

Hydrolysis optimization of porang (*Amorphophallus oncophyllus*) glucomannan using beta-mannanase by response surface methodology

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Abstract

Porang (*Amorphophallus oncophyllus*) is a native tuber from Indonesia which contains a high amount of glucomannan. The enzymatic hydrolysis of glucomannan may change its functional properties for a wider application in food products. This study aimed to obtain the optimal conditions for the enzymatic hydrolysis of porang glucomannan (PGM) by β -mannanase using response surface methodology (RSM). The conditions for enzymatic hydrolysis were optimized using four independent variables: pH (4-10), time (0-6 h), temperature (30-60°C), and concentration of enzyme-to-substrate ratio (E/S) (0.1-0.7%). Single-factor experiments were used to determine the center point of the RSM, and reducing sugar (RS) was used as an indicator of porang glucomannan hydrolyzate (PGH) in the RSM study. The optimal hydrolysis condition obtained by RSM was at pH 6.81, hydrolysis time of 3.03 h, hydrolysis temperature of 37.6°C, and E/S of 0.8%. The RS value obtained was 1.34±0.03 mg/mL. These data demonstrated that the experimental results were similar to the predicted responses from the RSM model (RS value of 1.35 mg/mL). The E/S had the most significant effect on the RS value of the four variables examined. TLC result showed that PGH is rich in mannotriose. Therefore, PGM was successfully hydrolyzed under optimal conditions and yielded mannotriose as the primary byproduct.

1. Introduction

Glucomannan, a source of water-soluble dietary fiber, is composed of D-mannose and D-glucose, which are linked by β -1,4-glycosidic bonds (Katsuraya *et al.*, 2003), and is often used as a thickener, gelling agent, forming textures, water binder, fat substitute, and forming film (Takigami, 2009). Glucomannan not only improves texture, but also provides a variety of health advantages, such as improving digestion (Chen *et al.*, 2006), anti-obesity (Birketvedt *et al.*, 2005), immunomodulatory activities (Gurusmatika *et al.*, 2017) and potential as a prebiotic source (Harmayani *et al.*, 2014).

Glucomannan can be extracted from various types of plants. One plant that contains a lot of glucomannan is the *Amorphophallus* plant. The method of extraction and the source of glucomannan will affect its functional properties. *Amorphophallus konjac* from Japan has been

widely commercialized as a source of glucomannan (Gille *et al.*, 2011). *Amorphophallus oncophyllus*, locally called porang in Indonesia, is rich in glucomannan that has good prospects to be used as a food ingredient and functional food (Harmayani *et al.*, 2014).

Glucomannan can dissolve in water and become a thick gel (Sumarwoto, 2007). However, the gel has a high viscosity that can cause choking if consumed directly or used in medicines (Tester and Al-Ghazzewi, 2013). In addition, glucomannan has a prolonged solubility rate because it has a large molecular weight (Luo *et al.*, 2012). High solubility of glucomannan is preferred in the food industry for simpler and wider applications that can be obtained by altering the glucomannan structure, which may affect its characteristics. One process that can be done to modify the structure of glucomannan is the hydrolysis of glucomannan. The hydrolysis process can also affect the

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prebiotic potential of glucomannan (Ariestanti *et al.*, 2019; Anggela *et al.*, 2021) since the hydrolysis process simplifies its structure. Therefore, it is easier to be used by colon bacteria (Tester and Al-Ghazzewi, 2013).

The hydrolysis process of glucomannan can be done using acids or enzymes, a biocatalyst widely used to increase the extraction yield of the desired target compound on certain materials. Enzyme-based extraction is regarded as a method that is energy-efficient and beneficial to the environment (Bhotmange *et al.*, 2017). β -mannanase is a hydrolase enzyme that can hydrolyze β -1,4-mannosidic bonds in the main chain of glucomannan and produce straight or branched chain oligosaccharides of a certain length (van Zyl *et al.*, 2010).

The process of enzymatic hydrolysis of glucomannan is influenced by several factors that affect the efficiency of the hydrolysis process. The traditional approach, which involves changing one parameter while keeping the other at a predetermined constant level, is one-dimensional, time-consuming, and frequently unsuccessful in identifying the optimal conditions. However, because of the high number of experiments needed, conducting tests with every factorial combination of the test variables is impossible. This problem can be solved using the response surface methodology (RSM). RSM is a statistically designed experimental protocol in which several factors were varied at the same time (Chen *et al.*, 2013). RSM has several advantages, such as being easy to implement, cheap, fewer required unit experiments, and less time consumption (Herrera-Calderon and Vega, 2020). RSM has proven successful and economical in various industries during optimization processes (Chen *et al.*, 2013).

This study aimed to obtain the optimal conditions for the hydrolysis of porang glucomannan (PGM), mainly for its pH, hydrolysis time, hydrolysis temperature, and concentration of enzyme-to-substrate ratio (E/S) using β -mannanase by RSM.

2. Materials and methods

2.1 Materials and chemicals

Porang was obtained from a local farmer in Nglanggeran, Gunung Kidul, Indonesia. Its glucomannan was obtained by direct extraction from the tubers using 50% ethanol with a ratio of tuber: ethanol 1:1.75 (Yanuriati *et al.*, 2017). β -mannanase was purchased from Mianyang Habio Bioengineering Co. Ltd. (Sichuan, China). All other analytical-grade chemicals were purchased from Merck KGaA, Darmstadt, Germany.

2.2 Hydrolysis of porang glucomannan

PGM powder (0.5 g) was added to a 50 mL citrate-phosphate buffer then mixed with β -mannanase to start the reaction. The E/S ranging from 0.1% to 0.7% (w/w) were used (Table 1). The mixture was incubated at various pH (4, 5, 6, 7, 8, and 10) for reaction times of 0, 0.5, 1, 2, 4, and 6 h while the temperature of the water bath was kept steady at a given temperature (30°C, 35°C, 40°C, 45°C, 50°C, and 60°C). The reaction was stopped by boiling the sample for 15 min. The sample was then vacuum filtered, and the filtrate was tested for the total sugar (TS) and reducing sugar (RS) to calculate the degree of polymerization (DP).

Table 1. The concentration of the enzyme to substrate ratio (E/S) used for the hydrolysis of PGM.

E/S	β -mannanase (mg)	PGM powder (mg)
0.1%	0.5	500
0.2%	1.0	500
0.3%	1.5	500
0.4%	2.0	500
0.5%	2.5	500
0.6%	3.0	500
0.7%	3.5	500

2.3 Determination of total sugar, reducing sugar, and degree of polymerization

The degree of polysaccharide degradation is indicated by total sugar (TS), reducing sugar (RS), and DP. Thus, TS, RS, and DP can be used to monitor the enzymatic process (Chen *et al.*, 2005; Chen *et al.*, 2013). The ratio of TS to RS formed after enzymatic hydrolysis yields DP. RS was analyzed using the Somogyi method (Somogyi, 1945). TS was determined by adding 5 mL 8% H₂SO₄ to 5 mL of the sample, mixing the sample, and then boiling for 2 h. The pH was then adjusted to 8-10 with 9% NaOH after the sample was cooled to room temperature. Then the sample was diluted with deionized water to a volume of 25 mL and analyzed using the Somogyi method (Chen *et al.*, 2013). DP value was then calculated with the following equation:

$$DP = \frac{TS}{RS}$$

2.4 Experimental design

The hydrolysis parameters were optimized using RSM, specifically Box-Behnken Design (BBD). A total of 4 variables BBD with 3 levels were used to obtain the maximum conditions in the production of porang glucomannan hydrolyzate (PGH) (Table 2). The independent variables were the pH, time, temperature, and E/S. RS was chosen as the response (Y) and the

single-factor experiment findings were used to determine the center point of the RSM (Level 0). All the experiments were done in triplicate, and experimental runs were randomized to reduce the impact of unanticipated variability in the observed responses. The behavior of the system was explained by the following quadratic equation:

$$Y = \beta_0 + \sum_{i=1}^4 \beta_i X_i + \sum_{i=1}^4 \beta_{ii} X_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^4 \beta_{ij} X_i X_j$$

Where Y is the response value (RS); β_0 is a constant; β_i is the linear regression coefficient; β_{ii} is the quadratic regression coefficient; β_{ij} is the interaction regression coefficient; X_i and X_j are the levels of the independent variables.

To determine the optimal conditions for the hydrolysis of PGM, the response value (RS) was analyzed using Design Expert software (Trial version) (Stat-Ease, Minneapolis, MN, USA). The p -values that are less than 0.05 were regarded as statistically significant.

2.5 Thin layer chromatography

The TLC method was used to assess the simple sugars in the PGH, which was modified from Safitri *et al.* (2014). PGH (4 μ L) and the standard solution (2 μ L) were spotted on a silica gel 60F₂₅₄ plates (Merck Art20-

20 cm, Darmstadt, Germany) and then eluted with *n*-butanol/acetic acid/water mixture (12:6:6). The standards used were glucose, mannose, mannobiose, mannotriose, mannotetraose, mannopentaose and mannohexaose (Megazyme, Wicklow, Ireland). The silica plate was then sprayed with a staining solution consisting of 0.4 g α -diphenylamine, 20 mL acetone, 3 mL phosphoric acid, and 0.4 mL aniline. The spots formed were visualized by heating the silica plate at 121°C for 15 min.

3. Results and discussion

3.1 Single factor experimental analysis

3.1.1 Effect of pH on total sugar, reducing sugar, and degree of polymerization

Hydrolysis of PGM was carried out at pH 4, 5, 6, 7, 8, and 10, while the time, temperature, and the E/S were kept constant at 4 h, 40°C, and 0.1%, respectively. The results showed that the higher the pH, the higher the RS value obtained up to pH 7 (Figure 1a). However, the RS decreased when the pH was increased from 8 to 10. This trend was the same as the TS results, while the DP at pH 4 was very high, but decreased when the pH was raised to pH 7, then increased once again when the pH was raised from 8 to 10. The lowest DP was obtained when the pH was between 6 and 7. According to Soni *et al.* (2016), β -mannanase isolated from *Aspergillus terreus*

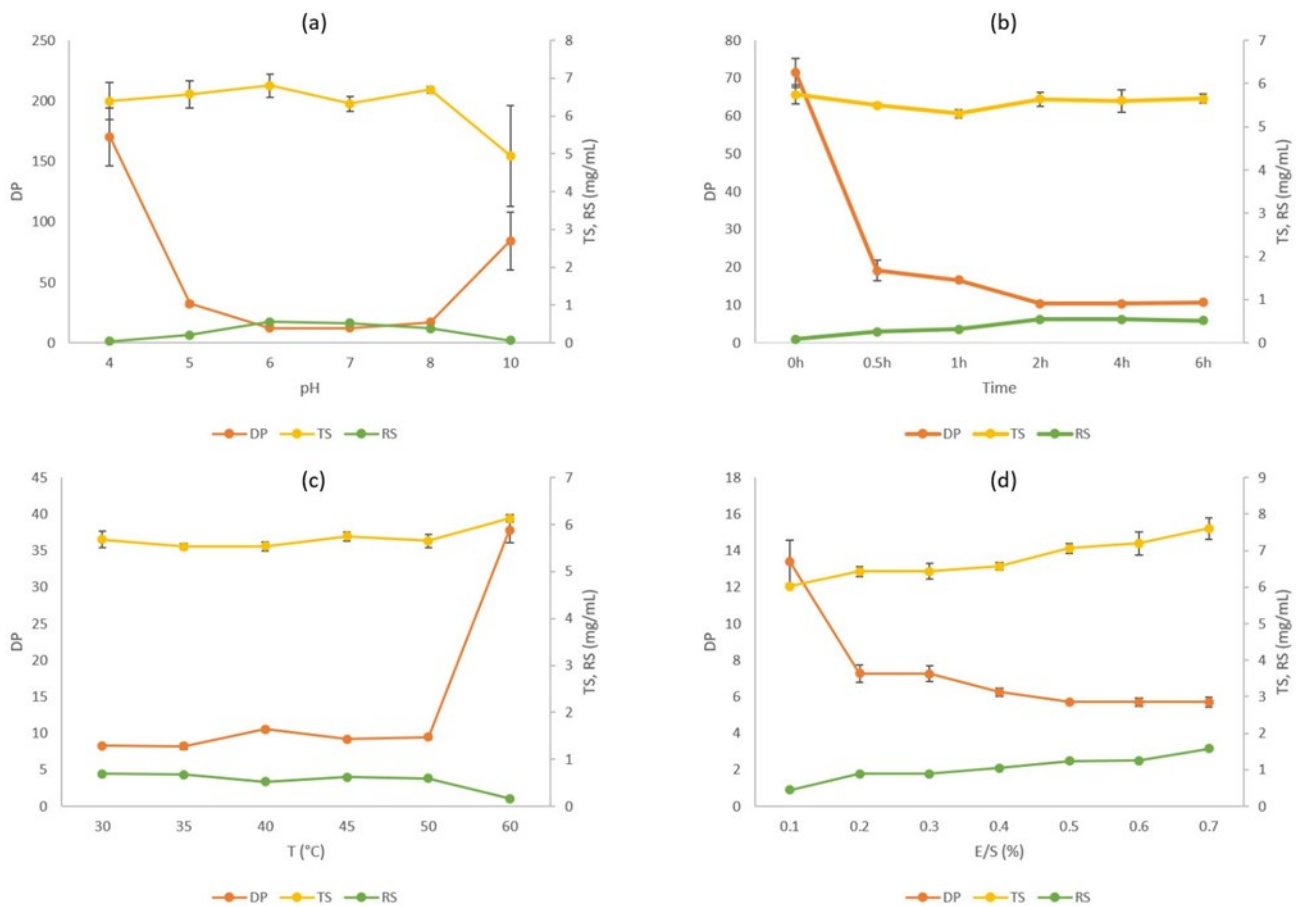


Figure 1. Single factor experimental on the RS, TS, and DP value of PGH (a) effect of pH, (b) effect of time, (c) effect of temperature, and (d) effect of E/S.

has an optimal pH of 7 and exhibits broad pH stability ranging from 4 to 8. The same result was obtained from the research by Wahyuni *et al.* (2016), where the optimal pH of β -mannanase from mannanase-producing bacteria from sago hump was pH 6 to 7. Chen *et al.* (2013) revealed that the optimal pH for producing glucoman-oligosaccharides or GMOs hydrolyzed by β -mannanase was 6.8. Therefore, pH 7 was chosen as the center point with 2 as the level change (Table 2).

3.1.2 Effect of time on total sugar, reducing sugar, and degree of polymerization

The times used for PGM hydrolysis were 0 h, 0.5 h, 1 h, 2 h, 4 h, and 6 h, while pH, temperature, and E/S were set at pH 7, 40°C, and 0.1%, respectively. According to the results in Figure 1b, the DP considerably decreased in the first half hour, followed by a modest decreased for up to two h, after which the DP became static. This result contrasts with the RS value, which increased on hydrolysis for up to 2 h and then became stagnant. Meanwhile, the TS was decreased slightly until the first hour of hydrolysis and increased after 2 h, and then it was static. Hydrolysis of konjac glucomannan (KGM) with mannanase showed the DP declined drastically at the first hour of hydrolysis (Takahashi *et al.*, 1984; Chen *et al.*, 2013). This finding was in line with the results of this study, where the DP value drastically dropped at the start of the reaction. A hydrolysis time of 2.5 h was selected as the center point, with 2 h as the level change (Table 2).

Table 2. Independent variables and the levels used in BBD.

Independent variables	Level		
	-1	0	1
pH	5	7	9
Time (h)	0.5	2.5	4.5
Temperature (°C)	30	45	60
E/S (%)	0.2	0.5	0.8

3.1.3 Effect of temperature on total sugar, reducing sugar, and degree of polymerization

The temperatures used for hydrolysis of PGM were 30°C, 35°C, 40°C, 45°C, 50°C, and 60°C, with the other conditions set as pH 7, time 4 h, and E/S of 0.1%. At 30°C to 50°C, the values of RS, TS, and DP obtained were not significantly different (Figure 1c). These findings were similar to the study by Chen *et al.* (2013), who reported that the β -mannanase has a broad optimal temperature. When the temperature increased to 60°C, the RS decreased while the DP and TS increased. The increase in DP is quite significant. This result contradicts some mannanases, which have optimal temperatures between 60°C and 65°C (Chauhan *et al.*, 2014; Seesom *et al.*, 2017; Liu *et al.*, 2018). However, the mannanase of *Penicillium occitanis* was reported to have decreased

its activity by 50% after incubation for 4 h at 60°C (Blibech *et al.*, 2011). In the enzymatic hydrolysis process, using a too-high temperature is not recommended because it can affect the denaturation of the enzyme (Chen *et al.*, 2013; Anggela *et al.*, 2020). Therefore, 45°C was chosen as the center point and 15°C as the level change (Table 2).

3.1.4 Effect of enzyme-to-substrate ratio on reducing sugar, total sugar and degree of polymerization

The E/S used was 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, and 0.7% with the hydrolysis conditions set at pH 7, time of 4 h and 40°C. Figure 1d demonstrated that as the E/S increased, the DP value decreased while the RS and TS values increased. However, the DP value stabilized at an E/S of 0.5%. This might be as a result of greater hydrolysis brought on by β -mannanase addition (Chen *et al.*, 2013; Liu *et al.*, 2015). Considering the cost, E/S of 0.5% was selected as center point with $\pm 0.3%$ as the level change (Table 2).

3.2 Optimization of hydrolysis with response surface methodology

3.2.1 Model fitting and statistical analysis

The results of the effect of pH, time, temperature, and E/S on the hydrolysis of PGM were used to determine the center point of RSM (Table 2). With 4 factors and 3 levels, a total of 29 runs were obtained. Table 3 presents the findings of the DP, TS, and RS values for all running hydrolysis. According to ANOVA analysis, the lack of fit obtained in the DP ($p=0.0053 < 0.05$) and TS ($p=0.0009 < 0.05$) responses was significant while the RS response was not significant ($p=0.8676 > 0.05$). When the obtained lack of fit value is not statistically significant, the model is indicated to be suitable or fit. Therefore, only the RS response will be considered for future optimization analyses. The RS values obtained ranged from 0.03 ± 0.01 mg/mL to 1.36 ± 0.01 mg/mL. The highest RS value was obtained under hydrolysis conditions of pH 7, time of 0.5 h, temperature of 45°C, and E/S of 0.8%.

From the 29 runs, the ANOVA analysis for RS was carried out in Table 4. The recommended model for the RS response was quadratic, with the equation obtained as follows:

$$Y = -7.7731 + 1.7039X_1 + 0.1152X_2 + 0.0972X_3 + 2.7438X_4 + 0.0125X_1X_2 - 0.0018X_1X_3 - 0.0463X_1X_4 - 0.0028X_2X_3 - 0.0146X_2X_4 - 0.0018X_3X_4 - 0.1198X_1^2 - 0.0153X_2^2 - 0.0009X_3^2 - 1.1391X_4^2$$

Where Y is the RS response, X_1 is the pH, X_2 is the hydrolysis time, X_3 is the hydrolysis temperature, and X_4 is the E/S.

F and p values were used to see the effect of each factor on the RS value. The p-value was used to

Table 3. Box-Behnken design with the independent variables.

Run	pH	t (h)	T (°C)	ES (%)	DP	TS (mg/mL)	RS (mg/mL)
1	7	2.5	30	0.2	13.12±0.37	6.53±0.24	0.50±0.02
2	5	2.5	45	0.8	7.10±0.03	6.49±0.07	0.91±0.01
3	7	0.5	30	0.5	6.57±0.04	6.82±0.14	1.04±0.02
4	9	4.5	45	0.5	12.66±0.06	6.40±0.15	0.51±0.01
5	9	2.5	30	0.5	13.16±0.14	6.57±0.02	0.50±0.01
6	7	2.5	45	0.5	5.23±0.09	6.70±0.23	1.28±0.02
7	7	2.5	30	0.8	5.70±0.14	6.80±0.10	1.19±0.04
8	7	4.5	60	0.5	14.47±0.30	6.66±0.17	0.46±0.00
9	9	2.5	45	0.8	8.12±0.23	6.50±0.02	0.80±0.02
10	7	0.5	60	0.5	7.85±0.09	6.64±0.07	0.85±0.02
11	5	2.5	30	0.5	9.44±0.05	6.50±0.03	0.69±0.01
12	7	0.5	45	0.2	11.00±0.15	6.28±0.04	0.57±0.01
13	7	2.5	60	0.8	8.20±0.27	6.85±0.09	0.84±0.04
14	5	2.5	60	0.5	14.50±0.34	6.31±0.15	0.44±0.02
15	7	4.5	30	0.5	6.82±0.21	6.76±0.05	0.99±0.04
16	9	2.5	45	0.2	22.54±0.08	4.83±0.03	0.21±0.00
17	5	0.5	45	0.5	12.23±0.28	6.28±0.16	0.51±0.00
18	7	2.5	45	0.5	5.38±0.10	6.98±0.19	1.30±0.02
19	7	4.5	45	0.2	12.26±0.23	6.82±0.10	0.56±0.02
20	9	2.5	60	0.5	26.04±4.54	0.79±0.04	0.03±0.01
21	7	0.5	45	0.8	4.89±0.03	6.65±0.03	1.36±0.01
22	7	4.5	45	0.8	5.19±0.09	6.78±0.13	1.31±0.05
23	5	4.5	45	0.5	10.37±0.31	5.82±0.25	0.56±0.01
24	9	0.5	45	0.5	17.92±0.20	4.77±0.10	0.27±0.00
25	7	2.5	60	0.2	37.80±0.34	6.59±0.11	0.17±0.00
26	7	2.5	45	0.5	7.14±0.11	6.71±0.18	0.94±0.02
27	7	2.5	45	0.5	6.65±0.05	6.60±0.09	0.99±0.02
28	5	2.5	45	0.2	30.07±0.26	6.48±0.08	0.22±0.00
29	7	2.5	45	0.5	7.82±0.28	6.81±0.05	0.87±0.04

determine the significance of each coefficient. The smaller the p -value, the more significant the effect of the coefficient (Guo *et al.*, 2010). Temperature, E/S, pH*pH, and temperature*temperature had a significant effect on RS ($p < 0.05$). The E/S and pH*pH had the greatest influence on the RS value. The ratio of E/S or E/S*E/S has been shown to have a major impact in various investigations of enzymatic processes (Chen *et al.*, 2013), which aligns with this result. The RS response was not significantly affected by the factor interaction ($p > 0.05$).

The coefficients of determination (R^2 value) obtained were 0.9158, indicating that 91.58% of the variability in the RS response can be explained using equation (3). A high R^2 value indicated that the model used matches the response. The results of the ANOVA also showed that the lack of fit value obtained was not significant ($p > 0.05$), which further indicates that the

model used was correct.

3.2.2 Analysis of response surface plot

Three-dimensional response surface and two-dimensional contour plots showed the interactions between two independent variables, while the other two variables have a fixed value of '0' (Figures 2 and 3). Figures 2a and 3a show the effect of pH and time on the RS, where temperature and E/S were at fixed values. The RS value increased with increasing pH. However, when the pH increased further, the RS value decreased, while time did not influence the RS. Figures 2b and 3b show the effect of pH and temperature on the RS response while the time and E/S were at fixed values, and both variables showed a quadratic effect. An increase in pH caused an increase in RS value. However, RS will decrease as the pH increases, as shown in the temperature effect on the RS value.

Table 4. ANOVA for the RS response.

Source	Sum of Squares	Df	Mean Square	F Value	p-value
Model	3.587601	14	0.256257	10.87964	< 0.0001
X ₁ (pH)	0.083338	1	0.083338	3.538187	0.0809
X ₂ (time)	0.003951	1	0.003951	0.167739	0.6883
X ₃ (temp.)	0.376041	1	0.376041	15.96515	0.0013
X ₄ (E/S)	1.45601	1	1.45601	61.81624	< 0.0001
X ₁ * X ₂	0.010088	1	0.010088	0.428313	0.5234
X ₁ * X ₃	0.011647	1	0.011647	0.494501	0.4935
X ₁ * X ₄	0.003092	1	0.003092	0.131267	0.7225
X ₂ * X ₃	0.029176	1	0.029176	1.238679	0.2845
X ₂ * X ₄	0.000308	1	0.000308	0.013086	0.9106
X ₃ * X ₄	0.000273	1	0.000273	0.011601	0.9158
X ₁ ²	1.481838	1	1.481838	62.91282	< 0.0001
X ₂ ²	0.024477	1	0.024477	1.039205	0.3253
X ₃ ²	0.316838	1	0.316838	13.45166	0.0025
X ₄ ²	0.068179	1	0.068179	2.894603	0.1110
Residual	0.329754	14	0.023554		
Lack of Fit	0.172431	10	0.017243	0.438412	0.8676
Pure Error	0.157323	4	0.039331		
Total	3.917355	28			

R² = 0.9158

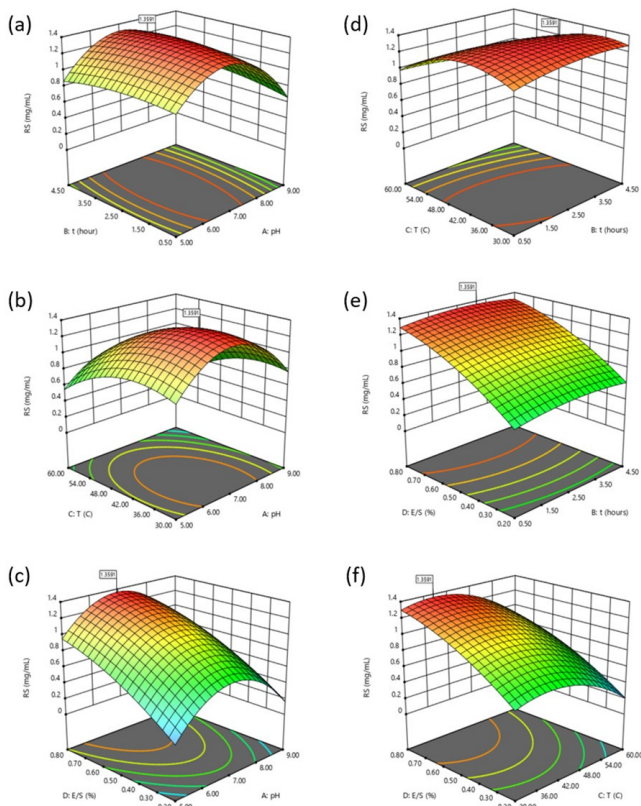


Figure 2. Response surface plot showing the effect of pH, time, temperature, and E/S on the response RS: (a) pH and time, (b) pH and temperature, (c) pH and E/S, (d) time and temperature, (e) time and E/S, (f) temperature and E/S.

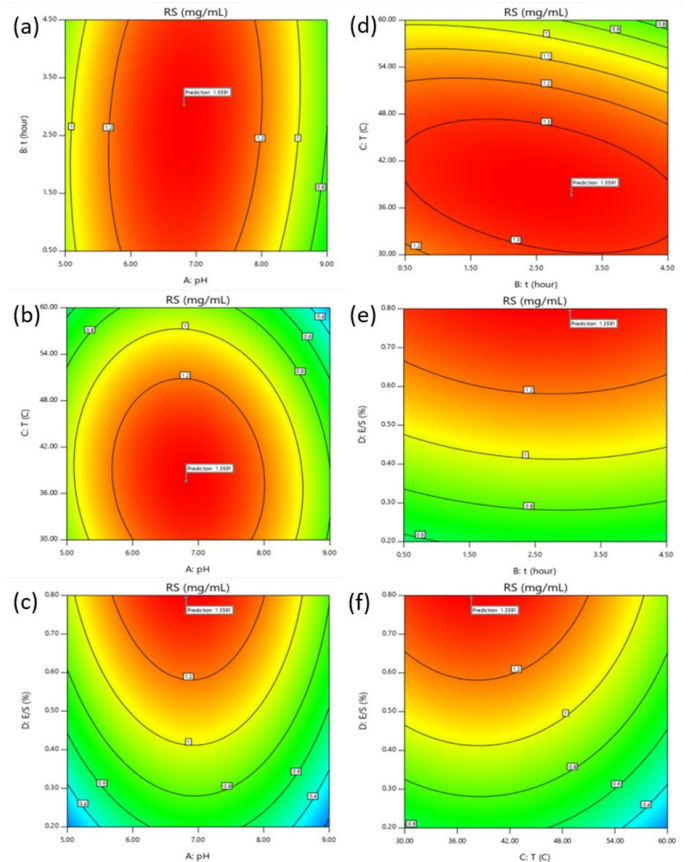


Figure 3. Contour plots showing the effect of pH, time, temperature, and E/S on the response RS: (a) pH and time, (b) pH and temperature, (c) pH and E/S, (d) time and temperature, (e) time and E/S, (f) temperature and E/S.

The effect of time and temperature on the RS response was shown in Figures 2d and 3d, where the pH and E/S were fixed. Temperature significantly impacted the RS over time; the higher the temperature, the longer the RS will continue to increase. The RS value will decrease, nevertheless, if the temperature increases further. The E/S showed a much greater influence on the RS than the pH (Figure 2c and 3c), the time (Figure 2e and 3e), and the temperature (Figure 2f and 3f), where 2 other variables were set at fixed values. The RS value increased as the E/S did.

From Figures 2 and 3, the optimal conditions for the enzymatic hydrolysis of PGM with β -mannanase were obtained at pH 6.81, time 3.03 h, hydrolysis temperature at 37.6°C, and E/S of 0.8%. The E/S had the most significant effect on the response of the four variables examined.

3.2.3 Verification of predictive model

Three independent validation experiments were performed under the predicted optimal conditions. The RS value obtained from the experiment was then compared with the RS value predicted by RSM, and the results are shown in Table 5. The RS value obtained from the hydrolysis using optimal conditions was 1.34 ± 0.03 mg/mL. This result was not much different from the RS value predicted by RSM, which was 1.35 mg/mL. These data indicated that the designed model could optimize hydrolysis of PGM using β -mannanase.

Table 5. Results of verification of predictive model.

Independent variables	Optimal condition	RS (mg/mL)	
		Predictive value	Experimental value
pH	6.81		
Time (h)	3.03	1.35	1.34±0.03
Temperature (°C)	37.6		
E/S (%)	0.8		

3.3 Thin layer chromatography analysis of porang glucomannan hydrolyzate

TLC analysis was conducted to qualitatively observe the simple sugars formed after the hydrolysis of PGM at optimal conditions. The results showed that mannotriose predominated in the PGH and that glucose and mannose were not found (Figure 4). These findings were consistent with Safitri *et al.* (2014), who used mannanase from *Streptomyces violascens* BF 3.10 to hydrolyze porang flour and produce manno-oligosaccharides dominated by mannotriose. In addition, the hydrolysis process of ivory nut mannan with β -mannanase from *Bifidobacterium adolescentis* can produce

mannotetraose, mannotriose, and mannobiose products, where mannotriose was also the most dominant product (Kulcinskaja *et al.*, 2013). The results demonstrated that the hydrolysis of PGM was effective and led to the production of oligosaccharide compounds dominated by mannotriose.

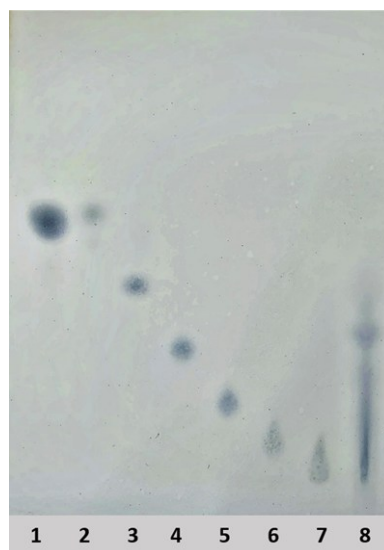


Figure 4. Thin layer chromatography analysis of the PGH by β -mannanase at the optimal condition from RSM (pH 6.81, reaction time 3.03 h, T 37.6°C and E/S 0.8%). Lane 1: standard glucose, Lane 2: standard mannose, Lane 3: standard mannobiose, Lane 4: standard mannotriose, Lane 5: standard mannotetraose, Lane 6: standard mannopentaose, Lane 7: standard mannohexaose, and Lane 8: PGH.

4. Conclusion

RSM was used to optimize experimental variables, such as pH, time, temperature, and E/S on the hydrolysis of PGM. The optimal conditions for hydrolysis obtained using RSM were at pH 6.81 for 3.03 h, hydrolysis temperature at 37.6°C, and E/S of 0.8%, with an obtained RS value of 1.34 ± 0.03 mg/mL. Based on TLC analysis results, the hydrolysis of PGM was successfully carried out under the optimal conditions and yielded mannotriose as the primary byproduct.

Conflict of interest

The authors declare no conflict of interest.

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