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Optimization of Conditions for Extracting Type 2 Jackfruit Seed Resistant Starch using Response Surface Methodology

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Abstract: The process of extracting resistant starch 2 (RS2) from jackfruit seed starch using alpha-amylase enzyme under different processing conditions to remove digestible starch. At a hydrolysis temperature of 37°C and an incubation time of 14 hours with a supplemented enzyme concentration of 300 UI/ml, the highest RS2 recovery was 85.95%. Optimal values through response surface method (RSM) were determined as hydrolysis temperature of 38.13°C, enzyme concentration of 314.26UI/ml, and incubation time of 14.37 hours, resulting in an RS2 content of 90.17%, which was significantly higher than the initial natural RS2 content (63.48%). The configuration of starch granules before and after hydrolysis to remove digestible starch showed no significant changes when observed under scanning electron microscopy (SEM). These results indicate that the enzymatic extraction of RS2 is an effective method that does not alter the structure of starch granules, making it suitable for use in food processing.

Keywords: resistant starch 2, jackfruit seed starch, surface response methodology, alpha-amylase

I. INTRODUCTION

Starch is a common carbohydrate found in various foods such as rice, wheat, potatoes, corn, and other grains. It provides energy to the body and is converted into glucose to fuel cells. Based on its composition and rate of glucose release, starch can be classified into rapidly digestible starch, slowly digestible starch, and resistant starch (RS) [1]. Among these, RS has recently gained significant attention in the food industry due to its increased satiety, blood sugar stability, and crucial role in combating metabolic diseases and obesity. RS cannot be digested in the small intestine but is fermented by colonic bacteria in the large intestine, producing short-chain fatty acids and promoting the growth of beneficial bacteria [2]. Food products with added RS are becoming popular among consumers, with many willing to pay extra for RS-rich products to increase their daily fiber intake. Thus, the demand for RS applications as a functional ingredient in supplements is growing exponentially. Resistant starch includes all starch and starch degradation products capable of resisting digestion in the small intestine and entering the colon intact in humans. It plays a crucial role in the colon to prevent colorectal cancer and hemorrhoids by producing short-chain fatty acids [3].

RS2 represents starch with a robust granular structure that can resist enzymatic digestion. RS2 is found in some starchy foods such as raw potatoes, green bananas, and high-amylose corn. The starch of these foods has a high amylose content and belongs to the B and C crystalline types, making it resistant to digestion. In raw starch granules, starch is tightly packed in a crystalline structure and relatively water-free. This rigid structure limits the accessibility of digestive enzymes, various amylase enzymes, and confers the natural resistant properties of RS2, such as ungelatinized starch found in green bananas and potatoes [4].

Currently, there are numerous studies aimed at creating various types of RS using physical, chemical, and enzymatic methods. Among these methods, enzymatic starch modification techniques have many advantages, such as mild reaction conditions, ease of control, no requirement for high-pressure or acidic equipment, ensuring environmental friendliness, and safety for users [5]. Das et al. used a combination of two enzymes, amylopullulanase and amyloglucosidase, to increase the resistant starch content of green bananas. The results showed that although both enzymes were capable of cleaving both α-1,4 and α-1,6 glycosidic bonds, their mode of action created a unique, simple, efficient biological processing system, and the resistant starch content increased from 385 g/kg to 806 g/kg [6]. Zhang and Jin studied increasing the RS content of corn by combining α-amylase and pullulanase, the results showed that compared to natural corn starch, RS corn showed increased crystallinity and a more compact structure. The density of the crystals increased significantly, enhancing resistance to starch hydrolysis enzymes. This is a promising method for producing RS-rich products [7].

Response Surface Methodology (RSM) is one of the statistical methods for solving multivariable problems and evaluating experimental results. In the RSM method, two experimental design models widely used are Central Composite Design (CCD) and Box-Behnken Design (BBD). Kaur et al. studied the extraction of RS from green banana peels using enzyme-assisted ultrasonic techniques, using Box-Behnken design and RSM to

investigate the influence of parameters on the process towards RS yield. The study showed that processing time, temperature, and liquid-solid ratio significantly influenced RS production yield [8].

In this study, RS was extracted from jackfruit seed starch using alpha-amylase enzyme to hydrolyze digestible starches, the remaining product was dried to low moisture, and the RS content was determined. Through the investigation of factors, RSM was applied to determine the optimal conditions for obtaining starch with the highest RS content. The structural characteristics of starch were evaluated by SEM to compare with the original starch structure.

II. MATERIAL AND METHODS

2.1. Material

Thai jackfruit seeds harvested in Daklak province, thoroughly cleaned, and starch extracted using the method described by Wong and colleagues [9]. The resistant starch test kit from Megazyme was purchased from Megazyme Int. Ireland Ltd., Co. (Wicklow, Ireland); enzyme α-amylase from *Aspergillus oryzae*, Merck. All other reagents met the analysis criteria.

2.2. Methods

2.2.1. Preparation of jackfruit seed starch

The jackfruit starch was obtained using the chemical method outlined by the research group of Wong and colleagues, as depicted in Figure 1. After removing the tough outer skin, the jackfruit seeds were ground into a paste and diluted in distilled water (1:2 ratio). This mixture was continuously stirred (500 revolutions per minute) for 3 hours at room temperature. Subsequently, the residue was separated using a sieve or filter cloth. The supernatant obtained was centrifuged at 8000g for 5 minutes, and the upper layer was decanted. The sediment at the bottom was treated with 0.1M sodium hydroxide to dissolve the remaining proteins. The supernatant was kept at room temperature for 18 hours with continuous stirring. Then, it was centrifuged at 8000g for 10 minutes (25°C) and washed twice with 0.1M sodium hydroxide. The upper layer was decanted, and the remaining brown layer was also removed. The sample was thoroughly washed with water and neutralized using 0.1M hydrochloric acid until reaching a pH of about 6.5 to 7.0. The starch sample was further washed three times with distilled water to remove excess salts and then centrifuged at 8000g for 10 minutes (25°C). The moisture content of the starch supernatant was adjusted to 70% before being dried in an oven at 40°C for 18 hours. The dried starch sample was powdered using a grinder. The starch recovery efficiency from jackfruit seeds was calculated by dividing the final starch mass by the mass of jackfruit seeds used in the extraction process[9].

2.2.2. Determining the resistant starch content

The resistant starch content is analyzed using AOAC 2002.02 method. It involves weighing 0.1 grams of starch sample into a test tube with 0.1M sodium acetate buffer (pH=4.5), adding porcine pancreatic α-amylase and amyloglucosidase from Aspergillus Niger, vortex mixing, and enzymatic hydrolysis in a shaking water bath at 37°C for 16 hours. The reaction is stopped by adding ethanol, and the resistant starch fraction is recovered via centrifugation. The combined liquid portions are quantified to determine the digested starch (DS). The residue is dried, dissolved in 2M KOH in an ice-water bath, adjusted to pH ~4.5 with acetate buffer, and the undigested starch is quantified, considered as the amount of RS hydrolyzed by amyloglucosidase in a shaking water bath at 37°C for 30 minutes. This is further diluted to 100ml, and glucose absorption is measured using a glucose oxidase-peroxidase (GOPOD) assay at 510nm wavelength against a standard sample to determine the RS starch content, calculated as glucose content × 0.9. The total starch content of the sample is obtained by adding DS and RS [10].

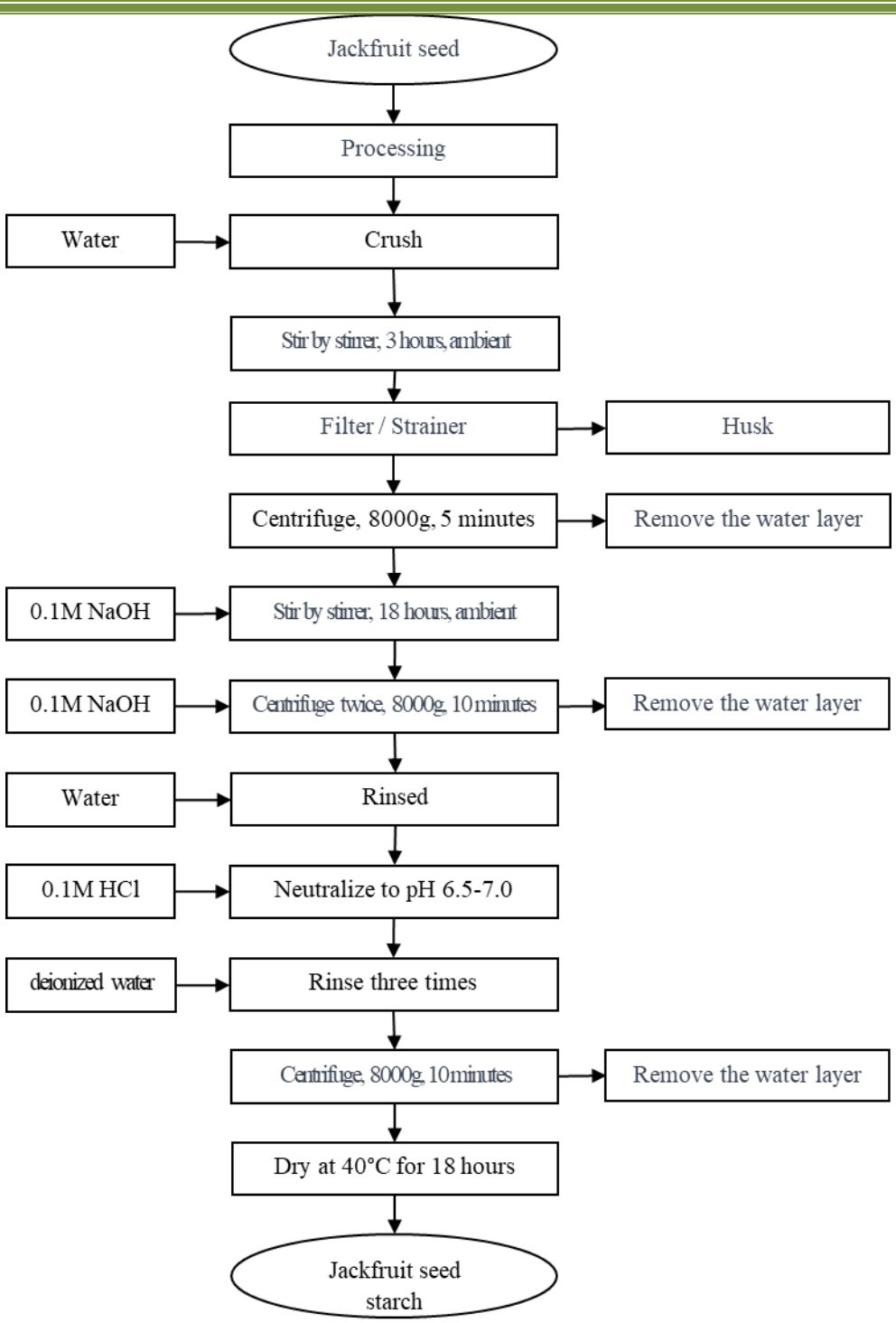


Figure 1. Process Flowchart for Extracting Jackfruit Seed Starch

2.2.3. Scanning electron microscopy (SEM) analysis.

Experimental SEM to analyze particle surface, shape and size using a JEOL JSM-5410LV instrument (JEOL, USA) equipped with a large field detector. The acceleration voltage is 15 kV in low vacuum mode (0.7-0.8 torr). The sample was affixed onto a copper stub using adhesive tape and then coated with gold in a vacuum environment. Images were taken at 2000x magnification [11].

2.2.4. Experimental Design

Starch solution was supplemented with enzyme at various concentrations, hydrolysis temperatures, and hydrolysis times as presented in Table 1. The average values of three experimental replicates were recorded. Statistical analysis of the data was conducted using Microsoft Excel 2016 and Statgraphics 19-X64 software.

Table 1. Technological parameters of jackfruit seed starch hydrolysis process

Factors Investigated	Fixed Factor(s)	Result
Hydrolysis temperature: 32, 37, 42, 47, and 50^{o}C	Enzyme supplementation concentration: 300UI/ml; hydrolysis time is 16 hours	Selected the temperature
Enzyme supplementation concentration: 200, 300, 400, 500, and 600UI/ml	Chosen temperature; hydrolysis time is 16 hours	Selected the enzyme supplementation concentration
Hydrolysis time: 12, 14, 16, 18, and 20 hours	Chosen temperature, chosen concentration	Selected the hydrolysis time.

Investigate the simultaneous effects of temperature, additional enzyme concentration and hydrolysis time on the RS content formed by the response surface method with Box-Behnken design. Optimize the hydrolysis process according to fixed factors and variable factors as shown in Table 2.

Table 2. Factors that change during the optimization experiment

Factor	Symbol	Levels		
		-1	0	+1
Temperature (^{o}C)	X_1	32	37	42
Concentration of added enzyme(UI/ml)	X_2	200	300	400
Hydrolysis time (hour)	X_3	12	14	16

The second-order polynomial regression model describes the influence of the above selection factors on the overall RS as the following equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_1 3 X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 (1)$$

In which: Y is the highest RS content (%) formed [6]. Data analysis and construction of response surface graphs according to the 3 factors selected above were performed using Design-expert 12.0 software.

III. RESULTS AND DISCUSSION

3.1. Investigation of factors influencing the RS formation hydrolysis process

3.1.1. Hydrolysis temperature

Temperature has a significant impact on enzyme reactions. Enzyme activity only increases up to a certain temperature limit; beyond that limit, enzyme activity decreases, leading to denaturation. The research results are illustrated in Figure 1, where increasing the incubation temperature from 32°C to 37°C resulted in a considerable increase in starch recovery efficiency. However, after increasing the temperature from 37°C to 52°C, the recovery efficiency showed a sharp decrease (from 83.95% to 66.16%). These results indicate that the enzyme operates optimally at a temperature of 37°C, yielding the highest recovery efficiency. At temperatures lower than this, the enzyme activity is still weak, and as the temperature exceeds 37°C, the enzyme activity gradually decreases, leading to a corresponding decrease in starch hydrolysis capability.

Furthermore, the pancreatic α-amylase enzyme in this study typically has an optimum temperature within the range of the body's operating temperature. The hydrolysis process with pancreatic *α-amylase* is carried out at temperatures higher than 37°C; therefore, significant enzyme inactivation may occur [12]. Under high-temperature conditions, enzymes undergo denaturation and lose their catalytic activity. The extent of enzyme activity reduction corresponds to the degree of protein denaturation. Additionally, enzymes lose their activity under the influence of other denaturing agents such as strong acids or bases, or heavy metal salts.

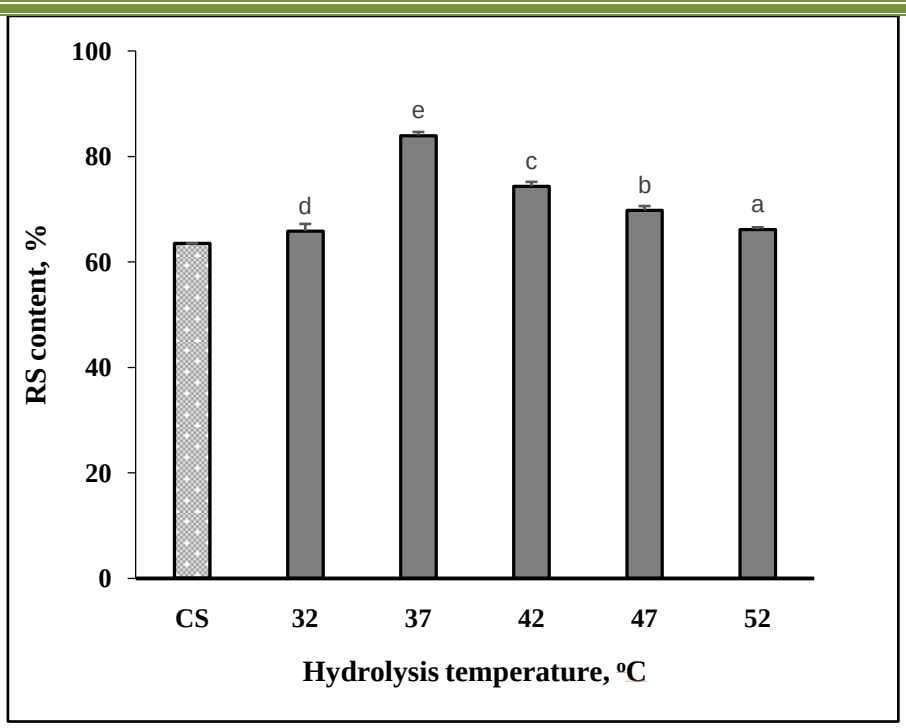


Figure 1. Influence of incubation temperature on resistant starch recovery capability
Different letters indicate significant differences within the same column at a 95% confidence level.

Miłek and Lamkiewicz investigated the starch hydrolysis process using *Bacillus spp.* α-amylase. The optimum temperature was determined to be within the range of 323.67 ± 1.48 K to 354.00 ± 2.27 K, with activation energy ranging from 18.01 ± 7.22 kJ/mol to 102.85 ± 20.53 kJ/mol, and the values of activation reduction energy ranging from 79.76 ± 8.77 kJ/mol to 162.85 ± 32.23 kJ/mol. The obtained results can be applied in starch hydrolysis industries. Enzyme activity varies with temperature. Initially, the enzyme activity increases with temperature. At a certain temperature, known as the optimum temperature, enzyme activity is at its maximum. Enzyme activity decreases beyond the optimal temperature[13].

Based on the fact that temperature directly affects enzyme activity by increasing the kinetic energy of beneficial molecules, thereby enhancing the effective collision between molecules, leading to the breakdown of enzyme-substrate complexes, product formation, and release of the enzyme to initiate a new cycle of activity. Studying the effect of temperature on enzyme reaction rate allows us to determine the maximum operating temperature of the enzyme (the temperature at which it typically becomes inactive) as well as optimize the enzyme process to operate at the maximum activity temperature and enzyme stability [14]. In the range of 20 to 50°C, the starch hydrolysis rate catalyzed by α-amylase enzyme from *A. oryzae* is proportional to the reaction temperature, reaching maximum speed at 50°C [15].

3.1.2. Enzyme concentration

The content of resistant starch (RS) in jackfruit seed starch initially increased and then decreased as the amount of pancreatic *α-amylase* added gradually increased from 300 to 600 UI/mL, as shown in Figure 2. The maximum RS content obtained was 83.78% when pancreatic *α-amylase* was added at a level of 300 UI/mL. This result follows a similar trend to that of Liu and colleagues. When there is an excess of substrate, the reaction rate increases with increasing enzyme concentration, but when the enzyme concentration saturates with substrate concentration, the reaction rate does not change or tends to decrease [16].

The reason for this phenomenon is that when the enzyme concentration increases, the viscosity of the solution also increases, making it difficult for the enzyme to act on the substrate. Additionally, the enzyme is inhibited by the hydrolysis products, thereby reducing the amount of digestible starch in jackfruit seed starch without reducing the recovery efficiency of resistant starch. According to Zhang and colleagues, although their study was on corn starch, the use of high enzyme concentrations tends to inhibit the crystallization process of amylose, thereby reducing the formation of RS [7].

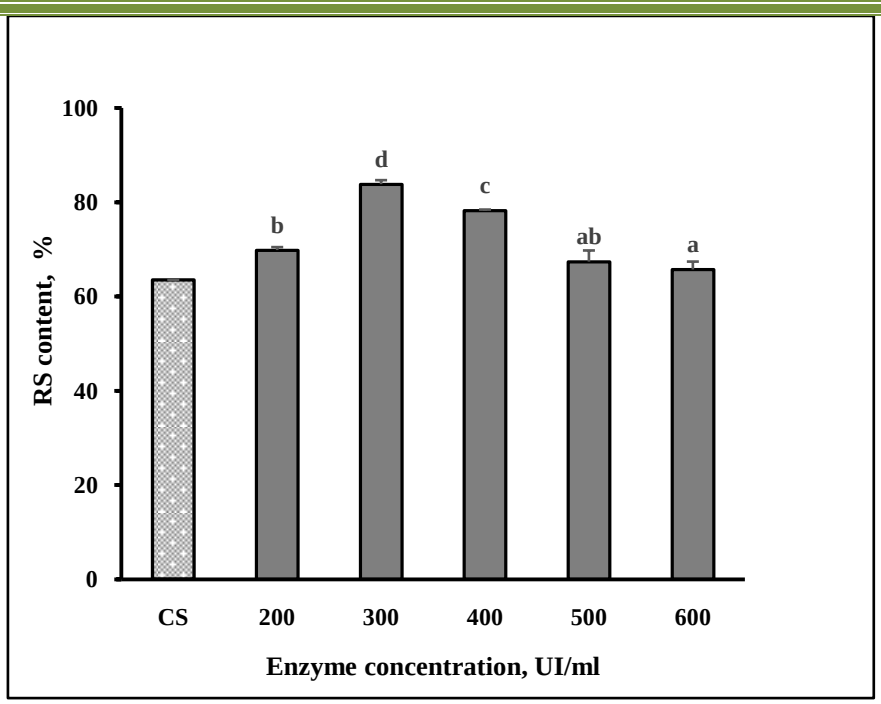


Figure 2. The influence of supplemented enzyme concentration on the recovery ability of resistant starch.
Different letters indicate significant differences within the same column at a 95% confidence level.

3.1.3. Hydrolysis time

Based on the results in Figure 3, when the incubation time is increased from 12 to 14 hours, the recovery efficiency of resistant starch tends to increase from 80.01% to 84.37%. However, when the incubation time is further increased to 20 hours, the recovery efficiency of resistant starch does not increase but tends to decrease gradually (from 84.37% to 75.16%). These results indicate that extending the hydrolysis time of the enzyme is necessary to completely hydrolyze the digestible starch, thereby leading to a higher recovery efficiency of resistant starch. This result is consistent with a study by Yangjin Liu and colleagues, where the hydrolysis of corn starch with heat-resistant alpha-amylase enzyme showed that as the time increased from 15 minutes to 25 minutes, the RS content gradually increased, reaching a maximum at 25 minutes, but continued increase in hydrolysis time up to 55 minutes resulted in a decrease in RS [16].

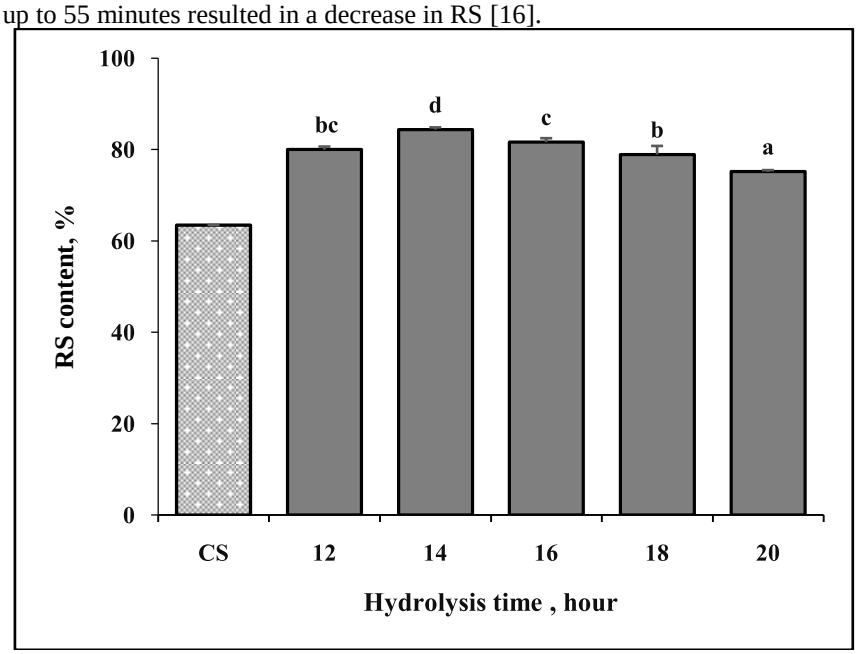


Figure 3. The effect of hydrolysis time on the recovery of resistant starch.
Different letters indicate significant differences within the same column at a 95% confidence level.

A study by Zhang and Jin combined two types of enzymes, α-amylase and pullulanase, to hydrolyze digestible starch and recover resistant starch from corn starch. The highest recovered RS content was 58.87% under the following conditions: temperature of 90°C; pH level of 5.5; hydrolysis time of 15 minutes; and supplemented *α-amylase* amount of 4 μ/g. Zhang suggested that reaction time is one of the most important factors in removing digestible starch and forming RS by enzymes. In the initial stage of the reaction, RS gradually increases over time. However, at a certain point, amylose molecules will be completely hydrolyzed into glucose molecules, which will prevent the formation of RS. This may be due to the tendency of the hydrolysis reaction to reach equilibrium due to the consumption of substrate [7].

Miao and colleagues proposed that initially, the RS content increases as enzymes remove maltose to accelerate the enzyme reaction rate and continue to form α-1,6 glycosidic bonds. Then, the decrease in RS content may be due to the inhibition of this hydrolysis product [17].

Therefore, in this experiment, a 14-hour incubation time with an enzyme concentration of 300 UI/mL and a temperature of 37°C achieved the highest RS recovery efficiency of 84.37%.

3.2. Investigation of optimal conditions

The surface response methodology, aimed at minimizing the number of experiments while still ensuring reliable results, has been widely applied in optimizing food processing processes [18], [19]. The results indicate that the influence of investigated factors such as hydrolysis temperature, supplemented enzyme concentration, and hydrolysis time all affect the formation of RS2. Under conditions of 37°C temperature, enzyme concentration of 300 UI/ml, and 14 hours of hydrolysis, the obtained RS2 is 85.95%, which is higher than the initial experiment (Table 3).

Table 3. Experimental Design Matrix

Experiment	Temperature (°C)	Enzyme Concentration (UI/mL)	Hydrolysis Time (hour)	RS Content (%)
1	37	300	14	84.47
2	42	400	16	73.57
3	37	300	14	85.95
4	32	200	16	64.89
5	32	200	12	63.61
6	37	432	14	73.81
7	42	400	12	66.74
8	37	300	17	79.74
9	42	200	16	64.11
10	30	300	14	70.38
11	37	300	11	74.63
12	42	200	12	64.17
13	44	300	14	81.74
14	32	400	12	64.34
15	37	300	14	84.48
16	32	400	16	66.37
17	37	300	14	82.65
18	37	300	14	85.03
19	37	300	14	82.13
20	37	168	14	67.94

The second-degree polynomial model of RS2 function (Y) includes variables such as hydrolysis temperature (X_1), enzyme concentration (X_2), and hydrolysis time (X_3).

$Y(\%) = - 466.24 + 14.67X_1 + 0.36X_2 + 30X_3 - 0.21X_1^2 – 0.0008X_2^2 – 1.12X_3^2$ (2).

The statistical data show that the factors investigated influence the content of resistant starch (RS) according to the second-degree regression model; as the values of the factors within the investigated range increase, the RS content also increases, and there is interdependence among them. The smaller the p-value of the model and the larger the Lack of Fit (insignificant lack of fit), the higher the model's fit. The results of statistical analysis based on the Fisher index, with coefficients having P-values < 0.05 being significant (Table 4), show that the influence of each individual independent variable (X_1, X_2, X_3) and the quadratic terms (X_1^2, X_2^2, X_3^2) are all significant when included in the model. The Fisher test value (Fisher) of the model is 32.04 with a p-

value < 0.0001, indicating that the model is entirely statistically significant with 99.99% confidence. The Lack of Fit of the model is 0.1073, demonstrating that the model fits well with the experimental data. Additionally, the R2 (coefficient of determination) values of the model greater than 0.9 indicate a high correlation between the predicted model and the experimental values [20], [16].

Table 4. Significance Test of Equation Coefficients

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	1312.22	9	145.80	32.04	< 0.0001
X_1- Tempareture	51.64	1	51.64	11.35	0.0071
X_2- Concentration	42.09	1	42.09	9.25	0.0124
X_3- Time	24.63	1	24.63	5.41	0.0423
X_1X_2	12.05	1	12.05	2.65	0.1347
X_1X_3	1.50	1	1.50	0.3288	0.2341
X_2X_3	7.30	1	7.30	1.60	< 0.0001
X_1^2	193.07	1	193.07	42.42	< 0.0001
X_2^2	475.10	1	475.10	104.40	< 0.0001
X_3^2	148.36	1	148.36	32.60	0.0002

According to Table 4, the experimental condition that has the greatest impact on the RS2 content is the quadratic term of enzyme concentration (X_2^2), followed by the hydrolysis temperature of starch (X_1^2), and the quadratic term of hydrolysis time (X_3^2). All other independent factors are significant ($p > 0.05$). The order of the independent variables based on their main effects on increasing the RS2 content is $X_1 > X_2 > X_3$, indicating that the enzyme hydrolysis temperature is the most important variable influencing the RS2 content, followed by the concentration and hydrolysis time. However, the simultaneous correlation between X_1, X_2, and X_3 is not statistically significant for this model.

Table 5. Values and Metrics Assessing Model Fit

Std. Dev.	2.13	**R^2**	0.9665	**Lack of Fit**	F = 3.31
Mean	74.04	**Adjusted R^2**	0.9363		P = 0.1073
C.V. %	2.88	**Predicted R^2**	0.7729	**Adeq Precision**	14.2913

The results of the ANOVA analysis in Table 5 show an R^2 value of 0.9665, indicating a high level of accuracy of the model, with 96.65% of the variation in starch hydrolysis and conversion of resistant starch to glucose being explained by the related factors, with only 3.35% attributable to error (random error). Additionally, the coefficient of variation (C.V.%) in the experiment is low at 2.88%, indicating precise experimentation and high repeatability. The predicted R^2 value is 0.7729, which is close to the adjusted R^2 of 0.9363. The goodness of fit, representing the signal-to-noise ratio, is 14.2913, which is greater than 4, indicating sufficient signal [6], [21].

Surface Response Analysis

From the experimental database and regression equation (2), a response surface model can be utilized to predict optimal points demonstrating the highest RS2 recovery efficiency. The response surface (Figure 4) illustrates a three-dimensional bell-shaped surface and displays contour lines indicating the presence of positions with the highest RS2 recovery efficiency within the investigated range.

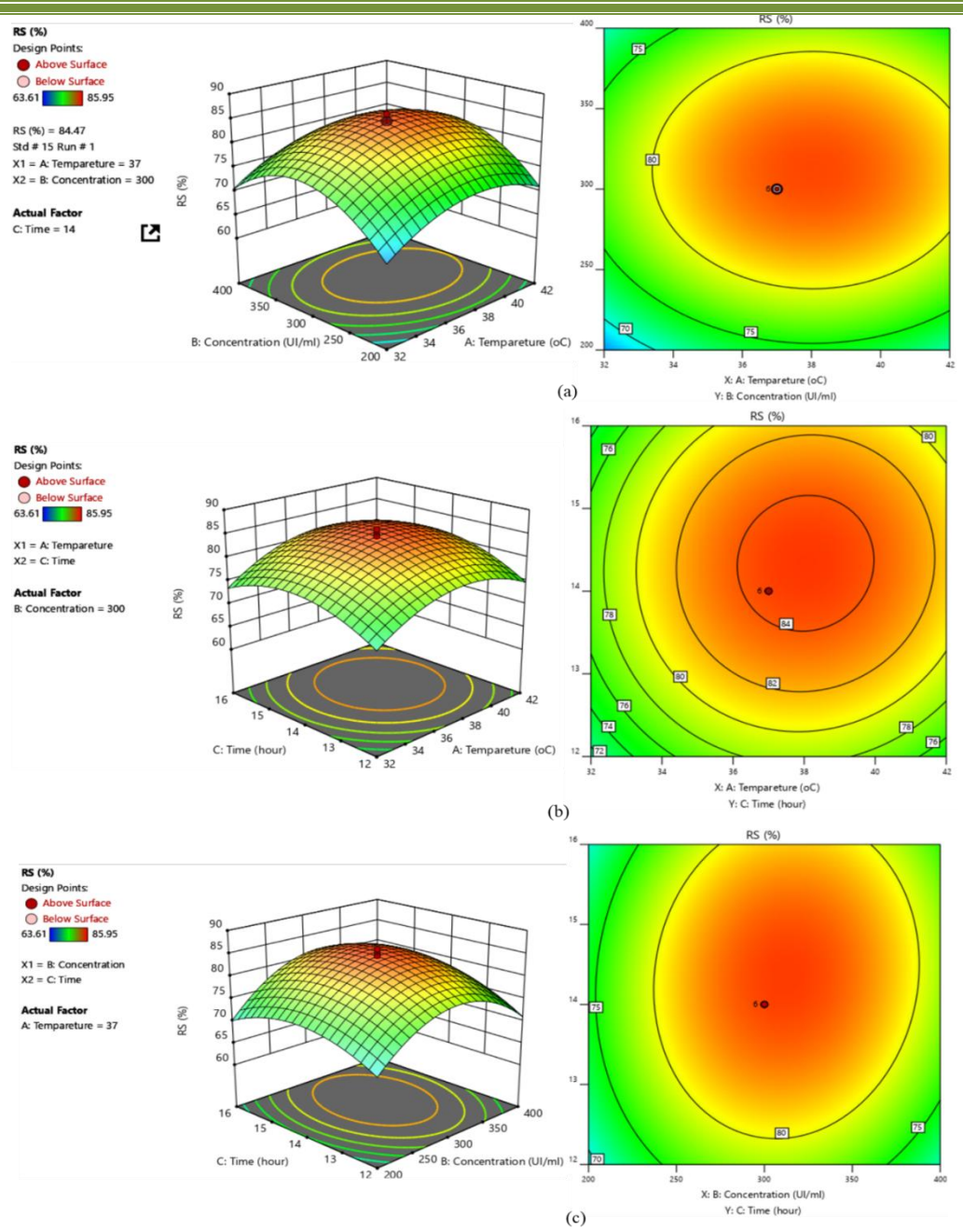


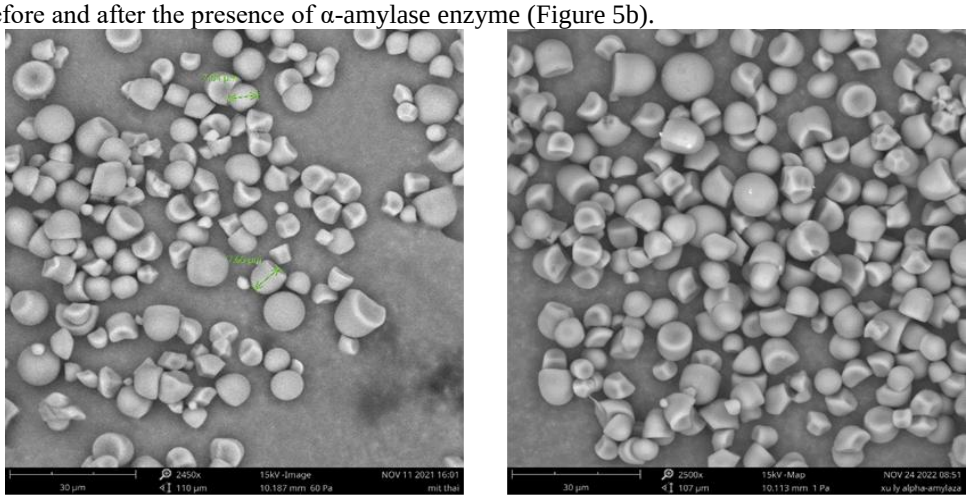
Figure 4. The response surface graph illustrates the dependency of the recovery efficiency of RS2-resistant starch on the hydrolysis temperature and enzyme concentration (a); hydrolysis temperature and hydrolysis time (b); hydrolysis time and enzyme concentration (c).

The model aims to find the optimal processing conditions to achieve the highest RS2 content, predicted by the model to be Y_{max} = 84.95% at a hydrolysis temperature of 38.13°C, enzyme concentration of 314.36 UI/mL, and hydrolysis time of 14.37 hours. For the six experiments according to the model, Y_{max} = 85.95% at a hydrolysis temperature of 37°C, enzyme concentration of 300 UI/mL, and hydrolysis time of 14 hours, the investigated factors show no significant difference. According to the study by Nguyen and Nguyen in 2018, optimizing the conditions of heat and moisture treatment for sweet potatoes and purple potatoes using the response surface method, the maximum predicted RS content of processed sweet potato starch (43.9%) obtained under optimal conditions is moisture content 34.76%, preheating temperature 100.11°C, and soaking time 6.01 hours; the maximum predicted RS content of processed purple potato starch (36.8%) obtained under optimal conditions is moisture content 30.06%, preheating temperature 109.68°C, and soaking time 6.59 hours using a second-order model within the range of various process variables. The experimental RS content of processed

sweet potato and purple potato starch obtained under optimal processing conditions is 42.4% and 35.4%, respectively; this confirms that the models are valid and complete because the predicted data and experimental data are not significantly different [22].

3.3. Scanning Electron Micrograph of RS2

The surface of RS2 starch particles was investigated using SEM. The surface of natural starch particles appeared as irregularly shaped circles or ellipsoids of varying sizes, with some particles showing cut marks (Figure 5a). The surface of the enzymatically hydrolyzed sample exhibited significant indentations and cut marks, with variations in particle size. However, overall, there were no significant changes observed in the particles before and after the presence of α-amylase enzyme (Figure 5b).



(a) **(b)**

Figure 5. Morphology of jackfruit seed starch particles before (a) and after (b) treatment with alpha-amylase enzyme.

The SEM results differ from the study by Liu and colleagues on the modification of corn starch by enzyme mixtures [16]. The enzymatic hydrolysis process can help break the α-1,4 glucan bonds of starch particles into linear chains and shorten the starch chains. When there is an excess of α-amylase, it tends to attack the linear chains of jackfruit seed starch. Subsequently, these starches form smaller particles during aging and aggregation. After dissolving amylose, amylose crystals are formed in the subsequent aging process [23].

IV. CONCLUSION

When using hydrolytic enzymes to remove digestible starch, it leads to a significant increase in the resistant starch (RS) content in jackfruit seed starch. Through the survey, it is shown that temperature has the greatest influence on the hydrolysis process of digestible starch, followed by the concentration of supplemental enzymes and hydrolysis time. The RS2 content formed under optimal conditions does not differ significantly from the results of previous surveys. This indicates that the Response Surface Methodology (RSM) model is suitable for the selected experiments. The obtained starch has a higher RS2 content compared to the initial RS2, from 63.48% to 85.95%. The starch granule structure remains unchanged and serves as the basis for producing resistant starch from jackfruit seeds, which can be beneficial for future health-oriented food processing technologies.

REFERENCES

[1]. T. M. Ali and A. Hasnain, “Morphological, physicochemical, and pasting properties of modified white sorghum (Sorghum bicolor) starch,” *International journal of food properties*, vol. 17, no. 3, pp. 523–535, 2014.

[2]. E. Fuentes-Zaragoza, M. J. Riquelme-Navarrete, E. Sánchez-Zapata, and J. A. Pérez-Álvarez, “Resistant starch as functional ingredient: A review,” *Food Research International*, vol. 43, no. 4, pp. 931–942, 2010.

[3]. A. P. Nugent, “Health properties of resistant starch,” *Nutrition Bulletin*, vol. 30, no. 1, pp. 27–54, 2005.

[4]. M. G. Sajilata, R. S. Singhal, and P. R. Kulkarni, “Resistant starch–a review,” *Comprehensive reviews in food science and food safety*, vol. 5, no. 1, pp. 1–17, 2006.

[5]. X. Li *et al.*, "Partial branching enzyme treatment increases the low glycaemic property and α-1, 6 branching ratio of maize starch," *Food chemistry*, vol. 164, pp. 502–509, 2014.
[6]. M. Das, N. Rajan, P. Biswas, and R. Banerjee, "Dual enzyme treatment strategy for enhancing resistant starch content of green banana flour and in vitro evaluation of prebiotic effect," *LWT*, vol. 160, p. 113267, 2022.
[7]. H. Zhang and Z. Jin, "Preparation of products rich in resistant starch from maize starch by an enzymatic method," *Carbohydrate polymers*, vol. 86, no. 4, pp. 1610–1614, 2011.
[8]. B. Kaur, K. B. Venkatrao, P. S. Panesar, H. K. Chopra, and A. K. Anal, "Optimization of ultrasound-assisted enzymatic extraction of resistant starch from green banana peels and its structural characterization," *Journal of Food Science and Technology*, vol. 59, no. 12, pp. 4663–4672, 2022.
[9]. K. T. Wong, G. Y. Y. Poh, K. K. T. Goh, M. S. M. Wee, and C. Jeyakumar Henry, "Comparison of physicochemical properties of jackfruit seed starch with potato and rice starches," *International Journal of Food Properties*, vol. 24, no. 1, pp. 364–379, 2021.
[10]. B. V. McCleary, M. McNally, and P. Rossiter, "Measurement of resistant starch by enzymatic digestion in starch and selected plant materials: collaborative study," *Journal of AOAC International*, vol. 85, no. 5, pp. 1103–1111, 2002.
[11]. O. S. Kittipongpatana and N. Kittipongpatana, "Resistant starch contents of native and heat-moisture treated jackfruit seed starch," *The Scientific World Journal*, vol. 2015, 2015.
[12]. J. Mi\lek, "Determination of activation energies and the optimum temperatures of hydrolysis of starch by α-amylase from porcine pancreas," *Molecules*, vol. 26, no. 14, p. 4117, 2021.
[13]. J. Mi\lek and J. Lamkiewicz, "The starch hydrolysis by α-amylase Bacillus spp.: an estimation of the optimum temperatures, the activation and deactivation energies," *Journal of Thermal Analysis and Calorimetry*, vol. 147, no. 24, pp. 14459–14466, 2022.
[14]. T. Baks, F. H. Kappen, A. E. Janssen, and R. M. Boom, "Towards an optimal process for gelatinisation and hydrolysis of highly concentrated starch–water mixtures with alpha-amylase from B. licheniformis," *Journal of Cereal science*, vol. 47, no. 2, pp. 214–225, 2008.
[15]. J. L. M. Soto, L. M. García, J. V. González, A. B. Nicanor, and L. G. Cruz, "Influence of starch source in the required hydrolysis time for the production of maltodextrins with different dextrose equivalent," *African Journal of Biotechnology*, vol. 11, no. 69, pp. 13428–13435, 2012.
[16]. Y. Liu *et al.*, "Optimization of Corn Resistant Starch Preparation by Dual Enzymatic Modification Using Response Surface Methodology and Its Physicochemical Characterization," *Foods*, vol. 11, no. 15, p. 2223, 2022.
[17]. M. Miao, S. Xiong, B. Jiang, H. Jiang, S. W. Cui, and T. Zhang, "Dual-enzymatic modification of maize starch for increasing slow digestion property," *Food Hydrocolloids*, vol. 38, pp. 180–185, 2014.
[18]. Y. Song, X. Li, and Y. Zhong, "Optimization of butter, xylitol, and high-amylose maize flour on developing a low-sugar cookie," *Food Science & Nutrition*, vol. 7, no. 11, pp. 3414–3424, 2019.
[19]. N. T. N. Giang, T. V. Khai, and N. M. Thuy, "Optimization of moromi fermentation parameters to nutritional content of oyster mushrooms sauce (Pleurotus spp.) by using response surface methodology," *Food Research*, vol. 5, no. 5, pp. 149–156, 2021.
[20]. X. Guan and H. Yao, "Optimization of Viscozyme L-assisted extraction of oat bran protein using response surface methodology," *Food chemistry*, vol. 106, no. 1, pp. 345–351, 2008.
[21]. D. Kundu, S. Karmakar, and R. Banerjee, "In silico optimization of enzyme mediated debittering of Assam lemon: biochemical and sensory evaluation studies," *Journal of food science and technology*, vol. 56, pp. 2233–2243, 2019.
[22]. K. K. Nguyen and T. L. P. Nguyen, "Optimisation of heat-moisture treatment conditions for producing high amounts of resistant starches from purple sweet potato and yam starches using response surface methodology," *Vietnam Journal of Science, Technology and Engineering*, vol. 60, no. 4, pp. 8–14, 2018.
[23]. J. Bartz, E. da Rosa Zavareze, and A. R. G. Dias, "Study of heat–moisture treatment of potato starch granules by chemical surface gelatinization," *Journal of the Science of Food and Agriculture*, vol. 97, no. 10, pp. 3114–3123, 2017.

