Central Composite Design for Optimization of Starch-Based Bioplastic with Bamboo Microfibrillated Cellulose as Reinforcement Assisted by Potassium Chloride

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Abstract. Background: Sago starch is a natural resource which has the potential to be used as a raw material for biocomposite such as bioplastic. In addition, bamboo cellulose is able to improve the characteristics of sago starch bioplastics. Potassium chloride can act as dispersing agent to increase efficiency in solution preparation of bamboo microfibrillated cellulose. This paper is aimed to observe the best condition for optimal tensile strength of sago starch-based bioplastics reinforced with bamboo cellulose dispersed in potassium chloride as assistance and durability assessment of the bioplastic by soil burial. To achieve this goal, bioplastic was prepared by using matrix of sago starch-based reinforced bamboo cellulose (1-5 %-w/w) assisted by sonication and dispersing agent of potassium chloride with 1-3 %-w/w. The bioplastic with optimum tensile strength was then analysed for durability by burying the sample in soil. Optimum tensile strength of bioplastics was obtained at 28.6 MPa with the optimum concentration of bamboo cellulose micro fibril and potassium chloride of 5 %-w/w and 3 %-w/w, respectively. Post durability test, the non-reinforced and without dispersing agent bioplastic experienced a mass loss of 67.95 %, while the bioplastic reinforced with bamboo cellulose assisted by potassium chloride experienced mass loss of 33.33 % to 41.25 % (more durable).

1. Introduction
Currently, community and industry have begun to focus on solving environmental issues such as energy savings, pollution free areas, suppressing environmental pollution, and reducing the use of fossil (unrenewable) resources [1,2]. The biggest environmental pollution is produced by plastic waste as it is a synthetic material that is made using petroleum-based materials that is difficult to decompose in soil. Due to the advantages such as durability, high tensile strength and relatively cheap price of synthetic plastic, plastics are in high demand and remain widely used as food packaging.

Plastic has now begun to be developed from natural materials called bioplastics. Bioplastics are developed as an alternative to synthetic plastic as they are considered more environmentally friendly [3]. Developments of bioplastics have been carried out by using protein from egg white [4], gluten [5], and sago starch [6,7]. Sago starch is a promising material because it has a high amylopectin content of ± 73% [8]. In addition, Indonesia is the largest producer of sago starch in the world after Brazil with an area of 1.25 million Ha or about 50% of the total production in the world [8]. However, these starch-based bioplastics still have weaknesses in their characteristics such as weak mechanical properties, poor long-term stability, and sensitivity to water [7]. Microfibrillated Cellulose (MFC) from biomass began to be developed as a reinforcing filler in the manufacture of biocomposites. Biomass as referred to in
this case include pear skin [9], bagasse [10], and bamboo [11]. Bamboo is a biomass with high cellulose content of ±55% from its total content and higher than cellulose found in woods [12].

Efforts to improve the mechanical properties of bioplastics made from sago starch were carried out by adding bamboo MFC as a reinforcement [13]. This study obtained result of tensile strength value of 21.9 MPa. However, SEM analysis shows that the pores on the surface of the bioplastic are still randomly distributed with a non-uniform size and diameter. KCl salt was used as a dispersing agent in the manufacture of bamboo MFC solutions to accelerate the homogenization process [14]. At 0.5% w/w of bamboo MFC concentration along with 0.5% and 2.0% w/w of KCl, MFC solution was able to be produced in less than 1 hour. Of what specific use MFC solution can be applied for is arbitrary. Therefore, in this study the manufacture of bamboo MFC solution with the addition of KCl salt as a dispersing agent for application in sago starch bioplastics was carried out. The purpose of this study was to determine the effect of the addition of KCl dispersing agent on the value of tensile strength and morphological properties of sago starch bioplastics. Bioplastic results with the best tensile strength values are optimized using Central Composite Design (CCD) in Design Expert 8.0.6 with data response in the form of tensile strength. The optimum bioplastics produced were analyzed physically (durability).

2. Materials and Methods
This research was conducted at the Advance Material Laboratory (AMaL) Chemical Engineering at Diponegoro University. The first stage is the preparation of bamboo raw materials (Dendrocalamus asper). The second stage is the cellulose isolation process. The third stage is a preliminary experiment. The fourth stage is optimization using Central Composite Design and the fifth stage is the optimum product analysis.

2.1. Preparation
Bamboo was sliced into flat and thin pieces. The bamboo slices were then chopped to form flakes and ground using a mini crusher. Bamboo powder produced was then sieved to 100 mesh and then washed with 90 °C water three times to remove dust and other impurities [14,15].

2.2. Cellulose Separation
The process of cellulose separation was carried out through four steps, namely; dewax, delignification, bleaching and drying. Dewax was carried out by extraction using toluene-alcohol solvents (2:1) for 6 hours at 110 °C to remove wax on bamboo. Delignification was done by mixing bamboo powder with potassium hydroxide (KOH) at a temperature of 90 °C while being stirred for 4 hours [16]. Bleaching was done by combining the filtered delignification product with Hydrogen Peroxide (H2O2) under constant stirring at 55 °C for 3 hours then washed to a neutral pH. Drying was done by inserting the filtered product from bleaching into an oven with a temperature of 85 °C. After that, the powder was processed using a planetary ball mill (PBM) into powder and sieved to get a homogenous size of 345 mesh or 45 µm.

2.3. Preliminary
Bamboo MFC solution was made by mixing bamboo MFC powder into KCl solvent with an indiscriminate concentration until a suspension solution is formed, this is called preliminary I. This step was also done to determine the best mixing time using ultrasonic homogenizer by comparing the production of bamboo MFC solution with water solvent and with KCl solvent at the same concentration, followed by dissolving 4 g of sago starch in 100 ml of water. MFC concentration was varied by 1; 1.5; 2; 2.5; 3; 3.5; 4; 4.5; and 5 (%-w/w), while KCl concentration was performed with 1, 2, and 3%-w/w. Bioplastic solution was made by mixing MFC solution with starch solution which was then ultrasonic homogenized for 30 minutes. Furthermore, the perfectly mixed solution was gelatinized through heating 90 °C to form a gel. Glycerol was added and stirred for 15 minutes. Bioplastics produced were tested for their tensile strength for getting optimum condition of MFC and KCl. This step is called preliminary
II. Mechanical Tests (Tensile Strength) were carried out in the Food Technology Laboratory of Soegijapranata Catholic University Semarang using a Lloyd Instruments Texture Analyzer plus.

2.4. Optimization
Optimization was done to study bioplastic products statistically. This procedure was carried out by entering the boundary conditions of the concentration of bamboo MFC and KCl on the Central Composite Design (CCD). The boundary conditions were obtained from preliminary II results, i.e. by using optimum MFC concentration (1 % w/w as low level and 5 % w/w as high level) and KCl concentration (1 % w/w as low level and 3 % w/w as high level). These variables level have been conducted by central composite design with alpha of 1.414 resulting 13 experiment running (Table 1). The results could then achieve an experimental design by central composite design.

2.5. Analysis
The results of the measurement of the tensile strength using the Central Composite Design (CCD) design would produce the optimum tensile strength value. This was done to achieve the third goal. The optimum results were then analyzed for physical structure (durability). The following is an explanation of the analysis that was carried out to characterize bioplastics products made from sago starch.

Physical Test (Durability). Durability is a test of the ability of a degraded material in the soil to the effect of decomposing microbes, soil moisture and various other chemical factors. There were 3 bioplastics tested, namely sago starch bioplastics, sago starch bioplastics with the addition of bamboo MFC, and bamboo bioplastics-MFC starch with the addition of KCl. Burial was carried out for 45 days and observations were made by weighing and measuring the water content of bioplastic every day to find out its mass loss. Mass loss is defined as follows:

\[
\text{(\%) mass loss} = \left(\frac{M_0 - M_a}{M_0}\right) \times 100
\]  

(1)

Where \(M_0\) and \(M_a\) respectively are the weight of bioplastics before being buried and after being buried.

3. Results and Discussion

3.1. Preliminary
This process is called preliminary which is done randomly so that the bamboo MFC does not settle and the solution is perfectly mixed with a 10 ml base. The solution has been processed in the ultrasonic homogenizer.

The concentrations of bamboo MFC used 1; 1.5; 2; 2.5; 3; 3.5; 4; 4.5; and 5 (% w/w) with KCl concentrations of 1; 2; and 3 (% w/w). Producing a bamboo MFC solution takes a time since cellulose is undissolved in water. Therefore, the homogenization process is usually carried out with homogenizer such as ultrasonic homogenizer. The addition of potassium chloride (KCl) dispersing agent can reduce homogenization time so as to reduce high energy consumption [14].

In the first hour, the two mixtures still showed deposits from bamboo MFC. After the mixing was continued for up to three hours, the precipitate in the mixture was reduced especially in the mixture with KCl solvent. The mixture with KCl solvent was more suspended than the mixture with water as solvent. The mixture with just water as solvent took up to four hours to obtain a suspension solution much like the mixture with KCl solvent. This confirms that homogeneous conditions can be achieved in a shorter time using KCl of varying concentrations than without KCl. The high viscosity of bamboo MFC is due to the gel network on the fiber that is formed regularly, a suspension phenomenon. The characteristic of dispersed bamboo MFC is related to the principle of electrostatic repulsion [14]. When bamboo MFCs are spread on pure water, the surface of the solution will be slightly negatively charged so as to allow repulsion between the fibrils. The addition of KCl that dissociates to K\(^+\) and Cl\(^-\), will reduce the repulsion
force between the fibrils, specifically caused by K\textsuperscript{+}, that changes the fibrous interaction to be more stable. Decrease in viscosity can occur if KCl is used excessively due to damage to the gel tissue which disrupts the repulsion balance between fibrils [17]. Thus, the use of the right salt composition is very influential in making a bamboo MFC solution. Furthermore, preliminary II was conducted to find out the best composition in producing the best tensile strength value. From the results of the tensile strength test, the best result is 33.10 MPa with 3 % w/w of bamboo MFC solution and 0.59 % w/w of KCl composition as shown in Table 1.

3.2. Bioplastics Optimization Using Central Composite Design (CCD)

This procedure was carried out by entering the boundary conditions of the concentration of bamboo MFC and KCl on the Central Composite Design (CCD). The boundary conditions were obtained from the results of preliminary II, namely the concentration of bamboo MFC upper level of 5% and the lower level of 1%, KCl concentration of the upper level of 3% and the lower level of 1%. This level was chosen on the consideration of the value of the tensile strength obtained and was adjusted to its constituent components. The experimental design and measurement results of tensile strength values are presented in Table 1, while the prediction result denoted with coded equation from ANOVA (as in equation (2)).

\[
\text{Tensile strength (MPa)} = 14.43 - 5.37 \times (\text{KCl}) - 0.68 \times (\text{MFC}) + 2.13 \times (\text{KCl}) \times (\text{MFC}) + 5.35 \times (\text{KCl})^2 + 5.63 \times (\text{KCl})^2 \times (\text{MFC}) + 7.71 \times (\text{KCl})^2 \times (\text{MFC})^2
\] (2)

| Table 1. Central Composite Design (CCD) experimental design |
|------------------------------------------------------------|
| Factor (A) KCl (% w/w) | Factor (B) MFC (% w/w) | Data Responses (experiment) Tensile Strength (MPa) | Prediction (MPa) | Error % |
|-------------------------|-------------------------|-----------------------------------------------|-----------------|--------|
| R1                      | 1                       | 17.55                                         | 14.66           | 16.47  |
| R2                      | 1                       | 19.20                                         | 23.02           | 19.90  |
| R3                      | 3.41                    | 17.96                                         | 17.49           | 2.59   |
| R4                      | 2                       | 9.91                                          | 14.43           | 45.61  |
| R5                      | 2                       | 14.47                                         | 14.43           | 0.28   |
| R6                      | 2                       | 17.51                                         | 14.43           | 17.59  |
| R7                      | 2                       | 13.68                                         | 14.43           | 5.48   |
| R8                      | 3                       | 28.15                                         | 31.96           | 13.53  |
| R9                      | 0.59                    | 33.10                                         | 32.64           | 1.40   |
| R10                     | 2                       | 16.55                                         | 14.43           | 12.81  |
| R11                     | 3                       | 17.98                                         | 15.08           | 16.13  |
| R12                     | 2                       | 5.83                                          | 13.91           | 30.64  |
| R13                     | 2                       | 21.49                                         | 16.25           | 24.36  |

Optimization method using CCD was done to determine the variables that significantly influence the performance of bioplastics. Bamboo KCl and MFC concentrations function as variables and tensile strength as data response. Data processing results in the form of Analysis of Variance (ANOVA) are presented in Table 2. Values of ‘Prob>F’ less than 0.0500 indicate model terms are significant.
### Table 2. Analysis of Variance (ANOVA) in response to tensile strength data.

| Source      | Sum of Squares | Df | Mean Square | F Value | p-value Prob > F | p-value Prob > F |
|-------------|----------------|----|-------------|---------|------------------|------------------|
| Model       | 419.37         | 7  | 59.91       | 8.17    | **0.0172**       | Significant      |
| A-KCl       | 114.55         | 1  | 114.55      | 15.61   | **0.0108**       | Significant      |
| B-MFC       | 28.70          | 1  | 28.70       | 3.91    | 0.1049           | Not Significant  |
| Residual    | 36.68          | 5  | 7.34        |         |                  |                  |
| Lack of Fit | 1.70           | 1  | 1.70        | 0.19    | 0.6818           | Not significant  |
| Pure Error  | 34.98          | 4  | 8.75        |         |                  |                  |
| Cor Total   | 456.06         | 12 |             |         |                  |                  |

| Std. Dev    | 2.71           | R-Squared | **0.9196** |
| Mean        | 18.58          | Adj R-Squared | 0.8070  |
| C.V. %      | 14.58          | Pred R-Squared | **0.6411**  |
| PRESS       | 163.67         | Adeq Precision  | 9.028   |

The F value and probability value (p-value) indicate the highest potential influence on the response of the factors used. This predicts and determines the optimum conditions of the response. Value (prob > F) less than 0.05 will show a significant response [18]. The value of prob > F of MFC concentration variable more than 0.05 indicates that the MFC factor does not affect the response of the data with the results of the tensile strength in the sample optimum condition is 28.613 MPa. The addition of MFC did not significantly influence the response of the data even though there were changes in tensile strength values, but the changes were not significant [19]. The value (prob > F) of KCl concentration variable of less than 0.05 indicates that the KCl factor of bamboo has a significant effect on the response of the data.

The gelatinization process can form a series of matrices (starch), cross linking (citric acid) and plasticizers (glycerol) consisting of OH group bonds. The phenomenon of increasing value of tensile strength is due to the fact that sago starch as a matrix will form a framework arranged with glycerol which is added as plastic. The added bamboo MFC will fill the spaces of the skeleton and when the gelatinization process occur during heating the bamboo MFC will attach perfectly to the sago starch matrix. The matrix and filler sequence are intertwined because of the bonding of the OH group since both starch and cellulose have many OH groups. Addition of citric acid is also carried out, though it results in a very flexible bioplastic so that tensile strength cannot be measured. This happens because the composition that includes citric acid is less suitable as it increases the bond length of the OH group and makes the bioplastic more flexible.
Optimized data is also displayed in the predicted vs. Actual and 3D graphics. Predicted vs. Actual is used to find out whether the ANOVA model is eligible or not. ANOVA model is said to meet the requirements (significant) if the plot on the graph provides a linear shape closed to diagonal line. Figure 2 shows the Predicted vs. Actual of the response of the tensile strength produced. Experiments in this study show a linear graph meaning that the plot on the graph approaches the diagonal line. So, it can be concluded that the ANOVA model meets the requirements (significant), while three points did not closely approach the diagonal line. These results can be seen also from Table 1.
Figure 3. 3D Model optimization of tensile strength response

The optimization results are presented in the form of a 3D graph in figure 3. From the figure it is known that the optimum tensile strength value is 28.613 MPa with a concentration of 5 % - w/w of bamboo MFC and 3 % - w/w KCl.

3.3. Characterisation of Optimum Bioplastics

Physical measurements structure and morphology were performed on the same bioplastic. The measurements results are presented in figure 4. Based on calculation of the lost mass using the equation, it is found that bioplastic R01 experienced a mass loss of ± 67.95%, from its initial weight ($M_0$) of 0.078 g to 0.025 g ($M_a$). Bioplastics R02 experienced a mass loss of ± 41.25%, originally from 0.080 g to 0.047 g and R2 bioplastics had a mass decline of ± 33.33% from 0.081 g to 0.054 g.

Figure 4. The measurement results of sago starch bioplastic mass
Durability test was carried out to determine the ability of bioplastics produced to be degraded in the soil. The degradation was characterized through mass loss of bioplastic measured every day during the durability test process (45 days). Figure 4 implies that there is a decrease in mass in bioplastics which indicates that there was contact between the environment and the tested bioplastic. This phenomenon can clearly be seen in bioplastics R01 that possesses the greatest mass reduction. Judging from its constituent structure, bioplastic R01 has more OH groups because it was composed only of sago starch which is a material rich in amyllopectin. Amorphous properties of sago starch are caused by branching (1–6) glycosidic tissue with OH groups spreading in each branch. The more OH groups exist, the easier it is to come into contact with water. Therefore, R01 bioplastic had larger mass loss. In bioplastic R02, a decrease occurred however not as significant as R01 bioplastic. In bioplastics R02, bamboo MFC was added as a reinforcing filler. Although, structurally, the bamboo MFC is rich in OH groups, in reality bamboo cellulose possesses a structure which is difficult to mix with water. The addition of bamboo MFC will make the starch matrix more resistant to water. This makes the resulting bioplastic more durable, but since it consists of cellulose, the bioplastic will not pollute the environment. R2 bioplastic experienced mass reduction slower than the two previous bioplastics. Structurally, R2 bioplastic contains KCl which was used as a dispersing agent. Salt can absorb water (hygroscopic) and can suppress water activity (aW) in the material so that microbes cannot breed [20]. KCl possesses high osmotic pressure, so it can absorb water content in bioplastics. Another uniqueness is that KCl salts have hypertonic properties, where the greater concentration of water is in the outside of bioplastics and bioplastics themselves contain low concentrations of water, both of which are separated by semipermeable surfaces. The transfer of molecules from low concentrations to high concentrations is referred to as hypertonic. The water content in the bioplastic R2 moves to the environment so that bioplastics are drier hence the mass decrease of bioplastic is more stable.

4. Conclusion
Potassium chloride (KCl) as a dispersing agent helps reduce the time to make bamboo MFC solution up to ± 1 hour during ultrasonication process. The K⁺ cation is able to stabilize the surface of the mixture so that the homogenization process can reach effectively and efficiently.

Bamboo MFC as a reinforcing agent can significantly increase the tensile strength value where the results of statistical analysis using CCD ($R^2 = 0.9196$) obtained optimum tensile strength of 28.613 MPa, with the optimum condition of 5 % w/w bamboo MFC and 3 % w/w KCl concentration. Physical measurement results (durability) concluded that KCl was able to subdue water activity in bioplastics so as to suppress the proliferation of microorganisms which in turn result in more stable decrease of bioplastic mass.

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