Optimization of the ultrasonic treatment for improving catalytic activity of glucoamylase preparation

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ABSTRACT

In this work, ultrasonic treatment was used for improving the catalytic activity of glucoamylase preparation Dextrozyme GA. The ultrasonic temperature, power and time were optimized by a Central Composite Circumscribed design for maximizing of the catalytic activity of the preparation. The optimal ultrasonic temperature, power and time were 30°C, 20 W/mL and 33 sec, respectively. Under these conditions, the maximum glucoamylase activity was 83.142 ± 0.213 KU/mL and this value increased 11 % in comparison with that in the control without ultrasonic treatment. Our results also showed that V_{max} and K_M of the sonicated Dextrozyme GA preparation were higher than those of the control. The ultrasonic treatment would be a potential method for improving the catalytic activity of the glucoamylase preparation in starch hydrolysis.

Keyword: *glucoamylase, optimization, ultrasonic treatment,*

1. INTRODUCTION

In food industry, ultrasonic treatment can be considered as a potential method for enzyme inactivation. Ultrasound generated cavitation that could cause the change in protein structure and reduce enzyme activity [1]. Under mild treatment conditions, however, ultrasound could increase enzyme activity. This phenomenon was observed for different enzymes including amylase [2], [3], cellulase [4], dextranase [5], pectinase [6]... It was explained that slight modification of protein conformation facilitated the formation of enzyme-substrate complex and that resulted in an improved catalytic activity of the sonicated enzyme preparation [1].

Recently, our study showed that the ultrasonic treatment of glucoamylase preparation Dextrozyme GA under certain circumstance could improve the enzyme activity [7]. Nevertheless, optimal sonication conditions

for maximizing catalytic activity of enzyme preparations have never been reported.

The objective of this study was to optimize the sonication conditions for maximizing glucoamylase activity of the preparation as well as to compare kinetic parameters of the sonicated and unsonicated glucoamylase preparations.

2. MATERIALS AND METHODS

2.1. Materials

Dextrozyme GA produced from a genetically modified strain of an *Aspergillus sp.* with an activity of 270 amyloglucosidase units per gram was purchased from Novozymes, Denmark. The maltodextrin with dextrose equivalent (DE) of 20 used as substrate, 3,5-dinitrosalicylic acid and citrate phosphate buffer were purchased from Merck - Schuchardt OHG and KGaA (Germany).

2.2. Experimental methods

Samples of 15 mL enzyme preparation were taken in 50 mL beakers and sonicated with a horn type ultrasonic probe (Sonic Vibra-Cell VC 750, The United States) at the frequency of 20 kHz. During the sonication, the beakers were placed in a thermostatic water bath (Memmert, Germany) for temperature regulation. At the end of the treatment, the amylase activity of the sonicated and unsonicated samples was determined.

2.2.1. Optimization of ultrasonic treatment for improving the catalytic activity of the glucoamylase preparation

Our preliminary study showed that the catalytic activity of Dextrozyme GA was strongly improved when the ultrasonic temperature, power and time were 30°C, 20W/mL and 30s, respectively. These values were therefore selected as central conditions for optimization experiment.

Ultrasonic treatment of Dextrozyme GA preparation was optimized by Central Composite Circumscribed design with 3 variables and 5 levels (Table 1). The dependant variable was amylase activity (KU/mL). The software Modde (version 5.0) was used to generate the experimental planning and to process data. The experiment included 20 points (Table 2).

Table 1. Independent variables and their levels in the response surface design

Independent variables	Coded level				
	$-2^{3/4}$	-1	0	+1	$+2^{3/4}$
X_1 – Ultrasonic temperature (T – °C)	13	20	30	40	47
X_2 – Ultrasonic power (P – W/mL)	12	15	20	25	28
X_3 – Ultrasonic time (t – sec)	5	15	30	45	55

The second order polynomial equation was as follow:

$$Y_{coded} = b_0 - b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}1X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (1)$$

Where Y_{coded} was the response variable (amylase activity), X were the coded independent variables (Table 1), and b were the regression coefficients.

The analysis of variance was conducted, the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significance of each coefficient in the equation was tested using the Student t-test. The regression coefficients were used for statistical calculations to generate the response variable.

2.2.2. Comparison of kinetic parameters of the sonicated and unsonicated glucoamylase preparations

In this experiment, the ultrasonic temperature, power and time were selected from the results of section 2.2.1. The sonicated and unsonicated enzyme preparations in phosphate buffer were used. Kinetic parameters Km and Vmax of the sonicated and unsonicated enzyme preparations were determined by Lineweaver-Burk method using various maltodextrin concentration (0.05-0.20% w/v). The experiment was carried out at 65^{o}C and pH 4.0.

2.3. Analytical methods

Amylase activity was assayed by the modified method of Bernfeld (1955). 0.5mL of 0.4% (w/v) maltodextrin solution was mixed with 0.5mL of citrate phosphate buffer (pH 4.0) and 0.1mL of the enzyme solution (The dilution factor for the enzyme preparation was 10000 times) [8]. The mixture was incubated at 65°C for 5 min. The reaction was stopped with 1mL of 3,5-dinitrosalicylate reagent. The mixture was then kept in boiling water for 5min, cooled to the ambient temperature and measured for absorbance at 540nm using UV–visible spectrophotometer (Genesys 6, The United States). One unit (U) of glucoamylase preparation was defined as the amount the enzyme that liberates 1μmol of reducing sugar as glucose in 1mL for 1min under the assay conditions.

2.4. Statistical analysis

All experiments were performed in triplicate. The experimental results were expressed as means ± SD. The data was analyzed for statistical significance by Analysis of Variance (ANOVA). Multiple Range Test with the Least Significant Difference ($LSD_{0.05}$) was applied in order to determine which means are significantly different from which others by using STATGRAPHICS © Plus for windows 3.0.

3. RESULTS AND DISCUSSION

3.1. Optimization of the ultrasonic treatment for improve amylase activity of glucoamylase preparation

Table 2 presents the amylase activity in function of ultrasonic temperature, power and time. The estimated effects of the independent variables on amylase activity are shown in Table 3. All the quadratic and cross-product coefficients were significant ($P < 0.05$). One linear coefficient was eliminated in the refined equation as its effect was not significant. Neglecting the insignificant term, the regression equation for coded values and actual experimental values were given as Equation (2) and Equation (3), respectively.

Table 4 presents ANOVA of the fitted model. According to the ANOVA table, the regression model is significant at the considered confidence level since a satisfactory correlation

coefficient was obtained and the F-value was 15 times more than the F listed value.

$$Y_{coded} = 83.260 - 0.161X_2 + 0.248X_3 - 0.723X_1^2 - 0.918X_2^2 - 0.688X_3^2 - 0.263X_1X_2 + 0.262X_1X_3 - 0.263X_2X_3 \quad (2)$$

$$Y_{actual} = 34.695 + 1.841P + 1.856t - 0.029T^2 - 0.037P^2 - 0.003t^2 - 0.011PT + 0.003T.t - 0.004Pt \quad (3)$$

Table 2. Experimental planning and results of amylase activity for ultrasonic treatment

Run	X_1	X_2	X_3	Amylase activity (KU/mL)
1	-1	-1	-1	80.626 ± 0.106
2	1	-1	-1	80.871 ± 0.184
3	-1	1	-1	81.301 ± 0.106
4	1	1	-1	80.442 ± 0.106
5	-1	-1	1	81.117 ± 0.106
6	1	-1	1	82.344 ± 0.184
7	-1	1	1	80.565 ± 0.106
8	1	1	1	80.933 ± 0.106
9	-1.682	0	0	81.178 ± 0.106
10	1.682	0	0	80.994 ± 0.213
11	0	-1.682	0	80.749 ± 0.213
12	0	1.682	0	80.380 ± 0.213
13	0	0	-1.682	80.749 ± 0.281
14	0	0	1.682	81.669 ± 0.213
15	0	0	0	83.203 ± 0.281
16	0	0	0	83.449 ± 0.184
17	0	0	0	83.326 ± 0.281
18	0	0	0	83.224 ± 0.221
19	0	0	0	83.414 ± 0.191
20	0	0	0	83.117 ± 0.231

Table 3. Estimated effects of independent variables on amylase activity of the ultrasonic samples

Factor	Coefficient estimate of coded factors	Std. Err.	t- value	P- value
X_o	83.260	0.080	1575.76	$1.7.10^{-26}$
X_1	0.041	0.053	1.40	0.37*
X_2	-0.161	0.053	4.89	$0.9.10^{-3}$
X_3	0.248	0.053	6.83	$0.1.10^{-3}$
X_{11}	-0.723	0.052	20.92	$6.1.10^{-8}$
X_{22}	-0.918	0.052	26.33	$7.1.10^{-9}$
X_{33}	-0.688	0.052	19.65	$1.1.10^{-7}$
X_1X_2	-0.263	0.070	5.36	$0.5.10^{-3}$
X_1X_3	0.262	0.070	6.03	$0.3.10^{-3}$
X_2X_3	-0.263	0.070	6.03	$0.3.10^{-3}$

** Non significant variables*

Table 4. Analysis of variance of the regression model in experiments of sonication treatment

Source of variation	Degree of freedom	Sum of squares	Mean square	F-value	p-value
Regression	9	25.39	2.82	72.71	0.00
Residual	10	0.39	0.04		
Total Corrected	19	25.78	1.35		
F listed value				$F_{7,5} = 4.88$	
Lack of Fit	5	0.314	0.06	3.63	0.092

Surface response graph, obtained by using the fitted model presented in Eq. (3), is presented in Fig. 2. The interaction of ultrasonic temperature and power, ultrasonic power and time, ultrasonic temperature and time on the catalytic activity of the glucoamylase preparation were described by parabolic shape. These interactions have never been reported not only for glucoamylase preparation but also for other enzyme preparations.

Based on the developed model (equation (3)) for ultrasonic treatment, the optimum conditions for improving amylase activity were determined using Modde 5.0 software. The model predicted that as the ultrasonic temperature, power and time are 30^{o}C, 19.3 W/mL and 33 sec, respectively, the catalytic activity of glucoamylase preparation would achieve the maximum of 83.300 KU/mL.

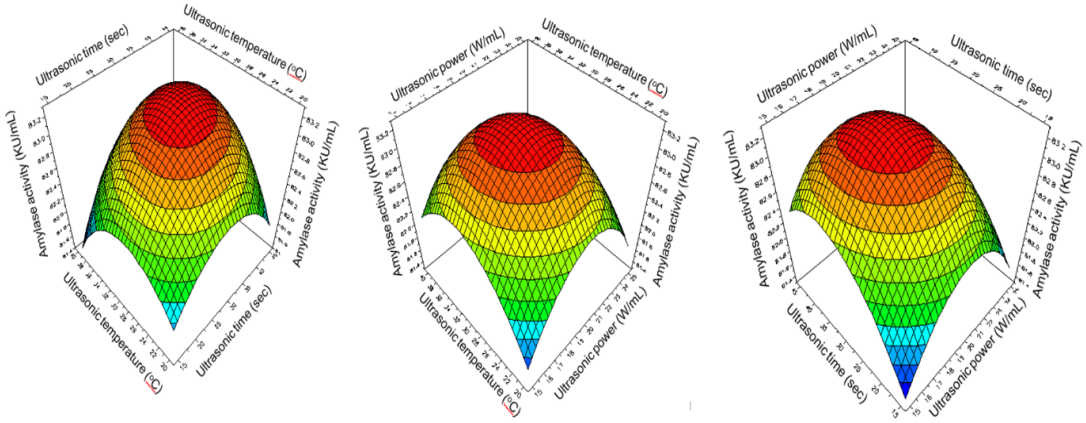

Figure 1. Fitted surface for amylase activity of ultrasonic treatment of glucoamylase as a function of ultrasonic temperature, power and time.

In order to verify the accuracy of the model, three independent replicates were carried out for measuring amylase activity under the optimal conditions: ultrasonic temperature of 30°C, power of 20 W/mL and time of 33 sec. The experimentation shows that the amylase activity was 83.142 ± 0.213 KU/mL. The experimental value was therefore nearly similar to the theoretical value (83.300KU/mL) from the model. Simultaneously, the catalytic activity of the unsonicated glucoamylase preparation was also tested as control. The amylase activity of the control was 74.857 ± 0.106 KU/mL. Thus, sonication increased catalytic activity of the glucoamylase preparation by 11% in comparison with the control without ultrasonic treatment.

3.2. Comparison of catalytic characteristics of the sonicated and unsonicated enzyme preparations

V_{max} and K_m of the sonicated and unsonicated glucoamylase preparations are presented in Table 5.

Table 5. Kinetic parameters of the sonicated and unsonicated glucoamylase preparations

Sample	V_{max} (μM/min)	K_m (μM)
Ultrasonic treatment	517 ± 1	0.154 ± 0.001
Control	413 ± 2	0.141 ± 0.002

Sonication increased V_{max} of the glucoamylase preparation. The obtained results in section 3.1 showed that ultrasonic treatment improved the amylase activity and that resulted in higher V_{max}. However, sonication also augmented K_m of the glucoamylase preparation. High K_m indicates that V_{max} will only be reached if the substrate concentration is high enough to saturate the enzyme [9]. It should be noted that high substrate concentration would improve economic efficiency in starch hydrolysis [9]. Similar increase in both V_{max} and K_m value was recently reported by Bashari et al. (2013) who

compared kinetic parameters of sonicated and unsonicated dextrinase preparation [5].

4. CONCLUSIONS

Ultrasonic technology was performed for improvement in catalytic activity of glucoamylase preparation. A central composite circumscribed design was used to estimate and optimize the experimental variables: ultrasonic temperature, power and time. The optimal of ultrasonic conditions were determined as follows: ultrasonic temperature of 30°C, ultrasonic power of 20 W/mL and ultrasonic time of 33 sec. Under these conditions, the glucoamylase activity was 83.142 ± 0.213 KU/mL and this value increased 11% in comparison with that in the control without ultrasonic treatment. In addition, sonication increased enzyme kinetic parameters including V_{max} and K_m. Increase in enzyme activity of commercial preparations is an important benefit for industrial application. Further research needs to be conducted to clarify the impact of ultrasound on enzyme structure and catalytic activity.

Tối ưu hóa quá trình xử lý siêu âm để làm tăng hoạt tính xúc tác của chế phẩm glucoamylase

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TÓM TẮT

Trong nghiên cứu này, quá trình xử lý siêu âm được sử dụng để làm tăng hoạt tính xúc tác của chế phẩm glucoamylase Dextrozyme GA. Nhiệt độ, công suất và thời gian siêu âm được tối ưu hóa bằng phương pháp quy hoạch thực nghiệm theo phương pháp quay bậc hai của Box và Hunter, cấu trúc có tâm để hoạt tính xúc tác của chế phẩm glucoamylase đạt cực đại. Giá trị nhiệt độ, công suất và thời gian siêu âm tối ưu lần lượt là 30°C, 20 W/mL và 33 giây. Khi đó, hoạt tính glucoamylase cao nhất là 83.142 ± 0.213 KU/mL và giá trị này cao hơn 11% so với mẫu đối chứng không qua xử lý siêu âm. Kết quả nghiên cứu cũng cho thấy các giá trị K_m và V_{max} của chế phẩm enzyme đã qua xử lý siêu âm đều cao hơn so với mẫu đối chứng. Xử lý siêu âm có thể được xem là một giải pháp tiềm năng để làm tăng hoạt tính xúc tác của chế phẩm glucoamylase trong quá trình thủy phân tinh bột.

***Từ khóa**: glucoamylase, tối ưu hóa, xử lý siêu âm.*

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