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Optimization of Enzymatic Clarification from Corncob

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ABSTRACT

A major content that was 22.76% total carbohydrate of the corncob could be simply hydrolyzed into reducing sugars by using alpha-amylase. The clarification process using alpha-amylase was optimized by response surface methodology (RSM) in this work. Independent variables including: enzyme amount of 0.05-0.2 %w, time of 60-240 min and temperatures of 80-100 °C were investigated. Their effects were found on the reducing sugar (Glucose content) by a second order central composite design (CCD). The optimum condition was 0.2 %w alpha-amylase, 87.6 °C for 150 min. It could provide the highest amount of 6.21 g/L glucose content in the clarified product.

1. Introduction

Ethanol is an alternative energy source as the clean and safe transportation fuels that can be produced domestically in response today's high-energy demand. This renewable energy has been interesting and rapidly developing to be used for substituting on fossil fuels and reducing pollution. Agricultural residues are used economically as raw materials for the ethanol production (Liu *et al*., 2010; Chena *et al*., 2007). The raw materials can be conveniently classified into three types: (i) sugars such as sugar beet, sweet sorghum and sugar cane, (ii) easily degradable carbohydrates such as corn, rice, wheat barley and corncob, (iii) cellulose such as rice bran, rice straw, wood chips, sawdust and waste from industries (Kreuger *et al*.,

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2011; Zhu *et al.,* 2006). The production steps are pretreatment, hydrolysis and fermentation, respectively. The pretreatment are necessary for increasing the porosity of the materials that are active to next steps. Then the hydrolysis, cellulose and/or carbohydrate molecules are converted into reducing sugars or broken down into corresponding monomers. After that the fermentation is carried out to transform the reducing sugars (fermentable sugars) into ethanol (Balata *et al.,*2008). Corncob is a cheap raw that can give a suitable reducing sugar (glucose) content for ethanol production. The components per 100 g of corncob are 22.76 g total carbohydrate, 7.11 g crude fiber, 1.17 g crude protein, 0.15 g fat, 0.44 g ash and 75.48 g moisture (Agro-Industry department center for export, 2011). The alpha-amylases breaks down the long-chain carbohydrates by acting at random locations of the carbohydrate chain. In this process, the pH is adjusted to be about 6.0 - 6.5 and the reaction is performed for about 2 hours at 95°C (Aiyer, 1995; Das *et al.,* 2011; Reed and Nagodawithana, 1995). The optimum condition for the hydrolysis of prebiotic extracted jackfruit seeds using alpha-amylase enzyme was 0.17 %w enzyme amount at 80 °C for 240 min. The highest reducing sugar content in the product was 3.04 g/L (Bancha *et al.,*2011).

Response surface methodology (RSM) is a statistical technique to identify the effect of individual variable for the optimization of multivariable system. It is widely used in optimizing the bioprocesses by the statistical experimental design method. This method can be employed to determine the optimum processes i.e. pretreatment, hydrolysis and fermentation. In addition, it can enhance production yield, reduce process variability, save time and cost (Wang *et al.,* 2008; Bandaru *et al.,* 2006).

Crucial factors (alpha-amylase amount, time and temperature) for clarification process from corncob in this work would be optimized by RSM to obtain the highest glucose content in the product.

2. Materials and Methods

2.1 Materials and chemicals

The corncob of sweet corn, sugar specie, was obtained from a local market in Hat-Yai, Songkhla province, Thailand. The composition of the corncob is shown in Table 1. Alpha-amylase from *Aspergillus oryzae* was purchased from the Sigma-Aldrich company. Dinitrosalicylic solution (DNS) was used for the analysis of the glucose (reducing sugar) in the products (Miller, 1959). DNS was the mixture of 1% dinitrosalicylic acid, 0.2% phenol, 0.05% sodium sulfite, 1% sodium hydroxide and 20% sodium potassium tartrate that were a laboratory grade.

Table 1: The components of the corncob content.

Test Items	Test Method	Results
Protein	AOAC (Kjeldahl Method)	1.17 %
Crude Fat	AOAC (Soxhlet Extraction Method)	0.15 %
Moisture	AOAC (Loss on Drying at 95-100 °C Method)	75.48 %
Ash	AOAC	0.44 %
Crude Fiber	AOAC (Fritted Glass Crucible Method)	7.11 %
Total Carbohydrate	Calculation	22.76 %
Energy	Calculation	97.07 kcal
Total Sugar	Modified Phenol Sulfuric Method	3.71 %
Reducing sugar	Modified dinitrosalicylic acid method	1.68 g/L

2.2 Pretreatment and pre-hydrolysis (Clarification)

The corncob was firstly cut into small pieces and crushed to be about 2 mm particle size. The 20 g crushed corncob and 100 mL clean water were put into 250 ml screw-capped bottles, and added with 0.05-0.2 %w alpha-amylase. An initial pH was adjusted to be 6.0 by ammonia solution. Then the bottles were immersed in an oil bath at a studied temperature in the range of 80-100 °C for a heating time in the range of 60-240 min with a constant shaking rate of 80 rpm. After that, the clarified products were separated by a fabric filter to get the clear liquid phase product before the analysis of reducing sugar content by a UV-vis spectrophotometer.

2.3 Analytical method

DNS method using a double beam UV-Vis spectrophotometer (Model HP 8453) with UV-Visible Chem-Station software was used to analyze the reducing sugar that was assayed in term of glucose. The reflective light was measured at 520 nm on the spectrophotometer (Chongkhong *et al.,* 2012).

2.4 Experimental design and optimization

Central composite design (CCD) was employed to assign important parameters for investigation. Time (X_1, min), temperature (X_2, °C) and alpha-amylase amount (X_3, %w) were chosen as the independent variables that are shown in Tables 2 and 3. The reducing sugar concentration in the product (Y, g/L) was the dependent output variable. For statistical calculation, the variables were coded according to Equation (1).

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$$x_i = \frac{(X_i - \bar{x}_i)}{\Delta x_j},\ i = 1, 2, 3, \ldots, k \qquad (1),$$

where x_i and X_i are the dimensionless value and the real value of the independent variable, $\bar{x}_i$ is the real value of the independent variable at the center point and Δx_j is the step change.

Table 2: Independent variables for the experimental design.

Variables	Coded levels				
	-1.68	-1	0	1	1.68
Time (min)	60	95	150	205	240
Temperature (°C)	80	85	90	95	100
Alpha-amylase (%w)	0.05	0.08	0.13	0.17	0.2

Table 3: The central composite design matrix employed for the three independent variables (Actual values are given in Table 2)

Run no.	X_1	X_2	X_3
1	1	-1	-1
2	0	0	0
3	-1	1	1
4	-1.68	0	0
5	0	-1.68	0
6	0	0	0
7	-1	-1	-1
8	0	0	1.68
9	1.68	0	0
10	-1	1	-1
11	-1	-1	1
12	0	1.68	0
13	0	0	-1.68
14	1	-1	1
15	1	1	-1
16	1	1	1
17	0	0	0

The 17 experiments (N) were estimated by $N = 2^n + 2n + n_0$ that their operating conditions were performed in Table 3. This design consists of the following three portions:

(1) A complete 2^n factorial design, when n is number of test variables.

(2) n_0 center point ($n_0 \geq 1$).

(3) An additional design, the experimental point at a distant ±α from center, while the distance of the axial point was ± 1.68 ($2^{n/4} = 1.682$ for $n = 3$) calculated by Equation (2).

$$\alpha = (2^n)^{1/4} \qquad (2),$$

where α is the distance of the axial points and n is the number of independent variables. The coefficient of the polynomial model was calculated by Equation (3).

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 \quad (3),$$

where Y is the predicted reducing sugar, X_1, X_2, X_3 are the independent variables, b_0 is the offset term, b_1, b_2, b_3 are the linear effects, b_{11}, b_{22}, b_{33} are the square effects, and b_{12}, b_{23}, b_{13} are the cross effects of the interaction terms (Khuri and Mukhopadhyay, 2010; Bezerra *et al.*, 2008).

3. Result and Discussion

3.1 Components of corncob

Components of the fresh corncob are shown in Table 1. The major components are 22.76 % carbohydrate that can be hydrolyzed to fermentable sugars before transforming into ethanol and 75.48 % moisture that can support the good growth of microorganisms and save water material used in the fermentation process. This showed that the corncob was a potential material for the ethanol fermentation.

3.2 Response surface analysis for the optimization of three factors

The important factors for this clarification, hydrolysis process, to produce the reducing sugars are time, temperature and alpha-amylase amount. This method evaluates the effects of the hydrolysis process, design model used to study interaction of the three factors and to find the optimum condition. The experimental conditions are shown in Tables 2 and 3. The results for central composite design (CCD) are shown in Table 4, the second-order polynomial equation giving the reducing sugar as a function of time (X_1,min), temperature (X_2,°C) and alpha-amylase amount (X_3,%w) was shown as Equation (4).

$$Y=-100.01+0.061X_1+2.235X_2+19.90X_3-0.0000176X_1^2-0.01190X_2^2 +85.42X_3^2-0.000540X_1X_2 -0.040X_1X_3-0.339X_2X_3 \quad (4),$$

The RSM predicted and experimental values of the reducing sugar are given in Table 4. To test the fit of the CCD model, the regression equation and determination coefficient (R^2) were

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estimated. The value of R^2 is 0.906 implied that it was a quite good fit, and that 90.6% of the variation could be explained by the model.

Table 4: Experimental and RSM predicted results

Run no.	X_1	X_2	X_3	Reducing sugar (g/L)	
				Experimental	RSM predicted
1	205	85	0.08	5.64	5.37
2	150	90	0.13	5.26	5.35
3	95	95	0.17	5.25	5.31
4	60	90	0.13	4.82	5.06
5	150	80	0.13	4.37	4.47
6	150	90	0.13	5.36	5.35
7	95	85	0.08	4.76	4.71
8	150	90	0.20	6.17	6.18
9	240	90	0.13	5.30	5.35
10	95	95	0.08	5.18	4.84
11	95	85	0.17	5.71	5.48
12	150	100	0.13	3.75	3.85
13	150	90	0.05	5.20	5.47
14	205	85	0.17	5.61	5.75
15	205	95	0.08	4.90	4.93
16	205	95	0.17	5.15	5.00
17	150	90	0.13	5.39	5.35

From Table 5, the fitting model is predicted by the analysis of variance (ANOVA). The ANOVA of the quadratic regression model indicates that the model is highly significant, because of Fisher's F-test (F-model, mean square regression: mean square residual = 7.53) and a very low probability value (P-model > F = 0.00718). As illustrated in Table 6, some effects of factors and their interactions on reducing sugar concentrations are significant ($p<0.05$) in the ANOVA that indicates a significant effect of the corresponding factors on the response. The p-values from the t-test analysis given in Table 6 are used to determine the significant levels of three process parameters and their interactions on the reducing sugar. The most significant parameter is temperature. The effect of alpha-amylase amount is less significant ($p> 0.05$) so this interaction can be deleted from Equation (4) without significant effect on the accuracy of predicted reducing sugar concentration. (Yu *et al.,* 2009; Wang *et al*., 2013).

Table 5: ANOVA for the full quadratic model

ANOVA					
Source of variation	%Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	Probe > F
Regression	4.397	91	0.489	7.532	0.00718
Residual	0.454	9	0.06487		
Total	4.851	100			

Table 6: Coefficients, t-statistics and significant probability of the model for Equation (4).

Term	Coefficient	Value	Standard Error	t -value	P - value
Constant	b0	-100.01	19.81	-5.048	0.00148
Time (min)	b1	0.06053	0.03149	1.922	0.09605
Temperature (°C)	b2	2.235	0.414	5.397	0.00101
Alpha-amylase (%w)	b3	19.90	38.45	0.518	0.621
Time x Time	b4	-1.76 E-05	2.68 E-05	-0.655	0.533
Temperature x Temperature	b5	-0.01190	0.00226	-5.256	0.00118
Alpha-amylase x Alpha-amylase	b6	85.42	38.89	2.196	0.06408
Time x Temperature	b7	-0.000540	0.000334	-1.617	0.150
Time x Alpha-amylase	b8	-0.04026	0.03705	-1.087	0.313
Temperature x Alpha-amylase	b9	-0.339	0.408	-0.830	0.434

3.3 Interactions among the factors

3.3.1 The effects of alpha-amylase amount and temperature

Figure 1 shows the effects of alpha-amylase amount and temperature on reducing sugar content. The reducing sugar content of clarified product increased with increasing amount of alpha-amylase and temperature in the range of 84.4 to 91.1°C. However, the conversion rate was reduced for a further increase in temperature.

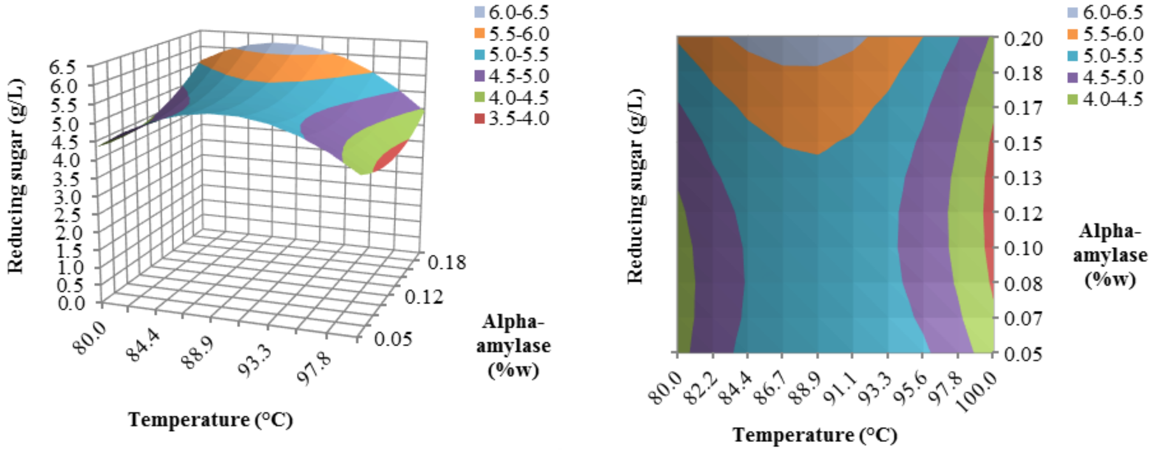

Figure 1: Response surface and contour plot of temperature vs. alpha-amylase on reducing sugar content for 150 min.

3.3.2 The effects of heating time and temperature

The effects of heating time and temperature on reducing sugar content are shown in Figure 2. The reducing sugar increased with an increase in both time and temperature. However a higher temperature from 88.9 to 100 °C caused a reduction in the sugar content. To obtain an optimum reducing sugar content the clarification process should be operated at a temperature in the range of 84.4 to 88.9°C for a time in the range of 150 to 240 min.

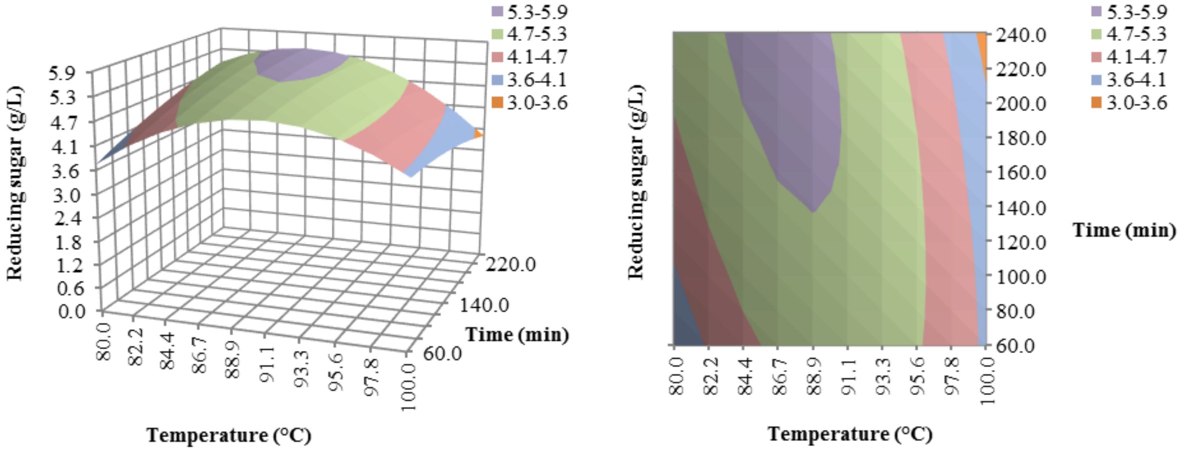

Figure 2: Response surface and contour plot of temperature vs. time on reducing sugar content with 0.13 %w alpha-amylase.

3.3.3 The effects of heating time and alpha-amylase amount

The interaction of time and alpha-amylase amount on reducing sugar content (Figure 3) implies that the clarification process should be carried out for a time in the range of 100 to 180 min with 0.17-0.2 %w alpha-amylase to achieve a maximum content of reducing sugar.

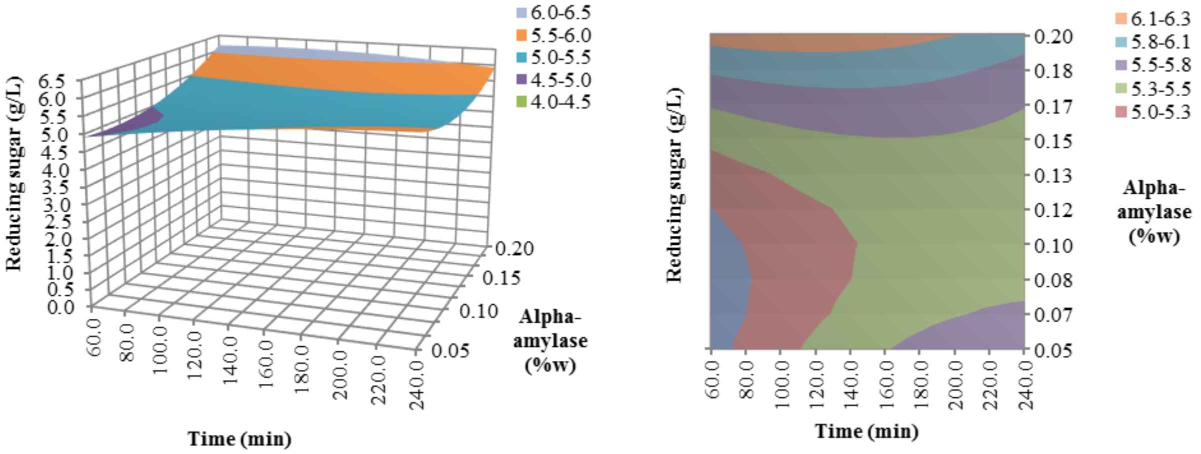

Figure 3: Response surface and contour plot of time vs. alpha-amylase on reducing sugar content at 90 °C.

The results of the influence and interaction of the factors using CCD indicated that the highest yield could be reached near the center point of the operating conditions as on the contour curves. The optimum condition was at 87.6 °C for 150 min with 0.2 %w alpha-amylase which could provide 6.21 g/L for experimental and 6.25 g/L for predicted reducing sugar contents. These showed that the model, Equation (4), could be useful.

4. Conclusion

A clarification step before liquefaction and fermentation steps of the ethanol production from the corncob has been evaluated. The ranges of time, temperature and alpha-amylase amount were established to optimize the operation condition by RSM which could save experimental time and cost. The optimum condition were an alpha-amylase amount of 0.2 %w, a temperature of 87.6 °C and a time of 150 min. that gave the highest amount of 6.21 g/L reducing sugar content.

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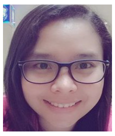

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