

# Optimization of Operating Conditions in Lignin Isolation Process of Rice Straw Using Box-Behnken Design Methodology

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## ABSTRACT

Exposure to sunlight causes ultraviolet (UV) radiation, which can damage the body cells. Those negative effects can be prevented by using sunscreen, which contains lignin. Lignin has been proven to absorb or reflect UV radiation effectively. Rice straw was being used as a non-synthetic active ingredient in sunscreen production. This research shows that during the operating conditions of the isolation process, several variables affect yield production, such as the mass of rice straw, temperature and time of isolation. Therefore, this research was carried out to optimize the lignin isolation process using the Box-Behnken Design (BBD) methodology. Rice straw dregs that have undergone sample preparation were subsequently isolated through hydrolysis using 5% (w/v) NaOH. After that, the product was carried out to the next process, namely acidification, which used 72% (v/v) H<sub>2</sub>SO<sub>4</sub>. The resulting precipitate was then filtered and dried in an oven. Subsequently, the fiber content of the product was analyzed using the Van Soest analysis method, while the process conditions were evaluated using the BBD methodology. In this research, the highest lignin yield obtained was 17.04%, and the optimal process conditions used were 30 g mass of straw, 140°C, and 3 hours reaction time.

**Keywords:** Box-Behnken methodology, isolation, lignin, optimization, sunscreen, ultraviolet radiation.

## 1. INTRODUCTION

Exposure to sunlight can produce ultraviolet (UV) radiation. UV light radiation triggers skin cancer, accelerated skin aging, and free radicals that can damage body [1]. Free radicals are reactive because they are caused by unpaired electrons [2]. The adverse effects of sun exposure and free radicals can be prevented using antioxidants and sunscreen [3].

Antioxidants stabilize free radicals by complementing the lack of electrons from free radicals to inhibit chain reactions. Antioxidant compounds can inhibit oxidation reactions by binding free radicals and highly reactive molecules. They are essential in protecting the body from the effects of free radicals that can cause cell damage [1].

Lignin is a natural phenolic polymer compound. Lignin has been known to have a

phenolic hydroxyl group, so it allows lignin to act as an antioxidant that has been proven to be effective in absorbing or reflecting most of the UV radiation [3]. In general, lignin is co-distributed with hemicellulose in lignocellulose, which forms part of plants' primary and secondary cell walls and is covalently connected to cellulose structures. Lignin plays a role in maintaining the stiffness and strength of the stem, makes the cell wall resistant to water, and protects against pathogens [4]. So, lignin can act as an active ingredient for sunscreen.

Rice straw is composed of cellulose (32–47%), hemicellulose (19–27%), and lignin (5–24%) [5]. This content can make rice straw an active ingredient with lignin content as a raw material for sunscreen. Several researchers have researched lignin isolation, using various lignin sources and isolation

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methods to produce different yields. In the Klason method, the yield produced is greater (24.9%) than using the Wilsatter method (22.6%) [6]. The nanoprecipitation method was reported in 2015 to produce lignin nanoparticles. There are two types of lignin used, namely dioxane lignin nanoparticles (DLNP) and alkali lignin nanoparticles (ALNP), which are made from two different lignin sources, namely hardwood dioxane lignin (DL) and softwood alkali lignin (AL). DLNP and ALNP have higher oxidant activity when compared to their parent polymers, namely DL and AL. The UV protective potential of DLNP and ALNP was validated by monitoring the survival rate of *Escherichia coli* in UV light. DLNP and ALNP were more efficient than DL and AL in protecting *E. coli* against UV irradiation-induced death. However, after irradiation for different periods, DLNP offered significant protection for *E. coli* against UV compared to ALNP [7]. Then, Irawan et al. [8] synthesized lignin from coconut fibre using the alkali delignification method and then carried out the isolation process under acidic conditions. The results showed that the yield produced was high, namely 37.16%. In this study, coconut fibre was chosen as the raw material for making lignin because of its high lignin content and its abundant amount in nature. Lignin synthesis by extraction from sawdust, then hydrolyzed and identified by FTIR, has also been carried out by Kusumo et al. Isolation was carried out with a sample weight of 10 grams, a time of 2 hours, a temperature of 110°C and variations in NaOH concentration. Meanwhile, the yield of lignin produced was 0.106% [9]. In addition, from research conducted by Sari et al., the yield of lignin from rice straw was also obtained by 22% of the total lignin content in rice straw of 24% [3].

Based on these studies, the yield of lignin obtained has different values using different operating conditions. Therefore, it can be concluded that the operating conditions in obtaining lignin or isolating lignin are one of the important variables in producing high lignin yield using response surface

methodology, especially using box-Behnken methodology. This method has been used in a lot of experiments which optimized 3 factors of operating condition with less total run of experiment than the other methodology [10-21]. Thus, in this study, process parameters or operating conditions were optimized in lignin isolation using the Box Behnken Design (BBD) Methodology to obtain the highest lignin yield.

## 2. METHODS

The materials were 99.5% Ethanol, NaOH, and 98% H<sub>2</sub>SO<sub>4</sub>. These materials were purchased in Merck and used without purification. While, biomass used in this work was rice straw.

This research consisted of 2 stages. First, rice straw was extracted with soxhlet using 80% (v/v) ethanol to remove impurities. The second stage was the lignin optimization process, which performed variable inputs. The mass of straw was 10-30 g, the temperature was 80-140°C, and the time of 1-6 hours in the Design Expert 13<sup>th</sup> trial version software as can be seen in Table 1.

**Table 1.** Experimental data variables.

Factors	Unit	Low Level	High Level
A- Mass of Rice Straw	gram	10	30
B- Temperature	°C	80	140
C- Reaction Time	hours	1	6

17 experimental variables were obtained for the optimization process by hydrolysis using 5% (w/v) NaOH. This hydrolysis process was conducted in a reflux extractor. Hydrolysis results in the form of black leachate that was acidified by using 15 ml of 72% (v/v) H<sub>2</sub>SO<sub>4</sub>. Then, the solid precipitated was separated using a centrifuge. The solid separation was taken by filtering the solution using filter paper, which was then washed with equates until neutral. The resulting lignin was removed from the moisture content, and the yield could be calculated. Then, the estimated yield value was input as the response in Design-Expert 13<sup>th</sup> trial version software,

which was to be analyzed using the BBD methodology.

### 3. RESULTS AND DISCUSSION

Research on lignin isolation or delignification processes has been widely conducted on various types of biomasses. The delignification method can be done physically, chemically, and biologically. Although both can lower lignin levels, physical and biological delignification treatments are less effective than chemical treatments. Physical and biological delignification reduces lignin levels in sengon wood powder by about 17-21% and in palm leaf fronds by about 5-6.5% while chemical delignification can reduce lignin levels in sengon wood belts by 35% and 29% in palm leaf fronds [22]. This suggests that the use of chemicals is more effective in breaking lignocellulose bonds and dissolving lignin from biomass.

The chemical delignification process can use a wide variety of compounds such as acidic or alkaline compounds. The most widely used acid compound is  $H_2SO_4$ . Some studies have also used  $HNO_3$ ,  $HCOOH$ , and  $HCl$ . However, these three types of acids are less effective in separating lignin from biomass. Meanwhile, the most widely used alkaline compounds are  $NaOH$ ,  $Ca(OH)_2$ , and  $NH_4OH$ . The use of  $NH_4OH$  is generally chosen if the focus of the desired product is cellulose as this base is known to be quite selective in removing lignin and hemicellulose simultaneously whereas  $NaOH$  is known to be effective in separating lignin from lignocellulose biomass. Unlike the previous two alkaline compounds which can remove lignin in a relatively short time, the process of delignification with  $Ca(OH)_2$  can take weeks but the cost required is lower and does not produce a residue of sodium salt compounds [23].

Research on the comparison of the effectiveness of the delignification process using acid and alkaline compounds has been conducted. In the process of delignification of various types of grass, it is known that

$NaOH$  has a higher ability than  $H_2SO_4$  in separating lignin from biomass [23]. The use of  $NaOH$  in elephant grass can separate 88% of lignin and 94% lignin in king grass [24,25]. This result is likely due to  $NaOH$ 's ability to break down lignocellulose bonds which can also simultaneously act as a catalyst in the hydrolysis process

Besides the selection of compounds used for the delignification process, the determination of the process conditions also greatly affects the results. To obtain lignin from teak wood powder require 250ml  $NaOH$  12%, 10 grams sample, and hydrolysis time of 2 hours at  $110^\circ C$  [9]. In addition to the  $NaOH$  concentration and time factors, the temperature and mass of the raw materials also have a significant influence. Each type of sample responds differently to variations in  $NaOH$  concentration, sample mass, temperature, and operating time. For example, to isolate lignin maximally in the Palm Kernel Shell (PKS), 25%  $NaOH$  (w/v), a mass ratio 1:40, and hydrolysis temperature of  $130^\circ C$  for 120 minutes are required. Meanwhile, in Ground Nutshell (GNS) samples, lignin can be isolated maximally using 20%  $NaOH$  (w/v) with a mass ratio of 1:20, and a hydrolysis temperature of  $130^\circ C$  for 120 minutes [26].

The research design for the optimization of lignin isolation operating conditions can use BBD methodology, while the data analysis can be carried out using the Response Surface Methodology (RSM) method. In this study, optimal process parameters were obtained and analyzed using the BBD methodology. This method combines mathematical and statistical techniques to find the optimal condition of response influenced by independent variables. The parameters of straw mass, temperature, and hydrolysis time were statistically analyzed using the BBD method. The number of experiments and yield responses, both predictive and experimental analysis, are shown in Table 2.

**Table 2.** Factor matrix and yield response to lignin isolation process.

Run	Factor			Responses (yield %)		
	<i>m</i> (g)	<i>T</i> (°C)	<i>t</i> (h)	Observed	Predicted	Residual
1	20	140	6	8.65	9.11	-0.4582
2	10	110	6	2.10	3.03	-0.9309
3	20	140	1	6.85	8.62	-1.77
4	20	80	1	0.5950	1.55	-0.9509
5	30	110	1	9.16	8.75	0.4098
6	20	110	3.5	6.05	5.90	0.1479
7	20	110	3.5	5.83	5.90	-0.0771
8	20	110	3.5	2.65	5.90	-3.25
9	20	110	3.5	8.26	5.90	2.35
10	10	140	3.5	3.90	2.29	1.61
11	30	110	6	9.50	10.41	-0.9067
12	30	80	3.5	4.47	3.71	0.7662
13	20	110	3.5	5.83	5.90	-0.0771
14	30	140	3.5	17.04	15.45	1.59
15	20	80	6	4.70	4.33	0.3656
16	10	110	1	1.81	1.42	0.3856
17	10	80	3.5	2.96	2.17	0.7904

Note: *m* is mass of rice straw (g), *T* is the temperature (°C), and *t* is time reaction (hours).

### 3.1. Summary Statistic Model

Design-Expert analyzes graph models such as linear, 2FI (two-factor interaction), quadratic, and cubic from the response surface on the data. The recommended model is shown with bold data in Table 3.

**Table 3.** Model summary of statistics.

Source	Std Dev.	Sequential p-value	R <sup>2</sup>	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>
Linear	2.22	0.0004	0.7417	0.6820	0.5220
<b>2FI</b>	<b>1.70</b>	<b>0.0401</b>	<b>0.8834</b>	<b>0.8135</b>	<b>0.6620</b>
Quadratic	1.72	0.4860	0.9161	0.8082	0.5875
Cubic	2.00	0.7591	0.9356	0.7423	

The 2FI (two-factor interaction) model has a sequential p-value of less than 0.05 and has smaller standard deviation than the other models. In addition, the adjusted R<sup>2</sup> value is 0.8135, and the predicted R<sup>2</sup> value is 0.6620, where this value is close to 1. Moreover, the

coefficient of determination (R<sup>2</sup>) has a value of 0.8834, where this value is close to 1, which means that the degree of relationship between the experimental data and the optimization model is closely related [10,14,27,28]. Thus, the summary statistical model recommends that Design-Expert use the 2FI (two-factor interaction) model to optimize the parameters of the lignin isolation process from rice straw.

### 3.2. Lignin Variant Analysis and Isolation Parameters

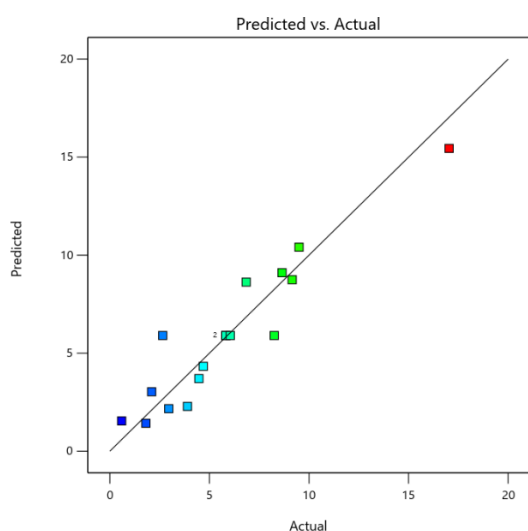
The ANOVA results in Table 4 show that response parameters significantly affect the yield of lignin isolation. The significance of this model is determined from the p-value and F-value of the ANOVA, where a p-value of 0.0004 and F-value of about 12.63 also show that the model is significant. A p-value less than 0.05 and the F-value results indicate significant models [13].

**Table 4.** Analysis of ANOVA variants on response results.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
<b>Model</b>	218.72	6	36.45	12.63	0.0004	significant
A	108.01	1	108.01	37.42	0.0001	
B	70.28	1	70.28	24.35	0.0006	
C	5.34	1	5.34	1.85	0.2038	
AB	33.77	1	33.77	11.70	0.0065	
AC	0.0007	1	0.0007	0.0003	0.9876	
BC	1.32	1	1.32	0.4590	0.5135	
<b>Residual</b>	28.86	10	2.89			
<b>Cor Total</b>	247.58	16				

A comparison between the experimental results and the predicted model is also used to confirm the model using the graphical method, as shown in Figure 1.

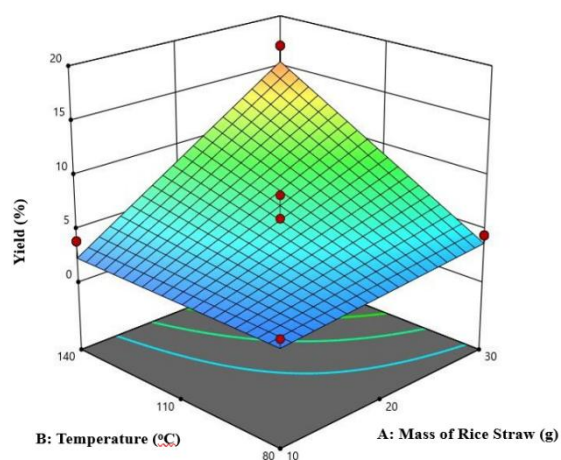
Figure 1 shows that the relationship between the experimental data and the optimization model cuts past linear regression. This figure proves that the results of model optimization and experiments have a slope close to 1, so the line looks straight. A straight line strongly correlates the experiment and the predicted model value [17]. Thus, the line confirms that the optimization model can indicate the optimum yield from lignin isolation from rice straw.



**Figure 1.** Comparison of data between prediction model and lignin isolation experiment from rice straw.

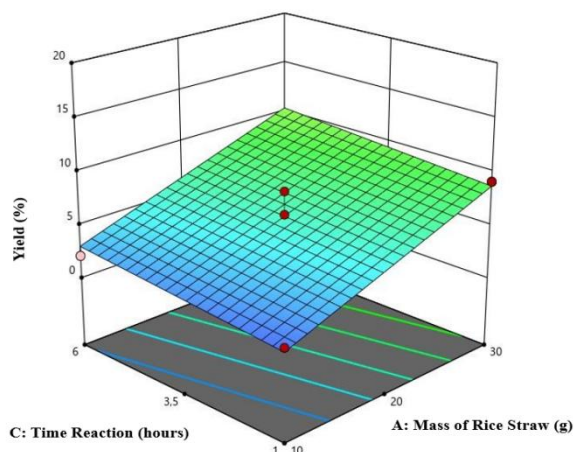
### 3.3. Effect of Interaction of Lignin Isolation Process Parameters from Rice Straw

Figure 2 shows the influence of the interaction between the hydrolysis process temperature and the mass of the straw used. The high process temperature can affect the yield of lignin obtained and the mass of rice straw; the higher the temperature, the higher the yield value.

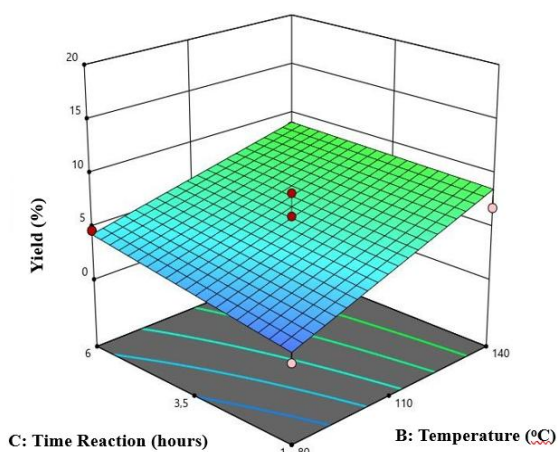


**Figure 2.** Interaction between process parameters A and B.

The interplay between hydrolysis duration and straw quantity on the lignin yield was illustrated in Figure 3. As the isolation process extends, the resulting lignin yields increases. Similarly, a greater mass of straw input leads to a higher lignin yield.



**Figure 3.** Interaction between process parameters A and C.



**Figure 4.** Interaction between process parameters B and C.

Meanwhile, Figure 4 demonstrates how the combination of hydrolysis time and temperature affects the outcome. The illustration indicates that employing both a high process temperature and a prolonged duration of hydrolysis can lead to significant yield values.

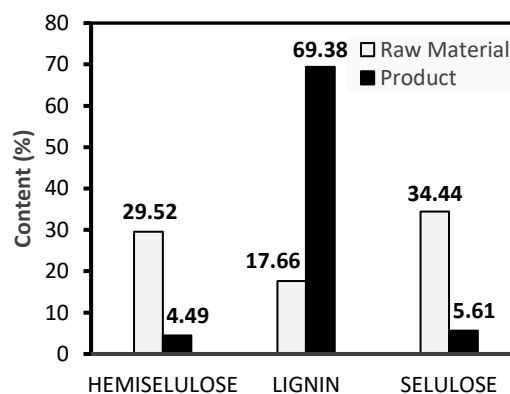
### 3.4. Verification and Validation for Optimization of Lignin Isolation Process Parameters from Straw

After an optimization study was carried out on the parameters of the lignin isolation process from rice straw using the BBD response surface methodology, the optimum parameters to produce the highest lignin yield were obtained by using a straw mass of 30 g

at a temperature of 140°C, and a reaction time of 3.5 hours with a lignin yield of 17.04%. This design also has suggested using 30 g of rice straw, 140°C of temperature process and 5.9961 hours time reaction which has produced 15.7028% with a desirability value of 0.919.

### 3.5. Lignin Content of Product

Rice straw contains a complex lignocellulosic structure consisting of cellulose, hemicellulose, and lignin components. This complex lignocellulosic structure must first be degraded to release lignin from the lignocellulosic structure. After that, hydrolysis is carried out to separate lignin from cellulose and hemicellulose. The Van Soest Test was conducted to determine the lignin content obtained from the isolation process. The graph is presented in Figure 5.



**Figure 5.** Content of raw material and isolation product.

Figure 5 shows that rice straw has a hemicellulose 29.52%, lignin 17.66%, cellulose 34.44%. The quality of the straw itself can cause a difference in content from the straw sample and depends on the sample selection. Van soest analyzed result that the lignin content after the isolation process has increased by 69.38%. This is because NaOH can degrade the lignin structure and separate parts of the hemicellulose. In this process, the OH<sup>-</sup> ions from NaOH will break the bonds of the basic structure of lignin, while the Na<sup>+</sup> ion will bind with lignin to form sodium

phenolate; phenolic salts are easily soluble. Dissolved lignin is characterized by a black color in the solution [3]. The percentage of hemicellulose before and after the isolation process decreased by 4.49%. This is because hemicellulose is soluble in solution-soluble alkali.

#### 4. CONCLUSION

Optimization of the lignin isolation process from rice straw using the Box-Behnken Design Methodology has suggested optimal parameters to produce the highest lignin. The highest yield of lignin from this experiment was obtained with the process parameter, namely straw mass 30 g at a temperature of 140°C, and a reaction time of 5.9961 hours with the yield of lignin by 15.7028% with a desirability value of 0.919.

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