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Article

Development of novel biodegradable bioplastics for packaging film using mango peels

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SUMMARY

Plastic pollution and greenhouse gases emitted by fruit waste in landfills are detrimental to human and environmental health. Implementing biodegradable, eco-friendly, and sustainable bioplastic films derived from fruit wastes could solve both issues. In this study, bioplastic film produced from mango peels was developed through an environmentally-friendly, aqueous, hydrolysis process using a 5% vinegar solution. We hypothesized that natural biopolymers in mango peels would be released from the destructed peel cell wall to form bioplastic upon acid treatment, and the bioplastic film properties could be tailored through the processing conditions. The film properties, including roughness, transparency, thickness, tensile strength, elongation, water vapor transmission rate (WVTR), degradation, and water resistance, were characterized. Then, the mechanical performance was optimized through adjusting the hydrolysis temperature, duration, and the plasticizer type and concentration. Bioplastic films with the best surface morphology and tensile strength were formed at 50°C for 48 hours. Among the three plasticizers evaluated, 20% polyglycerol-3 showed the best results to improve film elongation without compromising strength. When the film was dip-coated in a hydrophobic solution, the water resistance was significantly enhanced, as evidenced by the improvement of the WVTR and the water contact angle value. We also evaluated the in-soil degradation of the films and the effect of adding chitosan to inhibit bacterial growth. Overall, our results showed the developed bioplastic film had tensile strength equivalent to commercial petroleum-based plastic food wrap, was biodegradable, bacteriostatic, and has the potential to be used as packaging film.

INTRODUCTION

Petroleum-based plastics pollute the environment and contribute to climate change while harming humans and animals through microscopic plastic particles called nanoplastics. Nanoplastics travel through the bloodstream, invading cells and getting absorbed by major organs, contributing to numerous chronic diseases and cancers (1, 2). Furthermore, nearly 45% of all cultivated crops are wasted annually (3). All of these hard-grown crops end up rotting in landfills, which contribute to 14.3% of total greenhouse gas emissions (3). However, if this food waste went into the production of plastic, plastic pollution would cease to be such a grave issue, and the problems with food waste might receive a solution too.

There have been many methods reported to convert agricultural waste into bioplastic. The most traditional way to create bioplastics is to chemically synthesize monomers, such as lactic acid, a monomer made from fermented plant starch (4). Because plant cells are mainly made of natural polymers such as cellulose, hemicellulose, pectin, and small amounts of protein and lignin, another popular method is extracting and purifying natural polymers found within plant cell walls using acidic or alkaline solutions (5). Five percent hydrochloric acid has been used to convert vegetable wastes, including carrot, radicchio, parsley, and cauliflower, into bioplastics (6). Through microbial fermentation, polyhydroxyalkanoates and bacterial cellulose are synthesized by microorganisms that store lipids within cells as energy storage materials. These biopolymers are then processed and purified to be used in bioplastic films (7). Generally, biopolymers obtained through these processes are hydrophilic and have short chain lengths, which result in highly soluble polymers. Although lignin, purified cellulose nano crystals, essential oils, and proteins have been added to enhance bioplastic qualities, the mechanical properties and water resistance of bioplastics are still inferior to petroleum-based plastics (8-11).

The bioplastic field currently lacks an eco-friendly process that can create cost-effective bioplastics with improved mechanical properties, water vapor barrier properties, and durability that are comparable to petroleum-based plastics. The Environmental Protection Agency and Ocean Conservancy reported that Americans used 14.53 million tons of plastic in containers and packaging in 2018, and plastic food wrappers were the second most common beachline litter (12). Furthermore, mangoes are the second most consumed tropical fruit in the world, and its demand in US increased by 263% from 2003 to 2014 (13). Mango biowaste, including the peel, comprises 35-60% of the entire mango and contributes to several million pounds of waste annually (13). This waste has a significant impact on the environment, especially when there is poor disposal management. In such cases, mango peels decompose in landfills and produce methane gas, a major contributor to global warming (14).

Mango peels contain high cellulose, hemicellulose, pectin, and lignin content, which are valuable biopolymers, and thus, mango peels have the potential to make extremely beneficial products (15, 16). Therefore, the goal of this experiment was to convert mango peel fruit waste into a bioplastic film with the potential to be used in the packaging industry through a mild, environmentally-friendly, water-based hydrolysis process using a 5% vinegar solution. We hypothesized that, using a vinegar treatment, we could disrupt the cell walls of mango peels and release biopolymers to be transformed into

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bioplastics. Furthermore, we hypothesized that the bioplastic properties could be optimized with process conditions and the addition of plasticizers or other natural polymers. This study systematically investigated how the hydrolysis temperature, duration, and plasticizer type and concentration impacted the mango peel bioplastic film properties. Moreover, instead of adding components such as lignin or essential oils into the bioplastic film, as others have reported, we implemented a novel method of surface modification to improve mango peel film water resistance (8, 10). To our knowledge, this surface coating modification technique has not been reported in the bioplastic field. In addition, we investigated the addition of chitosan, a natural polymer with a broad spectrum of antimicrobial activity, into the mango peel film with the intent of extending the shelf life of packaged food. Overall, we successfully transformed mango peels into bioplastic films using an eco-friendly acetic acid hydrolysis process. The developed mango peel films were translucent, biodegradable, bacteriostatic, and exhibited good tensile strength.

RESULTS

Bioplastic Film Formation

In this study, we aimed to create a bioplastic film from biopolymers extracted from mango peel. To obtain the film with optimal properties, we screened process conditions. Specifically, we investigated the hydrolysis temperatures 35 °C, 50 °C, and 70 °C and durations ranging from 6 to 72 hours.

When the mango peel powder was first added into the vinegar, the mixture was a suspension. Over time, the suspension became viscous, suggesting the plant cell wall was disrupted, and the liberated biopolymers dissolved in the solution. The mixture was observed to become more viscous as the hydrolysis time increased past 24 hours. Additionally, the grain size in the suspension became smaller after 24 hours, suggesting that more biopolymers were released into the solution and were partially hydrolyzed. With an increase of time, it is most likely that more pectin and sugars, such as fructose and xylose, were liberated from the mango peel cell wall, and after the partial hydrolysis of biopolymers, such as cellulose and hemicellulose, more glucose was released (17). To further confirm the hypothesis that cell walls of mango peel can be disrupted and thus biopolymers can be released to be transformed into bioplastics using a vinegar treatment, we measured the viscosities of the resulting hydrolysate solutions under various conditions. At 50 °C, the viscosity of the supernatant after 6 hours of hydrolysis was 24 cp, and it increased to 60 cp after 12 hours, 240 cp after 24 hours, and 282 cp after 48 hours, indicating more biopolymers were released from plant cell walls with increased hydrolysis duration. However, the viscosity dropped to 246 cp after 72 hours, suggesting that partial hydrolysis of released biopolymers and thermal degradation may have caused viscosity to decrease.

To remove residual acid, we first tried a membrane dialysis method. The hydrolysate solution incubated at 50 °C for 48 hours was dialyzed using a dialysis membrane tubing with MWCO 3.5 KD (Spectrumlabs, part number 132724) for 48 hours and then poured into a Petri dish to form a polymeric film. However, the films made from dialyzed solution were much coarser and too brittle to be handled. This is likely because the dialysis tubing removed molecules with a molecular weight less than 3500 da, which includes pectin, glucose, xylose, and fructose. This may indicate that pectin and sugars act as plasticizers and can improve the elasticity and flexibility of the films. Due to the negative impact of dialysis, the hydrolysate solutions were not dialyzed prior to casting the films we tested in this research.

Freestanding films were formed after casting the mango peel hydrolysate in vinegar onto Petri dishes and letting the aqueous solution evaporate. We assessed the transparency of mango peel films by placing East Ridge High School logos under the films. The obtained mango peel films were translucent, and longer hydrolysis times resulted in better translucency. The exception were the films formed under 35 °C for 6 hours, which had numerous defects because the released biopolymers were not enough to form a strong film under this mild condition (**Figure 1**). The East Ridge High School logo placed under the other films could clearly be seen and read. However, incubation at 70 °C and hydrolysis durations longer than 48 hours produced brown films, which impacted the translucency of the film.

Under a microscope, all obtained bioplastic films were revealed to be not smooth or homogenous. Microparticles, most likely cellulose and other non-soluble components, were dispersed throughout the polymeric matrix. The mango peel films showed coarser surfaces and larger particles at lower hydrolysis temperatures and shorter incubation durations. However, higher hydrolysis temperatures and longer incubation durations produced films with smoother surfaces and finer particles, which was consistent with measurements of surface roughness (**Figure 2A**). The surface roughness of the mango peel films, Ra, was observed to have a statistically significant difference among all three hydrolysis temperatures for each incubation duration, with the surface roughness decreasing as hydrolysis temperature increased (p <0.001). The surface roughness also significantly decreased with increasing hydrolysis duration up to 48 hours at all three temperatures (p < 0.001). No statistically significant difference was observed in Ra between the 48-hour and 72-hour groups for any hydrolysis temperature (p > 0.05).

Mechanical Properties of Bioplastic Film

We characterized the tensile strength of the mango peel films at break and compared their tensile strength to that of two commercial petroleum-based food packaging films, Kirkland Signature® food wrap and Freshrite® cling wrap.

The mango peel films formed using hydrolysate made at 35 °C hydrolysis temperature demonstrated a tensile strength that continually increased with hydrolysis duration up to 72 hours. The films formed using hydrolysate made at 50 °C and 70 °C hydrolysis temperatures showed a tensile strength that reached a maximum at a hydrolysis time of 48 hours and then declined as hydrolysis time increased. Interestingly, the hydrolysis conditions 50 °C and 48 hours resulted in the highest film tensile strength, which was significantly higher than all other conditions (p < 0.001) (**Figure 2B**). Therefore, 50 °C/48 hours was identified and chosen for the following studies.

The tensile strength is dependent on the thickness of the film, which can be adjusted based on the amount of hydrolysate added to the Petri dish. As the film thickness increased, the tensile strength increased. When the mango peel film was thicker than 65 µm, the tensile strength was

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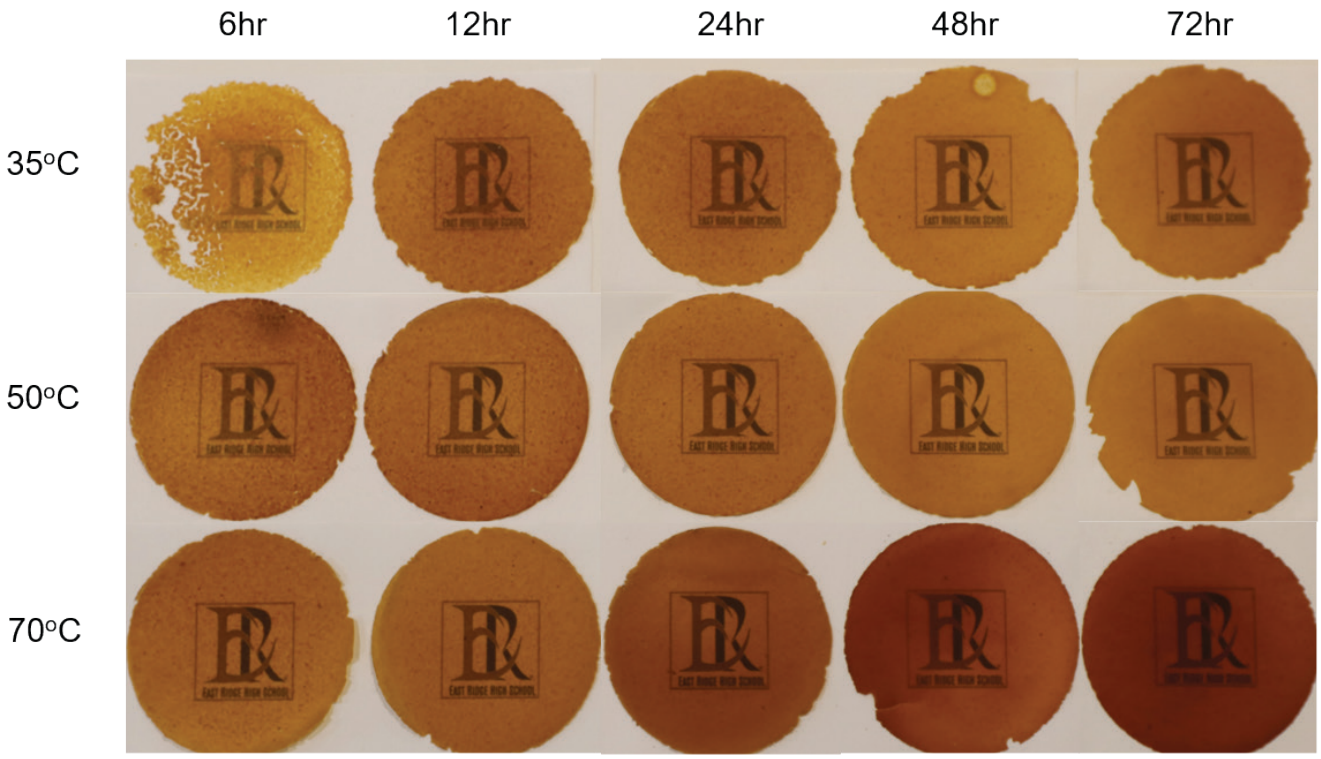

Figure 1: Photograph of mango peel films hydrolyzed under different conditions. Photos showing visual observation of mango peel film. The films were prepared from hydrolysis conducted at 35 °C, 50 °C, and 70 °C for 6, 12, 24, 48, or 72 hours using 5% vinegar solution. The East Ridge High School logo is placed under films to assess transparency. The films' thickness is between 150 and 200 µm, with a diameter of 90 mm.

equivalent to or greater than the commercial petroleum-based food packaging films. Mango peel films with a thickness greater than 105 µm had significantly higher tensile strength as compared to commercial films ($p < 0.001$) (**Figure 3**). However, the commercial petroleum-based films were only around 10 µm thick. This indicates that more material is required for the mango peel films to achieve similar strength as commercial films.

Plasticizer Effects

As packaging film, flexibility is important for the films to take on different sizes, shapes, and formats to meet demand. We have characterized film elongation, which reflects film flexibility, and we measured elongation by the percent the film was stretched at break. Almost all mango peel films were brittle and had low elongation. The films formed at 50 °C for 48 hours had a maximum elongation of 12.5% (**Figure 4A**). To use the bioplastic films for packaging, the film flexibility needed to be improved. We thus investigated three water soluble, non-toxic plasticizers to improve the mango peel film properties: glycerol, polyglycerol-3, and polyglycerin-10 at the commonly used concentration range of 10-30% (18).

All three plasticizers significantly enhanced the flexibility and elongation of the film ($p < 0.001$), as the film elongation at break increased by two to three times, depending on the plasticizer type and concentration. For example, the 30% glycerol mango peel film elongated 43.39%, whereas the control elongated 12.5% (**Figure 4A**). Although an increased plasticizer concentration resulted in better elongation, it negatively impacted the tensile strength of the film, resulting in a weaker film (**Figure 4B**). However, the reduced tensile strength of the mango peel film was still higher than that of the petroleum plastic food wraps (**Figure 3**). The 30% glycerol mango peel film had the lowest tensile strength, 10 N/0.5 inch, which is still twice that of petroleum plastic food wraps (5 N/0.5 inch). The commercial petroleum-based food wraps still had higher elongation than the plasticizer-containing mango peel films. For example, Kirkland Signature® food wrap and Freshrite® cling wrap had elongations of 99% and 168%, respectively. Compared to the mango peel film with no plasticizers added, we found that glycerol improved elongation the most but also reduced the tensile strength the most out of the three plasticizers. Given its mechanical performance, cost, and stability in the film, polyglycerol-3 was chosen for the following studies. Depending on the test, either 20% or 30% polyglycerol-3 formulation was used.

Water Resistance Improvement

The developed mango peel has poor water resistance. To address this issue, we utilized a novel method of dip-coating the mango peel films into a hydrophobic coating solution to modify the surface properties of the film and improve the water resistance of the bioplastic. This idea was inspired by the technology used in Tech Suits during swimming competitions to reduce the drag in water (19). The PM-3869 coating solution we used contains hydrophobic C18 groups. By dipping the film into the solution, a thin layer of hydrophobic coating was formed, which enhanced the water barrier properties without negatively impacting the mechanical properties of the film (**Table 1**). The water contact angle and WVTR were used

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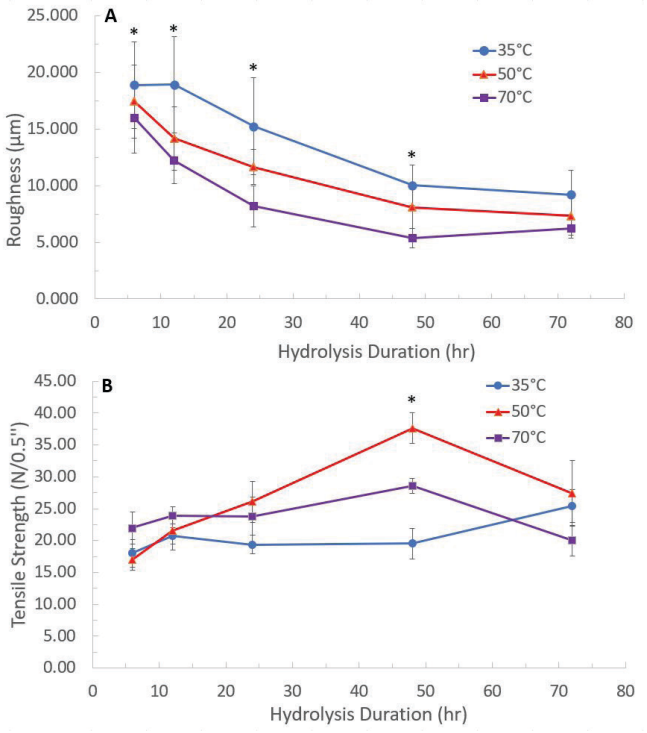

Figure 2: The mango peel film roughness and tensile strength as a function of hydrolysis duration and temperature. (A) Mean ± SD Ra, a roughness specification measuring the arithmetic average of the absolute values of the peaks and valleys of the surface (n = 18). The overall mean roughness at three hydrolysis temperatures for each hydrolysis duration was statistically significant (*p < 0.001). The mean roughness among the 6-hour, 12-hour, 24-hour and 48-hour groups for all three temperatures was also statistically significant (*p < 0.001) (B) Mean ± SD tensile strength, the peak force required to break film prepared under different temperatures and durations (n = 6). The film created at 50 °C for 48 hours had a significantly higher tensile strength than all other conditions (*p < 0.001). The films' thicknesses were between 150-200 µm.

to characterize the interaction between the mango peel film and water. Uncoated, the water contact angle of the film was 38.9 °. However, the contact angle of the coated film increased to 59.8 °, suggesting that the surface became less hydrophilic (**Table 1**). This was consistent with the WVTR data, which was reduced by 50% after coating the film in the hydrophobic solution.

In-Soil Degradation

In the last few decades, the need to protect our environment against plastic pollution and to use environmentally-friendly renewable resources has been brought to the global spotlight, thus making biodegradable materials desirable (20). Mango peel films developed using 30% polyglycerol-3, which was considered the worst-case scenario in terms of degradation, with and without the hydrophobic coating were used to study in-soil degradation. Film degradation started quickly with a 60% weight loss after week one. After 4 weeks, there was an 80% weight loss, and after 7 weeks, all films were completely degraded. The hydrophobic coating did not impact the biodegradation profile of mango peel films (**Figure 5**).

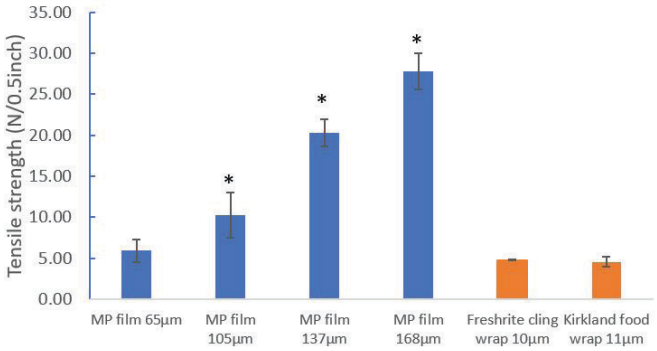

Figure 3: Tensile strength as a function of film thickness. Bar chart showing mean ± SD tensile strength of films with different thicknesses. Mango peel (MP) films were prepared under 50 °C/48 hr hydrolysis conditions and are compared to commercial petroleum-based packaging films, Kirkland Signature® food wrap and Freshrite® cling wrap (n = 3 − 6). Mango peel films with thickness higher than 105 µm had significantly higher tensile strength than both commercial films (*p < 0.001).

Microbiological Testing

Packaging film with antimicrobial properties can improve food safety and quality by preventing microbial growth, and thus it is more desired. Chitosan, a natural biopolymer derived from the shells of crustaceans, has gained attention in recent years due to its biocompatibility, biodegradability, and broad-spectrum antimicrobial activity (21). To obtain a packaging film with antimicrobial properties, we explored the idea of adding chitosan into the mango peel film as chitosan dissolves in acetic acid and is compatible with the mango peel acetic acid-based hydrolysate being studied. The zone of inhibition against *Escherichia coli* (*E. coli*) was used to assess the antimicrobial efficacy of bioplastic films with different levels of chitosan added. No measurable zone of inhibition was observed in any of the samples tested, indicating that they do not kill bacteria. The mango peel film with no added chitosan was observed to have bacteria growth underneath. However, no bacteria growth underneath the film samples containing chitosan occurred, which suggested that the films containing chitosan effectively inhibited the growth of *E. coli* (**Figure 6**).

DISCUSSION

The objective of the study was to develop a bioplastic film from biopolymers extracted from mango peel using hydrolysis. Our study confirmed that biopolymers were liberated from mango peel cell walls upon a vinegar treatment, forming standalone bioplastic films. Higher hydrolysis temperature and longer hydrolysis duration improved the film surface smoothness and tensile strength, and adding plasticizers or natural polymers enhanced the film properties. This supports our hypothesis that the cell walls of mango peels can be disrupted to release the biopolymers inside the cell walls to form bioplastic films with a vinegar treatment. In addition, it supports our hypothesis that the film properties can be optimized with process conditions and the addition of plasticizers or other natural polymers.

The mango peel film surface smoothness was enhanced by increasing the hydrolysis temperature and duration. It is possible that acetic acid refines the crystalline cellulose grains of the mango peel and hydrolyzes and liberates more

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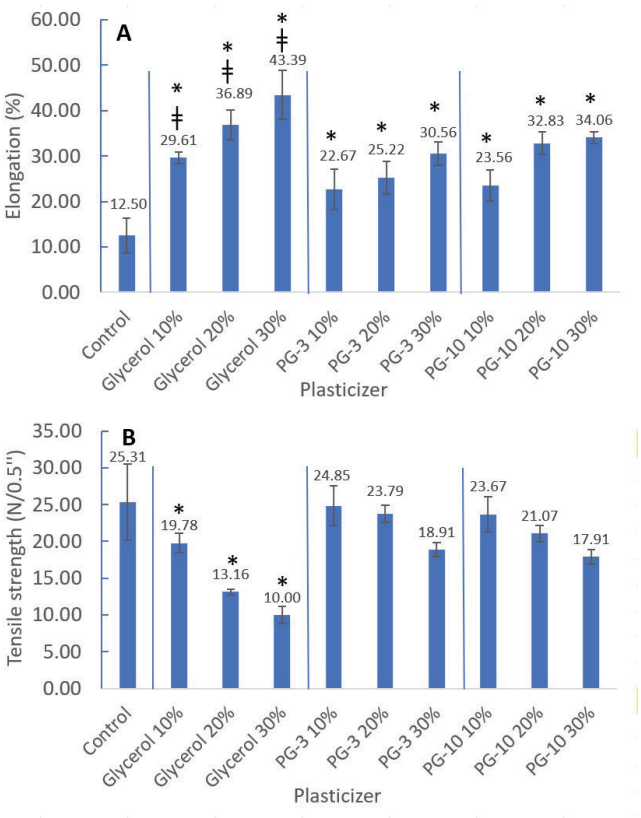

Figure 4: Effects of plasticizer type and concentration on mango peel film elongation and tensile strength. PG-3: polyglycerol-3, PG-10: polyglycerin-10 (A) Bar chart showing mean ± SD elongation, % film stretched at break (n = 6). All three plasticizer groups at three concentrations levels had greater elongation than control group (no plasticizer) (*p < 0.001). Glycerol group has statistically higher elongation than polyglyerol-3 and polyglycerin-10 group under same concentration (‡p < 0.001). (B) Bar chart showing mean ± SD tensile strength, the peak force required to break film (n = 6). All three glycerol groups had lower tensile strength than the control group, polyglyerol-3 and polyglycerin-10 groups (*p < 0.001). The tensile strength difference between the polyglycerol-3, polyglycerin-10 and control groups is not statistically significant (p > 0.05).

amorphous cellulose and hemicellulose when either the temperature is higher or the duration is longer (6). It was observed that there were always insoluble, fine particles left in the solution, regardless of the hydrolysis conditions.

After 48 hours at 50 °C, or after 24 hours at 70 °C, the color of the mixture and casted film began to change from a golden yellow (the color of the original mango peel powder) to a brownish yellow. At the end of the reaction, the mixture was a raw sienna color. Additionally, 70 °C resulted in weaker films than those created in 50 °C after 24 hours. This color transformation was likely caused by thermal degradation of the biopolymers in the mixture, which resulted in a decrease in film tensile strength performance. At 35 °C, the mixture remained a golden yellow throughout the entire 72-hour process. Because the reaction conditions were mild, biopolymers were likely slowly liberated from the plant cell walls and did not degrade. This resulted in a consistent increase of the film tensile strength that corresponded to the increasing hydrolysis time.

Film	Water contact angle	WVTR (g/m²/24 hr)	Tensile at break (N/0.5 inch)
No coating	38.9°	543.7 ± 19.4	18.00 ± 2.93
With hydrophobic coating	59.8°	284.8 ± 11.2	20.84 ± 1.33

Table 1: Mango peel film properties with and without hydrophobic coating (n = 3 − 6). Table showing water contact angle, wetting behavior of a solid surface with water; water vapor transmission rate (WVTR) and tensile at break. Formulation: 20% polyglycerol-3 in mango peel hydrolysate

Adding plasticizers into the hydrolysate solutions enhanced the flexibility and elongation of the mango peel films. Generally, glycerol or glycerol oligomers are abundant in hydroxyl groups, which reduce the hydrogen bonding between polymer chains and increase the flexibility of the polymer (22). Additionally, glycerol or glycerol oligomers have good moisture retention capabilities, further improving the polymer flexibility because water also plays the role of a plasticizer (23). Both polyglycerol-3 and polyglycerin-10 are mixtures of glycerol oligomers, but polyglycerin-10 contains longer chains of glycerol oligomers than polyglycerol-3. In contrast to polyglycerol-3 and polyglycerin-10, glycerol has a short molecular chain and remarkable water affinity due to its stronger hydrogen bonds with water. This effectively hindered the crystallinity of the mango peel films, resulting in the best plasticizer effects. Though polyglycerol-3 and polyglycerin-10 did not improve the film elongation as much as glycerol, the tensile strength of the films was not compromised as much as with glycerol. We also found that adding plasticizer into the films did not significantly change the film roughness and transparency. While adding plasticizer to the film significantly improved the elongation, the elongation of the mango peel films was still lower than that of commercial petroleum-based food wrap.

We assessed the in-soil degradation of the developed mango peel films. After the first week covered in soil, the mango peel films shrank significantly and over 60% of the weight was lost. This was likely caused by the leaching out of low molecular weight materials, such as pectin, sugars, and the added polyglycerol-3. After the second week, the

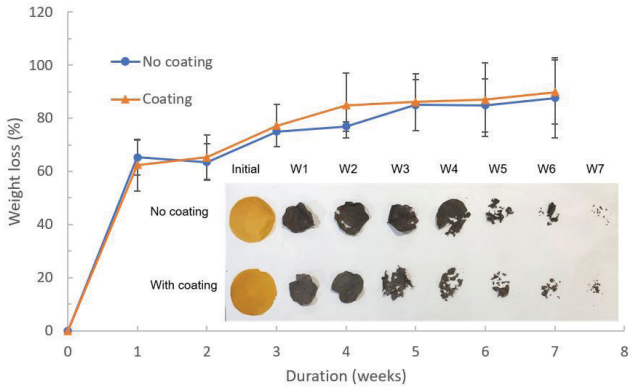

Figure 5: Mango peel film in-soil degradation. Graph showing the weight loss over time of mango peel films with or without a hydrophobic coating when buried in soil (n = 2). Photos showing degraded dried film over time (n = 1). Formulation: 30% polyglycerol-3 in mango peel hydrolysate

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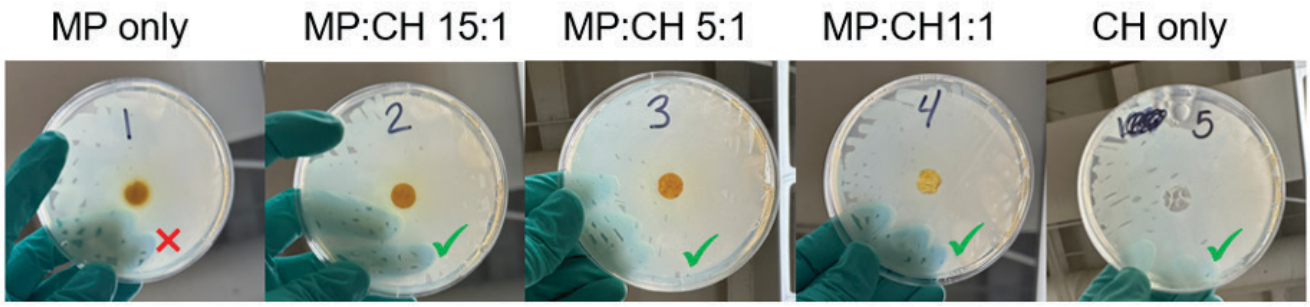

Figure 6: Zone of inhibition results against *E. coli*. Photos showing agar plate inoculated with *E. coli* with mango peel film placed on top for 24 hours. A red ✕ denotes that bacteria grew under the sample. A green check mark ✓ denotes that no bacteria grew under the sample. MP: mango peel hydrolysate, CH: chitosan. Formulation: 20% polyglycerol-3 in mango peel hydrolysate, no hydrophobic coating was used.

mango peel films had holes, which indicated degradation and led to more weight loss over time. By the end of week four, only pieces of the film were remaining. At the end of week seven, only tiny scraps were left. The data strongly indicate that the mango peel film developed is biodegradable, and that the hydrophobic coating does not negatively impact the degradation of the films. Compared to petroleum-based plastics, the developed mango peel bioplastic film easily degrades in soil, which could help decrease the impact that plastic makes on oceans, the environment, and the atmosphere. However, the impact of the plasticizers and hydrophobic coating on the stability and shelf life of the film is an area that needs to be investigated.

Based on the zone of inhibition testing, the mango peel films containing chitosan are bacteriostatic. The benefit of bacteriostatic bioplastic films is the potential of extending the shelf life of packaged food. As chitosan is commercially available with a variety of molecular weights, which impact its antimicrobial activity, the mango peel/chitosan bioplastic properties including mechanical strength, surface smoothness, biodegradation as well as antimicrobial performance may be impacted by the type and concentration of chitosan. Future work will focus on maximizing the mango peel/chitosan bioplastic properties.

In summary, a novel and eco-friendly acetic acid hydrolysis process was developed to transform mango peels into bioplastics, which have the potential to be used as packaging film. The process conditions, including hydrolysis temperature and duration, were optimized to achieve the desired film performance. It was found that intermediate temperature (50 °C) and relatively long duration lengths (48 hours) for hydrolysis led to the best bioplastic film results. Among the three plasticizers studied, polyglycerol-3 was chosen due to its low cost, excellent stability, and ability to enhance elongation while minimally compromising the tensile strength. A higher water contact angle and reduced WVTR indicated that the hydrophobic coating was able to significantly improve the film's water resistance. Adding chitosan into the mango peel films improved their antimicrobial properties, which effectively inhibited the growth of *E. coli* underneath. Overall, the developed mango peel films were translucent, biodegradable, bacteriostatic, and exhibited good tensile strength at break. Because this bioplastic is derived from renewable biomass and has a smaller carbon footprint than traditional plastics, it offers potential environmental benefits. However, there are some limitations as well. For example, the flexibility of the developed bioplastic is inferior to its petroleum-based counterpart. Thus, future work will focus on improving the flexibility and elasticity of the mango peel film and understanding how chitosan impacts the overall performance of mango peel films.

MATERIALS AND METHODS

Materials

The mango peels were obtained from ripe Ataulfo mangoes, which were purchased at local grocery stores. The dried mango peels were ground to powder using a Blendtec food blender (Orem, Model: ES3), then sifted through a 500 µm sieve (VWR International, catalog no. 57334-458) and stored in a container in a desiccator prior to use. Acetic acid, or 5% acidity vinegar solution (Heinz, item no. 013000581608) was used to hydrolyze mango peel. Glycerol (Sigma-Aldrich, catalog no. G9012), polyglycerol-3 (Inovyn, EC number: 915-741-3) and polyglycerin-10 (Spiga Nord S.p.A. Kosher grade) were used as plasticizers. The hydrophobic solution (3M, product no. Scotchgard PM-3869) and chitosan (Sigma-Adrich, catalog no. 419419) were used to improve the bioplastic properties.

Acid Hydrolysis and Film Preparation

Hydrolysis was conducted in the 5% vinegar solution with varying temperatures and durations using a magnetic hot plate stirrer with temperature probe (VWR, catalog no. 76549-918). Three grams of mango peel powder were added into 60 g of acetic acid solution at three separate temperatures: 35 °C, 50 °C, and 70 °C, each for 6 hours, 12 hours, 24 hours, 48 hours, and 72 hours while the solution was continuously stirred at 200 rpm. After, 20 g of the obtained hydrolysate solutions were poured into a 100 mm x 15 mm Petri dish (Fischer scientific, catalog no. FB0875712) and air-dried on a leveled drying shelf. Each condition was run in triplicate. After the films dried, they were stored in a room with 45% relative humidity to precondition the films for at least 48 hours before further characterization.

In some cases, plasticizers were added into the hydrolysate solution to modify the elongation and elasticity of the bioplastic film. Once hydrolysis was completed, 10%, 20% or 30% of glycerol, polyglycerol-3, or polyglycerin-10 with respect to the mango peel dry weight was added to the solutions, and the mixture was continuously stirred for an hour. The films were

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then cast and dried, as previously mentioned.

A hydrophobic coating solution PM-3869 was applied to the dried, cast films by dip coating to improve water barrier properties. The complete process of developing bioplastic films from mango peels is illustrated (**Figure 7**).

Adding Chitosan into Mango Peel Films

Adding chitosan into the mango peel films was explored to improve the antimicrobial properties of bioplastic. First, chitosan was dissolved in 5% acidity vinegar to make a 1 wt% solution. The mango peel hydrolysate vinegar solution and the chitosan solution were precalculated, weighed and then mixed to obtain target dry weight ratios of 15:1, 5:1, and 1:1, respectively, then cast into film.

Characterization of Hydrolysate Solution Viscosity

The hydrolysate solutions after reaction were characterized using a viscometer. First, the hydrolysate solution was centrifuged for 20 minutes at 2000 rpm (Beckman Counter, model no: ALLEGRA-6R) and the supernatant was collected. The viscosity of the supernatant was measured using a viscometer with a spindle LV-4 (Brookfield, model no: DV2TLVTJO).

Characterization of Film Thickness and Surface Roughness

The thickness of the bioplastic film was measured using a micrometer (VWR, catalog no. 470110-398). The surface roughness was characterized through a profilometer with a 5 µm probe tip made by Mitutoyo (model number: 178-561-12A). Ra, a roughness specification that measures the arithmetic average of the absolute values of the peaks and valleys of the surface, was used in this study. Eighteen measurements were taken for each sample condition.

Characterization of Film Tensile Strength and Elongation

The tensile strength and the elongation of the bioplastic films at the breaking point were measured using a self-built apparatus using an FGE-10XY force gauge (VWR, item no. 97024-778) mounted on a manual horizontal force test stand made by Baoshishan (model number: APW). The bioplastic film sample was first cut into three 0.5-inch-wide strips that were approximately 3 inches long. The gauge clamp and the fixing clamp were set 30 mm away from each other, and each end of the film sample strip was aligned and fastened to a clamp. The test began with the sample strip loose, with no tension applied. Then, the handwheel was slowly turned to move the gauge clamp away and pull the sample strip apart. The peak force was recorded as the tensile strength when the sample broke. The stretched film length at break was also recorded. The ultimate elongation percentage of the film was calculated by subtracting the final length of the film by 30 mm then dividing the result by 30 mm and multiplying by 100. Each film sample was run in a replicate of six.

Characterization of Film WVTR

The WVTR was measured by calculating the weight loss of water in a 4 oz glass bottle after 24 hours. The glass bottle had a cap with an oval shaped hole with a surface area of 0.8 inch^2 (5.16 X 10^{-4} m^2). The film sample was first cut into a 1.5-inch diameter circle and placed over the opening of the glass bottle, which contained 50 mL of water. The cap was then placed over the film, tightened, and the bottle was immediately weighed as W_1 grams. The bottle was placed in an ambient condition, and after 24 hours, it was weighed again as W_2 grams. The WVTR was calculated with Equation 1:

$$WVTR = \frac{(W_1 - W_2) \times 24}{5.16 \times 10^{-4} m^2 \times T} \quad (1)$$

where T is the test period in hours, and the unit of WVTR is g/m^2/24 hr.

Characterization of Film Water Contact Angle

The static contact angle was measured with a Pocket Goniometer PGX (FIBRO System AB, S/N: #51120). A 5 µL water droplet was placed onto the film, and images were captured at 20 seconds after contact. Then, the angle between the water droplet and the film surface was measured at the point of contact.

Characterization of Film In-Soil Degradation

The study of the degradation of selected bioplastic

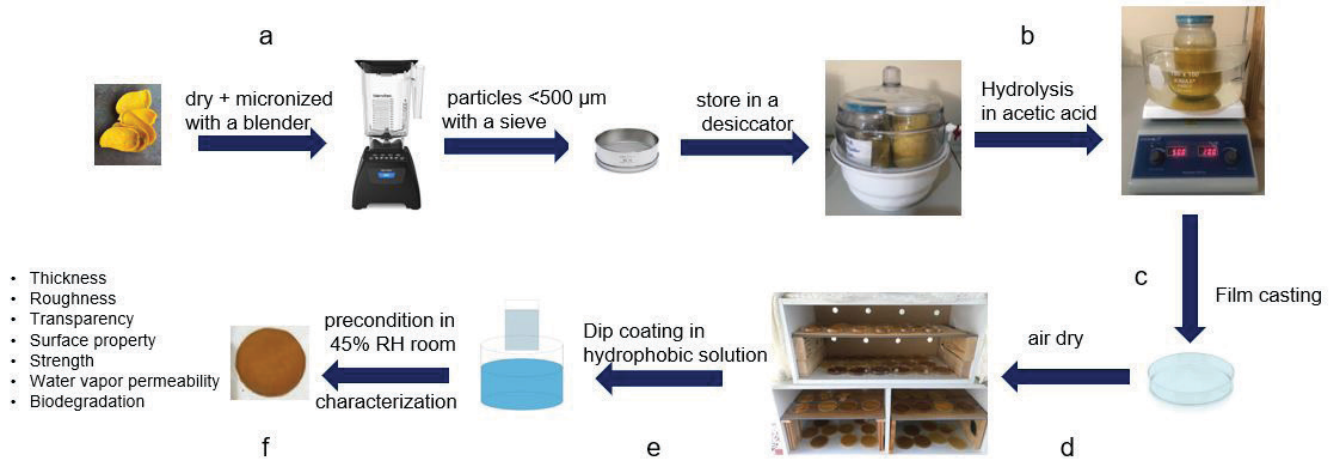

Figure 7: The complete process of bioplastic film making from mango peel. Mango peel grinding (a), hydrolysis in vinegar (b), film casting (c), film drying (d), film post treatment (e) and film characterization (f).

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films in soil was conducted in garden soil over a period of 7 weeks. The dried film samples, each 90 mm in diameter, were weighed (W_1), and placed into separate nylon mesh bags. The mesh bags containing the film samples were then buried under between 1 and 4 inches of garden soil which was stored in a room at temperature around 20 °C. After each week, predetermined samples were taken out and the soil attached to the samples was carefully removed with a brush. After the samples dried, they were re-weighed (W_t). Finally, the weight loss of each sample was determined using Equation 2 and was plotted as a function of time. Each week, pictures of the dried films were taken. Each sample was run in duplicate for this degradation study.

$$weight\ loss\% = \frac{(W_1 - W_t)}{W_1} \times 100\% \quad \textbf{(2)}$$

Film Microbiological Testing (Zone of Inhibition)

The zone of inhibition, a quick and qualitative test to assess the antimicrobial activity of a specimen against the microorganisms being grown on an agar plate, was used in this study. A 10^8 CFU/ml suspension of the *E. coli* 8739 strain (Fischer Scientific, Catalog no. 23-021178) in Phosphate Buffer (Butterfield's Buffer) was first prepared. A sterile cotton swab was then dipped into the *E. coli* suspension and the whole plate was inoculated with *E. coli* by applying the swab over the entire agar surface. A 10 mm diameter sample was cut with a disinfected die and was then placed onto the center of the inoculated plate using disinfected forceps. Then, the plates were incubated at 35 °C for 24 hours. Afterwards, the diameters of the zones where no bacteria grew were measured.

Data Analysis

Data were statistically analyzed using MiniTab. A one-way ANOVA was applied to assess the data collected at different variables levels. Tukey's HSD comparison procedures were applied, assuming equal variances with a significance level of 0.05.

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