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Research Article

Barrier Property, Antimicrobial Susceptibility, and Biodegradability of Waste Cassava Peel Starch/Waste Shrimp Shell Chitosan/Sorbitol Bioplastic Films

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ABSTRACT

Barrier properties, antimicrobial susceptibility potential, and biodegradability of bioplastics are critical indicators of bioplastic viability in industrial use, especially when raw materials to the production were sourced from food waste, such as waste cassava peel starch and shrimp shell chitosan. This study aims to investigate these properties from the created bioplastic film primarily consisting of cassava peel starch (CPS) and shrimp shell chitosan (SSCHT), with sorbitol (SOR) as a plasticizer, utilizing green methods and a constrained D-optimal mixture design. Films were assessed via water uptake, water vapor transmission rate, morphology, antimicrobial susceptibility, and biodegradability. Models were generated in terms of water uptake ($p = 0.0684$) and water vapor transmission rate ($p = 0.0013$) and ranged between 88.49% to 326.35% and 435.67 to 559.09 $g/m^2/24h$ respectively. CPS ($p = 0.0008$) had a significant effect on water uptake levels due to its hydroxyl groups, which form hydrogen bonds that retain water. On the other hand, water vapor transmission rate was significantly affected by CPS ($p = 0.0001$) and SOR ($p = 0.0001$). Although SSCHT ($p = 0.0787$) was statistically insignificant its acetyl group reduced the hydrophilic nature of CPS. CPS and SOR were found to positively affect weight loss through biodegradation due to increased hydrophilicity and microbial colonization. Scanning electron microscopy (SEM) at 300x magnification revealed visibly smooth morphology of films, while at 1500x and 6500x magnification the films had visible crevices possibly due to greater SSCHT concentrations lower WVTR, and higher CPS concentrations raising water absorption levels. Antimicrobial susceptibility tests showed that increased SSCHT led to more effective inhibition against

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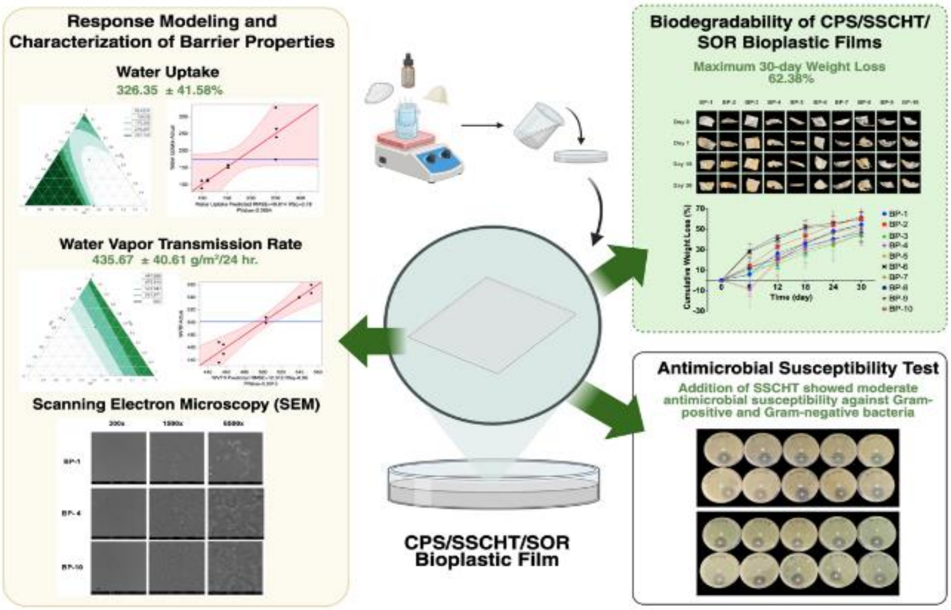
E. coli and P. aeruginosa. This study provides insight into the possibilities of bioplastic films created from valorized food waste and used for packaging applications and these findings suggest the viability of waste-derived bioplastics in packaging applications that demand water resistance and antimicrobial activity.

Keywords: *Bioplastic, Water vapor, Water uptake, Antimicrobial, Biodegradability*



Background

A significant amount of bioplastic applications is found in the area of food packaging, where effective packaging shields food from microbial contamination, air, water vapor, and UV light (Westlake et al., 2022). Research has extensively focused on the use of cassava starch to create plastic packaging materials, including combining starch with various polymeric substances, as well as incorporating reinforcing materials, and adding polyphenols and essential oils to enhance antimicrobial properties (Silveira et al., 2025). However, a significant disadvantage of starch-based bioplastics is the moisture sensitivity, particularly in humid conditions due to the hydrophilic nature of starch from its hydroxyl groups (Ulyarti et al., 2020). These polar hydroxyl groups form hydrogen bonds with water molecules, making starch-based films prone to absorbing atmospheric moisture. Consequently, this leads to swelling, high water vapor transmission rate (WVTR) and water uptake (WU) which limits their functionality in packaging applications that require long-term storage or moisture control, such as food packaging. Thus, their use is restricted in situations where water resistance, structural durability, and effective barrier performance are essential.

To address these limitations, various tactics have been used to improve their water stability. Chitosan, as noted by Tan et al. (2022), is a biopolymer with excellent barrier and film-forming properties, making it suitable for food packaging. It possesses strong antibacterial action, both Gram-positive and Gram-negative bacteria against a variety of harmful and spoiling microbes (Ahmed et al., 2025). The most used method for creating films or coatings for food packaging continues to consist of chitosan solution in a mild acid, such as acetic acid, in which the casting procedure is the most extensive of all the techniques applicable (Cazón & Vázquez, 2019). Dasumiati et al. (2019)

found that basic waste cassava peel reinforced with a chitosan composition of 5% can be produced as wrap packaging for direct consumption. To improve the brittleness of the films, plasticizers like glycerol, sorbitol, and glucose can be added to the biopolymer matrix to increase the films' elasticity. However, adding plasticizer to starch films makes them more sensitive to moisture, which causes the films to lose their structural integrity (Shapi'i et al., 2022). In addition to mechanical and barrier properties, biodegradability remains a critical advantage of starch and chitosan-based films. These materials naturally decompose under environmental conditions without leaving toxic residues unlike conventional plastics, which makes them an eco-friendly alternative (Oberlintner et al., 2021; Jiang et al., 2020). However, the rate of biodegradation can vary depending on the film's composition, environmental factors such as humidity, and soil microbial activity (Payanthoth et al., 2024).

Despite growing interest in bioplastic developments, there is still limited information on the barrier properties of bioplastic films derived from waste materials such as cassava peels and shrimp shells. Consequently, the potential of agricultural and marine waste as raw material for bioplastics remains underutilized. Important characteristics such as WVTR, WU, biodegradability and antimicrobial effectiveness are not well documented for these waste-based films, even though these properties are essential for future application in plastic packaging industries.

Research Objectives

The purpose of this study is to characterize and analyze the barrier properties, antimicrobial activity, and biodegradability of a bioplastic film produced from waste cassava peel starch (CPS), shrimp shell chitosan (SSCHT), and sorbitol (SOR). The study will measure the film's performance using water uptake, water vapor transmission rate, antimicrobial susceptibility, biodegradability test, and scanning electron microscopy. The specific objectives are as follows:

1. Determine the water uptake and water vapor transmission rate of the CPS/SSCHT/SOR bioplastic films under varying CPS, SSCHT, and SOR compositions prepared using the D-optimal mixture design of experiment;
2. Model the water uptake and water vapor transmission rate of the CPS/SSCHT/SOR bioplastic films under varying CPS, SSCHT, and SOR compositions using Scheffe cubic model;
3. Conduct morphological analysis of the barrier properties of the CPS/SSCHT/SOR bioplastic films using scanning electron microscopy (SEM);
4. Investigate the biodegradability of the CPS/SSCHT/SOR bioplastic films; and
5. Determine the antimicrobial susceptibility of the CPS/SSCHT/SOR bioplastic films against a gram-positive and a gram-negative bacteria.

Scope and Limitations

The scope of this study is limited to the characterization of barrier, antimicrobial, and biodegradability properties of the produced CPS/SSCHT/SOR food packaging films. The study analyzes how the films can withstand the passage of water vapor and water absorbance, their potential to inhibit bacterial growth. Morphological characterization of the bioplastic films was done using scanning electron microscopy. The antimicrobial susceptibility of the bioplastic films was investigated against one gram-positive bacteria (Pseudomonas aeruginosa) and one gram-negative bacteria (Escherichia coli), using a paper disk diffusion assay according to Agustin & Padmawijaya (2017). Natural biodegradation will be observed using a soil burial test in accordance with the study by Tan et al. (2022). in a limited amount of time. JMP 18.1.1. was utilized as the primary software tool for the data analysis and construction of the mixture design of this study.

Significance of the Study

The study explores the viability of a bioplastic film primarily made of valorized food components used specifically for food packaging. While previous researchers have developed starch/chitosan bioplastic films, these studies commonly utilized commercial or laboratory-grade materials. The utilization of starch and chitosan derived from cassava peel and shrimp shells, respectively, in the development of bioplastic food packaging films would help justify the feasibility and potential of food waste as bioplastic materials in terms of water barrier properties, antimicrobial susceptibility and biodegradability. Furthermore, the modeling of water uptake and water vapor transmission rate, and the characterization of antimicrobial susceptibility and biodegradability are the main factors this study focuses on because these characteristics directly affect the effectiveness and feasibility of the bioplastic film as food packaging. According to Tan et al. (2022) and Priya et al. (2021) the bioplastic film's water uptake quality and water vapor transmission rate is important because low levels entail a stronger and firmer bioplastic, which is needed in the context of food packaging. Additionally, antimicrobial susceptibility capabilities of the bioplastic film will determine how well it can combat common strains of bacteria present in food (Agustin & Padmawijaya, 2017), which aids in preserving food's shelf life. Meanwhile, the assessment of biodegradability ensures that the produced bioplastic films will do as little harm to the environment as possible after disposal. Testing each factor ensures which CPS/SSCHT/SOR film concentration is most feasible in food packaging.

Methods

Experimental Phases

The experimental phases in this study were comprised of five phases. The first phase involved the production of bioplastic films using the 10-point D-optimal mixture design of CPS, SSCHT, and SOR. The second phase involved the response modeling of water uptake and water vapor transmission rate, which are essential barrier properties of bioplastics. The next phase involved the morphological characterization of the CPS/SSCHT/SOR bioplastic films using scanning electron microscopy. The fourth phase involved the biodegradability characterization of the bioplastic films using compost burial test, while the fifth phase involved the antimicrobial susceptibility screening of the CPS/SSCHT/SOR bioplastic films against Gram-positive and Gram-negative bacteria Pseudomonas aeruginosa and Escherichia coli, respectively. The methodological flowchart of the experimental phases is shown in Figure 1.

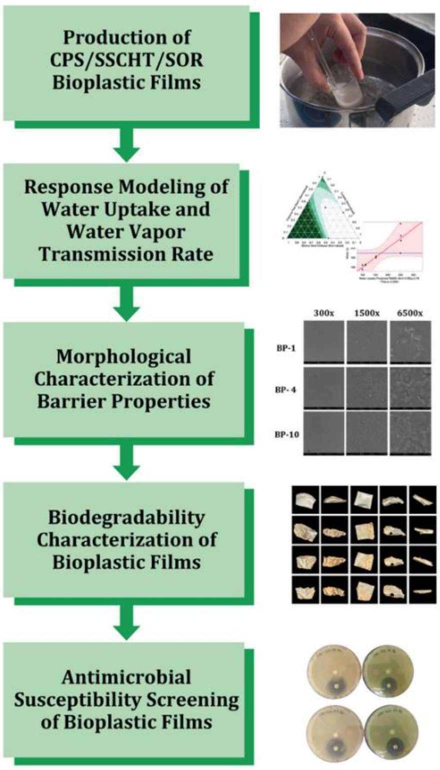


Figure 1. Experimental phases for the determination of barrier properties, biodegradability, and antimicrobial susceptibility of CPS/SSCHT/SOR bioplastic films.

Production of Cassava Peel Starch/Waste Shrimp Shell Chitosan/Sorbitol Bioplastic Films

The production of bioplastic films begins with the extraction of cassava peel starch (CPS) and shrimp shell chitosan (SSCHT), following the procedure described in Picar et al. (2025). The resulting CPS is a white powder that had a dry basis yield of 7.90 ± 1.91%, and the resulting SSCHT is a fine, bone-colored powder that had a yield of 6.09 ± 0.61% with an 80.22% degree of deacetylation.

The method of the production of bioplastic films follows Maulida et al. (2016) with some modifications described in Picar et al. (2025). CPS and sorbitol (SOR) are mixed with water and heated in a double boiler beaker setup at 70-80 °C until homogenized. SSCHT is mixed with 12.5 mL of acetic acid, then added to the beaker and stirred until a thick, viscous, translucent consistency is achieved. The mixture is then poured into a petri dish, dried at room temperature for a few hours, then oven-dried for 24 hours. A d-optimal mixture design, as applied in Picar et al. (2025), was used to generate 10 runs for the water vapor transmission and water uptake tests as seen in Table 1.

Table 1. Ten-point constrained D-optimal mixture design for the barrier property response modeling of the CPS/SSCHT/SOR bioplastic films (Picar et al., 2025).

Run	In real concentrations			In pseudo-components		
	Starch (x_1)	Chitosan (x_2)	Sorbitol (x_2)	Starch (x_{1*})	Chitosan (x_{2*})	Sorbitol (x_{3*})
BP-1	0.77	0.05	0.18	0.58	0.42	0.00
BP-2	0.70	0.05	0.25	0.00	0.42	0.58
BP-3	0.75	0	0.25	0.42	0.00	0.58
BP-4	0.82	0	0.18	1.00	0.00	0.00
BP-5	0.77	0.05	0.18	0.58	0.42	0.00
BP-6	0.76	0.025	0.215	0.50	0.21	0.29
BP-7	0.76	0.025	0.215	0.50	0.21	0.29
BP-8	0.70	0.05	0.25	0.00	0.42	0.58
BP-9	0.82	0	0.18	1.00	0.00	0.00
BP-10	0.75	0	0.25	0.42	0.00	0.58

Water Uptake

The determination of water uptake percentage follows the research design of Tan et al. (2022). A puncher was used to obtain small, circular films for testing to ensure a uniform sample size. The CPS/SSCHT/SOR bioplastic films were initially weighed before being submerged into a small petri dish filled with distilled water for 10 minutes. Then, the films were removed, blotted dry with tissue paper, and then weighed. This process was repeated across three trials, each consisting of 10 runs for the different concentrations and the results were reported as the mean ± standard deviation of the triplicates. Water uptake (WU) was calculated using Equation 1, and the average value was reported.

$$WU = \frac{W - W_0}{W_0} x\, 100\% \quad \text{(Eq. 1)}$$

Wherein:
W is weight of the sample after immersion in grams
W_0 is weight of the dry sample in grams
A is the area available for transmission of water vapor in m2
t is the duration of the test in days

Morphological Characterization of CPS/SSCHT/SOR Bioplastic Films

Samples were gold-sputter coated using a VPS-020 system and analyzed at 15 kV. This process of imaging a sample surface using a high-energy electron beam in a raster scan pattern is known as scanning electron microscopy wherein electrons interact with the sample's constituent atoms to produce signals that reveal information about the sample's composition, surface topography, and other characteristics like electrical conductivity (Opoku, 2019). To observe general morphology, Kusumastuti et al. (2020) used a voltage of 15 kV and a magnification of 300x, in which gold was sputter-coated onto the mixed polymer using VPS-020. This magnification was also utilized by Aziz et al. (2019), identifying the polymerization structure of the sample. Moreover, the study of Ginting et al. (2018) used a 1500x magnification scanning electron microscope with a 100-mesh starch size to observe granules. It revealed that the starch granules were elliptical or spherical, with a size of 20 µm. Furthermore, Dianursanti and Khalis (2018) utilized 6000x to observe the sample with the best mechanical properties such as tensile strength and elongation.

Antimicrobial Susceptibility Screening

The Kirby-Bauer Disk Diffusion susceptibility test method was applied to determine antimicrobial activity against Gram-negative bacteria, particularly E. coli and P. aeruginosa. The objective is to ascertain the pathogenic aerobic sensitivity or resistance and facultative anaerobic bacteria to different types of antimicrobials. Mueller-Hinton agar is used to cultivate the pathogenic bacterium in the existence of different filter paper disks treated with antimicrobials. The growth surrounding the disks, whether present or not, is an indirect indicator of the compound's capacity to inhibit that organism (Hudzicki, 2009). Following the model of Agustin & Padmawijaya (2017), bioplastic films punched 7 mm diameter in size were set on solid nutritive media (nutrient agar) that had been equally swabbed with a suspension of either gram-positive (Pseudomonas aeruginosa) or gram-negative (Escherichia coli) bacteria. The Petri dish was then incubated for 24 hours. The culture used for the test determines the incubation conditions. The inhibitory zone, also known as the clear zone, must be identified surrounding the bioplastics samples on the agar plate following the incubation time (Agustin & Padmawijaya, 2017).

Biodegradability Test

The biodegradability rate was determined following the model of Tan et al. (2022). Ten films representing ten runs of the D-optimal mixture design done in triplicates were buried in individual pots containing loam soil purchased from a local plant nursery (Sta. Rosa, Laguna) at a depth of 3 cm for 30 days. Every 48 hours, the samples were carefully retrieved, brushed off of surface soil, dried, and weighed using an analytical balance. This process was repeated until the end of the testing period. However, for analysis, only the initial (W) and final weights (W0) were considered to determine the total weight loss percentage (WL), with the use of Equation 3 below. The average of these values will be reported.

$$WL\,(\%) = \frac{IW\,(g) - FW\,(g)}{IW\,(g)} \qquad \text{(Eq. 3)}$$

Wherein:
WL is weight loss in percentage
IW is initial weight in grams
FW is final weight in grams

Statistical Analysis

Ternary plots were graphed using the software JMP Pro 18.0.2 from JMP Statistical Discovery LLC with the fit model function using the various tests' average and standard deviation values. In addition to visualization, the software provided regression coefficients and analysis of variance (ANOVA) utilizing the Scheffe’s Cubic model with a significance level of 5% ($p \leq 0.05$). Additionally, the p-value will be used to assess statistical significance and determine the correlation between variables. The assumptions for ANOVA were tested using the Shapiro-Wilk and Anderson-Darling tests for normality. Assumptions for regression analysis were verified using an actual predicted plot generated by the Scheffé cubic model.

Results and Discussion

The main aim of the study is to investigate the barrier properties, which are essential in the characterization of industrial-grade alternatives to synthetic plastics. In this study, two barrier properties, namely water uptake and water vapor transmission rate, were examined to observe the effect of mixture composition on the barrier properties of the bioplastics. The summary of water uptake and water vapor transmission rate is shown in Table 2.

Table 2. Water uptake (%) and water vapor transmission rate (g/m2/24 hr.) of CPS/SSCHT/SOR bioplastic films formulated using D-optimal mixture design

Run number	In real concentrations			Barrier Properties	
	CPS (%)	SSCHT (%)	SOR (%)	Water Uptake (%)	Water Vapor Transmission Rate ($g/m^2/24$ hr.)
BP-1	77	5	18	263.38 ± 89.61	435.67 ± 40.61
BP-2	70	5	25	156.82 ± 9.70	464.25 ± 45.60
BP-3	75	0	25	110.50 ± 11.86	546.10 ± 24.59
BP-4	82	0	18	326.35 ± 41.58	539.17 ± 110.26
BP-5	77	5	18	238.74 ± 15.80	468.15 ± 10.50
BP-6	76	2.5	21.5	109.24 ± 9.98	498.90 ± 22.65
BP-7	76	2.5	21.5	113.78 ± 19.25	507.56 ± 27.87
BP-8	70	5	25	149.37 ± 13.92	449.53 ± 54.02
BP-9	82	0	18	173.22 ± 2.89	539.61 ± 30.02
BP-10	75	0	25	88.49 ± 13.69	559.09 ± 80.36

Effect of Mixture Composition on Water Uptake

The histogram shows a negatively skewed distribution, with most of the data located at the higher values and a tail extending to the lower values. The box plot and quantile (Q-Q) plot further support this trend by showing points deviating from the normal line, as shown in Figure 2. Furthermore, the Shapiro-Wilk ($p = 0.3580$) and Anderson-Darling ($p = 0.3908$) tests revealed an insignificant p-value. This indicates that the assumptions of normality cannot be rejected despite the negative skewness of the histogram. Suggesting that the data and parametric analyses follow a normal distribution.

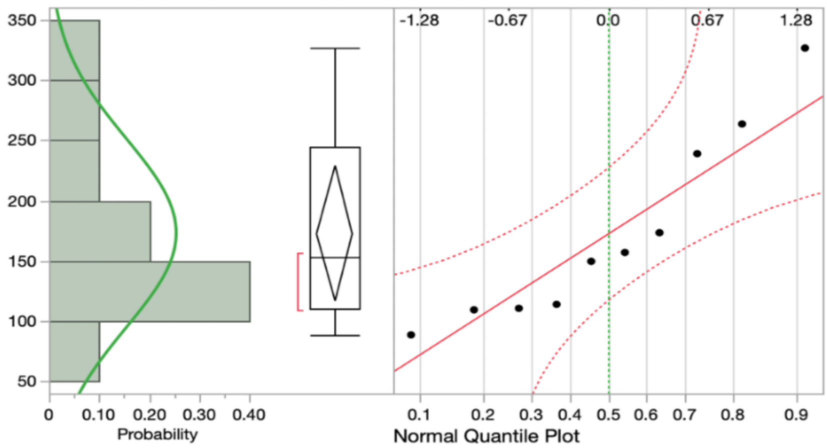
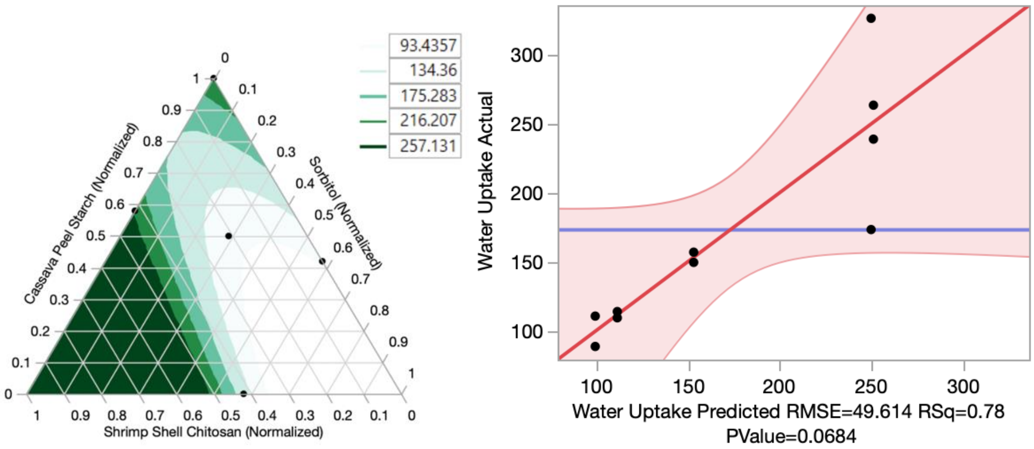


Figure 2. Graph of histogram and quantile plot of water uptake

The ternary plot, as shown in Figure 3, shows the relationship between water uptake levels and bioplastics with varying SSCHT, CPS, and SOR compositions. The ternary plot summarizes how the varying compositions influence the bioplastic's ability to absorb water, which confirms key trends found in the research of Tan et al. (2022), where starch-based bioplastics were utilized. Table 2 summarizes the variability of water uptake across varying compositions, with values ranging from 88.49% to 326.35%. The highest water uptake levels (326.35%) are seen in run 4. Meanwhile, the lowest water uptake levels (88.49%) are seen in run 10 as shown in Table 2. These results show that higher concentrations of CPS increase the water uptake level, and moderate concentrations of SSCHT and SOR may have a slight effect on water uptake levels but are not as pronounced as CPS. Moreover, the ternary plot confirms these trends since the distinct regions associated with higher concentrations of CPS have higher water uptake levels. Furthermore, the research of Tan et al. (2022) states that since starch is rich in hydroxyl (-OH) groups, which form hydrogen bonds with water molecules, increasing water retention. Polysaccharides like cassava starch have polar functional groups, leading to higher water uptake levels when high CPS concentrations (Tan et al., 2022).



(a) (b)

Figure 3. Plot of (a) ternary diagram showing the predicted water uptake based on mixture composition and (b) actual water uptake against predicted water uptake

The analysis of variance (ANOVA), as seen in Table 3, shows that CPS (p = 0.0008) is a statistically significant factor influencing water uptake levels. Furthermore, its positive parameter estimate of 249.78 reveals that increasing CPS concentrations lead to higher water uptake levels. On the other hand, it is revealed that SSCHT (p = 0.0637) and SOR (p = 0.8924) were statistically insignificant, which entails that their inclusion in the bioplastic does not affect the water uptake levels. Additionally, their t-ratios suggest SSCHT and SOR have a weak relationship with the response variable. Therefore, only CPS plays a crucial role in contributing to water uptake levels, while SSCHT and SOR have no significant impact on water uptake levels. This is further supported by the p-value (p = 0.0684) and F ratio (4.3811) as seen in Table 4, which entails that not all components play a statistically significant role in affecting the bioplastic film's water uptake levels.

Table 3. Regression coefficients and parameter estimates of water uptake in terms of shrimp shell chitosan, cassava peel starch, and sorbitol

Parameters	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}	β_{123}
Parameter Estimates	1263.65	249.79	-9.33	-1742.79	-1527.87	-	-
***p*-value**	0.0637	0.0008*	0.8924	0.1074	0.1631	-	-

Table 4. ANOVA of water uptake

Source	**DF**	**Sum of squares**	**Mean of squares**	**F ratio**	**Prob > F**
Model	4	43177.399	10784.3	4.3811	0.0684
Error	5	12307.859	2461.6	-	-
U. Total	9	55445.258	-	-	-

Effect of Mixture Composition on Water Vapor Transmission Rate

The histogram shows a somewhat symmetrical distribution and the box plot and quantile (Q-Q) plot support this somewhat symmetrical distribution with most points following the normal line as shown in Figure 4 This entails that the data are normally distributed, which is further supported by the Shapiro-Wilk tests (p = 0.1737) and Anderson-Darling test (p = 0.1664) that reveal insignificant p-values. These indicate that there is not enough evidence to reject normality, which means a normal distribution for water vapor transmission rate against the composition of the CPS/SSCHT/SOR film.

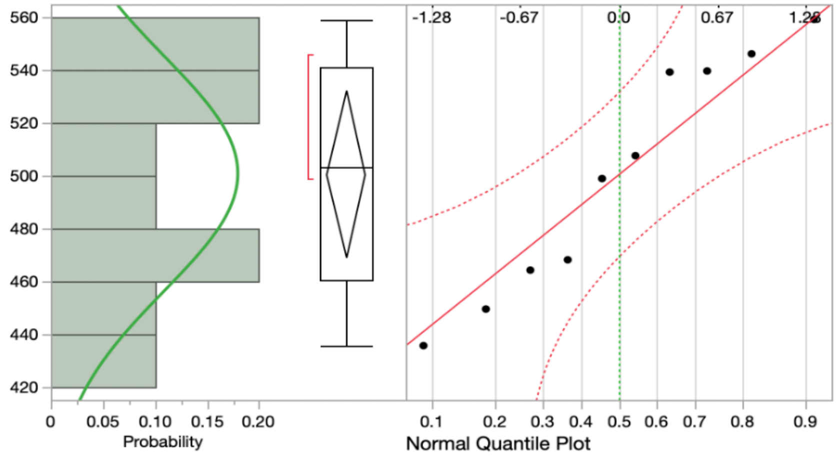


Figure 4. Graph of histogram and quantile plot of water vapor transmission rate

The ternary plot shown in Figure 5 reveals how SSCHT, CPS, and SOR influence the water vapor transmission rate (WVTR). It shows that higher CPS concentrations increase WVTR levels, while SSCHT reduces WVTR levels due to the film's enhanced water resistance caused by SSCHT. Table 2 shows that runs with high CPS concentrations exhibit the highest WVTR levels, such as runs 3, 4, and 9. According to Abdullah et al. (2020), this is attributed to starch's ability to increase bioplastic permeability due to its hydrophilic nature. Conversely, runs with higher SSCHT concentrations, such as run 1, had the lowest WVTR. The trend aligns with the expectation that starch-based bioplastics become more water vapor-resistant as SSCHT concentrations increase (Abdullah et al., 2020).

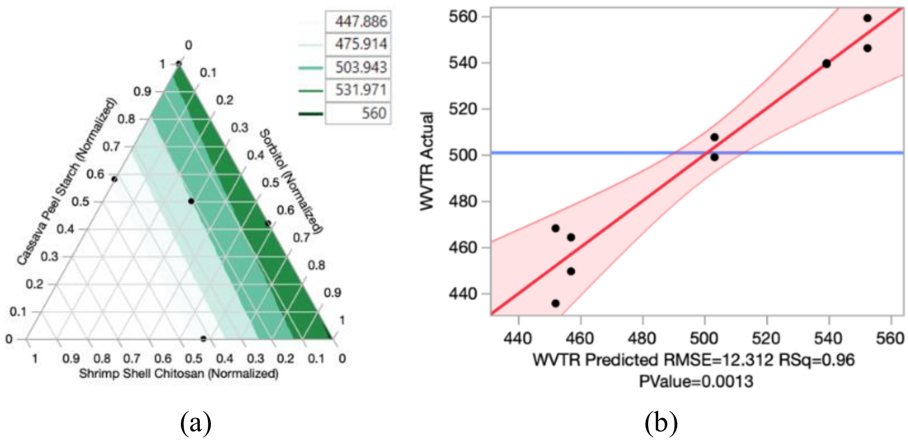


Figure 5. Plot of (a) ternary diagram showing the predicted water vapor transmission rate based on mixture composition and (b) actual water vapor transmission rate against predicted water vapor transmission rate

Moreover, ANOVA further confirms the statistical significance, as seen in Table 5, of CPS ($p = 0.0001$) and SOR ($p = 0.0001$) concerning WVTR levels on bioplastic films, since both had statistical significance. Although SSCHT ($p = 0.0787$) revealed statistical insignificance according to Abdullah et al. (2020), SSCHT plays a significant role in modulating water vapor transmission rates. This is caused by the acetyl group (CH_3) in SSCHT, which causes the bioplastic film to have hydrophobic properties, which reduce the hydrophilic -OH groups of starch. Additionally, it was found that the interaction and relationship between CPS and SSCHT ($p = 0.7680$) concentrations were insignificant, which entails that their effects on the WVTR and water permeability act independently rather than synergistically on the bioplastic films. These results show that increased SSCHT concentrations promote water resistance in the bioplastic and decrease WVTR levels. However, higher CPS concentrations can counteract the SSCHT's effects due to the bioplastic's water vapor permeability. This statistical data aligns with the study of Abdullah et al. (2020), which entails that the balance between SSCHT and CPS is crucial for improved WVTR levels and maintaining structural integrity. Furthermore, the p-value ($p = 0.0013$) and F ratio (28.0369) as seen in Table 6 further supports the notion that all three components play a statistically significant role in affecting WVTR.

Table 5. Regression coefficients and parameter estimates of water vapor transmission rate in terms of shrimp shell chitosan, cassava peel starch, and sorbitol

Parameters	β_{1}	β_{2}	β_{3}	β_{12}	β_{13}	β_{23}	β_{123}
Parameter Estimates	291.23	539.39	562.17	68.74	34.96	-	-
***p*-value**	0.0787	0.0001*	0.0001*	0.7680	0.8861	-	-

Table 6. ANOVA of water vapor transmission rate

Source	**DF**	**Sum of squares**	**Mean of squares**	**F ratio**	**Prob > F**
Model	4	16993.376	4248.34	28.0269	0.0013*
Error	5	757.904	151.58	-	-
U. Total	9	17751.280	-	-	-

Morphological Characterization of Bioplastic Films

The surfaces of the CPS/SSCHT/SOR bioplastics were analyzed using Scanning Electron Microscopy at 300x magnification 15kV, showing that the bioplastic films had a smooth surface as shown in Figure 6. An open drying method was utilized which is a rapid drying process along with even distribution of materials. This entails a better interaction of components as the synthesis' microstructure was stronger, and the particle arrangement and bonding were close to one another (Aziz et al., 2019). According to Thuppahige et al. (2023), cassava peel chambers with thickened cell walls and distinct structure resembled honeycombs. As with earlier studies, there are a lot of starch granules in the peel.

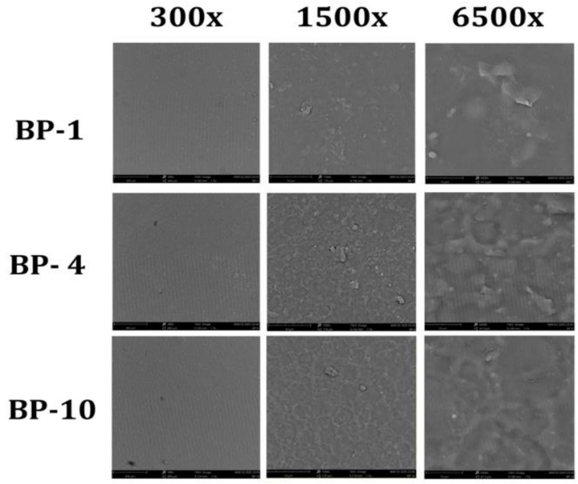


Figure 6. Scanning electron micrographs of CPS/SSCHT/SOR films in 300x, 1500x, and 6500x magnifications

In contrast to this, micrographs revealed a porous nature despite the smooth surface of the starch granules. This could be due to initial porosity differences and different drying techniques (Thuppahige et al., 2023). Furthermore, Picar et al. (2025) revealed that BP-4 had the highest CPS concentration of 82% indicating a high water uptake level potentially leading to swelling, making it susceptible to surface cracks. Moreover, BP-10 had the highest WVTR with a CPS concentration of 75%. This entails that bioplastics may crack under considerable amounts of tension from the evaporating water (Picar et al., 2025). At 6500x magnification, minor surface cracks were visible. It is possible because greater SSCHT concentrations lower WVTR, and higher CPS concentrations raise water absorption levels. Due to surface pores, the samples' low component adhesion lowers their mechanical qualities and affects their tensile characteristics (Kowser et al., 2025). Furthermore, when cassava peels were mesh-pretreated, the fibrous particles lost their original long-rod shape and instead took on a sphere-like appearance (Zhang et al., 2024).

Antimicrobial Susceptibility Test of CPS/SSCHT/SOR Bioplastic Films

The antimicrobial properties of the bioplastic films were evaluated using the disk diffusion method against E. coli and P. aeruginosa. Escherichia coli was selected for this study due to its wide usage for testing the efficacy of many antimicrobial agents and is a common indicator for fecal pollution (Suryanegara et al., 2021). Pseudomonas aeruginosa is a known biofilm-forming bacterium, which can affect the effectiveness of antimicrobial components, making it a suitable model for evaluating the antimicrobial efficacy of the bioplastic (Li et al., 2023). The summary of the antimicrobial susceptibilities of the bioplastics against P. aeruginosa and E. coli is shown in Table 7.

Table 7. Real concentrations of CPS/SSCHT/SOR films and their corresponding inhibition against E. coli and P. aeruginosa

Bioplastic Compositions			Antimicrobial Susceptibility	
CPS (%)	SSCHT (%)	SOR (%)	*E. coli*	*P. aeruginosa*
77	5	18	**+++**	**+++**
70	5	25	**+++**	**+++**
76	2.5	21.5	**+**	**+**
75	0	25	**–**	**–**
82	0	18	**–**	**–**

Unlike conventional antimicrobial plastics that diffuse into the agar medium and create clear zones of inhibition, the tested bioplastic films did not produce measurable inhibition zones, as shown in Figure 7. This suggests that the antimicrobial components within the films remained localized on the bioplastic surface rather than diffusing into their surrounding medium. The reduction in bacterial colonies was observed exclusively on the surface of the bioplastic itself. This observation aligns with the findings of Savitskaya et al. (2017), who reported that chitosan, when incorporated into cellulose composite films, does not readily diffuse due to its strong integration within the polymer matrix. Furthermore, chitosan has limited solubility in neutral and basic environments, which restricts its application under such conditions. In this study, the agar medium used for the disk diffusion test was at neutral pH, which may have hindered chitosan's diffusion-based antimicrobial activity due to its reduced solubility (Aranaz et al., 2021). Consequently, the disk diffusion test may not be suitable for evaluating the antimicrobial activity of the bioplastic, as their antimicrobial effect relies more on direct contact than on diffusion.

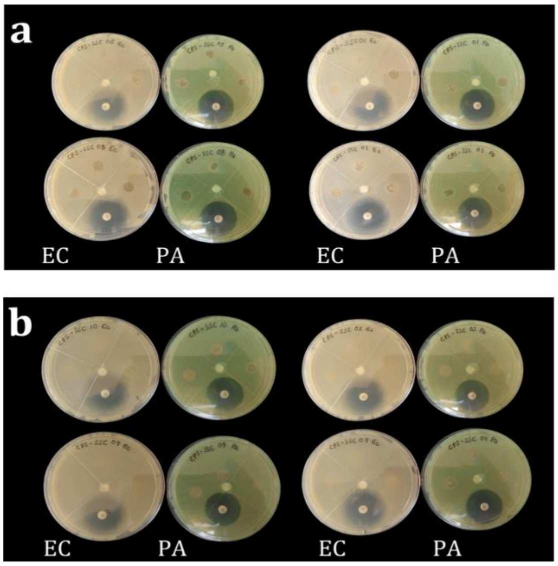


Figure 7. Paper disk diffusion test results of bioplastic films against E. coli and P. aeruginosa, where (a) represents films with chitosan and (b) represents films without chitosan

Despite the absence of inhibition zones, a qualitative assessment of bacterial growth on the bioplastic surfaces revealed differences between films that had chitosan and those without. The antimicrobial effectiveness of the bioplastic films was assessed by categorizing bacterial presence on the film surface into three qualitative groups: +++ (no visible bacterial growth), + (reduced bacterial presence), and – (significant bacterial presence). Table 6 summarizes the results of ten trials for different formulations. Films with chitosan concentrations of 2.5% or higher exhibited a notable reduction in bacterial adhesion to their surface. In contrast, formulations lacking chitosan could not prevent the growth of bacterial colonies on their surface.

Chitosan demonstrates antimicrobial activity linked to its amino groups. In acidic conditions, chitosan's glucosamine monomer gains a positive charge (-NH_3^+), enabling it to bind to negatively charged microbial membranes. This leads to leakage of intracellular proteins (Suryanegara et al., 2021; Tanpichai et al., 2020). The effectiveness of this mechanism is influenced by the degree of deactylation (DD); a higher DD provides more free amino groups that can interact with -COO- groups on microbial cell membranes (Aranaz et al., 2021). Additionally, chitosan can enter bacterial cells, bind to DNA, and inhibit mRNA synthesis, thereby disrupting bacterial growth and reproduction (Suryanegara et al., 2021; Savitskaya et al., 2017; Aranaz et al., 2021).

Biodegradability of CPS/SSCHT/SOR Bioplastic Films

Figure 8 shows the physical changes of ten different bioplastic samples across a 30-day period of soil burial. Initially, at Day 0, all samples appear intact with smooth surfaces and a translucent white coloring. By Day 7, noticeable swelling and distortion are observed in most films, likely due to water uptake from the soil, causing the bioplastics to expand in size. This expansion is accompanied by varying degrees of surface roughness, discoloration, and early signs of biodegradation, signifying the beginning of microbial colonization. As biodegradation progresses, most examples exhibit changes in texture and structure, most notably the crumpling and tearing of BP-2, BP-5, BP-8 and BP-10. By Day 30, the samples proceeded to shrink, likely due to the evaporation of water and the breakdown of the material caused by the microbes in the soil. According to Folino et al. (2020), the biodegradation of bioplastics commonly undergoes three stages, 1) bio-deterioration: wherein the polymers undergo physical, mechanical, and chemical change, 2) bio-fragmentation: where microbes begin the breakdown of the material, and lastly 3) assimilation: where the materials get converted into residual products such as CO2, H2O, and biomass. It is important to note that the test likely did not progress to the assimilation stage, possibly due to the limited duration of the experimental period. Future studies should extend the testing period to capture the full biodegradation process, including assimilation, which likely was not achieved within the 30-day timeframe.

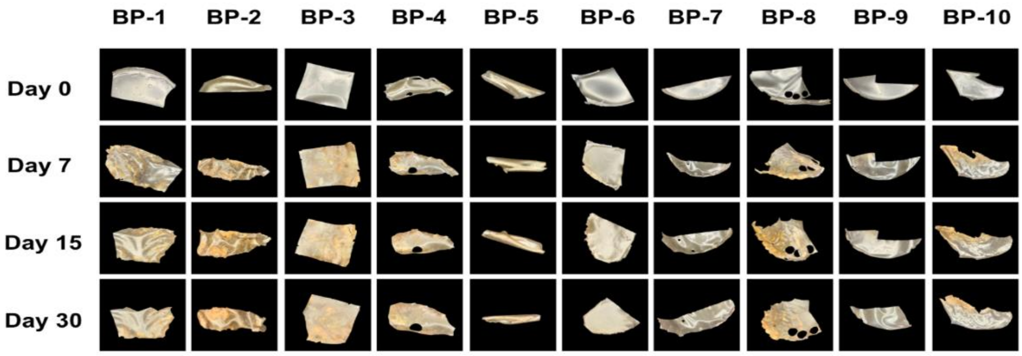


Figure 8. Macroscopic images of biodegraded CPS/SSCHT/SOR films during the 30-day soil burial test.

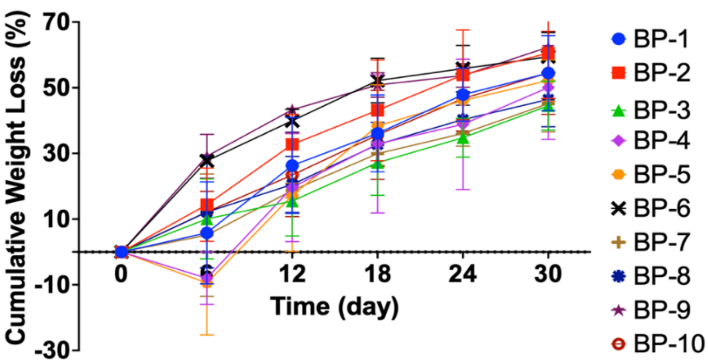


Figure 9. Graph of cumulative percentage weight loss over time during the soil burial test.

The biodegradability analysis of the bioplastic formulations (BP-1 to BP-10) revealed differing degradation rates over a 30-day period, as illustrated in the graph in Figure 8. Compositions BP-9 and BP-2 showed the highest weight change of 62.38% and 60.56%, respectively, while BP-3 and BP-7 exhibited the least weight change of 44.50% and 45.16%, respectively, as shown in Figure 9. These findings suggest that CPS, SSCHT, and SOR can be strategically combined to develop bioplastics with effective biodegradation. The impact of these individual components on biodegradability can be attributed to their physicochemical properties. CPS, known for its hydrophilicity, enhances microbial access by promoting water absorption, which leads to faster absorption. These observations are consistent with the findings of Tan et al. (2022), who reported that starch-based films degrade faster than those reinforced with chitosan due to the greater water affinity of starch matrices. Sorbitol further contributes to biodegradation by disrupting the internal hydrogen bonding within starch structures, leading to increased porosity and exposure to microbial activity (Lusiana et al., 2019). As a hydrophilic plasticizer, sorbitol attracts water, which facilitates microbial colonization and the formation of bioplastic film that further promotes biodegradation (Arief et al., 2021). Additionally, chitosan can undergo enzymatic degradation through enzymes like chitosanase and lysozyme, which cleave through its glycosidic bonds, leading to depolymerization and deacetylation. This process gradually breaks chitosan into smaller fragments, including monomers such as glucosamine, which microorganisms can utilize (Wrońska et al., 2023). Moreover, chitosan can act as a nutrient source that promotes microbial colonization on the film surface, further aiding degradation (Kusumastuti et al., 2020).

To ensure the reliability of the data distribution, normality was assessed using the Shapiro-Wilk and Anderson-Darling tests. Both tests confirmed that the data did not significantly deviate from a normal distribution. This is further supported by Figure 10, where the histogram, box plot demonstrates a relatively symmetric distribution of weight loss values, while the Q-Q plot shows that most data points align closely with the theoretical normal distribution line, indicating that the assumption of normality.

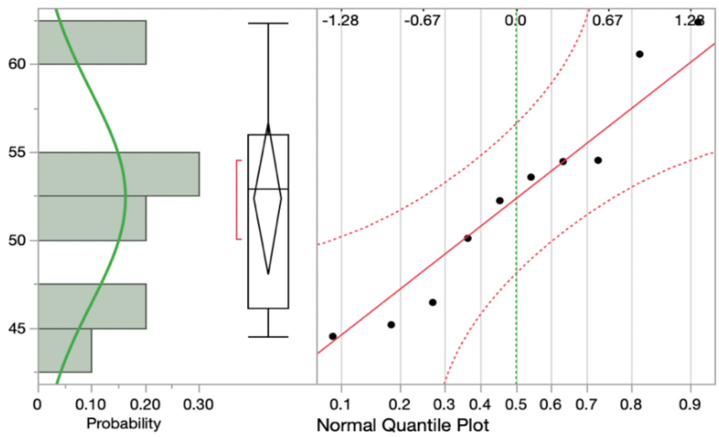


Figure 10. Graph of histogram and quantile plot of biodegradability weight loss

Table 8. ANOVA of cumulative weight loss at the end of the 30-day soil burial test.

Source	DF	Sum of squares	Mean of squares	F ratio	Prob > F
Model	4	68.6697	17.1674	0.3258	0.8501
Error	5	263.4401	52.6880	-	-
U. Total	9	332.1098	-	-	-

Following the verification of normality, ANOVA was conducted to evaluate the significance of the compositional effects on biodegradation rate. The ANOVA results (Table 8) indicate a general trend of biodegradability, although not statistically verified due to the high p-value (0.85) and a low F-ratio (0.3528), suggest that the variance in the proportions of the components within the bioplastic formulations does not significantly affect the biodegradation rate. Instead, it appears that bioplastics as a whole share an inherent biodegradability likely due to their material properties, such as hydrophilicity, porosity, and microbial accessibility. While no statistically significant differences were found in degradation rates ($p > 0.05$), descriptive trends suggest enhanced biodegradation in samples with higher CPS and SOR content.

The key findings demonstrate that water uptake levels in bioplastics are influenced by the concentration of CPS. Run 4 showed the highest water uptake levels, while run 10 had the lowest. Higher CPS concentrations result in higher water uptake, leading to increased WVTR levels. However, SSCHT lowers these levels due to the film's increased water resistance. The porous nature of starch granules suggests high water uptake, potentially causing swelling and surface cracks. BP-10, with a 75% CPS content, exhibited the highest WVTR, suggesting that bioplastics could break under strain. The tested bioplastic films did not produce measurable inhibition zones, consistent with Savitskaya et al.'s (2017) findings. However, chitosan demonstrates antimicrobial activity linked to its amino groups. Furthermore, as biodegradation progresses, the films change texture and structure, with BP-2, BP-5, BP-8, and BP-10 showing the most crumpling and ripping.

Conclusion and Recommendations

This study explored the CPS/SSCHT/SOR bioplastic film's varying barrier and antimicrobial properties, including water uptake, water vapor transmission, antimicrobial susceptibility, and biodegradability. The findings demonstrated that due to CPS being hydrophilic, larger quantities of it resulted in higher levels of WVTR and water uptake. SSCHT, on the other hand, decreased WVTR levels primarily due to its improved water resistance. The films' morphology at 300x demonstrated the bioplastic's smooth surface; however, at 1500x and 6500x, the presence of a few surface pores and crevices became apparent because of higher CPS concentrations, lower WVTR, and higher SSCHT concentrations, which increased the levels of water absorption, and SOR, as a plasticizer, contributes to increased flexibility and reduced tensile strength, which correlates with higher porosity seen under SEM. Additionally, surface porosity may enhance microbial attachment, facilitating biodegradation, though it may also minimize mechanical integrity. Regarding antimicrobial activity, chitosan was notable for its linkage of amino groups. Its efficiency was influenced by the high degree of deacetylation (DD), which provided more free amino groups that could interact with -COO- groups on microbial cell membranes. Still, given the localized antimicrobial action observed and the neutral pH of the medium, further studies should use contact-based assays to evaluate antimicrobial efficacy. Furthermore, the limited solubility of chitosan in neutral environments highlights the need for alternative antimicrobial testing approaches, such as direct contact tests or confocal microscopy. For biodegradability, high levels of CPS with moderate SOR produce the best formulations. Higher SSCHT concentrations tend to have lower or inconsistent degradation responses. As a plasticizer, sorbitol influences mechanical properties such as elongation as it increases the flexibility of the bioplastic. This research suggests that organic waste be valorized for the composition of antimicrobial bioplastics, but further research will be necessary to determine the ideal ratio for high mechanical strength and sustainability for food packaging, mechanical strength under real packaging conditions to assess the shelf-life of packaged goods using these bioplastics, and pilot-scale trials. Consumer safety evaluations will be essential for future commercialization.

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