Research Article

Packaging Influences Antioxidants and Antioxidative Enzyme Activities of Tomato during Storage under Refrigerated Conditions

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Abstract

Present study was conducted to evaluate the effect of packaging on biochemical parameters and antioxidative enzymes in tomatoes stored under refrigerated conditions for extending their shelf life. Tomatoes harvested at pink stage were placed in open trays, low-density polyethylene (LDPE) bags and high-density polyethylene (HDPE) bags and were stored under refrigerated conditions. Tomato packaged in polyethyene bags showed more retention in quality and antioxidative attributes along with the higher enhancement of activities of antioxidative enzymes viz. catalase, peroxidase, glutathione reductase, superoxide dismutase, dehydro-ascorbate reductase and mono-dehydro-ascorbate reductase as compared to tomatoes placed in open trays. HDPE packaging was found to be most efficient in maintaining the quality attributes as it showed highest antioxidative enzyme activities with slowest changes in biochemical and antioxidant parameters and extended the shelf life of tomatoes by 21 days. In conclusion, modified atmosphere provided by HDPE packaging reduced oxidative stress by enhancing the activities of antioxidative enzymes resulting in quality retention and shelf life enhancement of tomatoes.

Keywords: Antioxidative enzymes, ascorbic acid, lycopene, modified atmosphere packaging, phenolics, shelf life, tomatoes

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Introduction

Tomato (*Lycopersicon esculentum*) is an important horticultural crop having an estimated worldwide production of over 120 million metric tons [1]. India produces about 7.4 million metric tons of tomatoes annually for an area of 0.24 million hectares. Like most fruits and vegetables, tomatoes have tendency to rapidly deteriorate soon after harvesting. Water comprises about 90% of their fresh weight, which makes tomatoes highly perishable. Tomatoes contain many compounds having antioxidant properties which are carotenoids, phenolic compounds, vitamin – C and small amount of vitamin-E. The major carotenoid present in tomatoes is lycopene which accounts more 80% of total carotenoids present in fully red ripe fruits [2] and has the highest antioxidant activity than all the dietary antioxidants [3]. Phenolic compounds, the secondary metabolites present in tomatoes are substituted by sugar moieties [4].

After harvesting, tomato fruits are still alive, respire and ripen. Several biochemical processes occur in tomato during ripening like synthesis of different pigments, softening of cell wall, degradation of starch with involvement of several classes of enzymes. Although ripening makes fruit edible and flavourful, it also initiates the gradual deterioration of fruit quality [5]. Activated oxygen species like superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) are produced during these biochemical reactions are responsible for oxidative damage during senescence and fruit ripening [6]. Antioxidative system may provide protection to fruit tissues from these potentially toxic ROS. Anti-oxidative enzymes such peroxidase (POX), superoxide dismutase (SOD) and catalase (CAT) are the main protective enzymes engaged in removal of free radicals and active oxygen species [7]. The onset of ripening, sigmoid by ethylene burst is closely related to superoxide dismutase and catalase activities. Peroxidase is a hydrogen donor oxido-reductase and has a role in many important physiological processes like control of growth by lignifications, cross-linking of structural proteins and pectins in cell wall [8]. Increased fruit mono-dehydro-ascorbate reductase (MDHAR) activity and a lower level of fruit ascorbate pool have been correlated with decrease in loss of firmness [9]. Glutathione reductase (GR) plays a role in endogenous H_2O_2 via an oxido-reduction cycle involving ascorbate and glutathione [10]. The extent of the activity of these antioxidative enzymes during post-harvest storage in tomatoes will be of great interest.

Antioxidants in the tomatoes are strongly influenced by geographical, agronomical and environmental and storage conditions [9, 11]. Polyethylene packaging of fruits during retail marketing has become a common practice these days. The plastic film used affects the gaseous atmosphere around the fruits and thus influence its storage life. The present investigation was untaken to evaluate the effect on packaging on activities of antioxidant enzymes and associated biochemical changes during storage of tomatoes under refrigerated conditions in order to find out the suitable packaging material for extending shelf life of tomatoes.

Materials and Methods

This study was conducted in the laboratories of Department Processing and Food Engineering, Punjab Agricultural University, Ludhiana. Tomato of varieties Punjab Ratta, raised in the fields of Department of Vegetable Crops, Punjab Agricultural University, Ludhiana, following the recommended agronomic and cultural practices.

Collection and storage of tomatoes

Tomatoes were harvested at pink stage, washed, sorted and treated with disinfectant sodium hypochlorite (200ppm). Ten tomatoes of each variety were placed in open trays, low-density polyethylene (LDPE) bags and high-density polyethylene (HDPE) bags of thickness 100 gauges with two perforations (1mm) in each bag. These samples were then stored under refrigerated conditions. Ten bags of each type were taken at weekly intervals, pooled and analysed for various biochemical parameters and anti-oxidative enzyme activities till edible state.

Biochemical quality analysis

Total soluble solids concentration (i.e. brix %) of expressed juice was determined by placing few drops of tomato pulp on hand held 'ERMA' refractometer (brix range = 0-32% at 20°C). Lycopene and carotenoid content of tomato sample were estimated using method described by Sozzi *et al.* [12] and expressed as mg/100g fresh weight. Ascorbic acid content was determined according to the method of AOAC, [13] using dichlorophenolindophenol as a dye and was expressed as mg/100g of fresh weight. For estimation of phenolic compounds, methanolic extracts of tomato were used. From methanolic extracts, total soluble phenols were estimated by spectrophotometric method reported by Swain and Hillis, [14]. The concentration of total phenols was determined from the standard curve prepared simultaneously using gallic acid (10-50μg). Total soluble flavonoids were estimated by the method of Balabaa *et al.* [15]. Concentration of flavonoids was determined from standard curve prepared simultaneously using rutin (40-200μg) as standard. Ion leakage of tomatoes sample was measured by the method of Bailey *et al.* [16].

Extraction and estimation of antioxidative enzymes

Sample (0.5gm) was homogenized in 5ml of cold (4°C) extraction buffer (0.1M Sodium phosphate buffer (pH 7.0) containing 1% (w/v) insoluble PVP and 1Mm EDTA) and centrifuged at 20,000g for 10 minutes. Supernatant was collected and analysed for activities catalase, superoxide dismutase, glutathione reductase and dehydro- ascorbate reductase. Enzyme catalase was assayed by the method of Chance and Mahley [17] and activity was expressed as μmoles of H_2O_2 decomposed min^{-1} mg^{-1} FW using 0.0394 as extinction coefficient. Activity of superoxide dismutase was measured by the method of Xing *et al*. [18] and was expressed as the amount of enzyme required for the 50% inhibition of photochemical reduction of nitro blue tetrazolium. For assaying the activity of glutathione reductase, method of Esterbauer and Grill [19] was used and activity was expressed as μmol NADPH oxidized min^{-1} mg^{-1} FW with 6.22 as the extinction coefficient. Dehydro-ascorbate reductase (DHAR) enzyme was assayed according to the method of Foyer *et al*. [20] and activity was expressed as nmoles min^{-1} mg^{-1} FW. For extraction peroxidase, tomato (0.2gm) was homogenized in 2ml of cold (4°C) extraction buffer buffer (0.1M Tris-HCl buffer containing 1mM EDTA, 1% (w/v) PVP and 10μM β-mercaptoethanol) and centrifuged at 20,000g for 10 minutes and supernatant was collected and analysed for peroxidase activity using guaiacol and H_2O_2 as substrates [21].The activity is expressed as units min^{-1} mg^{-1} FW. The unit of enzyme was defined as a change in O.D by 1.0 under standard conditions. For extraction of mono-dehydro-ascorbate reductase, 0.2gm of tomato sample was homogenized in 2ml of cold (4°C) extraction buffer (50mM Hepes buffer, pH 7.6) and centrifuged at 20,000g for 10 minutes. Supernatant was collected and analysed for enzyme activity by the method of Hossain *et al*. [22]. The activity of enzyme was expressed as the amount of enzyme that causes the oxidation of 1μmol of ascorbate to mono-dehydro-ascorbate in 1 minute.

Statistical analysis

All experiments were done in triplicate. Critical difference at 5% level was performed using Completely Randomized Designs (CRD) in the CPCS software. Standard deviation was calculated manually for all the experiments.

Results and Discussion

Changes in biochemical and antioxidant quality parameters

Total soluble solids and titrable acidity

Total soluble solids (TSS) indicate tomato sweetness, although sugars are not the sole soluble components [23]. Irrespective packaging material, total soluble solid contents increased during storage (**Table 1**) which might be due to the degradation of polysaccharides during maturity as indicated by Monerruzzaman *et al*. [24]. Titrable acidity decreased dramatically in tomatoes as the ripening progresses and was mainly due depletion of organic acids especially malic and citric acid during metabolic activities of living tissues [25]. Increase in TSS and decrease in titrable acidity was found to be lower in the fruits packed in polyethylene bags (Table 1). Suppression of respiration and delayed ripening during controlled atmosphere storage in packed tomatoes might be reason of slow increase in TSS. Higher retention of water by packaging material and the concentration effect caused by water loss in open trays may be reflected in titrable acidity values [26]. Amongst the packaging material, HDPE packed tomatoes showed a better response in maintaining the TSS and titrable acidity as compared to tomatoes stored in LDPE bags.

Table 1 Effect of packaging on total soluble solids, titrable acidity, total sugars and electrolyte leakage in tomatoes stored under refrigerated conditions

Parameter	**S→ P↓**	**0**	**7**	**14**	**21**	**28**	**35**	**42**	**CD ($p\leq0.05$)**
TSS(%)	Open	3.30±0.080	3.63±0.063	4.20±0.060	5.00±0.060	5.40±0.077	n.d	n.d	S:0.34
	LDPE	3.30±0.080	3.52±0.060	3.45±0.090	4.80±0.077	5.00±0.062	5.20±0.064	n.d	P:NS
	HDPE	3.30±0.080	3.40±0.052	3.80±0.082	4.61±0.053	5.20±0.064	5.40±0.062	5.60±0.093	S×P:NS
Titrable acidity	Open	2.24±0.069	1.99±0.072	1.74±0.062	1.55±0.060	1.36±0.057	n.d	n.d	S:0.17
	LDPE	2.24±069	2.04±0.042	1.88±0.045	1.74±0.071	1.60±0.049	1.48±0.058	n.d	P:NS
	HDPE	2.24±0.069	2.09±0.055	1.90±0.055	1.79±0.052	1.51±0.051	1.39±0.052	1.16±0.044	S×P:NS
Electrolyte leakage (%)	Open	22.6±0.65	39.8±0.68	53.7±0.73	66.9±0.75	83.7±0.84	n.d	n.d	S:14.92
	LDPE	22.6±0.65	32.1±0.66	49.2±0.55	56.5±0.75	70.85±0.81	79.8±0.83	n.d	P:NS
	HDPE	22.6±0.65	30.5±0.72	42.6±0.77	51.4±0.74	67.0±0.82	77.4±0.86	85.8±0.87	S×P:NS

S: Days of Storage, P: Packaging; Values are mean ± S.E. of three determinations.
n.d. (values not determined due to deterioration of tomatoes); NS (non-significant)

Electrolyte leakage

Irrespective of packaging, electrolyte leakage also increased with increase in storage period (Table 1) which might be due to the loss of membrane integrity with ripening of the fruit [16]. Marangoni *et al*. [27] reported that correlated ion leakage to losses in linolenic acid, a fatty acid, particularly prone to oxidation. Under packaging conditions the increase in electrolyte was slow as compared to tomatoes in open trays, thereby, lowering the loss of membrane integrity in packed tomatoes. Maintenance of firmness of the tomato fruit for a longer period in packed tomatoes as compared to open tomatoes had been reported [28].

Lycopene and ascorbic acid

Lycopene and ascorbic acid content increased gradually thorough the ripening process during storage as is evident from **Table 2** and packaging significantly lowered the rate of increase in these parameters. Ripening of tomatoes was accompanied by chloroplasts degradation and carotenoid biosynthesis, as chloroplasts were converted into chromoplasts [29] resulting in an increase in lycopene during storage. The decrease in lycopene content under packaging conditions could be attributed to delay in ripening due to reduced respiration rates of packed fruits. According to Mathooko [26], modified atmosphere created due to packaging suppressed the synthesis of ascorbic acid but did not impair the fruit capability to synthesize the vitamin C and might inhibit the activity of L-galactano-f-lactone dehydrogenase, which was required for the synthesis of ascorbic acid resulting in lower ascorbic acid in packed tomatoes. Amongst packaging material, increase in lycopene and ascorbic acid content for longer period was found in for HDPE-packed tomatoes.

Table 2 Effect of packaging on lycopene, ascorbic acid, phenols and flavonol content tomatoes stored under refrigerated conditions

Parameter (mg/100g FW)	S→ P↓	0	7	14	21	28	35	42	CD (p≤ 0.05)
Lycopene content	Open	0.53±0.040	1.89±0.048	2.44±0.057	4.67±0.045	6.73±0.073	n.d	n.d	S: 0.92
	LDPE	0.53±0.040	1.45±0.052	1.79±0.061	2.87±0.048	3.91±0.066	5.39±0.053	n.d	P: 0.65
	HDPE	0.53±0.040	1.25±0.048	1.91±0.053	2.25±0.053	3.50±0.067	5.36±0.059	7.68±0.23	S×P:NS
Ascorbic acid content	Open	7.22±0.58	11.97±0.60	13.70±0.52	15.67±0.63	15.92±0.63	n.d	n.d	S: 1.02
	LDPE	7.22±0.58	10.90±0.58	12.29±0.46	14.04±0.63	14.88±0.54	15.13±0.53	n.d	P: 0.72
	HDPE	7.22±0.58	9.77±0.55	11.64±0.75	13.93±0.61	14.65±0.62	15.04±0.63	16.24±0.65	S×P:NS
Phenols	Open	122.1±1.46	177.1±1.45	211.4±1.50	245.9±1.49	258.2±1.48	n.d	n.d	S: 9.84
	LDPE	122.1±0.46	139.9±1.44	164.7±1.45	186.5±1.46	206.6±1.55	217.7±1.51	n.d	P: 6.96
	HDPE	122.1±1.46	148.9±1.37	172.2±1.38	193.3±1.32	212.5±1.50	220.1±1.55	235.1±1.58	S×P: 17.05
Flavonoid content	Open	127.2±1.49	178.8±1.57	206.3±1.61	248.1±1.68	233.5±1.75	n.d	n.d	S: 12.55
	LDPE	127.2±1.49	148.5±1.49	169.0±1.54	193.9±1.58	212.8±1.59	208.2±1.56	n.d	P: 12.54
	HDPE	127.2±1.49	155.0±1.58	182.5±1.58	204.8±1.62	234.1±1.71	227.9±1.70	221.7±1.67	S×P: 30.72

S: Days of Storage, P: Packaging; Values are mean ± S.E. of three determinations.
n.d. (values not determined due to deterioration of tomatoes) ; NS (non-significant)

Total Phenols and flavonoids

Phenols and flavonoids are the major components of total phenolic contents in tomatoes. Total phenols increased dramatically with progression of storage period in packed as well as unpacked tomatoes. Irrespective of packaging conditions, the flavonoid content first increased up to certain storage period and then declined with advancement in storage period. The change in phenol and flavonoid content was rapid in tomatoes placed in open trays than in packed tomatoes (Table 2). Slower changes in phenolics might be because of hypoxic conditions created under modified atmosphere due to packaging resulted in reduction in the rate of biochemical processes of the fruits [18].

Activity of anti-oxidative enzymes

Catalase activity

The activity of enzyme catalase had increased with the storage under all conditions (**Figure 1**) reached to a high level in fully ripe tomato fruits and finally declined in over-ripped fruit. The increase in catalase activity was found to be associated with decrease in H_2O_2 - dependent oxidative processes that contributed to fruit softening [30]. Increase in catalase activity was found upto 21-, 28- and 35 days in open, LDPE packed and HPPE packed tomatoes respectively. Increase in catalase activity during storage was also reported by Xing *et al*. [18].

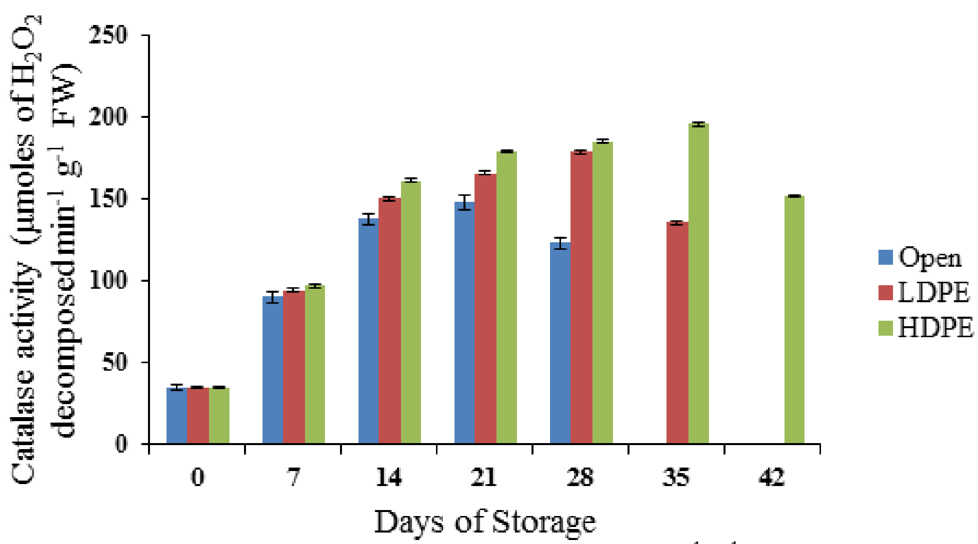

Figure 1 Changes in catalase activity (µmoles of H_2O_2 decomposed min^{-1} g^{-1} FW) of tomato fruits stored under refrigerated conditions

The lower activity of catalase at the end of storage period (Figure 1) resulted in reduced scavenging of H_2O_2, which could therefore be responsible for reduction of membrane integrity in hence, increased electrolyte leakage (Table 1) that led to the large decrease in firmness during later stages of storage. The decrease in firmness during storage had been reported by Kaur and Bhatia [28]. The increase in activity was highest in HDPE packed tomatoes and for a longer storage period followed by LDPE packed tomatoes (Figure 1) thus, protecting the fruits from the damaging effect reactive oxygen species resulting in slower increase in electrolyte ion leakage (Table 1) therefore, prolonging the shelf life of tomatoes under refrigerated conditions.

Glutathione reductase and peroxidase activity

It was observed that storage of tomato resulted in an increased activity of glutathione reductase with the progression of storage period (**Figure 2**). The increased activity could be related to the fact that during ripening, the SH content of the fruits increased as glutathione reductase is the enzyme that reduces glutathione disulphide to sulfhydryl form which is an important cellular antioxidant and protect the thiol groups on enzymes and regenerate ascorbate that further reduces oxidative stress [31]. The activity of peroxidase was found to be related to glutathione reductase, as peroxidase enzymes use glutathione as an electron donor for its role in detoxification of H_2O_2 [32]. In our experiments, activity of peroxidase enzyme first increased with increase in storage period then showed slight decline declined thereafter. This increase was observed till 21 days for open tray tomatoes and 28-35 days for packaged tomatoes (**Figure 3**). The decrease in activity might be related to the loss in firmness of fruit as peroxidase mediates an increase in exocarp tissue stiffness over course of fruit growth [33]. Increase in activities of these enzymes was more in packed tomatoes as compared to open tray tomatoes throughout the storage period (Figure 2, 3) and highest increase in activities had been observed in HDPE packed tomatoes indicating that the respective packaging conditions provided better shelf life.

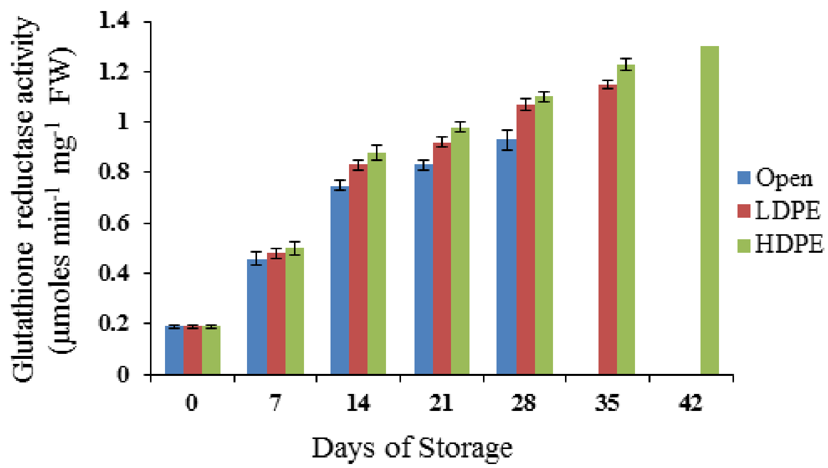

Figure 2 Changes in glutathione reductase activity (µmoles NADPH oxidized min^{-1} mg^{-1} FW) of tomato fruits stored under refrigerated conditions

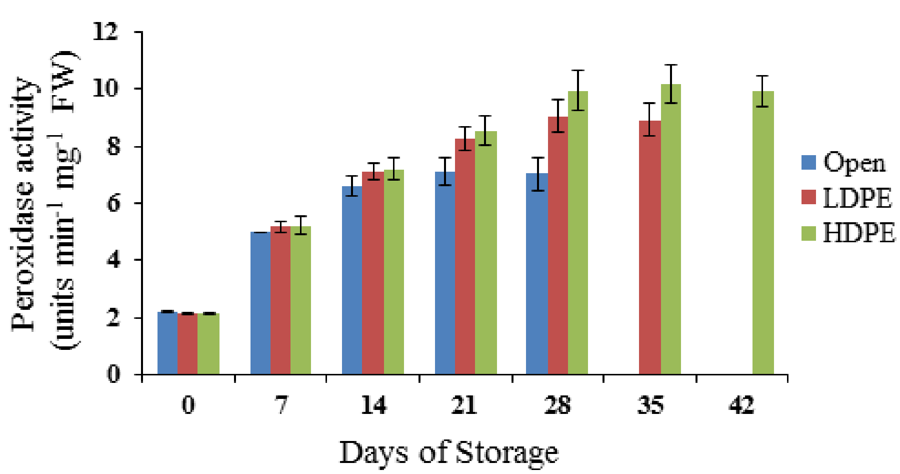

Figure 3 Changes in peroxidase activity (units min^{-1} mg^{-1} FW) of tomato fruits stored under refrigerated conditions

Superoxide dismutase activity

The SOD enzyme is believed to function in the elimination of hydroxyl ion produced during ripening that cause serious damage in cellular components and metabolic dysfunction [34]. Activity of SOD first increased with increase in storage time then decreased after a certain period in all treatments (**Figure 4**) similar to CAT activity (Figure 1) as they both are key components of cellular antioxidant systems. The SOD catalyzed the dismutation of superoxide anions to produce hydrogen peroxide which was then removed by CAT. These two enzymes were reported to extend food freshness by protecting the integrity of membranes and gene encoding the enzyme reported to up regulate post-harvest [18]. Rapid increase in activity was observed in packed tomatoes as compared to open trays tomatoes and HDPE packaging proved to be better than LDPE as it showed higher activity of the enzyme.

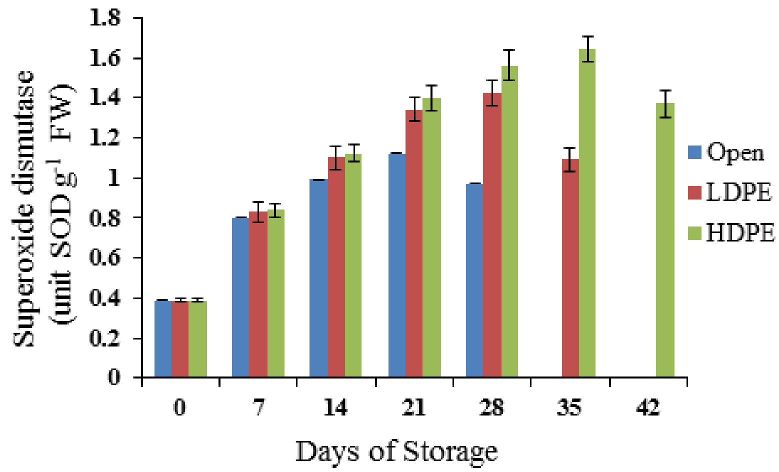

Figure 4 Changes in superoxide dismutase activity (unit SOD g^{-1} FW) of tomato fruits stored under refrigerated conditions

Dehydro-ascorbate reductase activity and Mono-dehydro-ascobate Reductase activity

Both DHAR and MDHAR are involved in maintaining the ascorbate levels in the cells during ripening. The activity of DHAR and MDHAR had first increased and then decreased with the increase in storage period (**Figures 5** and **6**). Our results are consistent with the results of Jimenez *et al*. [35] who reported that increase in activities of DHAR and MDHAR were in parallel with the increase in ascorbate content which plays an important role in preventing senescence [36]. The activities of both the enzymes increased much faster in packed tomatoes than the open trays tomatoes. Increased activity of DHAR and MDHAR and lower oxidation level of ascorbate pool in fruits are correlated with decline in loss of firmness [8]. This increase in activities of the enzymes under packed conditions as compared to open tray tomatoes resulting in retaining freshness of tomatoes for a longer storage period under packed conditions. However, HDPE packaging was found better than LDPE as it showed higher activities of the enzymes.

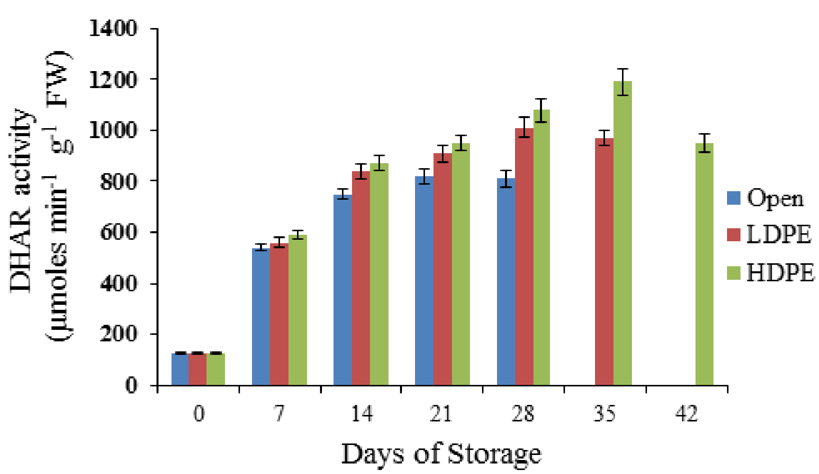

Figure 5 Changes in DHAR activity (nmoles min^{-1} g^{-1} FW) of tomato fruits of stored under refrigerated conditions

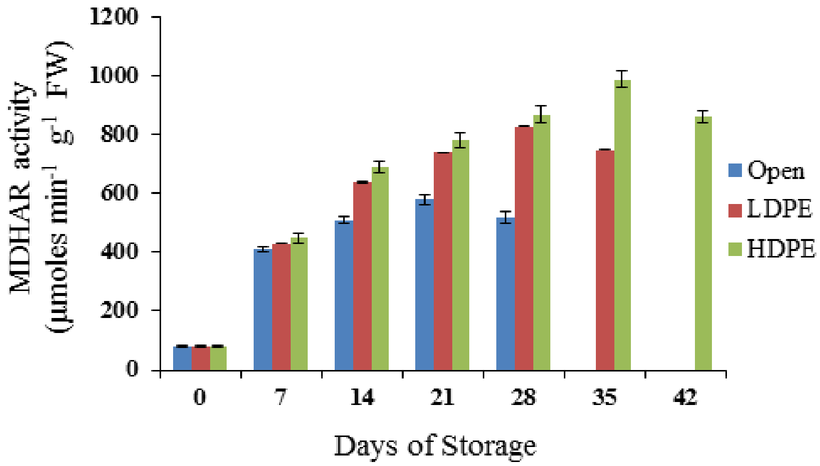

Figure 6 Changes in MDHAR activity (nmoles min^{-1} g^{-1} FW) of tomato fruits stored under refrigerated conditions

Conclusions

Tomato fruits packaged in polyethene bags showed higher enhancement of activities of antioxidative enzymes with less change in quality attributes as compared to fruits placed in open trays. HDPE bags for tomatoes were found to be most efficient in maintaining the quality attributes as it showed slowest changes in the various physiological and biochemical parameters and highest increase in the enzymatic activities and can be suitable for enhancing shelf life of tomatoes under refrigerated conditions.

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