

Article

Synthesis and Characterization of Cellulose *Nata de Coco* Inserted Nanoparticles Zn-Moringa Leaf Extract (*Moringa oleifera*) as Biodegradable Plastic Banana Fruit Packaging

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Abstract

Synthesis and characterization of cellulose nata de coco inserted nanoparticles of *zinc-moringa leaf extract* as biodegradable plastic for banana fruit packaging has been carried out. This study aims to determine the characteristics, biodegradability and preservation activity of biodegradable plastic cellulose nata de coco inserted zinc nanoparticles-moringa leaf extract. The research process was carried out through the nata de coco synthesis stage, zinc nanoparticle synthesis-moringa leaf extract and the manufacture of *biodegradable* plastics. The success of this synthesis is evidenced by being characterized using a UV-Vis spectrophotometer, FTIR spectrophotometer and digital optical microscope. The results of UV-Vis spectrophotometer analysis showed that *the zinc* nanoparticles formed had an estimated particle size of 85.72-92.92 nm and were stable. The results of FTIR analysis on Moringa leaf extract showed the presence of -OH, C-H, C=O, C=C and C-O groups. In *the biodegradable* plastic film nata de coco-NPZn-moringa leaf extract, there was a peak shift in several functional groups marked at the peaks of wavenumbers 555.50 and 432.05 cm⁻¹ which are ZnZP groups. Characterization using a digital optical microscope showed that *zinc* nanoparticlesThe synthesis is spherical, non-uniform, and has been well distributed on the cellulose surface of nata de coco. Biodegradation test on nata *de coco*-NPZn-Moringa leaf extract biodegradable plastic film using activated sludge system, test results show biodegradable plastic can be *degraded* 100% for 15 days. Preservation activities show that packaged bananas are still able to survive up to 8 days of post-harvest storage period. These results show the effectiveness of biodegradable plastic preservation of *nata de coco*-NPZn-moringa leaf extract biodegradable plastic which is quite good.

Keywords: *Biodegradable Plastic, Nata de Coco Cellulose, ZnNP Nanoparticles, Banana Fruit Packaging*

1. INTRODUCTION

Plastic is still the main choice as a packaging material for food products because it has a number of advantages including flexibility, ease of formation, transparent, resistance to damage, and affordable price [1]. The use of plastic as packaging involves various types of products, ranging from electronic equipment, household appliances, office equipment, to food and beverages. Plastic packaging is not only dominant in the industrial sector, but is also commonly used by traditional traders and households as storage containers for vegetables and fruits, with the aim of extending the shelf life of such products [2].

The plastic currently used is a type of synthetic polymer made from petroleum (*non-renewable*) and cannot be degraded by microorganisms in the environment [3]. Plastic waste processing can produce toxic fumes that are potentially harmful to health if the combustion process is not complete, causing plastic to break down in the air as dioxin. Dioxin is a compound that is very dangerous if inhaled by humans, can trigger serious diseases such as cancer, hepatitis, liver swelling, nervous system disorders, and depression [4]. Efforts to overcome the problem of plastic waste continue, one of which is through plastic recycling and the development of environmentally friendly plastics, known as plastics *Biodegradable*. Indonesia, as a country with a wealth of natural resources, has the potential to produce various biodegradable biopolymer materials.

Biodegradable plastic is a type of plastic that can be naturally decomposed by microorganisms into environmentally friendly compounds. This plastic is generally made from basic materials such as corn, chitosan, sweet potatoes, and cassava, with the main content in the form of starch or cellulose. One of the natural ingredients used is nata de coco cellulose, which is a fermentation product of *Acetobacter xylinum* bacteria on a sugar-containing substrate, forming a chewy nata cellulose sheet [5]. Nata de coco cellulose has advantages over plant cellulose, including a high level of purity because it is lignin-free, relatively fast production, and cellulose produced in sheet form [6].

Biodegradable plastics have the potential to be an alternative packaging material to conventional plastics, due to their physical and mechanical characteristics that resemble conventional plastics, such as flexibility, lightness, and the ability to degrade naturally. According to Bica *et al.* (2010), biodegradable plastic can be applied as packaging for bananas. Bananas are one of the national leading fruit commodities that are easily available, have economic value, and high nutritional value. The nutritional content of plantain peel is quite complete such as carbohydrates, fats, proteins, calcium, phosphates, iron, B vitamins, and vitamin C [7]. However, there are challenges in providing good quality bananas to local and export consumers, especially since bananas are susceptible to unsuitable environmental conditions, such as high temperatures and air humidity that can accelerate the process of spoilage and post-harvest losses [8]. One smart solution is to use biodegradable plastic packaging with the addition of green synthesis plant extract nanoparticles, which have antioxidant and antimicrobial properties to improve biodegradable plastic characteristics, such as strength, resistance, antimicrobial properties, and stability to heat and cold [9].

Green synthesis of nanoparticles has become a major concern of many researchers, by harnessing biological resources to synthesize nanoparticles. The use of plant extracts as metal ion reducing agents has the advantages of being relatively short, environmentally friendly, requires little energy, and is easily available compared to the use of microbes. Plant extracts are believed to act as reducing agents and stabilizing agents in the biosynthetic process of nanoparticles [10]. Some researchers have successfully biosynthesized zinc (Zn) nanoparticles using plant extracts, and the results are used as antibacterial and antimicrobial compounds. This is based on the entry of zinc metal ions and the size of very small nanoparticles (nanometers) so that reactive oxygen species can form in the bacterial cell wall resulting in damage to the bacterial cell wall [11].

Moringa is a plant that has many benefits. There have been many studies concerning Moringa plants that explain that Moringa leaves contain many secondary metabolite compounds, namely alkaloids, flavonoids, tannins, terpenoids, and saponins [12]. Some studies show that the compounds contained in Moringa *leaves* (*Moringa oleifera*) have antimicrobial activities such as anti-inflammatory, antifungal, antibiotic and anticancer as well as antioxidants so that they can be used in the preservation process [13]. Based on these properties, Moringa leaf extract can be used as a nanoparticle reducing bioreducer that can be applied to the manufacture of bioplastics as antimicrobial packaging to extend the shelf life of fruits. Therefore, in this study, the Synthesis and Characterization of Nata de Coco Cellulose Insertion of Zn-Moringa Leaf Extract Nanoparticles (*Moringa oleifera*) as Biodegradable Plastic Banana Fruit Packaging will be carried out.

2. MATERIALS AND METHODS

2.1 Materials

The materials used in this study were, coconut water, *starter Acetobakter xylinum*, moringa leaves, granulated sugar, acetic acid (CH_3COOH) 25%, zinc nitrate hexahydrate 0.1 M ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), ethanol 96% ($\text{C}_2\text{H}_5\text{OH}$), aquades (H_2O), zwavelzure ammonium (ZA) and whatman filter paper No. 42.

2.2 Instrument

The instruments used in this study are UV-Vis spectrophotometers (Spectroquant Pharo 300 M), Fourier Transform Infrared (Shimadzu) and digital optical microscopes (Leica Microsystems).

2.3 Method

2.3.1 Synthesis of Nata de Coco

Nata de coco cellulose is synthesized using pre-filtered coconut water. The filtered coconut water is put into a clean and sterile container, then 100 g of granulated sugar and 15 g of *Zwavelzure ammonium* (ZA) are added to one liter of coconut water. This mixture is heated to boiling point to kill microorganisms that may be present in coconut water, while stirring slowly so that the sugar can dissolve. Upon reaching the boiling point, the mixture is cooled, and then 10 mL of acetic acid is added to the inoculum of *A. xylinum* as much as 250 mL. The resulting fermentation medium is then poured into sterilized containers, covered with newsprint, and attached rubber bands. The fermentation process is carried out for 10 days at room temperature.

2.3.2 Synthesis of Zn Nanoparticles-Moringal Leaf Extract

The synthesis of zinc nanoparticles (ZnNP) using Moringa leaf extract bioreducing agents through *the green synthesis* method is based on the approach that has been carried out by Daphedar and Taranath (2018). In this method, zinc nanoparticles are synthesized by reducing the 0.1 M $\text{Zn}(\text{NO}_3)_2$ precursor solution using Moringa leaf extract as a bioreducing agent. Solution of $\text{Zn}(\text{NO}_3)_2$ and Moringa leaf extract are reacted in ratios of 1:1, 1:2, 1:3, 1:4, 1:5, and 1:6 (v/v) in a beaker. The mixing process is carried out with *a magnetic stirrer* for 30 minutes at room temperature. Next, the solution mixture is centrifuged at a rate of 3000 rpm for 10 min to separate the precipitate from the filtrate. The resulting filtrate is then stored in vials for each comparison, while the centrifuged precipitate is placed in a petri dish and dried in an oven at 50°C until ready to be characterized.

2.3.3 Making Biodegradable Plastic Inserting Zn Nanoparticles-Moringa Leaf Extract

The manufacture of biodegradable plastics adopts methods from studies conducted by Oktariana *et al.* (2012). Nata de coco cellulose is thoroughly cleaned, then pressed using a *hand press* to form a thin film layer. The thin film of nata de coco is then soaked in a 3% glycerol solution for three days and dried at room temperature for 24 hours. After that, the film was soaked in a solution of zinc-extract nanoparticles of moringa leaves at each ratio (ZEK05,

ZEK10, ZEK15, ZEK20, ZEK25, and ZEK30) (v/v). The soaked film is then removed from the solution and dried again at room temperature for 24 hours and produces a *biodegradable plastic film*.

2.3.4 Mechanical Test

The mechanical test of a biodegradable plastic sample consists of several tests, namely tensile strength tests, elongation tests and elasticity tests. This mechanical test is carried out using a Universal Testing Machine (UTM) tool by clamping the tip of the sample using a tensile testing machine. Then recording the initial length of the sample is carried out. The record button on the computer is pressed, then rotated the control of the test equipment to pull the sample up until it breaks. The measurement of the tensile strength test is determined by the following equation:

$$\sigma = \frac{F}{A} \text{ (MPa)}$$

The elongation test is performed in the same way as the tensile strength test using the following equation:

$$\varepsilon = \frac{\Delta L}{L_0} \times 100\%$$

The gradient of the initial linear part of the stretch stress curve is the modulus of elasticity. The modulus of elasticity is a measure of the rigidity of a material. The greater the modulus of elasticity, the smaller the stretch of elasticity produced due to applying voltage. Elasticity can be expressed in the following equation:

$$\text{Modulus Young (E)} = \frac{\sigma}{\varepsilon}$$

2.3.5 Water Resistance Test

Water resistance testing is carried out to determine the formation of bonds in the polymer, which can be identified through an increase in the weight of the polymer after undergoing absorption of water molecules on the film sheet. The water resistance test was carried out using *biodegradable* plastic measuring 3×3 cm which has a known initial weight (w_0). The film is put in a beaker filled with pure water for 5 minutes. After a period of 5 minutes, the plastic film is removed from the test tube, and the surface is cleaned using paper towels. The weight of the sample (w) that has permeated the water is then measured [14]. The percentage of absorbed moisture content can be calculated using the formula.

$$\text{Water Absorbability} = \frac{\text{Final Weight}_{(w)} - \text{Initial Weight}_{(w_0)}}{\text{Initial Weight}_{(w_0)}} \times 100\%$$

Then, the percent of water absorbed is calculated again in the following calculation to get the percent water resistance.

$$\text{Water Resistance} = 100\% - \% \text{Water Absorbability}$$

2.3.6 Antibacterial Test

Antibacterial activity tests were carried out using the disc method, in which *Staphylococcus aureus* and *Escherichia coli* bacteria were used as representatives of the gram-positive and gram-negative bacterial groups. Each bacterium was diluted using a sterile 0.9% NaCl solution of 900 μ L to achieve turbidity equivalent to a standard 0.5 Mc solution. The next step involves making agar media in 48 petri dishes by pour *plate*, which is then allowed to freeze. Test samples that have been cut with paper holes 5 mm in diameter are placed on the surface agar that has been frozen. As a positive control, *Streptomycin* was used, while the negative control used sterile aquades. Next, bacteria were incubated for 1×24 hours at 37°C, and then observed to determine the presence of a clear zone as an indicator of bacterial inhibition. The measurement of the diameter of the clear zone is calculated using a certain equation.

$$Z = \frac{(D1-D_{rk}) + (D2-D_{rk}) + (D3-D_{rk})}{3}$$

with, Z, D1, D2, D3 and D_{fk} are clear zone, vertical diameter, horizontal diameter, diagonal diameter and film diameter respectively. Antibacterial inhibitory criteria are based on the diameter of the clear zone, namely very strong (> 20 mm), strong (10–20 mm), medium (5–10 mm) and classified as weak (< 5 mm).

2.3.7 Preservation Test Activity

Preservation tests are carried out to see the ability of bioplastics to protect products. Bioplastic quality testing is carried out by applying to fruit, namely by wrapping a type of fruit that is easily damaged or mechanically changed. Bananas are one of the fruits that experience changes during the storage period, namely *browning* on bananas. The preservation test was carried out by packaging bananas with bioplastics that have optimal characteristics, carried out for 8 days at room temperature and observed every 2 days [15].

2.3.8 Biodegradation Test

The biodegradation test was carried out by inserting borehole water into an activated mud tank as much as 4 L. Then added 6 g of glucose (C₆H₁₂O₆) as a source of carbon (C), 1 g of KNO₃ as a source of nitrogen (N) and 0.5 g KH₂PO₄ as a source of phosphorus (P). Then added microbial starter (*indigenous microbia*) taken from the swamp location of Halu Oleo Kendari University Hospital building as much as 300 g. The mixture is then stirred until all dissolved. The aerator on the activated sludge tank is turned on, then after 24 hours, biodegradable plastic measuring 3×3 cm with a thickness of 0.1 mm is weighed as the initial weight and put into the tank. Then observations begin to be made after 24 hours for 28 days or until all are degraded with an interval of 24 hours or 72 hours or adjust to the speed of degradation. The percent weight loss of the polymer can be calculated using the Equation:

$$\text{Biodegradability} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100\%$$

3. RESULT AND DISCUSSION

3.1 Synthesis of Nata de Coco

The synthesis of nata de coco is carried out through a fermentation process using coconut water media with the help of a starter from *Acetobacter xylinum* bacteria. Nata de coco has the characteristics of white, gel-shaped, chewy texture, high fiber content, cholesterol-free, and low in calories. *A. xylinum* bacteria produce nata when grown in media containing carbon and nitrogen, and are able to thrive in acidic environments. To prevent contamination and growth of other bacteria, fermentation is carried out in a sterile medium. Because *A. xylinum* bacteria are aerobic, the fermentation process is carried out using a hollow cover to keep oxygen available.

A. xylinum bacteria secrete extracellular enzymes that convert sugar into cellulose. *A. xylinum* cells take up glucose from a sugar solution, which then combines with fatty acids to form precursors on the cell membrane. These precursors undergo polymerization and bind to acceptors to form cellulose. These bacteria also produce extracellular enzymes that organize sugary substances into thousands of fiber chains or cellulose. With the growth of millions of bacteria in coconut water, millions of sheets of cellulose threads appear dense, white to transparent, known as nata. The image of nata de coco synthesis can be seen in Figure 1.



Figure 1. Synthesis of *nata de coco*

3.2 Synthesis of Zn Nanoparticles-Moringa Leaf Extract

The content of secondary metabolites in plants can act as a reducing agent in the process of reducing Zn metal nanoparticles. The synthesis of zinc nanoparticles (ZnNP) with Moringa leaf extract bioreducing agents was synthesized using the reduction method by reacting $\text{Zn}(\text{NO}_3)_2$ 0.1 M solution as a precursor and Moringa leaf extract as a bioreducing agent by varying the Moringa leaf extract in the sample code ZEK05, ZEK10, ZEK15, ZEK20, ZEK25 and ZEK30. Moringa leaf extract contains flavonoid compounds that act as reducing and stabilizing agents in the reaction system. Flavonoid compounds have hydroxyl groups (OH) that reduce Zn^{2+} ions to Zn^0 [16].

Bioreducing compounds in addition to functioning as metal ion reducers also function as *capping agents* that will interact electrostatically to form covalent covalent covalent bonds enveloping the nanoparticles so that agglomeration does not occur. *Capping agent* is a substance that plays a role in stabilizing *zinc* nanoparticles that have been synthesized from an agglomeration process as a phenomenon of Zn nanoparticle size growth caused by the attraction between nanoparticles. Indicators of the formation of Zn nanoparticles can be characterized by color changes. The color formed from the synthesis process of Zn nanoparticles comes from oxidized flavonoid compounds. The more concentrated the color produced shows that more flavonoid compounds are oxidized and the more Zn^{2+} is reduced to Zn^0 , so that more Zn nanoparticles are formed.

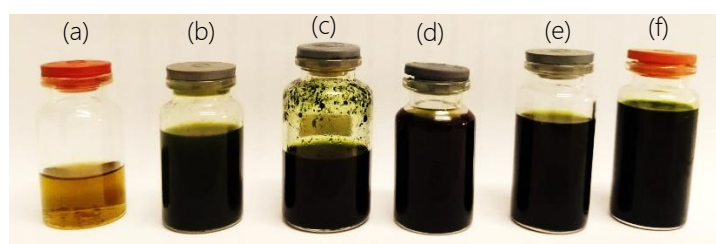


Figure 2 Reaction products with volume ratio of precursors and bioreducing agents a) ZEK05, b) ZEK10, c) ZEK15, d) ZEK20, e) ZEK25

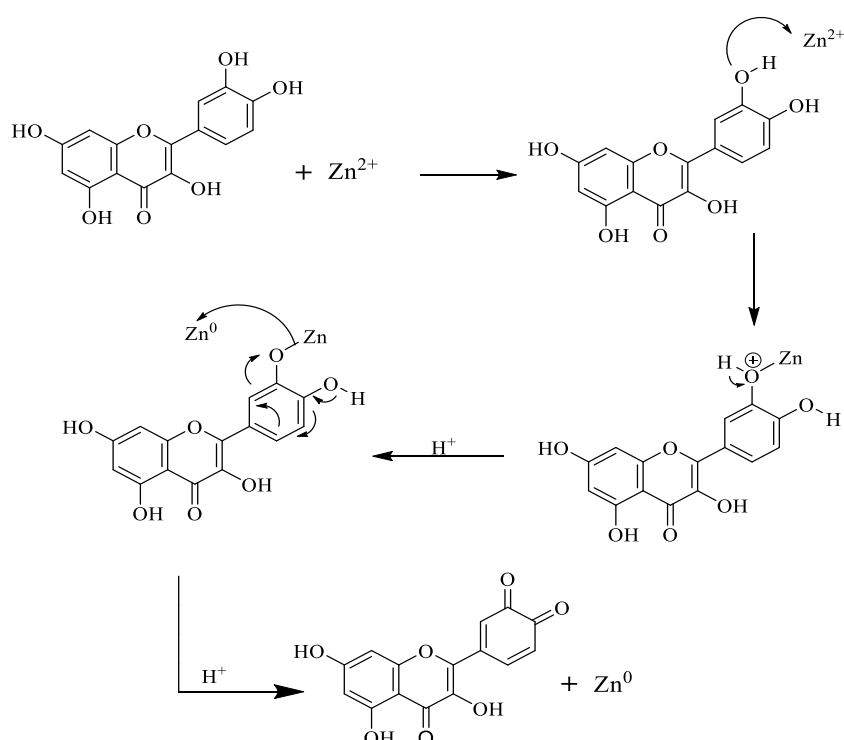


Figure 3 Proposed nanoparticle reaction mechanism.

3.3 Making Biodegradable Plastic Inserting Zn Nanoparticles-Moringa Leaf Extract

Cellulose is a biopolymer that can be produced from agricultural products. Agriculturally produced biopolymers have thermoplastic properties, which allow them to be molded or molded into bioplastics. Cellulose from nata de coco that has undergone fermentation is cleaned with running water, then pressed using a hydraulic power press at 100°C to remove moisture content in cellulose fibers. A thin film of nata de coco is then soaked in a 3% glycerol solution for three days. The addition of glycerol serves as a *plasticizer* that can impart physical and mechanical properties to the film. *Plasticizers* are non-volatile materials, can change the dimensional structure of objects, lower protein chain bonds, and fill empty spaces in products. Films made of proteins and polysaccharides tend to be brittle, so they require *plasticizers* to increase their elasticity. Plasticizer molecules reduce the binding power of protein chains, increase elasticity, and flexibility of film materials. The more glycerol used the more parts of the bioplastic will be degraded. However, excessive addition of glycerol can reduce the physical properties of bioplastics [17]. The thin film of nata de coco that has been soaked in glycerol is then re-soaked in a solution of Moringa leaf extract Zn-nanoparticles to ensure the composition of Moringa leaf extract Zn-nanoparticles into nata de coco film. Next, the biodegradable plastic film is dried at room temperature for 24 hours. Visual differences between biodegradable plastic film membranes from each comparison of Moringa leaf extract Zn-nanoparticles can be observed in the attached Figure.

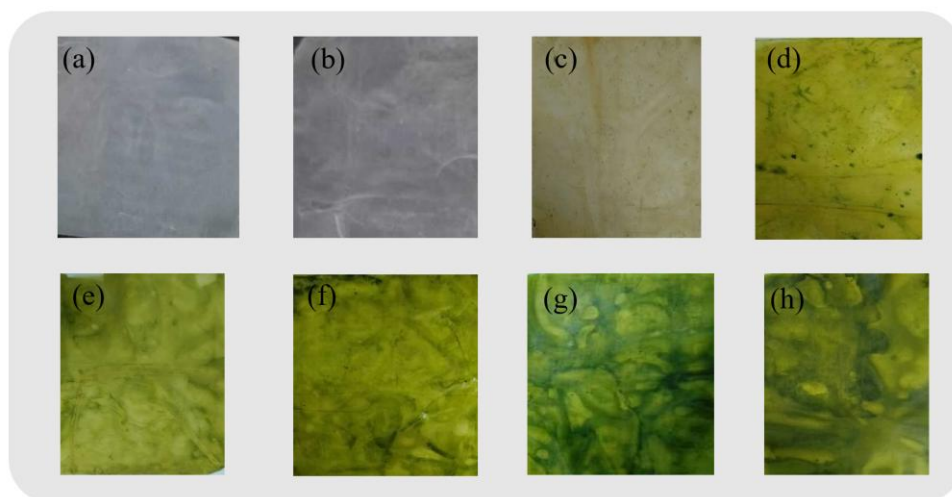


Figure 4 Biodegradable plastic film inserted Zn nanoparticles – basil leaf extract (a) Nata de Coco film before reacting, (b) Nata de Coco film + glycerol, (c) ZEK05, (d) ZEK10, (e) ZEK15, (f) ZEK20, (g) ZEK25, (h) ZEK30

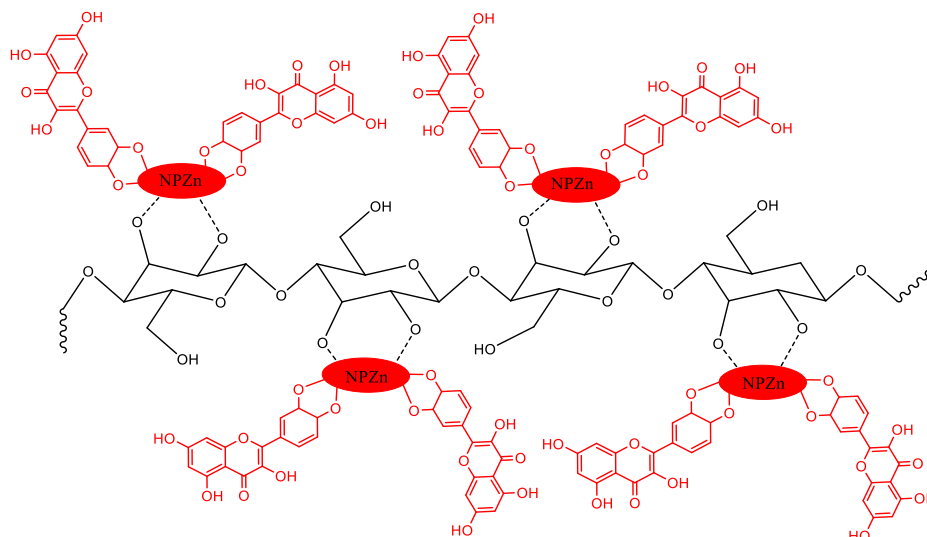


Figure 5 Illustration of the interaction of cellulose and Zn-nanoparticles of Moringa leaf extract

A schematic illustration of Zn nanoparticles inserted into bacterial cellulose is shown in Figure 4, when the bacterial cellulose membrane is immersed in a zinc nanoparticle solution, Nata de Coco pores can allow Zn^{2+} guest molecules to seep into the three-dimensional network structure until an adsorption equilibrium is formed. So zinc ions combine at a stable absorption site through electrostatic interaction between Zn^{2+} and OH groups in bacterial cellulose fibers, because oxygen atoms have free electron pairs that can bind Zn^{2+} through electron pair sharing [18].

3.4 Characterization

3.4.1 Functional Group Analysis Using Fourier Transform Infrared (FTIR)

Functional group analysis using FTIR was carried out to determine the functional groups in the biodegradable plastic constituent compounds produced, Nata de Coco cellulose, Moringa leaf extract, and Zn-Moringa leaf extract nanoparticles. The results of FTIR analysis are shown in Figure 6. Based on Figure 6, it can be seen that there is a shift in several functional groups in *biodegradable* plastics with the addition of ZnNP-Moringa leaf extract. Such a shift indicates a polymerization reaction of *biodegradable* plastic materials during blending and indicates that the interaction that occurs is a physical interaction.

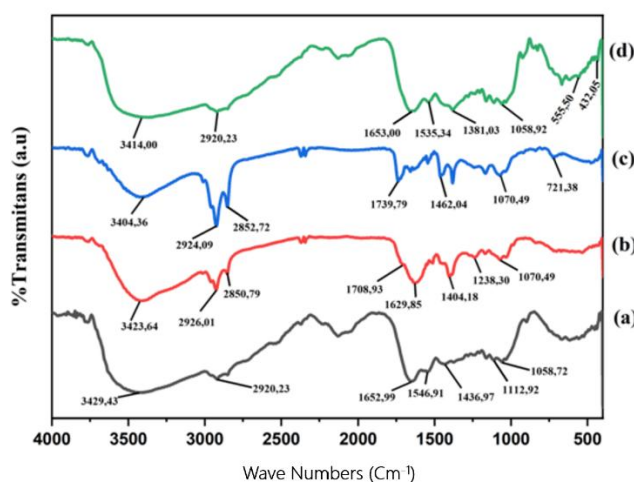


Figure 6. FTIR Spectrum (a) Nata De Coco Cellulose, (b) Moringa Leaf Extract (c) ZnNP-Moringa Leaf Extract (d) Biodegradable plastic film-ZnNPs

Figure 6(a) shows that the characteristic absorption peak of nata de coco cellulose shows the presence of an -OH group at wavenumber 3429.43 cm^{-1} and a C-O group at wavenumber 1058.72 cm^{-1} . Uptake from this group indicates the presence of glycoside bonds and C-O bonds in the cellulose ring. The absorption peak at wavenumber 2920.23 cm^{-1} indicates the presence of a C-H group, while absorption at wavenumber 1652.99 cm^{-1} indicates the presence of a C=O group. Wavenumber 1546.91 cm^{-1} , absorption is seen indicating the presence of a C=C group, and the CH_2 group is indicated by absorption at wavenumber 1436.97 cm^{-1} [19].

Figure 6(b) shows the presence of a bound -OH group at wavenumber 3423.64 cm^{-1} , while at wavenumbers 2926.01 cm^{-1} and 2850.79 cm^{-1} shows the aliphatic C-H vibrational band. Then the wavenumber 1708.93 cm^{-1} indicates the presence of a C=O group while the wavenumbers 1629.85 cm^{-1} and 1404.18 cm^{-1} indicate an aromatic C=C group, and in the wavenumber region 1238.30 cm^{-1} and 1070.49 cm^{-1} is the C-O group of alcohol. Based on the basic structure of flavonoid types among khalkon and flavonol compounds, only khalkon has C-H bonds on aliphatic chains while flavonols do not have aliphatic chains. Thus, based on the results of FTIR spectra identification, isolates from Moringa leaf extract contain flavonoid compounds that refer to khalkon type flavonoids [20].

Figure 6(c) is the FTIR spectrum of Moringa leaf extract Zn nanoparticles. The results of the analysis showed a shift in the spectral wavenumber of Moringa leaf extract before and after reduction, at absorption of 3404.36 cm^{-1} showed the OH group where there was a shift in wavenumber from 3423.64 cm^{-1} . Absorptions of 2924.09 cm^{-1} and

2852.72 cm^{-1} indicate aliphatic C-H groups. The wavenumber 1739.79 cm^{-1} is the vibration of the C=O group which has shifted the wavenumber from 1709.93 cm^{-1} . Then the vibration of the C=C group shifted the wavenumber from 1404.18 cm^{-1} to 1462.04 cm^{-1} . while the C-O group of alcohol did not shift the wavenumber, which is 1070.49 cm^{-1} . The wavenumber shift shows that there is an interaction between the OH group and Zn^{2+} ions, due to the Zn nanoparticle reduction process. This proves that Moringa leaf extract is able to reduce Zn^{2+} to Zn^0 .

Figure 6(d) shows the FTIR spectra of biodegradable plastic cellulose nata de coco-ZnNP-moringa leaf extract. The wavenumber absorption shift in the OH group that occurs at wavenumber 3414.00 cm^{-1} peaks widen due to the addition of glycerol. Peak absorption widening also occurs in aliphatic C-H groups, namely at wavenumber 2920.23 cm^{-1} . The missed peak widening indicates that each component with its functional groups interacts well. The results of functional group identification also showed ZnNP absorption, namely at wavenumbers 555.50 cm^{-1} and 432.05 cm^{-1} [21]. It can be concluded that the bioplastics produced still have properties such as their constituent components, namely plastic, easily decomposed or biodegradable.

3.4.2 Analysis of Stability and Size of Zn Nanoparticles Using UV-Vis Spectrophotometer

The formation of Zn nanoparticles can be known by the color change that occurs in the solution. The formation of Zn nanoparticles is not only seen from the change in the color of the solution but can also be seen from the appearance of the maximum wavelength (λ_{max}). Analysis of Zn nanoparticle formation was performed using a UV-Vis spectrophotometer in the wavelength range of 200-500 nm [22]. The shift of absorption peaks to higher wavelengths or commonly referred to as hypsochromic shifts is a phenomenon that occurs due to the addition of bio-reducing volume resulting in greater absorbance. the higher the absorbance value of synthesized colloids indicates an increasing concentration of ZnNPs formed every increase in time [23]. This suggests that the ability of a bio-reducing agent to reduce Zn^{2+} will differ based on its volume. The results of the UV-Vis spectrum are shown in Figure 7.

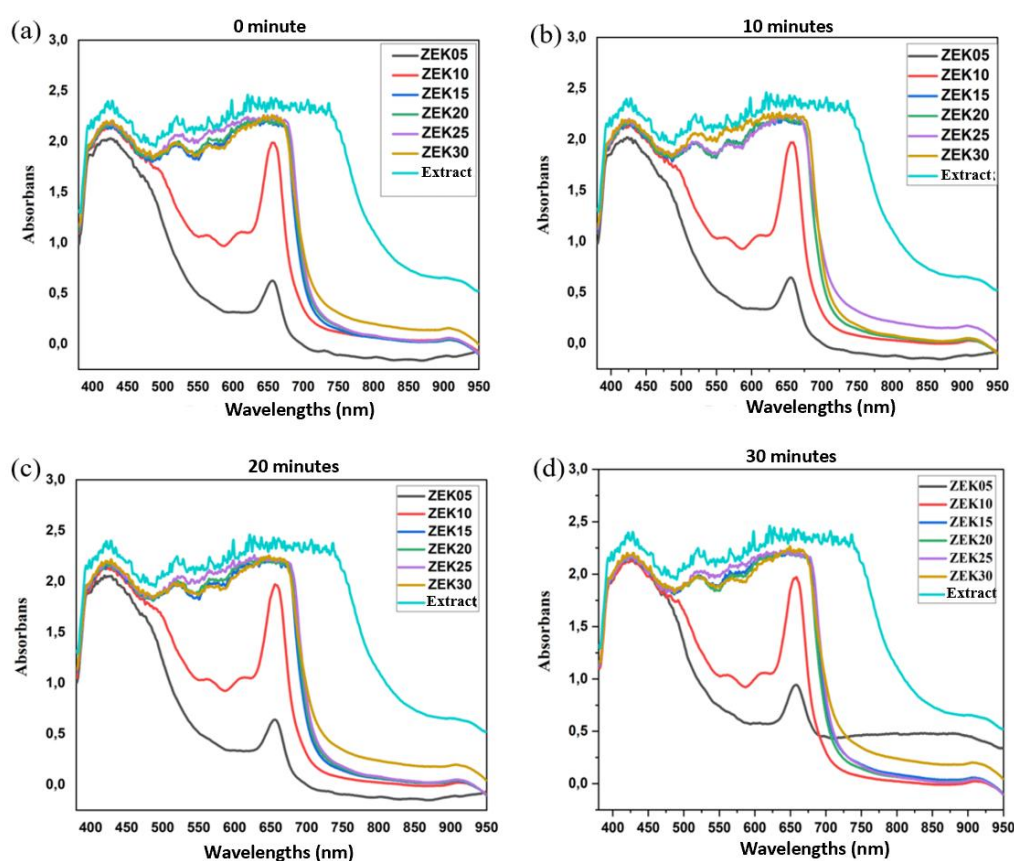


Figure 7. absorption spectrophotometer UV-Vis Moringa leaf extract and NPZn-Moringa leaf extract

The stability of Zn-Moringa leaf extract nanoparticles can be known through the peak of SPR absorption in the time span of 0, 10, 20 and 30 minutes. The results showed that at the peak of SPR uptake, Zn nanoparticles did not experience significant changes for 30 minutes, indicating that the Zn nanoparticles produced had good stability. The addition of the volume of Moringa leaf extract bio-reducing agent shows a slight shift in wavelength.

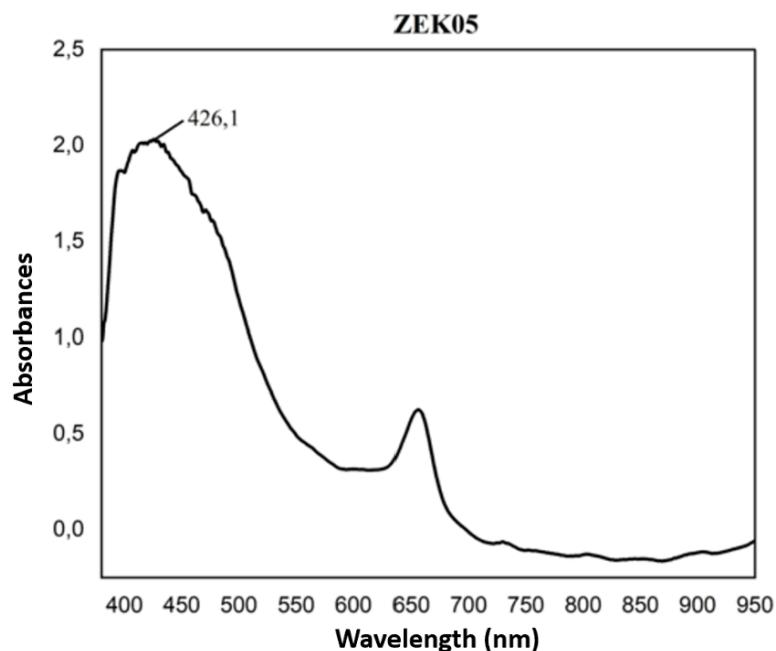


Figure 8. Example of UV-Vis Spectrum Illustration for Particle Size Estimation of NPZn-Moringa Leaf Extract

The measurement results of the UV-Vis spectrophotometer from colloidal Zn-Moringa leaf extract produce two parameters, namely wavelength and absorbance (λ , A). Both parameters can be used to measure the diameter of synthesized ZnNPs using absorption values at λ_{max} . The maximum wavelength is related to the particle size where the larger the nanoparticle size, the higher the wavelength produced because the excitation energy is getting smaller with the smaller the distance traveled by electrons to excite from ground state to excitation state is getting smaller [24]. Figure 4.8 shows that colloidal Zn nanoparticles have a maximum absorption peak (λ) at a wavelength of 422.1 nm indicating the typical wavelength of zinc nanoparticles. A high peak size indicates an increasing amount of Zn formed. The results of particle size estimation in each sample can be seen in Table 1.

Table 1. Results of particle size estimation of Zn-Moringa leaf extract nanoparticles

Nanoparticle composition	λ_{spr} (nm)	λ_0 (nm)	L1	L2	D (nm)
ZEK05	422.1	380.5	6.53	0.0216	85.72
ZEK10	426.1	380.5	6.53	0.0216	89.97
ZEK15	429.1	380.5	6.53	0.0216	92.92
ZEK20	429.1	380.5	6.53	0.0216	92.92
ZEK25	429.1	380.5	6.53	0.0216	92.92
ZEK30	429.1	380.5	6.53	0.0216	92.92

Based on Table 1. It can be seen that the particle size of Zn-Moringa leaf extract nanoparticles obtained varies between 85.72-92.92 nm. The size of the Zn-Moringa leaf extract nanoparticles obtained meets the nanoparticle size criteria, which ranges from 1-100 nm.

3.4.3 Morphological Analysis Using Digital Optical Microscope

Analysis using digital optical microscopy aims to identify the morphology of bacterial cellulose, synthetic zinc nanoparticles, and biodegradable plastic film inserted Zn-Moringa leaf extract nanoparticles. Morphological imaging is carried out using a digital microscope with a magnification of 1000x. The capture results are then processed using the *Image J* application to produce clearer images. The results of the analysis obtained are presented in Figure 9.

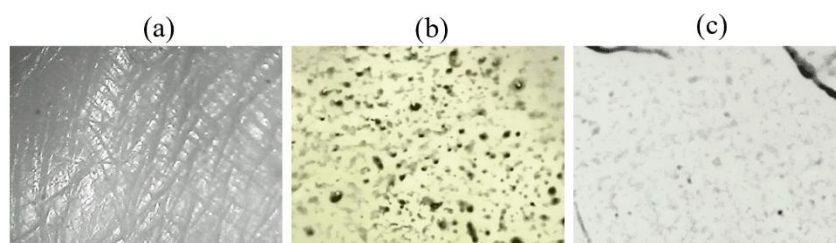


Figure 9. Cintra optical microscope (a) Cellulose Nata de Coco Bacteria, (b) Zinc nanoparticle powder Moringa leaf extract, (C) biodegradable plastic film inserted ZnNP nanoparticle – Moringa leaf extract

Figure 9(a) shows that the cellulose surface of Nata de Coco bacteria has a fine fibrous structure, dense and without cracks. In figure 9(b) the morphology of Zn nanoparticles scattered and different particle sizes show that Zn nanoparticles have a shape that appears spherical and not uniform. Figure 9(c) shows the presence of Zn nanoparticles dispersed and deposited into Nata de Coco fibers. This is seen by the presence of coarse wrinkles in the form of small particles scattered onto the cellulose surface of Nata de Coco bacteria.

3.5 Biodegradable Plastic Test

3.5.1 Mechanical Test

Mechanical properties analysis is carried out to see the ability of a material or component to receive maximum load or tensile force without any damage to the material. Mechanical properties were tested on nata de coco and nata de coco composite film Zn nanoparticles – Moringa leaf extract. The analysis of mechanical properties tested in this study is tensile strength, elongation and modulus of young biodegradable plastics. The results of mechanical test measurements can be seen in Table 2.

Table 2 Biodegradable Plastic Mechanical Test Results

Membrane	Stress σ (MPa)	Strain ϵ (%)	Young' s Modulus E (MPa)
<i>Nata de Coco</i> Films	23,81	1,8	13,23
<i>Biodegradable plastic film</i> inserted Zn nanoparticles-Moringa leaf extract	52,12	4,06	12,84

Based on Table 2, an increase in tensile strength of 52.12 MPa occurred with the addition of Zn nanoparticles to Moringa leaf extract, which exceeded the tensile strength of nata de coco film by 23.81 MPa. The addition of these nanoparticles has an impact on the mechanical properties of such composites. Nanocomposite materials play a crucial role in improving and limiting material properties. The nanoparticles fill in the blanks and interact with the nata de coco cellulose film. The more interactions between particles, the stronger the bonds between particles, so the mechanical properties of the material are further improved [25].

Elongation is tested by comparing the increase in length with the initial length of the material before the tensile test is performed. From Table 2, it can be concluded that the percentage of elongation of *biodegradable* plastic films is greater at 4.06% compared to nata de coco films at 1.8%. One factor affecting elongation is the addition of glycerol

to nata de coco films. Glycerol acts as a plasticizer to increase elasticity by reducing the degree of hydrogen bonding and increasing the distance between polymer molecules. Glycerol enters the intermolecular bonds of cellulose, increasing flexibility, and the percentage of film elongation [26].

Young's modulus is directly proportional to tensile strength and inversely proportional to elongation. Based on data from Table 2, the elasticity values of *biodegradable* plastic film and nata de coco film are 12.84 MPa and 13.23 MPa. *Biodegradable* plastic films have *almost the same young modulus* as cellulose films. This is influenced by the growth of *A. xylinum* bacteria and the addition of glycerol. Glycerol can decrease the value of *young's modulus* or film stiffness, according to the purpose of using plasticizers to make elastic films. Plastic films with a high *young modulus* are rigid and strong, suitable for resisting external damage [27].

3.5.2 Water Resistance Test

Water resistance tests are carried out to determine the level of water resistance in the biodegradable plastic produced. Biodegradable plastics are expected to be resistant to water or in other words less water absorbed by biodegradable plastics. Here is a graph showing the water resistance value of biodegradable plastic film:

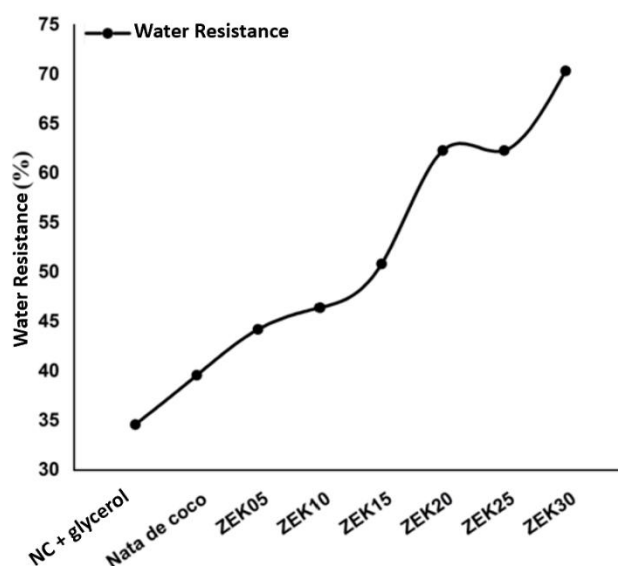


Figure 10 Percentage of Water Resistance in Each Sample

Figure 10 showed the highest water resistance value found in the nanoparticle composition in the ZEK30 sample code with a water resistance value of 70.37% while the lowest water resistance value was nata de coco film reacted with glycerol plasticizer without the addition of ZnNPs of 34.62%. Based on the results of the water resistance test, it can be seen that the water resistance content of biodegradable plastic films is directly proportional to the addition of ZnNPs. The higher the ratio of ZnNPs, the higher the water resistance value of biodegradable plastic films. This is because the more ZnNPs boosters added, the higher the water resistance of the biodegradable plastic.

The value of water resistance can be influenced by the addition of glycerol as a plasticizer that has hydrophilic properties so that it can increase the flexibility of plastic but increase the amount of empty space to be occupied by water molecules. The cellulose content of nata de coco and the addition of ZnNPs nanoparticles can affect the resulting polymer matrix. The high cellulose content gives the film a high density, as its structure is linear whereas the addition of ZnNPs nanoparticles can fill the empty space on the film. The more uniform the constituent components of a film matrix, the more homogeneous and dense the surface of the film. The denser a biodegradable plastic is, the lower the water absorption, and vice versa [28]. The results showed that the percentage of water resistance was not entirely good because the biodegradable plastic film produced still did not meet the Indonesian National Standard

(SNI 7188.7: 2 016), water resistance or hydrophobicity for biodegradable plastic was 99%. While in this study, biodegradable plastic with the best water resistance in ZEK30 samples was 70.37%.

3.5.3 Antibacterial Activity Test

Antibacterial testing of biodegradable plastics inserted in Zn-extract nanoparticles of Moringa leaves was carried out using the disc method. The bacteria used are *S. aureus* for Gram positive and *E. coli* for Gram negative. The presence of antibacterial activity is characterized by the appearance of a clear zone around the disc after incubating for 24 hours. The larger the diameter of the clear zone formed, the better the antibacterial activity of a substance [29]. The results of the antibacterial activity test are shown as in Figure 11.

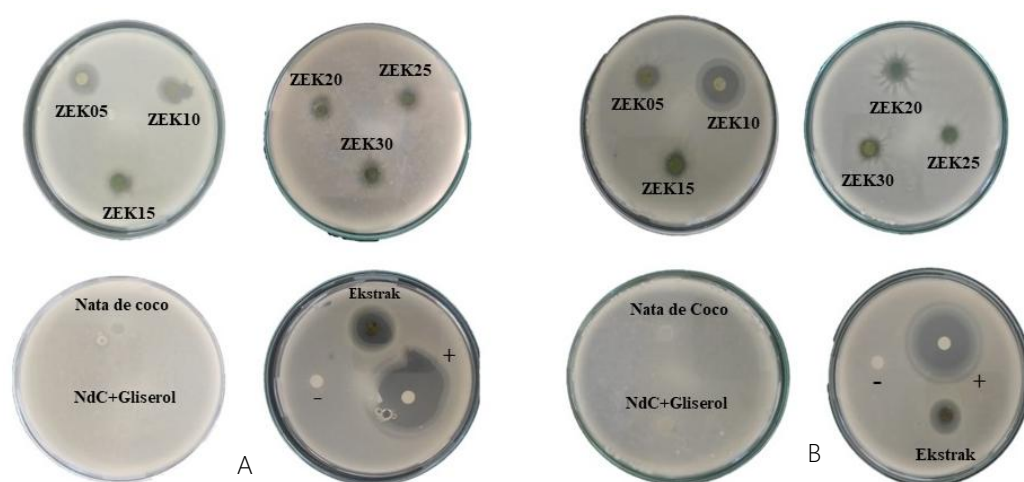


Figure 11 Antibacterial Test Results (a) *S. aureus* and (b) *E. coli* on Samples ZEK05, ZEK10, ZEK15, ZEK20, ZEK25, ZEK30, Nata de Coco, Nata de Coco-Glycerol and Moringa Leaf Extract

Based on Figure 11, it can be seen that the antibacterial activity of ZnNPs nanoparticles is characterized by the presence of clear zones around the disc paper. Differences in particle size can cause differences in antibacterial activity, where Zn particles with nanometer size are proven to have good antibacterial activity. This is in accordance with research conducted by Prasetyaningtyas *et al.* (2020) that antibacterial nanoparticles will increase when their size gets smaller. The smaller the particle size of ZnNPs, the larger the surface area and increased contact with bacteria or fungi. The concentration, shape and size of nanoparticles as well as the time of contact with bacteria are factors that influence the antibacterial activity of nanoparticles. The diameter of the clear zone can be seen in Table 3.

Table 3 Clear zone measurement results

Test Bacteria	Test Parameters	Clear Zone Diameter (mm)	Information
<i>S. aureus</i>	ZEK05	13,45	Strong
	ZEK10	12,03	Strong
	ZEK15	11	Strong
	ZEK20	10,02	Strong
	ZEK25	8	Keep
	ZEK30	7,33	Keep
	Moringa leaf extract	13,67	Strong
	Nata de Coco	-	-
	Nata de Coco-glycerol	-	-
	<i>Streptomycin</i> (+)	22,08	Very powerful
	Aquades (-)	-	-

<i>E. coli</i>	ZEK05	11,33	Strong
	ZEK10	10,02	Strong
	ZEK15	8,05	Keep
	ZEK20	7,38	Keep
	ZEK25	7,08	Keep
	ZEK30	6,48	Keep
	Moringa leaf extract	13,67	Strong
	Nata de Coco	-	-
	Nata de Coco-glycerol	-	-
	<i>Streptomycin</i> (+)	30	Very powerful
	Aquades	-	-

Based on Table 3, the diameter of the inhibitory zone against *S. aureus* bacteria shows a larger size compared to *E. coli*. This difference indicates that ZnNPs nanoparticles are more effective in inhibiting the growth of *S. aureus* compared to *E. coli*. The reason behind this different effectiveness can be attributed to the different cell wall structure between the two types of bacteria. Gram-positive bacteria such as *S. aureus* are more susceptible to the influence of ZnNPs nanoparticles when compared to Gram-negative bacteria. Gram positive bacteria have one plasma membrane with a thick cell wall consisting of peptidoglycan layer, while Gram negative bacteria have a more complex cell wall structure with a peptidoglycan layer between the outer membrane and plasma membrane [30].

Antibacterial activity tests also showed that nata de coco films, both before and after reaction with glycerol, showed no clear zones around the disc paper. This indicates that the film has no antibacterial activity effect against biodegradable plastics, while Moringa leaf extract shows a fairly strong inhibitory zone. These results confirm that Moringa leaf extract plays a role in antibacterial activity. The negative controls used in the test also showed that sterile aquades had no effect on antibacterial activity. The use of negative control aims to ensure that the diameter of the inhibitory zone formed is the result of the active compounds of *S. aureus* and *E. coli* bacterial isolates rather than being affected by the solvent used.

3.5.4 Preservation Test

Packaging aims to simplify the distribution process, protect commodities against mechanical damage (shock, pressure and friction) and microbial infections and dust. Biodegradable plastic film preservation tests are carried out to determine the ability of *biodegradable* plastic films to protect a product. The preservation test was carried out by wrapping the tip banana fruit (*Musa paradisiaca Forma Typica*) using biodegradable plastic film-ZnNPs ZEK05, ZEK10, ZEK15, ZEK20, ZEK25, ZEK30 and nata de coco film and without packaging with a storage period of 8 days at room temperature. Once every 2 days observed changes in texture and color in bananas. The results of the preservation test can be seen in Table 4.

The success rate of preservation tests is positively correlated with the antimicrobial properties possessed by *bio-degradable* plastics. The stronger the antimicrobial properties possessed, the more effectively the plastic protects the product. The data in Table 4 show the rapid color change of bananas during storage. Color is a crucial parameter observed by consumers in food products, so it is a very important factor. Brown discoloration in bananas can be caused by the process of respiration and the potential for *chilling injury* during storage. The use of *biodegradable plastics* with ZnNPs nanoparticles slows down the discoloration of bananas, in contrast to nata de coco films without nanoparticles and bananas without packaging.

Table 4. Preservation Test Results

Day 1-	Comparison	Color	Texture	Mold growth
0	ZEK05	Green	Hard	Not
2		Greenish-yellow	hard	Not
4		Yellow	A little hard	Not
6		Brownish-yellow	A little mushy	Not
8		Blackish yellow	Soft	Not
0	No straining	Green	Hard	Not
2		Yellow	A little hard	Not
4		Blackish yellow	Soft	Not
6		Predominantly black	Soft	Little
8		Black	Soft	Moldy

The texture test results showed that on the 3rd day, bananas without packaging and packaging without nanoparticles experienced softness in some parts, while on the 6th day, the texture of bananas was soft and showed signs of fungal growth. By the 8th day, the banana fruit has rotted and molded. Meanwhile, bananas packed with ZEK20, ZEK25, and ZEK30 biodegradable plastic films began to undergo texture changes on day 6, and by day 8, the texture of bananas had softened with signs of rot and mold. Bananas packed with ZEK05, ZEK10, and ZEK15 biodegradable plastic films underwent texture changes on days 7 and 8, but showed no signs of fungal growth.

3.5.5 Biodegradation Test

Biodegradation tests are carried out through an aerobic biological treatment process using activated sludge as a medium. The selection of activated sludge as a medium is based on the concept that, in handling plastic waste by the method of disposal to landfills in landfills, plastic waste will not pose a threat to aquatic ecosystems. This is because the main problem arises when plastic waste is in the waters, which can then move passively and create environmental problems. The creation of this media involves taking muddy soil from the sewers of Musholla FMIPA Halu Oleo University. Glucose, KNO_3 , and KH_2PO_4 are added to activated sludge tanks as sources of carbon, nitrogen and phosphorus, then allowed to stand for 1-2 days to give microbes an adaptation time before entering an accelerated growth phase. The *biodegradable* plastic is then fed into activated sludge tanks during the growth phase, and changes are observed. Mass reduction was measured every 3 days for 28 days, with a *biodegradable* plastic sample size of 3×3 cm.

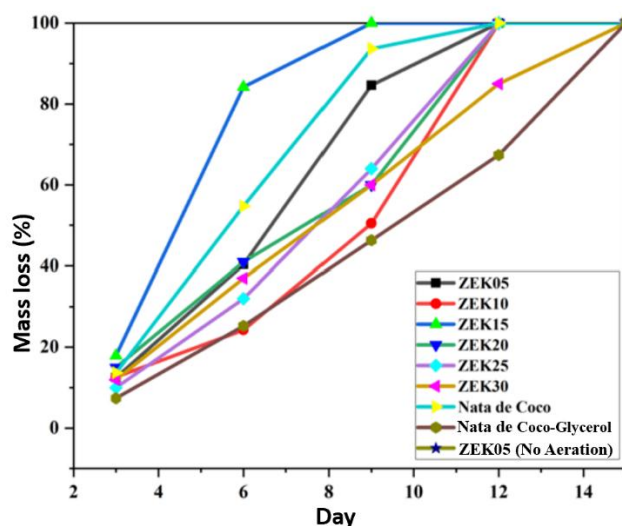


Figure 12. Difference (%) mass loss of biodegradable plastics

The results of the biodegradation test are directly proportional to the value of water resistance, where the greater the value of water resistance, the more difficult it will be to degrade. This is due to the difficulty of water absorption in plastics that have high water resistance values. Figure 12 shows an increase in the percentage of mass loss of *biodegradable* plastics every 3 days during the degradation process. The higher the percentage of mass loss on the film, the better the biodegradability rate. The results showed that ZEK15 samples achieved the highest mass loss percentage of 100% on day 7, while ZEK30, nata-glycerol, and ZEK05 samples without aeration had the lowest mass loss percentages, namely 23%, 20%, and 65% on day 13. ZEK05 samples and nata de coco films were completely degraded on day 10 with a mass loss of 100%, while samples of ZEK10, ZEK20, and ZEK25 achieved 100% mass loss on day 12. Research has also shown that biodegradable plastic films degrade faster with the help of aeration than without aeration. This is due to the greater number of aerobic bacteria in the presence of aeration. Table 5 provides information on the magnitude of mass loss in biodegradable plastics during the degradation process.

Table 5. Results of mass loss of biodegradable plastic for 15 days

Sample	Mass loss (%)				
	Day 1-				
	3	6	9	12	15
ZEK05	12,5	40,38	84,62	100	100
ZEK10	12,62	24,27	50,46	100	100
ZEK15	17,89	84,21	100	100	100
ZEK20	15	41	60	100	100
ZEK25	10	32	64	100	100
ZEK30	12	37	60	85	100
Nata de Coco	13,68	54,74	93,68	100	100
Nata de Coco-Glycerol	7,36	25,26	46,32	67,36	100
ZEK05 (No aeration)	22,64	31,13	33,96	43,4	58,49

The longer the incubation time of the colony of microorganisms, the number of degrading bacteria produced will increase. Direct observation in biodegradation tests shows damage to *biodegradable* plastics manifested by discoloration to blackness and erosion of the film surface. This damage is caused by the action of microorganisms that attack *biodegradable plastic film molecules*. The degrading bacteria of activated sludge secrete cellulase enzymes, produced by the hydrolytic system of microorganisms, capable of breaking β -glycosidic bonds in nata de coco cellulose. The enzyme results in the erosion of the surface of the biodegradable plastic through the process of hydrolysis, resulting in a decrease in the weight of the biodegradable plastic. The aeration process also affects the decomposition of biodegradable plastics. Aeration creates air bubbles that supply oxygen and mixes microorganisms, substrates, and nutrients in the tank, resulting in stirring that allows collisions between all particles. Aeration time and stirring strength affect the performance of activated sludge, so balancing oxygen demand with oxygen supply is important.

The decomposing ability of biodegradable plastics is also affected by the use of cellulose and glycerol containing -OH groups, which can undergo a hydrolysis reaction after absorbing water from activated sludge media. The hydrophilic nature accelerates water absorption, allowing microorganisms to degrade biodegradable plastic samples faster. The addition of ZnNPs in the biodegradation process does not affect the course of the process, because it has acted as a cofactor or micronutrient for degrading bacteria. This is due to the interaction that has been established between biodegradable plastics and bacteria or fungi during the preservation process, so that the antibacterial activity of ZnNPs nanoparticles is reduced due to saturated or stable conditions. ZnNPs only serve as cofactors or micronutrients for

cellulose-degrading bacteria from plastics in activated sludge, facilitating the degradation of cellulose plastics through hydrolysis of glycosidic bonds in polyglucose.

4. CONCLUSION

The characterization results showed that each biodegradable plastic component with its functional group experienced good interaction and showed that the absorbance value for 30 minutes tended to be stable and showed the estimated particle size with the smallest size was 68.28 nm. Biodegradable plastic film has a solid surface and bow which shows good film forming ability. But there are coarse wrinkles in the form of various small particles on the plastic film that show Zn nanoparticles have successfully adhered to the plastic film. Biodegradation tests on biodegradable plastic cellulose nata de coco-NPZn-Moringa leaf extract showed that biodegradable plastic is able to degrade 100% for 15 days. This shows that biodegradable plastics made from cellulose nata de coco inserted zinc nanoparticles have excellent *biodegradability*. The results of the preservation activity test showed the effectiveness of nata de coco-NPZn-Moringa leaf extract biodegradable plastic preservation which was quite good in ZEK05 samples. This can be seen from the test results on the color and texture indicators of packaged bananas that are still able to last up to 8 days of storage.

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