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Article

Comparing the biodegradability of petroleum-based plastic with a novel, sustainable bio-plastic alternative

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SUMMARY

The convenience and affordability of single-use plastic products makes them an attractive option for consumers. The toxic constituents of these traditional plastics, however, are known to cause a variety of health issues in thousands of species. These environmental hazards, along with the issue of white plastic and microplastic pollution, causes increased interest in biodegradable alternatives to petroleum-based hydrocarbons. In this research, a novel bioplastic inclusive of bamboo tannins and chitosan is selected from more than 60 trial formula variations based on resulting strength, fatigue, and transparency attributes. The biodegradability of the finalized bioplastic is compared to that of conventional polyethylene, in addition to investigating its solubility and water absorbance. Biodegradation rates of the bio-based plastic exceeded that of the petroleum-based formula, as determined with Fourier Transform Infrared Spectroscopy (FTIR-ATR) analysis. The behavior of the experimental product in water deviated from the initial hypothesis, with substantial weight increase of approximately 193% after 60 minutes. A cost analysis displayed a difference of $0.0016 between the two products, with the natural additives of the experimental being more expensive. This research displays the potential of a legitimate, fully biodegradable plastic alternative to current marketplace bioplastics.

INTRODUCTION

The reliance on single-use, synthetic plastic polymers dominates hundreds of industries despite the variety of environmental and health risks they pose (1). Accumulating at a rate of eight million metric tons per year, the practice of recycling plastics, such as polystyrene and polyethylene, is being abandoned, and facilities are resorting solely to incineration or landfill usage to dispose of plastics (2). Such methods endanger thousands of species via air pollution, water contamination, and the production of toxic residue (3). Plastic substitutes with at least 20% of their constituents considered renewable are currently seen as the key to combating white pollution (4). Additionally, bioplastics are evolving in the commercial market which appeal to environmentally conscious consumers; however, most of these new products are not capable of complete degradation (5-6).

A majority of bioplastics include corn or cassava starches, which are converted to polylactic acid prior to use (7). The incorporation of carbohydrates is responsible for the tendency of such substances to break down with prolonged contact with water or soil (8). However, utilizing a food source as the backbone of such a ubiquitous material may not be ideal due to the potential increase in land and fertilizer usage. In this research, a bioplastic film incorporating tannins, a derivative of *Bambusa sp*. (bamboo), chitin, derived from *Gryllus sp*. (field cricket), and agar agar, originating from seaweed, was developed over the course of nine months with over 60 formulas tested.

Hydrolyzable tannins, a group of amorphous substances commonly found within bamboo, form complexes with a variety of proteins and carbohydrates, mainly those rich in proline (PRPs), such as gelatin and agar (9-10). While there is some debate as to whether tannins cause the precipitation of proteins or the formation of a complex of weak and strong chemical bonds, it is crucial that the solution is the proper acidity to reach its isoelectric point in order for any notable interactions to occur (11). Bamboo was selected as the source material from which to extract tannins because of its sustainability and wide availability (12). Certain species of the *Bambusa* genus are beginning to be considered nuisances because of their rapid growth and ability to repopulate in a multitude of conditions (13). These features make bamboo particularly attractive for costing models.

Chitosan, derived from the carbohydrate chitin, is an example of a substance that interacts with tannins (14). An abundant polysaccharide, chitin's occurrence in nature is only second to cellulose. It can be found in the cell walls of fungi, marine invertebrates, insect exoskeletons, as well as numerous plants and animals (15). Chitin is a natural polymer with a varied cellulose structure, containing an N-acetyl group instead of a hydroxyl group at the second carbon (16). Its low solubility usually requires a deacetylation process for conversion to chitosan **(Figure 1),** which is necessary for proper complex formation (17-18).

In this investigation, the degradation rate of the innovative bioplastic in both water and soil, in addition to its absorbance, was compared against a traditional, petroleum-based film after initial formula selection. Serving as a potential substitute for single-use plastic wraps/films, poor transparency and mediocre strength, biodegradation, and fatigue resistance

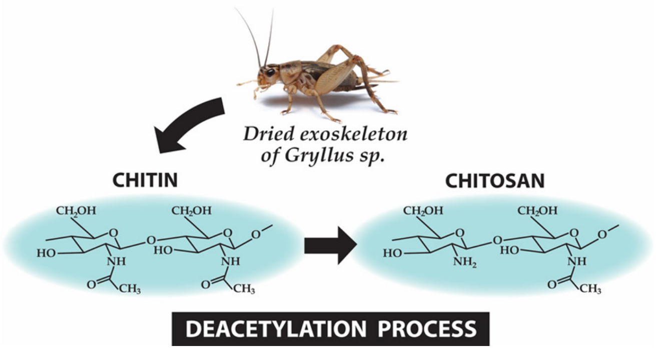

Figure 1. Converting chitin to chitosan. The reaction requires the degree of deacetylation to be between 60% and 90%. Once deacetylated, the product is then soluble in organic acids, such as acetic acid.

are acceptable, but low solubility and water absorption are imperative. We hypothesized that the bioplastic formula would biodegrade much more rapidly than the polyethylene film but have little solubility and absorption. The ideal biodegradable film for application in the packaging industry should undergo a lag phase, sustained from synthesis to disposal, before the biodegradation process accelerates.

RESULTS

Part I: Preliminary Testing and Selection

The nature of this research required a two-part procedure for an adequate product comparison. The first component involved the formation of a novel bioplastic, primarily incorporating chitosan, tannins, and agar agar. Additional elements, such as acetic acid and glycerin, were included to ensure successful interactions that generated a stable material. The final product was developed through a process of trial and error with more than 60 created bioplastics **(Figure 2, Table 1).**

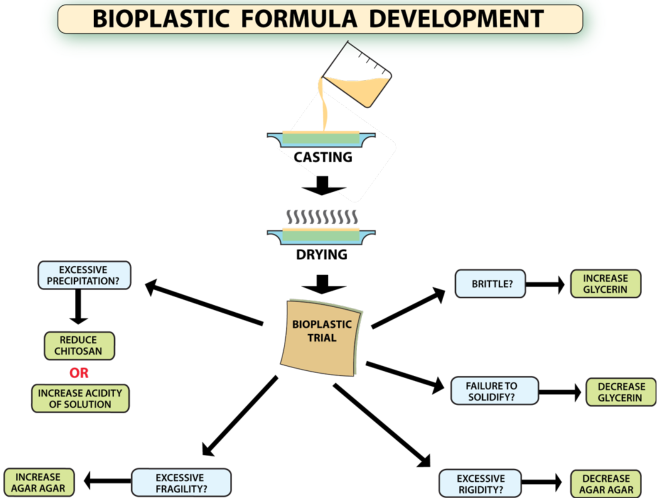

Figure 2. Flow chart demonstrating the experimentation process. Trial and error led to the creation of the novel formula. Frequent adjustments were made to ensure the product was pliable and able to withstand handling during strength, flexibility, transparency, biodegradation, water absorption, and solubility tests.

The experimental bioplastic formula used in the comparison was selected based off the results of three preliminary tests—strength, transparency, and fatigue—for nine final film compositions. A grading scale was constructed to simplify the selection process **(Table 2)**. Data collected from a simulated tensile strength test and UV-spectrophotometry were favorable for obtaining a basic profile of each film tested and determining which is most suitable for further examination. Fatigue resistance was used to simulate repetitive use as well as gauge flexibility; a value greater than 100 represented a material with adequate flexibility for sustained usage and comfortable handling **(Figure 3A)**. The minimum strength requirement was arbitrarily decided to be

Table 1. Characteristics/scores of nine bioplastic formulations.

Trial	Agar agar	Tannin	Chitosan	Acetic Acid	AA Treatment	Glycerin	Solidification	Transmittance (%)	Strength (N)	Fatigue (Cycles)	Final Score
1	4.000%	2.000%	0.100%	8.000%	No	0.150%	Yes	6.20%	8.92	>100	7
2	4.000%	2.000%	<0.001%	8.000%	N/A	0.100%	No	N/A	N/A	N/A	0
3	4.000%	2.000%	<0.001%	<0.001%	N/A	1.000%	No	N/A	N/A	N/A	0
4	4.000%	2.000%	<0.001%	8.000%	N/A	2.000%	No	N/A	N/A	N/A	0
5	4.000%	2.000%	2.000%	8.000%	No	0.200%	Yes	<0.01%	20.14	29	5
6	7.500%	2.000%	<0.001%	8.000%	N/A	0.150%	Yes	18.65%	7.19	>100	8
7	7.500%	2.000%	2.000%	8.000%	No	0.150%	Yes	<0.01%	20.34	1	4
8	7.500%	2.000%	2.000%	8.000%	No	0.100%	Yes	<0.01%	6.43	<1	1
9	7.500%	2.000%	0.100%	8.000%	Yes	0.150%	Yes	12.52%	11.72	>100	8

Note: The final experimental plastics were ranked based on ability to solidify, transparency, and flexibility using a grading scale for each attribute. The final score was the summation of points from the three areas of evaluation. No points were awarded for solidification; however, a failure to solidify resulted in a final score of zero. The formula with the highest point total was selected for further experimentation. Formula #9 was selected due to the preference of a strong film for biodegradation comparison.

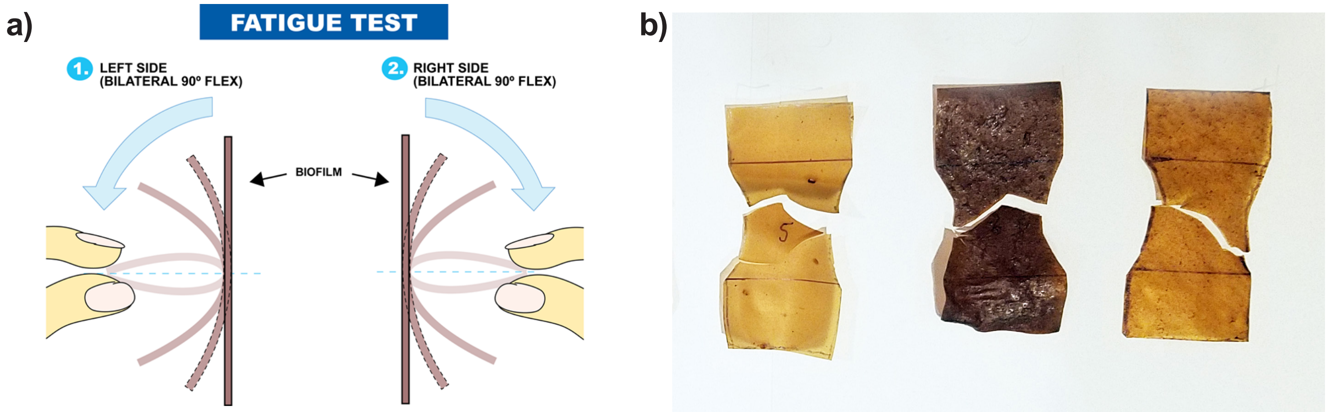

Figure 3. Preliminary flexibility and strength testing procedures. a) Each 70 mm x 20 mm x 0.1mm bioplastic trial was subjected to a flex test to examine its ability to withstand fatigue. One 90-degree bilateral flex to each side was termed one cycle. The trial was given the full 5 points if it was able to complete 100 cycles with no signs of breakage. **b)** Samples were cut into a dog bone shape to maintain a strong grip and limit error. Breakage occurred within a range of approximately 500-1850 mL of water. The maximum weight (in kilograms) each sample could sustain was multiplied by the gravitational constant to obtain the force in newtons.

9.81 N, meaning that the optimal film would be able to support the weight of one kilogram, sufficient for its intended use **(Figure 3B)**. Transparency is trivial when compared to the other mechanical properties but was considered when tasked with designing an aesthetically pleasing commercial product. Trial 9, with a 75:1 ratio of agar agar to chitosan, received the highest rating with the ability to withstand approximately 11.72 newtons of force and the maximum number of flexes, in addition to its fair transmittance of 12.52% **(Table 1)**.

Part II: Water Absorption and Solubility Comparison

After initial testing, ten 10 mg samples of both the selected bioplastic and polyethylene film were used for an *in vitro* biodegradation test, using soil as the source of microorganisms. Subsequent to the 168-hour period, Fourier Transform Infrared Spectroscopy (FTIR) with the Attenuated Total Reflection attachment (ATR) was utilized to determine the percent degradation, using unexposed samples as controls. The FTIR-ATR analysis of three peaks for the biodegraded bioplastic film produced an average peak ratio (control:experimental) of 1.000:0.778 **(Figure 4A, areas #1-3)**, while a peak ratio of 1.000:0.978 was calculated for the petroleum-based plastic **(Figure 4B, areas #1-2)**. Negligible change was seen in the polyethylene film, unlike the tannin and chitosan-based formula, which achieved approximately 22% biodegradation in the 168-hour period. A *t*-test suggested that the difference in biodegradation rates was not significant ($p = 0.0635$; $\alpha = 0.05$).

Fifteen 10 mg samples of each plastic type were submerged in distilled water for one of five possible timespans, then reweighed using an analytical balance to conclude the water absorption testing. The turbidity of the remaining solution, in addition to FTIR analysis as a secondary method, was used to calculate percent solubility. Solubility was examined to determine if the material would break down when deposited into a marine system, while water absorption was assessed to estimate the film's practicality as a consumable wrapping or similar product. Absorbance significantly increased across all bioplastic trials, which was confirmed by a *t*-test assuming unequal variances ($p = 0.0191$; $\alpha = 0.05$). The average percent absorption for the bioplastic samples for 1, 5, 15, 30, and 60 minutes were 110%, 168%, 176%, 206%, and 193% respectively **(Figure 5A)**. Around the 60-minute mark, the bioplastic samples reached peak solubility, with the average turbidity of the remaining solutions increasing from 20 FAU at the 1-minute mark to 66 FAU after the full 60

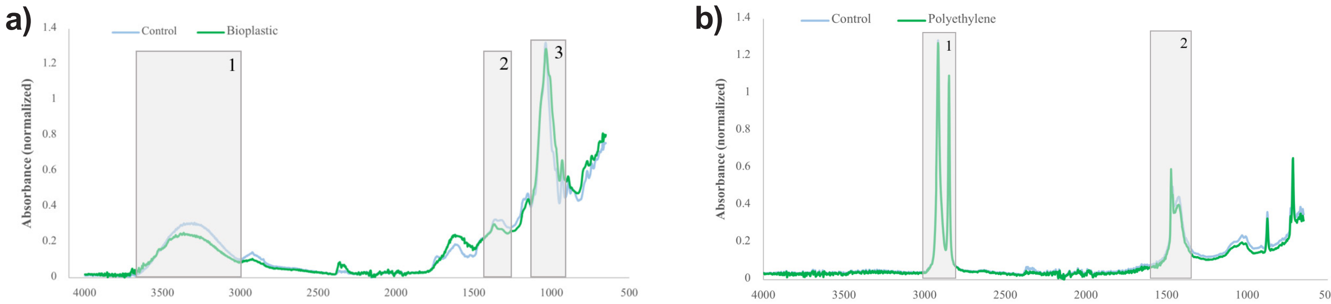

Figure 4. Results of biodegradation analysis. FTIR-ATR spectra of the biodegraded a) bioplastic (1.000:0.778) and **b)** polyethylene (1.000:0.978) samples after an exposure period of 168 hours at 25±2°C. Unexposed bioplastic and polyethylene samples were used as controls to calculate the peak ratios. 64 iterations of each sample were taken to reduce error from outside factors (sound, carbon dioxide, etc). Shaded regions indicate analyzed peaks used to produce the final ratio of the control to the experimental.

minutes, translating to 5.5% and 18.2% solubility. The FTIR supported these results by producing an average peak ratio of 1.000:0.9373:0.9349:0.7133 (control:5min:15min:60min) **(Figure 5B, areas #1-2)**. Unlike the experimental bioplastic film, the polyethylene samples were consistently insoluble (*p* = 0.006; α = 0.05) and had low absorption values **(Figure 5A)**.

DISCUSSION

The chitosan and tannin bioplastic formula designed in this investigation demonstrates potential after analyzing its effectiveness compared to petroleum-based plastic film. The mean biodegradation rates of each material suggested that the 10.0 mg sample of the experimental bioplastic biodegraded 11 times faster than its petroleum-based counterpart. The average biodegradation rate of approximately 22% per week is particularly impressive when considering other biodegradable plastics currently on the retail market which, in fact, do not fully biodegrade as implied (19). The product's ability to biodegrade is mainly due to the utilization of organic constituents in the formula (20). The unnatural bonding in polyethylene is responsible for its indestructible nature, as few microorganisms are equipped with the metabolic pathways needed to weaken the long chains of such interactions (21). Evidence of this is seen in the FTIR spectra between 1400 and 1600 cm^{-1} (region III), where a peak indicating carbon-carbon double bonding is visible for polyethylene (22). The presence of C-H bonds (1050 cm^{-1}) in the bioplastic, commonly observed in organic compounds, allows for increased biodegradation (23).

Though the usage of environmentally friendly constituents was responsible for rapid biodegradation, it was also responsible for the film's water absorbance and dissolution in distilled water (24). Shortly after submersion, the absorption of the bioplastic plateaued and eventually decreased. The slight decrease in weight may have been due to dissolution. We inferred that this trend would continue to increase inversely with time. The FTIR-ATR analysis confirmed these findings, suggesting that a minimum exposure time of 60 minutes is necessary to achieve considerable dissolution. Distortion of the carbon-carbon bond within the film as a result of its introduction to water is evident near 1600 cm^{-1}, indicated by the varying peaks (25). The FTIR-ATR analysis differed from the colorimetry solubilities by an average of 12.4%. The discrepancy is likely due to excess water remaining on the samples at the conclusion of testing, resulting in continuous dissolution; however, the FTIR result still mirrors the incremental differences over the 60-minute period that the colorimetry provided. A sharp peak is seen in the fingerprint region near 1,020 cm^{-1} within the FTIR spectra of the experimental plastic, a possible indicator of the presence of aliphatic amines, molecules that interact with water to form hydrogen bonds (26-27). Another peak is visible in region I (4,000 to 2,500 cm^{-1}), unlike the polyethylene samples, revealing that the suggested hydrogen bonding is present (28). The existence of O-H or N-H single bonds indicate polarity, justifying the film's solubility in a polar solute (29).

The pricing of polyethylene was approximately $0.0031 per square centimeter, while the bioplastic was approximately $0.0047 per square centimeter. When considering the additional cost for maintaining a landfill for the polyethylene products (including after closure costs), the price rises to $0.0035, meaning the bio-based film costs 34% more than its competitor (30).

The data supports the hypothesis that the bioplastic has an enhanced biodegradation capability, displaying an average degradation rate of 22% per week. However, the results do not support the hypothesis in that the water absorption and solubility percentages were significantly higher in the bio-based formula. Excessive water absorption may inhibit its application as a single-use product because of the weakened structure and durability. A product with solute capabilities is of value, under certain conditions. Rapid dissolution is a hindrance that must be addressed in order to consider its usage as a legitimate alternative. Despite this, the bioplastic

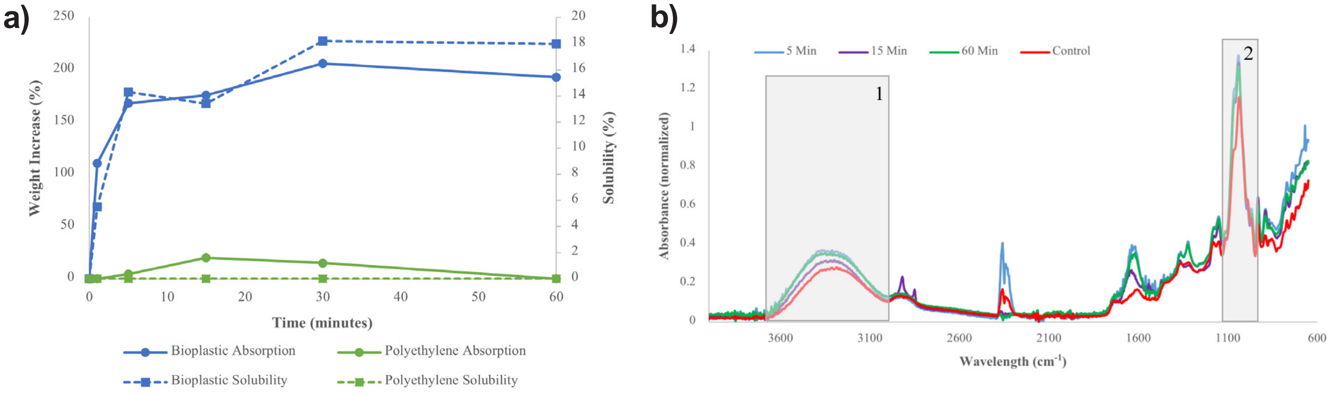

Figure 5. Results of water absorption/solubility testing. a) Both plastic films were subjected to distilled water at 22°C for five exposure times — 1, 5, 15, 30, and 60 minutes (n = 3). Mean absorbance values of the bio-based plastic were significantly higher than those of the polyethylene film (*p* = 0.0191; α = 0.05). **b)** Subsequent to solubility testing, further evaluation of the experimental samples determined moisture effects on the structural integrity of the bioplastic film. Shaded regions indicate peaks that were investigated to produce the final ratio of the control to the experimental samples (by increasing exposure time: 0.9373:0.9349:0.7133).

film was successful in that it displayed the basic mechanical properties fundamental to plastic packaging and achieved considerable biodegradation within the experiment's timeframe.

Adjustments to the formula of the bioplastic could be considered as certain properties made testing cumbersome (i.e. stiffness due to lack of glycerin). With the improvements, tensile strength testing in addition to film elongation testing at breaking point will be warranted choices. Supplementary testing may also be conducted to test the film's effectiveness as a wrapping for consumables, which may be applied as a spray or an immersive solution. Besides its application in the food and packaging markets, this biodegradable, moldable plastic may be useful in the medical industry as well. One of its uses may include bioabsorbable surgical implants which assist with tendon-to-bone repair, or for improved methods of implanted drug delivery due to its biodegradability and possible limitation of bacterial growth. Coupled with copolymer blending, this novel bioplastic formula shows much future ability.

MATERIALS AND METHODS

Part I: Bioplastic Formulation and Selection

Formula Constituents

The solution-casting method was utilized to synthesize the bioplastic in this research. The primary constituent of the most successful formula was agar agar (7.50 wt%), which acted as the base substance of the film. Hydrolyzable tannins derived from members of the subfamily *Bambusoidae* (common bamboo) were incorporated into the mixture (4.00 wt%), obtained through boiling of sheared, young culms approximately 2.50 cm in diameter in distilled water for upwards of 200 hours. The ability of tannins to form a complex of chemical bonds with proline-rich proteins and carbohydrates, including chitin, was the reasoning for its inclusion (31). Dried exoskeleton of *Gryllus sp.* was ground into a fine powder using a spice mill and added to the formula; however, due to the incomplete solubility of granular particles, 0.300 g of commercial grade powdered chitosan (X002ATKNGV, MarkNature) was dissolved within 5.00 mL of 5% acetic acid and added as a supplementation. In an effort to reduce the pH of the solution and effectively reach the isoelectric point calculated prior to experimentation (approximately 5), acetic acid was selected as the solvent for the chitosan solution (32). Additionally, glycerin, a plasticizer responsible for the flexibility of the product, was included at a concentration of approximately 0.200 wt%. A solution was produced with all additives using distilled water as the solvent at a ratio of 11:2. The solution was heated to 95±5°C with constant stirring, then filtered through a sieve with a 2 mm aperture diameter. Once cast into silicone molds, gelatinization occurred, and the films dried at room temperature (23°C) for 48–72 hours. A similar procedure applied to all films, with only the concentrations being altered according to **Table 1**. Formulas that failed to produce a solidified product or displayed excessive brittleness were ineligible to proceed to preliminary testing. The final experimental formula described was selected from the favorable trials using a grading scale which considered transparency, strength, ability to solidify, and ability to withstand fatigue **(Table 2).**

Table 2. The grading scale of the three preliminary tests.

Score	Transmittance (%)	Force (N)	Number of Cycles
0	<0.01	<0.01	<1
1	0.01-12.50	0.01-10.0	1-25
2	12.51-25.00	10.1-20.0	26-50
3	25.01-37.50	20.1-30.0	51-75
4	37.51-50.00	30.1-40.0	76-100
5	>50.00	>40.1	>100

Note: Each bioplastic received a score of 0–5 based on the percent transmittance of light, the force of weight (in newtons) each dog-bone shaped samples could withstand, or the number of flexing cycles that were completed before tearing/breaking was imminent.

Transparency Testing

Transparency was determined using the transmittance (%T) obtained through UV-spectrophotometry using a Shimadzu UV-1201 spectrophotometer at 600 nm. Each of the nine formulas were cut to fit the spectrophotometer cell (approx. 70 mm x 20 mm x 0.1 mm), and the resulting transmittance was an average of three trials. Testing was conducted in low-light areas in order to reduce the amount of error from stray light.

Strength Testing

Strength was estimated by determining the maximum force of weight (N) each 60 mm x 30 mm x 0.1 mm dog bone-shaped bioplastic sample could withstand. Each solidified sample (6 in total) was suspended vertically at each wide dog bone end between two 5.1 cm spring clamps. A container was attached via nylon cording to the lower clip, freely suspended. Water was added in 100 mL increments into the container attached to the apparatus. After breakage, the force in newtons was calculated by multiplying the mass of water by gravitational acceleration **(Figure 3B)**.

Fatigue Testing

Each film's ability to withstand fatigue was tested by performing 90-degree bilateral flexes in series until tearing was visible **(Figure 3A)**. Testing concluded if a sample was able to withstand one hundred bilateral flexes in series without disturbing the film's structural integrity.

Part II: Polyethylene Comparison

Biodegradation Rate Analysis

Five months prior to collection, several polyethylene plastic products were buried near the location of the soil sample extraction to mimic the conditions of a typical landfill and attract native soil microorganisms known to gradually biodegrade plastics, such as *Pseudomonas putida* (33). Ten

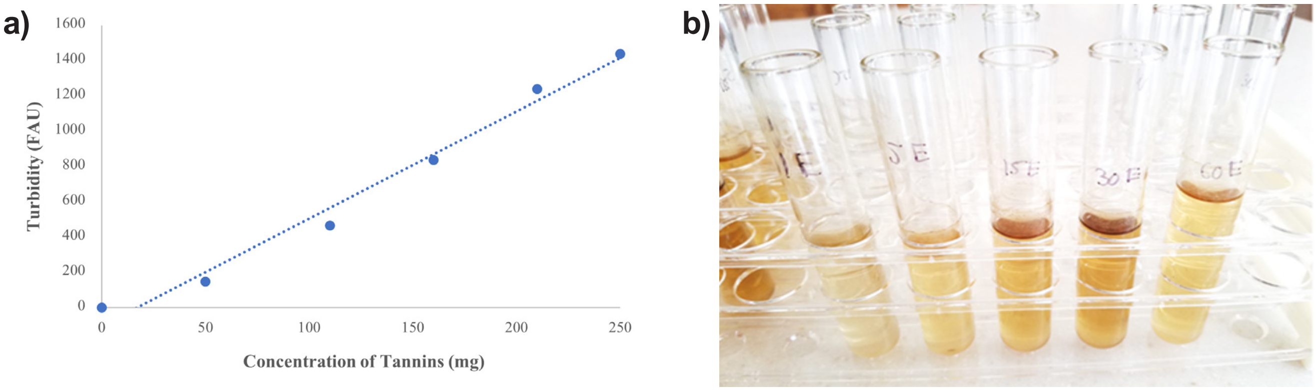

Figure 6. Tannins standard curve used in calculating solubility of bioplastic samples. a) The equation of the standard curve (y = 6.072x - 101.03) was utilized to calculate the concentration of tannins within the remaining solution of the absorption trials. The R^2 value was 0.9829, indicating strong correlation between the two variables. **b)** The turbidity of the remaining solution at the conclusion of the water absorption test was sufficient for estimating the solubility of the plastics (n = 3).

10 mg strips of the experimental bioplastic and traditional polyethylene film were placed in borosilicate test tubes containing 8.00 g of soil. 0.50 mL of distilled water was added for proper moisture content, and trials were incubated at 25±2°C for 168 hours, with an additional 0.50 mL of distilled water introduced to the system at the 85-hour mark to maintain moisture. Samples were removed and allowed to dry. The comparison of peak ratios from the FTIR-ATR analysis determined the average percent biodegraded. A *t*-test (assuming unequal variances) was utilized to detect a significant difference in the percentages between the two products (α = 0.05).

Water Absorbance Testing

Fifteen 10 mg samples of the novel bioplastic and an LDPE polyethylene plastic film shopping bag were obtained. An *in vitro* water absorption test was performed using borosilicate test tubes in accordance with the ASTM D570-98 procedure (with modification) (34). Samples were weighed using an Ohaus analytical balance (+/- 0.0001 g) then submerged in 8.00 mL of distilled water at 22°C for 1, 5, 15, 30, and 60 minutes. After exposure, samples were dried to remove excess water on the film's surface, then reweighed. A *t*-test assuming unequal variances was performed on the weight differences after the full 60-minute period to compare the absorbances of the two plastics examined (α = 0.05).

Solubility Testing

Solubility was determined via colorimetry using a LaMotte Smart3 Colorimeter (absorption method) and compared using a second *t*-test. Testing was conducted in a dark room to minimize the error in the turbidity values (FAU) by reducing light interference. Prior to processing experimental solutions, a standard curve was created using the turbidity of six standards, with each successive standard containing an increasing ratio of hydrolyzable tannins to distilled water **(Figure 6A)**. The curve determined the turbidity, and therefore, the concentration of tannins of the remaining solutions at the conclusion of the water absorption testing **(Figure 6B)**. Fourier Transform Infrared Spectroscopy (FTIR) with the Attenuated Total Refraction (ATR) attachment was utilized to further assess the bioplastic samples after being submerged for 5, 15, and 60 minutes. Sixty-four iterations were captured for each test to limit any error from interferences such as sound and carbon dioxide.

Cost Analysis

A cost analysis was performed to compare the pricing (per square centimeter) of the novel bioplastic formula with current single-use plastic products. Only the major components of each film were included in the calculation (i.e. agar agar, tannins, and chitosan for bioplastic, and petroleum for polyethylene).

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