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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Influence of $CaCl_2$ on Biochemical Composition, Antioxidant and Enzymatic Activity of Apricot at Ambient Storage

Sartaj Ali[1], Tariq Masud[2], Kashif S. Abbasi[3], Talat Mahmood[2] and Ijlal Hussain[4]
[1]Department of Agriculture and Food Technology,
Karakoram International University, Gilgit, Baltistan, Pakistan
[2]Department of Food Technology, PMAS Arid Agriculture, Rawalpindi, Pakistan
[3]Department of Agriculture, University of Haripur, Khyber Pakhtunkhawa, Pakistan
[4]Department of Agriculture, Provincial Directorate of Agriculture, Gilgit, Baltistan, Pakistan

Abstract: Fresh fruits are increasingly focused in the recent years for their nutritional and health benefits. Research involving simple and safe preservation technologies is at a high demand to satisfy consumer concerns and harvest maximum market potential. The present study was therefore undertaken to assess the response of chemical and bioactive compounds (pH, acidity, ascorbic acid, total phenolics, carotenoids, free radical scavenging capacity and enzyme activity) in apricot under the effect of calcium treatments (1, 2, 3 and 4%) at ambient storage. The results revealed that ascorbic acid, phenolic compounds and carotenoids were least affected in 3 and 2% calcium as compared to control, 1 and 4% $CaCl_2$. Similarly, higher antioxidant capacity and lower enzymatic activity was retained in the same concentrations. Titratable acidity and ascorbic acid decreased in all treatments during storage and higher reduction was observed in control, 1 and 4% as compared to 3 and 2% calcium, respectively. pH, phenolics, carotenoids, antioxidant and enzymatic activity increased initially and it was rapid up to 6th day, followed by a slight decrease during subsequent storage. The overall results demonstrated higher compositional losses in control followed by 1 and 4% calcium treated fruits, while 3 and 2% treatments exerted pronounced effects on keeping quality of apricot during ambient storage up to 12th day.

Key words: Apricot fruit, bioactive compounds, antioxidants, enzyme activity, ambient storage

INTRODUCTION

Apricot is one of the most important stone fruit of Rosaceae family and widely grown in the Mediterranean countries, Himalayan regions of South Asia and China. Pakistan is the 3rd major producer of apricot in the world followed by Turkey and Iran (FAO, 2010). It is a soft delicious fruit having stone inside the fleshy pericarp; with an attractive color and flavor. It is highly nutritious fruit having bioactive compounds with numerous health benefits (Leccese *et al.*, 2010). These compounds also impart color and aroma thus improving the consumer acceptability of the commodity, hence apricot is considered as a highly aromatic fruit and very much liked the world over. It contains significant amounts of phenolic compounds and carotenoids and posses a higher antioxidant activity (Ali *et al.*, 2011). The climacteric nature of apricot makes it susceptible to post harvest decay and spoilage as a result of rapid ripening, accompanied by flesh softening and complete senescence (Egea *et al.*, 2007). Apricots are considered vulnerable to dehydration and shrinkage, owing to their non waxy skin. Two main physiological disorders appear in apricot that are internal browning and break down of tissues which reduce their storage life and market value (Manolopoulou and Mallidis, 1999). Phenolic compounds, carotenoids and enzymes are compartmentalized within the cellular structure of fruits. As a result of tissue destruction, these contents liberate and breakdown occurs with increased metabolic activities, resulting in quantity and quality loss (Paliyath and Droillard, 1992). The levels of phenolic contents reduce with the increased enzyme activity because these compounds are utilized as substrates by many enzymes (de Rigal *et al.*, 2000). Therefore cellular integrity of the commodity is important towards the conservation of nutritional contents. In post harvest management, chemical preservatives are widely used to maintain the final quality of fruits. Calcium chloride is an example of such chemicals that have considerable impact on the shelf life of different fruits and vegetables (Choudhury, 2004). It is an important component of cellular structure and its deficiency cause internal tissue browning. Previous studies have suggested pre and post harvest application of calcium salts to maintain nutritional status and to slow down the ripening process of fruits (Souty *et al.*, 1995). It modifies intra-cellular and extra-cellular processes which delay ripening and softening, lowers the rates of color change, CO_2 and ethylene production and thus maintain overall quality (White and Broadley, 2003; Conway, 1987). The increasing awareness of consumer regarding health improving foods has attracted the attention of researchers towards poorly

Corresponding Author: Sartaj Ali, Department of Agriculture and Food Technology, Karakoram International University, Gilgit, Baltistan, 15100, Pakistan

studied bioactive compounds during storage. Apricot is among the less focused fruits and very limited information is available on post harvest storage quality. Researchers are interested in extending shelf life of economically important fruits so as to increase their useful life. Post harvest shelf life determines the suitability of any fruit for its distant marketing. The core purpose of post harvest treatment of fruits is to present a good quality fruit to the end user in markets for away from the production regions (Fuchs *et al*., 1995). At the cross road of famine and health foods, it is important to explore new avenues to feed the growing world populations and achieve food security in the future. In the developing countries like Pakistan, sophisticated technologies and cold chains are far away from the access of farmers and marketing entrepreneurs due to their poor economies and infrastructures. Therefore it is imperative to introduce and disseminate post harvest technologies that are cheap and easily adaptable by the growers and entrepreneurs for comparatively better returns of their produce. Although, Pakistan has enormous potential of apricot production and is among the main producers in the world, however no single study is available on the storage life of apricot from this region. There is an increasing cultivation of apricot year by year in Gilgit-Baltistan of Pakistan and the fruit from this region has good quality and high market value (MFC, 2005). Keeping in view the above facts this study was planned to ascertain the effect of post harvest calcium application on bioactive compounds, free radical scavenging capacity and enzymatic activity of apricot at ambient storage.

MATERIALS AND METHODS

Collection of fruit sample and experimental plan: The fruit was harvested at commercial maturity stage and transported to the Food Technology Laboratory of the Department of Food Technology PMAS, Arid Agriculture University Rawalpindi immediately after picking. Fruits were cleaned and washed to remove all foreign matter such as dust and dirt. Grading and sorting was carried out to select uniform and blemish free fruit for treatments and subsequent storage. The cleaned and graded fruits were divided into five lots for treatments. One lot was kept as control and the others were treated by dipping with 1, 2, 3 and 4% solutions of $CaCl_2$ for 3 minutes, air dried and put into card board cartons. Sponge cubes of equal size were cut and dipped into saturated solution of potassium permanganate and placed in the same cartons of treated samples, sealed and stored at ambient conditions. The data for different quality parameters as described below was recorded at two day intervals during subsequent storage.

Analyses of biochemical characteristics: The following parameters were analyzed during storage at two days intervals: The pH of fruit samples assessed by pH meter according to AOAC (2000) and titratable acidity of fruit samples was determined by titration with 0.1 N NaOH following method No. 981.12, AOAC (2000). Ascorbic acid was determined by titration with 2, 6-dichlorophenol indophenols dye as described in AOAC (2000); method No. 967.21.

Total Phenolic Compounds (TPC): TPC were measured by using the Folin-Ciocalteu (FC) assay (Sponas and Wrolstad, 1990). Ten fruits randomly selected from each variety were crushed and homogenized in a homogenizer. The fruit puree (5g) was diluted to 30ml with deionized water and clarified by centrifugation at 10000g for 15 min. The extract was filtered through a 0.45mm membrane filter. Filtrate (0.5ml), 5ml 0.2N FC reagent and 4ml of 7.5% sodium carbonate solution were added to a 25ml volumetric flask and filled to volume by deionized water. The contents were allowed to stand for 5-8 minutes at 50°C and the absorbance was measured at 765 nm using a CE-2021, Spectrophotometer (CECIL Instruments Cambridge, England). Total phenolics were quantified from a calibration curve using Gallic acid as standard. The concentrations were expressed as milligram GAE per 100g on dry weight basis.

Total Carotenoids (TC): TCs were extracted by using the procedure reported by Rodriguez-Amaya (1999). Briefly, five grams of sample was homogenized with 100ml of methanol/petroleum ether (1:9, v/v) and the mixture was transferred to a separating funnel. Petroleum ether layer was filtered through sodium sulphate, transferred to a volumetric flask and total volume was made up to 100ml with petroleum ether. Finally, the total carotenoid content was measured by a spectrophotometer (CE-2021, 2000 series CECIL Instruments Cambridge, England) at a wave length of 450nm and the results were expressed as beta-carotene equivalents (milligrams per 100g of dry weight).

Free Radical Scavenging Capacity (FRSC): Antioxidant activity was measured as % DPPH (2, 2-diphenyl-l-picrylhydrazyl) free radical reducing power described by Brand-Williams *et al*. (1995). Five gram of ground frozen tissue was taken in triplicate, homogenized and extracted with 10 ml methanol (MeOH) for 2 hours. From the above extract, 0.1ml was taken in a test tube and 3.9ml of DPPH solution (6×10^{-5} mol/L) was added. The mixture was incubated at room temperature for 30 minutes and then absorbance was measured at 517nm in a UV-Spectrophotometer (UNICO 2100 Series Japan). The DPPH solution was freshly prepared daily, stored in a flask covered with aluminum foil and kept in dark at 4°C between the measurements. Blank sample was prepared containing the same amount of MeOH and DPPH solution and measured daily. Radical scavenging activity was calculated as % of inhibition of DPPH radical by the following formula:

$$\text{Inhibition (\%)} = \frac{\text{A blank} - \text{A Sample}}{\text{A blank}} \times 100$$

Enzyme assay: Enzyme extraction was carried out according to the method described by Abbasi *et al.* (1998) with some alterations. 5 gram of frozen apricot pulp of ten fruits was pasted with a mortar and pestle and suspension was made with 15ml of 100mM KH_2PO_4 buffer (pH 7.8) with 0.5% (v/v) Triton X-100 and 1g polyvinyl polypyrrolidone (PVPP). The above homogenate was then centrifuged (18000xg) for 30 minutes at 4°C and the supernatant was collected and stored at -2°C. Three replications from each treatment were taken for the data.

Polyphenol Oxidase (PPO) Assay: Polyphenol oxidase was determined based on the oxidation of catichol (Abassi *et al.*, 1998). A reaction mixture of 2.5ml of 0.1M sodium citrate buffer (pH 5.0), 0.3ml of 0.02M catichol in sodium citrate buffer (pH 5.0) and 0.2ml enzyme extract made in to a 3ml total volume. Absorbance of the above mixture at 420nm was recorded by means of spectrophotometer. PPO activity was determined based on change in optical density over a period of 3 minutes and expressed as U g^{-1} protein (enzyme unit per gram of protein).

Peroxidase (POD) assay: Peroxidase activity was determined according to Abbasi *et al.* (1998). An assay mixture of 3ml total volume was prepared with 2.1ml, 15mM $NaKPO_4$ buffer (pH 6.0), 600 µl substrate, consist of 300 µl 1mM H_2O_2 and 300 µl 0.1mM guaiacol and 300 µl enzyme extract. Activity was calculated at 470nm on the basis of change in optical density over a 3 minute period and expressed as U g^{-1} of protein on fresh weight basis.

Catalase (CAT) assay: CAT activity was determined according to the method described by Abbasi *et al.* (1998). To complete the reaction two solutions were used as buffer A and B. the buffer A consist of 2.7ml, 15M KPO_4 (pH 7.0), while buffer B consist of 2.7ml, 12, 5mM H_2O_2 in 15M KPO_4 (pH 7.0) to the cavettes containing buffer A and B 300 µl enzyme extract was added and kept in dark. Optical density was measured at 240nm by as spectrophotometer at 45 sec and 60 sec starting from the time when the enzyme extract was added to the cavettes. The difference in the optical density of two time intervals (45 and 60 seconds) was noted and used to calculate the catalase activity and expressed as U g^{-1} of protein on fresh weight basis.

Statistical analysis: The data obtained was subjected to two-way analysis of variance (ANOVA), by considering treatments and storage time as source of variance. The means were separated by Duncan Multiple Range test according to Steel *et al.* (1996) at a probability level of p-0.05, using MSTAT-C software (Michigan State University, 1991).

RESULTS AND DISCUSSION

Changes in biochemical composition and antioxidant potential: The results pertaining to the biochemical composition, antioxidant and enzymatic activity in apricot at ambient storage are presented in (Fig. 1-9). Fruit pH increased during storage in all treatments with the progress in ripening. Figure 1 clearly indicates that the values started decline at the advanced ripening stage. This is because; sugar fermentation takes place and organic acid concentration increase with the removal of moisture. Maximum pH was maintained by 3 and 2% $CaCl_2$ as these concentrations retarded degradation of sugars and organic acids. pH represents the hydrogen ion concentration and is an important factor to measure the free acid content in any commodity. An increase in pH is associated with the degradation of acids during storage (Pesis *et al.*, 2002). Similarly, lower pH values indicate sugar break down and fermentation which cause increase in acid content. A slight change in the hydrogen ion concentration results in to change in

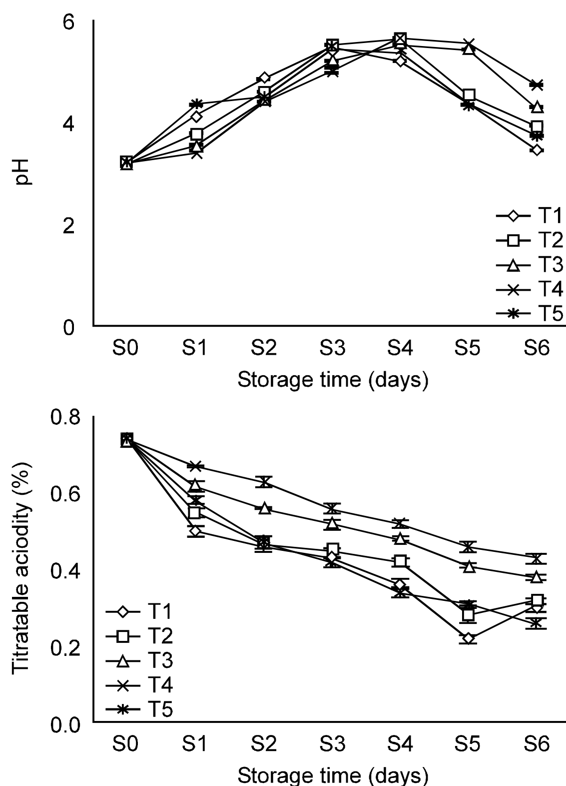

Fig. 1, 2: Effect of $CaCl_2$ on fruit pH and titratable acidity of apricot at ambient storage. The values are means of three replications with standard error. S: Storage days i.e., 0, 2, 4, 6, 8, 10, 12

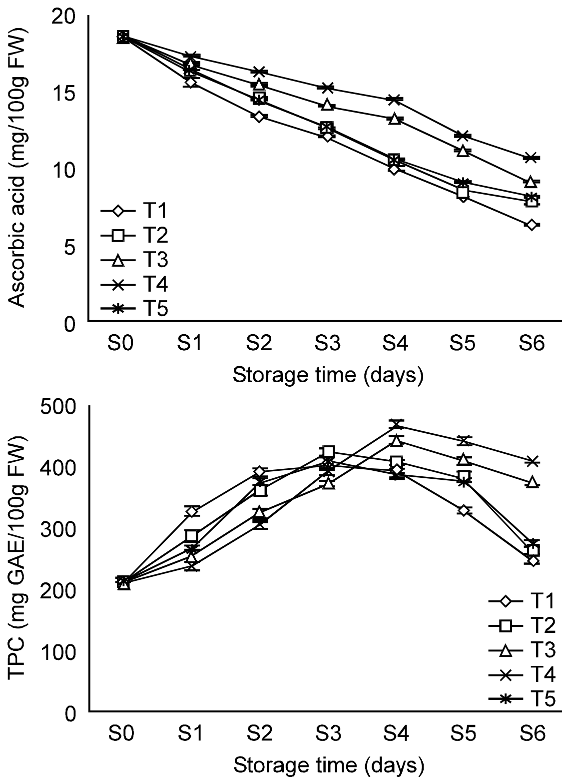

Fig. 3, 4: Effect of $CaCl_2$ on ascorbic acid and total phenolic contents of apricot at ambient storage. The values are means of three replications with standard error. S: Storage days i.e., 0, 2, 4, 6, 8, 10, 12

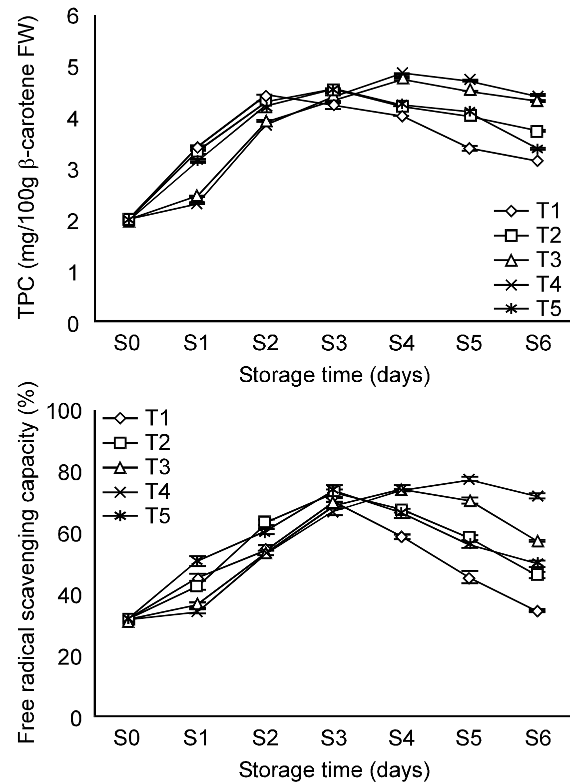

Fig. 5, 6: Effect of $CaCl_2$ on total carotenoids and antioxidant activity of apricot at ambient storage. The values are means of three replications with standard error. S: Storage days i.e., 0, 2, 4, 6, 8, 10, 12

pH value (Ball, 1997). The results pertaining to titratable acidity showed a declining pattern in all treatments (Fig. 2). Maximum decrease in TA was found in control (52.70%) followed by 1 and 4% $CaCl_2$ treated samples (43.24%), while least decrease was observed in 3% followed by 2% $CaCl_2$ (25.67, 28.37%, respectively). Treatment means showed lower acidity levels in control followed by 1 and 4%, while 3, 2% calcium concentrations maintained higher TA contents up to 12th day at ambient storage with significant differences at $p<0.05$ (Table 1). A slight increase in acidity values of control and 1% calcium treatments in the last intervals might be due to sugar fermentation at senescent stage. Titratable acidity represents the organic acid content present in the commodity and often related to the maturity. These acids significantly contribute to the quality and taste of the fruit. The acid content of fruits decreased with the ripening as a result of respiration and fermentation (Ball, 1997). During ripening, sugar contents increase, while malic acid starts degrading followed by citric acid (Salunkhe and Desai, 1984) and that might be result in to lower acidity values. In our findings, it is evident that the calcium treatments partially affected the titratable acidity as compared to control. The acidity retention in 3%, 2% calcium treatments may be attributed to the reduction in metabolic activities of organic acids. Similar observations have also been made by Ishaq *et al.* (2009), where $CaCl_2$ maintained higher acidity values in apricot during storage. The data recorded for ascorbic acid showed that 3 and 2% $CaCl_2$ maintained maximum values respectively (Fig. 3). Significant effect among treatments was noticed during ambient storage up to 12 days at $p<0.05$ (Table 1). Ascorbic acid losses were higher in control (53.70%) followed by 1 and 4% $CaCl_2$ (44.94, 43.17%, respectively). Minimum losses were found in 3 and 2% $CaCl_2$ (15, 18.65%), respectively. The initial value for ascorbic acid 18.6mg/100g decreased to 8.61, 10.24, 15.13, 15.81 and 10.57mg/100g in control, 1, 2, 3 and 4% $CaCl_2$. An overall decreasing trend was observed throughout the storage period in all the treatments, however, 3 and 2% calcium concentrations were effective in retaining higher ascorbic acid content (Fig. 3). Ascorbic acid is an important indicator of quality and sensitive to oxidation during post harvest storage (Veltman *et al.*, 2000). Watada *et al.* (1987) has also reported vitamin C losses associated with the ripening

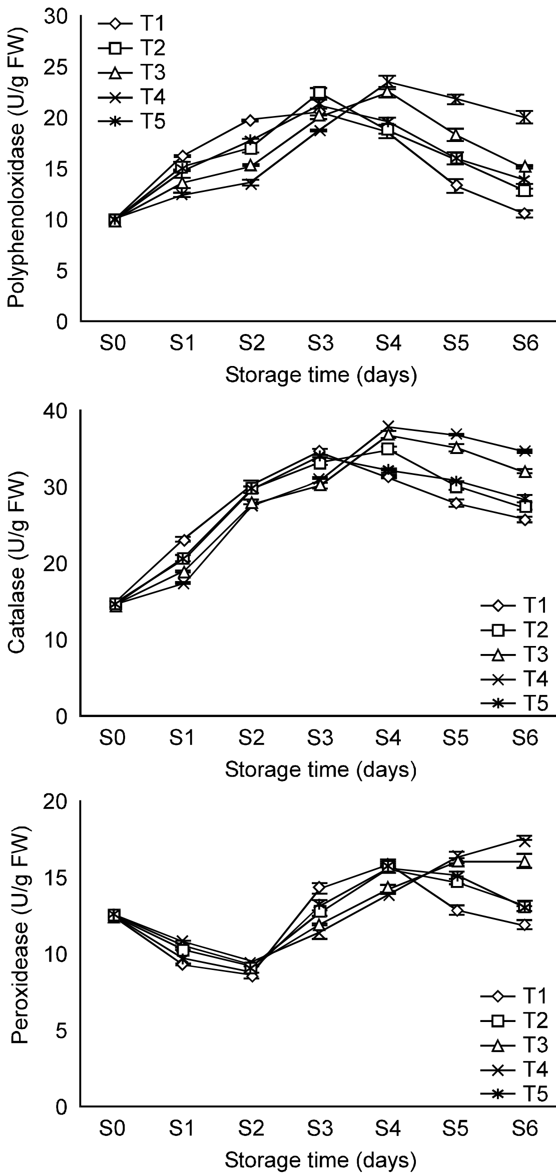

Fig. 7, 8, 9: Effect of $CaCl_2$ on PPO, POD and CAT activity of apricot at ambient storage. The values are means of three replications with standard error. S: Storage days i.e., 0, 2, 4, 6, 8, 10, 12

and senescence of fruits. It may loss with the increasing storage time and exposure to high O_2 concentrations. However, calcium has been reported to be effective in delaying ripening and oxidation of tissues that intern ascorbic acid. It has been observed by Ruoyi *et al.* (2005) that calcium chloride maintained AA content during 50 days storage of peaches. Total phenolic compounds increased during storage in all the treatments at the initial intervals (Fig. 4). The rate of increase in phenolic contents was slow in 3 and 2% $CaCl_2$, while a rapid increase was observed in control (100%) followed by 1 and 4% $CaCl_2$. All the treated samples significantly differed ($p<0.05$) as compared to control, however, partially significant trends were found for 1 and 2% $CaCl_2$ concentrations (Table 1). Maximum retention of phenolic compounds was found in 3 and 2% calcium (351.12, 340.33 mg GAE/100g FW respectively) and the differences were statistically same. Phenolic concentration normally increases with the maturity of fruit and attains maximum accumulation at full ripe stage. However, some phenolic constituents decrease with the advancement in the stage of maturity (Dragovic-Uzelac *et al.*, 2007). Our results are in agreement with previous reports that phenolic compounds increased towards ripening. A slight decline was observed in control, 1 and 4% calcium at the 6th day of storage and the losses were higher in control set during the subsequent storage intervals. A similar declining trend in 2 and 3% calcium was witnessed after 8th day during storage (Fig. 4). This might be due to the fact that full ripening is accompanied by the destruction of cellular structure and enhanced enzymatic activity where phenolic compounds are utilized as substrates. In general, phenolic compounds show stability during storage (Kevers *et al.*, 2007). Furthermore, the maximum retention in phenolic compounds can be inferred by the reduced respiration, softening and acidity loss in calcium treated samples (Tzoutzoukoua and Bouranisb, 1997). Apricot is among the carotenoid rich fruits. The contents increased during storage regardless of treatment in all samples at the initial storage intervals (Fig. 5). A rapidly increasing trend was observed in control up to 4th day, while 1 and 4% calcium concentrations showed increasing behavior up to 6th day with a slight decline in the later stages. A slow

Table 1: Effect of $CaCl_2$ on biochemical composition of apricot at ambient storage

Treatments	pH	TA	AA	TPC	TC	AoA	PPO	POD	CAT
Control	4.39e	0.43d	12.04d	327.17c	3.52d	48.71c	15.54c	12.11c	26.71d
1% $CaCl_2$	4.45c	0.46c	12.75c	330.86bc	3.74b	54.75b	16.07bc	12.51b	27.17c
2% $CaCl_2$	4.53b	0.53b	14.09b	340.33b	3.76b	56.12b	16.39b	12.67b	27.90b
3% $CaCl_2$	4.57a	0.57a	15.00a	351.12a	3.80a	58.52a	17.14a	13.05a	28.47a
4% $CaCl_2$	4.42d	0.44c	12.90c	325.07c	3.67c	55.81b	16.17b	12.50b	27.12cd
LSD	0.02	0.02	0.14	10.22	0.04	1.60	0.62	0.32	0.42

TA: Titratable acidity (%), AA: Ascorbic acid (mg/100g FW), TPC: Total phenolic compounds (mg GAE/100g FW), TC: Total carotenoids (mg β-carotene/100g FW), AoA: Antioxidant activity (%), PPO: Polyphenoloxidase activity (U/g protein FW), POD: Peroxidase activity (U/g protein FW), CAT: Catalase activity (U/g protein FW).
The data shows treatment means of 12 days storage recorded at 2 days intervals.
The values with same letters within the column are statistically same at $p<0.05$.

pattern of increase was noticed in 2%, 3% $CaCl_2$ that persisted up to 10th day and the results were statistically same at end of 12 days storage. This phenomenon might be due to the delay in ripening by calcium application. The results were partially significant among the treatments at $p<0.05$ (Table 1). It can be observed that a slow decline occur in the contents at the later stages of storage that might be due to the conversion of carotenoids in to volatile compounds. The mean values show comparatively higher contents in 2 and 3% calcium treated samples (3.80, 3.39 mg β-carotene E/100 g) followed by 1%, 4% calcium treatments (3.09, 3.01 mg β-carotene E/100 g) and the lowest in control (2.47 mg β-carotene E/100 g). Previously, it has been shown that carotenoids are stable during storage (Radi *et al*., 1997). They are resistant to heat, changes in pH and water leaching. The increase in carotenoid concentration is responsible for color development in fruits and maximum color is attained at full ripe stage (Leccese *et al*., 2010). Antioxidant capacity in terms of percent DPPH free radical reducing power indicated and increasing trend throughout storage (Fig. 6). 3 and 2% calcium treated samples demonstrated higher antioxidant capacity (57.28, 56.13%) and statistically significant ($p<0.05$) on conclusion of 12 days storage (Table 1). Similarly, non significant results were found for 1 and 4% calcium chloride (47.57, 47.40%), however all treated samples significantly differed as compared to control. Maximum activity for control, 1 and 4% calcium treated sample was obtained at the sixth day of storage followed by decline, while 3 and 2% calcium treatments maintained a comparatively stable activity during subsequent storage. This might be due to the fact that apricot was harvested at commercial maturity stage and hence; continued its ripening process resulting in increasing amounts of phenolics and carotenoids. It is evident from previous findings that phenolic compounds significantly contribute towards antioxidant capacity in apricot. Ali *et al*. (2011) and Drogoudi *et al*. (2008) have reported a good correlation between phenolic content and antioxidant activity in apricot cultivars. It has further been established that ascorbic acid also contribute to antioxidant potential along with phenolics (Kevers *et al*., 2007). With prolonged storage, phenolic contents reduce through enzymatic activity and oxidation; as a result, decrease in antioxidant activity in apricot occurs (Kevers *et al*., 2007).

Changes in enzymatic activity: Effect of calcium treatments on PPO activity in apricot during ambient storage have been shown in (Fig. 7). It revealed that PPO activity rapidly increased during initial days of storage and was partially significant ($p<0.05$) among the treatments (Table 1). Highest activity was attained at the 6th day in control followed by 1 and 4% calcium, while a steady increasing trend in PPO activity was observed in 3 and 2%, respectively followed by decline after 9th day (Fig. 7). Apparently, 3%, 2% calcium concentrations maintained a reduced PPO activity towards the end of storage period. Enzymatic activity increased with the advances in ripening as a result of cellular destruction with the liberation of enzymes. PPO is responsible for the browning of tissues through oxidation of phenolic compounds to quinones and further polymerization to melanin pigments (Chengl and Crisosto, 1995; Whitaker, 1995). In higher plants, senescence is characterized by the destruction of cellular structures and liberation of compartmentalized constituents like enzymes that are responsible for compositional degradation of fruits (Paliyath and Droillard, 1992). It has been established that PPO activity is substrate dependent. Thus adverse browning does not occur in healthy intact tissues (Macheix *et al*., 1990). The biological barriers are disrupted by the injury or senescence and enzymes become active when they come in contact with substrate (Yoruk and Marshall, 2003). Calcium chloride effectively maintained cellular integrity and as a result lower PPO activity was observed in 3 and 2% calcium treatments. Similar results have been reported by Akhtar (2010) on loquat fruit during cold storage, where calcium treated samples maintained a lower PPO activity. POD activity increased in all samples during storage; however, it was rapid in control followed 1 and 4% calcium chloride (Fig. 8). The results were significant for treated samples as compared to control but non-significant trend was observed among treatments as 1, 4, 3 and 2% at $p<0.05$ (Table 1). Highest activity was found for control at the 6th day of storage. 3 and 2% $CaCl_2$ controlled a steady level of activity with a slight decline from the 9th day of storage to onward. It has been established that POD activity is related to ripening process and increase with the advance in senescence (Tian *et al*., 2004). Bhattacharya *et al*. (2009) has also reported an increased activity of POD during initial storage followed by a rapid decline in eggplants. The exogenous calcium application has been reported effective against cell wall degrading enzymes (White and Broadley, 2003). Our findings also agree with pervious work of researchers that demonstrated irregular POD activity in a variety of fruits. Ding *et al*. (2006) found a fluctuating POD activity in loquat fruit during low temperature storage. The initial values for catalase activity increased in all the samples during storage regardless of treatments (Fig. 9). Highest activity was found in control and 1% calcium at the 6th day of storage and reduced afterward. While other treatments maintained significantly higher CAT activity during storage. It can be observed from Fig. 9 that 3% calcium kept higher activity followed by 2% and the differences were significant at $p<0.05$ (Table 1). CAT activity normally decreases during storage (Wang, 2005). However, Akhtar (2010) has reported that calcium

treatments maintained a higher CAT activity in loquat fruit during cold storage for ten weeks. In our study we also found that the effective concentrations of $CaCl_2$ (3%, 2%) maintained a maximum catalase activity in apricot fruit at ambient storage. This might be due to the reason that calcium retarded higher respiration rates in the treated samples as compare to control and lower $CaCl_2$ concentrations.

Conclusion: This study investigated the response of biochemical constituents (i.e., phenolics, carotenoids and ascorbic acid), free radical scavenging potential and enzymatic activity of apricot fruit to different calcium concentrations at ambient conditions. Significant improvement in shelf life of apricot was observed in the treated samples. The contents were least affected at 3 and 2% calcium concentrations, while higher losses were witnessed in 1 and 4%, respectively. The effective concentrations slowed down ripening and delayed onset of climacteric peak which in turn maintained compositional quality and physical integrity of the fruit. It can be concluded from the results that 3% calcium can be used to extend shelf life and marketability of fresh apricot fruit.

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