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Optimizing Fructooligosaccharides Production from Beneng Taro (*Xanthosoma undipes* K.Koch) using Enzymatic Hydrolysis

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ABSTRACT

Fructooligosaccharides (FOS) are a prominent class of prebiotics that confer significant health benefits. Utilizing local resources like Beneng taro (*Xanthosoma undipes* K.Koch) for FOS production offers a sustainable strategy to enhance food security and add economic value to underutilized regional crops. This study investigated the potential of Beneng taro as a novel substrate for FOS production utilizing a crude inulinase preparation from *Aspergillus niger*, based on the hypothesis that optimizing enzymatic conditions can maximize FOS yield and polymerization quality. To systematically evaluate multi-factor interactions with minimal experimental runs, a D-Optimal Response Surface Methodology (RSM) was justified and employed. The experimental setup incorporated three independent variables (crude inulinase dosage, hydrolysis temperature, and hydrolysis time) with all trials performed in triplicate to ensure statistical robustness. The statistical model successfully predicted the optimal hydrolysis conditions, achieving 0.733 as desirability value. Verification experiments performed under these optimized conditions validated the model's predictive accuracy, yielding a high-quality product with the target total sugar, reducing sugar, and degree of polymerization. These findings demonstrate the viability of Beneng taro as a sustainable substrate for optimized prebiotic production, bridging local agricultural potential with functional food development.

INTRODUCTION

Fructo-oligosaccharides (FOS) are low-molecular-weight β-D-fructans characterized by fructosyl units linked via β-(2,1) glycosidic bonds. Beyond their well-established prebiotic potential in selectively stimulating beneficial intestinal microbiota like *Bifidobacterium* spp. and *Lactobacillus* spp., recent global clinical studies have highlighted their broader therapeutic impacts. These include enhancing mineral bioavailability (particularly calcium and magnesium), mitigating metabolic syndromes through glucose regulation, reducing serum lipid levels, and exerting systematic immunomodulatory effects that reinforce the mucosal barrier. Consequently, the global demand for high-purity FOS has surged within the functional food and pharmaceutical sectors[1].

However, a critical gap exists between this expanding global demand and the domestic market dynamics in developing countries like Indonesia, where FOS application remains heavily restricted. This limitation is primarily driven by prohibitive production costs and a strict reliance on imported

specialty ingredients, which creates a significant vulnerability in the national functional food supply chain. From a methodological standpoint, industrial-scale FOS production is predominantly achieved through enzymatic synthesis using sucrose as a substrate, catalyzed by fructosyltransferases. However, a critical appraisal of this approach reveals inherent efficiency bottlenecks: the synthesis concurrently generates a high concentration of glucose, which acts as a potent competitive inhibitor of the enzyme, leading to low-purity final products[2]. Remediating this requires costly, complex downstream purification steps, such as the incorporation of glucose oxidase or active carbon chromatography[3]. In contrast, the enzymatic hydrolysis of plant-derived inulin offers a superior alternative by yielding high-purity FOS with minimal glucose byproducts. Globally, substrates like chicory and Jerusalem artichoke dominate this sector; however, these crops are geo climatically unsuited for tropical cultivation, leaving a profound research gap in identifying viable, high-yield tropical alternatives within the Indonesian agricultural context[4].

Beneng taro (*Xanthosoma undipes* K.Koch), an underutilized local crop in Indonesia, presents a highly promising yet unexplored tropical substrate for inulin extraction. It contains high levels of carbohydrates (81.81 %) and starch (56.29 %), with a reported inulin content of 1.72 mg per gram of fresh weight [5] [6]. Extraction using 30 % ethanol yields up to 12 % inulin, demonstrating its feasibility as a sustainable raw material for FOS production [7]. Enzymatic hydrolysis of inulin using inulinase which produced by *Aspergillus niger*, *Aspergillus flavus, Kluyveromyces marxianus, and Saccharomyces cerevisiae* offers a precise and selective method to generate high-purity FOS [8][9]. Enzymatic hydrolysis of this inulin using fungal endo-inulinase from *Aspergillus niger* offers a addition significantly increased LAB viability (8.46 ± 0.02 log CFU/mL) compared to control (7.86 ± 0.05 log CFU/mL) in yogurt[10].

Studies on inulin hydrolysis from dahlia tubers have reported FOS yields ranging from 22.45 % to 29.15 %, achieved after 24 h of hydrolysis at 45 °C using crude inulinase at concentrations of 1, 2, and 3 U g^{-1}[11]. Optimal FOS production depends critically on the coordination of enzyme dosage, pH, temperature, and reaction time, as inulinase activity is highly sensitive to these parameters [12]. Notably, current research on FOS production in Indonesia is restricted to inulin extracted from dahlia tubers, with insufficient optimization of key process variables. This presents a critical knowledge gap and an opportunity for innovation.

To bridge these domestic and methodological gaps, this study systematically optimizes the enzymatic hydrolysis parameters of Beneng taro inulin using a D-Optimal Response Surface Methodology (RSM) framework. The primary objective is to map the interactive effects of crude inulinase dosage, hydrolysis temperature, and reaction time. This objective is directly linked to the hypothesis that precise coordination of these variables will suppress over-hydrolysis into

monosaccharides, maximize the structural yield of high-purity FOS (DP 3–5), and establish Beneng taro as a statistically robust, economically viable local substrate for domestic prebiotic production.

MATERIALS AND METHODS

Materials

Materials used for inulin extraction were Beneng taro tubers from Talaga Warna village, ethanol p.a (Smartlab), water, and salt. Materials for the production of crude inulinase enzyme were the stock culture of Aspergillus niger from the Microbiology and Food Safety Laboratory of Food Technology, Sultan Ageng Tirtayasa University, Potato Dextrose Agar (Liofilchem), MgSO4 (Merck), (NH4)2SO4 (Merck), physiological saline solution (NaNO3, KCl, K2SO4), and micronutrients (ZnSO4, H3BO3, Fe2(SO4)2, CoCl2, CuSO4, ammonium molybdate, and EDTA). Materials for FOS production were crude inulinase enzyme, and inulin. Materials used for analysis were H2SO4 (Mallinckrodt), K2SO4 (Merck), distilled water, NaOH (Merck), HCl (Merck), CH3COONa (Merck), glacial CH3COOH (Merck), 3.5 dinitrosalicylic acid (Sigma Aldrich), Na-K-tartarate (Merck), standard fructose (Merck), phenol (Merck).

Methods

Preparation of Inulin from Beneng Taro [13]

Beneng taro tubers were peeled, washed, and diced into 3 cm cubes. A 1 kg sample was soaked in 2 L of 10% (w/v) NaCl solution for 2 hours, rinsed with distilled water, and blended with distilled water at a 1:2 (w/v) ratio. The slurry's pH was adjusted to 6.5 using 0.1M NaOH or HCl, and heated at 85 ^{0}C for 30 minutes under continuous stirring. After cooling, the mixture was filtered through a double-layered cheesecloth. To 1,5 L of the collected filtrate, 30% (v/v) ethanol was added at a 1:0.4 volumetric ratio, and the mixture was stored at -10 ^{0}C for 18 hours. The frozen filtrate was thawed at room temperature for 2 hours, then centrifuged at 3,000 rpm for 15 minutes. The resulting precipitate was collected, dried in a cabinet dryer at 50 ^{0}C until constant weight, and passed through an 80 mesh sieve.

Preparation of Crude Inulinase Enzyme [11]

The production process began with the cultivation of *Aspergillus niger* isolates in a tilted agar medium containing Potato Dextrose Agar (PDA). One spore of the culture was suspended in 10 ml of 0.9% NaCl. The spore suspension was then inoculated in an erlenmeyer containing sterile inulin fermentation media 2% (v/v). Subsequently incubated in an incubator for 84 hours at 37℃. After 84 hours, it was centrifuged for 10 minutes at 1000 rpm and the crude inulinase enzyme was obtained.

FOS Preparation

The 5% (w/v) inulin substrate pH 6 was put into a jar and crude inulinase enzyme was added according to the dosage. After the enzyme was added, put the jar into an incubator for hydrolysis process in a predefined dosage, temperature, and time. After the hydrolysis was complete, the product was heated for 15 minutes to inactivate the crude inulinase enzyme. The amount of enzyme added is based on the following calculation:

$$\text{Amount of enzyme (ml)} = \frac{\text{Enzyme dosage}\left(\frac{\text{U}}{\text{g}}\right) \times \text{amount of substrate (g)}}{\text{enzyme activity } \left(\frac{\text{U}}{\text{ml}}\right)}$$

Experimental design

This study optimized the production of fructo-oligosaccharides from beneng taro inulin by enzymatic hydrolysis. Data analysis used Design Expert 13.0.5 with Response Surface Method - D'optimal (RSM). The experiment consisted of three variables which were enzyme dosage, temperature, and hydrolysis time. Eighteen (18) experimental units were obtained, including all data on the lower, middle, and upper limits of variables, units, research levels ($p<0.05$), lack of points using a value of 5 and a replicate value of 3.

Response design

Inulin Yield [14]

Crude Inulinase Activity Test [15]

Reducing sugar content [16]

Total sugar content [17]

Degree of polymerization [30]

Optimization

This study optimized the production of fructo-oligosaccharides (FOS) from Beneng taro inulin via enzymatic hydrolysis. Experimental design and statistical analysis were performed using Design-Expert software version 13.0.5, employing Response Surface Methodology (RSM) with a D-Optimal design framework. The D-Optimal criterion was justified to maximize the precision of parameter estimates within a highly constrained experimental space while minimizing the total number of runs.

The optimization systematically evaluated three independent variables: crude inulinase dosage (1–3 U/g), hydrolysis temperature (45–60 °C), and reaction time (6–60 hours). The ranges for these independent variables were selected based on preliminary kinetic screening, targeting the

optimal stability window of *Aspergillus niger* endo-inulinase to prevent early thermal denaturation while ensuring complete substrate breakdown.

The design generated 18 distinct experimental runs, which strategically incorporated the lower, middle, and upper limits of each factor. To ensure high statistical robustness and reliability, the model configuration specified error rate of 0.05, 5 lack-of-fit check points to evaluate model curvature, and 3 true replicates at the design center to estimate pure experimental error. Commercial FOS (purity 95%, Sigma-Aldrich) was utilized as the positive validation control alongside a blank substrate control (taro inulin without enzyme) to account for any baseline non-enzymatic thermal degradation.

Verification

The verification stage is carried out after the optimum formula results are obtained, which consists of making the best formula obtained from the optimization results of Design Expert 13.0.5. The verification stage aims to see the suitability of the predicted response value produced by the program with the actual value obtained. Verification is carried out with two replicates and the results will be compared with the response value predicted by design expert 13.0.5 [18]. The optimal formula that was verified then analyzed using FTIR with diamond ATR Alpha II brand Bruker to determine the characterization of compound groups from the optimal FOS formula[19]

RESULTS

Table 1. Recapitulation of response data and factors

Formula	Faktor			Respon		
	Enzyme dosage (U/g)	Hydrolysis temperature (℃)	Hydrolysis time (hour)	Total sugar level (%)	Reducing sugar level (%)	DP
1	1	45	33	84,7916	20,578	4,12
2	3	60	6	81,9462	19,8921	4,12
3	3	45	60	49,4025	22,0261	2,24
4	2	60	33	45,6679	21,5307	2,12
5	3	55	33	57,7607	22,1023	2,61
6	2	45	6	77,5004	20,6542	3,75
7	3	55	33	57,8496	22,1785	2,61
8	1	60	60	41,9334	19,2824	2,17
9	2	45	33	70,7426	21,7975	3,25
10	2	55	60	43,7118	20,8829	2,09

Formula	Faktor			Respon		
	Enzyme dosage (U/g)	Hydrolysis temperature (℃)	Hydrolysis time (hour)	Total sugar level (%)	Reducing sugar level (%)	DP
11	1	55	33	69,32	20,4256	3,39
12	1	55	6	89,4153	19,9683	4,48
13	2	60	33	45,8458	21,6069	2,12
14	2	55	60	48,6911	20,8067	2,34
15	3	45	6	81,5906	20,4256	3,99
16	2	55	6	71,8096	20,1969	3,56
17	3	45	33	58,472	22,1785	2,64
18	3	60	60	41,0442	21,7212	1,89

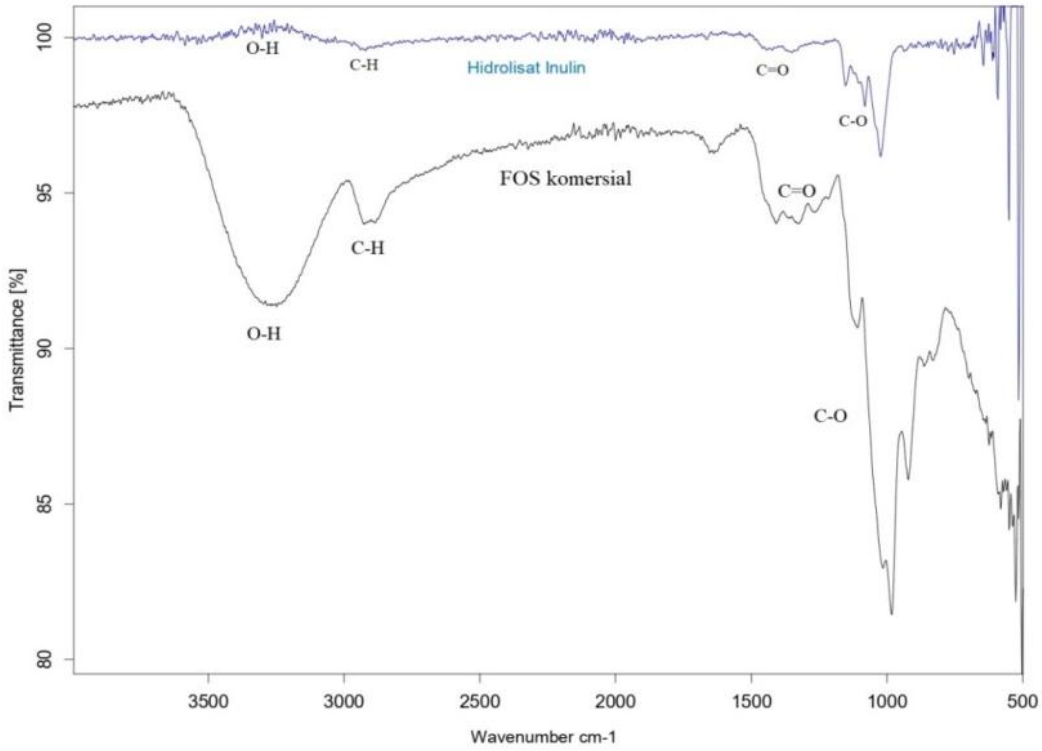

Figure 1. FTIR spectrum of the optimal formula with commercial FOS

DISCUSSION

Inulin yield

The extraction yield of 11.58% represents a highly efficient recovery rate for non-traditional tropical tubers. Rather than being a mere reflection of mass retention, this yield demonstrates that the

thermodynamic conditions applied during the thermal processing 85 ^{o}C and subsequent eco-friendly ethanol precipitation (30% v/v) successfully disrupted the rigid cell wall matrix of Beneng taro without inducing significant thermal depolymerization of the fructan chains. This outcome aligns closely with the maximum predictable recovery threshold (approximately 12%) reported for *Xanthosoma* species under optimized laboratory precipitation [13].

When critically evaluated against alternative local carbohydrates, this recovery is slightly lower than the 16.63% yield reported for Gembili (*Dioscorea esculenta*) tubers [14]. This discrepancy is not an indicator of protocol inefficiency, but is mechanistically driven by the structural and morphological differences of the native starch-inulin matrices. Taro possesses a highly dense mucilage layer composed of water-soluble glucomannans, which increases slurry viscosity and physically traps a portion of the solubilized inulin within the fibrous residue during filtration[10]. Conversely, Gembili tubers exhibit a more porous cellular structure with lower mucilage interference, facilitating unhindered aqueous mass transfer[20]. From an industrial feasibility standpoint, achieving an 11.58% yield using a low ethanol concentration (30%) underscores the commercial viability of Beneng taro as an alternative to global inulin sources like chicory (*Cichorium intybus*), which requires complex, energy-intensive frost-induction parameters to crystallize long-chain fructans. Economically, Beneng taro is a climate-resilient, high-yield per hectare underutilized crop in tropical regions. Utilizing it shifts the supply chain from expensive imported prebiotics to a localized circular bioeconomy framework.

Crude inulinase activity

The crude inulinase activity of 0.7765 U mL^{-1} achieved after 84 hours of incubation underscores the exceptional efficiency of Beneng taro inulin as an inductive carbon source. Rather than serving as a basic substrate, the unique structural configuration of Beneng taro fructans acts as a potent molecular inducer for the expression of the *InuA* and *InuB* genes in *Aspergillus niger*, which regulate endo- and exo-inulinase secretion [11] [22]. When critically evaluated against global benchmarks, this activity significantly outperforms bacterial and alternative fungal systems, such as *Acremonium* sp. CBS-3 (0.1296 U mL^{-1}) [23] and *Lactobacillus plantarum* B1765 (0.047 U mL^{-1}) [24]. This disparity is mechanistically rooted in the superior secretome capacity of *A. niger*, which bypasses the complex intracellular transport bottlenecks typically observed in bacterial hosts, allowing direct, unhindered enzyme secretion into the extracellular matrix[25].

A critical analysis of the fermentation kinetics reveals that the initial, unadjusted medium pH of 3.36 represents a major physiological bottleneck. At this highly acidic threshold, which falls far below the typical fungal optimal window of 4.5 to 7.0 [12] [27], the excess accumulation of hydronium ions induces localized electrostatic repulsion at the enzyme's binding surface. Mechanistically, this suboptimal pH alters the ionization state of crucial catalytic amino acid residues (such as glutamate

and aspartate) at the active site, disrupting the precise formation of the enzyme-substrate complex through hydrogen bonding and destabilizing the transition state during catalysis growth [28]. This explains why the recorded activity remained submaximal despite the extended 84-hour incubation, confirming that active pH-stat maintenance is required to prevent premature catalytic inactivation. Furthermore, the deployment of a 2% (w/v) substrate concentration balances the kinetic tradeoffs defined by Michaelis-Menten dynamics. At this concentration, the availability of inulin chains satisfies the saturation requirements of the active sites without triggering the high-viscosity mass transfer limitations common to complex fructan glues [26]. While industrial agricultural byproducts like beet-sugar molasses can yield higher crude activities 383.73 U mL^{-1} due to their trace mineral matrices [29], Beneng taro inulin provides a chemically clean inducer that minimizes the co-synthesis of non-targeted invertases. This high-purity profile is vital for industrial downstream processing, as it suppresses the over-hydrolysis of FOS into terminal monosaccharides.

From an industrial and economic standpoint, utilizing Beneng taro as a domestic fermentation substrate provides a dual-benefit strategy for the global prebiotic sector. Conventionally, commercial inulinase production relies on pure, imported chicory inulin, which inflates manufacturing costs. Transitioning to an underutilized, climate-resilient tropical tuber like Beneng taro significantly reduces raw material procurement expenses while mitigating the carbon footprint associated with long-distance ingredient shipping. Chronologically, because inulinase functions as a primary metabolite tightly coupled with the logarithmic growth phase [25], the 84-hour window captures the enzyme during active biosynthesis. However, the multi-day optimization trends observed in other fungal strains like *Rhizopus oryzae* [28] and *Penicillium* sp. [27] suggest that extending the fermentation toward 120 hours would allow the system to exploit the full cell-mass capacity before nutrient depletion triggers proteolytic degradation. This makes Beneng taro a highly feasible, low-cost, and environmentally sustainable vehicle for green, localized enzyme manufacturing.

Total sugar

The quadratic response surface model established for total sugar content exhibits exceptional statistical adequacy and predictive power. Rather than merely presenting a functional mathematical fit, the highly significant ANOVA regression ($p < 0.0001$) coupled with a non-significant lack-of-fit ($p = 0.0956$) indicates that the experimental space accurately captures the underlying biocatalytic dynamics without major confounding variables. The high coefficient of determination ($R^2 = 0.9726$) and an adequate precision value well above the critical threshold of 4 confirm that this empirical model can be reliably used to navigate the multi-factor optimization space. The final predictive regression equation in terms of actual factors is designated as follows:

$$\text{Total sugar} = 58{,}70 - 6{,}69A - 9{,}41B - 14{,}45C + 7{,}04AB - 2{,}49AC - 3{,}34BC + 8{,}45A^2 - 3{,}20B^2 + 5{,}19C^2$$

Where :

A = Enzyme dosage (U/g)

B = Hydrolysis temperature (℃)

C = Hydrolysis time (Hour)

A critical, holistic interpretation of the linear coefficients (A, B, C) reveals a paradox: independently, increasing each variable exerts a strong negative effect on the final total sugar content. Mechanistically, this downward trend does not signify a failure of hydrolysis, but rather indicates a shift toward over-saccharification. Extended reaction times (C = -14.45) and elevated temperatures (B = -9.41) promote the exhaustive cleavage of both internal β-(2,1) and terminal glycosidic bonds. This drives the dynamic conversion of high-molecular-weight inulin polymers into highly volatile or reactive monomeric components that are prone to non-enzymatic degradation or thermal caramelization during prolonged processing[30].

However, the significant positive interaction coefficient for AB (+7.04) counteracts these individual antagonistic effects, illustrating a profound kinetic synergy between enzyme dosage and temperature. From a biochemical perspective, higher thermal energy reduces the viscosity of the Beneng taro inulin slurry by decreasing the structural rigidity of the native polysaccharide matrix. This matrix relaxation increases the mass transfer coefficient, allowing the crude endo-inulinase to diffuse rapidly and access hidden sterically hindered active sites [31]. Consequently, when higher enzyme dosages (A) are matched with synchronized temperature elevations (B), the rate of targeted FOS generation surpasses the rate of thermal denaturation, maximizing product recovery within a shorter operational window.

The prominent negative linear impact of hydrolysis duration (C) represents a major operational boundary. The sharp decline in total sugar concentration observed over extended periods (from 6 to 60 hours) mirrors the classic kinetics of substrate depletion and product inhibition. As the *Aspergillus niger* endo-inulinase progressively breaks down the inulin backbone into shorter-chain prebiotics, the local concentration of terminal fructose and glucose units rises. These accumulation products can act as competitive or non-competitive inhibitors that block the active site pockets of the enzyme. Furthermore, prolonged exposure to even mild thermal environments prompts structural unfolding of the fungal enzyme, which triggers irreversible catalytic inactivation. This behavior aligns with global bioprocessing studies on non-structural fructans, where prolonged incubation shifts the product equilibrium away from desired oligosaccharides toward unrecoverable monosaccharide degradation products [30] [31]. This demonstrates that FOS production from local tropical substrates must be tightly regulated through optimized, short-duration stop-points to preserve structural integrity and prevent total carbohydrate loss.

Reducing sugar

The empirical quadratic model generated for the reducing sugar response demonstrates high statistical rigor and predictability. The highly significant regression variance ($p < 0.0001$) combined with a non-significant lack-of-fit ($p = 0.1046$) validates that the experimental framework is exceptionally robust. The close agreement between the predicted R^2 (0.9706) and adjusted R^2 (0.9931), along with a high adequate precision ratio (55.1271), indicates that the signal-to-noise ratio is well-optimized for steering industrial scale-up. The resulting predictive regression equation in terms of actual experimental factors is formulated as follows:

Reducing sugar level=21,58+0,7186A-0,1106B+0,2374C-0,0920AB+0,6323AC+0,0774BC-0,3571A^2+0,06997B^2-0,9851C^2

A critical examination of the linear coefficients indicates that both crude inulinase dosage (A=+0.7186) and hydrolysis time (C=+0.2374) act as major driving forces for the accumulation of reducing sugars. This accumulation is chemically directly linked to the progressive depolymerization of Beneng taro inulin, where endo-inulinase cleaves internal glycosidic bonds to yield shorter-chain fructo-oligosaccharides (FOS) and free terminal reducing ends [24].

However, the prominent negative coefficient for temperature (B=-0.1106) reveals a major thermodynamic constraint. Elevated thermal environments induce irreversible structural changes within the *Aspergillus niger* endo-inulinase secretome, forcing the catalytic protein matrix away from its native, highly active fold [32][33]. Although some fungal inulinases can technically maintain structural stability at 60 °C, their catalytic efficiency drops due to a disruption of the hydrogen-bonding network required for stable substrate docking at the active pocket. This localized denaturation explains why the lowest reducing sugar yield was observed at the maximum temperature boundary 60 °C, confirming that higher thermal energy suppresses enzymatic performance within this specific bioprocess window.

The model also highlights a strong positive interactive synergistic effect between enzyme dosage and hydrolysis duration (AC=+0.6323). Mechanistically, a higher initial enzyme loading expands the total surface area of available active sites, creating a high substrate-binding capacity that accelerates initial reaction rates [34]. As the reaction time extends toward the mid-point (33 hours), these active sites continuously break down the high-molecular-weight inulin fraction, maximizing the release of reducing sugars without causing early exhaustion. This kinetics pattern matches global profiles of industrial enzymatic hydrolysis, where long-term yield optimization depends strictly on balancing enzyme density with reaction time to maintain high catalytic turnover [11][24]. However, the negative quadratic terms (A^2=-0.3571 and C^2=-0.9851) reveal a strict boundary threshold for optimization. While initial increases in enzyme dosage and reaction duration boost reducing sugar accumulation, exceeding these optimal limits triggers a kinetic plateau followed by a decline.

From a bioprocess engineering perspective, excessive enzyme concentration creates sterically hindered environments where adjacent protein chains block or crowd out active sites, physically preventing the taro inulin substrate from entering the binding pocket. Similarly, extended reaction times beyond the optimum window drive competitive product inhibition. As the concentration of short-chain FOS and monomeric byproducts rises, these terminal units remain weakly bound within the active site, blocking fresh inulin polymers from entering [34]. This critical interpretation shows that FOS production cannot be optimized by simply maximizing individual process parameters; instead, it demands a coordinated approach to balance enzyme density against product-driven catalytic inhibition.

Degree of polymerization

The degree of polymerization (DP) serves as a critical structural metric that directly indicates the molecular weight distribution and functional identity of the hydrolyzed fructans. Mathematically determined by the ratio of total sugars to reducing sugars, the DP values obtained in this study ranged from 1.89 to 4.48. From a functional food classification standpoint, this specific range conclusively proves that the optimization framework successfully drove the conversion of high-molecular-weight Beneng taro inulin (DP>9) into high-purity fructo-oligosaccharides (FOS), which typically require a DP threshold between 2 and 9 to exert optimal prebiotic properties.

To precisely map the structural breakdown of these fructan chains, a quadratic empirical model was established. The ANOVA regression for the DP response was highly significant ($p<0.0001$) with a non-significant lack-of-fit ($p=0.0980$). Combined with a robust coefficient of determination ($R^2=0.975$), a tight alignment between predicted and adjusted R^2 values, and an adequate precision ratio of 17.2458, the model demonstrates high reliability for targeted structural engineering. The final predictive equation in terms of actual process factors is expressed as follows:

$$DP=2{,}71–0{,}4129A–0{,}4279B–0{,}7460C+0{,}3556AB–0{,}1815AC–0{,}1815BC+0{,}4648A^2–0{,}1543B^2+0{,}3946C^2$$

A critical interpretation of the linear coefficients reveals that all three process variables—enzyme dosage (A=-0.4129), temperature (B=-0.4279), and hydrolysis duration (C=-0.7460)—exert a powerful negative impact on the DP value. Among these, reaction time (C) acts as the dominant kinetic driver behind chain shortening. Mechanistically, as the endo-inulinase from *Aspergillus niger* continuously targets internal β-(2,1) glycosidic bonds, the long-chain prebiotic backbone undergoes progressive depolymerization. This cleavage pattern exponentially increases the concentration of free terminal reducing ends relative to the total carbohydrate mass, causing the downward shift in DP toward short-chain oligomers. At the upper boundary limits (3U/g, 60^{o}C, and 60 hours), the system reached a suboptimal DP floor of 1.89. This structural collapse signifies the transition from target FOS

production to over-saccharification, where the oligosaccharide products are further degraded into free fructose monomers (DP=1). This rapid structural decline is driven by the elevated kinetic energy at 60^{o}C, which accelerates molecular collisions and molecular motion. This thermal acceleration forces the substrate-enzyme complex through a high-frequency turnover rate, overwhelming the structural control of the process. Furthermore, the significant positive quadratic coefficients for enzyme dosage (A^2=+0.4648) and time (C^2=+0.394) establish a critical parabolic threshold for industrial bioprocessing. While combining a low enzyme loading (1U/g) with a short operational window (6 hours) preserves a higher structural integrity (DP=4.48), extending these variables triggers an intense, non-linear rate of structural degradation.

This kinetic behavior mirrors global prebiotic optimization profiles, where initial processing hours show rapid decreases in chain length (e.g., transitions from unhydrolyzed inulin down to intermediate oligomers) before settling into a plateau governed by product accumulation [24]. The negative interaction terms (AC=-0.1815 and BC=-0.1815) further confirm that prolonged exposure, when coupled with either high enzyme concentrations or elevated temperatures, creates an aggressive catalytic environment. This synergy rapidly bypasses the desired FOS spectrum, highlighting that precise time-gated stop-points are required during the processing of tropical *Xanthosoma* substrates to arrest hydrolysis before valuable prebiotics are lost to monomeric saccharification.

Optimization

The optimization process is carried out to obtain the optimal formula based on the results of the analysis of the responses that have been carried out. In the optimization process, the weighting of the importance of each response refers to the FOS product analysis data so that the optimal formula produced has the desired criteria.

The enzyme dosage factor was targeted to minimize because it was desired to use the least enzyme dosage possible to obtain optimal results and minimize production costs. The hydrolysis temperature factor was set to be in range because the use of 45-60°C is the optimal temperature for inulinase enzyme. The hydrolysis time factor is chosen as minimized because it is desired to use the minimum hydrolysis time as possible to shorten the hydrolysis period and get optimal results. The total sugar content response was determined to be in range based on the total sugar content of different commercial FOS. Reducing sugar content is targeted maximized. The degree of polymerization was determined to be in range to get a formula close to DP FOS. FOS optimization can be seen in Table 3. The optimal formula of the FOS product resulting from the optimization stage by Design Expert 13.0.5.0 was composed of a 1.229 U/g dosage of crude inulinase enzyme, 45℃

hydrolysis temperature, and 14.332 hours hydrolysis time. In this study, the optimal formula has a desirability value of 0.733, indicating that the optimal formula meets the optimization target of 73.3%.

Verification

The formula verification stage systematically validated the empirical accuracy of the generated response surface model. Rather than serving as a basic post-optimization check, comparing the experimental datasets with the Design-Expert 13.0.5.0 software output establishes the predictive limits of the bioprocess. Experimental validation under the optimized parameters (1.23U/g enzyme dosage, 45^{o}C temperature, and 14.33 hours duration) yielded a total sugar content of 85.91%, reducing sugar level of 20.58%, and a structural DP of 4.17. All observed values sat strictly within the calculated 95% Confidence Interval (CI) and 95% Prediction Interval (PI) bounds. Because the PI incorporates both pure experimental error and the variance between individual future observations under identical operating parameters, this tight alignment mathematically confirms that the model has high reproducibility and is fully capable of navigating scale-up bioprocess dynamics without systemic bias.

When compared to the industrial standard (commercial FOS), the verified taro hydrolysate exhibits unique structural profile characteristics. While the commercial FOS demonstrated a lower average DP (2.43) and higher reducing sugars (36.67%), the Beneng taro hydrolysate maintained a higher intermediate prebiotic length DP (4.17) and a significantly lower sugar fraction. From a nutritional standpoint, this structural difference is highly advantageous. A higher intermediate DP within the FOS spectrum indicates a higher concentration of oligosaccharides over free mono- and disaccharides, which ensures sustained, slow-rate fermentation kinetics in the distal colon rather than premature, gas-inducing gasification in the proximal gut.

To verify these structural profiles, Fourier-Transform Infrared (FTIR) spectroscopy was performed to evaluate the functional group arrangements and carbohydrate linkages. The critical carbohydrate fingerprint region (1250 to 900 cm^{-1}) shows an overlapping absorption pattern between the optimized hydrolysate and the commercial standard, confirming the successful synthesis of authentic FOS. Specifically, the intense absorption bands captured at 1133.57cm^{-1}, 1034.99cm^{-1}, and 930.26cm^{-1} directly correspond to the stretching vibrations of C-O-C and C-O-H structures, alongside the characteristic C-C glycosidic linkages that form the functional backbone of β-(2,1) fructans [35]. The slight variations in peak intensity between the samples reflect the higher average DP of the taro hydrolysate, as longer oligomeric chains alter the dipole moments within the sugar rings compared to highly hydrolyzed commercial monomers.

Beyond the fingerprint region, the broader spectral shifts reveal how the enzymatic breakdown affects the physical state of the carbohydrate matrix. The broad, intense band at 3270.66cm^{-1}

corresponds to the O-H stretching vibrations of structural hydroxyl groups, which are directly involved in extensive inter- and intramolecular hydrogen bonding networks. The adjacent medium bands between 2926.30cm^{-1} and 2885.55cm^{-1} match the asymmetric stretching of aliphatic C-H bonds within the furanose rings. The weaker absorption intensity observed in the 3500-2000cm^{-1} region for the optimized formula compared to the raw unhydrolyzed matrix points to a major structural shift: as the *Aspergillus niger* endo-inulinase breaks down the long-chain inulin polymers, it alters the water-binding capacity and the spatial orientation of the peripheral hydroxyl groups. This reduces the density of the crystalline hydrogen networks and shifts the product into a more amorphous, highly soluble prebiotic state.

CONCLUSION

This study confirmed the hypothesis that precise bioprocess tuning can arrest enzymatic hydrolysis within the optimal prebiotic window (DP=2-9), maximizing FOS production from Beneng taro inulin. By synchronizing a low enzyme dosage (1.229 U/g), a mild thermal state (45 ℃), and a time-gated stop-point (14.332 hours), the system achieved a high multi-response desirability score of 0.733 while successfully preventing over-saccharification into free fructose. These outcomes validate Beneng taro as a highly feasible, climate-resilient tropical alternative to expensive imported chicory inulin, supporting localized circular bioeconomies.

However, a key limitation of this study is its reliance on small, batch-scale laboratory environments, which do not account for the complex fluid dynamics and mass transfer limitations found in industrial setups. To support commercial scale-up, practical recommendations for future research should focus on deploying continuous immobilized enzyme reactors to enable catalyst recycling and lower operational costs. Additionally, future work should include *in vivo* clinical evaluations to verify the precise gut microbiota fermentation pathways of this localized prebiotic ingredient.

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