



RESEARCH ARTICLE

# Modulation of physicochemical properties and antioxidant capacity of papaya fruits by edible coatings under ambient storage

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## Abstract

In recent years, fresh papaya has gained a lot of popularity due to its status as a superfood and its application in functional foods and beverages. Despite its significant production, the per-capita availability of this fruit is low because of its extreme perishability. Therefore, an attempt was made to prolong the postharvest life of papaya fruits by using edible coatings, specifically carboxymethyl cellulose (CMC), chitosan, gum arabica and sodium alginate. Of the many edible coatings used in this study, CMC 1 % permitted papaya fruits to be kept at ambient conditions for 14 days. The application of CMC coating slowed down the process of ripening and significantly preserved fruit quality by lowering the rates of respiration, ethylene evolution and physiological weight loss. At the end of the storage period, CMC-coated papaya fruits had superior marketable fruit quality parameters, including total sugars (9.80 %), titratable acidity (0.23 %), ascorbic acid content (52.76 mg/100 g) and antioxidant activity (13.95 mg AEAC/100 g) with the highly favourable sensory properties up to 14 days. Our findings enable us to extend the postharvest shelf life of papaya fruits, allowing them to reach consumers with good sensory qualities and at reasonable prices.

**Keywords:** carboxymethyl cellulose; papaya; physicochemical properties; shelf life; surface coating

## Introduction

Papaya (*Carica papaya* L.) is a highly valued fruit crop in tropical and subtropical regions of the world due to its flavour and nutritional content (1). India produces around 5.95 MMT of papaya annually on 0.149 M ha of land (2). This fruit's export potential, particularly in non-native countries, is driving demand for it in order to guarantee year-round availability on the global market (3). In several Indian states, production has somewhat declined over the last decade, mostly due to pathological diseases that occur in the field, followed by postharvest decay and quality loss (4). The perishability and high moisture content of papayas result in a shorter shelf life and significant postharvest losses every year in developing nations (5). Thus, it is essential to regulate postharvest deterioration and ripening during storage and distribution to preserve the maximum quality of natural produce until it is consumed (6).

There have been several attempts to minimise papaya postharvest losses, but only a few techniques are considered ecologically acceptable. These methods consist of ozonation, edible coatings, temperature reduction and modified atmospheric packaging (7). Growing customer concerns about safety have prompted postharvest horticulturists to focus more

on reducing fruit and vegetable postharvest losses through the use of natural substitutes like edible coatings and environmentally friendly techniques like hot water treatment. Reducing the need for chemical fungicides, which can be harmful to human health and promote the emergence of disease resistance, is a major advantage of these tactics (8).

Unfavourable physicochemical changes, such as softening of the flesh, desiccation and weight loss through respiration and transpiration, as well as deterioration of quality due to microbial assault resulting in changes in sugar and acid content, are the main reasons for the fruits' perishability. A coating of edible material prolongs the shelf life of highly perishable fruits, including papaya, by creating a semi-permeable membrane that replicates the effects of MAP (9). Furthermore, the selective gas exchange barrier properties of different edible coatings have the potential for use in extending the storage life of perishables (10).

Plant extracts rich in bioactive compounds, lipids, milk proteins and polysaccharides are examples of naturally occurring renewable resources that are used to make edible coatings. Fresh fruits and vegetables can have edible coatings applied to their surface that are edible without being noticeable

to the tongue and usually consist of thin layers of less than 0.3 mm (11). Pectin, carnauba wax, shellac, cassava starch, *Aloe vera* and many others are common edible coatings.

The effectiveness of several polysaccharide-based edible coatings was assessed in our laboratory through systematic preliminary studies. Chitosan, sodium alginate, Gum arabica and CMC, which are readily soluble and reasonably priced, were shown to create a stable and consistent matrix that maintained good structural integrity throughout the storage period. The natural biopolymer chitosan ( $\beta$ -(1,4)-2-amino-2-deoxy-D-glucose) is produced by deacetylating chitin, which is present in the exoskeleton structure of marine invertebrates, fungi, insects, yeast and algae (12). Commercial cellulose derivative CMC is produced from agricultural crop residue. Gum arabica is sourced from *Acacia Senegal* or *Acacia seyal*. Sodium alginate is biosynthesised from brown seaweed (10).

To the best of our knowledge, there was limited information available on the effectiveness of the above edible coatings in preserving the quality and extending the storage life of papaya fruit. Consequently, this study was undertaken to evaluate the impact of these edible coatings on prolonging the storage life of papaya fruit while maintaining its physicochemical properties, antioxidant capacity and sensory quality under ambient storage conditions.

## Materials and Methods

### Plant material

Papaya cv. Arka Prabhat fruits were freshly harvested at the breaker stage from fruit orchards at ICAR-IIHR, Bengaluru (13° 71 N latitude, 72° 291 E longitude and 890 above mean sea level altitude) and taken to the Research Laboratory of the Division of Post-Harvest Technology and Agricultural Engineering for further treatments. Fruits were carefully washed, allowed to air dry, sorted to remove any blemishes and graded for size uniformity across treatments to ensure consistency.

### Treatment details

The experimental setup involved randomly dividing the fruits into lots of 15 for each treatment. Four different edible coating treatments were applied to the individual fruit sets, whereas untreated fruits were used as the control group. The fruits were subjected to coating treatments with 1 % CMC, 1 % Chitosan, 10 % Gum arabica and 1 % Sodium alginate. Treatment concentrations were identified through preliminary laboratory testing and the best-performing ones were chosen for the final experiment. Sodium alginate, gum arabica and CMC were all dissolved separately and then gradually combined with distilled water while being constantly stirred with a glass rod until the appropriate volume was reached. The coating solution for the partly water-soluble chitosan is made using a 1 % glacial acetic acid solution in distilled water. Fruits were coated by dipping in coating solutions taken in separate containers, drained out and then surface dried and stored in paper-lined plastic crates for further examinations.

### Storage

The crates with treated fruits were stored at ambient temperature (23.4 - 34.4 °C, 25 % - 82 % RH). The experimental

setup was maintained in triplicate, containing 5 fruits for each replication. The physiological observations were recorded daily and the quality parameters were obtained at different edible stages till the end of their storage life.

## Observations recorded

### Physical and physiological parameters

An Instron-universal testing machine (Model 4201, USA) was used to measure the force needed to puncture the fruit using an 8 mm probe. The firmness of the fruits was then computed and expressed as kg/cm<sup>2</sup>. The physiological loss in weight was calculated as a percentage (PLW %) of the initial weight (13). The respiration rate and ethylene evolution rate of treated and untreated papaya fruits were measured by enclosing individual fruits (in 5 replicates) in hermetically sealed containers of known volume for 45-60 min, following the headspace gas measurement method (13). Respiration rate and ethylene evolution rates were expressed as mg CO<sub>2</sub>/kg/hr and  $\mu\text{L C}_2\text{H}_4/\text{kg/hr}$ , respectively. The coefficient of ripening of fruits was calculated by assigning a score to each ripening stage of the fruits based on visual colour assessment to calculate the ripening rate. The stages categorised were defined as follows: breaker (1.0), quarter ripe (2.0), semi-ripe (3.0), three-quarters ripe (4.0) and fully ripe (5.0) and then the coefficient of ripening was determined using the following formula:

Coefficient of ripening =

$$\frac{\sum (\text{No. of fruits at a particular ripening stage} \times \text{Score of the ripening stage})}{\text{Total number of fruits observed}}$$

(Eqn. 1)

### Quality parameters

At specified intervals during storage, three fruits were randomly selected from each treatment and analysed to assess biochemical changes. The fruits were peeled, the seeds were removed and the pulp was cut into small pieces. The collected pulp was immediately frozen and stored at -18 °C until further chemical analysis. Before analysis, the samples were thawed and homogenised using a VirTishear homogeniser (Virtis Company Inc., Gardiner, NY, USA). Quality attributes, including total soluble solids (TSS in °B), reducing and total sugars, titratable acidity (%), ascorbic acid (mg/100 g), total carotenoids (mg/100 g) and lycopene (mg/100 g) were determined using the standard methods of analysis (14). Total phenols were estimated and expressed as mg of gallic acid equivalents (GAE), flavonoids were estimated and expressed as mg of catechin equivalents (CE) and antioxidant capacity (FRAP) is estimated and expressed as mg of ascorbic acid equivalent antioxidant capacity (AEAC) (15-17). The spoilage percentage (%) was recorded through visual observations and calculated using the formulae:

Percentage of fruits showing spoilage symptoms (%) =

$$\frac{\text{Number of fruits with spoilage symptoms}}{\text{Total number of fruits}} \times 100$$

(Eqn. 2)

## Sensory evaluation

The sensory evaluation of fruits was carried out for fruit appearance, pulp colour, pulp texture, taste and overall acceptability by ten semi-trained panellists. Potential bias was minimised by coding the fruit samples with 3-digit numbers and randomising servings. The evaluations were conducted under uniform lighting and ambient conditions in a quiet room to avoid distractions, while the panellists were instructed to rinse their mouths with water between samples to neutralise taste carry-over. Assessments were conducted using a hedonic scale from 1 to 5, where 1 = very bad, 2 = poor, 3 = fair, 4 = good, 5 = very good (13).

## Statistical analysis

The data recorded under different parameters were subjected to ANOVA in a factorial completely randomised design (FCRD) and completely randomised design (CRD) with 5 % level of significance ( $p=0.05$ ) and were analysed using online statistical software GRAPES. Wherever significant differences were observed at  $p \leq 0.05$ , mean separation was carried out using Duncan's multiple range test (DMRT) at the 5 % probability level (18).

## Results and Discussion

### Physiological loss in weight and respiration rate

Weight loss results in a direct loss of economically viable produce as well as a decrease in consumer acceptability. Weight loss from the continuous respiration and transpiration processes is the main reason for the decline in quality of fresh horticultural crops after harvest (19). The respiration rate (RR) and physiological weight loss (PLW) of papaya fruits are evaluated for various edible coating treatments during ambient storage and depicted in Fig. 1. Throughout the duration of the storage period, the fruits' PLW (%) increased steadily as RR increased, regardless of the treatments employed in the experiment. When compared to the control, edible coated fruits showed repressed RR and transpiration loss, which led to a lower rate of PLW because the coatings act as a physical barrier for gas exchange. The control had the highest respiratory peak on day 7 (157.46 mg CO<sub>2</sub>/kg/hr) and thereafter it started to decline.

On the other hand, CMC 1 % coated fruits successfully decreased PLW (12.17 %) until 14 days of ambient storage by reaching a lower climacteric peak of 118.23 mg CO<sub>2</sub>/kg/h more slowly than control and other treatments, *i.e.*, on the 10<sup>th</sup> day of storage. Chitosan 1 % coating, which came after CMC, similarly resulted in the lowest PLW (13.46 %) up until the 14<sup>th</sup> day of storage. By partially blocking the lenticels and stomatal apertures, CMC 1 % improved the physical barrier surrounding the fruit and decreased the rates of respiration and transpiration (20). The higher rate of PLW (24.05 %) in the control on the 14<sup>th</sup>

day, in comparison to coated fruits, may be due to higher moisture loss and increased respiration through an uninterrupted air column. These findings are consistent with studies in papaya and golden berries (21-23).

### Ethylene evolution rate and coefficient of ripening

Ethylene is the hormone responsible for the ripening of climacteric fruits. Understanding the rate at which ethylene evolves is essential for managing the produce during storage since the ethylene evolution rate (EER) and the coefficient of ripening are intimately related. Fig. 2 illustrates the effect of various edible coating treatments on EER and the ripening coefficient of papaya fruits over time. Irrespective of the treatment used, the EER increased with the advancement in the storage period, indicating progressive ripening of fruits. Among all the treatments, the lowest EER peak of 48.28  $\mu\text{L C}_2\text{H}_4/\text{kg/hr}$  was reported in CMC 1 % followed by Chitosan 1 % (52.33  $\mu\text{L C}_2\text{H}_4/\text{kg/hr}$ ) on the 10<sup>th</sup> day of storage. Whereas a higher EER peak of 68.32  $\mu\text{L C}_2\text{H}_4/\text{kg/hr}$  was recorded in control fruits on the 7<sup>th</sup> day of storage itself, indicating rapid ripening. CMC 1 % coating resulted in a lower rate of ripening (4.60 score/5.0) on the 14<sup>th</sup> day of storage. In contrast, control fruits exhibited a faster ripening rate, attaining a score of 5.0 by day 12. Among coating treatments, sodium alginate recorded a higher ethylene peak of 60.91  $\mu\text{L C}_2\text{H}_4/\text{kg/hr}$  on the 9<sup>th</sup> day of storage and attained a ripening score of 5.0 on the 14<sup>th</sup> day. The ACC-oxidase enzyme, which is essential for the synthesis of ethylene in the plant, requires oxygen to function and edible coatings that function as a barrier for gaseous exchange decrease this availability (24, 25). CMC 1 %, an effective gas barrier, significantly reduced EER in the current investigation. Similar results were reported in papaya and sapodilla (20, 26).

### Fruit firmness

One of the key quality factors that determines the storage of papaya fruits and consumer acceptance is fruit firmness. As storage progresses, a decreasing tendency in fruit firmness has been observed, irrespective of the treatment (Table 1). Fruit softening results from the activity of pectinase and hydrolase enzymes, which degrade the fruit cell wall during storage (27). But the fruits coated with CMC 1 % maintained better fruit firmness (3.19 kg/cm<sup>2</sup>), followed by Chitosan 1 % (2.95 kg/cm<sup>2</sup>) up to 14 days of storage. In contrast, control fruits could be stored only for 7 days in an edible quality state and had a lower fruit firmness of 2.24 kg/cm<sup>2</sup>. Gum arabica 10 % (2.59 kg/cm<sup>2</sup>) and Sodium alginate 1 % (2.43 kg/cm<sup>2</sup>) showed intermediate firmness values. CMC 1 %, a polysaccharide-based coating, strengthens cell walls, preserves the structural and functional integrity of the membrane by binding into the lamella and thus maintains fruit firmness (28). Edible coatings can help fruits stay firmer by reducing gaseous exchange, which in turn reduces the activity of enzymes that break down cell walls. Similar results

**Table 1.** Physical and biochemical properties of edible coated papaya fruits stored at ambient conditions

Treatments	Texture (kg/cm <sup>2</sup> )	Reducing sugars (%)	Total sugars (%)	TSS (°B)	Acidity (%)	Ascorbic acid (mg/100 g)
Control	2.24e $\pm$ 0.062	7.63d $\pm$ 0.026	9.15c $\pm$ 0.232	10.28d $\pm$ 0.026	0.15c $\pm$ 0.000	39.73d $\pm$ 1.14
Chitosan 1 %	2.95b $\pm$ 0.036	8.14ab $\pm$ 0.072	9.67ab $\pm$ 0.062	11.42ab $\pm$ 0.484	0.22a $\pm$ 0.010	50.63b $\pm$ 0.91
CMC 1 %	3.19a $\pm$ 0.135	8.23a $\pm$ 0.062	9.80a $\pm$ 0.135	11.63a $\pm$ 0.062	0.23a $\pm$ 0.010	52.76a $\pm$ 0.57
Gum arabica 10 %	2.59c $\pm$ 0.036	8.09b $\pm$ 0.046	9.40bc $\pm$ 0.288	11.13bc $\pm$ 0.154	0.20b $\pm$ 0.010	46.60c $\pm$ 1.30
Sodium alginate 1 %	2.43d $\pm$ 0.062	7.88c $\pm$ 0.129	9.31c $\pm$ 0.082	10.92c $\pm$ 0.243	0.19b $\pm$ 0.006	45.73c $\pm$ 0.25

\*The mean values are presented with DMRT ranking followed by  $\pm$  standard deviation. Treatments denoted by the same letter(s) are not significantly different from each other.

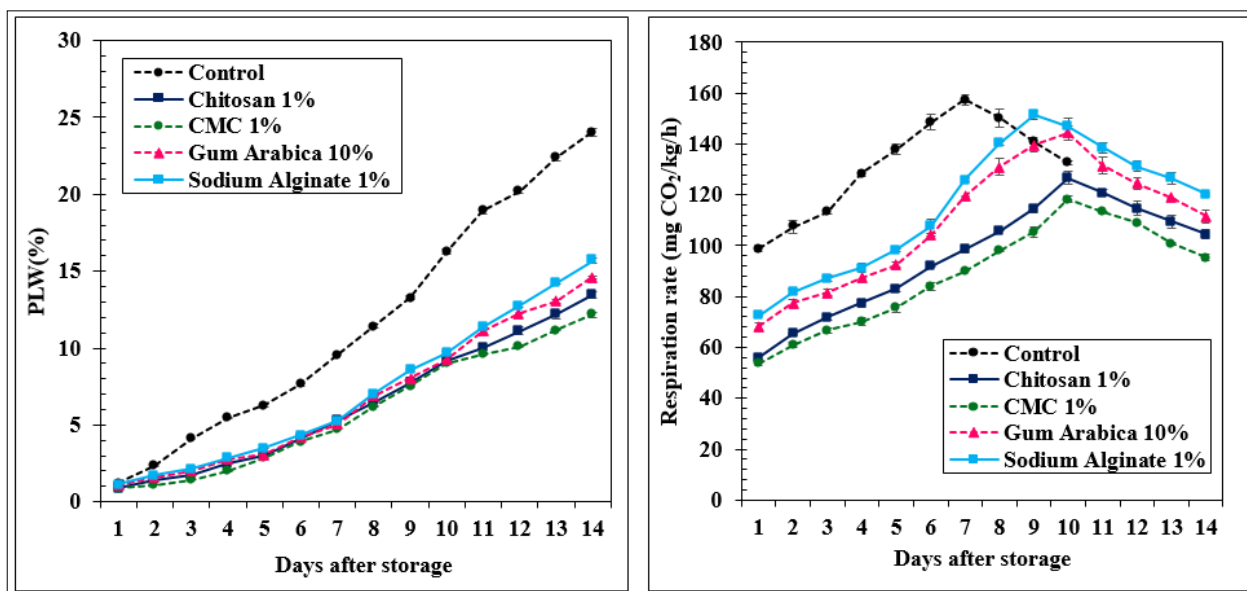


Fig. 1.

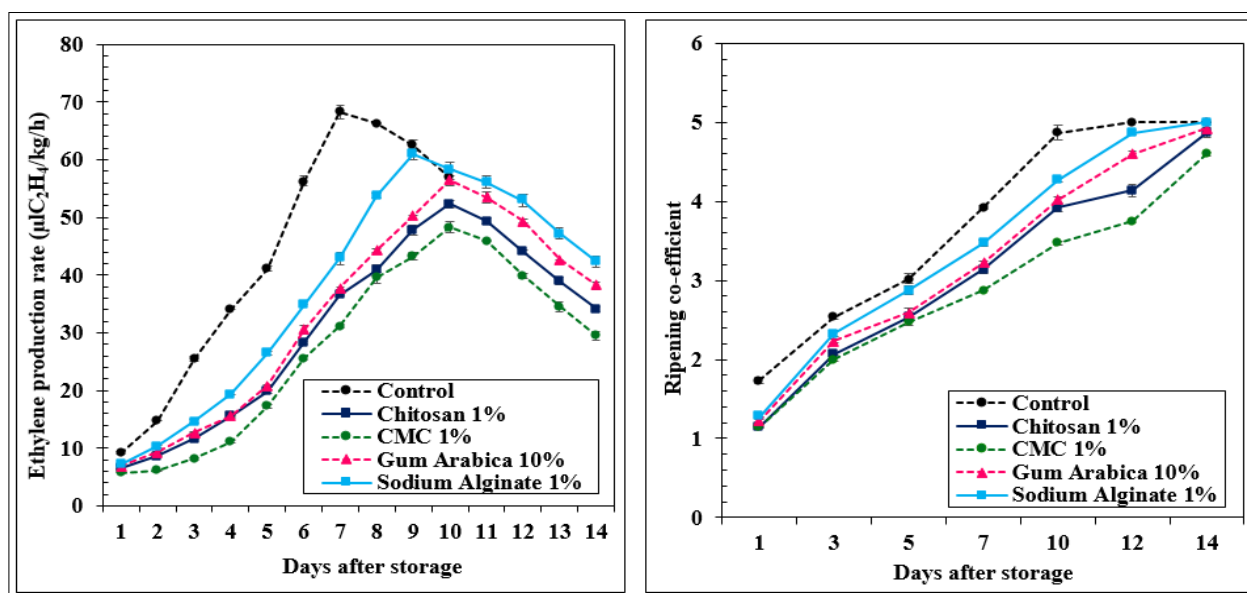


Fig. 2. Effect of different edible coatings on ethylene production rate and coefficient of ripening of papaya fruits stored at ambient conditions.

were noticed in papaya and Bartlett pear (20, 29).

### Reducing and total sugars

Reducing and total sugars are among the key factors that determine the taste, quality, shelf-life and nutritional status of the fruits. As the fruit ripened, sugars increased in all the treatments initially. However, they serve as a substrate for the physiological process of respiration and a gradual decrease in the amount of these sugars is observed with the progression of storage in all the treatments (Table 1). Since CMC 1 % coating is effective in reducing the RR, it helped in maintaining a good amount of reducing sugars (8.23 %) and total sugars (9.80 %) for a longer duration, i.e., 14 days under ambient storage conditions. While control fruits managed to retain a meagre amount of reducing sugars (7.63 %) and total sugars (9.15 %) on the 14<sup>th</sup> day of storage. Similar findings have been reported during ambient storage of edible coated papaya (20).

### Total soluble solids

Total soluble solids (TSS) are reduced during storage when sugars are used in climacteric respiration since they constitute a component of TSS (30). In comparison to control and other treatments, CMC 1 % and Chitosan 1 % were successful in preserving

a good amount of TSS (11.63 °Brix and 11.42 °Brix, respectively) at the end of storage at ambient conditions (Table 1). CMC, which is effective in reducing RR, preserved higher sugar content, thereby maintaining better TSS content. Similar outcomes were noted in goldenberries and papaya (9, 23).

### Titrateable acidity and ascorbic acid

As storage progresses and sugar reserves deplete, the metabolism changes to use organic acids as an alternate substrate for respiration. Titrateable acidity thus decreased as storage advanced due to a decrease in the quantity of organic acids (Table 1). But even after 14 days of storage, CMC 1 % retained a good amount of ascorbic acid content (52.76 mg/100 g) and titrateable acidity (0.23 %). In comparison, control fruits showed a substantial decrease in titrateable acidity (0.15 %) and ascorbic acid (39.73 mg/100 g) at the end of storage, highlighting the efficacy of the coating treatment. This discrepancy in the ascorbic acid decline pattern (Table 1) may be the result of varying degrees of oxidation in various coating applications. The ascorbic acid concentration of fruits may decrease during storage due to the actions of oxidising enzymes such as catalase, peroxidase, polyphenol oxidase and ascorbic acid oxidase (31). Nevertheless, the fact that CMC 1 % maintained a higher



ascorbic acid level than other treatments may be because it can decrease gaseous exchange, which in turn lowers oxidation caused by oxidising enzyme activity (32).

### Lycopene and total carotenoids

As papaya fruit ripens, pigments such as lycopene and total carotenoids build up with the degradation of chlorophyll (33). On the 14<sup>th</sup> day of ambient storage, the level of lycopene (2.88 mg/100 g) and total carotenoids (4.89 mg/100 g) was higher in control fruits with a higher rate of ripening. CMC 1 % coating treatment, which slows down the rate of ripening, had slower accumulation of pigments over time (Table 2), with the total carotenoids value of 4.41 mg/100g and lycopene value of 2.30 mg/100 g after 14 days of storage. The endogenous ethylene is the major trigger responsible for the breakdown of chlorophyll and pigment accumulation in the fruit pulp, which was also reported to be reduced by the coating treatments (20, 29).

### Total phenols and flavonoids

The secondary metabolites like flavonoids and phenols, which scavenge ROS, electrophiles and chelated metal ions, gradually decreased as a result of oxidation until papaya fruits reached the edible ripe stage (4). By maintaining total phenols and flavonoids for a duration of 14 days, coating treatments assisted in extending storage under ambient conditions (Table 2). The CMC 1 % coating treatment demonstrated an even better outcome by maintaining a good level of flavonoids (9.43 mg/100 g) and total phenols (201.33 mg GAE/100 g) until the end of storage, demonstrating the advantages of this treatment over control and other treatments in prolonging the storage life. The experiment demonstrated and reported that a CMC 1 % coating effectively lowers fruit metabolic rate, particularly fruit respiration and ethylene evolution, over a prolonged duration. As a result, the fruits' higher levels of flavonoid and total phenol content were preserved (34).

### Antioxidant (FRAP) activity

The antioxidant activity, which reduces oxidative stress and postpones the degradation of bioactive components, significantly preserves fruit quality during storage. As storage advances, it will decline (4). After 14 days of ambient storage, fruits coated with 1 % CMC maintained a good FRAP value (13.95 mg AAE/100 g), demonstrating strong antioxidant activity. The benefits of this coating treatment were illustrated by the control fruits' low antioxidant activity (12.26 mg AAE/100 g) on the same day of storage (Table 2). This effect of preserving antioxidant activity by CMC is also due to controlled respiration and ethylene evolution in coated fruits (35).

### Sensory properties

Consumer preference was measured using a five-point hedonic scale and the CMC 1 % coating treatment on the 14<sup>th</sup> day of ambient storage conditions received higher scores for overall acceptability

(4.3), pulp texture (4.1), taste (3.9) and fruit appearance (4.1) (Fig. 3). It also showed improved sensory qualities through the 14<sup>th</sup> day of storage. On day 14 of storage, the chitosan 1 % coating also showed better acceptance. The reduced acceptance of sodium alginate 1 % and the control group, especially in terms of flavour and appearance during ambient storage, demonstrated the efficacy of the treatments in preserving the appearance and sensory quality of papaya fruits (Fig. 4). Similar results have been recorded in CMC-coated strawberry fruits and goldenberry fruits (23, 36).

### Spoilage percentage

Spoilage percentage reflecting microbial decay and tissue breakdown during storage is a critical indicator of postharvest fruit quality and shelf life (Fig. 5). In papaya, spoilage is accelerated by high respiration and moisture content, especially under ambient conditions. The percentage of papaya fruits showing spoilage symptoms (%) at ambient storage was significantly influenced by different treatments, with notable differences observed across the storage period. The Control treatment had the highest spoige throughout the storage period, with 69.33 % spoilage at the 14<sup>th</sup> day of storage, indicating that the papayas in the control group spoiled the fastest. CMC 1 % exhibited the lowest spoilage throughout the storage period, with 21.33 % spoilage at 14 days after storage, making it an effective treatment in reducing spoilage. Chitosan 1 % also performed well, with 32 % of spoilage on the same day of storage, showing effective control over spoilage, though slightly higher than CMC 1 %. The effectiveness of CMC 1 % and Chitosan 1 % treatments can be attributed to their ability to reduce microbial activity and moisture loss by forming protective films on the fruit surface (37). Research reported a decrease in spoilage rate in CMC-treated strawberries and the delayed microbial growth in CMC-coated goldenberries (23, 38).

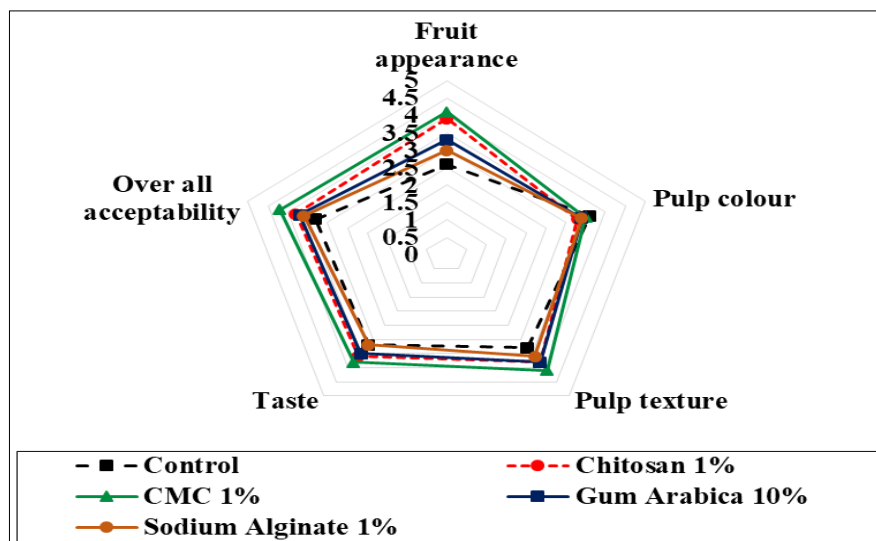
### Conclusion

Post-harvest handling of papaya fruit is quite complex because of thin skin, faster ripening and microbial attack. Compared to other coating treatments under ambient storage conditions, the edible coating of CMC 1 % preserved fruit freshness and quality for up to 14 days by significantly lowering the PLW, respiration, ethylene production and ripening rates. The marketable fruit quality parameters assessed at the end of storage are highly desirable in the CMC 1 % coating treatment. This investigation confirmed the potential of CMC coating in extending the storage life up to 14 days at ambient storage without compromising the fruit quality. It is anticipated that this treatment will reduce the post-harvest loss of papaya fruit significantly by enhancing its marketable period for the fruit growers/traders. Continued investigations into the application of CMC coating and its combination with other preservation techniques in the management of post-harvest losses

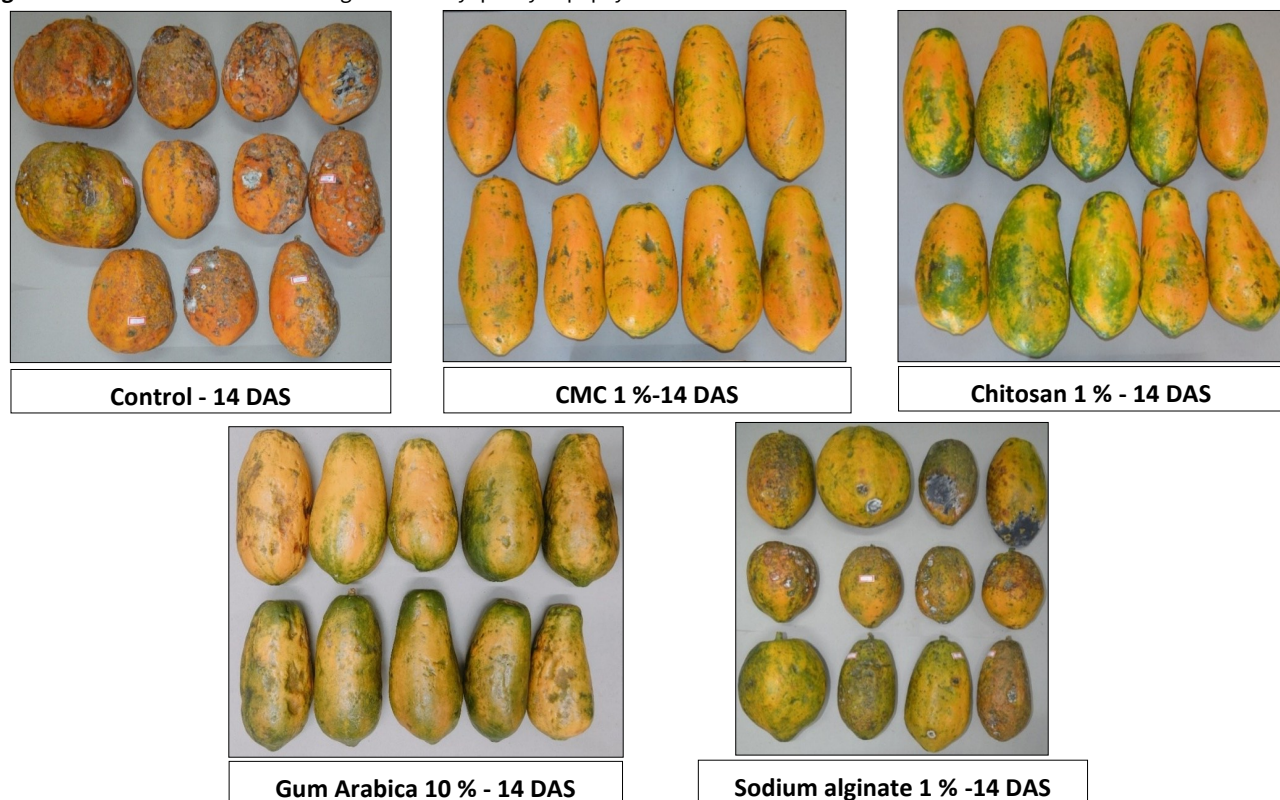
**Table 2.** Phytochemical and pigment properties of edible coated papaya fruits stored at ambient conditions

Treatments	Total phenols (mg GAE/100 g)	Total flavonoids (mg CE/100 g)	Total antioxidant capacity (mg AEAC/100 g)	Total carotenoids (mg/100 g)	Lycopene (mg/100 g)
Control	163.44d ± 2.36	7.22c ± 0.24	12.26c ± 0.41	4.89a ± 0.17	2.88a ± 0.01
Chitosan 1 %	194.79ab ± 8.43	9.17a ± 0.07	13.31b ± 0.07	4.30c ± 0.19	2.38cd ± 0.06
CMC 1 %	201.33a ± 3.81	9.43a ± 0.01	13.95a ± 0.24	4.41bc ± 0.06	2.30d ± 0.05
Gum arabica 10 %	181.67c ± 6.88	8.31b ± 0.33	13.02b ± 0.58	4.59abc ± 0.19	2.45bc ± 0.1
Sodium alginate 1 %	185.59bc ± 3.34	8.06b ± 0.12	12.87bc ± 0.3	4.67ab ± 0.20	2.51b ± 0.01

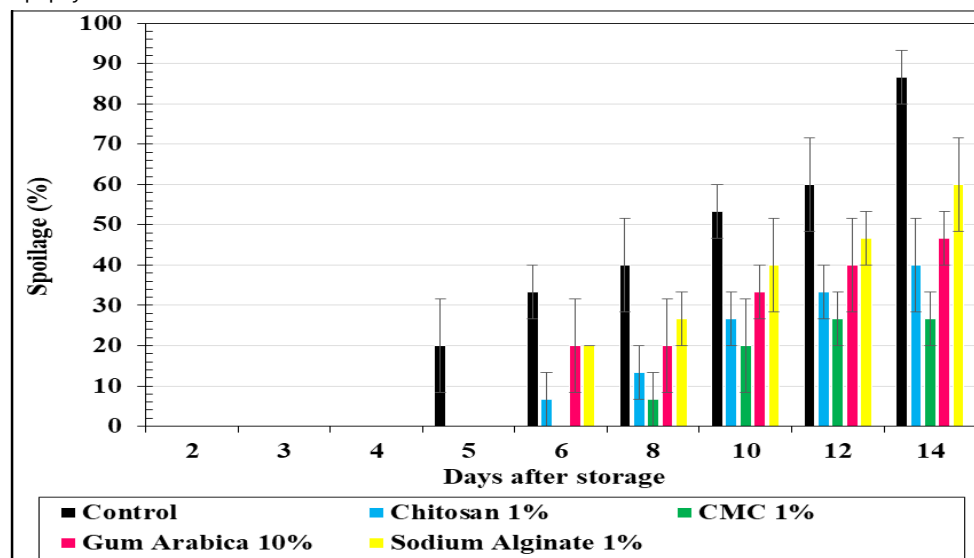
\*The mean values are presented with DMRT ranking followed by ± standard deviation. Treatments denoted by the same letter(s) are not significantly different from each other.



**Fig. 3.** Effect of different edible coatings on sensory quality of papaya fruits stored at ambient conditions.



**Fig. 4.** Edible coated papaya fruits stored at ambient conditions.



**Fig. 5.** Effect of different edible coatings on percentage of papaya fruits showing spoilage symptoms (%) stored at ambient conditions.

in papaya will have profound implications for the food industry and global food security.

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## Authors' contributions

MP contributed to investigation, formal analysis, data curation and writing the original draft. DVSR contributed to resources, methodology, supervision and reviewing. SVRR contributed to conceptualization, supervision, visualization and reviewing and editing of the manuscript. KSS contributed to reviewing and editing. CV contributed to resources and reviewing. PP contributed to supervision, methodology, reviewing and editing. SS contributed to reviewing and editing. All authors reviewed and approved the final version of the manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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