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Full Length Research Paper

Combined effects of hot air and calcium chloride on quality and antioxidant enzymes activity in 'red delicious' apple fruits

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In order to increase the storage life of 'red delicious' apple fruits; they were treated by pressure infiltration with 0, 2 and 4% calcium chloride ($CaCl_2$) solutions and then heat**ed for 0, 24 and 48 h at 38°C. Fruits were stored at 0°C for up to 6 months after treatment. Some quality characteristics including hydrogen peroxide level and antioxidant enzymes activity such as superoxide dismutase (SOD) and Catalase (CAT) were evaluated at 2 months interval. The results showed that the combination of 4% $CaCl_2$ with heat treatment for 48 h improved firmness during storage. The combination of hot air and $CaCl_2$ increased SOD and CAT activities in comparison with untreated fruits. Higher activities of antioxidant enzymes lead to decrease of hydrogen peroxide thus protect cell membranes from damage.**

Key words: *Malus domestica* Borkh, red delicious, $CaCl_2$, catalase, fruit, heat, hydrogen peroxide, superoxide dismutase.

INTRODUCTION

There has been increasing interest in usage of heat treatments to control insect pests, prevent fungal rots, increase resistance to chilling injury, delay fruit ripening and extend post-harvest shelf life of fruits and vegetables (Lurie, 1998). Shahram et al., (2003) reported that α-farnesene accumulation and oxidation were slower in the skin of heated fruits. Leverentz et al., (2000) found that pre-storage heating (38°C for four days) extended the post-harvest life of apple fruit throughout a delay in rotting. Also, Saftner et al., (2003) reported that exposure of apples to hot air accelerated the loss of green peel colour and titratable acidity, but maintained firmness and delayed respiration increasing, ethylene production and volatile levels following cold storage. Inhibition of solubilisation of the carbonate-soluble pectin fraction is one of the main factors contributing to firmness retention due to heat treatment. However, loss of natural sugar side chains during heat treatment also leads to closer packing of the pectin strands, which hinders enzymatic cleavage, resulting in firmer fruit (Drake and Spayed, 1983; Lamikanra and Watson, 2007; Roy et al, 1994). Pre-harvest and post-harvest treatments with Calcium salts have been effective in controlling several physiological disorders, reducing the incidence of fungal pathogens and maintaining fruit firmness (Dris and Niskanen, 1999; Hernández-Muñoz et al., 2008). García et al. (1996) showed that dipping strawberries in 1% calcium chloride ($CaCl_2$) solution was the most effective treatment for increasing the calcium content of the fruits, controlling the post-harvest decay and maintaining the firmness and soluble solids content. The positive effects provided by calcium chloride can be explained by: (1) the complex of calcium ions with cell wall and middle lamella pectin, (2) the stabilization of the cell membrane by calcium ions, and/or (3) effect of calcium on cell turgor pressure (Luna-Guzman and Barrett, 2000). Heat allows the formation of COO^- groups from the pectin content of the fruits with which Ca^{++} ions can form salt-bridge cross-links (Stanley et al. 1995, Food Sci., 60: 327-333). This

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makes the cell wall less accessible to the enzymes that cause softening (Lamikanra and Watson, 2007). In recent years, increasing interest has focused on enzymatic and non-enzymatic antioxidants in relation to storage, shelf life and nutritional quality of fruits and vegetables in post-harvest. Fruits and vegetables employ diverse enzymatic antioxidants such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), peroxidase (POD, EC 1.11.1.7) and ascorbate peroxidase (AsPX; EC 1.11.1.11), in various combinations to regulate and maintain active oxygen species (AOS) at controlled steady-state concentrations (Lurie, 2003). Non-enzymatic antioxidants also play a role in resistance to physiological disorders caused by oxidative stresses (Petkovsek et al., 2007).

Therefore, the objectives of this investigation were to evaluate the effects of hot air and calcium chloride for maintaining the quality of 'red delicious' apple and also examined the relationship between the antioxidant system dynamics with post-harvest quality of treated fruit.

MATERIALS AND METHODS

Plant material and treatments

'Red delicious' apple fruits *(Malus domestica* Borkh.*)* were randomly harvested in the pre-climacteric stage from a commercial orchard in Aghajari, East Azerbaijan province of Iran. Apples were selected based on shape, size and free of fungal infection. Fruits were divided into three groups (each group 108 apples) for calcium chloride treatment. Fruits were pressure infiltrated (3 min at 68.95 kPa) with 0, 2 and 4% solutions of $CaCl_2$ (Merck, Darmstadt, Germany). Pressure infiltration was done using the method of Hrirahrara (1982). After calcium chloride treatment, each group of apples divided into three equal parts (each part 36 apples) and then exposed to hot air (38°C) with in the period of 0, 24 and 48h. Heat treatments were conducted inside a gas-tight, temperature-controlled, forced-air chamber (Yahria and Ortega, 2000). All the treatments were replicated 3 times. After treatment, fruits were placed in polyethylene boxes and stored in cold storage at 0°C and 95% relative humidity for up to 6 months. At 2 months interval, sampling was done from stored fruits for evaluation of total soluble solids (TSS), titratable acidity (TA), pH, dry matter, flesh firmness, hydrogen peroxide (H_2O_2), CAT and SOD activities.

Fruit quality evaluation

Twelve samples were selected from each treatment every 2 months of storage period. Apple juice was extracted by a Pars Khrazar E17 electric juicer (Pars Khrazar Inc., Tehran, Iran) for measuring fruit quality parameters such as TSS, TA and pH.

Total soluble solids (TSS) was measured with an Atago N-1 handheld digital refractometer (Atago Co., Ltd., Tokyo, Japan) and expressed as Brix percent. Titratable acidity (TA) was determined by titration with 0.1N NaOH up to pH 8.1, using 10 ml of fruit juice diluted with 90 ml of distilled water and results were calculated as percentage of malice acid. The pH of juice was recorded by Hanna B 417 pH meter (Hanna Instruments Inc., Woonsocket, Rhode Island, USA). Dry matter was determined by drying specified weight of fruits in Hreraeus T5042 EK oven (Kendra Laboratory Products GmbH, Hanau, Germany) at 72°C for 48h. Firmness was measured after removing a small area of peel, using McCormick FT-327 penetrometer (Facchrini Srl., Alphronsine, Italy) fitted with a 11 mm tip. Values were expressed as Newton (N). Measurement for each characteristic was performed in the three replications.

HR_2O_2 measurement

HR_2O_2 measurement was done according to Duan et al. (2008). Frozen apple tissues (0.5 g) were homogenised with 5 ml of 0.1% (w/v) trichrloracetic acid (TCA) in an ice bather. The homogenate was centrifuged at 12,000xg for 15 min at 4°C (model 3-16k, Sigma Co., Ltd., Munich, Germany), and 0.5 ml of supernatant was mixed with 0.5 ml of potassium phosphate buffer (10 mM, pH 7.0) and 1 ml of potassium iodide (1 M).

The reaction was developed for 1 h in darkness and absorbance measured at 390 nm. Hydrogen peroxide concentration was calculated using a standard curve prepared with known concentrations of H_2O_2 and values were expressed as mM.

SOD and CAT extraction and activity assay

For SOD extraction, frozen apple tissue (0.5 g) was homogenised with 15 ml of potassium phosphate buffer (50 mM, pH = 7.0) containing 10% polyvinyl-polypyrolidone (PVPP) and 0.1 mM ethylene di-amine tetra acetic acid (EDTA). The homogenate was centrifuged at 15000xg for 15 min at 4°C (Gong et al., 2001). The supernatant was used for SOD activity assay. SOD activity was determined by measuring inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) using the method of Beauchamp and Fridovichr (1971). The reaction mixture (3 ml) was composed of 13 mM methrionine, 0.075 mM NBT, 0.1 mM EDTA, 0.002 mM riboflavin, and 0.1 ml of enzyme extract in 50 mM phosphate buffer (pH= 7.8). Riboflavin was added at last and the reaction was initiated by placing the tubes below a light source of two 20 W florescent lamps at a 6 cm distance for 7 min. The reaction was stopped by switching off the light and covering the tubes with black cloth.

Absorbance was read at 560 nm with a UNICCO UV-2100 spectrophotometer (UNICCO Instrument Co., Ltd., Shanghais, China). Two tubes without enzyme extract were taken as control. The activity of SOD was presented as units/mg protein. One unit of SOD activity was defined as the amount of enzyme that reduced the absorbance reading of samples to 50% in comparison with tubes lacking enzymes.

For CAT extraction, fresh apple flesh tissue (0.5 g) was homogenised in 15 ml of Tris-HCl buffer (pH = 8.5) including 2 mM EDTA, 10% (w/v) PVPP. The homogenate was centrifuged at 15000xg for 15 min at 4°C. Supernatant was used for the CAT activity measurement. CAT activity was determined by following the disappearance of H_2O_2 in the enzyme reaction mixture. Foundation of CAT activity is based on hydrogen peroxide decomposition to water and oxygen (Brennan and Frenkel, 1977). The extracted enzyme (0.25 ml) was added to 2 ml of assay mixture (50 mM Tris-HCl buffer pH = 6.8, containing 5 mM H_2O_2). The reaction was stopped by adding 0.25 ml of 20% (w/v) titanic tetrachloride (in concentrated HCl) after 10 min in 20°C. A blank was prepared by adding of 0.25 ml of 20% (w/v) titanium tetrachloride at zero time to stop the enzyme activity. The absorbance of solution was read at 415 nm against distilled water. CAT activity was determined by comparing absorbance against a standard curve of H_2O_2. The activity of CAT was expressed as katal/mg protein (1 katal = 1 mol/sec).

Table 1. Effects of calcium chloride and hot air treatments on fruit quality and activity of antioxidant enzymes in 'red delicious' fruits after 6 months storage in 0°C.

Treatment	TSS (% Brix)	TA (%)	pH	Dry matter (%)	Firmness (N)
$CaCl_2$, 0%	14.622±0.224	0.3558±0.016^{c}	4.237±0.025^{a}	16.393±0.298	63.608±13.867^{c}
2%	14.619±0.217	0.3916±0.018^{b}	4.204±0.023^{a}	15.964±0.287	69.806±16.329^{b}
4%	14.376±0.231	0.4471±0.021^{a}	4.136±0.020^{b}	16.312±0.306	75.171±12.602^{a}
Significance	NS	**	**	NS	**
Hot air, 0h	14.763±0.214	0.4502±0.021^{a}	4.113±0.023^{b}	16.289±0.328	66.472±14.181^{b}
24 h	14.459±0.206	0.4079±0.019^{b}	4.225±0.018^{a}	16.379±0.275	69.904±16.986^{ab}
48 h	14.394±0.249	0.3365±0.012^{c}	4.24±0.024^{a}	16.001±0.285	72.209±14.622^{a}
Significance	NS	**	**	NS	**

TSS = total soluble solids; TA = titratable acidity. Different letters in each column indicate significant difference using DMRT ($P \leq 0.05$). Each value represents the mean of 27 determinations ± S.D (Standard deviation). NS. ** indicate not significant and significant levels at $P \leq 0.01$, respectively.

Statistical analysis

A factorial experiment based in a completely randomized design with three replications was used. Analysis of variance of all parameters was done with MSTATC statistical software (Version 1.42, Crop and Soil Sciences Department, Michigan State University, USA). Treatment means were separated using Duncan's multiple range tests (DMRT, $P \leq 0.05$).

RESULTS AND DISCUSSION

Effects of calcium chloride and heat treatments on quality characteristics

During storage, dry matter and TA decreased but TSS and pH increased. After 6 months of storage, dry matter had no significant difference among treatments. There were no significant differences in total soluble solids between calcium chloride and heat treated apples (Table 1). Calcium chloride and heat treatments significantly affected TA of 'red delicious' apples. The TA of the 4% $CaCl_2$ treated apples was higher than that of the 2% $CaCl_2$ treated and control fruits. Heated fruits had significantly lower TA than non-heated fruits (Table 1). Calcium chloride and heat treatments significantly affected pH of 'red delicious' apples. The pH of the 4% $CaCl_2$ treated apples was lower than that of the 2% $CaCl_2$ treated and control fruits. Heated fruits had significantly higher pH than non-heated fruits and there was no significant difference between the 24 and 48 h heat treatments (Table 1).Results confirm the influence of heat and $CaCl_2$ on fruit texture firmness. Application of Calcium chloride generally increased firmness of the apple texture. Apples treated with 4% $CaCl_2$ had higher firmness in comparison with 2% $CaCl_2$ treated and control fruits. Heat treated fruits were significantly ($P < 0.01$) firmer than the non-heated fruits (Table 1). Fruits treated with 4% calcium chloride and heat treatments for 48 h were firmer than the other treatments after 6 months of storage (Figure 1).

Effects of calcium chloride and heat treatments on antioxidant enzymes activity

Comparison of enzyme activities in apples following 2, 4 and 6 months of storage, demonstrated that enzymes' activities, depending on the pre-storage treatment, increase or decrease or remain unchanged. Calcium chloride and heat treatments significantly affected SOD activity of 'red delicious' apples. SOD activity of heat treated fruits increased with increasing the time of heating (Table 2). SOD activity of the 2% $CaCl_2$ treated apples was significantly higher than that of the 4% $CaCl_2$ treated and control fruits (Table 2). SOD activity increased until 4th months of storage, thereafter, its activity decreased at the end of storage that is, 6th months (Figure 3C).

CAT activity was significantly higher at 2 and 4% $CaCl_2$ treated fruits than controls and there was no significant difference between 2 and 4% $CaCl_2$ treated fruits (Table 2). HR_2O_2 level decreased in both calcium chloride and hot air treatments (Figure 2A). CAT activity of the heat treated fruits increased with increase in time of exposure to heat. Correlation between CAT activity and heat treatment duration was positive, so that by increasing heat treatment duration CAT activity increased. The highest level of CAT and SOD activities were observed in apples treated with 4% $CaCl_2$ and exposed 48 h to hot air (Figure 2B and C). In addition, CAT activity increased during the storage period (Figure 3B).

H_2O_2 level significantly decreased in calcium chloride treated fruits. Heat treatment had no significant effect on the level of H_2O_2 (Table 2). The H_2O_2 level increased during the storage period (Figure 3A). Our data indicate the possible effects of $CaCl_2$ and heat treatments on quality improvement of apples. Calcium chloride and heat treatments had no effect on TSS and dry matter. Similar results have been reported by Zhranga et al. (2007) and Le et al. (2010).

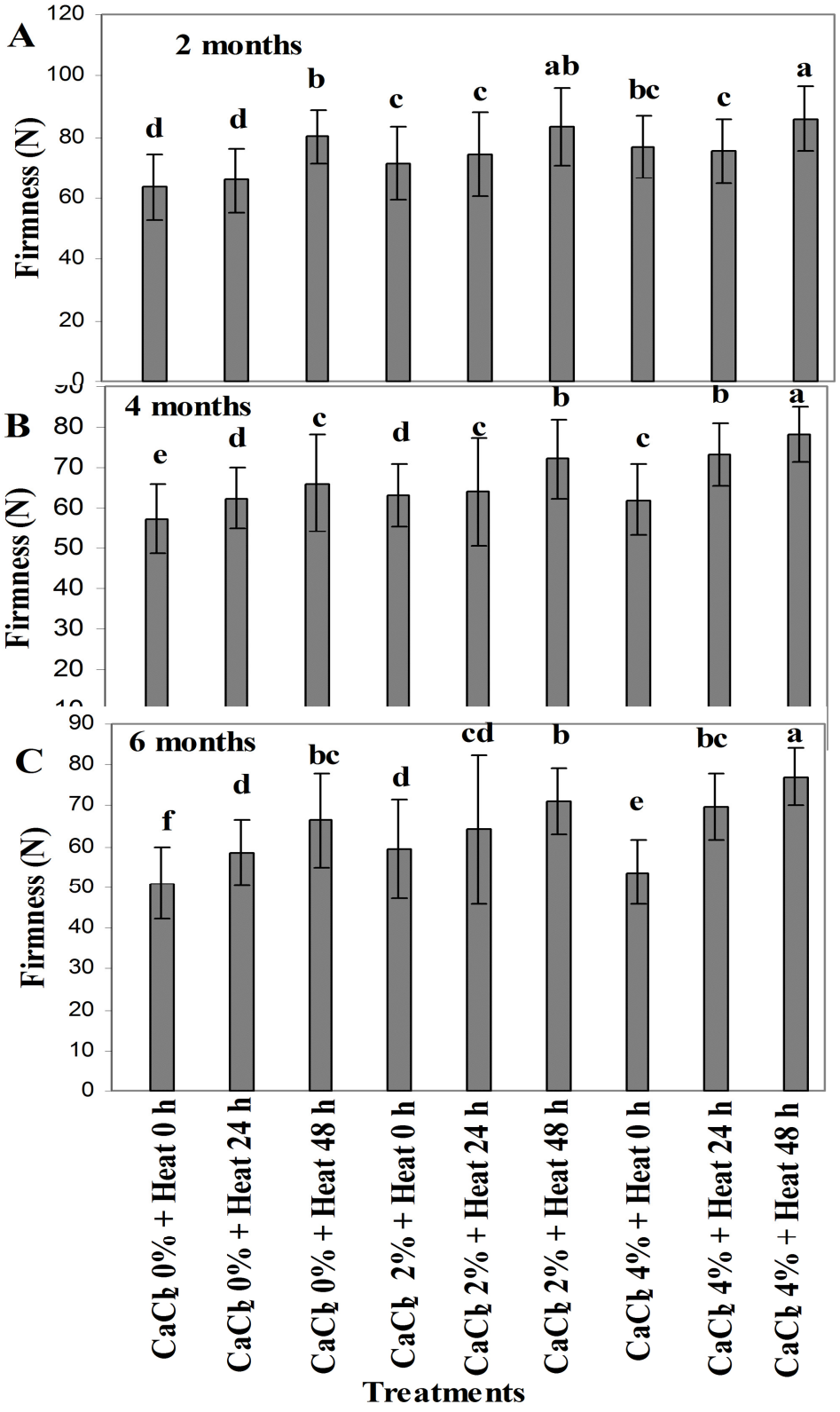

Figure 1. Effect of calcium chloride and hot air treatments on the firmness of 'red delicious' fruits after 2, 4 and 6 months storage at 0°C. Measurements were made with a penetrometer. Different letters above each bar indicate significant difference using DMRT ($P \leq 0.05$). Each data point is the average of 9 determinations ± S.E (Standard Deviation).

Table 2. Effect of calcium chloride and hot air treatments on antioxidant enzymes activity and HR_2O_2 in 'Red Delicious' apple after 6 months of storage at 0°C.

Treatment	SOD (Units / mg protein)	CAT (katal/mg protein)	H_2O_2 (mM)
$CaCl_2$, 0%	155.444±10.934[c]	5.314±0.545[b]	1.656±0.052[a]
2%	180.722±12.936[a]	6.097±0.579[a]	1.341±0.024[b]
4%	170.889±11.760[b]	5.908±0.597[a]	1.238±0.066[c]
Significance	**	**	**
Hot air, 0h	135.75±9.313[c]	4.564±0.451[c]	1.393±0.026
24 h	177.139±11.641[b]	6.031±0.577[b]	1.430±0.077
48 h	194.167±12.674[a]	6.725±0.626[a]	1.411±0.061
Significance	**	**	NS

SOD = Superoxide dismutase; CAT = Catalase; H_2O_2 = Hydrogen peroxide. Different letters in each column indicate significant difference using DMRT ($P \leq 0.05$). Each value represents the mean of 27 determinations ± S.D (Standard deviation). NS, ** indicate not significant and significant levels at $P \leq 0.01$, respectively.

PH value increased in apples, which were heated for 24 or 48 h at 38°C. The pH value decreased with increasing $CaCl_2$ concentration as the lowest level of pH was obtained in samples which were treated with 4% $CaCl_2$. These results are confirmed by Mahmud et al. (2008) and Wojcik (2001). Ferguson (1984) supposed that calcium ions interact with free carboxyl groups in the membrane phospholipids and cell wall pectin's and chemical alteration of fruit ripening delayed, conesquently, organic acid degradation decreases and as a result pH level reduces. Apples treated with 4% $CaCl_2$ had higher titratable acidity in comparison with 2% $CaCl_2$ treated and control fruits. Titratable acidity is directly related to the amount of organic acids in fruit, which are an important parameter in fruit quality. $CaCl_2$ retards ripening by inhibition of pectin esterase (PE) and polygalacturonase (PG) activities which are involved in the enzymatic reactions of respiration and thereby $CaCl_2$ application brings about decrease in degradation of organic acids. These results are similar with the studies of Chruni et al. (2010) in fresh-cut dragon fruit. Titratable acidity of the heat treated fruits increased with prolongation of exposure time to heat. Similar observations were reported by Gholamian et al. (2008) in heated peach fruits.

It was found that $CaCl_2$ treatment improved the firmness of apple. This improvement had positive correlation with $CaCl_2$ concentration. Luna-Guzman and Barrett (2000) observed that 2.5% $CaCl_2$ at 25°C was effective in delaying the softening of fresh-cut cantaloupes. A close relationship has been established between fruit calcium levels with firmness and quality of fruits (Chrardonnet et al., 2003). Ishraqi et al. (2009) reported that 3% $CaCl_2$ dips improved firmness in apricot fruits. Post-harvest application of $CaCl_2$ by increasing Ca^{2+} concentration in apple tissue, can inhibit fruit ripening and decay (Ferguson, 1984). Tissue firming has been attributed to the cross-linking between the carboxyl groups of adjacent polyuronide chains and divalent calcium ions creating the so-called 'egg-box model'. The beneficial effect of calcium treatments on greater turgor pressure and firmness of tomato pericarp discs has also been reported (Luna-Guzman et al., 1999).

Flesh firmness of heat treated apples was better than non-heated fruits. Lurie et al. (1996) found that during the heat treatment, cuticular wax softens and fills in cracks on the fruit peel surface. Similar observations were reported by Ekinci and Çelik (2006) in heated apples. It has also been reported that cell wall degrading enzymes are destroyed by heat treatment (Obenland and Neipp, 2005). Highest firmness was observed in apples which were treated with 4% $CaCl_2$ and exposed to 48 h hot air that calcium ions infiltration enhanced into the apple tissue during the heating time.

Fruits have self defence mechanism to protect from oxidative stress by the activation of much antioxidant defence system. The development of oxidative stress in fruit mainly depends upon its cellular antioxidant levels, physical atmosphere of the fruits and its post-harvest handling (Niranjana et al., 2009). Activated oxygen species in plant cells, can react with unsaturated fatty acids to cause peroxidation of membrane lipids in the plasma membrane or in intracellular organelles (Gechrev and Hille, 2005). Peroxidation damage of the plasmalemma leads to leakage of cellular contents, rapid dehydration and cell death in plant tissue (Scandalios, 1993).

SOD activity was higher in $CaCl_2$ treated fruits; similar result was reported by Schrmitz-Eiberger et al. (2002). Also, SOD activity increased with increasing heating duration. Increasing SOD activity was concomitant with superoxide radical scavenging activity increasing and decreasing of cell membrane damage and oxidative stress (Mittler, 2002). Moreover, increasing SOD activity trend activates other antioxidant enzymes which are very dynamic in H_2O_2 scavenging such as catalases (Yörök et

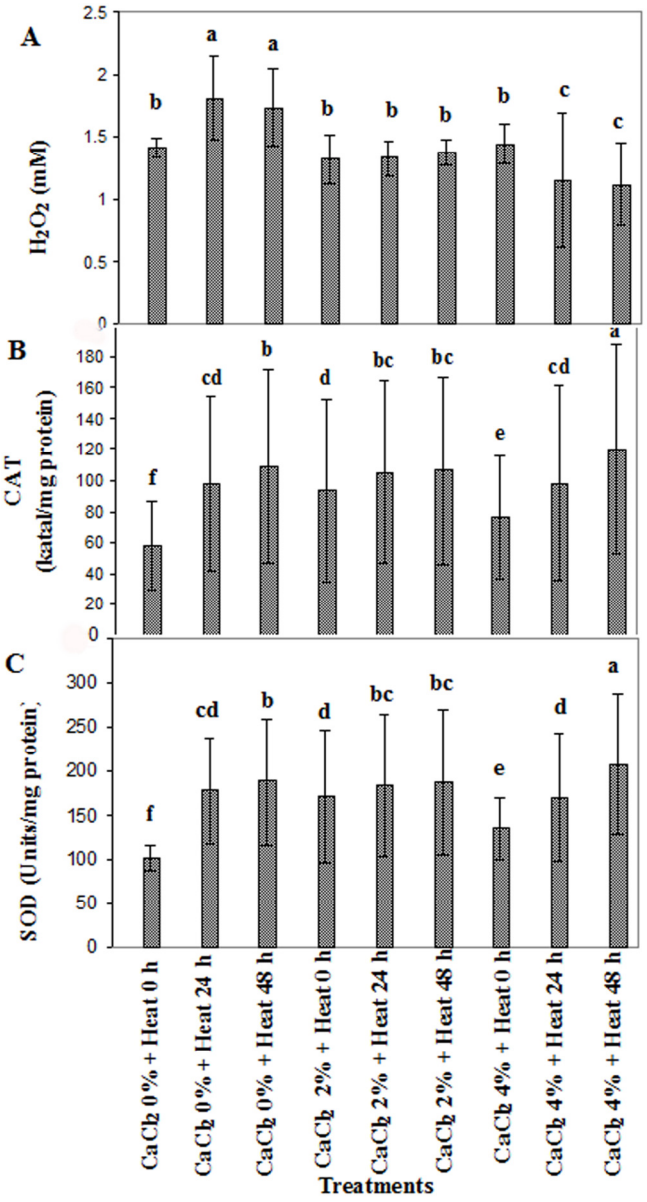

Figure 2. Effects of Calcium chloride and hot air on the activity of antioxidant enzymes in 'Red Delicious' fruits after 6 months storage at 0°C. Different letters above each bar indicate significant difference using DMRT ($P \leq 0.05$). Each data point is the average of 9 determinations ± S.E (Standard Deviation). Different letters above each bar indicate significant difference using DMRT ($P \leq 0.05$).

al., 2005) and peroxidises (Lata, 2008). Catalase decomposes hydrogen peroxide, a powerful and potentially harmful oxidizing agent, to water and molecular oxygen. Catalase also uses hydrogen peroxide for oxidation toxic materials such as phenols, formic acid, formaldehyde and alcohols (Karra-Chraabouni et al., 2003). Whole of scientific testimonies indicate that catalase plays an important role in plant defence system, aging and senescence (Mura et al., 2007). CAT activities increased in apples which were treated with $CaCl_2$.

Higher activity of CAT decreases the amount of H_2O_2 in sub cellular and increases the stability of cell membranes (Yamazaki et al., 2003). CAT activity increased during storage which is important in reactive oxygen species (ROS) scavenging system (Arora et al., 2002). Apples treated with 4% $CaCl_2$ and exposed to 48 h hot air had

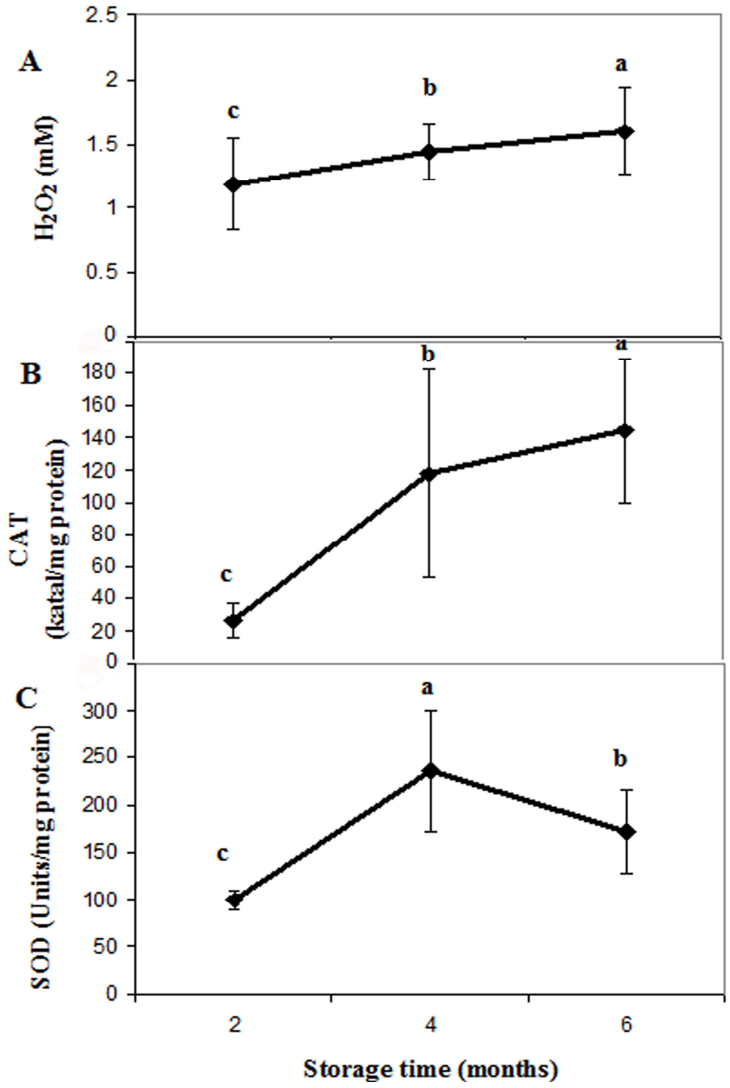

Figure 3. Storage time effects on the activity of the antioxidant enzymes in 'Red Delicious' apple. Each data point is the average of 27 determinations ± S.E (Standard Deviation). Different letters above each diamond symbol indicate significant difference using DMRT ($P \leq 0.05$).

the highest ROS scavenging capacity especially for H_2O_2. This investigation confirmed that, $CaCl_2$ and heat treatments maintained the quality of apple fruit under storage conditions and this as associated with the higher activities of SOD and CAT in treated fruits. Higher activities of antioxidant enzymes lead to successful scavenging of ROS thus protect cell membranes from damage.

Conclusion

In general, the data obtained from the present experiment showed that diverse natural antioxidant enzymes and their steady state and dynamic function positively affect biochemical streams in cellular and sub cellular level and overall organ. These primitive actions change the routine biochemical pathways in favour of organ and plant growth, development and post-harvest biology. Owing to the mentioned effects, optimum and balanced levels of the photochemical highly promoted the quality attributes of apple fruit under storage conditions and could be a useful trend for post-harvest biology studies of other fruits and horticultural crop.

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REFERENCES

Arora A, Sairam RK, Srivastava GC (2002). Oxidative stress and antioxidative system in plants. Curr. Sci., 82(10): 1227-1237.

Beauchramp C, Fridovichr I (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem., 44(1): 276-287.

Brennan T, Frenkel C (1977). Involvement of hydrogen peroxide in the regulation of senescence in pear. Plant Physiol., 59(3): 411-416.

Chrardonnet CO, Chrarron CS, Sams CE, Conway WS (2003). Chemical changes in the cortical tissue and cell walls of Calcium-infiltrated 'Golden Delicious' apples during storage. Postharvest Biol. Technol., 28(1): 97-111.

Chruni SHR, Awang Y, Mohramed MTM (2010). Cell wall enzymes activities and quality of Calcium treated fresh-cut red flesh dragon fruit (*Hylocereus polyrhrizus*). Int. J. Agric. Biol., 12(5): 713-718.

Drake SR, Spayed SE (1983). Influence of Calcium treatment on Golden Delicious apple quality. J. Food Sci., 48(2): 403-405.

Dris R, Niskanen R (1999). Calcium chloride sprays decrease physiological disorders following long term cold storage of apple. Plant Foods Hum. Nutr., 54(2): 159-171.

Duan WE, FAN PG, Wang LJ, Li WD, Yan ST, Li SHR (2008). Photosynthetic response to low sink demand after fruit removal in relation to photoinhribition and photo protection in peach trees. Tree Physiol., 28(1): 123-132.

Ekinci N, Çelikv S (2006). The effects of heat application on the quality of Golden Delicious and staking Golden Delicious apple varieties. J. Agron., 5(3): 509-514.

Ferguson IB (1984). Calcium in plant senescence and fruit ripening. Plant Cell Environ., 7(6): 477-489.

García JM, HRerrera S, Morilla A (1996). Effects of postharvest dips in Calcium chloride on strawberry. J. Agric. Food Chem., 44(1): 30-33.

Gholamian M, Moallemi N, Alamzadeh N, Sadrzadeh M (2008). Effect of $CaCl_2$ and UV-C irradiation on quality and storage life of peach fruits. Iran. J. Hort. Sci., 9(1): 35-44.

Gechrev T, HRille J (2005). Hydrogen peroxide as a signal controlling plant programmed cell death. J. Cell Biol., 168(1): 17-20.

Gong Y, Toivonen PMA, Lau OL, Wiersma PA (2001). Antioxidant system level in 'Braeburn' apple is related to its browning disorder. Bot. Bull. Acad. Sinica., 42(4): 259-264.

HRernández-Muñoz P, Almenar E, Valle VD, Velez D, Gavara R (2008). Effect of chritosan coating combined with postharvest Calcium treatment on strawberry (*Fragaria × ananassa*) quality during refrigerated storage. Food Chem., 110(2): 428-435.

HRirahrara K (1982). Calcium chloride infiltration of apples. Chricago, FMC Corporation. hrttp://www.patentgenius.com/patent/4353930.hrtml [accessed 21 August 2010].

Ishraq S, Rathrore HRA, Masud T, Ali S (2009). Influence of postharvest Calcium chloride application, ethylene absorbent and modified atmosphere on quality characteristics and shelf life of apricot (*Prunus armeniaca* L.) fruit during storage. Pak. J. Nutr., 8(6): 861-865.

Karra-Chraabouni M, Pulvin S, Meziani A, Thromas D, Touraud D, Kunz W (2003). Bio oxidation of n-hexanol by alcohols oxidise and Catalase in biphasic and micelle systems without solvent. Biotechnol. Bioeng., 81(1): 27-32.

Lamikanra O, Watson MA (2007). Mild heat and Calcium treatment effects on fresh-cut cantaloupe melon during storage. Food Chem., 102(4): 1383-1388.

Lata B (2008). Apple peels antioxidant status in relation to genotype, storage type and time. Sci. Hort., 117(1): 45-52.

Leverentz B, Janisiewicz WJ, Conway WS, Saftner RA, Fuchrs Y, Sams CE, Camp MJ (2000). Combining yeasts or a bacterial bio control agent and heat treatment to reduce postharvest decay of 'Gala' apples. Postharvest Biol. Technol., 21(1): 87-94.

Le TN, Shriesh CC, Lin HRL (2010). Effect of vapor heat and hot water treatments on disease incidence and quality of Taiwan native strain mango fruits. Int. J. Agric. Biol., 12(5): 673-678.

Luna-Guzman I, Barrett DM (2000). Comparison of Calcium chloride and Calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes. Postharvest Biol. Technol., 19(1): 61-72.

Luna-Guzman I, Cantwell M, Barrett DM (1999). Fresh-cut cantaloupe: effects of $CaCl_2$ dips and heat treatments on firmness and metabolic activity. Postharvest Biol. Technol., 17(3): 201-213.

Lurie S (1998). Postharvest heat treatments. Postharvest Biol. Technol., 14(3): 257-269.

Lurie S (2003). Antioxidants. In: Hodges DM ed. Postharvest oxidative stress in horticultural crops. 1st edition. New York, Haworth Press. Pp.131-150.

Lurie S, Fallik E, Klein JD (1996). The effect of heat treatment on apple epicuticular wax and Calcium uptake. Postharvest Biol. Technol., 8(4): 271-277.

Mahmud TMM, Al Eryani-Raqeeb A, Seyed Omar SR, Mohamed Zaki AR, Al Eryani AR (2008). Effects of different concentrations of applications of Calcium on storage life and physicochemical characteristics of Papaya (*Carica papaya* L.). Am. J. Agric. Biol. Sci., 3(3): 526-533.

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. Trends in Plant Sci., 7(9): 405-410.

Mura A, Pintus F, Medda R, Floris G, Rinaldi AC, Padiglia A (2007). Catalase and antiquation from *Euphrorbia chraracias*: Two proteins involved in plant defence. Biochem., 72(5): 501-508.

Niranjana P, Gopalakrishrna KPR, Sudhrakar DVR, Madhrusudhran B (2009). Effect of pre-cooling and heat treatment on antioxidant enzymes profile of mango and banana. Afr. J. Food, Agric. Nutr. Dev., 9(5): 1210-1225.

Obenland DM, Neipp PM (2005). Forced hot air treatment of stone fruit to inhibit the development of maleness. Acta Hort., 682(5): 1171-1178.

Petkovsek MM, Stampar F, Veberic R (2007). Parameters of inner quality of the apple scab resistant and susceptible apple cultivars *(Malus domestica* Borkhr*)*. Sci. Hort. 114(1): 37-44.

Roy S, Conway WS, Watada AE, Sams CE, Erbe EF, Wergin WP (1994). Heat treatment affects epicuticular wax structure and postharvest Calcium uptake in Golden Delicious apples. Hortcult. Sci., 29(9): 1056-1058.

Saftner RA, Abbot JA, Conway WS, Barden CL (2003). Effects of 1-methrylcyclopropane and heat treatments on ripening and postharvest decay in 'Golden Delicious' apples. J. Am. Soc. Hort. Sci., 128(1): 120-127.

Scandalios JG (1993). Oxygen stress and superoxide dismutase. Plant Physiol., 101(1): 7-12.

Schrmitz-Eiberger M, Haefs R, Noga G (2002). Calcium deficiency-influence on the antioxidative defence system in tomato plants. J. Plant Physiol., 159(7): 733-742.

Shahram Z, Lers A, Lurie S (2003). Effect of heat or 1-methrylcyclopropane on antioxidative enzyme activities and antioxidants in apples in relation to superficial scald development. J. Am.Soc. Hort. Sci., 128(6): 761-766.

Wojcik P (2001). 'Jonagold' apple fruit quality as influenced by fall sprays with Calcium chloride at higher rates. J. Plant Nutr., 24(12): 1925-1936.

Yahria EM, Ortega D (2000). *In vitro* mortality of *Anastrephra ludens* and *A. obliqua* with insecticidal atmospheres at higher temperatures. Postharvest Biol. Technol., 20(3): 295-302.

Yörök IHR, Demir HR, Ekici K, Savran A (2005). Purification and properties of Catalase form Van apple (Golden Delicious). Pak. J. Nutr., 4(1): 8-10.

Zhranga HR, Wanga L, Zhrengb X, Donga Y (2007). Effect of yeast antagonist in combination with heat treatment on postharvest blue meld decay and Rhrizophus decay of peaches. Int. J. Food Microbiol., 115(1): 53-58.