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OPTIMIZATION OF PULLULANASE-ASSISTED HYDROLYSIS TO PRODUCE RESISTANT STARCH - ENRICHED INSTANT RICE FROM ST25 CULTIVAR

Anh Nguyen Hoang, Hang Nguyen Vo My, Duc Pham Minh, Phat Nguyen Thuan, Thao Phan Thi Thu, Anh Nguyen Van, Duy Le Nguyen Doan*

Faculty of Food Science Technology, Ho Chi Minh City University of Industry and Trade

*Email: *duylnd@huit.edu.vn*

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ABSTRACT

ST25 rice, a premium Vietnamese cultivar known for its high eating quality, contains primarily carbohydrates (82.4%), with moderate protein (6.74%), and low lipid (0.76%) content. This study optimized the enzymatic hydrolysis of ST25 rice using pullulanase to enhance its resistant starch (RS) content. Key variables-substrate concentration (10-50% w/v), enzyme activity (10-50 U/g), hydrolysis temperature (45-65 °C), time (2-10 h), and pH (3.5-5.5)-were evaluated. Optimal conditions were identified using response surface methodology (RSM) with a Box-Behnken design, yielding a maximum RS content of 9.25% at 39.09% substrate concentration, 18.28 U/g enzyme activity, and 5.94h hydrolysis. The experimental RS value (9.32%) closely matched the model prediction (R^2 = 94.45%). Following hydrolysis, the rice underwent gelatinization, dual-stage enzymatic degradation, microwave treatment, and retort sterilization. The final instant rice product contained 45.9% starch, 5.17% protein, 1.7% lipid, and 44.1% moisture, while meeting food safety standards (no detection of *E. coli*, Salmonella, or spoilage organisms).

Keywords: Pullulanase enzyme, instant rice, optimization, resistant starch.

1. INTRODUCTION

Rice (*Oryza sativa*), a staple food crop for over 50% of the global population, plays a crucial role in ensuring food security, particularly in Asia, which accounts for 95% of world rice production. In Vietnam, rice remains the primary dietary staple for more than 99.4 million people. Recent estimates by the FAO indicate that global rice production in early 2025 reached 532.7 million tons, with a corresponding consumption of 530.7 million tons, a global trade volume of 58.5 million tons (According to "Rice Market Report 2025"). The ST25 rice cultivar, notable for its superior eating quality, comprises primarily carbohydrates (82.4%), alongside proteins (6.74%), lipids (0.76%), and minimal ash and sugars [1]. Amid rising rates of diabetes, obesity, colorectal cancer worldwide in Vietnam, the health implications of excessive rapidly digestible carbohydrate intake have become increasingly evident (According to "Diabetes") [2]. Consequently, there is growing consumer demand for functional foods rich in dietary fiber, resistant starch (RS), with projections indicating significant growth in the global markets for prebiotic and low-glycemic index products [3], [4].

Resistant starch (RS) is a type of carbohydrate that resists hydrolysis by enzymes in the small intestine and thus remains undigested, contributing to blood glucose regulation. RS is classified into five types based on its resistance mechanisms during digestion: RS1, found in whole grains and legumes, is physically protected by a fibrous cell wall; RS2 occurs naturally in high-amylose foods such as raw potatoes, green bananas, and some legumes; RS3 forms when starchy foods like rice or sweet potatoes are cooked and subsequently cooled, promoting amylose retrogradation; RS4 is chemically modified starch produced through industrial processes; and RS5 results from the formation of amylose-lipid complexes during food processing [5]. Notably, resistant starch provides only 2 kcal/g, compared to 4 kcal/g for regular starch, making it an attractive ingredient for low-energy functional foods. In the large intestine, RS is fermented by gut microbiota into short-chain fatty acids (SCFAs), which serve as an energy source for colonocytes and have been linked to numerous health benefits, including improved digestive health, prevention of colorectal cancer, glycemic control, weight management, and cardiovascular protection [6].

In the context of ready-to-eat convenience foods, instant rice has garnered attention; however, challenges remain in improving its nutritional quality, notably RS content [7], [8]. Pullulanase-assisted debranching has been identified as an effective enzymatic strategy to enhance RS3 formation through selective cleavage of α-1,6 linkages in amylopectin, followed by retrogradation to form digestion-resistant structures [9], [10]. Despite advancements in RS enhancement techniques, there is limited research on integrating pullulanase-mediated hydrolysis into an optimized processing workflow to produce prebiotic instant rice using high-quality cultivars such as ST25. Moreover, comprehensive optimization of enzymatic conditions specific to instant rice development, tailored for health-conscious consumers and individuals with metabolic disorders, remains underexplored. The study aims is to optimize pullulanase treatment conditions to produce resistant starch-enriched instant rice, which serves as a precursor for the development of prebiotic-rich instant rice. Key processing parameters-substrate concentration, enzyme activity, hydrolysis temperature, time, and pH-were systematically optimized to maximize RS yield. The goal is to establish a foundation for producing a low-calorie, shelf-stable, RS-enriched instant rice suitable for dietary strategies supporting metabolic health.

2. MATERIALS AND METHODS

2.1. Materials

ST25 rice, harvested during the 2023-2024 Winter-Spring season, was sourced from Soc Trang Province and milled prior to use. Pullulanase enzyme (700 U/mL), derived from Bacillus licheniformis, was provided by Biozym Enzyme Vietnam. The following analytical-grade reagents were used in the experiments: acetic acid, sodium acetate, ethanol (99%), resistant starch (RS) assay kit, maleic acid, and potassium hydroxide.

2.2. Experimental design method

The production process for prebiotic instant rice was adapted from the method described in reference [11], the optimization of cooking temperature and the water-to-rice ratio (KDML105, PT, SH) contributes to the reduction of the glycemic index [12]. The application of extrusion technology facilitates the production of instant low glycemic rice derived from broken rice, foxtail millet, barnyard millet, and quinoa [13], incorporating several modifications that align with the research. ST25 rice, after milling and cleaning, was hydrolyzed in a thermal stabilization tank using pullulanase enzyme. The effects of five

processing parameters-substrate concentration, pH, hydrolysis temperature, enzyme activity, and hydrolysis time-were systematically investigated. The hydrolyzed rice was then rinsed with distilled water and steamed at 100 °C for 20 minutes. The steamed rice underwent two stages of retrogradation. In the first stage, it was cooled at 4 °C for 24 hours. It was then subjected to microwave treatment at 270 W for 50 seconds, followed by a second retrogradation phase at 4 °C for another 24 hours. Finally, the rice was sterilized at 115 °C for 15 minutes and hermetically packaged to ensure microbial stability during storage. The final product is designed for easy rehydration, requiring only boiling water or brief microwave heating, providing a convenient, shelf-stable meal with enhanced nutritional value. Post-processing, the sterilized ST25 rice was evaluated for amylose content, resistant starch levels, and overall product quality, establishing key parameters for the development of prebiotic instant rice.

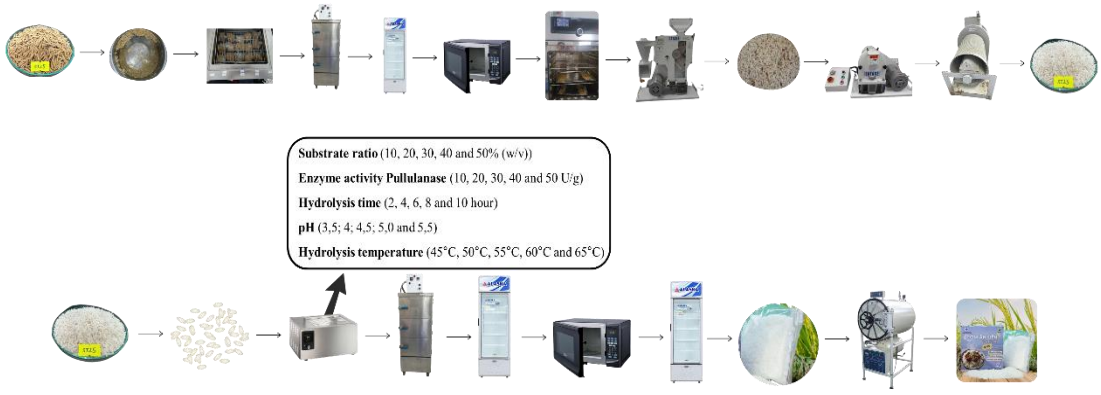

Figure 1. Schematic of prebiotic instant rice production process

2.2.1. Effect of parameters on hydrolysis process by Pullulanase enzyme

The influence of five key factors on the hydrolysis process-substrate concentration, pH, temperature, enzyme activity, and reaction time-was systematically investigated using a single-factor experimental design. Each variable was examined individually while maintaining the others at constant intermediate levels to determine optimal conditions for resistant starch (RS) production. Specifically, pH levels were tested at 3.5, 4.0, 4.5, 5.0, and 5.5; hydrolysis temperatures at 45, 50, 55, 60, and 65 °C; substrate concentrations at 10, 20, 30, 40, and 50% (w/v); enzyme activities at 10, 20, 30, 40, and 50 U/g; and hydrolysis times at 2, 4, 6, 8, and 10 hours. After the hydrolysis process, the Resistant Starch (RS) and Amylose Content (AM) of obtained products were determined.

2.2.2. Optimization of hydrolysis process by Pullulanase enzyme

Three most effected factor were selected to optimize hydrolysis process by Pullulanase enzyme. For this purpose, the experimental design for modeling was conducted using the Box-Behnken model with three parameters: Substrate ratio (X1), Pullulanase enzyme activity (X2), and hydrolysis time (X3) as independent variables, while the % RS content (Ymax) is the dependent response. The proposed polynomial regression equation is formulated as follows:

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

Where: βo - The intercept coefficient.

βi - The linear terms

βii - The quadratic terms

βij - The interaction terms

2.3. Analytical method

2.3.1. Determination of Amylose Content (AM):

Accurately weigh 100 mg of sample into a test tube, add 1 mL of 95% ethanol, 9 mL of 1N NaOH, heat in a water bath for 10 minutes. Cool to room temperature, allow the mixture to stand for 2 hours. Dilute to 100 mL with distilled water. Transfer 5 mL of this solution into a 100 mL volumetric flask containing 50 mL of distilled water. Add 5 mL of 0.09N NaOH, 1 mL of 1N acetic acid, and 2 mL of 0.2% iodine solution. Dilute to volume with distilled water, allow the mixture to stand for 20 minutes, and measure the absorbance at 620 nm. Prepare amylose-amylopectin mixtures at 0%, 10%, 20%, 30%, 35% (v/v), treat identically, and construct a calibration curve of amylose content vs. absorbance [TCVN 5716-2:2017].

2.3.2. Determination of Resistant Starch (RS):

Resistant starch was quantified using the Megazyme Resistant Starch Assay Kit (Megazyme International, Wicklow, Ireland), following AOAC Method 2002.02. Weigh 100 mg of sample and add 4 mL of an enzyme mixture containing pancreatic α-amylase (10 mg/mL) and amyloglucosidase (AMG, 3 U/mL). Incubate in a shaking water bath (200 rpm) at 37°C for 16 hours. After hydrolysis, add 4 mL of absolute ethanol, mix vigorously to inactivate enzymes, and centrifuge at 1500×g for 10 minutes. Wash the pellet twice with 50% ethanol. Dissolve the residue in 2 mL of 2M KOH under vigorous stirring for 20 minutes in an ice bath, then neutralize with 8 mL of 1.2M sodium acetate buffer (pH 3.8). Add 0.1 mL of AMG (3300 U/mL) and incubate at 50°C for 30 minutes. Centrifuge at 1500×g for 10 minutes, collect the supernatant, and react 0.1 mL with 3 mL of GOPOD reagent at 50°C for 20 minutes. Measure absorbance at 510 nm. All samples were analyzed in triplicate [TCVN 13287:2021]. The amount of RS is calculated using the following formula: $RS\ (\%) = \Delta E \times F/W \times 90$

In which:

- ΔE: Absorbance minus blank

- F: is the conversion factor from absorbance to micrograms of the standard sample divided by the absorbance of the GOPOD sample.

- W: is the mass of the sample.

- 90: conversion factor from free D-glucose to anhydro-D-glucose.

2.4. Data analysis

Data were analyzed using analysis of variance (ANOVA). Significant differences between means ($p < 0.05$) were determined using Fisher's Least Significant Difference (LSD) test. Optimization and statistical analyses were performed using Minitab software (version 2022).

3. RESULT AND DISCUSSION

3.1. Effect of buffer pH on resistant starch content

The effect of pH on resistant starch (RS) content was evaluated at five levels: 3.5, 4.0, 4.5, 5.0, and 5.5, under fixed conditions (substrate ratio: 40% w/v; pullulanase activity: 20 U/g; hydrolysis time: 4 h; temperature: 55 °C).

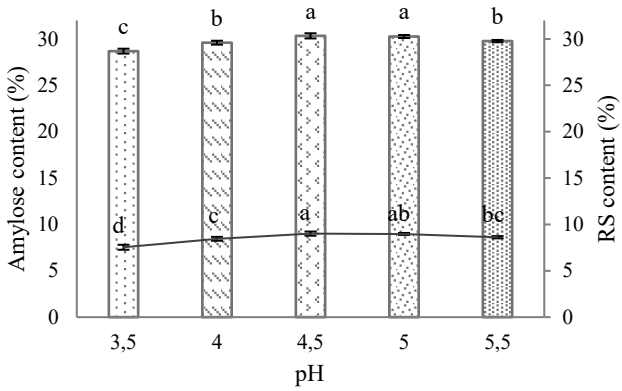

Figure 2. Effect of buffer pH on resistant starch content.
Different digits in the same column indicate significant differences, with significance level α = 0.05%

As shown in Figure 2, RS content increased progressively from 7.54% at pH 3.5 to a maximum of 9.01% at pH 4.5. This enhancement is attributed to the influence of pH on enzyme structure and activity, particularly at the active site. Optimal pH promotes favorable enzyme-substrate interactions, increasing hydrolysis efficiency and RS formation. Beyond the optimal pH, enzyme activity may decline due to denaturation. These findings align with previous studies. For example, Cuihong Dai et al. (2023) [14] reported a maximum RS yield of 20.3% for corn starch at pH 5.0. Additionally, Biozyme data indicate an optimal pH range of 4.0-5.0 for pullulanase activity. In this study, pH 4.5 not only yielded the highest RS content (9.01%) but also elevated amylose levels (30.34%), making it the optimal pH condition selected for subsequent experiments.

3.2. Effect of hydrolysis temperature on resistant starch content

The impact of hydrolysis temperature on amylose and resistant starch (RS) formation was evaluated under the following conditions: substrate ratio 40% (w/v), pullulanase activity 20 U/g, pH 4.5, hydrolysis time of 4 hours. Temperatures tested included 45, 50, 55, 60, 65 °C.

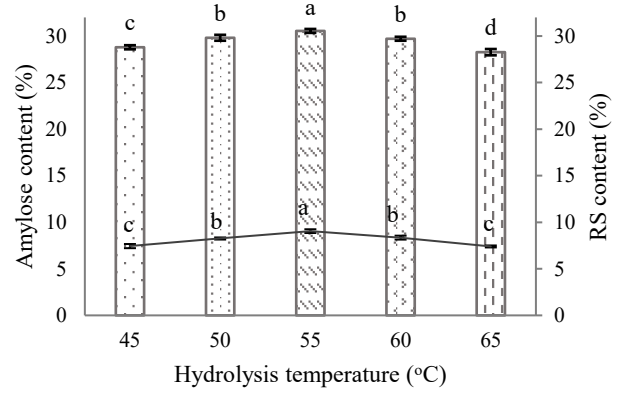

Figure 3. Effect of pullulanase enzyme hydrolysis temperature on RS content
Different digits in the same column indicate significant differences, with significance level α = 0.05%

As shown in Figure 3, RS content increased from 7.44% at 45 °C to a peak of 9.05% at 55 °C, with a corresponding amylose content of 30.55%. Beyond this point, RS levels declined to 8.35% at 60 °C and 7.40% at 65 °C. This trend reflects the thermal sensitivity of enzymes. While moderate heating enhances enzymatic activity by increasing molecular motion and substrate collisions, excessive temperatures can lead to denaturation, reducing catalytic efficiency. The optimal temperature is the point at which enzyme activity and thus product yield is maximized. The current results indicate that 55 °C is the optimal hydrolysis temperature for pullulanase, consistent with Biozyme's reported range (55-65 °C). Previous studies have also demonstrated similar findings: Zhang et al. (2019) reported optimal pullulanase activity at 50 °C for corn starch, while Reddy (2013) observed peak performance at 60 °C for red bean starch. For sweet potato starch, 55 °C has been identified as suitable [15], [16]. Based on these results, 55 °C was selected as the optimal hydrolysis temperature for subsequent experiments.

3.3. Effect of substrate ratio on resistant starch content

To evaluate the impact of substrate concentration on pullulanase activity, experiments were conducted using substrate ratios: 10%, 20%, 30%, 40%, 50% (w/v), with constant conditions: enzyme of 20 U/g, temperature of 55 °C, pH 4.5, hydrolysis time of 4 hours.

The results, shown in Figure 4, revealed significant variations in amylose and resistant starch (RS) content across different substrate concentrations. RS content increased steadily from 7.13% at 10% substrate to a maximum of 9.09% at 40%, corresponding to an amylose content of 31.18%. This enhancement is likely due to sufficient enzyme accessibility at moderate substrate concentrations, enabling efficient cleavage of α-1,6-glycosidic bonds in amylopectin, thereby promoting linear amylose formation and subsequent RS generation.

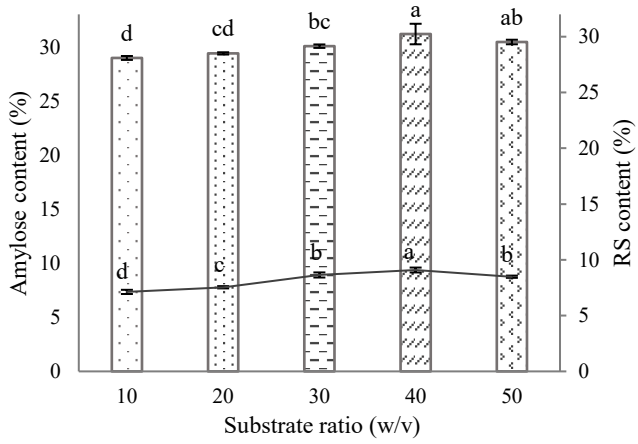

Figure 4. Effect of substrate ratio on resistant starch content
Different digits in the same column indicate significant differences, with significance level α = 0.05%

However, at 50% substrate concentration, RS content decreased to 8.49%, suggesting enzyme saturation or substrate inhibition, which can impede enzyme-substrate interactions and reduce hydrolysis efficiency. These findings are consistent with those of Medina et al. [17], who observed optimal hydrolysis at a 40% (w/v) concentration when using legume-derived starches (lentils, green beans, red beans). Based on these results, a substrate concentration of 40% (w/v) was identified as optimal for maximizing resistant starch formation and was selected for further optimization experiments.

3.4. Effect of pullulanase enzyme activity on resistant starch content

The influence of pullulanase activity on resistant starch (RS) formation was assessed at enzyme levels of 10, 20, 30, 40, and 50 U/g, under constant conditions: 40% (w/v) substrate ratio, 55 °C hydrolysis temperature, pH 4.5, and 6 hours of reaction time.

As shown in Figure 5, both amylose and RS content increased with enzyme activity, reaching a maximum at 20 U/g (AM: 31.60%, RS: 9.13%). Beyond this point, RS levels slightly declined at 30 U/g (RS: 8.86%), 40 U/g (RS: 8.77%), and 50 U/g (RS: 8.49%), though the differences were not statistically significant.

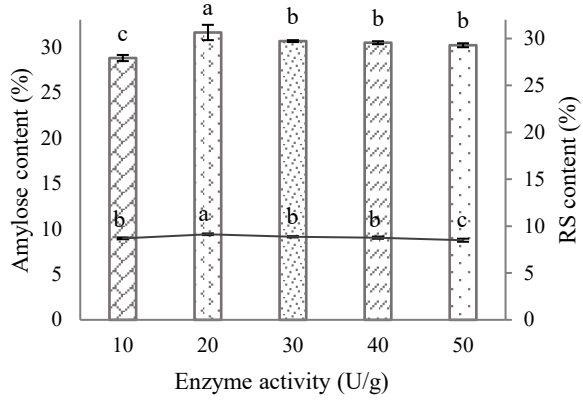

Figure 5. Effect of pullulanase enzyme activity on resistant starch content
Different digits in the same column indicate significant differences, with significance level α = 0.05%

The initial increase in RS content can be attributed to the enzymatic cleavage of α-1,6-glycosidic linkages in amylopectin, yielding linear amylose chains that readily retrograde into double-helical structures resistant to enzymatic digestion. However, at enzyme activities above 20 U/g, the starch matrix likely became saturated with enzyme, resulting in limited additional hydrolysis and negligible RS yield improvement, as suggested by Suriya et al. [10] and Zhang et al. [18] in studies involving red bean and corn starch, respectively. Similar trends have also been reported for sweet potato starch hydrolysis using pullulanase [16]. Given the plateau in RS yield beyond 20 U/g, this enzyme activity level was selected as optimal for further experimentation, balancing performance with cost-effectiveness.

3.5. Effect of pullulanase enzyme hydrolysis time on resistant starch content

To determine the optimal hydrolysis duration for resistant starch (RS) formation, experiments were conducted at time intervals of 2, 4, 6, 8, and 10 hours. Hydrolysis conditions were standardized based on prior optimization: pH 4.5, temperature 55 °C, substrate concentration 40% (w/v), and enzyme activity of 20 U/g.

As shown in Figure 6, both amylose and RS content increased with hydrolysis time, reaching a peak at 6 hours (RS: 9.20%, AM: 30.97%). This increase is attributed to pullulanase-mediated cleavage of α-1,6-glycosidic bonds in amylopectin, facilitating the formation of linear amylose chains that retrograde into resistant structures. These findings align with studies by Mohan Das et al. (2022) on green banana flour [19], as well as Wu et al. (2009) and Gonzalez et al. (2004), who reported optimal RS yields with pullulanase after approximately 5-6 hours of hydrolysis [20], [21].

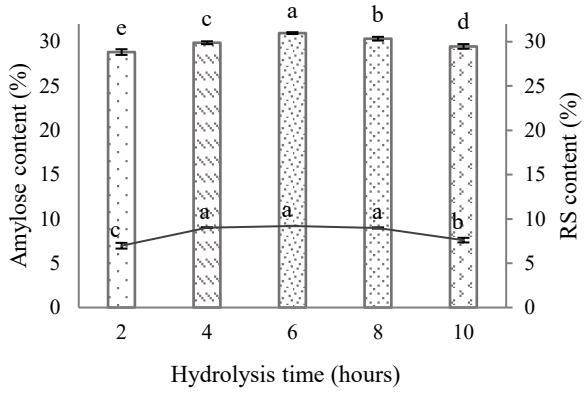

Figure 6. Effect of Pullulanase enzyme hydrolysis time on resistant starch content.
Different digits in the same column indicate significant differences, with significance level α = 0.05%

Beyond 6 hours, RS content declined-dropping to 8.98% at 8 hours and 7.61% at 10 hours-suggesting excessive hydrolysis may degrade short-chain amylose molecules crucial for RS formation. These results underscore the time-dependent nature of enzyme-substrate interactions and emphasize that overextended hydrolysis may compromise RS yield.

Therefore, a hydrolysis time of 6 hours is considered optimal under the specified experimental conditions, offering a balance between enzymatic modification and maximal resistant starch production.

3.6. Optimizing factors affecting the hydrolysis ability of pullulanase enzyme

Single-factor experiments investigating five variables-substrate concentration, pullulanase activity, hydrolysis temperature, hydrolysis time, and buffer pH-revealed that substrate concentration (30-50% w/v), enzyme activity (10-30 U/g), and hydrolysis time (4-8 h) significantly influenced resistant starch (RS) yield. Hydrolysis temperature and pH were optimized at 55 °C and pH 4.5, respectively, aligning with Biozym Enzyme Vietnam's recommended conditions. Therefore, the three most influential parameters-substrate concentration (X_1), enzyme activity (X_2), and hydrolysis time (X_3)-were selected for multivariate optimization. A Box-Behnken design with three center-point replicates was used for response surface modeling and was presented in Table 1.

Table 1. Experimental value and value of the optimization model

Substrate concentration - X1 (%)	Activity enzyme - X2 (U/g)	Hydrolysis time - X3 (hours)	% RS Experimental	%RS Module
30	10	6	8.81	8.79
50	10	6	8.47	8.52
30	30	6	8.63	8.59
50	30	6	8.52	8.53
30	20	4	8.72	8.78
50	20	4	8.45	8.45
30	20	8	8.52	8.53

Substrate concentration - X1 (%)	Activity enzyme - X2 (U/g)	Hydrolysis time - X3 (hours)	% RS Experimental	%RS Module
50	20	8	8.52	8.53
40	10	4	8.83	8.80
40	30	4	9.15	9.15
40	10	8	9.18	9.16
40	30	8	8.58	8.61
40	20	6	9.22	9.24
40	20	6	9.29	9.24
40	20	6	9.31	9.24
30	10	6	8.74	8.79
50	10	6	8.56	8.52
30	30	6	8.57	8.59
50	30	6	8.45	8.53
30	20	4	8.8	8.78
50	20	4	8.56	8.45
30	20	8	8.61	8.53
50	20	8	8.63	8.53
40	10	4	8.91	8.80
40	30	4	9.25	9.15
40	10	8	9.07	9.16
40	30	8	8.49	8.61
30	10	6	8.87	8.79
50	10	6	8.41	8.52
30	30	6	8.71	8.59
50	30	6	8.58	8.53
30	20	4	8.67	8.78
50	20	4	8.38	8.45
30	20	8	8.44	8.53
50	20	8	8.58	8.53
40	10	4	8.75	8.80
40	30	4	9.07	9.15
40	10	8	9.21	9.16
40	30	8	8.66	8.61

The resulting quadratic model was statistically significant ($p < 0.05$ for all terms), with a high coefficient of determination ($R^2 = 0.945$) and adjusted $R^2 = 0.930$, indicating a strong correlation with the experimental data. Among the three factors, hydrolysis time had the most substantial effect on RS yield, followed by enzyme activity and substrate concentration.

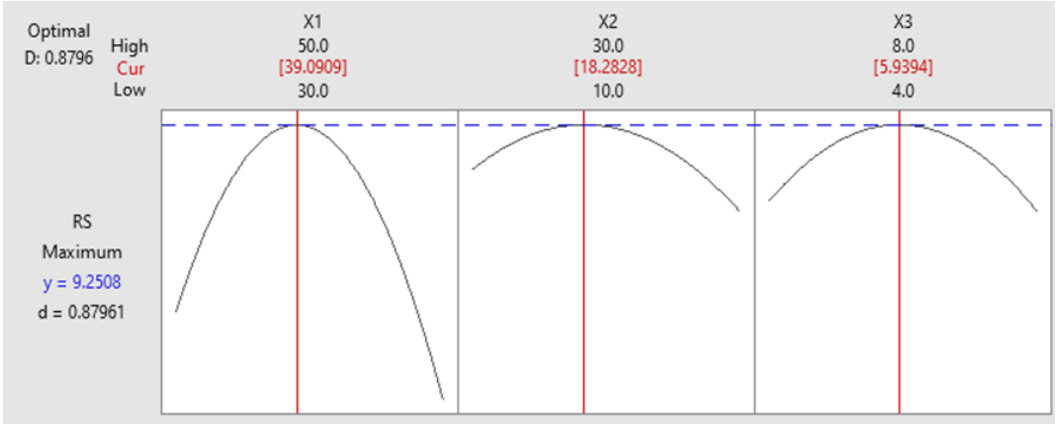

Figure 7. Results of optimal parameters of the hydrolysis process

The regression equation from the model was after eliminating the non-significant coefficients obtained:

$$Y = -0.213 + 0.3526X_1 + 0.0976X_2 + 0.5685X_3 - 0.00494X_1^2 - 0.001382X_2^2 - 0.04372X_3^2 + 0.000517X_1X_2 + 0.00400X_1X_3 - 0.01129X_2X_3$$

From the regression equation, it is evident that all three factors (substrate concentration, enzyme activity, hydrolysis time) significantly influence the increase in resistant starch content. Based on this model, the optimal hydrolysis conditions predicted were: substrate concentration of 39.09% (w/v), enzyme activity of 18.28 U/g, and hydrolysis time of 5.94 h. Validation under these conditions yielded an RS content of $9.32 \pm 0.13\%$, closely matching the model prediction (9.25%) with a deviation of only 0.07%, which was not statistically significant. These parameters-substrate concentration: 39.09%, enzyme activity: 18.28 U/g, hydrolysis time: 5.94 h, pH 4.5, and temperature 55 °C were selected for further experiments.

3.7. Production of resistant starch-enriched instant rice

For the production of prebiotic instant rice, ST25 rice was initially subjected to enzymatic hydrolysis using pullulanase to debranch amylopectin and enhance the amylose content. The rice is subsequently steamed at 100 °C to gelatinize the starch, followed by a two-stage retrogradation process under refrigerated conditions to promote starch crystallization. A brief microwave treatment restructures the starch granules, enhancing their resistance to enzymatic digestion. Finally, the product undergoes retort sterilization (115 °C, 15 min), is hermetically packaged for extended shelf life [11]. The final product contains 9.32%, resistant starch (RS).

An instant rice (untreated rice): Rice will be subjected to a preliminary washing to remove impurities, soaked (75°C, 15 minutes), steamed (100°C, 20 minutes), dehydrated (4°C, 24 hours), and the cooked rice will be dried at 55°C for 160 minutes and reconstituted with the ratio of (1 part rice: 3 parts water) for 6 minutes using a microwave with a power of 350W. The final product contains 1.18%, resistant starch (RS) [22].

3.7.1. Microbiological analysis of prebiotic instant rice products

The final prebiotic instant rice product was subjected to microbiological testing, the results confirmed its compliance with national food hygiene standards. Pathogenic microorganisms were not detected in the final product. Specifically, Escherichia coli was absent in 1 g of the sample, with a limit of detection (LOD) of 1 CFU/g, according to TCVN 7924-2:2008. Salmonella spp. was also undetected in 25 g of the sample, with an estimated limit of detection at 50% ($eLOD_{50}$) of 1.1 CFU/25 g (TCVN 10780-1:2017). Furthermore, both total yeast, mold counts were below the detection threshold of 1 CFU/g (TCVN 8275-2:2010). The total aerobic plate count was also below 1 CFU/g (TCVN 4884-1:2015). These results demonstrate that all tested microbial parameters were below their respective detection limits, confirming that the product meets stringent food safety, microbiological quality standards.

3.7.2. Nutritional composition analysis of prebiotic instant rice products

The nutritional composition of prebiotic instant rice and instant rice (not subjected to enzymatic hydrolysis) was analyzed. The results indicate that both samples exhibit similar levels of key nutritional components, including starch (45.9% vs. 48.0%), protein (5.17% vs. 5.44%), lipid (1.79% vs. 1.69%), and moisture content (44.10% vs. 42.9%). Notably, the resistant starch (RS) content of prebiotic instant rice at 9.32% is significantly higher than that of instant rice at 1.18%, corresponding to an increase of about 6.9 times compared to instant rice. This RS level aligns with the recommended daily intake in the United States (6-12g/day) [23].

4. CONCLUSION

The prebiotic instant rice product was developed from ST25 rice via enzymatic hydrolysis of branched-chain starches using pullulanase. Optimal processing parameters for the pullulanase treatment were established based on the yield of digestible resistant starch (RS). Specifically, ST25 rice was hydrolyzed under the following optimized conditions: substrate ratio of 39.09% (w/v), pH 4.5, temperature 55 °C, enzyme activity of 18.28 U/g, and hydrolysis time of 5.94 hours. Under these conditions, the resistant starch content in the final product reached 9.32%, representing an increase of 2.67% compared to the native rice (RS: 6.65%) [24]. The results confirm that pullulanase-mediated hydrolysis is an effective approach for enhancing the resistant starch content in ST25 rice, making it suitable for the production of prebiotic instant rice. The developed product provides a promising foundation for future functional food innovations targeting dietary management, weight control, metabolic health, and diabetes prevention.

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TÓM TẮT

TỐI ƯU HÓA THỦY PHÂN HỖ TRỢ PULLULANASE ĐỂ SẢN XUẤT CƠM ĂN LIỀN GIÀU TINH BỘT KHÁNG TỪ GIỐNG ST25

Nguyễn Hoàng Anh, Nguyễn Võ Mỹ Hằng, Phạm Minh Đức, Nguyễn Thuận Phát,

Phan Thị Thu Thảo, Nguyễn Văn Anh, Lê Nguyễn Đoan Duy*

Khoa học Công nghệ thực phẩm, Trường Đại học Công Thương Thành phố Hồ Chí Minh

*Email: *duylnd@huit.edu.vn*

Gạo ST25, một trong những giống lúa nổi tiếng về chất lượng cảm quan. Trong đó, hàm lượng carbohydrate (82,4%), với hàm lượng protein tương đối (6,74%) và lipid thấp (0,76%). Dựa vào nghiên cứu này đã tối ưu hóa được quá trình thủy phân gạo ST25 bằng enzyme Pullulanase giúp tăng cường hàm lượng tinh bột kháng (RS). Các biến đổi chính gồm – nồng độ cơ chất (10-50% w/v), hoạt độ enzyme (10-50 U/g), nhiệt độ thủy phân (45-65 °C), thời gian (2-10 giờ), và pH (3,5-5,5) đã được đánh giá. Xác định các điều kiện tối ưu hóa bằng phương pháp bề mặt đáp ứng (RSM) với thiết kế Box-Behnken, hàm lượng RS tối đa là 9,25% ở nồng độ cơ chất 39,09%, hoạt độ enzyme 18,28 U/g và thời gian thủy phân 5,94 giờ. Giá trị RS thực nghiệm (9,32%) gần như trùng khớp với dự đoán từ mô hình (R^2 = 94,45%). Sau quá trình thủy phân, gạo sau khi qua các công đoạn hồ hóa, phân giải enzyme hai giai đoạn, xử lý vi sóng và tiệt trùng bằng túi retort. Sản phẩm cơm ăn liền cuối cùng chứa 45,9% hàm lượng tinh bột, 5,17% protein, 1,7% lipid và 44,1% độ ẩm. Bên cạnh đó sản phẩm cơm ăn liền cũng đáp ứng các tiêu chuẩn an toàn thực phẩm (không phát hiện *E. coli*, *Salmonella* hoặc các vi sinh vật gây hư hỏng).

Từ khóa: Enzyme Pullulanase, cơm ăn liền, tối ưu hóa, tinh bột kháng.