Response surface optimization of enzymatic hydrolysis of germinated brown rice for higher reducing sugar production

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Abstract

The hydrolysis of germ rice by the use of α-amylase and glucoamylase enzymes will help increase the reducing sugar content, reduce viscosity, and improve the yield of milk solution compared to the traditional extraction method. The liquefaction experiment was arranged with two factors, which are substrate ratio: α-amylase concentration and α-amylase concentration: different hydrolysis time. The saccharification experiment was carried out based on a multivariate model according to the Central Composite Design method. As a result, a 1 : 2 substrate ratio, 0.5% α-amylase concentration (approx. 11U/g starch) and 50 minutes hydrolysis time were selected as the basis for the next experiment. Analysis of variance in the regression model showed that the quadratic model was significant ($p < 0.0001$). Lack of fit ($p > 0.05$) this indicates that the model is suitable for all data. The reliability of the model $R^2 = 0.993$ shows that the built regression model fits the data set 99.3%. CV = 1.19% indicated a better precision and reliability of the experiments carried out. Optimal conditions for hydrolysis of glucoamylase concentration of 0.399% (approx. 119.863U/g starch), temperature of 59.813°C and hydrolysis time of 160.468 minutes gave the highest DE content at 25.245% and higher than the non-enzymatic method (DE = 8.985 ± 0.062).

Keywords: *nutrition drink, starch hydrolysis, reducing sugar, germinated brown rice.*

1. INTRODUCTION

Brown rice is rice with only the husk removed, the bran layer has not been milled. Brown rice is a food with more nutrients than white rice in terms of fiber, essential amino acids, minerals, protein and vitamins [1-2]. However, due to its dark color, hard texture and unappealing taste like white rice, brown rice is less commonly used as white rice [3]. So far, there have been quite a few studies on the changes in nutrient content during the germination of brown rice [4-9]. Especially the active ingredient gamma-amino butyric acid (GABA) is very good for human health [10-12]. Currently, there are not many researches to develop food products from germinated brown rice, author Watanabe et al., [13] and Morita et al.,

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[14] research on the application of germinated brown rice flour in bread production, author Bolarinwa & Muhammad [15] studied the application of germinated brown rice flour in biscuit production. Therefore, this study was conducted with the aim of studying the hydrolysis conditions of germinated brown rice applied in the processing of pineapple flavored germinated brown rice milk and jelly to create products beneficial to human health. There are many methods to hydrolyze starch, in which the enzyme method has been widely applied to enhance the extraction efficiency of hydrolyzate, contribute to reducing viscosity, easy to filter the solution, improving extract color and fluid quality (deposition, flavor intensity) during production [16]. To facilitate hydrolysis, some starch hydrolyzing enzymes are added in concentrations ranging from 0.1 to 0.5% [17-18].

The efficiency of starch hydrolysis depends on many factors such as substrate concentration: water, temperature, time, enzyme concentration, etc. In this study, the author conducted hydrolysis of germinated brown rice starch through two stages, liquefaction and saccharification. Because of the influence of many factors in the hydrolysis process, if the experimental arrangement is full of factors, it is costly and time consuming. Therefore, the author uses the central composite model and the response surface model according to the Central composite design (CCD) in the design of the saccharification experiment, this model has the advantage of reducing the number of experimental units but the results are still statistically significant.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Germinated brown rice

Germinated brown rice brand Vibigaba, Loc Troi group, made in An Giang, Vietnam. Nutritional value for 1kg of product according to the manufacturer: carbohydrates ≥ 600 g, protein ≥ 70 g, lipids ≥ 20 g, digestible fiber ≥ 30 g, GABA: 120 - 200 mg, inositol ≥100 mg, calcium ≥ 50 mg, vitamin ≥ B1 3 mg, vitamin E ≥ 3 mg, glycemic index Gl (%): 58 ± 4.3 (compared to glucose), humidity ≤ 14.5%, plate rate < 7%.

2.1.2. α-amylase (thermostable α-amylase)

Novozyme brand, declared activity 120 KNU-S/g, (KNU is the amount of enzyme which breaks down 5.26 g starch), colour: amber, physical from: liquid, approximate density 1.25 (g/mL), viscosity 1 - 25 (cPs), organnism: *bacillus licheniformis*.

2.1.3. Glucoamylase

Trade name: Leafgluco L, Origin: India, light brown liquid, pH 3.0 - 5.0, temperature 60 -65°C, declared activity 300U/mL, organism *Aspergillus sp*.

2.1.4. Chemicals

Dinitrosalicylic Acid (DNS) from Himedia-India, Sodium hydroxide, Sodium Potassium tartrate, D-glucose from Xilong-China.

2.1.5. Equipment

UV-Vis spectrophotometer (Genesys 20, Thermo Scientific - USA), 4-digit electronic balance (Ohaus, USA), Brix meter (Atago, Japan), and some other necessary equipment and tools in the laboratory experience.

2.2. Design of experiments

2.2.1. Investigate the substrate ratio (rice : water) and α-amylase concentration affecting the liquefaction process

The germinated brown rice is finely ground in a mill, then mixed with water in the ratios (rice : water) 1:1, 1:2, 1:3, 1:4 and 1:5, gelatinised at a temperature 80°C for 10 minutes, conduct liquefaction of germinated brown rice starch with α-amylase concentrations 0.3%; 0.4%; 0.5% and 0.6% (approx. 7U/g starch; 9U/g starch; 11U/g starch and 13U/g starch). The survey of conditions is evaluated on DE value and Brix.

2.2.2 Investigate the α-amylase concentration and time affecting the hydrolysis process

After selecting the appropriate substrate concentration from section 2.2.1, the hydrolysis time of the enzyme was investigated at 20, 30, 40, 50, 60 and 70 minutes with *α-amylase* concentrations of 0.3; 0.4; 0.5 and 0.6%. The survey of conditions is evaluated on DE value and Brix.

2.2.3 Investigation of saccharification conditions with the addition of glucoamylase

In this study, the saccharification experiment was investigated simultaneously with 3 factors, each factor has 5 levels (Table 1). In which the values "0″, "+1″ and "-1″ are the values at the center, the value of the upper boundary point and the value of the lower boundary point, respectively. The values of "+α and -α″ are the upper pole values and the lower pole values called "the star points" of the variables considered in the experiment of the experimental design. Because it is not certain that all survey points are within a predetermined range, it is necessary to survey to the upper and lower pole points to evaluate the survey area more effectively. Therefore, the central composite design model was selected for this experimental design. The data were coded for three factors as follows: glucoamylase concentration (X_1), hydrolysis temperature (X_2) and hydrolysis time (X_3) as independent variables and reducing sugar content (Y_{max}) as response dependent. Experimental units in the factorial and axial treatments were repeated 2 times and 4 central treatments. Thus, the experiment was performed with 32 experimental units, including 16 factorial points, 12 axial points (with $\alpha = \pm 1.5$) and 4 central points. The proposed applied polynomial regression equation is as Eq. (1):

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum \sum_{i<j=1}^{n} \beta_{ij} X_i X_j + e \quad (1)$$

Where Y is the dependent variable, β_o is the intercept coefficient; β_i is the coefficient of the quadratic equation, β_{ii} is the coefficient of the quadratic equation of the variable X_i, and β_{ij} is the interaction coefficient and e is the random error.

Table 1. *Data coding for saccharification experiments according to Central composite design*

Coded symbols	***Independent variable***	***Units***	***Levels***				
			-1.5	***-1***	***0***	***1***	***+1.5***
X_1	Enzyme	%	0.15	0.2	0.3	0.4	0.45
X_2	Temperature	°C	35	40	50	60	65
X_3	Time	minute	30	60	120	180	210

Glucoamylase concentrations 0.15; 0.2; 0.3; 0.4 and 0.45% (approx. 45U/g starch; 60U/g starch; 90U/g starch; 120U/g starch and 135U/g starch).

2.2.4 Determination of Reducing sugar content (DE)

The DE value is calculated by the formula: DE (%) = (Reducing sugar content in terms of glucose/dry matter content of the sample) × 100. In which, reducing sugar content is determined by DNS method (3,5 dinitrosalicylic acid) (Miller.,1959) [19], add 1 mL of sample to 3 mL of DNS solution, then heat at 95°C for 15 min and cool rapidly to room temperature. The absorbance of the test sample was measured at 570 nm. Calculate the reducing sugar content based on the glucose standard curve (y = 0.2131x + 0.0528, where y is the reducing sugar content and x is the absorbance).

2.2.5 Statistical analysis

Using the ANOVA test method to test the reliability at the 5% level of significance to evaluate the difference of the results of the experiments, the results are repeated 3 times, using SPSS 20 IBM statistical software and Microsoft Excel 16. The central composite design was designed by Design-Expert 11 from Stat-Ease software.

3. RESULTS AND DISCUSSION

3.1. Effect of substrate ratio and α-amylase concentration in the liquefaction process

3.1.1. Effect of substrate ratio and α-amylase concentration on DE

The results showed that there was an effect between the substrate ratio and α-amylase concentration on the reducing sugar ($p < 0.05$). When changing the substrate ratio from 1 : 5 to 1 : 1 and gradually increasing the α-amylase concentration from 0.3 - 0.6%, the DE content gradually increased and reached the highest at 0.6% with the 1 : 2 substrate ratio.

Table 2. *Effect of substrate ratio and α-amylase concentration on DE*

Substrate ratio	***α-amylase concentration (%)***				
	0.3	***0.4***	***0.5***	***0.6***	***Average***
1:1	3.54 ± 0.350	4.84 ± 0.928	6.63 ± 0.249	7.03 ± 0.304	5.5 ± 1.535
1:2	4.06 ± 0.516	5.77 ± 0.401	6.78 ± 0.142	6.92 ± 0.320	5.8 ± 1.235
1:3	4.27 ± 0.707	4.59 ± 0.860	5.45 ± 0.860	5.83 ± 0.636	5.04 ± 0.874*
1:4	3.28 ± 0.235	4.12 ± 0.258	4.47 ± 0.522	5.00 ± 0.455	4.22 ± 0.732*
1:5	2.76 ± 0.052	3.40 ± 0.110	4.56 ± 0.404	4.50 ± 0.516	3.81 ± 0.843*
Average	3.58 ± 0.670*	4.55 ± 0.915*	5.58 ± 1.102	5.86 ± 1.115	

*Note: *. The mean difference is significant at the 0.05 level.*

However, post-ANOVA by Tukey method showed that there was no statistically significant difference between two 1 : 1 and 1 : 2 substrate ratios, as well as two α-amylase concentrations 0.5 and 0.6% for DE content ($p > 0.05$). Under conditions of suitable substrate concentration, the reaction rate is directly proportional to the enzyme concentration. However, when the enzyme concentration increases to a limit, the reaction rate does not increase anymore, this is consistent with Nguyen Duc Luong's theory [20]. At the 1 : 2 substrate ratio, the average DE value is higher than 1 : 1, so the 1 : 2 ratio was chosen, and to reduce the cost of the production process while still ensuring the desired DE content, an α-amylase concentration 0.5% was chosen for the liquefaction process.

3.1.2. Effect of substrate ratio and α-amylase concentration on Brix

There was an influence between the substrate ratio and α-amylase concentration to the product Brix ($p < 0.05$). When changing the substrate ratio from 1 : 5 to 1 : 1 and gradually increasing the α-amylase concentration from 0.3 - 0.6%, the Brix value increases gradually. The Brix value reaches the highest at 1 : 1 and 1 : 2 substrate ratio with Brix values of (Brix = 27.24 ± 1.774) and (Brix = 27.43 ± 1.442), respectively by Tukey method (Table 3). However, there was no statistically significant difference between these two rates, so the one for the higher Brix value was chosen for this experiment.

Table 3. *Effect of substrate ratio and α-amylase concentration on Brix*

Substrate ratio	***α-amylase concentration (%)***				
	0.3	***0.4***	***0.5***	***0.6***	***Average***
1 : 1	24.67 ± 0.577	27.47 ± 0.462	28.33 ± 0.577	28.50 ± 1.500	27.24 ± 1.774
1 : 2	25.73 ± 0.643	27.00 ± 0.500	27.67 ± 0.577	29.33 ± 0.577	27.43 ± 1.442
1 : 3	24.00 ± 0.000	24.73 ± 0.404	25.67 ± 0.577	25.00 ± 1.000	24.85 ± 0.813*
1 : 4	16.33 ± 0.577	18.20 ± 0.529	19.00 ± 1.000	19.33 ± 0.577	18.22 ± 1.352*
1 : 5	15.33 ± 0.577	16.33 ± 1.155	18.33 ± 0.577	19.23 ± 0.404	17.31 ± 1.736*
Average	21.21 ± 4.616*	22.75 ± 4.802*	23.80 ± 4.475	24.28 ± 4.548	

*Note: *. The mean difference is significant at the 0.05 level.*

Similarly, when increasing the α-amylase concentration from 0.3 - 0.6%, the Brix value increased, reaching the highest at 0.5 and 0.6%, but no statistically significant difference ($p > 0.05$). To reduce research costs, α-amylase concentration 0.5% was prioritized. So, an α-amylase concentration 0.5%, corresponding to a 1 : 2 substrate ratio was chosen as the basis for the next experiment.

3.2. Effect of hydrolysis time and α-amylase concentration in the liquefaction process

3.2.1. Effect of hydrolysis time and α-amylase concentration on DE

There was an interaction between α-amylase concentration and hydrolysis time to reducing sugar content ($p < 0.05$), Table 4 data showed that reducing sugar content of hydrolyzate increases when increasing α-amylase concentration from 0.3 - 0.6% and hydrolysis time from 20 to 50 min.

Table 4. *Effect of hydrolysis time and α-amylase concentration on DE*

Time	***α-amylase concentration (%)***				
(minute)	***0.3***	***0.4***	***0.5***	***0.6***	***Average***
20	1.48 ± 0.235	2.56 ± 0.469	3.20 ± 0.248	3.17 ± 0.592	2.60 ± 0.809*
30	2.16 ± 0.081	3.31 ± 0.301	5.22 ± 0.784	5.67 ± 0.592	4.09 ± 1.548*
40	4.72 ± 0.543	5.77 ± 0.401	6.52 ± 0.121	6.38 ± 0.471	5.84 ± 0.821*
50	5.84 ± 0.219	6.25 ± 0.214	8.78 ± 0.755	9.03 ± 0.465	7.48 ± 1.556*
60	5.53 ± 0.516	6.52 ± 0.210	7.80 ± 0.916	7.59 ± 0.193	6.86 ± 1.058*
70	4.67 ± 0.504	6.28 ± 0.104	7.11 ± 0.477	6.97 ± 0.540	6.26 ± 1.080*
Average	4.07 ± 1.733*	5.12 ± 1.638*	6.44 ±1.941	6.47 ± 1.906	

*Note: *. The mean difference is significant at the 0.05 level.*

When increasing the hydrolysis time from 20 to 50 minutes, the DE value increases proportionally. If the hydrolysis time is continued to be extended to 60 and 70 minutes, the DE value begins to decrease. Since enzyme concentration and hydrolysis time are directly proportional to the reaction rate, at first, the substrate is hydrolyzed to create a large amount of low-molecular dextrin, so when increasing the enzyme concentration and hydrolysis time, the product obtained is larger. Then these dextrins are separated to continue to form shorter chains and are slowly degraded to glucose and maltose, so when prolonging the time up to 60, 70 minutes, the products obtained increase slowly or decrease. This is consistent with Nguyen Duc Luong's theory [20]. At hydrolysis time 50 minutes for highest DE value at 0.5 and 0.6% α-amylase concentrations with a DE of 8.783 and 9.033 respectively. However, there was no statistically significant difference between these two enzyme concentrations ($p > 0.05$).

3.2.2. Effect of hydrolysis time and α-amylase concentration on Brix

Table 5. *Effect of hydrolysis time and α-amylase concentration on Brix*

Time	***α-amylase concentration (%)***				
(minute)	***0.3***	***0.4***	***0.5***	***0.6***	***Average***
20	23.50 ± 0.500	24.33 ± 0.289	24.83 ± 0.289	25.50 ± 0.500	24.54 ± 0.838*
30	23.67 ± 0.577	25.10 ± 0.000	25.80 ± 0.173	26.33 ± 0.577	25.23 ± 1.104*
40	25.73 ± 0.643	27.00 ± 0.500	28.67 ± 0.577	29.00 ± 1.000	27.60 ± 1.502*
50	26.67 ± 0.577	28.00 ± 0.000	31.00 ± 1.000	31.67 ± 0.577	29.33 ± 2.229*
60	26.67 ± 0.577	28.00 ± 0.000	30.00 ± 0.866	30.00 ± 1.000	28.67 ± 1.600
70	26.67 ± 0.577	28.00 ± 0.000	30.00 ± 1.000	30.00 ± 1.000	28.67 ± 1.614
Average	25.82± 1.826*	27.07 ± 1.874*	28.72 ± 2.701	29.08 ± 2.556	

*Note: *. The mean difference is significant at the 0.05 level.*

The ANOVA test showed that there was an interaction between time and α-amylase concentration affecting Brix ($p < 0.05$). Table 5 data showed that Brix reached the highest value after hydrolysis with a time of 50 minutes at α-amylase concentration 0.5 and 0.6% giving a Brix value of 31,000 and 31,667 respectively, but no significant difference ($p > 0.05$). So, an α-amylase concentration 0.5% was chosen as the basis for the next experiment

analysis. The results of liquefaction experiment are similar to those of the previous study by Tu *et al*., 2016 [21], but there are differences compared with Ngoc Hanh *et al*., 2014 [17] because of different sources of raw materials and enzymes.

3.3. Effect of glucoamylase concentration, temperature and hydrolysis time on saccharification

The efficiency of starch hydrolysis by enzymes depends on many conditions, especially glucoamylase concentration, temperature and hydrolysis time. Therefore, this study was conducted with the aim of optimizing the parameters of the hydrolysis process to produce the highest reducing sugar content.

Table 6. *Predicted and experimental value of response*

Sample	*Run order*	*Space type*	X_1	X_2	X_3	*DE (%)*	
						Experimental	*Predicted*
M1	2	Factorial	1	1	1	25.239	25.04
M2	7	Factorial	-1	1	-1	18.984	18.98
M3	8	Factorial	1	-1	-1	18.37	18.19
M4	10	Factorial	1	-1	-1	18.057	18.19
M5	11	Factorial	1	1	-1	23.501	23.45
M6	13	Factorial	-1	-1	1	18.246	18.38
M7	14	Factorial	-1	-1	-1	16.306	16.49
M8	15	Factorial	-1	-1	-1	16.494	16.49
M9	19	Factorial	1	-1	1	20.873	20.38
M10	20	Factorial	-1	-1	1	18.808	18.38
M11	22	Factorial	1	1	-1	23.313	23.45
M12	23	Factorial	1	1	1	25.227	25.04
M13	25	Factorial	-1	1	1	20.436	20.27
M14	27	Factorial	-1	1	-1	18.996	18.98
M15	30	Factorial	1	-1	1	20.061	20.38
M16	31	Factorial	-1	1	1	20.248	20.27
M17	1	Axial	0	-1.5	0	18.106	18.11
M18	4	Axial	-1.5	0	0	18.794	18.88
M19	5	Axial	0	1.5	0	23.464	23.47
M20	6	Axial	1.5	0	0	23.501	23.73
M21	9	Axial	0	0	-1.5	17.982	18.00
M22	17	Axial	0	-1.5	0	17.982	18.11
M23	18	Axial	0	0	-1.5	18.248	18.00
M24	21	Axial	1.5	0	0	23.688	23.73
M25	24	Axial	0	0	1.5	20.123	20.60
M26	26	Axial	0	1.5	0	23.252	23.47
M27	28	Axial	0	0	1.5	20.497	20.60
M28	29	Axial	-1.5	0	0	18.87	18.88
M29	3	Center	0	0	0	21.248	21.42
M30	12	Center	0	0	0	21.436	21.42
M31	16	Center	0	0	0	21.624	21.42
M32	32	Center	0	0	0	21.624	21.42

Carrying out the analysis of the research results, the ANOVA test results are shown in Table 7 and Figure 1 showed actual and predicted DE content from the model.

Table 7. *ANOVA table for the adjusted model of response from enzymatic hydrolysis of germinated brown rice*

Source	***SS[a]***	***df[b]***	***MS[c]***	***F-value***	***p-value***	
Model	185.9678	9	20.66309	348.9329	< 0.0001	significant
X_1	65.32034	1	65.32034	1103.05	< 0.0001	
X_2	79.81993	1	79.81993	1347.901	< 0.0001	
X_3	18.83907	1	18.83907	318.1311	< 0.0001	
$X_1 X_2$	7.713118	1	7.713118	130.2497	< 0.0001	
$X_1 X_3$	0.09015	1	0.09015	1.522344	0.2303	
$X_2 X_3$	0.361502	1	0.361502	6.104595	0.0217	
X_1^2	0.038057	1	0.038057	0.642652	0.4313	
X_2^2	1.148645	1	1.148645	19.3969	0.0002	
X_3^2	13.09844	1	13.09844	221.1903	< 0.0001	
Residual	1.30	22	0.0592			
Lack of Fit	0.4600	5	0.0920	1.86	0.1555	not significant
Pure Error	0.8428	17	0.0496			
R²	0.993					
Adjusted R²	0.9902					
Predicted R²	0.9848					
Adequate Precision	62.7975					

Note: [a] sum of squares, [b] degree of freedom, [c] mean of squares

The experimental data were statistically analyzed using the SAS package for analysis of variance and the results are shown in Table 7. The variance of the quadratic regression model showed that the model is significant with *p-value* < 0.0001. Lack of fit *p-value* > 0.05, this indicates that the model is suitable for all data. The reliability of the model R^2 = 0.993 showed that the survey factors explained most of the experimental results. R^2adj. = 0.990 equivalent to R^2, indicating that the survey factors explained most of the experimental results. The value of the coefficient of variation CV = 1.19% indicated a better precision and reliability of the experiments carried out.

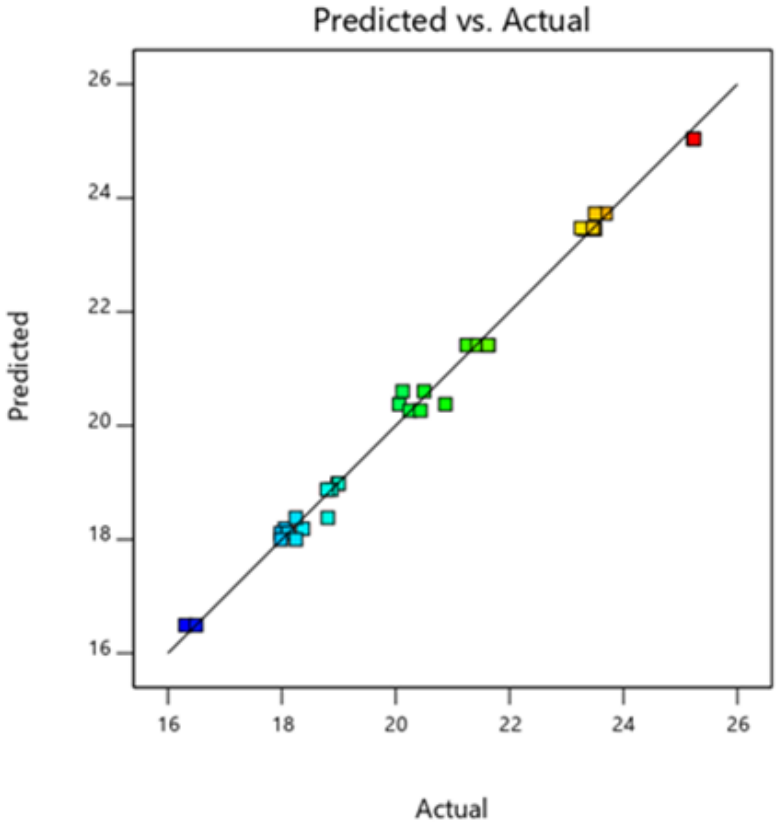

Figure 1. *Actual and predicted DE content from the model*

The application of response surface methodology yielded the following regression Eqation (2).

$$Y = 21.4162 + 1.6164X_1 + 1.7868X_2 + 0.868X_3 + 0.6943X_1X_2 - 0.1503X_2X_3 - 0.2783X_2^2 - 0.9398X_3^2 \quad (2)$$

The results of the regression equations found by solving the equations in the model are only coding variables that take on values when $p < 0.05$, so it is necessary to convert to real variables.

The regression equation for real variables has the form:

$$DE = 4.085 - 17.0129E_1 + 0.2787t_2 + 0.0859T_3 + 0.6943E_1t_2 - 0.0003t_2T_3 - 0.0028t_2^2 - 0.0003T_3^2 \quad (3)$$

Where: E_1 (%): Real variable of enzyme value; t_2 (°C): Real variable of temperature value; T_3 (minute): Real variable of time value.

From the mathematical equation, we optimize the hydrolysis process at which the amount of reducing sugars is the highest required. As a result of three factor optimization, we obtain the results in the Table 8.

Table 8. *Optimal condition results for three factors*

Optimum conditions			DE (%)
Temperture (°C)	***Time (minute)***	***Glucoamylase concentrations (%)***	
59.813	160.468	0.399	25.245

By using the central composite design and response surface methodology, we have accurately determined the glucoamylase concentration, temperature and processing time at which the DE content can be guaranteed to be the highest (Figure 2). This result is similar to the hydrolysis time, higher glucoamylase concentration, but lower DE content compared to Minh Thuy et al., 2015 [18] possibly due to a different enzyme activities and raw materials.

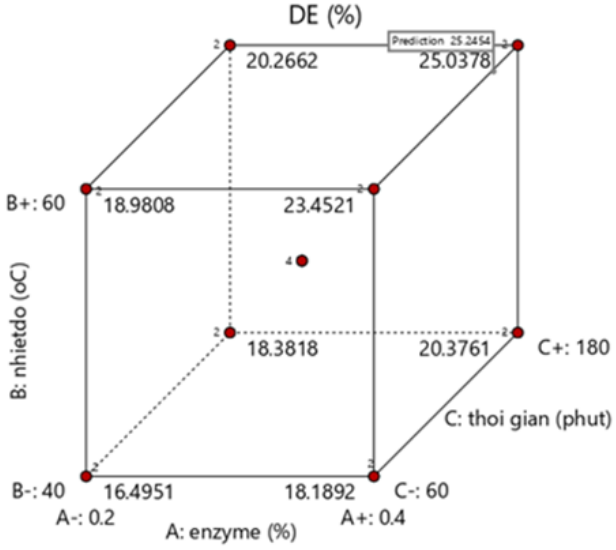

Figure 2. *Cube graph showing DE content by factors of temperature, enzyme concentration and time*

Verification test

To verify the accuracy of the value obtained from the regression equation, three experiments were independently repeated at the condition of enzyme concentration = 0.4%, temperature of 60°C and time = 160 minutes. The results in the Table 9 showed that the results obtained from the experiment reached the DE value of 25.191%, equivalent to the theoretical DE value of 25.245%.

Table 9. *DE results from regression equations and experiments*

		Optimal sample	***Actual sample***	***Control***
Variable	***Glucoamylase, %***	0.399	0.4	-
	Temperature, °C	59.813	60	60
	Time, minute	160.468	160	160
Response	***DE, %***	$25.245^{a} \pm 0.003$	$25.191^{a} \pm 0.005$	$8.985^{b} \pm 0.062$

Note: [a,b]. with the same letter, there is no statistically significant difference at the 0.05 level

The test results once again confirm the high accuracy of the built models. The models can be used to predict DE values under different hydrolysis conditions. Statistical analysis results showed that there is no statistically significant difference between these two values at the 95% level. Meanwhile, the natural sample had a DE value 2.8 times lower than the theoretical DE value. It proves that the intrinsic enzyme activity in the germinated brown rice material after the drying process of semi-finished products for preservation has decreased significantly, so it is necessary to add extra enzymes from the outside to increase the hydrolysis efficiency. So, saccharification conditions were chosen as follows: glucoamylase concentration 0.4% (approx. 120U/g starch), hydrolysis temperature 60°C, and hydrolysis time 160 minutes.

4. CONCLUSION

The enzyme α-amylase is very effective in the starch liquefaction stage, with the selected conditions, the substrate : water ratio is 1 : 2, the concentration of α-amylase 0.5% (approx. 11U/g starch), the hydrolysis time is 50 minutes and temperature of 80°C gives the highest Brix value and reducing sugar content (31 and 8.78%, respectively). The saccharification can be carried out at 0.399% glucoamylase (approx. 119.863U/g starch), the hydrolysis temperature is 59.813°C and the hydrolysis time is 160.468 minutes for high DE content highest. The results showed that the enzyme method gave higher reducing sugar content (DE = 25.245% ± 0.003) than the non-enzymatic method (DE = 8.985 ± 0.062). Optimal model results allow application in small-scale processing under controlled conditions such as research conditions.

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Tối ưu hóa quá trình thủy phân gạo lứt nảy mầm bằng enzyme tạo đường khử cao sử dụng mô hình bề mặt đáp ứng

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Tóm tắt

Quá trình thủy phân gạo mầm bằng cách sử dụng enzym α-amylase và glucoamylase sẽ giúp tăng hàm lượng đường khử, giảm độ nhớt, nâng cao sản lượng dịch sữa so với phương pháp chiết truyền thống. Thí nghiệm dịch hóa được bố trí hai nhân tố là các tỷ lệ cơ chất : nồng độ α-amylase và nồng độ α-amylase : thời gian thủy phân khác nhau. Thí nghiệm đường hóa được thực hiện dựa trên mô hình đa biến theo phương pháp Central Composite Design. Kết quả, tỷ lệ cơ chất 1 : 2, nồng độ α-amylase 0.5% (khoảng 11U/g starch) và thời gian thủy phân 50 phút được chọn làm cơ sở cho thí nghiệm tiếp theo. Phân tích phương sai trong mô hình hồi quy cho thấy mô hình bậc hai có ý nghĩa ($p < 0,0001$). Lack of fit ($p > 0,05$) điều này chỉ ra rằng mô hình phù hợp cho tất cả dữ liệu. Độ tin cậy của mô hình $R^2 = 0,993$ cho thấy mô hình hồi qui đã xây dựng phù hợp với tập dữ liệu 99,3%. CV = 1,19% chứng tỏ độ chính xác và độ tin cậy của các thí nghiệm được thực hiện tốt. Điều kiện tối ưu cho quá trình đường hóa ở nồng độ glucoamylase 0,399% (khoảng 119.863U/g starch), nhiệt độ 59,813°C và thời gian 160,468 phút cho hàm lượng DE cao nhất là 25,245% và cao hơn so với phương pháp không dùng enzym (DE = 8,985 ± 0,062).

Keywords: *nước uống dinh dưỡng, thủy phân tinh bột, đường khử, gạo lứt nảy mầm*