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Preparation of Antibacterial Chitosan Membrane and Potential Application as Coating in Maintaining the Quality of Jackfruit

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ABSTRACT

Chitosan, a byproduct of the food industry, has garnered increasing attention due to its advantages in food preservation. This study reports on the preparation of an antibacterial chitosan membrane: 1.0% (w/v) chitosan and 200 rpm stirring at 60°C for 30 minutes, resulting in the highest inhibition zone of *E. Coli* (21 mm). Regarding its mechanical properties, the membrane exhibited a tensile strength of 21.95 MPa and elongation of 26.42%. The membrane's physical properties included a moisture content of 23.88 ± 1.39%, solubility of 24.05 ± 1.20%, swelling degree of 216.4 ± 5.64%, and transmittance of 86.18 ± 3.79%. The chitosan membrane functions as a barrier covering the surface of jackfruit and maintains its quality, including color and texture, even after seven days of preservation. In contrast, the non-preserved jackfruit exhibited signs of discoloration. Additionally, the non-preserved sample showed a total aerobic microorganism approximately twice as high as the chitosan membrane preserved sample.

Keywords: chitosan, membrane, preservation, jackfruit.

Introduction

In recent years, the chitosan membrane has gained recognition as an effective method for preserving food products due to its convenience, popularity, low cost, and safety.[1] Chitosan, a straight-chain biopolyaminosaccharide, shares similarities with cellulose and serves as a diverse biofilm-forming substance. Chitosan can be found in nature or chemically synthesized from chitin, the Crustacean shell's primary substance, such as crabs and prawns. It is also found in insects and certain mushrooms through a process called deacetylation.[2,3] Various applications are associated with the chitosan membrane, which includes medical, food, cosmetic, and waste management sectors. The chitosan membrane's barrier prevents microbial invasion into cells, slows the evaporation rates, and consequently decelerates the natural maturation of products. The polymeric film formed by chitosan on the surface hinders the entry of oxygen and nutrients into cells, thereby inhibiting and slowing the growth of aerobic bacteria.[4] Chitosan with a higher degree of deacetylation exhibits enhanced antibacterial activity due to increased free amine groups in the polymer chain.[5] Besides, in its free macromolecular form, chitosan exhibits activity against fungi, including *Aspergillus niger*, *Alternaria alternata*, *Rhizopus oryzae*, *Phomopsis asparagus*, and *Rhizopus stolonifera*.[6] In 2007, Chien et al. reported that mango samples dipped in chitosan demonstrated lower weight loss and fewer aerobic microorganisms after a week of storage than undipped samples.[7] In 2016, Teja *et al*[8]. addressed the effects of different coatings (aloe vera and pectin) on weight, ascorbic acid, and pH on the shelf-life extension of fresh-cut jackfruit. [8]

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Specifically, aloe vera coating was deemed more effective for preserving fresh-cut jackfruit due to its good moisture resistance, reduced ascorbic acid loss, weight loss during storage, and high protein content, as well as vitamin A and vitamin C.[8]

Jackfruit, scientifically known as *Artocarpus heterophyllus* Lam., belongs to the mulberry family and thrives in tropical and subtropical regions. It stands out as an easily cultivable and highly commercial fruit due to its sweet aroma, the abundance of vitamins, proteins, amino acids, minerals, traces of elemental nutrients, and fiber. In terms of its health and medical applications, jackfruit contains valuable nutrients such as lignans, isoflavones, and saponins. Its antibacterial, antifungal, and anti-inflammatory properties also strengthen the immune system.[9,10] In Vietnam, jackfruit cultivation is widespread and has a high yield. However, the consumption and preservation of jackfruit are currently facing several challenges, resulting in the necessity for developing effective preservation methods.

As previously mentioned, chitosan demonstrates various characteristics, including antifungal, antibacterial, and the ability to restrict water loss. The application of chitosan in the food sector has garnered notable attention. The present study aims to identify a suitable preservation method to preserve separated jackfruit's shelf life from its peel. It utilizes a chitosan-based membrane as an economical and non-toxic material that benefits consumers and the environment.

Materials and Methods

Sample collection, chemicals, and reagents

Thai jackfruit (*Artocarpus Heterophyllus*) was sourced from Tien Giang province (Vietnam) in 2022. The selection of jackfruit was carefully carried out based on the following criteria: size, roundness, fragrance, even ripening, and undamaged. After collection, the jackfruit was peeled and the seeds removed, preparing it for the subsequent preservation experiments involving chitosan membranes.

Chitosan powder (average deacetylation degree of 80-85%) was procured from Chitosan Kien Giang Co., Ltd., Vietnam. Additionally, the study used other chemicals, including acetic acid (>99%, Xilong Scientific Co., Ltd), glycerol (>99%, Xilong Scientific Co., Ltd), LB Broth Power Miller agar (HiMedia India), Mueller Hinton Broth media (MHB, HiMedia, India).

Membrane preparation and coating treatment

Chitosan films were produced by dissolving 0.5, 1.0, 1.5, and 2.0 g of chitosan in 100 mL of a 1% v/v acetic acid and 30% w/w glycerol solution.[11] The mixture was heated to 60^{o}C with continuous stirring at 200 rpm for 20, 30, 40, and 50 minutes. The mixture was centrifuged, and 20 g of membrane fluid was poured into a 90-mm-diameter glass petri dish. The membrane was left to air dry at room temperature for 72 hours.

Thai jackfruit was immersed in a 1.0% chitosan solution (following the same method used for membrane/film preparation) for 3 minutes before being allowed to dry at room temperature. The jackfruit was then stored at 10 ^{o}C for seven days before undergoing further tests.

Analytical methods

Evaluation of the antibacterial ability of chitosan membranes

The agar plate diffusion method was employed to evaluate the antibacterial activity of the chitosan films. In brief, 50 µL of *E. coli* bacteria, cultured for 24 hours and with turbidity measured at 600 nm (absorbance ranging from 0.4-0.5), was put into the MHB agar. Each petri dish was controlled alongside a positive control antibiotic, and four wells were perforated with a diameter of 6 mm. Subsequently, 100 µL of sample solution was added into each agar well, followed by incubation at 37°C for 24 h.[12] The antibacterial effectiveness was determined by measuring the diameter of the inhibition zone, where a larger ring indicated a higher level of antibacterial ability.

Determination of membrane moisture and solubility

The sample was weighed, and the initial weight (W_0) was recorded using an analytical balance (±0.0001g) to determine the moisture content of the membrane. It was then subjected to drying at 105 °C for 24 hours. After drying, the sample was cooled in a desiccator, and its weight (W_1) was recorded.[13] The moisture was calculated based on the equation:

$$\text{Moisture (\%)} = \frac{W_0 - W_1}{W_0} x\ 100$$

The membrane solubility was determined using a sample that was dried to constant weight (W_d) in an oven at 105 °C for 24 hours. Next, the dried membrane was immersed in distilled water at room temperature for 24 hours. Finally, the film was dried to constant weight in an oven (W_s).[13] The solubility of the film was calculated using the equation:

$$\text{Solubility (\%)} = \frac{W_d - W_s}{W_d} x\ 100$$

Determination of membrane transmittance

The membrane was cut into 2 x 3 cm pieces and placed into a cuvette. Water was then added to prevent the formation of air bubbles. Distilled water was used as a blank. An automatic spectrophotometer (UV-2602) was configured to measure transmittance at 600 nm, facilitating the determination of the transmittance.[11]

Determination of membrane swelling

The membrane sample was cut into squares measuring 3 x 3 cm, and its weight (W) was recorded. Then, the cut film was immersed in 30 mL of distilled water at the ambient temperature for 60 minutes, and its weight (W_t) was recorded.[13] The swelling was calculated using the equation:

$$\text{Swelling (\%)} = \frac{W_t - W}{W} x\ 100$$

Tensile strength and elongation of the membranes

The tensile strength and elongation of chitosan films represent the mechanical properties of the films concerning storage time. The chitosan film's tensile strength (MPa) was determined using a tensile speed of 80 mm/min, and the elongation (%) was evaluated at the Quality Assurance and Testing Center in Ho Chi Minh City, Vietnam.

Evaluation of chitosan surface by scanning electron microscopy (FE-SEM)

The microstructure of the chitosan film was imaged using a field-emission scanning electron microscope SU 8010, Hitachi, at the Institute for Nanotechnology, Linh Trung Ward, Thu Duc City (Vietnam). The microscopy image of the chitosan surface was recorded at 5000X magnification, with an electron beam generating voltage was 5.0 kV.

Sensory evaluation of the jackfruit

In the present study, the sensory evaluation was conducted to assess taste, color, and appearance changes. The method adhered to Vietnam's National Standard (TCVN 12387:2018) and was performed under ambient temperature and good lighting conditions.[14]

*Weight loss of jackfrui*t

The weight loss was determined by weighing all samples over the storage days using an analytical balance.[15] The percentage of weight loss was calculated using the equation:

$$\text{\% weight loss} = \frac{W_1 - W_2}{W_1} x\ 100$$

whereas W_1: initial sample weight (g); W_2: sample weight during storage (g).

Total dissolved solid content

The increase in sweetness observed during the storage period could be attributed to the elevated sugar concentration in jackfruit influenced by dehydration. One gram of the sample was ground to analyze this, and 1 mL of distilled water was added. The mixture was then boiled for 5 minutes and cooled to ambient temperature. A refractometer was utilized to assess the dissolved solid content following TCVN 4414:1987.[16]

Total aerobic microorganisms of jackfruit during storage

The diluted sample solution was inoculated in a glass petri dish containing 15 mL of LB agar under sterile conditions according to TCVN 11039-1:2015 guidelines.[17] To ensure even distribution, a glass triangle cell coated bar was used to spread the agar evenly in the petri dish before allowing it to dry. Subsequently, the petri dish was incubated at 37^{o}C for 24 hours. The plate count technique was employed to determine the total count of aerobic microorganisms. The total count was achieved using the following equation:

$$\text{A (CFU/mL)} = \frac{N}{n_i V f_{i+\cdots+} n_i V f_i}$$

Whereas A represents the number of bacterial cells present in 1 mL of sample; N: the total number of colonies counted on the selected plates; n_i: number of plates at dilution i; V: volume of sample solution (mL) inoculated into each plate and f_i: corresponding dilution.

Data processing methods

The experimental data were performed in triplicates and presented as average values. All charts and calculations were processed using Microsoft Excel 2016.

Results and Discussion

Effects of chitosan concentrations on the antibacterial ability of the membranes

Literally, *E. coli* bacteria have been commonly used for the assessment of the antibacterial ability in different sections due to the ease of cultivation as well as rapid growth rate, resulting in saved experimental time. In the present study, acetic acid was used as a dissolution medium for chitosan to obtain a homogeneous and stable system.[18] In the presence of glycerol, the chitosan-glycerol gel was formed. The concentrations of acetic acid and glycerol were kept constant while the chitosan was varied to understand the effects of the chitosan contents (0.5%, 1.0%, 1.5%, and 2.0%) in different membranes on the antibacterial efficacy against *E. coli* (Figure 1) since the chitosan-based conjugates have been reported to perform the antimicrobial function.[19,20] The results indicated that all chitosan films exhibited reduced bacterial growth compared to the water-controlled sample, in which the 1.0% chitosan showed the highest inhibitory zone diameter, although smaller than ampicillin. This result is consistent with a prior report on papaya fruit disease control using 1.0% chitosan.[21] However, in comparison to the research conducted by Ngoc et al. on oranges (utilizing 1.15% chitosan and 0.39% polyvinyl alcohol), the observed effect was less potent.[22] As a result, the 1.0% chitosan concentration was chosen for further experiments.

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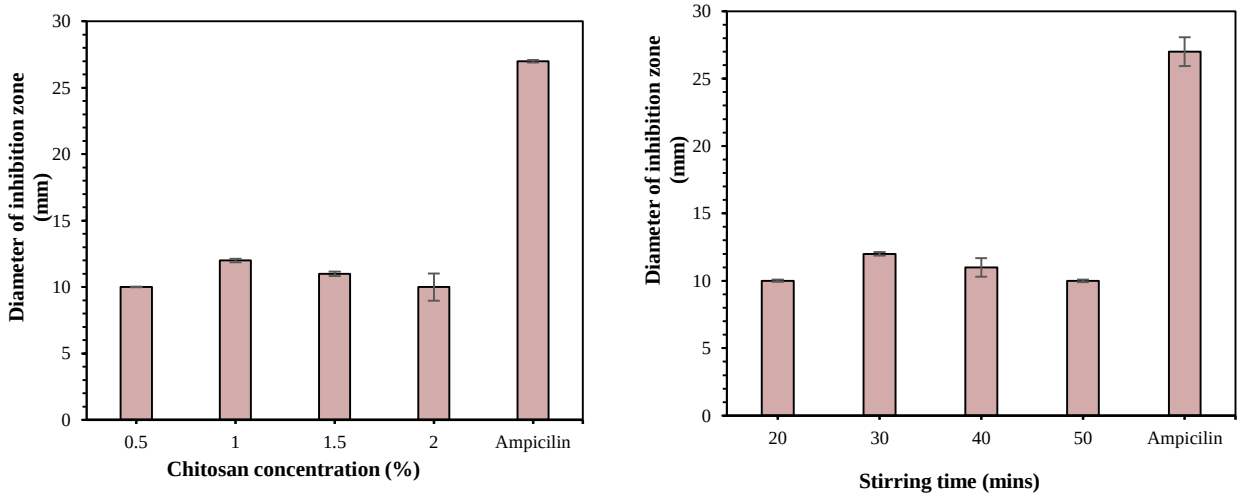

Figure 1: Effects of chitosan concentrations (left) and stirring time (right) on the antibacterial ability.

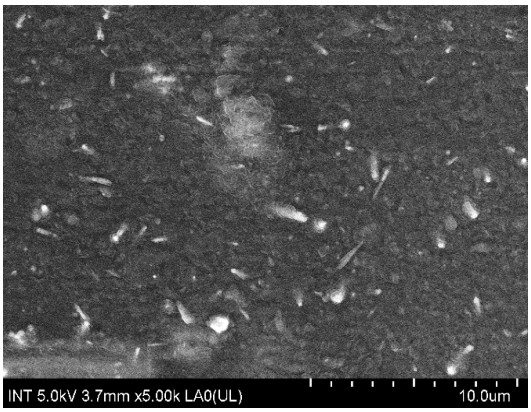

Figure 2: FE-SEM image of 1.0% chitosan membrane.

Table 1: Results of mechanical and physical properties of 1.0% chitosan films

	The present study	Other studies	References
Tensile strength (MPa)	21.95 ± 0.05	31.89	[23]
Elongation (%)	26.42 ± 0.23	30.51	
Moisture (%)	23.88 ± 1.39	24.29 ± 2.03	[7]
Solubility (%)	24.05 ± 1.20	17.52 ± 0.91	
Swelling (%)	216.4 ± 5.64	208	[24]
Transmittance (%)	86.18 ± 3.79	84	[26]

Effects of stirring time on the antibacterial ability of the membranes

During the chitosan membrane preparation, the mixture of chitosan and solvent underwent continuous stirring to ensure the proper solubility of chitosan in the solvent. Prolonged stirring resulted in enhanced homogeneity and smaller particle sizes. The diameters of the *E. coli* inhibition zone obtained through varying stirring times (20, 30, 40, and 50 minutes) are shown in Figure 1, with the 30-minute duration demonstrating the largest inhibition zone diameter. The *E. coli* antibacterial activity of chitosan was also reported by Leceta et al., using a chitosan concentration of 1.0% and a stirring period of 20 minutes at ambient temperature.[23] In this study, a stirring time of 30 minutes was chosen for further experiments.

Based on the *E. coli* antibacterial ability assessment results, the optimal parameters for membrane fabrication were determined as 1.0% chitosan concentration and 30 minutes of stirring time at 60°C with a continuous stirring speed of 200 rpm. In the present study, a 1% chitosan film was prepared to test its mechanical and physical properties.

Mechanical and physical properties of the chitosan membranes

Table 1 illustrates the mechanical properties of 1.0% chitosan films produced through a simple heating-stirring method. These physical and mechanical properties represent durability during food preservation. Tensile strength and elongation are commonly tested as the mechanical parameters for strength and maintainability used for packaged products. In comparison to the findings of Leceta *et al*., 1.0% chitosan membranes in this study exhibited relatively lower tensile strength and elongation.[23]

Films with low moisture content and solubility benefit food packaging and preservation, especially in high-humidity environments.[11] The moisture content and solubility results of chitosan films are detailed in Table 1.

Moisture and solubility

In the current study, the moisture content of the chitosan membrane was found to be 3.88 ± 1.39%, similar to a recent report in the literature,[11] while the solubility was at 24.05 ± 1.20%. These results demonstrated the presence of hydrogen bonding interactions between glycerol and chitosan during the biofilm formation.

Swelling

The swelling ability is a significant property of biofilms. Like other film-forming agents, the swelling behavior of chitosan within wet environments depends on the presence of hydrophilic groups and the degree of cross-linking between macromolecules. Owing to their rapid diffusion into the water, the membranes experience swell before they decompose. The results presented in Table 1 showed that the observed swelling closely resembles findings from another publication.[24]

Transmittance

The membranes or films intended for preservation must protect the food from light effects, especially UV radiation.[25] Light exposure can accelerate ripening and induce spoilage during storage. The transmittance of the chitosan membrane in the present study was approximately 80%, which aligns with previous findings by Gasti et al. for the transmittance of Poly (Vinyl Alcohol) films and Guar Gum at 600 nm.[26] Figure 2 depicts the transmission electron microscopy image of the 1.0% chitosan film. The film surface exhibited a relatively flat structure with no layering, air bubbles, and cracks. However, when the film surface was magnified, white particles remained visible and were not entirely dissolved into the solution. In general, the surface of 1.0% chitosan film displayed a stable structure in terms of shape and remained intact without fracture.

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Storage days	0	1	3	5	7
Undipped jackfruit samples					
1% Chitosan dipped jackfruit samples					

Figure 3: Fresh jackfruit after 7 days of cold storage at 10^0C: undipped and dipped in 1% chitosan.

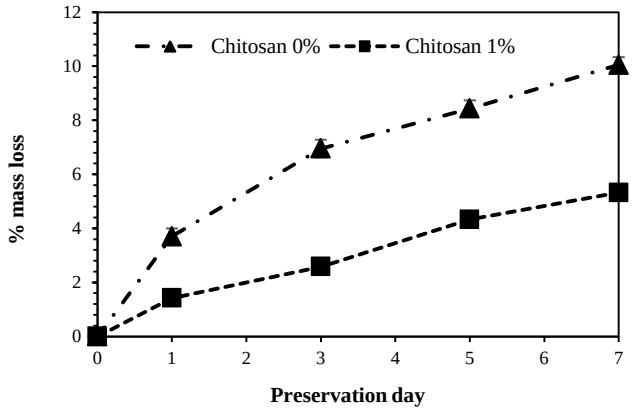

Figure 4: Jackfruit's mass loss after seven days of preservation

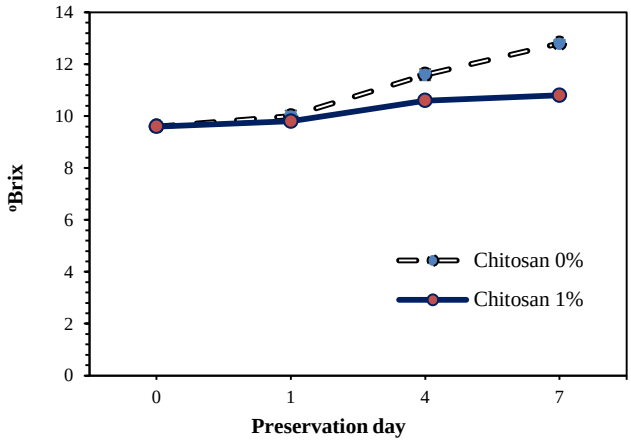

Figure 5: Total dissolved solid content of jackfruit after seven days of preservation.

Application of chitosan film in preserving Thai jackfruit
Sensory evaluation
Figure 3 and Table 2 present the sensory evaluation results for jackfruit during storage at 10^0C for seven days. The non-coated samples (without chitosan film) exhibited signs of rapid color changes compared to the coated samples (with chitosan film). Regarding fragrance and viscosity, the coated samples treated with 1.0% chitosan solution predominantly preserved their distinctive jackfruit flavor and retained their firm texture. There was no viscosity observed within the 7-day storage period. Meanwhile, the non-coated samples exhibited wrinkling, intensified fragrance, and increased viscosity for non-coated samples. The obtained results initially indicated the chitosan membrane's effectiveness in maintaining jackfruit quality during storage.

Mass loss
Figure 4 illustrates the gradual decline in the natural weight of jackfruit pulp over time during the storage period. However, the sample without chitosan protection had a higher mass loss than the sample protected with 1.0% chitosan. Submerging the jackfruit pulp in chitosan solution led to the surrounding membrane reducing the jackfruit pulp's transpiration rate and respiration. As a result, after a seven-day storage period, the unpreserved jackfruit pulp displayed a greater weight loss than the sample preserved with the optimized chitosan solution.

Total dissolved solids content
Ripening comprises changes related to the biochemical composition of the fruit, involving the conversion of starch into sugar and dehydration that increases the sugar concentration during fruit storage. Figure 5 illustrates the changes in jackfruit's total dissolved solids content (^{o}Brix). After seven days of storage, the control sample's total dissolved solid content showed a significant increase from 9.6 to 12.8, while the 1.0% chitosan-coated sample exhibited a more modest increase from 9.6 to 10.8. This result aligns with the study by Hong et al. on guava,[27] indicating that the total dissolved solid content of the guava coated with 1.0% chitosan displayed minimal increase throughout a storage period of 12 days. The chitosan coating slowed the metabolic and ripening processes. By covering the sample, the film reduced internal air volume by either reducing O_2 or increasing CO_2, thereby inhibiting ethylene growth.

Total aerobic microorganisms
Chitosan is widely used to extend the post-harvest shelf life of agricultural produce. This polysaccharide can form a semi-permeable coating on the product's surface, enhancing the product's quality by reducing transpiration, retaining moisture and fragrance, and preventing the penetration and growth of microorganisms. Figure 6 shows the outcomes concerning the total count of aerobic microorganisms in jackfruit. Undipped chitosan samples rapidly increased from $2.2x10^3$ to $5.4x10^4$ (CFU/mL) throughout one week's storage. Meanwhile, preserving jackfruit with a 1.0% chitosan solution has reduced microorganisms' growth. This result resonates with another report on maintaining sliced mangoes.[7]

Conclusion

Chitosan has been observed to yield positive effects on preserving quality, slowing down the spoilage process and thus significantly prolonging the shelf life of Thai jackfruit. The optimized parameters for membrane fabrication were as follows: a chitosan solution of concentration of 1.0%, 30% (w/v) glycerol, and a stirring time of 30

minutes at 60°C with a stirring speed of 200 rpm. The membrane exhibited a tensile strength of 21.95 MPa, an elongation rate of 26.42 ± 0.23%, a moisture content of 23.88 ± 1.39%, solubility of 24.05 ± 1.20%, swelling of 216.4 ± 5.64%, and transmittance of 86.18 ± 3.79%. Jackfruit cultivated in Vietnam was immersed in a 1% chitosan solution and stored for seven days. The jackfruit treated with chitosan retained its color, reduced weight loss, maintained sweetness, and had better firmness and shape than undipped samples. In addition, the total number of aerobic microorganisms found in the undipped sample on the final day of storage was twice that of the dipped sample (54000 *vs* 24800 CFU/mL, respectively).

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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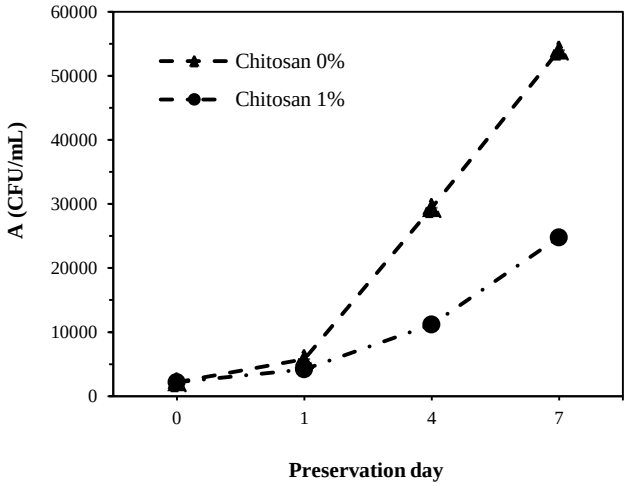

Figure 6: The total number of aerobic microorganisms after seven days of preservation.

Table 2: Sensory evaluation of jackfruit within seven days of preservation

Day	Color		Smell		Shape and Texture	
	Without chitosan film	Chitosan film	Without chitosan film	Chitosan film	Without chitosan film	Chitosan film
0	Glossy, yellow	Glossy, yellow	Faint flavor	Faint flavor	Firm texture, non-viscous	Firm texture, non-viscous
1	Light yellow	Glossy, yellow	Faint flavor	Specific jackfruit	Firm texture, non-viscous	Firm texture, non-viscous
3	Dark yellow	Light yellow	Sweet flavor	Specific jackfruit	A slightly soft, slightly viscous, wrinkles appeared	Firm texture, non-viscous
5	Dark yellow	Light yellow	Intense flavor	Sweet flavor	Soft, viscous, wrinkles appeared	Slightly soft, non-viscous, wrinkles appeared
7	Darker yellow	Dark yellow	Intense flavor	Sweet flavor	Soft, slightly viscous, wrinkles appeared	Slightly soft, non-viscous, wrinkles appeared

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