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Effect of $CaCl_2$ and Exogenous Putrescine on Post-harvest Life and Quality of Peach (*Prunus persica* (L.) Batsch) Fruit, cv. 'J. H. Hale'

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ABSTRACT

The effect of 40, 60 and 80 mM calcium chloride ($CaCl_2$) and 0.5, 1 and 2 mM putrescine (Put) on post-harvest life and quality of peach (*Prunus persica* L. cv. 'J. H. Hale') fruit was studied. This experiment was carried out by immersing fruits in solutions at 25°C for 5 min, then transferring them into storage at 1-2°C and 75% relative humidity, together with untreated fruits (dry) as well as fruits immersed in distilled water only. Ethylene production, soluble solids content (SSC), titratable acidity (TA), pH, flesh firmness, weight loss and ascorbic acid (AA) of fruits were measured at regular intervals (5 days for cold storage and 2 days for shelf life) throughout the experiment. Quality of fruits was also tested through a taste panel at the end of experiment. During the whole storage time in which 5 measurements were performed, 60 mM $CaCl_2$ was the best treatment for keeping fruit quality in terms of flesh firmness, SSC, AA, TA, pH and weight loss. The highest score in terms of fruit quality, as given by panelists, corresponded to 60 mM $CaCl_2$ and 2 mM Put. Among the Put solutions, 2 mM could maintain the quality of fruits preventing ethylene production at the highest rate compared to other treatments. The results of a microbiological test showed that 80 mM $CaCl_2$ was the best treatment to control yeast and mold populations significantly ($P < 0.05$). Put solutions were better than $CaCl_2$ treatments in terms of preventing ethylene production by fruits. Lower quality fruit was observed when fruit was immersed in distilled water only.

Keywords: ethylene, fruit quality, shelf life, storage time, yeast and mold population
Abbreviations: AA, ascorbic acid; **CFU**, colony forming unit; **FW**, fruit weight; **PA**, polyamine; **Put**, putrescine; **ROS**, reactive oxygen species; **SE**, standard error; **SSC**, soluble solid content; **TA**, titratable acidity

INTRODUCTION

Peach is a 'climacteric' fruit having a short storage-life. Post-harvest decay is the major factor limiting the extension of storage and/or shelf-life of fruits. Rapid ripening also shortens the shelf-life of commodities and represents a serious constrain for efficient handling and transportation (Bonghi *et al*. 1999). Considering the varying response of different products to post-harvest treatments, development of suitable techniques becomes necessary to achieve optimum quality for any product. In recent years, the use of physiologically active compounds has attracted food industries and consumers (Alzamora *et al*. 2005).

Calcium is one of the important elements affecting quality and post-harvest life of many fruits. Calcium deficiency in some fruits causes certain diseases such as bitter pit, cork spot, water core and senescent breakdown. Fruits with calcium deficiency fall earlier than the expected time of ripening and cannot be kept in store longer compared to those with enough calcium (Valero and Serrano 2010). Calcium reduction in the cell middle lamella is the major factor of cell wall softening in apple during post-harvest storage (Stow 1999). Several other roles have been reported for calcium including tissue firmness preservation in apple (Chardonnet *et al*. 2003), peach (Prussia *et al*. 2005), strawberry (Saftner *et al*. 2003; Verdini *et al*. 2008) and fresh-cut lettuce (Martin-Diana *et al*. 2006); fruit ripening and senescence retardation (Picchioni *et al*. 1996; Lester and Grusak 1999); reduction of ethylene production (Ben-Aries *et al*. 1982); CO_2 and respiration rate reduction (Singh *et al*. 1993; Luna-Guzmán 1999); flesh browning control (Hewajulige *et al*. 2003; Saftner *et al*. 2003; Manganaris *et al*. 2007) and molds decay prevention (Smilanick and Sorenson 2001; Lara *et al*. 2004; Verdini *et al*. 2008). Internal breakdown and flesh browning in peach fruits have also been observed in cold storage due to calcium deficiency (Hewajulige *et al*. 2003).

Polyamines (PAs) are biological active compounds with low molecular weight and aliphatic nitrogen groups occurring in all living things including animals and plants (Valero *et al*. 2002). Several studies have shown that PA accumulation occurs under abiotic stresses including drought, salinity, extreme temperature, hypoxia, UV-B irradiation, heavy metals, mechanical wounding and herbicide treatments (Groppa and Benavides 2008). At the cellular pH values, PAs behave as cautions. In order word, these compounds have positive molecular charge, and this makes them to react with anionic macromolecules such as DNA, RNA, Phospholipids and certain proteins (Heby and Persson 1990). A strong incorporation of PAs with the cell wall poly-anionic structures such as pectins and their exchange with pectic polysaccharides contributing to pectin signal modulation in pathogenesis and differentiation have been reported (D'Orazi and Bagni 1987). Many beneficial roles of PAs in post-harvest physiology of fruits and vegetables could also be found in literatures which include senescence retardation and decreasing ethylene production (Saftner and Baldi 1990; Kakkar and Rai 1993; Bregoli *et al*. 2002; Torrigiani *et al*. 2004); tissue firmness and membrane safety preservation (Kramer *et al*. 1991; Wang *et al*. 1993; Pon-

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appa *et al*. 1993; Serrano *et al*. 2003); cold damage reduction (Shen *et al*. 2000; Marténez-Téllez *et al*. 2002; Mirdehghan *et al*. 2006) and mechanical damage resistance induction (Valero *et al*. 1998; Martínez-Romero *et al*. 2000; Perez-Vicente *et al*. 2002). PAs should not be taken only as protective molecules but rather like double-faced molecules that likely serve as major area for further research efforts (Hussain *et al*. 2011).

The aim of this research was to study the effect of different $CaCl_2$ and putrescine (Put) concentrations on postharvest life and certain quality characteristics of peach fruit, cultivar 'J. H. Hale' during storage period. 'J. H. Hale' is one of the most desirable peach cultivars to consumers in Iran with increased production rate.

MATERIALS AND METHODS

Plant material

Peach (*Prunus persica* (L.) Batsch) fruits, cv. 'J. H. Hale' were harvested in late August from a private orchard (Moghan Industry and Culture Co., Azerbayjan, Iran). About 130-140 g fruits with uniform size were selected at commercial maturity stage when 1/2 to 2/3 fruit surface had red color. Fruits were then packed in polyethylene bags, put into 10 kg plastic baskets and transferred to supplementary curriculum laboratories of Tabriz University. All damaged and physiologically injured fruits were finally eliminated and divided into 24 lots of 30-35 fruits.

Treatments

Experiment was conducted in a complete randomized design, including eight treatments (0.5, 1 and 2 mM Put (Merck, Germany); 40, 60 and 80 mM $CaCl_2$ (Merck), distilled water for the control and dry treatment (without water, Put or $CaCl_2$) and three replications in each treatment. Fruits were immersed in Put and $CaCl_2$ solutions as well as distilled water all containing 2 g l^{-1} Tween-80 (Sigma Chemical Co., USA) surfactant at 24-25°C for 5 min. Fruits were then dried in room temperature for 2 hrs and transferred into cold storage with 1-2°C and 75% relative humidity. Quality parameters were measured weekly (5 days cold storage + 2 days shelf life at 25°C) during a 35-day storage period.

Weight loss

In order to determine fruit weight loss during the storage time, 4 fruits from any lot were separated at the beginning of storage and weighed using a digital balance (Sartorius, GM612, Germany). Fruit weight loss was then measured weekly (5 days cold storage + 2-days shelf-life) through the following equation:

$$\text{Fruit weight loss (\%)} = 100 \times \frac{\text{(pre-storage FW - after-storage FW)}}{\text{pre-storage FW}}$$

Fruit firmness

Flesh firmness determination was carried out using a Penetrometer (Model FDK32; Wagner, Milan, Italy) to measure the force required for a 11 mm-probe to penetrate the fruit tissue after removing the peel and expressed as 'Newton'.

pH, soluble solids content and titratable acidity

The pH of fruit juice (blended 3 fruits) was measured using a PHS-25C pH-meter (Shanghai, China). SSC was determined using a portable refractometer (ATAGO N1, Tokyo, Japan) at 25°C and expressed as percentage (%). TA was determined by titration to pH 8.2 with 0.1 N NaOH and expressed as g malic acid per 100 g fruit weight.

Ascorbic acid

AA was determined by 2,6-dichlorophenolindophenol (Merck) titration method (AOAC 1984) and expressed as mg ascorbic acid per 100 g fruit weight.

Taste panel

In order to compare the quality of treated fruits with the control and dry treatment, a taste panel examination was carried out by 10 adults, aged 24-36 years including 5 males and 5 females. Fruit appearance, firmness, juiciness, taste and sweetness were evaluated by the panelists at the end of storage period based on a ranking of 1 to 5, where 5 = superb; 4 = very nice; 3 = nice; 2 = average; 1 = bad.

Microbiological analysis

One fruit from each lot was considered for microbiological analysis at the beginning as well as the end of the experiment. To do this, 10 g fruit was sampled by slicing from peel to deep flesh in various parts and homogenized in 90 ml peptone water using a sterile mortar under aseptic condition. 1 ml of the homogenized solution was placed on Potato Dextrose medium (pH = 3.5) and transferred into incubator at 30°C for 3 days. The number of fungi and yeasts colonies formed on the medium was then counted and only 30-300 colony forming units (CFU) were taken into account and expressed as Log CFU g^{-1} FW^{-1}.

Ethylene production

Ethylene production was measured by placing one fruit in 1 l glass jar tightly filled in with a rubber cap for 1 h. One ml of the holder atmosphere was withdrawn using a gas syringe, and the ethylene was quantified by a gas chromatograph (Shimadzu, C-R4A, Japan) apparatus. Results were expressed in nl of ethylene released per gram of fruit flesh per hour (nl g^{-1} h^{-1}).

Statistical analysis

The data was analyzed using SAS statistical software and means were compared by Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

pH

The pH of fruit juice was generally increased during storage and the various treatments significantly reduced the extent of this increase (**Fig. 1**). At the end of storage the highest and the lowest pH values were belonged to distilled water and 60 mM calcium chloride respectively. Increase in pH value may be due to the breakup of acids with respiration during storage which has been reported in peach cv. 'J. H. Hale (Toğrul and Arslan 2004); 'Tokhm-Sefid', a local variety of apricot (Zokaee-Khosroshahi and Esna-Ashari 2007; Liu *et al*. 2009) and green asparagus (Bhowmik *et al*. 2002).

Fruit firmness

Fruit firmness continuously decreased during storage and various treatments significantly reduced fruit softening (**Fig. 2**). The highest and the lowest decrease in fruit firmness were belonged to distilled water and 60 mM calcium chloride respectively at the end of storage. However, 'Put' treatments could also maintain fruit firmness during storage which is in agreement with the results of Zokaee-Khosroshahi and Esna-Ashari (2007) and Liu *et al*. (2009) in apricot (*Prunus armenica* L.) and Zokaee-Khosroshahi *et al*. (2007) in strawberry (*Fragaria ananassa* Duch.). Maintaining flesh firmness by calcium and PAs has already been reported in different fruits including apples (Kramer *et al*. 1991; Wang *et al*. 1993; Chardonnet *et al*. 2003), Peaches (Bregoli *et al*. 2002; Prussia *et al*. 2005) and strawberries (Ponappa *et al*. 1993; Saftner *et al*. 2003; Shafiee *et al*. 2010). The mechanism involved seems to be the result of their bounds with pectin compounds leading to a physically stabilized cell wall which is detectable immediately after treatment (Van–Buren 1979; Heby and Persson 1990; García *et al*. 1996). Significant effect of polyamines and $CaCl_2$ treatments on maintaining fruit firmness has shown that

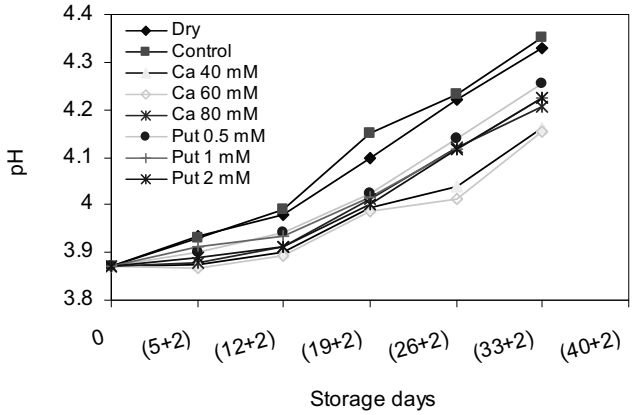

Fig. 1 The effect of various treatments on pH of fruit juice during storage (Cold storage + Shelf life). Data are the mean ± S.E.

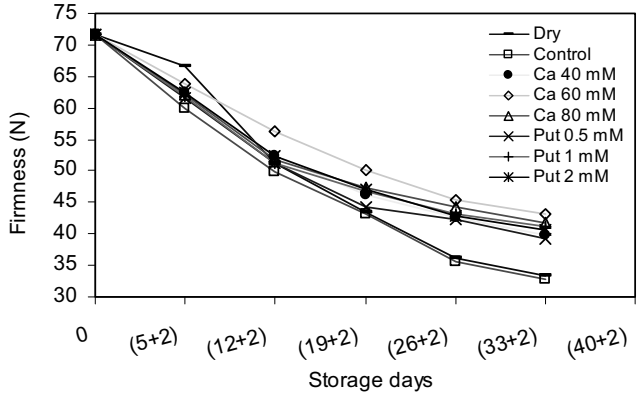

Fig. 2 The effect of various treatments on fruit firmness during storage (Cold storage + Shelf life). Data are the mean ± S.E.

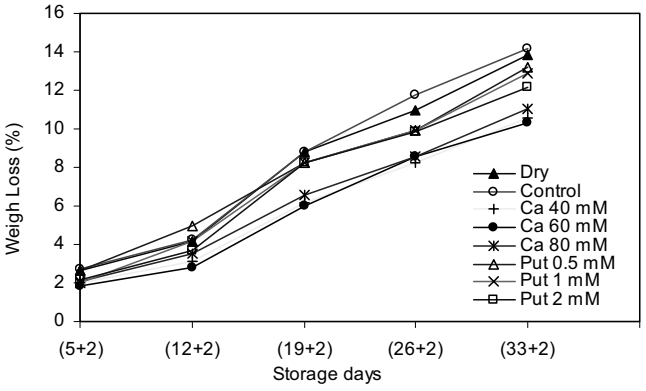

Fig. 3 The effect of various treatments on fruit weight loss during storage (Cold storage + Shelf life). Data are the mean ± S.E.

these compounds have similar abilities in terms of causing senescence delay in fruit softening in bounding cell wall or cell membrane (Ponappa *et al*. 1993).

Weight loss

Considerable weight loss occurred during storage (**Fig. 3**) but, various treatments showed significantly less weight loss than the control. Fruits treated with 40 and 60 mM $CaCl_2$ showed lowest weight loss than the other treatments. However, no significant difference in weight loss was found between the 40 and 60 mM $CaCl_2$ treatments. The maximum weight loss observed in the control was probably because of loosing piles from the fruit surface during immersion in water may affect peach storage life and the mechanism by which calcium prevents fruit weight loss is

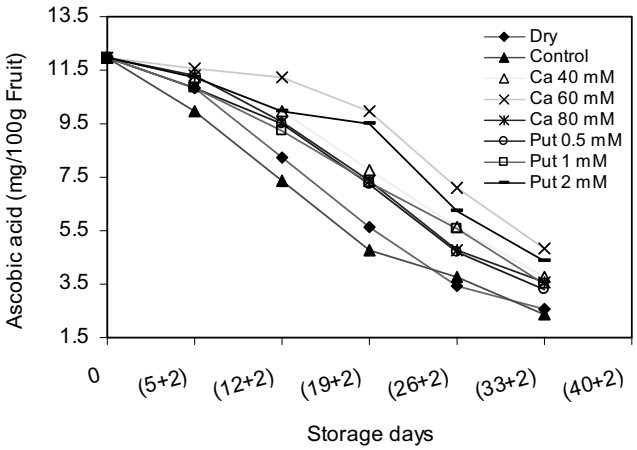

Fig. 4 The effect of various treatments on ascorbic acid content of fruits during storage (Cold storage + Shelf life). Data are the mean ± S.E.

due to the inhibition of respiration. Singh *et al*. (1993) reported that pre-harvest calcium (nitrate and chloride) treatment in mangoes (*Mangifera indica* L.) delayed fruit ripening and reduced respiration rate resulting in fruit quality improvement during storage. Immersion of fresh-cut cantaloupe (*Cucumis melo* L., var. *reticulatus*) chunks in $CaCl_2$ has also decreased CO_2 production and respiration rate (Luna-Guzmán *et al*. 1999). The effects of 'Put' and '$CaCl_2$' treatments on the prevention of fruit weight loss in this study are in agreement with the results of Valero *et al*. (1998), Martínez-Romero *et al*. (2002), Serrano *et al*. (2003), Zokaee-Khosroshahi and Esna-Ashari (2007), Shafiee *et al*. (2010) and Chen *et al*. (2011).

Ascorbic acid (AA)

Continuous reduction in ascorbic acid content of treated and untreated fruits during storage is shown in **Fig. 4**. Various treatments significantly affected the amount of AA; however, the lowest extent of reduction was observed in 60 mM $CaCl_2$ treatment while the highest one belonged to the control. Reducing trend in AA content in fruits was decreased with increasing Put concentration, most likely because Put treatments prohibit fruit ethylene production followed by metabolism retardation. Calcium-treated papaya (*Carica papaya* L.) fruit led to maintain higher AA concentration during storage compared to non-treated ones (Mahmud *et al*. 2008). Declining AA content during storage has also been reported by Toğrul and Arslan (2004) in peach and Tulio Jr. *et al*. (2002) in *Corchorus olitorius* which are in agreement with the results of this study. Reactive oxygen species (ROS) production is a common plant response to both abiotic and biotic stresses (Miller *et al*. 2008). ROS is characterized by the accumulation of toxic molecules $\bar{O}_2$, H_2O_2 and OH^- in tissues which are capable of causing damage to plant cell, membranes and macromolecules (Apel and Kirt 2004), due to increase in oxidative metabolism during ripening, especially in climacteric fruits, ROS are increased damaging cell membranes. Plants therefore need to prevent ROS damages to their cells membranes through developing enzymatic and/or non-enzymatic anti-oxidant agents such as ascorbate peroxidase, vitamin C and vitamin E (Spinardi 2005). Anti-oxidant agents react themselves with reactive oxygen species and prevent their oxidative damages to the cell membranes.

Titratable acidity and soluble solids content

TA and SSC were decreased in all treated and untreated fruits during storage (**Figs. 5**, **6**), but the difference between treatments was significant in this respect. TA and SSC were highest in 60 mM $CaCl_2$ treatment at all measuring times, while their lowest amounts belonged to the control. These

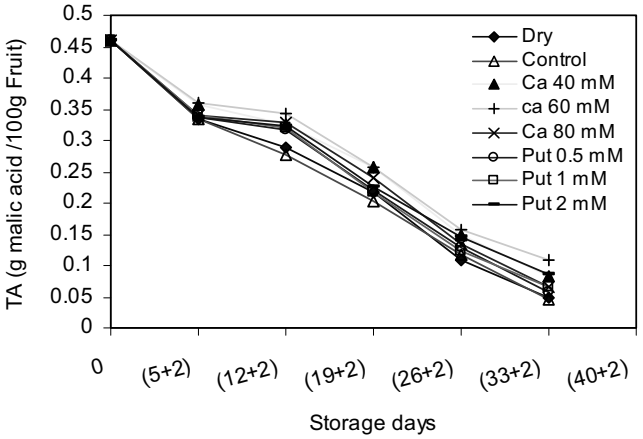

Fig. 5 The effect of various treatments on titrable acidity of fruits during storage (Cold storage + Shelf life). Data are the mean ± S.E.

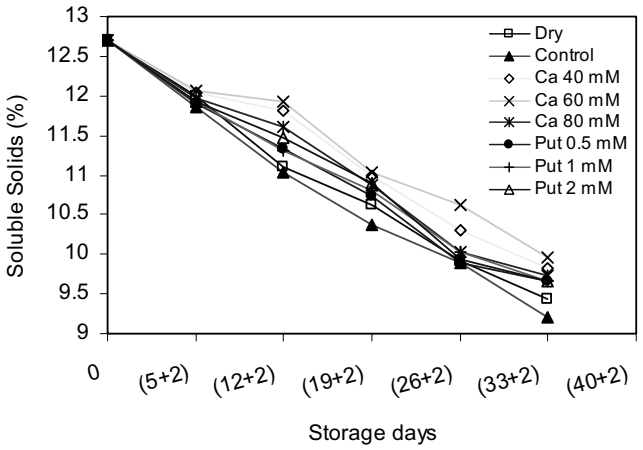

Fig. 6 The effect of various treatments on soluble solids content of fruits during storage (Cold storage + Shelf life). Data are the mean ± S.E.

results are in agreement with the findings of Huang *et al*. (2005) in litchi (*Litchi chinensis* Sonn), Manganaris *et al*. (2007) in peach (*Prunus persica* (L.) Batsch), Zokaee Khosroshahi and Esna-Ashari (2007) and Liu *et al* (2009) in apricot (*Prunus armenica* L.) and Chen *et al*. (2011) in strawberry (*Fragaria ananassa* Duch.) Decreasing total SSC seems to be the result of carbohydrates and pectins breakdown, partial protein hydrolysis and decomposition of glycosides into subunits during respiration (Lara *et al*. 2004; Mahmud *et al*. 2010).

Microbiology test

All treated and untreated fruits showed a considerable increase in yeast and mold population during the storage compared to the harvesting time (**Fig. 7**), but there was significant difference between the treatments in terms of yeasts and fungi CFUs counted at the end of storage. The highest increase in yeast and mold populations was observed in dry fruits showing significant difference with all treatments. No significant difference was found between the control, 0.5 and 1 mM Put as well as 60 and 80 mM $CaCl_2$. The best result in this respect belonged to 60 and 80 mM $CaCl_2$. These findings are in agreement with the results of Manganaris *et al*. (2005) who applied a calcium pre-harvest spray on peach and found a decrease in brown rot and also Saftner *et al*. (2003) who reported a reduction in yeast and mold population in honeydew (*Cucumis melo* L., cv. Inodorus Group) chunks using calcium treatments.

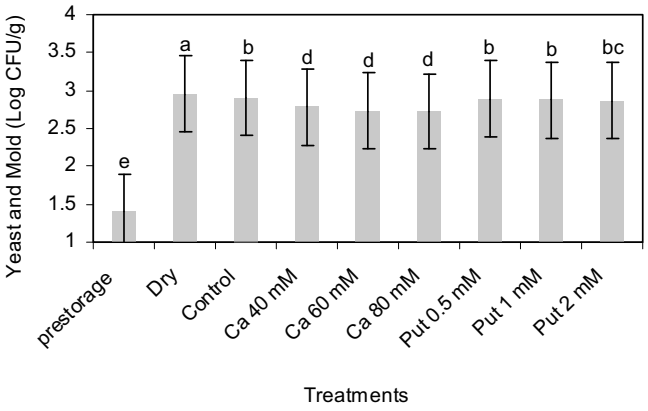

Fig. 7 Effects of various treatments on yeast and mold population during storage.

It is assumed that calcium compounds strongly preserve the cell wall which acts as a powerful barrier against pathogens attacks and prevents microorganisms to penetrate into the cells and tissues and to increase their population. Such a cell wall preservation effect of calcium has been reported by Smilanick and Sorenson (2001); Lara *et al*. (2004), Hernández-Munoz *et al*. (2006) and Chen *et al*. (2011). Campanella *et al*. (2002) have also shown that calcium salts not only decrease the growth of *Phytophthora nicotinae in vitro* but also reduce the severity of pathogen inoculation *in vivo*.

Taste panel

Scores given by the panelists to some fruit quality characteristics were significantly different. Untreated (dry) and control fruits lost their quality 28 days after storage when they transferred from cold storage to the shelves. They also became very soft and sweet gaining a different taste (like apricots) when kept in the cold storage (1-2°C) for 35 days. Untreated (dry) and control fruits did therefore not obtain any score by the panelists and omitted from panel taste (**Fig. 8**). In summary, panelists gave the highest scores to 60 mM $CaCl_2$, whereas 40 mM $CaCl_2$ and 2 mM Put obtained the second place. No significant difference observed between 80 mM $CaCl_2$ and 1 mM Put treatments.

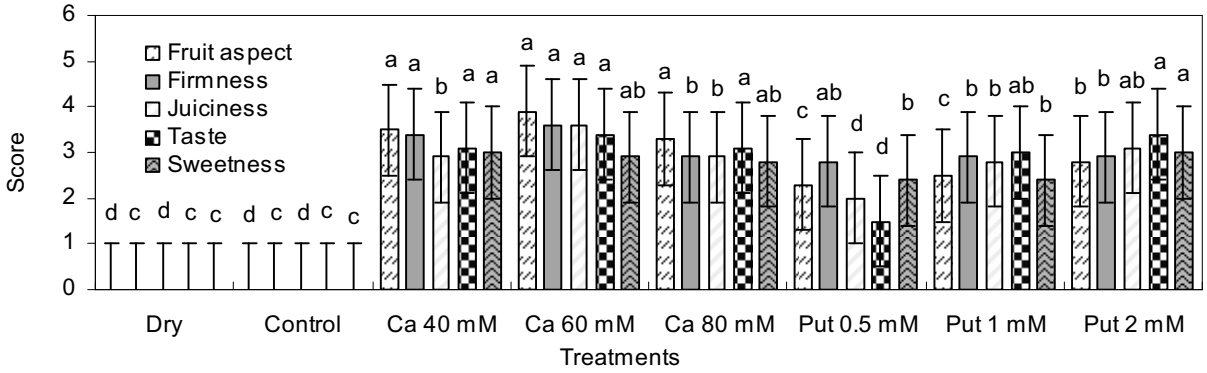

Fig. 8 Scores given by the panelists to some fruit quality characteristics at the end of storage.

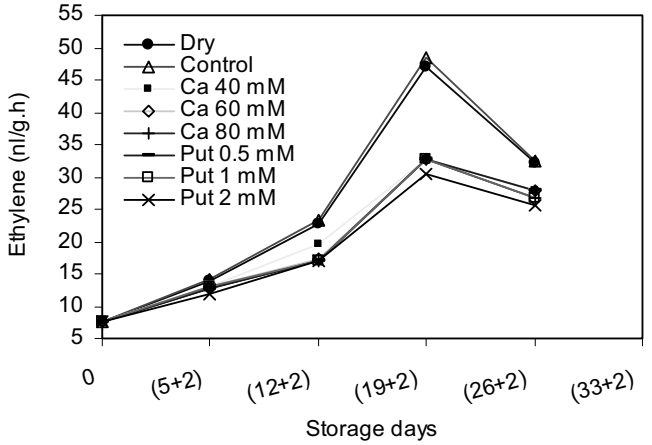

Fig. 9 Ethylene production rate by peach fruit, cv. 'J.H.Hale' during storage (Cold storage + Shelf life). Data are the mean ± S.E.

Ethylene production

Ethylene production rate in treated and untreated fruits increased during storage (**Fig. 9**). The effects of various treatments on fruit ethylene production were significantly different at 4 measuring times. Untreated (dry) and control fruits produced the highest rate of ethylene, while the best result was obtained from 2 mM Put treatment in terms of reducing ethylene production. The solutions of 1 mM Put and 80 mM $CaCl_2$ were remained the next place.

Through reducing ethylene production PAs prevent ethylene-related effects such as chlorophyll loss, senescence, membrane deterioration and RNAse and Protease activities in plants (Evans and Malemberg 1989). Post-harvest application of PAs on a number of fruits like avocadoes (*Persea americana* L.), pears (*Pyrus communis* L.) and tomatoes (*Lycopersicom esculentum* Miller.) has shown a high percentage of reducing ethylene production through inhibition of ACC synthase (Saftner and Baldi 1990; Kakkar and Rai 1993). In some other fruits like apricots (*Prunus armenica* L.), peaches (*Prunus persica* L.), nectarines (*Prunus persica* L.), strawberries (*Fragaria ananassa* Duch.) and several varieties of plums (*Prunus domestica* L.) decreasing ethylene production by PAs has also reported (Valero *et al*. 2002; Bregoli *et al*. 2002; Torrigiani *et al*. 2004; Zokaee-Khosroshahi and Esna-Ashari 2007; Zokaee-Khosroshahi *et al*. 2007).

The results of this study are supported by the above findings but in contrast, are not in agreement with the repot of Kramer *et al*. (1991) regarding ethylene production in apples. There is an inverse relation between ethylene production rate and the concentration of calcium in apple fruit tissues (Faust 1989). Ben-Aries *et al*. (1982) reported that calcium and spermine reduce cell membrane fluidity through the inhibition of ethylene biosynthesis in apple (*Malus domestica* Borkh.) discs, and this happens more with calcium if the temperature is below 12°C, whereas spermine can prevent ethylene production more at the temperatures above 12°C. Luna-Guzmán *et al*. (1999) showed that $CaCl_2$ treatment on fruits decreased CO_2 and ethylene production and this supports our findings in this study. Put solutions were more effective in terms of inhibiting ethylene production than $CaCl_2$, but dry fruits and those treated with distilled water (control) produced significantly more ethylene compared to Put and $CaCl_2$ treatments.

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