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## Development of Yam-Starch-Based Bioplastics with the Addition of Chitosan and Clove Oil

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### Abstract

The potency of yam starch (*Dioscorea alata*) as film-forming material together with the potency of chitosan and clove oil as antibacterial materials has led the authors to produce active bioplastics based on these materials. This research was performed to determine the effect of chitosan and clove oil on the physical, mechanical, and barrier properties of yam starch's active bioplastics. The best bioplastics produced were further tested for their antimicrobial properties. This study used a completely randomized design with five levels of chitosan concentration (1%, 1.2%, 1.4%, 1.6%, and 1.8%) and five levels of clove oil concentration (0.3%, 0.6%, 0.9%, 1.2%, and 1.5%). Each treatment was repeated four times. The results showed that the concentrations of chitosan and clove oil had a significant effect on the thickness and water vapor transmission rate of film but did not affect its solubility and compressive strength much. The use of 1% chitosan or 1.5% clove oil produced biofilms with the lowest water vapor transmission rate. Both plastics also exhibited strong antibacterial activity against *Staphylococcus aureus* with chitosan bioplastics having a larger inhibition zone than that of clove oil bioplastics.

**Keywords:** active bioplastics, antibacterial, chitosan, clove oil, yam starch

### Introduction

Synthetic plastics are slowly being replaced with biodegradable plastics, because the latter material is not only good for our environment but also safer to pack foods. Bioplastics can be produced using protein [1, 2], starch [3-5], or hydrocolloids [6, 7]. Among these materials, starch is the cheapest and most abundant resource. Many starch sources have been used as bioplastics raw materials, such as cassava [3], corn [8], potato [9], and sago [10]. Yam (*Dioscorea alata*) is another potential source of starch for bioplastics [5, 11-13]. It grows easily in various soil types and with little maintenance. As a single material, yam starch produces a high water vapor transmission rate and low-strength bioplastics [5, 12]. These parameters in yam starch bioplastics can be improved by adding hydrophobic material, such as chitosan [11, 14, 15] or oil, into a starch-based film-forming solution [13, 16-18].

Research interest in chitosan as raw material for bioplastics is increasing because of its nontoxic, bio-functional, biodegradable, and antimicrobial properties

[11, 14, 15, 19-25]. Chitosan is a linear polysaccharide of N-acetyl-D-glucosamine and D-glucosamine [15] obtained from the deacetylation of chitin. Chitosan is not water soluble but can easily dissolve in acid solution. The molecular weight and degree of deacetylation, which is the ratio of D-glucosamine units to the sum of N-acetyl-D-glucosamine and D-glucosamine units present in the chain, are factors that affect chitosan solubility in water [26]. As starch exhibits hydrophilic properties, blending yam starch with chitosan, which is hydrophobic, can produce less hydrophilic bioplastics, which in turn decreases the water vapor transmission rate. Furthermore, a higher concentration of chitosan produced higher tensile strength film [19]. Chitosan also has antimicrobial properties, as reported by many researchers [11, 19-21, 24, 25]. The addition of chitosan in the production of yam starch bioplastics was expected to not only improve the mechanical properties but also add an antimicrobial attribute to the film. Another study reported the effect of adding 1%–5% chitosan solution on the antimicrobial properties of bioplastics, but reports on their physical and mechanical properties were based on a subjective assessment [11]. Other reports on the effect of chitosan

concentration on the physical, barrier, and mechanical properties of bioplastics were published using pure chitosan film without starch using 0.5%–1.5% chitosan solution [19].

Clove oil is an essential oil that is classified as “Generally Recognized as Safe” and shows acceptable sensory properties. Owing to its antimicrobial activity, clove oil has been considered as an additive in active bioplastic production [22, 27, 28]. In contrast to chitosan, in solid state, the liquid state of clove oil facilitates incorporation into a starch-based film-forming solution. However, chitosan must be dissolved in acid solution before incorporation into starch paste; clove oil can be added directly to starch paste and mixed thoroughly. Another study has reported the effect of clove oil concentration on pectin-based films using clove oil concentrations of 0.5%–1.5% [27], but the addition of clove oil to yam starch-based bioplastics has not been reported. This background led the authors to conduct a comparative study of the characteristics of yam-starch-based antimicrobial film produced using chitosan and clove oil.

## Material and Methods

**Materials.** This research was performed at the Faculty of Agricultural Technology and Energy and Nanomaterial Study Center, University of Jambi. This research consisted of two experiments, both using a completely randomized design. The first experiment was designed to determine the effect of chitosan, whereas the second was to determine the effect of clove oil, on the characteristics of active bioplastics from yam starch. These experiments used five levels of chitosan and clove oil concentrations. Each experiment was repeated four times. The bioplastics were analyzed for their thickness [29], transparency [30], water solubility [31], water vapor transmission rate (WVTR) [30], compressive strength [12], and surface and cross-section of microstructures using SEM, clove oil composition using Gas Chroma-tography-Mass Spectrometry, and activity against *Staphylococcus aureus* using the disk diffusion method [27].

Yam starch was obtained by extracting white yam tuber. The tuber was grown in Jambi City and harvested 9 months after planting. Chitosan (94% deacetylated) was provided by the Chimultiguna Company. The clove oil brand “Happy Green” was obtained from steam distillation of clove buds. Glycerol, calcium chloride, magnesium nitrate, sodium chloride, nutrient agar, and nutrient broth were of analytical grade and supplied by Merck.

**Starch Extraction.** Starch extraction of yam tubers was performed using the procedure reported by Ulyarti [32]. After being soaked in 15% table salt solution for 30 min to remove mucus and washing thrice, the slices of tuber were smoothed using a blender (Phillip HR2115) with the addition of water. The smoothies were filtered using a

200-mesh filter, precipitated for 6 h, and redissolved in water to purify the starch. The sediment was dried in a drying oven at 50°C for 24 h. The dry starch was sieved using a 200-mesh filter, packed, and kept at room temperature (30–32 °C).

## Bioplastics Preparation [12]

**Active bioplastics using chitosan (Chi treatment).** A solution of 1%, 1.2%, 1.4%, 1.6%, and 1.8% chitosan was prepared by dissolving 1, 1.2, 1.4, 1.6, and 1.8 g of chitosan, respectively, in a 100-mL solution of 1% CH<sub>3</sub>COOH. A 4.5-g of yam-starch sample was stirred in 112.5-g distilled water for 10 min. A 30-g chitosan solution and 3-g glycerols were added to this mixture, which was then heated to 80°C and held at this temperature for 30 min under continuous stirring using a magnetic stirrer. A 25-g sample of the filmogenic solution was placed in a Petri dish (diameter, 9.2 cm) and dried in a drying oven at 50 °C for 24 h. The films were equilibrated in a desiccator at room temperature and 52% relative humidity (RH) for 2 d before analysis [30]. This RH was obtained by a saturating desiccator using saturated Mg(NO<sub>3</sub>)<sub>2</sub> salt.

**Active bioplastics using clove oil (CO treatment).** A 4.5-g yam-starch sample was stirred in distilled water for 10 min, the amount of water depending on the treatment. The final weight of the mixtures was maintained at 150 g. A 3 g sample of glycerols was added to this mixture. The mixture was then heated to 80 °C and maintained at this temperature for 10 min. To this solution, a certain amount of clove oil was added (0.45, 0.9, 1.35, 1.8, or 2.25 g depends on the treatment). The heating was continued for another 20 min. The next steps were similar to the procedures for obtaining active bioplastics using chitosan.

**Film Thickness.** Film thickness was measured using a micrometer at five points in the film. The random measurements were taken at different parts of the film.

**Transparency.** Bioplastics were cut into a rectangle (50 mm × 10 mm) and placed inside a spectrophotometer cell. The percent transmittance (%T) was obtained using a UV-Vis spectrophotometer at a wavelength of 600 nm [30]. The transparency of the bioplastics was calculated using the formula below:

$$\text{Transparency} = \log T/\text{Thickness} \quad (1)$$

**Solubility.** Bioplastics were cut into 2 cm × 2 cm squares and soaked in water for 24 h. The film was stirred periodically. The amount of undissolved film was weighed, and the percent solubility was calculated [31].

**WVTR.** A test tube containing CaCl<sub>2</sub> was sealed using sample bioplastics. The tube was weighed and placed inside a desiccator that had been previously saturated

using saturated sodium chloride (RH, 75%). The amount of water absorbed by  $\text{CaCl}_2$  was plotted as a function of time [30]. The WVTR was calculated using the formula below:

$$\text{WVTR} = \frac{\text{Slope}}{A} \quad (2)$$

**Compressive Strength.** An LFRA Brookfield Texture Analyzer was used to measure the compressive strength of the films. A TA-7 60 mm probe was attached in its place. The texture analyzer was run using a 2-g trigger, 2-mm distance, and 2-mm/s speed [12]. A 5 cm × 2 cm film sample was used for the measurement.

**Antibacterial Activity.** A 0.1 mL aliquot of *S. aureus* culture in nutrient broth media (grown for 24 h) was transferred to a sterile Petri dish. A 14 mL aliquot of nutrient agar media was placed in the Petri dish, which was capped and slowly shook. Once the media turned to a solid, a piece of bioplastics (diameter, 1 cm) was placed in the center of a Petri dish. The Petri dish was covered, wrapped with paper, and incubated at 37 °C for 24 h. The inhibition zone was the clear area around the film, determined by measuring the diameter of the clear area [27].

**Data Analysis.** The data were analyzed using ANOVA and Duncan's new multiple range test (DnMRT).

## Results and Discussion

**Color and Transparency.** Yam-starch bioplastics with the addition of chitosan or clove oil have a similar color of "dark moderate orange" to "dark orange" according to colorhexa.com. The range of chitosan concentrations used in this experiment did not appear to change the bioplastic's color, but the range of clove oil concentrations caused the change shown in Figure 1. This result is consistent with the statistical analysis for transparency shown in Table 1, which indicates that chitosan concentration did not affect transparency, whereas clove oil concentration did. However, the increase in chitosan and clove oil concentrations in the film-forming solution tended to decrease the transparency of yam-starch-chitosan bioplastics ( $p < 5\%$ ) and decreased the transparency of yam-starch-clove-oil bioplastics ( $p > 5\%$ ). This result agrees with the result reported by Nisar *et al.* [27]. The higher concentration of clove oil and chitosan increased the interaction between the polymer chains or clove oil and the plasticizer, which modifies the refractive index and limits light passage through bioplastics [19].

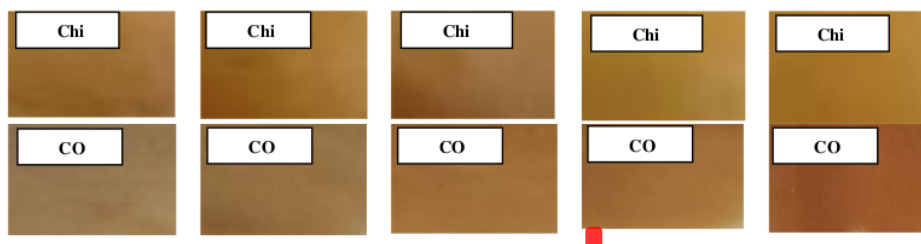


Figure 1. Yam Starch Bioplastics using Several Concentrations of Chitosan (Chi) and Clove Oil (CO)

Table 1. Physical, Mechanical, and Barrier Properties of Yam-Starch Bioplastics using Chitosan and Clove Oil

| Treatment | Thickness (mm)              | Transparency (%)           | WVTR (g/m <sup>2</sup> /day) | Water Solubility (%/mm)    | Compressive Strength (N/m <sup>2</sup> ) |
|-----------|-----------------------------|----------------------------|------------------------------|----------------------------|------------------------------------------|
| Chi 1%    | 0.130 ± 0.007 <sup>a</sup>  | 14.99 ± 6.95 <sup>a</sup>  | 45.00 ± 35.12 <sup>a</sup>   | 77.48 ± 6.07 <sup>a</sup>  | 192.9 ± 67.8 <sup>a</sup>                |
| Chi 1.2%  | 0.140 ± 0.006 <sup>ab</sup> | 10.75 ± 0.86 <sup>a</sup>  | 120.00 ± 21.60 <sup>bc</sup> | 67.86 ± 13.82 <sup>a</sup> | 147.2 ± 20.1 <sup>a</sup>                |
| Chi 1.4%  | 0.150 ± 0.006 <sup>bc</sup> | 11.20 ± 2.45 <sup>a</sup>  | 142.50 ± 26.30 <sup>c</sup>  | 65.42 ± 7.12 <sup>a</sup>  | 170.0 ± 19.7 <sup>a</sup>                |
| Chi 1.6%  | 0.160 ± 0.006 <sup>c</sup>  | 11.25 ± 2.20 <sup>a</sup>  | 97.50 ± 12.58 <sup>b</sup>   | 75.71 ± 4.95 <sup>a</sup>  | 191.9 ± 68.8 <sup>a</sup>                |
| Chi 1.8%  | 0.170 ± 0.010 <sup>d</sup>  | 13.90 ± 4.34 <sup>a</sup>  | 87.50 ± 28.72 <sup>b</sup>   | 69.34 ± 13.87 <sup>a</sup> | 132.7 ± 31.5 <sup>a</sup>                |
| CO 0.3%   | 0.146 ± 0.017 <sup>a</sup>  | 12.28 ± 1.20 <sup>c</sup>  | 36.57 ± 3.97 <sup>c</sup>    | 21.48 ± 7.30 <sup>a</sup>  | 86.5 ± 19.8 <sup>a</sup>                 |
| CO 0.6%   | 0.159 ± 0.013 <sup>ab</sup> | 10.42 ± 1.10 <sup>bc</sup> | 33.95 ± 2.90 <sup>bc</sup>   | 20.57 ± 4.80 <sup>a</sup>  | 78.6 ± 18.8 <sup>a</sup>                 |
| CO 0.9%   | 0.165 ± 0.015 <sup>ab</sup> | 8.67 ± 1.30 <sup>b</sup>   | 29.22 ± 3.14 <sup>b</sup>    | 19.77 ± 4.60 <sup>a</sup>  | 74.9 ± 5.0 <sup>a</sup>                  |
| CO 1.2%   | 0.169 ± 0.012 <sup>b</sup>  | 8.58 ± 1.40 <sup>b</sup>   | 23.95 ± 3.14 <sup>a</sup>    | 18.33 ± 2.60 <sup>a</sup>  | 72.5 ± 11.7 <sup>a</sup>                 |
| CO 1.5%   | 0.176 ± 0.004 <sup>b</sup>  | 5.95 ± 2.90 <sup>a</sup>   | 21.32 ± 2.33 <sup>a</sup>    | 16.05 ± 3.50 <sup>a</sup>  | 68.3 ± 12.8 <sup>a</sup>                 |

Treatment Chi = Chitosan; CO = Clove Oil

Note: Among the same treatments, the numbers in the same column followed by the same superscript are not significantly different ( $p > 5\%$ )

The transparency of yam-starch-chitosan bioplastics is higher than that of yam-starch-clove-oil. At least two

possible explanations can be proposed for this phenomenon. Lipid in the clove oil was first broken into small

droplets during the mixing process to produce a starch-oil emulsion, but the coalescence of these droplets can subsequently be enhanced during the drying process. The creaming effect and the coalescence promote surface coarseness in the yam-starch-clove-oil bioplastics and reduce the ability of light to pass through [27]. The second reason is provided by the moisture content of bioplastics, which is directly proportional to opacity [19]. Although chitosan solution contains water, during the drying process, lipid can prevent water from escaping from the bioplastics surface, leading to a higher moisture content of yam-starch-clove-oil bioplastic than yam-starch-chitosan bioplastic. The presence of water prevents light transmission through bioplastics and lowers their transparency.

**Thickness.** The addition of chitosan or clove oil significantly increased film thickness ( $p > 0.5\%$ ), as seen in Table 1, because of the increase in the total soluble solid of film-forming solution. The increase in thickness as the total soluble solid increases has been reported [7, 27, 33]. The bioplastics produced in this study were thicker than that of bioplastics reported by other authors: 0.08 mm for potato-starch-based film [9], 0.1 mm for chitosan-based film [23], 0.094 mm for citrus-pectin-based film [27], and 0.076 mm for pectin-based film [29]. Another factor that influence the thickness of bioplastic is the amount of film-forming solution (either volume or mass) poured per area of the molds. The higher the amount of film-forming solution that is poured, the thicker the bioplastic produced will be.

**WVTR.** The variation in chitosan and clove oil concentrations had different effects on the WVTR of yam-starch bioplastics. The WVTR was decreased by a higher concentration of clove oil but increased with an increase in chitosan concentration to 1.4% and decreased with a further increase in chitosan concentration. Whether water vapor can move in and out of the film depends on the relative polarity of the polymer used in the film-forming solution. The more cationic and hydrophilic the polymer is, the higher the WVTR of the film will be [34]. Chitosan and clove oil have a hydrophobic nature, leading to a lower WVTR.

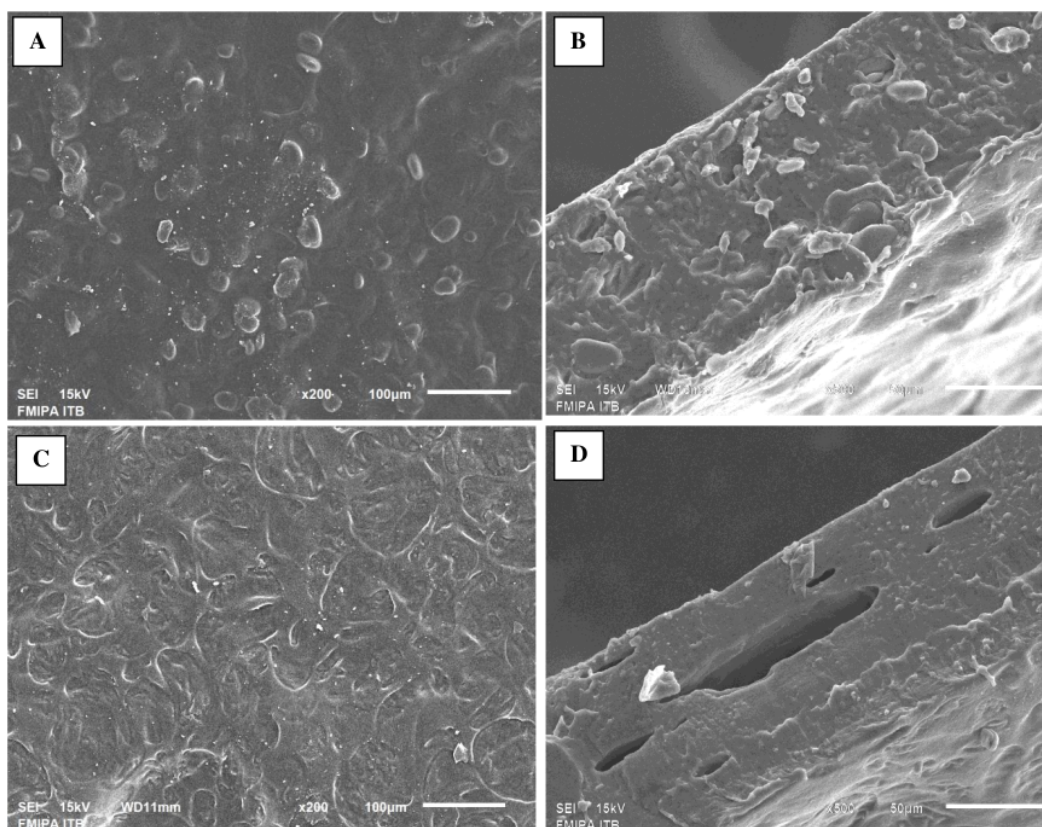
**Water Solubility.** Water solubility depends on the thickness of bioplastics. The thicker the bioplastics are, the more time is needed to dissolve bioplastics material and therefore, decrease the solubility (Table 1). For materials with similar thickness, yam-starch-clove-oil bioplastics have a lower solubility than yam-starch-chitosan because of the supply of hydrophobic material by the presence of lipid in clove oil. The solubility of yam-starch-chitosan in this study was higher than the reported value of 32.3% for chitosan-based film [19]. The difference can arise

from the presence of yam starch, which contains many OH groups, leading to a more hydrophilic nature of bioplastics. The solubility value of yam-starch-clove-oil obtained in this experiment was similar to data reported by other authors, such as 17.44% for pectin film with the addition of 1.5% clove oil [27].

**Compressive Strength.** Among the mechanical parameters usually used to describe the quality of bioplastics, compressive strength is one parameter mentioned in the Japanese Industrial Standard (JIS). Yam-starch-chitosan bioplastics have achieved the minimum compressive strength for acceptable quality, 100 N, whereas yam-starch-clove-oil bioplastics have not. (Table 1). The lower value for the compressive strength of yam-starch-clove-oil bioplastics can be owing to the less compact nature of the film. Yam-starch-clove-oil showed empty space in the polymer matrix structures, as seen in the cross-section of bioplastics (Figure 2), which causes higher brittleness [35].

**The Selection of the Best Bioplastics.** The quality parameters provided by the JIS were used to select the best treatment. The thickness should be below 0.25 mm, the WVTR should be lower than 5 g/m<sup>2</sup>.day, and the compressive strength should be higher than 100 N. Statistical analysis showed that the solubility and compressive strength were not affected by the concentration of clove oil or chitosan (Table 1); therefore, the limiting factors for deciding the concentration to produce the best bioplastics come from the thickness and WVTR. All treatments produced bioplastics with a standard thickness according to the JIS; however, none of the bioplastics were in the range of the acceptable WVTR. Therefore, the chosen clove oil and chitosan concentration was 1.5% and 1%, respectively, which gave the lowest WVTR for each bioplastic. The chosen bioplastics were further analyzed for their microstructure and antimicrobial properties.

**Surface and Cross-Section Morphology.** The morphology of the bioplastics surface and cross-section provide information on the compactness of the film-forming materials in the bioplastics. As shown in Figure 2, chitosan and clove oil could not mix well with starch and glycerol. Yam starch-chitosan bioplastic is more acidic because of the state of chitosan solution used in the experiment. As the solubility of starch strongly depends on acidity, it seems that chitosan has a different affinity with the starch-glycerol matrix; therefore lumps are produced, as seen in the surface and cross-section of bioplastics. The surfaces of both bioplastics are rough, leading to low transparency. The cross-section of yam starch-chitosan bioplastics showed a more compact matrix compared to that of yam starch-clove oil, promoting low mechanical properties in yam starch-clove oil.



**Figure 2.** SEM Images of the Surface of Yam-Starch-Chitosan (A), Cross-Section of Yam-Starch-Chitosan (B), Surface of Yam-Starch-Clove-Oil Bioplastics (C) and Cross-Section of Yam-Starch-Clove-Oil Bioplastics (D)

**Antimicrobial Activity of Bioplastics.** The antimicrobial activity of bioplastics was determined from the diameter of the inhibition zone for *S. aureus*. The inhibition zone of bioplastics was higher with the addition of 1% chitosan (41 mm) than with the addition of 1.5% clove oil (12 mm). A higher inhibition zone (30 mm) of bioplastics with the addition of clove oil at the same concentration was reported [27]. An inhibition zone above 30 mm was reported for the addition of clove oil above 1% [36]. The different results may be due to the interaction between clove oil and film-forming compounds. Furthermore, the quality of the clove oil used can also play a role. Differences in the quality of clove oil resulted in different amounts of the active component in the oil. Eugenol,  $\beta$ -caryophyllene, and acetogenol are major inhibitory compounds in clove oil, but the clove oil used in the current experiment contained 54.9% eugenol and 38.2% triacetin/1,2,3-propanetriol, as determined by a GC-MS report analysis.

## Conclusion

Chitosan and clove oil can be used as active components in the production of antimicrobial bioplastics from yam starch. Chitosan and clove oil affect the thickness and WVTR of bioplastics but not their solubility and compressive strength. The transparency of bioplastics was influenced by clove oil, whereas chitosan had no such effect. Considering its mechanical properties and inhibitory capacity against *S. aureus*, chitosan is more promising than clove oil for antimicrobial bioplastics.

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