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Beeswax Coating Loaded with Putrescine to Enhance the Quality and Shelf Life of Plum cv. Satluj Purple

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Abstract

Climacteric ripening in plum fruit is characterized by enhanced respiration rate accompanying by an autocatalytic ethylene production that results in fruit decay, loss of flavour and easily susceptible to softening. The present investigation was carried out to enhance the quality and storability of plum cv. Satluj Purple with different concentrations of beeswax and putrescine i.e. beeswax (5 %) + putrescine (1 mM), beeswax (5 %) + putrescine (2 mM), beeswax (5 %) + putrescine (3 mM), beeswax (10 %) + putrescine (1 mM), beeswax (10 %) + putrescine (2 mM) and beeswax (10 %) + putrescine (3 mM), while control fruits were dipped in distilled water. All the treated and control fruits were dried, packed in CFB boxes with 5 % ventilation and stored for 15 days under ambient conditions. The experiment was laid out in Completely Randomized Design replicated thrice. Fruits from each treatment were analyzed for physico-biochemical characteristics at an interval of three days. Results revealed that physiological loss in weight, spoilage and carotenoids content increased with the advancement of storage period whereas fruit firmness, TA, total phenols, ascorbic acid and chlorophyll 'a' and chlorophyll 'b' showed declining trend with the increase in storage intervals. Other parameters like TSS, total and reducing sugars showed an increasing trend at earlier stages of storage afterwards started declining towards the end of storage period. Based on this, it was concluded that treatment beeswax (5 %) + putrescine (3 mM) and beeswax (10 %) + putrescine (3 mM) was effective in delaying the ripening process and can be used to extend the shelf life of plum fruit.

Keywords: Plum; Beeswax; Putrescine; Quality; Shelf life; Ambient storage

Introduction

Plum, *Prunus domestica* popularly known as 'Alubukhara' (as a varietal name) is a temperate deciduous stone fruit that belongs to the family Rosaceae of the genus *Prunus* and sub family Prunoidae. Plum fruit has high nutritive value and this nutritive value was determined by the substances like carbohydrates (sucrose, glucose and fructose), organic acids (citric and malic acids), tannins, aromatic substances, vitamins (C, A, B_1 and B_2), minerals like potassium, phosphorus, calcium and magnesium (Ertekina et al., 2006). Plum fruit is known for its cooling effect and considered best to cure Jaundice (Chirhah and Baruah, 2019). Fruits are highly perishable due to its climacteric nature and is characterized by enhanced respiration rate accompanying an autocatalytic ethylene production during fruit development (Eum et al., 2009). Enzymes are essential biocatalysts in physiology and metabolism of plants. Most of the enzymes remain active after harvest such as polyphenol oxidase, chlorophyllase, peroxidase and lipoxygenase, pectinases, cellulase and hemicellulase that leads to changes in quality attributes

such as color, flavour, texture and nutritional value (Terefe et al., 2014). Various innovative techniques that are of low cost and high efficiency could be used such as beeswax and application of putrescine as they targeted to reduce transpiration, ripening process, spoilage and fruit senescence.

The use of food grade wax coating on fruits is safe and its application on fruits is approved by Prevention of Food Adulteration Act (PFA), 2008. Beeswax is important hydrophobic lipid based edible wax forming protective layer against moisture losses and impart glossiness to the fruits (Trevisani et al., 2017). Polyamines (PAs) are the natural compounds with aliphatic nitrogen structure that occurs in free and conjugated forms (Zhang et al., 2019). Polyamines that are commonly found in plant cells include Putrescine (PUT), Spermine (SPE) and Spermidine (SPD) (Sawhney et al., 2003). PAs that exist in free forms act as anti-senescence agent, reduce rate of respiration, inhibits the biosynthesis of ethylene, retard colour changes, induce mechanical resistance and decrease chilling symptoms (Valero et al., 2002). Polyamine and ethylene share a common precursor S-adenosylmethionine (SAM) and shows opposite effects in relation to senescence (Nichols et al., 1983). Putrescine (PUT) treatment decreases the synthesis of ethylene by decreasing the enzyme activities of ACC synthase (ACS); ACC oxidase (ACO) and increased the shelf life of fruits (Khan et al., 2007). Thus, the present study was carried with the objectives to enhance the quality and storability of plum cv. Satluj Purple using beeswax coating loaded with putrescine.

Materials and Methods

Uniform and healthy fruits of plum cv. Satluj Purple were harvested at colour break stage from Orchard, Khalsa College Amritsar and shifted to the Horticultural laboratory, P.G. Department of Agriculture, Khalsa College Amritsar. Fruits were washed and then seven treatments were performed with different concentration of beeswax and putrescine *i.e.*, beeswax (5 %) + putrescine (1 mM), beeswax (5 %) + putrescine (2 mM), beeswax (5 %) + putrescine (3 mM), beeswax (10 %) + putrescine (1 mM), beeswax (10 %) + putrescine (2 mM), beeswax (10 %) + putrescine (3 mM) and control (water dip). Putrescine (1 mM) solution was prepared by dissolving 0.161 g putrescine in 1 L of distilled water. Beeswax (5 %) was prepared by heating 5 g of beeswax at 70 °C and 2 ml of oleic acid was added, followed by addition of 6 ml of triethanolamine with constant stirring to melt beeswax. Then 100 mL of distilled water was added slowly with continuous stirring for 5 minutes. Firstly, fruits were dipped in putrescine solution and then coated with beeswax with the help of paint brush. Afterwards coated fruits were dried and packed in corrugated fiber board (CFB) boxes with 5 % ventilation lined with newspaper.

Observations

Physiological weight loss (%)

The weight of plum fruit was recorded under each replication before storage and after every 3 days of storage interval. The physiological loss in weight was calculated in the given formula and expressed in terms of per cent.

$$\text{PLW (\%)} = \frac{\text{Initial fruit weight} - \text{Final fruit weight}}{\text{Initial fruit weight}} \times 100$$

Fruit firmness (lbf)

Firmness of randomly selected plum fruits was measured with the help of penetrometer having stainless steel probe. About one square centimeter of the peel in each fruit was removed from the shoulder end on both sides with the help of peeler and firmness of pulp was recorded and expressed in terms of lbf.

Spoilage (%)

The spoilage percentage of fruits was calculated by counting the number of spoiled fruits in each replication after three days of storage interval and total number of fruits per replication and expressed in per cent.

$$\text{Spoilage (\%)} = \frac{\text{Number of fruits spoiled}}{\text{Total number of fruits}} \times 100$$

TSS (%)

Total soluble solids content of juice was determined with the help of ATAGO digital hand refractometer. The juice of randomly selected fruits was extracted and strained through filter paper. One drop of juice was placed on the surface of prism and by pressing start button TSS value was displayed within seconds.

Total sugars and reducing sugars (%)

Total sugars and reducing sugars were estimated by the method given in AOAC (2000) with following formula:

$$\text{Total sugars (\%)} = \frac{\text{Fehling factors (0.05)}}{\text{Volume of filtrate used}} \times \frac{\text{Dilution made}}{\text{Weight of sample taken}} \times \frac{\text{Final volume made}}{\text{Volume of juice taken}} \times 100$$

$$\text{Reducing sugars (\%)} = \frac{\text{Fehling factors (0.05)}}{\text{Volume of filtrate used}} \times \frac{\text{Dilution made}}{\text{Weight of sample taken}} \times 100$$

Titratable acidity (%)

Two ml of strained juice was titrated against 0.1 N NaOH solution using an indicator i.e., phenolphthalein. The end point was recorded with change in colour from colourless to light pink. The acidity was noted in per cent of malic acid.

$$\text{Titratable acidity (\%)} = \frac{0.0067 \times \frac{N}{10}\ \text{NaOH used (ml)}}{\text{Volume of juice taken (ml)}} \times 100$$

Total phenols (mg/100g FW)

Folin-Ciocalteu (FC) reagent was used for the estimation of total phenols (Swain and Hills 1959). 0.5 ml juice of plum cv. Satluj Purple was diluted with 10 ml distilled water and 0.1 ml sample was taken from the diluted solution. To this 0.1 ml diluted solution, 1.5 ml freshly prepared FC reagent (10 ml FC: 90 ml distilled water) and 4 ml saturated Na_2CO_3 was added and final volume was made to 10 ml with distilled water. The mixture was placed for 30 minutes in dark and absorbance was noted at 738 nm using spectrophotometer (Spectronic 200+, Thermo scientific, USA).

Ascorbic acid (mg/100g FW)

Ascorbic acid was estimated by indole phenol dye method.

$$\text{Dye factor} = \frac{0.5}{\text{Titre}} \times 100$$

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made}}{\text{Aliquot taken} \times \text{Volume of sample}} \times 100$$

Carotenoids and chlorophyll content (mg/100g FW) (Barnes et al 1992)

Dimethyl sulphoxide reagent was used for the estimation of chlorophyll and carotenoids. Tissue (0.1 g) was taken and dipped in 5 ml DMSO solution. For pigment extraction the samples were kept in water bath at 60-70 ℃ for 1hr. The absorbance was recorded at 480, 645 and 663 nm using spectrophotometer (Spectronic 200, Thermo scientific, USA). The contents of chlorophyll a, chlorophyll b and carotenoids were calculated using formulas given below and were expressed as mg/100g FW.

$$\text{Chl a} = \frac{12.47 \times A663 - 3.62 \times A\ 645}{1000 \times W} \times V$$

$$\text{Chl b} = \frac{25.06 \times A645 - 6.5 \times A\ 663}{1000 \times W} \times V$$

$$\text{Carotenoids} = \frac{1000 \times A480 - 1.29 \times \text{Chl a} - 53.78 \times \text{Chl b}}{220 \times 1000 \times W} \times V$$

W = Fresh weight of samples (g), V = Volume of extract, A_{480}, A_{645} and A_{663} are absorbance of samples at 480, 645 and 663 respectively.

Statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) with three replications for each treatment. Means were separated using LSD test. Differences were considered significant at $p \leq 0.05$ using statistical analysis system software Statistix 10.

Results and Discussion

Physiological loss in weight (%)

Weight loss is a primary quality attribute in fruits that relates to texture and associated with loss of cell membrane integrity and cellular breakdown (Nilaprapruck et al., 2016). The per cent loss in weight increased significantly ($p \leq 0.05$) with the advancement in storage period (Fig. 1a). The minimum PLW was registered in fruits treated with beeswax @ 5 % + putrescine 3 mM and maximum PLW was observed in untreated fruits. The hydrophobic nature of beeswax acts as an additional barrier for the movement of water molecules between inner and outer environment of fruits to reduce water loss (Eshetu et al., 2019). Putrescine treatment maintained the membrane integrity and delayed the removal of epicuticular waxes that have an important role in exchange of water through the skin (Martinez-Romero et al., 2002). Similar findings for the reduction in the weight loss by beeswax was reported in sapodilla (Foo et al., 2018) and with putrescine reduction in weight loss was noticed in lemon (Valero et al., 1998), pomegranate (Atukari et al., 2020). PLW showed significantly ($p \leq 0.01$) positive correlation with spoilage, total sugars and carotenoids whereas it shows negative correlation with firmness, titratable acidity, ascorbic acid and total phenols (Table 1). The increase in weight loss decreases the firmness due to reduction in the stabilization of cell membrane integrity (Mirdehglam et al., 2007).

Fruit firmness (lbf)

Polysaccharides present in the cell wall like pectin, hemicellulose and starch contribute to the fruit firmness. Degradation of such compounds by hydrolyzing enzyme like pectin methylesterase and polygalacturonase results in softening of fruit during storage (Kim et al., 2013). Fruit firmness decreased with the advancement in storage period and all treatments had significant effect on fruit firmness (Fig. 1b). Beeswax @ 5 % + putrescine @ 3 mM retained maximum fruit firmness whereas in control minimum fruit firmness was found. Edible coatings modify the internal gas composition of fruit that influences the cell wall degrading enzymes activities and reducing fruit softening (Gunaydin et al., 2017). Similarly, Baez-Sanudo et al. (2009) reported that coating with wax had strong effect on retention of firmness in banana. The application of putrescine increased fruit firmness due to cross-linking properties of putrescine to pectic substances that result in rigidification of cell wall and this binding also blocks the access of degradative enzymes to cell wall, which reduces the softening of tissues (Valero et al., 1998).

Spoilage (%)

Spoilage per cent increased as with the advancement in storage period (Fig. 1c). Results demonstrated that different treatments significantly ($p \leq 0.05$) showed effect on spoilage of plum fruit. It was noted that minimum spoilage was recorded in beeswax @ 5 % + putrescine @ 3 mM and beeswax @ 10 % + putrescine @ 3 mM whereas maximum spoilage recorded in untreated fruits. Mladenoska (2012) reported that there was lowest deterioration in coated fruits due to antifungal properties of beeswax by restricting permeation which induced fungal and bacterial infection. Similar results were reported by Mezemir et al. (2017) in sweet orange and Foo et al. (2018) in sapodilla. Putrescine treatment has antipathogenic effect which reduced the decay incidence in fruits (Khosroshahi et al., 2007). Polyamines conjugated to phenolic compounds and hydroxycinamic acids amides showed a good correlation for resistance against pathogen and also polyamines induce pathogenesis related proteins (Walter, 2003).

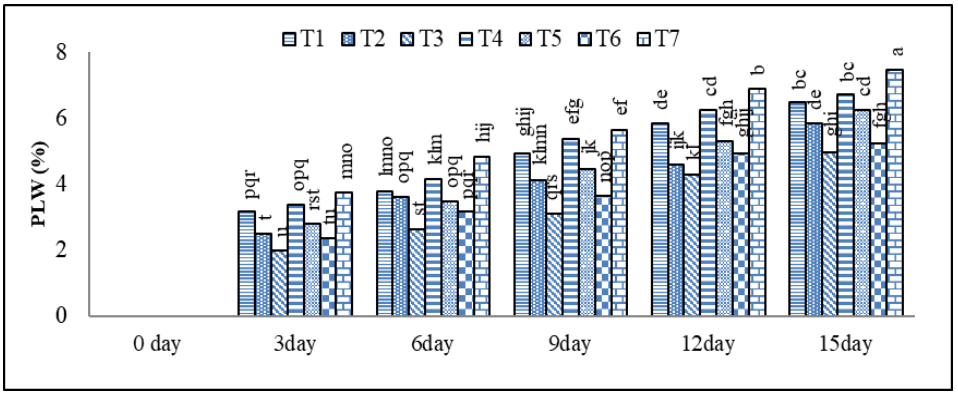

Fig. 1a

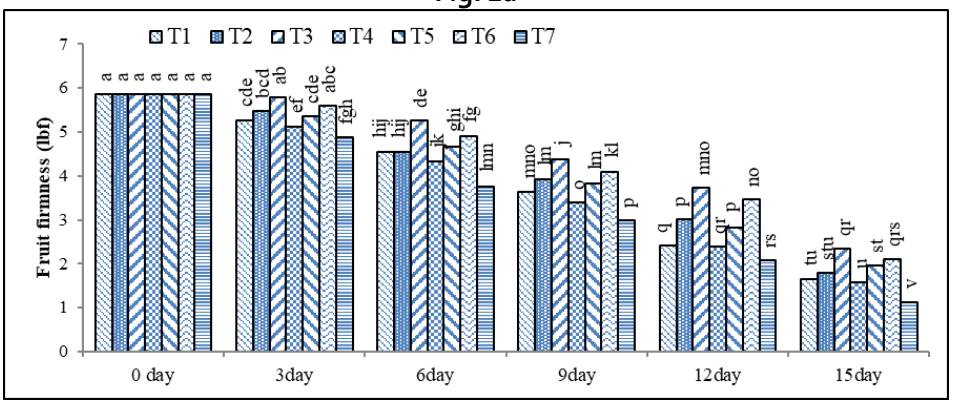

Fig. 1b

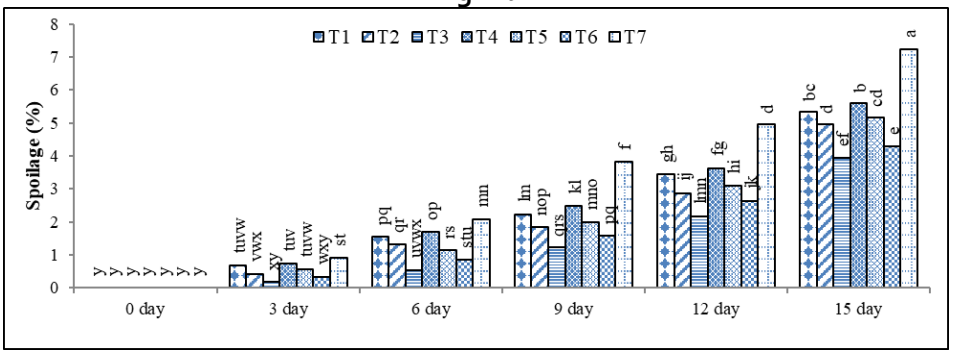

Fig. 1c

TSS, total sugars and reducing sugars (%)

The most striking chemical changes that occur during the postharvest ripening of fruits were hydrolysis of starch and accumulation of sugars (Archana and Suresh, 2018). In the present findings, treatments and storage intervals showed significant effect on TSS, total sugars and reducing sugars. Results demonstrated that TSS, total sugars and reducing sugars increased slowly to 12th day in beeswax @ 5 % + putrescine @ 3 mM and beeswax @ 10 % + putrescine @ 3 mM while it increased upto 9th day in beeswax @ 5 % + putrescine @ 2 mM and beeswax @ 10 % + putrescine @ 2 mM but in control it increased rapidly upto 6th day and then decline towards the end of storage intervals (Fig 2 a, b and c). At the end of storage period, the highest TSS, total sugars and reducing sugars were recorded in fruits treated with beeswax @ 5 % + putrescine @ 3 mM and beeswax @ 10 % + putrescine @ 3 mM whereas minimum were recorded in untreated fruits. The decline in sugars at the end of storage might be due to the consumption of sugars along with organic acids for metabolic activities (Parshant and Masoodi, 2009). The slow rate of increase in total sugars and reducing sugars might be due to the wax coating which affects the

activity of mitochondria and some enzymes like pectinases (Wills and Rigney, 1979). Similar trend was observed in papaya fruit (Hazarika et al., 2017) and peach (Kaur, 2011).

TSS was significantly positive correlated with total sugars and carotenoids while negative correlated with titratable acidity at 1 % level of significance. TSS also showed negative correlation at 5 % level of significance with ascorbic acid and total phenols. Increase in TSS resulted in increased total sugars due to rapidly hydrolysis of starch to sugars (Sirisha, 2003). Retardation in the degradation of TSS retained the titratable acidity, ascorbic acid due to reduction in respiration and ethylene synthesis (Valero et al., 2002). Total sugars showed significantly ($p \leq 0.01$) positive correlation with carotenoids but total sugars were non-significantly correlated with ascorbic acid and total phenols (Table 1). Increase in ripening process resulted in more degradation of chlorophyll and biosynthesis of carotenoids (Carrillo-Lopez et al., 2000).

Titratable acidity (%)

The titratable acidity decreased as with the advancement in storage period due to the utilization of acids in tricarboxylic acid cycle during the respiration process (Rokaya et al., 2016). Irrespective of storage intervals, the maximum titratable acidity was found in beeswax @ 5 % + putrescine @ 3 mM and beeswax @ 10 % + putrescine @ 3 mM whereas minimum titratable acidity was recorded in control (Fig. 3). The higher titratable acidity in wax treated fruits might be due to lower utilization of organic acids in the respiration process whereas untreated fruits had minimum acids due to rapidly organic acids utilization during the storage period (Sonkar et al., 2009). The maintenance of highest titratable acidity with putrescine treatment was reported by Razzaq et al. (2014) in mango fruits and Khan et al. (2007) in plum fruits.

Table 1. Correlation among various fruit attributes

	PLW	Firmness	Spoilage	TSS	Titratable acidity	Total sugars	Ascorbic acid	Total phenols	Carotenoids
PLW	**1.00**								
Firmness	-0.92**	**1.00**							
Spoilage	0.89**	-0.96**	**1.00**						
TSS	0.49**	-0.24***	0.12***	**1.00**					
Titratable acidity	-0.93**	0.91**	-0.91**	-0.35**	**1.00**				
Total sugars	0.33**	-0.10***	-0.02***	0.89**	-0.23***	**1.00**			
Ascorbic acid	-0.95**	0.98**	-0.97**	-0.26*	0.93**	-0.10***	**1.00**		
Total phenols	-0.93**	0.96**	-0.97**	-0.26*	0.94**	-0.10***	0.97**	**1.00**	
Carotenoids	-0.93**	-0.89**	0.89**	0.41**	-0.93**	0.31**	-0.91**	-0.91**	**1.00**

Total phenols (mg/100 g FW)

Total phenolic content decreased with the advancement in storage intervals due to the activities of enzymes i.e., polyphenoloxidase. The maximum total phenolic content was noticed in fruits treated with beeswax @ 5 % + putrescine @ 3 mM followed by beeswax @ 10 % + putrescine @ 3 mM whereas minimum total phenolic content was noticed in untreated fruits (Fig. 4). Similar results were reported by Shiri et al. (2012) in grapes, Amin et al. (2021) in mango and Davarynejad et al. (2013) in plum. The treatment with polyamines i.e., putrescine delayed the polyphenoloxidase enzyme activities and also provides the defense mechanism against pathogens (Khaliq et al., 2019). The maintenance of phenolics compounds with the beeswax coating might be due to the low oxygen permeability that slowed down the enzymatic degradation and oxidation in coated fruits (Riaz et al., 2021).

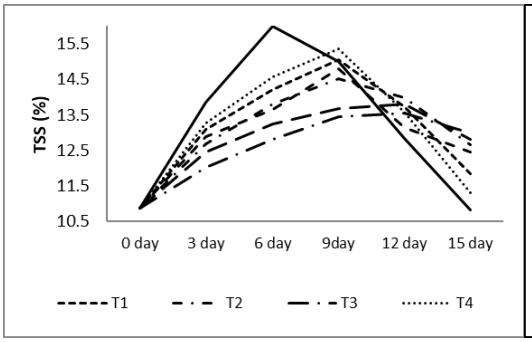

Fig. 2a

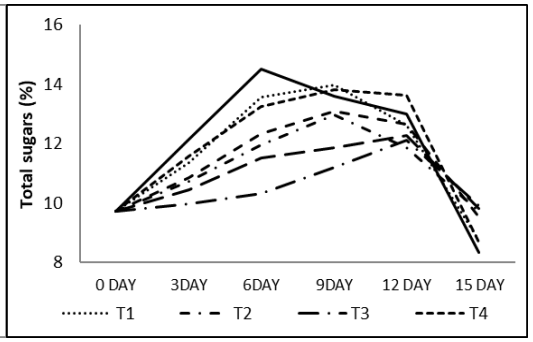

Fig. 2b

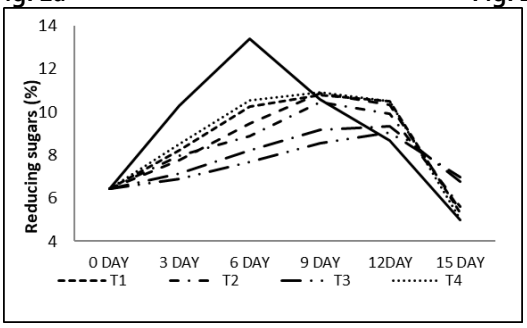

Fig. 2c

Ascorbic acid (mg/100 g FW)

Ascorbic acid one of the important nutrients that is very sensitive to degradation due to oxidation as compared to other nutrients during storage period (Veltman et al., 2000). The ascorbic acid content decreased significant ($p \leq 0.05$) progressively with the increase in storage intervals (Fig. 5). The highest ascorbic acid content was recorded in fruits treated with beeswax @ 5 % + putrescine @ 3 mM and beeswax @ 10 % + putrescine @ 3 mM while the lowest ascorbic acid was estimated in untreated fruits. The maximum retention of ascorbic acid content in wax treated fruits might be due to lower degradation of ascorbic acid content in the storage (Sonkar et al., 2009). The lower level of ascorbic acid content in control fruits might be due to increased respiration rate which accelerates the deteriorative oxidation reaction and loss of ascorbic acid content (Davey et al., 2000). The treatment with putrescine also ascribed to delay or decreased activity of ascorbate oxidase (Ishaq et al., 2009). Similar results were reported in citrus (Hassan et al., 2014, Rokaya et al., 2016) and in plum (Davarynejad et al., 2013).

Carotenoids (mg/100g FW)

Carotenoids are the stable compounds which remain intact in the tissue even after considerable senescence has occurred (Wills et al., 2007). The carotenoids content increased significantly with the advancement in storage period (Fig. 6). The minimum carotenoids content was recorded in fruits treated with beeswax @ 5 % + putrescine @ 3 mM followed by beeswax @ 10 % + putrescine @ 3 mM while the maximum carotenoids content was estimated in untreated fruits.

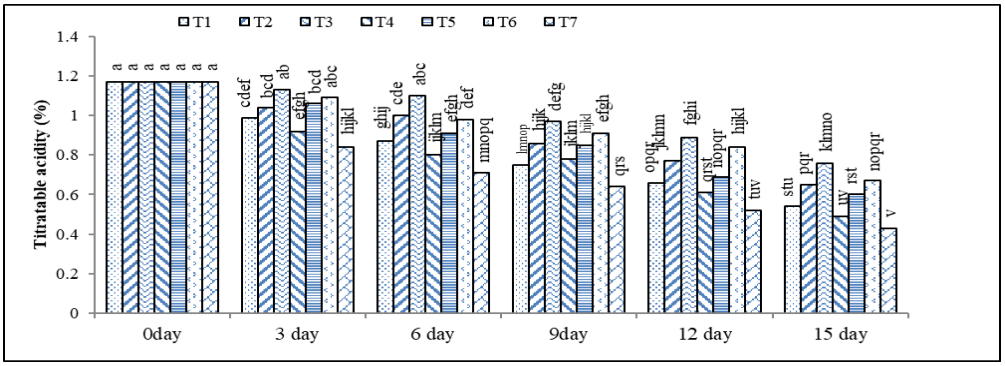

Fig. 3

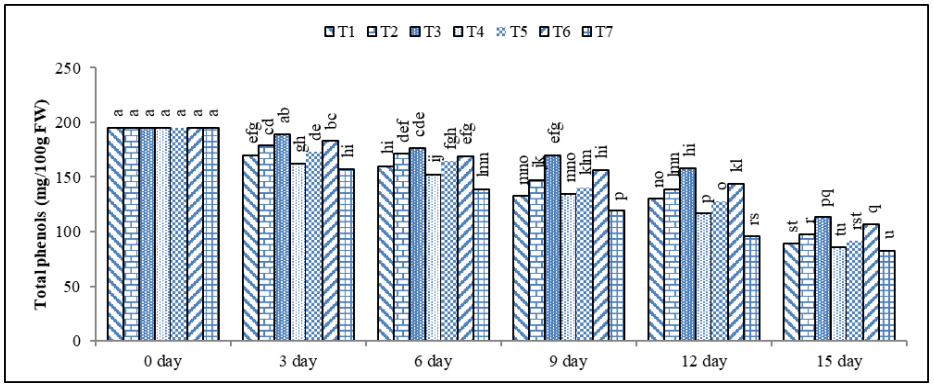

Fig. 4

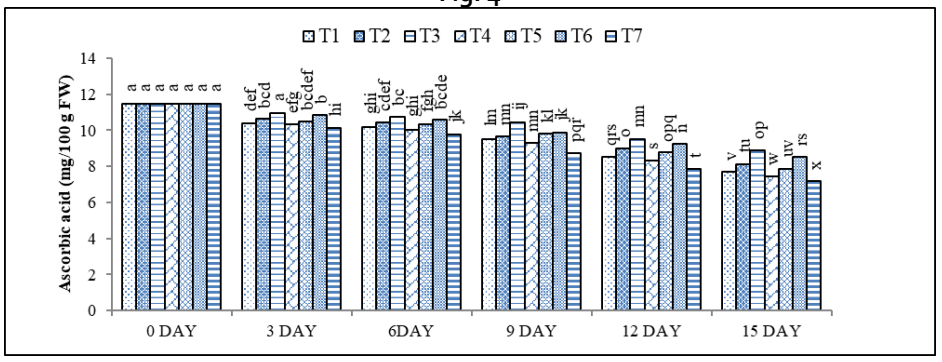

Fig. 5

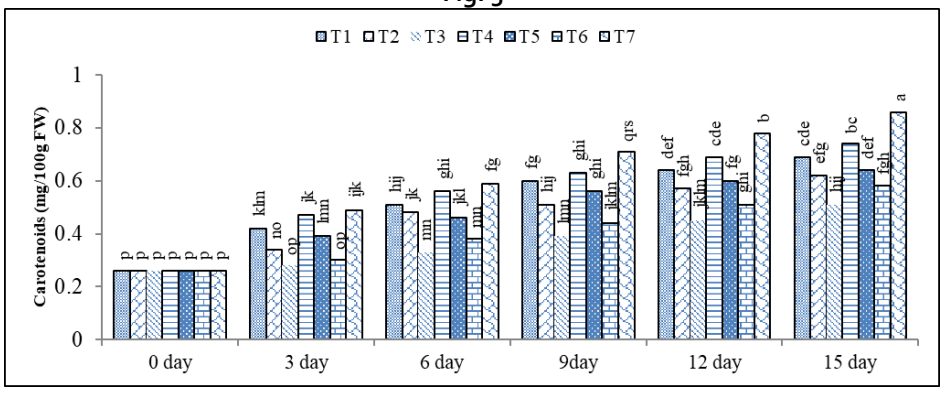

Fig. 6

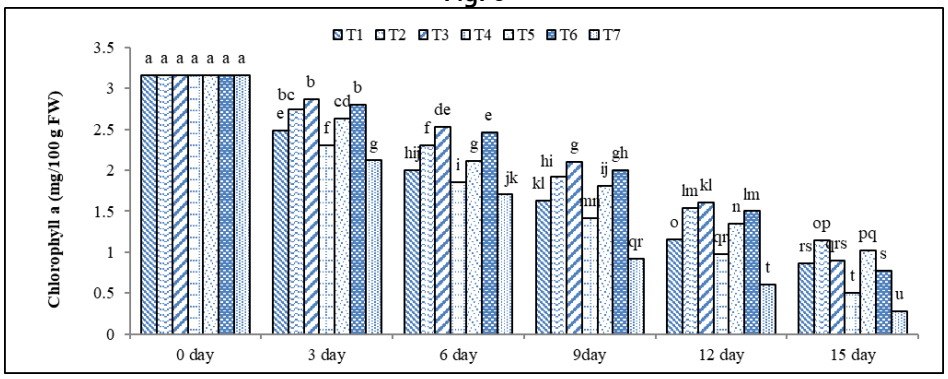

Fig. 7

The disappearance of chlorophyll was related to the synthesis and pigments revelation ranging from yellow to red (Shankhu et al., 2022). The coating helps in reducing the synthesis of carotenoids, by creating modified internal atmosphere in coated fruits which delay ripening, degradation of chlorophyll and senescence (Carrillo-Lopez et al., 2000). The present findings are

in agreement with the results of Baraiya et al. (2014) in carambola, and Shankhu et al. (2022) in papaya.

Chlorophyll a and Chlorophyll b content (mg/100g FW)

The chlorophyll content decreased with the increase in storage period due to the breakdown in chlorophyll structure and also due to the increased in chlorophyllase enzyme activity of pigments (Wills et al., 1998). The highest chlorophyll 'a' and chlorophyll 'b' content were recorded in fruits treated with beeswax @ 5 % + putrescine @ 3 mM and beeswax @ 10 % + putrescine @ 3 mM while the lowest chlorophyll 'a' and chlorophyll 'b' were estimated in untreated fruits. The polyamines prevent chlorophyll loss in thylakoid membranes by stabilizing the photosystem complex and slow down the loss of apoprotein of the light-harvesting chlorophyll a/b-protein complex of photosystem II from thylakoid membranes (Cheng et al., 1984). Similar results were reported in carambola (Baraiya et al., 2014; Gol et al., 2013) and in banana (Nilpraprucк et al., 2016).

Conclusion

From the present study, it can be concluded that postharvest treatment of beeswax and putrescine significantly improved the quality and storage life of plum cv. Satluj Purple. Fruits treated with beeswax @ 5 % + putrescine @ 3 mM and beeswax @ 10 % + putrescine @ 3 mM were found to be most effective in reducing the physiological weight loss, spoilage and carotenoids content along with maintaining the highest firmness, TSS, TA, total sugars, reducing sugars, ascorbic acid, total phenolic content and chlorophyll 'a' & 'b' as compared with other treatments during the entire storage period.

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