OPTIMIZING THE HYDROLYSIS PROCESS PARAMETERS WITH THE COMBINATION OF CELLULASE AND PECTINASE FOR INCREASING THE RECOVERY EFFICIENCY OF TOTAL PHENOLIC CONTENT AND DRY MATTER FROM PUMPKIN FIBROUS STRANDS BY-PRODUCTS (*CUCURBITA MOSCHATA*) BY RESPONSE SURFACE METHODOLOGY

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ABSTRACT:

This study focused on the optimization of enzyme hydrolysis by using the combination of cellulase and pectinase to improve the recovery efficiency of dry matter (Y1) and total phenolic content (TPC, Y2) from the pumpkin fibrous strands. Experimental design by response surface method (RSM) with central composite circumscribed (CCC) design was used to optimize the parameters of the hydrolysis process, including temperature (40-50^oC), enzyme concentration (0.1-0.2%), and time (60-120 minutes). The optimal hydrolysis parameters were found at the temperature of 44.9^oC, the enzyme concentration of total cellulase and pectinase of 0.177% and the time of 105.34 minutes. At this condition, Y1 and Y2 reached 55.99 ± 0.62% and 56.61 ± 0.71%; these increased by 22.09% and 26.71%, respectively, compared with the control. This study's results open up the potential to effectively use the pumpkin fruit co-products.

Keywords: pumpkin fibrous strands, hydrolysis, TPC, RSM.

1. Introduction

Pumpkin (Cucurbita moschata) is grown in many countries around the world, it is a kind of vegetable rich in nutrients and bioacitive compounds [18-19]. The pumpkin fruit contains 3-4% of the fibrous strands, 4-6% of the seeds, 10-12% of the peel and 79-82% of the pulp. The pulp of pumpkin is mainly used for food processing while the waste of pumpkin can create up to 17-20% [10]. This wastes a good source of raw materials [11]. The by-products from the fibrous strands of pumpkin contain many bioactive compounds such as carotenoids, polyphenol compounds, vitamin C, minerals, etc [2]. The enzymatic hydrolysis of this co-product source is a first potential choice because of the safe, friendly, and effective biological treatment. The combination of cellulase and pectinase could affect the cellulose molecules of the cell wall and the intercellular pectin adhesive layer in the plant tissue structure. These make the extraction process easier and the cell fluid recovery more efficient [13-14].

The combination of pectinase and cellulase for the hydrolysis process of the fibrous strands from the pumpkin fruits was investigated to obtain the highest yield and biological compounds in the extract which has not yet been reported so far. The hydrolysis process was optimized by the response surface method (RSM) to predict the optimal response variables of the progress [3].

2. Materials and methods

2.1. Material

Chemicals: Foline-Ciocalteu, Na_2CO_3, and C_2H_5OH were supplied by Merck, Germany; Acid gallic was supplied by Sigma-Aldrich, USA.

Plant materials: the F1 hybrid pumpkin variety were grown in Cau Ngang district, Tra Vinh province.

Enzymes: Commercial enzymes, Cellulase from Trichoderma reesei and pectinase from Aspergillus aculeatus were purchased from Novozymes, Denmark.

2.2. Methods

Sample preparation: The pumpkin fruits were washed, peeled, split in half and collected the fibrous strands. Next, the fibrous strands were added to pure water at a ratio of fibrous strands: pure water = 1:2. Then, the mixture was pureed in 2 minutes. The mixture was then homogenized for 2 minutes.

Experimental procedure: The hydrolysis process was conducted in a shaken water bath. The samples were then heated in a water bath at 85°C for 10 minutes to inactivate the enzymes and filtered. The extract was used to determine the dry matter content, and TPC.

Experimental design: CCC design was used to analyze the interaction between the factors of the hydrolysis process including temperature, enzyme concentration, and incubation time using Design Expert (version 11.0.7.1, Stat-Ease, Inc, Minneapolis, MN, USA) statistical software. The experimental plan is shown in Table 1.

Determination of TPC: TPC was determined by Foline-Ciocalteu method and measured at 650 nm by a spectrophotometer machine (UV-1800 Shimadzu, Japan) [4]. The results were expressed as milligrams of gallic acid equivalent to 100 grams (mg GAE/100 g) of the sample. All of the determinations were performed in triplicate.

The recovery efficiency (Y) of components: Y was calculated by Eq. (2).

$$Y(\%) = \left[\frac{\textit{The yield in the extract}}{\textit{The yield in the raw sample}}\right] \times 100 \quad (2)$$

Data analysis: The predicted result of the input variable parameters on the predicted response of the hydrolysis process was shown as the following regression equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \beta_{ij} X_i X_j + e$$

In which, Y is the predicted response, β_0 is the constant coefficient, X_i X_j are the input variable parameters that influence the response variable of Y; β_i is the i[th] linear coefficient; β_{ii} the i[th] quadratic

Table 1. The variable parameters in the CCC design of the RSM

Variable parameters	Sympol	Unit	Code level				
			-α	-1	0	+1	+α
Temperature	X_1	°C	36.59	40	45	50	53.41
Total C/P concentration	X_2	% v/ w	0.066	0.1	0.15	0.2	0.234
Time	X_3	minutes	39.55	60	90	120	140.45

coefficient and β_{ij} is the ij[th] interaction coefficient, k is the number of factors and e is the random error.

3. Results and discussion

Three factors of the hydrolysis process including the temperature, the enzyme concentration of total C/P and the incubation time were optimized according to the CCC design. The results of the recovery efficiency (%) are presented in Table 2.

A model that shows a good correlation requires a good fit between the actual and theoretical data [8, 17]. The deviation between adjusted R^2 and predicted R^2 must be within 20% to be suitable. The reliability of the experiment is low if the C.V.% value is high [15, 21].

The results observed in our research showed that R^2 for Y1 (0.9998), and Y2 (0.9993) achieved greater than 80%. The ratio of Adeq Precision > 4 indicated an adequate signal. The difference between adjusted R^2 and predicted R^2 was less than 20%, and the adjusted coefficient of determination (Adj R^2) was found to be a high value (>90%). In addition, the C.V.% reached a low value, showing that the model is reliable for predicting response variables [15, 21].

The results of ANOVA analysis also showed that all three factors significantly impacted the ability to the response variables. From statistical analysis of ANOVA with coefficients according to the response variables of the model which have p-value < 0.05, it was put into the regression equation to improve the model as following Table 3.

Where, X_1, X_2, X_3 are temperature (^oC), enzyme concentration (%), and incubation time (minute) respectively.

For raising the temperature in the range from 40 to 46^oC, Y1 and Y2 were recovered more effectively. When the temperature was less than the optimal

Table 2. Full experimental central composite design with levels of the input variables and output the response values

Run	Independent factors			Y (%)	
	X_1 (^oC)	X_2 (%)	X_3 (minutes)	The dry matter	TPC
1	40	0.1	60	44.80	46.00
2	50	0.1	60	44.51	46.83
3	40	0.2	60	48.43	50.86
4	50	0.2	60	49.69	51.31
5	40	0.1	120	49.81	49.96
6	50	0.1	120	48.32	49.53
7	40	0.2	120	51.73	53.61
8	50	0.2	120	51.82	52.68
9	36.59	0.15	90	47.23	47.59
10	53.41	0.15	90	47.11	47.00
11	45	0.07	90	47.01	47.85
12	45	0.23	90	53.07	54.66
13	45	0.15	39.55	46.84	50.33
14	45	0.15	140.45	53.01	55.00
15	45	0.15	90	54.44	55.54
16	45	0.15	90	54.54	55.89
17	45	0.15	90	54.59	55.73
18	45	0.15	90	54.62	55.78
19	45	0.15	90	54.68	55.69
20	45	0.15	90	54.56	55.63
p-value				<0.0001	<0.0001
Lack-of-fit				>0.05	>0.05
F-value				4445.8	1584.34
R^2				0.9998	0.9993
Adjusted R^2				0.9995	0.9987
Predicted R^2				0.9989	0.9965
Adeq Precision				109.0766	105.4073
C.V.%				0.1538	0.2503

Note: X_1, X_2, and X_3 are the factors of the hydrolysis process: the temperature, the enzyme concentration and incubation time.

Table 3. The quadratic regression model predicts the recovery efficiencies

The responses	2nd Order polynomial equation
Y1	$-199.63 + 9.41X_1 + 184.59X_2 + 0.52X_3 + 1.57X_1X_2 - 0.002X_1X_3 - 0.28X_2X_3 - 0.11X_1^2 - 647.12X_2^2 - 0.002X_3^2$
Y2	$-233.01 + 10.95X_1 + 267.96X_2 + 0.39X_3 - 0.44X_1X2 - 0.002X_1X_3 - 0.21X_2X_3 - 0.12X_1^2 - 628.83X_2^2 - 0.001X_3^2$

temperature, it might be the cause of the enzyme that did not work well. This could lead to a decrease in the interaction between the enzymes and substrates. However, If the temperature was higher the optimal temperature, Y1 and Y2 decreased as shown at temperatures above 50°C. These results are consistent with previous research that reported that the hydrolysis process with the combination of pectinase and cellulase is highly effective at temperatures from 40-45°C [12].

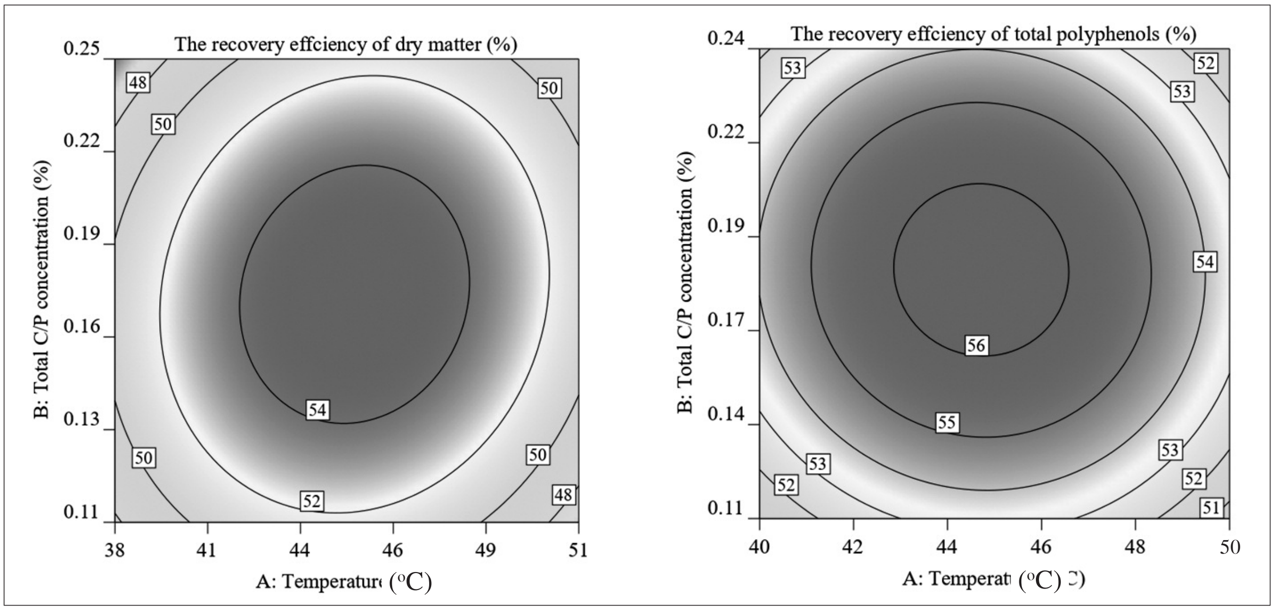
Besides the temperature, the concentration of total C/P also had a significant effect on the hydrolysis process. When gradually increasing the amount of enzyme used to a certain concentration, leading to an increase in the reaction rate of the enzyme with the substrate.

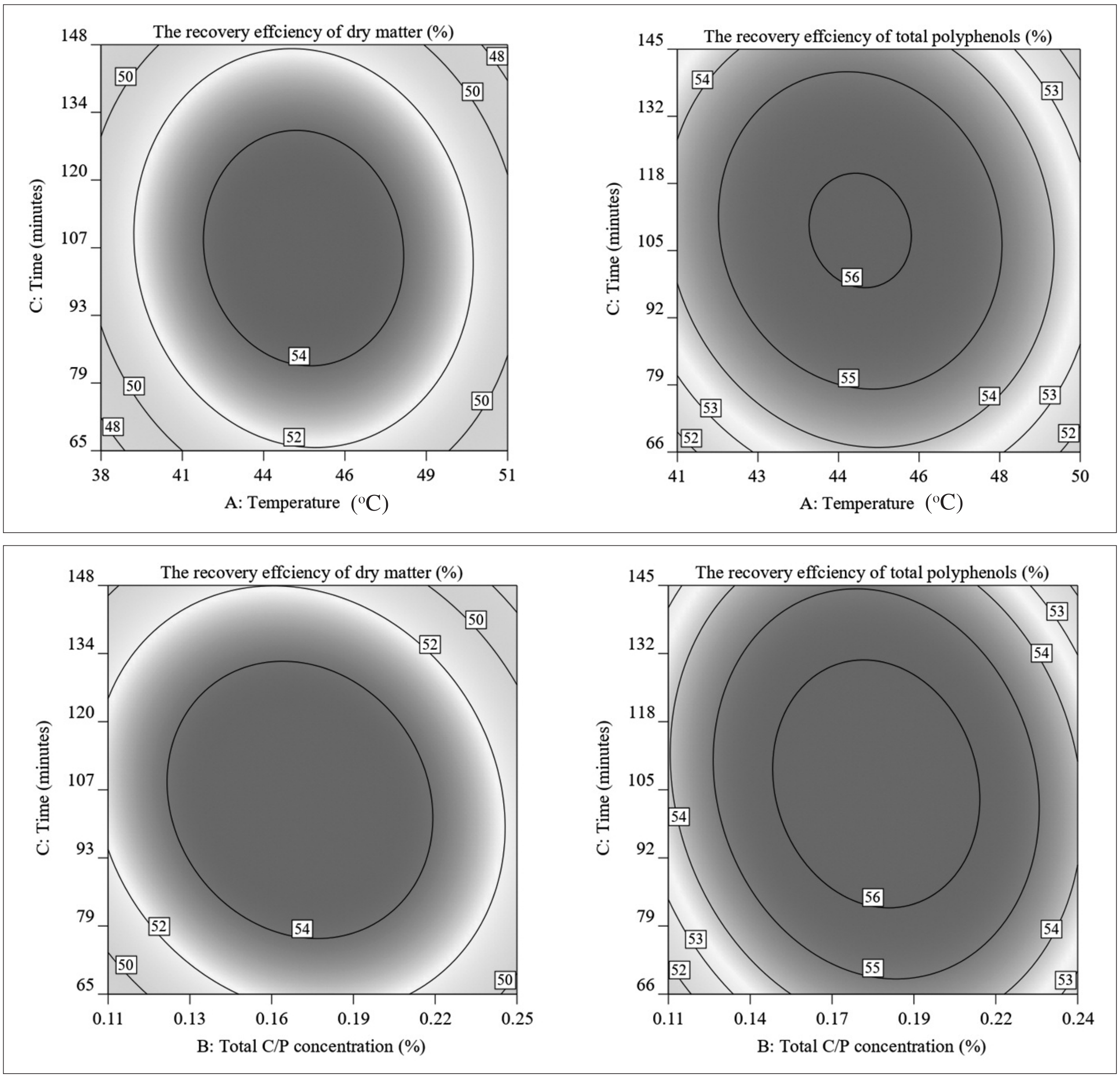
This clearly shows that the increase in enzyme concentration of total C/P increased in the range from 0.1 to 0.15% which caused to increase in the response variables (Table 2). This may be related to the number of enzyme molecules per unit of substrate that will promote hydrolysis and increase economic efficiency [1][16].

This result is consistent with the previously reported study, the bioactive compounds tended to decrease as increasing the enzyme concentration from 0.2 to 0.25% [20]. The hydrolysis time is also a factor to consider in the process because when the reaction time is short, the hydrolysis will not occur completely. On the contrary, when the reaction time is too long, the extract quality may be affected by unwanted reactions, the increase of microorganisms, and the waste of time [6].

Y1 and Y2 increased when increasing the incubation time from 60-90 minutes, then gradually decreased (Table 2). That may be due to the hydrolysis process, substrate is exposed to the enzyme, and the breakdown of the cell wall releases phenolic compounds and soluble compounds resulting in an increase in Y1 and 2.

Fig. 1: Contour plots of Y1 and Y2 (%) by RSM





When the hydrolysis process is prolonged, Y2 decreased, that may be due to the decomposition of phenolic compounds under atmospheric conditions in the presence of oxygen [9]. Our finding is consistent with the previous study, when the hydrolysis time was too long, the dry matter content and bioactive compounds were significantly affected for the hydrolysis of Date syrup using the combination of C/P [5].

From the response surface model, the optimal hydrolysis condition was predicted at the incubation temperature of 44.886°C, the enzyme concentration of 0.177%, and the incubation time of 105.339 minutes. At this condition, the hydrolysis process was predicted the highest recovery efficiency of dry matter (55.438%), and TPC (56.677%) (Table 4).

To evaluate the accuracy of the model, the experiments were performed at the conditions: the fibrous strands/water ratio of 1/2; C/P ratio of 1/2; natural pH of raw materials, incubation temperature of 44.9°C, the enzyme concentration of total C/P of 0.177% and the hydrolysis time of 105.34 minutes. The obtained results indicated that there was no significant difference ($p > 0.05$) between the experimental recovery efficiency compared with the predicted results from the regression equation. This proved that the built equation was suitable. In comparison to the control, Y1 and Y2 increased by 22.09% and 26.71 (Table 4). Our finding is in agreement with the previous study showing the use of C and P enzymes to hydrolyze pineapple pulp improved dry matter recovery efficiency by 14% compared to the control sample [7].

Table 4. The results from the model prediction and experiment

Response (%)	Optimal value	Actual value*	Control*
Y1	55.438[a]	55.99 ± 0.62[a]	33.9 ± 0.5[b]
Y2	56.677[a]	56.61 ± 0.71[a]	29.9 ± 0.5[b]

4. Conclusions

RSM with CCC design is suitable to optimize the factors of temperature, enzyme concentration and time for the hydrolysis process of the fibrous strands with the combination of cellulase and pectinase. The highest recovery efficiency of the hydrolysis process with the combination of C/P compared to the control. Our finding provides a better understanding of the nutrient value and the application capacity of both cellulase and pectinase to recover the nutritional ingredients that are beneficial to health from the fibrous strands, which is a potential co-product source for product development to increase the economic value of the pumpkin fruits ■

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TỐI ƯU HÓA CÁC YẾU TỐ CỦA QUÁ TRÌNH THỦY PHÂN VỚI SỰ KẾT HỢP CỦA CELLULASE VÀ PECTINASE ĐỂ TĂNG HIỆU SUẤT THU HỒI HÀM LƯỢNG POLYPHENOL TỔNG SỐ VÀ CHẤT KHÔ TỪ PHỤ PHẨM RUỘT BÍ ĐỎ (*CUCURBITA MOSCHATA*) BẰNG PHƯƠNG PHÁP BỀ MẶT PHẢN ỨNG

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TÓM TẮT:

Nghiên cứu này tập trung vào việc tối ưu hóa quá trình thủy phân enzyme bằng cách sử dụng kết hợp cellulase và pectinase để cải thiện hiệu suất thu hồi chất khô (Y1) và hàm lượng polyphenol tổng số (TPC, Y2) từ ruột bí đỏ. Phương pháp bề mặt đáp ứng (RSM) với thiết kế xoay tâm (CCC) được sử dụng để tối ưu hóa các thông số của quá trình thủy phân bao gồm nhiệt độ (40-50^{o}C), nồng độ enzyme (0,1-0,2%) và thời gian (60-120 phút). Các thông số tối ưu được tìm thấy tại nhiệt độ 44,9^{o}C, nồng độ enzyme kết hợp cellulase và pectinase là 0,177% và thời gian 105,34 phút. Ở điều kiện này, Y1 và Y2 đạt 55,99 ± 0,62% và 56,61 ± 0,71%, tăng lần lượt 22,09% và 26,71% tương ứng so với mẫu đối chứng. Kết quả nghiên cứu này mở ra tiềm năng sử dụng hiệu quả phụ phẩm từ quả bí đỏ.

Từ khoá: ruột bí đỏ, thuỷ phân, hàm lượng polyphenol tổng số, phương pháp bề mặt đáp ứng.