# Effects of Cold Storage Period on Fruit Antioxidants of Three Apple Cultivars Pre-treated with 2% $CaCl_2$ and 20°C Hot-air Treatment per 24 Hours

**[a]Elisabeta Susaj, [a]Irena Kallço, [b]Lush Susaj, [c]Belul Gixhari**
[a]University "Fan S. Noli", Faculty of Agriculture, Department of Plant Production, Korçë, Albania
[b]Agricultural University of Tirana, Faculty of Agriculture and Environment, Department of Horticulture, Kodër Kamëz, 1029, Tirana, Albania
[c]Agricultural University of Tirana, Faculty of Agriculture and Environment, Department of Plant Production, Kodër Kamëz, 1032, Tirana, Albania

**Corresponding author:**
Elisabeta Susaj
University "Fan S. Noli", Faculty of Agriculture, Department of Plant Production, Korçë, Albania

## Abstract

The study on the effect of cold storage period on postharvest fruit quality of three apple cultivars ("Red Delicious", "Golden Delicious" and "Granny Smith"), pre-treated with 2% $CaCl_2$ and 20°C hot-air treatment per 24 hours, was conducted during 2011-2012 vintage-storage season. Apple fruits of three cultivars were dipped in 2% $CaCl_2$ solution, under 250 mm Hg pressure, and were exposed on hot air treatment at 20°C and a relative humidity of 90% for 24 hours. Apple fruits were stored at 0°C up to 6 months. Three cold storage periods (2, 4 and 6 months), three apple cultivars (variants) and three replications for variant, with a plot size of 10 kg for variant in each replication, were used. Fruits were sampled for enzymatic assays every 2 months. There was found that Peroxidase ($H_2O_2$) and Catalase (CAT) levels were significantly increased during cold storage period for three cultivars, while Superoxide Dismutase (SOD) was significantly increased up to the end of 4$^{th}$ month for all cultivars and was significantly decreased after 4$^{th}$ month cold storage period. "Granny Smith" apple cultivar showed the highest levels of all antioxidant enzymes, with less negative effects on antioxidant levels during cold storage.

**KEYWORDS*:*** antioxidant enzymes, apple, $CaCl_2$, hot treatment, cold storage.

## INTRODUCTION

Apple (*Malus domestica* Borkh) is one of the most widely cultivated tree fruits in the world with 7500 cultivars all over the world. Due to its high nutritional value, apple ranks thirds in consumption after citrus and banana (UIE, 2013). Apple tree occupies a large area under cultivation, especially in the north-eastern and south eastern regions of Albania. Apple production in Albania is over 35% of fruit production (MoAFCP, 2012). Korça is one of the most known Albanian areas for qualitative apple production, because of a long tradition and specific agro-climate conditions. Apple tree in Korça occupies over 3000 ha under cultivation, with an annual production over 13 thousand tons apple or ≈62% of the overall Albanian apple production (ProMali,

2012). Production potencial and storage facilities in Korça provide a qualitative market supply throughout the year.

Fruits after harvest and before consumption, encounter several stress factors simultaneously like heat shock or storage at low temperature. Under optimum conditions, cellular homeostasis has been achieved by a coordinated action of several biochemical pathways. Stress factor may affect biochemical pathways. Reactive Oxygen Species (ROS) have been continuously produced due to various metabolic activities and production increases under stress conditions along with the activation of various defence genes, which may include ROS scavenging, and stress proteins (Al-Saikhan *et al.*, 1995; Noctor & Foyer, 1998). Protection from such damages could be characterized by activation and deactivation of antioxidant defence enzymes, such as Superoxide Dismutase (SOD), Peroxidase (POX), Catalase (CAT), Ascorbate peroxidase (APX) and Glutathione Reductase (GR) and also by natural antioxidants, such as Ascorbic acid, Beta carotene, and Glutathione (D'Angelo *et al.*, 2007; Gechev & Hille, 2005). Jan and Rab (2012) have found that storage duration affect significantly physic-chemical changes of apple fruit cultivars, but different cultivars remain with a better quality than some others. Storage methods affect apple fruit shelf life and fruit quality such are fruit decay and brown rot of apple (Balla & Holb, 2007). In recent years there is an increased interest related to storage, shelf life and nutritional quality of postharvest fruits and vegetables, especially on enzymatic and non-enzymatic antioxidants (Lurie, 2003). 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 and modifications in the activities of catalase, peroxidase, and superoxide dismutase in squash (Wang, 1995) and pear (Ju *et al*., 1994), superficial scald in apple (Du & Bramlage, 1994; Rao *et al*., 1998) and senescence of pear and apple fruits (Du & Bramlage, 1994; Lata, 2007).

Antioxidant compounds are found in all higher plants, and they include ascorbic acid, α-tocopherol, β-carotene, glutathione and some flavonoids**.** 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 (Breusegem *et al.*, 2001). Oxidative stress is involved in many biological systems, among which are fruit ripening and senescence. Free radicals play an important role in senescence and ageing processes (Masia, 1998). 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 (Lurie, 2003), and non-enzymatic antioxidants play a role in resistance to physiological disorders caused by oxidative stresses (Lata *et al.*, 2005; Lata, 2007; Wolfe et al., 2003). Masia (1998) has found that superoxide dismutase and catalase are the most efficient antioxidant enzymes, influencing patterns of fruit ripening and senescence in on-tree and cold-stored apple fruits of Fuji and "Golden Delicious" cultivars. Pre-treatment methods of mango fruit affect significantly antioxidant enzyme (CAT, POX and SOD) activities during storage, compare to untreated fruits (Niranjana *et al.*, 2009). Postharvest calcium and hot-water dip treatments affected significantly the catalase, peroxidase and superoxide dismutase of chilled storage Lisbon lemon fruit (Safizadeh *et al.*, 2007) and other fruits and vegetables (Poovaiah, 1986). The major chemical changes in the cortical tissue and cell walls of calcium-infiltrated "Golden Delicious"

apples occur after six months cold storage, indicating that this stage is critical for quality maintenance (Chardonnet *et al.,* 2003).
Aazami *et al.* (2010) have found that $H_2O_2$ and catalase (CAT) levels increased during the increase of cold storage period, while superoxide dismutase (SOD) levels increased up to 4 months storage period, then decreased from 4 to 6 months storage period for "Red Delicious" apple cultivar, and the highest CAT levels were measured on 4% $CaCl_2$ and 24 and 48 h before cold storage heat treatments.
The concentration of polyphenolic compounds, such as flavones and anthocyanins, and the antioxidant activity in apples differ with cultivar, maturity stage, environmental growing conditions, the part of the fruit and storage conditions, as well (Vieira *et al.,* 2009; Tsao *et al*., 2005).
Different countries are using different postharvest treatment and storage methods (Kader, 2002; Dris & Jain, 2004). There is a need for each country to find the best postharvest treatment storage and method for different fruits and cultivars.

## MATERIAL AND METHODS

The study on the effects of cold storage period on postharvest fruit quality of three apple cultivars, "Red Delicious", "Golden Delicious" and "Granny Smith", postharvest pre-treated with 2% $CaCl_2$ and 20$^o$C hot-air treatment per 24 hours, was conducted during 2011-2012 vintage-storage season. "Red Delicious" and "Golden Delicious" were harvested in September 20, while "Granny Smith" was harvested in October 15. A cold storage period of 2, 4 and 6 months was applied.

***Plant material***

Apple fruits were harvested at the fruit maturity stage from a 10 years apple orchard, under the ownership of Saimir Ahmetli, in Menkulas, Korça, in the south-eastern part of Albania. Three cold storage periods (2, 4 and 6 months), three variants (apple cultivars) and three replications for variant, with a plot size of 10 kg apple fruits for each treatment were used. Apples were selected with an equal size and were placed on wooden crates with 10 kg each. After selection, apples were dipped on 2% $CaCl_2$ solution for 30 seconds, as floating and penetration under 250 mm Hg, and were exposed to hot air at 20$^o$C and a relative humidity of 90% for 24 hours. Thereafter, apples were stored at 0$^o$C and 90% relative humidity (RH) up to 6 months. Fruits were sampled for enzymatic assays every 2 months.

***Enzymes extraction***

Enzymes extraction was carried out at the Microbiology Lab of the Agriculture Faculty at the University "Fan S. Noli" Korçë. SOD and CAT extraction was used 5 g frozen apple tissue which 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 15 000 g for 15 min at 4$^o$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 Fridovich (1971). Three ml of reaction mixture 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 mixture (Brennan & Frenkel, 1977). 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% titanic 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. There was examined the relationship between the antioxidant system dynamics (postharvest quality) in relation to different cold storage periods of "Red Delicious", "Golden Delicious" and "Granny Smith" apple cultivars, post-harvest pre-treated with 2% $CaCl_2$ solution and 20°C hot-air treatment for 24 hours. Differences between cold storage periods on the antioxidant enzymes activities of three cultivars were tested by LSD test (Papakroni, 2001).

## RESULTS AND DISCUSSION

***Effect of cold storage period on $H_2O_2$ activity.*** Cold storage period affected significantly main enzymes activities of three apple cultivars. Results showed a linear increase of $H_2O_2$ levels from 2 up to 6 months for all apple cultivars under cold storage conditions. There were observed significant differences of $H_2O_2$ activity as the cold storage period was increased from 2nd to the 4th month for "Red Delicious", "Golden Delicious" cultivars, while for "Granny Smith" apple cultivar differences from 4th to the 6th month storage periods were not significant. The highest $H_2O_2$ activity was observed for 6 months storage period for all apple cultivars, showing the highest ROS scavenging capacity, but the values were different for different cultivars. The highest value of $H_2O_2$ was measured for "Granny Smith" apple cultivar after 6 months cold storage (1.62 µmole), while the lowest value was measured for "Golden Delicious" after 2 months cold storage (1.13 µmole) (Table 1 and Figure 1).

**Table 1.** Effects of cold storage period on the antioxidant enzyme $H_2O_2$ activity of "Red Delicious", "Golden Delicious" and "Granny Smith" apple cultivars pre-treated with 2% $CaCl_2$ solution and hot-air treatment at 20°C for 24 hours (different letters indicate significant difference at $P<0.05$).

| Apple cultivar | Cold storage period | | |
|---|---|---|---|
| | 2 months | 4 months | 6 months |
| Red Delicious | 1.19 c | 1.44 b | 1.56 a |
| Golden Delicious | 1.13 c | 1.27 b | 1.48 a |
| Grany Smith | 1.31 b | 1.53 a | 1.62 a |

***Effect of cold storage period on SOD activity***

SOD activity significantly increased in response to 2% $CaCl_2$ solution and 24 hours hot-air treatment from 2 to 4 months cold storage periods. This accompanies the vital role of SOD as initial defence system against Reactive Oxygen Species (ROS). SOD levels showed an increasing pattern until the 4th month for all apple cultivars, without significant differences between cultivars, thereafter, its level decreased to the end of cold storage period (6th month). "Red Delicious" and "Golden Delicious" cultivars showed the same pattern, where SOD activity was significantly decreased and there were observed not significant differences between them up to the end of 6th month. Increasing SOD activity was concomitant with increased superoxide radical scavenging activity and decreased membrane damage and oxidative stress (Mittler, 2002). This trend activates other antioxidant enzymes which are very dynamic in $H_2O_2$ scavenging such as catalases (Yörök, *et al.*, 2005) and peroxidases (Brabara, 2008). There were observed significant differences of SOD activity as the cold

storage period was increased from 2nd to the 6th month for "Red Delicious", "Golden Delicious" cultivars, while for "Granny Smith" apple cultivar differences from 4th to the 6th month storage periods were not significant which means that "Granny Smith" can stay for a longer period under cold storage than "Red Delicious" and "Golden Delicious". The highest SOD activity was observed for 4 months storage period for all apple cultivars (Table 2 and Figure 1).

**Table 2.** Effects of cold storage period on the antioxidant enzyme SOD activity of "Red Delicious", "Golden Delicious" and "Granny Smith" apple cultivars pre-treated with 2% $CaCl_2$ solution and hot-air treatment at 20°C for 24 hours (different letters indicate significant difference at P<0.05).

| Apple cultivar | Cold storage period | | |
|---|---|---|---|
| | 2 months | 4 months | 6 months |
| Red Delicious | 100.05 c | 235.44 a | 171.55 b |
| Golden Delicious | 96.32 c | 227.44 a | 158.32 b |
| Grany Smith | 117.32 b | 237.12 a | 219.04 a |

***Effect of cold storage period on CAT activity.*** CAT activity showed a concomitant increase with $H_2O_2$ during cold storage conditions. This trend led to the greater ROS scavenging capacity. There were observed that CAT activity was significantly increased in response to cold storage period up to 6 months for three apple cultivars, especially after 2 months of cold storage by high scavenging capacity of $H_2O_2$. Yamazaki *et al.* (2003) have found that higher activity of CAT decreases $H_2O_2$ level in cellular level and increase the stability of membranes and preserves $CO_2$ fixation because several enzymes of the Calvin Cycle within chloroplasts are extremely sensitive to $H_2O_2$. A high level of $H_2O_2$ directly inhibits $CO_2$ fixation. Low antioxidant activity of SOD and CAT in contrast with high content of superoxide radical and $H_2O_2$ ultimately led to an elevated level of hydroxyl radical. The later radical attacks all biomolecules, disrupts cell metabolism and causes membrane deterioration (Yahaia et al., 2007). The highest CAT activity was observed after six months cold storage for "Granny Smith" apple cultivar by 14.03 µmole/min/mg protein (Table 3 and Figure 1).

**Table 3.** Effects of cold storage period on the antioxidant enzyme CAT activity of "Red Delicious", "Golden Delicious" and "Granny Smith" apple cultivars pre-treated with 2% $CaCl_2$ solution and hot-air treatment at 20°C for 24 hours (different letters indicate significant difference at P<0.05).

| Apple cultivar | Cold storage period | | |
|---|---|---|---|
| | 2 months | 4 months | 6 months |
| Red Delicious | 1.56 c | 7.08 b | 8.67 a |
| Golden Delicious | 1.45 b | 6.89 a | 7.82 a |
| Grany Smith | 1.73 c | 11.21 b | 14.03 a |

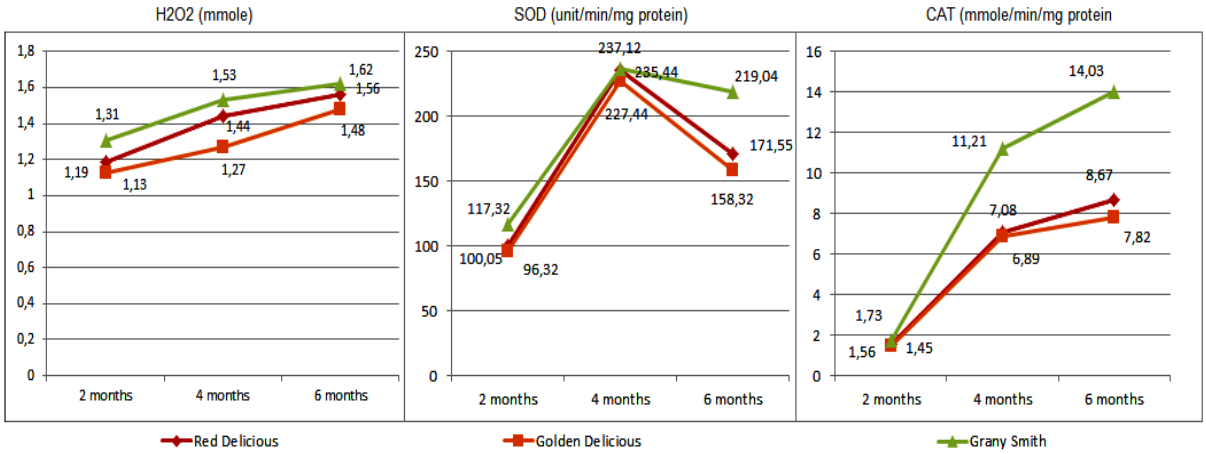


**Figure 1.** Effects of cold storage period on the antioxidant enzyme CAT activity of "Red Delicious", "Golden Delicious" and "Granny Smith" apple cultivars pre-treated with 2% $CaCl_2$ solution and hot-air treatment at 20°C for 24 hours.

## CONCLUSIONS

The observed data showed that cold storage period significantly affected antioxidant enzymes activity of "Red Delicious", "Golden Delicious" and "Granny Smith" apple cultivars, pre-treated with 2% $CaCl_2$ solution and hot-air treatment at 20°C for 24 hours. Diverse natural antioxidant enzymes, their state and dynamic function positively affect biochemical streams of apple fruit during postharvest cold storage. Peroxidase and Catalase levels were significantly increased during cold storage period for three cultivars, while Superoxide Dismutase (SOD) was significantly increased up to the end of 4th month for all cultivars and was significantly decreased after 4th month cold storage period. "Granny Smith" apple cultivar showed the highest levels of all antioxidant enzymes, with less negative effects on antioxidant levels during cold storage conditions.

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