

A comparison of starches and plasticizers for biopolymer synthesis and degradation

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SUMMARY

The production and disposal of plastic waste causes widespread environmental and economic harm. Most plastics are made of crude oil, which does not biodegrade and gathers in landfills, resulting in pollution. Biopolymers made from renewable materials like starch, on the other hand, degrade more rapidly and are cost-effective and versatile. In this study, we aimed to compare several starch-based polymers to identify viable alternatives to plastic made from crude oil. While multiple studies have investigated individual starch-based biopolymers, we conducted a direct comparison of different ratios of various types of starch and additives used to modify the properties of the resulting biopolymers to identify the polymer with the highest rate of degradation by sodium hydroxide. We hypothesized that potato starch and glycerol would lead to a biopolymer most susceptible to degradation due to its less stable chemical composition. However, our results indicated that the biopolymer made from potato starch and citric acid was the most susceptible to degradation, as it took the shortest time to reach complete degradation (53 min). Other biopolymers took up to 143 minutes to degrade. Starch-based biopolymers rapidly degrade and should be considered as an alternative to plastic made from crude oil. Our study shows that the type of starch and additive used affect the rate of degradation, making starch-based biopolymers highly versatile for different applications.

INTRODUCTION

Plastics are prevalent in our everyday lives and are found in everything from cups and bags to components of airplanes and cars. According to an industry report by Plastic Europe, 330 million tons of plastic were manufactured worldwide in 2016 (1). Most plastics are made from crude oil in a process that releases numerous pollutants that contribute to global warming (2). Of the approximately 80 million tons of plastic packaging used annually, over 40% is thrown away in landfills (3). The plastic in landfills does not break down easily via biodegradation or hydrolysis, leading to large amounts of persistent plastic waste that enters the ecosystem, causing the deaths of millions of animals (4). Biopolymers, created by combining starch from plants and additives called plasticizers to make biodegradable plastic, are a way to tackle these problems (4, 5). Plasticizers are molecules that bind to polymers, decreasing intermolecular interactions and resulting in

greater mobility (4, 5). These biopolymers have potential applications in paper, pharmaceuticals, beverages, biomedical products, the agricultural field, and textiles (4, 5). Compared to the manufacturing of plastics derived from crude oil, the manufacturing of biopolymers emits fewer greenhouse gases, and their byproducts are generally less toxic (5, 6). Due to the easy attainability of raw materials, biopolymers are also an excellent option for the recycling of solid biomass residue (7, 8).

Not only is starch low in cost and high in renewability, but it is also completely biodegradable, making it an ideal candidate for biopolymer development (9). Starch-based biopolymers also hold great potential due to their absorbance, biocompatibility and non-toxicity (10, 11). Starch molecules are connected by intermolecular interactions between oxygen in amylopectin and the hydroxyl group in amylose. The linear amylose provides the biopolymer with strength and greater elongation while the branched amylopectin affects a biopolymer's flexibility (Figure 1) (11). Starches from various sources all contain different amylose to amylopectin ratios that result in distinct properties (12). The amylose content in potato starch varies between 11.9%-20.1% with an amylopectin content of 63.4-88.6%, while the amylose content of arrow-root starch ranges from 16%-27% with an amylopectin content of ~62.3% (13-15). Thus, potato starch is estimated to have an amylose to

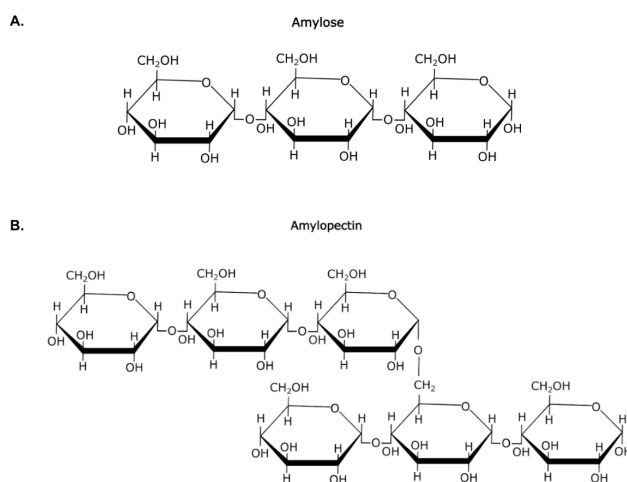


Figure 1: Chemical structure starch molecules. A. amylose; B. amylopectin. The linear structure of amylose provides strength, elongation, and higher water content to biopolymers, while the branched amylopectin is more flexible and prone to hydrolysis by enzymes.

amylopectin ratio ranging from 0.01-0.30 while arrowroot starch has a ratio of 0.363 (16). Higher concentrations of amylose lead to a biopolymer with a higher crystallinity, while higher concentrations of amylopectin lead to a more amorphous structure (13). The large number of hydroxyl groups in the linear structure of amylose makes it highly hydrophilic, increasing the water content of biopolymers with a high amylose content (13, 17-19). Finally, the structure of amylose makes it less accessible to enzymes, meaning that biopolymers with higher amylose to amylopectin ratios are more resistant to hydrolysis (13, 17, 18).

One of the main challenges when using starch-based biopolymers is that they are hydrophilic and water-soluble (19). In aqueous environments, the glycosidic linkages between the glucose subunits of the starch molecule are broken in a process of hydrolysis. Hydrogen bonds between starch molecules are also disrupted, allowing the breakdown of the starch-based biopolymers (20). Hydrolysis can be reduced via starch modification, starch blends, or the addition of plasticizers. Strong intermolecular and intramolecular hydrogen bonds link the chains of amylopectin and amylose in starch. When a plasticizer is added, starch-plasticizer interactions replace the starch-starch interactions (21, 22). The compatibility between the polymer and plasticizer contributes to the biopolymer's solubility, polarity, dielectric constant, and hydrogen bonding (23).

Two widely used plasticizers are citric acid and glycerol, but they differ in how they interact with starch molecules (24, 25). Glycerol can form only relatively weak hydrogen bonds with starch molecules. In contrast, citric acid contains carboxylic acid groups which allow it to form strong covalent ester bonds with both starch and glycerol in addition to hydrogen bonds with starch (25, 26). This suggests that glycerol would be better suited to biopolymers which are intended to dissolve in water. Tarique *et al.* demonstrated in 2021 that glycerol increased the thermal stability and flexibility of arrowroot starch biopolymers while also increasing water solubility, suggesting an increased rate of degradation during hydrolysis (21, 27). Previous studies have shown that citric acid increases the stability of a dry rice and potato starch biopolymer in water and high heat (28). Thus, either citric acid or glycerol may be suitable as a plasticizer depending on the application of the biopolymer. For example, when developing starch-based biopolymers for food packaging, glycerol would not be well-suited for high-

moisture foods.

In this study we compare multiple biopolymers prepared from different combinations of starch type and plasticizer. We expected each polymer to hydrolyze at different rates. We hypothesized that the higher amylopectin content and lower amylose content of potato starch would result in biopolymers made from this starch undergoing degradation at a faster rate compared to those made from arrowroot starch. We also hypothesized that the chemical properties of glycerol, such as its hydrophilic nature and low molecular weight, will lead to biopolymers containing it to degrade faster (21).

As different applications require different biopolymer lifetimes, exploring how different starches affect the rate of degradation is relevant for the field of materials science. This experiment is valuable because the hydrolysis of starch-based biopolymers is a major hurdle to starch-based polymer applications in food packaging and agriculture (19, 30). Our experimental results indicated that the type of starch and plasticizer used affect the rate of degradation of biopolymers. The biopolymer made from potato starch and citric acid took the shortest time of all biopolymers tested to reach complete degradation (53 min). We also observed variation in the textures of the biopolymers, with potato starch and citric acid being more brittle and arrowroot starch-based biopolymers being more flexible. These different characteristics indicate that different biopolymers may have different uses and applications.

RESULTS

Five biopolymers were tested: citric acid and glycerol, arrowroot starch and citric acid, potato starch and citric acid, arrowroot starch and glycerol, potato starch and glycerol. The control biopolymer contains both plasticizers and no starch (**Figure 2**).

The texture of a biopolymer is influenced by factors such as its crystallinity, surface porosity or roughness, all of which can influence the applications and characteristics of the biopolymer (31). Both biopolymers made from glycerol had a thicker texture as compared to those made from citric acid. On the other hand, those made from citric acid were more brittle. Biopolymers made from arrowroot starch were more flexible than the ones made from potato starch, which broke more easily. Thus, the potato starch and citric acid biopolymer was the most brittle. The control polymer with no starch added was brittle and had a glass-like texture.

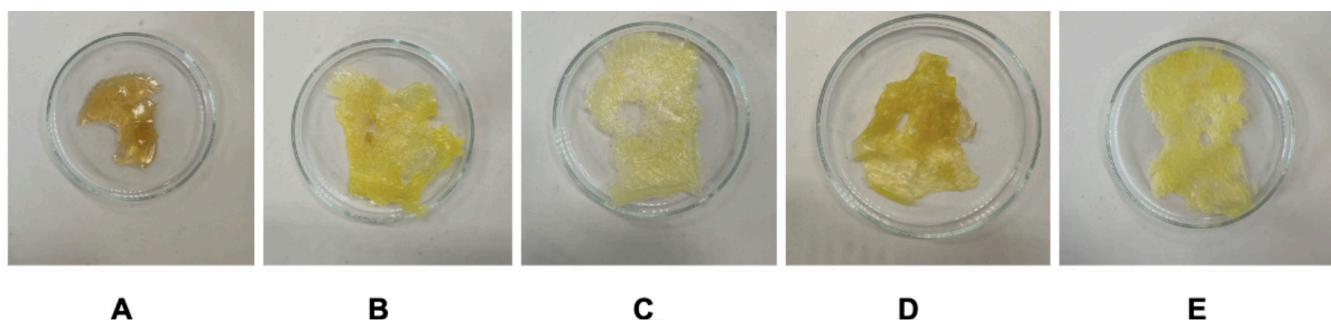


Figure 2: Starch-based biopolymers synthesized for this study. Photograph of each starch-based biopolymer, taken before colorimeter analysis. A. citric acid and glycerol; B. arrowroot starch and citric acid; C. potato starch and citric acid; D. arrowroot starch and glycerol; E. potato starch and glycerol.

Biopolymer degradation was first measured using a colorimeter analysis, in which a decrease in absorbance corresponds to polymer degradation (**Figure 3**). The arrowroot starch and glycerol biopolymer experienced a greater decrease (0.67 to 0.08 AU) when compared to the arrowroot starch and citric acid biopolymer. (0.36 to 0.30 AU) (**Figure 3A**). In contrast, the potato starch and glycerol biopolymer experienced a smaller decrease in absorbance in the first 5 minutes (0.30 to 0.08 AU) when compared to the potato starch and citric acid biopolymer (0.88 to 0.29 AU) (**Figure 3B**).

All the biopolymers measured reached their points of greatest degradation by 5 minutes except for the arrowroot starch and citric acid biopolymer (**Figure 3A**). However, the large error bars for this sample make our observation of the timing less reliable. This suggests a similar rate of degradation may have been the case for all biopolymers studied. We further explored the nature of the polymers in a degradation analysis where each biopolymer was degraded to completion.

The degradation analysis allowed us to evaluate the water solubility of the biopolymer. There was a notable difference in timing of degradation between the five biopolymers (**Table 1**). While the potato starch and citric acid biopolymer took the shortest time to degrade (53 min) out of all four starch-based biopolymers, the potato starch and glycerol biopolymer took the longest to degrade (143 min). The arrowroot starch and citric acid biopolymer took an intermediate 94 minutes to degrade. Additionally, unlike the final product of the biopolymers made from citric acid, the degraded product of both biopolymers containing glycerol was thick and viscous (**Figure 4**).

In the degradation analysis, the potato starch and citric acid biopolymer experienced complete degradation, the point at which no visible biopolymer remained in the tube, in the shortest period. When comparing the biopolymers made from arrowroot starch, the arrowroot starch and glycerol biopolymer degraded in half the time when compared to the arrowroot starch and citric acid biopolymer. This differs from the analysis of biopolymers made from potato starch, where the potato starch and glycerol biopolymer took longer to

Biopolymer	Arrowroot + citric acid	Potato + citric acid	Arrowroot + Glycerol	Potato + Glycerol	Citric acid + Glycerol
Time of degradation (min)	94	53	61	143	22.24

Table 1: Degradation Analysis. 1g of each biopolymer was dissolved in 10cm³ of sodium hydroxide and the time of complete degradation (no solid biopolymer visible) was recorded.

degrade than the potato starch and citric acid biopolymer.

DISCUSSION

Our results suggest that biopolymers containing potato starch and citric acid degrade faster than their counterparts. This supports our hypothesis that potato starch produces a biopolymer more susceptible to degradation than other biopolymers tested in this study. The impact of glycerol or citric acid as a plasticizer did not show any consistent results but invites discussion on the different factors which contribute to the degradation time of biopolymers.

The difference in amylose and amylopectin content in the two different starches may also explain the different biodegradability of the biopolymers. The high amylopectin content in potato starch makes its structure more accessible to enzymes and the lower amylose content reduces strength (11, 13, 19). The lower amylopectin and higher amylose content in arrowroot starch by contrast could explain its reduced biodegradability and the stronger yet more brittle properties observed (11, 13, 17).

The control biopolymer made of two plasticizers, citric acid and glycerol, was the most brittle and dissolved at the fastest rate. This suggests that the starch component delays degradation, making starch-based biopolymers practical for extended application and promising for use as biodegradable bioplastics. This is consistent with research showing that starch is one of few plant derivatives which has gained attention due to its promising physio-chemical properties (28). These results are relevant when exploring the applications of different biopolymers. The brittle nature of biopolymers made of potato starch or citric acid can be paired with biopolymers with flexible qualities to combat this

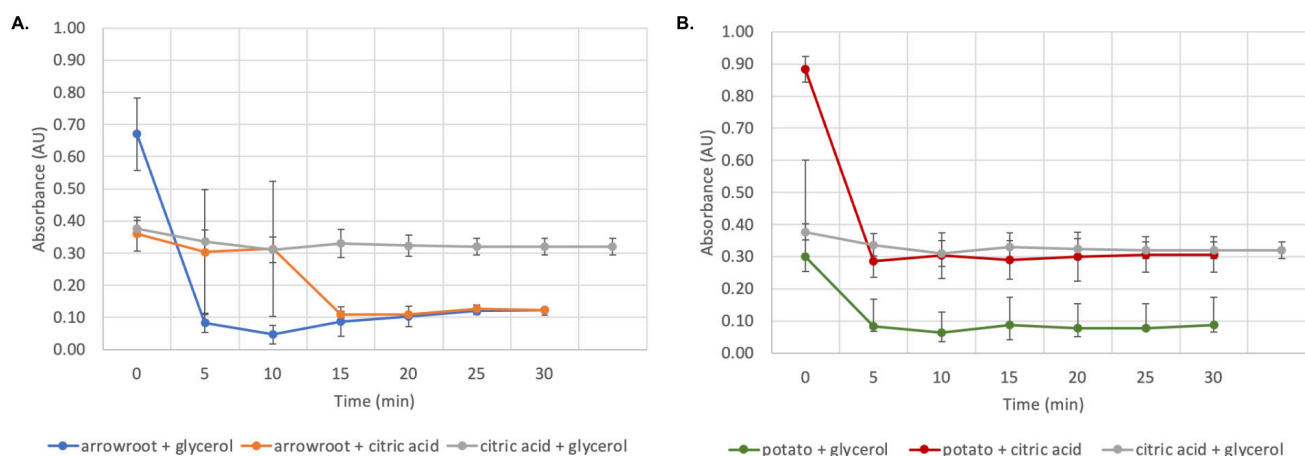


Figure 3: Colorimeter analysis results. Line graphs showing mean absorbance of biopolymers over time ($n=3$, \pm s.d.). 0.1g of each biopolymer was mixed with 1 M sodium hydroxide and absorbance was measured using a colorimeter. A. arrowroot biopolymers; B. potato starch biopolymers.

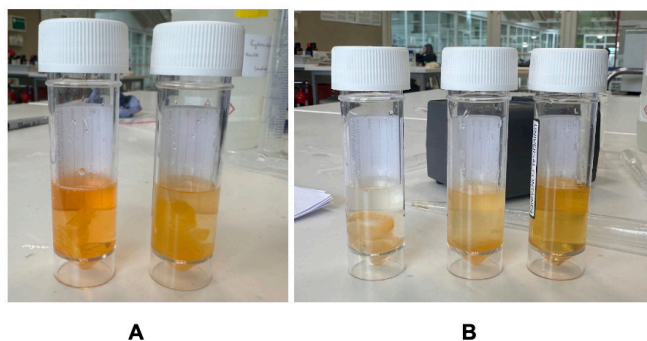


Figure 4: Degradation of biopolymers at 15 minutes. A. Arrowroot and citric acid biopolymer (LEFT), potato starch and citric acid biopolymer (RIGHT); B. Potato starch and glycerol biopolymer (LEFT), arrowroot and glycerol biopolymer (CENTER), citric acid and glycerol biopolymer (RIGHT). 1g of each biopolymer was dissolved in 10cm³ of 1 M sodium hydroxide.

issue. Such starch-based biopolymers can also be used for single-use plastic packaging such as trays (32). On the other hand, the flexible qualities of the arrowroot starch can be used as textiles.

In the colorimeter analysis, there were no major differences in the rates of degradation between the biopolymers, except for the arrowroot starch and citric acid biopolymer. Most degradation took place within the first five minutes, but the arrowroot starch and citric acid biopolymer absorbance did not complete degradation until 15 minutes. However, the large standard deviation calculated for that biopolymer makes these results difficult to interpret. It is possible that the biopolymer completed degradation in a similar time but measurement was affected by unknown errors. Possible sources of error include human measurement error. In future experiments, earlier time points should be included in this analysis. Additionally, the absorbance should be recorded every minute for the first five minutes.

We were only able to conduct one trial for the degradation analysis. In the future, we would ideally replicate these analyses and calculate averages and standard deviations. The degradation analysis also took place in a sealed tube as compared to an open environment, as it would in nature. This limits the scope of our findings results especially due to the nature of the degraded substance. The sodium hydroxide used to accelerate degradation also does not perfectly emulate the natural degradation a biopolymer may face in the environment. Additionally, the substance remaining after hydrolysis is composed of starch and plasticizer dissolved in water. This solution may be viscous, especially in the case of the potato starch and glycerol biopolymer, which can affect the rate of degradation (27). The byproducts of the hydrolysis surround the biopolymer, limiting the surface area exposed to sodium hydroxide (27). The outer layers of the polymer may become more saturated with water, hindering further access to the inner parts of the biopolymer, and slowing down the rate of degradation (27). This could explain the result in the degradation analysis where the potato starch and glycerol biopolymer took the greatest time to degrade, contradicting the results of the colorimeter analysis and our hypothesis.

To expand on the experiment, another colorimetric analysis could be conducted using a larger sample size. This analysis could be conducted simultaneously with the repeated degradation analysis. Every 15 minutes, a sample of the degrading solution would be tested in the colorimeter for its absorbance until the biopolymer is fully degraded. This would allow the tracking of the rate of biopolymer degradation.

While potato starch was found to increase the degradability of biopolymers, more research should be done to investigate the other factors influencing degradation in addition to the plasticizer and starch used. Further investigation of the role of different starches and their compatibility with other biopolymers may lead to the creation of starch blends which lack the downsides of each biopolymer alone. For example, a starch blend could be developed where the easily hydrolyzed nature of a potato starch-based biopolymer could be paired with biopolymers such as Polylactic Acid (PLA) which do not experience hydrolysis and are not soluble in water (4, 22). This could lead to versatile and green alternatives to crude oil-based plastics which could be widely adopted.

MATERIALS AND METHODS

100% arrowroot and potato starch powder obtained from a local supermarket were used to create several biopolymers. Two recipes for each starch-based polymer were also used: one with glycerol as the plasticizer and another using citric acid. A control polymer was created using both citric acid and glycerol but without any starch. This polymer allowed us to evaluate the properties of the plasticizers alone, in the absence of starch. All non-starch chemicals were sourced from common lab stock. Colorimeter analysis is widely used to analyze biopolymer concentration (24, 29). Colorimeter analysis involves the use of a color reagent to measure the light absorbance of the solution at a specific wavelength, thereby determining the concentration of a chemical compound in a solution. According to Beer's Law, concentration and absorbance are directly proportional.

Biopolymers were prepared as follows: 3.2 g of starch powder and 1.5 g of plasticizer were weighed on an

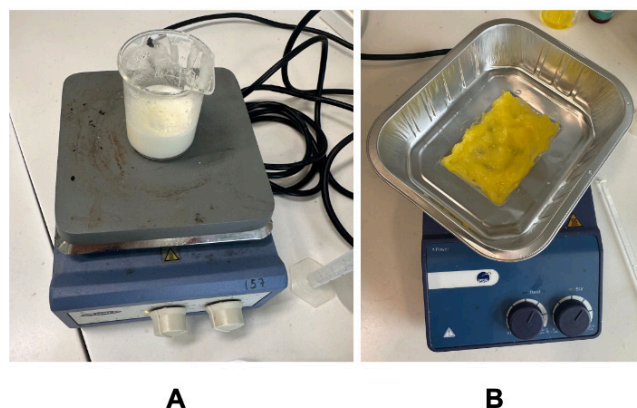


Figure 5: Biopolymer synthesis. A. Arrowroot starch and citric acid solution mixing on a hotplate set at 75°C; B. Arrowroot starch and citric acid solution drying on a hot plate set to a medium-high setting.

electronic balance and mixed with 10mL distilled water and 1 mL 2 M ethanoic acid. The solution was mixed at 75 °C on a hotplate until resistance to stirring was observed to increase indicating thickening (Figure 5A). Two drops of yellow dye were added, and the solution was transferred to an aluminum tin and left on a hot plate until dry at 250 °C (Figure 5B).

All colorimeter analyses used 0.1g of biopolymer measured at 5 minutes intervals for a total of 30 minutes. The biopolymers were added to colorimeter-calibrated 5 cm³ cuvettes two-thirds filled with 1 M sodium hydroxide. Sodium hydroxide was chosen to accelerate the degradation of the biopolymers due to its properties as a strong base. Each biopolymer was measured three times.

For the degradation analysis, 1 g of each biopolymer was placed in a universal container filled with 10 cm³ of 1 M sodium hydroxide (Figure 4). Initially, the biopolymer was visible as a globular mass. When the biopolymer was no longer visible (only liquid remained), the biopolymer was considered fully degraded and the time of degradation was recorded.

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