Synthesis and Characterization of Biofilm from Jatropha Curcas L., Chitosan, and Gelatin by Blending Method

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Abstract. Based on the rapid development of science, it is known that the Jatropha Curcas L., Chitosan, and Gelatin are natural materials which have various benefits. The aim of this research is to determine the characteristic of blend of Jatropha Curcas L., Chitosan, and Gelatin as a biomedical application. The blending material is obtained in the form of film / layer as a preliminary review of the research This research is experimental and the process in this research consists of 2 steps i.e. variation of gelatin composition and variation of chitosan composition. The samples obtained were characterized by tensile test to determine the value of tensile strength, strain, and Young's modulus. The best result of it was tested by FTIR, then it's solubility also was tested. Based on the tensile strenght test, gelatin which can improve the chitosan properties are rigid and brittle. The composition of 2: 8: 12 which has a value of tensile strength, strain, and Young's modulus is low while the composition of 2:12:10 is high. Based on the FTIR test, there are extension and shift of OH group at wave number 3428,4; 3447.2; and 3447.2 cm-1. In the solubility test was found that the composition of 2: 8: 12 has the highest solubility while the composition of 2:12:10 has the lowest solubility.

Keywords: Jatropha Curcas; Chitosan; Gelatin; Film; Tensile Strenght

1 Introduction

Chitosan has a antifungal and antimicrobial activities, the activities are believed to originate from its polycationic nature [1], [2]. Despite its attractive properties, the film made only from chitosan has poor mechanical properties so that has bad printability [3], [4]. Forming films from chitosan mixed with other hydrocolloids and hydrophilic is an alternative to improve its mechanical properties [2], [5]–[7].

Gelatin is one of the important macromolecule material. It has many benefits which used in medicine, food, and chemical industry. It is good properties as a plasticizer, such as: the gel property, water holding capacity, film-formation, emulsification and foaming ability [8]. despite its attractive properties, the film made only from gelatin show brittleness, extremely fast degradation, and less flexibility [8]–[10]. Polymer blending is a method commonly used to obtain the benefits of each materials [11].

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The natural material derived from plants which have been used as medicine [12]. The antimicrobial activities on the jatropha curcas which is extracted with etyl acetate showed the highest efficacy [13]. The development of science and technology provide an innovative opportunity to maximize the properties of medicinal plants. This research is innovating to maximize the jatropha curcas by adding chitosan, and gelatin to improve its mechanical properties.

Saraswathy does a mixture of chitosan-gelatin materials.The report of that study is combination of material (chitosan-gelatin) that has been used does not occur chemical reactions, meaning that the original characteristics of each component is not lost [14]. The other studies concern to the chitosan mechanical properties reported by Judawisastra et al. is obtained the value of tensile strenght (TS) 38.4 Mpa and the value of elongation at break (EAB) 16.2% [15]. The research combination of chitosan and gelatin reported that the optimum of variation obtained was chitosan 60:40 gelatin with tensile strenght (TS) value of 16,60 MPa and elongation at break (EAB) 25,3% [16].

This study focuses on determining the effect of the composition variation of chitosan and gelatin added to the jatropha curcas. This research was conducted as an early stage to find out some integrated materials with their respective functions that complement each other as candidates of biomedical applications especially absorbable surgical suture. The integrated material in this research is obtained in the form of film / layer as a preliminary review of the research. The results obtained from this study are characterization of functional groups using FTIR, tensile strenght and elongation break, and solubility of the sample in pbs solution.

2 Materials and Methods

This research is experimental and the process in this research consists of 2 steps i.e. variation of gelatin composition and variation of chitosan composition. Variation of gelatin composition is the first step done to seek the best composition based on its mechanical properties. The second step is a variation of chitosan composition with the best sample reference in the first step.

2.1 Materials

The material used in this research is Jatropha Curcas obtained from Balai Penelitian Tanaman Pemanis dan Serat (BALITTAS) Karangploso Malang, chitosan 7% *w/v*, gelatin 10% *w/v*, aquabides, acetic acid 1% *v/v* and phosphat buffer saline (PBS) solution.

2.2 Stem Sap Removal of Jatropha Curcas

Stem sap removal of jatropha curcas was planned to do at 08.00-10.00. Stem sap of jatropha curcas were taken, done by cutting the skin of stem.

2.3 Preparation of Gelatin Solution 3%

Gelatin solution 3% was prepared by adding gelatin powder 0,15 gram, and then dissolved with acetic acid 1% until 50 ml in beaker glass (60 °C).

2.4 Preparation of Chitosan Solution 5%

Chitosan solution 5% was prepared by adding chitosan powder 0.4 gram, then dissolved with acetic acid 1% until ml in a beaker glass and then with magnetic stirred.

2.5 Film/Layer Formation

Films were prepared by blending method. They were prepared with formula given, jatropha curcas L.: chitosan: gelatin 2:8:8, 2:8:9, 2:8:10, 2:8:11, 2:8:12 (v/v) and then stirred homogeneously with a magnetic stirrer at 60 °C for 10 minutes. The homogeneous solution is poured into some petri dish. Then shake eachm petri dish until the solution establishes a layer that is almost the even. Next dry the sample at room temperature for 3 days until completely dried and film/coating formed.

Based on the variation of gelatin composition, was selected the best value of tensile strength and elongation at break for chitosan variation. Making a film in chitosan variation was performed by using the same method as gelatin variation. The variation of chitosan composition was done with Jatropha Curcas L.: Chitosan: Gelatin as follows 2:8:10; 2:10:10; 2:11:10; 2:12:10 (v/v) [16].

2.6 Mechanical Properties

The tensile strength test was done at UB's agricultural technology faculty by using IMADA's Tensile Strength tool.

2.7 Functional Groups Analysis

The functional group analysis was performed in the science and technology faculty of Chemistry Department of Maulana Malik Ibrahim Malang State University using Fourier Transform Infrared (FTIR) merc. scimitar series

2.8 Solubility Test

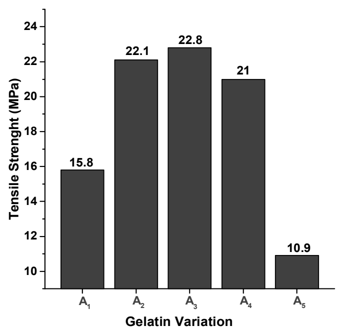
Solubility test of this research is used to find out the time required by sample for perfect degradation in phosphate buffer solution (PBS). In this study, PBS solution was used as a substitute for body fluids. Each of the samples was cut with the size of 1x1 cm size then it was immersed in PBS solution inside the test tubes. The samples solubility time was performed in 28 days. The degradation rate of each sample was stated with the percentage (W/W), as calculated with the following formula [17].

$$\eta\% = \frac{\omega_1 - \omega_2}{\omega_1} \times 100\% \quad (1)$$

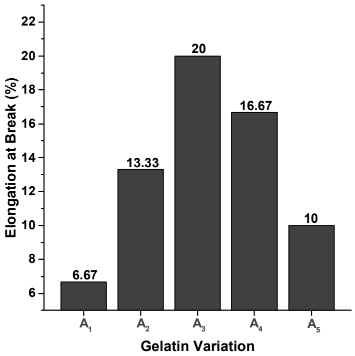
3 Result and Discussion

3.1 The Mechanical Properties of The Variation of Gelatin Composition

The first step from mechanical properties is the variations of gelatin composition. Based on the data obtained, the increasing of gelatin composition can decrease the TS value and increase the sample EAB value. This is proved by the diagrams obtained in Fig 1 (a) and 1 (b).



(a)



(b)

Figure 1. Graph of relation of mechanical properties to variation of gelatin composition (a) tensile strenght (TS) (b) elongation at break (EAB) *Composition of Jatropha curcas L.: chitosan: gelatin (A_1=2:8:8, A_2=2:8:9, A_3=2:8:10, A_4=2:8:11, A_5=2:8:12).

The highest TS and EAB values of Jatropha Curcas L.: Chitosan: Gelatin were obtained on sample A_3. The film has strong and elastic mechanical properties. This is also supported by data obtained on Young's modulus of A_3 sample. The composition of the jatropha curcas, chitosan, and gelatin in the A_3 sample is in the optimum composition. The result of optimum mechanical properties can be linked from higher interactions between biopolymers so it generate the better mechanical properties. Pranoto et al. reported an optimum level for the interaction between polysaccharides-protein in which gelatin as a protein presents the main and the dominant phases in the used film system the increased mechanical properties with increased gelatin proportions may be an important advantage in some applications [18].

The result of measurement on each variation of gelatin composition for Young's modulus (E) is depicted in diagram form shown in Figure 2. The presence of an increasing gelatin composition can decrease the sample's stiffness, the greater gelatin composition makes the sample more elastic. The highest E values are obtained in A_1 so it can be said that A_1 is the most rigid sample among the other samples. The presence of gelatin as plasticizer in this study can improve the elasticity of material on sample A_3 and A_5.

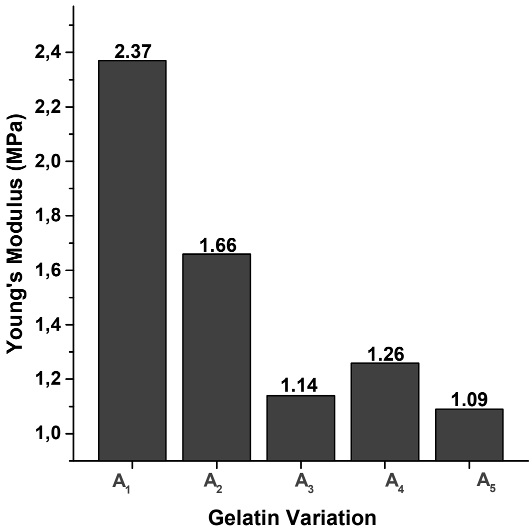
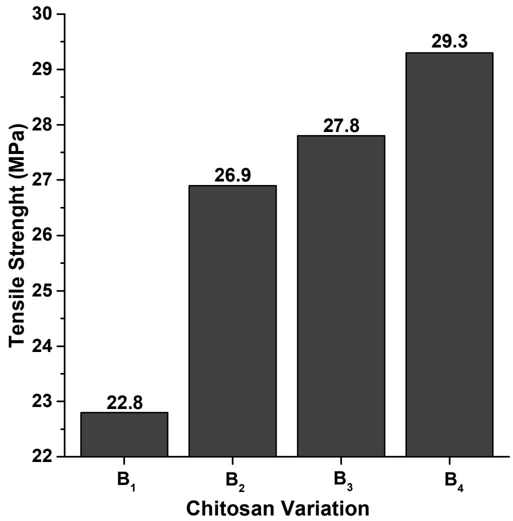


Figure 2. Graph of relation of mechanical properties to variation of gelatin composition modulus Young (E) *Composition of Jatropha curcas L.: chitosan: gelatin (A_1=2:8:8, A_2=2:8:9, A_3=2:8:10, A_4=2:8:11, A_5=2:8:12).

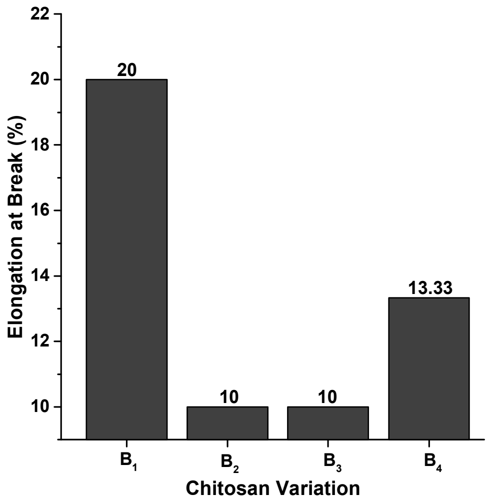
3.2 The Mechanical Properties of The Variation of Chitosan Composition

Based on the variation of gelatin composition, the maximum yield was obtained at 2:8:10 (A_3), so it is composition was considered as reference (B_1) for the variation of chitosan composition. The second step is a variation of chitosan composition that is 2:10:10 (B_2), 2:11:10 (B_3), and 2:12:10 (B_4) (v / v) by using the procedure as in the first step. This is to know the best composition of chitosan because basically chitosan as a material has quite high rigid properties. Figures 3 (a) and (b) show the diagram of TS and EAB values obtained on the variation of chitosan composition. Based on the variation of chitosan composition that has been done can be said that the sample B_4 has the highest TS value but not with its EAB value which shows B_4 has a strong properties but not elastic.

Figure 4 show the diagram of Young's modulus (E). the increasing chitosan composition produces more rigid samples, and obtain plastic sample properties. The highest E value is obtained on B_3 so it can be said that B_3 is the most brittle sample among others. This is likely due to the difference in TS and EAB values of B_3 films higher than B_4. Overall, the mechanical properties of the film depend on several parameters, one of them is the material composition.



(a)



(b)

Figure 3. Graph of relation of mechanical properties to variation of chitosan composition (a) tensile strenght (TS) (b) elongation at break (EAB) *Composition of Jatropha curcas L.: chitosan: gelatin (B_1=2:8:10, B_2=2:10:10, B_3=2:11:10, B_4=2:12:10).

Judawisastra et al. (2012) in his research by extending the process of demineralization of chitosan, it's obtained TS value of 38.4 Mpa and EAB value of 16.2% [15]. Another study conducted by Hosseini et al. with variation of chitosan:gelatin reported that the optimum variation obtained was chitosan 60:40 gelatin with TS value of 16,60 MPa and EAB 25,3% [16]. The results obtained in this study almost closer to TS and EAB value by Hosseini et al. on some variations. The mechanical properties are required for sewing operation thread i.e., tensile strength that is not too high and elongation at break that is sufficient [16]. It is intended that the material obtained is more easily degraded in the body (in vivo), resulting in an end product that is biocompatible to the human body.

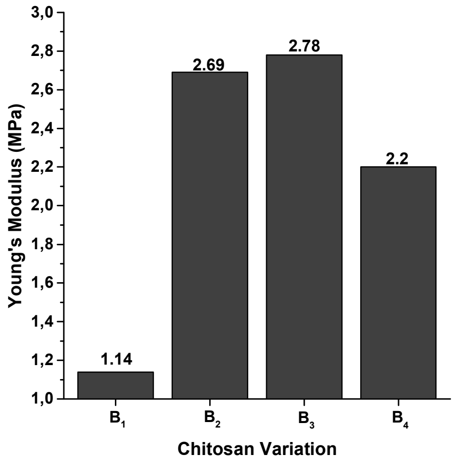


Figure 4. Graph of relation of mechanical properties to variation of chitosan composition Young's modulus (E) *Composition of Jatropha curcas L.: chitosan: gelatin (B_1=2:8:10, B_2=2:10:10, B_3=2:11:10, B_4=2:12:10).

3.3 Functional Group Analysis

Functional group analysis is performed to identify the functional groups of the compounds contained in the sample. The analysis was performed for samples A_3, A_5, and B_4

because they have the TS value of respectively i.e., low, medium, and high based on the test of its mechanical properties. FTIR spectral data is shown at figure 5. Spectra A_3, A_5, and B_4 don't show significant difference. In the three samples happened the extension and shift of the OH group in the wave numbers 3428,4; 3447.2; and 3447.2 cm^{-1}. It indicates the formation of intermolecular hydrogen bonds between gelatin and chitosan. The aromatic compounds in the film appear at the wave numbers 3734,7; 3745,6; and 3744.6 cm^{-1} which are typical spectra of C-H sp^2 Aromatic group and 1453.1; 1458.4; and 1457.7 cm^{-1} which are the aromatic C = C group. The presence of such compounds is due to the inclusion of hydrophobic aromatic groups. The aromatic compounds may be derived from active compounds of jatropha curcas.

The spectra of amide compound I, amide compound II and amide compound III on A_3, A_5, and B_3 obtained decreased intensity. The decrease in amide I shows that the presence of chitosan causes the decrease in the helix content in the sample [19]. The decrease in the intensity of amide II confirms the presence of electrostatic interactions between carboxyl group of gelatin and amino group of chitosan, but also indicates the formation of hydrogen bonds in which the -NH gelatin groups are involved [20]. The intensity change in amide III shows the interaction of the amino group of chitosan with the carboxyl group of gelatin through the electrostatic interaction [19].

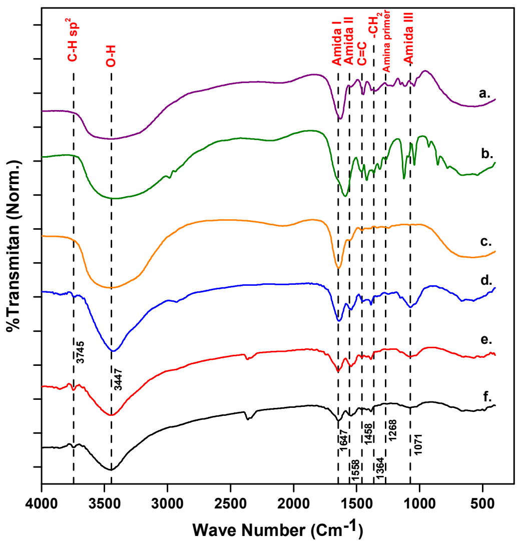


Figure 5. FTIR spectra (a) jatropha curcas L., (b) chitosan, (c) gelatin, (d) A_3 film, (e) A_5 film, dan (f) B_4 film *Composition of jatropha curcas L.:chitosan:gelatin (A_3=2:8:10, A_5=2:8:12, B_4=2:12:10).

According to Qiaio et al. gelatin as a protein is a polyampholyte type in which the carboxyl group (-COO-) can interact ionically with the amine group ($-NH_3^+$) present in the chitosan chain in acetic acid solutions [21]. A number of gelatin groups such as -NH and OH is able to form hydrogen bonds with the -OH and $-NH_2$ groups in the chitosan chain. Thus interaction between gelatin and chitosan is produced by electrostatic interaction and based on its hydrogen bond as described by Sionkowska (2004) [22].

3.4 Solubility Test

The solubility test was performed by immersing the sample in phosphate buffered saline (PBS) solution. In this study used PBS solution was used because the solution has the same properties as liquid in the human body. The samples were immersed in a PBS solution within 28 days and controlled every week to determine the weight loss in the solution. The results of the solubility test in this study are shown in Figure 6 for variation of gelatin composition and figure 7 for variation of chitosan composition.

Based on these data it indicates that A_1 has the largest mass loss value. This is possibly because the TS value is not too large and the EAB value is too small in the sample based on its mechanical properties. Based on figure 6 the low solubility in the variation of gelatin is available at the sample A_2 and A_5 but sample A_5 and A_2 have the different case because the sample A_5 has high solution firstly in the second week then becomes stable in the fourth week. So it can be said that the sample A_2 has low solution in the variaton of gelatin composition, this is possible because the sample A_2 has the high value of TS and the quite low value of EAB

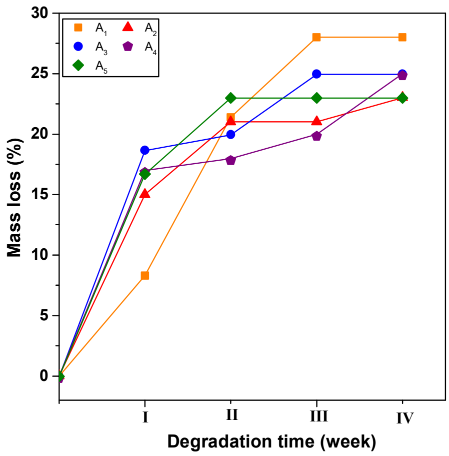


Figure 6. Solubility test of gelatin composition variation.

Based on variation of chitosan composition, the highest solubility is found in the films of B_1 and B_2. This is because the film has a low TS value and a fairly high EAB value. In the B_1 sample at week III the solubility tends to be high. This is because sample B_1 has the lowest TS value the variation of chitosan composition. The lowest solubility is B_4 film because it has a high TS value and has a fairly low EAB value, so the B_4 sample is more difficult to be degraded by PBS solution.

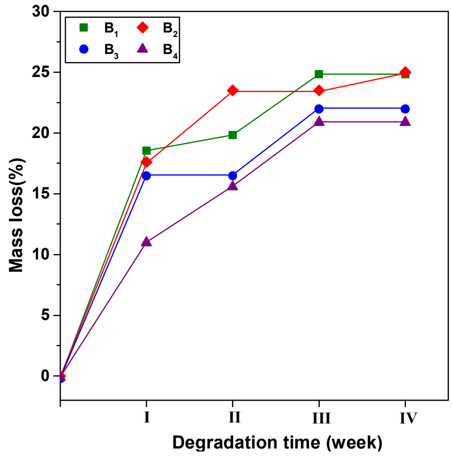


Figure. 7. Solubility test of chitosan composition variation.

4 Conclusion

Based on the variation of gelatin composition, the presence of gelatin can increase the elongation of chitosan but can not decrease stiffness in chitosan significantly. Composition A_3 (2: 8:10) has the highest TS and EAB values. Based on the variation of chitosan composition it is known that the highest TS value is obtained at B_4 (2:12:10). The higher chitosan composition produces an increasing TS value. The curves show typical behavior of strong and hard chitosan.

Spectra A_3, A_5, and B_4 don't show any significant difference. In the three samples the extension and shift of the OH group in the wave number are 3428,4; 3447.2; and 3447.2 cm^{-1}. The spectra of amide compound I, amide compound II and amide compound III on A_3, A_5, and B_4 have decreased intensity.

In the solubility test of PBS solution it's found that, composition A_1 has the highest solubility whereas composition B_4 has the lowest solubility.

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