

Synthesis of Antibacterial and Biodegradable Bioplastic Based on Shrimp Skin Chitosan and Durian Skin Cellulose with the Microwave Assistance

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Abstract

This study aimed to obtain the best composition in the synthesis of antibacterial bioplastics made from chitosan from shrimp skin (SS) and cellulose from durian skin (DS). The research method began with the isolation of chitin from SS. Then it was deacetylated using a microwave (MW) at 60 °C for 15 minutes at 400 watts of power in an alkaline solution. The extraction of cellulose from DS through delignification using the MW for 20 minutes, 300 watts of power in Na₂SO₃ solution. Synthesis of bioplastics is made from variations in the composition of chitosan (8, 12 and 16% w/w), cellulose and glycerol as a plasticizer. The characterization of bioplastics with FTIR obtained functional groups O–H, C–H, C=O, C–N and N–H amines, and SEM characterization obtained bioplastic has fibre and pore size 15.429 µm. The best bioplastic characteristics were the composition of 12 % chitosan, with tensile strengths of 13.28 Mpa, water resistance of 79 % and the ability to degrade 52.67% after 15 days have met international plastic standards (ASTM 5336). The antibacterial activity of bioplastics against *Escherichia coli* and *Staphylococcus aureus* with the Disc diffusion method showed the presence of moderate zones category of inhibition so that the resulting bioplastics can be recommended as food packaging that is environmentally friendly and antibacterial.

Keywords: Antibacterial, bioplastic, cellulose, chitosan, microwave.

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1. INTRODUCTION

The high demand for plastic causes problems for the environment. Plastic is a carbon-based polymer material with long-chain molecules and is flexible or elastic and difficult to decompose (Kamsiati et al., 2017). According to the Indonesian Aromatic and Plastic Olefin Industry Association (INAPLAS), plastic consumption in Indonesia in 2015 reached 17 kg/capita/year from food packaging plastic. Synthetic plastics contain phthalate compounds that can be leached or free from plastic and evaporate quickly and contaminate food and beverages and have the potential to cause endocrine system (hormonal) disorders or are called *endocrine-disrupting chemicals* (EDC) (Ilmiawati et al., 2016). The

use of conventional plastics can cause problems because they are difficult to degrade in the environment, which is the basis for alternative efforts to develop bioplastics. Bioplastics are environmentally friendly plastics that can be decomposed by microorganism activity.

Some or almost all of its components come from renewable raw materials, namely from compounds found in plants such as cellulose, starch and lignin, and in animals such as chitin and chitosan (Situmorang et al., 2019). The results of bioplastic degradation can be used as animal feed or compost. In addition, the quality of the soil will increase because the decomposition products will be faster by microorganisms and will increase the

nutrients in the soil (Asngad et al., 2018). The raw materials for bioplastics are chitosan from shrimp shells and cellulose from durian skins with the help of a microwave (MW). MW is an excellent method to help produce high yields and fast reaction rates in the synthesis process (da Silva et al., 2020; Lalegani et al., 2020). Therefore, the microwave synthesis method has been declared as an environmentally friendly green chemical synthesis method (Kumar et al., 2020). Furthermore, the effect of microwaves in synthesizing chitosan increases the degree of deacetylation and low molecular weight of chitosan (Setyawati et al., 2016) and cellulose synthesis plays an essential role in the morphology of the resulting cellulose that the cellulose has a fibre-like shape and irregular morphology (Ma et al., 2012).

Chitosan can increase the strength of plastics and has water barrier properties when mixed with cellulose. Cellulose itself is an unbranched polymer of glucose. This polymer form allows cellulose to be bonded to each other to form solid fibres (Lim & Hudson, 2004; Yin et al., 2021). In plastic production, additive substances called plasticizers are added (Pratiwi et al., 2016). The plasticizers used to the polymer to obtain the desired plastic characteristics such as clear, robust and wide temperature tolerance range and flexibility. Chitosan can increase the mechanical strength of plastics and reduce water absorption (Haghighi et al., 2020; Yin et al., 2021). Another significant property of chitosan is its antibacterial properties, namely the inhibition of bacterial growth (Dewi et al., 2017). Meanwhile, cellulose has thermoplastic properties that can be formed or moulded into packaging bioplastics and can be naturally decomposed by microorganisms into environmentally friendly compounds (Pratiwi et al., 2018). The bioplastics using chitosan from shrimp shells (SS) and cellulose from durian shells (DS) will improve the quality of bioplastics as antibacterial food packaging.

2. MATERIALS AND METHODS

Instrumentation and Materials

The instruments used in this research were microwave (SHARP R899RS-iN), Fourier Transform Infrared Spectroscopy (FTIR) Shimadzu type IRPrestige-21, Scanning Electron Microscopy (SEM) merk FEI type Inspect-S50. The materials used in this research were shrimp shells (SS), durian shells (DS),

distilled water (H_2O), nutrient agar (NA), nutrient broth (NB), bacterial isolates (*Escherichia coli* and *Staphylococcus aureus*) and chemicals from Merck, Germany include sodium hydroxide (NaOH), hydrochloric acid (HCl), acetone (C_3H_6O), sodium sulfite (Na_2SO_3), hydrogen peroxide (H_2O_2), glycerol ($C_3H_8O_3$), sodium chloride (NaCl), acetic acid ($C_2H_4O_2$).

Synthesis of Chitosan from Shrimp Shells (SS)

Shrimp shells (SS) waste was cleaned and dried in the sun, then mashed with a size of 250 μm . The stages of chitosan synthesis include deproteinization, demineralization, decolourization and deacetylation, which are modifications of research by Zaeni et al. (2017). The deproteinization stage using a ratio of sample and solvent 1:10 with 4% NaOH solution and heated at a temperature of 65 $^{\circ}C$. Then the sample was filtered and washed with distilled water until the pH was neutral and dried at a temperature of 80 $^{\circ}C$ for six hours. The demineralization stage, using 1.5 M HCl solution with a ratio of 1:10 w/v (sample: solvent), was stirred and heated at a temperature of 75 $^{\circ}C$ for one h. Then filtered, the resulting residue was washed using distilled water to a neutral pH, then dried at a temperature of 80 $^{\circ}C$ for six hours. The decolourization stage is a colour removal process using acetone (1:15) as a solvent and then washing the residue until the pH is neutral with distilled water. The resulting residue was dried in an oven at a temperature of 80 $^{\circ}C$ for six hours. In the deacetylation stage, weigh 4 g of chitin and add a 50% NaOH solution (1:20). The mixture was heated in MW at 450 watts for 15 min. Chitosan was washed until the pH was neutral and then dried at a temperature of 45 $^{\circ}C$ for 24 h. FTIR tested the resulting chitosan (Zaeni et al., 2017).

Synthesis of Cellulose from Durian Shells

Cleanse the DS from the flesh, then dry them. The dried DS was mashed with a size of 250 μm . The cellulose synthesis stages include pre-treatment, delignification and bleaching, which is a modification of the research by Sari et al. (2018). In the pre-treatment stage, durian peel powder was put in an Erlenmeyer, and 1.5% NaOH solution (1:10) was added, then heated in MW at 300 watts of power for 20 min. Next, the mixture was filtered and neutralized using distilled water to pH seven

and then dried for six hours at 100 °C. The delignification stage used a ratio of sample and solvent 1:10 by adding 20% Na₂SO₃ (Lismeri et al., 2019) and heating in MW at 300 watts of power for 20 min (Pooja & Padmaja, 2017). Furthermore, separated and washed with distilled water to pH 7, then dried for six hours at a temperature of 100°C (Sari et al., 2018). The bleaching stage uses 2% H₂O₂ with a sample and solvent ratio of 1:15, heated at a temperature of 60 °C for 2 hours. Then filtered, and the residue was washed until the pH was neutral. Next, it was dried at 100 °C for six hours (Lismeri et al., 2016). The last step is to analyze the lignocellulosic content, including cellulose, hemicellulose and lignin, using the Cheson Datta method (1981) and test the FTIR characteristics.

Manufacturing of Bioplastic

The manufacture of bioplastics begins with making a solution of 8, 12, and 16% concentrations dissolved in 1% acetic acid. The durian skin cellulose of 6 g was added to 100 ml of distilled water and then heated and stirred at a temperature of 70-80 °C at a speed of 600 rpm for one hour to form a thick solution. The cellulose solution was allowed to cool, then added each of the chitosan solutions with varying concentrations of 8, 12 and 16%, then reheated for 15 minutes at a temperature of 85 °C. After homogeneously mixed, each was cooled for a few minutes and added 5 ml of glycerol. The mixed material is heated at a temperature of 50 and stirred with a magnetic stirrer for 15 minutes to obtain a thickened solution (forming a gel). Next, print it on a glass plate with the size of 20 × 20 cm evenly until there are no bubbles on the surface, then dry at a temperature of 55 °C for 48 hours (Alam et al., 2018). Bioplastics were characterized using FTIR and SEM to determine the surface morphology of the test samples.

The Characteristics and Biodegradation of Bioplastic

The bioplastic characteristic tests include solubility, thickness (ASTM D988), tensile strength (ASTM D-638), elongation (ASTM D-638) and biodegradation test. The biodegradability test used the soil burial test method based on the modified method from Widiatmono et al. (2021) research, namely burying bioplastic samples in humus soil for

15 days by checking samples every five days of burial. The determined percentage of biodegradability (% mass loss) by calculating the residual weight fraction of the bioplastic sample using equation 1. W_i is the sample mass before biodegradation, and W_f is the sample mass after biodegradation.

$$\% \text{ mass loss} = \frac{W_i - W_f}{W_f} \times 100\% \quad (1)$$

The Antibacterial of Bioplastic

The antibacterial activity testing was performed using *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) bacterial cultures. The sterilized NA media were *Staphylococcus aureus* and *Escherichia coli*, respectively, inoculated, and the turbidity was adjusted with a solution of 0.5 Mac. Farland. Next, make the crucible using the pour plate method and allow it to solidify. Tests were carried out using bioplastic films from the best formulations. The bioplastic film was made with a diameter of 5 mm and attached to the agar surface, and incubated at 37 °C with the petri dish inverted.

Furthermore, observing the zone of inhibition was carried out in the presence of inhibition, and the diameter of the inhibition was measured using a calliper. The diameter of the inhibition zone was measured to determine the amount of inhibition using a calliper and calculated using the following formula.

$$Z = \frac{(D_1 - D_{fk}) + (D_2 - D_{fk}) + (D_3 - D_{fk})}{3} \quad (2)$$

Z is the zone of inhibition, D_1 is the vertical diameter, D_2 is the horizontal diameter, D_3 is the diagonal diameter and D_{fk} is the bioplastic diameter. Therefore, the criteria for antibacterial inhibition were based on the diameter of the clear zone, namely very strong (> 20 mm), strong (10-20 mm), moderate (5-10 mm), and classified as weak (< 5 mm). Positive control is chitosan and negative control is bioplastic without chitosan.

3. RESULTS AND DISCUSSION

The FTIR Characterization of Chitosan and Cellulose

Functional group analysis of the FTIR spectrum was carried out to identify the functional groups present in chitosan and

cellulose. Figure 1a shows the FTIR spectrum of chitosan, the peak in the absorption region of 3446 cm^{-1} is the OH group. Absorption around 2920 and 2883 cm^{-1} can be attributed to C-H symmetric and asymmetric stretching. The presence of an N-acetyl group at the absorption of about 1656 cm^{-1} showed a stretch of C=O amide I, the absorption of 1579 cm^{-1} an N-H bending of amide II and the absorption of 1321 cm^{-1} a C-N amide III stretching. It is an uptake characteristic of all three distinct N-acetyl groups and may overlap with other bands. The peak at 1589 cm^{-1} corresponds to the N-H bending of the primary amine (Lim & Hudson, 2004; Queiroz et al., 2015). The CH_2 bending and CH_3 symmetrical deformation were confirmed by peaks at 1421 and 1379 cm^{-1} , respectively. The absorption band at 1155 cm^{-1} is attributed to the C-O-C asymmetric strain. The peaks at 1082 and 1029 cm^{-1} correspond to C-O stretching. All absorption regions were found in the spectrum of the reported chitosan samples following the results of previous studies (Song et al., 2013; Vino et al., 2012).

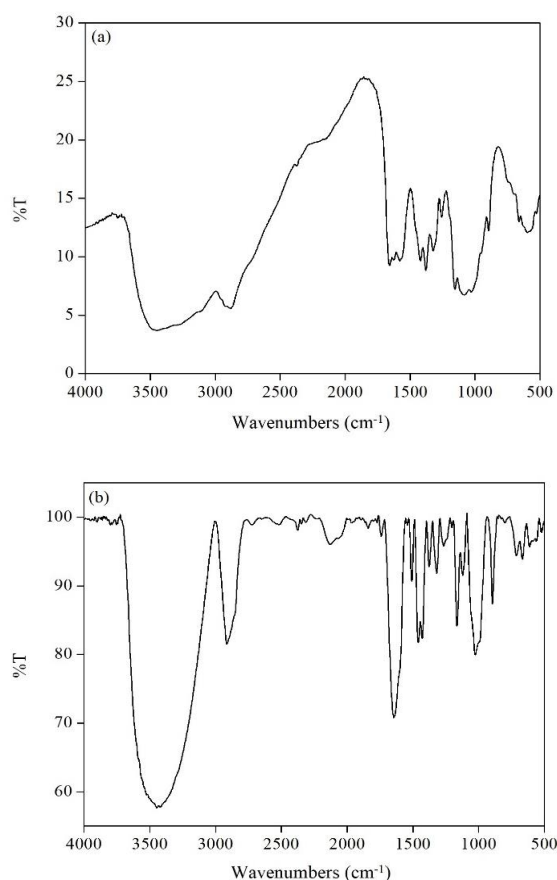


Figure 1. The FTIR characterization (a) chitosan and (b) cellulose

The level of chitosan from shrimp shells using the MW method is 32%, with a degree of deacetylation (DD) chitosan of 96.4%. According to Mashuni et al. (2021), if the DD is $>70\%$, the polymer is called chitosan. The deacetylation process involves the removal of an acetyl group ($-\text{COCH}_3$) from the chitin molecular chain, leaving an amine group ($-\text{NH}_2$) with high reactivity. The DD is an essential parameter in producing chitosan because it can affect its physical properties to determine the appropriate application for the final product of chitosan. The DD indicates the number of free amine groups in polysaccharides that distinguish between chitin and chitosan (Baharuddin & Isnaeni, 2020; Setyawati et al., 2016).

The cellulose content of DS using the microwave method was 60% higher than that of lignin at 16.25% and hemicellulose at 23.75%. Figure 1b shows the FTIR spectrum of cellulose, the peaks observed in the range of wave numbers $3446\text{--}2900\text{ cm}^{-1}$ are characteristic of the stretching vibrations of O-H and C-H bonds in polysaccharides. The peak width at 3331 cm^{-1} is a characteristic of the strain vibration of the hydroxyl group in polysaccharides (Hospodarova et al., 2018; Poletto et al., 2011; Rosa et al., 2010). This peak also includes the intermolecular and intramolecular hydrogen bonding vibrations in cellulose (Hospodarova et al., 2018; Popescu et al., 2011). The peak at 2914 cm^{-1} is associated with the CH stretching vibrations of all the hydrocarbon constituents in the polysaccharide (Poletto et al., 2011; Rosa et al., 2010). A typical band defined for cellulose was observed in $1630\text{--}900\text{ cm}^{-1}$. The peak at 1645 cm^{-1} vibration comes from OH by water molecules absorbed in cellulose (García-Ramón et al., 2021; Hospodarova et al., 2018). The peaks at 1429 , 1377 , 1319 , 1065 and 894 cm^{-1} belong to the stretching and bending vibrations of CH_2 , CH, OH and C-O bonds in cellulose (Fackler et al., 2011; Xu et al., 2013). The peaks are 1429 to 1460 cm^{-1} associated with the amount of crystalline cellulose structure, while the band at 894 cm^{-1} is related to amorphous regions in cellulose. (Poletto et al., 2014).

The Solubility of Bioplastic

Figure 2a shows the different values of water absorption against plastic resistance obtained. Bioplastic without chitosan (BS) has

a low water resistance value of 20%, so the solubility of bioplastics in water is most significant and causes bioplastics to be very easily destroyed. It shows that bioplastics are not resistant to water. The BSK 12% showed an increase in the value of water resistance due to the hydrophobic nature of chitosan. The added chitosan will reduce the moisture of the bioplastic. The BSK 8% showed an increase in the value of resilience. It can be compared to the bioplastic water resistance standard, 99% based on the Indonesian national standard (SNI). In this study, the value close to water resistance based on SNI is BSK 12%, so it is excellent to be used as bioplastic. The higher the water resistance value in bioplastic, the better the quality of the plastic so that the durability of the product to be packaged will be longer. Conversely, the lower the plastic water resistance, the greater the plastic damage level and solubility in water, so that the packaged product will not last long in storage.

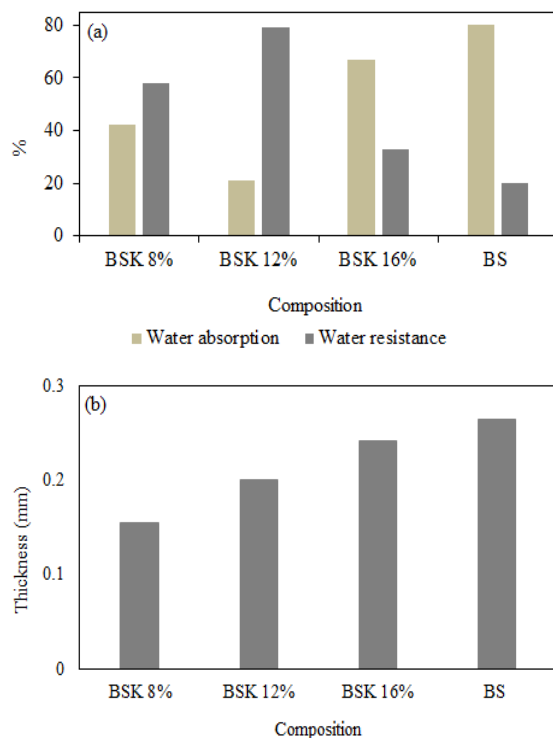


Figure 2. The characteristics of bioplastics from cellulose based materials with variations in the composition of chitosan of 8, 12 and 16%, respectively: (a) Solubility of water absorption and water resistance. (b) Thickness (BSK: bioplastics of cellulose and chitosan; BS: bioplastics of cellulose without chitosan)

The Thickness of Bioplastic

Figure 2b shows that the more chitosan composition, the thickness of the bioplastic

tends to increase. The bioplastic compositions of BSK 8%, BSK 12%, BSK 16% and BS each produced a thickness of 0.200; 0.242; 0.264 and 0.154 mm. A similar study was reported for bioplastics by Santana et al. (2018) dan Zavareze et al. (2012). It is caused by cellulose that accumulates on the sidelines of the chitosan matrix, causing its thickness to increase. The results obtained indicate that more chitosan will increase the total volume of substances and water in the bioplastic, increasing the thickness level. This mechanical test showed that the bioplastic, which had the highest concentration of chitosan at 16%, had the best thickness.

The Tensile Strength of Bioplastic

The characterization of the tensile strength test is one of the crucial parameters in bioplastics to determine the strength of bioplastics against external forces that can damage bioplastics (Santana et al., 2018). The strength of bioplastics includes tensile strength (stress) and elongation (strain) at the break. The tensile strength of bioplastics from chitosan is the maximum strength or tensile strength of bioplastics obtained before breaking or tearing. The measurement of the tensile strength of bioplastics is intended to determine the magnitude of the force required to obtain the maximum stress in each area of the bioplastic film.

Table 1 shows the tensile strength of bioplastics. The BSK composition of 8% produces a tensile strength of 14.85 MPa and an elongation of 3.77%, while BSK of 16% has a lower tensile strength of 0.30 MPa and an elongation of 4.25%. The BS composition without the addition of chitosan resulted in tensile strength of 1.53 MPa and an elongation of 1.36%. This research showed that the addition of chitosan to the bioplastic composition would produce better tensile strength and elongation. The tensile strength values were within the range recorded by López & García (2012) for ahipa and cassava, and Basiak et al. (2017) for wheat and cassava are the same as the results in this study. According to Pratiwi et al. (2016), chitosan causes the formation of interactions with cellulose polymer chains in the form of hydrogen bonds, where these polymer chain interactions are formed to increase the speed of the viscoelastic response in the polymer to increase the mobility of the polymer chains

(Pratiwi et al., 2016). Increasing the mobility of this polymer chain causes the tensile strength value to increase. The increase will be valid as long as polymer chain interactions are still formed.

Table 1. The tensile of bioplastic

Bioplastic	Stress σ (MPa)	Strain ϵ (%)
BSK of 8%	14.85	3.77
BSK of 12%	13.28	4.81
BSK of 16%	13.30	4.25
BS	1.53	1.36

The Biodegradation of Bioplastic

The biodegradation test was carried out by dumping biodegradable plastic into the soil for 15 days. Figure 3 shows the results of bioplastic degradation, namely 60-100%, meaning that most of the plastic has been degraded. This biodegradation ability is due to cellulose and glycerol, which contain O-H groups that can bind with and the ability to bind moisture from the air so that they are

rapidly degraded. The biodegradation test has met the international standard (ASTM 5336) that plastic takes 60 days to decompose completely, so it can conclude that the bioplastic from SS waste and DS waste has met the standard.

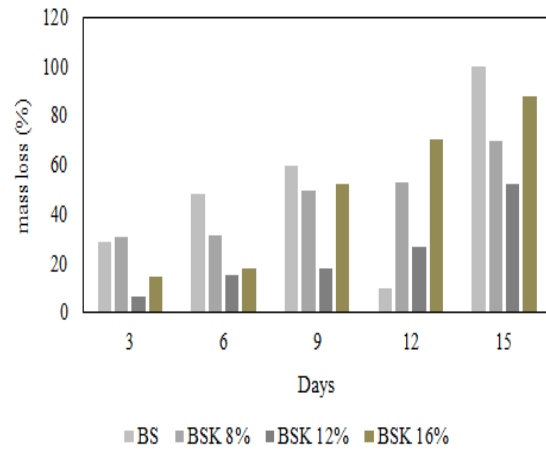


Figure 3. The Biodegradation of bioplastic

The Characterization of FTIR and SEM Bioplastics

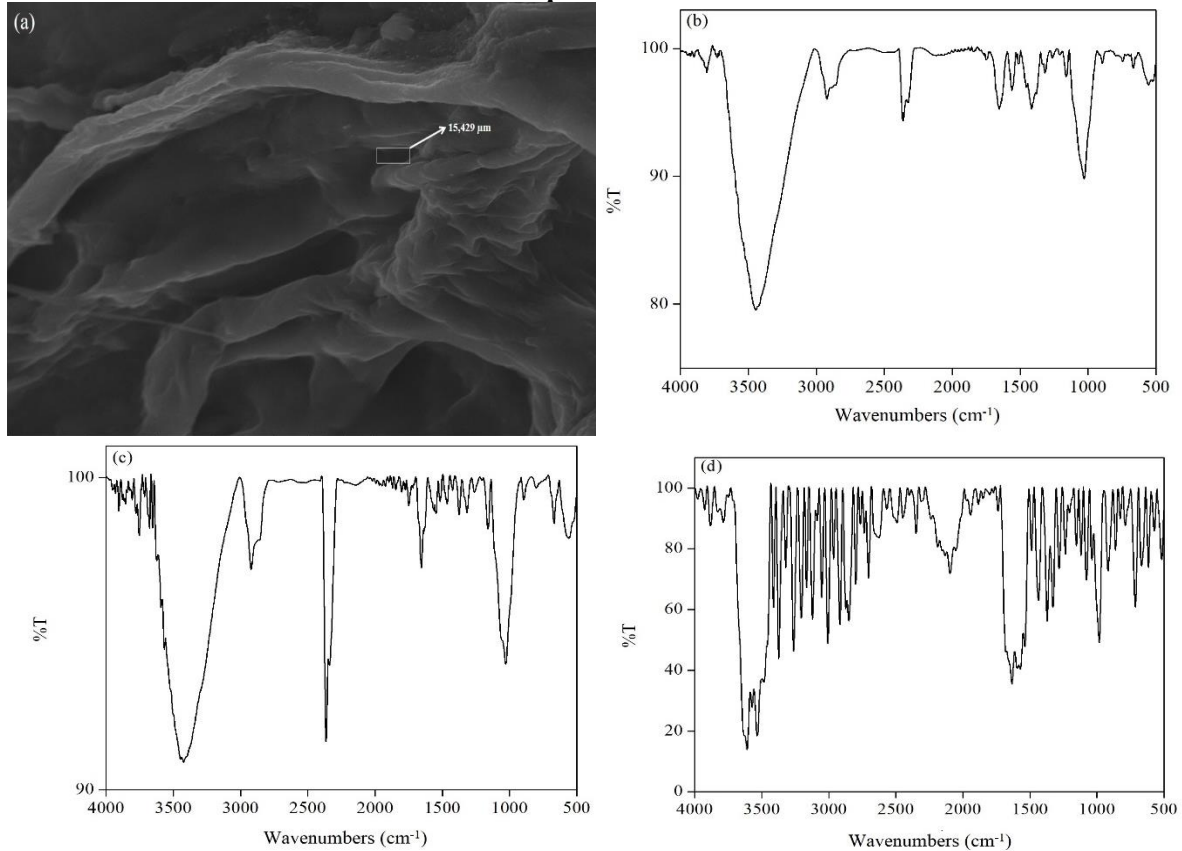


Figure 4. Characterization of bioplastics (a) SEM, (b) BSK of 8% FTIR, (c) BSK of 12% FTIR and (d) BSK of 16% FTIR

Figure 4a shows the SEM results of the surface morphology of bioplastics made with the composition of chitosan, cellulose and glycerol, which have a non-smooth surface due to the fibre content in cellulose. In addition, there is a buildup (clot) at several points on the surface due to the insoluble nature of cellulose. The results of the bioplastic morphology analysis showed a pore size of 15.429 μm which was obtained by further research using ImageJ software. It is stated that the greater the concentration of chitosan used, the more visible pores in the bioplastic. Therefore, chitosan affects the size and number of pores by 15.429 μm .

Figure 4a-b, FTIR of bioplastics of all compositions, shows a solid and wide absorption band in the absorption area around 3444 and 3423 cm^{-1} , indicating -OH groups' presence (Hospodarova et al., 2018; Santana et al., 2018). It is caused by the increase in the -OH group due to the increase in chitosan concentration. Figure 4d, the composition of SBK 16% is a sample containing 16% chitosan content so that it shows there is an N-H bending of the N-H primary amine at the absorption of 3535 and 1595 cm^{-1} (Lim & Hudson, 2004; Queiroz et al., 2015).

The Antibacterial Activity of Bioplastic

Bioplastic with *Staphylococcus aureus* bacteria at a concentration of 16% had the largest zone of inhibition diameter. The size of the zone of inhibition in *S. aureus* bacteria is 10.9 mm, categorized as strong (active). The diameter of the inhibition zone formed is influenced by the concentration of the compound or active substance contained in the fraction. Bioplastics can inhibit bacteria of Gram-positive (*S. aureus*) more than Gram-negative (*E. coli*). It can be seen in the diameter of the clear zone that has been measured. The difference in the level of sensitivity between bacteria *E. coli* and *S. aureus* causes the zone of inhibition resulting from bioplastics in these two bacteria to be different.

This interaction may moderate the antibacterial capacity of the $-\text{NH}_2$ group. The presence of amine groups in bioplastics is responsible for the antibacterial action (Hisbiyah et al., 2021; Priscilla et al., 2020; Wu et al., 2015). It explained that the nitrogen atom in the compound is involved in the formation of hydrogen bonds with the target

enzyme indicating an antibacterial active site (Bonilla et al., 2013; Priscilla et al., 2020). When the $-\text{NH}_2$ group already reacts with another molecule, the antibacterial action may be reduced due to the inhibitory effect by forming hydrogen bonds between the $-\text{NH}_2$ group and the target bacterial enzyme (Kongkaoroptham et al., 2021).

Table 2. The antibacterial activity of bioplastic

Parameters	Zone of inhibition (mm)	Categories
<i>Escherichia coli</i>		
Positive control	10.23	Strong
Negative control	0	-
BSK of 8%	5.23	Moderate
BSK of 12%	6.56	Moderate
BSK of 16%	9.90	Moderate
<i>Staphylococcus Aureus</i>		
Positive control	11.90	Strong
Negative control	0	-
BSK of 8%	7.23	Moderate
BSK of 12%	9.23	Moderate
BSK of 16%	10.90	Strong

4. CONCLUSION

Bioplastics made from chitosan and durian skin cellulose, insulated using the MW method, produce quality bioplastics. The best bioplastic was obtained at the composition of 12% chitosan with a water resistance value of 79%, a tensile strength of 13.28 MPa and elongation of 4.81%. Bioplastic has typical characteristics of SNI 7818:2014. Furthermore, bioplastic can be degraded by 52.67% after 15 days. This value already meets the requirements of international plastic standards (ASTM 5336). In addition, bioplastics have antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, so the resulting bioplastics can be recommended as environmentally friendly and antibacterial.

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