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Application of *Choerospondias axillaris* fruit extract in edible coating films

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Choerospondias axillaris fruit methanolic extract along with natural gums have been studied for its application in edible coating films as a bioactive agent for the protection of orange fruit against decay, to prevent post-harvest losses. Edible coating minimize migration of components within the food system or between the food and its surrounding environment. They provide an alternative to synthetic packaging and thereby bring on a significant role in environmental protection.*C. axillaris* fruit methanolic extract was checked for its antibacterial properties and the extract was then added in edible coating films for its effectiveness against the decay of orange fruits. The effect of coatings on the extension of shelf-life of orange fruits was studied at 4 and 25 °C for 30 days. During storage, changes in various physiological and chemical parameters such as weight loss, change in pH, total soluble solids and decay rate of coated and uncoated samples were observed at regular intervals. The results indicated that the methanolic extract of *C. axillaris* showed considerable antimicrobial activity against various gram-positive and gram-negative bacteria and when added in the coating solution along with gum acacia was efficient in delaying weight loss, pH, total soluble solids and decay rate.

Keywords: Anti-microbial activity, *Choerospondias axillaris*, Edible coating, Methanolic extract.

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Introduction

Current fruit market possesses very high commercial value for citrus fruits. Oranges are considered as one of the very best sources of bioactive compounds, providing various health benefits upon consumption[1]. Because of nutrient composition and high moisture content, fruit becomes susceptible to softening, browning/colour change, bacterial and fungal contamination[2,3]. Oranges are one of the most widely consumed fresh fruit globally. Therefore, the application of new engineering sciences to increase the shelf life of this fruit is essential. To overcome the concerns, the use of edible coating is recommended to enhance the shelf life of the fruit. To figure out the purpose naturally occurring polysaccharides, proteins, antioxidants and antimicrobials can be used as constituents for edible coating films[3]. Edible coatings are made up of thin layers of palatable constituents which cover the outside surface of food by dipping or spraying methods, which provides a barrier to various gases and water vapour, therefore, decreases respiration, enzymatic browning, loss of water, and damaging effects of microbes[4,5].

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In the edible coating films chitin, chitosan[6], whey protein[7], casein[8] have been widely studied, for their properties to act as excellent gas and solute barrier, but possess poor moisture barrier. Addition of plasticizers[9] (sorbitol or glycerol) increases their resistance towards moisture transfer. Although guar gum is extremely used in industrial and food processing, it has not been fully explored as an constituent in edible coating film and it possesses important nutritional and medicinal effects. Research has been done on several films and their application on fresh product, but very less has been known about the application of guar gum, gum acacia and xanthum gum on whole fruits and vegetables and for fresh-cut fruits and vegetables.

Gum acacia, Guar gum, Chitosan and *Choerospondias axillaris* fruit methanolic extract were selected for their application as edible coating on Orange. *C. axillaris* commonly known as a Lupsi/ Lapsi fruit belongs to the family *Anacardiaceae* and categorized as one of the underutilized and unexplored fruits. The fruit is native to Asia from India, China, Japan to Bhutan and Nepal[10]. This underutilized plant has changed its conception by expanding the usage beyond food and fibre to being an enormous source of therapeutically active

secondary metabolites[11]. The edible coating containing methanolic extract of *C. axillaris* can be compared to chitosan for suitability towards preservation of orange. Various formulations were made and investigated for their effectiveness. The coating solutions were applied to fresh oranges and the quality and shelf-life were studied at 4 °C and room temperature (25 °C) for 30 days.

Material and Methods

Plant material

Fresh fruits of oranges were procured from Mother Dairy, Delhi, which was seven days old postharvest and grown without chemical fertilizers. Upon arrival, the fruits were selected based on their size, weight and maturity stage, and free of visual defects or injuries. The samples were soaked in tap water for five minutes, followed by washing in distilled water at room temperature.

C. axillaris fruits were collected from Kalimpong, West-Bengal, India. It was identified by the National Institute of Science Communication and Information Resources (NISCAIR), Delhi. About 100 g fresh fruits were washed in water, crushed and extracted with methanol at 150 rpm at 45 °C for 24 h in an incubator shaker. The supernatant was collected and vacuum dried. The extract yield was measured, dissolved in dimethyl sulfoxide (DMSO) and was stored at −20 °C till further use.

Antimicrobial activity

The antibacterial activity of the *C. axillaris* methanolic extract was tested against Gram-positive and Gram-negative foodborne bacterial strains by agar well diffusion method and MIC[12]. The MICs can be defined as the minimum concentration of the extract for which the absorbance of a well was insignificant and is stated in mg/mL extract using tetracycline as the positive control.

Coating preparations

Four coating solutions (blends) were prepared and labelled as Coating A, Coating B, Coating C and Coating D. Table 1 summarizes the various components of the preparation coating solutions. Chitosan was prepared first by dissolving Chitosan (1% w/v) in 1% (v/v) glacial acetic acid in distilled water (incubated at 37 °C for 5 h). Tween-80 (0.1% v/v) was added as an emulsifier. Guar gum and Gum acacia (1% w/v) as a main edible component and glycerol (1% v/v) as a plasticizer were used for film preparation. The pH of the final solution was adjusted to neutral with 0.1 M sodium hydroxide.

Coating application

The samples after been cleaned with distilled water were divided into five batches under the label Coating A, Coating B, Coating C, Coating D and control with 30 oranges in each batch. Dipping method[13] was used for coating the samples. Oranges were dipped in their respective coating solutions for 1min each and the residual coatings were allowed to drip off. The coated oranges were left at room temperature (RT) until the coating was completely dry. The coated and uncoated samples were then incubated at 4 and 25 °C for 30 days and the oranges were examined for change in various parameters (weight loss percentage, pH, total soluble solids and decay percentage) at regular intervals.

Weight loss percentage

Six samples from each batch were weighed at zero time of the storage beginning and then on day 3, 7, 10, 13, 18, 23, 27, and 30 using a digital balance (Sartorius, GC 1603 S-OCE). The results were expressed as a percentage loss of the initial weight[14].

pH

The change in pH of the coated and uncoated oranges was recorded every 5th day during the storage period of 30 days. A sample from each batch was crushed and the pH of the pulp juice was measured using a digital pH meter (Thermo scientific, Orion 2 Star pH Benchtop)[15].

Total soluble solids (TSS)

Total soluble solids refer to the amount of sugars and acids together with small amount of vitamins, minerals and amino acids present in fruits. The filtered juice of the coated and uncoated oranges was used to determine the total soluble solids (every 5th

Table 1 — Composition of coating A, coating B, coating C and coating D

Coating A	Coating B	Coating C	Coating D
Chitosan (1 %)	MeOH ext. Lupsi (1 %)	Chitosan (1 %)	MeOH ext. Lupsi (1 %)
Guar gum (1 %)	Guar gum (1 %)	Gum acacia (1 %)	Gum acacia (1 %)
Glycerol (1 %)	Glycerol (1 %)	Glycerol (1 %)	Glycerol (1 %)
Tween 80 (0.1 %)	Tween 80 (0.1 %)	Tween 80 (0.1 %)	Tween 80 (0.1 %)

day). The TSS for each sample was determined in triplicates using a handheld refractometer with a scale of 0-32 °Brix. The instrument was calibrated using distilled water before readings were taken. The result was expressed in degree Brix (°Brix)[16].

Decay percentage

The various batches of coated oranges were observed during the storage period of 30 days for any incidence of infection. The number of decayed fruits[17] in both coated and uncoated batch was recorded every 5th day throughout the storage period and the decay percentage was calculated.

Results and Discussion

Antibacterial activity

Microbial contamination is a very common problem in the fruit/food preservation and protection and food preservation has been the technology used since ages to prevent spoilage of food[11]. Methanolic extract of *C. axillaris* showed the highest inhibitory activity against *Bacillus subtilis* (0.625 mg/mL) and *Salmonella enteric* (0.625 mg/mL) by showing the lowest MIC values (Table 2).

Weight loss

Weight loss which occurs in orange fruit during ripening is due to both transpiration as well as respiration. Transpiration is associated with the water vapour pressure of the surrounding atmosphere and the orange surface. Weight loss contributed by respiration is a result of carbon atoms, in the form of carbon dioxide molecules, leaving the fruits[18]. Weight loss (%) of oranges coated, and those of control during storage at 4 and 25 °C are shown in Fig. 1. Weight loss of oranges increased by the storage period, which was higher for the coating C set of oranges at 4 °C. At 25 °C, coating A and B showed comparable results. Control sample after 21 days showed a maximum decrease of weight loss. Coating C and coating D at 25 °C have shown comparable data. Coating B at 4 °C with composition Guar-gum and

Table 2 — Antibacterial activity of methanolic extract *Choerospondias axillaris*

Microorganisms	Methanolic extract** *Choerospondias axillaris*	
	ZI±SD (mm)	MIC (mg/mL)
Staphylococcus aureus	10.5±0.5	6.25
Bacillus subtilis	13±0.1	3.12
Staphylococcus epidermis	10.3±0.5	6.25
Bacillus cereus	11.5±0.5	5
Shigellaflexeneri	15.6±0.1	1.25
Escherichia coli	13.6±0.1	6.25
Salmonella enteric	14±0.0	3.12
Proteus mirabilis	15±0.5	3.125

*<8.0 mm, weak activity; 8.0-9.0 mm, slight low activity; 9.1-10.0 mm, middle activity; 10.1-11.0 mm, slight high activity; 11.1-12.0 mm, moderate high activity; over 12.0 mm, very high activity.

** 20 mg/mL concentration was used for the methanolic extract of *Choerospondias axillaris*

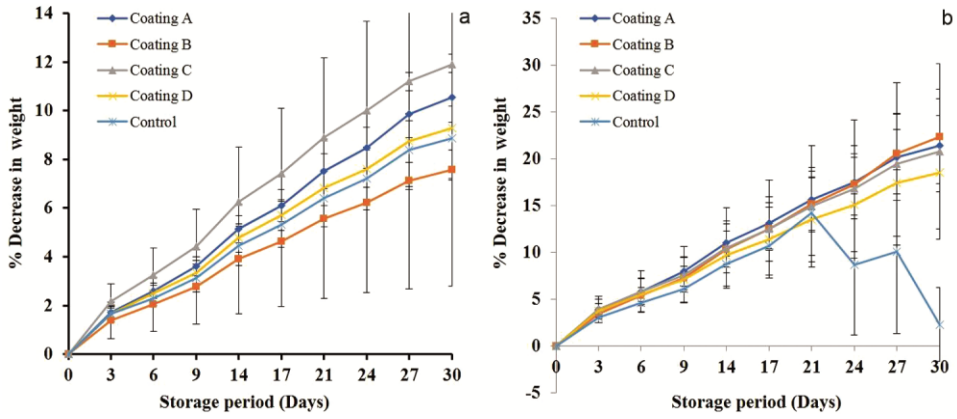

Fig. 1 — Effect of edible coatings on physiological loss in weight of orange fruits stored at 4 °C (a) and 25 °C (b) [Coating A, B, C, D show significant changes in % weight loss w.r.t. control (P <0.05) at 25 °C]

methanolic extract of Lupsi showed great potential. At 25 °C coating D with composition Guar-acacia and methanolic extract of Lupsi gave better results in comparison to other formulations. The data suggest that the methanolic extract of Lupsi fruit gave the appreciable results in the present study and somewhere methanolic extract of Lupsi fruit and Chitosan (Known antimicrobial compound) showed comparable activity. Weight loss causes wilting and shrivelling which decreases market value and acceptance by consumers. In the present study, oranges coated with 1% methanolic extract of Lupsi fruit were more effective than 1% Chitosan coating and weight loss percentage was more in control samples. A decrease in weight loss was probably due to the effects of coating which act as a semi-permeable barrier against O_2, CO_2, moisture and solute movement, thus reducing respiration, water loss and oxidation reaction rates[19]. This quality parameter is very crucial as each loss in weight leads to economic loss. Edible coatings provide a barrier to water loss by maintaining high relative humidity in the adjacent atmosphere of the orange fruit and consequently decreases the moisture gradient to the exterior[20].

pH

The pH of the coated and control oranges was measured every 5th day of the storage period at 4 and 25 °C. The pH increased with maturity and was higher in coating B at 4 °C oranges compared to other coatings as well as control samples (Fig. 2). The pH increase was nearly comparable in all coatings and control oranges at 25 °C. pH is the equilibrium measure of hydrogen ion concentration in a juice. Organic acids give most of the hydrogen ions in oranges which usually reduce with maturity, thereby increase pH[21]. The results of the present study revealed that the use of a coating solution slows down the change in pH, which means less loss of organic acids.

Total soluble solids (TSS)

Change in the TSS of coated and uncoated (control) orange fruits during the storage period of 30 days at 4 and 25 °C is shown in Fig. 3. The overall change in soluble solids concentration was relatively small in all coated and uncoated fruits. However, there is a chance of any changes in the constituents of TSS, such as the ratio of glucose/fructose and organic acids during storage[22]. Soluble solids content

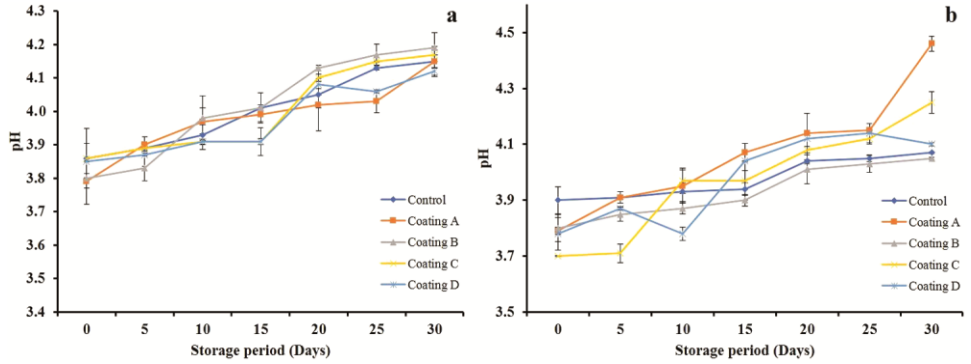

Fig. 2 — Effect of edible coatings on pH during the storage period of 30 days at 4 °C (a) and 25 °C (b)

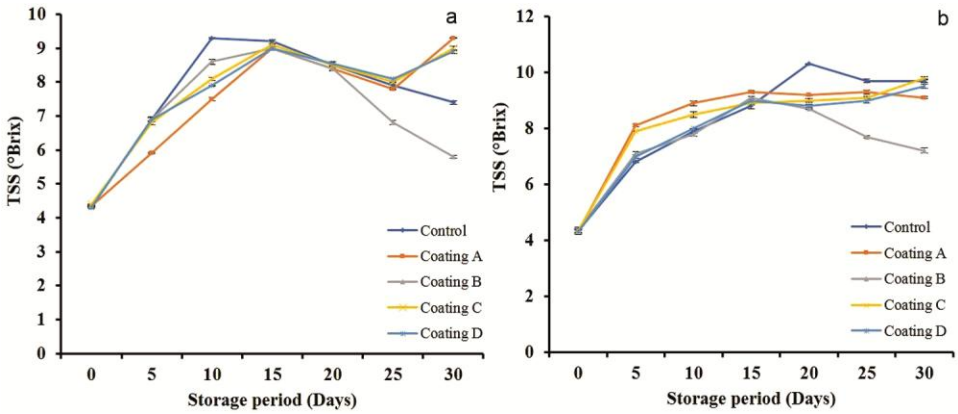

Fig. 3 — Effect of edible coatings on total soluble solids (°Brix) of oranges during the storage period of 30 days at 4 °C (a) and 25 °C (b)

increases with fruit maturity and indicate sweetness; even though sugars are not the only soluble constituent it measures. In our study, it was observed that the application of coating solution slows down the changes in TSS and this reduction is probably due to the slowing down of respiration rate and metabolic activities[23].

Decay percentage

No sign of decay was visible in the coated Oranges till the 21st day of storage at 25 °C. In control samples stored at 25 °C decay was observed at 9th day. On day 24th, decay in oranges was observed in coating C at 25 °C. At 4 °C, no signs of decay were observed in either coated or control. The rate of decay was relatively less in coating A, coating B and coating D than coating C and control samples at 25 °C. About 85.71 % oranges decayed at 25 °C in control samples up to the 30th day. The decrease in the decay percentage in coated fruits may have been due to the result of a coating on delaying senescence.

Conclusion

The present study revealed that *C. axillaris* methanolic extract in edible coating film along with gum acacia gave appreciable results as compared to other formulations. Delayed changes in weight loss, physiological, chemical properties and TSS were observed in coated fruits as compared to the control sample at 25 ºC. The fruit extract possesses significant antimicrobial activity, therefore prevented oranges against microbial decay. The methanolic extract may be used as a substitute or be alternative to commercial antimicrobial compounds used in handling citrus fruit for the domestic market and export. This study reveals that natural compounds can replace synthetic compounds for various formulations to increase shelf life. Further studies may be conducted on developing new formulations of natural gums at different concentrations and should be investigated for the effect on fruits and vegetables.

Conflict of interest

The authors have no conflict of interest to disclose.

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