weight loss and, therefore, fruit juice concentration. SA treatments at 1 and 2 mM reduced fruit SSC during storage, compared to control [51]. Treatment of Kiwifruits with MeSA maintained a lower SSC content than the control fruits at the end of cold storage [52].

However, a number of researchers reported that SA treatment had no effect on SSC of several fruits like grape and persimmon [53, 54]. Reduced ethylene production in SA treated fruits may result in lowered enzyme activity, leading to a decrease in sucrose synthesis [54].

Titratable Acids: The results in Table (4) showed that the higher salicylic acid and PEG concentrations recorded a significantly higher acidity (%) than the control and the lowest concentration. Titratable acidity at the banning of cold storage (after 15 days) at 5±1° C was recorded from 0.79 to 0. 87 mg/100 ml for the first season and from 0.87 to 0.93 mg/ 100 ml for the second season, acidity content significantly decreased with prolonging storage, in both seasons .After 45 and 60 days, TA content was higher in fruits treated with 0.6% PEF than other treated fruits in both seasons. Whereas the lower concentration of salicylic acid gave the less percentage of total acidity.

It is well known that most of the hypothesis in relation to fruit chilling injury suggested a sharp respiratory drop or membrane crystallization or cytoplasmic streaming inhibition which in consequently cause a dramatic drop in the required activating energy of several enzymes. The inhibition of such enzymes as a result of chilling injury may resulted in accumulation of their substances which are acidic metabolic substances. The accumulation of such middle substances result in the subsequent development of chilling injury symptoms Since most of this middle substances are acid it is logic, therefore, to assume that higher acidity level will be paralleled to the fruits having symptoms of chilling injury. It is suggested, therefore, that acidity increment is an accurate inductor for fruit chilling injury [44]. The fact that SA treated fruit showed a higher TSS may be attributed to the alleviated CI. The contents of organic acids in fruit are mainly dependent on the activities of their synthetic and hydrolytic enzymes, SA treatment was probably regulated the activities of related enzymes [55].

L- Ascorbic Acid (mg / 100 ml Juice): Generally, it was noticed that ascorbic acid content in fruits decreased slightly and gradually with low concentrations of salicylic acid and PEG while, decreased sharply with increasing the time of storage and increasing SA& PEG rates in both seasons.

The results presented in Table (5) indicated that, in both experimental seasons, ascorbic acid content It was found that T_1 (SA at 0.5 Mm) and T_4 (PEG at 0.2 %) gave the highest significant values of L-ascorbic acid compared to control during the all duration 0f storage. As for the changes in fruit ascorbic acid content during storage, results showed that there was a significant decrease in the juice ascorbic acid content with prolonging the storage time in both seasons.

Table 4: Effect of salicylic acid and polyethylene glycol postharvest treatments on Titratable acids % of " Valencia" oranges under cold storage at 5±1° C, during 2014 and 2015 seasons

Treatments	Days in cold storage at 5±1° C: 15	30	45	60
	Season 2014			
Control	0.86 a	0.74 a	0.70 bc	0.67 c
0.5 mM SA	0.87 a	0.76 a	0.64 c	0.53 d
1.0 mM SA	0.81 a	0.70 a	0.76 ab	0.78 b
1.5 mM SA	0.79 a	0.73 a	0.78 ab	0.87 ab
0.2 % PEG	0.85 a	0.77 a	0.70 c	0.61 cd
0. 4% PEG	0.84 a	0.74 a	0.69 c	0.64 c
0.6 % PEG	0.82 a	0.72 a	0.81 a	0.98 a
	Season 2015			
Control	0.91 a	0.86 a	0.81 bc	0.78 c
0.5 mM SA	0.93 a	0.83 a	0.72 c	0.64 d
1.0 mM SA	0.89 a	0.84 a	0.88 ab	0.95 b
1.5 mM SA	0.90 a	0.81 a	0.86 ab	0.90 b
0.2 % PEG	0.90 a	0.82 a	0.75 c	0.70 cd
0. 4% PEG	0.87 a	0.85 a	0.77 c	0.71 cd
0.6 % PEG	0.92 a	0.84 a	0.96 a	1.18 a

Values followed by the same letter (s) of each column are not significantly different at 5% level.

PEG: polyethylene glycol, SA: salicylic acid.

Table 5: Effect of salicylic acid and polyethylene glycol postharvest treatments on L- ascorbic acid % of " Valencia" oranges under cold storage at 5±1° C, during 2014 and 2015 seasons

Treatments	Days in cold storage at 5±1° C: 15	30	45	60
	Season 2014			
Control	61.50 a	53.23 bc	49.18 b	42.66 b
0.5 mM SA	61.96 a	58.11 a	54.35 a	50.08 a
1.0 mM SA	60.02 a	51.72 cd	43.67 cd	37.70 c
1.5 mM SA	60.31 a	50.22 cd	45.86 c	36.69 c
0.2 % PEG	60.71 a	56.79 a	52.33 ab	48.11 a
0. 4% PEG	61.11 a	55.85 ab	50.11 b	44.56 b
0.6 % PEG	59.46 a	49.39 d	40.84 d	33.13 d
	Season 2015			
Control	63.66 a	56.49 bc	45.66 c	40.75 bc
0.5 mM SA	63.12 a	61.22 a	59.09 a	55.55 a
1.0 mM SA	64.08 a	50.61 d	42.11 d	35.76 d
1.5 mM SA	62.15 a	54.73 c	46.28 c	38.08 cd
0.2 % PEG	64.11 a	59.35 ab	50.56 b	45.06 b
0. 4% PEG	63.72 a	60.06 a	56.69 a	50.82 a
0.6 % PEG	65.07 a	48.39 d	40.60 d	30.02 e

Values followed by the same letter (s) of each column are not significantly different at 5% level.

PEG: polyethylene glycol, SA: salicylic acid

L-ascorbic acid content was decreased with advanced in storage duration in treated or untreated fruits. This finding could be attributed to the conversion of L-ascorbic acid to dehydroascorbic acid and decreasing the active form of ascorbic acid. No significant differences were noticed between different treatments and controls till 15 days of cold storage. However, after 45 days, fruits treated with 0.6% PEG exhibited the least value of L-ascorbic than all treatments. Low temperature storage cause a decline ascorbic acid content [56]. The end of cold storage 0.6% PEG treated fruits contained less values of L-ascorbic than others. This finding is correlated to the previously mentioned about acidity, T.S.S and chilling injury symptoms where this treatment recorded less fruit quality characters. The reduction in L-ascorbic in the treated fruits was from 59.46 mg to 33.13 mg/100 g fresh weight (about 44% losses in L-ascorbic acid) in the first season and from 65.07 to 30.02 mg/100 g fresh weight (about 53% losses in L-ascorbic acid) in second season. On contrary low concentrations contained high values of L-ascorbic acid than other treatments without significant differences between them. This finding was noticed during all storage duration. L-ascorbic acid reduced from 61.96 to 50.08mg (19.2%), 60.71 to 48.11mg (20.7%), 63.12 to 55.55 mg (12.0%) and from 61.96 to 45.06mg (29.8%) in the treated fruits with 0.5mM SA and 0.2% PEG during both seasons under study, respectively.

SA treatments could be used to reduce deterioration and chilling injury symptoms in some fruit [13, 57, 15]. Both pre- and post-harvest SA treatments have been reported as being effective in fruit quality maintenance and storage life extension of strawberry [58]. Lu *et al.* [59] reported that SA delayed the decline of ascorbic acid (AsA) content and prevented AsA destruction, so high contents of AsA in treated pineapple could improve the fruit quality [58, 59].

Total Phenols (mg / g Fresh Weight) and Total Flavonoid (µG /G Fresh Weight): Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions [60]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decaying peroxides [61]. However, the role of phenolic compounds in plant abiotic stresses particularly low temperature stress, has received much less attention.

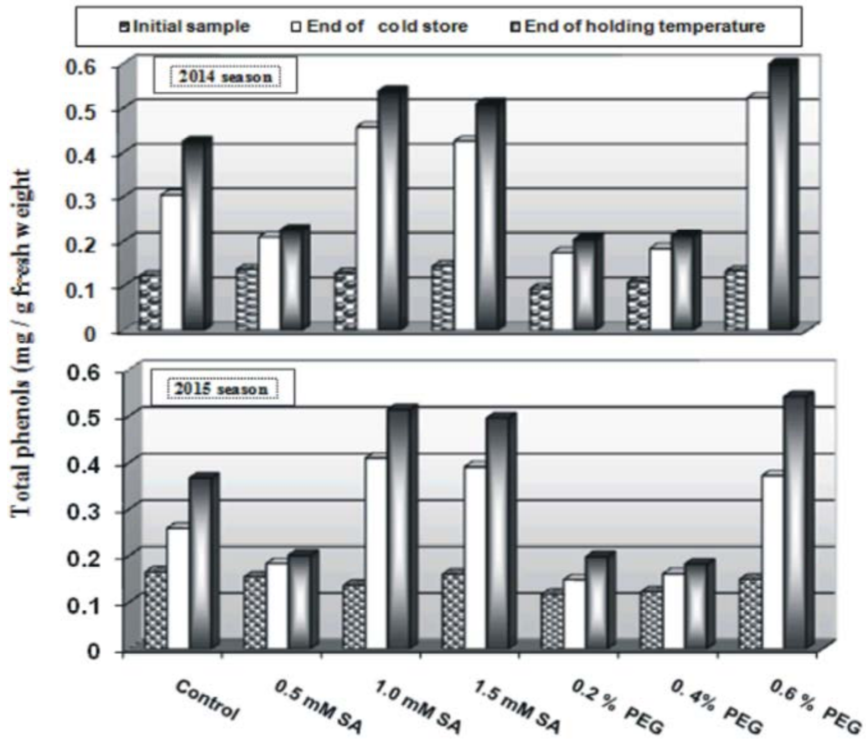

Fig. 1: Effect of salicylic acid and polyethylene glycol postharvest treatments on Total phenols (mg / g fresh weight) of " Valencia" oranges under cold storage at 5±1° C, during 2013 and 2014 seasons

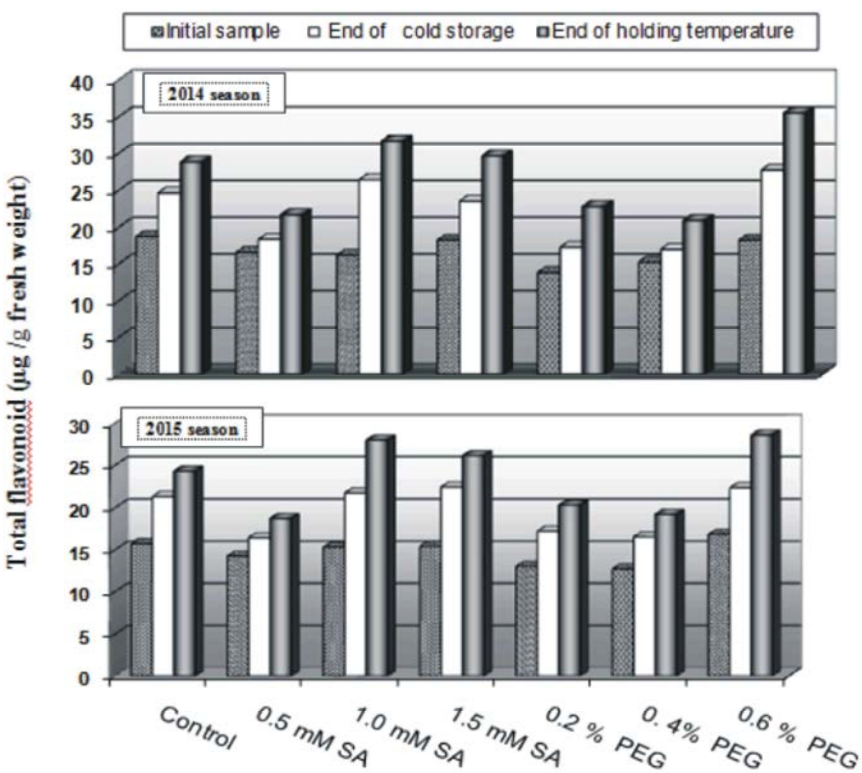

Fig. 2: Effect of salicylic acid and polyethylene glycol postharvest treatments on total flavonoid (µg /g fresh weight % of " Valencia" oranges under cold storage at 5±1° C, during 2013 and 2014 seasons

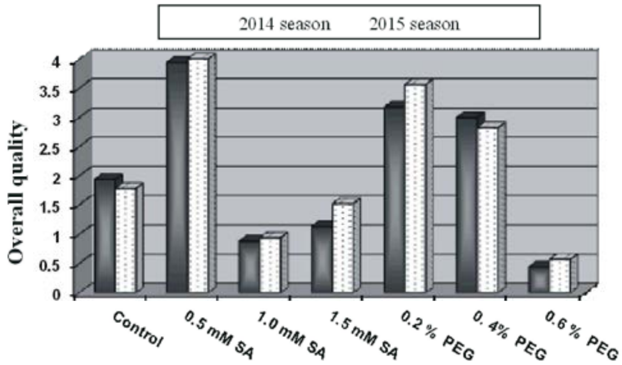

Fig. 3: Effect of salicylic acid and polyethylene glycol postharvest treatments on Overall quality of " Valencia" oranges the end of market conditions at 20± 2 °C, during 2014 and 2015 seasons

Results presented in Figure (1& 2) revealed that, in both experimental seasons, it was noticed that total phenol (mg/g) and total flavonoid (µg /g) content in fruits increased with increased by prolonging storage periods in both seasons. At time zero total phenols ranged from (0.094 to 0.145 mg / g fresh weight) and total flavonoid from (13.8 to 18.7 µg /g fresh weight) in treatments. In cold storage the maximum values of total phenols (0.523 mg / g) and total flavonoid (27.7 µg /g) were found in fruit treated with 0.6 % PEG while the minimum values of total phenols and total flavonoid (0.172 mg / g and 16.9 µg /g) were found in treated fruits with 0.2 % PEG.

End of holding temperature these compounds (total phenols and total flavonoid) increased more with high salicylic acid and PEG concentrations which reached (0.631 & 539 mg /g and 31.7 & 35.5 µg /g) respectively. No significant differences were reported among T_1 (SA at 0.5 Mm), T_4 and T_5 (PEG at 0.2 and 0.4%) treatments in both seasons which gave less significant values. The study indicated the beneficial effect of post-harvest treatments (SA and PEG) immersion on 'Valencia oranges' orange fruit quality. Also, they suggest that the chilling stimulates the biosynthesis of phenolics by enhancing PAL activity and PPO synthesis. Further characterization of the browning substrates involved would help to better understand the specific biosynthetic pathway associated with IB development. Moreover, SA treatments inhibited the activities of PPO and PAL, thus reduced TP content production and delayed conversion [62]. The amount of total phenolics was relatively depended on the flavonoid content in Citrus. Increase of phenolic compounds in tissues during chilling treatments may be partially due to chilling adaptation as defense mechanisms for scavenging ROS and also to mediate these stresses [63]. Consequent decrease in freezing temperatures was also probably due to polymerization and oxidation of phenolic compounds. It is assumed that freezing temperatures with disruption of cell membranes may trigger the release of oxidative and hydrolytic enzymes that would destroy the antioxidants. Probably, deactivating these enzymes avoid the loss of phenolics and, therefore, lead to the increase of total phenolics content.

At the end of an additional week of storage at 20°C, external appearance and eating quality of oranges declined slightly but remained good. The overall quality of the oranges in both external appearance and eating quality remained acceptable after 2 months of storage. Sensory evaluation greatly affected with some pre storage treatments of orange fruits. However the treated fruits with 0. 2% PEG and 0.5 Mm SA got high scores for color, juiciness and taste during sensory evaluation compared with treated fruits with high concentrations of PEG and SA which exhibited unmarketable, sensory evaluation. No significant differences among untreated fruits and fruits treated with 0. 4 % PEG and 1.0 Mm SA which achieved values moderate.

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