Indonesian Journal of Chemical Research

http://ojs3.unpatti.ac.id/index.php/ijcr Indo. J. Chem. Res., 13 (1), 46-56, 2025

Optimization Of Hydrolysis Of Gayam Peel Waste Using The Acid Method Into Glucose

Sani, Umar Wirayudha*, Ainur Rofiq, Suprihatin

Department of Chemical Engineering, Faculty of Engineering and Science, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Jl. Rungkut Madya, Gn. Anyar, Surabaya 60294, Indonesia

Corresponding Author: umarwirayudha09@gmail.com

Received: March 2025
Received in revised: April 2025
Accepted: May 2025
Available online: May 2025

Abstract

This study aims to optimize the hydrolysis process of gayam fruit peel waste using the acid method to produce glucose, which serves as a raw material for bioethanol production. Gayam peel waste contains a high amount of cellulose, which can be converted into glucose through acid hydrolysis. The process began with a delignification step using an 8% NaOH solution at 80°C for 60 minutes to reduce the lignin content. This was followed by hydrolysis using hydrochloric acid (HCl) with varying concentrations (1, 2, 3, 4, and 5 N) and durations (90, 120, 150, 180, and 210 minutes). Optimization was performed using the Response Surface Methodology (RSM) with Minitab 18 software. The RSM results indicated that the highest glucose yield was achieved at a 5 N HCl concentration and 156.67 minutes of hydrolysis time, with a predicted glucose content of 24.37%. Validation using UV-Vis spectrophotometry and the Nelson-Somogyi method showed an actual glucose content of 4.7217% or 47.217 ppm. These findings demonstrate that gayam fruit peel waste can be efficiently converted into reducing sugars and holds significant potential as an environmentally friendly raw material for bioethanol production.

Keywords: cellulose, glucose, hydrolysis, RSM

INTRODUCTION

Indonesia has abundant biodiversity, including the gayam plant (Inocarpus fagifer), which is typically used only for its seeds, while the fruit peel is often discarded as waste. However, studies have shown that gayam fruit peel contains high levels of cellulose, making it a promising raw material for glucose production through acid hydrolysis (Santhi et al., 2022). With the increasing demand for energy and the depletion of fossil fuel reserves, the development of alternative energy sources that are more environmentally friendly and sustainable is essential. One solution that has been explored is bioethanol, a renewable fuel produced through glucose fermentation. Bioethanol offers several advantages over fossil fuels, including reduced environmental impact and the potential to be produced from various biomass sources, including agricultural and industrial waste (Khurniawati et al., 2019).

To efficiently produce bioethanol, raw materials rich in glucose are necessary, and this can be achieved through the conversion of cellulose. Cellulose, a complex polymer, must be broken down into glucose monomers via hydrolysis, which can be accomplished using either an acid catalyst or enzymes. Acid hydrolysis is an attractive method due to its relatively lower cost and faster processing time compared to enzymatic hydrolysis. Previous studies have demonstrated the effectiveness of acid hydrolysis in converting biomass into glucose. For instance, the hydrolysis of elephant grass using hydrochloric acid resulted in a glucose content of 90.54% when using 3 N acid for 210 minutes (Khurniawati et al., 2019). Similarly, Ayuni and Hastini (2020) reported that hydrolysis of coconut fiber with 4 M HCl for 90 minutes resulted in an optimal glucose yield of 1,445.74 mg/L.

These studies indicate that the acid concentration and hydrolysis time are key factors influencing the efficiency of cellulose-to-glucose conversion. However, there has been limited research focusing on the hydrolysis of lesser-known biomass sources, such as gayam fruit peel. While much research has been dedicated to more common feedstocks like elephant grass and coconut fiber, the potential of gayam fruit peel remains underexplored. The use of this biomass could significantly expand the range of materials available for bioethanol production.

Therefore, this study aims to fill this gap by utilizing gayam fruit peel waste as an alternative cellulose source for glucose production through acid hydrolysis. Unlike previous studies that have focused on single-variable optimization approaches, this research applies Response Surface Methodology (RSM) to evaluate the effects of two critical parameters—acid concentration (1 to 5 N) and hydrolysis time (90 to 210 minutes) on glucose yield. RSM offers a more comprehensive and statistically robust method for optimizing these process parameters (Wyantuti et al., 2020).

In conclusion, the novelty of this research lies in the utilization of an underutilized agricultural waste—gayam fruit peel—as a new feedstock for glucose production, and the application of RSM to optimize hydrolysis conditions. Scientifically, this study expands the range of potential lignocellulosic materials for bioethanol production, while practically, it contributes to agricultural waste valorization, supports local bioethanol development, and promotes environmentally sustainable energy solutions.

METHODOLOGY

Materials and Instrumentals

The materials needed in this study are gayam fruit peel waste, NaOH, HCl, Aquadest. While the equipment used in this study are clamps, stands, thermometers, beaker glass, magnetic stirrer, 80 mesh sieve, oven, filter paper, stirring rod, glass funnel, measuring cup, analytical balance, baking pan, aluminum foil, Erlenmeyer flask. Then the instruments used in this experiment include initial content testing using Acid Detergent Fiber, and Neutral Detergent Fiber. then instrument for testing glucose levels using a refractometer and UV-Vis spectrophotometer of the type UV-1800, then using Minitab 18 software to optimize glucose results, then testing pH using a pH meter.

Methods

Preparation of Materials

The initial step involves drying the gayam tree fruit peel in the sun for 3 days. After drying, the material is cut into small pieces. Next, the size of the fruit peel is reduced using a chopper, and sieving is performed to achieve a uniform sample size. Sieving is done using an 80-mesh screen. The resulting powder is then analyzed for its content using Acid Detergent Fiber and Neutral Detergent Fiber methods.

Delignification

In the process of hydrolyzing cellulose into reducing sugars, lignin content plays a crucial role. This hydrolysis reaction is influenced not only by reaction conditions such as time, concentration, and temperature but also by the physical properties of cellulose and steric factors that can hinder acid penetration into cellulose molecules. For this reason, a delignification process is necessary to remove lignin (Bioetanol et al., 2017). Delignification was carried out according to the procedures outlined in previous studies (Ayuni & Hastini, 2020; Herawati et al., 2021). The delignification process was performed using an 8% sodium hydroxide (NaOH) solution at 80°C for 60 minutes to remove lignin, which inhibits the accessibility of cellulose to hydrolysis. The delignification process serves to break down the lignin content bound to lignin-cellulose, which otherwise obstructs the hydrolysis process (Permana et al., 2024). The results of delignification were then analyzed using Acid Detergent Fiber and Neutral Detergent Fiber methods to assess the reduction in lignin content. After delignification, the sample was washed to a neutral pH and dried again before proceeding to the hydrolysis stage.

Hydrolysis

The hydrolysis process can be carried out in two ways: using enzymes or acid catalysts. Hydrolysis using acid catalysts can produce more efficient results because it not only dissolves hemicellulose components but also reduces the crystallinity of cellulose and increases the material's porosity (Telussa et al., 2023). Hydrolysis was performed using hydrochloric acid (HCl) solutions with varying concentrations (1, 2, 3, 4, and 5 N) and different hydrolysis times (90, 120, 150, 180, and 210 minutes). This process aims to convert cellulose into glucose by breaking the glycosidic bonds within the polysaccharide structure (Hayati et al., 2022). The hydrolysis mixture was then filtered to separate the residue from the hydrolysis solution, and the glucose content was analyzed using a refractometer.

Data Analysis

Acid Detergent Fiber and Neutral Detergent Fiber Testing

Neutral detergent fiber (NDF) is a substance that is insoluble in neutral detergents and represents the largest component of plant cell walls, consisting of cellulose, hemicellulose, lignin, silica, and fibrous protein. Acid detergent fiber (ADF) is a fraction of NDF that is insoluble in acid detergents and consists of cellulose and lignin. The calculation formulas for cellulose, lignin, and hemicellulose are as follows:

$$\%Hemicellulose = \%NDF - \%ADF \quad (1)$$

$$\%Cellulose = \%ADF - \%dissolved\ ash - \%lignin \quad (2)$$

$$\%Lignin = \frac{Residue\ ADF - Acid\ Insoluble\ Ash}{sample\ weight} \times 100\% \quad (3)$$

The determination of cellulose, lignin, and hemicellulose content was carried out in stages using the Van Soest method, through the application of Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) analyses (Bina et al., 2023).

Refractometry Glucose Content Results Testing

Glucose content was analyzed using two methods: refractometry and UV-Vis spectrophotometry. A refractometer was used for the initial measurement of the hydrolysis solution concentration, which was later optimized. The working principle of the refractometer is based on the phenomenon of light refraction, where light changes direction or is refracted when passing through a medium with a different density. The results of the refractometer analysis are expressed on a scale of 0 to 32 percent Brix (Adityarini et al., 2020).

Optimization Respon Surface Method (RSM)

After analyzing the initial refractometry results, optimization was conducted using the Response Surface Methodology (RSM) with Minitab 18 software. In this study, a custom design experimental method was applied within the RSM framework to optimize the variables of hydrolysis catalyst concentration and hydrolysis time. Statistical analysis was performed using ANOVA to evaluate the effect of these variables on the hydrolysis outcome and to assess the adequacy of the model and response variables (Setiawan & Pradipta, 2024). The regression model obtained from the RSM analysis was used to determine the relationship between the research variables and the resulting glucose content. The outcome of the RSM optimization provided the predicted optimum conditions for the hydrolysis catalyst concentration and hydrolysis time that are expected to yield the highest glucose content.

Spectrophotometry UV-Vis Glucose Content Testing Results

UV-Vis spectrophotometric testing was carried out on the results obtained under the optimum operating conditions determined by the Response Surface Methodology (RSM). This analysis was conducted for the quantitative determination of glucose content based on a previously established standard curve (Esati et al., 2024). In the initial stage, the maximum wavelength was determined to obtain the highest absorbance value of the analyzed sample. Wavelength measurements were conducted at 740 nm, which corresponds to the specific absorption area of the Nelson-Somogyi reagent (Vifta, 2018). The glucose standard curve was generated by measuring the absorbance at various glucose concentrations: 0, 0.1, 0.2, 0.3, 0.5, and 1 g/100 mL. The results of this analysis were used to determine the glucose content in gayam fruit peel waste by comparing the sample absorbance values to those of the standard solutions.

Liquid Glucose Standard Test from Gayam Skin Based on Indonesian National Standards

Tests were conducted based on the quality parameters of SNI 2978:2021 to evaluate the quality of the liquid glucose product obtained from the optimization process. A series of analyses were performed, including glucose level determination using the DNS method, % Brix measurement using a refractometer, pH testing, as well as organoleptic evaluation of the sample's color and odor. These assessments were carried out to determine whether the sample meets the quality standards or is classified as off-grade glucose.

RESULTS AND DISCUSSION

Initial Content Analysis and Delignification

Initial content analysis of gayam fruit peel waste was conducted using Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) methods. The dried gayam fruit peel, as shown in Figure 1, was first collected, cleaned, and processed prior to chemical analysis and pretreatment. This agricultural waste contains significant lignocellulosic components, making it a promising raw material for glucose production through hydrolysis.

Figure 1. Dried Gayam Fruit Peel Waste Used as Raw Material for Cellulose Hydrolysis.

Based on the results of the Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) tests, the cellulose content was found to be 35.242%. This indicates that gayam fruit peel has potential as a raw material for cellulose hydrolysis into reducing sugars (glucose). A delignification process is then carried out to reduce the lignin content. In its natural form, cellulose is bound by hemicellulose and protected by lignin, making it difficult to degrade or hydrolyze.

Therefore, delignification is a crucial step to increase the amount of glucose that can be obtained. This process alters the structural bonds between lignin and cellulose, allowing alpha-cellulose to be more easily isolated (Sumiati et al., 2023). The composition of the material after delignification is as follows. Initial analysis results of gayam fruit peel waste raw material content can be seen on Table 1.

Table 1. Initial Analysis Results of Gayam Fruit Peel Waste Raw Material Content

Component	%Content	
	Before Delignification	After Delignification
Cellulose	35.242	37.536
Hemicellulose	11.328	9.9223
Lignin	12.36	4.603

Based on Table 1, it can be observed that after the delignification process using an 8% NaOH solution for 60 minutes, the lignin content decreased from 12.36% to 4.603%. During this process, cellulose and hemicellulose remained as residues for further analysis, while lignin was separated and removed as a filtrate. As a result, the lignin content was significantly reduced compared to the pre-delignification condition. However, the remaining 4.603% lignin may still hinder the acid hydrolysis process by limiting cellulose accessibility and reducing the efficiency of the acid. Therefore, further lignin reduction through extended or multi-stage pretreatment is recommended to enhance hydrolysis efficiency and increase glucose yield.

Effect of Hydrolysis Catalyst Concentration and Hydrolysis Time on Glucose Content Results

Hydrolysis is carried out to break down cellulose compounds into glucose using hydrochloric acid as a catalyst, derived from gayam fruit peel waste. A refractometer is used to analyze the glucose content, operating on the principle of light refraction (Fawaid et al., 2021). The results of the glucose content analysis from the hydrolysis process showed an increase in glucose levels across the specified variables. The variables include hydrolysis catalyst concentration, hydrolysis time, and the resulting glucose content, as presented in the following table:

Table 2. Results of glucose content analysis using a refractometer

Catalyst Concentration (N)	Hydrolysis Time (Minute)				
	90	120	150	180	210
1	4.8%	4.9	6.0%	5.3%	5.1%
2	7.3%	10.4%	11.3%	10.5%	10.0%
3	14.2%	14.8%	19.7%	15.7%	15.0%
4	18.1%	18.5%	20.0%	19.8%	18.9%
5	22.5%	22.9%	25.3%	23.5%	23.1%

Based on Table 2, the highest glucose content was recorded at 25.3% under the operating conditions of a maximum hydrolysis catalyst concentration of 5 N hydrochloric acid (HCl) and a hydrolysis time of 150 minutes. The H^+ ions from HCl facilitate the conversion of fiber components in the gayam fruit peel powder into free radical groups. These radical groups, no longer bound to fibers, react with OH^- groups from water, leading to the formation of glucose (Lestari, 2022). Conversely, the lowest glucose content, 4.8%, was obtained under conditions of 1 N catalyst concentration and a hydrolysis time of 90 minutes. Based on the data in the table, the relationships between hydrolysis catalyst concentration and glucose content, as well as between hydrolysis time and glucose content, are as follows:

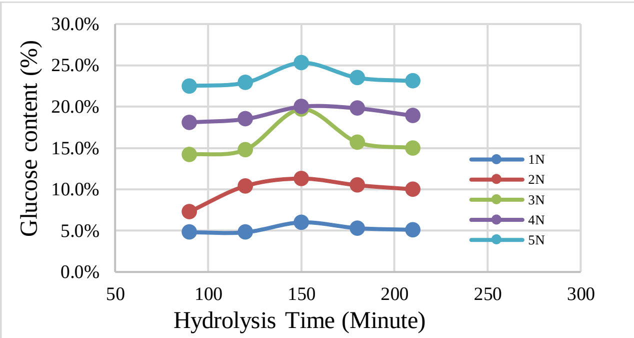

Figure 2. Graph of the Relationship between Hydrolysis Time and Glucose Content

Based on Figure 2, which shows the graph of the relationship between hydrolysis time and glucose content, it can be observed that the glucose content increases as the hydrolysis time extends from 90 to 150 minutes. This indicates that a longer hydrolysis time tends to result in higher glucose production. The increase is attributed to the extended contact time between the acid and the gayam fruit peel, allowing for more effective degradation of hemicellulose and cellulose into glucose and other sugar compounds, thereby optimizing the hydrolysis process. However, after reaching the optimum time of 150 minutes—with the highest glucose content of 25.3%—the glucose yield began to decline. This aligns with previous studies, which state that after reaching the optimal hydrolysis time, the sugar yield decreases due to the decomposition of the formed sugars into derivative compounds, such as 5-hydroxymethylfurfural (HMF). HMF may subsequently undergo further reactions to form compounds like formic acid (Świątek et al., 2020). Additionally, residual lignin that has not been fully removed through delignification may degrade and produce phenolic compounds, which can also contribute to the reduction in sugar yield.(Ayuni & Hastini, 2020).

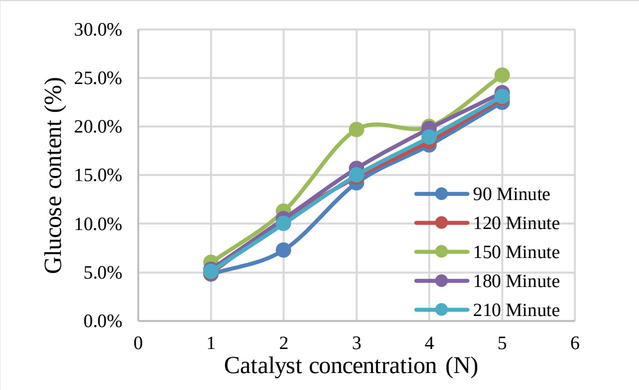

Figure 3. Graph of the Relationship between Hydrolysis Catalyst Concentration and Glucose Content

Based on Figure 3, which illustrates the relationship between hydrolysis catalyst concentration and glucose content, it can be observed that the catalyst concentration is directly proportional to the glucose yield. As the concentration of the catalyst used in the hydrolysis process increases, the resulting glucose content also rises. This is because a higher catalyst concentration increases the hydrolysis rate; under acidic conditions, the reaction rate constant is directly proportional to the concentration of H^+ ions (Sadimo et al., 2016). The highest glucose content from the hydrolysis of gayam fruit peel waste was achieved at a catalyst concentration of 5 N, with values of 22.5%, 22.9%, 25.3%, 23.5%, and 23.1%. However, if hydrolysis were to be continued using an acid concentration higher than 5 N, a decrease in glucose content would likely occur. This is supported by experimental observations showing that at 5 N HCl, the glucose yield no longer increased significantly and began to exhibit a declining trend. Although concentrations above 5 N were not tested in this study, the decrease at 5 N suggests that the optimum condition had been reached, beyond which the efficiency of the hydrolysis reaction declined. This reduction in efficiency is attributed to the lower water content at higher acid concentrations, which affects the availability of OH^- ions (from water ionization) needed to bind carbonium ions during the cellulose hydrolysis process, ultimately reducing the glucose yield (Harianja et al., 2015).

Optimization of Glucose Content Response Surface Methodology

The optimization of reducing sugar (glucose) content was conducted using the Response Surface Methodology (RSM). A custom experimental design was employed to analyze the effects of two main variables: hydrolysis catalyst concentration (A) and hydrolysis time (B), on reducing sugar (glucose) yield (Y). A total of 25 experimental runs were performed based on the design, which were then optimized and reduced to 13 key data points. From the 25 experimental results, a normal probability plot of the residuals was generated, as shown in the Figure 4.

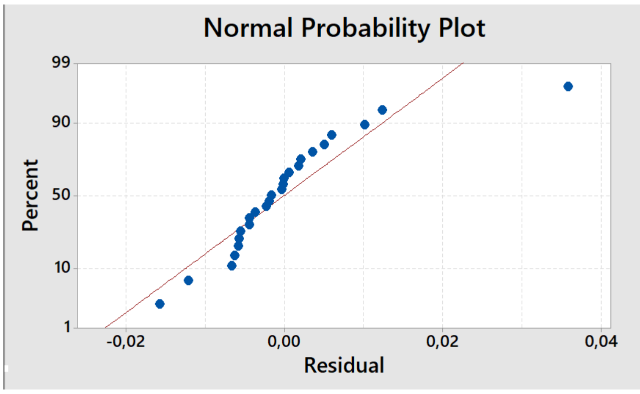

Figure 4. Normal Probability Plot 25 Data Residual Before Optimization Evaluation Response Surface Methodology (RSM)

A normal Probability Plot is used to evaluate whether the residuals in a regression model are normally distributed (Sembiring et al., 2023). If the residuals follow a normal distribution, the points on the plot will align closely with the diagonal reference line. Based on the figure above, 25 residual data points were plotted. Most of the points are distributed near the diagonal line, indicating that the residuals exhibit a pattern that approximates a normal distribution (Hermawan, 2022). However, a few points deviate slightly from the line. Such deviations may indicate the presence of outliers or suggest that the residual distribution is not perfectly normal. Therefore, further evaluation of the model is necessary. From the original 25 data points, 13 were selected based on their residuals being closest to the diagonal line, resulting in a refined normal probability plot of the residuals, as shown in the following figure.

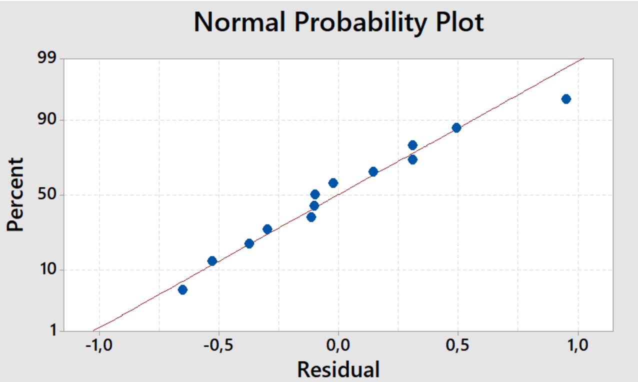

Figure 5. Normal Probability Plot 13 data residual after optimization evaluation Response Surface Methodology (RSM)

Based on the Figure 5, it can be observed that the residual points, which were reduced from 25 to 13, are more closely aligned with the diagonal line. This indicates that the residuals exhibit a pattern that closely approximates a normal distribution (Ahmad Fadhilah et al., 2025). These 13 data points were then input into Minitab 18 software. The selected data represent the variables of hydrolysis catalyst concentration and hydrolysis time, along with their corresponding response variable, namely glucose content, as shown below.

Based on the Table 3, the highest reducing sugar (glucose) content was found in the 7th run, with the use of a 5 N acid concentration and a hydrolysis time of 150 minutes, resulting in 25.3%. In contrast, the lowest glucose content was observed in the 1st experiment, using a 1 N acid concentration and a hydrolysis time of 90 minutes, yielding 4.8%. Table 3 shows that both acid concentration and hydrolysis time have a significant impact on the reducing sugar (glucose) content. To determine the extent of the influence of these two factors, an ANOVA statistical analysis was performed. This analysis compares the means of the influencing factors and identifies significant differences between these means (Putri et al., 2023). The results of the ANOVA analysis are presented in Table 4.

Table. 3 Variable Data and Optimization Response Response Surface Methodology (RSM):

Hydrolysis Time (Menit)	Catalyst Concentration (N)	Glucose Content (%)
90	1	4.8
90	4	18.1
120	1	4.9
120	2	10.4
150	1	6.0
150	4	20.0
150	5	25.3
180	1	5.3
180	3	15.7
180	4	19.8
180	5	23.5
210	2	10.0
210	4	18.9

The Model F value obtained is 401.27, indicating that the model is significant. A p-value < 0.05 suggests that the model is significant. Conversely, if the p-value > 0.05, the variable is considered to have no significant effect on the response (Khasanah et al.,

Table. 4 Anova Analysis

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	665.032	133.006	401.27	0.000
Linear	2	559.446	279.723	843.91	0.000
Time (Minute)	1	0.25	0.25	0.75	0.414
Concentration (N)	1	535.305	535.305	1614.99	0.000
Square	2	3.404	1.702	5.13	0.042
$Time^2$ (Menit)	1	3.404	3.404	10.27	0.015
$Concentration^2$ (N)	1	0.398	0.398	1.2	0.309
2-Way Interaction	1	0.008	0.008	0.02	0.880
Time*Concentration	1	0.008	0.008	0.02	0.880
Error	7	2.32	0.331		
Total	12	667.352			

2023. In this case, the variables of concentration, concentration², and time² have a significant impact on the model. After conducting the ANOVA analysis, a regression equation was derived, which shows that the glucose content response is influenced by the hydrolysis catalyst concentration and hydrolysis time. The resulting equation is as follows:

$$y = -8.38 + 0.1172\,x1 + 5.323\,x1 - 0.000383\,x2^2 - 0.129\,x1^2 + 0.00051\,x1.x2$$

Explanation: y = Glucose content (%)

x_1= hydrolysis catalyst concentration (N)

x_2= hydrolysis time (Minute)

Table. 5 Summary Model Analysis of Response Surface Regression

S	R-sq	R-sq(adj)	R-sq(pred)
0.57573	99.65%	99.40%	97.38%

Based on Table 5, it can be concluded that the R-sq value or coefficient of determination is 99.65% or 0.9965. A high R-sq value indicates that the model obtained is accurate in predicting data, conversely a low R-sq indicates that the model is inaccurate in illustrating the relationship between variables. (Djimtoingar et al., 2022). However, the R-sq value that is said to be good for the accuracy of predicting data must be at least 0.75. (Muzakhar et al., 2024). R-sq(adj) is the R-sq that takes into account the number of factors in the model. If there are independent factors that do not need to be input into the model, the R-sq(adj) value tends to decrease or remain the same as R-sq (Venkatachalam et al., 2021). The resulting model is used to navigate the glucose content design space in RSM. The coefficient of determination (R-sq) value of 99.65% or 0.9965 which is close to 1 indicates that the model prediction results are very close to the experimental results (Chicco et al., 2021).

Table. 6 Optimization Results of Glucose content Response Surface Method (RSM)

Parameter

Response	Goal	Lower	Target	Weight	Importance
Glucose content	Maximum	4,8	25,3	1	1

Solution

Solution	Time (Minute)	Concentration (N)	Glucose content Fit (%)	Composite Desirability
1	159,32	5	25,427	0,94215

Multiple Response Prediction

Variable	Setting
Time	159,32
Concentration	5

Response	Fit	SE Fit	95% CI	95% PI
Glucose content	25.427	0.322	(23.824; 25.916)	(22.138; 26.421)

Based on Table 6, it can be seen that the optimization results of the maximum glucose content were obtained on the hydrolysis time variable for 159.32 minutes and the hydrolysis catalyst concentration was 5 N with a predicted glucose content of 25,427% and a standard error (SE) of 0.322 was obtained and also a composite desirability of 0.94215, where the smaller the composite desirability value or approaching the value of 0, the response value obtained is not optimal, conversely if the composite desirability value approaches 1, the response value obtained is optimal. (Hidayat et al., 2021). In addition, the results of the response surface method (RSM) analysis were also obtained in the form of visual surface plots and contour plots as follows:

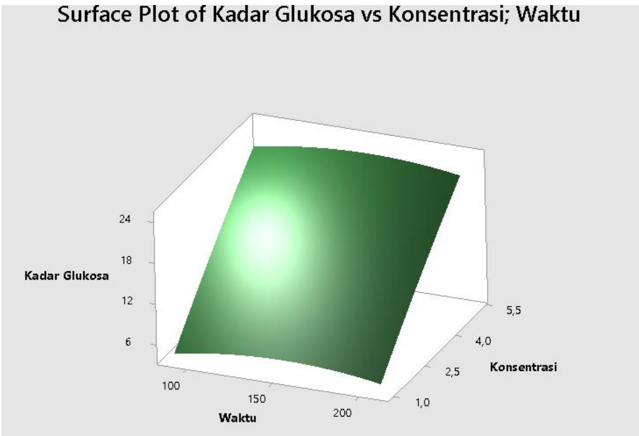

Figure 6. Surface Plot of Glucose content (%) Vs Hydrolysis Catalyst Concentration (N) and Hydrolysis Time (Minutes)

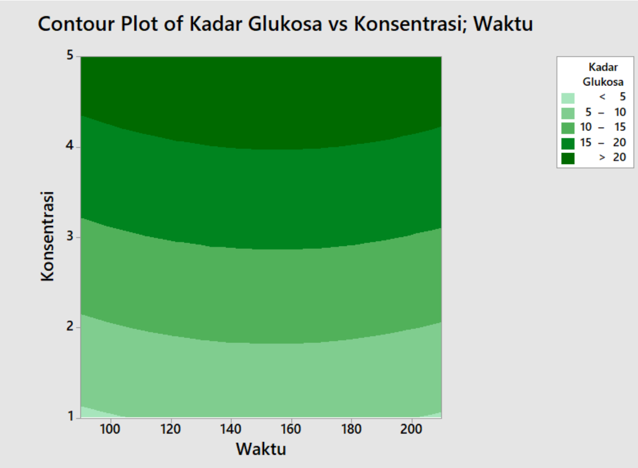

Figure. 7 Contour Plot of Glucose content (%) Vs Hydrolysis Catalyst Concentration (N) and Hydrolysis Time (Minutes)

The response surface model is visually represented through surface and contour plots. Surface plots are three-dimensional graphs that illustrate how variations in factors affect the response, revealing maximum, minimum, or intermediate values (Winarni et al., 2021). The surface plot shown in Figure 6 is projected into a contour plot in Figure 7. A contour plot is a two-dimensional representation that explains the relationship between two variables and the corresponding response (Widarsaputra et al., 2022).

Based on Figure 7, the light green zone represents areas of low glucose content, while the darker green zone indicates higher glucose levels. The peak glucose concentration occurs when the hydrolysis catalyst concentration ranges from 4 to 5 N and the hydrolysis time ranges from 100 to 200 minutes. Figures 6 and 7 both illustrate the relationship between the hydrolysis catalyst concentration and hydrolysis time with the glucose content. From both plots, it can be observed that increasing the catalyst concentration results in a proportional increase in glucose content, suggesting improved reaction efficiency as the concentration of the catalyst increases. This is attributed to the higher catalyst concentration accelerating the hydrolysis rate, considering that the hydrolysis rate constant is linearly related to the concentration of H^+ ions under acidic conditions (Sadimo et al., 2016).

Furthermore, both plots indicate that glucose content increases with longer hydrolysis time up to an optimum point. Beyond this point, further increases in hydrolysis time lead to a decrease in glucose yield. This decline is due to the degradation of sugars into byproducts such as 5-hydroxymethylfurfural (HMF), which may further react to form compounds like formic acid. In addition, incomplete delignification may result in the breakdown of lignin into phenolic compounds, which can also contribute to reduced sugar yields (Ayuni & Hastini, 2020).

Glucose Content Analysis with UV-Vis Spectrophotometer

The analysis of optimal reducing sugar content resulting from the response surface method (RSM) optimization—at a hydrolysis catalyst concentration of 5 N and a hydrolysis time of 159.32 minutes—was carried out through quantitative analysis using the Nelson-Somogyi method. This method is based on the oxidation of glucose by Nelson's reagent, which subsequently reacts with arsenomolybdate to form a blue-green molybdenum complex. The intensity of the resulting color is then measured using UV-Vis spectrophotometry to determine the glucose concentration with high accuracy (Sari & Razali, 2021).

Prior to glucose analysis using UV-Vis spectrophotometry, instrument calibration was performed using standard glucose solutions. In the initial step, the maximum wavelength was determined to obtain the highest absorbance value for the analyzed sample. Wavelength measurements were conducted within the 400–780 nm range, which corresponds to the specific absorption region of the Nelson-Somogyi reagent (Vifta et al., 2018). The standard glucose curve was constructed by measuring absorbance at various glucose concentrations: 0, 0.1, 0.2, 0.3, 0.5, and 1 g/100 mL. The resulting calibration data were then used to determine the glucose content in gayam fruit peel waste by comparing the absorbance values of the sample with those of the standard solutions, as shown in the following Table 7.

Table. 7 Absorbance Test Results of Standard Sample Solutions

Concentration (g/100ml)	Absorbance
0	0,0201
0,1	0,0451
0,2	0,0595
0,3	0,0793
0,4	0,0953
0,5	0,1197
1	0,2259

The glucose standard curve illustrates the correlation between glucose concentration and absorbance values measured at a wavelength of 740 nm. The test results yielded a standard glucose calibration curve equation along with its corresponding regression coefficient. The standard calibration curve, derived from the measurement of a pure glucose solution, is presented in the following figure.

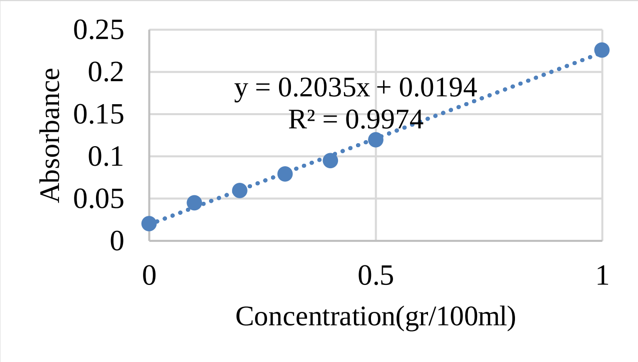

Figure 8. Glucose Standard Calibration Curve

In Figure 8, the glucose standard calibration curve yields the equation y = 0.2035x-0.0194, with an R^2 value of 0.9974. An R^2 value close to 1 indicates that the model has a high level of goodness of fit (Chicco et al., 2021). After determining the regression equation, the absorbance value of the sample (0.2596) was substituted into the equation to calculate the glucose concentration. The calculation results showed that the glucose concentration in the sample was 1.1804 g/100 mL. A correction was then applied for the dilution factor, with a dilution volume of 10 mL and a sample volume of 2.5 mL, resulting in a final glucose content of 4.7217% or 47,217 ppm.

The hydrolysis results of gayam fruit peel waste showed a higher glucose yield compared to the study conducted by Ayuni and Hastini (2020), where coconut fiber was hydrolyzed using 4 N HCl for 90 minutes and produced a glucose content of 1.44% or approximately 1,445.744 ppm. In contrast, the current study achieved an optimum glucose content of 4.7217% or 47,217 ppm by using 5 N HCl and a hydrolysis time of 159,.2 minutes. The improved glucose yield in this study can be attributed to differences in raw material cellulose content, pretreatment methods, and hydrolysis conditions, particularly the acid concentration and duration.

Liquid Glucose Standard Test Results from Gayam Skin Based on Indonesian National Standards Liquid glucose obtained from the optimization process was evaluated based on the quality parameters of SNI 2978:2021. The glucose content was determined using the DNS method, which requires a minimum standard of ≥35%; however, the analysis results showed only 4.72%, indicating low conversion efficiency of cellulose to glucose. The Brix value, measured using a refractometer to determine the concentration of dissolved solids, was 25.3%, which falls below the standard minimum of 70%, suggesting a low glucose concentration. The pH was measured using a pH meter and found to be 1.5, which does not meet the standard range of 4.0–6.0.

Organoleptic evaluation of the sample's color showed a cloudy brown appearance, while the standard requires the product to be colorless to light yellow. Despite this, the sample's color still falls within an acceptable range. The odor, also assessed organoleptically, should be neutral without acidic or fermentative characteristics; however, the sample retained an acidic smell, indicating the presence of residual catalyst. These results show that the glucose product does not meet SNI requirements and is therefore classified as off-grade glucose, meaning it is not certified under the Indonesian National Standard. Although off-grade glucose has a lower market value, it can still be utilized as a raw material for bioethanol production (Khurniawati et al., 2019).

CONCLUSION

Based on the research results, it can be concluded that gayam fruit peel waste can be hydrolyzed into glucose, while the optimum conditions for glucose hydrolysis were obtained from the optimization of the response surface method (RSM) through Minitab 18 software with a Custom model with a variable concentration of HCl hydrolysis catalyst of 5 N and a hydrolysis time of 159.32 minutes with a predicted glucose content of 25.427% and analyzed using Uv-Vis spectrophotometry with the Nelson Somogyi method, the glucose content was 4.7217% or 47217 ppm.

ACKNOWLEDGMENTS

The author would like to thank Ir. Sani. MT. for his guidance during the writing of this journal.

REFERENCES

Adityarini, D., Suedy, S. W. A., & Darmanti, S. (2020). Kualitas Madu Lokal Berdasarkan Kadar Air, Gula Total dan Keasaman dari Kabupaten Magelang. *Buletin Anatomi Dan Fisiologi*, *5*(1), 18–24.

Ahmad Fadhilah, S., Fatkhurin, E., & Ketut Sari, N. (2025). Indonesian Journal of Chemical Research Optimization of the Esterification Process of Crude Palm Oil (CPO) with Natural Zeolite Catalyst Using Response Surface Methodology (RSM). *J. Chem. Res*, *12*(3), 220–228.

Ayuni, N. P. S., & Hastini, P. N. (2020). Serat Sabut Kelapa Sebagai Bahan Kajian Pembuatan Bioetanol Dengan Proses Hidrolisis Asam. *Jurnal Sains Dan Teknologi*, *9*(2), 102–110.

Bina, R. M., Syaruddin, Sahara, L. O., & Sayuti, M. (2023). Kandungan Selulosa, Hemiselulosa, dan Lignin Dalam Silase Ransum Komplit Dengan Taraf Jerami Sorgum (Sorghum bicolor (L.) Moench) yang Berbeda. *Gorontalo Journal of Equatorial Animals*, *2*(1), 48–50.

Bioetanol, P., Baku, B., Bekas, K., Metode, M., Asam, H., Fermentasi, D., Ramayanti, C., & Giasmara, K. R. (2017). Bioethanol Production From Waste Paper Using Separate Hydrolysis and Fermentation. *J. Chem. Res*, *5*(1), 17–21.

Chicco, D., Warrens, M. J., & Jurman, G. (2021). The coefficient of determination R-squared is more informative than SMAPE, MAE, MAPE, MSE and RMSE in regression analysis evaluation. *PeerJ Computer Science*, *7*(e623), 1–24.

Djimtoingar, S. S., Derkyi, N. S. A., Kuranchie, F. A., & Yankyera, J. K. (2022). A review of response surface methodology for biogas process optimization. *Cogent Engineering*, *9*(2115283), 1–35.

Esati, N. K., Oriana, E., La, J., & Sinarsih, N. K. (2024). Penetapan Kadar Glukosa Ubi Jalar Ungu Dengan Metode Semikuantitatif dan Kuantitatif. *Jurnal Farmamedika (Pharmamedica Journal)*, *9*(1), 9–15.

Fawaid, E. S. A., L I Sari, C Pujiastuti, N K Erliyanti, A D Priyanto, & E A Saputro. (2021). Aplikasi Portable Brix Meter untuk Perhitungan Indeks Bias Minyak Atsiri Daun Jambu Kristal. *Biomedical and Mechanical Engineering Journal (BIOMEJ)*, *1*(1), 11–14.

Harianja, J. W., Idiawati, N., & Rudiyansyah. (2015). Optimasi Jenis Dan Konsentrasi Asam Pada Hidrolisis Selulosa Dalam Tongkol Jagung. *JKK*, *4*(4), 66–71.

Hayati, N., Masrullita, M., Ishak, I., Suryati, S., & Sulhatun, S. (2022). Pembuatan Glukosa Dengan Memanfaatkan Limbah Bonggol Jagung. *Chemical Engineering Journal Storage (CEJS)*, *2*(1), 1–11.

Herawati, N., Roni, K. A., Fransiska, S., & Rifdah. (2021). Pembuatan Bioetanol Dari Rumput Gajah Dengan Proses Hidrolisis Asam. *Jurnal Redoks*, *6*(1), 35–51.

Hermawan, A. R. (2022). Humantech Jurnal Ilmiah Multi Disiplin Indonesia. *Jurnal Ilmiah Multi Disiplin Indonesia*, *1*(9), 1278–1285.

Hidayat, I. R., Zuhrotun, A., & Sopyan, I. (2021). Design-Expert Software sebagai Alat Optimasi Formulasi Sediaan Farmasi. *Majalah Farmasetika*, *6*(1), 99–120.

Khasanah, K., Nawangsari, D., & Kusuma, I. Y. (2023). Solid Dispersion of Acetosal Using Polyvinyl Pyrrolidone (PVP) K-30 in Tablets with Direct Compressing Method. *Indo. J. Chem. Res.*, *10*(3), 183–194.

Khurniawati, Fathoni, M. U., & Sari, N. K. (2019). Pembuatan Bioetanol Berbasis Glukosa Off Grade Dengan Proses Fermentasi Menggunakan Fermiol Manufacture Of Glucose-Based Bioetanol Off Grade With The Fermentation Process Using Fermiol. *Jurnal Teknik Kimia*, *13*(2), 48–52.

Lestari, Y. P. I. (2022). Optimasi Konsentrasi Hcl Pada Proses Hidrolisis Untuk Pembuatan Mikrokristalin Selulosa (Mcc) Dari Eceng Gondok. *Majalah Farmasi Dan Farmakologi*, *1*(10), 1335–1344. \

Muzakhar, S. S. A., Assidiqie, G. I., Siregar, A. S. B., Aparamarta, H. W., Fahmi, F., & Gunawan, S. (2024). Optimization of Esterification in the Synthesis of Surfactants Feedstock from Polar Lipid Fraction of Crude Palm Oil. *Journal of Fundamentals and Applications of Chemical Engineering (JFAChE)*, *4*(2), 30.

Permana, H. A., Delvitasari, F., Hartari, W. R., & Maryanti, M. (2024). Pengaruh Konsentrasi NaOH dan Suhu Delignifikasi pada Kandungan Lignoselulosa Tandan Kosong Kelapa Sawit. *Jurnal Agro Industri Perkebunan*, *12*(1), 51–58.

Putri, D. R., Aziz, A. D., & Rizqi, M. N. (2023). Analisis Rasio Keuangan Dan Financial Distress Sebelum Dan Sesudah Covid-19 Subsector Food

And Beverage. *Jurnal Maneksi*, *12*(3), 564–572.

Sadimo, M. M., Said, I., & Mustapa, K. (2016). Pembuatan Bioetanol Dari Pati Umbi Talas (*Colocasia Esculenta [L] Schott*) Melalui Hidrolisis Asam Dan Fermentasi. *Jurnal Akademika Kimia*, *5*(2), 79–84.

Santhi, M., Arnata, I. W., & Wrasiati, L. P. (2022). Isolasi Selulosa Dari Serat Sabut Kelapa (Cocos Nucifera L.) Pada Variasi Suhu dan Waktu Proses Bleaching Dengan Asam Perasetat. *Jurnal Rekayasa Dan Manajemen Agroindustri*, *10*(3), 248–258.

Sari, N., & Razali, M. (2021). Penetapan Kadar Glukosa Reduksi dari Sirup Glukosa Hasil Hidrolisa Selulosa dari Limbah Buah Mengkudu (Morinda Citrifolia L) dengan Asam Klorida. *Journal of The Indonesian Society of Integrated Chemistry*, *13*(2), 98–104.

Sembiring, E., Lubis, A., & Effendi, I. (2023). Pengaruh Penggunaan Sosial Media Instagram Dan Electronic Word Of Mouth Terhadap Minat Beli Produk Erigo Pada Mahasiswa Fakultas Hukum Universitas Sumatera Utara. *Jurnal Ilmiah Akuntansi Keuangan Dan Bisnis (JIKABI)*, *2*(1), 8–15.

Setiawan, O., & Pradipta, A. T. (2024). Penggunaan Metode Response Surface Methodology Box Behnken Untuk Pemodelan dan Optimasi Proses Fenton pada Pengolahan Limbah Cair Home Industri Sarung Tenun Tradisional Medangan Gresik. *METANA*, *20*(2), 97–107.

Sumiati, T., Yuningtyas, S., & Haloho, L. E. B. (2023). Delignifikasi Lignoselulosa Daun Nanas Sebagai Sumber Alfa Selulosa. *Jurnal Farmamedika (Pharmamedica Journal)*, *8*(2), 130–137.

Świątek, K., Gaag, S., Klier, A., Kruse, A., Sauer, J., & Steinbach, D. (2020). Hidrolisis Asam pada Biomassa Lignoselulosa: Pembentukan Gula dan Furfural. *Catalysyt*, *10*(4), 1–18.

Telussa, I., Fransina, E. G., Singerin, J., & Taipabu, M. I. (2023). Bioethanol Production From Tropical Marine Microalgae Ambon Bay Navicula sp. of The Inner Ambon Bay Strain. *Indo. J. Chem. Res.*, *10*(3), 136–142.

Venkatachalam, M., Shum-Chéong-sing, A., Caro, Y., Dufossé, L., & Fouillaud, M. (2021). Ovat analysis and response surface methodology based on nutrient sources for optimization of pigment production in the marine-derived fungus talaromyces albobiverticillius 30548 submerged fermentation. *Marine Drugs*, *19*(5), 1–22.

Vifta, R. L., & Advistasari, D. Y. (2018). Analisis Penurunan Kadar Glukosa Fraksi n-Heksan Buah Parijoto (*Medinilla speciosa* B.) secara in vitro dengan Metode Spektrofotometri UV-Vis. *J. Chem. Sci*, *7*(3), 249–253.

Widarsaputra, A. Y., Prawatya, Y. E., & Sujana, I. (2022). Response Surface Methodology (Rsm) Untuk Optimasi Pengolahan Keripik Nanas Menggunakan Mesin Vacuum Frying. *Integrate: Industrial Engineering and Management System*, *6*(2), 70–77.

Winarni, S., Sunengsih, N., & Ginanjar, I. (2021). Multi responses taguchi optimization using overlaid contour plot and desirability function. *Journal of Physics: Conference Series*, *1776*(1), 1–8.

Wyantuti, S., Aristantia, R., Hartati, Y. W., & Bahti, H. H. (2020). Penerapan Desain Eksperimen Plackett-Burman dan Box-Behnken pada Analisis Voltametri Pulsa Diferensial untuk Penentuan Kadar Senyawa Kompleks Gd-DTPA. *ALCHEMY Jurnal Penelitian Kimia*, *16*(1), 140–151.