BIODIVERSITAS
Volume 23, Number 5, May 2022
Pages: 2601-2608

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230542

Cassava starch/bacterial cellulose-based bioplastics with *Zanthoxylum acanthopodium*

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Manuscript received: 5 March 2022. Revision accepted: 27 April 2022.

Abstract. *Gea S, Pasaribu KM, Sarumaha AA, Rahayu S. 2022. Cassava starch/bacterial cellulose-based bioplastics with* Zanthoxylum acanthopodium. *Biodiversitas 23: 2601-2608.* The use of conventional plastics has become one of the biggest environmental problems because of their difficulties in decomposing. Bioplastics are plastics that are decompose easily in nature as they are naturally sourced. The purpose of this research was to create antimicrobial bioplastics from cassava starch with the addition of bacterial cellulose (BC) as a reinforcement material and *Zanthoxylum acanthopodium* (*andaliman*) as an antibacterial agent. Furthermore, this study determines the optimal concentration of BC and the antibacterial effect of *Z. acanthopodium* extract in bioplastics produced by the solution casting method. The addition of BC to bioplastics changed the properties of bioplastics, according to FTIR, XRD, TGA, SEM, and tensile strength analysis. Mechanical analysis showed an increase in tensile strength with higher amount of BC. The best tensile strength was observed in CS2BCA sample (2.34 MPa). The antibacterial test of bioplastic samples showed good inhibition zone (10.8 mm) against *Bacillus cereus*.

Keywords: Antibacterial, bacterial cellulose, bioplastics, cassava starch, *Zanthoxylum acanthopodium*

INTRODUCTION

Conventional plastics are widely used today, they are petroleum-based materials and are known to be stable, water-resistant, light-transparent, flexible, strong, and inexpensive (Zimmermann et al. 2020). However, the process of producing petroleum-based plastics is known to have quite high negative impacts, particularly on the environment, such as carbon dioxide (CO_2) emission and an increase in the amount of plastic waste which is unable to be degradable biologically was become main reason many studies with the main aim is replacing conventional plastics into environmentally-friendly and renewable plastics was conducted (Byun and Kim 2013). Currently, the alternative way to promote reaching that goals is replacing petroleum with natural biodegradable materials as raw materials to produce bioplastics, which, according to several reports, are more environmentally friendly than conventional plastics (Song et al. 2009).

Bioplastics are biopolymers that can be easily decomposed by soil microorganisms and weather change effects like solar radiation or moisture (Gironi and Piemonte 2011; Selvamurugan and Pramasivam 2019). They can even decompose into biomass via enzymatic reactions by microorganisms. Bioplastics are biodegradable because they are made from natural polymers such as carbohydrates, proteins, natural rubber, recyclable food waste, and biomass (Folino et al. 2020). Research into the use of biopolymers as a base material for food wrapping has become an intriguing topic to date (Rydz et al. 2018; Atta et al. 2021; Gzyra-Jagieła et al. 2021). According to the data, the average consumption of plastic, both newly produced and recycled plastics, per capita in Indonesia, is 22.5 kg/capita/year. It is approximated that 35% of this amount is used for food packaging with annual growth rate of 5-7 percent (KLHK RI 2020). However, if only one component is used, the use of bioplastic as a food packaging material is still quite limited (Jabeen and Nayik 2015). Specifically, several additional compounds are required for food packaging to optimize bioplastics as an active food packaging (Harnkarnsujarit et al. 2021). In this study, starch derived from cassava is used as a based biopolymer material in producing bioplastics. Cassava starch was chosen as a matrix because it is one of the largest agricultural products in Indonesia with up to 90% starch content on a dry basis. The use of starch as base material in bioplastics to be used as food packaging has attracted attention because of its abundant availability, economical price, biodegradability, and edibility (Luchese et al. 2017; Ayetigbo et al. 2018; Zuhra et al. 2020).

However, bioplastics formed solely from starch components are known to have low mechanical properties in terms of tensile strength, elongation, and elastic modulus, and thus do not meet the requirements for being classified as active food packaging (Marichelvam et al. 2019; Carina et al. 2021). Reinforcing materials are required to improve the mechanical properties of bioplastics to overcome these issues. Bacterial cellulose (BC) was one of the biopolymers that many studies suggested had the potential to be used as a reinforcing

agent. Furthermore, the addition of bacterial cellulose was chosen not only for its potential to improve mechanical properties, but nano-sized fibers neatly and regularly arranged in BC can also act as a barrier for contaminants in bioplastics, particularly bioplastics used as food packaging (Gea et al. 2018; Ludwicka et al. 2020; Pasaribu et al. 2020; Hasibuan et al. 2021).

In addition to having good mechanical properties and being able to contain contaminants in packaged foods, another requirement for bioplastics to be categorized as active food packaging is to have antibacterial properties so that they can prevent the spoilage process in packaged foods (Jabeen et al. 2015; Zahra et al. 2016; Huang et al. 2019; Mahmud 2021; Wang et al. 2021).

Several reports have shown that *Zanthoxylum acanthopodium* (locally known as *andaliman*) successfully inhibits the growth of bacteria, such as *Bacillus stearothermophilus*, *Pseudomonas aeruginosa* and *Vibrio cholera*, *Salmonella typhi*, *Bacillus subtilis* and *Staphylococcus aureus* (Liren 2011; Majumder et al. 2014). In this study *Z. acanthopodium* was promoted as an antibacterial agent in the manufacture of bioplastics for application as food packaging.

Zanthoxylum acanthopodium is one of the typical Indonesian spices commonly found in North Sumatra. Its fruit is often used by the Bataknese for cooking that gives spicy taste in food. Besides that, the bark, stems and leaves of *Z. acanthopodium* are medicinal, usually used to treat stomachache, toothache, cough, and backache. *Z. acanthopodium* also showed biological activities, such as larvicidal, anti-inflammatory, analgesic, antioxidant, antifungal, and antibacterial (Majumder et al. 2014; Pasaribu et al. 2020; Tarigan and Stevani 2021). Thus, through the study of producing cassava starch/bacterial cellulose-*Z. acanthopodium* extract films with antibacterial properties, bioplastics with promising innovation in active packaging systems that protect the surface of materials from microorganisms such as parasites, bacteria, germs, and fungi can be produced.

The aim of this study was to characterize the mechanical properties of cassava starch/bacterial cellulose-based bioplastics and to test the effectiveness of *Z. acanthopodium* extract as a natural antibacterial agent.

MATERIALS AND METHODS

Materials

The materials used in this study were: cassava roots, coconut water, and *Z. acanthopodium* (fruits) purchased from traditional market in Medan, Indonesia. Glucose, urea, distilled water, glacial acetic acid, NaOH, NaOCl, glycerol, and ethanol were purchased from Merck KGaA, Darmstadt, Germany without any purification. *Acetobacter xylinus* starter was purchase from CFM Research Centre Collection, Medan, Indonesia.

Procedures

Cassava starch extraction

The amount of 250 g of cassava was peeled and washed with clean water. Roots were cut into small pieces and mashed using a blender. At the time of refining, 500 mL of water was added to facilitate refining. Next, cassava was filtered to separate the filtrate (starch suspension) from the residue. The filtrate was allowed to stand for 24 hours to precipitate. The decantation process was carried out to separate the precipitate from the liquid phase. Finally, the precipitate was dried in an oven at 40°C and the dried cassava starch was sieved by using a 100 mesh sieve.

Bacterial cellulose synthesis

Bacterial cellulose was synthesized by the static method. First, 2 L of coconut water (with contaminant removed) was poured into a 2000 mL beaker glass and added 20 g glucose and 10 g urea. The mixture was homogenized, covered with aluminum foil, and heated on a hot plate until boiling. Then, the solution was allowed to cool at room temperature, followed by the addition of glacial acetic acid ($CH_3COOH_{(l)}$) until the pH became 4. *Acetobacter xylinus* starter was added into it and inoculated for 7 days at room temperature.

Bacterial cellulose purification

The BC sheets formed were separated from the media and washed with distilled water. To purify the BC sheets from bacteria, BC sheets were soaked in 1 M NaOH solution, while stirred and heated at 80°C for 1 hour. Finally, the sheets were neutralized by using aquadest and stored at 4°C for further use.

Production of bacterial cellulose slurry

Neutral BC sheets were mashed with a home blender and placed in a beaker glass containing aquadest. The mixture was homogenized by using a homogenizer for 2 hours to obtain smaller and homogeneous size BC. The cellulose slurry dispersed in water was filtered and stored.

Extraction of Zanthoxylum acanthopodium

Extraction procedure was carried out according to (Muzafri et al. 2018) with some modifications. *Z. acanthopodium* fruit was separated from the stem and dried at room temperature. The dried *Z. acanthopodium* fruit was grinded with a blender into a fine powder. The powder was transferred to a maceration bottle where ethanol solvent was added until the *Z. acanthopodium* powder was completely submerged. The mixture was left for 3 days with occasional shaking. After 3 days, the mixture was filtered to obtain *Z. acanthopodium* extract from the residue. The filtrate was evaporated in a rotary evaporator at 70°C to obtain a thick extract. The viscous extract was stored for further use.

Bioplastics production

Five grams of cassava starch were added to a beaker containing 100 mL of distilled water, then BC was added based on the mass variation determined, followed by 2 mL of glycerol. The mixture was heated on a hotplate and

stirred at 80-90°C for 30 minutes. Then, 1 mL of *Z. acanthopodium* extract was added to the mixture. The mixture was transferred to a 15 x 15 cm mold and dried at room temperature. Finally, the bioplastics obtained were characterized. In this study, bioplastics were produced with different variations of BC mass, such as 0 g, 0.25 g, 0.5 g, 0.75 g and 1 g. They were labelled as CS0BCA, CS1BCA, CS2BCA, CS3BCA, and CS4BCA (Table 1).

Characterizations

Fourier transform infrared spectroscopy (FTIR)

A Thermo Scientific Nicolet iS50 FT-IR spectrometer with an Attenuated Total Reflectance (ATR) diamond was used to perform FTIR graph (Thermo Scientific, USA). All spectra were derived from 32 scans with a 4 cm^{-1} resolution. The wavenumber ranges from 400 to 4000 cm^{-1} throughout the wavenumber range. The IR spectra were measured after dry sheets were deposited on the ATR crystal. Each sample experiment was repeated at least three times.

X-ray diffraction (XRD)

The crystallinity of the samples was studied utilizing the SmartLab system's wide angle X-ray scattering (Rigaku Corporation, Model HD 2711N). Cu-Ka radiation (k 5 0.15406 nm) produced at 40 kV and 44 mA was used for XRD. 2θ Scans were performed at a rate of 28/min sequentially between 10-50°C.

Scanning electron microscopy (SEM)

Morphological imaging with a Merlin Field Emission (FE)-SEM was used to establish the visual structure of the films (Carl Zeiss NTS GmbH, Germany). The cellulose-based films were sliced into small 1 cm^2 pieces and mounted to SEM sample holders covered with carbon tape. Sputtering (30 mA, 30 s) was used to coat film samples on the holders with gold to increase sample conductivity. Images were captured with three different sides.

Thermogravimetric analysis (TGA)

The Pyris1 TGA was used to perform TGA on the films (PerkinElmer Shelton, CT). The inert environment was maintained by a nitrogen flow of 20 mL/min, thermograms of samples were recorded between 37-600°C at a heating rate of 10°C/min. The first derivatives of thermograms (DTG), percentage weight loss, and decomposition temperatures were calculated using Pyris software**.**

Table 1. Treatment variation for each sample

Sample treatments	Sample labels
5 g cassava starch + 0 g bc +1 mL *Zanthoxylum acanthopodium* extract	CS0BCA
5 g cassava starch + 0.25 g bc +1 mL *Zanthoxylum acanthopodium* extract	CS1BCA
5 g cassava starch + 0.5 g bc +1 mL *Zanthoxylum acanthopodium* extract	CS2BCA
5 g cassava starch + 0.75 g bc +1 mL *Zanthoxylum acanthopodium* extract	CS3BCA
5 g cassava starch + 1 g bc +1 mL *Zanthoxylum acanthopodium* extract	CS4BCA

Antibacterial activity test

The disc diffusion (Kirby Bauer) test was employed to test the antibacterial activity of bioplastics film. Bioplastics with a diameter of 1 cm were swabbed evenly on the surface of solid nutritive media (nutrient agar) containing a suspension of Gram-positive bacteria (*Staphylococcus aureus*) or Gram-negative bacteria (*Escherichia coli*). Petri dish was then incubated for 18 to 24 hours at 37°C. The incubation time is determined by the culture used in the test. After the incubation period, the inhibitory zone (clear zone) around the bioplastics samples on the agar plate must be determined.

Tensile test

A Lloyd LS5 materials testing equipment (AMETEK measurement and calibration technologies, USA) was used to evaluate tensile strength of the films. Young's modulus, and strain at break at 23°C and 50% RH with a load cell of 10 kN. For overnight, all samples were stabilized in the provided circumstances. The initial grasp distance was 30 mm, and the grip separation rate was 10 mm/min. A lab film knife was used to cut the samples into 15 mm width of the specimens. The thicknesses of each specimen were measured thrice a time with a digital caliper.

RESULTS AND DISCUSSION

Functional group analysis

The FTIR data for bioplastic samples showed a wide absorption peak at 3265.1 cm^{-1} wavenumber indicating hydroxyl group (O-H) stretch derived from cassava starch, BC, and *Z. acanthopodium* extract. The absorption region at 2929.7 cm^{-1} was indicating C=H stretching, the peak at 1640.0 cm^{-1} indicated C-O presence, absorption at 1000-1200 cm^{-1} which was C-O strain derived from ester compounds and carboxylic acids. Moreover, a new absorption region was only observed in CS1BCA and CS2BCA samples at 1334.4 cm^{-1}, which indicated the presence of C-H bending showing BC characteristics (Figure 1). This data showed the difference in transmittance related to the addition of BC. The increase in the amount of filler in bioplastics has caused decreases in transmittance, which means that there was a higher absorption at that wavenumber. This finding was in accordance with the previous research, which indicates that the addition of fillers interfere the interactions of the hydrogen bonds between compounds in bioplastics during the production process (Abe et al. 2021; Wongphan et al. 2022; Wadaugsorn et al. 2022).

XRD analysis

X-ray diffraction analysis was carried out to analyze crystal orientations (texture), and other structural parameters. The diffraction patterns were measured, recorded, and plotted against the diffraction angle (2θ). The results of XRD analysis of bioplastic samples with the addition of different BC concentrations are presented in Figure 2. X-ray diffraction (XRD) is a powerful nondestructive technique for characterizing crystalline

materials. It provides information on structures, phases, preferred crystal orientations (texture), and other structural parameters, such as average grain size, crystallinity, strain, and crystal defects (Bunaciu et al. 2015).

XRD diffractogram of CS0BCA bioplastic showed the presence of 2θ diffraction peaks at 15.1° and 19.82° which indicated the presence of a crystalline phase. Cassava starch is a semi-crystalline material with the existence of crystalline and amorphous units (Alashwal et al. 2020; Dome et al. 2020). XRD diffraction pattern of CS1BCA and CS2BCA samples showed changes in 2θ angular shift and smaller peak intensities than CS0BCA. This was due to the effect of glycerol on the intercalation and growth of the reinforcing layer that caused the peaks to experience reductions. This is consistent with previous studies which showed the addition of material with hydroxyl group-cassava starch and BC, with the addition of glycerol consisting of the same group, would undergo substitution reaction with other functional groups and reduced the crystallinity (Bumbudsanpharoke et al. 2022). CS3BCA and CS4BCA samples had changes in their intensity at 2θ were due to the increase in BC concentration causing higher intensity angle changes indicating that the mixture had more rigid structures. Both CS3BCA and CS4BCA samples showed typical peaks which could be interpreted as interactions of hydrogen bonds between BC and other materials (Alashwal et al. 2020). As a result, the higher the BC in cassava combination, the more crystalline the substance became.

Morphological analysis

SEM analysis was carried out to observe the changes in the morphological structure on the surface of bioplastics. This analysis aimed to compare bioplastics before and after the addition of BC.

The results of the morphological analysis showed that the bioplastic surface became denser with the addition of BC (Figure 3b, c, d, e) when compared to the bioplastic surface that was not added with BC (Figure 3a). The presence of lumps was observed on all sample surfaces, this was due to cassava starch which was not completely dissolved during the gelatinization process. Incomplete melting of starch caused the presence of granules dispersed in the matrices (Katekhong et al. 2022). Bioplastics with a tighter surface absorb less water, resulting in a slower natural decomposition process (Krishnamurthy and Amritkumar 2019; Lee and Yeo 2021; Tan et al. 2022). SEM results showed no significant changes in the surface of the bioplastic with the addition of 0.25-1 g of BC (Figure 3b-e). However, the surface density had reduced, which showed that bioplastics had higher water absorption capacity thus it could be more easily degraded. Result showed cracks in the surface of CS3BCA and CS4BCA samples. These cracks could be caused by weak bonds that occurred between the components that made up bioplastics (Syuhada et al. 2020; Triawan et al. 2020; Ruggero et al. 2021). As a result of the SEM morphology, it appears that the CS2BCA sample has optimum conditions for use as a bioplastic when compared to other samples.

Thermogravimetric analysis

Thermogravimetric analysis was the method used to determine the rate of decomposition of materials through changes in weight over certain temperature. The results of the TGA analysis are presented in Figure 4.

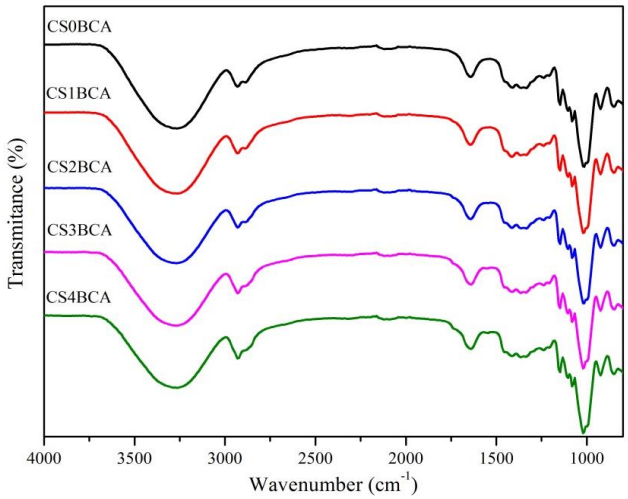

Figure 1. FTIR spectrum of bioplastic samples

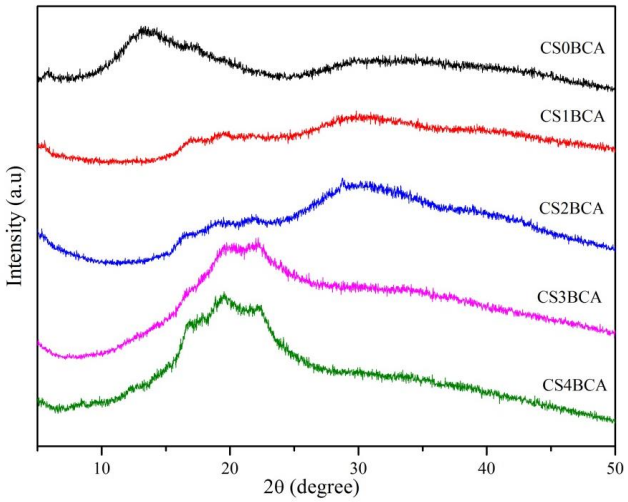

Figure 2. XRD curve of bioplastic samples

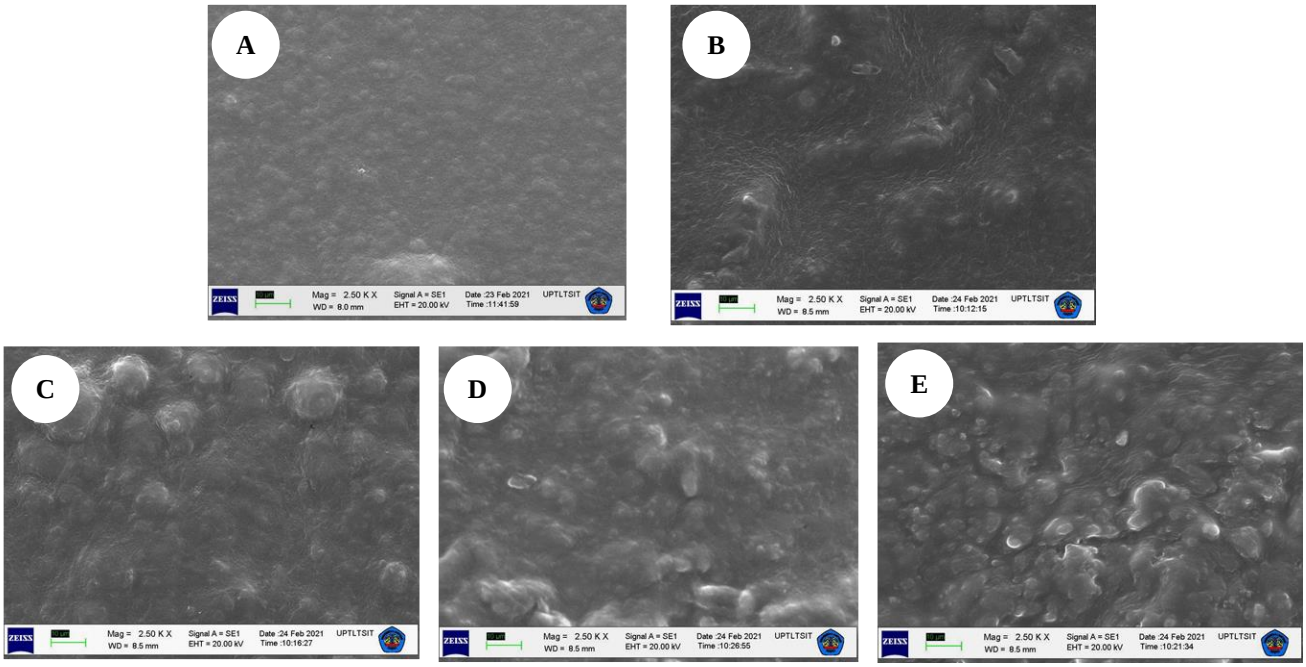

Figure 3. SEM images of bioplastic samples. A. CS0BCA, B. CS1BCA, C. CS2BCA, D. CS3BCA, and E. CS4BCA

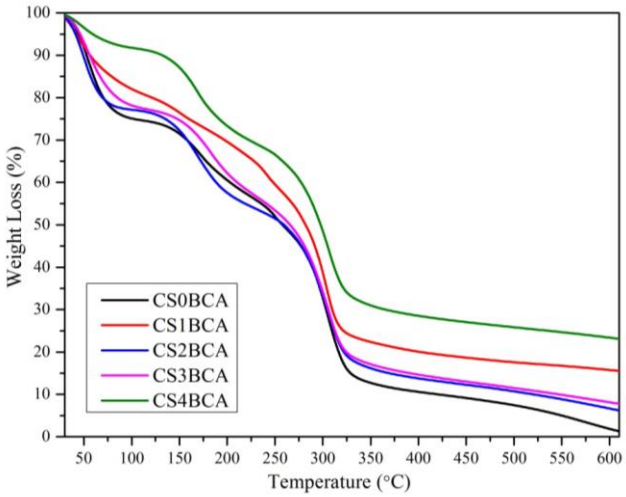

Figure 4. TGA curve of bioplastic samples

Result showed that there were three stages of bioplastic mass reduction. In the first stage, when the temperature reached 200°C, mass reduction in the samples was affected by the release of water molecules. Moreover, volatile compounds, which may be mostly derived from *Z. acanthopodium* extract, were released at this stage. Next, second stage occurred at 350°C, when the sample experienced significant mass reduction, such as 87.3%, 77.7%, 84%, 83% and 69.14% in sample CS0BCA, CS1BCA, CS2BCA, CS3BCA, and CS4BCA, respectively. Volatile compounds were also removed in the second stage. This stage became the main thermal degradation process due to the occurrence of significant mass reduction. High mass reduction took place at this due to the degradation of amylose particles from cassava starch. Finally, the third stage occurred at 550°C, when the mass reduction was constant with no more extreme mass reduction. Bioplastic CS0BCA, CS1BCA, CS2BCA, CS3BCA, and CS4BCA samples experienced 94.99%, 83.30%, 91.22%, 90.17%, and 75.38% mass reduction, respectively. At this stage, charcoal was formed and underwent combustion with the presence of oxygen at the surface and burned the charcoal and including remaining volatile substances, leaving only carbon.

From the curve, all samples were observed to have similar degradation patterns, where significant reduction in mass occurred in the first stage, followed by high mass loss rate in the second stage, and finally a horizontal-line degradation rate in the third stage. However, with the increase in BC concentration, thermal resistance of bioplastics increased. This means that bioplastics had more regular structure with higher BC concentration. Moreover, this could be indicated by increasing residual mass with higher BC concentration as shown in Table 2.

Table 2. Bioplastic residue mass

Samples	Residual mass (%)	T_{max} (°C)
CS0BCA	0.38	306.2
CS1BCA	15.1	303.6
CS2BCA	5.4	306.5
CS3BCA	7.16	305.6
CS4BCA	22.64	305.4

Antibacterial activity test

Antibacterial activity tests were conducted to determine the ability of bioplastics to protect food from harmful pathogens. Bioplastics with the addition of *Z. acanthopodium* extract were tested for their antibacterial activity against Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*Bacillus cereus*). The results are summarized in Table 3.

Bioplastic with the addition of *Z. acanthopodium* extract had good antibacterial activity against *B. cereus* (Gram-positive) compared to *Escherichia coli* (Gram-negative). This may be due to the low concentration (1 mL) of *Z. acanthopodium* extract given to bioplastics, resulting in a lack of antibacterial properties against *E. coli* bacteria. Previous research found that a 10% concentration of *Z. acanthopodium* extract could inhibit *E. coli* bacteria by 0.65 mm (Muzafri et al. 2018). Parhusip (2004) reported that 10% concentration of *Z. acanthopodium* extract in the sample produce 17.1 mm inhibition zone against *B. cereus* bacteria. Incorporated plant extract provided antimicrobial activity to the films, producing active packaging (Klinmalai et al. 2021). Figure 5 shows the results of antibacterial analysis of bioplastics.

Mechanical properties

The purpose of this test was to compare the mechanical properties of bioplastic samples at various concentration of BC. The analysis of mechanical properties was carried out by ASTM D822-02 using a 10 kN UTM tensile tester with at 2 mm/min tensile speed rate. Mechanical properties test results are presented in Figure 6.

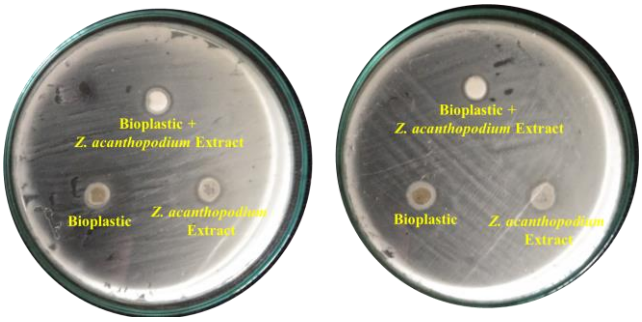

Figure 5. Antibacterial activity against bacteria (A) *Escherichia coli* and (B) *Bacillus cereus*

Table 3. Antibacterial activity of *Zanthoxylum acanthopodium* extract with bioplastic samples

Bacteria	Samples	Average diameter of inhibition zone (mm)	Antibacterial activity
Escherichia coli	Bioplastic without *Zanthoxylum acanthopodium* extract	-	No activity
	Zanthoxylum acanthopodium extract	0.7	Weak
	Zanthoxylum acanthopodium extract + bioplastic	0.53	Weak
Bacillus cereus	Bioplastic without *Zanthoxylum acanthopodium* extract	-	No activity
	Zanthoxylum acanthopodium extract	11.1	Strong
	Zanthoxylum acanthopodium extract + bioplastic	10.8	Strong

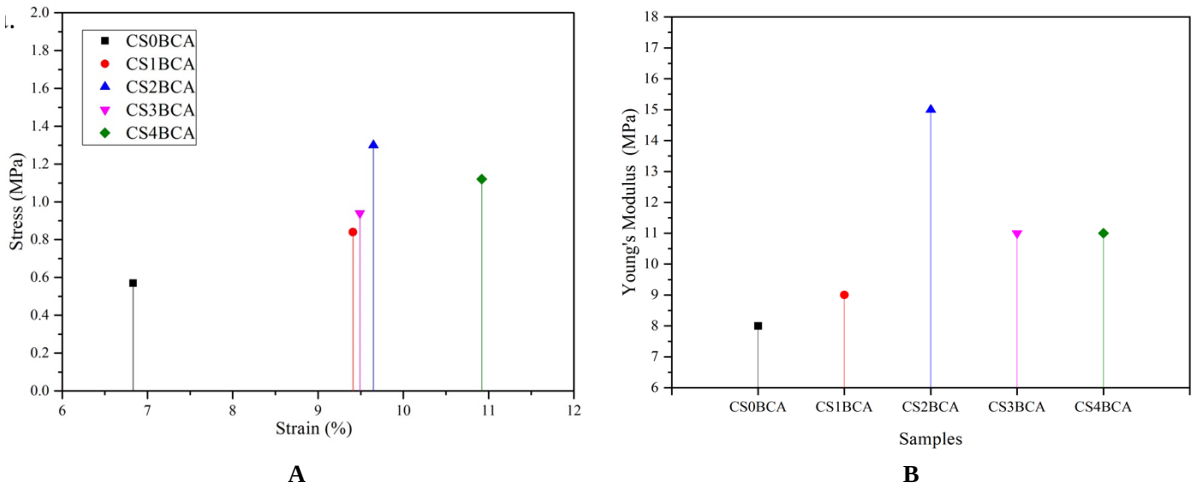

Figure 6. A. Stress-strain and B. Elastic modulus graphs of bioplastics samples

The lowest tensile strength was observed in sample CS0BCA at 0.57 MPa, which was due to the absence of filler in the bioplastic film. On the other hand, CS1BCA, CS2BCA, CS3BCA and CS4BCA showed an increase in tensile strength, as follow 0.84 MPa, 1.30 MPa, 0.94 MPa, and 1.12 MPa, respectively. The addition of BC improved the mechanical properties of bioplastic films, as BC had straight and long polymer chains that could strengthen bioplastic films. However, the data showed that at a certain BC concentration the tensile strength could decrease. The highest tensile strength in this research was observed in CS2BCA sample. This shows that bioplastic formulation had optimum conditions for production, indicating that continuous addition of BC did not necessarily increase tensile strength. Addition of extract caused non-homogeneity which reduced mechanical properties. Dispersed particles disrupted connectivity of the film matrices and reduced tensile properties (Chatkitanan and Harnkarnsujarit 2021; Harnkarnsujarit 2017). Likewise, elastic modulus value increased with the tensile strength, in which sample CS0BCA, CS1BCA, CS2BCA, CS3BCA, and CS4BCA showed elastic modulus of 8 MPa, 9 MPa, 15 MPa, 11 MPa, and 11 MPa, respectively. The increase in elastic modulus was also supported by the increase in the elongation value percentage, where the value increased from 6.83% in CS0BCA, to 10.92% in CS4BCA. The increase in elongation percentage indicated that the bioplastic product would be difficult to tear due to the mixture of BC with starch and glycerol, which made the bioplastic more difficult to break (Iswendi et al. 2021).

In conclusion, this study successfully produced cassava starch/bacterial cellulose-*Z. acanthopodium* films, which could potentially to be used as an environmentally friendly bioplastic for food packaging. The addition of bacterial cellulose and *Z. acanthopodium* altered the transmittance of FTIR absorption and influenced the crystallinity of bioplastic films as analyzed by XRD. TGA analysis revealed that the CS2BCA sample had the lowest residual mass, with a value of 5.4 %. The SEM image shows the change in surface morphology caused by the addition of BC. Tensile test revealed that CS2BCA sample had the highest tensile strength (2.34 MPa). The antibacterial activity test on bioplastics demonstrated good inhibition against *B. cereus* bacteria with an inhibition zone of 10.8 mm. Consequently, bioplastic based on cassava starch/bacterial cellulose-*Z. acanthopodium*, which have antibacterial properties, can be a promising innovation in active packaging systems due to its superiority in protecting food from microorganisms.

ACKNOWLEDGEMENTS

The authors would like to thank the head of Cellulosic and Functional Materials Laboratory, Universitas Sumatera Utara, Medan, Indonesia for facilitating the experimental procedures of this research.

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