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Optimization of pyrodextrin hydrolysis from rice starch to produce resistant maltodextrin

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Abstract

Resistant maltodextrin (RMD) is a short-chain glucose polymer characterized by high resistance to hydrolysis by human digestive enzymes. RMD typically exhibits a low dextrose equivalent (DE < 12), high resistance to digestion (> 85%), excellent solubility (> 95%), and low metabolizable energy (< 2 kcal/g). RMD has been reported to exert beneficial effects on metabolic disorders such as diabetes and obesity through the production of short-chain fatty acids (SCFA) and modulation of inflammatory responses. Therefore, RMD is widely applied in the food and nutraceutical industries. This study aimed to investigate key factors affecting the enzymatic hydrolysis of pyrodextrin derived from IR50404 rice starch and to optimize processing conditions for the production of resistant maltodextrin. Pyrodextrin was prepared by acid heat treatment of rice starch with the addition of 0.33 M HCl acid solution to achieve a pH of 2.3 before pyrolysis at 170°C for 105 minutes, yielding a product with the following characteristics: resistant starch content 70.82%, water solubility 96.73%, whiteness 64.27%, and dextrose equivalence index DE 4.87. Single-factor experiments were conducted using thermostable α-amylase (2860 U/mL) to establish the experimental range for multivariate analysis. A Box–Behnken design with three independent variables: enzyme concentration (0.2–0.4%), temperature (90 - 100°C), and hydrolysis time (60 - 90 min) was conducted. The response variables included DE and resistant maltodextrin content (%). Experimental data were analyzed using Design-Expert 7.1, and optimization was performed using the desirability function approach. Optimal hydrolysis conditions were obtained at an enzyme concentration of 0.24%, temperature of 94.63°C, and reaction time of 84.89 min, resulting in an RMD product with a DE of 7.44 and a resistant content of 85.41%. The findings indicate that rice is a promising raw material for the production of resistant maltodextrin.

1. Introduction

The rapid growth of functional foods and medical nutrition has increased demand for digestion-resistant carbohydrates. Resistant maltodextrin (RMD) is a short-chain glucose polymer highly resistant to hydrolysis by human digestive enzymes. Typically prepared from starches (corn, cassava, potato, rice) via enzymatic or thermo-acid plus enzymatic routes, RMD exhibits desirable physicochemical traits-low viscosity, high solubility, storage stability, and low discoloration-attributable to low molecular weight and the presence of digestion-resistant glycosidic linkages. RMD favorably modulates metabolic conditions (e.g., diabetes, obesity) through short-chain fatty acid (SCFA) generation and anti-inflammatory effects, and is widely used in beverages, dairy products, and desserts for its nutritional value and textural functionality without excessive quality loss (Fei Li *et al.,* 2023). Mechanistically, compared with resistant dextrins (RD), RMD shows a much lower hydrolysis rate (< 8% vs ≈ 34–37% for RD). Morphologically, RMD typically displays porous particle surfaces; structurally, "new" glycosidic linkages such as

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α-1,2; β-1,2; β-1,4; β-1,6 appear in place of only α-1,4/α-1,6 found in native starch (Chen *et al.,* 2023).

RMD is characterized by a low dextrose equivalent (DE < 12), a high digestion-resistant fraction (> 85%), high water solubility (> 95%) (Lefranc-Millot, 2008; Roquette Frères, 2020), and low metabolizable energy (< 2 kcal/g) (Lefranc-Millot, 2008; Baer *et al.*, 2014).

A two-stage process is commonly used: (i) acid-catalyzed thermal pretreatment (usually HCl) to form pyrodextrin, thereby disrupting glycosidic bonds, increasing solubility, and sensitizing starch to enzymes (Toraya-Avilés *et al*., 2017); (ii) enzymatic hydrolysis (mainly α-amylase) to cleave polysaccharide chains, controlling DE and the resistant fraction (Chang *et al.,* 2022; Chen et al., 2023).

Numerous studies have produced RMD from tuber starches (cassava, potato, sweet potato) following this thermo-acid plus enzymatic logic. For cassava starch pyrodextrinized with HCl and hydrolyzed by thermostable α-amylase, products with DE ≈ 24.45 and resistant fraction 56.06% were reported (Toraya-Avilés *et al.*, 2017); other heat/hydrolysis conditions yielded DE 8 –12 (Trithavisup *et al.,* 2022). With makal starch (*Xanthosoma yucatanensis*) pyrodextrinized by HCl, then hydrolyzed by α-amylase, RMD reached DE 13.89% and resistant fraction 90.73% (Barbosa-Martín *et al.*, 2024). Using three starch sources (potato, cassava, sweet potato) and HCl-based thermo-acid treatment followed by α-amylase and amyloglucosidase, RMD products had 97–100% solubility and 80–85% resistant fraction (highest for potato, 84.96%) (Chen *et al., 2023)*. Rice starch is abundant, cost-effective, hypoallergenic, and its amylose/amylopectin architecture is amenable to modification toward RMD (Chang *et al., 2022)*. In Vietnam, rice starch from the IR50404 cultivar has been investigated for the production of resistant starch type 3 (RS3), taking advantage of this cultivar's high amylose content (29.14%) and its initial resistant starch content of 2.92% (Thang *et al.*, 2024).

There remains limited work on RMD from rice starch. Building on our published thermo-acid pretreatment (HCl) to generate pyrodextrin from IR50404 rice starch (Lam *et al.,* 2020), we conducted one-factor, multifactor and optimization studies for enzymatic hydrolysis with thermostable α-amylase, targeting low DE and high resistant fraction RMD, suitable for food applications.

2. Materials and methods

2.1 Raw materials

R50404 rice variety was supplied by Hoa Phat Vietnam Food Processing Company Limited. The refined starch contained > 90% total carbohydrate, < 0.5% protein, < 0.02% lipid; amylose 29.14%; resistant starch 2.92% (Thang *et al*., 2024).

For the preparation of pyrodextrin, rice starch (4 - 5% moisture) was measured at 33 g/sample. 0.33 M HCl acid solution was titrated (acid/starch ratio by weight) until the starch reached a pH of 2.3. The starch was mixed in batches using a grinder (Sharp EM-ICE 2). The acid solution was then added gradually over a 10-minute mixing period, followed by incubation at room temperature for 10 h before pyrolysis at 170°C for 105 minutes. The pyrodextrin was cooled and packaged for testing.

2.2 Reagents and enzymes

Thermostable α-amylase (EC 3.2.1.1) with an activity of 2860 U/mL was supplied by Novozymes A/S (Denmark). The enzyme activity was verified by a national food testing laboratory in the United States. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) of analytical grade were purchased from Sigma-Aldrich (USA).

2.3 Hydrolysate preparation

For all single-factor and multifactor experiments, 100 g pyrodextrin powder per batch was dispersed with distilled water to 33% (w/w), heated to 60 - 70°C with stirring to fully dissolve, and adjusted to pH 5.8 with 1 N NaOH prior to enzymatic hydrolysis.

2.4 Single-factor experiments

2.4.1 Effect of enzyme concentration on resistant maltodextrin quality attributes

A total of five experiments were conducted corresponding to five levels of thermostable α-amylase concentration (0.1, 0.2, 0.3, 0.4, 0.5%), w/w on a dry dextrin basis. Common conditions for all trials were a hydrolysis temperature of 95±1°C and a reaction time of 60 min. Each experiment was performed in triplicate.

2.4.2 Effect of hydrolysis temperature on resistant maltodextrin quality attributes

Five experiments were conducted at five hydrolysis temperatures (85, 90, 95, 100, 105°C). The common conditions across trials were the enzyme concentration determined from the experiment in section 2.4.1 and a hydrolysis time of 60 min. Each experiment was performed in triplicate.

2.4.3 Effect of hydrolysis time on resistant maltodextrin quality attributes

Five experiments were conducted at five hydrolysis

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times (40, 60, 80, 100, 120 min). The common conditions across trials used the enzyme concentration and temperature determined from experiments in sections 2.4.1 and 2.4.2. Each experiment was performed in triplicate.

The overall objectives of the single-factor experiments (2.4.1—2.4.3) were evaluated by the following indices: DE, digestive-resistant fraction (%), and solubility (%). For Experiment group 2.4.3, metabolizable energy (kcal/g) was additionally analyzed. For all three experimental groups, upon completion of hydrolysis, the slurry was adjusted to pH 3 (with 1 N HCl) and held at 97°C for 10 min to inactivate α-amylase. The hydrolysate was then purified by ion exchange with continuous cation-exchange chromatography to remove glucose, followed by concentration and spray-drying to obtain the RMD product, which was subsequently analyzed for quality attributes.

2.5 Multifactor experimental method

The rationale for selecting variables and their variation ranges was based on the single-factor results. The multifactor experimental design for enzymatic hydrolysis followed a Box–Behnken design with three coded independent variables over the investigated ranges: enzyme concentration, X_1 (−1 to +1); temperature, X_2 (−1 to +1); and hydrolysis time, X_3 (−1 to +1). The objective (response) functions were dextrose equivalent (DE), Y_1, and digestive-resistant fraction (%), Y_2. Optimization was performed using a desirability function multi-objective approach according to the algorithm proposed by Derringer and Suich (2018).

2.6 Determination of dextrose equivalent

Dextrose equivalent (DE) is determined by the Lane–Eynon titration method according to Vietnamese Standard TCVN 10376:2014 (ISO 5377:1981). The method is based on the ability of Fehling's solution to reduce Cu(II) ions in an alkaline environment by reducing sugars in the sample. The volume of Fehling's solution consumed for the reaction is used to calculate the reducing sugar content converted to dextrose and expressed as DE (%).

2.7 Determination of resistant fraction

The resistant fraction was quantified using the method of Englyst and Hudson (1996) with minor adaptations. Specifically, 1.00 g of pyrodextrin sample was weighed accurately and mixed with 50 mL of 0.08 M phosphate buffer (pH 6.0), followed by the addition of 0.1 mL thermostable α-amylase (Termamyl 120L, Novo Laboratories, Inc., USA) and incubation at 95°C for 30 min. The mixture was cooled to room temperature, and the pH was adjusted to 7.5±0.1 with 0.275 M NaOH (approximately 10 mL). Then, 0.5 mL of protease solution was added, and the reaction proceeded at 60°C for 30 min. The reaction mixture was cooled, and the pH was adjusted to 4.5; the solution was then further adjusted to pH 4.5±0.2 with 0.325 M HCl (approximately 10 mL). Next, 0.3 mL amyloglucosidase was added, and the mixture was incubated at 60°C for 30 min. The reaction was terminated by heating to 90°C for 15 min. The resulting mixture was filtered and diluted to 100 mL with distilled water, and the released glucose was determined by the pyranose-oxidase method using the K-GLUC kit (GOPOD format) (Novozymes, Denmark). The resistant dietary fiber content (%) was calculated as: Resistant fraction (%) = 100 - [Glucose (%) × 0.9], where 0.9 converts glucose to glucan (Englyst and Hudson, 1996).

2.8 Determination of solubility

An aliquot of 0.50 g pyrodextrin powder was mixed with 30 mL of water in a centrifuge tube and vortexed to homogeneity. The suspension was heated in a water bath to 90°C for 30 min, then centrifuged at 1,500 rpm for 30 min. The supernatant was decanted into a pre-weighed aluminum dish and dried to determine the mass of dissolved solids. Solubility (%) was calculated as: Solubility (%) = [mass of dissolved starch after drying (g) / initial sample mass (g)] × 100 (Hung *et al.*, 2016).

2.9 Determination of metabolizable energy

Metabolizable energy (ME) was calculated using the EU 1169/2011—Annex XIV energy conversion factors: carbohydrate 4 kcal/g, fibre 2 kcal/g, protein 4 kcal/g, and fat 9 kcal/g. The calculation was as follows: ME (kcal/100 g) = 4×available carbohydrate + 2×TDF + 4×protein + 9×fat; where available carbohydrate = 100 - moisture - ash - protein - fat – TDF (European Parliament and Council, 2011).

2.10 Experimental data processing methods

The research data were statistically processed using Microsoft Excel 2016 and Minitab 16. Multifactor experimental data analysis and optimization were conducted with Design-Expert 7.1 (Design-Expert version 7.1, 2007).

3. Results and discussion

3.1 Experimental results of single-factor enzymatic hydrolysis of pyrodextrin

3.1.1 Effect of enzyme concentration

The single-factor experimental results on the effect

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of α-amylase concentration on dextrose equivalent (DE), digestive-resistant fraction, and solubility at five enzyme levels (0.1%, 0.2%, 0.3%, 0.4%, 0.5%) under identical conditions of 95±1°C for 60 min, a pyrodextrin/water ratio of 33%, and pH 5.8 are presented in Figure 1.

Figure 1 shows that as the enzyme concentration increases from 0.1% to 0.5%, the DE value rises progressively, reflecting a marked increase in the extent of starch hydrolysis. Within the lower concentration range (0.1 - 0.3%), DE increases rapidly from 5.65 to 7.45, indicating vigorous catalysis when the enzyme readily accesses α-1,4-glycosidic linkages in amylose. When the enzyme concentration exceeds 0.3%, the rate of DE increase slows (7.45 to 8.60). As explained by Gupta *et al.* (2003), this behavior arises as the reaction system approaches enzyme saturation, with the substrate becoming the limiting factor. In parallel, solubility also increases from 97.05% to 98.18% with higher enzyme concentrations. This rise can be interpreted, following Crabb *et al.* (1997), as a consequence of polymer chain scission yielding smaller dextrin, maltose, and glucose molecules that dissolve more readily. While DE increases from 5.65% to 8.60%, the digestive-resistant fraction also increases slightly from 74.38% to 79.12%. This may be attributed to structural disruption followed by reorganization during cooling; moreover, as discussed by Zhang *et al.* (2009), hydrolysis can promote the formation of recrystallized (retrograded) regions, thereby increasing the resistant fraction through the development of more stable crystalline structures that are less susceptible to enzymatic attack.

Thus, α-amylase concentration directly influences hydrolysis efficiency and the physicochemical properties of pyrodextrin. Beyond an optimal threshold, further increases in enzyme do not appreciably raise DE and instead escalate processing costs. The present results indicate that an enzyme range of approximately 0.2–0.4% is appropriate for subsequent multifactor experiments and process optimization.

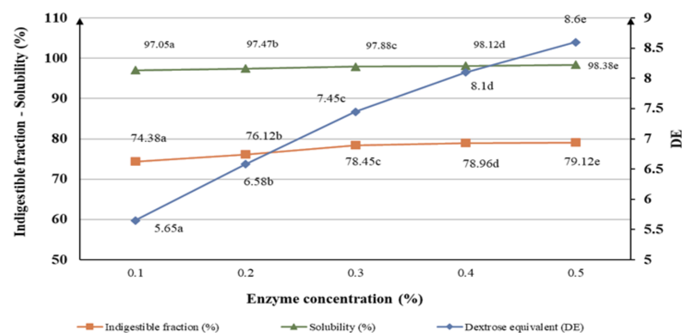

Figure 1. Effect of enzyme concentration on the quality characteristics of maltodextrin. Values with different letters are statistically significantly different ($p<0.05$) in the same evaluation criterion (indigestible fraction, solubility and dextrose equivalent (DE)).

3.1.2 Effect of hydrolysis temperature

The experimental results for hydrolysis temperature at five levels (85°C, 90°C, 95°C, 100°C, 105°C) under identical conditions of enzyme concentration 0.3% (per Section 3.1.1), time 60 min, pyrodextrin/water ratio 33%, and pH 5.8-are presented in Figure 2.

Figure 2 shows that, as the hydrolysis temperature rises from 85°C to 105°C, the DE increases from 6.13 to 8.21, solubility increases from 97.11% to 98.41%, and the digestive-resistant fraction increases from 75.26% to 79.44%. This indicates that the rise in DE reflects more intensive starch chain scission at higher temperatures, generating additional reducing ends and shorter, more soluble chains. These results are consistent with the findings of Kapuśniak and Jane (2007) on enzymatic hydrolysis of pyrodextrin, which showed that DE and solubility increase with processing temperature.

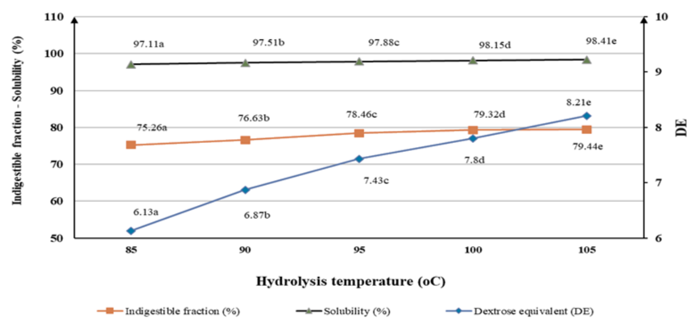

Figure 2. Effect of hydrolysis temperature on the quality characteristics of maltodextrin. Values with different letters are statistically significantly different ($p<0.05$) in the same evaluation criterion (indigestible fraction, solubility and dextrose equivalent (DE)).

Additionally, Baks *et al.* (2008) noted that higher temperatures increase enzyme reaction rates within the limits of activity, so the higher DE observed at 95–100°C is reasonable. Solubility rises in tandem with DE because α-1,4 and α-1,6 linkages are cleaved, reducing molecular weight and enhancing diffusivity in water. The study by Słomińska *et al.* (2002) reported that, during the hydrolysis of potato, corn, and pea starch at 90–100°C, solubility increased almost linearly with dextrose equivalent (DE). Moreover, Trinh (2015) found that the digestive-resistant fraction also increases slightly with temperature. This phenomenon may result from retrostructuring (retrogradation) or the formation of cross -links among glucose chains at elevated temperatures, creating regions that are difficult to digest yet remain soluble. According to Liu *et al.* (2009), rice starch subjected to thermal treatment at 100–120°C exhibits higher enzyme resistance due to structural rearrangement.

Thus, elevated hydrolysis temperature simultaneously promotes two processes: (1) chain

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scission, which increases DE and solubility; and (2) molecular reorganization, which generates digestion-resistant regions. Based on these results, a hydrolysis-temperature investigation window of 90 - 100°C is appropriate for subsequent multifactor experiments and process optimization.

3.1.3 Effect of hydrolysis time

The experimental results for the effect of hydrolysis time at five levels (40 min, 60 min, 80 min, 100 min, 120 min)-under identical conditions of enzyme concentration 0.3% and hydrolysis temperature 95°C (per Sections 3.1.1 and 3.1.2), with a pyrodextrin/water ratio of 33% and pH 5.8-are summarized in Figure 3. In this Figure 3a depicts the relationship of enzyme concentration to DE and the digestive-resistant fraction, while Figure 3b depicts the relationship of enzyme concentration to metabolizable energy (ME).

Figure 3a shows that, as hydrolysis time increases from 40 to 120 min, the DE value rises from 6.22 to 8.98, indicating progressively greater cleavage of glycosidic chains within the starch structure. This concurred with Liu *et al.* (2015), who explained that during α-amylase- or amyloglucosidase-catalyzed starch hydrolysis, prolonging reaction time affords enzymes more opportunity to access and break α-1,4-glycosidic linkages. However, the rate of DE increase tends to diminish at later stages because the more readily hydrolyzable substrate has already been cleaved, leaving starch molecules with more stable structures or sites that are difficult to access.

The digestive-resistant fraction increases sharply from 74.35% to 82.37% as hydrolysis time extends to 100 min, then declines slightly to 81.55% at 120 min. According to Chakraborty *et al.* (2021), this trend arises because early hydrolysis can facilitate crystalline reorganization, generating more digestion-resistant regions (RS type III) via amylose retrogradation under high temperature (95°C) and prolonged holding. However, as noted by Chung *et al.* (2009), excessive reaction time may allow enzymes to disrupt newly formed crystalline domains, causing a slight decrease in the resistant fraction. Meanwhile, solubility also increases from 97.08% to 98.68% with longer hydrolysis, reflecting greater proportions of low-molecular-weight starch fragments or readily soluble dextrins. Sasaki *et al.* (2000) attributed this to continued enzymatic scission of amylose and amylopectin chains, yielding more soluble intermediates through reduced crystallinity and an increased number of free hydroxyl groups. This trend is consistent with the observations of Chang and Lin (2002), whereby prolonging the hydrolysis time increases solubility but can concurrently reduce the viscosity and gel-forming properties of starch.

Figure 3b shows that, as hydrolysis time is prolonged from 60 to 120 min, metabolizable energy tends to decrease markedly from 3.12 to 2.46. This is plausibly due to the rising resistant fraction, which reduces metabolizable energy.

In summary, hydrolysis time substantially affects RMD quality indices: increasing time elevates DE and solubility and yields a maximum in the resistant fraction at under 100 min. Based on these results, a hydrolysis-time investigation window of 60 - 90 min is appropriate for subsequent multifactor experiments and process optimization.

3.2 Results of multifactor experiments and enzymatic hydrolysis optimization

The results of the multi-factor experiment, including 15 experiments, are summarized in Table 1. Data processing was carried out using Design-Expert 7.1 software, and the results of ANOVA analysis to check the significance of regression coefficients and the compatibility of experimental models (Y_1, Y_2) are summarized in Table 2.

3.2.1 Multifactor effects on dextrose equivalent (Y_1)

Regression analysis for response Y_1 yielded an F-value = 25.35 ($p < 0.05$), indicating the model is statistically significant with 99.88% confidence ($p < 0.0012$). Significance of regression coefficients was

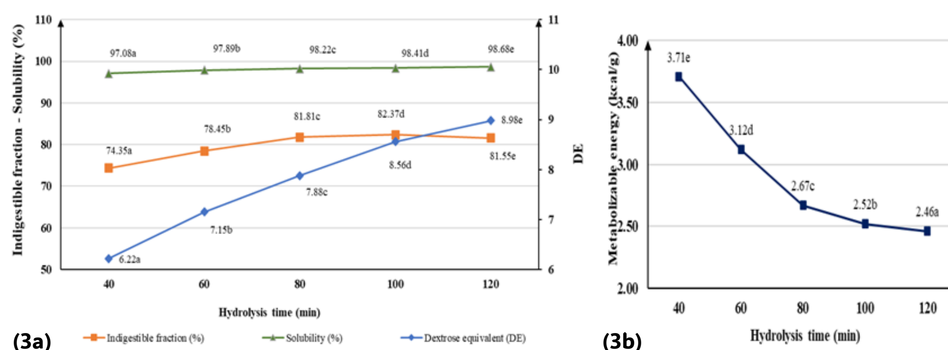

Figure 3. Effect of hydrolysis time on the quality characteristics of maltodextrin. Values with different letters are statistically significantly different (p<0.05) in the same evaluation criterion (indigestible fraction, solubility and dextrose equivalent (DE)).

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Table 1. Summary of multifactor experimental data.

No.	X_{-1}	X_2	X_3	Y_1	Y_2
1	0.4	90	75	7.82	83.9
2	0.3	95	75	7.83	88.87
3	0.2	100	75	6.74	77.61
4	0.3	90	90	6.97	79.94
5	0.3	100	90	8.12	86.52
6	0.3	90	60	6.77	78.57
7	0.3	100	60	6.85	77.19
8	0.3	95	75	7.98	89.16
9	0.3	95	75	7.85	89.28
10	0.2	90	75	6.98	77.47
11	0.4	95	60	7.25	82.28
12	0.4	100	75	8.48	86.14
13	0.4	95	90	8.38	87.21
14	0.2	95	60	6.67	76.27
15	0.2	95	90	6.85	79.46

evaluated by the F-test; $p < 0.05$ denotes that A, B, C, AB, BC, AC, B^2, and C^2 are statistically significant, whereas A^2 ($p > 0.05$) is not. Nevertheless, A^2 was retained in the model for optimization purposes. In addition, the lack-of-fit $F = 5.67$ ($p > 0.05$) shows that the lack of fit is not significant. The model coefficient of determination $R^2 = 0.9786$ indicates that 97.86% of the variability in Y_1 is explained by the independent variables, while the Predicted R-Squared (0.6879) is consistent with the Adjusted R-Squared (0.94).

The regression equation for response Y_1 in coded variables (1a) and in actual variables (1b) is as follows:

$$Y_1 = + 7.89 + 0.59A + 0.21B + 0.35C + 0.23AB + 0.24AC + 0.27BC - 0.14A^2 - 0.25B^2 - 0.46C^2 \quad (1a)$$

$$Y_1 = - 62.761 - 35.862X_1 + 1.614 X_2 - 0.089X_3 + 0.45X_1X_2 + 0.0791X_1X_3 + 1.783X_2X_3 - 13.58X_1^2 - 9.833X_2^2 - 5.148X_3^2 \quad (1b)$$

Figure 4A and the regression coefficients in equation (1a) show that enzyme concentration (A) exerts the greatest influence on DE, followed by hydrolysis time (C) and, lastly, temperature (B). When temperature and hydrolysis time are fixed at the center level (0), and the enzyme concentration is increased from −1 to +1, DE rises continuously, then tapers within the range +0.5 to +1. Increasing the enzyme concentration raises the number of active sites participating in the reaction. This enhancement accelerates the hydrolysis rate, resulting in the generation of more reducing sugars and ultimately leading to a higher dextrose equivalent (DE). At enzyme concentrations around 0.35–0.40%, the reaction system reaches near saturation, with most substrate molecules already bound to the enzyme. Since the substrate becomes the limiting factor, further increases in enzyme concentration have minimal effect on hydrolysis rate, resulting in a slower increase in dextrose equivalent (DE).

Figure 4B, for the interaction between increasing enzyme concentration (0.2 - 0.4%) and rising hydrolysis temperature (90 - 100°C), the DE value increases from 7.17 to 8.25. This indicates that both factors positively influence the extent of pyrodextrin hydrolysis. The result accords with studies on hydrolyzing rice starch by thermostable α-amylase, in which DE increases with enzyme concentration and temperature (Sringam,1994; Chang *et al.*, 2022). A DE of 7–8 reflects mild hydrolysis, appropriate for producing resistant maltodextrin.

Figure 4C, for the interaction between raising enzyme concentration from 0.2% to 0.4% and extending hydrolysis time from 60 to 90 min (at 95°C), DE increases from 6.912 to 8.199. This rise reflects greater cleavage of α-1,4-glycosidic linkages under both factors. Similar findings were reported by Fathoni *et al.* (2023),

Table 2. Regression analysis results for objective functions Y_1 and Y_2.

Source	Response Y_1		Response Y_2	
	F-value	p-value	F-value	p-value
Model	25.35	0.0012	86.60	< 0.0001
A	109.03	0.0001	243.20	< 0.0001
B	13.49	0.0144	16.63	0.0096
C	38.31	0.0016	105.21	0.0002
AB	8.03	0.0365	2.55	0.1711
AC	8.95	0.0304	1.83	0.2344
BC	11.35	0.0199	38.02	0.0016
A^2	2.70	0.1612	108.40	0.0001
B^2	8.85	0.0310	160.69	< 0.0001
C^2	31.43	0.0025	159.20	< 0.0001
Lack of Fit	5.67	0.1536	15.23	0.0622
Coefficient of Determination R^2	0.9786		0.9936	
Adjusted R^2(R^2_{Adj})	0.9400		0.9822	
Predicted R^2(R^2_{Pred})	0.6879		0.9017	

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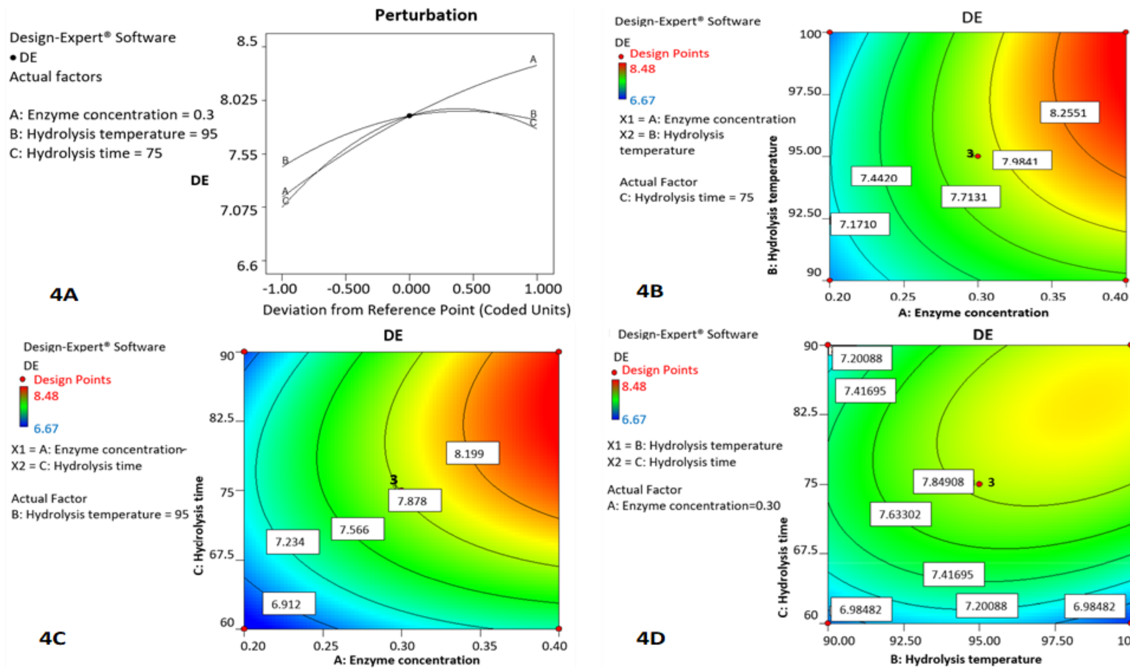

Figure 4. Effects of experimental factors on objective function Y_1.

where increases in enzyme level and reaction time led to a clear rise in the DE of corn starch. In the hydrolysis of rice-starch pyrodextrin, the modest DE increase (6.9 to 8.2) suggests an early reaction stage, with much of the dextrin structure not yet fully cleaved. Such a low DE (< 10) aligns with the goal of producing resistant maltodextrin, because the product retains structures that are difficult for enzymes to digest. This is consistent with Li *et al.* (2022), who noted that the formation of α-1,2 and β-1,6 linkages during thermal and enzymatic treatment reduces the hydrolyzability of dextrins.

Figure 4D, for the interaction between hydrolysis temperature (90 - 100°C) and hydrolysis time (60 - 90 min) with other factors held constant, the product's DE increases from 6.98 to 7.84. This trend is consistent with Hoover and Zhou (2003), who showed that temperature promotes the extent of glycosidic-bond hydrolysis within pyrodextrin structures. At higher temperatures, the reaction's activation energy is more readily overcome, intensifying starch chain scission and thereby increasing reducing sugars and DE. Similarly, Shin *et al.* (2004) reported that raising the processing temperature from 90 to 110°C accelerates hydrolysis and reduces starch crystallinity, leading to higher DE. Meanwhile, extending hydrolysis time (60 - 90 min) prolongs enzyme-substrate contact, further enhancing glucosidic chain cleavage and contributing to DE elevation. However, when temperature or time exceeds the optimal threshold, re-association phenomena or degradation of low-molecular-weight sugars may occur, lowering efficiency and adversely affecting the digestion-resistant properties of maltodextrin.

Thus, within the surveyed ranges of enzyme concentration (0.2 - 0.4%), temperature (90 - 100°C), and time (60 - 90 min), there is a direct proportional relationship of enzyme level-temperature-time with DE. Nevertheless, to preserve the characteristics of resistant maltodextrin (low DE, stable branched architecture), optimization is required to balance hydrolysis efficiency with digestion resistance.

3.2.2 Multifactor effects on the content of digestion-resistant dietary fiber (Y_2)

The regression analysis for response Y_2 yielded an F-value = 86.6 ($p < 0.05$), indicating that the model is statistically significant with 99.99% confidence ($p < 0.0011$). The significance of regression coefficients was assessed by the F-test; $p < 0.05$ shows that coefficients A, B, C, BC, A^2, B^2, and C^2 are statistically significant, whereas AB and AC ($p > 0.05$) are not. Nevertheless, AB and AC were retained in the model for optimization. In addition, the lack-of-fit F = 15.23 ($p > 0.05$) indicates that the lack of fit of model Y_2 is not significant. The coefficient of determination (R^2) for model Y_2 is 0.9936, explaining 99.36% of the variation in the objective function Y_2 as a function of the independent variables. Meanwhile, the Predicted R-Squared (0.9017) is consistent with the Adjusted R-Squared (0.9822).

The regression equation for response Y_2 in coded variables (2a) and in actual variables (2b) is as follows:

$$Y_2 = + 89.1 + 3.59A + 0.94B + 2.36C + 0.52AB + 0.44AC + 2.01BC - 3.53A^2 - 4.3B^2 - 4.28C^2 \quad (2a)$$

$$Y_2 = - 1419.43 - 135.57X_1 + 31.316 X_2 - 0.381X_3 + 1.04X_1X_2 + 0.146X_1X_3 + 0.013X_2X_3 - 352.79X_1^2 - 0.171X_2^2 - 0.00475X_3^2 \quad (2b)$$

Examining Figure 5A and the regression coefficients of equation (2a) shows that: enzyme concentration (A)

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exerts the greatest influence, followed by hydrolysis time (C) and, lastly, temperature (B) on the content of the digestive-resistant fraction. This pattern is broadly consistent with the increasing trend observed for the degree of hydrolysis (DE). When temperature and hydrolysis time are fixed at the center level (0), the resistant fraction increases continuously as enzyme concentration rises from −1 to +1; however, the rate of increase tapers when the enzyme level advances from +0.5 to +1. Conversely, when enzyme concentration and hydrolysis time are fixed at the center (0) and temperature is raised from −1 to +1, DE tends to increase sharply from −1 to 0, then more gradually from 0 to +0.5, and begins to decline markedly from +0.5 to +1.

Figure 5B shows that: when enzyme concentration is increased within 0.2–0.4% and hydrolysis temperature within 90–100°C at a fixed time of 60 min, the digestive-resistant fraction rises from 79.4% to 87.96%. This agrees with Wang *et al.* (2001) and Chen *et al.* (2023), who reported that higher enzyme levels facilitate selective hydrolysis of easily digestible fractions, leaving structures capable of retrogradation or branching that are less susceptible to enzymatic digestion. Moreover, according to Li *et al.* (2024), higher hydrolysis temperatures disrupt starch granule architecture and promote post-reaction rearrangement of amylose-amylopectin chains, contributing to crystalline networks more resistant to digestive enzymes. However, if enzyme level or temperature exceeds the optimal range, resistant structures may be degraded through over-hydrolysis or enzyme inactivation. Meanwhile, Toraya-Avilés *et al.* (2016) likewise reported findings consistent with published studies on the production of resistant maltodextrin from cereal and tuber starches via enzymatic hydrolysis combined with thermal treatment.

Figure 5C shows that when enzyme concentration is increased from 0.2 to 0.4% and hydrolysis time is extended from 60 to 90 min at a fixed temperature of 95°C, the content of the digestive-resistant fraction rises from 78.22% to 87.97%. This accords with studies indicating that higher enzyme levels accelerate cleavage of α-1,4 and α-1,6 linkages, generating smaller dextrin fragments and branched structures that are difficult to digest, thereby increasing the resistant fraction. Likewise, longer hydrolysis provides sufficient time for enzymatic action, promoting molecular reorganization and the formation of atypical linkages, which in turn elevates the resistant portion (Igual *et al.*, 2021; Trithavisup, 2023). Conversely, operation at 95°C promotes starch gelatinization, optimizes enzyme–substrate contact, and favors the development of resistant structures; however, a thermostable enzyme is required to avoid inactivation (Wang *et al.*, 2018).

Figure 5D further shows that, as the hydrolysis temperature for pyrodextrin increases from 90°C to 100°C and the hydrolysis time is extended from 60 to 90 min (at a fixed enzyme level of 0.3%), the digestive-resistant fraction rises markedly from 81.25% to 87.48%. This increase indicates that temperature and time act synergistically to shape molecular architecture and resistance to digestive enzymes. At higher temperatures, starch granule structure is more extensively disrupted, enabling deeper enzyme penetration and selective cleavage of glycosidic bonds; concurrently, post-hydrolysis amylose retrogradation fosters more robust structures against enzymatic attack.

These findings align with Wang *et al.* (2023), who

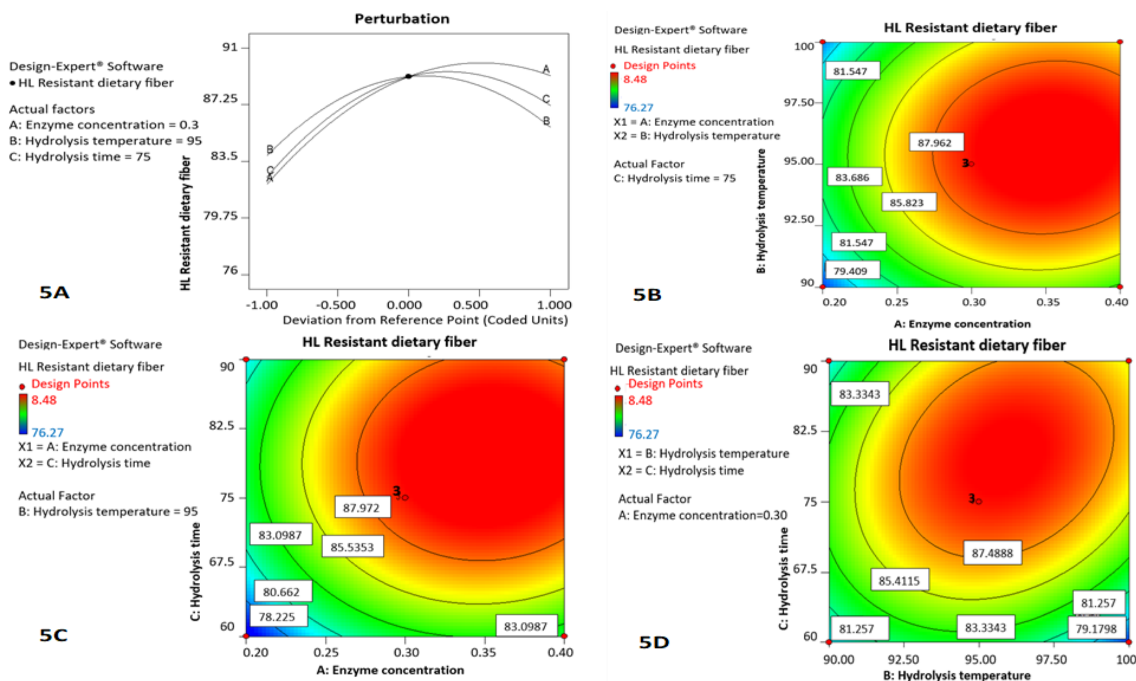

Figure 5. Effects of experimental factors on objective function Y_2.

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reported that partial hydrolysis of waxy rice starch by maltogenic α-amylase increased the proportion of resistant starch due to recrystallization of short amylose segments. Similarly, Qin *et al.* (2011) demonstrated that hydrolysis by α-amylase from *Bacillus licheniformis* enhanced crystallinity and the proportion of B-type regions in high-amylose rice starch, thereby reducing starch digestibility.

3.2.3 Optimization of pyrodextrin hydrolysis

The multifactor investigation shows how variations in experimental factors drive changes in the attributes related to the objective functions in the desired directions, while the countervailing constraints set on a negotiated basis are specified in Table 3.

Table 3. Constraint conditions for the factors and experimental objectives.

Factors and objectives	Desired target	Importance level
Enzyme concentration (%)	In range (0.2 – 0.4)	5/5
Hydrolysis temperature (°C)	In range (90 - 100)	5/5
Hydrolysis time (min)	In range (60 - 90)	4/5
Dextrose equivalent (DE)	Minimize	5/5
Resistant fraction (%)	Maximize	5/5

Based on the importance coefficients reflecting the influence of the experimental factors, the prioritized selections were: enzyme concentration (5/5), hydrolysis temperature (5/5), and hydrolysis time (4/5). For the objective functions, the priorities and targets were: dextrose equivalent, DE (5/5), minimized; and digestion-resistant dietary fiber content (5/5), maximized.

Data optimization using Design-Expert 7.1, subject to the constraints in Table 3, identified the optimal hydrolysis conditions as: enzyme concentration 0.24%, temperature 94.63°C, and hydrolysis time 84.89 min, corresponding to objective values of DE = 7.44 and digestive-resistant fraction = 85.41%.

Experimental validation with three replicates under the optimal conditions (summarized in Table 4) yielded DE = 7.53±0.18 and a digestive-resistant fraction = 84.12±1.38%, indicating that the optimized model agrees with the experimental results, with no statistically significant difference ($p > 0.05$).

The optimized result with DE = 7.44 indicates that the maltodextrin/hydrolysate possesses a low dextrose equivalent, i.e., many polymer segments remain not fully hydrolyzed to short sugars or maltose, which is favorable for forming the digestion-resistant fraction. A resistant fraction of 85.41% aligns well with Chen *et al.* (2023), who reported RMD from tuber starches (potato, cassava, sweet potato) with resistant fractions ranging 80–85%, the highest for potato (84.96%, $p < 0.05$). The hydrolysis conditions of 94.63°C for 84.89 min using thermostable α-amylase is comparatively high versus many enzymatic hydrolyses (70–90°C); this likely enhances starch disruption, crystal breakdown, and gelatinization, thereby accelerating hydrolysis and supporting the formation of digestion-resistant linkages. The 0.24% enzyme level is moderately high, ensuring sufficient catalytic activity at elevated temperature, which confirms the appropriateness of selecting a thermostable enzyme. The combination of the three factors-temperature, time, and enzyme concentration-suggests the process involves not only hydrolysis but also starch modification/ gelatinization, followed by hydrolysis and possible transglycosylation, generating atypical linkages (e.g., β-1,2; β-1,4; α-1,2; α-1,6), as documented for RMD/RD by Chen *et al.* (2023) and Zarski *et al.* (2024).

Moreover, hydrolyzing rice-starch pyrodextrin from IR50404 (amylose 29.14%, resistant starch 2.92%) implies that the A-type crystalline starch underwent gelatinization, hydrolysis, and restructuring. Newly formed shorter amylose chains tend to realign into stable crystalline regions (retrograded starch, RS3). Such reorganization creates hydrogen-bonded networks and distinctive branched structures (e.g., β-1,2; β-1,4), reducing digestive-enzyme accessibility and yielding a higher resistant fraction than starches from potato, cassava, or sweet potato. Specifically, potato starch (B-type crystals; large granules; lower amylose ~20%; abundant phosphate esters) shows high resistance when raw (RS2) but drops markedly after cooking. Cassava/ sweet-potato starches (mixed A/B type; amylose lower than rice) retrograde less, so RS3 formation is more limited.

Therefore, these initial findings indicate that hydrolyzing rice-starch pyrodextrin with thermostable α-amylase is well-suited to the goal of producing resistant maltodextrin.

Table 4. Experimental results under the optimal conditions.

Response		Optimal conditions			Predicted value	
		X_{-1}	X_2	X_3	Optimal conditions	Experimental value (mean±SD, n=3)
Y_1	Dextrose equivalent (DE)	0.24	94.63	84.89	7.44	7.53±0.18
Y_2	Resistant fraction (%)				85.41	84.12±1.38

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4. Conclusion

From the single-factor investigations, hydrolysis with thermostable α-amylase (activity 2,860 U/mL) proved effective for generating RMD with low DE, high digestive-resistant content, high solubility, and low metabolizable energy within the ranges of enzyme concentration (0.2 - 0.4%), temperature (90 - 100°C), and hydrolysis time (60 - 90 min). The optimal technological conditions for hydrolyzing pyrodextrin from IR50404 rice starch to produce maltodextrin were identified as enzyme concentration 0.24%, temperature 94.63°C, and hydrolysis time 84.89 min, yielding an RMD product with DE 7.44 and a digestive-resistant fraction of 85.41%. These findings provide a basis for further process refinement at pilot scale-encompassing purification and decolorization steps-toward an efficient, high-quality RMD production process.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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