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EFFECT OF PACKAGING FILMS ON CELL WALL HYDROLYZING ENZYMES IN RELATION TO SHELF LIFE ENHANCEMENT OF TOMATOES STORED UNDER AMBIENT CONDITIONS

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ABSTRACT

The effect of packaging films on post-harvest life span of tomato in relation to their effect on the activities of potential cell wall hydrolyzing enzymes were investigated. Tomato cv. 'Punjab Upma' and 'Punjab Ratta' picked at pink stage were placed in open trays, high density polyethylene (HDPE)-, low density polyethylene (LDPE)- and polypropylene (PP)-bags and stored under ambient conditions till their edible state. Irrespective of the variety, there was enhancement in the activities of cell wall hydrolyzing enzymes i.e. polygalacturonase, pectin methylesterase, cellulase and β-galactosidase. Further, there was decrease in firmness with increase in storage period. However, less changes were noticed in tomatoes packaged in polythene bags in comparison to those kept in open trays. Tomato cv. 'Punjab Ratta' displayed slow increase in the activities of cell wall hydrolases and less decrease in firmness as compared to cv. 'Punjab Upma'. In both the varieties, LDPE packaging showed slowest changes in enzymatic activities and firmness and were efficient in enhancing the shelf life of tomatoes.

Key words: Cell wall hydrolyzing enzymes, firmness, packaging, post-harvest, shelf life, tomatoes

INTRODUCTION

Tomato is most widely used vegetable crop and is ranked highest with respect to their contribution of nutrients to the diet in comparison to other crops (Chapagain and Weisman, 2004). They are a major source of antioxidants *viz.*, carotenoids, vitamin C, phenolics and tocopherols. After harvest, the tomato fruits continuously respire and their quality changes on ripening. Although ripening makes fruit edible and flavoured, it also initiates gradual deterioration of fruit quality (Abeles, 1992) depending on their storage temperature and conditions, thus causing overripening characterized by the loss of firmness, other quality attributes and restricted shelf life.

Ripening is a complex process involving major transitions in fruit development and metabolism. The textural changes primarily occur in cell wall structure in middle lamella and primary cell wall, which during ripening leads to cell separation and tissue softening (Bartley and Knee, 1982) due to pectin depolymerization and loss of Ca^{2+} by cell wall degrading enzymes c (Fischer and Bennett, 1991). Polygalacturonases (PG) and pectin methylesterases (PME) are important enzymes involved in fruit softening which solubilize polygalacturonic acid in pectin fraction of cell wall during ripening process. However, evidences show that PG's are not exclusively responsible for structural changes in fruit cell wall. Much less is known about the action of other hydrolytic enzymes (e.g. glycosidases, cellulases). Degradation of cellulosic components of cell wall by C_x-cellulases [carboxy-methyl cellulases E.C. 3.2.1.4] results in the disorganization of fibrillar cellulose (Fischer and Bennett, 1991). In tomato fruits, the loss of galactosyl residues during fruit ripening has been observed in pectic-galactan. β-galactosidases (E.C. 3.2.1.23) play important role in softening of tomato fruit and are characterized by their ability to hydrolyze terminal, non-reducing β-D-galactosyl residues from numerous substrates (Tateishi, 2008). Complex series of modifications in ripening of fruit cell walls may be due to the interplay of several classes of enzymes which loosen cell integrity during ripening.

The deterioration of tomato fruit following harvest is influenced by many factors including storage conditions (Znidarcic and Pozrl, 2006) and packaging material (Batu and Thompson, 1998). Storage temperature is important for shelf life of tomatoes. High temperature accelerates fruit ripening by increasing respiration rate that reflects enhanced tissue metabolism (Assi *et al.*, 2009). Packaging of fruits in polyethylene bags has become a common practice in these days that influences its storage life. Modified atmosphere packaging (MAP) of fresh produce relies on the modification of atmosphere inside the package achieved by natural interplay of respiration rate of commodity and permeability of packaging films (Mangaraj *et al.,* 2009). Modified atmosphere packaging greatly improves moisture retention and inflicts greater influence on fruit preserving quality as well as on the activity of decay causing organism to increase the storage life of fresh produce (Mangaraj and Goswami, 2009). Packaging of fruits in plastic film provides modified atmosphere that affects gaseous atmosphere around the fruit and thus influences its storage life. The present study was planned to evaluate the post-harvest changes in the activities of cell wall hydrolases and texture of tomatoes stored in different packaging films under ambient storage conditions so as to identify a suitable packaging material for increasing their shelf life.

MATERIALS AND METHODS

Plant material and sample preparation

The present study was conducted in the Department of Processing & Food Engineering, PAU, Ludhiana (India) in the year 2009-2010. Two tomato varieties *viz*., 'Punjab Upma' and 'Punjab Ratta' were raised in the fields of Department of Vegetable Crops, as per the agronomic and cultural practices adopted and recommended by PAU, Ludhiana. Tomatoes varieties were harvested at pink stage (when 30-60% tomato surface was pink or red in colour**)**, washed, sorted and treated with sodium hypochlorite (200 ppm**)**. Fifteen tomatoes of each variety were placed in open trays, low density polyethylene bags, high density polyethylene bags and polypropylene bags (thickness 100 gauges) with two perforations (1 mm) in each bag and stored under ambient conditions. The temperature and relative humidity varied from 25.2-36.0^{o}C and 60-90%, respectively, during the experiment. Sampling was done in triplicate and samples were analyzed for post-harvest cell wall hydrolases activity after every 3 days till edible state.

Estimation of firmness

Textural profile analysis was recorded with the help of Texture Analyzer (TA.XT plus). Texture analysis was performed under the following conditions: test: TPA; probe: 75 mm compression plate (P/75); pre-test speed: 1 mm sec^{-1}; test speed: 1 mm sec^{-1}; post-test speed: 1 mm sec^{-1}; distance: 25% strain. Tomato fruits were compressed and firmness determined from the height of force peak (distance between base line and peak point of curve) on the 1st compression cycle (first byte i.e. pressing for one time).

Extraction and estimation of cell wall hydrolyzing enzymes

Polygalacturonase and cellulase were extracted by homogenizing 2 g tomato fruit in 15 mL extraction buffer [200 mM sodium acetate buffer (pH 5.8) containing 1M sodium chloride, 1% (w/v) insoluble polyvinylpyrrolidone, 0.1% (w/v) sodium hydrosulfite, 5 mM dithiothreitol, 1 mM phenyl methyl sulfonyl fluoride and 0.05% (w/v) sodium azide]. Solid material was removed by centrifugation at 6600 rpm for 15 min at 4°C and supernatant dialyzed against 50 times volume of 20 mM sodium acetate buffer (pH 5.2). Sodium azide was added to a final concentration of 0.05% of extract volume. The extracts were assayed for enzyme activities (Ianneta *et al*., 1999).

For polygalacturonase and cellulose assay, 0.2 mL substrate (polygalacturonic acid and carboxy-methyl cellulose, respectively) and 0.6 mL 50 mM sodium acetate buffer (pH 5.2) were added to 0.2 mL test solution in a test tube and incubated at 37°C for 20 min. The reaction was stopped by adding1 mL Nelson's alkaline-Cu reagent and the reducing sugars liberated measured as per Nelson (1944) using UV visible double beam spectrophotometer (ELICO). The enzyme activity was expressed as µg reducing sugars produced min^{-1} g^{-1} fresh weight and specific activity expressed as µg reducing sugars produced min^{-1} mg^{-1} protein.

For the extraction of pectin methyl esterases, 1 g fruit sample was homogenized in 15 mL cold (4°C) 8.8% sodium chloride. The mixture was centrifuged at 9400 rpm for 10 min and supernatant collected. The pH of supernatant was adjusted to7.5 with 0.1N NaOH and was analyzed for enzyme activity. For assay, 2 mL pectin, 0.15 mL bromothymol blue and 0.85 mL distilled water were mixed and incubated at 25°C in a circulating water bath for 15 min. Initial absorbance was determined at 620 nm against water blank. Reaction was started by adding 100 µL enzyme solution and rate of decrease in absorbance measured at 20, 40, 60 and 80 sec intervals. The enzyme activity was determined from linear part of the curve by subtracting initial absorbance value measured using UV visible double beam spectrophotometer (ELICO). Enzyme activity was expressed as A_{620} min^{-1} and specific activity as A_{620} min^{-1} mg^{-1} protein (Hagerman and Austin, 1986). For extraction and assay of β-D-galactosidases, 1 g sample was homogenized with 5 mL ice cold 10 mM tris-HCl buffer and centrifuged at 6600 rpm for 20 min at 4°C and supernatant collected. For measuring enzyme activity, 0.25 mL 0.1M sodium acetate buffer (pH 5.5) in a cuvette was mixed with 0.01 mL ρ-nitrophenol-β-D-galactoside and incubated at 55°C for 10 min. Reaction was started by adding 0.74 mL enzyme preparation and then incubating at 55°C for 10 min followed by the addition of 4 mL 0.1 M NaOH. The intensity of yellow colour developed was measured at 410 nm using UV visible double beam spectrophotometer. The concentration of ρ-nitrophenol produced was calculated from a standard curve using ρ-nitrophenol as standard (1.4-14 µg mL^{-1}). The enzyme activity was expressed as µg ρ-nitrophenol produced min^{-1} g^{-1} fresh weight (Biswas, 1985). Protein concentrations from enzyme extracts were measured as per Lowry *et al.* (1951).

Statistical analysis

All the experiments were done in triplicate. The critical difference at 5% level was analyzed by using completely randomized design in CPCS software. Standard deviation was calculated manually for all the experiments.

RESULTS AND DISCUSSION

Changes in firmness

The reduction of pericarp firmness i.e. the softening of tomato fruits was affected by storage period. Irrespective of the variety used and storage conditions, the firmness of tomato fruits decreased with increase in time of storage period, but those sealed in plastic films softened more slowly than those stored unwrapped which may be due to the modified atmosphere created by plastic films (Fig.1). Similar results had been obtained by Batu and Thompson (1998). Edusei and Cornelius (2015) reported that tomatoes stored in polyethylene bags remain significantly ($p<0.05$) firmer than unwrapped tomatoes under ambient conditions. Post-harvest firmness changes may occur due to the moisture loss and/or due to enzymatic changes as the hemicellulose and pectin fractions become more

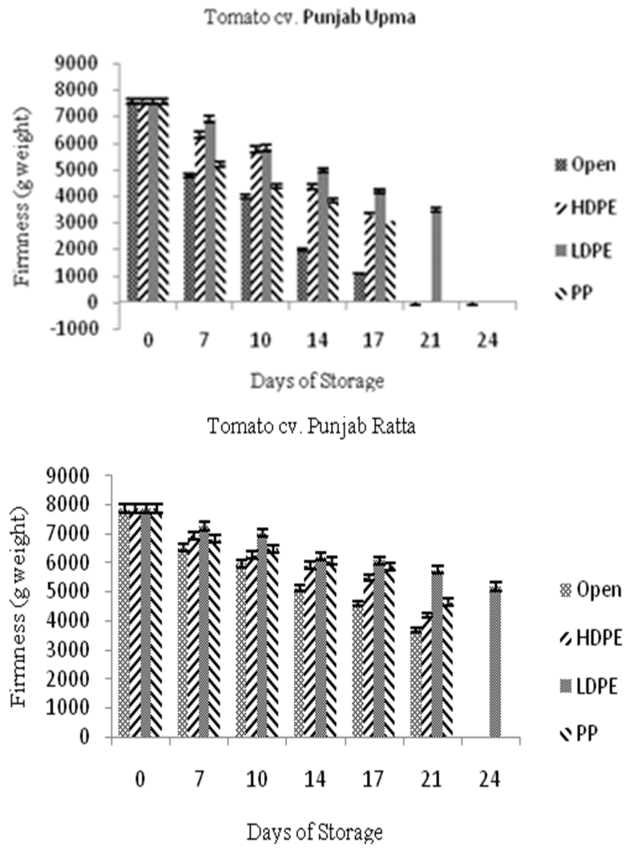

Fig. 1: Changes in firmness (g weight) of tomato fruits of cv. 'Punjab Upma' and 'Punjab Ratta' stored under different packaging conditions

soluble during post-harvest storage and result in disruption and loosening of cell wall. In both the varieties less decline in firmness was seen in LDPE bags kept under ambient conditions followed by HDPE bags in 'Punjab Upma' and PP bags in 'Punjab Ratta'. Decrease in firmness with storage time was less in cv. 'Punjab Ratta' than 'Punjab Upma'. After 17 days of storage, decrease in firmness in cv. 'Punjab Upma' was 84.3, 56.7, 45.6 and 59.5% for the tomatoes placed in open trays, HDPE, LDPE and PP bags whereas in cv. 'Punjab Ratta' it was 41.7, 30.4, 22.7 and 35.3%, respectively, after the same period of storage.

Polygalacturonase (PG) and pectin methylesterase (PME) activity

PG and PME are the key enzymes involved in fruit softening during ripening. PG is a primary determinant of cell wall polyuronide degradation but this alone does not account for tomato fruit softening (Giovannoni *et al.,* 1989). The PG activity increased with increase in storage time irrespective of the variety tested (Table 1) and it reached high level in fully ripe tomato fruit.

Table 1: Changes in polygalacturonase activity (µg reducing sugars produced min^{-1} g^{-1} fresh weight) of tomato fruits cv. 'Punjab Upma' and 'Punjab Ratta' stored under variable conditions

	Punjab Upma				Punjab Ratta			
P→ S↓	Open	HDPE	LDPE	PP	Open	HDPE	LDPE	PP
0	0.26±0.02 (0.07±0.03)	0.26±0.02 (0.07±0.03)	0.26±0.02 (0.07±0.03)	0.26±0.02 (0.07±0.03)	0.19±0.04 (0.11±0.02)	0.19±0.04 (0.11±0.02)	0.19±0.04 (0.11±0.02)	0.19±0.04 (0.11±0.02)
7	2.35±0.06 (1.71±0.04)	2.32±0.04 (1.47±0.03)	2.15±0.05 (1.32±0.03)	2.32±0.08 (1.36±0.06)	1.88±0.13 (0.37±0.02)	1.20±0.08 (0.35±0.05)	1.11±0.06 (0.60±0.05)	1.47±0.06 (0.33±0.08)
10	4.96±0.08 (2.94±0.08)	4.62±0.08 (2.45±0.02)	3.16±0.09 (1.80±0.05)	4.07±0.12 (2.56±0.45)	3.26±0.20 (1.99±0.04)	2.46±0.11 (1.54±0.04)	1.70±0.07 (0.73±0.07)	1.85±0.10 (0.84±0.06)
14	8.28±1.42 (4.53±0.08)	6.74±0.65 (3.72±0.08)	4.70±0.32 (3.05±0.06)	7.12±0.10 (2.73±0.55)	5.78±1.03 (2.08±0.12)	3.82±0.08 (1.82±0.08)	1.97±0.08 (1.08±0.04)	3.35±0.04 (1.41±0.06)
17	8.30±0.20 (4.60±0.21)	6.68±0.23 (4.27±0.18)	6.24±0.54 (3.27±0.07)	7.20±0.22 (2.95±0.05)	7.57±0.24 (3.22±0.02)	5.91±0.10 (2.71±0.04)	4.31±0.08 (2.31±0.07)	4.91±0.06 (2.06±0.02)
21	nd	nd	6.36±0.07 (4.0±0.05)	nd	9.56±0.62 (3.86±0.09)	7.49±0.31 (3.45±0.10)	5.23±0.08 (3.00±0.14)	6.19±0.10 (3.32±0.09)
24	nd	nd	nd	nd	nd	nd	5.43±0.07 (3.21±0.16)	nd
$CD_{0.05}$	Punjab Upma: P: 0.014; S: 0.016; P × S: 0.032				Punjab Ratta: P: 0.014; S: 0.018; P × S: 0.036			

P = Packaging; S = Days of storage; The values are mean ± SD of three determinations;
nd = Values not determined due to the deterioration of tomatoes. The values in parenthesis indicate specific activity (µg of reducing sugars produced min^{-1} mg^{-1} protein) of the enzyme].

Table 2: Changes in pectin methylesterases activity (change in O.D. min^{-1} g^{-1}fw) of tomato fruits cv. 'Punjab Upma' and "Punjab Ratta" stored under variable conditions

	Punjab Upma				Punjab Ratta			
P→ S↓	Open	HDPE	LDPE	PP	Open	HDPE	LDPE	PP
0	0.28±0.07 (0.15±0.02)	0.28±0.07 (0.15±0.02)	0.28±0.07 (0.15±0.02)	0.28±0.07 (0.15±0.02)	0.28±0.03 (0.12±0.04)	0.28±0.03 (0.12±0.04)	0.28±0.03 (0.12±0.04)	0.28±0.03 (0.12±0.04)
7	3.43±0.21 (1.40±0.05)	3.42±0.06 (0.43±0.06)	2.27±0.05 (0.58±0.04)	3.02±0.02 (0.6±0.08)	1.97±0.10 (1.36±0.02)	1.37±0.04 (0.95±0.06)	1.20±0.18 (0.57±0.04)	1.50±0.10 (0.27±0.02)
10	5.33±0.14 (1.68±0.04)	4.22±0.07 (1.65±0.06)	3.70±0.11 (2.13±0.09)	4.60±0.07 (1.05±0.07)	4.98±0.09 (1.88±0.04)	3.40±0.09 (1.65±0.06)	1.94±0.04 (1.03±0.04)	1.99±0.15 (1.27±0.04)
14	9.35±1.26 (4.43±0.03)	8.59±0.16 (3.68±0.04)	5.55±0.05 (3.23±0.07)	8.38±0.08 (3.77±0.06)	6.66±0.12 (2.11±0.03)	4.32±0.03 (1.93±0.02)	2.67±0.54 (1.87±0.04)	4.86±0.67 (2.11±0.09)
17	10.26±0.09 (4.87±0.12)	9.22±0.07 (4.03±0.10)	6.46±0.03 (4.87±0.09)	8.86±0.09 (3.90±0.11)	8.09±0.72 (4.98±0.05)	6.11±0.63 (2.54±0.08)	4.56±0.07 (2.35±0.10)	5.58±0.03 (2.88±0.09)
21	nd	nd	7.49±0.54 (5.40±0.05)	nd	8.43±1.86 (5.29±0.20)	7.74±0.18 (3.53±0.12)	5.39±0.46 (2.84±0.09)	6.82±0.25 (3.00±0.14)
24	nd	nd	nd	nd	nd	nd	5.69±0.88 (2.95±0.08)	nd
$CD_{0.05}$	Punjab Upma: P: 0.021; S: 0.024; P × S: 0.04				Punjab Ratta: P: 0.082; S: 0.101; P × S: 0.202			

P = Packaging; S = Days of storage; The values are mean ± S.D. of three determinations;
nd = The values not determined due to the deterioration of tomatoes.
The values in parenthesis indicate specific activity (O.D. min^{-1} mg^{-1} protein) of the enzyme

Increase in PG activity during storage has been reported by Kramer *et al.* (1992) and Jeffery *et al.* (1984). In present study, a progressive increase in PME activity during storage was observed in both the varieties (Table 2). According to Tieman and Handa (1994) PME determines tissue integrity during fruit senescence, perhaps by regulating calcium binding to cell wall. The fruit firmness declined concomitantly with increase in PG activity.

The increase in PME activity (Table 2) corresponded with similar increase in PG activity as PME catalyzes pectin de-esterification before PG could depolymerize the pectin resulting in fruit softening. Hobson (1964) reported that solubilization of pectic substances occurs in two steps wherein demethoxylation by PME is followed by chain shortening and degradation by hydrolytic PG. In both the varieties tested, packaging of tomatoes resulted in less increase in PME and PG activities as compared to unwrapped tomatoes. This could be related to increase shelf life of packaged tomatoes while maintaining their visual appearance and firmness. Amongst packaging films, LDPE packaging proved efficient in suppressing the activities of these two hydrolytic enzymes, thus maintaining the firmness.

β-galactosidase activity

An increase in β-galactosidase activity was observed with increase in storage period in both the varieties (Table 3). In cv. "Punjab Upma" highest β-galactosidase activity was observed in open trays i.e., 415.6 µg p-nitrophenol producedmin^{-1}g^{-1}fresh weight, whereas in cv. 'Punjab Ratta' the activity was 569.9 µg p-nitrophenol producedmin^{-1}g^{-1}fw. In both the varieties, packaging retarded the increase in β-galactosidase activity as compared to the tomatoes placed in open trays at respective storage period. Similar results have also been reported by Ali *et al.* (2004) and Barbarinsa *et al.* (2015). However, this retardation in increase in activity was more in cv. 'Punjab Ratta' than cv. 'Punjab Upma'.

In both the varieties, LDPE packaging under ambient conditions proved most efficient films in maintaining β-galactosidase activity at lower level. An increase in β-galactosidase activity corresponded with decrease in firmness which might be due to β-galactosidase action in cell wall degradation that eventually resulted in cell wall separation and fruit softening. Tong *et al.* (1986)

Table 3: Changes in β-galactosidase activity (µg of p-nitrophenol produced min^{-1} g^{-1}fw) of tomato fruits cv. of Punjab Upma and Punjab Ratta variety stored under variable conditions.

	Punjab Upma				Punjab Ratta			
P→ S↓	Open	HDPE	LDPE	PP	Open	HDPE	LDPE	PP
0	16.7±0.32 (11.1±0.28)	16.7±0.32 (11.12±0.28)	16.7±0.32 (11.1±0.28)	16.7±0.32 (11.1±0.28)	25.0±0.50 (19.5±0.32)	25.0±0.50 (19.5±0.32)	25.0±0.50 (19.5±0.32)	25.0±0.50 (19.5±0.32)
7	218.2±5.03 (44.5±1.20)	100.1±2.05 (29.2±0.6)	22.2±0.43 (16.7±1.20)	98.7±1.79 (63.9±1.09)	83.4±0.10 (52.8±0.80)	66.7±0.82 (25.0±0.62)	40.3±0.57 (27.8±0.82)	55.6±0.94 (20.8±0.60)
10	243.2±4.08 (67.0±1.78)	105.6±2.05 (32.0±0.97)	51.4±0.76 (41.7±0.90)	154.3±2.04 (87.6±1.00)	147.3±2.19 (59.8±0.67)	126.5±2.25 (36.1±0.54)	75.0±0.86 (41.7±0.79)	127.9±2.06 (66.7±1.50)
14	265.5±3.14 (107.2±2.04)	159.8±2.16 (65.3±0.95)	87.6±1.04 (50.0±0.87)	269.7±3.04 (107.0±2.05)	400.3±6.10 (180.7±2.40)	143.2±2.07 (79.2±0.98)	77.8±0.92 (68.1±0.88)	193.2±2.21 (107.0±2.05)
17	415.6±6.13 (177.9±3.14)	226.6±3.05 (90.3±2.17)	98.7±1.50 (63.9±0.87)	279.4±3.29 (143.4±2.78)	422.6±5.12 (191.8±2.56)	207.1±3.40 (90.3±1.94)	101.5±2.50 (72.3±0.90)	218.2±3.10 (114.0±2.47)
21	nd	nd	152.9±2.24 (105.4±2.48)	nd	569.9±7.00 (244.6±3.10)	253.0±3.15 (109.8±1.00)	148.7±1.80 (111.2±1.67)	261.3±3.30 (139.0±1.75)
24	nd	nd	nd	nd	nd	nd	201.55±3.25 (122.3±1.84)	nd
$CD_{0.05}$	Punjab Upma: P: 0.015; S: 0.017; P × S: 0.034				Punjab Ratta: P: 0.016; S: 0.020; P × S: 0.040			

P = Packaging; S = Days of storage; The values are mean ± SD of three determinations;
nd = Values not determined due to the deterioration of tomatoes; The values in parenthesis indicate specific activity (µg ρ-nitrophenol produced min^{-1} mg^{-1} protein) of the enzyme

proposed that β-galactosidase might be responsible for the solubilization of galactose during fruit ripening. However, Sozzi *et al.* (1998) opined that β-galactosidase action was not in itself enough to explain the variations in tissue firmness during ripening.

Cx-cellulase activity

Cx-cellulase activity increased in both the varieties during tomato fruit ripening throughout the storage period (Table 4). Incv. 'Punjab Upma' maximum Cx-cellulase activity was 5.80 (day 17) µg reducing sugars produced min^{-1} g^{-1} fresh weight in open trays; whereas in 'Punjab Ratta', the activity

Table 4: Changes in cellulase activity (µg reducing sugars produced min^{-1} g^{-1} fw) of tomato fruits cv. Punjab Upma and Punjab Ratta variety stored under variable conditions

	Punjab Upma				Punjab Ratta			
P→ S↓	Open	HDPE	LDPE	PP	Open	HDPE	LDPE	PP
0	0.67±0.08 (0.22±0.02)	0.67±0.08 (0.22±0.02)	0.67±0.08 (0.22±0.02)	0.67±0.08 (0.22±0.02)	0.28±0.06 (0.17±0.05)	0.28±0.06 (0.17±0.05)	0.28±0.06 (0.17±0.05)	0.28±0.06 (0.17±0.05)
7	1.43±0.02 (0.62±0.08)	1.10±0.02 (0.39±0.09)	1.05±0.09 (0.45±0.07)	1.20±0.07 (0.38±0.02)	1.76±0.08 (0.92±0.03)	1.04±0.06 (0.66±0.05)	0.73±0.07 (0.59±0.07)	0.98±0.08 (0.65±0.04)
10	2.35±0.02 (0.87±0.12)	1.81±0.02 (0.72±0.06)	1.47±0.04 (0.68±0.09)	1.80±0.06 (0.66±0.12)	2.78±0.06 (1.25±0.02)	1.84±0.12 (0.95±0.04)	1.50±0.05 (0.87±0.03)	1.54±0.09 (1.06±0.03)
14	3.09±0.35 (1.10±0.05)	2.27±0.38 (1.28±0.05)	2.07±0.21 (1.05±0.03)	2.50±0.08 (1.09±0.02)	3.47±0.04 (2.13±0.05)	2.81±0.04 (1.27±0.06)	1.86±0.09 (1.05±0.08)	3.19±0.06 (1.76±0.09)
17	5.80±0.04 (2.14±0.18)	3.46±0.03 (1.86±0.06)	2.75±0.08 (1.73±0.06)	4.12±0.52 (2.10±0.04)	4.24±0.10 (2.86±0.06)	3.40±0.05 (1.73±0.05)	2.81±0.04 (1.78±0.04)	3.34±0.08 (2.18±0.05)
21	nd	nd	3.04±0.15 (1.92±0.08)	nd	5.63±0.04 (3.10±0.08)	4.32±0.05 (2.07±0.09)	3.20±0.06 (1.97±0.06)	4.57±0.54 (2.48±0.04)
24	nd	nd	nd	nd	nd	nd	3.25±0.10 (2.01±0.04)	nd
$CD_{0.05}$	Punjab Upma: P: 0.016; S: 0.018; P × S: 0.036				Punjab Ratta: P: 0.015; S: 0.018; P × S: 0.037			

P = Packaging; S = Days of storage; The values are mean ± SD of three determinations;
nd = Values not determined due to the deterioration of tomatoes; The values in parenthesis indicate specific activity (µg of reducing sugars produced min^{-1} mg^{-1} protein) of the enzyme

was 5.63 (day 21) µg reducing sugars produced$min^{-1}g^{-1}$fw in open trays. Irrespective of the variety, the increase in Cx-cellulase activity was significantly less in all packagings as compared to open tomatoes. The increase in cellulose activity during storage has also been reported by Iannetta *et al.* (1999). Hobson (1968) has reported that cellulase activity increased with normal ripening but the action of this enzyme was not the primary cause for the loss in firmness.

Conclusion: Tomatoes packed in polythene bags showed lesser increase in activity of cell wall hydrolases and lesser decrease in firmness as compared to open trays tomatoes indicating that packaging was efficient tool for maintaining tomato quality. Amongst packaging films, LDPE packaging showed slowest changes in activities of cell wall hydrolases and firmness in both the varieties and found to be most effective for enhancing shelf life of tomatoes under ambient storage conditions.

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