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Response surface optimization of acid modification on mung bean starch

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Abstract

This research shows the dependence of number average degree of polymerization (DP_n) of mung bean starch (MBS) on starch slurry concentration, acid/starch slurry ratio, time and temperature in acid modification process. Optimizing these factors to earn as low as possible DP_n was evaluated by using the Response Surface Methodology (RSM) with reduced Central Composite Face-Centered (reduced CCF) model. Optimal result is DP_n of 795.6 (y) with 29.76% starch slurry (x_1), 4.9:1 acid/starch slurry ratio (x_2), treating in 120 min of time (x_3) and 47.2°C of temperature (x_4). Pasting viscosity of MBS decreased after the treatment and DE (dextrose equivalent) was not detected.

Introduction

Scientific name of mung bean is *Vigna radiata.* Mung bean originated from northeastern India - Burma and Central Asia (Mogotsi, 2006). In most of the countries, mung bean was consumed in some different forms, for instance whole bean, bean sprouts, green bean soups, desserts, porridge and noodles. It was a source of quality nutrients, nearly 63% carbohydrate (Muhammad, 2013). MBS is extracted from ground mung beans. Amylose content of MBS is high, from about 33.6 - 37.9% (Xu *et al*., 2013) to 40.69% (Thao and Noomhorm, 2011). This makes MBS starch have potential use widely in food industry.

Optimization of conditions for processing is one of the most critical stages in the development of an efficient and economic bioprocess. Statistical methodologies involved used mathematical models for designing fermentation processes and analyzing the process results (Bas *et al*., 2007). RSM, originally described by Box and Wilson (1951), is a collection of mathematical and statistical techniques which are useful for designing experiments, building models, and analyzing the effects of several independent factors. The main advantage of RSM is the reduced number of experiments needed to evaluate multiple factors and their interactions. Also, the study of the individual and interactive effects of these factors will be helpful in the effort to find the target value. Therefore, RSM provides an effective tool for investigating the factors affecting the desired response if there are many factors and their interactions in the experiment. RSM can be employed to optimize the experimental process by determining a suitable polynomial equation for describing the response surface.

In the main aim of this study, we researched into the effect of starch slurry concentration, acid/starch slurry ratio, time and temperature on DP value of MBS and focused on explain the relationship of these factors and find out the optimal formula to modify MBS by 0.5N HCl.

Material and Methods

Material

MBS (81% starch) was the product of Pine Tree brand, Thailand. The MBS was white, smooth powder, no odor, taste or mould. Moisture content was 9.68 ± 0.06%. Protein was not detected. MBS was preserved in LDPE bag at 29.5 ± 1.3°C, relative humidity 71 ± 11.37%.

Acid modification

A demand of starch slurry was acid modified as described by Ali and Kempf (1986) using 0.5N HCl at an exact temperature, for a certain time. The slurry was stirred continuously during the treatment period, neutralized with 1N NaOH at the end. The slurry was then repeatedly washed with water and centrifuged to recover the starch during 5 minutes with speed 5000 r/m. This starch was dried at 50°C, 3 hour until the moisture content was lower than 12%.

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Number average degree of polymerization (DP_n)

Modified MBS was formed with the process below:

MBS → Mixing with distilled water and HCl → Modifying → Neutralizing → Centrifuging → Drying → Grinding → Sifting → Modified MBS

1g acid modified MBS was weighed on an analytical balance with 0.0001 mg readability, dispersed in 35 ml distilled water and added 60 ml KIO_4 saturated solution, mixed well. The slurry was then placed in dark at room temperature for 24 h. After the reaction, 7 ml ethylene glycol was added, mixed in 10 min. Then, the slurry was titrated with 0.01N NaOH.

The formula to calculate DP_n:

$$DP_n = \frac{a.3.1000}{162.C_{N\,NaOH}.V_{NaOH}}$$

a (g) = weigh of acid modified MBS.

$C_{N\,NaOH}$ (N) = Normal concentration of NaOH.

V_{NaOH} (ml) = Volume of NaOH.

(Mejzler *et al*., 1962)

Experimental design

Response surface methodology (RSM) was used to determine optimum conditions for modifying MBS by acid. Four factors including starch slurry concentration (x_1), acid/starch slurry ratio (x_2), treatment time (x_3), treatment temperature (x_4) (obtained DP_n of starch - y) were determined using optimization method (Table 1). Influence of factors to target function was described according to equation below:

$$y = b_o \sum_{i=1}^{n} b_i x_i + (\sum_{i=1}^{n} b_{ii} x_i)^2 + \sum_{i<j}^{n} b_{ij} x_i x_j \quad (1)$$

In this study, n-value was 4 so equation (1) can be written:

$$y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{44}x_4^2 \quad (2)$$

Using model in this case was reduced Central Composite Face (CCF). The star points are at the center of each face of the factorial space, so α = ±1. CCF designs provide relatively high quality predictions over the entire design space and do not require using points outside the original factor range. Requires 3 levels for each factor (Natrella *et al*., 2003; Cheynier *et al*., 1983). Reduced CCF design valid for four factors. The design is composed of the fractional part of the design reduced from 24 to 20 runs. The design contained a total of 23 experimental trials with the 3 replications of the central points.

Table 1. Codes and actual levels of the in dependent variables for design of experiment

Independent variables	Symbols	Coded levels		
		-1	0	+1
Starch slurry concentration (%)	X_1	20	30	40
Acid/starch slurry ratio (v/w)	X_2	3:1	4:1	5:1
Time (min)	X_3	60	90	120
Temperature (°C)	X_4	40	50	60

Statistical analysis

The DP_n was determined by actual response value. The data reported represented its mean. Statistical significance was evaluated using the Analysis of Variance (ANOVA) and p-value < 0.05 was considered as significant. Optimum parameters were defined by the software Modde version 5.0.

Dextrose equivalent (DE)

DE was determined according to methods: 945.66 of AOAC (2000).

Paste viscosity

Paste viscosity was measured and modified slightly by using a Brookfield DV-III, spindle number 5 at a spin rate of 250 rpm. Starch samples suspension in water (10% solid) was heated from 31 to 95°C at a heating rate of 3.2°C min^{-1}, held at 95°C for 10 min, cooled to 50°C at 2°C min^{-1}, and then held at 50°C for 10 min (Chung *et al*., 2003). Recorded parameters were: peak viscosity (PV), hot paste viscosity (HPV, minimum viscosity at 95°C), cool paste viscosity (CPV, final viscosity at 50°C), breakdown (BD = PV - HPV), and setback (SB = CPV - HPV) (Sodhi *et al*., 2009).

Results and Discussion

Optimization

Number average degree of polymerization (DP_n) was determined according to Table 2. The p-value was used as a tool to check the significance of each of the coefficients, which in turn indicated the pattern of the interactions between the variables. The smaller value of p was more significant to the regression. According to the ANOVA table (Table 3), the regression model was significant at the considered confidence level (95%) since the regression had p-value < 0.05. After eliminating insignificant factors which had p-value > 0.05 from Table 4, the regression equation of DP_n was determined as below:

$$y = 874.019 - 34.466x_1 - 99.415x_2 - 52.941x_4 + 83.088x_1^2 + 82.22x_2^2 + 63.172x_4^2 - 38.761x_1x_2 - 37.105x_2x_3 + 44.775x_2x_4 + 47.916x_3x_4 \quad (3)$$

With first-level coefficients, then y was inversely proportional to the variables x_1, x_2 and x_4. This meant the increase in starch slurry concentration (x_1), ratio

Table 2. Three level factorial composite design and experimental responses of dependent variable y (Number average degree of polymerization, DP_n)

Run No	Coded levels x_1	x_2	x_3	x_4	Real values X_1	X_2	X_3	X_4	DP_n Observed	Predicted(*)
1	-1	-1	-1	-1	20	3	60	40	1254.18	1251.73
2	+1	-1	-1	-1	40	3	60	40	1377.47	1377.53
3	-1	+1	-1	-1	20	5	60	40	1104.35	1115.08
4	+1	-1	+1	-1	40	3	120	40	1347.7	1344.92
5	-1	+1	+1	-1	20	5	120	40	903.508	913.191
6	+1	+1	+1	-1	40	5	120	40	916.185	904.806
7	-1	-1	-1	+1	20	3	60	60	938.8	950.243
8	-1	+1	-1	+1	20	5	60	60	980.23	992.696
9	+1	+1	-1	+1	40	5	60	60	983.837	983.901
10	-1	-1	+1	+1	20	3	120	60	1078.75	1088.43
11	+1	-1	+1	+1	40	3	120	60	1266.21	1255.54
12	+1	+1	+1	+1	40	5	120	60	982.4	994.531
13	-1	0	0	0	20	4	90	50	974.198	922.641
14	+1	0	0	0	40	4	90	50	978.998	991.574
15	0	-1	0	0	30	3	90	50	1060.94	1055.65
16	0	+1	0	0	30	5	90	50	890.52	856.825
17	0	0	-1	0	30	4	60	50	910.53	878.21
18	0	0	+1	0	30	4	120	50	863.449	856.788
19	0	0	0	-1	30	4	90	40	994	990.133
20	0	0	0	+1	30	4	90	60	919.364	884.25
21	0	0	0	0	30	4	90	50	832.415	874.019
22	0	0	0	0	30	4	90	50	831.974	874.019
23	0	0	0	0	30	4	90	50	840.72	874.019

(*): Running the software Modde version 5.0 to predict obtained model

Table 3. Analysis of variance (ANOVA) for the fitted quadratic polynomial model for DP_n

y	DF	SS	MS	F	p	SD
Total	23	2.40301e+007	1.04479e+006			
Constant	1	2.34638e+007	2.34638e+007			
Total Corrected	22	566356	25743.5			160.448
Regression	14	554439	39602.8	26.5851	**0.000**	199.004
Residual	8	11917.3	1489.66			38.5961
Lack of Fit	6	11868.7	1978.12	81.4824	0.012	44.4761
Pure Error	2	48.5533	24.2767			4.92714

of acid/starch slurry (x_2) and temperature (x_4) resulted in the decrease of DP_n (y), and vice versa. Among these four factors, acid/starch slurry ratio had greatest influence in the modification, then the temperature, concentration of starch slurry and treatment time in turn.

With second-level coefficients, y was directly proportional to the x_1^2, x_2^2, x_4^2, x_2x_4, x_3x_4 and inversely to x_1x_2, x_2x_3. Thus, beside the single effects of starch slurry concentration, acid/starch slurry ratio and temperature on the DP_n, there were also the interaction between the acid/starch slurry ratio with starch slurry concentration (x_1x_2), with treatment time (x_2x_3) and temperature (x_2x_4). In addition, the interaction between time and temperature (x_3x_4). With the single variables, the important role increased respectively temperature (x_4^2), acid/starch slurry ratio (x_2^2) and starch slurry concentration (x_1^2). With interactive variables, the interaction between time and temperature (x_3x_4) had the greatest impact on DP_n, subsequently the interaction between acid/starch slurry ratio with temperature (x_2x_4), with the time (x_2x_3) and starch concentration (x_1x_2).

The regression equation reflected the accuracy of the experimental model, and this was confirmed by the correlation coefficient R^2 = 97.9%, meaning that

Table 4. Results of regression analysis of the full factorial model

y	Coeff. SC	Std. Err.	P	Conf. int (±)
Constant	874.019	14.2865	5.66404e-012	32.9447
x_1	34.4664	11.9561	0.0204249	27.5707
x_2	-99.4145	12.2052	3.83533e-005	28.1451
x_3	-10.7114	11.9561	**0.396466**	27.5707
x_4	-52.9411	12.2052	0.00248641	28.1451
$x_1 * x_1$	83.0882	24.1531	0.00882305	55.6971
$x_2 * x_2$	82.22	24.1531	0.00930371	55.6971
$x_3 * x_3$	-6.52056	24.1531	**0.794018**	55.6971
$x_4 * x_4$	63.1721	24.1531	0.0308663	55.6971
$x_1 * x_2$	-38.7611	13.3003	0.0194602	30.6704
$x_1 * x_3$	5.21455	11.8055	**0.670398**	27.2235
$x_1 * x_4$	5.11197	13.3003	**0.710733**	30.6704
$x_2 * x_3$	-37.1045	13.3003	0.0235671	30.6704
$x_2 * x_4$	44.7752	13.0107	0.0088056	30.0028
$x_3 * x_4$	47.9162	13.3003	0.00695547	30.6704
N = 23	Q^2 =	0.759	Cond. no. =	5.8726
DF = 8	R^2 =	0.979	Y-miss =	0
	R^2_{Adj} =	0.942	RSD =	38.5961

Table 5. Result of optimal condition

Result	x_1	x_2	x_3	x_4	y
Predictive model	29.7556	4.897:1	119.984	47.2066	795.599
Experimentation	30	4.9:1	120	47	803.7±2.7

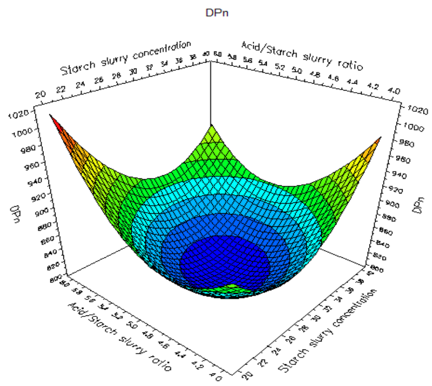

Figure 1. Response surface plot of starch slurry concentration versus acid/starch slurry ratio

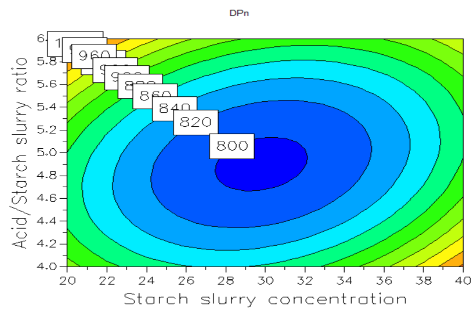

Figure 2. Isoresponse contour plot of starch slurry concentration versus acid/starch slurry ratio

equation (3) could explain 97.9% of the actual data. The predictive power of the model was represented by Q^2 which the estimation achieved 75.9%. From Table 4, we found that all the factors affecting the DP_n. The value of $Q^2 > 0.5$, $R^2 > 0.8$ and $R^2 - Q^2 < 0.3$, we assumed that the regression model was good (Eriksson *et al*., 2008).

Using Modde 5.0, the optimal condition, which obtained from regression equation, was 29.76% starch slurry, 4.9:1 acid/starch slurry ratio, treating in 120 min, at 47.2°C (Table 5). We re-tested with the received parameters in three times. The result showed

Table 6. Pasting viscosity parameters

Sample	Parameter				
	PV (cP)	HPV (cP)	CPV (cP)	BD (cP)	SB (cP)
Native MBS	396.8	166.4	-	230.4	-
Modified MBS	54.4	48	96	6.4	48

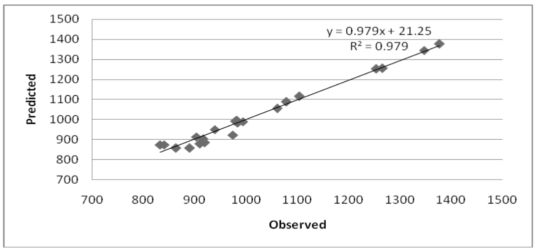

Figure 3. Parity plot showing the distribution of experimental versus predicted values by the mathematical model of the y values.

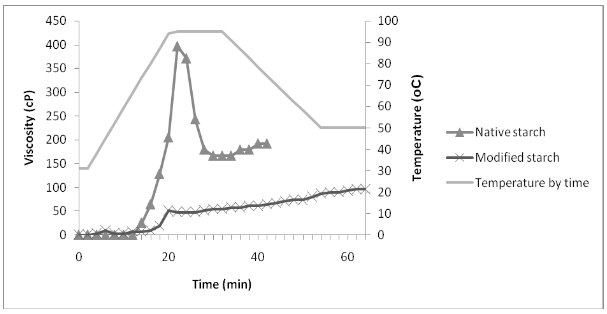

Figure 4. Pasting viscosity of native MBS and acid modified MBS

that the difference between the experimentation and the predicted model was 1.02% (<5%). This indicated a fine accuracy of predictive model.

In Figures 1 and 2, the three-dimensional and two-dimensional contour plots were displayed according to equation (3). The graphs determined the contribution of the starch slurry concentration and acid/starch slurry ratio on DP_n. The contours around the stationary point were elliptical and became elongated more and more along either axis. The lowest DP_n (y_{min}) was received when acid/starch ratio was from 4.7:1 to 5.1:1, starch slurry concentration was from 27% to 32% (w/w), treating in 120 min, at 47°C. In the Figure 3, the predicted results and experimental results had linear correlation with each other. The correlation coefficient R^2 = 0.979. This meant the DP_n results obtained from the experimentation were suitable to 97.9% with the predictive model.

Dextrose equivalent (DE)

Native MBS had high starch content (81%) and DE was 0 because the hydrolysis of starch did not take place and starch chains should not be cleaved into dextrose (Orthoefer and Eastman, 2004). According to the research of Singh and Ali (1997), at the same starch slurry concentration and treatment temperature, the development of hydrolysis time and acid amount would increase DE value. Meanwhile, we applied the method of Ali and Kempf (1986) for the purpose of finding out the optimal parameters for modified starch. The product was the starches having smaller DP_n obtain some suitable properties such as: high solubility at room temperature, low viscosity. Our conditions were 4.9:1 acid/starch slurry ratio and 120 min of treatment, so the starch hydrolysis could not thoroughly break all of linkages down to create glucose or maltose. Therefore, the DE of acid modified MBS in this study was not in the limit of detection.

Paste viscosity

In Figure 4 and Table 6, the viscosity of starch before and after acid modification had a significant change. Peak viscosity (PV) and hot paste viscosity (HPV) of native MBS recorded respectively 396.8 and 166.4 cP. The native starch samples were quickly frozen into hard gel around 75°C during the cooling period. This happened mainly because of amylose degradation (Gupta, 2011).

For modified starch samples, the peak viscosity, hot paste viscosity, cool paste viscosity were collected full value respectively 54.4, 48 and 96 cP. The breakdown dropped from 230.4 to 6.4 cP after the modification. The decrease in peak viscosity of acid modified starches might be the result of the decrease in swell ability and increase in solubility (Sohdi *et al.*, 2009). Li and Corke (1999) observed that higher amylose containing starch showed a lower value of breakdown (BD) indicating great resistance to shear thinning. Thus, the MBS after modification had higher amylose content, soluble rate and capacity of the starch particles increased leading to lower value of breakdown.

The reduction of viscosity corresponded with the reduction of DP_n. This relationship agreed to the result of Manphakdee (1999), who researched on the acid hydrolysis of rice flour. When starch was modified by acid, the solubility of starch improved. Because during the modification, H^+ ions had change to penetrate and hydrolyze within starch granules. DP_n decreased, so short starch chains increased and starch molecular weight reduced. This made it easy for starch to diffuse in water (Hoang and Hanh, 2007).

Conclusion

Conventional optimization studies are time consuming and expensive. To overcome these problems, a RSM was used for the optimization of process conditions. From the present study, it is evident that the use of statistical process condition optimization approach, full factorial model has helped to locate the most significant conditions with minimum effort and time. Using the optimal method of target function, we exposed regression equation and this equation can be applied on actual model:

$$y = 874.019 - 34.466x_1 - 99.415x_2 - 52.941x_4 + 83.088x_1^2 + 82.22x_2^2 + 63.172x_4^2 - 38.761x_1x_2 - 37.105x_2x_3 + 44.775x_2x_4 + 47.916x_3x_4$$

Based on the regression equation, four factors including the starch slurry concentration (x_1), acid/starch slurry ratio (x_2), treatment time (x_3) and temperature (x_4) effected the acid modification of MBS. These factors had independent and interactive effects on DP_n. The optimal parameters were 29.76% starch slurry, 4.9:1 acid/starch slurry ratio, 120 min and 47.2°C of treatment. The model had high R^2, R^2_{adj} and Q^2. We have every confidence in practical applications of acid modified MBS.

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