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Microbial fermentation optimization to increase glucomannan content in porang flour with a mixed-level factorial design

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Abstract. *Sandjaya NA, Priharto N, Esyanti RR. 2025. Microbial fermentation optimization to increase glucomannan content in porang flour with a mixed-level factorial design. Biodiversitas 26: 6541-6559.* Porang (*Amorphophallus muelleri*) flour is a promising source of glucomannan, a polysaccharide widely applied in food, pharmaceutical, and industrial sectors. However, its use is limited by the presence of starch and calcium oxalate impurities, which degrade product quality and safety. Conventional purification methods, such as mechanical separation, solvent extraction, and enzymatic treatment, can improve glucomannan content but are constrained by cost, chemical residues, or low efficiency. This study aimed to develop a sustainable microbial fermentation to enrich glucomannan and reduce oxalate content. Four α-amylase producers (*Bacillus subtilis, Bacillus cereus, Aspergillus niger,* and *Aspergillus oryzae*) were evaluated. Optimization to increase glucomannan content was conducted using a Mixed-Level Factorial Design (MLFD) with varying concentrations of substrate, inoculum, and microbial species. Microbial growth kinetics and α-amylase activity profiles were monitored to determine fermentation efficiency, while statistical modeling was applied to identify significant factors influencing glucomannan yield. The findings showed that the optimum conditions, with the highest increase in glucomannan and calcium oxalate content, were achieved with *B. subtilis* at a substrate concentration of 4% (w/v) and an inoculum concentration of 1% (v/v). These findings demonstrate that microbial fermentation with *B. subtilis* provides an eco-friendly and cost-effective alternative to chemical and enzymatic extraction for glucomannan enrichment. It has the potential to scale as a sustainable processing method to support the increased value-added valorization of porang as a strategic Indonesian commodity.

Keywords: α-amylase, *Amorphophallus muelleri*, glucomannan enrichment, microbial fermentation, process optimization

INTRODUCTION

Indonesia is rich in underutilized tuber crops that combine ecological suitability for marginal lands with the potential to increase rural incomes. Porang (*Amorphophallus muelleri*) has emerged as a strategic crop due to its ability to grow on low-input soils, support smallholder livelihoods, and produce tubers rich in glucomannan. Glucomannan is a hemicellulosic polysaccharide with applications in food, pharmaceuticals, and biomaterials (Rafani et al. 2021). Globally, demand for glucomannan is rising due to its functional properties (high viscosity, gelation, water-holding) and health benefits as a soluble dietary fiber, creating a commercial opportunity for value-added porang products (Lovegrove et al. 2017; Sun et al. 2023).

The commercialization of porang products is constrained by intrinsic impurities in porang flours, specifically residual starch and calcium oxalate, which reduce the glucomannan fraction, impair functional quality, and pose safety concerns (Witoyo et al. 2020). In particular, calcium oxalate limits direct food uses and may reduce its value (Bargagli et al. 2020). To address these issues, several extraction and purification strategies have been developed, each with its own advantages or disadvantages. Mechanical polishing and sieving are inexpensive and scalable but typically yield only modest glucomannan with oxalate levels that may still exceed safety thresholds. Chemical approaches (maceration, solvent-assisted, or ultrasound-assisted extraction) increased glucomannan content but are energy-intensive, rely on organic solvents that may leave residues, and raise environmental and regulatory concerns (Wardhani et al. 2015, 2020). Enzymatic purification using commercial α-amylase yields the highest purities; however, the use of enzymes increases production costs, which is a limitation for small- and medium-scale producers (Wardhani et al. 2019; Haryani et al. 2023). These limitations highlight the need for efficacy, safety, cost-effectiveness, and environmentally friendly processes.

Microbial fermentation offers a promising alternative that combines in situ enzyme production with low-cost operation and minimal solvent use. Many microbes naturally secrete α-amylase, enabling selective hydrolysis of starch (without cleaving the β-1,4 bonds of glucomannan), while some strains produce oxalate-degrading activities (Conter et al. 2019). *Bacillus* sp. is notable for robust extracellular α-amylase secretion under submerged fermentation and for genetic/physiological traits that support industrial enzyme production (Miłek and Lamkiewicz 2022; Vojnović et al. 2024). Filamentous fungi such as *Aspergillus niger* and *A. oryzae* produce carbohydrate-active enzymes, with *A. oryzae* being widely used in food fermentations (Naili et al. 2016; Tanaka and Gomi 2021). Beyond enzyme supply, microbial processes can be regulated by medium, inoculum, and time, to limit undesirable side activities (e.g., β-mannanase) and minimize glucomannan degradation (Norizan et al. 2020; Nath and Kango 2024). Fermentation can also reduce oxalate through enzymatic conversion or acidification pathways, thereby providing dual benefits of purification and detoxification (Conter et al. 2019; Ferdian and Perdana 2021).

The systematic application of microbial fermentation to increase glucomannan yield in porang flour remains underdeveloped, particularly in Indonesia, where most research focuses on mechanical or chemical extraction and only a limited number of studies have examined fermentation for oxalate reduction (Wardhani et al. 2019; Ferdian and Perdana 2021). Moreover, process parameters, such as substrate load, inoculum size, and strain selection, interact non-linearly in viscous, high-polysaccharide media, complicating empirical optimization (Tang et al. 2020). To address these challenges, multivariate experimental designs are needed to reveal factor interactions and identify robust operating windows. To our knowledge, this is the first systematic application of MLFD to optimize microbial fermentation for glucomannan enrichment in porang flour.

The present study fills this gap by evaluating four α-amylase-producing strains, *Bacillus subtilis*, *Bacillus cereus*, *A. niger*, and *A. oryzae,* and applying a Mixed-Level Factorial Design (MLFD) to optimize substrate concentration, inoculum level, and microbial type for maximal glucomannan enrichment and oxalate reduction. Applying MLFD here is novel for porang processing because it allows explicit assessment of interactions among biological and process variables that determine enzyme production, substrate accessibility, and mass transfer in viscous media. We hypothesize that selected α-amylase-producing microbes can selectively remove starch without significant loss of glucomannan, and fermentation can concurrently reduce calcium oxalate content. Furthermore, MLFD optimization will identify conditions that approach enzymatic extraction yields while offering better sustainability and lower cost. Therefore, the objectives are to screen and validate the performance of strains on porang flour, optimize fermentation parameters using MLFD, quantify the effects on glucomannan and oxalate, and compare fermentation with conventional extraction methods to assess industrial feasibility.

MATERIALS AND METHODS

Procedures

Materials

Porang flour (*A. muelleri*) was obtained from Selera Organik Jogja, Indonesia. Microbial strains (*B. subtilis*, *B. cereus*, *A. oryzae*, and *A. niger*) were obtained from the culture collection of the School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia (SITH ITB). Analytical-grade chemicals and reagents (e.g., media components, DNS reagents) were procured from commercial suppliers (Himedia, Merck, and others) and used without further purification.

Preparation of fermentation medium using porang flour substrate

Porang flour was dissolved in sterile distilled water to prepare the fermentation substrate at 2.5% (w/v) (Wardhani et al. 2019). Bulk organic carbon of the porang slurry was estimated using a modified Walkley-Black procedure adapted from Rosydiati (2019), because Walkley-Black is optimized for soils, these measurements are reported as approximate, relative organic carbon values and were used only for comparative purposes. The Walkley-Black calibration/standard curve for this matrix is provided in Supplementary Figure S1.

The nitrogen content was determined by measuring the total urea nitrogen (organic N) and ammonium nitrogen (N-NH_4), calculated using the method of Sulaeman et al. (2005), with the following equation:

$$N\ content\ (\%) = \frac{(titrant\ for\ blank\ (mL) - titrant\ for\ sample\ (mL))\ x\ N\ H_2SO_4\ x\ Ar\ N\ x\ 100\%}{sample\ tested\ (mg)}$$

The C/N ratio was adjusted by adding glucose to increase the C value, adding ammonium nitrate to increase the N value in the fungal medium, and adding sodium nitrate to increase the N value in the bacterial medium. The C/N ratio was adjusted to optimize α-amylase production, with values of 1 g C/g N for bacteria and 2 g C/g N for fungi (Naili et al. 2016).

Fermentation

All treatments were conducted as independent biological experiments in triplicate (n = 3). Each analytical measurement was performed in technical triplicate. Non-inoculated medium for each condition served as a negative control. Fermentations were performed in 250 mL Erlenmeyer flasks using porang flour as the primary substrate and four microorganism species (listed in Materials). Submerged fermentation (SmF) conditions (temperature, pH, agitation) were set according to literature-optimized parameters for each species (Supplementary Table S1). Bacterial inocula were prepared from 24-hour cultures and adjusted to 3×10^8 cells·mL^{-1}; fungal inocula were prepared from 48-hour cultures and adjusted to an OD600 ≈ of 1.5.

Growth of bacteria and fungi

Growth curves were constructed to follow population dynamics and to determine harvest timing. Bacterial cell density was measured every 4 h for 48 h using a hemocytometer/counting chamber and reported as cells·mL^{-1}. Fungal biomass was sampled every 6 h by collecting 1 mL aliquots, centrifuging at 10,000 rpm for 10 min, and determining pellet dry weight to obtain dry cell mass. Medium pH was recorded in parallel and plotted together with biomass data versus cultivation time.

Enzyme extraction and activity assay of α-amylase

The α-amylase assay was performed by centrifuging 2 mL of fermentation medium at 10,000 rpm for 15 minutes at 4°C. The supernatant obtained contained an enzyme (Hari Krishna et al. 2000). α-Amylase activity was determined by measuring reducing sugars using the DNS method, with glucose as the standard (Shah et al. 2014).

$$\alpha - \text{amylase activity}\ (U/ml) = \frac{\text{Glucose released}\ (\mu mol)}{\text{Enzyme solution volume}\ (ml)\ x\ \text{Incubation time}\ (min)}$$

Optimization using the Mixed-Level Factorial Design (MLFD) method

Optimization process was performed using a Mixed-Level Factorial Design (MLFD) with three factors: substrate concentration (two levels: 1% and 4% w/v), inoculum concentration (two levels: 1% and 3% v/v), and microorganism species (four levels: *B. subtilis*, *B. cereus*, *A. niger*, and *A. oryzae*). All other cultivation parameters (temperature, pH, agitation) were set according to literature-optimized conditions for each species (Supplementary Table S1). The "microorganism species" factor combines the microorganism species and its species-specific optimal growing conditions (including temperature, pH, etc) in producing α-amylase; therefore, results must be interpreted as the joint effect of both. The experimental matrix and factor-level assignments are listed in Supplementary Table S2; experimental runs were generated and analysed in Minitab 22 using a general linear model (Response ~ Species + Substrate + Inoculum + SpeciesSubstrate + SpeciesInoculum + Substrate*Inoculum). Response variables were: DNS-derived glucomannan content, glucomannan extraction efficiency, and extraction rate. All treatments were performed as independent biological triplicates (separate flasks started independently); analytical measurements were conducted in duplicate. Optimum conditions from the MLFD analysis were confirmed with three independent biological validation runs.

Conventional extraction method benchmark

Benchmarks were performed on the same porang flour: (i) Mechanical polishing/sieving (Sitompul et al. 2018) with a 100-mesh sieve; (ii) IPA maceration (80% v/v IPA, solvent: sample 8:1 mL/g) (Wardhani et al. 2015); (iii) Ultrasonic-assisted extraction (80% IPA, 3 cycles × 10 min) per Wardhani et al. (2020); (iv) Enzymatic extraction using commercial α-amylase at the optimal dose and conditions described by Haryani et al. (2023). Flour from chemical and enzymatic extraction was then dried in an oven at 40°C until constant mass, then ground and sieved through a 100-mesh sieve.

Glucomannan content testing using the DNS method

Glucomannan was estimated by measuring the reducing sugars released after acid hydrolysis using the 3,5-dinitrosalicylic acid (DNS) assay as described by Shah et al. (2014). Glucose concentrations were determined from a standard curve (Supplementary Figure S2) and converted to glucomannan content using the published equation:

$$\text{Glucomannan content}\ (\%) = \frac{5000f\,(5(\text{glucose in the hydrolysate}\ (mg)) - \text{glucose in extract}\ (mg))}{\text{mass of the glucomannan flour tested}(mg)}$$

Because DNS detects reducing-sugar equivalents rather than intact polysaccharide mass, results are reported as DNS-derived glucomannan equivalents and interpreted as operational estimates. Where available, DNS results were compared to a konjac glucomannan standard hydrolysed under identical conditions. We acknowledge this limitation and recommend biochemical validation (e.g., monosaccharide HPLC, mannose-specific assays, or molecular-weight/viscometric analysis) in follow-up work.

Determination of calcium oxalate content

The calcium oxalate content was determined using the method described by Rachmaniah et al. (2024), as follows:

$$\begin{array}{c}\text{Calcium oxalate content}\\ (mg/\ 100\ g)\end{array} = \frac{\text{Volume}\ KMnO_4\ \text{titrant}\ (ml)x0{,}00225\ x\ \text{dilution factor}}{m_{sample}\ (g)\ x\ \text{the redox reaction of}\ KMnO4\ (=5)}\ x\ 10^5$$

Measurement of water and ash content

The moisture and ash content were calculated based on the method by Sukma et al. (2022) as follows:

$$\text{Water content}\ \ (\%) = \frac{(W_1 - W_0) - (W_2 - W_0)}{W_2 - W_0}\ x\ 100\%$$

$$\text{Ash content}\ (\%) = \frac{W_3 - W_0}{W_2 - W_0}\ x\ 100\%$$

Where: W_0 is the mass of the empty crucible, W_1 is the mass of the crucible containing the sample, W_2 is the mass of the crucible containing the dry sample, and W_3 is the mass of the crucible containing the ashed sample. All masses are measured in grams.

Replication

A biological replicate is an independently initiated fermentation (separate media preparation and inoculum), while a technical replicate is a repeated analytical measurement from a single fermentation sample. The experimental unit for hypothesis testing was the biological replicate (n = 3) for conditions with independent fermentations; analytical determinations were measured in technical triplicate. Conditions for which only technical repeats were available are identified in the run-level data and are interpreted as measures of analytical precision rather than independent biological variability. Non-inoculated media were included as negative controls for each condition.

Data analysis

Experimental designs and statistical analyses were performed in Minitab Statistical Software v22. The mixed-level factorial design (MLFD) was analysed using a general linear model and ANOVA to identify significant main effects and interactions for the response variables (DNS-derived glucomannan equivalents, extraction efficiency, and extraction rate). Model performance and fit were assessed using R^2, adjusted R^2, predicted R^2, desirability values, and residual diagnostics. Significant factor means were compared with Tukey's HSD ($\alpha = 0.05$). The predicted optimum conditions were tested in independent biological validation runs (n = 3). Graphical outputs (growth and enzyme activity curves, response optimizer plots) were produced in Microsoft Excel 2021 for visualization.

RESULTS AND DISCUSSION

Composition profile of untreated porang flour

Porang flour contained ~22.8% glucomannan (22.8 ± 0.6%) and calcium oxalate of ~2.9 mg/100 g (2.9 ± 0.1 mg/100 g), indicating a need for purification to meet food-safety and functionality targets.

The growth curves of microorganisms and α-amylase activity

All strains produced extracellular α-amylase, with differing kinetics and activities (Figures 1-5): *Bacillus subtilis* had the earliest peak activity (12 h) among the other microorganisms, and exhibited the highest overall activity. These differences likely reflect species-specific secretion capacities and growth rates, explaining the superior starch hydrolysis observed with *B. subtilis* treatments.

Multi-level factorial design optimization

ANOVA revealed a significant interaction between substrate concentration and inoculum concentration on glucomannan yield (substrate × inoculum, $p < 0.01$). A simpler Generalized Linear Model (GLM) explained 78% of the data variation ($R^2 = 0.78$) but had limited predictive ability ($R^2_{pred} = 0.29$). Essential parameters of the second-order MLFD model summary for each response are provided in Table 1. Full ANOVA tables and factorial regressions are provided in Supplementary Tables S3-S6, and diagnostic plots (residuals, main-effect, and interaction plots) are in Supplementary Figures S3-S10. Desirability optimization indicated *B. subtilis* at 4% (w/v) substrate and 1% (v/v) inoculum as the optimal condition (predicted glucomannan = 25.6763%; desirability = 0.9018). Experimental validation (three independent biological replicates) produced 25.51 ± 0.58% glucomannan (mean ± SD, n = 3), closely matching the model prediction (mean absolute error ≈ 0.17 percentage points; relative error ≈ 0.66%), as shown in Table 2.

Comparison with the conventional extraction method

Fermentation with *B. subtilis* produced glucomannan levels (25.5%) comparable to those of enzymatic extraction (27.7%) while achieving the low calcium oxalate content (1.1 mg/100 g) among biological methods. The complete measurements of the quality parameters of extracted porang flour obtained using various extraction methods are shown in Supplementary Table S7.

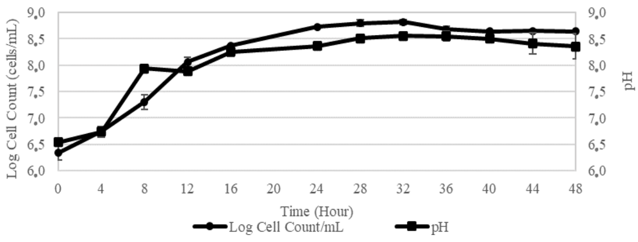


Figure 1. Growth curve of *B. subtilis* (n = 3 biological and technical replication). Conditions: 2,5% w/v substrate, 1 C/N ratio, pH 8, 37°C, 150 rpm agitation, 2% inoculum concentration

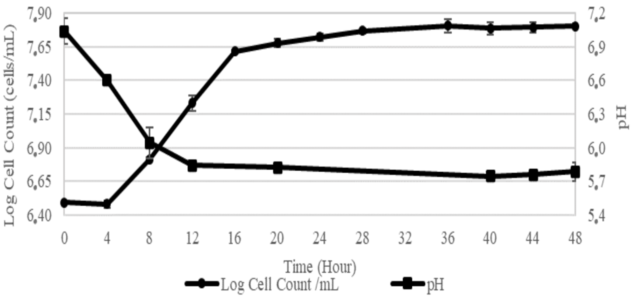


Figure 2. Growth curve of *B. cereus* (n = 3 biological and technical replication). Conditions: 2,5% w/v substrate, 1 C/N ratio, pH 7.5, 37°C, 150 rpm agitation, 2% inoculum concentration

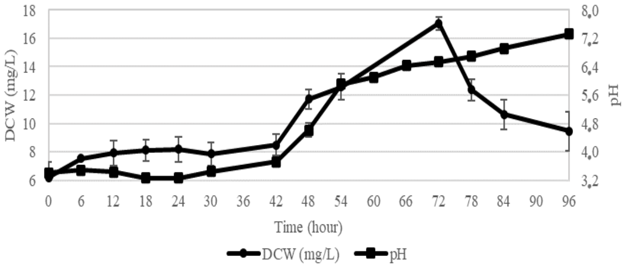


Figure 3. Growth curve of *A. niger* (n = 3 biological and technical replication). Conditions: 2,5% w/v substrate, 2 C/N ratio, pH 4.5, 32.5°C, 150 rpm agitation, 2% inoculum concentration

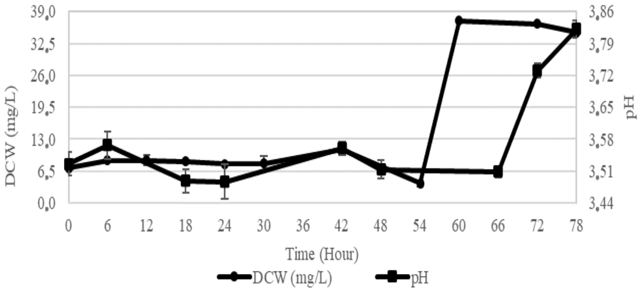


Figure 4. Growth curve of *A. oryzae* (n = 3 biological and technical replication). Conditions: 2,5% w/v substrate, 2 C/N ratio, pH 4.5, 32.5°C, 150 rpm agitation, 2% inoculum concentration

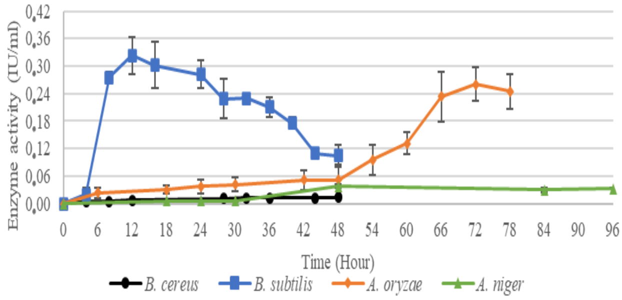


Figure 5. Activity curve of α-amylase enzyme (n = 3 biological and technical replication). *Bacillus subtilis* shows earlier and higher peak activity compared to other strains

Table 1. Results of the second-order MLFD model summary analysis for each response

Response	S	R-sq	R-sq(adj)	R-sq(pred)
Glucomannan Content (%)	3.51484	85.32%	26.58%	0.00%
Glucomannan Extraction Efficiency (% glucomannan recovery/mL culture)	1.42647	95.00%	74.98%	0.00%
Glucomannan Extraction Rate (% glucomannan recovery/h)	0.159623	73.29%	0.00%	0.00%

Table 3. Initial composition of untreated porang flour

Component	Composition
Organic Carbon	7.3 ± 0.8 g/L
Nitrogen	0.9 ± 0,1 g/L
Glucomannan	22.8 ± 0.6%
Calcium Oxalate	2.9 ± 0.1 mg/100 g
Water	11.0 ± 0.1%

Table 2. Validation of the optimizer

	Glucomanan content (%)				
	Predicted	**Observed run 1**	**Observed run 2**	**Observed run 3**	**Mean + SD (n=3)**
Fermentation	25,6763	24,9237	26,0977	25,5107	25,5107 ± 0,5870

Discussion

Composition profile of untreated porang flour

The porang flour used in all experiments was carbohydrate-dominant, with starch and glucomannan as the major polysaccharides, and contained measurable levels of calcium oxalate, moisture, and ash (Table 3). The content of porang flour is significantly influenced by environmental conditions, the available nutrients during cultivation, the harvest time of porang tubers, and the processing method of the tubers into porang flour (Sitompul et al. 2018; Nurlela et al. 2019). The carbon content in porang flour originates from macronutrient compounds, including hydrocarbon-based compounds, such as starch, glucose, lactose, sucrose, cellulose, and glucomannan (Lovegrove et al. 2017). The nitrogen content in porang flour is closely related to the amino acids in the protein fraction, alkaloids, amines, ammonia, phospholipids, and nitrogen glycosides, nitrates, nitrites, nucleic acids, urea, vitamins, free amino acids, and small peptides (Zurak et al. 2023). The organic compounds are more closely related to values observed in fermentation, microbial growth, and metabolic responses (Stutter et al. 2020).

Calcium oxalate is a component of porang flour that negatively affects its quality, as it can cause crystallization in the kidneys and other health problems if consumed (Bargagli et al. 2020). Other components present in porang flour at relatively high levels include water and ash, which are influenced by the drying and polymerization processes, as well as inorganic residue (Mukkun et al. 2022; Iskandar et al. 2024). High ash content alters solution characteristics and leads to gel precipitation, thereby reducing the gel's strength and clarity (Sun et al. 2023). The organic C/N ratio indicated sufficient nutrient availability to support microbial growth; however, the relatively high content of oxalate, water, and ash suggested the need for a pretreatment or selective removal step before producing high-value products.

Interpretation of microbial performance and enzyme kinetics

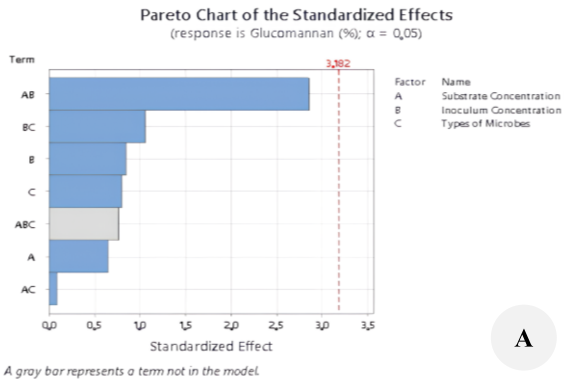
Four microbial isolates (*B. subtilis, B. cereus, A. niger,* and *A. oryzae*) produced extracellular α-amylase when grown in porang-flour medium; however, their kinetics differed markedly (Figures 1-4). The superior performance of *B. subtilis* is attributed to its rapid onset and high extracellular α-amylase activity, which promotes efficient starch hydrolysis while avoiding excessive degradation of glucomannan. The shift in peak enzyme activity to the early stationary phase indicates a low content of simple sugar in the porang flour medium. The peak enzyme activity typically occurs when simple sugars in the fermentation medium are depleted, triggering a decrease in amylolytic gene expression and an increase in protein secretion (Strach et al. 2023). Fungi require incubation to accumulate hyphal biomass and secrete hydrolase. Fungal peak enzyme activity is influenced by the fermentation method, the content of inducing compounds, and aeration (Tanaka and Gomi 2021). In viscous, glucomannan-rich media, mass-transfer limitations exacerbate differences between fast-secreting bacteria and slower-secreting fungi; thus, strain selection must consider both secretion kinetics and medium rheology. Optimal timing of enzyme peak activity is key; it ensures a balance between high enzyme output, fast processing, and protection of the glucomannan compound. The findings showed that maximum α-amylase activity occurred at 12 h for *B. subtilis*, 28 h for *B. cereus*, 48 h for *A. niger*, and 72 h for *A. oryzae,* thereby maximizing amylolytic activity while minimizing incubation time (Figure 5).

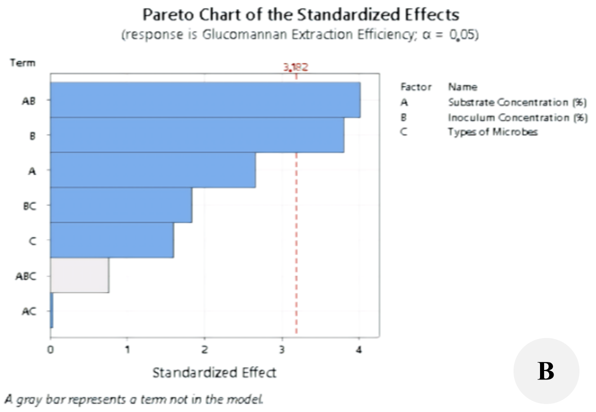
Fermentation Mixed-Level Factorial Design (MLFD) optimization result

A Mixed-Level Factorial Design (MLFD) evaluated substrate (1-4% w/v), inoculum (1-3% v/v), and microbial type for three responses: glucomannan content (%), extraction efficiency (% glucomannan recovery/mL culture), and extraction rate (% glucomannan recovery/h). The whole experimental run matrix is provided in Supplementary Table S3.

In several Run Orders, a negative response value indicated a decrease in glucomannan content of fermented porang flour. This is due to microorganisms' potential to secrete additional enzymes that can degrade various fractions and alter the flour matrix structure (Manurung and Yanuriati 2024). In addition, the majority of *Bacillus* sp. and *Aspergillus* sp. groups can secrete the enzyme β-mannanase, which hydrolyzes the β-1,4-glycosidic bonds in glucomannan into oligomers that are removed during extraction (Nath and Kango 2024; Zhang et al. 2024). In the reduced analysis (second-order model), the substrate-inoculum interaction was the dominant factor across all responses (Figure 6), underscoring the need to optimize both factors jointly. Increasing substrate increases nutrient availability but also viscosity, which impairs oxygen transfer and enzyme-substrate diffusion (Tang et al. 2020). The number of microbial cells producing enzymes is directly related to the amount of enzyme production (Ben Hadj Hmida et al. 2024). Increasing the inoculum at high substrate concentrations produces saturation/competition effects that reduce extraction performance. Increasing incubation time at low substrate concentration cannot increase the extraction rate, as limited substrate availability prevents it (Singh et al. 2022). The multi-response desirability optimizer identified *B. subtilis* at 4% (w/v) substrate and 1% (v/v) inoculum as the best compromise (composite desirability ≈ 0.90) (Figure 7). Compared to other microbes, *B. subtilis* exhibits a more stable enzyme profile characterized by a low level of glucomannan-degrading enzyme production (Vojnović et al. 2024). The low inoculum concentration may suppress the glucomannan-degradation process, possibly due to the β-mannanase produced by *B. subtilis,* without significantly suppressing the activity of the α-amylase enzyme (Hu et al. 2021).

The MLFD provided helpful insight into factor interactions (notably substrate × inoculum). Yet, its predictive performance was limited: the predicted R^2 was low, indicating limited ability to forecast responses beyond the experimental runs. A decrease in predictability is likely due to biological variability (varying enzyme secretion), substrate heterogeneity, viscosity-induced mass-transfer limitations, and the inherent nonlinearity of enzymatic/biological responses, which a two-level factorial design cannot fully capture. Future research should explore pilot-scale validation and refined optimization (RSM) approaches to improve predictive accuracy and to confirm industrial feasibility.





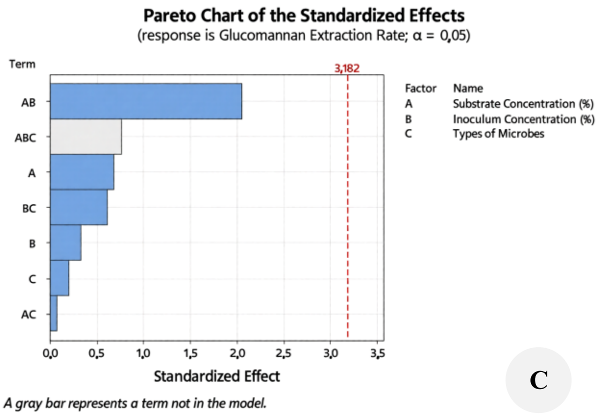


Figure 6. Pareto chart of A. Glucomannan content, B. Glucomannan extraction efficiency, C. Glucomannan extraction rate response

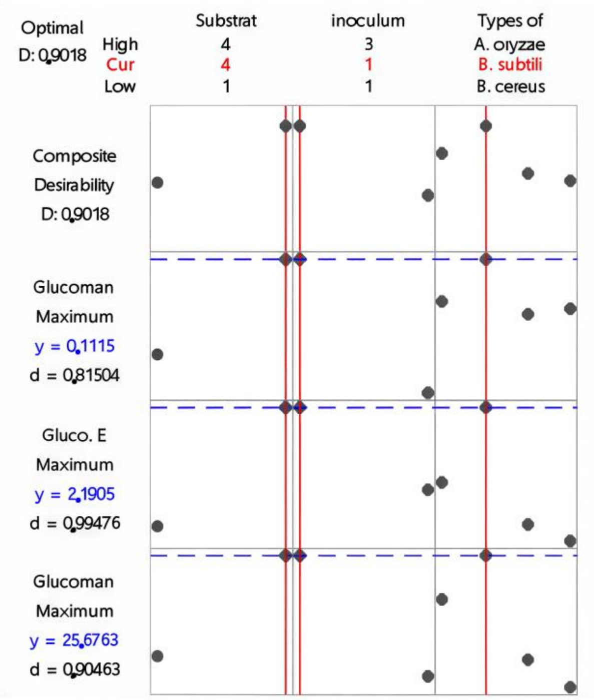


Figure 7. Response optimizer graph

Table 4. Comparison of the quality of porang flour from various extraction methods (mean ± SD, n = 3 technical replicates)

Method	Glucomannan content (%)	Increase in glucomannan content (%)	Calcium oxalate content (mg/100 g)	Water content (%)	Ash content (%)
Initial flour	22,8 ± 0,6^{b}	-	2,9 ± 0,1^{a*}	11,0 ± 0,1^{a*}	5,6 ± 0,1^{b*}
Mechanical	24,2 ± 2,6^{ab}	6,1^{ab}	0,6 ± 0,1^{e}	7,3 ± 0,3^{b}	4,4 ± 0,0^{d*}
Maceration	23,3 ± 2,8^{ab}	2,1^{ab}	1,4 ± 0,1^{bc}	7,2 ± 0,5^{b}	5,2 ± 0,4^{bc*}
Ultrasonication	25,4 ± 0,4^{ab}	11,3^{ab}	1,7 ± 0,1^{b}	7,2 ± 0,6^{b}	4,9 ± 0,4^{cd*}
Enzymatic	27,7 ± 1,0^{a}	21,5^{a}	1,3 ± 0,1^{cd}	9,3 ± 1,5^{a}	2,3 ± 0,3^{e}
Fermentation	25,5 ± 0,8^{ab}	11,8^{ab}	1,1 ± 0,2^{d}	2,2 ± 0,1^{c}	8,2 ± 0,1^{a*}

Note: values with the same letter indicate insignificant differences. The "*" sign indicates a parameter value that does not meet the standard quality requirements of SNI 9211:2023 (Supplementary Table S8)

Comparison of glucomannan extraction method

The results on flour quality from various extraction methods indicate that enzymatic extraction is the most effective (Table 4). Ultrasound-Assisted Extraction (UAE) increases the mass transfer rate between the solvent and the sample and the dissolution efficiency, while still being significantly influenced by the solvent (Iskandar et al. 2024). The solvent used (IPA 80%) is effective in degrading and dissolving impurities, such as cellulose and oxalic acid, but remains less selective towards certain impurities in porang flour (Wardhani et al. 2020). Enzymatic extraction delivered the highest absolute glucomannan purity, reflecting the use of a purified α-amylase preparation, which ensures higher enzyme concentration and greater specificity for rapid and selective starch hydrolysis under stabilized conditions (Zaferanloo et al. 2014). This enzyme property enables a significant reduction in the starch content and the release of purer glucomannan compounds from the flour matrix (Wardhani et al. 2019). Fermentation, by contrast, produces a crude enzyme cocktail: enzyme titers fluctuate, proteases and ancillary hydrolases (e.g., β-mannanase) may reduce enzyme stability or directly hydrolyze glucomannan, and high medium viscosity can limit enzyme-substrate contact (Norizan et al. 2020; Helmi and Eni 2024). These mechanistic differences explain why purified enzymatic treatment still holds a narrow efficiency advantage under controlled lab conditions, even though fermentation offers sustainability and cost benefits. In addition to glucomannan enrichment, the fermentation method has been shown to significantly reduce calcium oxalate content. The oxalate decarboxylase enzyme and the organic acids secreted by *B. subtilis* play a crucial role in the breakdown of calcium oxalate (Conter et al. 2019; Mukherjee and Mohan 2021). The decrease in water content in the fermentation product is attributed to metabolic mechanisms of microorganisms that bind water and consume organic components (He et al. 2019). At the same time, the modest increase in ash observed reflects the accumulation of microbial biomass and inorganic residues, the release of bound minerals during organic-matter degradation, and concentration effects as soluble starch is removed. The slight increase in ash content may affect gel clarity or pH-sensitive applications and could be mitigated in pilot-scale processes through simple washing or centrifugation steps (Fitria et al. 2025).

Optimized microbial fermentation by *B. subtilis* at 4% substrate concentration and 1% inoculum, offers a viable, lower-impact route to increase glucomannan content in porang flour and reduce oxalate levels. These new approaches align with global demand for eco-friendly bioprocessing approaches and support Indonesia's strategy to increase value-added porang exports by reducing solvent use, lowering chemical residues, and decreasing dependence on purchased commercial enzymes. However, these results derive from shake-flask experiments; transferring to pilot or production bioreactors will require re-optimization (mixing, aeration, and mass transfer), techno-economic analysis, and control strategies to regulate enzyme titers (e.g., fed-batch feeding, partial enzyme concentration, or strain selection). The limitations observed in this study include a lab-scale scope, a limited strain set, and constrained MLFD predictive power. With targeted optimization and pilot validation, fermentation has the potential to rival enzymatic methods while delivering sustainability and cost benefits that are well-suited to Indonesian porang value chains. Future work should implement RSM or non-linear modelling to capture curvature and improve predictions; explore additional strains or engineered variants with reduced glucomannan-degrading activity; test partial enzyme concentrations or in-process removal of deleterious activities; and validate optimized conditions in bench-scale bioreactors with techno-economic and life-cycle assessment to ascertain industrial feasibility.

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SUPPLEMENTARY TABLES

Supplementary Table S1. Fermentation conditions for each microorganism

	Bacteria		Fungi	
	B. subtilis	***B. cereus***	***A. niger***	***A. oryzae***
Medium substrate	Porang flour			
Medium concentration	2.5% (w/v)			
Medium volume	120 mL		135 mL	
C/N ratio	1		2	
N source	NH_4NO_3		$NaNO_3$	
pH	8	7.5	4.5	4.5
Pasteurization temperature	90℃			
Pasteurization duration	15 minutes			
Pasteurization cycle	2			
Fermentation temperature	37℃		32.5℃	
Agitation	150 rpm			
Inoculum concentration	2% (v/v)			
Fermentation duration	48 h		78 h	96 h

Supplementary Table S2. Optimization model with multi level factorial design approach

Run order	Substrate concentration (%)	Inoculum concentration (%)	Type of microbes
1	1	1	*A. niger*
2	4	1	*A. niger*
3	1	3	*A. niger*
4	4	3	*A. niger*
5	1	1	*A. oryzae*
6	4	1	*A. oryzae*
7	1	3	*A. oryzae*
8	4	3	*A. oryzae*
9	1	1	*B. subtilis*
10	4	1	*B. subtilis*
11	1	3	*B. subtilis*
12	4	3	*B. subtilis*
13	1	1	*B. cereus*
14	4	1	*B. cereus*
15	1	3	*B. cereus*
16	4	3	*B. cereus*

Supplementary Table S3. Full factorial design multilevel model

Substrate concentration (%)	Inoculum concentration (%)	Types of microbes	Glucomannan (%)	Glucomannan extraction efficiency	Glucomannan extraction rate
1	1	*B. cereus*	17.84	-4.14	-0.18
1	1	*B. subtilis*	19.47	-2.79	-0.28
1	1	*A. niger*	11.91	-8.08	-0.23
1	1	*A. oryzae*	11.01	-8.75	-0.16
1	3	*B. cereus*	17.52	-1.47	-0.19
1	3	*B. subtilis*	21.49	-0.37	-0.11
1	3	*A. niger*	20	-0.70	-0.06
1	3	*A. oryzae*	27.22	1.09	0.06
4	1	*B. cereus*	20.53	-1.90	-0.08
4	1	*B. subtilis*	25.51	2.25	0.22
4	1	*A. niger*	19.58	-2.40	-0.07
4	1	*A. oryzae*	19.21	-2.67	-0.05
4	3	*B. cereus*	19.35	-0.96	-0.12
4	3	*B. subtilis*	18.16	-1.29	-0.39
4	3	*A. niger*	15.49	-1.81	-0.15
4	3	*A. oryzae*	17.7	-1.26	-0.07

Supplementary Table S4. General factorial regression on glucomannnan response

Factor Information

Factor	Levels	Values
Substrate Concentration (%)	2	1; 4
Inoculum Concentration (%)	2	1; 3
Types of Microbes	4	*B. cereus; B. subtilis; A. niger; A. oryzae*

Analysis of Variance

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Model	12	215.325	85.32%	215.325	17.944	1.45	0.424
Linear	5	52.994	21.00%	52.994	10.599	0.86	0.59
Substrate Concentration (%)	1	5.141	2.04%	5.141	5.141	0.42	0.565
Inoculum Concentration (%)	1	8.802	3.49%	8.802	8.802	0.71	0.461
Types of Microbes	3	39.051	15.47%	39.051	13.017	1.05	0.483
2-Way Interactions	7	162.331	64.32%	162.331	23.19	1.88	0.326
Substrate Concentration (%)*Inoculum Concentration (%)	1	100.724	39.91%	100.724	100.724	8.15	0.065
Substrate Concentration (%)*Types of Microbes	3	4.732	1.87%	4.732	1.577	0.13	0.938
Inoculum Concentration (%)*Types of Microbes	3	56.875	22.53%	56.875	18.958	1.53	0.367
Error	3	37.063	14.68%	37.063	12.354		
Total	15	252.387	100.00%				

Model Summary

S	R-sq	R-sq(adj)	PRESS	R-sq(pred)	AICc	BIC
3.51486	85.32%	26.58%	1054.23	0.00%	506.85	97.66

Coefficients

Term	Coef	SE Coef	95% CI	T-Value	P-Value	VIF
Constant	18.875	0.879	(16,079; 21,672)	21.48	0	
Substrate Concentration (%)						
1	-0.567	0.879	(-3,363; 2,230)	-0.65	0.565	1
4	0.567	0.879	(-2,230; 3,363)	0.65	0.565	*
Inoculum Concentration (%)						
1	-0.742	0.879	(-3,538; 2,055)	-0.84	0.461	1
3	0.742	0.879	(-2,055; 3,538)	0.84	0.461	*
Types of Microbes						
B. cereus	-0.06	1.52	(-4,91; 4,78)	-0.04	0.97	1.5
B. subtilis	2.28	1.52	(-2,56; 7,13)	1.5	0.231	1.5
A. niger	-2.13	1.52	(-6,97; 2,71)	-1.4	0.256	1.5
A. oryzae	-0.09	1.52	(-4,93; 4,75)	-0.06	0.956	*
Substrate Concentration (%)*Inoculum Concentration (%)						
1 1	-2.509	0.879	(-5,306; 0,287)	-2.86	0.065	1
1 3	2.509	0.879	(-0,287; 5,306)	2.86	0.065	*
4 1	2.509	0.879	(-0,287; 5,306)	2.86	0.065	*
4 3	-2.509	0.879	(-5,306; 0,287)	-2.86	0.065	*
Substrate Concentration (%)*Types of Microbes						
1 *B. cereus*	-0.56	1.52	(-5,41; 4,28)	-0.37	0.737	1.5
1 *B. subtilis*	-0.11	1.52	(-4,95; 4,73)	-0.07	0.947	1.5
1 *A. niger*	-0.23	1.52	(-5,07; 4,62)	-0.15	0.892	1.5
1 *A. oryzae*	0.9	1.52	(-3,95; 5,74)	0.59	0.597	*
4 *B. cereus*	0.56	1.52	(-4,28; 5,41)	0.37	0.737	*
4 *B. subtilis*	0.11	1.52	(-4,73; 4,95)	0.07	0.947	*
4 *A. niger*	0.23	1.52	(-4,62; 5,07)	0.15	0.892	*
4 *A. oryzae*	-0.9	1.52	(-5,74; 3,95)	-0.59	0.597	*
Inoculum Concentration (%)*Types of Microbes						
1 *B. cereus*	1.12	1.52	(-3,73; 5,96)	0.73	0.516	1.5
1 *B. subtilis*	2.07	1.52	(-2,77; 6,92)	1.36	0.266	1.5
1 *A. niger*	-0.26	1.52	(-5,10; 4,59)	-0.17	0.876	1.5
1 *A. oryzae*	-2.93	1.52	(-7,78; 1,91)	-1.93	0.15	*
3 *B. cereus*	-1.12	1.52	(-5,96; 3,73)	-0.73	0.516	*
3 *B. subtilis*	-2.07	1.52	(-6,92; 2,77)	-1.36	0.266	*
3 *A. niger*	0.26	1.52	(-4,59; 5,10)	0.17	0.876	*
3 *A. oryzae*	2.93	1.52	(-1,91; 7,78)	1.93	0.15	*

Supplementary Table S4. General factorial regression on glucomannnan response (Continued)

Means

Term	**Fitted mean**	**SE mean**
Substrate Concentration (%)		
1	18.31	1.24
4	19.44	1.24
Inoculum Concentration (%)		
1	18.13	1.24
3	19.62	1.24
Types of Microbes		
B. cereus	18.81	1.76
B. subtilis	21.16	1.76
A. niger	16.75	1.76
A. oryzae	18.78	1.76
Substrate Concentration (%)*Inoculum Concentration (%)		
1 1	15.06	1.76
1 3	21.56	1.76
4 1	21.21	1.76
4 3	17.67	1.76
Substrate Concentration (%)*Types of Microbes		
1 *B. cereus*	17.68	2.49
1 *B. subtilis*	20.48	2.49
1 *A. niger*	15.95	2.49
1 *A. oryzae*	19.11	2.49
4 *B. cereus*	19.94	2.49
4 *B. subtilis*	21.84	2.49
4 *A. niger*	17.54	2.49
4 *A. oryzae*	18.45	2.49
Inoculum Concentration (%)*Types of Microbes		
1 *B. cereus*	19.19	2.49
1 *B. subtilis*	22.49	2.49
1 *A. niger*	15.75	2.49
1 *A. oryzae*	15.11	2.49
3 *B. cereus*	18.44	2.49
3 *B. subtilis*	19.83	2.49
3 *A. niger*	17.74	2.49
3 *A. oryzae*	22.46	2.49

Fits and Diagnostics for All Observations

Obs	**Glucomannan (%)**	**Fit**	**SE Fit**	**95% CI**	**Resid**	**Std Resid**	**Del Resid**	**HI**	**Cook's D**	**DFITS**
1	17.84	15.55	3.17	(5,47; 25,63)	2.29	1.51	2.49	0.8125	0.76	5.17805
2	19.47	19.3	3.17	(9,22; 29,39)	0.17	0.11	0.09	0.8125	0	0.18526
3	11.91	12.44	3.17	(2,36; 22,53)	-0.53	-0.35	-0.29	0.8125	0.04	-0.60786
4	11.01	12.93	3.17	(2,85; 23,01)	-1.92	-1.26	-1.51	0.8125	0.53	-3.14366
5	17.52	19.82	3.17	(9,73; 29,90)	-2.29	-1.51	-2.49	0.8125	0.76	-5.17805
6	21.49	21.66	3.17	(11,58; 31,74)	-0.17	-0.11	-0.09	0.8125	0	-0.18526
7	20	19.46	3.17	(9,38; 29,54)	0.53	0.35	0.29	0.8125	0.04	0.60786
8	27.22	25.3	3.17	(15,22; 35,38)	1.92	1.26	1.51	0.8125	0.53	3.14366
9	20.53	22.83	3.17	(12,74; 32,91)	-2.29	-1.51	-2.49	0.8125	0.76	-5.17805
10	25.51	25.68	3.17	(15,59; 35,76)	-0.17	-0.11	-0.09	0.8125	0	-0.18526
11	19.58	19.05	3.17	(8,96; 29,13)	0.53	0.35	0.29	0.8125	0.04	0.60786
12	19.21	17.29	3.17	(7,21; 27,37)	1.92	1.26	1.51	0.8125	0.53	3.14366
13	19.35	17.06	3.17	(6,97; 27,14)	2.29	1.51	2.49	0.8125	0.76	5.17805
14	18.16	18	3.17	(7,91; 28,08)	0.17	0.11	0.09	0.8125	0	0.18526
15	15.49	16.03	3.17	(5,94; 26,11)	-0.53	-0.35	-0.29	0.8125	0.04	-0.60786
16	17.7	19.62	3.17	(9,54; 29,70)	-1.92	-1.26	-1.51	0.8125	0.53	-3.14366

Supplementary Table S5. General factorial regression on glucomannnan extraction efficiency response

Factor Information

Factor	Levels	Values
Substrate Concentration (%)	2	1; 4
Inoculum Concentration (%)	2	1; 3
Types of Microbes	4	*B. cereus; B. subtilis; A. niger; A. oryzae*

Analysis of Variance

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Model	12	115.865	95.00%	115.865	9.6554	4.75	0.113
Linear	5	60.986	50.00%	60.986	12.1973	5.99	0.086
Substrate Concentration (%)	1	14.351	11.77%	14.351	14.3507	7.05	0.077
Inoculum Concentration (%)	1	29.414	24.12%	29.414	29.4136	14.46	0.032
Types of Microbes	3	17.222	14.12%	17.222	5.7407	2.82	0.208
2-Way Interactions	7	54.878	44.99%	54.878	7.8398	3.85	0.148
Substrate Concentration (%)*Inoculum Concentration (%)	1	32.83	26.92%	32.83	32.8301	16.13	0.028
Substrate Concentration (%)*Types of Microbes	3	0.449	0.37%	0.449	0.1496	0.07	0.97
Inoculum Concentration (%)*Types of Microbes	3	21.6	17.71%	21.6	7.1999	3.54	0.163
Error	3	6.104	5.00%	6.104	2.0348		
Total	15	121.969	100.00%				

Model Summary

S	R-sq	R-sq(adj)	PRESS	R-sq(pred)	AICc	BIC
1.42647	95.00%	74.98%	173.638	0.00%	477.99	68.81

Coefficients

Term	Coef	SE Coef	95% CI	T-Value	P-Value	VIF
Constant	-2.202	0.357	(-3,337; -1,067)	-6.17	0.009	
Substrate Concentration (%)						
1	-0.947	0.357	(-2,082; 0,188)	-2.66	0.077	1
4	0.947	0.357	(-0,188; 2,082)	2.66	0.077	*
Inoculum Concentration (%)						
1	-1.356	0.357	(-2,491; -0,221)	-3.8	0.032	1
3	1.356	0.357	(0,221; 2,491)	3.8	0.032	*
Types of Microbes						
B. cereus	0.084	0.618	(-1,882; 2,050)	0.14	0.901	1.5
B. subtilis	1.653	0.618	(-0,313; 3,618)	2.68	0.075	1.5
A. niger	-1.041	0.618	(-3,007; 0,924)	-1.69	0.19	1.5
A. oryzae	-0.695	0.618	(-2,661; 1,271)	-1.13	0.342	*
Substrate Concentration (%)*Inoculum Concentration (%)						
1 1	-1.432	0.357	(-2,567; -0,298)	-4.02	0.028	1
1 3	1.432	0.357	(0,298; 2,567)	4.02	0.028	*
4 1	1.432	0.357	(0,298; 2,567)	4.02	0.028	*
4 3	-1.432	0.357	(-2,567; -0,298)	-4.02	0.028	*
Substrate Concentration (%)*Types of Microbes						
1 *B. cereus*	0.26	0.618	(-1,706; 2,225)	0.42	0.702	1.5
1 *B. subtilis*	-0.08	0.618	(-2,046; 1,886)	-0.13	0.905	1.5
1 *A. niger*	-0.195	0.618	(-2,161; 1,771)	-0.32	0.773	1.5
1 *A. oryzae*	0.016	0.618	(-1,950; 1,981)	0.03	0.982	*
4 *B. cereus*	-0.26	0.618	(-2,225; 1,706)	-0.42	0.702	*
4 *B. subtilis*	0.08	0.618	(-1,886; 2,046)	0.13	0.905	*
4 *A. niger*	0.195	0.618	(-1,771; 2,161)	0.32	0.773	*
4 *A. oryzae*	-0.016	0.618	(-1,981; 1,950)	-0.03	0.982	*
Inoculum Concentration (%)*Types of Microbes						
1 *B. cereus*	0.454	0.618	(-1,512; 2,419)	0.73	0.516	1.5
1 *B. subtilis*	1.636	0.618	(-0,330; 3,602)	2.65	0.077	1.5
1 *A. niger*	-0.636	0.618	(-2,602; 1,330)	-1.03	0.379	1.5
1 *A. oryzae*	-1.454	0.618	(-3,419; 0,512)	-2.35	0.1	*
3 *B. cereus*	-0.454	0.618	(-2,419; 1,512)	-0.73	0.516	*
3 *B. subtilis*	-1.636	0.618	(-3,602; 0,330)	-2.65	0.077	*
3 *A. niger*	0.636	0.618	(-1,330; 2,602)	1.03	0.379	*
3 *A. oryzae*	1.454	0.618	(-0,512; 3,419)	2.35	0.1	*

Supplementary Table S5. General factorial regression on glucomannnan extraction efficiency response (Continued)

Means

Term	**Fitted Mean**	**SE Mean**
Substrate Concentration (%)		
1	-3.149	0.504
4	-1.255	0.504
Inoculum Concentration (%)		
1	-3.558	0.504
3	-0.846	0.504
Types of Microbes		
B. cereus	-2.118	0.713
B. subtilis	-0.549	0.713
A. niger	-3.243	0.713
A. oryzae	-2.897	0.713
Substrate Concentration (%)*Inoculum Concentration (%)		
1 1	-5.937	0.713
1 3	-0.361	0.713
4 1	-1.178	0.713
4 3	-1.331	0.713
Substrate Concentration (%)*Types of Microbes		
1 *B. cereus*	-2.81	1.01
1 *B. subtilis*	-1.58	1.01
1 *A. niger*	-4.39	1.01
1 *A. oryzae*	-3.83	1.01
4 *B. cereus*	-1.43	1.01
4 *B. subtilis*	0.48	1.01
4 *A. niger*	-2.1	1.01
4 *A. oryzae*	-1.97	1.01
Inoculum Concentration (%)*Types of Microbes		
1 *B. cereus*	-3.02	1.01
1 *B. subtilis*	-0.27	1.01
1 *A. niger*	-5.24	1.01
1 *A. oryzae*	-5.71	1.01
3 *B. cereus*	-1.22	1.01
3 *B. subtilis*	-0.83	1.01
3 *A. niger*	-1.25	1.01
3 *A. oryzae*	-0.09	1.01

Fits and Diagnostics for All Observations

Obs	**Gluco. extraction efficiency**	**Fit**	**SE Fit**	**95% CI**	**Resid**	**Std Resid**	**Del Resid**	**HI**	**Cook's D**	**DFITS**
1	-4.14	-5.14	1.29	(-9,23; -1,05)	1	1.62	3.67	0.8125	0.87	7.63424
2	-2.79	-2.73	1.29	(-6,82; 1,36)	-0.06	-0.09	-0.08	0.8125	0	-0.15874
3	-8.08	-7.81	1.29	(-11,90; -3,72)	-0.27	-0.43	-0.36	0.8125	0.06	-0.7537
4	-8.75	-8.07	1.29	(-12,16; -3,98)	-0.68	-1.09	-1.15	0.8125	0.4	-2.39557
5	-1.47	-0.47	1.29	(-4,56; 3,62)	-1	-1.62	-3.67	0.8125	0.87	-7.63424
6	-0.37	-0.42	1.29	(-4,52; 3,67)	0.06	0.09	0.08	0.8125	0	0.15874
7	-0.7	-0.96	1.29	(-5,05; 3,13)	0.27	0.43	0.36	0.8125	0.06	0.7537
8	1.09	0.41	1.29	(-3,68; 4,51)	0.68	1.09	1.15	0.8125	0.4	2.39557
9	-1.9	-0.9	1.29	(-4,99; 3,19)	-1	-1.62	-3.67	0.8125	0.87	-7.63424
10	2.25	2.19	1.29	(-1,90; 6,28)	0.06	0.09	0.08	0.8125	0	0.15874
11	-2.4	-2.66	1.29	(-6,75; 1,43)	0.27	0.43	0.36	0.8125	0.06	0.7537
12	-2.67	-3.34	1.29	(-7,43; 0,75)	0.68	1.09	1.15	0.8125	0.4	2.39557
13	-0.96	-1.96	1.29	(-6,05; 2,13)	1	1.62	3.67	0.8125	0.87	7.63424
14	-1.29	-1.23	1.29	(-5,33; 2,86)	-0.06	-0.09	-0.08	0.8125	0	-0.15874
15	-1.81	-1.54	1.29	(-5,63; 2,55)	-0.27	-0.43	-0.36	0.8125	0.06	-0.7537
16	-1.26	-0.59	1.29	(-4,68; 3,50)	-0.68	-1.09	-1.15	0.8125	0.4	-2.39557

Supplementary Table S6. General factorial regression on glucomannnan extraction rate response

Factor Information

Factor	Levels	Values
Substrate Concentration (%)	2	1; 4
Inoculum Concentration (%)	2	1; 3
Types of Microbes	4	*B. cereus; B. subtilis; A. niger; A. oryzae*

Analysis of Variance

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Model	12	0.20978	73.29%	0.20978	0.01748	0.69	0.725
Linear	5	0.03422	11.96%	0.03422	0.00685	0.27	0.904
Substrate Concentration (%)	1	0.01180	4.12%	0.01180	0.01180	0.46	0.545
Inoculum Concentration (%)	1	0.00276	0.96%	0.00276	0.00276	0.11	0.764
Types of Microbes	3	0.01966	6.87%	0.01966	0.00655	0.26	0.853
2-Way Interactions	7	0.17556	61.34%	0.17556	0.02508	0.98	0.559
Substrate Concentration (%)*Inoculum Concentration (%)	1	0.10759	37.59%	0.10759	0.10759	4.22	0.132
Substrate Concentration (%)*Types of Microbes	3	0.00861	3.01%	0.00861	0.00287	0.11	0.947
Inoculum Concentration (%)*Types of Microbes	3	0.05937	20.74%	0.05937	0.01979	0.78	0.58
Error	3	0.07644	26.71%	0.07644	0.02548		
Total	15	0.28622	100.00%				

Model Summary

S	R-sq	R-sq(adj)	PRESS	R-sq(pred)	AICc	BIC
1.42647	73.29%	0.00%	2.17425	0.00%	407.9	-1.28

Coefficients

Term	Coef	SE Coef	95% CI	T-Value	P-Value	VIF
Constant	-0.1158	0.0399	(-0,2428; 0,0112)	-2.9	0.062	
Substrate Concentration (%)						
1	-0.0272	0.0399	(-0,1542; 0,0998)	-0.68	0.545	1
4	0.0272	0.0399	(-0,0998; 0,1542)	0.68	0.545	*
Inoculum Concentration (%)						
1	0.0131	0.0399	(-0,1139; 0,1401)	0.33	0.764	1
3	-0.0131	0.0399	(-0,1401; 0,1139)	-0.33	0.764	*
Types of Microbes						
B. cereus	-0.0271	0.0691	(-0,2471; 0,1929)	-0.39	0.721	1.5
B. subtilis	-0.0221	0.0691	(-0,2421; 0,1979)	-0.32	0.77	1.5
A. niger	-0.0106	0.0691	(-0,2306; 0,2093)	-0.15	0.887	1.5
A. oryzae	0.0598	0.0691	(-0,1601; 0,2798)	0.87	0.45	*
Substrate Concentration (%)*Inoculum Concentration (%)						
1 1	-0.082	0.0399	(-0,2090; 0,0450)	-2.05	0.132	1
1 3	0.082	0.0399	(-0,0450; 0,2090)	2.05	0.132	*
4 1	0.082	0.0399	(-0,0450; 0,2090)	2.05	0.132	*
4 3	-0.082	0.0399	(-0,2090; 0,0450)	-2.05	0.132	*
Substrate Concentration (%)*Types of Microbes						
1 *B. cereus*	-0.0131	0.0691	(-0,2331; 0,2068)	-0.19	0.861	1.5
1 *B. subtilis*	-0.0293	0.0691	(-0,2492; 0,1907)	-0.42	0.7	1.5
1 *A. niger*	0.0107	0.0691	(-0,2093; 0,2306)	0.15	0.887	1.5
1 *A. oryzae*	0.0317	0.0691	(-0,1882; 0,2517)	0.46	0.677	*
4 *B. cereus*	0.0131	0.0691	(-0,2068; 0,2331)	0.19	0.861	*
4 *B. subtilis*	0.0293	0.0691	(-0,1907; 0,2492)	0.42	0.7	*
4 *A. niger*	-0.0107	0.0691	(-0,2306; 0,2093)	-0.15	0.887	*
4 *A. oryzae*	-0.0317	0.0691	(-0,2517; 0,1882)	-0.46	0.677	*
Inoculum Concentration (%)*Types of Microbes						
1 *B. cereus*	0.0003	0.0691	(-0,2197; 0,2203)	0	0.997	1.5
1 *B. subtilis*	0.0978	0.0691	(-0,1221; 0,3178)	1.42	0.252	1.5
1 *A. niger*	-0.034	0.0691	(-0,2539; 0,1860)	-0.49	0.657	1.5
1 *A. oryzae*	-0.0642	0.0691	(-0,2841; 0,1558)	-0.93	0.422	*
3 *B. cereus*	-0.0003	0.0691	(-0,2203; 0,2197)	0	0.997	*
3 *B. subtilis*	-0.0978	0.0691	(-0,3178; 0,1221)	-1.42	0.252	*
3 *A. niger*	0.034	0.0691	(-0,1860; 0,2539)	0.49	0.657	*
3 *A. oryzae*	0.0642	0.0691	(-0,1558; 0,2841)	0.93	0.422	*

Supplementary Table S6. General factorial regression on glucomannnan extraction rate response (Continued)

Means

Term	**Fitted Mean**	**SE Mean**
Substrate Concentration (%)		
1	-0.1429	0.0564
4	-0.0886	0.0564
Inoculum Concentration (%)		
1	-0.1026	0.0564
3	-0.1289	0.0564
Types of Microbes		
B. cereus	-0.1429	0.0798
B. subtilis	-0.1379	0.0798
A. niger	-0.1264	0.0798
A. oryzae	-0.056	0.0798
Substrate Concentration (%)*Inoculum Concentration (%)		
1 1	-0.2118	0.0798
1 3	-0.0741	0.0798
4 1	0.0065	0.0798
4 3	-0.1837	0.0798
Substrate Concentration (%)*Types of Microbes		
1 *B. cereus*	-0.183	0.113
1 *B. subtilis*	-0.194	0.113
1 *A. niger*	-0.143	0.113
1 *A. oryzae*	-0.051	0.113
4 *B. cereus*	-0.103	0.113
4 *B. subtilis*	-0.081	0.113
4 *A. niger*	-0.11	0.113
4 *A. oryzae*	-0.061	0.113
Inoculum Concentration (%)*Types of Microbes		
1 *B. cereus*	-0.129	0.113
1 *B. subtilis*	-0.027	0.113
1 *A. niger*	-0.147	0.113
1 *A. oryzae*	-0.107	0.113
3 *B. cereus*	-0.156	0.113
3 *B. subtilis*	-0.249	0.113
3 *A. niger*	-0.106	0.113
3 *A. oryzae*	-0.005	0.113

Fits and Diagnostics for All Observations

Obs	**Glucomannan extraction rate**	**Fit**	**SE Fit**	**95% CI**	**Resid**	**Std Resid**	**Del Resid**	**HI**	**Cook's D**	**DFITS**
1	-0.178	-0.252	0.144	(-0,710; 0,206)	0.074	1.07	1.12	0.8125	0.38	2.32693
2	-0.279	-0.165	0.144	(-0,623; 0,293)	-0.113	-1.64	-4.14	0.8125	0.9	-8.61744
3	-0.227	-0.246	0.144	(-0,704; 0,212)	0.019	0.27	0.22	0.8125	0.02	0.46361
4	-0.164	-0.184	0.144	(-0,642; 0,273)	0.02	0.3	0.24	0.8125	0.03	0.50982
5	-0.189	-0.115	0.144	(-0,573; 0,343)	-0.074	-1.07	-1.12	0.8125	0.38	-2.32693
6	-0.11	-0.223	0.144	(-0,681; 0,235)	0.113	1.64	4.14	0.8125	0.9	8.61744
7	-0.059	-0.04	0.144	(-0,498; 0,418)	-0.019	-0.27	-0.22	0.8125	0.02	-0.46361
8	0.061	0.082	0.144	(-0,376; 0,540)	-0.02	-0.3	-0.24	0.8125	0.03	-0.50982
9	-0.081	-0.007	0.144	(-0,465; 0,451)	-0.074	-1.07	-1.12	0.8125	0.38	-2.32693
10	0.225	0.112	0.144	(-0,346; 0,569)	0.113	1.64	4.14	0.8125	0.9	8.61744
11	-0.067	-0.049	0.144	(-0,507; 0,409)	-0.019	-0.27	-0.22	0.8125	0.02	-0.46361
12	-0.05	-0.03	0.144	(-0,487; 0,428)	-0.02	-0.3	-0.24	0.8125	0.03	-0.50982
13	-0.124	-0.198	0.144	(-0,656; 0,260)	0.074	1.07	1.12	0.8125	0.38	2.32693
14	-0.388	-0.274	0.144	(-0,732; 0,184)	-0.113	-1.64	-4.14	0.8125	0.9	-8.61744
15	-0.152	-0.171	0.144	(-0,629; 0,287)	0.019	0.27	0.22	0.8125	0.02	0.46361
16	-0.071	-0.091	0.144	(-0,549; 0,366)	0.02	0.3	0.24	0.8125	0.03	0.50982

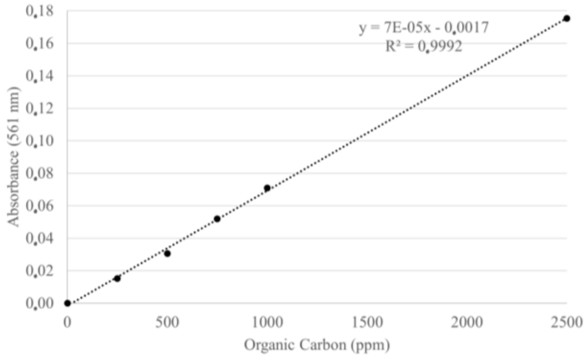
Supplementary Table S7. Quality parameter of extracted porang flour

Metode	Replication	Glucomanan content (%)	Calcium oxalate content (mg/100g)	Moisture content (%)	Ash content (%)
Untreated flour	1	22.1891	3.0150	10.8238	5.6974
	2	23.4717	2.8350	11.0523	5.6080
	3	22.7779	2.9700	10.9727	5.6130
Mechanic extraction	1	22.7157	0.6300	6.9788	4.3770
	2	22.6623	0.4950	7.5066	4.3552
	3	27.2339	0.5400	7.3303	4.4022
Maceration	1	20.4566	1.3050	6.6859	5.0556
	2	23.3827	1.4850	7.2893	4.8701
	3	26.0065	1.4400	7.6069	5.5745
UAE	1	25.5574	1.6650	6.5976	5.2401
	2	25.6597	1.7550	7.2316	5.0067
	3	24.9254	1.6650	7.7199	4.5043
Enzymatic	1	26.9493	1.2600	10.1515	1.9163
	2	28.7948	1.4400	10.2444	2.3175
	3	27.4162	1.2600	7.5484	2.5567
Fermentation	1	24.9237	1.0800	2.1001	8.2846
	2	26.0977	0.9000	2.2250	8.0754
	3	25.5107	1.2600	2.1632	8.1800

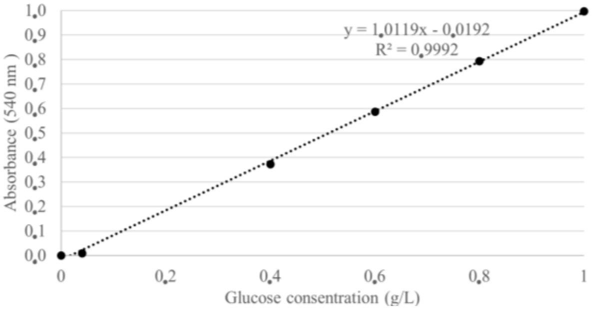
Supplementary Table S8. Quality requirements for glucomannan flour according to SNI (Indonesian National Standard) 9211-202

Test parameter	Requirement	Unit
Color	Normal	-
Odor	Normal	-
Foreign matter		
Other plant skins, soil, stones, sand, and others	None	-
Insects in any stage and visible fragments	None	-
Fineness, passing through mesh 40	≥90	% mass fraction
Moisture content	≤10,0	% mass fraction
Ash content (dry weight basis)	≤3,0	% mass fraction
Glucomannan content (dry weight basis)	≤85	% mass fraction
Calcium oxalate	≤2,5	mg/100 g
Sulfur oxide (as sulfite)	≤7	mg/kg
Heavy metal contaminants		
Lead (Pb)	≤0,25	mg/kg
Cadmium (Cd)	≤0,05	mg/kg
Tin (Sn)	≤40	mg/kg
Mercury (Hg)	≤0,03	mg/kg
Arsenic (As)	≤0,1	mg/kg

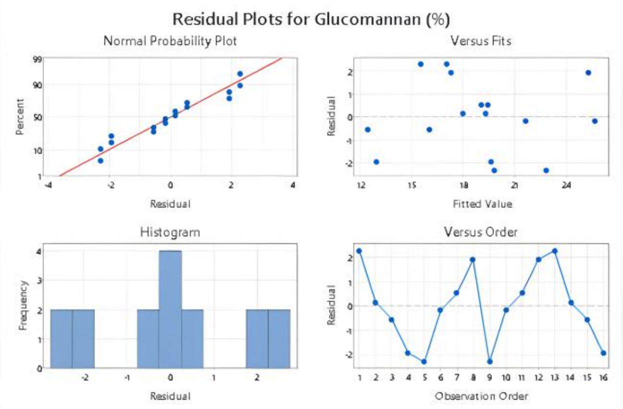
SUPPLEMENTARY FIGURES



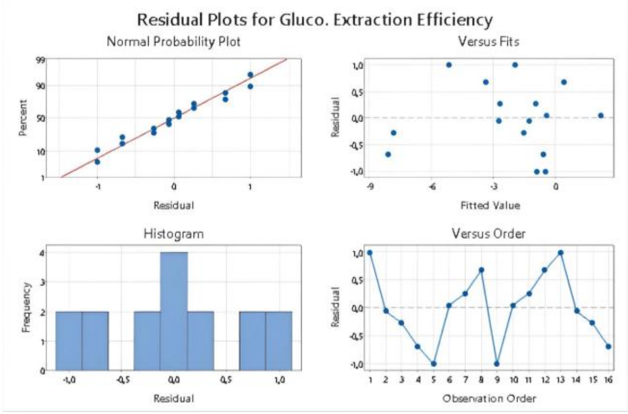
Supplementary Figure S1. Organic carbon standard curve



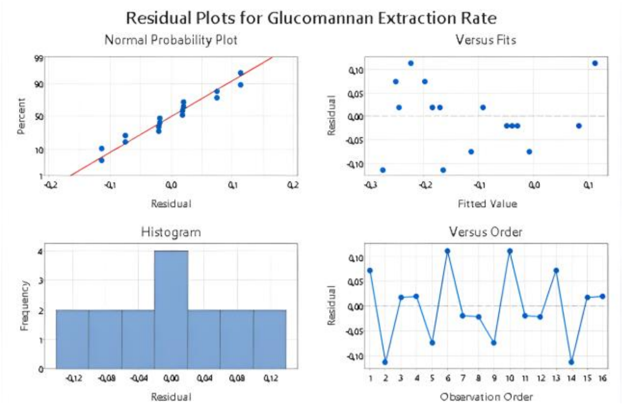
Supplementary Figure S2. Glucose standard curve



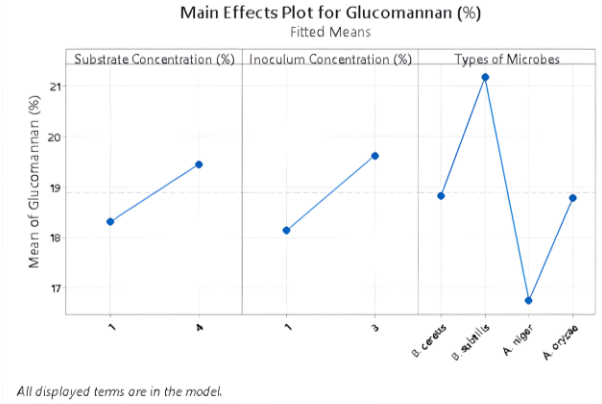
Supplementary Figure S3. Residual plots for glucomannan



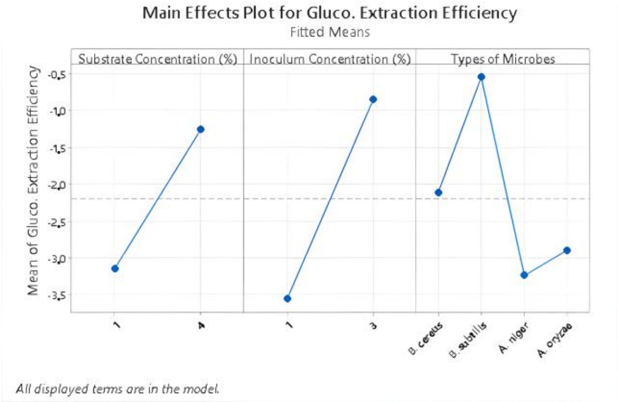
Supplementary Figure S4. Residual plots for glucomannan extraction efficiency



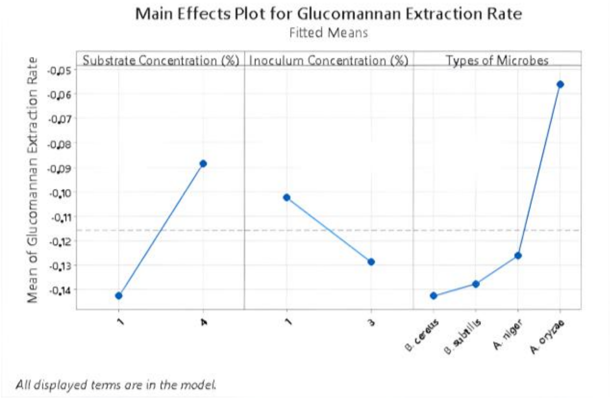
Supplementary Figure S5. Residual plots for glucomannan extraction rate



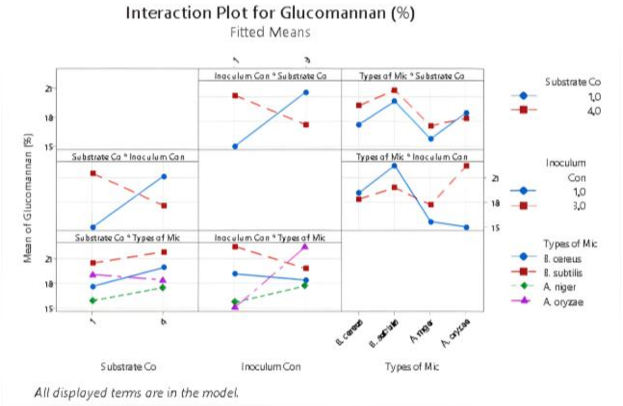
Supplementary Figure S6. Main effects plot for glucomannan



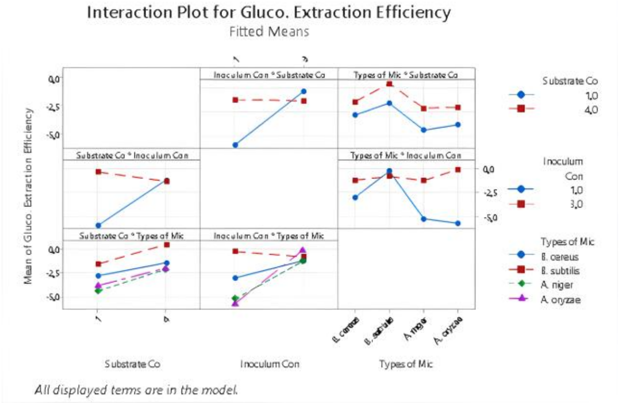
Supplementary Figure S7. Main effects plot for glucomannan extraction efficiency



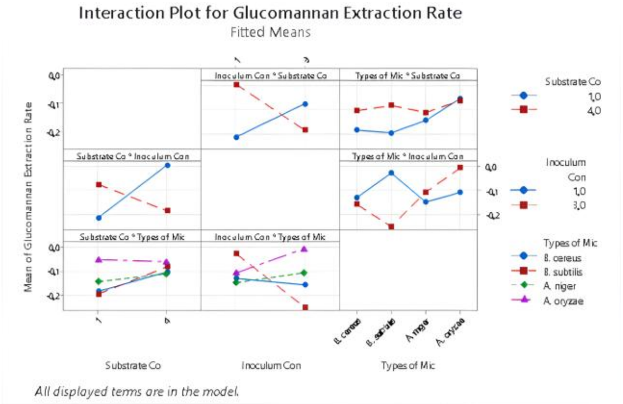
Supplementary Figure S8. Main effects plot for glucomannan extraction rate



Supplementary Figure S9. Interaction plot for glucomannan



Supplementary Figure S10. Interaction plot for glucomannan extraction efficiency



Supplementary Figure S11. Interaction plot for glucomannan extraction rate