OPTIMIZATION OF JACKFRUIT SEED STARCH HYDROLYSIS PROCESS USING GLUCOAMYLASE: RESPONSE SURFACE METHODOLOGY APPROACH

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ABSTRACT

Jackfruit seeds, an underutilized by-product of jackfruit processing, are rich in starch and represent a promising raw material for bioconversion into fermentable sugars. This study investigated the enzymatic hydrolysis of jackfruit seed starch using glucoamylase to produce reducing sugars. The effects of incubation temperature and reaction time were initially evaluated using the one-factor-at-a-time (OFAT) approach, followed by optimization through response surface methodology (RSM) with a central composite design (CCD). The optimal hydrolysis conditions were 70.75°C of incubation temperature and 4.8 h of reaction time, resulting in the highest reducing sugar concentration of 9.286 ± 0.228 mg/mL. The findings highlight the potential of using jackfruit seeds as a substrate for the enzymatic hydrolysis to produce fermentable sugars, contributing to sustainable waste valorization and bioethanol production.

Keywords: *Glucoamylase, jackfruit seed, enzymatic hydrolysis, reducing sugar, response surface methodology.*

1. INTRODUCTION

Glucoamylase (EC 3.2.1.3) is a starch-hydrolyzing enzyme capable of cleaving α-1,4-glucosidic linkages at the non-reducing ends of polysaccharides to release glucose. This enzyme is primarily produced by fungi such as *Aspergillus niger*, *Rhizopus niveus*, and *R. delemar*, whose enzymes are known for their thermal stability and high catalytic efficiency (Wang et al., 2020; Carrasco et al., 2017). Due to these outstanding properties, glucoamylase has been widely applied in various industrial sectors, including the production of glucose, ethanol, beer, textiles, and pharmaceuticals (Tong et al., 2021). In ethanol production, glucoamylase is often combined with *Saccharomyces cerevisiae* to convert starch into glucose, which is subsequently fermented into ethanol (Xian and Feng, 2018). Moreover, the enzyme plays a critical role in the development of low-alcohol beer, sake, and other value-added food products (Blanco et al., 2014).

Jackfruit (*Artocarpus heterophyllus* Lam.) is a widely cultivated tropical fruit in Southeast Asia. Its seeds account for approximately 10–15% of the fruit's weight and are rich in starch, protein, and essential nutrients (Ho et al., 2022). However, jackfruit seeds are often treated as waste during food processing, contributing to resource inefficiency and increased pressure on agricultural waste management systems. Utilizing these seeds as a starch-rich substrate for fermentable sugar production represents a sustainable solution that supports waste valorization and bio-based production. Interestingly, there is a lack of studies on fermentable sugar preparation from jackfruit utilization, especially its seed. Nuriana and Wuryantoro (2015) reported that enzymatic hydrolysis of jackfruit seed starch yields higher sugar content than acid hydrolysis, underscoring the advantages of enzymatic methods in efficiency and selectivity. Optimizing enzyme reaction conditions is a crucial step in improving the conversion of starch into sugars. In this context, response surface methodology (RSM) is a statistical tool that enables rapid evaluation of both the effects and the significance of experimental variables on the response (Kim et al., 2023). Thus, in this study, RSM was applied to optimize the reaction conditions of glucoamylase for sugar production from jackfruit seeds. Two key factors affecting hydrolysis efficiency—incubation temperature and reaction time—were investigated. The aim was to determine the optimal conditions to maximize the yield of reducing sugars..

2. MATERIALS AND METHODS

2.1. Materials

Jackfruit seeds were collected from local markets in Buon Ma Thuot City, Vietnam. The seeds were washed, blended, and filtered to obtain a starch-containing slurry at a ratio of 100 g seeds per 500 mL of water. Glucoamylase from *Aspergillus niger* (100,000 IU/g) was purchased from Hunan NHY Bioengineering Company (China) and used as the hydrolytic enzyme. The 3,5-dinitrosalicylic acid (DNS) reagent was purchased from Merck (Germany). All other reagents used were of analytical grade (China).

2.2. Determination of Reducing Sugar Content

Reducing sugar concentration was determined using the DNS method (Miller, 1959) with modification. The reaction mixture contained 1

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mL of the hydrolyzed solution and 2 mL of DNS reagent, which was heated in a boiling water bath (100 °C) for 10 minutes to develop a reddish-orange color. After cooling to room temperature, absorbance was measured at 540 nm using a UV-Vis spectrophotometer. Glucose standards were used to establish the calibration curve.

2.3. Effect of Temperature and Reaction Time

The jackfruit seed slurry was gelatinized at 100 °C for 10 minutes before enzymatic hydrolysis. The reaction mixture included 0.05 g of glucoamylase in 100 mL of gelatinized jackfruit seed starch solution. Hydrolysis was performed at temperatures ranging from 30 to 100 °C (in 10 °C increments) for 30 minutes to assess the effect of temperature. Reactions were conducted at the optimum temperature (as determined in the previous step) for 0, 1, 2, 3, 4, and 5 hours to investigate the effect of time. The data are presented as percentage relative to the highest value in each experiment. The conditions that yielded the highest reducing sugar values were selected for further experiments (Doan et al., 2024).

2.4. Optimization Using Response Surface Methodology

A central composite design (CCD) was used to optimize the two variables: incubation temperature (X_1) and reaction time (X_2). Each variable was studied at five levels: -1.414, -1, 0, +1, and +1.414. The experimental design included 13 runs with five replicates at the center point (Table 1). The experiments were randomized and analyzed using SigmaXL. The response variable (Y) was the concentration of reducing sugars (mg/mL). The second-order polynomial regression model used was:

$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_1X_2 + \beta_4X_1^2 + \beta_5X_2^2$

where Y is the amount of reducing sugar (mg/mL); X_1 is the incubation temperature (°C); X_2 is the reaction time (h); and β_0 to β_5 are the regression coefficients.

Table 1. Levels of independent variables used in CCD.

Factor	Level				
	-1.414	**-1**	**0**	**+1**	**+1.414**
Incubation temperature (°C)	56	60	70	80	84
Reaction time (h)	1.2	2	4	6	6.8

2.5. Statistical Analysis

All experiments were conducted in triplicate. Data were analyzed using SigmaXL for statistical modeling and optimization.

3. RESULTS AND DISCUSSION

3.1. Effect of temperature on reducing sugar yield

Enzymes are protein-based biological catalysts whose activity is affected by temperature (Robinson, 2015). While higher temperatures typically increase reaction rates, excessive heat can lead to a decline in enzyme activity due to thermal denaturation. The effect of temperature on reducing sugar formation is illustrated in Figure 1. The hydrolysis process was evaluated at temperatures ranging from 30 °C to 100 °C, with relative reducing sugar contents of 21.56 ± 3.54%, 65.92 ± 4.70%, 87.66 ± 0.75%, 96.26 ± 1.59%, 100.00 ± 3.04%, 98.75 ± 3.46%, 86.36 ± 8.62%, and 57.03 ± 8.37%, respectively. There was a near-linear increase in sugar concentration up to 70 °C, where the maximum relative reducing sugar content of 100.00 ± 3.04% occurred, followed by a gradual decrease at higher temperatures, indicating optimal activity of crude glucoamylase at around 70 °C. Likewise, glucoamylase from *A. niger* has an optimal temperature of 60–70 °C (Xian and Feng, 2018). The reaction at a high temperature provides benefits such as enhanced solubility and dispersibility of compounds and reduced risk of microbial contamination (Doan et al., 2021). Therefore, with an optimal hydrolysis temperature of around 70 °C, the enzymatic hydrolysis of jackfruit seed slurry using glucoamylase offers practical advantages for industrial applications.

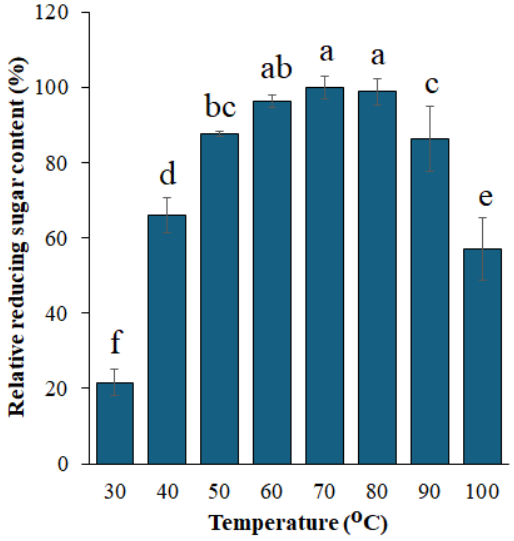

Figure 1. Effect of temperature on the amount of reducing sugar. The data was the mean ± standard deviation (SD). Different letters indicate the significant difference at $p < 0.05$.

3.2. Effect of incubation time on reducing sugar yield

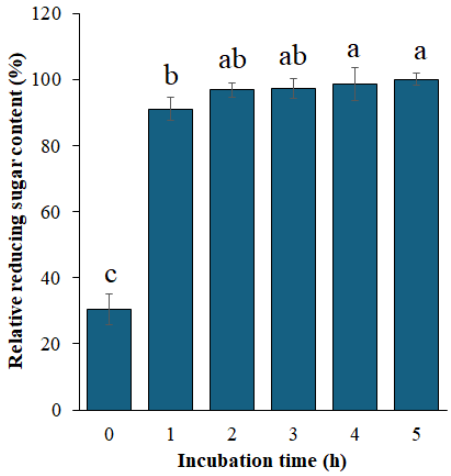

Figure 2. Effect of incubation time on the amount of reducing sugar. The data was the mean ± standard deviation (SD). Different letters indicate the significant difference at p < 0.05.

The reaction rate generally increases with time as the enzyme has more opportunity to interact with the substrate. The endpoint of the reaction is reached when the reaction rate slows down or ceases entirely. Besides, prolonged exposure may reduce the thermal stability of the enzyme, resulting in diminished catalytic activity over time. When the hydrolysis time was increased from 0 to 3 hours, the amount of reducing sugar rose significantly due to more effective contact between the enzyme and the substrate (Figure 2). The reducing sugar concentration was 30.48 ± 4.54% at the initial reaction, then increased to 97.23 ± 3.12% after 3 hours of hydrolysis. However, extending the reaction time more than 3 hours slightly increased the reducing sugar concentrations. At 4 and 5 hours, the reducing sugar concentrations were 98.68 ± 4.98% and 100 ± 1.76%, respectively, showing no significant difference.

3.3. Optimization via response surface methodology

Response Surface Methodology (RSM) is an experimental approach based on mathematical modeling to evaluate the relationships between multiple variables and a response. The results obtained from RSM are expressed through a second-order polynomial regression equation, which can be visualized using surface and contour plots. The optimal condition corresponds to the extremum point on these response surfaces. In this study, optimization experiments were conducted, as shown in Table 2. The effects of incubation temperature (°C) and incubation time (hours) were assessed using a Central Composite Design (CCD) with five coded levels. The reducing sugar concentrations obtained from the 13 experimental runs ranged from 6.497 to 8.379 mg/mL and are summarized in Table 2.

Table 2. Experimental design and amount of reducing sugar

Run	Coded variable		Actual variable		Amount of reducing sugar (mg/mL)
	X_1	X_2	X_1 (°C)	X_2 (h)	
1	-1	1	60	6	7.050
2	0	1.414	70	6.8	7.936
3	1.414	0	84	4	6.809
4	1	-1	80	2	6.497
5	0	0	70	4	8.288
6	-1	-1	60	2	6.678
7	-1.414	0	56	4	6.557
8	1	1	80	6	7.433
9	0	0	70	4	7.966
10	0	0	70	4	8.379
11	0	0	70	4	8.318
12	0	0	70	4	8.298
13	0	-1.414	70	1.2	7.091

Note: X_1, incubation temperature (°C); X_2, reaction time (h).

The regression analysis result for reducing sugar concentration are presented in Table 3. The regression coefficients were evaluated for statistical significance using both t-values and p-values. The most statistically significant terms in the model were the quadratic effects of temperature (X_1^2) and time (X_2^2), with t-values of –13.736 and –6.858, and corresponding p-values of <0.0001 and 0.0002, respectively. These results indicate that the curvature effects of both variables play a critical role in shaping the response surface. The linear term for incubation time (X_2) also exhibited a high level of significance (t = 5.557, p = 0.008), confirming that increasing incubation time within the experimental range has a strong positive effect on reducing sugar production. In contrast, the linear term for temperature (X_1) showed a t-value of 1.239 and a p-value of 0.255, indicating a statistically non-significant effect in its linear form. Similarly, the interaction term

(X_1X_2) yielded a t-value of 1.771 and a p-value of 0.119, suggesting that the combined influence of temperature and time is not statistically significant at the 95% confidence level. These results imply that the reducing sugar concentration is primarily influenced by the nonlinear (quadratic) effects of both temperature and time, while the linear effect of time also contributes significantly.

Table 3. Regression coefficients for the model

Term	Coefficient	SE Coefficient	T-value	p-value
Constant	8.250	0.071	115.757	<0.0001
X_1	0.070	0.056	1.239	0.2553
X_2	0.313	0.056	5.553	0.0009
X_1X_2	0.141	0.080	1.770	0.1201
X_1^2	-0.829	0.060	-13.726	<0.0001
X_2^2	-0.414	0.060	-6.853	0.0002

Note: X_1, incubation temperature (°C); X_2, reaction time (h).

Based on the regression analysis of the experimental data, the following second-order polynomial equation was established to predict the reducing sugar concentration:

$Y = 8.249 + 0.070X_1 + 0.312X_2 + 0.141X_1X_2 – 0.829X_1^2 – 0.414X_2^2$

Where: Y is the reducing sugar concentration (mg/mL); X_1 is the incubation temperature (°C); X_2 is the reaction time (h).

The ANOVA results in Table 4 indicate that the model is statistically significant and fits for predicting reducing sugar concentration with a high F-value (49.998) and very low p-value (<0.0001), highlighting their dominant influence on the model. The adequacy of the model was further evaluated through the lack-of-fit test. A non-significant lack-of-fit ($p > 0.05$) is desirable, as it indicates a good agreement between the experimental data and the model's predictions. As shown in Table 4, the lack-of-fit F-value was 0.910 with a p-value of 0.5114, confirming that the lack-of-fit is not statistically significant. This result suggests that the model fits the experimental data well and can be reliably used to predict the optimal conditions for the hydrolysis process. According to Guan and Yao (2008), a good predictive model typically has a coefficient of determination (R^2) greater than 0.8. In this study, the R^2 value was 0.973, indicating that the model explained 97.3% of the variability in the amount of reducing sugar, with only 2.7% unexplained. The adjusted R^2 was also high at 0.953, meaning the model can account for up to 95.3% of the variation in the response variable, confirming its strong predictive performance.

Table 4. Analysis of Variance (ANOVA) for the Model.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	p-value
Model	5	6.349	1.270	49.998	<0.0000
Error	7	0.178	0.025		
Lack of Fit	3	0.072	0.024	0.910	0.5114
Pure Error	4	0.106	0.026		
Total (Model + Error)	12	6.526	0.544		

Note: R^2 = 0.973; adjusted R^2 = 0.953.

To further visualize the effects of the two independent variables on reducing sugar concentration, contour (2D) and response surface (3D) plots were generated based on the fitted regression model (Figure 3). As illustrated in Figure 3, both incubation temperature and reaction time significantly influenced the production of reducing sugars. The reducing sugar concentration increased markedly as the temperature rose from 56 °C to approximately 71 °C and as the reaction time extended from 1.2 to 4.8 hours, reaching a predicted maximum value of 8.310 mg/mL. Beyond this temperature threshold, further temperature increases led to a decline in sugar yield, likely due to enzyme denaturation or substrate depletion. According to the model, the optimal hydrolysis conditions using glucoamylase for jackfruit seed slurry were determined to be 70.75 °C and 4.8 hours, resulting in a predicted maximum reducing sugar concentration of 8.314 mg/mL.

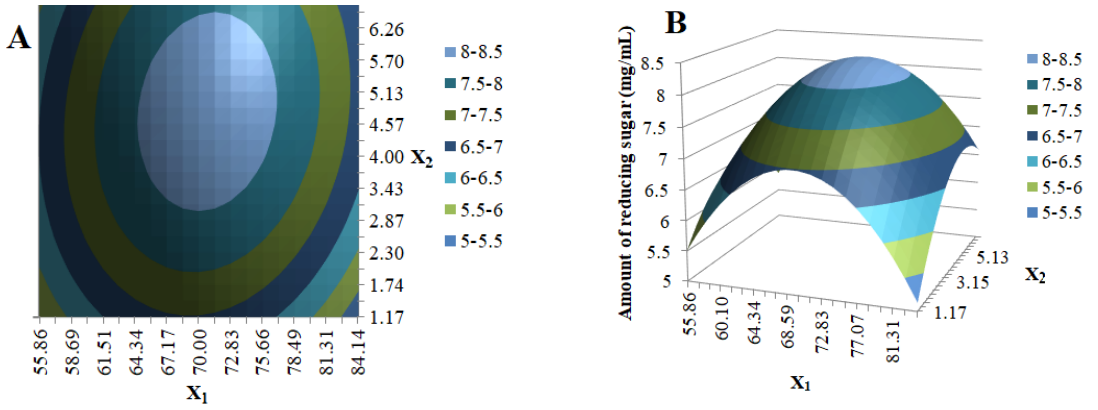

Figure 3. Contour (A) and 3D response surface (B) plots for the effects of incubation temperature and reaction time on the amount of reducing sugar. X_1, incubation temperature (ºC); X_2, reaction time (h).

Hydrolysis was experimentally carried out in triplicate under the suggested optimal conditions to validate the model. The actual reducing sugar concentration obtained was 9.286 ± 0.228 mg/mL, higher than the model prediction (11.69%). Nevertheless, the close agreement between predicted and actual values demonstrates the model's overall reliability and practical applicability in optimizing the enzymatic hydrolysis of jackfruit seed starch. Studies have explored the enzymatic hydrolysis of jackfruit seeds to optimize sugar production. Pham et al. (2016) reported optimal conditions for hydrolyzing jackfruit seed starch using α-amylase at a concentration of 0.05%, a reaction temperature of 90 °C, pH 6.5, and a reaction time of 60 minutes, resulting in a reducing sugar yield of 8.82 mg/mL. In another report, Hoang et al. (2024) found that reducing sugar of jackfruit seed drink after hydrolysis by α-amylase was 2.06%, and by a mixture of α-amylase and glucoamylase was 9.48%, both higher than the pre-hydrolysis value of 0.60%.

4. CONCLUSIONS

In this study, the application of RSM-CCD successfully identified the optimal conditions for the enzymatic hydrolysis of jackfruit seed slurry. The optimal incubation temperature and reaction time were determined to be 70.75 ºC and 4.8 hours, respectively. Under these conditions, the experimental reducing sugar yield reached 9.286 mg/mL, confirming the effectiveness of the optimization approach and the potential of jackfruit seeds as a valuable substrate for bioconversion.

TỐI ƯU HÓA QUÁ TRÌNH THỦY PHÂN TINH BỘT HẠT MÍT BẰNG GLUCOAMYLASE: TIẾP CẬN THEO PHƯƠNG PHÁP ĐÁP ỨNG BỀ MẶT

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TÓM TẮT

Hạt mít, một loại phụ phẩm của ngành công nghiệp chế biến mít, có thành phần cấu tạo phần lớn là tinh bột. Vì vậy, hạt mít đang trở thành nguồn nguyên liệu tiềm năng dùng để chuyển hóa thành đường ứng dụng trong lĩnh vực lên men. Trong nghiên cứu này, glucoamylase đã được sử dụng để thủy phân dịch hạt mít nhằm tạo ra sản phẩm đường khử. Các điều kiện thủy phân như nhiệt độ và thời gian thủy phân đã được khảo sát bằng phương pháp một yếu tố tại một thời điểm (OFAT). Sau đó, phương pháp đáp ứng bề mặt (RSM) với kiểu thiết kế có tâm (CCD) đã được sử dụng để tối ưu hóa điều kiện thủy phân dịch hạt mít. Kết quả ghi nhận, dịch thủy phân hạt mít sử dụng glucoamylase tại điều kiện nhiệt độ và thời gian ủ tối ưu lần lượt 70,75 ºC và 4,8h cho hàm lượng đường khử đạt 9,286 ± 0,228 mg/mL. Những phát hiện này cho thấy tiềm năng sử dụng hạt mít làm cơ chất cho quá trình sản xuất đường có thể lên men được bằng enzyme, đóng góp vào nâng cao giá trị chất thải và ứng dụng sản xuất ethanol sinh học.

Từ khóa: *Glucoamylase, hạt mít, thủy phân bằng enzyme, đường khử, phương pháp đáp ứng bề mặt.*

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