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ORIGINAL ARTICLE

Beeswax + Low Density Polyethylene Packaging Retard Ripening Related Changes and Preserved Postharvest Quality of Guava During Storage

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Abstract

Guava is a climacteric fruit, showing an increased rate of respiration and metabolic activities within a short period, leading to rapid senescence. Keeping this in view, this current experiment has been planned whereby the guava fruits coated with beeswax at 2.5% and 5.0% concentrations along with packing with low density polyethylene (LDPE) were assayed for shelf life and storability. After coatings, the fruits were packed in ventilated corrugated fiberboard (CFB) boxes and stored in the cold chamber (6–8 °C, 90–95% relative humidity [RH]) and at ambient conditions (6–24 °C, 40–75% RH). Quality analysis after 5-day and 3-day intervals in cold storage and ambient storage conditions, respectively, revealed that fruit firmness, ascorbic acid (AsA), acidity, sugars, total phenols, and pectin content decreased during storage, whereas weight loss (WL) and decay incidence was maintained in beeswax treated fruit compared to the control. The highest mean value of firmness, AsA, titratable acidity (TA), sugars, total phenolic content (TPC), pectin content and overall sensory quality (SQ) and lowest decay incidence, WL, and pectin methyl esterase (PME) activity were obtained from Beeswax 5% + LDPE treated guava fruits up to 20 days and 9 days of storage under cold and ambient conditions, respectively.

Keywords Guava (*Psidium guajava* L.) · Beeswax · LDPE packing · Ripening · Storage

Introduction

Guava (*Psidium guajava* L.), a climacteric fruit, is a globally popular fruit in the subtropical and tropical regions. It is a highly nutritious fruit, rich in minerals such as calcium, iron, and phosphorus, vitamins like A and B1, and vitamin C (Formiga et al. 2022). The white-fleshed guava cv. 'Shweta' is a popular variety of Punjab because of its unique taste and high yielding capacity. Edible coatings and packaging of fruits have been increasingly used for long-term storage and in the preservation of fruit qualities by regulating physiological, biochemical, and enzymatic changes, as it is more environmentally friendly alternatives to harmful chemical treatments. However, little is known about the effects of storage on this eco-friendly approach on guava biochemical and enzymatic during storage. Coatings extend the shelf-life of fresh fruit without causing anaerobiosis and reduce decay without affecting the quality of the fruit. So far, various attempts have been made to evaluate the effectiveness of various edible coating materials in terms of maintaining the freshness and extending the shelf-life of different fruits. The main function of edible films and coatings is to provide a defensive barrier between food and the surrounding environment for moisture, oxygen, flavor, aroma, etc. The application of edible coatings to fruits and vegetables is a very simple technology that minimizes the loss of moisture and allows respiration regulation.

The guava (*Psidium guajava* L.) is a highly perishable crop with minimum postharvest life. Guava cv. 'Shweta' is the choicest cultivar under North-West Indian conditions. Once the guava is harvested, stress on cells and tissues results in the production of reactive oxygen species (ROS) which damages the plasma membrane and leads to homeostasis (Singh and Pal 2008). Therefore, fresh guava fruits need to be consumed as early as possible for better palatability and acceptability otherwise its fruit becomes mealy

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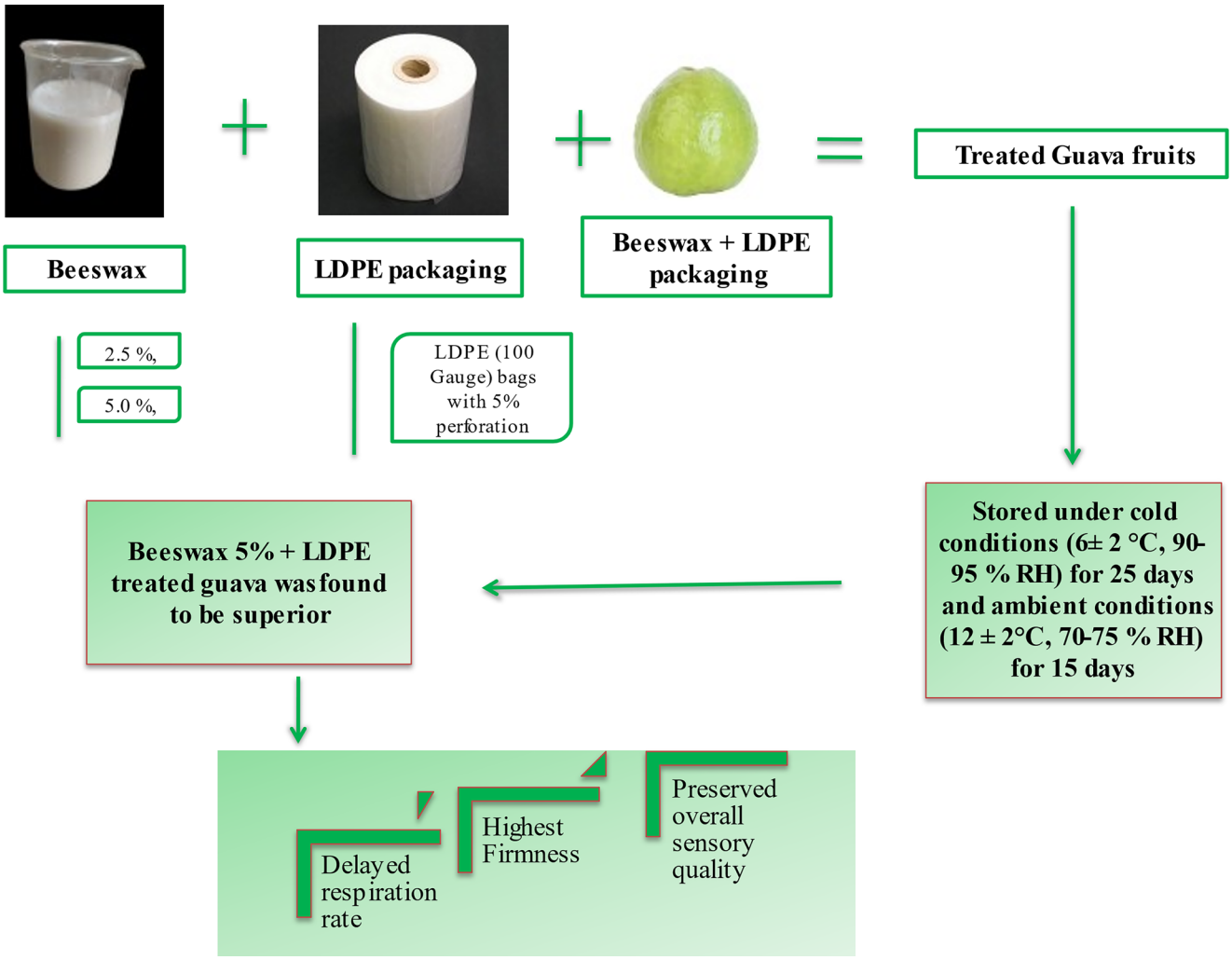
Graphic abstract



and starts emitting off-flavor. Previous research findings suggest that the shelf life of guava fruits is usually less than 1 week under ambient conditions and up to 2 weeks under a controlled environment. Most of the practices intended to augment the postharvest life of fruits are focused to reduce the respiration rate and catabolism ultimately.

The edible coating forms a fine film of innate layers on the surface of the fruit. Indeed, it barricades against microbial contamination, and moisture loss preserves textural properties and retards respiration as well as transpiration rate in fruits. Lipid-based beeswax coatings exhibits key barrier characteristics to preventing moisture loss from fruits as well as a positive effect on quality maintenance of fruit for long-term storage (Adhikary et al. 2022). Polymer packaging is a good choice to extend shelf-life of fruit which demonstrated notable benefit in ameliorating physical damage due to the properties of thermal stability, toughness, water vapor and oxygen permeability etc. (Li et al. 2017). However, to the best of our knowledge, there are no reports documented on the beeswax coating along with low density polyethylene (LDPE) packaging and their application in guava fruit preservation. In this study, the effect of LDPE packaging films in guava fruit cv. 'Shweta' coated with beeswax coating on the physiochemical characteristics was investigated. In this study, we addressed the hypothesis that the application of beeswax and LDPE packing in guava during storage reduces damage (and decay) of postharvest loss, through several biochemical and transcriptional modifications in sugar transport, secondary metabolism, antioxidant activity, cell wall organization, and pathogen defence. Keeping in view the same, in the present research, an attempt was made to prolong the storage life of guava fruit cv. 'Shweta' under North-West Indian conditions.

Materials and Methods

Sample Collection

Physiologically mature guava fruits harvested during the 4th week of January were collected from Regional Fruit Research Station Punjab Agricultural University, Bahadurgarh, Patiala (Punjab). All the Guava plants were of the same age and uniform cultural practices were followed in the orchard. It was ensured that all the fruits used in the experiments were homogeneous (145 ± 5 g, having a firmness of 105 ± 2 N) and free from external injury. The fruits were then transported in cushioned plastic crates to the postharvest laboratory of the Fruit Science Department, Punjab Agricultural University, Ludhiana, India. The fruits were again sorted, graded, washed with running water, and subjected to dipping in sodium hypochlorite (100 ppm) solution for 5 min.

Application of Treatments

Guava fruits were dipped in beeswax emulsion (beeswax 2.5%, beeswax 5.0%) that was prepared by a method prescribed by Salman et al. (2008) with some modifications as per the requirements. The coated fruits were then packed in LDPE (100 Gauge) bags with 5% perforation as Beeswax 2.5% + LDPE packing and Beeswax 5% + LDPE packing, respectively. Afterward, the treated fruits were placed in ventilated corrugated fiberboard (CFB) boxes and stored under cold conditions (6 ± 2 °C, 90–95% relative humidity [RH]) and ambient conditions (12 ± 2 °C, 70–75% RH). Every treatment had three replications, where 20 fruits kept for each replication for each storage interval. The non-treated fruits were also subjected to physical and biochemical analysis beginning from day zero. The stored fruits were assessed for physico-chemical and enzymatic characteristics on 05, 10, 15, 20, 25 days of cold storage and 03, 06, 09, 12, and 15 days from ambient storage conditions.

Determination of Physical Changes

Weight loss (WL; %) was calculated by recording the initial weight and final weight of fruits. It was calculated by the following Eq. 1:

$$\text{WL}\ (\%) = \frac{\text{Initial weight} - \text{Final weight} \times 100}{\text{Initial weight}}. \quad (1)$$

Firmness (N) was recorded using a "Penetrometer" (Model FT-327, QA Supplies, Norfolk, VA, USA). The device has a probe (8-mm diameter) made of stainless steel which is used to penetrate peeled guava flesh and the force used is recorded as newton (N).

Sensory Quality Evaluation

Sensory quality (SQ) was evaluated by five major attributes, viz., colour, texture, odour, taste and overall acceptability to the consumers and rated by a 1- to 9-point hedonic scale (Amerine et al. 1965). A total of 10 judges were appointed to evaluate the SQ evaluation of the pear fruit sample based on their interest, availability and previous experience (Adhikary et al. 2022). At each storage for SQ evaluation, judges randomly picked the fruit of each treatment in four replicates to reduce biases.

Decay Incidence

The decay incidence was recorded during storage span by visualisation. Decay incidence was given ranks between 0 and 4, where 0 symbolised no sign of decay incidence, 1 symbolised 1–25% decay incidence, 2 symbolised 26–50% decay incidence, 3 symbolised 51–75% decay incidence, and 4 symbolised 76–100% decay incidence. The decayed fruit was eliminated to avoid further contamination at each storage interval. It was calculated by the following Eq. 2:

$$\text{Decay incidence} = \frac{\text{number of decay groups}}{\text{number of decayed fruit falling into this group} \times \text{maximum no. of decay groups}} \times 100 \quad (2)$$

Estimation of Soluble Solid Content and Titratable Acidity

Extracted juice from 10 fruits of each treatment was used to measure soluble solid content (SSC) and titratable acidity (TA). For the assessment of SSC a digital hand refractometer was used (PAL-1 by Atago Co., L., Tokyo, Japan) and expressed in percentage. TA was assessed by our previously followed method (Adhikary et al. 2021).

Estimation of Ascorbic Acid

Ascorbic acid (AsA) content in fruit juice was determined by the oxidation of ascorbic acid with 2,6-dichlorophenol indophenol dye (DCPIP) dye and was expressed in mg 100 mL^{-1} of juice (Ranganna 1986). For AsA analysis, 2 mL of lemon juice, and 5 mL of metaphosphoric acid were added and then titrated against DCPIP dye until the distinct pink colour appeared and persisted for 15–20 s.

Estimation of Total Phenolics Content

Total phenolics content (TPC) was assayed according to our previously determined method (Adhikary et al. 2021) spectrophotometrically (Thermo Scientific SPECTRONIC 20 D+, USA) at an absorbance of 760 nm. The values were computed according to the standard gallic acid curve measured in micrograms of gallic acid equivalents kg of fresh fruit weight.

Determination of Pectin Content

To quantify the amount of pectin, 10 g of pulp was dried in an oven and then mixed with 100 ml acidified water (pH 1) and kept at temperature of 60 °C for 1.5 h in a shaking incubator at 150 revolutions per minute. The extract was heated and then 96% ethanol was added to make precipitates of pectin and kept overnight. In the morning, centrifugation is performed for 20 min at 2500 revolutions per minute followed by moisture removal from pectin by ethanol washings. After removing the supernatant, the pellets were dried in an oven at 45–50 °C and the final weight was noted (Sharma et al. 2013). This was calculated by the following Eq. 3:

$$\text{Pectin}(\%) = \frac{Pectin\ obtained \times 100}{weight\ of\ sample\ taken} \tag{3}$$

Pectin Methylesterase Activity

The activity of pectin methylesterase (PME) was observed using the procedure given by Chen et al. (2019) with some modifications and expressed as mg per kg fresh weight.

Sugars

Total sugars were quantified from juice through the procedure standardized by AOAC (2005). Sugars are expressed in percent and measured by the Eq. 4 given as under:

$$\text{Total sugars}\ (\%) = \frac{Fehlig\ factor\,(0.05)}{Volume\ of\ filtrate\ used} \times \frac{Dilution\ made}{weight\ of\ sample\ taken} \times \frac{Final\ volume\ made \times 100}{Volume\ of\ filtrate\ taken} \tag{4}$$

Statistical Analysis

The data were analyzed by analysis of variance (ANOVA). The sources of variation, like treatments and storage intervals were analysed by the Least Significant Difference test (LSD) at $p < 0.05$ level of significance. All analyses were performed using statistical analysis software (SAS) version 9.3 developed by Institute Inc., Cary, NC, USA.

Results and Discussion

Weight Loss

WL is considered a major factor during storage since it causes shrivelling and browning of guava fruits. Progressive increment in WL was slow in coated fruits than in uncoated fruits under cold and ambient storage conditions (Fig. 1a, b). The lowest mean WL was recorded in Beeswax 5% + LDPE treated fruits followed by Beeswax 2.5% + LDPE treatment, whereas the highest WL was observed in the control fruits under cold (6.73%) as well as under ambient (8.10%) storage conditions. Beeswax 5% + LDPE treated fruits remained marketable for 9 and 20 days under ambient and cold storage when compared to 3 and 5 days for untreated fruits. According to standards given by Mahajan et al. (2009), more than 5% of WL renders fruits unmarketable. The reduction in WL in sealed fruit was due to retardation in evaporation and respiration processes. The role of beeswax coating in reducing moisture loss may be due to of continuous hydrophobic phase, which is highly repellent to moisture. The fact that the beeswax coating, coupled with LDPE sealing, was more effective in reducing WL may be due to the effect of blocking of stomatal apertures and lenticels of the fruits, which reduces respiration and transpiration rate (Adhikary et al. 2020). Beeswax-treated Murcot tangor (Samra et al. 2014) and plum (Navarro-Tarazaga et al. 2011) fruits showed reduced WL during storage. On the 60th day of cold storage, pear fruit coated with beeswax 3% exhibited a minimum comparison to 40% higher loss in uncoated fruits (Adhikary et al. 2022). A similar trend in reduction of WL in individually high-density polyethylene (HDPE) film packed grapefruits, 'Shamouti' oranges and lemons was observed probably due to saturated humidity and no air circulation inside the sealed package (Jawandha et al. 2012).

Fruit Firmness

Firmness is an important quality parameter for determining the intactness of the cell wall constituents and the overall acceptability of fruit. Softening of fruit during storage and transportation is a limitation that compromises the quality and commercialization of fruits. For this reason, the firmness of guava fruit reduced from 105 N to the range of 30.65 N and 24.68 N during cold storage and ambient storage, respectively. Loss of firmness was more striking in uncoated fruits than in all other treatments throughout the storage. The maximum firmness is recorded in fruits

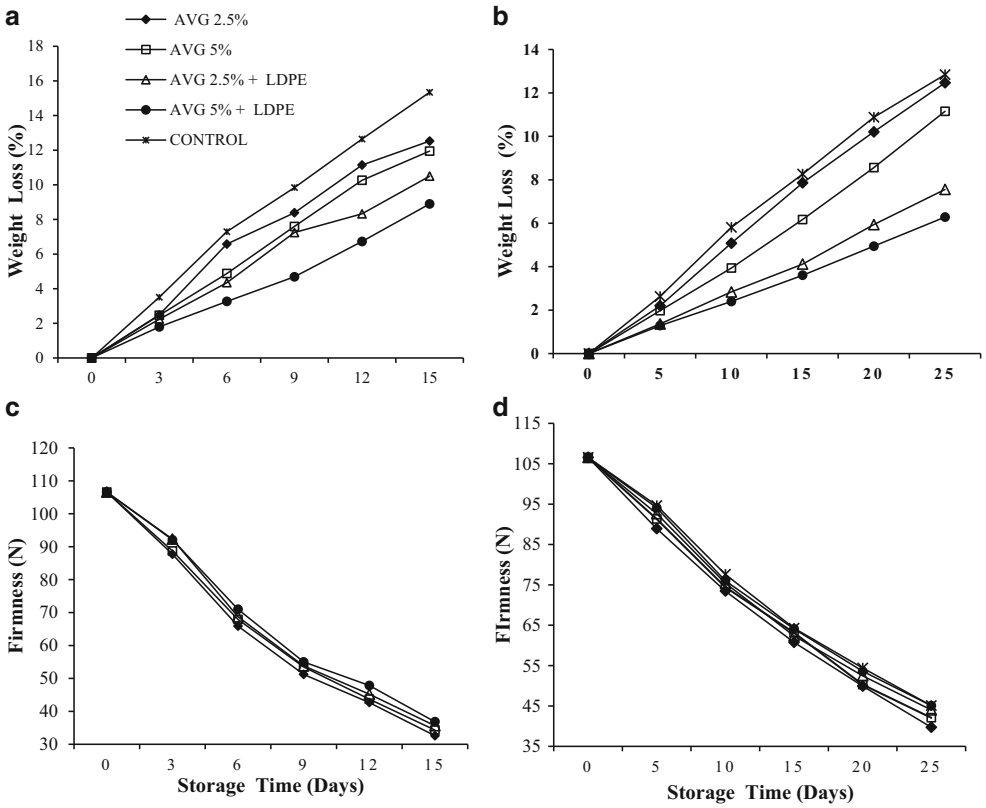


Fig. 1 The effect of beeswax and low density polyethylene (*LDPE*) packing on weight loss and firmness of guava cv. 'Shweta' under **a** and **c** ambient (12 ± 2 °C, 70–75% relative humidity [*RH*]) and **b** and **d** cold storage (6–8 °C, 90–95% RH) conditions for 15 days and 25 days, respectively. Results are expressed as mean ± SE of four replicates at $P \leq 0.05$

treated with Beeswax 5% + LDPE (Fig. 1c, d) and untreated fruits had the lowest firmness which is significantly lower than all other treatments under cold storage. The loss of firmness was due to lipid degradation in an uncontrolled manner which leads to quick deterioration of fruit. The decrease in firmness value is attributed to the increase in enzymatic activity of lipid peroxidation (LP), increase in PME activity, and decrease in polygalacturonase (PG) activity, which resulted in the loss of cell membrane fluidity, integrity, and solubilizations of pectin present in the cell wall (Germano et al. 2019). Similarly, under ambient conditions Beeswax 5% + LDPE appear to be most efficient in maintaining fruit firmness. The rate of loss of firmness is very high under ambient conditions as compared to cold storage for all treatments. Shahid (2007) reported that 5% beeswax along with 0.5% benlate is effective in maintaining firmness and texture. In contrast, these findings are similar to work by Ladaniya and Sonker (1997) on the waxing of 'Nagpur' mandarin. Beeswax 4% application can maintain the highest firmness in pear (Adhikary et al. 2022). Similarly, individual wrapping with LDPE packaging had potential merits in the maintenance of firmness (Mahajan et al. 2013; Rana et al. 2015).

Decay Incidence

Decay in guava fruits mainly occurs due to *Escherichia coli* (*E. coli), Micrococcus luteus (M. luteus), Proteus vulgaris (P. vulgaris), Enterobacter Aerogenes (E. aerogens), Bacillus subtilis (B. subtilis), Bacillus megaterium (B. megaterium), Bacillus cereus (B. cereus), Staphylococcus aureus (S. aureus), Shigella dysenteriae (S. dysenteriae), Klebsiella pneumoniae (K. pneumoniae), Staphylococcus epidermidis (S. epidermidis)* (Choudhary et al. 2016), *Phoma* spp., *Penicilium* spp., *Aspergillus* spp., *and Colletotrichum* spp. (Bishnoi and Sharma 2015), which cause green/blue mould rot, grey/brown rot, aspergillus rot, mucor rot, phomopsis rot, rhizopus rot, and soft rot, respectively. For both groups, there was a progression of decay incidence in fruit from day 0 to day 25. It was noticeable that decay incidence was maximum in untreated fruits. All other treatments were shown to be significantly effective in reducing the severity of decay incidence in both cold storage and ambient conditions (Table 1). There was no occurrence of decay incidence in fruits under any treatment up to 6 and 10 days under ambient and cold storage, respectively. Beeswax 5% + LDPE treated fruits had the lowest decay incidence of 5.78% under ambient conditions and the highest in control fruits, i.e., 19.31%. Under cold storage, the lowest decay incidence was observed in Beeswax 5% + LDPE treatment followed by Beeswax 5%, which was statistically at par

Table 1 Effect of beeswax and low density polyethylene (LDPE) packing with storage intervals on decay incidence (%) in guava cv. 'Shweta' under cold storage and ambient condition

Treatments	Storage interval (days)												
	Cold storage							Ambient storage					
	0	5	10	15	20	25	Mean	0	3	6	9	12	15	Mean
Beeswax 2.5%	0	0	0	3.71	12.60	30.80	*7.85*^{b}	0	0	0	8.33	26.13	45.63	*13.34*^{b}
Beeswax 5%	0	0	0	0	8.43	24.10	*5.42*^{bc}	0	0	0	4.16	16.87	36.11	*9.52*^{c}
Beeswax 2.5% + LDPE	0	0	0	4.16	12.14	28.84	*7.52*^{bc}	0	0	0	8.33	16.77	37.77	*10.47*^{bc}
Beeswax 5% + LDPE	0	0	0	0	8.33	19.90	*4.70*^{c}	0	0	0	0	7.87	26.85	*5.78*^{d}
Control	0	0	0	7.87	21.50	48.10	*12.91*^{a}	0	0	0	21.96	31.94	61.98	*19.31*^{a}
Mean	*0*^{d}	*0*^{d}	*0*^{d}	*3.35*^{c}	*12.70*^{b}	*29.01*^{a}	–	*0*^{d}	*0*^{d}	*0*^{d}	*10.71*^{c}	*20.93*^{b}	*41.58*^{a}	–
LSD ($p \leq 0.05$)	*Treatment (T)* = 2.98 *Days (D)* = 3.26 *T* × *D* = 7.30							*Treatment (T)* = 3.11 *Days (D)* = 3.41 *T* × *D* = 7.63						

and in control fruit decay incidence was the highest decay incidence at the end of the experiment. With time, the severity of decay incidence increased in all treatments at different rates. Results are in line with the study of Eshetu et al. (2019) with 2.0% of beeswax coated fruits of mango showed a reduced incidence of spoilage. Lima et al. (2010) investigated the effect of Modified Atmospheric Packaging (MAP) technology (Polyvinyl chloride [PVC]) on guava fruit cv. 'Paluma' and reported that the application of MAP can control the growth of viable mesophylic microorganisms and prolong the shelf life of guava fruits for up to 6 days at 3 °C.

Sensory Quality Evaluation

Sensory characteristics are important parameters for judging the quality of fresh guava and its marketability and acceptability to consumers. Fresh guava is characterised by its sweet taste and particular aroma due to the presence of volatile compounds such as (Z)-3-hexenal and cinnamyl acetate (Steinhaus et al. 2009). In this study, SQ deteriorates with the progression of storage in all treatments (Table 2). However, the fruits treated with Beeswax 5% + LDPE had the highest SQ rating followed by Beeswax 2.5% + LDPE for both the conditions. The lowest average SQ rating was observed in control fruits under both the conditions. The highest SQ rating was obtained from Beeswax 5% + LDPE treated guava fruit under ambient storage even at 15 days of storage. The application of packaging on fresh guava fruit improves or maintains the overall sensory characteristics better than unpackaged fruit due to the delay in ripening-associated changes (Yadav et al. 2022). Kaleemullah et al. (2019) and Adhikary et al. (2022) also reported maximum SQ in sweet oranges and pears fruits coated with beeswax at 8% and 3%, respectively, during long-term storage. The present results are lined with Navarro-Tarazaga et al. (2005) that beeswax and LDPE maintained the eating quality of plum fruits under cold storage conditions.

Soluble Solid Content

During the initial phase of ripening the total soluble solids (SSC) accumulation increased in guava fruit. The rise in SSC was higher in untreated fruit and increased only up to 25 days of cold storage and 12 days during ambient storage. The maximum SSC was observed in fruits treated with Beeswax 5% + LDPE followed by Beeswax 2.5% + LDPE; both were statistically at par under cold storage (Table 3). Under ambient conditions, SSC was higher in Beeswax 5% + LDPE (10.05) followed by Beeswax 2.5% + LDPE. The minimum amount of SSC was observed in untreated fruits, which was approximately 30 ± 5% lower than Beeswax 5% + LDPE in both the conditions. The amount

Table 2 Effect of beeswax and low density polyethylene (LDPE) packing with storage intervals on sensory quality (hedonic scale) in guava cv. 'Shweta' under cold storage and ambient condition

Treatments	Storage interval (days)												
	Cold storage						Ambient storage						
	0	5	10	15	20	25	Mean	0	3	6	9	12	15	Mean
Beeswax 2.5%	7.33	7.71	8.06	8.13	6.12	5.29	*7.10*^{d}	7.33	7.79	8.13	7.65	6.44	5.47	*7.13*^{d}
Beeswax 5%	7.33	7.65	7.89	8.20	6.65	5.59	*7.21*^{c}	7.33	7.46	8.21	7.89	6.54	5.77	*7.20*^{c}
Beeswax 2.5% + LDPE	7.33	7.49	7.82	8.31	7.10	5.72	*7.29*^{b}	7.33	7.72	8.15	8.34	6.86	5.93	*7.38*^{b}
Beeswax 5% + LDPE	7.33	7.57	7.87	8.62	7.76	6.25	*7.56*^{a}	7.33	7.63	7.85	8.25	7.24	6.42	*7.45*^{a}
Control	7.33	7.80	8.29	6.80	5.12	4.52	*6.64*^{e}	7.33	7.79	8.49	7.10	5.30	4.09	*6.68*^{e}
Mean	*7.33*^{c}	*7.66*^{b}	*8.02*^{a}	*8.05*^{a}	*6.73*^{d}	*5.46*^{e}	–	*7.33*^{d}	*7.70*^{c}	*8.21*^{a}	*7.77*^{b}	*6.63*^{e}	*5.48*^{f}	–
LSD ($p \leq 0.05$)	*Treatment (T)* = 0.05 *Days (D)* = 0.05 *T* × *D* = 0.13							*Treatment (T)* = 0.03 *Days (D)* = 0.04 *T* × *D* = 0.09						

of SSC keeps in increasing until the reserves of starch are available for conversion into soluble sugars. There was a decrease in SSC when starch gets exhausted and sugars begin utilised in the process of respiration (Wills et al. 1980). The results are in line with the study by Carrillo-Lopez et al. (2000), who reported peak of SSC was observed in tomatoes earlier in the uncoated one than the coated one. Excessive increase in SSC in control fruits indicates early quality deterioration, which may be attributed to the utilization of organic acid in pyruvate decarboxylation reaction. The rapid breakdown of complex polymer into simple sugars by hydrolytic enzymes might be due to higher respiration during subsequent storage. Mezemir et al. (2017) also reported similar results in sweet orange coated with beeswax and linseed oil coatings. Control fruits showed an increasing trend in SSC which decreased thereafter in shrink-wrapped guava fruit during 21 days of cold storage (Rana et al. 2015). In our experiment, individual wrapping decelerated the elevation in SSC. The combined effect of packaging and beeswax regime reduced the respiration rate, thus retarding compositional changes and maintaining SSC in guava fruits.

Titratable Acidity

A decreasing trend was noted in TA in all the fruit, regardless of the treatments was observed in both the storage conditions (Table 4). The highest TA was recorded in Beeswax 5% + LDPE treated fruits under cold storage which was 20% higher than control fruits at the end of storage. Under ambient conditions, the highest TA also was registered in Beeswax 5% + LDPE treated fruits which were not significantly higher than Beeswax 5%. The lowest TA was observed in untreated fruit which was 24% lower than Beeswax 5% + LDPE treated fruits. Jawandha et al. (1980) also noticed a continuing drop off in the number of acids in ber fruits kept under cold storage with time. Singh and Pal (2008) also observed a similar trend in the value of TA in guava fruits. The possible explanation for the continued decrease in acidity is the catabolism of organic acids like citric acid during the process of respiration. The results are in line with the findings of Eshetu et al. (2019) that fruits treated with beeswax 2.0% and chitosan 2.0% have a higher number of acids than control samples. Mukdisari et al. (2016) also reported maximum acidity in papaya fruits treated with beeswax at 6% at end of storage. Similarly, shrink wrapped guava fruit maintained higher TA during 21 days of cold storage as compared to control (Rana et al. 2015).

Ascorbic Acid

Observation revealed a declining trend followed in the AsA content in guava fruits throughout the storage period in both

Table 3 Effect of beeswax and low density polyethylene (LDPE) packing with storage intervals on soluble solids content (%) in guava cv. 'Shweta' under cold storage and ambient conditions

Treatments	Storage interval (days)													
	Cold storage							Ambient storage						
	0	5	10	15	20	25	Mean	0	3	6	9	12	15	Mean
Beeswax 2.5%	8.71	9.41	10.13	10.55	10.73	11.17	*10.11*^{b}	8.71	9.35	10.07	10.98	9.71	8.82	*9.60*^{d}
Beeswax 5%	8.71	9.48	10.16	10.64	10.86	11.13	*10.16*^{b}	8.71	9.52	10.12	11.30	10.17	9.02	*9.80*^{c}
Beeswax 2.5% + LDPE	8.71	9.69	10.81	11.08	11.18	11.30	*10.46*^{a}	8.71	9.48	10.37	11.35	10.54	9.22	*9.94*^{b}
Beeswax 5% + LDPE	8.71	9.77	10.51	10.98	11.39	11.78	*10.52*^{a}	8.71	9.39	10.22	10.57	11.24	10.17	*10.05*^{a}
Control	8.71	10.02	10.75	9.91	9.96	7.81	*9.42*^{c}	8.71	9.41	10.31	11.40	9.78	7.10	*9.45*^{e}
Mean	*8.71*^{e}	*9.48*^{d}	*10.19*^{c}	*10.51*^{b}	*10.95*^{a}	*10.98*^{a}	–	*8.71*^{e}	*9.35*^{c}	*10.18*^{b}	*11.14*^{a}	*10.23*^{b}	*8.91*^{d}	–
LSD ($p \leq 0.05$)	*Treatment (T)* = 0.11 *Days (D)* = 0.12 *T* × *D* = 0.27							*Treatment (T)* = 0.10 *Days (D)* = 0.11 *T* × *D* = 0.25						

Table 4 Effect of beeswax and low density polyethylene (LDPE) packing with storage intervals on titratable acidity (%) in guava cv. 'Shweta' under cold storage and ambient condition

Treatments	Storage interval (days)													
	Cold storage							Ambient storage						
	0	5	10	15	20	25	Mean	0	3	6	9	12	15	Mean
Beeswax 2.5%	0.511	0.481	0.419	0.322	0.280	0.216	*0.371*^{c}	0.511	0.451	0.382	0.302	0.251	0.222	*0.353*^{b}
Beeswax 5%	0.511	0.462	0.421	0.325	0.294	0.235	*0.374*^{c}	0.511	0.463	0.392	0.318	0.260	0.206	*0.358*^{a}
Beeswax 2.5% + LDPE	0.511	0.475	0.435	0.366	0.322	0.266	*0.395*^{b}	0.511	0.472	0.371	0.302	0.238	0.215	*0.351*^{b}
Beeswax 5% + LDPE	0.511	0.494	0.440	0.354	0.341	0.271	*0.401*^{a}	0.511	0.464	0.383	0.334	0.265	0.218	*0.362*^{a}
Control	0.511	0.475	0.369	0.285	0.228	0.182	*0.341*^{d}	0.511	0.460	0.337	0.291	0.224	0.189	*0.335*^{c}
Mean	*0.511*^{a}	*0.479*^{b}	*0.418*^{c}	*0.335*^{d}	*0.292*^{e}	*0.236*^{f}	–	*0.511*^{a}	*0.463*^{b}	*0.370*^{c}	*0.304*^{d}	*0.250*^{e}	*0.217*^{f}	–
LSD ($p \leq 0.05$)	*Treatment (T)* = 0.005 *Days (D)*=0.006 *T* × *D* = 0.013							*Treatment (T)* = 0.004 *Days (D)* = 0.004 *T* × *D* = 0.009						

Table 5 Effect of beeswax and low density polyethylene (LDPE) packing with storage intervals on ascorbic acid (mg/100 ml) in guava cv. 'Shweta' under cold storage and ambient condition

Treatments	Storage interval (days)												
	Cold storage						Ambient storage						
	0	5	10	15	20	25	Mean	0	3	6	9	12	15	Mean
Beeswax 2.5%	268.51	254.31	232.17	219.34	204.53	182.45	*226.88*^{d}	268.51	251.26	228.54	211.67	200.08	177.41	*222.91*^{d}
Beeswax 5%	268.51	263.41	239.84	220.14	199.83	189.68	*230.23*^{c}	268.51	258.17	241.49	225.98	207.72	195.71	*232.93*^{c}
Beeswax 2.5% + LDPE	268.51	260.34	250.93	232.11	215.43	197.20	*237.42*^{b}	268.51	257.20	246.65	227.68	209.43	191.32	*233.46*^{b}
Beeswax 5% + LDPE	268.51	261.46	252.81	240.18	221.58	203.48	*241.33*^{a}	268.51	263.41	249.45	233.56	219.37	196.33	*238.43*^{a}
Control	268.51	250.94	226.58	204.12	183.64	161.91	*215.95*^{e}	268.51	242.38	216.33	192.47	170.47	148.51	*206.44*^{e}
Mean	*268.51*^{a}	*257.39*^{b}	*240.29*^{c}	*224.72*^{d}	*205.99*^{e}	*186.67*^{f}	–	*268.51*^{a}	*254.76*^{b}	*238.56*^{c}	*221.28*^{d}	*203.28*^{e}	*183.81*^{f}	–
LSD ($p \leq 0.05$)	*Treatment (T)* = 0.70 *Days (D)* = 0.77 T × *D* = 1.73							*Treatment (T)* = 0.39 *Days (D)* = 0.43 *T* × *D* = 0.97						

conditions. Higher AsA content was found in all treatments than in control samples under cold and ambient storage (Table 5). The maximum AsA observed in fruits coated with Beeswax 5% along with LDPE showed followed by Beeswax 2.5% + LDPE treatment and the lowest AsA was obtained in control samples under cold storage. Guava fruits kept at room temperature had higher AsA in Beeswax 5% + LDPE treated than all other treatments and the minimum AsA content was recorded in control fruits. The decrease in AsA during storage may be due to its higher antioxidant enzyme activity during postharvest storage conditions (Li et al. 2016). The AsA content degraded due to its oxidation into dehydro-ascorbic acid by the action of the enzyme ascorbic acid oxidase (Adhikary et al. 2021). Dipping with $CaCl_2$ 4.5% followed by beeswax 3% coating maintained maximum Ascorbic acid (Seleshi et al. 2019). Results reported by Jacomina et al. (2003) are in line where AsA content was significantly higher in carnauba wax coated guava fruits than the control. In contrast, by the end of storage, AsA content in nano-TiO_2-LDPE packed strawberries was higher than that of the control (Li et al. 2016).

Total Phenolic Content

A steady decline in TPC with the progression of the storage period was recorded for all the treatments. Results indicated that Beeswax + LDPE treated fruit exhibited higher TPC when compared with untreated one (Fig. 2). The reason behind the gradual reduction in amount is the enzymatic oxidation of phenols by polyphenol oxidase and peroxidase (Bodelon et al. 2010). Under cold and ambient storage maximum TPC was observed in fruits coated with Beeswax 5% + LDPE and the minimum value was observed in control samples, respectively. Beeswax + LDPE was probably able to modify the internal atmosphere of the guava fruit to prevent the decrease in TPC contents. Therefore, Beeswax 5% + LDPE produced a small change in TPC throughout storage. The results obtained are in line with the study of Luo et al. (2015), which found 10% higher TPC in LDPE-packed fruits. The findings of a study on raspberry (Gallardo et al. 2012) and blueberry (Gallardo et al. 2015) fruits in contrast found an initial increase in TPC followed by a decrease and at the end higher content in control samples than wax-coated fruits.

Pectin Content

The postharvest ripening processes are associated with changes in the structure of the cell wall, which consist of a loss in firmness which is directly correlated with the pectin content. There was also a continuous drop in the level of pectin content during the storage of guava fruits under both storage conditions (Fig. 3a, b). Pectin content

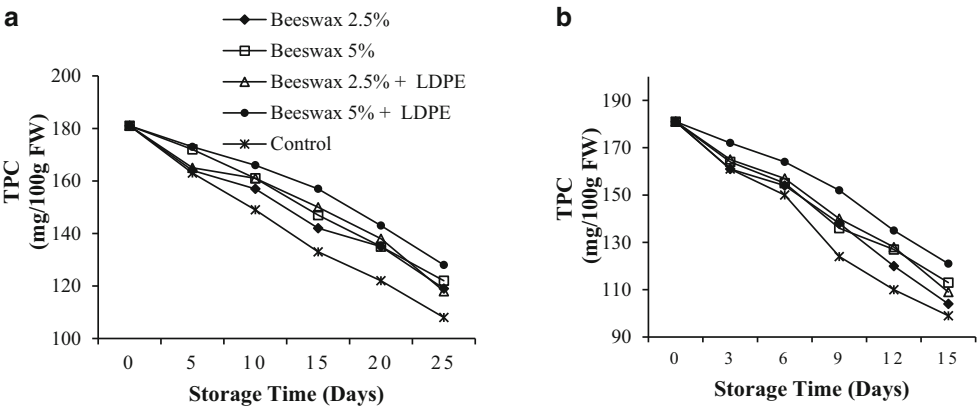


Fig. 2 The effect of beeswax and low density polyethylene (*LDPE*) packing on changes in phenolic content of guava cv. 'Shweta' under **a** ambient (12 ± 2 °C, 70–75% relative humidity [RH]) and **b** cold storage (6–8 °C, 90–95% RH) conditions for 15 days and 25 days, respectively. Results are expressed as mean ± SE of four replicates at $P \leq 0.05$

and firmness of fruit are directly related to each other and with a decrease in pectin, firmness also decreases. The major enzymes suspected to be behind the degradation of the calcium pectate present in the cell wall of fruits are exo-polygalacturonase (exo-PG), PME, endo-β-(1→4)-glucanase (EG) and β-galactosidase (GLB1) (Singh and Pal 2007). The maximum pectin content was found in Beeswax 5% + LDPE treated fruits followed by Beeswax 2.5% + LDPE and the lowest pectin content was found in untreated fruits in both the conditions. The results are similar to the study of Baswal et al. (2020) that the maximum pectin content was found in 'Kinnow' fruits coated with Carboxymethyl cellulose (CMC) (2.0 g L^{-1}) and Beeswax (10 g L^{-1}) even after 75 days of cold storage. Santos et al. (2020) also reported the highest of pectin in graviola fruits coated with beeswax 3% + $CaCl_2$ 3%.

Pectin Methylesterase Activity

Fruit softening is primarily caused by the degradative action of PME enzyme. In the present study, a consistent rise in PME activity was noted in all the treated and untreated fruit during the initial phase of ripening. However, the postharvest treatments of Beeswax + LDPE treatment exhibited a significant delay in the activities of PME enzyme. The highest PME activity in cold-stored fruits is observed in control fruits and minimum activity is observed in Beeswax 5% + LDPE treatment (Fig. 4a, b). In fruits

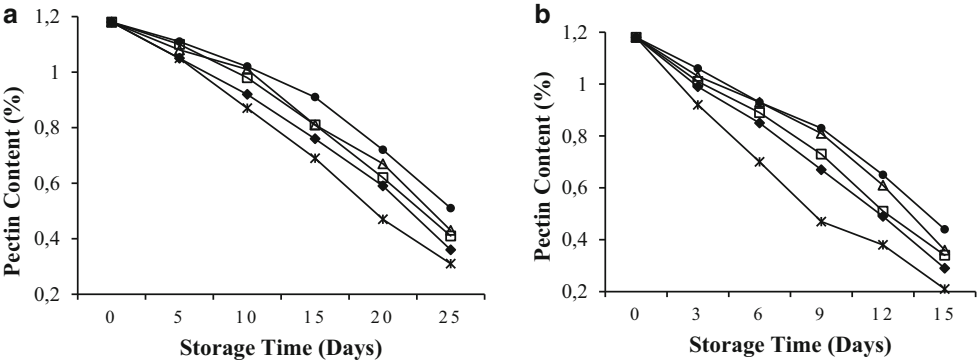


Fig. 3 The effect of beeswax and low density polyethylene (*LDPE*) packing on changes in pectin content of guava cv. 'Shweta' under **a** ambient (12 ± 2 °C, 70–75% relative humidity [RH]), and **b** cold storage (6–8 °C, 90–95% RH) conditions for 15 days and 25 days, respectively. Results are expressed as mean ± SE of four replicates at $P \leq 0.05$

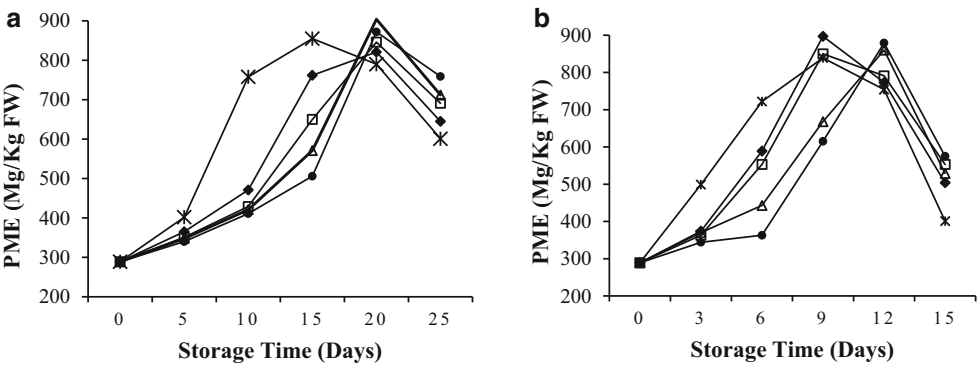


Fig. 4 The effect of beeswax and low density polyethylene packing on changes in pectin methyl esterase (*PME*) activity of guava cv. 'Shweta' under **a** ambient (12 ± 2 °C, 70–75% relative humidity [RH]) and **b** cold storage (6–8 °C, 90–95% RH) conditions for 15 days and 25 days, respectively. Results are expressed as mean ± SE of four replicates at $P \leq 0.05$

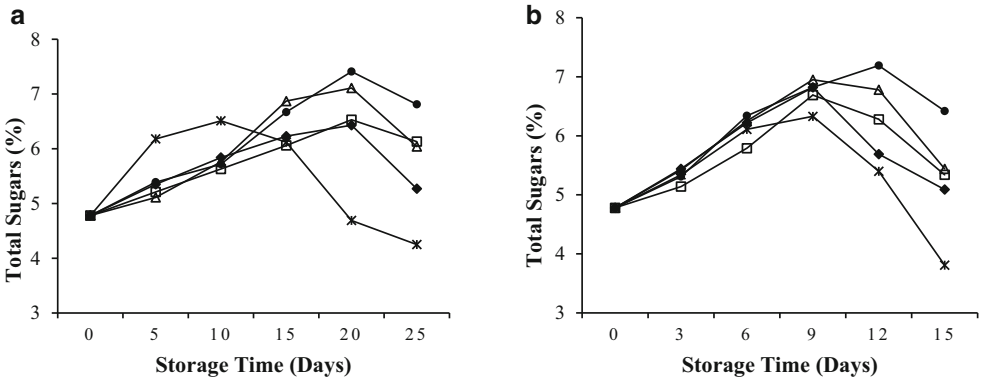


Fig. 5 The effect of beeswax and low density polyethylene packing on changes in total sugar content of guava cv. 'Shweta' under **a** ambient (12 ± 2 °C, 70–75% relative humidity [RH]) and **b** cold storage (6–8 °C, 90–95% RH) conditions for 15 days and 25 days, respectively. Results are expressed as mean ± SE of four replicates at $P \leq 0.05$

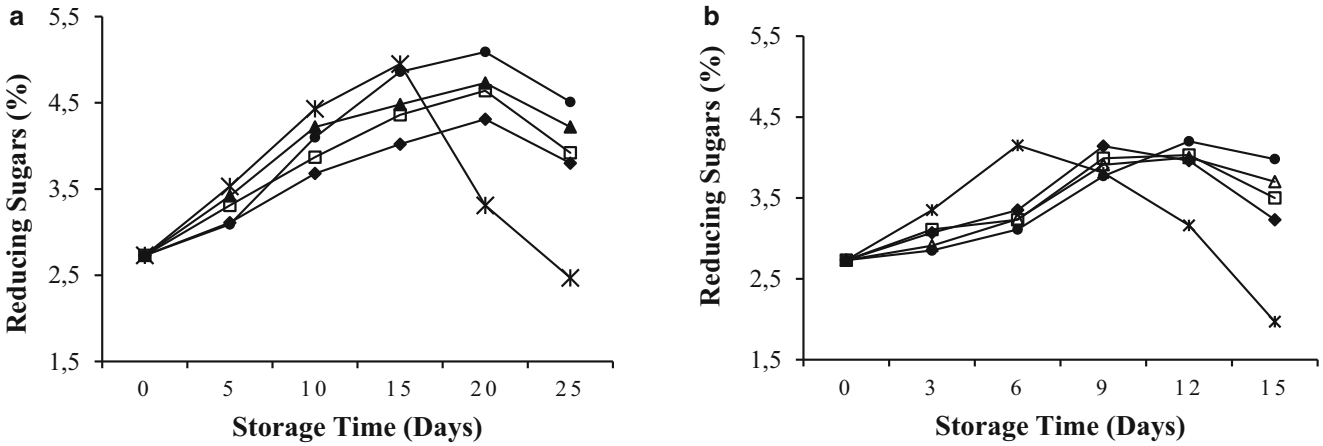


Fig. 6 The effect of beeswax and low density polyethylene packing on changes in reducing sugar content guava cv. 'Shweta' under **a** ambient (12 ± 2 °C, 70–75% RH) and **b** cold storage (6–8 °C, 90–95% RH) conditions for 15 days and 25 days, respectively. Results are expressed as mean ± SE of four replicates at $P \leq 0.05$

stored at ambient temperature maximum activity is again observed in untreated fruits and minimum activity is in Beeswax 5% + LDPE treated samples. The results are near the study of Baswal et al. (2020), which found peak activity of PME for control fruits is at 45 days, while for all other treatments it is on the 16th day of the experiment, and the activity of the enzyme is highest for control samples. Santos et al. (2020) reported a combination of beeswax 3% + $CaCl_2$ 3% reduced the activity of cell wall degrading enzymes in graviola. There was a positive correlation between the activity of PME enzymes and the reduction of firmness in 'Pedro Sato' guavas (Formiga et al. 2022). This observation is also in line with our observation. This behaviour can be explained by the importance of the degree of pectin esterification followed by the pectin degradation process (Wakabayashi 2000).

Sugars

During the initial phase of storage, the sugar accumulation increased in guava fruit. The rise in sugar content was higher in untreated fruit and increased only up to 15 days and 9 days cold and ambient storage, respectively. Under cold conditions total and reducing sugars started decreasing after 20 days of storage in all treatments except control samples in which the decline in total and reducing sugars commenced after 10 and 15 days, respectively, and in samples kept at room temperature both sugars are at peak on the 9th day of storage in most of the treatments except for Beeswax 5% + LDPE treatment in which maximum amount of both sugars is observed on 12th day of storage (Fig. 5a, b and 6a, b). The minimum quantity of both sugars was observed in control samples under both conditions of storage and the maximum sugars were noticed in Beeswax at 5% + LDPE under cold and ambient conditions. Control samples degraded sugars due to unrestricted respiration and ripening. Increase in the sugar content during the initial days of storage is due to dehydration and the conversion of starch and organic acid into simple sugars (Singh and Pal 2008). The results are in line with the findings of Azene et al. (2014) on fruits of papaya stored using LDPE.

Conclusion

This study showed that beeswax 5% combined with LDPE packaging could be applied as a postharvest treatment to maximize the storage life of guava fruits by regulating ripening-related changes during long-term storage. From the analysis of various quality parameters, it can be maintained in the long run. It can be concluded that beeswax 5% combined with LDPE packaging is effective in reducing loss of nutrients and maintaining freshness for a longer time, which would enhance marketing options and profitability of various fruits. Further, postharvest biotechnological research on gene regulation of anti-sense RNA activity within the beeswax-coated as well as LDPE-packed fruit needs to be analysed.

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Author Contribution Amanpal Singh Dhillon: Data curation (equal); Formal analysis (equal); Investigation (equal); (equal) J.S. Brar: Conceptualization (equal); Project administration (equal); Supervision (equal). Trina Adhikary: Conceptualization (equal); Writing—original draft (equal). Pankaj Das: Methodology (equal); Statistical Analysis (equal)

Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest A. Singh Dhillon, J.S. Brar, T. Adhikary and P. Das declare that they have no competing interests.

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