การเพิ่มประสิทธิภาพการสกัดโอลิโกแซ็กคาไรด์จากหัวมันแกวโดยใช้การออกแบบการทดลองแบบแพลกเกตต์-เบอร์แมน (Plackett-Burman Design)

Optimization of Oligosaccharide Extraction from Yam Bean Root Using Plackett-Burman Design

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อระบุปัจจัยสำคัญที่มีผลต่อประสิทธิภาพการสกัดโอลิโกแซ็กคาไรด์จาก หัวมันแกว (*Pachyrhizus erosus* (L.) Urban) โดยใช้การออกแบบการทดลองแบบแพลกเกตต์-เบอร์แมน (Plackett-Burman Design: PBD) การคัดเลือกปัจจัยที่สำคัญดำเนินการใน 5 ตัวแปร ได้แก่ ขนาดตัวอย่าง (2 มม. และ 10 มม.) อุณหภูมิในการสกัด (30°C และ 60°C) ระยะเวลาในการสกัด (30 และ 100 นาที) ความเข้มข้นของเอทานอล (30% และ 50% v/v) และค่า pH (5.0 และ 7.0) ผลการทดลองพบว่า ความเข้มข้นของเอทานอล ค่า pH และอุณหภูมิการสกัด มีผลอย่างมีนัยสำคัญต่อปริมาณโอลิโกแซ็กคาไรด์ที่ได้ โดยสภาวะที่ให้ผลผลิตโอลิโกแซ็กคาไรด์สูงสุด (36.00% โดยน้ำหนัก) คือ ขนาดตัวอย่าง 2 มม. อุณหภูมิ 60°C เวลา 30 นาที ความเข้มข้นของเอทานอล 50% โดยปริมาตร และค่า pH 7.0 การวิเคราะห์ทางสถิติด้วยโปรแกรม Design-Expert® 7.0.0 ยืนยันว่าแบบจำลองมีความเหมาะสม (R^2 = 0.90) และค่าคงเหลือมีการแจกแจงแบบปกติเป็นอิสระ และมีความแปรปรวนสม่ำเสมอ ผลการวิจัยนี้ชี้ให้เห็นถึงสภาวะที่เหมาะสมสำหรับการสกัดโอลิโกแซ็กคาไรด์ซึ่งสามารถนำไปใช้เป็นพื้นฐานในการขยายระดับการผลิตหรือศึกษาคุณสมบัติทางหน้าที่เพิ่มเติมในอนาคตได้

คำสำคัญ: หัวมันแกว การสกัดโอลิโกแซ็กคาไรด์ การออกแบบการทดลองแบบแพลกเกตต์-เบอร์แมน

Abstract

This study aimed to identify the key factors influencing the extraction efficiency of oligosaccharides from yam bean (*Pachyrhizus erosus* (L.) Urban) root using the Plackett-Burman design (PBD). The screening was conducted on five critical factors: sample size (2 mm and 10 mm), extraction temperature (30°C and 60°C), extraction duration (30 and 100 min), ethanol concentration (30% and 50% v/v), and pH (5.0 and 7.0). The experimental results revealed that ethanol concentration, pH, and extraction temperature had significant effects on oligosaccharide yield, with the highest yield (36.00% wt) obtained under the following conditions: 2 mm sample size, 60°C extraction temperature, 30 min extraction time, 50% ethanol, and pH 7.0. Statistical analysis using Design-Expert® 7.0.0 confirmed the adequacy of the model (R^2 = 0.90), and residuals exhibited normal distribution, independence, and homoscedasticity. The findings highlight optimal conditions for oligosaccharide extraction, which may serve as a foundation for future scale-up studies or further functional characterization.

Keywords: Yam bean root, Oligosaccharides extraction, Plackett-Burman design

1. Introduction

Yam bean (*Pachyrhizus erosus* (L.) Urban) is a leguminous root crop widely cultivated in tropical regions, including Thailand. The root is consumed fresh and processed into various food products due to its crisp texture and mild sweetness. It contains valuable carbohydrate components, notably inulin-type fructans and other oligosaccharides, which have attracted attention for their potential application as functional food ingredients (Bhanja et al., 2023). However, the extraction of such oligosaccharides is influenced by multiple factors, including solvent composition, temperature, and pH. Ethanol-water mixtures are frequently used in extraction because they offer an effective polarity range to isolate low molecular weight saccharides without degrading thermolabile compounds (Huang & Huang, 2020). Despite increasing interest in bioactive oligosaccharides, preliminary extraction studies often lack statistical rigor in evaluating the influence of multiple process variables. Conventional single-variable approaches are time-consuming and fail to reveal interactive effects between factors (Fan et al., 2022). Hence, a robust experimental design is essential for efficiently screening and identifying key variables influencing oligosaccharide extraction.

The Plackett-Burman Design (PBD) is a fractional factorial method suitable for identifying statistically significant factors among large variables using minimal experiments. This design is particularly effective for early-stage screening and has been widely applied in the food and biotechnology industries for optimizing extraction, fermentation, and formulation processes (Boateng & Yang, 2021). While PBD does not directly model interactions or curvature, it serves as a critical precursor to response surface methodology (RSM) or central composite designs (CCD) for further optimization (Du et al., 2021).

This study employed PBD to identify the most influential variables affecting the extraction of oligosaccharides from yam bean roots. Five factors—sample size, extraction temperature, extraction duration, ethanol concentration, and pH—were evaluated for their impact on extraction yield. The study aims to establish statistically validated conditions that maximize oligosaccharide recovery and to provide a framework for further functional assessment and process scale-up.

2. Materials and methods

2.1 Raw material preparation

Fresh yam bean roots (*Pachyrhizus erosus* (L.) Urban), aged 90 days, were harvested, washed with tap water, and peeled. The roots were sliced into thin pieces and dried at ambient temperature for 6 h. Subsequently, the slices were dried in a hot air oven at 55°C for another 6 h to reduce moisture content. The thoroughly dried samples were cut into two particle sizes: 2 mm and 10 mm. These samples were stored in a desiccator at room temperature until use.

2.2 Extraction and analytical procedures

Twenty grams of dried yam bean samples were immersed in ethanol-water at a solid-to-liquid ratio of 1:5 (w/v). The extraction process used specific temperature, time, ethanol concentration, and pH combinations according to the Plackett-Burman design. The mixture was stirred continuously during extraction. After extraction, the mixture was filtered through Whatman No.1 filter paper, and the filtrate was centrifuged at 4,000 rpm for 10 min. The supernatant was then evaporated at 40°C for 1 h using a rotary evaporator to reduce the ethanol content. The concentrate was further dried in a hot air oven at 50°C for 48 h, followed by freeze-drying at −55°C for 12 h to obtain a stable dry powder. The final extract was weighed to determine total yield, and the following analyses were conducted:

- Total sugar content was measured using the phenol-sulfuric acid method.
- Reducing sugar content was determined using the 3,5-dinitrosalicylic acid (DNS) method (Jain et al., 2020).
- Non-reducing sugar content was calculated using the equation:

Non-reducing sugar = Total sugar - Reducing sugar ....(1)

- Oligosaccharide yield (% wt) was used as the primary response and calculated as:

Yield of oligosaccharide (% wt) = $(W_o/W_d) \times 100$ ....(2)

where W_o is the dry weight (g) of oligosaccharide extract obtained, W_d is the dry weight (g) of the yam bean sample used.

2.3 Experimental design and statistical analysis

2.3.1 Plackett-Burman design (PBD)

The Plackett-Burman Design (PBD) was employed to identify the significant factors affecting the extraction yield of oligosaccharides from yam bean roots. Five independent variables were investigated:

sample diameter (X_1), extraction temperature (X_2), extraction duration (X_3), ethanol concentration (X_4), and pH (X_5). Each variable was tested at low (−1) and high (+1) levels, as shown in Table 1. The experimental design follows a first-order linear model without interaction terms:

$$Y = \beta_0 + \sum \beta_i X_i \qquad \text{.............................(3)}$$

where, Y is the response variable (oligosaccharide yield, wt%), β_0 is the intercept, and β_i represents the linear coefficient for the i-th factor, and X_i is the coded value of each variable. Each factor was tested at two levels (-1 and +1) (Table 1. and Table 2).

PBD is suitable for screening purposes, where the primary goal is to determine the most influential factors with statistical significance using a minimum number of experimental runs. While it does not account for interaction or quadratic effects, PBD serves as a valuable foundation for further optimization using response surface methodology (RSM) or central composite design (CCD).

The experimental results were analyzed using Design-Expert® version 7.0.0 (Stat-Ease, Inc., USA). Model validation was carried out through residual diagnostics, including normal probability plots, independence checks, and variance stability assessments to ensure the assumptions of linear regression were met. The R-squared (R^2) value was used to evaluate the goodness of fit of the model, while analysis of variance (ANOVA) was performed to assess the statistical significance of the independent variables. The impact of each variable on oligosaccharide yield was quantified using the following expression for the main effect:

$$E_{(Xi)} = \frac{2\left(\sum M_{i+} - M_{i-}\right)}{N} \qquad \text{.............................(4)}$$

where, E_{Xi} is the concentration effect of the tested variable, M_{i+} and M_{i-} are the response values (oligosaccharide yield in %wt) from experiments in which the variable of interest was at the high (+1) and low (−1) levels, respectively, N is the number of experimental runs. This first-order model enabled the identification of significant factors without considering interaction or quadratic effects, as is typical of screening designs like Plackett-Burman.

2.3.2 Statistical optimization and desirability function

The optimization function in the software was employed to determine the most suitable conditions for maximizing oligosaccharide yield. The analysis considered composite desirability (D), where values closer to 1 indicate greater optimization efficiency. This multi-response optimization ensured a balance between experimental feasibility and extraction performance. Using statistical tools and PBD design ensured the experiment was robust and reproducible. It provided a solid foundation for identifying the most influential parameters, guiding further process refinement using advanced optimization models.

Table 1. Levels of Independent Variables Used in the PBD

Code	Variable	-1 (Low Level)	+1 (Low Level)
X_1	Sample diameter (mm)	2	10
X_2	Extraction temperature (°C)	30	60
X_3	Extraction duration (min)	30	100
X_4	Ethanol concentration (% v/v)	30	50
X_5	pH	5.0	7.0

3. Result

3.1 The yield of oligosaccharide extract from yam bean root

The results in Table 2. illustrate the influence of various extraction parameters on oligosaccharide yield from yam bean root. The highest yield (36.00% wt) was achieved in experiment 5, which employed a 2 mm sample size, 60°C extraction temperature, 30-min extraction time, 50% (v/v) ethanol concentration, and pH 7.0. Conversely, the lowest yield (8.94% wt) was observed in experiment 12, where all parameters were set at their minimum levels.

Among the tested variables, ethanol concentration and pH were found to have the most pronounced positive effects, as shown by the Pareto chart. A smaller particle size (2 mm) also significantly contributed to a higher yield, likely due to an increased surface area, which enhances mass transfer and solvent penetration during extraction. These observations are consistent with the theoretical expectation that finer sample sizes promote solute-solvent interaction, thereby improving extraction efficiency. An additional extraction was carried out under the predicted optimal conditions—2 mm sample size, 60°C, 99.93 min, 50% ethanol, and pH 7.0 to validate the predictive model. The experimentally obtained yield (34.96% wt) was in close agreement with the model's predicted value (35.58% wt), demonstrating a relative error of less than 2%. This confirms the accuracy and practical applicability of the Plackett-Burman model for optimizing oligosaccharide extraction from yam bean root.

3.2 Statistical analysis

3.2.1 Model validation

Model validation was performed based on three key assumptions: normality of residuals, independence, and homoscedasticity (constant variance). Figure 1. illustrates the standard probability plot of the residuals. The residuals were approximately aligned along a straight diagonal line, indicating that the errors followed a normal distribution. No significant deviation or curvature was observed, thus supporting the normality assumption. Figure 2. displays the residuals versus predicted values to assess independence.

The plot showed a random scatter without any visible pattern or trend, confirming that the residuals were independently distributed. Figure 3. demonstrates the homogeneity of variance, showing residuals plotted against levels of the independent variables. The distribution of residuals was fairly symmetrical and balanced between positive and negative values, indicating stable variance across factor levels. These results collectively support the validity of the model assumptions. The residual analysis confirms that the experimental data meets the requirements for reliable statistical interpretation using regression analysis and ANOVA.

Table 2. PBD design matrix for evaluating factors influencing oligosaccharide yield from yam bean root

Run	X_1		X_2		X_3		X_4		X_5		Oligosaccharide
	code	actual	Code	actual	code	actual	code	actual	code	actual	Yield (% wt)
1	+1	10	+1	60	+1	100	-1	30	-1	5.0	**18.81**
2	-1	2	-1	30	-1	30	+1	50	-1	5.0	**22.50**
3	-1	2	+1	60	+1	100	-1	30	+1	7.0	**28.98**
4	+1	10	+1	60	-1	30	+1	50	+1	7.0	**25.83**
5	-1	2	+1	60	-1	30	+1	50	+1	7.0	**36.00**
6	+1	10	-1	30	+1	100	+1	50	+1	7.0	**24.70**
7	+1	10	-1	30	-1	30	-1	30	+1	7.0	**14.25**
8	+1	10	+1	60	-1	30	-1	30	-1	5.0	**11.01**
9	-1	2.	-1	30	+1	100	-1	30	+1	7.0	**17.15**
10	+1	10	-1	30	+1	100	+1	50	-1	5.0	**14.70**
11	-1	2	+1	60	+1	100	+1	50	-1	5.0	**25.45**
12	-1	2	-1	30	-1	30	-1	30	-1	5.0	**8.94**

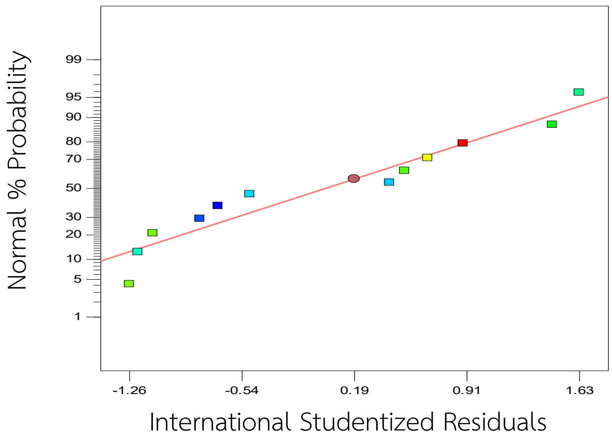

Figure 1. Normal probability of the residual plot

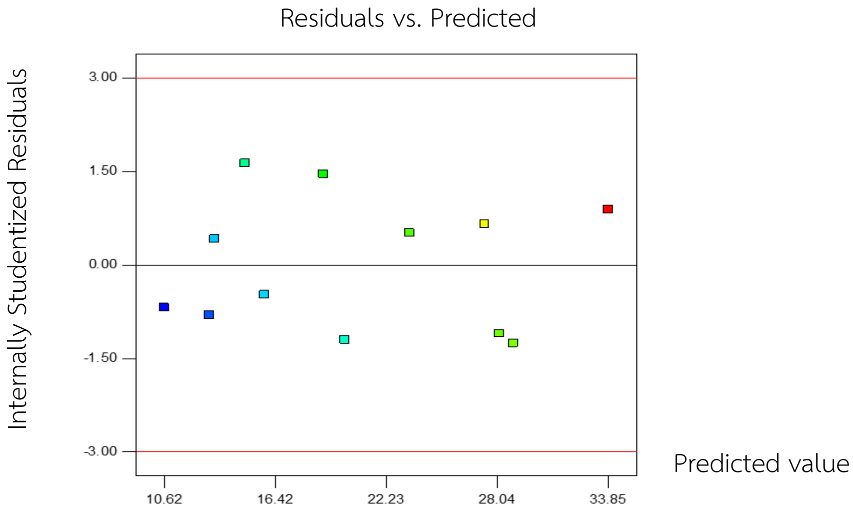

Figure 2. Studentized residuals and run number

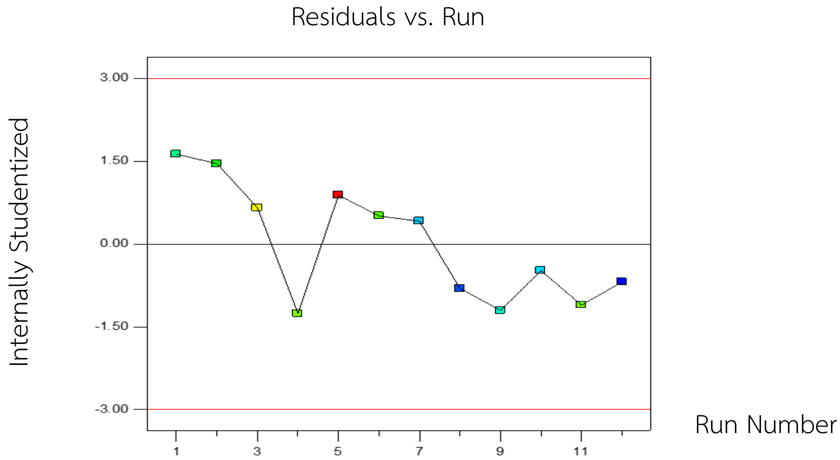

Figure 3. Studentized residuals and predicted values

3.2.2 Coefficient of decision and analysis of variance (ANOVA)

The coefficient of determination (R^2) was used to assess the goodness of fit of the regression model. The calculated R^2 value was 0.90, indicating that 90% of the variability in oligosaccharide yield could be explained by the five independent variables in the model (Table 3.). This reflects a high predictive power and model adequacy. Analysis of variance (ANOVA) was conducted to test the significance of each factor. The results showed that ethanol concentration, pH, and extraction temperature had statistically significant effects ($p < 0.05$) on oligosaccharide yield. These findings support using the Plackett-Burman model for screening influential variables in the extraction process.

3.2.3 Effect of independent factors

The oligosaccharide yield obtained from the experiments ranged from 8.94% wt to 36.00% wt, as shown in Table 2. The influence of the five independent variables—sample size, extraction temperature, extraction duration, ethanol concentration, and pH—was analyzed using a Pareto chart (Figure 4.), which graphically displays the standardized effects of each factor.

In the Pareto chart, effects that exceed the Bonferroni limit are considered statistically significant. In contrast, those above the *t*-value limit are likely significant, and those below the *t*-value limit are unlikely to be substantial. The results indicate that ethanol concentration, pH, extraction temperature, and sample size exceeded the Bonferroni limit, suggesting that they strongly influenced oligosaccharide yield.

Table 3. Coefficient of decision and analysis of variance (ANOVA)

Source	Sum of Squares	df	Mean Square	*F* Value	*p*-value Prob > *F*	Remark
Model	625.5352	5	125.107	10.53954	0.0062	significant
A - Sample diameter	73.55701	1	73.55701	6.196752	0.0472	
B - Extraction temperature	160.3083	1	160.3083	13.50505	0.0104	
C - Extraction duration	10.56563	1	10.56563	0.890093	0.3819	
D - Ethanol concentration	208.5834	1	208.5834	17.57194	0.0057	
E - pH	172.5208	1	172.5208	14.53388	0.0088	
Residual	71.22152	6	11.87025			
Cor Total	696.7567	11				
Std. Dev. = 3.44; Mean = 20.70; C.V. % = 16.65; PRESS = 284.89; R-Squared = 0.90; Adj R-Squared = 0.81						

Although not crossing the Bonferroni limit, extraction duration remained above the *t*-value threshold, indicating a moderate effect. The findings were consistent with the ANOVA results (Table 3.), which showed that all five variables had *p*-values less than 0.05, indicating statistical significance at the 95% confidence level.

The results confirm that optimizing these independent factors is critical for maximizing oligosaccharide recovery from yam bean root. The sign of the effect coefficients (Table 4.) provided further insights into the direction of influence:

- A negative coefficient for sample size suggests that increasing particle size reduced oligosaccharide yield, likely due to reduced surface area and mass transfer efficiency.

- Positive coefficients for ethanol concentration, pH, and extraction temperature indicate that increasing these variables enhances oligosaccharide extraction efficiency, likely by improving solute solubility and cell wall disruption.

The multiple regression analysis can establish models or equations that predict the experimental results of each factor. The results obtained from this experiment were a non-linear quadratic correlation model. Results of the oligosaccharide yield extract are shown as follows:

$$\gamma = 20.69 - (2.47X_1) + (3.65X_2) + (0.93X_3) + (4.16X_4) + (3.79X_5) \quad ....(5)$$

where, γ is the yield of the oligosaccharide extract, X_1 X_2, X_3, X_4 and X_5 represents sample diameter, extraction temperature, extraction duration, ethanol concentration, and pH.

3.3 Optimization of extraction conditions

The statistical analysis of experimental data was conducted using Design-Expert® version 7.0.0 (Stat-Ease, Inc., Minneapolis, MN, USA). The study was divided into three key components: model validation, coefficient of determination (R^2), and analysis of variance (ANOVA). These analyses were performed to evaluate model adequacy, identify significant factors, and confirm the reliability of the regression model. The numerical optimization function within the software was used to determine the most effective combination of extraction conditions. This function identifies the factor levels that maximize the desired response—in this case, oligosaccharide yield—while satisfying practical constraints. The composite desirability (D) index, which ranges from 0 to 1, was used to assess the overall optimization outcome. A value of 1.0 represents an ideal solution where all goals are fully met.

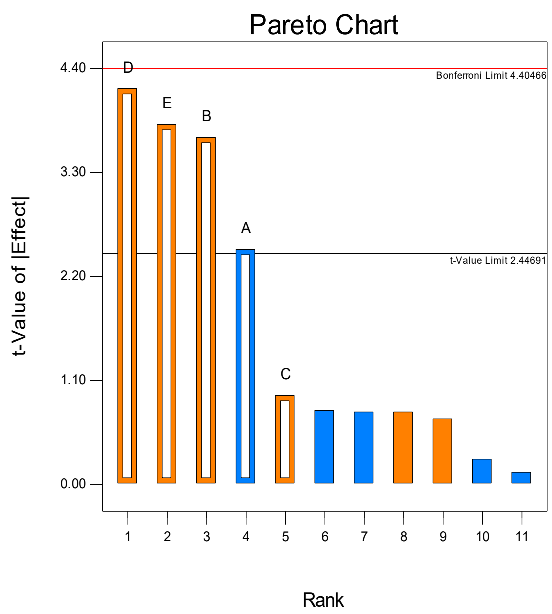

Figure 4. Pareto chart of the standardized effects of independent variables on the oligosaccharide yield

Table 4. Effect of independent factors

Term	Effect	Sum of Squares	% Contribution
Sample diameter (X_1)	-4.95	73.55	10.55
Extraction temperature (X_2)	7.31	160.30	23.00
Extraction duration (X_3)	1.87	10.56	1.51
Ethanol concentration (X_4)	8.33	208.58	29.93
pH (X_5)	7.58	172.52	24.76

The optimization results revealed the predicted optimal conditions for oligosaccharide extraction from yam bean root to be:

- Sample size: 2 mm
- Extraction temperature: 60°C
- Extraction duration: 99.93 min
- Ethanol concentration: 50% (v/v)
- pH: 7.0

Under these conditions, the model predicted an oligosaccharide yield of 35.58% wt with a composite desirability of 0.985, indicating a high level of agreement between the objectives and the optimal solution (**Figure 5**).

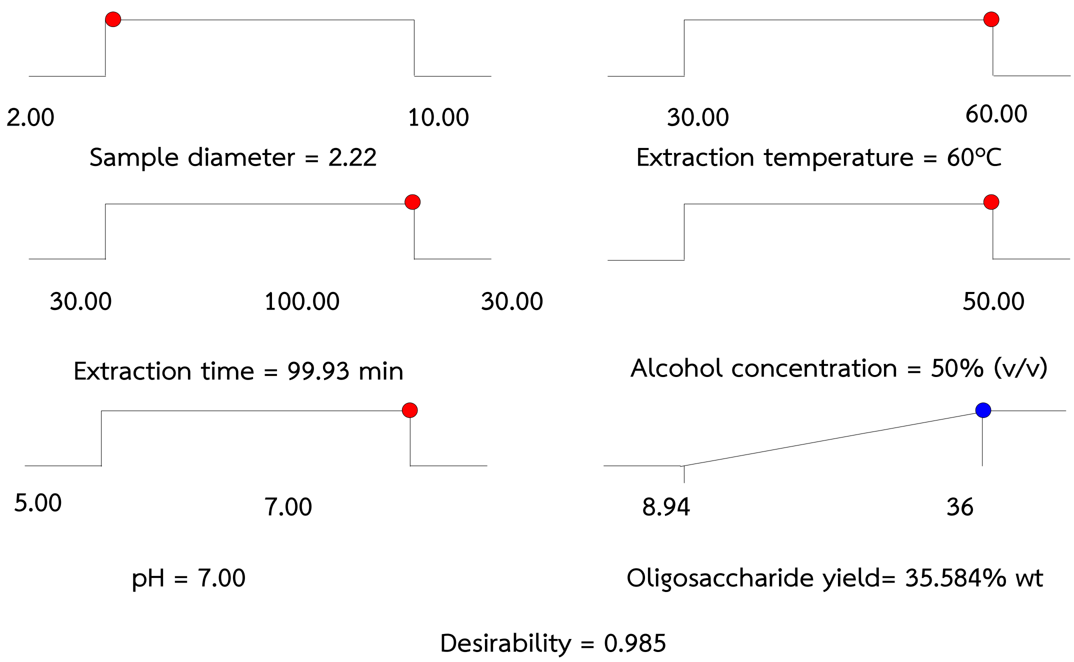

Figure 5. The optimal conditions for oligosaccharide yield

4. Discussion

The optimal conditions for oligosaccharide extraction from yam bean root were confirmed, highlighting the substantial influence of multiple factors on extraction efficiency and supporting the effectiveness of the Plackett-Burman Design in screening critical variables. Among these factors, ethanol concentration had a strong positive effect on oligosaccharide yield. Ethanol's amphiphilic nature facilitates the selective solubilization of oligosaccharides by adjusting the solvent polarity. Increasing ethanol content reduces the overall polarity of the solution, promoting the precipitation of high-molecular-weight polysaccharides and enhancing the solubility of smaller oligosaccharide fractions. This observation is consistent with (Huang & Huang 2020), who reported that ethanol concentration significantly affects carbohydrate solubility and fractionation during extraction.

Temperature also played a key role in improving yield. Elevated temperatures increase molecular mobility, solvent penetration, and diffusion rates, enhancing mass transfer between plant tissue and solvent. This trend aligns with the findings of (Poka et al. 2023), who demonstrated that higher temperatures facilitate more efficient oligosaccharide extraction from plant roots.

Extraction time was another contributing factor, as longer durations allow for more extensive contact between solvent and solutes, increasing the total recovery of sugars. This agrees with (Jaffur et al. 2024), who observed enhanced fermentable sugar recovery with prolonged extraction periods.

Sample size (particle diameter) was deliberately varied (2 mm vs. 10 mm) to study its effect on extraction performance. Smaller particles provided greater surface area and disrupted cellular structures more effectively, improving mass transfer and facilitating the release of target compounds. These results confirm the hypothesis that finer particle sizes enhance extraction efficiency, a trend also reported by (Fan et al. 2022) in studies on solvent extraction of bioactives. In contrast, larger particles may be more advantageous during industrial-scale processing by reducing clogging and energy input during milling. These findings underscore the relevance of particle engineering in optimizing both yield and downstream process compatibility.

The effect of pH was also significant. Lower pH values (more substantial acidity) enhanced the cleavage of glycosidic bonds in complex carbohydrates, thereby increasing oligosaccharide release. (Lu 2023; Wandee et al. 2021) previously supported this acid-assisted hydrolysis mechanism, which reported improved breakdown of plant-based carbohydrates under acidic conditions.

Although this study focused on oligosaccharide extraction from yam bean root, the findings provide comparative value when considering other plant sources. As summarized in Table 5., extraction efficiency and bioactive composition vary depending on plant material, method, and conditions. Therefore, the selection of raw material and process parameters must be tailored for each application. The insights gained from this work are relevant to research on functional carbohydrates and the design of scalable extraction protocols for use in functional foods and nutraceutical product development.

Table 5. Comparison of prebiotic extraction results from different plant sources

Plant source	Extraction method and Processing conditions	Reference
Jackfruit seeds (*Artocarpus heterophyllus*)	The extraction method using different solvents (e.g., distilled water, 50% ethanol, and 95% ethanol) demonstrated that 50% ethanol yielded the highest extraction efficiency.	(Wichienchot et al., 2011)
Sangyod rice (*Oryza sativa* L.)	Prebiotic extraction from Sangyod rice involves germination, drying, and milling processes. The obtained prebiotic powder is rich in non-digestible carbohydrates, serving as potential ingredients in health-promoting products.	(Sivamaruthi et al., 2023)
Mung bean seeds (*Vigna radiata* L.)	Oligosaccharides (RFOs) are the prebiotic extract of mung bean seeds. The ethanol extraction process has shown promising results in isolating these oligosaccharides, which contribute to the modulation of gut microbiota.	(Elango et al., 2022)
Carrot (*Daucus carota* L.)	A prebiotic extract from carrot residues is a polysaccharide developed as a functional ingredient. Studies indicate that consuming 300 mg daily enhances immune function and gut health.	(McKay et al., 2022)
Coconut residue (*Cocos nucifera* L.)	The extraction uses acid hydrolysis and enzymatic treatment. Oligosaccharides are used for the prebiotic extraction of coconut residue. The presence of these compounds was confirmed through Thin-Layer Chromatography analysis.	(Plangklang et al., 2023)

5. Conclusion and suggestions

5.1 Conclusion

This study successfully optimized the extraction of oligosaccharides from yam bean (*Pachyrhizus erosus*) roots using the Plackett-Burman Design (PBD). The statistical analysis revealed that ethanol concentration, pH, and sample size were the most influential variables affecting extraction efficiency. The optimal conditions identified were: 2 mm sample diameter, 60 °C extraction temperature, 99.93 min extraction duration, 50% (v/v) ethanol concentration, and pH 7.0, which yielded a maximum oligosaccharide content of 36% wt. An additional experiment was conducted under these optimal conditions to validate the predictive model. The experimentally obtained yield (34.96% wt) closely matched the predicted value (35.58% wt), with a relative error of less than 2%. This confirms the reliability and practical applicability of the PBD model for identifying effective conditions to enhance oligosaccharide extraction. The findings contribute to the design of statistically robust, scalable, and efficient extraction protocols for use in food and bioactive compound applications.

5.2 Suggestions for future research

5.2.1 Scale-up and industrial application

Further research should focus on scaling up the optimized extraction process to pilot or industrial scale, evaluating technical feasibility, cost-effectiveness, and process efficiency.

5.2.2 Structural and functional characterization

Advanced analytical techniques (e.g., HPLC, NMR, FTIR, MALDI-TOF) should be used to determine the extracted oligosaccharides' chemical structure and functional groups and assess their biological activity.

5.2.3 Stability and shelf-life assessment

The stability of oligosaccharide powders should be examined under varying temperature, humidity, and light exposure to evaluate shelf-life for commercial use in powdered or encapsulated forms.

5.2.4 Synergistic effects with probiotics

Studies should explore *in vitro* and *in vivo* interactions between the extracted oligosaccharides and probiotic strains (e.g., *Lactobacillus*, *Bifidobacterium*) to assess potential synbiotic effects.

5.2.5 Alternative extraction technologies

Comparative research involving ultrasound-assisted, microwave-assisted, or enzymatic hydrolysis may offer enhanced yield, reduced energy use, and improved selectivity.

6. References

Bhanja, A., Paikra, SK., Sutar, PP., & Mishra, M. (2023). Characterization and identification of inulin from Pachyrhizus erosus and evaluation of its antioxidant and in-vitro prebiotic efficacy. Journal of Food Science and Technology, 60(1), 328–339.

Boateng, ID., & Yang, XM. (2021). Process optimization of intermediate-wave infrared drying: Screening by Plackett–Burman; comparison of Box-Behnken and central composite design and evaluation: A case study. Industrial Crops and Products, 162, 113287.

Du, Y., Huang, P., Jin, W., Li, C., Yang, J., Wan, H., & He, Y. (2021). Optimization of extraction or purification process of multiple components from natural products: Entropy weight method combined with Plackett–Burman design and central composite design. Molecules, 26(18), 5572.

Elango, D., Rajendran, K., Van der Laan, L., Sebastiar, S., Raigne, J., Thaiparambil, N.A., ... & Singh, A.K. (2022). Raffinose family oligosaccharides: Friend or foe for human and plant health? Frontiers in Plant Science, 13, 829118.

Fan, L., Fan, W., Mei, Y., Liu, L., Li, L., Wang, Z., & Yang, L. (2022). Mechanochemical assisted extraction as a green approach in preparation of bioactive components extraction from natural products – A review. Trends in Food Science & Technology, 129, 98–110.

Huang, H., & Huang, G. (2020). Extraction, separation, modification, structural characterization, and antioxidant activity of plant polysaccharides. Chemical Biology & Drug Design, 96(5), 1209–1222.

Jaffur, BN., Kumar, G., & Khadoo-Jeetah, P. (2024). Enhancing deep eutectic solvent systems for efficient fermentable sugar recovery from lignocellulosic fiber. International Journal of Biological Macromolecules, 269, 131888.

Jain, A., Jain, R. & Jain, S. (2020). Quantitative Analysis of Reducing Sugars by 3, 5-Dinitrosalicylic Acid (DNSA Method). In: Basic Techniques in Biochemistry, Microbiology and Molecular Biology (pp. 181–183). Springer Protocols Handbooks. Humana, New York, NY.

Lu, Y. (2023). Effect of acid-assisted hydrolysis on oligosaccharide release from tuber polysaccharides. Carbohydrate Polymers, 315, 120097.

McKay, S., Teitsma-Jansen, A., Floris, E., Dekker, T., Smids, B., Khurshid, R., Calame, W., Kardinaal, A., Lutter, R., & Albers, R. (2022). Effects of dietary supplementation with carrot-derived rhamnogalacturonan-I (cRG-I) on accelerated protective immune responses and quality of life in healthy volunteers challenged with rhinovirus in a randomized trial. Nutrients, 14(20), 4258.

Plangklang T., Khuwijitjaru P., Klinchongkon K., Adachi S. (2023). Subcritical fluid process for producing mannooligosaccharide-rich carbohydrates from coconut meal and their in vitro fermentation. Food and Bioprocess Technology, 16, 1048–1060.

Poka, R., Chuenklin, S., & Tanwar, B. (2023). Influence of extraction temperature on the recovery of soluble carbohydrates from root vegetables: A kinetic approach. Journal of Applied Food Chemistry, 11(2), 101–110.

Sivamaruthi, BS., Alagarsamy, K., Thangaleela, S., Bharathi, M., Kesika, P., & Chaiyasut, C. (2023). Composition, microbiota, mechanisms, and anti-obesity properties of rice bran. Foods, 12(6), 1300.

Wandee, W., Sae-Lee, N., & Prinyawiwatkul, W. (2021). Acid-assisted release of functional oligosaccharides from plant biomass: Mechanisms and applications. Food Hydrocolloids, 113, 106505.

Wichienchot, S., Thammarutwasik, P., Jongjareonrak, A., Chansuwan, W., Hmadhlu, P., Hongpattarakere, T., Itharat, A., & Ooraikul, B. (2011). Extraction and analysis of prebiotics from selected plants from southern Thailand. Songklanakarin Journal of Science and Technology, 33(5), 517–523.