



Effects of calcium chloride and hot air on antioxidant system of 'Red Delicious' apple during storage

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Abstract

The effects of calcium chloride and hot air treatment on the postharvest quality of apple fruit (Red Delicious) was studied. Apple fruits were dipped in the calcium chloride (0, 2 and 4%) solution under pressure. Fruits were exposed to hot air at 20°C (control) and 38°C, and 95% relative humidity for 24 and 48 hrs and then stored at 0°C for 6 months. As a result, H₂O₂ and catalase (CAT) levels increased in storage period. Superoxide dismutase (SOD) and CAT levels increased with 4% calcium chloride and 24 and 48 h heat treatments. Finally the results indicated the potential improvement of apple shelf life by calcium chloride and hot air pretreatment with less negative effects on antioxidant levels during storage.

Key words: Apple, calcium chloride, heat treatment, antioxidant enzymes, storage.

Introduction

In recent years increasing interest focused on enzymic and non-enzymic antioxidants in relation to storage, shelf life and nutritional quality of postharvest fruits and vegetables ¹. The changes in antioxidant enzymes level in response to stress in plants is well known, and more recently such changes have been related to some postharvest disorders such as chilling injury in squash ² and pear ³, superficial scald in apple ^{4,5} and senescence of pear and apple fruits ^{4,6}.

Fruits and vegetables employ diverse enzymatic antioxidants, 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 ¹, and non-enzymatic antioxidants play a role in resistance to physiological disorders caused by oxidative stresses. Antioxidant compounds are found in all higher plants, and they include ascorbic acid, α -tocopherol, β -carotene, glutathione and some flavonoids ⁷. Under stress conditions, reactive oxygen species (ROS) typically are produced and these species are highly cytotoxic and can seriously react with vital biomolecules, such as lipids, proteins, nucleic acids etc., causing lipid peroxidation, protein denaturation and DNA mutation, respectively ^{8,9}. In this study, we examined the relationship between the antioxidant system dynamics in relation to the postharvest quality of 'Red delicious' apple under different combinations of calcium chloride and hot-air treatments.

Material and Methods

Apple fruits (Red Delicious) were harvested from an orchard at

the fruit mature stage during 2007/2008 season. Calcium chloride concentrations of 0, 2 and 4% were applied on fruits for 30 seconds as floating and penetration under 250 mmHg, 38°C and 90% relative humidity. Furthermore, calcium chloride treatment exposure times were 0, 48 and 72 h, respectively. Thereafter, fruits were stored at 0°C temperature and 90% RH for 6 months. Fruits were sampled for enzymatic assays every 2 months.

Enzyme extraction: For SOD and CAT extraction, frozen apple tissue (5 g) was homogenized with 15 ml of 0.05 M phosphate buffer (pH 7.0) containing 10% PVP and 0.1 M EDTA. The homogenate was centrifuged at 15,000g for 15 min at 4°C. The supernatant was used for SOD activity assay. SOD activity was determined by measuring inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) using the method of Beauchamp and Fridovich¹⁰. The reaction mixture (3 ml) was composed of 13 mM methionine, 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). The mixture in the tube was placed on a rotating tube holder in a light box for 7 min. The absorbance was read at 560 nm with a spectrophotometer (Unico, UV-2100). CAT activity was determined by following the disappearance of H₂O₂ in the enzyme reaction mixture¹¹. The enzyme extract (0.25 ml) was added to 2 ml assay mixture (50 mM tris-HCl buffer pH 6.8, containing 5 mM H₂O₂). The reaction was stopped by adding 0.25 ml of 20% titanous tetrachloride (in concentrated HCl) after 10 min at 20°C. A blank was prepared by addition of 0.25 ml of 20% titanium tetrachloride at zero time to stop the enzyme activity. The absorbance of the reaction solution was read at 415 nm against distilled water.

Results

Comparison of enzyme activities following 2, 4 and 6 months of storage demonstrated that enzymes activity, depending on the pre-storage treatment, can remain unchanged, increase or decrease. Significant differences were observed between the different concentration of calcium chloride and hot-air treatment (Table 1).

H₂O₂ activity decreased in both calcium chloride and hot-air treatments (Fig. 1). Treatments of 4% Ca as well as 24 and 48 h temperature exposure had the highest ROS scavenging capacity especially for H₂O₂. H₂O₂ levels increased with prolonged storage conditions. SOD activity significantly increased in response to

Table 1. Variance analysis for the effects of calcium chloride and hot-air treatments on the antioxidant enzymes of apple (Red Delicious).

Storage time	SOD	CAT	H ₂ O ₂	SOD/CAT
2	100.05	1.56	1.19	64.13
4	235.44	7.08	1.44	33.25
6	171.55	8.67	1.6	19.78
	**	**	*	**

Storage time month; SOD Unit/min/mg protein; CAT H₂O₂ μmole/min/mg protein; H₂O₂ mmole, ** Significant (P≤0.01), * Significant (P≤0.05).

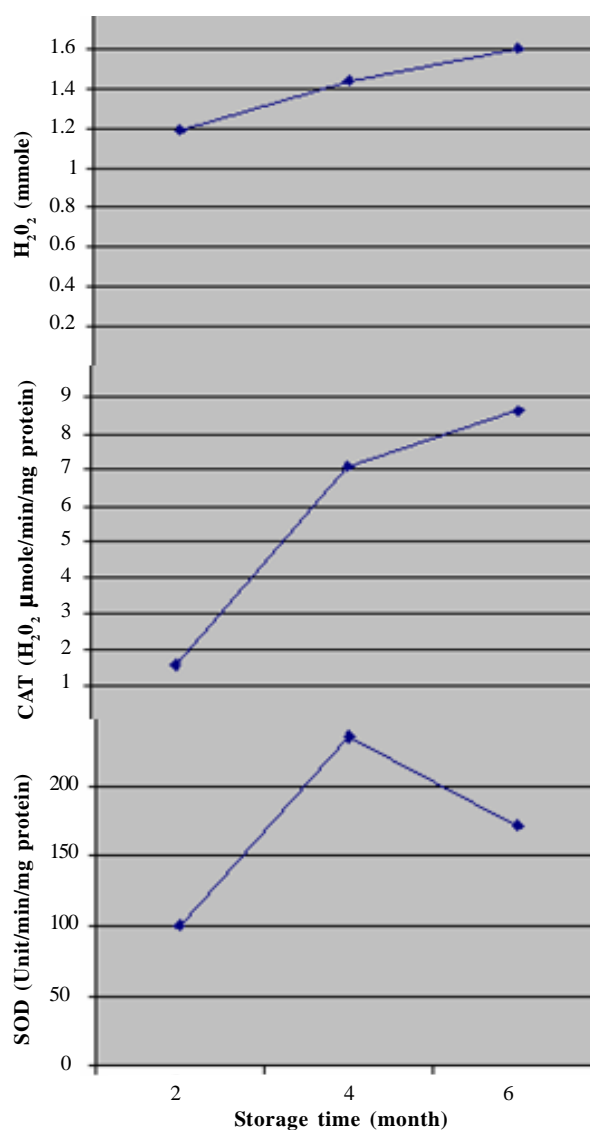


Figure 1. Effects of calcium chloride and hot-air treatments on the antioxidant enzymes of Apple (Red Delicious) during storage period.

4% Ca and 24 h thermal treatments (Fig. 1). This accompanies the vital role of SOD as initial defense system against ROS. SOD levels had increasing pattern until 4th month. Thereafter, its level decreased to the end of storage conditions, i.e. 6th month.

CAT activity had the highest level with 4% Ca and 24 and 48 h thermal treatments. In addition, CAT level had concomitant increase with H₂O₂ during storage condition. This trend led to the greater ROS scavenging capacity.

Discussion

Antioxidant compounds are important in prevention of different biotic and abiotic stresses in plants¹². 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 organelles¹³.

SOD activity increased with increasing calcium levels. Increasing SOD activity was concomitant with increased superoxide radical scavenging activity and decreased membrane damage and oxidative stress¹⁴. Moreover, this trend activates other antioxidant enzymes which are very dynamic in H₂O₂ scavenging such as catalases¹⁵ and peroxidases¹⁶. Higher activity of CAT decreases H₂O₂ level in cellular level and increase the stability of membranes and preserves CO₂ fixation because several enzymes of the Calvin cycle within chloroplasts are extremely sensitive to H₂O₂. A high level of H₂O₂ directly inhibits CO₂ fixation¹⁷. CAT levels increased during storage. This increase was evident especially after 4 months of storage by high scavenging capacity of H₂O₂. Low antioxidant activity of SOD and CAT in contrast with high content of superoxide radical and H₂O₂ ultimately led to an elevated level of hydroxyl radical. The later radical attacks all biomolecules, disrupts cell metabolism and causes membrane deterioration¹⁸. SOD and CAT activity was higher in control fruits and fruits treated with temperature and no Ca.

Conclusions

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 subcellular level and overall organ. These promotive actions change the routine biochemical pathways in favors of organ and plant growth, development and postharvest biology. Owing to above mentioned effects, optimum and balanced levels of these phytochemicals highly promoted of quality attributes of apple fruit under storage conditions and could be a useful trend for postharvest biology studies of other fruits and horticultural crops.

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