Effect of glycerol and zinc oxide addition on antibacterial activity of biodegradable bioplastics from chitosan-kepok banana peel starch

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Abstract. Bioplastic is a biopolymer plastic that can be degraded easily by microorganisms so it can be used as alternative replaced commercial plastic. This research aims to study the effects of additive (glycerol and zinc oxide) addition in the characteristic of antimicrobial activity and biodegradability bioplastic from chitosan and Kepok banana peel starch. In this research, bioplastics were synthesized by chitosan as the backbone and antimicrobial, Kepok banana peel starch as filler, glycerol as plasticizer, also ZnO as an amplifier. Bioplastics were characterized their antimicrobial activity using agar diffusion method (zone inhibition assay) and biodegradability test using microbe (EM4). The result showed the optimum composition of bioplastic is kitosan 4 - 30% starch – 5 mL glycerol – 5% ZnO gives the good antimicrobial activity towards gram positive and gram negative bacteria, and this bioplastic will be degraded within an hour and 12 min. Thus, this bioplastics may have potential to be use for food packaging by having biodegradable properties and also inhibit bacterial growth.

1. Introduction

In the last decades there has been an increasing interest in the development of renewable materials with biodegradable properties in attempt to contribute to the sustainable development and to reduce the environmental impact of non- biodegradable petroleum based plastics. Bioplastics that are made from renewable resources which are easily broken down by microorganism represent a new generation of plastics that can reduces the dependence on fossil fuels also at the same way can reduces the impact to the environment. In order to solve the problem, several studies have been reported the use of biodegradable starches from different sources to prepare films and coating with different properties. Starch is the most important polysaccharide polymer used to develop biodegradable films, as it has potential to form a continuous matrix. Starch exhibits some disadvantages such as strong hydrophilic character and poor mechanical properties as compared to conventional synthetic polymers, which make it an adequate for some packaging purposes [1, 2].

Nowadays, interest in antimicrobial polymer packaging was growing rapidly, especially in field of medicine, healthcare, and food industry. Antimicrobial polymer packaging is one of the most promising active packaging systems that used to protect surfaces from microorganism including parasites, bacteria, germs and fungi. Chitosan has been proved to be nontoxic, biodegradable, biofunctional, biocompatible and possess antimicrobial characteristics. Therefore chitosan have been
used as a packaging material for the quality preservation of a variety of food [3]. Starch based film is considered as an economical material for antimicrobial packaging when we blended it with chitosan as antimicrobial agents. Blended between chitosan with renewable and abundant agro-resource based such as polysaccharides, particularly starch can be utilized to reduce the manufacturing cost, improved moisture resistance and accelerated degradation [4].

The aim of the present work was to obtain bioplastics that have antimicrobial ability to restrain or inhibit the growth of spoilage and or pathogenic microorganisms. Blends between banana peel starch and chitosan using glycerol as a plasticizer will produce biodegradable plastics that can have antimicrobial activity towards microorganisms. The antimicrobial effect was tested on *Staphylococcus aureus* (gram positive bacteria) and *Escherichia coli* (gram negative bacteria) using zone of inhibition test on solid media.

2. Materials and Methods

2.1 Materials

Banana peel starch was isolated from white kepok banana that plenty found in Indonesia using water as a solvent extraction. Chitosan flakes with 85% de-acetylation degree were used as the backbone of bioplastics and also antimicrobial agents. Glycerol was used as plasticizer and purchased from PT. Brataco (Surabaya, Indonesia). NaOH flake technical was purchased from PT. Brataco (Surabaya, Indonesia). Glacial acetic acid was purchased from PT. Merck (Indonesia). EM4 (Effective Microorganism 4) solution containing mixed microbial cultures was purchased from PT. Songgolangit Persada (Indonesia). Deionised water was used during the experiments. Zinc oxide was used as the filler to strength the bioplastics film.

2.2. Preparation of Chitosan- Starch Bioplastic Film

Starch slurry was prepared by suspending kepok banana peel into water with ratio 1 : 2 (%w/v) for 10-12 h. Separation between slurry and filtrate was used by filtration method. Slurry was dried at 40°C for 12 h. Chitosan- starch bioplastics were prepared with 4% (w/v) chitosan and various ratio of starch (0, 10, 20, 30, 40, 50%) (w/w). Both mixtures were prepared by suspending into acetic acid glacial as the solvent, process was conducted at 55°C. Afterwards, 30 mL of glycerol was added in order to have chitosan- starch- glycerol ratio of 100 : 30 (v/v). Various additive glycerol (0, 5, 10 mL) and zinc oxide (1, 3, 5%) (w/v) was added to increase the biodegradability and antimicrobial activity of bioplastics. At the end, bioplastics casting were conducted by pouring the final mixtures into a petri dish and dried at 70°C. After dried, bioplastics were soaked with 4% (w/v) NaOH solution until it can
be easily remove out. Bioplastics were then rinsed under flowing water and dried at room temperature prior to analysis.

2.3. **Biodegradability Test**

Bioplastics were cut into 2 cm x 2 cm and then were put into petri dish. Afterwards, 20 mL microbial solution (EM4) which was previously diluted for 5 times was added. The degradation process such as color and dimension changing were monitored every 30 min.

2.4. **Antimicrobial Activity Test**

There are several methods to evaluate the antimicrobial activity of the bioplastics. This research was used disc diffusion (Kirby Baeur) test as antimicrobial activity test for bioplastics film. Bioplastics with 1 cm diameter were placed on solid nutritive media (nutrient agar) which contain a suspension of gram positive bacteria (*Staphylococcus aureus*) or gram negative bacteria (*Escherichia coli*) swabbed evenly on its surface. Afterwards, petridish was incubated for 18- 24 h. The incubation condition depends on the culture that was used on the test. After the incubation period, the inhibitory zone (clear zone) has to be determined around the bioplastic samples on the agar plate [5].

3. **Results and Discussion**

3.1. **Optimization of Starch Composition to Biodegradable Ability of Banana Peel Starch- Chitosan Blended Bioplastics**

Biodegradable test was also analyzed for commercial plastics as a comparison study. The result of biodegradability test towards banana peel starch- chitosan bioplastics was provided inside the Table 1 and Figure 1 as follows. Table 1 show the increasing rate of degradable ability of bioplastic when concentration of starch was increased in bioplastic and the visual degradation process of banana peel starch- chitosan bioplastic could be seen in Figure 1.

| Starch composition (%) | Degradation time |
|------------------------|------------------|
| 0                      | 2 h 16 min       |
| 10                     | 1 h 57 min       |
| 20                     | 1 h 42 min       |
| 30                     | 1 h 32 min       |
| 40                     | 1 h              |
| 50                     | 34 min           |
| Commercial plastic     | >2 weeks         |

Table 1. Biodegradability test of chitosan- starch bioplastics at various composition of starch (4% Chitosan)
3.2. Effect of Glycerol to Biodegradable Ability of Banana Peel Starch- Chitosan Blended Bioplastics

The effect of glycerol on the biodegradability of banana peel starch-chitosan based bioplastics was shown in Table 2.

Table 2. Biodegradability Test of Chitosan-Starch Bioplastics (4% Chitosan-30% Starch) with Addition of Glycerol

| Glycerol (mL) | Degradation time |
|---------------|------------------|
| 0             | 1 h 32 min       |
| 5             | 1 h 7 min        |
| 10            | 31 min           |

When glycerol was added in the 30% banana peel starch-chitosan bioplastics, the degradability of bioplastics film also increased. Glycerol can reduce intermolecular forces in starch or chitosan and thus decreased the strength of the films significantly [6]. The result indicated that addition of glycerol which acted as a plasticizer decreased the strength of banana peel starch-chitosan composite films.

3.3. Effect of Zinc Oxide to Biodegradable Ability of Banana Peel Starch- Chitosan Blended Bioplastics

Zinc oxide was loaded into banana peel starch-chitosan bioplastics as filler. The illustration of interaction between zinc oxide with starch-chitosan bioplastics was shown in Figure 2.
The interaction between zinc oxide with bioplastics was a complex metal-chitosan. Zn$^{2+}$ ion will become a bridge and substitute for the lost intra and intermolecular hydrogen bond. Biodegradability rates decreased as the increased of zinc oxide concentration in banana peel starch-chitosan bioplastics as could be references are clearly legible on the track of IOP Author Guidelines. Check in composite bioplastics will inhibit bacterial growing and can also decrease the degradation rate of bioplastics.

### Table 3. Biodegradability Test of Chitosan-Starch Bioplastics

| Zinc oxide (%) | Degradation time |
|----------------|------------------|
| 1              | 35 min           |
| 3              | 42 min           |
| 5              | 1 h 12 min       |

#### 3.4. Antimicrobial Activity of Banana Peel Starch-Chitosan Bioplastics

The results after 24 h incubation time was shown in Table 4 and Figure 3. The inhibitory effect was measured based on clear zone surrounding circular film disc. Measurement of clear zone diameter was started from the outside diameter of film bioplastics.

### Table 4. Antimicrobial Activity Test of Chitosan-Starch Bioplastics (4% Chitosan)

| Starch composition (%) | Clear zone diameter (mm) | | |
|------------------------|---------------------------|----------------|----------------|
|                        | Gram positive             | Gram negative |
| 0                      | 6.25                      | 8.875          |
| 10                     | 4.125                     | 8.75           |
| 20                     | 3.75                      | 7.375          |
| 30                     | 3.25                      | 6.625          |
| 40                     | 1.125                     | 5.375          |
| 50                     | 0.25                      | 2.75           |
| Biodegradable plastic  | 0                         | 0              |
| PE plastic             | 0                         | 0              |

**Figure 3.** Antimicrobial Activity Test for Banana Peel Starch-Chitosan Bioplastics against Gram Positive Bacteria (*S. aureus*) (a) 0-30% Starch Compositions (b) 40-50% Starch Compositions, Biodegradable Plastic (BP), and Polyethylene Plastic (PE)
If there is no clear zone surrounding, it means that there is no inhibitory zone, and the diameter was valued as zero. Films bioplastics showed fair antibacterial activity against gram positive (*S. aureus*) and gram negative (*E. coli*) bacteria. Commercial biodegradable plastics and polyethylene (PE) plastic was used as control for antimicrobial activity test. In general as shown on Table 4, chitosan bioplastics without starch addition will given the biggest clear zone against gram positive and gram negative bacteria [3]. Antimicrobial activity will decreased as the increased of starch addition on banana peel starch- chitosan bioplastics. The results were showed that banana peel starch- chitosan bioplastics have bigger clear zone against *E. coli* compare to *S. aureus*. It means banana peel starch-chitosan bioplastics have more effective antimicrobial activity against gram negative (*E. coli*) bacteria than gram positive (*S. aureus*) bacteria. Commercial biodegradable plastic and polyethylene (PE) plastic were not showed any antimicrobial activity.

**Table 5.** Antimicrobial activity test of chitosan- starch bioplastics (4% chitosan- 30% starch) with additive

| Additive      | Composition       | Clear zone diameter (mm) | Gram positive | Gram negative |
|---------------|-------------------|---------------------------|---------------|---------------|
| Glycerol      | 0 mL              | 3.25                      | 7.375         |               |
| Glycerol      | 5 mL              | 0.5                       | 7             |               |
| Glycerol      | 10 mL             | 0                         | 4.5           |               |
| Zinc oxide    | 1% (5 mL glycerol)| 4.5                       | 7.5           |               |
| Zinc oxide    | 3% (5 mL glycerol)| 8.5                       | 10.375        |               |
| Zinc oxide    | 5% (5 mL glycerol)| 8.75                      | 10.625        |               |

**Figure 4.** Antimicrobial activity test for banana peel starch- chitosan bioplastics (a) for addition 5 and 10 mL of glycerol (b) for addition 1,3,5% of zinc oxide

Addition of glycerol as a plasticizer into chitosan bioplastics was decreased the antimicrobial activity of bioplastics because glycerol filled the space between hydrogen bond and will weakness the chitosan strength that can affect chitosan antimicrobial activity. Zinc oxid (ZnO) is an efficient
antimicrobial agent since zinc oxide was able to exhibit an antimicrobial effect by diffusion of soluble species into agar medium [7]. Decrease of microbial population with respect to zinc oxide concentration on banana peel starch- chitosan bioplastics. The antimicrobial activity analysis also tested for banana peel starch- citosan bioplastics with addition of glycerol and zinc oxide as shown on Table 5 and Figure 4.

4. Conclusion

Biodegradability increased with the increase of banana peel starch concentration in starch-chitosan bioplastics. On contrary their antimicrobial activity will decreased. The starch- chitosan based bioplastics demonstrates more effective antimicrobial activity against gram negative (E. coli) bacteria than gram positive (S. aureus) bacteria. Addition of glycerol which acted as plasticizer will increase the biodegradability of bioplastics but at the same time will reduced their antimicrobial activity. Decreased of microbial growth will respected to zinc oxide concentration because of their antimicrobial ability. The banana peel starch- chitosan bioplastics may have potential applications for antimicrobial packaging by their fast biodegradable rate and antimicrobial activity that can inhibit bacterial growth to improve the food safety.

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