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Response Surface Optimization of Jackfruit Seed Starch Hydrolysis Using *Bacillus licheniformis* Alpha-Amylase for the Preparation of Maltose-Rich Starch Hydrolysate

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Abstract

Jackfruit seeds, a by-product of the jackfruit processing industry, comprise a substantial proportion of starch. As a result, jackfruit seeds are emerging as a viable source of fermentable sugars for fermentation processes. In this study, α-amylase from *Bacillus licheniformis* TKU004 was employed to hydrolyze gelatinized jackfruit seed starch slurry, and the hydrolysis conditions were systematically optimized using the Box–Behnken design (BBD) coupled with response surface methodology (RSM). Three independent variables, including incubation temperature (40–60 °C), enzyme-to-substrate ([E]/[S]) ratio (5–10 U/g), and reaction time (2–6 h), were evaluated, with dextrose equivalent (DE, %) as the response. The optimal hydrolysis parameters were determined to be 47 °C, an [E]/[S] ratio of 10 U/g, and a reaction time of 5.1 h, yielding a predicted DE of 31.72%. Experimental validation confirmed a DE of 32.85 ± 1.12%, in close agreement with the model prediction. HPLC (high-performance liquid chromatography) analysis of the hydrolysate revealed a composition of 14.20% glucose, 56.51% maltose, and 29.29% maltooligosaccharides, indicating that this process is well-suited for producing high-maltose syrup. In short, this study demonstrates the feasibility of valorizing jackfruit seed waste into value-added carbohydrate products through enzymatic hydrolysis with *B. licheniformis* α-amylase.

Keywords: amylase; *Bacillus licheniformis*; jackfruit seeds; starch hydrolysis; optimization

1. Introduction

The jackfruit tree (*Artocarpus heterophyllus* L.) is notable for its ability to produce large fruits directly from its stems. These fruits are uniquely versatile, serving as vegetables when unripe and as sweet fruit when ripe [1]. Jackfruit trees thrive and are widely cultivated throughout South and Southeast Asia, playing a significant role in agriculture and diets in these regions. FAO reports that global jackfruit exports rose from USD 2 billion in 2012 to USD 3.7 billion in 2023, with Vietnam holding a 25% market share [2]. Nevertheless, only a small proportion of the fruit is consumed directly, whereas a significant fraction is discarded during processing. Previous reviews suggest that approximately 60–70% of the fruit mass may be residual as by-products or waste, primarily comprising peel, rind, central core, fibrous rags, latex, and seeds. This substantial waste stream underscores the need for valorization strategies targeting specific fractions with industrial potential [1,3]. In industrial jackfruit processing, fruits are typically peeled, the central core is removed,

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and the edible bulbs are separated manually or mechanically. During this operation, seeds are detached from the bulbs as a secondary by-product and are commonly discarded or underutilized despite their valuable composition [1]. Indeed, jackfruit seeds account for 8–15% of the entire fruit's weight [4] and are high in protein, vitamins, minerals, and carbohydrates (mainly starch) [5]. Thus, jackfruit seed starch has recently attracted attention as a renewable material for food, fermentation, and packaging applications. Le et al. (2024) used jackfruit seed starch in lactic acid fermentation with a combination of *Lactobacillus plantarum* and *Bacillus subtilis* [6]. Van et al. (2023) evaluated the effects of jackfruit seed flour and jackfruit seed starch in cookie production [7]. In addition, jackfruit seed starch has been incorporated into biodegradable composite films due to its high amylose content, confirming its relevance as a plant-derived starch material for food-related applications [8,9].

Although jackfruit seed starch constitutes a promising renewable carbohydrate source, native starch is not readily assimilated in numerous biological processes due to its polymeric structure and limited direct bioavailability to microorganisms. Hydrolysis is therefore a vital step as it transforms starch into smaller saccharides, such as glucose, maltose, and maltooligosaccharides, which exhibit increased solubility and are more readily employed in fermentation and other bioconversion processes [10,11]. From this perspective, the production of defined starch hydrolysates from jackfruit seed starch is crucial for broadening the utilization of this agricultural by-product. Surprisingly, compared with other agricultural by-product starches, the enzymatic conversion of jackfruit seed starch into defined starch hydrolysates has received limited study. Several studies indicate that the starch in jackfruit seeds can be hydrolyzed using acids such as HCl [12] and H_2SO_4 [13] as well as amylase [12,14]. This represents a potential gap in expanding the use of jackfruit waste in fermentation, biofuel, and food industries.

The enzymatic method provides a more efficient, controlled, and environmentally friendly approach to starch hydrolysis than the acid method [15]. Amylase primarily hydrolyzes starch to produce shorter units (maltodextrin, maltose, and glucose) and is classified into α-amylase (EC 3.2.1.1), β-amylase (EC 3.2.1.2), and glucoamylase (EC 3.2.1.3) [16,17]. Among them, α-amylase acts as an endo-enzyme that randomly cleaves internal α-1,4-glycosidic linkages in amylose and amylopectin, producing soluble dextrins, maltose, maltotriose, and other maltooligosaccharides. It has been extensively studied for its applications in many industries, including food, pharmaceuticals, and biofuels [17,18], and accounts for 30% of the global enzyme market [19]. Bacterial sources of α-amylase, especially those from the genus *Bacillus*, are of particular interest for their excellent properties [20]. Among these enzymes, α-amylase from *Bacillus licheniformis* is widely used in enzymatic processes due to its efficient activity under relatively high temperatures and near-neutral pH [21]. These characteristics make them attractive biocatalysts for starch liquefaction and controlled hydrolysis processes [19]. Indeed, α-amylase from *B. licheniformis* is a commercially important thermostable enzyme widely used in starch processing, particularly for liquefying starch-rich substrates [22]. However, conventional production of glucose- or maltose-enriched syrups generally relies on a two-step liquefaction–saccharification strategy, in which α-amylase is combined with glucoamylase, β-amylase, pullulanase, or other amylolytic enzymes to enhance the formation of target sugars [23–26]. Thus, compared with multi-enzyme systems, the production of maltose-rich hydrolysates using a single *B. licheniformis* α-amylase remains underexplored.

In this study, α-amylase from *B. licheniformis* TKU004 was chosen because it shows good activity at 50 °C and pH 7 and produces maltose as the major product during starch hydrolysis. These properties suggested that the enzyme was suitable for developing a mild enzymatic process to produce a maltose-rich hydrolysate from jackfruit seed starch.

Previous studies have examined the enzymatic hydrolysis of starchy wastes, such as bread waste [24] and mango kernel [25]. However, although α-amylase from *Bacillus licheniformis* has been employed in the hydrolysis of jackfruit seed, to the best of our knowledge, no systematic statistical optimization (e.g., using RSM) for the enzymatic hydrolysis of jackfruit seed specifically using this α-amylase source has been reported to date. Addressing this gap, the present study aimed to explore optimal conditions for maximizing DE generated from jackfruit seed hydrolysis through systematic experiments and statistical analysis using RSM with a BBD. The final hydrolysate was analyzed by HPLC to determine its saccharide composition. The novelty of this work lies in demonstrating that jackfruit seed starch, an underutilized agricultural by-product, can be converted into a maltose-rich starch hydrolysate using only α-amylase. Importantly, this result shows that a high-maltose hydrolysate can be produced without additional saccharifying or debranching enzymes such as β-amylase or pullulanase, which are typically required in conventional methods. This suggests a simpler, more cost-effective enzymatic system for industrial applications.

2. Results and Discussion

2.1. Proximate Composition of Jackfruit Seed

The proximate analysis and chemical composition of fresh jackfruit seeds are presented in Table 1. Overall, the results confirm that jackfruit seeds contain a substantial carbohydrate fraction and a moderate protein content, while lipid and ash contents are relatively low. This compositional profile is generally consistent with previous reports on jackfruit seeds, although some variation has been observed among studies. Such differences have been attributed to cultivar, growing conditions, seed maturity, and analytical or extraction procedures [27–31].

Table 1. The proximate analysis and chemical composition of fresh jackfruit seeds.

Parameter	Fresh Jackfruit Seeds
Moisture (%)	43.75 ± 2.65
Total lipid (%)	2.32 ± 1.52
Total protein (%)	18.02 ± 0.98
Carbohydrate (%)	34.01 ± 2.70
Starch (%)	25.48 ± 0.98
Ash (%)	1.89 ± 0.36

Of particular importance in the present study is the starch fraction, which represents a major component of the seeds and supports their potential use as a substrate for enzymatic conversion. Although the starch content obtained here was lower than that reported in some earlier studies, it remained within the broad range described in the literature for jackfruit seeds [29–32]. This variability is not unexpected, as previous studies have shown that starch yield and composition can differ substantially depending on both the jackfruit variety and the starch isolation method [30].

The relatively high starch content observed in jackfruit seeds also compares favorably with other tropical seed by-products investigated as alternative starch sources, such as longan, durian, and avocado seeds [33–35]. Therefore, beyond their nutritional relevance, jackfruit seeds can be regarded as a promising low-cost agricultural residue for valorization through starch-based bioprocessing. In the context of the present work, this compositional characteristic provides a strong rationale for their use as feedstocks for producing fermentable saccharides and maltose-rich hydrolysates.

2.2. Box–Behnken Design and Response Surface Methodology

To explore the impact of reaction conditions on the hydrolysis of jackfruit seed by *B. licheniformis* amylase, RSM was used to optimize the independent variables: temperature (A, °C), [E]/[S] ratio (B, U/g), and reaction time (C, h). The dextrose equivalent (DE, %) is used to measure the impact of those independent variables. Table 2 shows the experimental settings and DE following the Box–Behnken design of RSM. The DE from 15 runs ranged from 5.28% to 29.19%. Significant shifts in DE were detected across all combinations, indicating that the studied factors had a significant impact. The three center points (runs 5, 9, and 12) exhibit DE values of 25.02%, 26.24%, and 25.93%, respectively, indicating satisfactory repeatability in the experiment.

Table 2. Box–Behnken design matrix showing observed DE values at different combinations of temperature, [E]/[S] ratio, and reaction time.

Run Order	A (°C)	B (U/g)	C (h)	DE (%)
1	40	7.5	6	20.72%
2	50	5	2	12.09%
3	50	10	6	29.19%
4	40	5	4	12.46%
5	50	7.5	4	25.02%
6	60	7.5	2	5.28%
7	40	7.5	2	5.72%
8	60	7.5	6	13.59%
9	50	7.5	4	26.24%
10	60	5	4	8.92%
11	50	10	2	19.68%
12	50	7.5	4	25.93%
13	50	5	6	17.67%
14	40	10	4	23.54%
15	60	10	4	16.61%

A = temperature (°C); B = enzyme-to-substrate ratio ([E]/[S], U/g); C = reaction time (h).

The quadratic regression model was constructed to describe the relationship among variables A, B, C, and the response (DE). The regression coefficients, standard errors, t-values, and statistical significance levels are presented in Table 3. Based on the regression results in Table 3, the model equation with encoded variables is written as follows:

$$Y\ (DE, \%) = 25.730 - 2.255 \times A + 4.735 \times B + 4.800 \times C - 0.848 \times A \times B - 1.673 \times A \times C + 0.983 \times B \times C - 9.339 \times A^2 - 1.009 \times B^2 - 5.064 \times C^2 \quad (1)$$

As shown in Table 3, the positive linear coefficients for the [E]/[S] ratio ($\beta_2 = 4.735$, $p < 0.001$) and reaction time ($\beta_3 = 4.800$, $p < 0.001$) indicate that increasing either factor within the investigated range significantly improved DE. Conversely, the negative linear coefficient for temperature ($\beta_1 = -2.255$, $p = 0.0068$) suggests that DE decreased as temperature increased toward the upper end. The large negative quadratic coefficient for temperature ($\beta_{11} = -9.339$, $p < 0.001$) and the significant negative quadratic term for time ($\beta_{33} = -5.064$, $p = 0.0011$) confirm a nonlinear, curved relationship between these variables and DE, indicating that optimal values lie within the range studied. In contrast, none of the two-factor interaction terms (AB, AC, BC) were statistically significant ($p > 0.05$), indicating that the effects of temperature, enzyme/substrate ratio, and reaction time on DE are mostly independent within the examined range.

Table 3. Regression coefficients and statistical analysis of the quadratic model.

Model Term	Coefficient Estimate	Standard Error	t-Value	*p*-Value	Significance
Intercept	25.730	0.829	31.026	<0.0001	***
Linear					
A	−2.255	0.508	−4.440	0.0068	**
B	4.735	0.508	9.324	0.0002	***
C	4.800	0.508	9.452	0.0002	***
Interaction					
AB	−0.848	0.718	−1.180	0.2911	
AC	−1.673	0.718	−2.329	0.0673	
BC	0.983	0.718	1.368	0.2296	
Quadratic					
A^2	−9.339	0.748	−12.493	<0.0001	***
B^2	−1.009	0.748	−1.350	0.2351	
C^2	−5.064	0.748	−6.774	0.0011	**

A = temperature (°C); B = enzyme-to-substrate ratio ([E]/[S], U/g); C = reaction time (h). Significance codes: *** $p < 0.001$; ** $p < 0.01$.

The ANOVA results in Table 4 revealed that the linear component FO has a highly significant impact (F = 65.33; $p = 0.0002$), showing that all three examined factors significantly influence DE. The quadratic component PQ also has high statistical significance (F = 63.36; $p = 0.0002$), confirming that the DE response has a clearly curved shape and that a quadratic model is necessary to describe the hydrolysis system. Conversely, the interaction component between two factors, TWI, is not statistically significant ($p = 0.1411$), suggesting that within the examined range, the effects of the factors on DE are mainly independent rather than strongly interactive.

Table 4. Analysis of variance (ANOVA) for the fitted quadratic polynomial model.

	Sum of Squares	df	Mean Square	F-Value	*p*-Value	Significance
Model	814.43	9	90.49	43.86	0.0003	***
Linear (FO)	404.36	3	134.79	65.33	0.0002	***
Interaction (TWI)	17.92	3	5.97	2.90	0.1411	
Quadratic (PQ)	392.15	3	130.72	63.36	0.0002	***
Residual	10.32	5	2.06			
Lack of Fit	9.51	3	3.17	7.89	0.1146	
Pure Error	0.80	2	0.40			
Total	824.75	14				

Significance codes: *** $p < 0.001$.

According to Table 4, the lack of fit was not statistically significant ($p = 0.1146$), indicating that the deviation between the model and the experimental data is not significantly greater than the experimental error. Therefore, the quadratic model obtained was suitable for describing the amylase-catalyzed starch hydrolysis of jackfruit seeds within the examined range.

R^2 and adjusted-R^2 further confirmed the model's precision. The model, which is recommended to be well-fit, must have an R^2 over 80% [36]. Based on the R^2 value, 98.75% of the variance in the model can be explained. The model's goodness of fit, as indicated by the adjusted R^2 value, is very high at 96.49%. This means the model can explain up to 96.49% of the variability in the response, confirming its significance. In conjunction with the non-significant results observed in the lack of fit test (Table 4), this statistical model may be utilized to predict the optimal conditions, namely temperature, [E]/[S] ratio, and reaction time for the hydrolysis process of jackfruit seed starch.

To evaluate the suitability of the quadratic regression model, the model residuals were examined through a normal Q–Q plot and a residual plot against predicted values. The results are presented in Figure 1. Figure 1a shows that the residual points are relatively close to the theoretical line on the normal Q–Q plot. This suggests that the model residuals approximately follow a normal distribution, satisfying an important assumption of regression analysis and ANOVA. Figure 1b shows that the residuals are randomly distributed around the zero line, without forming funnel shapes, curved patterns, or obvious abnormal clusters. This indicates that the variance of the residuals is relatively homogeneous across the entire range of predicted values. Additionally, no extreme outliers with residuals much larger than those of the other points were observed. These results further support the conclusion from the lack-of-fit test in Table 4 that the quadratic model is appropriate for describing the relationship between temperature, enzyme activity, hydrolysis time, and DE. Therefore, it can be concluded that the RSM model obtained has strong predictive and optimization capabilities for the amylase-mediated starch hydrolysis of jackfruit seeds within the surveyed range.

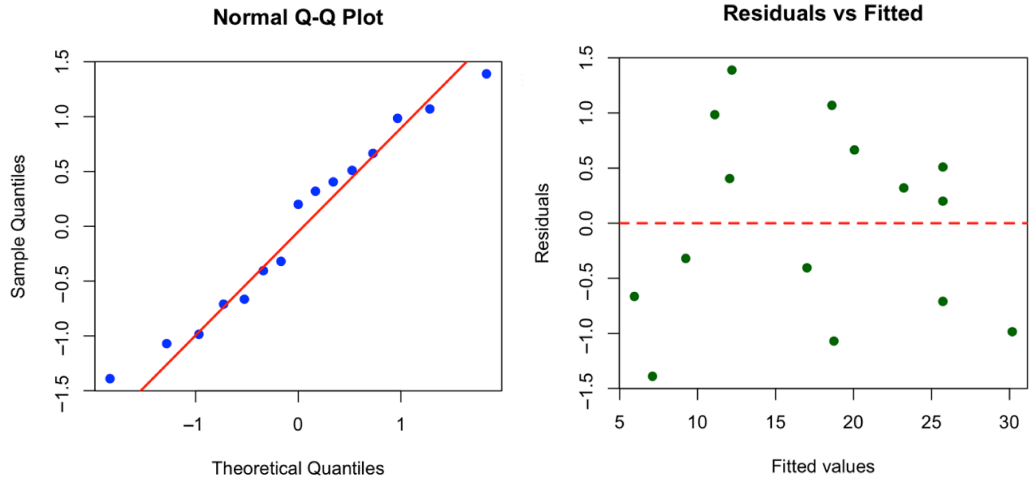


Figure 1. Diagnostic plots of the quadratic regression model: Normal Q–Q plot of residuals (**a**) and residuals versus fitted values (**b**). In the Normal Q-Q plot, blue dots are residuals, and the red line is the normal reference. In the residuals vs. fitted plot, green dots are residuals, and the dashed line marks zero residuals.

2.3. Response Surface Plots and Contour Plots

Three-dimensional response surface and contour plots were generated to visually evaluate the combined effects of the factors on DE (Figure 2). In the pairing of temperature and the [E]/[S] ratio, DE exhibited an increase as [E]/[S] increased; however, it only attained elevated values within the intermediate temperature range of approximately 46–50 °C. When the temperature reached 60 °C, a notable decrease in DE was observed, indicating a detrimental effect of high temperature on amylase activity (Figure 2a,d). This finding was consistent with the large negative quadratic coefficient for temperature (in Table 3) and with previously reported thermal inactivation profiles for *B. licheniformis* TKU004 α-amylase [37]. In the pairing of temperature and reaction time, high DE values were concentrated at intermediate temperatures (46–50 °C) and hydrolysis times of about 4.5–5.5 h (Figure 2b,e). Prolonged reaction at elevated temperatures likely accelerates enzyme denaturation, thereby limiting the increase in DE. For the [E]/[S] ratio–time pair (Figure 2c,f), DE increased when both factors were elevated simultaneously, with the highest response observed near 10 U/g and 4–6 h. A slight downward curvature along the time axis suggests that DE could plateau or marginally decline at extended reaction

times. Overall, Figure 2 demonstrated that optimal conditions for achieving high DE were a moderate temperature, a high enzyme/substrate ratio, and a moderate-to-long reaction time. These findings were consistent with the ANOVA results in Table 4, in which both linear and quadratic components were statistically significant, whereas the interaction between the two factors was not.

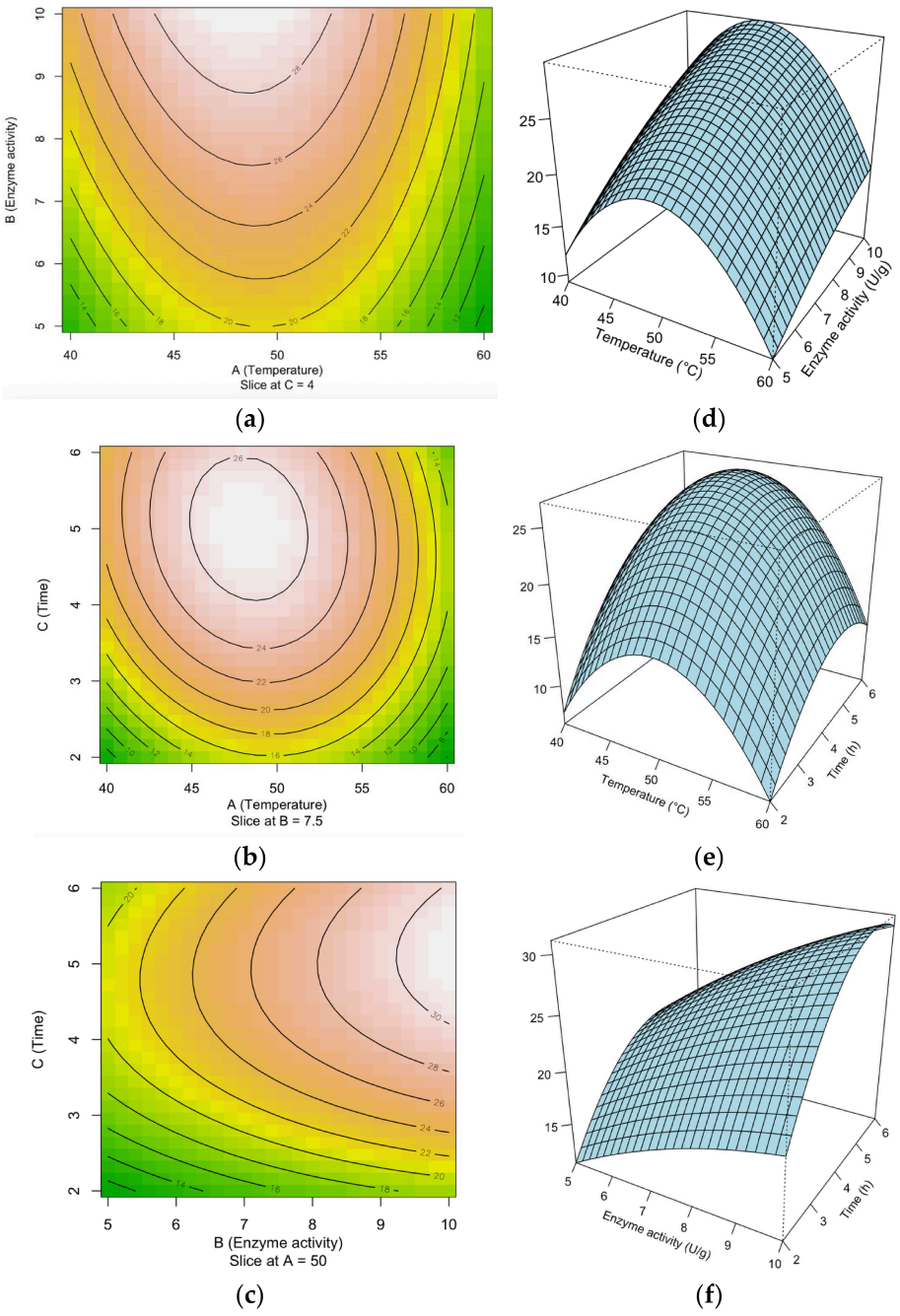


Figure 2. Contour plots and 3D response surface plots for the effects of three variates on DE formation: (**a**) contour plot for temperature (A, °C) vs. [E]/[S] ratio (B, U/g); (**b**) contour plot for temperature (A, °C) vs. reaction time (C, h); (**c**) contour plot for [E]/[S] ratio (B, U/g) vs. reaction time (C, h); (**d**) 3D response surface plot for temperature (A, °C) vs. [E]/[S] ratio; (**e**) 3D response surface plot for temperature (A, °C) vs. reaction time (C, h); and (**f**) 3D response surface plot for [E]/[S] ratio (B, U/g) vs. reaction time (C, h). The color gradient in contour plots represents the predicted DE (%), with green indicating lower values and light pink/white indicating higher values.

2.4. Model Validation

The quadratic equation identified the optimal conditions as 47 °C, a [E]/[S] ratio of 10 U/g, and an incubation period of 5.1 h, yielding a DE value of 31.72%. An experiment was conducted in triplicate to validate the model under these predicted optimal conditions. The results showed that the DE value was 32.85 ± 1.12%, which closely corresponds to the predicted values. In addition, the 95% prediction interval of the fitted quadratic model at this validation point was estimated to be 27.00–35.94%. The experimental mean value fell within this interval, further supporting the model's predictive reliability. Although no additional independent validation points beyond the original experimental design were included, the combination of experimental confirmation and prediction interval analysis indicates that the model adequately describes the hydrolysis system within the studied range. In comparison, the DE value here was similar to that of α-amylase–starch liquefaction systems for sweet potato starch, which produced about 30.52% DE [38].

2.5. Hydrolysate Analysis

The results of HPLC analysis (Figure 3) showed that the jackfruit seed starch hydrolysate product included some distinct peaks as follows: 14.98 min, 13.35 min, 12.5 min, 11.9 min, 11.43 min. The peaks at the positions of 14.98 min and 13.35 min were identified as glucose and maltose (respectively). The peaks at 12.5 min, 11.9 min, and 11.43 min may be maltooligosaccharides. This result indicated that the products in the jackfruit seed starch hydrolysate included: 14.196% glucose, 56.514% maltose, and 29.289% maltooligosaccharides. In a previous report, α-amylase from *B. licheniformis* TKU004 hydrolyzed starch, releasing maltose as the major product. Because maltose accounted for the majority of the hydrolysate (ratio > 50%), the jackfruit seed starch hydrolysate process in this study was well suited to preparing high-maltose syrup [39]. In short, jackfruit seeds, a by-product of jackfruit processing, can be used to produce maltose syrup by enzymatic hydrolysis using α-amylase from *B. licheniformis* TKU004.

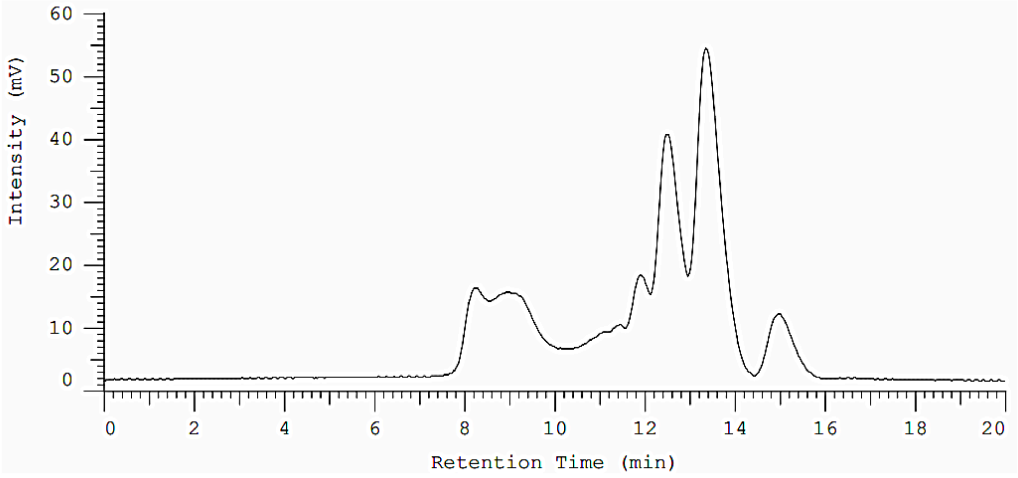


Figure 3. HPLC profile of jackfruit starch hydrolysate catalyzed by α-amylase from *B. licheniformis* TKU004.

The performance of the present process was compared with selected studies on the enzymatic hydrolysis of starch-rich substrates and by-products. Diopol et al. (2023) produced high-maltose syrup from rice bran starch through sequential liquefaction with α-amylase and saccharification with β-amylase, achieving a liquefaction DE of 15.6% and a final maltose content of 47.78% [26]. Araujo-Silva et al. (2018) obtained approximately 70% maltose conversion from cassava bagasse starch using β-amylase after 4 h at a substrate DE of 15.88 [40]. Although the maltose yield in the Araujo-Silva et al. (2018) study [40] was

higher, this difference is expected, given that β-amylase is an exo-acting enzyme specifically releasing maltose from the non-reducing end of starch chains, whereas α-amylase is an endo-acting enzyme that generates a mixture of maltose, maltotriose, and higher oligosaccharides. Achieving a maltose content of 56.51% with a single α-amylase under the present optimized conditions can therefore be considered a favorable result, particularly given that no supplementary debranching enzyme (such as pullulanase) or β-amylase was employed. Furthermore, a previous report on enzymatic hydrolysis of jackfruit seed starch using glucoamylase achieved a reducing sugar concentration of 9.286 ± 0.228 mg/mL at 70.75 °C over 4.8 h [14], with glucose as the predominant product, a profile fundamentally different from the maltose-enriched hydrolysate obtained in the present work. The ability of *B. licheniformis* TKU004 α-amylase to convert jackfruit seed starch into a maltose-rich hydrolysate (DE = 32.85%) under mild conditions highlights its potential as a biocatalyst for producing high-maltose syrup, a commercially important sweetener used in confectionery, beverages, and infant formula manufacture.

3. Materials and Methods

3.1. Materials

Jackfruits were purchased from local markets in Buon Ma Thuot, Vietnam. Seeds were manually extracted, washed with distilled water, and ground with deionized water at a ratio of 100 g seeds per 500 mL water using a laboratory blender. The resulting slurry was filtered through a double layer of cheesecloth to remove the solid residues. The filtered slurry was then heated at 100 °C for 20 min to achieve complete starch gelatinization, yielding a homogeneous starch paste that was used directly as the substrate for enzymatic hydrolysis. This condition was selected based on the typical gelatinization temperatures of jackfruit seed starch, which are generally below 100 °C [41], ensuring disruption of the granular structure and formation of a homogeneous paste suitable for enzymatic hydrolysis. The initial pH of the gelatinized jackfruit seed starch slurry was approximately 6.15. No buffer solution was added, and the hydrolysis reactions were conducted under the natural pH of the substrate system.

The strain *Bacillus licheniformis* TKU004 was detailed in a previous study [37,42,43]. The methodology for cultivating bacteria to produce amylase, as well as the purification process for the enzyme, followed Tran et al. (2025) [37]. The α-amylase was purified via ammonium sulfate precipitation and ion-exchange chromatography (High Q column, Bio-Rad Laboratories, Hercules, CA, USA), yielding an activity of about 560 U/mg and a 320-fold purification. The enzyme in this work is the purified α-amylase, with no other amylolytic enzymes added.

3.2. Proximate Composition Analysis

The moisture content was determined by dividing the mass of the jackfruit seeds after drying to a constant weight at 105 °C by the initial mass of the fresh jackfruit seeds. The ash content assay followed the method reported by Wong et al. (2021) [44]. Lipid content was determined by the Soxhlet method [44]. Protein content was determined by the Kjeldahl method [44]. The carbohydrate content assay followed the method reported by Thanh et al. (2020) [28]. The total starch content assay followed the method reported by Wong et al. (2021) [44].

3.3. Dextrose Equivalent Assay

The concentration of reducing sugars in the hydrolysate was determined by the 3,5-dinitrosalicylic acid (DNS) colorimetric method [11]. Briefly, 1 mL of appropriately diluted hydrolysate was mixed with 2 mL of DNS reagent and incubated in a boiling water

bath for 5 min. After cooling, the absorbance was measured at 540 nm using a UV–Vis spectrophotometer. Glucose was used as the standard to construct a calibration curve. The DE (%) was calculated as follows [45]:

$$\text{DE (\%)} = (\text{the reducing sugar content}/\text{the total solid content}) \times 100\% \quad (2)$$

where DE is the dextrose equivalent (%), the reducing sugar content is determined by the DNS method (mg), and the total solids content is the dry mass of the substrate used in the hydrolysis system (mg).

3.4. HPLC Analysis

The saccharide composition of the hydrolysate was analyzed by HPLC according to the method of Tran et al. (2025) [37] with minor modifications. Separation was performed on a KS-803 column maintained at 80 °C, using a flow rate of 1.0 mL/min and an injection volume of 10 µL. Sugars were detected with a refractive index (RI) detector. Glucose and maltose were identified by comparison of their retention times with those of authentic standards, and their relative proportions were estimated from the corresponding peak areas. Peaks eluting before maltose were assigned as maltooligosaccharides based on their retention behavior relative to the sugar standards.

3.5. Box–Behnken Design and Response Surface Methodology

The BBD was used to optimize three factors: incubation temperature, [E]/[S] ratio, and reaction time. Each factor was investigated at three levels: −1, 0, +1. Specifically, for temperature, the reaction temperatures were 40 °C (−1), 50 °C (0), and 60 °C (+1); for the [E]/[S] ratio, the levels were 5 U/g (−1), 7.5 U/g (0), and 10 U/g (+1); and for reaction time, the durations are 2 h (−1), 4 h (0), and 6 h (+1). The experiment is designed with 15 runs, with three replicates at the central point. The output variable used is the DE (%). The quadratic regression equation is as follows:

$$Y\ (\text{DE, \%}) = \beta + \beta_1 \times A + \beta_2 \times B + \beta_3 \times C + \beta_{12} \times A \times B + \beta_{13} \times A \times C + \beta_{23} \times B \times C + \beta_{11} \times A^2 + \beta_{22} \times B^2 + \beta_{33} \times C^2 \quad (3)$$

where

- Y is the response (DE, %).
- A, B, and C represent the independent variables: temperature, [E]/[S] ratio, and reaction time (respectively).
- β_0 is the constant.
- $\beta_{1–3}$ are the linear coefficients.
- β_{11}, β_{22}, and β_{33} are the quadratic coefficients.
- β_{12}, β_{13}, and β_{23} are the interaction coefficients.

3.6. Statistical Analysis

Analysis of variance (ANOVA) and regression analysis for the RSM model were performed using R (version 4.4.3) with the 'rsm' package.

4. Conclusions

This study demonstrated that gelatinized jackfruit seed starch can be efficiently hydrolyzed using α-amylase from *Bacillus licheniformis* TKU004, and that the process can be effectively modeled and optimized using Box–Behnken design coupled with response surface methodology. The fitted quadratic polynomial model exhibited high predictive reliability (R^2 = 0.9875, adjusted R^2 = 0.9649) with a non-significant lack-of-fit (p = 0.1146). The optimal conditions were identified as 47 °C, [E]/[S] = 10 U/g, and a reaction time of

5.1 h, yielding an experimentally validated DE of 32.85 ± 1.12%, in excellent agreement with the model prediction of 31.72%. Notably, HPLC characterization of the hydrolysate revealed a maltose-dominant saccharide profile (56.51% maltose, 14.20% glucose, 29.29% maltooligosaccharides), demonstrating that jackfruit seed starch can be converted into a high-maltose syrup using a single α-amylase without additional saccharifying or debranching enzymes. This finding is particularly significant, as conventional production of maltose-rich syrups typically requires multi-enzyme systems. Therefore, the present study provides a simplified and potentially more cost-effective enzymatic approach for industrial applications. These results highlight the potential of *B. licheniformis* TKU004 α-amylase as an efficient biocatalyst for valorizing jackfruit seed waste into high-value carbohydrate products, contributing to a more sustainable and economically viable food processing chain. Future work should evaluate the performance of the obtained maltose-rich hydrolysate as a substrate in fermentation or other bioprocesses to further validate its practical applicability.

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