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## Beeswax Coating Loaded with Putrescine to Enhance the Quality and Shelf Life of Guava Fruit cv. Allahabad Safeda

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Received:

13-11-2022

Accepted:

16-12-2022

Published:

24-12-2022

### Abstract

The present investigation was conducted in Department of Agriculture, Khalsa College, Amritsar during 2021-22. The fruits were harvested and treated with 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 plain water. All the treated and control fruits were packed in corrugated fibre boxes (CFB) and stored at ambient condition as well 95 % RH for 15 days. The experiment was laid out in Completely Randomized Design replicated thrice. The fruits from each treatment were analyzed for physico-biochemical characteristics at an interval of three days. The results revealed that physiological loss in weight, spoilage loss, TSS/TA and carotenoids of fruits increased with storage intervals whereas fruit firmness, TA, total phenols and chlorophyll 'a' and chlorophyll 'b' showed declining trend with the advancement of storage period. Other parameter like TSS showed an increasing trend at earlier stages of storage and later on start declining. The results reflected that beeswax @ 5 % + putrescine @ 3 mM showed a significant reduction in weight loss, spoilage loss and carotenoids while maintained highest firmness, TSS, titratable acidity, total phenol content, chlorophyll a and chlorophyll b as compared to other treatments as well as uncoated fruit.

**Keywords:** Guava; Quality; Shelf life; Putrescine; Beeswax; Ambient storage

### Introduction

Guava (*Psidium guajava* Linn.) is native to Tropical America and was introduced in India in early 17<sup>th</sup> century. It belongs to the family Myrtaceae and occupies fourth position in terms of area and production among fruit crops of India (Singh et al., 2017). The fruit is often referred to as "The fruit of the poor guy" or "Tropical apple" (Pradhan et al., 2021). The common names of *Psidium guajava* include Piara in Bengali, Araca in Brazil, Apple guava in English, Amarood and Jamba in India and Guayaba in Spanish (Uzzaman et al., 2018). It contains 2.3% protein, 12.16 % carbohydrates and 4.8% dietary fibre. It is a strong source of mineral calcium i.e. 17.63 mg/100 g. It is an excellent source of ascorbic acid, with 241.86 mg/100 g, making it a promising source for discovery and value addition in food products when combined with other fruits (Bogha et al., 2020). The fruit is also a moderate source of B complex vitamins such as niacin, vitamin-B<sub>6</sub> (Pyridoxine), vitamin K as well as minerals like magnesium, copper and manganese (Garasiya et al., 2013). Guava is perishable fruit and is susceptible to mechanical damage due to its delicate skin which offers very little protection against injury, which may lead to the development of



several diseases such as soft rot and anthracnose resulting in problem during storage and limiting the postharvest storage life (Kader 2002 and Jain et al., 2003). It is also a climacteric fruit with elevated respiratory activity and high rate of ethylene production which consequently leads to fast ripening and senescence process (Jain et al., 2003).

Polyamines (PA's), mainly putrescine (PUT), spermine (SPM) and spermidine (SPD) are compounds that represented in association with the plant growth and developmental processes such as cell division, fruit ripening and senescence (Pandey et al., 2000 and Theiss et al., 2002). Wax coatings create a modifying atmosphere around the fruit and provide a semi permeable barrier for raising carbon dioxide levels and reducing oxygen levels, thereby reduction in oxygen levels leads to reduction of respiration, water loss, oxidation reaction rates and metabolic activities especially respiration and transpiration (Sultan et al., 2021). Therefore, fruits coating increases their resistance to pathogens and increase their storage and marketing capabilities (Kaur et al., 2013 and Ghayempour et al., 2015).

### **Material and methods**

Physiologically matured fruits of guava cv. Allahabad Safeda were procured from Attari orchard, Amritsar. Uniform and healthy fruits of guava were randomly selected and treated with beeswax and putrescine before storage. For further the storage investigations, 1 kg guava fruit for each replication of all the treatments on every storage interval were packed. After application of coating fruits were dried and then packed in corrugated fiber board (CFB) boxes with 5 % ventilation lined with newspaper. The various physico-biochemical observations regarding storage potential and fruit quality were looked into on the day of storage and on 3, 6, 9, 12 and 15 days of storage.

#### ***Fruit quality measurements***

##### ***Physiological loss in weight***

The weight of fruits under each replication was recorded before storage and at each storage interval. The physiological loss in weight was calculated in the given formula and units expressed in terms of per cent.

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

##### ***Spoilage***

The spoilage percentage of fruits was calculated on the number basis by counting the spoiled fruits in each replication and total number of fruits per replication and expressed in per cent.

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

##### ***Fruit Firmness***

Firmness of randomly selected fruits was measured with the help of penetrometer having stainless steel probe.

##### ***Estimation TSS, Titratable acidity and TSS/TA***

Total soluble solids content of juice was determined with the help of ATAGO digital hand refractometer. Two ml of strained juice was titrated against 0.1 N NaOH solution using phenolphthalein as an indicator. The end point was noted with change in colour from colourless to light pink. The ratio of total soluble solids to the titratable acidity content of the fruits was calculated by dividing the TSS value of the fruit to its corresponding titratable acidity value.

##### ***Total phenols***

For estimation of total phenols, Folin Ciocalteu (FC) reagent was used (Swain and Hills 1959). Fruit juice of 0.5 ml guava cv. Allahabad Safeda 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<sub>2</sub>CO<sub>3</sub> 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 recorded at 738 nm using spectrophotometer (Spectronic 200+, Thermo scientific, USA).

### **Chlorophyll and Carotenoids Content (Barnes et al., 1992)**

Reagent: Dimethyl sulphoxide (DMSO)

Tissue (0.1 g) was taken and dipped in 5 ml DMSO solution. The samples were kept in water bath at 60-70 °C for 1hr for pigment extraction. 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 following formulas: Chlorophyll and carotenoids were expressed as mg/g FW tissue

$$\text{chl a} = \frac{12.47 \times A_{663} - 3.62 \times A_{645}}{1000 \times W} \times V$$

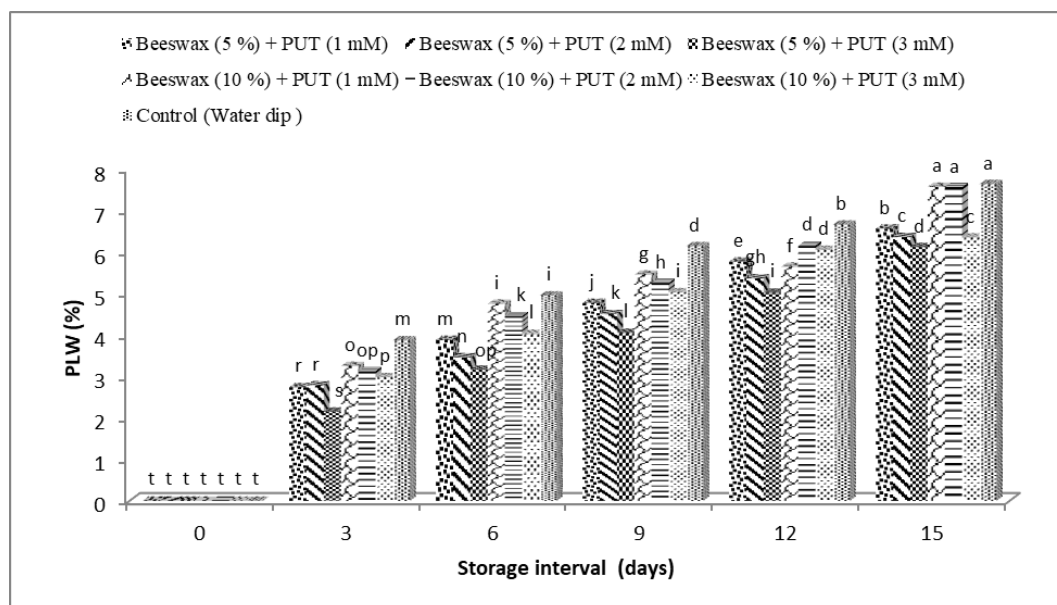
$$\text{chl b} = \frac{25.06 \times A_{645} - 6.5 \times A_{663}}{1000 \times W} \times V$$

$$\text{Carotenoids} = \frac{1000 \times A_{480} - 1.29 \times \text{Chla} - 53.78 \times \text{Chlb}}{220 \times 1000 \times W} \times V$$

W= Fresh weight of samples in gram, 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 the level  $p \leq 0.05$  using statistical analysis system software Statistix 10.



**Fig. 1A.** Effect of beeswax coating loaded with putrescine on PLW (%) of guava cv. Allahabad Safeda during ambient storage

### **Results and Discussion**

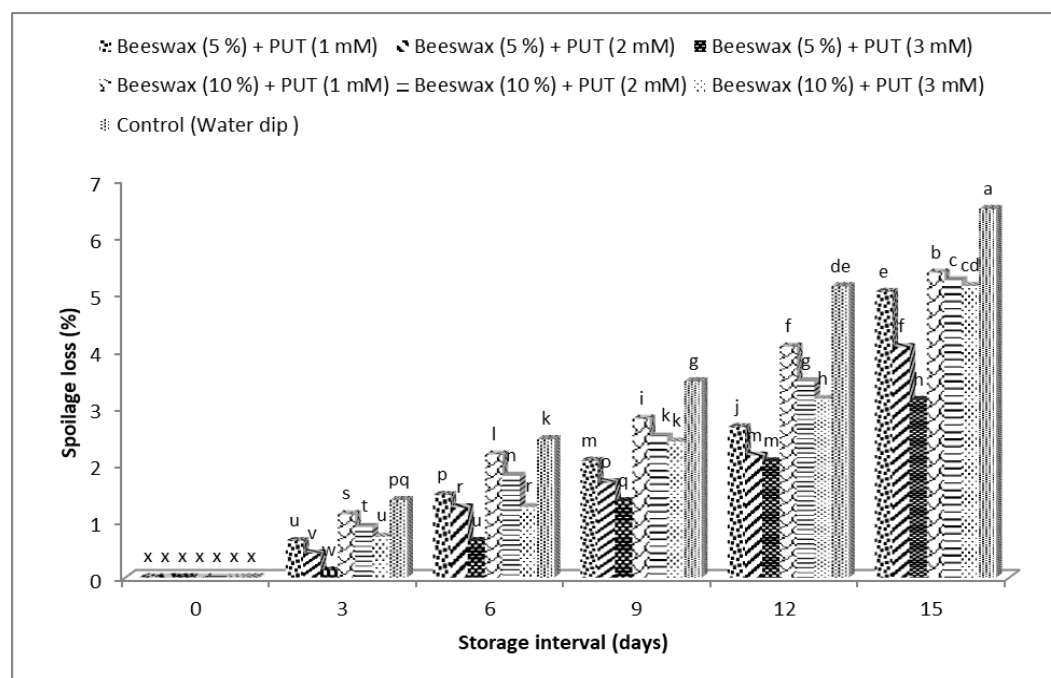
#### **Physiological loss in weight**

The per cent loss in weight increased significantly ( $p \leq 0.05$ ) with the increase in storage period is presented in (Fig. 1A). Minimum PLW was found in beeswax @ 5% + putrescine @ 3 mM and maximum was noted in untreated fruits. Beeswax coating provides a protection layer to the skin of the fruits so that respiration occurs more slowly and reduced fruit weight loss (Samson and

Straus 2010). Mirdehgha et al. (2007) investigated that the reduction in weight lost in putrescine treated fruits is the consequence of consolidation and stabilization of both cells' integrity and permeability of the tissues as polyamines forms linkage with cell membranes and preserves waxes of cuticle layer there by retard the removal of epicuticular waxes which play a very important role in water exchange through the skin. Similar to our results, putrescine treatment effectively reduced the weight loss during storage of mango Zahedi et al. (2019) and apricot fruit (Davarynejad et al., 2013).

### Spoilage

It was noticed from data that spoilage per cent increased with the progression of storage period. Data presented in Fig. 1B shows the effect of beeswax coating loaded with putrescine on the spoilage loss of guava fruit cv. Allahabad Safeda. It was found that minimum spoilage was registered in beeswax @ 5 % + putrescine @ 3 mM while maximum spoilage was observed in control. Seleshi et al. (2019) reported that the reduction of decay in  $\text{CaCl}_2$  dipping and beeswax coating could be due to the coatings film property which acted as a barrier for the growth of microbes. The polyamines conjugated to phenolic compounds and hydroxycinnamic acid amides have been shown to accumulate in cells during plant pathogen interaction (Walters 2003). Similar findings were also observed by (Mirdehghan et al., 2013a) in pistachio nut and (Mirdehghan et al., 2013b) in grape.

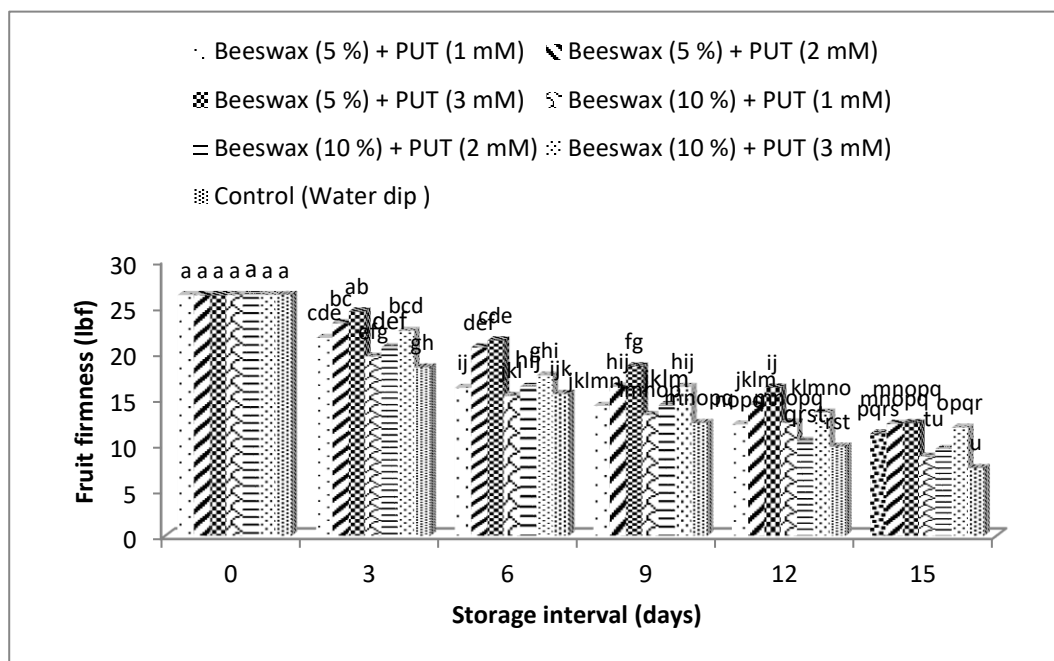


**Fig. 1B.** Effect of beeswax coating loaded with putrescine on spoilage loss (%) of guava cv. Allahabad Safeda during ambient storage

### Fruit Firmness

Firmness is the most reliable quality and shelf-life indicator for the guava fruit during storage. The result revealed that beeswax along with putrescine significant maintained the firmness shown in Fig. 1C. Loss of firmness was more striking in untreated fruits than all other treatments. The maximum fruit firmness was recorded in freshly harvested fruits and minimum fruit firmness was observed on 15<sup>th</sup> day of storage. Beeswax @ 5 % + putrescine @ 3 mM treated fruits showed maximum fruit firmness and minimum firmness was recorded in control. Polyamines reduced the activity of pectic acid degrading enzymes like cellulase, poly-galacturonase, pectin esterase etc. (Kramer 1989). Rigidification of cell wall is a reason for maintaining fruit firmness in treated fruits, which is result of cross linkage between polyamines and carboxyl group of pectic substances in the cell wall (Valero et al., 1998). Malik et al. (2006) found that high concentrations

of putrescine were found effective in maintaining fruit firmness in mango cv. Kensington Pride. Similar to our findings, PUT treatments had been reported to maintain the higher firmness of mango fruits (Wannabussapawich and Serayheap, 2018).



**Fig. 1C.** Effect of beeswax coating loaded with putrescine on fruit firmness (lbf) of guava cv. Allahabad Safeda during ambient storage

#### Total soluble solids

Data presented the effect of beeswax coating loaded with putrescine on the total soluble solids content of guava fruits shown in (Fig. 2A). The storage period and treatments displayed a significant effect on TSS content of fruits. TSS content in treated fruits increased upto 12<sup>th</sup> day and afterwards it started declining on 15<sup>th</sup> day of storage treatment (beeswax @ 5 % + putrescine @ 3 mM and beeswax @ 5 % + putrescine @ 2 mM). However, TSS increased upto 9<sup>th</sup> day of storage in beeswax @ 5 % + putrescine @ 1 mM followed by beeswax @ 10 % + putrescine @ 3 mM, beeswax @ 10 % + putrescine @ 2 mM and beeswax @ 10 % + putrescine @ 1 mM while in untreated fruits, it increased sharply upto 6<sup>th</sup> day afterwards it started declining. Abbasi et al. (2019) result showed that the putrescine treatments significantly slowed down the increased in TSS content of fruits which can be ascribed to its effect in delaying the conversion of starch into simple sugars, as well as other effects of putrescine decreasing ethylene biosynthesis thus delaying ripening process. Seleshi et al. (2019) reported that the lower respiration rate for CaCl<sub>2</sub> dipping and beeswax coatings might, therefore reduce the use of metabolites resulting in slow conversion of carbohydrates to sugars in nectarine fruit. Similar to our findings, effect of putrescine on gradual variation in TSS has also been reported in pomegranate (Barman et al., 2011) and in mango fruits (Malik et al., 2006).

#### Titrateable acidity and TSS/TA

The observations regarding effect of beeswax coating loaded with putrescine on acidity of guava fruits is given in (Fig. 2B). There was continuous decline in level of acidity throughout the storage period of storage interval, maximum titrateable acidity was estimated in beeswax @ 5 % + putrescine @ 3 mM and minimum acidity was recorded in untreated fruits. Oliveria et al. (2004) reported titrateable acidity was higher during initial stage and then decreased by the end of storage. Maruti et al. (2022) reported that papaya fruits treated with putrescine @ 4mM which reduced the respiration and transpiration rate that might have slowly decreased the acid content of fruits. Similar results have been reported in papaya by (Ali et al., 2011) and (Asgar et al., 2011). Progressed of storage period registered an increase in TSS/TA in all treatments is presented in

(Fig. 2C). Irrespective of storage intervals, the minimum TSS/TA was noticed in beeswax @ 5 % + putrescine @ 3 mM whereas maximum was recorded in untreated fruits. Mukdisari et al. (2016) reported that inhibition of respiration through beeswax coating caused the acid content of the fruit to be higher. High acid content reduced TSS/ TA ratio of beeswax coated fruits. Abbasi et al. (2019) reported that lower TSS/ TA from putrescine treated peach fruits might be explained by slower metabolic changes in organic acids and carbohydrates sugars. Khalek et al. (2018) reported that the lowest fruit contents in TSS/ TA by putrescine and hydroxypropyl methylcellulose treatment in guava cv. Maamoura were ascribed to a reduction in the respiration of the fruits and suppression of ethylene production which consequently affected acid and sugar ratio. The similar findings were observed in agreement with putrescine application on apricots (Martinez et al., 2002) and plum (Serrano et al., 2003).

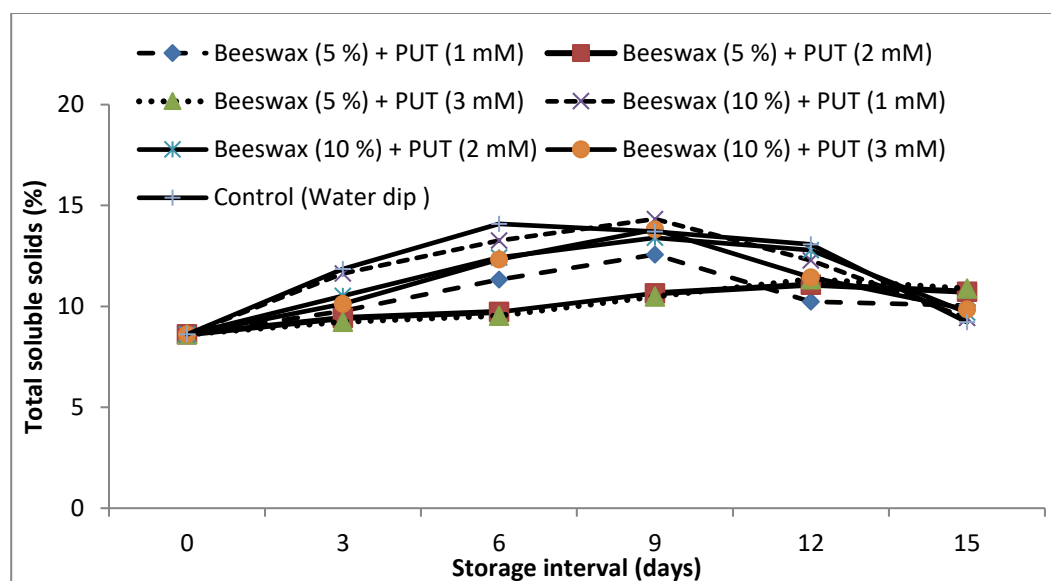


Fig. 2A. Effect of beeswax coating loaded with putrescine on total soluble solids (%) of guava cv. Allahabad Safeda during ambient storage

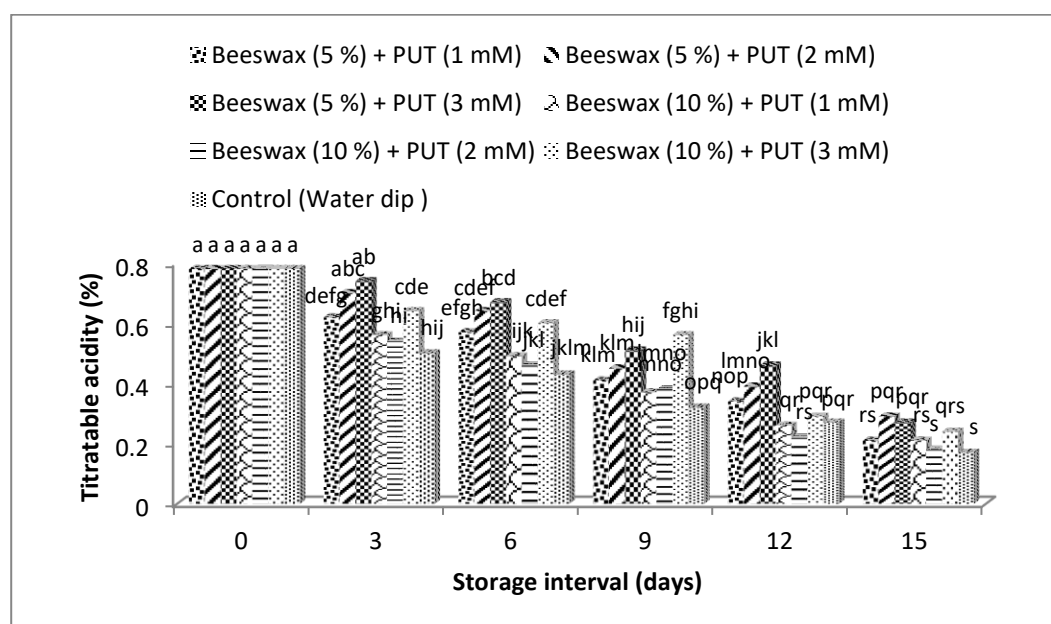


Fig. 2A. Effect of beeswax coating loaded with putrescine on TA and TSS/TA of guava cv. Allahabad Safeda during ambient storage



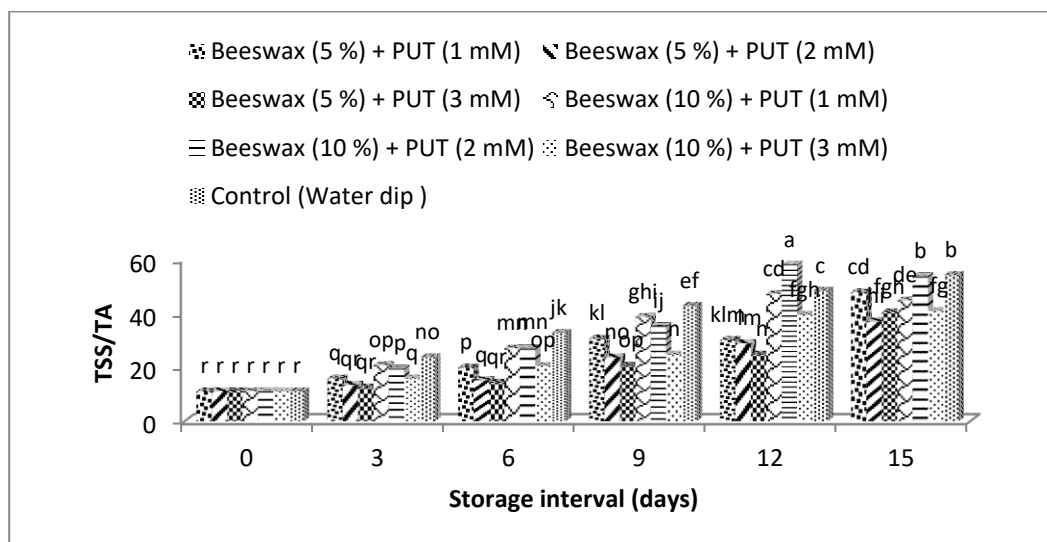


Fig. 2B. Effect of beeswax coating loaded with putrescine on TA and TSS/TA of guava cv. Allahabad Safeda during ambient storage

### Total phenols

Polyamines play very important roles in the antioxidant system and in protecting plasma membrane phospholipids against reactive oxygen species damages (Verma and Mishra 2005). The maximum total phenols were recorded in beeswax @ 5 % + putrescine @ 3 mM while minimum total phenols were noticed in untreated fruits presented in (Fig. 3A) Jhalegar et al. (2011) was observed papaya fruit treated with putrescine 4 mM had the highest total phenol content due to slower degradation rate of phenolics in polyamines treated fruits apparently indicates that they play important role in reducing activity of polyphenoloxidase enzymes due to reduces respiratory activity of the fruits. The role of putrescine treatment in maintenance of total phenols could be ascribed to a delayed in senescence process (Arora Sairam and Srivastava 2002).

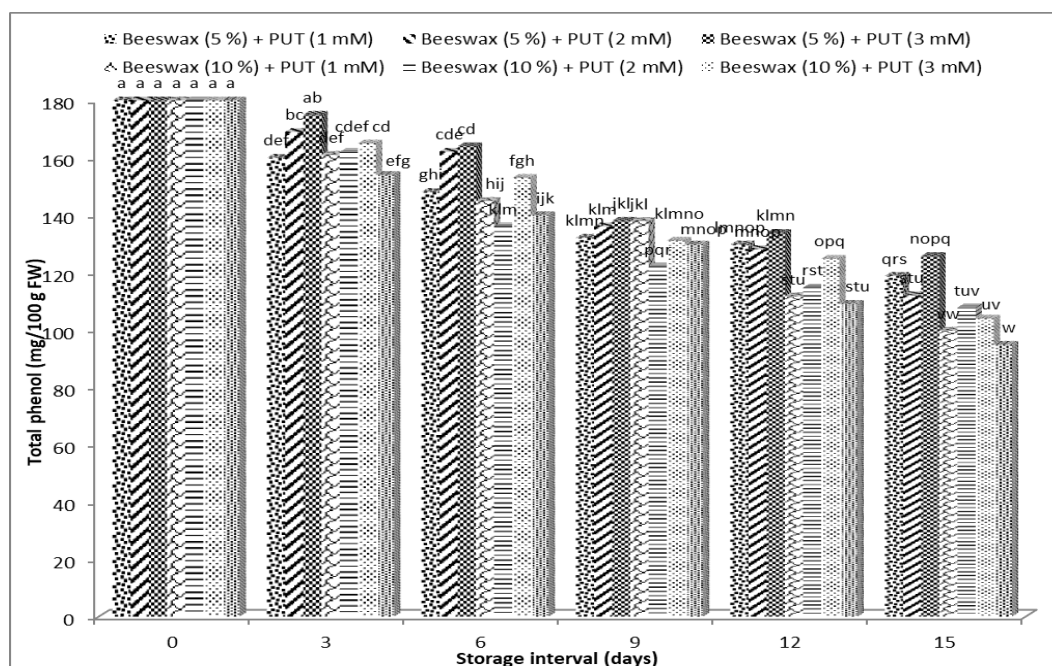
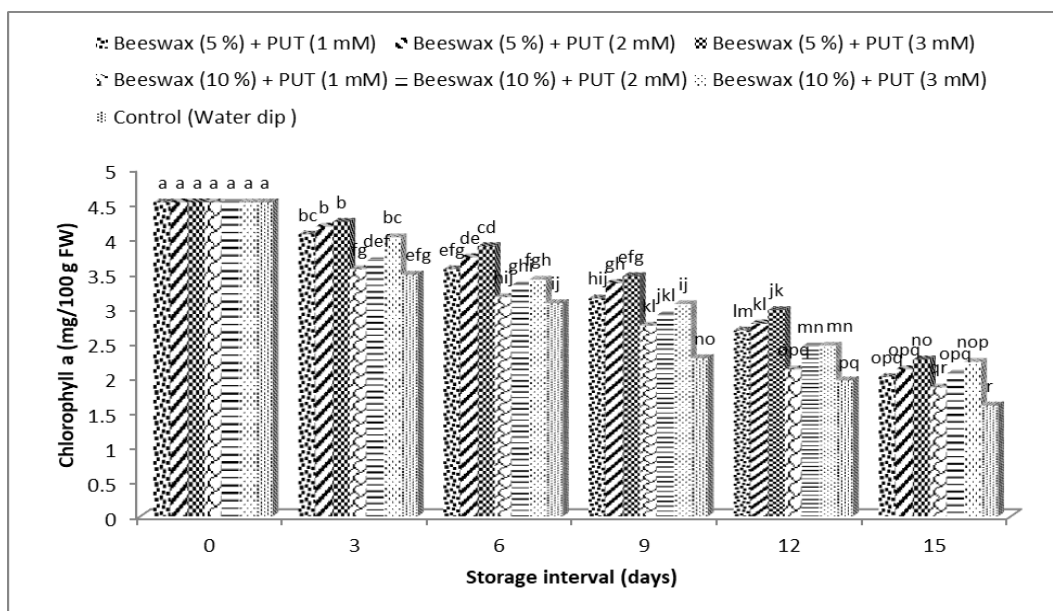
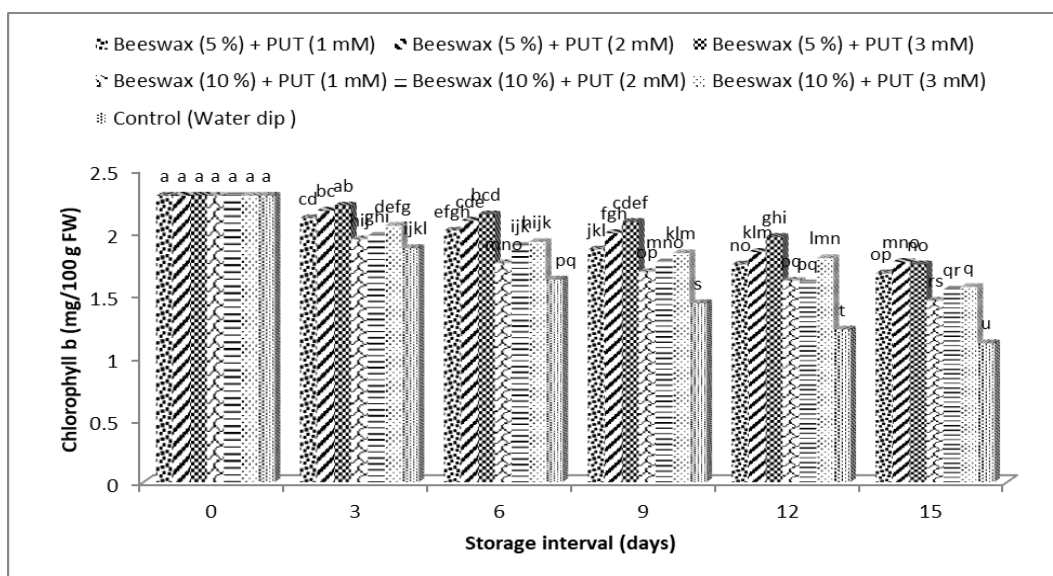


Fig. 3A. Effect of beeswax coating loaded with putrescine on total phenols (mg/100g FW) of guava cv. Allahabad Safeda during ambient storage



**Fig. 3B.** Effect of beeswax coating loaded with putrescine on chlorophyll a (mg/100 g FW) of guava cv. Allahabad Safeda during ambient storage



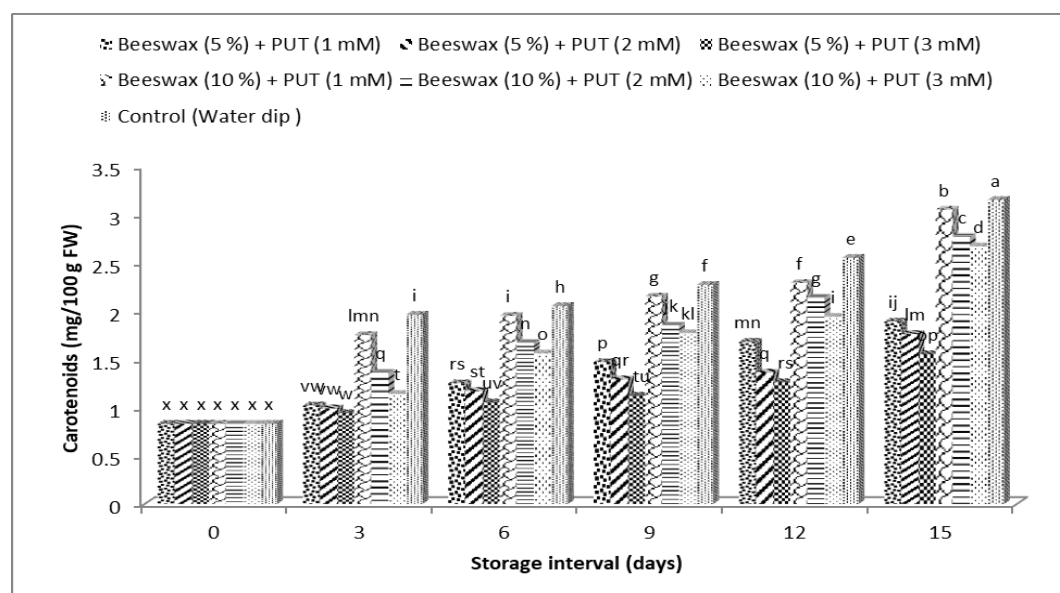
**Fig. 3C.** Effect of beeswax coating loaded with putrescine on chlorophyll b (mg/100 g FW) of guava cv. Allahabad Safeda during ambient storage

### Chlorophyll a, Chlorophyll b and Carotenoids

The result reveals that chlorophyll a and chlorophyll b contents decreased progressively with the increase in storage intervals in all treatments is presented in (Fig. 3B and 3C). During the storage time guava changed colour from green to yellow of the fruit peel (Rehman et al., 2020), due to chlorophyll degradation or qualitative and quantitative alterations of green pigment into another pigment as resulting of enzyme activities such as chlorophyll oxidase and peroxidase (Valiathan and Atmasevli, 2018). Polyamines slowed chlorophyll loss in muskmelon by inhibiting hydrolytic activities on chloroplast thylakoid membrane (Lester, 2000). The loss of chlorophyll content during storage interval could be attributed to the breakdown of the chlorophyll structure and increases in chlorophyllase enzyme activity of pigments (Wills et al., 1998). Data units



respect to the effect of beeswax coating loaded with putrescine on carotenoids content of guava fruits are presented in (Fig. 3D).



**Fig. 3D.** Effect of beeswax coating loaded with putrescine on carotenoids (mg/100 g FW) of guava cv. Allahabad Safeda during ambient storage

It was found that carotenoids increased progressively with the increase in storage intervals in all treatments. Irrespective of storage intervals maximum carotenoids were registered in control while minimum in beeswax @ 5 % + putrescine @ 3 mM. Polyamines treated fruits; beta carotene content increased rapidly due to the carotenoid pigments expressed concurrently with chlorophyll degradation. Whereas, beta carotene content of putrescine @ 4mM treated fruits recorded the lesser chlorophyll degradation of fruits during storage (Maruti et al., 2022). After chlorophyll degradation, yellow carotenoid pigments become visible (Wills et al., 1998). Similar results have been reported in papaya (Barrera et al., 2015).

### Conclusion

From the present study, it can be concluded that postharvest treatment with beeswax and putrescine significantly maintain the quality and shelf life of guava cv. Allahabad Safeda. Fruits treated with beeswax @ 5 % + putrescine @ 3 mM and beeswax @ 5 % + putrescine @ 2 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, TSS/TA, total phenolic content and chlorophyll a and b as compared with other treatments during the entire period.

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#### Author Contributions

PK, VS, MS and PKS conceived the concept, wrote and approved the manuscript.

#### Acknowledgements

Not applicable.

#### Funding

There is no funding source for the present study.

#### Availability of data and materials

Not applicable.

#### Competing interest

The authors declare no competing interests.

#### Ethics approval

Not applicable.



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**Citation:** Kaur P, Singh V, Singh M and Saini PK (2022) Beeswax Coating Loaded with Putrescine to Enhance the Quality and Shelf Life of Guava Fruit cv. Allahabad Safeda. *Environ Sci Arch* 1(STI-1): 22-33.