Food Science and Quality Management
ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online)
Vol.35, 2015

Influence of Packaging Material and Ethylene Scavenger on Biochemical Composition and Enzyme Activity of Apricot Cv. Habi at Ambient Storage

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Abstract
The present study was undertaken to evaluate the influence of different packaging materials and ethylene absorbent on biochemical composition, antioxidant and enzyme activity of apricot cv. Habi during ambient storage. Perforated (0.25%) polyethylene films of low, medium and high densities and wrapping paper were used along with $KMnO_4$ dipped sponge cubes. Biochemical characteristics (pH, ascorbic acid, phenolics and carotenoids), antioxidants and enzyme activity were determined at 2 day intervals during storage. The results demonstrated that low density polyethylene retained maximum chemical and bioactive compounds, higher antioxidant activity and lower enzyme activities during storage, while the maximum loss of quality was observed in control and paper wrapped sets followed by high and medium density packaging films. It was concluded that apricot harvested at commercial maturity stage and packed with low density polyethylene films along with ethylene scavenger ($KMnO_4$) can be successfully stored at ambient conditions up to two weeks.
Keywords: Apricot, biochemical composition, packaging material; ethylene absorbent, ambient storage

1. Introduction

Apricot is climacteric fruit in nature and demonstrates marked physiological changes during maturation or ripening. Continued respiration and evolution of ethylene are accompanied by losses in sensory quality and reduced shelf life. Environmental factors such as temperature, humidity, gas concentration and air circulation influence the postharvest physiology and concomitant changes in the chemical composition of fruit during storage (Brody, 1999). Packaging of fresh produce is meant to provide shelter against environmental factors involved in quality degradation of foods.

A wide range of packaging materials are used according to the nature of fruits and ease of handling of the material. Polyethylene films along with soft board or corrugated board cartons are now widely used in the packaging of fresh produce due to their flexible nature, efficient role in environmental protection and safety (Sammi and Masud, 2007). Sealed polyethylene packaging in soft or cardboard cartons with ethylene absorbent at ambient conditions have been found as effective as refrigerated storage for fruit (Scott *et al.,* 1970). Besides, extended shelf life during storage is related to lower levels of ethylene, since ethylene accumulation enhances biochemical changes. Ethylene inhibitors are normally used to mitigate harmful effects of ethylene gas in the packaging system. Amongst, potassium permanganate has been found useful in a number of studies (Ishaq *et al.*, 2009).

Apricot is widely grown in temperate climates and needs long distance transportation to reach main markets. It is among early summer fruits and sold at high prices in the market. Research is under way to work out efficient strategies for extending shelf life of fruits for getting maximum market share to the producers and provide healthy foods to the consumers. Apricot is the most abundant and popular fruit of Gilgit-Baltistan and has 20% share in the farm income (Ali *et al.,* 2011; Jasra and Rafi, 2002). Previously limited information regarding the effect of packaging on the chemical and bioactive composition of apricot at ambient storage is available in the literature (Isahaq *et al.,* 2009). However, packaging has been studied in combination with cold storage in peaches, cherries and nectarines (Di Vaio *et al.,* 2008; Diaz-Mula *et al.,* 2009). To work out ways for extending storage life of apricot for distant marketing, the present study was planned to investigate the influence of polyethylene film packaging along with ethylene absorbent on some biochemical attributes at ambient conditions.

2. Material and methods

2.1. Experimental material and treatment scheme

The effect of packaging material and ethylene scavenger on quality characteristics of apricot was assessed at ambient storage. Three types of polyethylene films of high, medium and low density (0.03, 0.06, 0.09 mm thickness with 0.25% perforation) and wrapping paper were used along with corrugated cartons (CC). Local apricot cultivar Habi was harvested at commercial ripening stage and transported to the Food Technology Lab. Arid Agriculture University, Rawalpindi. The fruit was graded, washed, air dried and then divided into five lots and packed with different packaging materials in the following scheme:

T_1 = Control, T_2 = Wrapping paper + CC+ $KMnO_4$,

T_3 = LDPE + CC+ $KMnO_4$, T_4 = MDPE + CC+ $KMnO_4$,

T_5 = HDPE + CC+ $KMnO_4$.

Treated samples were stored at ambient conditions and data was recorded at two day intervals on the following biochemical parameters.

2.2. Analyses of biochemical characteristics

2.2.1. Fruit pH, total soluble solids and ascorbic acid

Wedge shaped pieces of ten fruits were taken and extracted for a composite juice sample to determine pH, TSS and ascorbic acid. pH was assessed by a pH meter, total soluble solids with the help of a refractometer (PAL-3®, ATAGO Japan) as ^{o}Brix and ascorbic acid by titration with 2, 6-dichlorophenol indophenols according to AOAC (2000).

2.2.3. Total phenolic contents

Total phenolic contents (TPC) were measured by using the Folin-Ciocalteu (FC) assay (Sponas and Wrolstad, 1990). Ten fruit randomly selected from each variety were crushed and homogenized in a homogenizer. The fruit puree (5 g) was diluted to 30 ml with deionized water and clarified by centrifugation at 10000 g for 15 min. The extract was filtered through a 0.45 mm membrane filter. Filtrate (0.5 ml), 5 ml 0.2 N FC reagent, and 4 ml of 7.5% sodium carbonate solution were added to a 25 ml volumetric flask and filled to volume with deionized water. The contents were allowed to stand for 5-8 minutes at 50 ^{o}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 mg GAE^{-1} 100g on dry weight basis.

2.2.4. Total carotenoids

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

2.2.5. Antioxidant activity

Antioxidant activity was measured as % DPPH (2, 2-diphenyl-l-picrylhydrazyl) free radical reducing power as described by Brand-Williams *et al.* (1995). Five grams of ground frozen tissue were taken in triplicate, homogenized and extracted with 10 ml methanol (MeOH) for 2 hours. From the above extract, 0.1 ml was taken in a test tube and 3.9 ml of DPPH solution (6×10^{-5} mol/L) was added. The mixture was incubated at room temperature for 30 minutes and absorbance was measured at 517 nm in a UV-Spectrophotometer (UNICO 2100 Series Japan). DPPH solution was freshly prepared daily, stored in a flask covered with aluminum foil and kept in the 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:

$$\% \text{ Inhibition} = \frac{Ab - As}{Ab} \times 100$$

Ab = absorbance of blank, As =absorbance of sample

2.2.6. Enzyme assay

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

2.2.6.1. Polyphenol oxidase

Polyphenol oxidase (PPO) was determined based on the oxidation of catichol (Abassi *et al.,* 1998). A reaction mixture of 2.5 ml of 0.1 M sodium citrate buffer (pH 5.0), 0.3 ml of 0.02 M catichol in sodium citrate buffer (pH

5.0) and 0.2 ml enzyme extract made into a 3 ml total volume. Absorbance of the above mixture at 420 nm 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 enzyme units per gram (U/g) of proteinon fresh weight basis.

2.2.6.2. Peroxidase activity

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

2.2.6.3. Catalase activity

Catalase (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 2.7 ml, 15 M KPO_4 (pH 7.0), while buffer B consist of 2.7 ml, 12,5 mM H_2O_2 in 15 M 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 240 nm by as spectrophotometer at 45 and 60 seconds 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 enzyme units per gram (U/g) of protein on fresh weight basis

2.3. Statistical analysis

The data were recorded on triplicate samples and subjected to two-way analysis of variance (ANOVA) by considering packaging material and storage time as a source of variance according to Steel *et al.* (1997). The means were separated by least significance difference test (LSD) by using statistical software program Statistix 8.1 at a probability level of $p < 0.05$.

3. Results

3.1. Biochemical composition

During storage, pH values of fruit increased initially in all samples (Fig. 1). Control set and HDPE packed samples showed a rapid increase followed by paper and MDPE, whereas, LDPE demonstrated a slower increasing rates at the same intervals. The increasing pattern persisted up to the 6th day in control, paper, MDPE and HDPE packaging that declined during the subsequent stages. In contrast, declining in pH of LDPE packaging started after 8th day. The losses in pH were higher in Control samples followed by HDPE and paper packaging, whereas, significantly ($p < 0.05$) higher pH was found in LDPE followed by MDPE packaging at the 12th day.

Total soluble solids increased initially with a subsequent decline in all treatments in the later stages (Figure 2). Maximum increase in TSS was observed up to 8th day of storage followed by a general decline in all samples. A pronounced decrease was witnessed in control and Paper followed by HDPE and MDPE, while the trend was very slight in LDPE. The results were statistically ($p \geq 0.05$) same for control and paper packed fruits, while the differences were significant for polyethylene films. Higher TSS values were retained in LDPE followed by MDPE on the conclusion of 12 day storage.

Ascorbic acid content significantly decreased during storage in all samples regardless of treatments. Figure 3 shows the decreasing pattern of AA in apricot fruit during storage. Higher losses in AA were observed in control where initial contents (18.60 mg/100g) decreased to 6.33mg/100g FW followed by paper (6.50mg/100g FW) and HDPE (6.50 mg/100g), while LDPE and MDPE maintained a maximum AA (9.22, 8.85mg/100g FW) at the 12th day respectively.

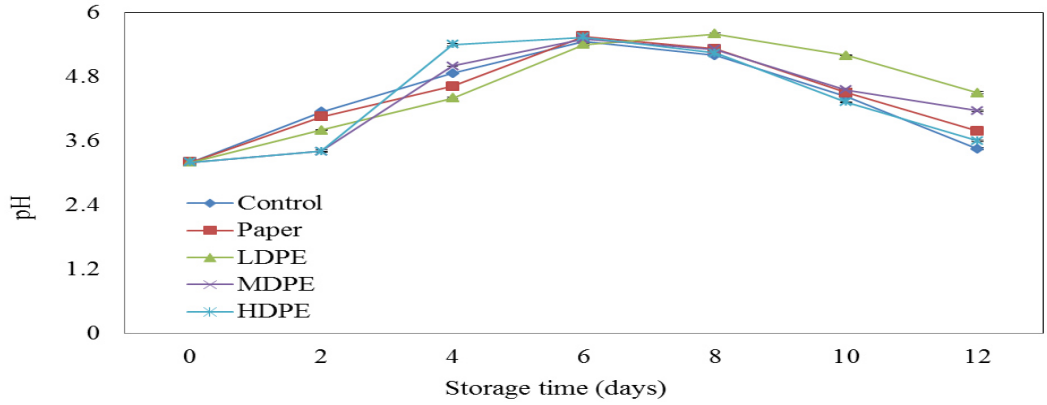

Figure 1. pH of apricot, lower values maintained in LDPE during storage

Total phenolic contents increased during initial intervals of storage and then declined at the later stages (Fig. 4). The variations in phenolic contents among different treatments were found significant ($p < 0.05$). Control, paper, MDPE and HDPE experimental sets showed an increase in TPC up to the 6th day of storage and declined during onward storage. LDPE continued an increasing trend up to 8th day and maintained significantly slower rates of increase in phenolic contents. A pronounced increase in phenolic contents recorded in all treatments as compared to the initial reading of 210.00mg GAE/100g FW. Carotenoid contents of apricot increased significantly during the initial storage intervals at ambient conditions (Fig. 5). A slight decline in the TC observed in the later stages of storage in all treatments. Control samples maintained an increasing trend up to second interval and then decreased. Paper and HDPE showed decline after the 6th day, while increase was recorded in LDPE and MDPE up to 8th day with a decreasing trend afterward. Maximum decline in TC recorded in control followed by paper and HDP, whereas LDPE had the highest content followed by MDPE at the conclusion of 12 days storage.

The free radical scavenging activity of apricot fruit under the effect of different packaging materials is presented in Figure 6. Mean interaction values for treatments and storage were significant at $p < 0.05$. AoA increased in all samples during the initial storage intervals (Fig. 6); however the pattern was rapid in control and paper packaging. Both samples continued an increasing trend up to the 6th day of storage and then declined afterward. LDPE, MDPE and HDPE demonstrated a slower rate of increase in AoA up to 8th day followed by a decline during the subsequent intervals and T_5 showed a rapid reduction in activity at the last intervals.

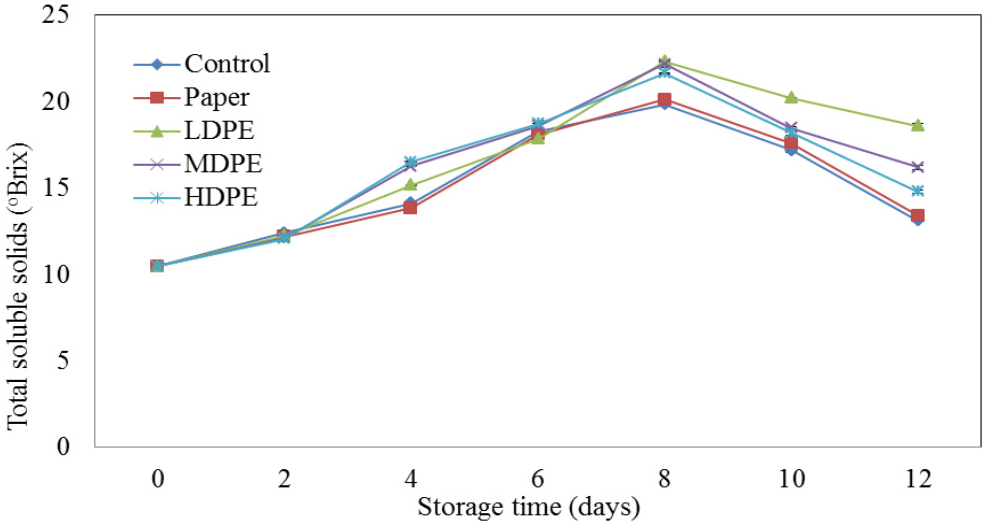

Figure 2. Total soluble solids in apricot, showing maximum loss in control and paper packaging during storage

3.2. Enzymatic activities

Polyphenol oxidase activity under the influence of different packaging material is presented in Figure 7. It shows storage behavior of apricot PPO at ambient conditions. PPO activity increased rapidly in all treatments during the first six days of storage followed by a decrease. Maximum reduction was observed in control followed by paper, HDPE and MDPE respectively, while least decrease was found in LDPE. Similarly LDPE also demonstrated a gradual increase in the activity followed by HDPE at the initial intervals. Overall comparison showed significant patterns among all treatments during storage ($p < 0.05$). The decrease in activity was slower in LDPE and MDPE respectively over the whole storage period.

Peroxidase activity dropped during the first two intervals and then showed an uprising trend in all samples (Fig. 8). The increasing trend again shifted to a decline after 8th day storage. The initial drop in POD activity was higher in control, paper, MDPE followed by HDPE. The initial values for POD activity (12.44 U/ g FW) reduced to 8.55, 8.53 U/ g FW in control and paper packed sets at day four and partly similar values were found for LDPE, MDPE and HDPE. Among all the experimental units, LDPE maintained a stable activity during the subsequent stages, whereas maximum activity was reduced in control, HDPE, paper and MDPE respectively. Catalase activity increased initially in all samples and the difference among treatments were significant ($p < 0.05$). Initial activity (14.62 U/g FW) increased to a maximum of 34.41, 35.25, 33.57 U/g FW in control, paper and HDP packaging up to 6th day and declined afterwards up to the 12th day respectively (Fig. 9). LDPE and MDPE packaging showed a slower rate of increase in CAT activity and the highest activity (36.85, 36.61 U/g FW) was obtained on the 10th day for both treatments. The decreasing pattern was faster in control followed by paper packaging and HDPE, whereas, a significantly higher CAT activity was observed in LDPE on the 12th day, which was followed by MDPE packaging.

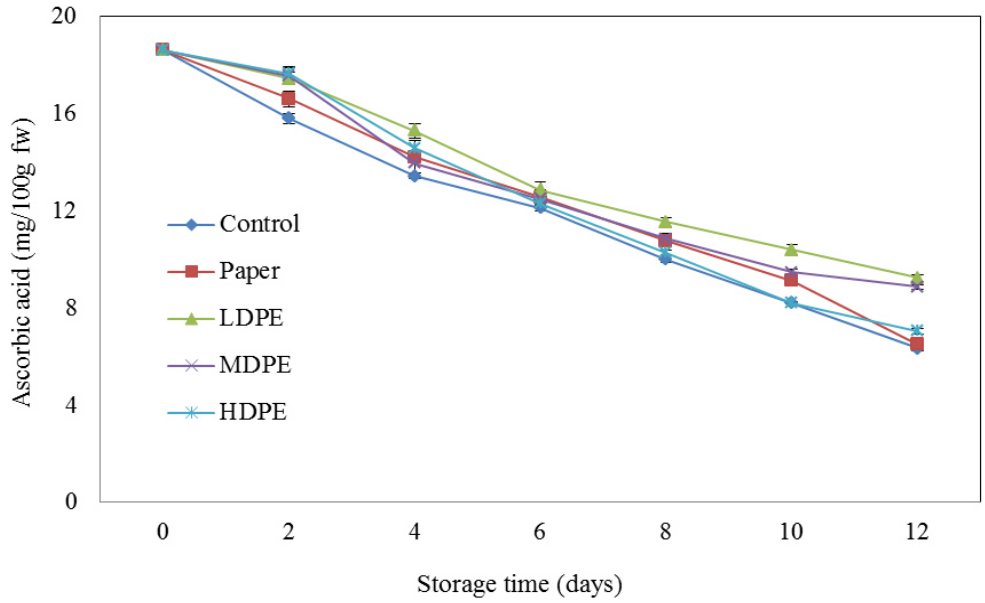

Figure 3. Ascorbic acid in apricot, showing maximum values in LDPE at ambient storage

4. Discussion

Fruit quality deterioration is accompnaid by a number of physiological and biochemical changes during storage. Postharvest treatments and storage environment influence the pace of deleterious activities responsible for quality loss. Effective packagings modify the storage environment and maintain fruit quality traits for extended periods during storage. The response of some biochemical attributes affected by different packaging films and ethylene absorbent are discussed here under:

In the present investigation, a progressive increase in pH values during the intial intervals was observed that declined slightly during the subsequent storage. The pace of changes in pH of treated samples was slow as compared to control, however, low density polyethylene with $KMnO_4$ signficantly maintainted lower values. This phenomenon might be attributed to the packaging that maintained a modified atmosphere and slowed down the ripening process. The increase in pH of fruit is in agreement with the study of Ghasemnezhad *et al.* (2010) who found increased pH values during storage in apricot. It has been further reported that packaging films significantly affected ripening rates in plum fruit (Diaz-Mula *et al.,* 2011). LDPE along with ethylene absorbent delayed ripening that is evident from the present results, where pH values changed slightly in the effective packaging.

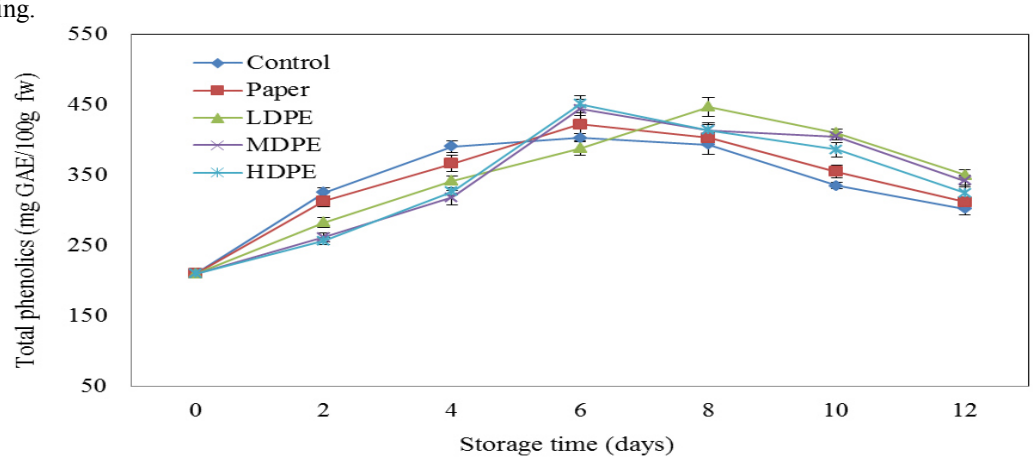

Figure 4. Total phenolic compounds in apricot, showing maximum retention in LDPE at ambient storage

The results showed that polyethylene packaging significantly affected TSS content. Among different films, LDPE effectively delayed ripening, since maintained a gradually increasing trend during storage. The increase in TSS is related to ripening of fresh commodities, while, slower rates of respiration may reduce metabolic activities and thus result in to lower TSS values (Rohani *et al.*, 1997). Ishaq *et al*. (2009) also reported a considerable increase in TSS during storage due to fully conversion of starches into soluble sugars. The decline in TSS during subsequent storage is attributed to fermentation of soluble sugars into alcohol, CO_2 and water. Majidi *et al.* (2011) also reported a decline in TSS content of tomato after peak increase during MAP and CAS.

Similar trends for TSS was also observed in plums in combine application of MAP and 1-MCP during storage (Erkan and Eski, 2012).

Ascorbic acid is sensitive to storage temperature and availability of free oxygen which may cause accelerated oxidation of AA into dehydro ascorbic acid (Piga *et al.,* 2003). The losses further associated with extended storage and respiratory process where organic acids are converted into sugars (Ishaq *et al.,* 2009). Packaging material does not allow free excess of oxygen to the commodity; hence retard deterioration of ascorbic acid by oxidation. Similarly, modified atmospheric conditions may increase CO_2 concentration in the packaging that also counters the deleterious effects of oxygen. The maximum losses of ascorbic acid in control and paper packed samples may be attributed to increased oxidation reactions in both treatments.

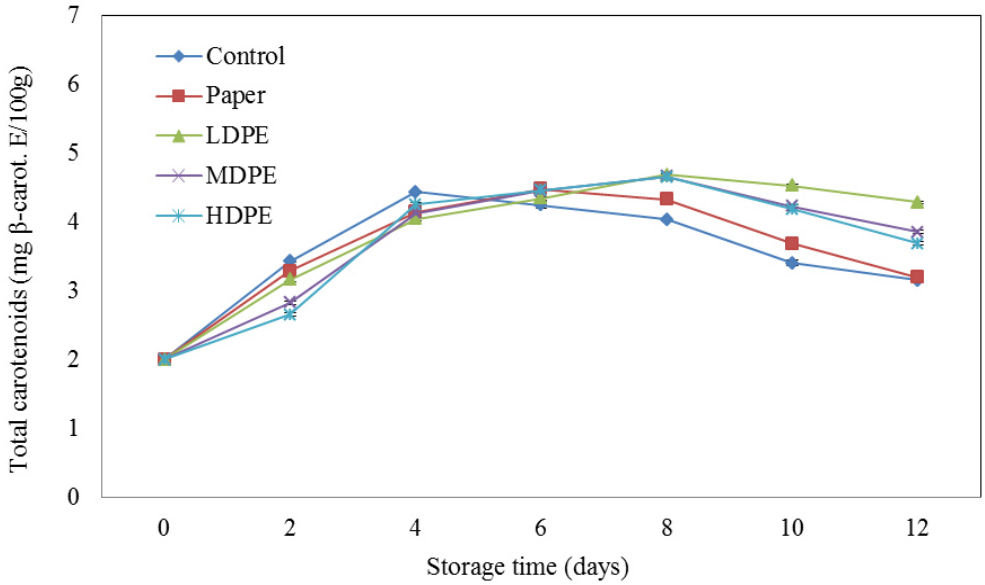

Figure 5. Total carotenoids in apricot, showing maximum amount in LDPE at ambient storage

The behavior of phenolic compounds in the present study showed an increasing trend during initial storage intervals. This phenomenon is in agreement with previous studies that phenolic contents increase during ripening and storage. Shiri *et al*. (2011) have shown that phenolic compounds gradually increased in PE and PVC packed fresh cut grape berries. Diaz-Mula *et al.* (2011) have also reported that MAP significantly delayed the ripening and maintained higher levels of phenolics in plum fruit. Metabolic activities mediated by enzymes lead to depletion of phenolic compounds and occurrence of browning in fruit tissues (Robert *et al.,* 2003). The reduction in phenolic levels is in line with the findings of Tian *et al.* (2004) who reported losses in phenolic contents in fruits during controlled atmospheric storage. Our results are in line with the above reports as an initial increase in phenolics may be linked to the ripening of fruit during storage, while the reduction in prolonged storage is related to advancing ripening and tissue senescence.

The increasing behavior of carotenoids in the current investigation might be attributed to the ripening of stored fruit. Fruits go through a series of physiological changes that modify color, flavor, texture and taste (Bureau *et al.,* 2009). Carotenoids have been shown stable in their natural environment; however, they are much labile to postharvest handling and processing (De Regal *et al.,* 2000). The results of the present study revealed that LDPE packaging delayed ripening and hence carotenoid contents increased gradually during storage. More losses of TC in HDPE and MDPE packaging might be due to the low permeability of these films that resulted into rising of humidity and temperature inside the packaging. While reduction in TC of control and paper packaged samples may be attributed to oxidation and tissue decay due to enzymes. Overall comparison of different packaging films showed that LDPE combined with ethylene absorbent retained higher TC in apricot at ambient storage.

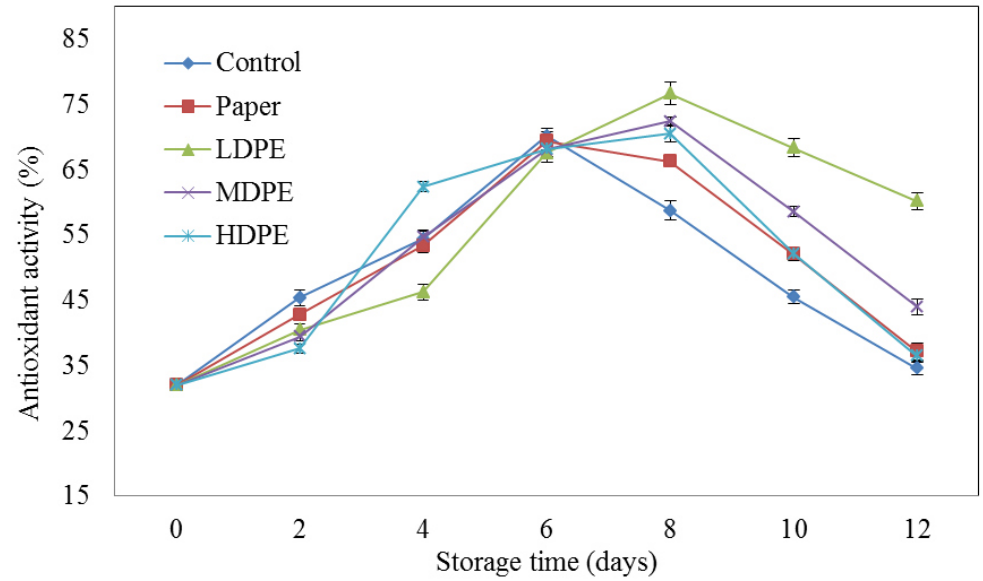

Figure 6. Antioxidant activity in apricot showing maximum activity in LDPE at ambient storage

The increase in AoA during storage is an indicator of ripening process where phytochemicals attained maximum accumulation. Similarly, a decrease in AoA during subsequent storage is attributed to the oxidation of phenolic contents and ascorbic acid. Higher antioxidant activity in the present study is comparable with previous reports that MAP retains ascorbic acid and other phyto-nutrients (Barth and Zhuang, 1996).

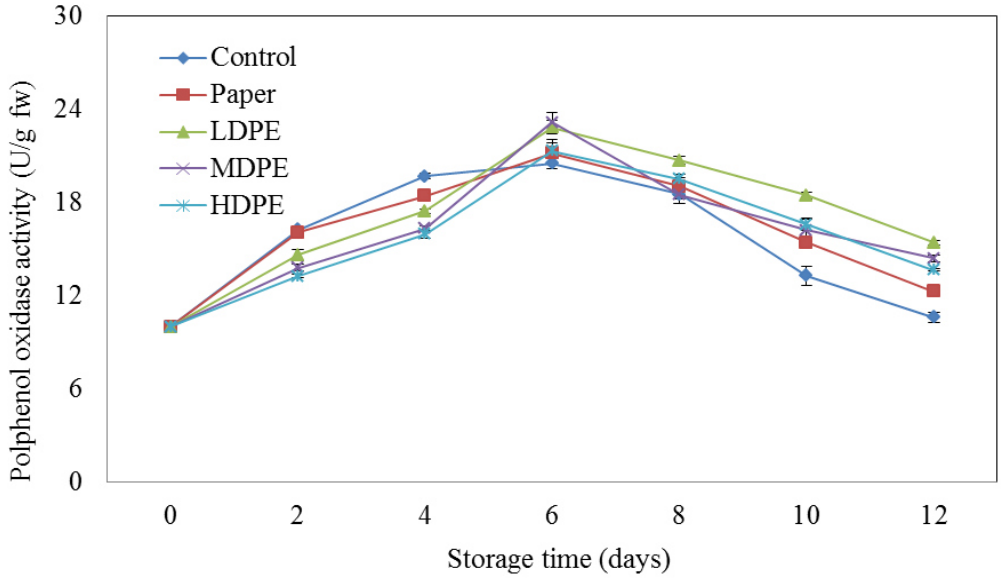

Figure 7. Polyphenol oxidase activity in apricot, showing higher activity in LDPE during storage

The efficiency of packaging films has been studied and perforated low density polyethylene was effective in maintaining carotenoids of Loquat fruit during storage as compared to low permeability bags (Ding *et al.,* 2002). It has further been suggested that modified atmosphere packaging retard the formation of free hydroxyl radicals in fruits and vegetables (Zhuang *et al.,* 1994). Our results demonstrated that radical scavenging capacity reduced with the advancement in senescence.

Polyphenol oxidase (EC 1. 10.3.1) is involved in undesired brown color of fruits and plant by-products (Whitaker 1996). Tissues browning occur due to enzymatic oxidation of phenolics in to quinones and melanin pigment where phenolic contents used as substrates in browning reactions. These biochemical reactions reduce market value, consumer acceptance and shelf life fresh produce. Peroxidase actively is also linked to polyphenol related oxidation of tissues during ripening and storage.POD generates hydrogen peroxide ions and indirectly enhances browning reaction by using antioxidants as substrates (Wongsheree *et al.,* 2009). PPO activity increase during the development stage and then gradually decrease towards ripening (Kadioglu and Yavru, 1998).

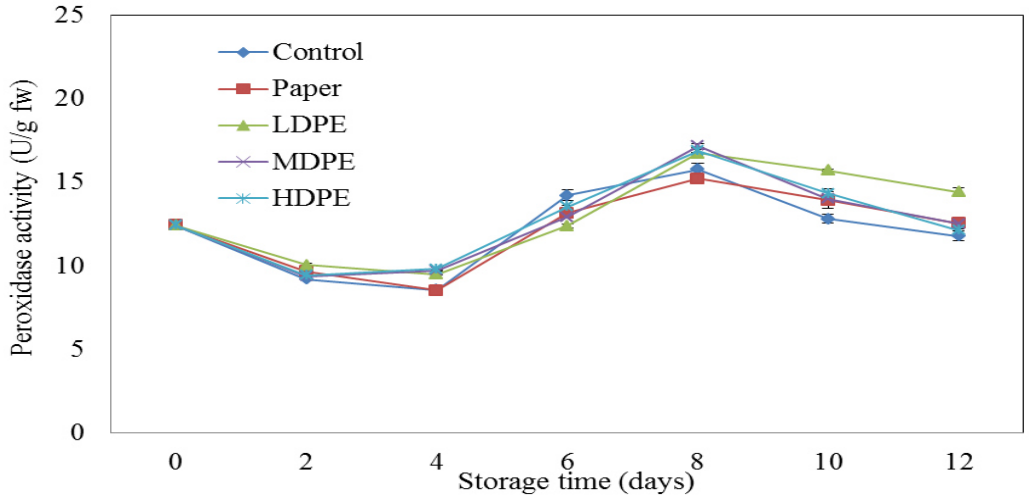

Figure 8. Peroxidase activity in apricot, showing more losses in HDPE during storage

The results of the present study indicated that polyethylene films significantly affected PPO activity and LDPE was more effective in delaying ripening of apricot fruit followed by MDP. Substrate reduction during the reaction leads to decay of fresh commodities (Rojas *et al.,* 2007). Among different postharvest techniques modified atmospheric packaging (MAP) is considered as a cost effective method for shelf life extension of fruits (Banaras *et al.,* 2005). POD activity in our study increased during storage that is in confirmation with previous studies on fruits of sweet cherry and mandarins as reported by Tian *et al*. (2004) and El-hilali *et al.* (2003). LDPE packaging maintained a slower ripening rate that resulted in a gradual increase in POD activity during storage of apricot at ambient storage. Catalase is the most important enzyme that decomposes free radicals and improves the antioxidant mechanism in fruits and vegetables. It degrades hydrogen peroxide into water and oxygen and prevents accumulation of H_2O_2 in plant tissues (Akhtar, 2009).

Ripening results in to the evolution of H_2O_2, consequently antioxidant defense enzymes activate and show increased activity. The increase in CAT activity during storage is in line with the previous reports by Akhtar, (2009) on loquat fruit during cold storage. The decrease in CAT activity might be attributed to reduced capacity of cells to scavenge hydrogen peroxide radicals. The findings of the present study revealed that the LDPE and MDPE packaging maintained freshness of fruit for a longer time by slowing down the ripening process.

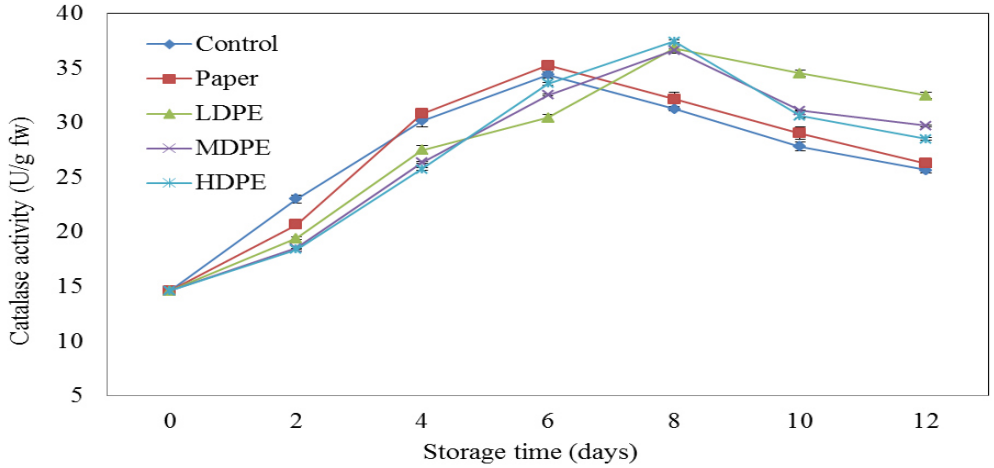

Figure 9. Catalase activity in apricot, showing maximum activity in LDPE during storage

Conclusion

The results of the present study revealed that packaging works as an effective postharvest management tool in extending shelf life of perishable commodities. Among different packaging films, LDPE was found effective in maintaining a modified atmosphere as compared to HDPE and MDPE at ambient temperatures. Different nutritional and functional parameters evaluated were retained by low density polyethylene films while quality deteriorating factors were contained for extended periods.

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