Arab Univ.
J. Agric. Sci.,
Ain Shams Univ.,
Cairo, 16(1), 137-159, 2008

EFFECT OF VAPOR GARD AND CALCIUM CHLORIDE TREATMENTS ON KEEPING QUALITY OF NAVEL ORANGES AT DIFFERENT STORAGE TEMPERATURES

[10]

Hanafy-Ahmed[1], A.H.; H.M. Rashad[1]; Samia, G. El-Oraby[2] and Amira, F. El-Wakil[2]

1- Plant Physiology Section, Agricultural Botany Dept., Faculty of Agriculture, Cairo University, Egypt

2- Fruit Handling Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt

Keywords: Citrus; Cold storage; Antitranspirant; Calcium chloride; Metabolic and physiological processes

ABSTRACT

The experiments were conducted in two successive seasons (2004 - 2005) on Washington Navel orange fruits to study the effect of cold storage conditions on reducing postharvest losses and improving keeping quality for the export market.Fruit samples were obtained from El–Fayoum governorate, Egypt and treated with vapor gard (VG) 2%, calcium chloride ($CaCl_2$) 4% or vapor gard 2% + calcium chloride 4%. Fruits were stored at 2 or 8 ºC for 60 days and the last group was stored at 2 ºC for 30 days and transferred to be stored at 8ºC for another 30 days at R.H. 85-90%.The results revealed that during storage fruits treated with Vapor gard (2%) alone or with calcium chloride (4%) + Vapor gard (2%) had been in good quality as well as it caused a pronounced increase in peel color and fruit firmness, while fruit weight loss and juice percentage were decreased. In addition, it caused a significant increase in ascorbic acid, total sugar and calcium concentration, but there is no significant effect in T.S.S. / acid ratio. While, free amino acid, total soluble phenols and free proline concentrations were decreased. Furthermore, the fruits storaged at 2º + 8ºC caused a pronounced increase in fruit firmness, while fruit weight losses, juice percentage and peel color were decreased. Also, it caused a pronounced decrease in T.S.S. / acid ratio, total sugar, total free amino acid, total soluble phenols and free proline concentrations, but there is no significant effect on calcium concentration. In addition, there is an increase in ascorbic acid concentration. Generally, the results revealed that Vapor gard either alone or combired with calcium chloride dipping treatments and stored at 2º + 8ºC had better results in improving fruit quality and decreasing total fruit losses compared with control or calcium chloride treatment alone at other storage temperatures and this was also accompanied by changes in various metabolic and physiological processes of orange fruits.

INTRODUCTION

Chilling injury is a physiological disorder which develops in citrus when they exposed to low temperature. Various treatments have been shown to be helpful in eliminating the chilling injury and maintaining the quality of different fruits and vegetables at low temperatures (**Wang, 1991**). Different postharvest treatments have been carried out to improve the physiological postharvest characteristics of fruits, such as exogenous calcium application on mandarin (**El-Hilali *et al* 2004**) as well as using antitranspirant agents such as Vapor gard (VG) (a polymer of B – pinene) which considered as one of the most successful compounds currently used in horticulture (**El- Sabrout, 1996 and Agusti *et al* 1997**).

Thas, the aim of this research is to study the effect of some safe postharvest treatments such as Vapor gard and Calcium chloride as supplemantry

(Received October 23, 2007)
(Accepted November 19, 2007)

refrigeration treatments on fruit quality as well as the changes of chemical constituents during storage of Navel orange fruits.

MATERIALS AND METHODS

This study was conducted in seasons 2004 and 2005 on Washington Navel oranges (20 years old) budded on Sour orange rootstocke and grown in a private orchard at El-Fayoum Governorate, Egypt. Washington orange fruits were at maturity stage (October) when fruits at the peel color break. The maturity stage is characterized by T.S.S. / acid ratio was 7.8-8.3 and juice % was 45.1-46.8 according to harvest indices of maturity (**Spiegel-Roy and Goldschmidt, 1996**).

All fruits were washed with water and soap mixture and rinsed with water to remove the residue of soap. Finally, the fruits were air dried. The fruits were divided into four groups: a) Fruits without treatment (control), b) Fruits were dipped in 2% Vapor gard (pinolene) (di – 1 – p – menthene 96 %) for 5 minutes and dried, c) Fruits were dipped in 4 % calcium chloride ($CaCl_2$) for 5 minutes and dried, d) Fruits were dipped in 4 % $CaCl_2$ for 5 minutes and dried then immersed in Vapor gard (2 %) for 5 minutes and dried.

Fruits of each group were packed in carton boxes, each box contained 5kg arranged in one layer. Each group consisted of 27 boxes. Boxes of each group were divided into three sub- groups. The boxes of the first sub-group (9 boxes) were stored at 2ºC for 60 days. The second sub-group (9 boxes) were stored at 8ºC for 60 days .Meanwhile, boxes of the third sub-group were stored at 2ºC for 30 days then at 8ºC for another 30 days. The relative humidity during storage reached 85-90%. From each sub-group, five samples were taken during storage period, i.e. 0, 15, 30, 45, and 60 days for fruit physical and chemical analysis.

Fruits physical properties

A number of parameters were recorded and calculated such as, losses in fruit weight percentage, peel color, fruit firmness and juice percentage. Peel color and fruit firmness were measured according to **McGuire, (1992)** and **Harold, (1985**), respectively. Penetrating cylinder of 1 mm of diameter to a constant distance 5 cm inside the skin (to the flesh by a constant speed 2 mm / sec.) the results were expressed as the resistance force to the penetrating tester, in units pressure gm / cm^2 (**Harold, 1985**).Juice percentage was extracted and weighed, then calculated as percentage of fruit weight (w /w).

Fruit chemical properties

Peel and flesh fruits were extracted by ethanol (80 %) to determine total sugars, total free amino acids and total soluble phenols. Total sugars were estimated by using the phenol sulphuric acid method according to **Dubois *et al* (1956**). Total free amino acids were determinated by using ninhydrin reagent according to **Moore and Stein, (1954**). Total soluble phenols were determined using the folin ciocalteau colorimeteric method (**Swain and Hillis, 1959**). Total soluble solids of juice were determined by Abb digital refractometer.

The obtained juice was used to determine titratable acidity by titration with 0.1 N sodium hydroxide in the presence of phenolphthalein as an indicator according to **Chen and Mellenthin, (1981)** and calculated as mg of citric acid per 100 ml juice. Ascorbic acid (Vitamin C) was determined in above filtered juice samples by using 2,6 dichlorophenol endophenol and expressed as mg / 100 ml fruit juice as described by **A.O.A.C. (1995)** .

Free proline was measured colorimetrically in the extraction of fresh weight using ninhydrin reagent according to **Bates *et al* (1973)**.

The wet digestion of 0.5 g dry plant materials (rind and flesh of orange fruits) was carried out with sulphuric and perchloric acid as reported by **Piper, (1947)** for determination of calcium. Calcium concentration was determined by using atomic absorption spectrophotometer (Thermo Jarrellash, A A S C A N I).

Polyamines

Free polyamines as putrescine (put) were only determined in the second season in peel and flesh fruits during the middle and end of storage duration by dansyl chloride reagent and quantified with spectrophotofluroimeter according to **Galston, (1983)**.

All data were arranged in complete randomized design as factorial experiment and statistically analyzed according to **Snedecor and Cochran, (1980).**

RESULTS AND DISCUSSION

Data presented in **Table (1)** revealed that there was a significant increase in fruit weight loss at high storage temperature (8ºC) comparing with the other temperatures storage (2 and 2+8 ºC), in both seasons. The fruit weight loss was significantly increased with the advanced storage periods. In addition, the results revealed that calcium chloride and Vapor gard mixture gave the lowest decrease in fruit loss as compared to the other treatments in both seasons.

These results are in agreement with **Mansour *et al* (1987**) who reported that weight loss percentage of Navel oranges was increased as the storage period prolonged. **Attia, (1995**) reported that in Balady orange fruits, the water loss percentage was increased gradually with progress of storage period. This result was attributed to increasing the rate of fruit respiration and evaporation during storage. **Erkan and Pekmezei, (2000**) working on navel orange fruits, found that the longer storage period gave the higher weight loss. Moreover, the weight loss increased with rising storage temperature. The weight loss of the orange fruits stored at 7ºC was higher than fruits stored at 5ºC and /or 3ºC. Moreover, **Lidster, (1981)** found that, the postharvest application of vapor gard (di -1-p-menthene) on cherry fruits caused significantly reduce in the fruit weight loss and was generally associated with a reduction in the incidence of discolored stems and surface pitting . Furthermore, **Mansour *et al* (1987)** reported that there was no consistent relationship between calcium content and weight loss, however, the increase in weight loss in some calcium treatments could be due to the higher juice content, which showed a positive relationship with calcium content. In addition, they observed that fruits with higher calcium content were greener in color as compared with the control fruits, and this could be an enhancing effect on weight loss. On the other hand **Chikaizumi *et al* (1997**) reported that postharvest dips of $CaCl_2$, $CaCO_3$, Ca $(NO_3)_2$ or Ca $(CH_3COO)_2$ at (50 or 200 p.p.m.) had no obvious effects on Navel orange fruits.

Concerning the effect of storage temperature on peel color of Navel orange, the data presented in **Table (2)** indicated that fruits stored at 2ºC had light orange color, while the fruits stored at 8ºC had good orange color, but fruits stored at 2ºC + 8ºC were in between, for both seasons. In this respect, **Erkan and Pekmezei, (2000)** working on Navel orange, indicated that the a* value of the skin increased with increasing storage period and storage temperature until about the 80th day and then continuously decreased during further storage. In addition, the b* value of the skin increased during the storage period.

Concerning the effect of treatments (vapor gard, calcium chloride and the mixture of them) on color, the data showed slight differences between the treatments. Good color was observed in fruits treated with VG and / or $CaCl_2$ + VG. Similar trend was obtained in second season. The external color is one of the most appreciated quality factors of citrus fruits (**Lambrinos and Manolopoulou, 2000**).

Data in **Table (3)** revealed that peel puncture resistance was decreased gradually with prolonged storage period up to the end of storage period, among all treatments in two seasons. The higher value of this character was recorded for fruits stored in descending order as follow; 2ºC, 2ºC + 8ºC and 8ºC. These findings may support the results of fruits weight loss and similar with that obtained by **El-Oraby and Ali, (2002)** on grapefruit and **Hassan, (2004)** on lemon fruits.

Regarding the effect of treatments on peel texture, the results indicated that the fruits treated with VG or $CaCl_2$ + VG had significant higher value than control or fruits treated with $CaCl_2$ alone. In this regard, **Martinez – Romero *et al* (1999)** and **Knee, (1978)** mentioned that calcium involved in the texture of fruits, i.e. the stability of the cell wall may be related to the cooperative binding of polygalacturonate chains with calcium ions,making the cell wall of the fruit less accessible to enzymes that cause softening or to cell wall degrading enzymes produced by fungal pathogens. In the addition, fruits calcium infiltrated at harvest stage remained firmer during storage, probably due to fewer breakdowns in the cell wall, as has been reported by **Sams and Conway, (1984)** on apple. Moreover, **Conway *et al* (1994)** observed that calcium chloride solution infiltration has only a slight effect on maintaining firmness, presumably because Ca-tissue concentration was not increased sufficiently.

Data in **Table (4)** revealed the effect of postharvest treatments on juice percentage in both studied seasons. In general, the effect of postharvest treatments did not have clearly effects on juice percentage. However, the values increased with increasing storage period at overall temperatures. These results indicated a direct correlation between fresh fruit weight and juice percentage. These results were agreed with that reported by

Lambrions and Manolopoulou, (2000), who reported that the juice content showed a steady decrease (6%) in navel orange during storage at 5ºC plus shelf-life. **Erkan and Pekmezei, (2000)** working on navel orange fruits, found that the juice content of the fruits increased at the beginning and decreased during the storage period. **Mansour *et al* (1987)** reported that, juice content as percentage of navel orange fruits weight was increased by calcium chloride application and decreased gradually by storage.

Data in **Table (5)** revealed that total soluble solids (TSS) / acid ratio increased significantly during storage period at the three different storage temperatures. Fruits stored at 2ºC had the lowest percentage of TSS/acid ratio followed by 2º + 8ºC and then 8ºC.

Concerning the effect of different postharvest treatments the data indicated that, no significant effect could be detected on TSS / acid ratio with different postharvest treatments. These results were in agreement with those reported by **Attia, (1995)** on balady orange; **Rodriguez-Felix *et al* (2001)** on Valencia orange, **El–Oraby and Ali, (2002)** on grapefruits and **Hassan, (2004**) on lemon. In this respect, **Mansour *et al* (1987)** reported that, TSS were enhanced by calcium spray over control and storage of the Navel orange fruits. The reduction of TSS increased as the storage period prolonged, but the reduction in TSS was in proportional to storage period more than calcium spraying. Also, the authors reported that, calcium spraying increased juice acidity as compared with un-sprayed ones. Also, acidity decreased by storage and as the period of storage prolonged the reduction became more obvious. Moreover, **Manolopoulou – lambrinou and Papadopoulou, (1995)** reported that, mandarin fruits sprayed with vapor gard reduced the increase in soluble solids as compared to the control and thiabendazole (T.B.Z.) treatments and the storage temperature did not affect the total soluble solids. In this respect, **El-Sabrout, (1996)** noticed that, there was a decrease in TSS% by increasing the concentration of VG for Navel orange fruits. This result could be due to the inhibitory effect of these chemicals on photosynthesis and photophosphorylation processes caused by their high concentrations (**Weller and Ferree, 1978**). Furthermore, VG application decreased juice acidity as compared with the control (**El-Sabrout, 1996**). **Lambrinos and Manolopoulou, (2000**) found that the soluble solids of oranges stored at 5ºC showed an approximate 7.7% increase after 90 days of storage plus 1 week of shelf life. Also, the acidity progressively decreased against time (33% decreases at the end of shelf – life). In addition, **Echeverria and Ismail, (1990)** reported that, TSS content also tended to increase with storage duration. Furthermore, the acidity of fruit juice was significantly higher in the control compared to the other treatments, whereas it declined with storage time which may be attributed to the use of such acids as substrates for respiration (**Echeverria and Valich, 1989**).

Data concerning the effect of vapor gard, $CaCl_2$ and its combination on ascorbic acid concentration at three different temperatures of storage are presented in **Table (6)**. From the obtained results, it could be noticed that the three different temperatures storage had significant higher values of juice's ascorbic acid concentration at 2ºC or 2º + 8ºC than storage at 8ºC. Moreover, there was a significant decrease in ascorbic acid with the advanced storage periods.

Concerning the effect of different treatments on ascorbic acid concentration, the obtained results indicated that VG treatment has positive effect on ascorbic acid concentration when compared with the other treatments. In this respect, **Khader, (1992)** working on mango fruits, found that postharvest application of vapor gard at 2.5% significantly retarded the degradation of ascorbic acid and reduced alpha-amylase and peroxidase activities during storage at 15ºC. **Mansour *et al* (1987)** reported that vitamin C of navel orange increased by calcium spray treatments during storage, beside, storage of the fruits resulted in a clear reduction in vit. C content and this reduction increased as the period of storage extended. **Manolopoulou-Lambrinou and Papadopoulou, (1995**) working on Encore mandarin fruits, observed that the fruits sprayed with vapor gard 1% had decreased ascorbic acid content of the juice. Also, the temperature treatments (2, 4, 7 and 10 ºC with 90% R.H.) did not affect significantly the vitamin (C) content, a fact which is opposed to what was reported by **Nagy, (1980)** that the greatest and most rapid loss is observed in high storage temperatures. **El-Sabrout,** (**1996**) revealed that vitamin (C) content increased significantly with increasing the concentration of vapor gard application in Washington Navel orange as compared with the control in both years. **Lambrinos and Manolopoulou, (2000)** working on Navel orange, reported that vitamin (C) content decreased considerably during storage at 5ºC, After 90 days of storage at 5ºC plus 1 week of shelf-life, the fruits

showed a 10% decrease. **Mohamed *et al* (2003)** showed that, juice ascorbic acid content of Valencia orange and Marsh grapefruits gradually decreased with prolonged storage periods. However, postharvest treatments with $CaCl_2$, Ca $(NO_3)_2$ and Na_2CO_3 had no effect on ascorbic acid content of Marsh seedless grapefruit during storage. While, juice ascorbic acid content of Valencia orange fruits treated with these chemical compounds were significantly higher as compared to untreated fruits.

Data of total sugar concentrations in the peel and flesh of orange fruit as affected by different treatments and storage temperatures at various periods are presented in **Table (7)**. The presented results indicated that low storage temperature increased the concentrations of the total sugars in peel and flesh gradually by advanced storage up to 45 days and decreased after that. Similar results were reported by **Nawar and Ezz, (1994)** who stated that, reducing sugars in peel grapefruit and orange fruit increased progressively with increasing storage duration, although the rate of such increment was diminished during the last few weeks of storage. In this respect, **Hulme, (1970)** reported that non reducing sugars in peel displayed a completely different behaviour, it showed a simultaneous decrease as storage period increased. The increase in the concentration of reducing sugars and the corresponding decrease in non-reducing sugars could probably due to sucrose hydolysis; the main non reducing sugar in citrus fruit peel. In this connection, **Purvis and Grierson, (1982)** and **Purvis and Yelenosky, (1982)** reported that, the increase in the reducing sugars of grapefruit peel resulted from the hydrolysis of sucrose, which might be regulated by temperature, although, it would be difficult to expect invertase activity under the prevailing low storage temperature. In this respect, **Attia, (1995)** working on Balady orange fruits, reported that sugars represented the main constituents of total soluble solids. TSS increased as well as sugars percentage increased during storage at 5ºC.

Concerning the effect of postharvest chemical treatments application on total sugars concentration, data of both peel and flesh fruits revealed that application of $CaCl_2$ increased total sugars concentration as compared to the other treatments. Similar results were reported by **Hanafy-Ahmed, (1996)** working on lettuce plants. who pointed out that, using calcium chloride as foliar application tended to increase the concentration of calcium, total sugars, total free amino acids and total soluble phenols concentrations. Also, the author reported that the increments in sugars and free amino acids concentrations under $CaCl_2$ treatments may play an osmotic function.

Data of total soluble phenols concentration in peel and flesh were presented in **Table (8)**. The results revealed that storage at 8ºC significantly increased concentration of total soluble phenols as compared to the other storage temperatures. Furthermore. There were a significant increase with the advanced storage period. Also, the results in **Table (8)** indicated that, low of total soluble phenols were recorded by the fruits treated with V.G or $CaCl_2$ either alone or in combination as compared with the control (untreated) fruits. While, an opposite trend was obtained by **Ikeda *et al* (2004)**. Furthermore, **Martins *et al* (2004)** reported that, the "Chimarrita" peaches during cold storage at 0ºC had higher phenolic compounds and polyphenol oxidase activity. Moreover, **Lovaas and Olsen, (1998)** mentioned that stress environmental factors such as drought and / or mineral excess induce oxidation of cellular components of plants. Oxidative stress leads to an increase in phenolic compounds (**Rosemann *et al* 1991**). Many phenolic compounds are capable of inhabiting ATP synthesis in mitochondria **(Stenlid, 1970)**, inhibiting enzyme activity (**Van Sumere *et al* 1975**), antagonizing plant hormones biosynthesis (**Stenlid, 1976**) and inhibiting ions absorption through alterations in the permeability of the membrane (**Glass, 1974 and Hanafy Ahmed , 1991**).In addition, **Morgna (1977)** reported that, the major determinate of phenol production is the supply of the prerequisites for its synthesis, namely the amino acid precursor's phenylalanine and tyrosine, a supply of carbon skeletons and energy sources.

The results in **Table (9)** indicated that, increasing storage temperature brought about a slight increase in total free amino acid concentration with a significant increase by the advanced storage periods. In addition, the application of $CaCl_2$ significantly increased total amino acid concentration of fruits as compared with the other treatments in both peel and flesh fruit samples.

These results were in agreement with those reported by **Nawar and Ezz, (1994)** on orange and grapefruits. They pointed out that concentration of free amino acid in the peel fruits increased with increasing cold storage period. They suggested that, sugars are proposed to act as cryoprotectants, whereas the high free amino acids content provides the necessary pool; for the synthesis of new

proteins. Thus, the increased reducing sugars and

free amino acids in response to cold storage might be regarded as a low temperature surviving mechanism. Similar suggestion were mentioned by **Yelenosky (1977)** concerning the potentials of freezing survival in citrus. **Leopold and Kridemann (1981**) reported that subjecting subtropical fruit individuals to chilling temperatures; from 0 to 10ºC was associated with a matching increase in amino acids. Furthermore, **Guy *et al* (1981)** found that, in comparison with high temperature (25ºC), low temperature (10ºC) caused greater retention of ^{14}C into amino acid fraction rather than into protein. **Goss, (1973)** suggested that amino acids can serve as source of carbon and energy when carbohydrates become deficient in the plant, amino acids are determinate releasing the ammonia and organic acid from which the amino acids was originally formed. **Rabe, (1990)** indicated that nitrogenous compounds can accumulate in higher plants as a result of stress. **Mangne and Larher (1992)** reported that the change in amino acid concentrations in apples during storage showed two distinct phases. The first, 40 days long, was characterized by a decrease in amino acid concentration due to respiration or to the synthesis of enzymes required for ripening (**Frenkel *et al* 1968**). The second was corresponded to an accumulation of amino acids, presumably as a consequence of intracellular protein hydrolysis and cell wall protein breakdown. **Aly, (2006)** working on Banzahir limes and Marsh grapefruits, found that increase of peel free amino acid content was noticed with storage duration. **Steponkus (1971)** indicated that higher concentration of free amino acids have sugar binding capacity, which protects protein from being denaturanted at low temperatures. The author also added that higher free amino acid concentration provides the necessary conditions to synthesis new proteins. This could lead to more protection to cell walls and reduced cell membrane damage or cell collapase (**Aly, 2006**). In this respect , **Hanafy-Ahmed (1996**) working on lettuce plants, suggested that increasing reducing sugars, total free amino acids and total soluble phenols concentrations due calcium chloride foliar application could be explained on the assumption that such plants might have less efficiency to condensate simple organic compounds into more complex ones. Moreover, the author assumed that the high levels of total free amino acids and soluble phenols might be due to the increase in the metabolic activity of plants to synthesize Shikimic acid.

The data in **Table (10)** indicated that, concentration of proline in peel and flesh fruits tended to increase with increasing cold storage duration till 45 days and then decreased. The lowest concentration of proline was noticed at 8 ºC as compared with fruits held at 2 ºC or 2 º + 8 ºC. These results appeared to agreement with those reported by **Nawar and Ezz, (1994)** on grapefruit and orange fruits.

Concerning, the effect of postharvest treatments on proline concentration, it is clear that fruits treated with vapor gard recorded the lowest concentration of proline. This might be due to that fruits treated with VG were free from chilling injury symptoms. Proline is considered as a cytoplasm protective osmolyte necessary for adaptation to stress (**Rudulier *et al* 1984**). Furthermore, **Lahrer *et al* (1993)** assumed that the proline enhancement under stress conditions may be positively related to the membrane integrity. **Venekamp, (1989**) revealed that an increased proline synthesis could be an attempy to limit cytoplasm acidifaction under stress conditions. In additions. **Purvis (1981)**, **Purvis *et al* (1979**) and **Purvis and Grierson, (1982)** found significant correlation between the accumulation of reducing sugars and proline and increased resistance of the fruit to chilling injury. **Steponkus, (1971)** presented an evidence that cold acclimation of Hedera helix is a 2-phase process, the first phase being the accumulation of sugars and the second phase is though to involve the production of proteins which due to an altered composition or configuration have a greater capacity to bind sugars and are thus protected from denaturation at freezing temperatures. Furthermore, **Purvis, (1981)**, **Purvis and Yelenosky, (1982)** and **Nordby *et al* (1987)** found that during cold acclimation of young grapefruit trees, considerable change occurred in proline and soluble carbohydrates of fruit peel flavedo tissue. They suggested that these changes might indicate the relationship for the susceptibility of grapefruit rind to chilling injury. Moreover, **Aly, (2006)** indicated that the concentration of proline in the peel of either Banzahir limes or Marsh grapefruit tended to show a marked increase with increasing cold storage duration, **Syvertsen and Smith, (1983)** reported that proline increased in grapefruit flavedo or peel tissues in response to low temperatures.

Data of total calcium concentrations in peel and flesh fruits are presented in **Table (11)**. The results indicated that, increasing temperature of storage increased the calcium level in both peel and flesh. The highest calcium concentration was

obtained at (8ºC) when compared with that at low storage temperature (2ºC).

Concerning the effect of postharvest treatments on calcium concentration, the results showed that, a significant increase in ca concentration was recorded with calcium applications either alone or combined with vapor gard. The highest Ca concentration was obtained by using $CaCl_2$ treatment with significant increase with advanced storage periods. These results are in confirm with those reported by **Conway *et al* (1994)** and **El-Hilali *et al* (2004)**. In this respect, calcium probably enters the fruit primarily through the lenticels (**Betts and Bramalage, 1977**) and through cracks in the cuticle and the epidermis (**Clements, 1935**). Furthermore, **Conway *et al*** (**1994**) reported that, infiltration apple fruits with 2 or 4% $CaCl_2$ solution resulted in a higher Ca concentration than in non infiltrated fruits. In this respect, the mechanism by which increased tissue Ca reduces decay and maintains firmness is hypothesized to be related to Ca ions in the cell wall (**Demarty *et al* 1984**). Moreover, many authors suggested that Ca is involved in controlling (reducing) respiration (**Faust and Shear, 1972**) and can reduce ethylene production (**Lougheed *et al* 1979**). In addition, **Mengel and Kirkby (1978)** reported that, calcium played role in both cell division and cell expansion. Also they mentioned that this might be induced through its effect on membrane permeability, structural abnormalities, selective ions transport, increase in surface potential of membranes, influence the activity of several enzymes, maintenance of the plasma and vacuolar membranes, membrane integrity and formation of mitochondria.

Data of polyamines concentrations in peel and flesh of orange fruits as affected by different storage temperatures and different postharvest applications at the 30 and 60 days storage periods are presented in **Table (12)**. The data revealed that low storage temperature increased concentration of polyamines for the flesh fruit during 30 days of storage. Meanwhile, the fruits held at 8 ºC or 2 º + 8 ºC had higher values of polyamine at 60 days of storage period. Moreover, the results indicated an increase in polyamine concentration for $CaCl_2$ treatment of flesh fruits compared with the other treatments during both storage periods. On the other hand, for peel fruits the VG treatment produced the highest concentration of polyamines after 30 days of storage but $CaCl_2$ treatment was the highest after 60 days. These results are in accordance with those obtained by **Yuen and Tridjaja, (1995)** who revealed an increase in putrescine during cold storage for lime and orange but not for grape fruit. Moreover, in citrus, the increase in polyamines in response to chilling injury was detected. Stress had been reported as a relationship between chilling injury severity and the polyamine levels (**McDonald *et al* 1993**). In this connection, **Willadino *et al* (1996)** pointed out that putrescine (Put) is synthesized in plants from arginine and/or ornithine by the action of the biosynthetic enzymes ADC or ODC. Polyamines could affect DNA and RNA synthesis and degradation; regulate rates of transcription, inhibit activities of protease, ribonuclease, peroxidase and polygalacturonase; stabilize ribosomal structure and maintain membrane integrity (**Smith, 1990**).

Wang *et al* (1993) working on apple, reported that treatment with 3% Ca appeared to increase spermidine (SPD) and spermine (SPN) at 8 week and SPN at 16 week, the fruit cell walls contained much less SPN than putrescine or spermidine. The level of SPN in the cell wall also declined steadily with time in storage at 0ºC. In addition, **Martinez-Romero *et al* (1999)** working on lemon, found that, concentration of putrescine showed significant decrease from their initial values (84.6 ± 7.0 $nmolg^{-1}$) during storage at 15ºC. Also, Calcium treated lemons showed significant higher levels of spermidine than control fruits after 7 days of storage. Other reports have shown that polyamines inhibit ethylene biosynthesis in fruit tissue (**Hyodo and Tanaka, 1986 and Ke and Romani, 1988).** The relative effectiveness of polyamines in retarding senescence of plant tissues is thought to be related to the number of positive charges per molecule (**Galston and Kaur-Sawhney, 1987**).

Gonzalez-Aguilar *et al* (2000) working on mandarin fruits, stated that after cold storage, a general decline in polyamines (PA_s) values occurred under all temperature conditioned (from 2 to 37ºC). PA content increased again after transferring cold storage fruits with chilling sensitivity, although the basis of cold-induced damage still remains unclear. They have focused on the involvement of PAs in the chilling tolerance of citrus, Alteration in the content of PAs has been considered to be a response of different plant tissues to stresses, including chilling (**McDonald and Kushad, 1986**). The authors reported that, after storage at 2ºC, the level of spermine fruit conditioned at 30 and 37ºC decreased to levels similar to those of non-conditioned fruit, but the differences in the chilling injury index were maintained. Moreover, many reports suggested that putrescine

Table 12. Effect of vapor gard, $CaCl_2$ or its combination as a pre-storage treatments on Polyamines concentrations of Navel orange fruits (nmol/g F.W.) stored for different periods at various temperatures during the 2nd season (2004 - 2005)

Temp.	Treatments	In Fruits' Peel		In Fruits' Flesh	
		Storage period (days)			
		30	60	30	60
2 °C	Control	1440	1560	1540	1650
	Vapor gard (2%)	1460	1910	1810	1550
	$CaCl_2$ (4%)	1780	1530	2410	810
	Vapor gard + $CaCl_2$	1440	2340	1250	1200
8 °C	Control	1070	1870	1250	2200
	Vapor gard (2%)	330	3330	1200	3160
	$CaCl_2$ (4%)	1200	3440	510	2530
	Vapor gard + $CaCl_2$	1060	3230	1560	2530
2° + 8 °C	Control	1400	1940	1450	1720
	Vapor gard (2%)	1850	2470	1520	2850
	$CaCl_2$ (4%)	1480	3520	1590	3270
	Vapor gard + $CaCl_2$	1370	1780	1430	1890

(Put) accumulation may be a protective response to many kinds of stress that cause physiological injury of tissue (**Wang, 1994, and Gonzalez-Aguilar *et al* 1995**). It was possible that polyamine increaseed cell viability during chilling injury by retarding membrance senescence (**Guye *et al* 1986**).

Generally, fruits treated with vapor gard or calcium chloride and vapor gard mixture gave afavourite characters from physiological view, including fruit weight loss, peel color, fruit firmness and juice percentage. Also, both treatments induced higher concentrations of: total sugar, total free amino acids, soluble phenols, free proline, ascorbic acid, calcium, polyamine as well as TSS/ acid ratio, reflecting an improvement in fruit chemical characteristics during storage. Thus, it could conclude that, for commercial range or the export market, it may be usefull to treat orange fruits with vapor gard or mixture of calcium chloride and vapor gard and their temperature storage at 2 + 8 ºC to decrease fruit weight loss as well as to increase fruit firmness and fruit's quality.

REFERENCES

Agusti, M.; V. Almela; S. Zaragoza; R. Gazzola and E. Primo-Millo (1997). Alleviation of peel pitting of "Fortune" mandarin by the polyterpene pinolene**. Journal of Horticultural Science, 72(4): 653-658.**

Aly, Z. Ekbal (2006). Effect of different methods and concentrations of proline preharvest and postharvest application on the control of chilling injury of Banzahir limes and Marsh grapefruits during cold storage**. Annals of Agric. Sci., Moshtohor, 44(3): 1239-1252.**

A.O.A.C. (1995). Official Methods of Analysis, 16th Ed. Association of Official Analytical Chemists. Washington, DC.

Attia, M.M. (1995). Effect of postharvest treatments on fruit lossed and keeping quality of Balady oranges through cold storage**. Alex. J. Agric. Res., 40(3): 349-363.**

Bates, L.S.; R.D. Waldre and L.D. Teare (1973). Rapid determination of free proline for water stress studies**. Plant and Soil, 39: 205-207.**

Betts, H.A. and W.J. Bramalage (1977). Uptake of calcium by apples from postharvest dips in calcium chloride solutions**. J. Amer. Soc. Hort. Sci., 102: 785-788.**

Chen, P.M. and W.M. Mellenthin (1981). Effect of harvest date on ripening capacity and postharvest life of 'Anjou' pears**. J. Amer. Soc. Hort. Sci., 106(1): 38 – 42 .**

Chikaizumi, E.; A. Hino and F. Mizutani (1997). Effect of calcium compounds, calcium competitive inhibitors, lime sulfur and abscisic acid incidence of "Kohansho" disorder in "Shirayanagi" navel orange (Citrus sinensis obseck var. brasiliensis tanaka) fruit. **Bulletin of the Experimental Farm College of Agriculture, Ehime University, Japan. 19: 9-15.**

Clements, H.F. (1935). Morphology and physiology of the Pome lenticels of *Pyrus malus*. **Bot. Gaz., 97: 101-117.**

Conway, W.S.; C.E. Sams; C.Y. Wang and J.A. Abbott (1994). Additive effects of post harvest calcium and heat treatment on reducing decay and maintaining quality in apples**. J. Amer. Soc. Hort. Sci., 119: 49-53.**

Demarty, N.; C. Morvan and M. Thellier (1984). Calcium and the Cell wall**. Plant Cell Env., 7: 441-448.**

Dubois, M.; F. Smith; K.A. Gilles; J. K. Hamilton and P.A. Robers (1956). Colorimetric method for determination of sugar and related substances. **Anal. Chem., 28(3): 350 – 356.**

Echeverria, E. and M. Ismail (1990). Sugars unrelated to Brix changes in stored citrus fruits. **Hort. Science, 25(6):710 .**

Echeverria, E. and J. Valich (1989) . Enzymes of sugar and acid metabolism in stored "Valencia" oranges **. J. Amer. Soc. Hort. Sci ., 114: 445-449.**

El-Hilali, F.; A. Ait-Oobahou; A. Remah and O. Akhayat (2004). Effect of preharvest spray of Ca and K on quality, peel pitting and peroxidases activity of "Fortune" mandarin fruit in low temperature storage**. Acta Hort., 632: 309-315.**

El-Oraby, G. Samia and Ali, M. Gihan, (2002). Maturity and cold storage of three new cultivars of grapefruits**. J. Agric. Res., Tanta Univ., Special Issue for 2nd Inter. Conf., Hort. Sci., 10-12 Sept. 2002, 28 (3/1): 327-343.**

El-Sabrout, M.B. (1996). Effect of some growth retardants on the physiological and biochemical aspects in washington Navel orange trees**. Alex. J. Agric. Res., 41(3): 257-273.**

Erkan, M. and M. Pekmezci (2000). The effects of different storage temperature and postharvest treatments on storage and chilling injury of "Washington Navel" oranges**. Acta Hort., 518: 93 – 100.**

Faust, M. and C.B. Shear (1972). The effect of calcium on respiration of apples. **J. Amer. Soc. Hort. Sci., 97: 437-439.**

Frenkel, C.; I. Klein and D.R. Dilley (1968). Protein synthesis in relation to ripening of Pome fruits**. Plant Physiol., 43: 1146-1153.**

Galston, A.W. (1983). Polyamines as modulators of plant development**. Bioscience, 33: 382 – 388.**

Galston, A.W. and R. Kaur-Sawhney (1987). Polyamines and Senescence in Plants, pp. 167 - 181. In: Thomson W.W.; E.A. Nothnagel and R.C. Huffaker (eds.). Plant senescence; Its biochemistry and physiology, Amer. Soc. Plant Physiol., Rockville, Md.

Glass, A.D.M. (1974). Influence of phenolic acids upon ion uptake**.** III. Inhibition of potassium absorption **. J. Exp. Bot., 25: 1104 -1113.**

Gonzalez-Aguilar, G.; M.T. Lafuente and L. Zacarias (1996). Changes in polyamines in "Fortune" mandarin with cold storage and temperature conditioning .Postharvest physiology, pathology and technology for horticultural commodities :recent advances. **Proceedings of an International Symposium Held at Agadir, Morocco, 16 -12 January (1994), 273 -278. (C.F. Hort. Abst., 66(8), 7226, 1996).**

Gonzalez-Aguilar, G.A.; L. Zacarias; M.A. Perez-Amador; J. Carbonell and M.T. Lafuente (2000). Polyamine content and chilling susceptibility are affected by seasonal changes in temperature and by conditioning temperature in cold stored "Fortune" mandarin fruit**. Physiol. Plant., 108: 140- 146.**

Goss, J.A. (1973). Amino acid synzthesis and metabolism**. Physiology of Plant and Their Cells. p. 202. Pergamon Press Inc., New York.**

Guy, C.L.; G. Yelenosky and H.C. Sweet (1981). Distribution of ^{14}C photosynthetic assimilates in valencia orange seedlings at 10 º and 25ºC**. J. Amer. Soc. Hort. Sci., 106 (4): 433-437.**

Guye, M.G.; L. Viegh and J.M. Wilson (1986). Polyamine titre in relation to chill. sensitivity in *Phaseolus sp***. J. Exp. Bot., 37: 1026-1043.**

Hanafy-Ahmed, A.H. (1991). Physiological studies on the nitrogen and phosphorus deficiencies in spinach plants (*Spinacia oleracea, L.).* 1. Growth and organic components**. Bull. Fac. of Agric. Univ. of Cairo, 42(1): 87-110.**

Hanafy-Ahmed, A.H. (1996). Physiological studies on tipburn and nitrate accumulation in lettuce plants**. J. Agric. Sci. Mansoura Univ., 21(11): 3971-3994.**

Harold, E.P. (1985). Evaluation of Quality of Fruits and Vegetables. p. 123. AVI Publications-West Port. Conn. U.S.A.

Hassan, M. (2004). Maturity indices and influence of some treatments on storage of lemon fruits. **Egyptian Journal of Applied Sciences, 19: 651 – 666.**

Hulme, A.C. (1970). Citrus fruits. In : **The Biochemistry of Fruits and their Products. Vol. 1, pp. 1-27.** Academic Press, New York.

Hyodo, H. and K. Tanaka (1986). Inhibition of 1-aminocyclopropane -1- carboxylic acid synthase activity by polyamines, their related compounds and metabolites of S-adenosylmethionine**. Plant Cell Physiol., 27: 391-398.**

Ikeda, F.; T. Baba and S. Ito (2004) . Effect of intermittent warming on the storage life of “Kiyomi” tangor in a low temperature room and antioxidative activity of fruit skin. **Horticultural Research Japan, 3(4): 421-424.**

Ke, D. and R.J. Romani (1988). Effects of spermidine on ethylene production and the senescence of suspension-cultured pear fruit cells**. Plant Physiol. Biochem., 26: 109-116.**

Khader, S. (1992). Effect of gibbrellic acid and vapor gard on ripening, amylase and peroxidase activities and quality of mango fruits during storage**. Journal of Horticultural Science, 67(6): 855-860.**

Knee, M. (1978). Properties of polygalacturonate

and cell cohesion in apple fruit cortical tissue**. Phytochemistry, 17: 1257-1260.**

Lahrer, F.; L. Leport and M. Chappart (1993). Effectors for the osmoinduced proline response in higher plants**. Plant Physiol. Biochem., 31: 911-922.**

Lambrinos, G. and H. Manolopoulou (2000). Effects of film thickness and storage temperature on water loss and internal quality of film packaged oranges. **Improving Postharvest Technologies of Fruits, Vegetables and Ornamentals, Proc. IIR. Conf., Murica, pp. 565-572.**

Leopold, A.C. and P.E. Kriedemann (1981). Plant Growth and Development. pp. 375 -399. Tata McGrow-Hill Publishing Company Ltd., New Delhi, India**.**

Lidster, P.D. (1981). Some effects of emusilifiable coatings on weight loss, stem discoloration and surface damage disorders in "Van" Sweet cherries**. J. Amer. Soc. Hort. Sci., 106(4): 478- 480.**

Lougheed, E.C.; D.P. Murr and S.R. Miller (1979). Effect of calcium and daminozide on ethylene production and softening of apple fruits**. Experientia, 35: 43-44.**

Lovaas, E. and J. Olsen (1998). No induction of polyamines and radical scavenging antioxidants in Nicotiana tabaccum exposed to iron excess, as investigated by the DPPH assay and differential spectroscopy. **J. Plant Physiol., 153: 401-408.**

Mangne, Ch. and F. Larher (1992). High sugar content of extracts interferes with colorimetric determination of amino acids and free proline**. Analytical Biochemistry, 200: 115-118.**

Manolopoulou-lambrinou, M. and P. Papadopoulou (1995). Effect of storage temperature on encore mandarin quality**. Acta. Hort., 379: 475-482.**

Mansour, M.F.; A.S. Atall and S.A. Ahmed (1987). Preliminary studies on the effect of spraying Navel orange trees with calcium chloride on yield, quality and postharvest behavior of fruits. **Minufiya J. Agric. Res., 12(2): 937-954.**

Martins, C.R.; R.F.F. Cantillano; R.M. Farias and C.V. Rombaldi (2004). Postharvest activity of phenolic compounds and pholyphenoloxidase of peaches produced at orchard with traditional cultivation and mulching. **Ciencia Rural 34(3): 749-754. (C.F. Hort. Abst., 74, 9, 7422, 2004).**

Martinez-Romero, D.; D. Valero; M. Serrano; F. Martinez-Sanchez and F. Riquelme (1999). Effect of postharvest putrescine and calcium treatments on reducing mechanical damage and polyamines and abscisic acid levels during lemon storage**. Journal of the Science of Food and Agriculture, 79: 1589-1595.**

McDonald, R.E. and M.M. Kushad (1986). Accumlation of putrescine during injury of fruits**. Plant Physiol., 82: 324-326.**

McDonald, R.E.; T.G. Mccollum and H.E. Nordyby (1993). Temperature conditioning and surface treatments of grapefruit affect expression of chilling injury and gas diffusion**. J. Amer. Soc. Hort. Sci., 118: 490-496.**

McGuire, R.G. (1992). Reporting of objective colour measurements**. Hort. Science, 27(12): 1254 – 1255.**

Mengal, K. and E. A. Kirkby (1978). Principles of Plant Nutrition, pp. 367-390. International Potash Institute Berne, Switzerland.

Mohamed, M.A.; A.A. Abdel-Haffeez and S.A. Mehaisen (2003). Effect of postharvest treatments with some safe compounds on fruit properties of Valencia orange and marsh seedless grapefruit fruit during storage**. Annals of Agric. Moshtohor, 41(3): 1253 - 1267.**

Moore, S. and W.H. Stein (1954). Amodified ninhydrin reagent for the photometric determination of amino acids and related compounds**. J.Biol. Chem., 211: 907 – 913.**

Morgna, V. (1977). Control at the level of substrate supply an alternative in the regulation of phenyl - propanoid accumulation in plant cells**. Phytochemistry, 16: 419 – 426.**

Nagy, S. (1980). Vitamin C contents of citrus fruits and their products**. J. Agric. Food Chem., 28(1): 8-18.**

Nawar, A. and Th. Ezz (1994). Surface pitting, proline, free amino acids and soluble sugars in grapefruit and orange fruit peel, in relation to chilling injury**. Alex. J. Agric. Res., 39(1): 357- 371.**

Nordby, H.E.; A.C. Purvis and G. Yelenosky (1987). Lipids in peel of grape fruit and resistance to chilling injury during cold storage**. Hort. Science, 22(5): 915-917.**

Piper, C.S. (1947). Soil and Plant Analysis. p. 368. Interscience Publishers, Inc. New York.

Purvis, A.C. (1981). Free proline in peel of grapefruit and resistance to chilling injury during cold storage. **Hort. Science, 16: 160-161.**

Purvis, A.C. and W. Grierson (1982). Accumulation of reducing sugars and resistance of grapefruit peel to chilling injury as related to winter temperatures. **J. Amer. Soc. Hort. Sci., 107(1): 139-142.**

Purvis, A.C.; K. Kawada and W. Grierson (1979). Relationship between midseason resistance to chilling injury and reducing sugar level in grapefruit peel**. Hort. Science, 14(3): 227-229.**

Purvis, A.C. and G. Yelenosky (1982). Sugar and proline accumulation in grapefruit flavedo and leaves during cold hardening of young trees**. J. Amer. Soc. Hort. Sci., 107(2): 222-226.**

Rabe, E. (1990). Stress physiology: The functional significance of the accumulation of nitrogen-containing compounds**. J. Hort. Sci., 65: 231-243.**

Rodriguez-Felix, A.; M.A. Villegas-Ochoa; G.A.

Camarena-Gomez and B.R. Martinez-Antunez (2001). Quality of "Valencia" oranges during low temperature storage. Revista. Chapingo. Serie**. Horticultura, 7(2): 259-274.**

Rosemann, D.; W. Haller and J. Sandermann (1991). Biochemical plant reponses to ozone**. Plant Physiol., 97: 1280-1286.**

Rudulier, L.D.; A.P. Strom; A.M. Dandrekar; L.T. Smith and R.C. Valentina (1984). Molecular biology of osmoregulation**. Science, 224: 1064-1069.**

Sams, C.E. and W.S. Conway (1984). Effect of calcium infiltration on ethylene production, respiration rate, soluble polyuronide content and quality of "Golden Delicious" apple fruit**. J. Amer. Soc. Hort. Sci., 109(1): 53-57.**

Smith, T.A. (1990). Plant polyamines-metabolism and function, **pp. 1-23.** In: Flores, H.E.; R.N. Arteca and J.C. Shannon (eds.). **Polyamines and Ethylene: Biochemistry, Physiology and Interactions.** Amer. Soc. Plant Physiol., Rockville, Md.

Snedecor, G.W. and W.G. Cochran (1980). Statistical Methods. 7th Ed., p. 50. Iowa State Univ. Press, Ames, Iowa, U.S.A.

Spiegel-Roy, P. and E.E. Goldschmidt (1996). Biology of Citrus, pp. 88-112. Cambridge Univ. Press, Cambridge, London.

Stenlid, G. (1970). Flavonoids as inhibitors of the formation of adenosine triphosphate in plant mitochondria**. Phytochemistry, 9: 2251-2256.**

Stenlid, G. (1976). Effect of flavonoide on the polar transport of auxins. **Physiol. Plant., 38: 262 – 266.**

Steponkus, P.L. (1971). Cold acclimation of Hedera helix. Evidence for a two phase process. **Plant Physiol., 47: 175-180.**

Swain, T. and W.E. Hillis (1959). The phenolic constituents of Prunus domestica. I. The quantitative analysis of phenolic constituent. **J. Sci. Food Agric., 10: 63- 69.**

Syvertsen, J.P. and M.L. Smith (1983). Environmental stress and seasonal changes in proline concentration of citrus tree tissue and juice**. J. Amer. Soc. Hort. Sci., 108(5): 861-866.**

Van Sumere, C.F.; J. Albercht; A. Dedonder; H. DcPooter and I. Pe (1975). Plant proteins and phenolics. In: **The Chemistry and Biochemistry of Plant Proteins pp. 211-264, (**Harborne, J. B. ed.), Academic Press**,** London.

Venekamp, J.H. (1989). Regulation of cytosol acidity in plants under conditions of drought**. Physiol. Plant., 76: 112 -117.**

Wang, C.Y. (1991). Reduction of chilling injury in fruits and vegetables**. Postharvest News and Information, 2: 165-168.**

Wang, C.Y. (1994). Combined treatment of heat shock and low temperature conditioning reduced chilling injury in zucchinisquash**. Postharv. Biol. Technol., 4: 65-73.**

Wang, Ch. Y.; W.S. Conway; J.A. Abbott; G.E. Kramer and C.E. Sams (1993). Postharvest infiltration of polyamines and calcium influences ethylene production and texture changes in 'Golden Delicious" apples. **J. Amer. Soc. Hort. Sci., 118(6): 801-806.**

Weller, S.C. and D.C. Ferree (1978). Effect of pinolen-base antitranspirant on fruit growth, net photosynthesis, transpiration and shoot growth of "Golden Delocious" apple trees**. J. Amer. Soc. Hort. Sci., 103: 17-19.**

Willadino, L.; T. Camara; N. Boget; M. Claprols; J. SaNtos and M. Torne (1996). Polyamines and free amino acid variations in NaCl-treated embryogenic maize callus from sensitive and resistant cultivars**. J. Plant Physiol., 149: 179-185.**

Yelenosky, G. (1977). The potential of citrus to survive freezes**. Proc. Intern. Soc. Citriculture, 1: 199-203.**

Yuen, C.M.C. and N.O. Tridjaja (1995). Chilling development of "Tahitian" lime, 'Emperor" mandarin, 'Marsh" grapefruit and "Valencia" orange**. J. Sci. Food Agric., 67: 335-339.**