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ASSESSING MECHANICAL PROPERTIES AND ANTIMICROBIAL ACTIVITY OF ZINC OXIDE-STARCH BIOFILM

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Abstract. Tapioca starch is able to form flexible transparent film eventhough the film was made without the use of additional chemicals. However, films made from tapioca starch still have various disadvantages in terms of functional properties. It is therefore an effort have to be done by adding chemical additif such as nanopartikel. The main objective of this study was to improve the functional properties of tapioca starch packaging by using ZnO nanoparticle as repair agents and glycerol as plasticizers. Some properties of the biofilm were analyzed including thickness, tensile strength, elongation, and water vapor permeability as well as antibacterial properties. The addition of nanoparticles was carried out in the amount of 0%, 1%, and 2% by weight of starch (w/w). While glycerol was used at 20% by weight of starch (v/w). The result of biofilm thickness can be determined the best treatment that was at 2% of ZnO where as the film had thickness value approaching JIS (Japanesse Industrial Standart) with 0,1087 mm. The highest tensile strength was reached at value of 2%, while the highest elongation was at 0% concentration. Water vapor permeability (WVP) allowed the best results at 2% concentration of ZnO with a value of 1.0146 × 10^9 gsm^-1 m^-1 Pa^-1. The results of this study indicated that tapioca starch film containing ZnO nanoparticle could be used as food packaging material with antibacterial properties. The inhibition index of the film was 7.67 mm from the film with 2% ZnO to bacteri of E. coli greater than film with 1% ZnO about 5.40 mm. Meanwhile, for Salmonella sp., after 24 h incubation, the film either 1% and 2% ZnO nanoparticle exhibited 6, 97 mm of clear zone of diameter.

Keywords: tapioka starch, ZnO, antibacterial, biofilm

Introduction
Increased use of plastic packaging from petrochemical-based synthetic polymer feedstocks has resulted in an increase in environmental pollution from plastic waste, because the plastic material is difficult to be degraded by the environment, either by solar radiation or by microbial decomposers. Various efforts have
been made to overcome these problems, some of them are considered less effective, including recycling which resulted lower quality than the original plastic. It is also difficult to separate plastic waste from various types of basic polymers. Another alternative, that is more studied lately, is the synthesis of biodegradable plastics. The synthesis of biodegradable plastics which is very effective and efficient, by means of blending various natural polymers. Natural polymers such as starch, chitin/chitosan and cellulose are potential sources for bioplastics. Basically, all natural polymers are biodegradable, but it have relatively low mechanical properties, fragile and easily damaged by thermal influences. Through the addition of plasticizers, natural polymers such as starch can be enhanced its mechanical strength [1]. In addition to the advantages of biodegradation, bioplastik can also be added antimicrobial (antibacterial) material to be able to avoid pathogenic bacteria on the food package that can interfere with human health.

Environmentally friendly packaging materials (biodegradable packaging) recently continues to be developed such as biofilm. Biofilm is a plastic made from materials that can be decomposed by organism. This biofilm could be corporated with active agent due to some purpose, known as antimicrobial packaging. Antimicrobial packaging is a package that can stop, inhibit, reduce or slow the growth of pathogenic microorganisms in food and packaging materials, thus this biofilm can potentially prevent pathogen contamination in various food ingredients. The ability of antimicrobials film packaging to control the growth of microbes in food, further, could prolong the shelf life and improve the quality of food [2]. Active agent incorporated into film could be in nano form. The nanometre scale of active agent will provide many opportunities, such as having deodorizing and antibacterial properties at finer sizes, but not on bulk particles or larger [3]. The nanoparticles can directly interact with microbial cells, for example interfering with trans-membrane electron transfer, interrupting and penetrating cell membranes, oxidation of cell components, producing secondary products such as reactive oxygen species and dissolved heavy metal ions. Among the nano active antimicrobial agent, ZnO had been wide applied due to its unique properties such as photo-catalytic properties, anti-UV properties, antibacterial properties, semiconductor properties as well as safe for humans [4].

The nanoZnO antimicrobial agent has a positive zeta potential that allows interaction with surface materials such as cellulose and other biopolymers so that it can be used to increase the mechanical strength of the biomaterials [5]. In addition, zinc oxide can be consumed and has no potential to pollute the environment. The advantages of ZnO for example are the low price, the abundant supply in nature, the chemical structure is stable, and non-toxic [6]. This research was aimed to asses the characteristics of ZnO-antibacterial biofilms and to measure the ability of ZnO in inhibiting the growth of pathogenic bacteria.

**Materials and Methods**

**Materials and apparatus**

Materials used in this study were tapioca starch and inorganic nanoparticles ZnO. The chemical used in the manufacture of film was glycerol as plasticizer. While the materials used for the antibacterial activity test, namely Nutrient Agar, Nutrient Broth, 70% alcohol and gram negative bacteria test culture and gram positive *Eschericia coli* and *Staphyllococcus aureus*. Equipment used in this research was film mold made of acrylic and glass ware set.

**Methods**

Biofilm was made from tapioca starch base incorporated with Zinc Oxide Nano Particle (ZnO nanoparticle) in concentration of 1 and 2% (b/v). An antimicrobial film with no active agent was also made as control. The film and then was analysed its mechanical properties including thickness, tensile strength and elongation as well as water vapour permeability. It was also measured the efficacy of the antimicrobial film to inhibit the growth of *Eschericia coli* and *Staphyllococcus aureus*.
Result and Discussions

Thickness
Film thickness is an important parameter, it will be considered in to calculate production cost. Thinner film is desired due to its less cost, as long as the film has fulfilled it mechanical properties as it is required. In addition, thickness is also affected by the height of the solution and the area of the mold [7]. The larger the volume used, in the same size of mold, the thicker films will be. The thickness of each film produced will affect the barrier and mechanical properties of the film produced. These properties are such as water vapor permeability, tensile strength and elongation. Figure 1 show that the higher the concentration of ZnO nanoparticles, the higher thickness of the biofilm will be produced. The film had a thickness in the range of 0.0833-0.1087 mm. As mention in JIS (Japanese Industrial Standard), plastic film for food packaging should has a maximum thickness of 0.25 mm, thus this film produced from this research still meet the standard.

Tensile strength and elongation
Tensile strength is the maximum stress that can be retained by the film before breaking. Good film should have tensile strength value that meets the standards. Moderate film, such as biodegradable film, should have a tensile strength 1-10 MPa [8]. The greater tensile strength indicates the stronger the film. Lower value of tensile strength indicate the more fragile the film. The data obtained from this research was presented in Figure 2. At 0% concentration of ZnO, the value obtained was 0.56 MPa, 1% concentration was 0.57 MPa, and at 2% concentration increased to 0.6 MPa. It seem that the tensile strength increased with the addition of ZnO nanoparticle concentration. Accordingly [9], ZnO nanoparticles, when it added into biofilms, could improve the mechanical properties of the film by giving a solid structure.
Elongation process is a change in maximum length during stretching until the film broke. Elongation is associated with the average flexibility of the mechanical properties of the film. Addition of ZnO has affected on the mechanical properties especially on the tensile strength, however it would be followed by decreasing the elongation. Elongation value at 0% concentration was 27.78%, 1% concentration was 22.22% and decreased to 16.67% for ZnO 2%. This change in mechanical properties is related to the interaction of ZnO with starch and also glycerol as a plasticizer. During the interaction process, the ZnO nanoparticles will give support for film tensile, hence, it will reduce elongation due to the decreasing plasticization of tapioca film[10]. Thus, this film could be categorized as moderate film with elongation value of 10-20% [8].

**Water vapor permeability**

Water vapor permeability (WVP) is a barrier property of film to prove the ability of films to retain the water vapor outside the package. The film is expected not to easily transmit water vapor from the surrounding [11]. The greater WVP value indicates that the film, in its application as packaging material, will be un-enough barrier, because moisture could penetrate into the film and it will cause the damage of the product in the packaging [12]. Addition of ZnO nanoparticles to tapioca starch films had showed a change in the value of water vapor permeability. The more ZnO concentration added into the film, the value of the permeability decreases, mean the barrier property of the film has increased.

![Figure 3. Water vapor permeability of ZnO-starch film](image)

**Film efficacy**

Antimicrobial activity was carried out by well diffusion method to evaluate the efficiency of concentration of ZnO nanoparticle in preventing the growth of pathogenic microbes. Pathogenic bacteria used in this study were *Escherichia coli* and *Salmonella sp*. Antimicrobial film is categorized as strong inhibition when it has clear zone diameter of 10-20 mm, moderate inhibition with about 5-10 mm clear zone and weak inhibition with <5 mm clear one [12]. The results showed that film with no ZnO do not have a clear zone which means it did not have antibacterial activity, while 1% ZnO gave 5.40 mm of the clear zone after 24 h of incubation, and 2% ZnO could inhibited 7.67 mm clear zone with the same period of incubation again *E. coli* (Figure 4). Furthermore, this film could inhibit *Salmonella* with moderate inhibitory as much as 6.97 mm (1% and 2% ZnO).
Conclusions

Nanoparticle of ZnO has affected the mechanical and antibacterial properties of biofilms. The highest tensile strength was achieved at 2% ZnO concentration, and from the elongation value, the film met the standard of moderate film. Higher ZnO addition has increased the efficacy of the film to inhibit pathogenic bacteria of *Escheresia coli* and *Salmonella sp.* Based on inhibitory of the diameter of clear zone, the ZnO-starch film was categorized as moderate antimicrobial film.

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