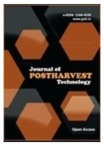

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RESEARCH ARTICLE

Effect of lysozyme based *Opuntia ficus-indica* mucilage edible coating on shelf life of papaya (*Carica papaya* L.) fruits

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ABSTRACT

Papaya is a highly perishable fruit with a very short postharvest life due to its susceptibility to mechanical injury, physiological disorders, and infection caused by several pathogens that can rapidly reduce fruit quality. The current investigation aims to assess the impact of *Opuntia ficus-indica* mucilage combined with lysozyme applied as edible coating on papaya fruit quality, sensory indices, and microbiological characteristics during storage at RT. *Opuntia ficus-indica* fruit polysaccharide was utilised as a component of an edible coating. The five edible antimicrobial coating formulations were created using lysozyme in varied concentrations: 0 (control), 0.2 (T1), 0.4 (T2), 0.6 (T3), 0.8 (T4), and 1 % (T5). The antimicrobial edible coated samples were subjected to sensory, chemical, and microbial evaluation and T5 sample showed higher overall acceptability. The uncoated papaya showed a significantly greater linear rise in weight loss after storage at RT than the coated papaya. The T5 sample was effective in maintaining fruit weight, TSS, pH, and acidity. The lysozyme-mucilage based coating had positive effect on reducing the *S. aureus* on papaya surface. Therefore, it is concluded that developed antimicrobial edible coating has a potential to reduce microbial load while keeping sensorial qualities and hence could be commercialized.

Keywords: Edible coating, lysozyme, *Opuntia ficus-indica, S. aureus*

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INTRODUCTION

Fruits and vegetables are crucial dietary staples essential to improving people's health (Hodges et al., 2011). Not only has India become a significant producer, but it is also the world's second-largest producer of fruits and vegetables. India produces 331 million tonnes of fruits and vegetables in an area of 27.23 million hectares, accounting for 11.38% and 11.78% of global production, respectively (Sah et al., 2022). *Carica papaya L.* is native to Mexico and Central America (Prasad and Poul, 2021). The macro and micro minerals Na, K, Ca, Mg, P, Fe, Cu, Zn, and Mn are found in the edible portion of ripe papaya fruit. *Carica papaya* is a source of carotenoids, vitamin C, thiamine, riboflavin, niacin, vitamin B6, and vitamin K (Parni and Verma, 2014).

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The post-harvest loss could be caused by several things, including pre-harvest factors, environmental factors, an inappropriate marketing chain, improper handling, poor transportation, post-harvest diseases and pests, chilling injuries, physiological disturbances, and senescence. These elements cause the fruit's quality, nutritional value, appearance, texture, firmness, and flavor to alter in several ways, resulting in poor marketing and a lower number of ways, which results in subpar marketing and lowers the fruit's nutritional importance (Prasad and Poul, 2021).

Several methods have been explored to reduce these losses, including edible coatings, irradiation, and low-temperature potential uses. The edible coating has recently grown in importance among these (Riaz et al., 2021). Against gases and water vapor, edible coatings can act as a semipermeable barrier. It can alter the rate of respiration in fruit tissues, reduce moisture and firmness loss, maintain color, transfer antibacterial, antioxidants, and other preservatives, restrict microbial development, and prolong fruit quality (Liguori et al., 2021). Additionally, as edible coatings are made of biodegradable raw ingredients, they have the added benefit of reducing synthetic packaging waste. The material used to prepare edible coatings and films shall be considered safe (GRAS) certified by the FDA and must comply with the rules relevant to the food product in concern because they will be consumed (Dhall, 2013).

Different natural ingredients, such as polysaccharides, proteins, and lipids, can be used to make edible coatings. These ingredients might be derived from sustainable agricultural resources or food processing waste (Aloui and Khwaldia, 2016). The importance of an *Opuntia ficus-indica* as a productive system for producing fruits and vegetables and biomass valorization may increase due to the loss of water resources and worldwide desertification (Gheribi and Khwaldia, 2019). Recent research was conducted on slices of kiwifruit, breba fig, strawberry, banana, tomato, and mandarin to examine an innovative edible coating for fruit storage focusing on the mucilage or polysaccharides obtained from cladodes of *Opuntia ficus-indica* (Liguori et al., 2021).

Muramidase, also known as Lysozyme, is an enzyme composed of 129 amino acids connected by four disulfide bonds. In order to preserve food, lysozyme kills specific microorganisms The components of bacterial cell walls, N-acetyl-glucosamine and N-acetyl-muramic, are broken into β-(1-4) links to accomplish this (Cunningham et al., 1991). Due to its bactericidal properties, lysozyme serves as a barrier in food preservation methods such as the inactivation of microorganisms through high levels of hydrostatic pressure and the reduction of thermal requirements in heat-treated products (Ercan and Demirci, 2016). Thus, the present research has been designed to investigate the option of extraction of polysaccharides from wild Opuntia cactus plant and lysozyme for the preparation of edible coating to improve the shelf life of papaya fruits along with marinating the quality by reducing microbial contamination over a longer storage period.

MATERIALS AND METHODS

Carica papaya L. was purchased from the farm level at Pune. The fruit was harvested at the stage of maturity but unripe. *Opuntia ficus-indica* fruit powder was purchased from Nisarg Organic farm in Gujrat. Lysozyme was obtained from Bioven ingredient Bihar. Citric acid, acetic acid, glycerol, and sunflower oil was made available at MIT School of Food Technology, Pune. Fruits were then washed under tap water, sanitized by immersion in 50 ppm of sodium hypochlorite for 2 min, and stored for further process (Abebe et al., 2017).

Preparation of edible coating

The edible coating was prepared by complete mixing of polysaccharides extract (1g), lysozyme (1g), acetic acid (1g), ascorbic acid (2g), citric acid (1g), glycerol (1.5g), sunflower oil (0.025) and distilled water (1ooml) for treatment 5. Five different concentrations of these edible coatings were prepared (Riaz et al., 2021).

Application of edible coating

The antimicrobial edible coating was sprayed on papaya fruit in the dispersion (Lara et al., 2020). Afterward, they were dried at room temperature for 2-4 h then stored in the room temperature (Riaz et al., 2021). In the formulation of antimicrobial edible coating, polysaccharide extract, acetic acid, citric acid, glycerol, and sunflower oil were kept constant. Only the lysozyme concentration was varied as 0.2 g in T1, 0.4 g in T2, 0.6 g in T3, 0.8 g in T4, and 1 g in T5. For comparison purposes, untreated papaya was also stored along with coated papaya.

Colour, appearance, overall acceptability

The color of papaya was observed visually up to the 5^{th} day of storage. The appearance of papaya was visually studied up to the fifth day of storage to observe microbial growth on the fruit's surface. The color and appearance of papayas influence their overall acceptability. The general acceptability of fruit is governed by its color and appearance.

pH, acidity, and total soluble solids

10 ml of the pulp was dispensed into a beaker and the pH was determined with a standardized pH meter. The pH meter was calibrated using a phosphate buffer of pH 4.0 and 7.0 (AOAC, 1990). For titratable acidity, 5 gm of fruit pulp was homogenized in 20 ml of distilled water. Then add few drops of phenolphthalein were added to the pulp as an indicator and titrated against 0.1 N NaOH. Titratable acidity was calculated using the equation:

$$\text{Titratable acidity (\%)} = \frac{\text{Titre value} \times \text{N of NaOH} \times \text{Eqv. wt of citric acid} \times 100}{\text{Volume of sample} \times 1000}$$

Total soluble solids (T.S.S.) of the pulp were measured with the help of an Erma hand refractometer at 20°C (AOAC, 2016).

Weight loss

The weight of the fruits was recorded immediately after the treatment (day 0) and up to storage time. Weight loss was expressed as the percentage reduction concerning the initial time, using the equation:

$$\text{\% Weight loss} = \frac{(\text{Initial weight of fruit} \times \text{Final weight of fruit}) \times 100}{\text{Initial weight of fruit}}$$

Microbial parameters

The fruit samples were microbiologically investigated for total plate count (TPC), yeast and mold count (YMC), and targeted microbe *Staphylococcus aureus*. Microbial analysis was carried out by using the spread plate method (Aneja, 2007).

Statistical analysis

All experiments were carried out in triplicates. The means and standard deviations of the data were calculated for each treatment. An analysis of variance (ANOVA) was carried out to determine any significant differences ($p < 0.05$). SPSS software was used for analysis.

RESULTS AND DISCUSSION

Visual color

The color of all samples was observed visually. During the storage period, it was observed that the color of the papaya changed from green to pale yellow. It was noticed that the color of the control sample rapidly changed from day 0 to day 5 compared to the treated samples. Among all the treatments given to papaya fruit, the T5 sample shows the greatest effect on retarding the change in color during coating application delayed the ripening process for about 5 days, while uncoated papaya reached this stage after 2 days only.

Appearance

The appearance of all samples was observed visually**.** During the storage period, it was observed that the appearance of papaya fruit rapidly changed from day 0 to day 5. The control sample has no treatment, therefore during storage, there is a significant change in appearance. Due to the application of an edible coating, the T5 sample exhibits a slower change in appearance.

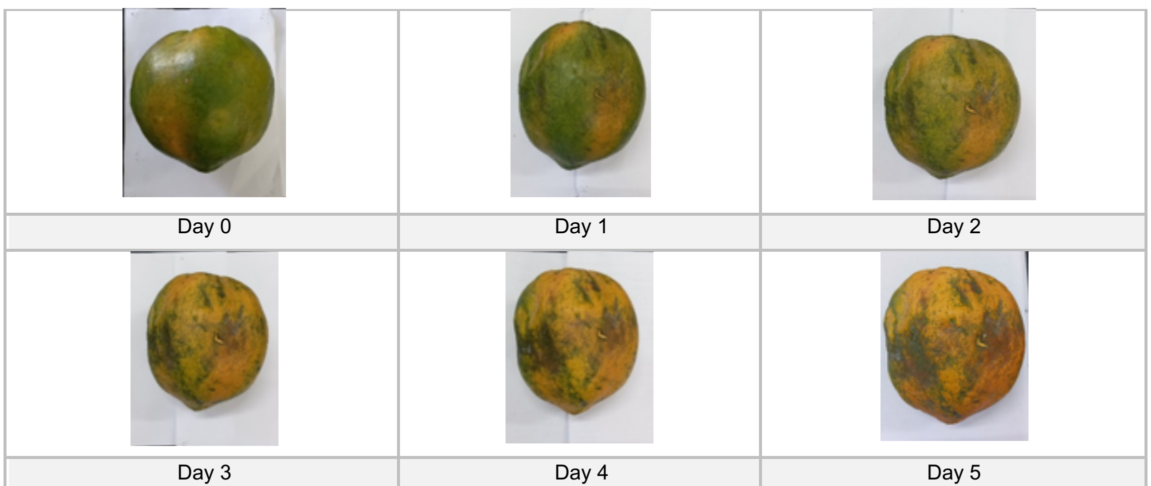

Fig. 1 Control samples of papaya during storage

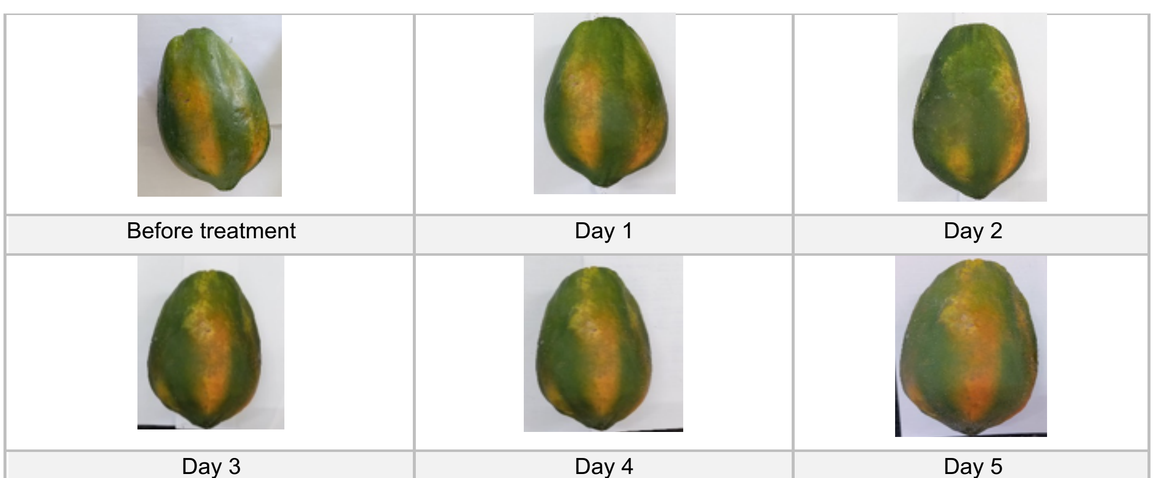

Fig. 2 Coated samples of papaya with T5 during storage

Overall acceptability

A fruit's colour and appearance determined whether it is generally regarded favorably. This led to the conclusion that the control sample is less acceptable than the T5 sample. The acceptance of the T5 sample was higher.

pH

The pH of all samples was analyzed after 5 days of coating. The average pH value of the control fruit was 7.26 ± 0.05, however, there was little change in the pH values of coated fruits after 5 days of storage. Result showed that pH of T1, T2, T3, T4, and T5 sample was 6.72 ± 0.01, 6.55 ± 0.00, 6.43 ± 0.15, 6.29 ± 0.00 and 6.15 ± 0.00 respectively. Fruit senescence was effectively delayed by coatings because they reduced pH fluctuations. According to the findings of Praseptiangga et al. (2016) the pH value of coated papaya on the 5th day of storage was found to be 6.25 ± 0.071.

Acidity

The titratable acidity of all samples was determined on the 5th day of storage. It was observed that the maximum values for the acidity (%) were recorded in the control as 0.11 ± 0.01. Similarly on the 5th day of storage T1 sample shows acidity as 0.11± 0.00, in T2 as 0.13 ± 0.01, in T3 as 0.11 ± 0.01, in T4 as 0.14 ± 0.00 and in T5 as 0.13 ± 0.01. Similar behavior was reported by Brishti et al. (2013), who applied papaya leaf extract and aloe-vera edible coating to papaya fruit and found that titratable acidity on the 4th day of coating in the control sample was 0.15%, while in the coated sample it was 0.17%.

Total soluble solids

The total soluble solids of all samples were measured. On 5th day of coating, total soluble solids were 7.96 ± 0.02 in the control sample, 7.66 ± 0.01 in T1, 7.50 ± 0.10 in T2,6.73 ± 0.01 in T3, 6.33 ± 0.01 in T4, and 6.13 ± 0.01 in T5. Sharmin et al. (2015) also studied the effect of aloe vera gel coating on papaya fruit at ambient temperature and found similar results.

Weight loss

The fruits were weighed at each sampling time to evaluate weight loss. It was observed that the loss of weight progressively increased with storage time from day 0 to day 5 and was linear for both cultivars and treatments. When compared to the control sample, the antimicrobial edible coating treatment significantly decreased the weight loss percentage during storage. From day 0 to day 5, the weight loss in the control sample increased from 7.87% to 34.13%. Similarly, T1, T2, T3, T4, and T5 show an increase in weight loss from 5.85 to 28.43%, 5.56% to 26.39%, 5.88% to 28.22%, 5.58% to 27.42%, and 4.03% to 18.83%, respectively.

Total plate count

Before any treatment, the bacterial burden was observed to be greater in all *Carica papaya* L. samples. The TPC count was found to be lower in treated samples compared to control samples after the edible coating was applied. The T5 sample was more successful than the other samples at reducing bacterial burdens. After the edible coating was applied, the bacterial burden in the T5 sample dropped from 4.61 log CFU/ml to 2.00 log CFU/ml. On day 0, the TPC count in the control sample was 4.48

log CFU/ml and grew to 4.79 log CFU/ml on day 1. Xu et al. (2019) studied the effectiveness of lysozyme coating and 1-MCP treatments on the storage and preservation of kiwifruit and found a similar behaviour.

Table 1: Total Plate count (Log CFU/ml)

Treatment	Before treatment	Day 1	Day 2	Day 3	Day 4	Day 5
Control	4.48 ±0.01^{e}	4.79 ± 0.00^{a}	5.08 ± 0.00^{a}	5.11 ± 0.00^{a}	Infinite	Infinite
T1	4.84 ±0.00^{b}	4.25 ± 0.02^{ab}	4.74 ± 0.01^{b}	5.10 ± 0.00^{a}	5.17 ± 0.00^{a}	Infinite
T2	5.04 ± 0.00^{a}	3.45 ± 0.15^{b}	4.33 ± 0.01^{c}	5.02 ± 0.00^{b}	5.14 ± 0.00^{b}	5.41 ± 0.00^{a}
T3	4.79 ± 0.00^{c}	3.41 ± 0.09^{b}	3.96 ± 0.02^{d}	4.93 ± 0.01^{c}	5.08 ± 0.00^{c}	5.30 ± 0.00^{b}
T4	4.80 ± 0.00^{c}	3.10 ± 0.17^{bc}	3.69 ± 0.00^{e}	4.44 ± 0.01^{d}	4.81 ± 0.01^{d}	5.23 ± 0.00^{c}
T5	4.61 ± 0.01^{d}	2.00 ± 1.73^{c}	3.30 ± 0.00^{f}	4.21 ± 0.01^{e}	4.45 ± 0.01^{e}	5.00 ± 0.00^{d}

CFU/ml is the unit of measurement. The results show the mean and standard deviation of three plate counts. According to the Duncan test, data inside a column followed by the same letter are not substantially different.

Yeast and mold count

Bacterial load was found to be higher in all samples of *Carica papaya* L. before any treatment was applied. After the application of the edible coating, it was noticed that the YM count was lowered in treated samples as compared to the control sample. Among that the T5 sample was more effective at reducing bacterial load than the other samples. On day 0, the initial bacterial count in the T5 sample was infinite. The bacterial load in the T5 sample decreased from infinite colonies to 3.91 log CFU/ml after the edible coating was applied on day 1. The YM count in the control sample was 4.94 log CFU/ml on day 0 and grew to 5.19 log CFU/ml on day 1. Wang et al. (2011) investigated the impact of lysozyme on *Momordica charantia L.* the findings indicated that lysozyme exerted antifungal activity.

Table 2: Yeast and Mold Count (Log CFU/ml)

Treatment	Before treatment	Day 1	Day 2	Day 3	Day 4	Day 5
Control	4.94 ±0.01^{a}	5.19 ±0.00^{a}	Infinite	Infinite	Infinite	Infinite
T1	4.85 ±0.02^{ab}	4.44 ± 0.01^{b}	4.56 ± 0.02^{a}	4.61± 0.02^{a}	5.12 ± 0.01^{a}	Infinite
T2	4.44± 0.02^{c}	4.26 ± 0.01^{c}	4.45 ± 0.01^{b}	4.60 ± 0.01^{a}	4.88 ± 0.01^{b}	5.18 ± 0.01^{a}
T3	4.50± 0.03^{bc}	4.18 ± 0.01^{d}	4.34 ± 0.03^{c}	4.49 ± 0.02^{b}	4.78 ± 0.01^{c}	5.09 ± 0.01^{b}
T4	Infinite	3.93 ± 0.02^{e}	4.25 ± 0.02^{d}	4.45 ± 0.01^{c}	4.69 ± 0.01^{d}	4.99 ± 0.01^{c}
T5	Infinite	3.91 ± 0.02^{e}	4.18 ± 0.01^{e}	4.42 ± 0.01^{c}	4.71 ± 0.01^{d}	4.93 ± 0.01^{d}

The units are log CFU/ml. The results show the mean S.D. of three plate counts. According to the Duncan test, data inside a column separated by the same letter are not substantially different.

Staphylococcus aureus

Bacterial load was found to be higher in all samples of *Carica papaya Linn*. before any treatment was applied. After the application of the edible coating, it was noticed that the *Staphylococcus aureus* count was lowered in treated samples as compared to the control sample. The T5 sample was more effective at reducing bacterial load than the other samples. In the T5 sample, the initial bacterial count was infinite on day 0. On the first day after applying the edible coating, the T5 sample showed 2.81 log CFU/ml of *Staphylococcus aureus* development. While the control sample's *Staphylococcus aureus* count increased from 4.49 log CFU/ml to 4.93 log CFU/ml on day 1. Wang et al. (2011) investigated the impact of lysozyme on *Momordica charantia* L. and findings indicated that lysozyme exerted antibacterial activity on *Staphylococcus aureus.*

Table 3: *Staphylococcus aureus* count (Log CFU/ml)

Treatment	Before treatment	Day 1	Day 2	Day 3	Day 4	Day 5
Control	4.49 ± 0.02^{d}	4.93 ± 0.00^{a}	5.14 ± 0.00^{a}	5.30 ± 0.00^{a}	Infinite	Infinite
T1	5.26 ± 0.00^{b}	4.51 ± 0.02^{b}	4.82 ± 0.01^{b}	5.03 ± 0.00^{b}	5.30 ± 0.00^{a}	Infinite
T2	Infinite	4.10 ± 0.03^{c}	4.32 ± 0.02^{c}	4.88 ± 0.00^{c}	5.14 ± 0.00^{b}	5.29 ± 0.00^{a}
T3	5.31 ± 0.00^{a}	3.60 ± 0.00^{d}	4.30 ± 0.01^{c}	4.79 ± 0.00^{d}	5.02 ± 0.00^{c}	5.16 ± 0.00^{b}
T4	5.21 ± 0.00^{c}	2.99 ± 0.01^{e}	4.09 ± 0.02^{d}	4.60 ± 0.00^{e}	4.98 ± 0.00^{d}	5.13 ± 0.00^{c}
T5	Infinite	2.81 ± 0.01^{f}	3.01 ± 0.00^{e}	4.08 ± 0.00^{f}	4.90 ± 0.00^{e}	5.04 ± 0.00^{d}

The units are log CFU/ml. The results show the mean S.D. of three plate counts. According to the Duncan test, data inside a column separated by the same letter are not substantially different.

CONCLUSION

Papaya can be preserved for a longer period by using polysaccharides that can be produced from natural sources like cactus to create edible coatings. As an antibacterial, lysozyme was also used as an ingredient of edible coating. Results stated that 1% of polysaccharides and 1% of lysozyme maintain the physical characteristics, sensory attributes, chemical parameters, and microbial characteristics. The prepared edible coating helps prolong the shelf life of papaya fruit by preventing the formation of gram-positive bacteria. Thus, the prepared coating was described as an “antimicrobial edible coating”.

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