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Original Article

ISOLATION OF CELLULOSE FROM SIWALAN FIBER (*BORASSUS FLABELLIFER* L.) USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Objective: This study investigated the isolation of α-cellulose powder from siwalan fibers (*Borassus flabellifer* L.).

Methods: The methods include delignification using 10% NaOH, and bleaching using 10% NaOCl. The present research used response surface methodology (RSM) to investigate the impact of temperature and duration on the bleaching process. Two factors were evaluated and optimized using the central composite design (CCD).

Results: The findings revealed that the hit quality and white degree were 91.5% and 52.58% when the temperature and time of bleaching were 40 °C and 30 min, respectively. Utilizing an analysis of variance (ANOVA), the most influential factors on the percentage of strike quality and whiteness degree were identified. Absorption at 3323.34 cm^{-1} for O-H, 2893.22 cm^{-1} for C-H, 1371.38 cm^{-1} for C-O-H, and 1157.28 cm^{-1} for C-O, according to FTIR characterization results, are characteristic cellulose peaks.

Conclusion: The cellulose characterization results acquired in this work show that the temperature in the bleaching process has a significant effect on the cellulose isolation results.

Keywords: α-cellulose, Siwalan fiber, Response surface methodology

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INTRODUCTION

Indonesia boasts the most biodiversity in the world, which provides enormous potential advantages for the nation. Hence, it is imperative to undertake endeavors aimed at devising strategies for the transformation of the nation's natural resources into higher-value commodities, encompassing veterinary foods, timber, paper, fibers, textiles, cosmetics, and pharmaceutical items [1].

Siwalan (*Borrasus flabellifer*) is a representative indigenous plant species found in arid regions of Indonesia, East Java (Madura, Tuban), NTT (Timor, Sumbawa, Rote, Sabu), Southeast Maluku, and South Sulawesi are the most affected. Following this, it can be observed that the Semanding district in Tuban City has the greatest proportion of the aforementioned plant. However, it is worth noting that the effective use of its plantation waste remains incomplete [2]**.** The use of trash as a food ingredient is primarily driven by its significant nutritional content. However, once subjected to processing, it is frequently discarded as a waste of little economic worth. In addition, the composition of the substance includes 68.94% cellulose, 14.03% hemicellulose, 5.37% lignin, and 0.6% wax [3]. Cellulose, an abundant polysaccharide, is a prominent component found in all plant products. It has the desirable characteristics of being both renewable and biodegradable. Photosynthetic processes routinely renew around half to one-third of plant tissues [4].

Nevertheless, environmental hazards are ubiquitous occurrences on a worldwide scale and are inherently intertwined with human existence. The occurrence of this phenomenon is frequently instigated by the human inclination to exploit the natural environment, coupled with the inappropriate use and disposal of waste materials. Hence, the optimization of siwalan waste utilization may be achieved by the advancement of research pertaining to the manufacture of cellulose derived from the peel of siwalan fruit. Cellulose, represented by the chemical formula $(C_6H_{10}O_5)n$, plays a pivotal role as a fundamental component in the manufacturing of many pharmaceutical products, cosmetics, biodegradable polymers, and materials used for packaging food. Cellulose is a commonly seen component in plants, acting as a core component that is critical to the development of cell walls. Moreover, the substance in question is classified as a natural polymer due to its linear molecular arrangement and crystalline morphology. However, it exhibits limited solubility. Cellulose is a polysaccharide comprised of glucose units that are connected by 1,4-glycosidic linkages, leading to the creation of an extensive and unbranched chain [5].

Various methods can be employed to isolate cellulose, which can be categorized into mechanical techniques such as high-pressure and ultrasonic approaches, chemical techniques including organoleptic, strong acid hydrolysis, alkaline solvent, ionic liquids, and oxidation procedures, as well as a biological approach involving the use of enzymes. The chemical cellulose separation procedure was employed as the methodology in this particular investigation [6]. The procedure was initiated by employing solid acids, including sulfuric acid, hydrochloric acid, and perchloric acid, for the process of hydrolysis. The amorphous component of prolonged plant fiber is effectively hydrolyzed by strong acids, resulting in its fragmentation. Additionally, they aid in the hydrolysis of hemicellulose, resulting in the production of xylose and other sugar compounds. Additionally, the subsequent phase is the elimination of lignin, known as delignification, by using a sodium hydroxide (NaOH) solution. In the third stage, a bleaching treatment is used to hasten the breakdown of lignin and other impurities that haven't been removed throughout the chemical process. Bleaching often involves the use of oxidizing chemical agents like hydrogen peroxide and hypochlorite. The obtained cellulose was then subjected to Fourier Transform Infrared Spectroscopy (FTIR) for characterization. A comparison of the cellulose isolate from the siwalan fruit's peel and the synthetic cellulose from Sigma-Aldrich® allowed researchers to assess the effectiveness of the synthesis [7].

Researchers aimed to investigated to isolation of α-cellulose powder from siwalan fibers (SF). Optimization was carried out based on a statistical approach using the Response Surface Method (RSM). The

RSM method is an effective model for optimizing processes in biochemistry, physics, and biotechnology [8-10]. This research uses the RSM method to determine the appropriate model to obtain the optimization point or target point for process variables in α cellulose isolation. The variable optimization step was conducted using the Central Composite approach (CCD) experimental approach, with the objective of identifying the optimal values for the process variables involved in the siwalan isolation process. Hence, the fundamental goal of this research is to look at how temperature and reaction duration affect cellulose yield, whiteness degree, and tensile strength of siwalan fiber insulation.

MATERIALS AND METHODS

Materials

Siwalan fiber (*Borassus flabellifer* L) was collected from Tuban, East Java, Indonesia. Grade of 10% sodium hydroxide (NaOH) and 10% sodium hypochlorite (NaOCl) were purchased from Merck.

Plant determination

Plant determination was intended to determine the identity of the sample. The experiment was conducted in the School of Life Sciences and Technology Laboratory at Bandung Institute of Technology (ITB).

Preparation of sample

The siwalan fibers obtained are weighed to determine their wet weight. Then washed and dried. The drying process is carried out with the help of sunlight. After the siwalan fibers are dry, then are reduced in size±60 mesh using a grinder/rolling machine. The obtained siwalan fiber powder is then sifted or filtered using a shave and sieve size of±60 mesh and weighed. After that, it is weighed to find out the dry weight. The obtained siwalan fiber powder is stored in sealed containers and stored in cabinets.

Cellulose isolation from siwalan fiber

The separation of cellulose begins with delignification. 50 g of siwalan powder was delignified by dissolving it in a 10% NaOH solution (w/v) for 30 min at 85 °C. Then it is filtered and washed under running water until it reaches a neutral temperature. The resulting fiber is then dissolved with 10% NaOCl with temperature variations of 40 °C to 60 °C and a time of 30 to 60 min. The bleaching process, the resulting fiber is washed and filtered using running water to a neutral temperature. The fibers are then desiccated in an oven at 60 °C for 24 h [11].

Response surface methodology

Two factors were revealed to evaluate the optimal condition for hit quality and whiteness index, namely temperature and time of bleaching process. Th temperature used an interval of 40–60 °C; and the independent variables used for this study were bleaching times ranging from 30 to 60 min. Central Composite Design (CCD) Expert® 7.0.0 software was used to disseminate the data. F-test or 95% confidence level analysis of variance (ANOVA) with p-value (P = 0.05) is used to determine whether the independent variable has a significant influence when compared to the dependent variable. Graph plots are used to see interactions between independent and dependent variables. Numerical optimization is calculated in the software.

Whiteness degree

The measurement of the whiteness level of the siwalan fiber and cellulose was conducted by assessing the lightness (L), yellowness (b), and redness (a) values using a chromameter (Konica Minolta CR-400, Japan). The quantification of whiteness was conducted by the use of Hunter's L, a, and b values inside the Hunter space formula. The white degree indicates the ability of the material to reflect light hitting the aforementioned material. The higher the white degree value, the whiter the resulting color [12]. Using the formula:

$$\text{Whiteness degree (\%)} = 100-[(100-L)^2+a^2+b^2]^{0.5} \quad \text{……… (1)}$$

Characterization using fourier-transform infrared spectrometry (FTIR)

The ATR-FTIR investigation was conducted with an FTIR spectrometer (FTIR bruker RS 1150000) it was equipped with an ATR (attenuated total reflectance) array made of diamond. The powder fibers, which had undergone water treatment, alkali treatment, and bleaching, as well as cellulose, were immediately positioned on the attenuated total reflectance (ATR) sampler. Fourier-transform infrared (FTIR) spectra were then obtained within the 4000-650 cm^{-1} wavenumber range [12].

RESULTS AND DISCUSSION

Plant determination

The outcomes of the research carried out at the Faculty of Life Sciences and Technology Laboratory at the Bandung Institute of Technology (ITB), based on number 5400/IT1.C11.2/TA.00/2023, explained that the sample used was the siwalan plant (*Borassus flabellifer* L.) from the Arecaceae family.

Experimental design

The study's goal was to explore the correlation between hit quality and worthiness index via the use of Response Surface Methodology (RSM). The investigation concentrated on determining the impact of parameter variables, namely temperature and duration of the bleaching process. The findings of the experimental design, as shown in table 1, indicate that the process factors under investigation have a considerable impact on both the hit quality and worthiness. This is evident from the model F-values of 8.65 and 7.06, which exceed the critical value at a confidence level of 0.05%.

Table 1: The result of the experimental design to isolation of cellulose from siwalan fiber

No	Temperature (°C)	Bleaching time (min)	Whiteness degree (%)	Hit quality (%)
1	40	30	52.58	91.5
2	80	30	36.57	30.2
3	40	60	60.49	72.5
4	80	60	23.87	50.2
5	31.7	45	69.87	84.9
6	88.3	45	25.01	58.1
7	60	23.8	24.72	36.2
8	60	66.2	40.82	88.2
9	60	45	42.49	32.2
10	60	45	37.68	32.4
11.	60	45	23.37	32.3
12.	60	45	26.36	32.4
13.	60	45	30.47	32.5

A total of 13 experimental runs were carried out in the current investigation, with five replications at center sites, as per the specified parameters [13]. From design experiment no. 9-13 shows the similarity of temperature conditions and bleaching time, namely at 60 °C and 45 min. This is because these conditions are the center points of the temperature range of 40-80 °C and 30-60 min [13]. In the context of

CCD design, it is common practice to replicate center points between 4 to 6 times in order to get a reliable estimation of experimental error, also known as pure error. By default, the system generates five central spots. The variable α encompasses a range of values, including negative, zero, and positive values for each element, namely-1, 0, and 1 [14]. From the results of the experimental design, it is known that the optimum conditions for the cellulose isolation process from siwalan fiber are at a temperature of 40 °C and a bleaching time for 30 min, with a hit quality of 91.5%. The degradation sequence of the fiber constituents was affected by increasing the heating temperature. The susceptibility of hemicellulose to degradation was shown to be greater than that of cellulose at lower temperatures. Consequently, the process of deacetylation was used to initiate the degradation of hemicellulose. This involved the cleavage of previously linked acetyl groups to the xylan backbone, resulting in the liberation of acetic acid. The depolymerization process was accelerated by the loss of side chains, leading to the disintegration of the polysaccharide. Consequently, the hydroxyl group experienced reduction, resulting in a high quality rating for the hit [15].

Thirteen experiments were conducted as shown in table 1, and the resulting data was analyzed using CCD expert software to find the fitting parameters of the regression model. The perturbation plot model serves as a visual depiction of the effects of several variables on a single Design of Experiment (DoE) point. The arrangement of axes in question provides a higher degree of freedom compared to contour plots and 3D graphs [16].

Based on these results, the use of the response surface approach in both research and industry offers many notable benefits: the statistical analysis conducted was executed seamlessly. Enhancement of a manufacturing system, method, or product via visualization, arrangement and config. uration of the experimental layout and design. Then, the presentation of predictions and the interaction between variables may be effectively conveyed through the use of visually intuitive representations, such as clear curves and other visual aids. As well as, the visualization of surface plots, graphs, and other visual aids allows for effective representation and visualization of answers or findings [17].

A variance analysis (ANOVA)

Whiteness degree

To evaluate the accuracy of the model created from the experiment's results, ANOVA was employed in the design of the experiment [18]. The ANOVA results showed that the bleaching temperature treatment had a very significant effect ($p<0.01$), the duration of the bleaching process had a statistically insignificant impact ($p<0.05$) on the level of whiteness observed in the cellulose extracted from siwalan fiber.

Based on the results in table 1 showed that the value of the whiteness degree of cellulose from siwalan fibers is highest obtained in a combination treatment of temperature 31.72°C and 45 min time of 69.87%. The combination of 60 bleaching temperature sessions and 45 min resulted in the lowest whiteness degree, measuring at 8.97%. The findings of this study indicate that there is an inverse relationship between the degree of whiteness of cellulose and the temperature and duration of the bleaching process. Specifically, higher temperatures and longer durations of bleaching result in a drop in the degree of whiteness of cellulose. Conversely, lower temperatures and shorter durations of the bleaching process lead to an increase in the degree of whiteness of cellulose. The level of whiteness index exhibits a decrease subsequent to reaching 40 °C, and afterwards continues to reduce as the temperature further increases. The observed result may be ascribed to a reduction in the stability of sodium hypochlorite. This is in accordance with Meghdad's research [19] which states that the higher the bleaching temperature, the lower the whiteness index of cellulose and conversely the lower temperature, the higher the whiteness index.

Characterization of cellulose from siwalan fiber using fourier-transform infrared spectrometer (FTIR)

The Fourier Transform Infrared (FTIR) analysis indicated the presence of absorption bands at certain wavenumbers, namely 3323.34 cm^{-1} for O-H, 2893.22 cm^{-1} for C-H, 1371.38 cm^{-1} for C-O-H, and 1157.28 cm^{-1} for C-O. These observed values are consistent with the characteristic peaks associated with cellulose. The findings indicated that the cellulose derived from siwalan fibers has certain resemblances to the conventional synthetic cellulose (fig. 2).

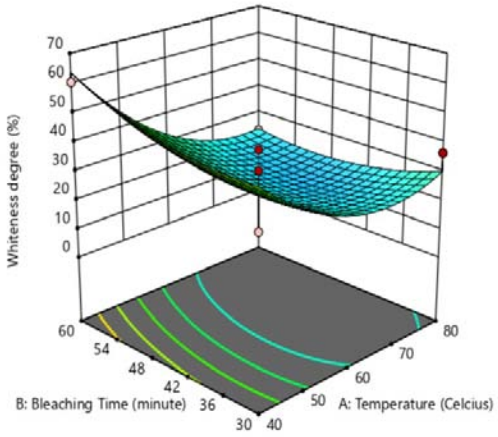

Fig. 1: Model graph of the effect between two factors (temperature and bleaching time) on whiteness degrees

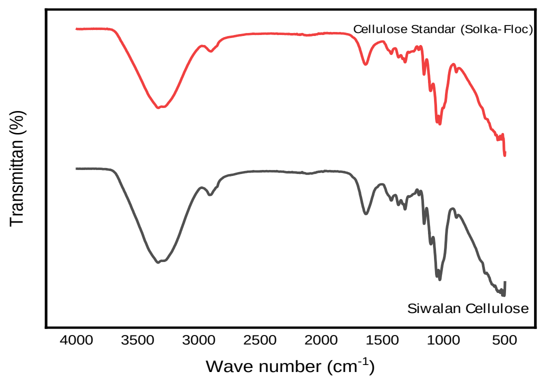

Fig. 2: Graphic spectra FTIR of siwalan fiber cellulose with 91.5 % of hit quality (black) and standard cellulose (Red)

The analysis of variance (ANOVA) findings revealed that the treatment, including variations in bleaching temperature, had a very significant impact ($p<0.01$) on the hit quality of siwalan cellulose, as determined using Fourier-transform infrared spectroscopy (FTIR). Conversely, the effect of bleaching duration was found to be negligible ($p<0.05$).

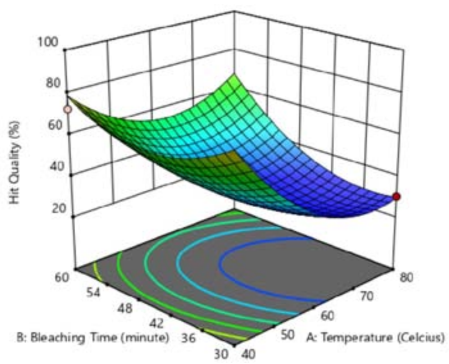

Fig. 3: Model graph of the effect between two factors (temperature and bleaching time) on Hit quality

The graph shown in fig. 3 illustrates the relationship between the parameters of temperature and bleaching time and their impact on Hit Quality. The analysis revealed that temperature has a statistically significant influence on Hit Quality, as indicated by a p-value of less than 0.05. The graph illustrates a negative correlation between bleaching temperature and hit quality, indicating that as the temperature increases, the quality of the hit decreases. At a temperature of 80 °C, the hit quality is around 30%, but at a temperature of 40 °C, the hit quality increases to almost 90%.

CONCLUSION

The present work successfully used the delignification and bleaching methods to extract cellulose from siwalan fiber. The current investigation used the Response Surface Methodology (RSM) technique to analyze the impacts of several factors in the bleaching process. The variables that were chosen for analysis were time and temperature. Utilizing the central composite design (CCD) allowed us to refine and modify the design matrix. The results of the study revealed that when exposed to a temperature of 40 °C for a length of 30 min, the ensuing quality of the bleaching process was measured at 91.5%, while the degree of whiteness achieved was recorded at 52.58%. The results of this investigation indicate that the temperature used during the bleaching procedure significantly influences the outcomes of cellulose isolation.

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AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

Declared none

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