www.foodandfeed.fins.uns.ac.rs
FOOD AND FEED RESEARCH
Journal of the Institute of Food Technology – FINS
University of Novi Sad
ISSN
2217-5369
(print version ceased in 2023)
2217-5660 (online)

UDK 621.798.1:[664.2:547.995
https://doi.org/10.5937/foodandfeedresearch0-67013

Original research

ECO-FRIENDLY ANTIMICROBIAL BIODEGRADABLE FILMS SYNTHESIZED FROM PINEAPPLE LEAF AND JAPANESE SWEET POTATO SKIN STARCH FOR FOOD PACKAGING APPLICATIONS

Monica Muniendra Babu, Selvakumar Thanganadar Appapalam, Millicent Mabel*

Rajalakshmi Engineering College, Department of Biotechnology, Thandalam, Chennai-105, India

ONLINE FIRST

Abstract: Biodegradable starch-based films produced from agricultural waste represent a sustainable alternative to petroleum-derived food packaging materials. In this study, starch extracted from pineapple leaves (*Ananas comosus*) and Japanese sweet potato skins (*Ipomoea batatas*) was incorporated with chitosan to develop antimicrobial biodegradable films using a gel-casting technique. The prepared films were characterised for their mechanical, thermal, swelling, solubility, structural, morphological, antimicrobial, and food-preservation properties. From the various formulations, the 10% starch-chitosan PA-L film displayed the best performance, with an optimum tensile strength of 19.8 ± 1.2 MPa, elongation at break of 25.3 ± 1.5%, thermal resistance of 95 ± 2°C, swelling degree of 120 ± 3%, and water solubility of 28.7 ± 1.5%. X-ray diffraction and Fourier transform infrared spectroscopy confirmed the successful formation of a semi-crystalline chitosan–starch composite through intermolecular interactions, while scanning electron microscopy revealed compact and homogeneous film morphology. The PA-L film demonstrated higher antimicrobial activity, producing inhibition zones of 14, 10, 10, and 8 mm against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, respectively. In comparison, the JSP-S film exhibited inhibition zones of 12, 0, 6, and 6 mm against the respective bacterial strains. In the shelf-life studies, the optimised PA-L film confirmed an extended storage life of lemons and ivy gourds for 10 days under ambient storage conditions by reducing the moisture loss and delaying visible spoilage. Weight loss was reduced from 32.7% to 6.7% for lemons and from 32.3% to 11.4% for ivy gourds when wrapped with the PA-L film. These findings suggest that starch-based biodegradable films that are derived from agricultural waste like pineapple leaves and Japanese sweet potato skins and reinforced with chitosan could serve as a sustainable food packaging material capable of extending the shelf life and maintaining the quality of fresh food produce.

Keywords: *starch-chitosan composite films; shelf-life; food preservation; lemon; Coccinia grandis; agricultural waste*

INTRODUCTION

The global food industry is under increasing pressure to adopt sustainable packaging solutions due to mounting environmental concerns and the need to preserve food quality (Guillard et al., 2018). Conventional plastic packaging, although extensively used, has

Corresponding author: Phone: 9176170575
E-mail address: millicentmable.m@rajalakshmi.edu.in

created significant environmental challenges owing to its non-biodegradable nature. The increased use of conventional plastics has worsened the problem of environmental pollution and micro-plastics, thereby posing a critical need for environmentally friendly and sustainnable packaging materials based on agricultural waste with superior properties. Among natural biopolymers, starch has considerable attention due to its biodegradability, renewability, low cost, excellent film-forming properties, and complete decomposition by naturally existing microorganisms during composting for 30-90 days depending on the composition of films and environment (Nguyen et al., 2024). Another important biodegradable polymer with antimicrobial, biocompatible, non-toxic, and film-forming properties is chitosan. Chitosan is also degraded by microorganisms and enzymes in the soil or compost environments during 4-12 weeks without any toxic residues (Su et al., 2023). Thus, the combination of starch and chitosan provides an environmentally friendly material for packaging with improved functional properties and reduced environmental pollution. Consequently, researchers have been actively seeking biodegradable alternatives that not only minimise environmental impact but also maintain food safety and quality (Asgher et al., 2020). Biodegradable films derived from renewable agricultural resources have emerged as promising substitutes for synthetic plastics. These materials, including starch and chitosan, have shown exceptional potential for food packaging applications (Nilsen-Nygard et al., 2021). Starch, an abundantly available polysaccharide, is valued for its film-forming properties, low cost, and biodegradability. It can be extracted from diverse plant-based agricultural waste, making it an ideal candidate for eco-friendly packaging solutions. Chitosan, a deacetylated derivative of chitin, is the second most abundant polysaccharide in nature after cellulose. Renowned for its antimicrobial and biocompatible properties, chitosan enhances starch-based films by improving their mechanical strength, barrier properties, and microbial resistance (Goy et al., 2009). The antimicrobial activity of chitosan is mainly due to its polycationic properties, which cause disruption of microbial cell membranes, resulting in leakage of the contents inside the cell (Kong et al., 2010). Organic food, prized for its nutritional benefits, is particularly vulnerable to spoilage caused by chemical, physical, or microbial factors. Spoilage leads to changes in a food's colour, texture, edibility, and nutritional value, thereby impacting its safety and quality (Rahman et al., 2007). Indicators of spoilage include alterations in flavor, appearance, or scent, often driven by interlinked mechanisms. Key factors such as temperature, pH, nutrient availability, air exposure, and chemical interactions play a significant role in accelerating food spoilage (Amit et al., 2017). Effective food preservation techniques aim to mitigate these factors, thereby maintaining the natural flavour, texture, and nutritional integrity of food. This study leverages agricultural waste, specifically pine-apple leaves *(Ananas comosus)* and Japanese sweet potato skin *(Ipomoea batatas)*, as sources of starch for the development of biodegradable films. These agricultural by-products, often discarded, are rich in starch, which can be utilised to create biopolymers. Employing such waste materials aligns with sustainable practices and promotes a circular economy by transforming waste into valuable resources (Zolek-Tryznowska et al., 2021). By combining the extracted starch with chitosan, biodegradable films were developed that not only address food spoilage issues but also reduce dependency on synthetic polymers. The effectiveness of biodegradable films depends on their functional properties, such as mechanical strength, thermal resistance, water solubility, and antimicrobial activity (Abdollahi et al., 2012). Mechanical strength determines the durability and flexibility of the films, while thermal resistance ensures their performance under varying storage conditions. Water solubility and swelling behaviour influence the films' biodegradability and suitability for different food types, particularly those with high moisture content (Khodaei et al., 2021). Incorporating chitosan into starch-based films enhances their antimicrobial properties, offering protection against foodborne pathogens such as Bacillus *subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. This dual functionality of extending shelf life and reducing microbial contamination is crucial for the preservation of perishable products (Cagri-Mehmetoglu et al., 2004). Despite considerable progress in starch-based biodegradable films, most reported studies have focused on conventional starch sources or a single agricultural by-product, with limited emphasis on systematically comparing

ONLINE FIRST

different waste-derived starches under identical fabrication conditions. Furthermore, the mechanical, antimicrobial and practical food preservation performance is not well understood in terms of the relationship with the structural characteristics of these films (Ranganathan et al., 2020). Therefore, this study aims to utilize two underutilized agricultural waste, pineapple leaves (*Ananas comosus*) and Japanese sweet potato skins (*Ipomoea batatas*), as sources of starch to prepare chitosan-reinforced biodegradable films by a gel casting technique.

Physicochemical, mechanical, thermal, structural (FTIR, XRD and SEM) and antimicrobial characterization were used to make a comprehensive comparison of the two film systems, and it was validated in real food packaging applications with lemon and ivy gourd. The comprehensive method used helps in gaining better insights into how starch from agricultural wastes can serve as eco-friendly alternatives to conventional food packaging films (Luchese et al., 2018).

MATERIALS AND METHODS

Isolation of starch from plant waste

The pineapple leaves (PA-L) and Japanese sweet potato skins (JSP-S) that are considered as waste were collected from the local markets of Chennai, Tamil Nadu. They were thoroughly washed and rinsed in distilled water. Then PA-L and JSP-S were crushed into small pieces and soaked in double-distilled water separately for one day to extract starch. From the supernatant, starch was collected by precipitation, and it was subsequently dried in a conventional oven at 70 ºC (Saman et al., 2019).

Starch iodine test

The two extracts (PA-L and JSP-S) were subjected to Iodine Starch Test to confirm the presence of starch by adding 2-3 drops of iodine solution.

Preparation of biodegradable films incorporating PA-L and JSP-S starch extracts

Biodegradable films were prepared by varying the concentrations of starch and chitosan simultaneously at 4%, 8%, 10%, 12%, and 16% (w/v), maintaining an equal starch-to-chitosan ratio (4:4, 8:8, 10:10, 12:12, and 16:16). For each formulation, the required amount of PA-L or JSP-S starch was dispersed in distilled water, while chitosan was dissolved separately in 1% (v/v) acetic acid at pH 4.5 under continuous stirring for 1–2 h at 30 °C until a homogeneous solution was obtained. The starch and chitosan solutions were then mixed thoroughly to obtain a uniform film forming solution. Approximately 20 mL of the final solution was poured into a sterile 90 mm diameter Petri dish and dried at room temperature (25 ± 2 °C) for 24 h (Luchese et al., 2018). After drying, the films were carefully peeled from the casting plates and stored in a desiccator for further characterization studies.

Characterization of the biodegradable film

Mechanical properties

The thickness of the two synthesized PA-L and JSP-S starch extracts incorporated in films was measured using a micrometer instrument. Other physicochemical characteristics like tensile strength and elongation at break was also evaluated using universal testing machine (Shimadzu, Japan) following ASTM D882 standard in triplicates with a film size of 60 x 10 mm at a crosshead speed of 5 mm/min.

Stability of the biodegradable film

The thermal resistance of the synthesized PA-L and JSP-S biodegradable films was evaluated by visual observation using a hot air oven (Memmert UN55, Memmert GmbH+ Co. KG, Schwabach, Germany). Thermal resistance of the synthesized biodegradable polymer films of PA-L and JSP-S was carried out by treating the film samples (20×20 mm) with different temperatures (4, 10, 37, 90, 100, and 120 °C) using a hot air oven/incubator.

The films were kept at each of these temperatures for 30 min and then cooled to room temperature. Thermal resistance was determined by observation of the physical change in the films in terms of any shrinkage, deformation, cracks, colour change, brittleness, or disintegration. If the films maintained their original shape and flexibility without undergoing any visible change in their physical form, then the films were said to be thermally stable (Rhim et al., 2013).

Analysis of swelling and solubility

The prepared films were completely dried and cut into 10×10 mm dimensions. The cut films

ONLINE FIRST

were soaked in 30mL of distilled water in a sterile beaker covered with aluminium foil and left at room temperature overnight (Diaz-Cru et al., 2022). The following formula was used for calculating film solubility and swelling degree:

Film solubility = [(F1-F3)/F1] ×100
Swelling degree = [(F2-F1)/F1] ×100
where,
F1- Initial dry mass (g)
F2- Residual film pieces after discarding water (g)
F3- Final dry mass after overnight incubation (g)

Fourier Transform Infrared spectroscopic (FTIR) spectral analysis

Each film's Fourier Transform Infrared spectroscopic (FTIR) characterization was carried out using PerkinElmer Spectrum Two FTIR spectrometer (USA). The spectra range was between 400 and 4000 cm^{-1} with an average of 100 scans and the respective films were analysed and compared (Nguyen et al., 2024).

X-Ray Diffraction Method (XRD)

The crystallographic structures of the film were assessed by X-ray diffraction (XRD) using X-ray diffractometer (Seifert JSO-Debyeflex 2002). It was run at a wavelength of 1.5406Å under 40 kV, 30 mA, and Cu K radiation conditions. The film was lyophilized at 50 °C and then XRD pattern was scanned in two ranges of 35 ° to 70 ° at a step size of 0.04° per second (Nguyen et al., 2024).

Scanning Electron Microscope (SEM)

The surface morphology of the films was examined using Scanning Electron Microscopy (SEM, JEOL, JSM-IT300 JEOL Ltd., Tokyo, Japan). Prior to imaging, the samples were affixed onto double-sided adhesive tape and coated with a thin layer of gold via sputtering in a low vacuum chamber. SEM analysis was conducted at an accelerating voltage of 30 kV (Homez-Jara et al., 2018).

Antibacterial study by disc diffusion method

The antibacterial properties of the biode-gradable films PA-L and JSP-S against the four bacterial strains of food-spoiling bacteria, including *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa,* and *Escherichia coli*, were assessed via agar disc diffusion test using three replications. The films were prepared as sterile discs of 10 mm diameter and were kept at 80 °C for 10 min in the hot air oven before performing the experiment. The film's integrity was not altered by this method. Bacterial cultures were grown in 4 mL of peptone water at 25 °C with agitation until attaining approximately10^8 CFU/mL concentration. The suspension was evenly spread on separate Mueller–Hinton agar (MHA) plates by using sterile cotton swabs. Then, the sterilized film discs were placed on the MHA plates, where they had been inoculated. The streptomycin disc (10 µg/disc) was used as the positive control, whereas sterile distilled water was chosen as the negative control. Plates were incubated for 24 h at 37°C (Jatav et al., 2022). Antibacterial activity was estimated by measuring the diameter of the inhibition zone (mm).

Shelf-life analysis of biodegradable film

Fruits and vegetables of uniform size without any defect were selected from fresh *Citrus limon* (lemon) and *Coccinia grandis* (ivy gourd). Fruits and vegetables were thoroughly washed, surface sterilized with 70% ethanol, rinsed with sterilized distilled water, and air-dried.

Fruits and vegetables were divided randomly into three treatments (n=3): (i) control fruits and vegetables without wrapping, (ii) fruits and vegetables wrapped individually with PA-L starch-chitosan films and sealed, (iii) fruits and vegetables wrapped individually with JSP-S starch-chitosan films and sealed. The wrapped fruits and vegetables were stored for 10 days under ambient laboratory condi-tions (25±2°C) with relative humidity of 62%.

Regular observations on physical quality change like colour change, firmness, shrinking, and visual deterioration were recorded. Percentage weight loss was estimated by taking the difference in weight before and after storage. Shelf life was determined based on physical appearance and weight loss percentage of wrapped and unwrapped fruits and vegetables (Ungureanu et al., 2023).

Statistical Analysis

All experiments were carried out in triplicates (n=3), and the data is expressed as mean ± standard deviation (SD) values. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to check for significant differences

ONLINE FIRST

between groups. A value of $p<0.05$ was considered significant (Montgomery et al., 2019).

RESULTS AND DISCUSSION

PA-L and JSP-S biodegradable film formulation

The extraction process yielded approximately 0.7 g of starch from JSP-S and d 0.9 g from PA-L, indicating a moderately effective recovery method, where PA-L exhibited a higher yield compared to JSP-S. The comparatively lower starch yield obtained from pineapple leaves and sweet potato skins could be due to the highly fibrous structure of these plant materials, along with the use of a basic water-based extraction process without the application of enzymatic or alkaline pretreatment. The isolated starches were incorporated into a chitosan biopolymer matrix to develop a biodegradable film. The starch–chitosan mixtures at various w/v % were stirred continuously for 2 hours to achieve a uniform and viscous solution, following a method similar to that described by Saman et al. (2019). The resulting homogeneous mixtures were processed using the gel casting technique to form bio-degradable film.

The compatibility between starch and chitosan resulted in uniform and flexible film structures. This outcome is consistent with the findings of Sun et al. (2017), who high-lighted the role of starch in enhancing the plasticity and film forming ability of chitosan-based materials.

The improved texture and handling properties of the films can be attributed to the intermolecular interactions between the hydroxyl groups of starch and the amino groups of chitosan. The physical appearance and integrity of the developed biodegradable film are presented in Fig. 1 a, b.

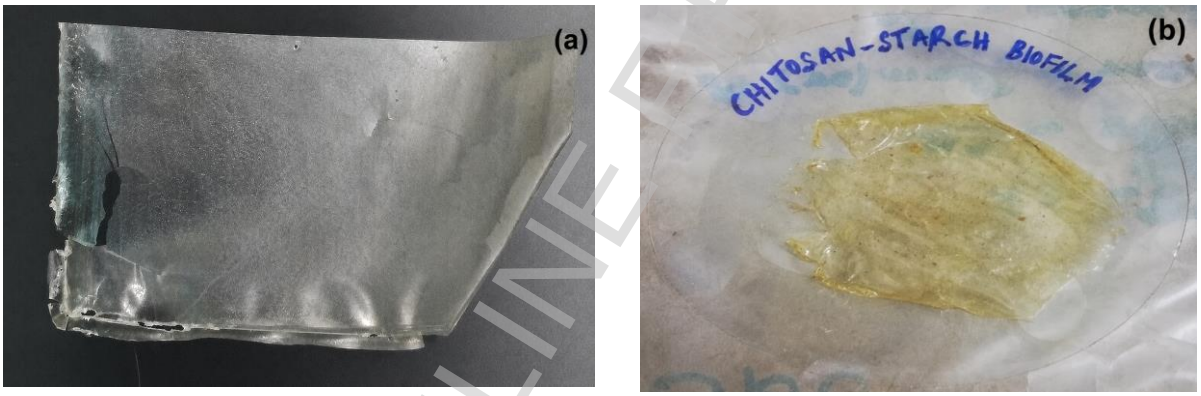


Figure 1. Biodegradable film prepared using **(a)** Pineapple leaf (PA-L); **(b)** Japanese sweet potato (JSP-S)

Table 1.
Mechanical, stability, and swelling properties of PA-L & JSP-S starch-based films

Chitosan (%)	Starch (%)	Tensile strength (MPa)		Elongation at break (%)		Thermal resistance (°C)		Swelling degree (%)		Solubility (%)	
		PA-L	JSP-S	PA-L	JSP-S	PA-L	JSP-S	PA-L	JSP-S	PA-L	JSP-S
4	4	12.4 ± 0.8^{d}	11.8 ± 0.7^{d}	18.2 ± 1.1^{d}	17.5 ± 1.0^{d}	82 ± 2^{d}	80 ± 2^{d}	145 ± 5^{b}	148 ± 5^{b}	38.5 ± 2.1^{b}	39.2 ± 2.0^{b}
8	8	15.6 ± 1.0^{b}	14.9 ± 0.9^{b}	21.5 ± 1.3^{b}	20.3 ± 1.2^{b}	88 ± 3^{b}	86 ± 2^{b}	132 ± 4^{c}	136 ± 4^{c}	32.4 ± 1.8^{c}	34.8 ± 1.7^{c}
10	10	19.8 ± 1.2^{a}	18.6 ± 1.1^{a}	25.3 ± 1.5^{a}	23.9 ± 1.4^{a}	95 ± 2^{a}	92 ± 2^{a}	120 ± 3^{d}	125 ± 3^{d}	28.7 ± 1.5^{d}	30.5 ± 1.6^{d}
12	12	16.2 ± 0.9^{b}	15.8 ± 0.8^{b}	20.1 ± 1.2^{c}	19.7 ± 1.1^{c}	90 ± 2^{b}	88 ± 3^{b}	135 ± 4^{c}	138 ± 5^{c}	34.2 ± 1.7^{c}	35.9 ± 1.8^{c}
16	16	13.5 ± 0.7^{c}	13.1 ± 0.6^{c}	17.8 ± 1.0^{d}	16.9 ± 0.9^{d}	85 ± 3^{c}	83 ± 2^{c}	150 ± 6^{a}	152 ± 6^{a}	40.6 ± 2.3^{a}	41.3 ± 2.2^{a}

**a = Highest mean value, b = Second highest mean value, c = Third highest mean value, d = Lowest mean value*

ONLINE FIRST

Table 2.
One-way ANOVA results for the mechanical and structural properties of PA-L and JSP-S starch-based biodegradable films

Parameter	PA-L F-value	PA-L p-value	JSP-S F-value	JSP-S p-value
Tensile strength	42.67	<0.001	39.82	<0.001
Elongation at break	31.84	<0.001	28.51	<0.001
Thermal resistance	26.11	<0.001	24.37	<0.001
Swelling degree	57.98	<0.001	53.14	<0.001
Solubility	44.53	<0.001	41.76	<0.001

Mechanical and structural properties of the biodegradable film

The mechanical properties of the film have shown substantial dependence on polymer concentration as well as on the type of starch used. Table 1 shows that, for both films, tensile strength increases with an increase in chitosan-starch concentration up to 10%, beyond which tensile strength decreases. Notably, PA-L film show higher tensile strength at 10% concentration, i.e., 19.8 ± 1.2 MPa, as compared to JSP-S film, which has a tensile strength of 18.6 ± 1.1 MPa at 10% concentration. This indicates that PA-L film has stronger intermolecular interactions as compared to JSP-S film. A similar observation was noted for elongation at break, where PA-L film has higher elongation at break as compared to JSP-S film. PA-L film has higher flexibility at 10% concentration, i.e., 25.3 ± 1.5%, as compared to JSP-S film, which has 23.9 ± 1.4% (Bof et al., 2015).

Thermal resistance analysis confirmed that PA-L film has higher thermal resistance at 10% concentration, i.e., 95 ± 2 °C, compared to JSP-S film, which has stability at 92 ± 2 °C that could be due to stronger hydrogen bonds. In addition to this, swelling degree and solubility also decreased with an increase in concentration up to 10%, which indicated improved water uptake and moisture resistance owing to the increased density of cross-linking. The lowest solubility was also recorded in PA-L film (28.7 ± 1.5%), followed by JSP-S film (30.5 ± 1.6%), which also indicated improved barrier characteristics in PA-L biodegradable film. However, at concentrations above the optimal concentration (≥12%), both PA-L and JSP-S films showed a reduction in tensile strength and elongation along with an improvement in swelling and solubility. These results are consistent with previous reports suggesting that the interaction of chitosan-starch plays an important role in the improvement of the film's mechanical strength and also showed reduced tensile strength and flexibility due to the disruption of the polymer structure by the starch component (Thakhiew et al., 2015). The effect of varying chitosan-starch concentration was statistically significant, as observed from one-way ANOVA (Table 2). Of all the formulations used in the experiment, the 10% concentration had the highest tensile strength, elongation, and thermal resistance while having the lowest swelling degree and solubility.

Antimicrobial analysis

The antimicrobial activity of biodegradable films was assessed against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa*. Streptomycin (20 ,µg/mL) was used as the positive control, while sterile discs without film served as the negative control. For PA-L film, the ZOI diameters were 14 mm for *B. subtilis*, 8 mm for *S. aureus*, and 10 mm each for *E. coli* and *P. aeruginosa* (Jatav et al., 2022). In contrast, JSP-S film exhibited lower ZOI values, measuring 12 mm for *B. subtilis*, 6 mm for *S. aureus*, and 6 mm for *P. aeruginosa*, with no inhibition against *E. coli* (Table 3). These findings align with earlier research on chitosan's antimicrobial properties, which are influenced by its molecular weight, degree of deacetylation, and the strain of microorganisms. The antimicrobial effect of the chitosan–starch films can largely be attributed to the polycationic characteristics of chitosan. The positively charged amino groups present in chitosan interact with the negatively charged components of bacterial cell membranes, which disrupts membrane integrity. This interaction enhances membrane permeability, causing leakage of cellular contents and ultimately resulting in bacterial cell death. Chitosan with a high degree of deacetylation was found to disrupt bacterial cell membranes by interacting with phospholipids, increasing permeability, and causing cell damage (Goy et al., 2009). Both Gram-positive and Gram-negative bacteria showed sensitivity to chitosan in this study, similar to the previous studies, which also have

ONLINE FIRST

Table 3.
Antibacterial activity of PA-L and SP-S starch-based biodegradable films against selected foodborne bacteria compared with positive and negative controls

Bacterial strain	Negative control (Sterilized water) (mm)	Positive control (Streptomycin 20 µg/disc) (mm)	PA-L film (mm)	SP-S film (mm)
Bacillus subtilis	0	30	14	12
Staphylococcus aureus	0	19	8	6
Escherichia coli	0	30	10	0
Pseudomonas aeruginosa	0	17	10	6

reported mixed results, with some demonstrating stronger effects against Gram-positive bacteria like *S. aureus* depending on chitosan's structural properties. The absence of *E. coli* inhibition in JSP-S film suggests that the antimicrobial effectiveness of these films may also depend on the composition of the starch extracts, the medium, and the concentration of active components (Ardila et al., 2017). These findings highlight the variability of chitosan's antimicrobial activity and the critical role of incorporated biopolymers in determining the overall effectiveness of biodegradable films. All the measurements were done in triplicate, and the results are expressed as mean ± standard deviation. Significant differences ($p < 0.05$) were observed between the control and treated samples.

Phase identification

The phase of the biodegradable films was identified using XRD (X-ray diffraction) (Fig. 2a). Both samples F1 and F2 (PA-L and JSP-S) show a broad peak at 22 ° and 23 ° implying the crystalline structure of starch integrated into the chitosan matrix. These peaks are primarily linked to the retrogradation of starch, a phenomenon where gelatinized starch reorganizes into a more ordered crystalline structure over time. Supporting this observation, Hatna et al., 2004 reported that incorporating PVA into starch films altered their crystalline regions, producing peaks between 20 ° and 25°, which were attributed to interactions between starch and PVA as well as retrogradation. Cellulose-starch blends exhibited XRD peaks between 18 ° and 22 °, indicating partial crystallinity arising from retrogradation and polymeric interactions (Baseer et al., 2022). Highest peak at 22 ° and 23 ° corresponds to the crystalline of starch in chitosan matrix (Diaz-Cruz et al., 2022). Notably, the retrogradation of starch-based chitosan biopolymers accelerates under high relative humidity (RH), leading to a decline in mechanical performance (Razali et al., 2019).

Functional group analysis

The FTIR analysis of the chitosan–starch biodegradable films (Figure 2b1 & 2b2) confirm successful blending and strong intermolecular interactions between the components. A broad band observed at ~3297 cm^{-1} corresponds to O–H stretching vibrations of hydroxyl groups present in both chitosan and starch, indicating extensive hydrogen bonding that contributes to the stability and hydrophilic nature of the composite. The peak at ~2920 cm^{-1} is attributed to C–H stretching vibrations of aliphatic chains, confirming the organic backbone of the matrix. The band observed around 1652–1654 cm^{-1} corresponds to the amide I (C=O stretching) of chitosan, indicating structural integrity of the polymer within the blend. A peak near 1739 cm^{-1} suggests the presence of carbonyl groups, which may arise from interactions between starch and chitosan or residual acetyl groups. The absorption bands at ~1411–1415 cm^{-1} and ~1154 cm^{-1} correspond to C–H bending and glycosidic linkages (C–O–C), respectively, confirming the presence and preservation of polysaccharide structures. Additionally, the strong band around ~1080 cm^{-1} is associated with C–O stretching vibrations of alcohol and ether groups, reflecting characteristic starch functionalities. The peaks observed near ~896–900 cm^{-1} and ~669–670 cm^{-1} are attributed to vibrations of the polysaccharide backbone and C–H bending, further confirming structural stability. Overall, the FTIR spectra confirm the successful formation of a chitosan–starch composite with preserved functional groups and strong hydrogen bonding interactions. The presence of a carbonyl peak around 1739 cm^{-1} suggests possible esterification or intermolecular interactions between hydroxyl and acetyl groups, contributing to improved structural properties (Xu et al., 2024). These findings are

ONLINE FIRST

consistent with previous reports by Del et al. (2020), which also highlighted hydrogen bonding and compatibility in chitosan–starch films. Similar improvements in functional properties due to polysaccharide blending were reported by Abdullah et al., 2023, while Wu et al., 2021 further confirmed the stability and synergistic behaviour of such biopolymer composites. The O-H and amide shifts seen indicate strong intermolecular hydrogen bonding, which plays a key role in contributing to increased mechanical stability and decreased solubility of the films.

Scanning Electron Microscope (SEM)

The SEM analysis of the biodegradable film shows a smooth, reasonably uniform matrix with slight surface imperfections (Figure 2C1 & 2C2). The image, which was taken at a working distance of 11.5 mm and a magnification of 20,000x utilizing secondary electron detection at an accelerating voltage of 30.00 kV, demonstrates the smooth integration of the starch and chitosan components inside the film structure. Strong connections between the chitosan and starch polymer chains are suggested by the dense and compact arrangement, which results in the creation of a durable biodegradable film matrix. The possible mechanical stability and barrier qualities of the film are connected to this structural homogeneity. Effective cross-linking or blending of the components is indicated by the absence of large cracks or gaps in the matrix, which may improve adhesion and resistance to environ-mental influences (Nguyen et al., 2024). Additionally, the surface characteristics point to continuous porosity, which could be important for uses like biodegradable packaging, antimicrobial coatings, and controlled drug release (Kraemer et al., 2025). These results are consistent with previous research on polysaccharide based biodegradable film, which highlights the structures' capacity to construct cohesive, flexible structures with a variety of uses.

Shelf-life study of fruits and vegetables using the biodegradable films

The biodegradable films demonstrated exceptional effectiveness in preserving the quality of lemon and Ivy gourd over a 10-day storage period, as shown in Fig. 3. Lemon, which is highly susceptible to pressure damage and rapid spoilage due to mould, and Ivy gourd, which typically has a short postharvest lifespan of 2–3 days, exhibited a significant extension in freshness with this biodegradable film. The wrapped fruit retained their natural colour, texture, and overall integrity without visible signs of deterioration.

ONLINE FIRST

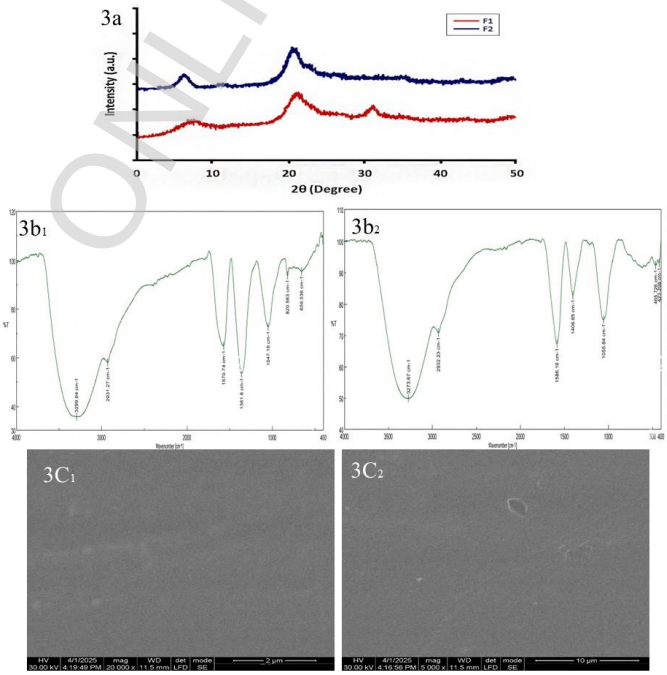


Figure 2. Structural and morphological characterization of the biodegradable film: XRD pattern (3a), FTIR spectra (3b1, 3b2), and FE-SEM images (3C1, 3C2)

In contrast, control samples showed spoilage by the third day, with lemon developing browning and rot, while Ivy gourd experienced extensive mould and yeast growth. Furthermore, weight loss percentages were calculated for both Ivy gourd and lemon with and without the wrapping (Fig. 4). The wrapped fruit and vegetable demonstrated significantly lower weight loss percentages compared to their counterparts. The rate of decay was substantially higher in fruit that was not wrapped with the film, as it exhibited a more rapid evaporation of water, which is summarised in Table 4.

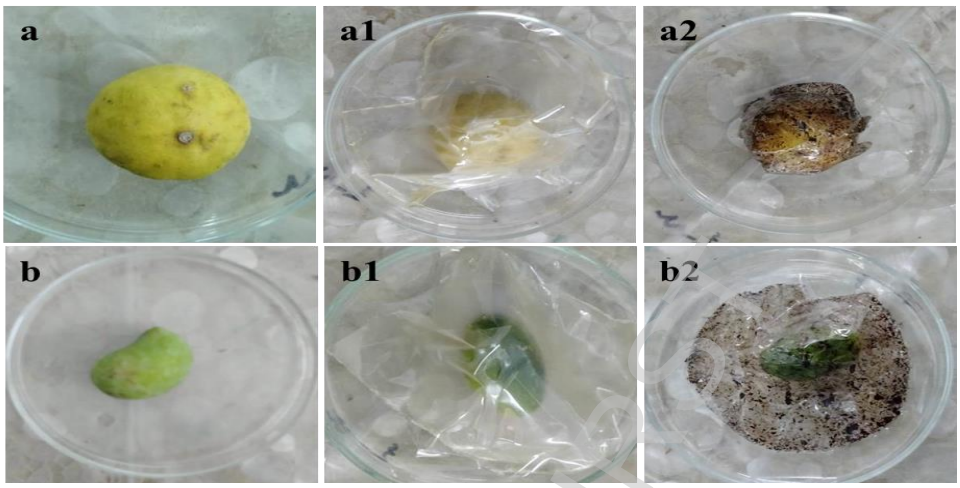


Figure 3. Comparative analysis of lemon and ivy gourd preservation: Images of lemon (a) and ivy gourd (b) on day 1: control samples (a, b), lemon and ivy gourd with PA-L starch-based film (a1, b1), and lemon and ivy gourd with JSP-S starch-based film (a2, b2)

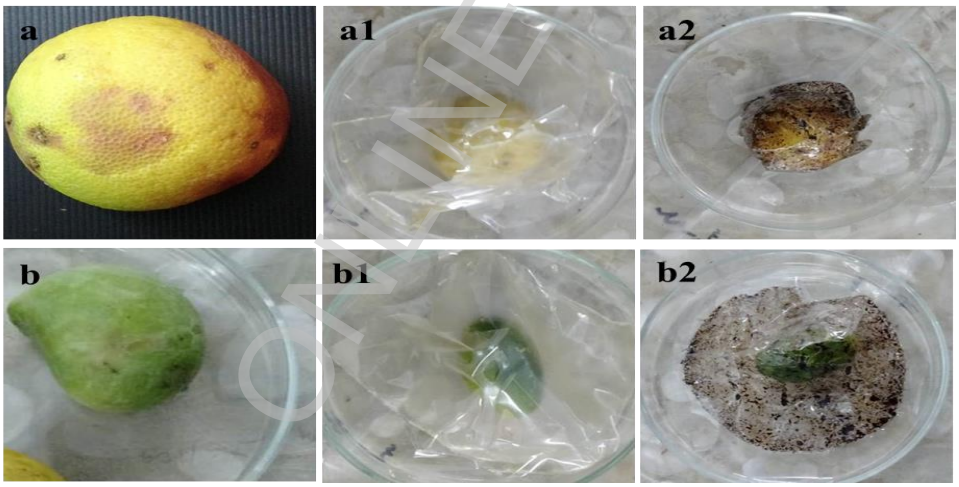


Figure 4. Preservation effect of biodegradable films after 10 days of storage: Images of lemon (a) and ivy gourd (b) after 10 days of storage at room temperature (25 ± 2 °C): control samples (a, b), lemon and ivy gourd wrapped with PA-L starch-based film (a1, b1), and lemon and ivy gourd wrapped with JSP-S starch-based film (a2, b2)

Table 4.
Effect of PA-L and JSP-S biodegradable film wrapping on weight loss during storage

Fruit/vegetable	Treatment	Initial weight (g)	Final weight (g)	Weight loss percentage (%)
Lemon	Control (Fig a)	29.89	20.12	32.7
	PA-L film (Fig a1)	30.23	28.20	6.7
	JSP-S film (Fig a2)	30.10	28.06	6.8
Ivy gourd	Control film (Fig b)	11.29	7.64	32.3
	PA-L film (Fig b1)	10.36	9.18	11.4
	JSP-S film (Fig b2)	11.24	9.85	12.36

Table 5.
Visual shelf-life assessment of lemon and ivy gourd wrapped with PA-L and JSP-S biodegradable films during storage at room temperature (25 ± 2 °C)

Storage days	Control		PA-L Film		JSP-S Film	
	Lemon	Ivy gourd	Lemon	Ivy gourd	Lemon	Ivy gourd
Day 1	+++++	+++++	+++++	+++++	+++++	+++++
Day 3	++++	++++	+++++	++++	+++++	++++
Day 5	+++	++	++++	+++	++++	+++
Day 7	++	+	+++	+++	+++	++
Day 10	+	+	+++	+++	+++	++

+++++ *Excellent;* ++++ *Very Good;* +++ *Good;* ++ *Average;* +*Poor*

This further confirms that this film iseffectively limiting the rate of gaseous exchange, and the hydrophilic nature of the film works as a moisturiser and prevents the water content from evaporating. The minimal weight loss observed in the wrapped fruit highlights the films' ability to prevent moisture loss and serves as an effective barrier against microbial contamination. These results align with previous research demonstrating the efficacy of biopolymer-based films, including chitosan, starch, and alginate, in inhibiting microbial growth and prolonging the shelf life of perishable produce such as tomatoes, strawberries, and cucumbers (Ungureanu et al., 2023). The antimicrobial properties of the biodegradable film, attributed to the capacity to disrupt microbial cell membranes, further contributed to decay prevention (Aloui et al., 2016). Visual Shelf-Life Assessment of lemon and ivy gourd wrapped with PA-L and JSP-S biodegradable films during storage at room temperature (25 ± 2 °C) (Table 5). The ability of these biodegradable films to maintain the freshness of lemon and Ivy gourd for up to 10 days underscores their potential as an eco-friendly and sustainnable alternative to synthetic preservatives.

By reducing food spoilage and extending the postharvest life of perishable fruits and vegetables, these biodegradable films offer a promising solution for minimising food waste and promoting environmentally responsible agricultural practices.

Barrier properties of biodegradable films

Film barrier performance like water vapour per-meability, was interpreted theoretically only using solubility and morphology of the film. Generally, starch-based films have high water vapour permeability owing to their hydrophilic characteristics. However, by adding chitosan, it is possible to improve the film barrier properties since it forms a denser polymer structure through hydrogen bonding. The dense film structure, as inferred from SEM for morphology, is likely to have less free volume for water vapour diffusion (Homez-Jara et al., 2018). At 10% w/v, it is possible that the film will have less solubility and hence less water vapour transmission. However, owing to its hydrophilic characteristics, it is probable that some permeability will still be achieved, especially in high humidity conditions.

CONCLUSION

The present study demonstrates the successful development of biodegradable starch-based composite films from pineapple leaf (PA-L) and Japanese sweet potato skin (JSP-S) agricultural wastes reinforced with chitosan for potential food-packaging applications. The developed films exhibited favourable mechanical properties, thermal resistance, and antimicrobial activity against selected foodborne microorganisms. Among the formulations investigated, the optimised films showed enhanced tensile strength, reduced water solubility, and improved structural integrity, indicating the beneficial role of chitosan in strengthening the polymer matrix. Furthermore, the films help to preserve the quality of lemon and ivy gourd for up to 10 days under ambient storage conditions, demonstrating their potential to reduce microbial spoilage and prolong the shelf life of fresh produce. The utilization of agricultural waste derived starch not only provides an eco-friendly and value-added approach for biomass utilization but also contributes to the development of sustainable packaging materials. The synergistic combination of starch and chitosan offers improved physicochemical and antimicrobial properties, making these biodegradable films promising candidates for food-packaging applications. Further optimisation and validation under prac-

ONLINE FIRST

tical packaging conditions will facilitate their future application as sustainable alternatives to conventional petroleum-based packaging materials.

AUTHOR CONTRIBUTIONS

Conceptualization, data curation, methodology, investigation, project administration, writing – review & editing, M.M.B..; Conceptualization, methodology, writing – review, M.M.; Resources, visualization, methodology, formal analysis, S. K. T. A.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

ACKNOWLEDGEMENTS

The authors would like to thank the biotechnology department, authorities, staff, and students for their assistance with the research.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

REFERENCES

Abdollahi, M., Rezaei, M., & Farzi, G. (2012). A novel active bionanocomposite film incorporating rosemary essential oil and nanoclay into chitosan. *Journal of Food Engineering, 111*, 343–350. https://doi.org/10.1016/j.jfoodeng.2012.02.012

Abdullah, N., Sahudin, S., & Kaharudin, N. (2023). Exploring the role of chitosan in fabricating biodegradable films for functional food packaging: a review. *Journal of Young Pharmacists, 15*, 64–73. https://doi.org/10.5530/097515050505

Aloui, H. & Khwaldia, K. (2016). Natural antimicrobial edible coatings for microbial safety and food quality enhancement. *Comprehensive Reviews in Food Science and Food Safety, 15*, 1080–1103. https://doi.org/10.1111/1541-4337.12226

Amit, S.K., Uddin, M.M., Rahman, R., Islam, S.M.R., & Khan, M.S. (2017). A review on mechanisms and commercial aspects of food preservation and processing. *Agriculture & Food Security, 6*, 51. https://doi.org/10.1186/s40066-017-0130-8

Ardila, N., Daigle, F., Heuzey, M.C., & Ajji, A. (2017). Effect of chitosan physical form on its antibacterial activity against pathogenic bacteria. *Journal of Food Science, 82*, 1–9. https://doi.org/10.1111/1750-3841.13635

Asgher, M., Qamar, S.A., Bilal, M., & Iqbal, H.M.N. (2020). Bio-based active food packaging materials: sustainable alternative to conventional petrochemical-based packaging materials. *Food Research International, 137*, 109625. https://doi.org/10.1016/j.foodres.2020.109625

Baseer, R.A., Dacrory, S., El Gendy, M.A.M., Ewies, F.E., & Kamel, S. (2022). A biodegradable film based on cellulose and thiazolidine bearing UV shielding property. *Scientific Reports, 12*, 7887. https://doi.org/10.1038/s41598-022-11457-5

Bof, J., Bordagaray, V., Locaso, D., & García, M. (2015). Chitosan molecular weight effect on starch–composite film properties. *Food Hydrocolloids, 51*, 1–10. Chitosan molecular weight effect on starch–composite film properties https://doi.org/10.1016/j.foodhyd.2015.05.018

Çagri-Mehmetoglu, A., Ustunol, Z., & Ryser, E.T. (2004). Antimicrobial edible films and coatings. *Journal of Food Protection, 67*, 833–848. https://doi.org/10.4315/0362-028X-67.4.833

Del Rosario Salazar Sánchez, M.H., Montoya, J.A.C., Castillo, H.S.V., Duque, J.F.S., Herrera, R.R., & Belmotes, F.A. (2020). Biogenerated polymers: an environmental alternative. *DYNA, 87*, 75–84. https://doi.org/10.15446/dyna.v87n214.82163

Díaz-Cruz, C.A., Caicedo, C., Jiménez-Regalado, E.J., Díaz de León, R., López-González, R., & Aguirre-Loredo, R.Y. (2022). Evaluation of the antimicrobial, thermal, mechanical, and barrier properties of corn starch–chitosan biodegradable films reinforced with cellulose nanocrystals. *Polymers, 14*, 2166. https://doi.org/10.3390/polym14112166

Goy, R.C., de Britto, D., & Assis, O.B.G. (2009). A review of the antimicrobial activity of chitosan. *Polímeros, 19*, 241–247. https://doi.org/10.1590/S0104-14282009000300013

Guillard, V., Gaucel, S., Fornaciari, C., Angellier-Coussy, H., Buche P., & Gontard, N. (2018). The next generation of sustainable food packaging to preserve our environment in a circular economy context. *Frontiers in Nutrition, 5*, 121. https://doi.org/10.3389/fnut.2018.00121

Hatna, S., Raj, B., & Rudrappa, S. (2004). Structure–property relation in polyvinyl alcohol/starch composites. *Journal of Applied Polymer Science, 91*, 630–635. https://doi.org/10.1002/app.13194

Homez-Jara, A., Daza, L.D., Aguirre, D.M., Muñoz, J.A., Solanilla, J.F., & Váquiro, H.A. (2018). Characterization of chitosan edible films obtained with various polymer concentrations and drying temperatures. *International Journal of Biological Macromolecules, 113*, 1233–1240. https://doi.org/10.1016/j.ijbiomac.2018.03.057

Jatav, J., Tarafdar, A., Saravanan, C., & Bhattacharya, B. (2022). Assessment of antioxidant and antimicrobial property of polyphenol-rich chitosan–pineapple peel film. *Journal of Food Quality, 2022*, 8064114. https://doi.org/10.1155/2022/8064114

Khodaei, D., Álvarez, C., & Mullen, A.M. (2021). Biodegradable packaging materials from animal processing co-products and wastes: an overview. *Polymers, 13*, 2561. https://doi.org/10.3390/polym13152561

Kong, M., Chen, X.G., Xing, K., & Park, H.J. (2010). Antimicrobial properties of chitosan and mode of action: A state of the art review. *International Journal of Food Microbiology, 144*, 51–63. https://doi.org/10.1016/j.ijfoodmicro.2010.09.012

Kraemer, M. V. S., Fernandes, A. C., Chaddad, M. C. C., Uggioni, P. L., Bernardo, G. L., & Proença, R. P. C. (2023). Is the List of Ingredients a Source of

ONLINE FIRST

Nutrition and Health Information in Food Labeling? A Scoping Review. *Nutrients*, *15*(21), 4513. https://doi.org/10.3390/nu15214513

Luchese, C.L., Pavoni, J.M.F., dos Santos, N.Z., Quines, L.K., Pollo, L.D., Spada, J.C., & Tessaro, I.C. (2018). Effect of chitosan addition on the properties of films prepared with corn and cassava starches. *Journal of Food Science and Technology*, *55*, 2963–2973. https://doi.org/10.1007/s13197-018-3214-y

Montgomery, D.C. (2019). *Design and Analysis of Experiments* (10th ed.). John Wiley & Sons, Inc.

Nguyen, V.N., Wang, S., Nguyen, T.H., Nguyen, V.B., Doan, M.D., & Nguyen, A.D. (2024). Preparation and characterization of chitosan/starch nanocomposites loaded with ampicillin to enhance antibacterial activity against *Escherichia coli*. *Polymers*, *16*, 2647. https://doi.org/10.3390/polym16182647

Nguyen, M.T.P., Escribà-Gelonch, M., Hessel, V., & Coad, B.R. (2024). A review of the current and future prospects for producing bioplastic films made from starch and chitosan. *ACS Sustainable Chemistry & Engineering*, *12*, 1750–1768. https://doi.org/10.1021/acssuschemeng.3c06094

Nilsen-Nygaard, J., Fernández, E.N., Radusin, T., Rotabakk, B.T., Sarfraz, J., Sharmin, N., Sivertsvik, M., Sone, I., & Pettersen, M.K. (2021). Current status of biobased and biodegradable food packaging materials: impact on food quality and effect of innovative processing technologies. *Comprehensive Reviews in Food Science and Food Safety*, *20*, 1333–1350. https://doi.org/10.1111/1541-4337.12715

Rahman, M.S. (2007). *Handbook of Food Preservation* (2nd ed.). CRC Press. https://doi.org/10.1201/9781420017373

Ranganathan, S., Dutta, S., Moses, J.A., & Anandharamakrishnan, C. (2020). Utilization of food waste streams for the production of biopolymers. *Heliyon*, *6*, e04891. https://doi.org/10.1016/j.heliyon.2020.e04891

Razali, M.H., Ismail, N.A., & Mat Amin, K.A. (2019). Fabrication and characterization of antibacterial titanium dioxide nanorods incorporating gellan gum films. *Journal of Pure and Applied Microbiology*, *13*, 1909–1916. .https://doi.org/10.22207/JPAM.13.4.03

Rhim, J.W., Park, H.M., & Ha, C.S. (2013). Bio-nanocomposites for food packaging applications. *Progress in Polymer Science*, *38*, 1629–1652. https://doi.org/10.1016/j.progpolymsci.2013.05.008

Saman W., Yuliasih I., & Poniso S. (2019). Dual modification of sweet potato crosslinking–hydroxypropylation as biodegradable film materials. *IOP Conference Series: Earth and Environmental Science*, *399*, 012060. https://doi.org/10.1088/1755-1315/399/1/012060

Su, C.Y., Li, D., Wang, L.J., & Wang, Y. (2023). Biodegradation behavior and digestive properties of starch-based film for food packaging – A review. *Critical Reviews in Food Science and Nutrition*, *63*, 6923–6945. https://doi.org/10.1080/10408398.2022.2036097

Sun, L., Sun, J., Chen, L., Niu, P., Yang, X., & Guo,Y. (2017). Preparation and characterization of chitosan film incorporated with thinned young apple polyphenols as an active packaging material. *Carbohydrate Polymers*, *163*, 81–91. https://doi.org/10.1016/j.carbpol.2017.01.016

Thakhiew, W., Champahom, M., Devahastin, S., & Soponronnarit, S. (2015). Improvement of mechanical properties of chitosan-based films via physical treatment of film-forming solution. *Journal of Food Engineering*, *158*, 1–10. https://doi.org/10.1016/j.jfoodeng.2015.02.027

Ungureanu, C., Tihan, G., Zgârian, R., & Pandelea, G. (2023). Bio-coatings for preservation of fresh fruits and vegetables. *Coatings*, *13*, 1420. https://doi.org/10.3390/coatings13081420

Wu, S., Wang, W., Zhang, R., Zhai, X., & Hou, H. (2021). Preparation and characterization of biodegradable trilayer films based on starch and polyester. *International Journal of Biological Macromolecules*, *183*, 1–10. https://doi.org/10.1016/j.ijbiomac.2021.05.051

Xu, L., Jiang, X., Zhao, Y., & Xia, L. (2024). Preparation of starch-based biodegradable film and the application in agriculture. *Journal of Agricultural Science*, *9*, 1. https://doi.org/10.5539/jas.v9n3p1

Zołek-Tryznowska, Z. & Kałuża, A. (2021). The influence of starch origin on the properties of starch films: packaging performance. *Materials*, *14*, 1146. https://doi.org/10.3390/ma14051146

ONLINE FIRST

EKOLOŠKI PRIHVATLJIVI ANTIMIKROBNI BIORAZGRADIVI FILMOVI SINTETISANI OD SKROBA IZ LISTA ANANASA I KORE JAPANSKOG SLATKOG KROMPIRA ZA PRIMENU U PAKOVANJU HRANE

Monica Muniendra Babu, Selva Kumar Thanganadar Appapalam, Millicent Mabel*

Rajalakšmi inženjerski koledž, Departman za biotehnologiju, Tandalam, Činaj, Indija

Sažetak: Biorazgradivi filmovi na bazi skroba proizvedeni iz poljoprivrednog otpada predstavljaju održivu alternativu materijalima za pakovanje hrane dobijenim iz nafte. U ovoj studiji, skrob ekstrahovan iz listova ananasa (*Ananas comosus*) i kore japanskog slatkog krompira (*Ipomoea batatas*) kombinovan je sa hitozanom radi razvoja antimikrobnih biorazgradivih filmova korišćenjem tehnike gel-castinga. Pripremljeni filmovi su karakterisani u pogledu bubrenja, rastvorljivosti, mehaničkih, termalnih, strukturnih, morfoloških, antimikrobnih karakteristika kao i svojstava očuvanja hrane. Od različitih formulacija, film PA-L sa 10% skroba i hitozana pokazao je najbolje performanse, sa optimalnom zateznom čvrstoćom od 19,8 ± 1,2 MPa, istezanjem pri kidanju od 25,3 ± 1,5%, termalnom otpornošću od 95 ± 2°C, stepenom bubrenja od 120 ± 3% i rastvorljivošću u vodi od 28,7 ± 1,5%. Rendgenska difrakcija i FTIR spektroskopija potvrdile su uspešno formiranje polukristalnog kompozita hitozan–skrob putem intermolekularnih interakcija, dok je skenirajuća elektronska mikroskopija otkrila kompaktnu i homogenu morfologiju filma. Film PA-L pokazao je višu antimikrobnu aktivnost, stvarajući zone inhibicije od 14, 10, 10 i 8 mm protiv *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* i *Staphylococcus aureus*, respektivno. Nasuprot tome, film JSP-S pokazao je zone inhibicije od 12, 0, 6 i 6 mm protiv istih bakterijskih sojeva. U studijama produženja roka trajanja, optimizovani PA-L film potvrdio je produženo skladištenje limuna i vrste povrća iz jugoistočne Azije (*Coccinia grandis)* za 10 dana u ambijentalnim uslovima, smanjujući gubitak vlage i odlažući vidljivo kvarenje. Gubitak mase smanjen je sa 32,7% na 6,7% kod limuna i sa 32,3% na 11,4% kod *C. grandis* kada su bili upakovani u PA-L film. Ovi rezultati ukazuju da biorazgradivi filmovi na bazi skroba, dobijeni iz poljoprivrednog otpada poput listova ananasa i kore japanskog slatkog krompira i ojačani hitozanom, mogu služiti kao održiv materijal za pakovanje hrane sposoban da produži rok trajanja i očuva kvalitet svežih prehrambenih proizvoda.

Ključne reči: *skrob-hitozan kompozitni film; rok trajanja; konzerviranje hrane; limun; Coccinia grandis; poljoprivredni otpad*

Received: *05 May 2026/* ***Received in revised form:*** *15 July 2026/* ***Accepted:*** *15 July 2026*

Available online: *October 2026*

ONLINE FIRST

This open-access article is licensed under the Creative Commons Attribution 4.0 International License. To view a copy of this license, visit https://creativecommons.org/licenses/by/4.0/ or send a letter to Creative Commons, PO Box 1866, Mountain View, CA 94042, USA.

© The Author(s) 0000