OPTIMIZATION OF CELLULASE-ASSISTED EXTRACTION OF POLYSACCHARIDE FROM COCONUT RESIDUE

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Abstract - In the present study, cellulase-assisted extraction of polysaccharides from coconut residue was optimized by response surface methodology (RSM). The optimal extraction parameters comprised an extraction temperature of 59.97°C, an extraction time of 5 h, pH value of 4.53, and cellulase amount of 4000 U/ml. Under these conditions, the relative yield was 6.87%, higher than the yield achieved with conventional methods: Hot water extraction(2.8%) and NaOH extraction(4.87%).

Index Terms - Cellulase, coconut, extraction, polysaccharide, response surface method

I. INTRODUCTION

Polysaccharides from natural plants have been used widely in functional foods, dietary supplements, health products and cosmetics [1-3]. Polysaccharides provide dietary fiber [3-5] and can be used as an emulsifier [2, 6]. Other health benefits, such as antioxidants, antibacterial, and antidiabetic activity have also been reported [7-9].

The coconut residue obtained after the extraction of milk is an inedible byproduct of the coconut milk and virgin coconut oil industry. The use of this residue is currently limited to fertilizer and animal food. Recently, the use of polysaccharides extracted from the coconut residue as dietary fiber have been reported [4].

Hot water and alkali extraction followed by precipitation with alcohol are the most common techniques used for polysaccharides extraction [9]. However, these methods require long extraction times and high temperatures giving low extraction yields [10-11]. The high temperature could also affect bioactivities of the extracted polysaccharides [5]. Enzyme-assisted extraction (EAE) methods are gaining more attention because of the need for eco-friendly extraction [12]. The degradation or disruption of cell walls and membranes, thus accelerating the mass transfer of target compounds into extraction solvent and as a consequence enabling a better release and more efficient extraction of bioactive compounds [13]. Enzymes such as pectinases, cellulases, and hemicellulases have been widely used for pretreatment of plant material prior to conventional methods for extraction [12], which can also significantly reduce the amount of solvent requirement. Application of EAE has been proved to be effective for natural ingredients, such as phenolic compounds, oils, and pigments when aiming for high extraction yields [14-15].

In the present study, cellulase-assisted extraction of crude polysaccharides was developedand the extraction conditions were optimized using a response surface method (RSM).

II. MATERIALS AND METHODS

A. Preparation of Coconut Meat Residue

Coconut residue obtained after extraction of coconut milk was collected from local market. The residue was dried at 60°C in an oven to a moisture content of 2-3% by weight. This coconut residue (CR) was then defatted with petroleum ether in a Soxhlet apparatus. Then the residue was washed three times with ethanol before drying at 60°C in an oven to a moisture content of 2–3% by weight. This sample of defatted coconut residue (DCR) was kept in an air-tight container at room temperature until being used. All the chemicals used were of analytical grade and purchased from Merck chemicalCo. (Darmstadt, Germany).

B. Extraction of polysaccharides

Extractions of crude polysaccharides (CP) from both CR and DCR were conducted using extraction methods explained in next sections. Each extraction method yielded a mixture and CP was obtained from the mixture by reducing the mixture to slurry of approximately 1/5 of the initial volume using a rotary evaporator under vacuum at 60°C (V-850 Buchi vacuum controller). If CR was used, fat layer was removed after this stage using filter paper (Whatman no. 42). After that the obtained slurry was centrifuged (6000 rpm, 15 min) to obtain supernatant which was then mixed with 95% ethanol at a ratio of 1:3 v/v. This mixture was then allowed to precipitate by keeping the mixture at 4°C overnight. The precipitate was collected by filtration (Whatman no. 42) and washed 3 times with 80% ethanol to obtain CP samples. All the extractions were done in triplicate.

For cellulase-assisted extraction (CAE), 10g of coconut residue was treated with cellulase (1000, 2000, 3000, 4000, 5000 U/ml), pH (3.5, 4, 4.5, 5.0, 5.5), time (2, 3, 4, 5, 6 h) and temperature (30, 40, 50, 60, 70°C). Temperature was controlled using a water bath. After the enzymolysis, the obtained mixture was heated in a water bath and stirred (90°C, 1500 rpm, 1 h).

For hot water extraction (HWE), 10g of coconut milk residue was added to 0.1M NaOH (1:5 w/v) and the

mixture was incubated at 55°C for 24 h. This mixture was then filtered and ethanol was added to the filtrate (pH 6.0-7.0).

For sodium hydroxide (NaOH) extraction (NaE), 10 g of coconut milk residue was added to 0.1M NaOH (1:5 w/v) and the mixture was incubated at 55°C for 24 h. This was then filtered and ethanol was added to the filtrate to give a mixture of pH 6.0-7.0.

C. Deproteinization

CP sample was deproteinized following the Sevag method as described in [16]. The CP sample was mixed with Sevag reagent (CHCl3:n-BuOH at 5:1 v/v) and stirred for 30 min. Then the mixture was centrifuged resulting in Sevag solution layer at the bottom, CP solution layer at the top and white protein layer in the middle. The PC solution was collected and further deproteinized with Sevag reagent for 5 times until no protein layer was observed. The CP solution was ensured to be free of proteins by UV absorption (260 and 280 nm). Ethanol was then added to the CP solution and the mixture was kept at 4°C overnight for CP to precipitate. The precipitates were then collected by centrifugation (6000 rpm, 15 min) and washed successively with acetone and ethanol several times. The washed precipitates then were subjected to lyophilization to get CP. The CP yield (%) was calculated using the following equation:

$$\text{Yield (\%)} = \frac{\text{Weight of CP (g)}}{\text{Weight of CR (g)}} \times 100 \quad (1)$$

D. Experimental Design

A Central composite experimental design was used to optimize cellulase-assisted extraction conditions of polysaccharides based on the single-factor experiment. Knowing that the enzyme amount (X_1), pH (X_2), extraction time (X_3), extraction temperature (X_4), and can significantly influence the yield of CP, a four-factor model at five levels was selected to evaluate their interactions.The uncoded levels of four independent input parameters for the extraction are listed in Table 1a. The extraction yield of crude polysaccharide (Y, %) was taken as the dependent variable of design experiment. The whole design consisted of 30 experimental points from single-factor experiments (Table 1b). An empirical second-order polynomial model was fitted to correlate the response and independent variables as follows:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i<j=2}^{4} \beta_{ij} X_i X_j$$

(2)where Y is the dependent variable, and β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for model intercept, linear, quadratic, and interaction terms, respectively; while X_i and X_j are the independent variables.

Numerical optimization was carried out to predict the exact optimum level of independent variables leading to the desirable response goal. In this regard, all theindependent variables were kept within range, while the Table 1a: Coded and real levels of input parameters involved in experimental design to estimate cellulase-assisted extraction of polysaccharide.

		Levels				
Independent variables	Symbol	-2	-1	0	1	2
Enzyme amount (U/ml)	X_1	1000	2000	3000	4000	5000
pH	X_2	4.0	4.5	5.0	5.5	6.0
Extraction time (hr)	X_3	2	3	4	5	6
Extraction temperature (^oC)	X_4	40	50	60	70	80

Table 1b: Central composite design matrix and the response values for the polysaccharide extraction yield.

No.	X_1	X_2	X_3	X_4	Yield (%)
1	2000	4.5	3	40	0.73
2	4000	4.5	3	40	1.1
3	2000	5.5	3	40	1.96
4	4000	5.5	3	40	3.9
5	2000	4.5	5	40	4.33
6	4000	4.5	5	40	6.73
7	2000	5.5	5	40	3.3
8	4000	5.5	5	40	3.16
9	2000	4.5	3	60	4.03
10	4000	4.5	3	60	3.07
11	2000	5.5	3	60	3.33
12	4000	5.5	3	60	3.4
13	2000	4.5	5	60	4.27
14	4000	4.5	5	60	7.43
15	2000	5.5	5	60	5.27
16	4000	5.5	5	60	6.83
17	1000	5	4	50	1.93
18	5000	5	4	50	5.1
19	3000	4	4	50	2.37
20	3000	6	4	50	3.33
21	3000	5	2	50	2.4
22	3000	5	6	50	5.23
23	3000	5	4	30	2.9
24	3000	5	4	70	4.37
25	3000	5	4	50	3.13
26	3000	5	4	50	3.1
27	3000	5	4	50	3.23
28	3000	5	4	50	3.23
29	3000	5	4	50	2.8
30	3000	5	4	50	2.87

response was maximized. Additional triplicate experimentswere carried out under optimal extraction conditions in order to determine the validity of optimized conditions. The average value of the validation experiment was compared with the predicted value of the developed condition in order to find out the accuracy and suitability of the optimized

conditions. [17].

The experimental design was analyzed and the predicted data were calculated using the estimate the response of the independent variables. According to the analysis of variance (ANOVA), the effect and regression coefficients of individual linear, quadratic, and interaction terms were measured. The regression coefficients were subsequently applied to generate dimensional maps from the regression equation.

III. RESULTS AND DISCUSSIONS

A. Effect of individual parameter on crude polysaccharide yield

Fig. 1 illustrates the effect of individual parameter on CP extraction yield.The yields of CP obtained from CR and DCR did not differ significantly so only yields obtained from DCR are reported in Fig.1.

The effect of enzyme amount on CP yield was investigated by fixing temperature, time, and pH (50°C, 4 h, pH 5.0). As shown in Fig. 1(a), CP yield increases with increasing enzyme amount reaching a peak (5.63 ± 0.02 %) when the enzyme amount was 4000 U/ml. After that a further increase in enzyme amount decreased CP yield. Therefore, 4000 U/ml was chosen as the optimal amount.

To find optimum pH for CAE, extractions at 50°C with 3000 U/ml enzyme and extraction time of 4 h were conducted at varying pH (pH 4.0, 4.5, 5, 5.5 and 6.0). The results are shown in Fig.1(b) and the optimum pH for CAE wasfound to be around 4.5-5.0. This range of optimum pHs was in accord with the previous study [18-20]. Hence, this pH range was used for further study.

Extraction times ranging from 2 to 6 h were tested, with the extraction temperature, extraction pH, and Design-Expert software (7.0.0 trail, State-East, Inc., Minneapolis, USA) in order to

cellulase amount fixed at 50°C 5.0, and 3000 U/ml, respectively. As shown in Fig. 1(c), CP yield initially increases with increasing extraction time, reaching a peak (5.27± 0.12%) at 6 h. The CP yield started to maintain a dynamic equilibrium with an increase of the extraction time up to 5 h (5.13± 0.04%), and no significant increase when the extraction time exceeded 5 h. Hence, 5 h was chosen as the optimal time.

To investigate the effect of different temperatures on the CP yield, the extraction process was performed at different temperatures (30, 40, 50, 60, and 70°C) with the cellulase amount, extraction time, and pH fixed at 3000 U/ml, 4 h and 5.0, respectively. From Fig. 1(d), CP yield varied with temperature in a similar manner to how enzyme activity varies with temperature. Thus, an extraction temperature of 60°C was considered optimal in the present study.

B. Optimization of the extraction by RSM

The experimental runs (Table 1a) were conducted and the data of the experimental yields were used to find out the coefficient of response surface equation, (2). By employing multiple regression analysis on the experimental extraction yield data, following polynomial model is developed:

$$Y = 1.75 + 0.15X_1 + 0.042X_2 + 0.28X_3 + 0.18X_4 + 0.034X_1^2 - 1.203\times10^{-3}X_2^2 + 0.057X_3^2 + 0.052X_4^2 - 0.015X_1X_2 + 0.058X_1X_3 - 0.031X_1X_4 - 0.15X_2X_3 - 0.023X_2X_4 - 0.045X_3X_4 \quad (3)$$

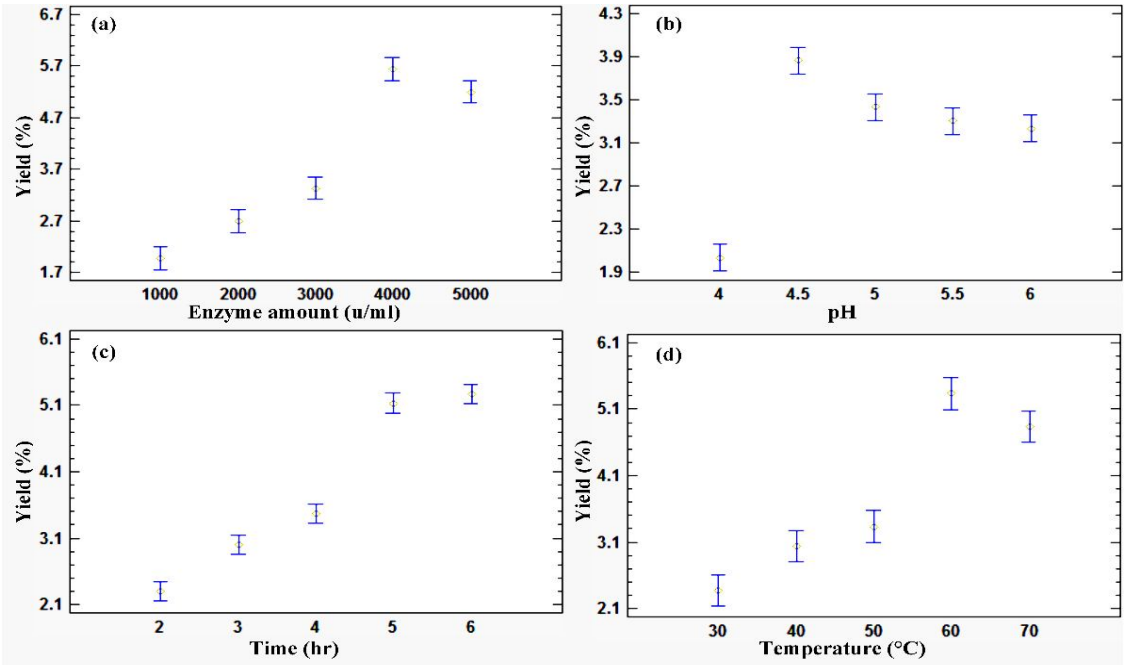


Fig. 1: Effects of (a) enzyme amount, (b) pH, (c) time and (d) temperature on the yield of polysaccharide from coconut residue. Data

are shown as a mean±sd (n = 3)

Table 2: ANOVA for RSM variables fitted to the model

Source	Sumof squares	df	Mean square	F-value	p-Value	Significance
Model	11.80	14	0.84	16.21	< 0.0001	Significant
X_1-Enzyme amount	1.72	1	1.72	33.06	< 0.0001	
X_2-pH	0.13	1	0.13	2.44	0.1227	
X_3-Time	5.73	1	5.73	110.12	< 0.0001	
X_4-Temperature	2.36	1	2.36	45.33	< 0.0001	
X_1X_2	0.011	1	0.011	0.21	0.6463	
X_1X_3	0.16	1	0.16	3.13	0.0810	
X_1X_4	0.047	1	0.047	0.90	0.3471	
X_2 X_3	1.03	1	1.03	19.84	< 0.0001	
X_2X_4	0.026	1	0.026	0.49	0.4842	
X_3X_4	0.099	1	0.099	1.90	0.1720	
X_1^2	0.096	1	0.096	1.86	0.1773	
X_2^2	1.190E-004	1	1.190E-004	2.289E-003	0.9620	
X_3^2	0.27	1	0.27	5.17	0.0259	
X_4^2	0.23	1	0.23	4.34	0.0408	
Residual	3.80	73	0.052			
Lack of fit	3.63	58	0.063	5.56	0.0003	Significant
Pure error	0.17	15	0.011			
Cor total	15.60	89				

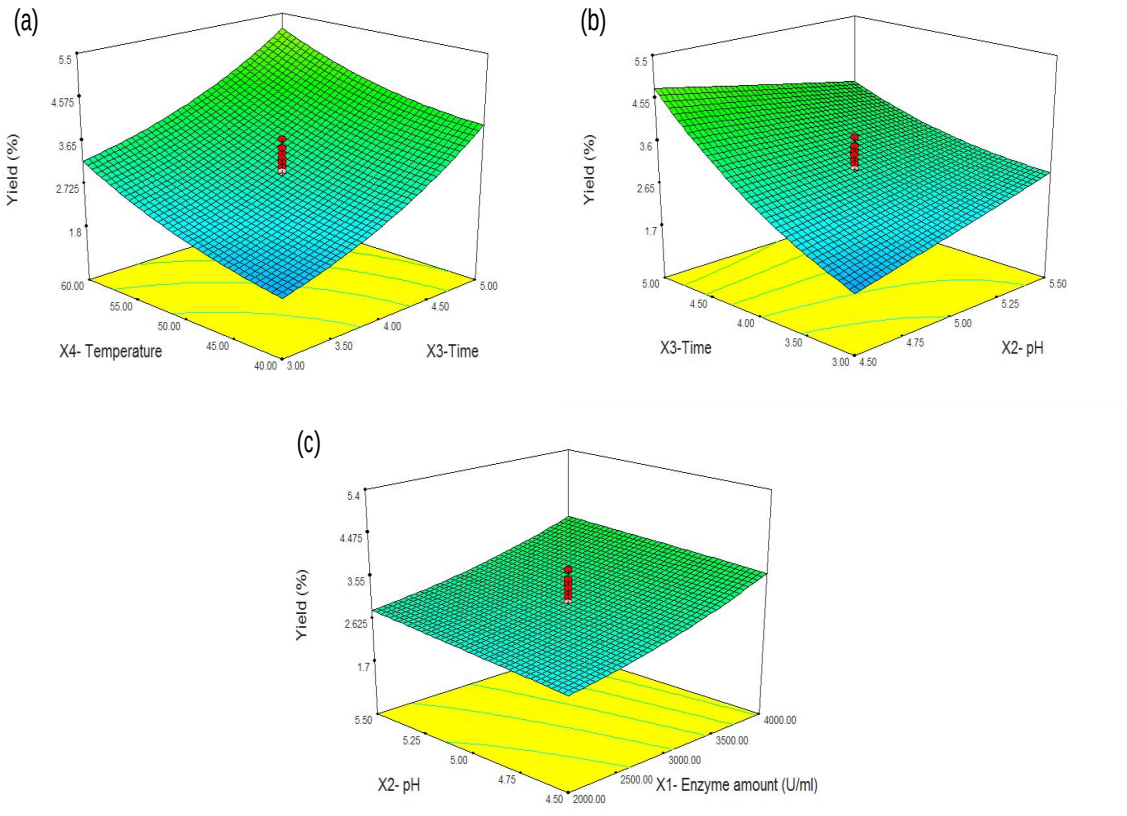


Fig. 2: Response surface (3D) showing effects of the extraction temperature, extraction time, pH value, and enzyme amount on the response

Table 3: Yields and fat contents of polysaccharides from coconut residue (Defatted Coconut residue - DCR and Not defatted Coconut residue - CR) by different extraction methods

%	DCR			CR		
	CAE	HWE	NaE	CAE	HWE	NaE
Yield	6.87±0.05	2.8±0.01	4.87±0.02	6.85 ± 0.1	1.24 ± 0.2	3.23 ± 0.1
Fat	0.3±0.13	2.2±2.1	0.7±1.1	0.6 ±0.04	38.5 ±0.9	33.8 ±0.1

The significance of the developed quadratic model is determined by ANOVA analysis. The ANOVA analysis followed by F-test is applied to estimate the significance of each term. According to Table 2, the determination coefficient of the quadratic regression model(R^2= 0.7566), indicates that the model was adequate for prediction within the range of the experimental variables. The individual, second order and interaction terms significantly affected the extracted polysaccharide yield ($p < 0.0001$; $p < 0.05$) are X_1, X_3, X_4, X_3^2, X_4^2 and X_2X_3.

The results also indicated that the extraction time was the most significant variable that affected the yield of crude polysaccharides, followed by the extraction temperature, enzyme amount and pH value. Fig. 1shows the effect of interaction of two input parameters among the various parameters on the extraction yield of crude polysaccharides. It is found that, all the input parameters were found to be involved in interactions within the range of input parameters studied in the present investigation.As show in Fig. 2, the optimal extraction conditions for crude polysaccharide extraction were predicted as follows: extraction temperature (59.97 ◦C), extraction time (5 h), pH value (4.53), and enzyme amount (4000 U/ml). Under these optimal conditions, the maximum predicted yield was 6.88%, and agreed the experimental yield of 6.87%.

A. Comparison with the conventional extraction methods

Yields of CP obtained from CAE the optimal condition was compared with the yields obtained from the traditional extraction methods as shown in Table 3. According to the table, experimental yield of CAE was higher than the yields achieved with HWE and NaE.

According to Table 3, CAE was superior to other methods, specially, the yield of CP did not have significant change between CR and DCR. This could save cost and time spent on defatting coconut residue. Moreover, less solvents used suggests that CAE is more environmental friendly.

Table 3 also shows that the DCR results in a reduction of fat content in CP as defatted by Soxhlet apparatus removes approximately 90% of fat in the dried coconut residue. However, fat content observed with CP obtained from CR samples using CAE was not significantly different from that associated with DCR. This could be because cellulase is effective in breaking up the coconut cell walls (30% cellulose, 70% hemicellulose [21]) to release the oil.

CONCLUSION

In this study, optimization of the cellulase-assisted extraction of polysaccharides from coconut residue conditions was conducted using RSM. The analysis showed that the extraction yield in term of a second-order polynomial model can be significantly enhanced with temperature, pH, cellulase amount, and extraction time. The highest yield (6.87 %) was obtained with an extraction temperature of 59.97◦C, an extraction time of 5 h, pH value of 4.53, and enzyme amount of 4000 U/ml. Comparing the optimized yield of CP from CAE with HWEand NaE, CAE gave the highest yield. Moreover, using CR in CAE did not affect fat content of the polysaccharides obtained.

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