The Effect of Microcrystal Cellulose Filling from Coconut Fiber and Betel Leaf Extract as Antimicrobials on Sago Starch Bioplastic Composite

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Abstract. Starch-derived bioplastics have weaker mechanical properties than conventional polymers and a short lifespan due to their susceptibility to microbial degradation. This study examines the effect of microcrystalline cellulose (MCC) and betel leaf extract on the characteristics of sago starch bioplastic composites. Microcrystalline cellulose extracted from coconut coir improves tensile strength, and betel leaf extract improves antibacterial properties. This study began by isolating alpha-cellulose from coconut coir and continued by isolating microcrystalline cellulose via the hydrolysis procedure employing hydrochloric acid. As an antibacterial, this research extracted betel leaf using 96% ethanol and a rotary evaporator. MCC variations of 0, 2, and 4(b) and betel leaf extract variations of 0, 3, and 6%(v) in 100 g of sago starch were examined. Up to 20% of the combination comprises glycerol as a plasticizer(v). MCC and betel leaf extract altered bioplastic composites' properties. A bioplastic composite with 2% MCC and 6% betel leaf extract can inhibit the growth of Bacillus cereus bacteria. It has a tensile strength of 274.531 kPa, an elongation at break of 3.72%, a density of 0.724 g/cm3, and a water absorption of 7.79%. It concludes that MCC and betel leaf extract had an antibacterial effect in starch bioplastic composite.

1. Introduction

Plastic Biodegradable Starch is a type of polysaccharide widely found in nature. Researchers have carried out various studies on manufacturing bioplastics using raw materials from different types of starches, such as corn starch [1], taro tuber starch [2], and sago starch [3]. Indonesia has the largest sago plantation area in the world—many sago plantation areas in western Indonesia in the Sumatra region, specifically in Riau. The area of sago plantations in the Riau region was 89,611 ha in 2016, more significant than in Papua, Maluku, and Southeast Sulawesi [4]. Starch in sago has a reasonably high content, namely 98.12% [5], so starch sourced from sago can be used as a raw material for biodegradable plastic, which can reduce environmental pollution.

The weakness of bioplastics made from sago starch is their low mechanical properties. It is possible to strengthen the structure of bioplastic compounds by adding plastic biodegradable starch-based fillers. Coconut coir fiber is a natural material that can be a sound reinforcement for environmentally friendly matrix polymers. Coconut fiber contains 36-43% cellulose, 0.2% hemicellulose, and 41-45% lignin [6]. Cellulose can be processed into tiny crystals or microcrystals to form microcrystalline cellulose (MCC). Microcrystalline cellulose is isolated from α-cellulose, which is pure cellulose that produces porous powder particles and is insoluble in water but floats when exposed to water [7].

Adding fillers in bioplastics, such as cellulose, increases the material's stiffness, so plasticizers are needed. Glycerol is used as a plasticizer because it has the advantage of a relatively high boiling point (290) and can increase the elasticity of bioplastic composites [8].

Starch-based bioplastics also have disadvantages, namely that they cannot utilize these materials for extended periods due to their susceptibility to natural degradation. [9]. Adding betel leaf extract can reduce microbial activity in bioplastics because betel leaves have many benefits, such as functioning as an antibacterial, analgesic, amebicide, fungicide, antiseptic, immunomodulator, and others. Betel leaves contain 4.2% essential oil, which mainly consists of bete phenol, which is the isomer of eugenol (26.8–42.5%), caryophyllene (6.2–11.9%), chavicol (5.1–8.2%), chavibetol (0.0–1.2%), estragole, and terpinene, which are antibacterial compounds. The essential oil in betel leaves contains 30% phenol and several derivatives. The contents of chavicol and chavibetol in betel leaves are derivatives of phenol, which are five times stronger at warding off bacteria than ordinary phenols [10]. Many people often use the maceration extraction process for extraction purposes. This extraction process uses a solvent in direct contact with the material, causing the solvent to diffuse into the material cells, and osmotic pressure facilitates the release of the active compound during extraction [11]. Research conducted using the maceration extraction method to carry out the betel leaf extraction process could yield 40% [12].

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Based on the description above, we must research the effect of adding microcrystalline cellulose (MCC) to enhance the mechanical properties and betel leaf extract as an antibacterial in sago starch-based bioplastic composites.

2. Method
The methods used in this research include isolating alpha-cellulose from coconut fiber, making microcrystalline cellulose from alpha cellulose, making betel leaf extract, and providing bioplastic composites. This research was conducted at the Chemical Industrial Process Laboratory, Faculty of Engineering, Department of Chemical Engineering, Universitas Sumatera Utara.

2.1. Materials and Tools
The equipment used in this research included a Beaker glass, an Erlenmeyer glass funnel, a measuring cup, filter paper, a sieve with mesh sizes 160 and 50, a digital balance, an oven, a blender, a hot plate, a thermometer, a compression molding, and a mixer.

The materials used in this research consisted of alpha-cellulose isolation material, the manufacture of microcrystalline cellulose from coconut fiber, the manufacture of bioplastic composites. The materials used in the isolation of alpha-cellulose from coconut fiber include 2% sodium hydroxide (NaOH) for the delignification process; sodium hypochlorite (NaOCl) 1.75% as bleach; distilled water as a solvent; and sodium hydroxide (NaOH) 17.5% for alpha-cellulose purification. Materials used to make microcrystalline cellulose include alpha-cellulose from coconut fiber, hydrochloric acid (HCl), a 2.5 N hydrolysis agent, and distilled water as a solvent. The ingredients used in making betel leaf extract consist of betel leaves and 96% ethanol as a solvent. The materials used to prepare bioplastic composites include sago starch, which is used as a raw material; distilled water as a solvent; and glycerol (C3H8O5), a plasticizer.

2.2. Isolation of Alpha Cellulose from Coconut Fiber
The coconut fiber is cut and blended, then sieved using a 160-mesh sieve to 75 grams. Coconut fiber powder was heated using 500 mL of a 2% sodium hydroxide (NaOH) solution at a temperature of 80°C for 2 hours. Strain and wash the coconut fiber dregs using distilled water until the filtrate is neutral. Purification of alpha-cellulose from samples using 500 mL of a 17.5% sodium hydroxide (NaOH) solution at a temperature of 80°C for half an hour. The pulp is filtered and washed with distilled water until the filtrate is neutral. After obtaining a neutral filtrate, the filtrate was bleached using 500 mL of 1.75% sodium hypochlorite (NaOCl) at a temperature of 60°C for 1 hour. The fiber is filtered and washed with Aquadest; after that, it is dried using an oven at a temperature of 60°C for 1 hour [13].

2.3. Making Microcrystalline Cellulose from Alpha Cellulose
Alpha-cellulose incorporated beaker glass as much as 1 g, and hydrolysis was carried out using 2.5 N hydrochloric acid (HCl) at a temperature of 75°C for 15 minutes. Then Aquadest was poured into a beaker glass, stirred using a spatula, and left for 24 hours. The microcrystalline cellulose obtained was washed with Aquadest in an oven at 60°C for 1 hour to reduce the water content [13].

2.4. Making Betel Leaf Extract
A sample of betel leaf powder was weighed at 50 g. Then 96% ethanol was added with a material-solvent ratio of 1:5. The sample was extracted for 30 minutes at a temperature of 40°C. The extract obtained was filtered using Whatman No. 1 filter paper. The extract is concentrated using a rotary evaporator at a temperature of 40°C with a rotation speed of 100 rpm until a thick betel leaf extract is obtained [14].

2.5. Provision of Bioplastic Composites
100 g of sago starch was weighed, then 20% glycerol plasticizer was added from 100 g of sago starch, and MCC was added with variations of 0%, 2%, and 4% from 100 g of sago starch. The solution was added with 2% betel leaf extract with variations of 0%, 3%, and 6% from 100 g of sago starch. Starch was placed in a glass beaker and added to 1000 mL of distilled water. The solution was stirred using a mixer at 70°C for 20 minutes. After stirring, the solution is poured into the mold. The bioplastic composite was dried for two days, then released from the mold and analyzed [14].

2.6. Density Analysis
The density analysis procedure for bioplastic films begins with the bioplastic film being cut to a size of 3 cm x 3 cm, then the thickness is measured, and the weight and volume are calculated. The density of bioplastic films is determined by dividing mass by volume with Equation 1 [14].

\[ \rho = \frac{m}{V} \]  

Note: \( \rho \) = density (gram/cm³); \( m \) = mass (gram); \( V \) = volume (cm³)

2.7. Water Absorption Analysis
The bioplastic film was cut to 3 cm x 3 cm in the water absorption analysis. The bioplastic film is soaked in water, and then the sample is weighed every 15 minutes.
until a constant mass is obtained. Water absorption in bioplastic films can be calculated using Equation 2 [14].

Water absorption = \frac{\text{final sample weight} - \text{initial sample weight}}{\text{initial sample weight}} \times 100\% \tag{2}

2.8. Analysis of the Properties of Tensile Strength and Elongation at Break

Tensile strength is one of the most important basic properties of biocomposite product materials and is often used to characterize a biocomposite product material. The tensile strength of a material is defined as the maximum load (Fmax) used to determine the specimen material divided by the initial cross-sectional area (AO). Biocomposite products are selected and cut to form specimens for tensile strength testing (tensile test) in accordance with ASTM D 882 standards. Tensile strength testing is carried out with a tensometer on each specimen. The tensometer is first conditioned to a load of 100 kgf at a speed of 50 mm/minute, then clamped firmly with the clamp on the tool. The machine is turned on, and the specimen will be pulled upwards. The specimen is observed until it breaks, and the maximum stress and strain are recorded. The calculation of tensile strength follows Equation 3 [8].

\sigma = \frac{F_{\text{max}}}{A_0} \tag{3}

Note: \sigma = \text{tensile strength (N/m}^2\text{)}; F_{\text{max}} = \text{maximum load (N)}; A_0 = \text{first cross-sectional area (m}^2\text{)}.

Elongation is the increase in the length of a material when tested under a tensile load, expressed in units of length, usually inches or millimeters. Percent elongation is the elongation of a test object expressed as a percent of its length. The percent elongation at break is the percent elongation at break of the test specimen. Measurements are carried out in the same way as tensile strength, namely based on ASTM D882 with the provisions of the Universal Testing Machine (UTM) model. Elongation is determined using Equation 4 [8].

\text{Elongasi} (\%) = \frac{\Delta l}{l_0} \times 100\% \tag{4}

Note: \Delta l = \text{change in length (cm)}; l_0 = \text{initial length (cm)}

2.9. Antimicrobial Activity Analysis

Microbes (Bacillus cereus) were inoculated on agar media in a petri dish to analyze antimicrobial activity. Bioplastic film extract samples were cut into discs with a diameter of 6 mm and placed on the surface of the cup. The plate was then incubated at 37°C for 24 hours. The inhibition zone marked with a transparent colored area around the well was measured using a caliper [9].

3. Results and Discussion

3.1. Density Analysis

The density of bioplastic composites is related to the physical properties of the biodegradable plastic film, which are related to water absorption capacity, thermal properties, gas and water permeability, and degree of crystallinity. A high-density value indicates that the film is biodegradable and can be more effective in preventing the substances contained in it from entering the air. Meanwhile, the density value is low for biodegradable plastic film, which has a lower regularity value, so it cannot prevent small molecules from entering [15]. The density value of the bioplastic composite can be seen in Figure 1.

![Fig. 1. Density vs. MCC rate for bioplastic composites](image)

It can be seen in Figure 1 that the density value of the bioplastic composite obtained with the highest results with a 2% MCC variation with 6% extract was 1.309 g/cm³. The lowest yield with a 0% MCC variation with 0% extract was 0.774 g/cm³. Adding MCC and betel leaf extract in bioplastic increases the density between molecules in bioplastics. This is because MCC and betel leaf extract have a higher density value than water, and adding MCC and betel leaf extract to the bioplastic composite increases the density value of the bioplastic composite. The density values of betel leaf extract and MCC from coconut fiber are 1.29 g/cm³ each and 1.2 g/cm³, respectively.

While making bioplastic composites, betel leaf extract, MCC, and glycerol are absorbed into sago starch. This improves the structure of the bioplastic composite so that the higher the concentration of MCC and betel leaf extract added, the higher the density value
of the bioplastic composite. However, adding too much MCC can cause low-density values due to MCC being hydrophilic, so its addition can decrease the density value of the bioplastic composite.

In bioplastic composite films, high-density values indicate that biodegradable plastic films can be more effective in preventing the substances contained in them from entering the air. Likewise, the low-density value of biodegradable plastic film has a lower regularity value, so it cannot prevent small molecules from entering [15]. This is in line with research on the effect of adding betel leaf extract on the density of sago starch bioplastic [14]. This research showed that the addition of betel leaf extract increased the density value of the sago starch bioplastic produced.

3.2. Water Absorption Analysis

Water absorption in starch-based bioplastic composites with the addition of MCC fillers from coconut fiber and betel leaf extract can influence the properties and characteristics of bioplastic composites. Therefore, it is necessary to analyze the ability of bioplastic composites to absorb water. Figure 2 shows the effect of soaking time on the water absorption properties of bioplastics for each sample variation.

The water absorption value of bioplastic composites is influenced by the percentage of added MCC filler from coconut fiber and betel leaf extract. Whereas with variations of 0%, 2%, and 4% addition of MCC and variations of 0%, 3%, and 6% betel leaf extract, the more betel leaf extract and MCC are added, the water absorption capacity will decrease as the soaking time increases. This happens because there are strong bonds in the polyphenolic compounds contained in betel leaves.

In samples with a ratio of MCC and betel leaf extract of 4%:0%, high water absorption values were obtained; this occurred with the addition of MCC. The water absorption value is inversely proportional to the water resistance value. The increasing concentration of MCC as a filler will reduce the water resistance value of the bioplastic composite. This shows that the more fillers are added to the bioplastic composite, the higher the water absorption value because there are more hydroxyl groups of molecules from the filler compound in the bioplastic composite.

![Fig. 2. Effect of time on the water absorption properties of bioplastic composites](image)

In samples with a ratio of MCC and betel leaf extract of 2%:6%, low water absorption values were obtained; this occurred with the addition of betel leaf extract. Based on research [9], adding eugenol extract contained in betel leaf extract to bioplastic composites can reduce the water absorption value due to the interaction of eugenol extract with starch.

3.3. Analysis of the Properties of Tensile Strength and Elongation at Break

The stiffness and elasticity of the bioplastic composite can be observed by measuring the maximum load applied and the changes in the length of the bioplastic composite, as shown in Figure 3. Bioplastic composites containing 4% MCC composition tend to break more quickly under maximum load due to the increased stiffness properties, which can reduce the bioplastic composite's elastic properties. On the other hand, bioplastics without MCC addition tend to change their shape (deform) before breaking under maximum load because bioplastics without filling have elastic properties.
Starch-based bioplastics generally have brittle properties and crack easily. Glycerol is an additive that can improve bioplastics’ mechanical properties so that they become soft, strong, and ductile [8]. Test tensile strength and elongation at break (Elongation at Break) was carried out to determine the effect of adding betel leaf extract and MCC on the tensile strength and elongation at break of bioplastic composites. Figure 4 shows the effect of adding MCC and betel leaf extract on the tensile strength properties of bioplastic composites.

The decrease in the tensile strength value was caused by changes in the molecular structure of the bioplastic composite due to the addition of MCC and betel leaf extract. The low tensile strength value is due to the low value of the filler material in the bioplastic composite, so it cannot withstand most of the forces applied to the bioplastic composite. Fillers are needed to make bioplastic composites to improve the properties of the resulting composite material. The presence of filler materials can improve the mechanical properties of bioplastic composites. This is related to the report from the research [16], where fiber functions are used to determine the composite material that will be produced, such as strength, stiffness, and other mechanical properties. Fibers that function as fillers can withstand most of the forces exerted on composite materials.

The test results show that bioplastic composites with high tensile strength values will break faster than those with lower tensile strength. This is caused by the nature of bioplastic composites made from starch, which are challenging but brittle and easily cracked. The brittle nature can cause the bioplastic composite film to become less elastic so that it will break more quickly. The effect of adding MCC and betel leaf extract on elongation at break (elongation at break) bioplastics can be seen in Figure 5.
Figure 5 shows that the increasing addition of MCC and betel leaf extract will reduce the elongation value at break. The bioplastic composite with 0% MCC variation and 0% betel leaf extract obtained the highest elongation at break value, namely 9.530%, while the lowest tensile strength value was in the bioplastic composite with 0% MCC variation and 6% betel leaf extract, namely 3.513%. The bond between glycerol plasticizer, starch, MCC, and betel leaf extract caused the decrease in elongation value at the break. The greater the glycerol concentration in the bioplastic composite, the more glycerol molecules will bind to starch and MCC. This will cause the bioplastic composite to become more elastic and flexible.

3.4. Antimicrobial Activity Analysis

Antibacterial analysis of bioplastics was carried out using the disc diffusion method. The bacterial isolate used in this analysis is Bacillus cereus. Bacillus cereus is a microorganism species that can reduce sago starch quality. Samples with varying percentages of MCC and betel leaf extract were placed.

In order to observe the antibacterial analysis of bioplastic composites properly, they are placed in the same petri dish. The preparation of samples with variations of MCC and betel leaf extract was carried out slightly apart to prevent the formation of clear zones. The results of the antibacterial activity analysis for bioplastic composite samples can be seen in Figure 6.

Figure 6 shows the antibacterial activity of all variations of bioplastic composites, which is shown by forming a clear zone around the sample. These results show that adding MCC and betel leaf extract can inhibit bacterial growth in Bacillus cereus. The diameter of this clear zone is then calculated using a caliper. The largest diameter of the inhibition zone was obtained from bioplastic composite samples with MCC variations of 2% and 6% betel leaf extract, namely 65 mm. Meanwhile, the minor zone of inhibition was obtained.
in the bioplastic composite sample with 0% MCC variation of 0% betel leaf extract, namely 15 mm.

Types of bacteria Bacillus cereus can degrade or break the bonds of plastic polymer chains. Bacillus cereus has the potential to degrade the test plastic because Bacillus cereus can grow in the medium it uses. Bacillus cereus is a bacterium that can harm human health through food consumption. Bacillus cereus is a gram-positive bacteria that forms spores and is spread in the environment, such as soil, water, and plants [17]. The addition of betel leaf extract influences the bacterial inhibitory properties of this bioplastic composite. Betel leaf extract contains bacteria-inhibiting compounds, namely polyphenolic compounds such as tannins and flavonoids, which act as inhibitors of the activity of microorganisms. These tannin and flavonoid compounds play a significant role in destroying bacterial cells. This research shows the best MCC and betel leaf extract variation is the 2% MCC variation with 6% betel leaf extract. This research shows that betel leaf extract can inhibit the bacterial growth of Bacillus cereus.

4. Conclusion

The results show that sago starch bioplastic composites treated with 2% microcrystalline cellulose fillers from coconut fiber and 6% betel leaf extract exhibit an antimicrobial effect. Based on the comprehensive findings presented in this research, it is evident that incorporating microcrystalline cellulose (MCC) from coconut fiber and betel leaf extract into sago starch bioplastic composites yields significant improvements in various properties. These enhancements include increased tensile strength, reduced water absorption, and notable antimicrobial activity against Bacillus cereus bacteria. The addition of MCC contributes to enhancing the mechanical properties of the bioplastic composites, particularly in terms of tensile strength. Furthermore, betel leaf extract serves as an effective antimicrobial agent, inhibiting the growth of Bacillus cereus bacteria. The synergistic effect of MCC and betel leaf extract leads to the development of bioplastic composites with enhanced antimicrobial properties and mechanical strength.

Looking forward, the findings of this study hold promise for several future applications and research directions. Firstly, the developed bioplastic composites show potential for use in biodegradable packaging materials, contributing to the reduction of environmental pollution caused by conventional plastics. Secondly, further research can explore the optimization of MCC and betel leaf extract concentrations to maximize the antimicrobial efficacy and mechanical properties of the bioplastic composites. Additionally, investigating the scalability and commercial viability of producing these bioplastic composites on a larger scale would be beneficial. In conclusion, this research underscores the promising potential of utilizing MCC from coconut fiber and betel leaf extract as additives in sago starch bioplastic composites. By addressing the challenges associated with mechanical strength and microbial degradation of bioplastics, this study paves the way for the development of sustainable and environmentally friendly materials with diverse applications in various industries.

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