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Optimization of sweet cassava (*Manihot esculents crantz.*) crude extract with high maltodextrin level using Response Surface Methodology

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Abstract

The purpose of this study was to optimize the conditions for extracting sweet cassava crude extract by using enzymatic hydrolysis extraction. The optimal extraction condition to prepare sweet cassava crude extract with the highest level of maltodextrin was determined using a Box-Behnken experimental design and the response surface method was applied to obtain the optimized conditions. Three factors at three levels were used in this experiment; enzyme concentration, temperature, and extraction time. In addition, the proximate composition, mineral content and cyanogenic potential were determined using standard methods.The results showed that the optimum extraction conditions were the following: enzyme concentration at 0.3% (w/v), extraction temperature at 95°C, and extraction time at 45 min gave the highest maltodextrin level of the sweet cassava crude extract. From the experiment, the results show that the high percentage yield of sweet cassava crude extract ($P<0.05$) was greater than the enzyme concentration and the extraction time. Furthermore, the cassava crude extract contained moisture content of 9.43 ± 0.05% protein, 3.25 ± 0.34% ash, 2.02 ± 0.09% crude fiber, 2.97 ± 0.41% fat, 1.15 ± 0.08% carbohydrate, 85.75 ± 0.53 and cyanogenic potential at 0.09 ± 0.01 mg HCN/kg.

Introduction

Maltodextrin is a mixture of saccharides with a molecular weight between that of polysaccharides and oligosaccharides with DE lower than 20 (not sweet), which is available as white powder or mostly in a concentrated solution. Maltodextrin is more soluble in water than native starches and it is also cheaper in comparison with other major edible hydrocolloids (Alexander, 1992). The maximum activities of the α-amylase are usually in the pH range between 4.8 and 6.5, but the activity-pH profile and location of the pH optima differ depending on the enzyme source. For production of low DE maltodextrin, BAN 480L (a type of α-amylase) may be used. The major steps in the enzyme conversion of starch are liquefaction and saccharification. In liquefaction, the enzyme hydrolyses the α-1,4-glycosidic bonds in starch (Bravo, 2006).

Sweet cassava (*Manihot esculenta Crantz*.) is an important agricultural plant in Thailand due to the fact that cassava has important used in agricultural sector and connections to manufactured products as well. The main production of the crop is a mostly in the northeast of Thailand, especially in Nakhon Ratchasima (Thailand Tapioca Starch, 2013). The mathematical modeling method seemed to be the most appropriate to apply to the optimization assessment to the extraction process which is widely used in response surface methodology (RSM). This experimental methodology combines mathematics with statistics in order to generate a mathematical model to describe the process, analyze the effects of the independent variables and optimize the processing operations (Myers, 1971). The purpose of the study was to determine the possibility of producing maltodextrin by using α-amylase from sweet cassava and then evaluating the different parameters in the process (enzyme concentration, temperature and hydrolysis time).

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Materials and methods

Sample preparation

Sweet cassava was obtained from Nakhon Ratchasima, Thailand. It was dried at 60°C in a tray dryer and then finely ground (GmbH & Co.KG D-42781, Haan, Germany) to powder, and kept in a vacuum package at 4°C until used.

Proximate analysis

Sweet cassava flour was analysed for moisture, ash, fat, crude fiber and protein by AOAC (2000) with the following results: moisture (AOAC 925.10), ash (AOAC 900.02A), protein (AOAC 928.08), fat (AOAC 963.15), and crude fiber (AOAC 978.10).

Dextrose equivalent determination

Dextrose equivalent determination modified from Lane and Eynon titration (Corn Refiner Association-Method E-26) was used. The determination of the soluble solids (° Brix) was conducted using the refractometer measurement method. The dextrose equivalent was then according to the following equation:

$$\text{Dextrose equivalent (DE)} = \frac{\text{Reducing Sugar in Starch}}{\text{Total Solid Content}} \text{ x } 100$$

Extraction

A suspension containing 30% dry matter was liquefied to make the starch susceptible to further enzymatic breakdown by α-amylase (EC 3.2.1.1, Merk, Darmstadt, Germany) modified as recommended by Kachenpakdee *et al*. (2016) to obtain the pH using starch hydrolysis by α-amylase adjusted to pH 6. The reaction was stopped by HCl 0.1 N at pH 4.2. The hydrolysate was separated by centrifugation (Hettich, Universal 32R, DJB Labcare Ltd.) at 8,000 rpm for 20 min to separate the soluble fraction from the insoluble fraction. The soluble fraction was dried by spray dryer. The powder was kept in a vacuum container at -20°C until used.

Experimental design

The response surface method was applied to identify the optimum levels of the three variables of the extraction enzyme – amylase concentration (w/v), extraction temperature (°C) and extraction time (min). The design of the independent and dependent variables include enzyme concentration (X_1;0.1,0.2 and 0.3 %w/wX_1; 0.1, 0.2 and 0.3 %w/w), extraction temperature (X_2; 90,95 and 100°CX_2; 90,95 and 100°C) and extraction time (X_3; 15, 30 and 45 minX_3; 15, 30 and 45 min). The experiments used the Box-behnken design. The order of the experiments was fully randomized. Data were analyzed by One-Way ANOVA. The data was fitted to the first-order model to obtain the regression coefficient. The model used in the response surface analysis is as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + e$$

The quality of the model was evaluated with R^2 and analyzed by ANOVA. The validity of the developed mathematical model was confirmed by three additional experiments that were performed under the optimum conditions.

Statistical analysis

All experiments were performed in triplicate and reported as the mean ± SD and the p-value at < 0.05 level of significance. The experimental data were analyzed using Design Expert® Software (Version 8.0.7.1, Stat-Ease Inc., Minneapolis, MN, USA) and an analysis of variance (ANOVA). SPSS® software (SPSS Inc., Chicago, IL, USA) was used to perform all the statistical calculations.

Results and discussion

Proximate analysis

The sweet cassava crude extract contained the following: 9.43 ± 0.05% protein, 3.25 ± 0.34% ash, 2.02 ± 0.09% crude fiber, 2.97 ± 0.41% fat, 1.15 ± 0.08% carbohydrate, 85.75 ± 0.53 and cyanogenic potential at 0.09 ± 0.01 mg HCN/kg.

In a previous study, Emmanuel *et al*. (2012) reported that the proximate composition was determined using standard methods. The cassava contained moisture content (33.14-45.86%), protein (1.17–3.48%), ash (1.71–2.34%), crude fiber (1.38-3.20%), fat (0.74-1.49%) and carbohydrates (83.42-87.35%).

Dextrose equivalent

The Dextrose Equivalent (DE) obtained from the sweet cassava hydrolysis (suspension of starch 30%) with varying α- amylase concentrations (0.1, 0.2 and 0.3%) at 100°C is shown in Table 1. Once the relationship between the DE and the hydrolysis time for each starch source under the hydrolysis conditions was established, one fraction was removed from the water bath every 5 min until 90 min of hydrolysis was completed. The results show that the hydrolysis time ranged from 15-45 min, DE<20 produced maltodextrin, but over 45 min, DE >20 produced syrup. So, this study selected the hydrolysis time to

be limited to 45 mins. Moore *et al*. (2005) reported that soluble solids in suspension of cassava and corn starch were evaluated during the enzyme action (α-amylase, THERMAMYL- 120L- NOVO Nordisk). A desirable DE was achieved after 15 min of hydrolysis at 100°C. During 30 min cassava starch and corn starch were used to produce maltodextrin.

Table 1. Dextrose equivalent (DE) obtained from sweet cassava hydrolysis (suspension of starch 30% with vary α- amylase (0.1, 0.2 and 0.3%(w/v) at 100°C.

Hydrolysis time (min)	%DE		
	0.1%	0.2%	0.3%
15	14.43±0.15	14.89±0.58	16.45±0.75
30	17.02±0.39	17.32±0.80	18.14±0.35
45	19.56±0.69	20.04±0.32	22.80±0.12
60	29.28±0.70	29.42±0.18	35.59±0.60
75	42.64±0.81	52.13±0.31	54.07±0.19
90	44.16±0.56	53.74±0.62	62.53±0.32

Optimization of maltodextrin produced by response surface methodology

The production of maltodextrin (Y) from sweet cassava was obtained from all the experiments listed in Table 3. According to Myer (1971), R^2 should be at least 0.80 for a good fit to the model. The high value of the coefficient of multiple determination (R^2 = 0.9172) exhibited that the model adequately represents the experimental results.

Table 3. ANOVA result for the response surface linear model on the yield of maltodextrin.

variables	coefficient
Intercept	+9.58
X_1	+1.10
X_2	-3.80**
X_3	+4.26**
R^2	0.92
Adjusted R^2	0.89
F	40.61**
Adeq. Prec.	20.95
Lack of fit	24.01

*p< 0.05 indicates statistical significance.
**p< 0.01 indicates statistical significance

RSM was used to determine the regression coefficients and statistical significance of the model terms. The model F-value (14.67) and Adequate Precision (20.9542) show that the model can be used to predict the maltodextrin yield. All the variables of extraction to determine the correlation between independent and dependent variables at the interactive had a significant effect (p <0.05), including the optimal extraction condition for obtaining the highest percentage yield as shown in Table 2.

Tables 2 and 3 show the ANOVA results for the suggested linear models for the maltodextrin responses. It can be seen that there was high statistical significance between the multiple regression relationships, the independent variables and the

Table 2. Experiment design and response of independent variables to the extract parameters.

Exp. No.[a]	Independent variables			Maltodextrin (Y, %(w/w))	
	α-amylase % (w/v) X_1	Temperature (°C) X_2	Time (min) X_3	Observed	Predicted
1	0.3	90	30	14.06±0.29b	14.48
2	0.1	100	30	3.98±0.22g	4.68
3	0.1	95	45	12.60±0.37c	12.74
4	0.2	95	30	11.01±0.05d	9.58
5	0.2	100	15	1.70±0.05h	1.53
6	0.1	90	30	12.51±1.15c	12.28
7	0.3	100	30	5.87±0.35f	6.89
8	0.2	100	45	8.59±0.02e	10.04
9	0.2	95	30	10.53±0.11d	9.58
10	0.1	95	15	4.05±0.20fg	4.22
11	0.2	90	15	9.37±1.26de	9.12
12	0.2	90	45	14.94±0.21b	17.63
13	0.2	95	30	11.40±0.06d	9.58
14	0.3	95	45	17.34±0.03a	14.93
15	0.3	95	15	5.02±0.11f	6.42
	X_1	X_2	X_3		
p-value	0.0608	<0.0001	<0.0001		

[a] Experiments were conducted in a random order.

maltodextrin responses. The probability (p) values of the regression models were less than 0.01, which is statistically significant. The R^2 values, adjusted R^2 values and predicted R^2 for the responses were 91.72, 89.46 and 84.39, respectively. This demonstrates a good correlation between the independent variables and the responses. The model was stronger and the predicted responses better as the R^2 values became closer to 1.0000. A regression model, with R^2 value greater than 0.8000, was considered to have a high correlation (Jaya *et al*., 2010). The ANOVA for the lack of fit test for responses was insignificant ($p > 0.05$) which demonstrates that the model was adequately fitted to the experimental data for the responses. The highest percentage of maltodextrin production was obtained when the enzyme concentration was at 0.3% (w/v), the extraction temperature at 95°C and the extraction time at 45 min that produced highest maltodextrin level at 17.34% (w/w). While replacing the value of the optimum conditions into

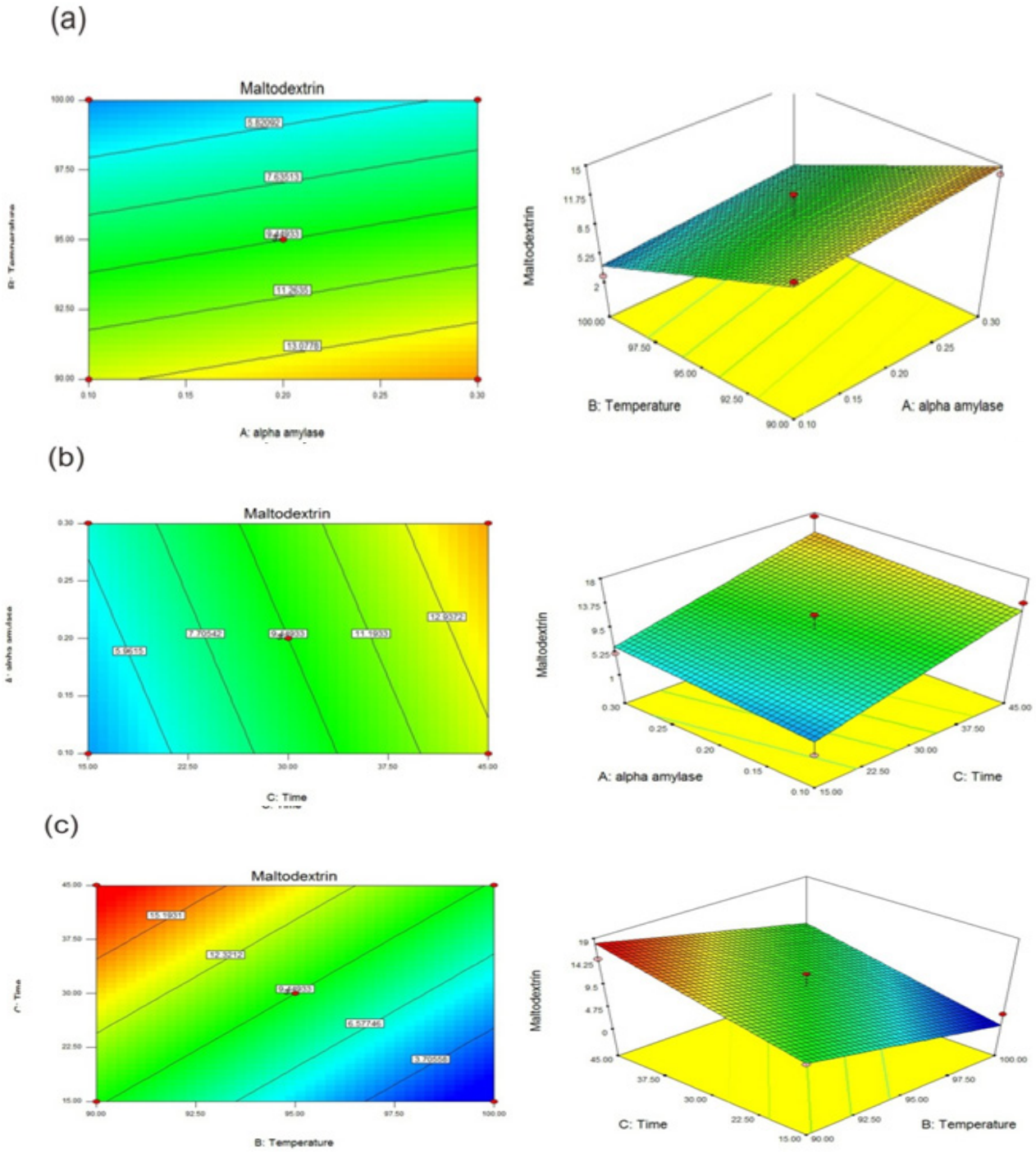

Figure 1. Response surface plots (a) the interaction effect of α-amylase concentrationsand extraction temperature on maltodextrin production at extraction time 45 min, (b) effect of α-amylase concentrations and extraction time on maltodextrin production at extraction temperature 95ºC, (c) effect of extraction temperature and extraction time on maltodextrin production at α-amylase concentrations 0.3%w/v.

the regression equation obtained:

Y= 9.58+ 1.10X1 -3.80X2 +4.26X3; linear

The equation showed predicted yield value is 14.94% (w/w). The results from the experiment show that the extraction temperature and extraction time affected to the maltodextrin percentage yield ($p < 0.01$) more than enzyme concentration. In addition, data was analyzed for correlation between independent variables and maltodextrin percentage. A statistical analysis showed p -value less than 0.01 which is significant.

The response surfaces are illustrated with three-dimensional plots which represent the responses according to two factors (enzyme concentration and extraction time) and with the other constant (extraction temperature). The highest maltodextrin level shown in relation to α-amylase concentration, extraction temperature, and extraction time are shown in Figure 1. This shows that maltodextrin decreased as the temperature increased from 90 to 100°C because of α-amylase Termamyl 120 L. Some studies on starch hydrolysis have used Bacillus a-amylases which showed reasonable activity at temperatures between 70 and 100°C (Manoj *et al*., 2005; Baskar *et al*., 2008). Maltodextrin started to increase when the enzyme concentration and extraction time increased.

Model verification

Optimization was carried out to determine the optimal parameters in the process of maltodextrin extraction using the response surface method optimization procedure as shown in Table 4. The selected optimum parameters in this study were an enzyme concentration of 0.2% (w/v), the extraction temperature at 95°C and the extraction time of 30 min. Based on the triplicate runs by using the recommended optimum parameters, the mean values were 10.99 ± 0.07 for maltodextrin production. The experimental values and predicted values and their p values were analyzed by using a t-test. The results show that there was no statistical significance ($p > 0.05$) in the experimental and predicted values of the responses, which indicates that the models were sufficient to predict the maltodextrin response. These results indicate that there is an excellent correlation between the experimental and predicted results which in turn proves the validity of the model.

Table 4. Experimental and predicted values of maltodextrin response from optimized paramaters.

Response	Experimental	Predicted	*p*- value
Maltodextrin	10.99±0.07	9.58	0.52

$p < 0.05$ indicates statistical significance.

Conclusion

The optimum conditions for the maximum production of maltodextrin from sweet cassava was determined using a randomized Box-Behken design and the data was statistically analyzed using ANOVA. The conditions show that enzyme concentration at 0.3% (w/v), extraction temperature at 95°C and extraction time at 45 min produce the largest amount on maltodextrin from sweet cassava crude extract. It is recommended that future research shouldstudy the functional properties of food products for the use of endurance athletes.

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