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Optimization of ultrasonic-assisted extraction process of *Poria cocos* polysaccharides by response surface methodology

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ABSTRACT

Polysaccharides production from *Poria cocos* was carried out using aqueous NaOH with the assistance of ultrasonic. Experimental design was used to investigate the effect of three parameters (extraction time, extraction concentration of NaOH, and ratio of aqueous NaOH to raw material) on polysaccharides yields. The ranges of the factors investigated were 1–3 min for extraction time (X_1), 0.5–1.0 mol/L for extraction concentration of NaOH (X_2), and 30–50 for ratio of aqueous NaOH to raw material (X_3). The statistical analysis of the experiment indicated that extraction concentration of NaOH had significant effect on *P. cocos* polysaccharides yields. The central composite design showed that polynomial regression models were in good agreement with the experimental results with the coefficients of determination of 0.9935 for *P. cocos* polysaccharides yield. The optimal condition for *P. cocos* polysaccharides yield within the experimental range of the variables studied was at 2.44 min, 0.789 mol/L, and 53.0. At this condition, the predicted yield of polysaccharides extracted was 82.3%.

1. Introduction

Poria cocos (Fuling), a fungus that grows on the roots of pine trees, is one of the most important traditional medicines in China and other Asian countries, and has many culinary and medical uses such as anti-inflammatory, antitumor, complement activating, and immune stimulating activities (Kanayama, Adachi, & Togami, 1983; Lee & Jeon, 2003; Yasukawa et al., 1998; Yu & Tseng, 1996). *P.* cocos sclerotium contains 93% dry weight of polysaccharides in which the largest chemical component is D-glucose (Wang et al., 2004). *Poria cocos* polysaccharides (PCP) have attracted considerably attention in the fields of biochemistry and pharmacology because of their biological activities (Chihara, Hamuro, Maeda, Aria, & Fukuoka, 1970; Kanayama et al., 1983; Lee et al., 2004).

Conventional techniques for solvent extraction of PCP have usually been based on correct choice of solvents and use of heat and/or agitation to increase the solubility of the compounds and the rate of mass transfer (Wang et al., 2004). They usually require long extraction times and extraction efficiency is low. Ultrasonic-assisted extraction (UAE) has been widely used to isolate bioactive substances from different parts of plants. Ultrasonic enhancement of extraction is attributed to disruption of cell walls, particle-size reduction, and enhanced mass transfer of the cell contents as a result of cavitation bubble collapse (Ma, Chen, & Hui, 1989; Vinatoru et al., 1997). Recent studies have shown that UAE with organic solvents or water is an alternative means of increasing the speed of sample extraction (Gimeno, Marcé, & Borrull, 2004; Schmeck & Wenclawiak, 2005).

The objective of this study was to investigate the significant variables (extraction time, concentration of NaOH, and ratio of aqueous NaOH to raw material), and further to optimize the levels of the extraction variables, for PCP production by employing response surface methodology (RSM). To the best of our knowledge, there were no reports of systematic studies of ultrasonic-assisted extraction process of PCP in the media of aqueous alkali.

2. Materials and methods

2.1. Materials and equipments

Poria cocos was purchased from Zhenan farm produce market, then ground to pass through 60 mesh screen and stored at 60 °C in loft drier. Acetone and ethylacetate were obtained from Quzhou Chemical Co. (Quzhou, China). Ethanol was obtained from Hangzhou Shuanglin Reagent Co. (Hangzhou, China). All other chemicals used in the experiment were of analytical grade. The extraction procedure was carried out in the ultrasonic washing equipment (LABUY-10LHT Hangzhou LABUY Company, Hangzhou, China).

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2.2. Preparation of P. cocos polysaccharides

The procedure for *P. cocos* polysaccharides extracts was carried out consulting the scientific literature on this subject (Wang et al., 2004).

Fresh sclerotium of *P. cocos* cultivated in Lishui (Zhejiang, China) was peeled, and the white body of the sclerotium was dried and powdered. The powder was defatted by Soxhlet extraction with EtOAc for 6 h and acetone for 6 h and extracted in water to remove soluble polysaccharides. Then the resulting residue was immersed in aqueous NaOH and extracted with the assistance of ultrasonic for some time. The extracted liquid fraction was collected, concentrated, decolorized with 30% H_2O_2, and deproteinated by the Sevage method 10 times and then dialyzed (regenerated cellulose tubing; Mw cut-off 8000, USA) against tap water for 5 days and distilled water for 4 days. The polysaccharide examined by UV spectroscopy (UV-160, Shimadzu, Japan) showed a main peak at 200 nm for polysaccharide, no absorption peaks at 280 nm for protein and 600 nm for pigment. The polysaccharides were finally lyophilized (Christ Alpha1-2, Germany) to obtain a white powder and weighted.

2.3. Experimental design

The extraction parameters were optimized using RSM. The central composite design (CCD) was employed in this regard. The range and center point values of three independent variables presented in Table 1 were based on the results of preliminary experiments. CCD in the experimental design consists of twelve factorial points and three replicates of the central point (Table 2). Extraction time (X_1), concentration of NaOH (X_2), and ratio of aqueous NaOH to raw material (X_3) were chosen for independent variables. Yield of polysaccharides was selected as the response for the combination of the independent variables given in Table 2. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

The variables were coded according to the equation

$$x_i = (X_i - X_0)/\Delta X \quad (1)$$

where x_i is the (dimensionless) coded value of the variable X_i, X_0 is the value of X_i at the center point, and ΔX is the step change. The behavior of the system was explained by the following quadratic equation:

$$Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=1+1}^{3} A_{ij} X_i X_j \quad (2)$$

where Y is the dependent variable (yield of polysaccharides in real value), A_0 is constant, and A_i, A_{ii}, and A_{ij} are coefficients estimated by the model. X_i, X_j are levels of the independent variables. They represent the linear, quadratic, and cross-product effects of the X_1, X_2, and X_3 factors on the response, respectively. The model evaluated the effect of each independent variable to a response. Analysis of the experimental design and calculation of predicted data were carried out using MATLAB Software (version 7.0) to estimate the response of the independent variables. Subsequently, three additional confirmation experiments were conducted to verify the validity of the statistical experimental strategies.

Table 1
Independent variables and their levels used for central composite rotatable design.

Independent variables	Levels[a]		
	−1	0	1
Extraction time (X_1)	1.0	2.0	3.0
Concentration of NaOH (X_2)	0.5	0.75	1.0
Ratio of aqueous NaOH to raw material (X_3)	30	40	50

[a] $x_1 = (X_1 - 2)/1$; $x_2 = (X_2 - 0.75)/0.25$; $x_3 = (X_3 - 40)/10$.

3. Result and discussion

3.1. Effect of different time on extraction yield of PCP

Extraction time is a factor that would influence the extraction efficiency and selectivity of the fluid. A longer extraction time also presents a positive effect on the yield of polysaccharides. It was reported that a long extraction time favors the production of polysaccharides (Hou & Chen, 2008; Liang, 2008). The effect of different time on extraction yield of polysaccharides is shown in Fig. 1. Extraction was carried out at different time conditions while other extraction parameters were same to ones. When extraction time varied from 1 to 4 min, the variance of extraction yield was relatively rapid, and polysaccharides production reached a maximum at 4 min, and then no longer changed as the extraction proceeded. This indicated that 4 min was sufficient to obtain the polysaccharides production. Thus, 4 min was favorable for producing the polysaccharides.

3.2. Effect of concentration of NaOH on extraction yield of PCP

The concentration of NaOH on extraction yield of polysaccharides is shown in Fig. 2. Extraction was carried out at different concentration of NaOH (0.25–1.25 mol/L) conditions while other extraction parameters were same to ones described in Section 3.1. The extraction yields of the polysaccharides significantly increased from 32% to 78.8% as the concentration of NaOH increased from 0.25 to 0.75 mol/L. However, when the concentration continued to increase, the extraction yields of the polysaccharides decreased, due to partly degradation of the polysaccharides (Luo, Xiao, & Wang, 2007).

3.3. Effect of different ratio of aqueous alkali to raw material on extraction yield of PCP

The effect of different ratio of aqueous alkali to raw material on extraction yield of polysaccharides is shown in Fig. 3. Extraction was carried out at different ratio of aqueous alkali to raw material (10–50) conditions while other extraction parameters were same to ones described in Section 3.1. The extraction yields of the polysaccharides significantly increased from 31% to 78.8% as the ratio increased from 10 to 40 shown in Fig. 3, due to the increase of the driving force for the mass transfer of the polysaccharides (Bendahou, Dufresne, Kaddami, & Habibi, 2007). However, when the ratio continued to increase, the extraction yields no longer changed.

Experimental results also showed that most PCP were extracted when the number of extraction was only one. Besides, the influence of ultrasonic power to the yield of polysaccharides was not obvious, but the yield of polysaccharides achieved maximum using the ultrasonic power 300 W(100%). The extraction temperature was kept at room temperature.

3.4. Optimization of the yield of the polysaccharides extract

Table 2 shows the process variables and experimental data. The results of the analysis of variance, goodness-of-fit and the adequacy of the models are summarized. The percentage yield ranged from 41.3% to 82.3%. The maximum yield of polysaccharides (82.3%) was recorded under the experimental conditions of ratio of aqueous alkali to raw material 50, extraction time 3.0 min and concentration of NaOH 0.75 mol/L. The application of RSM offers,

Table 2
Central composite arrangement for independent variables and their response.

No.	X_1 (extraction time, min)	X_2 (concentration of NaOH, mol/L)	X_3 (ratio of aqueous NaOH to raw material)	Extraction yield (%)
1	−1 (1.0)	−1 (0.5)	0 (40:1)	41.3
2	−1 (1.0)	0 (0.75)	−1 (30:1)	67.7
3	−1 (1.0)	0 (0.75)	1 (50:1)	75.2
4	−1 (1.0)	1.0 (1)	0 (40:1)	59.1
5	0 (2.0)	−1 (0.5)	−1 (30:1)	42.0
6	0 (2.0)	−1 (0.5)	1 (50:1)	46.6
7	0 (2.0)	1.0 (1)	−1 (30:1)	61.6
8	0 (2.0)	1.0 (1)	1 (50:1)	62.8
9	1 (3.0)	−1 (0.5)	0 (40:1)	44.7
10	1 (3.0)	0 (0.75)	−1 (30:1)	77.5
11	1 (3.0)	0 (0.75)	1 (50:1)	82.3
12	1 (3.0)	1.0 (1)	0 (40:1)	62.0
13	0 (2.0)	0 (0.75)	0 (40:1)	78.8
14	0 (2.0)	0 (0.75)	0 (40:1)	78.8
15	0 (2.0)	0 (0.75)	0 (40:1)	78.8

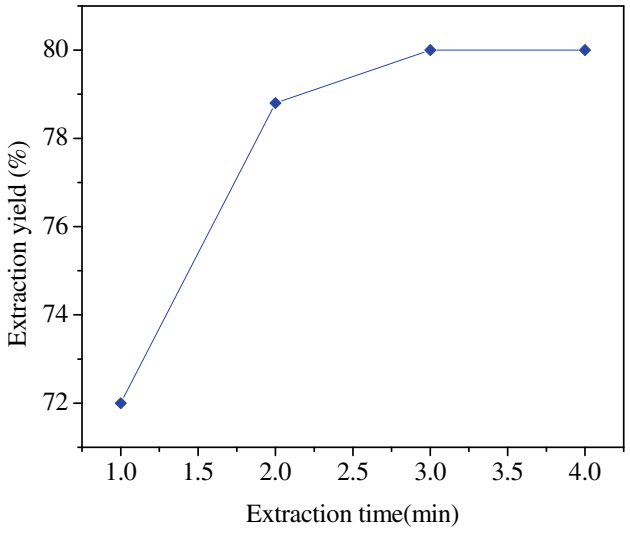

Fig. 1. Effect of extraction time on extraction yield.

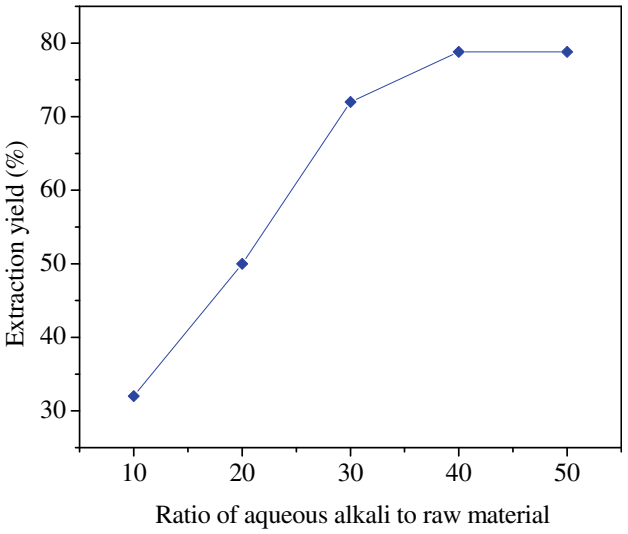

Fig. 3. Effect of ratio of aqueous alkali to raw material on extraction yield.

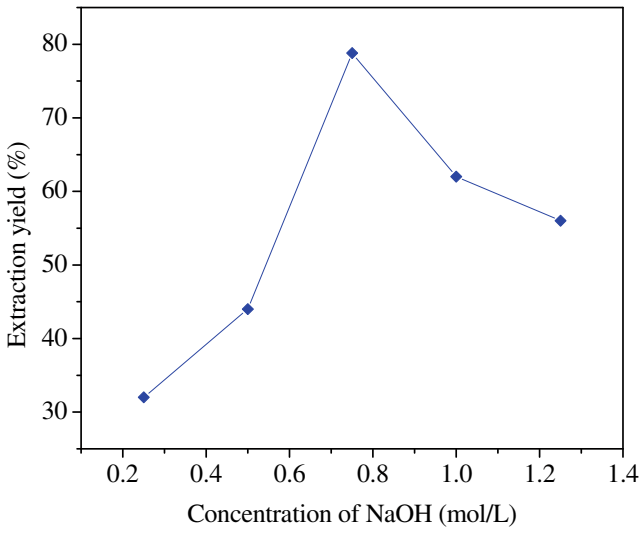

Fig. 2. Effect of concentration of NaOH on extraction yield.

based on parameter estimates, an empirical relationship between the response variable (extraction yield of polysaccharides) and the test variables under consideration. By applying multiple regression analysis on the experimental data, the response variable and the test variables are related by the following second-order polynomial equation:

$$Y = -2.2391 + 0.1517X_1 + 6.4345X_2 + 0.0128X_3 - 0.02300X_1^2 - 3.9560X_2^2 - 0.00008X_3^2 - 0.0050X_1X_2 - 0.00067X_1X_3 - 0.0034X_2X_3 \quad (3)$$

The correlation measure for testing the goodness-of-fit of the regression equation is the adjusted determination coefficient (R^2_{Adj}). The value of R^2_{Adj} (0.98) for Eq. (2) is reasonably close to 1, and indicates a high degree of correlation between the observed and predicted values. A very low value of coefficient of the variation (C.V.) (0.0681) clearly indicated a very high degree of precision and a good deal of reliability of the experimental values. Statistical testing of the model was performed in the form of analysis of variance (ANOVA), which is required to test the significance and adequacy of the model. The data showed a good fit with the Eq. (3), which were statistically acceptable at $P < 0.05$ level and adequate with satisfactory R^2 value ($R^2 = 0.99$).

Response surfaces were plotted using METLAB version 7.0 software to study the effects of parameters and their interactions on polysaccharides yield. Three-dimensional response surface plots and two-dimensional contour plots, as presented in Figs. 4–6, are

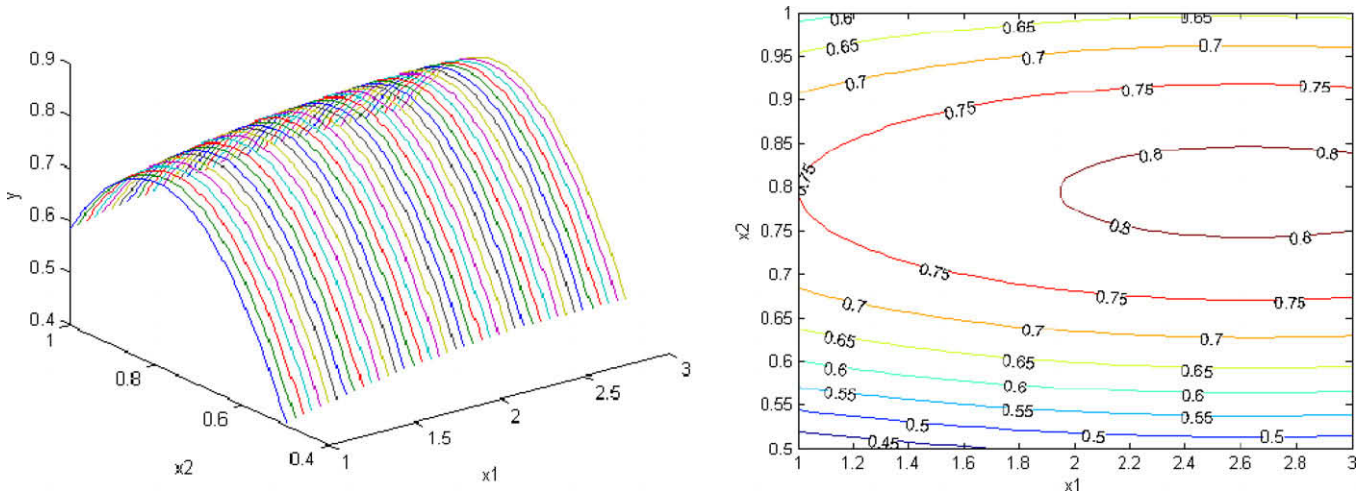

Fig. 4. Contour plot and response surface plot of $Y = f_1(X_1, X_2)$.

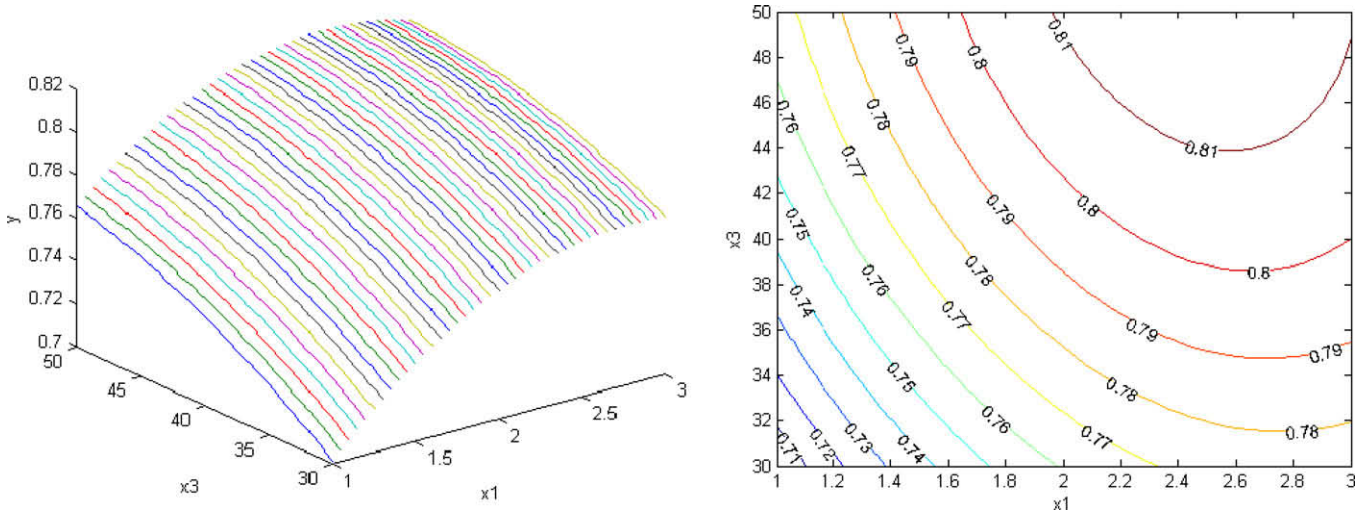

Fig. 5. Contour plot and response surface plot of $Y = f_2(X_1, X_3)$.

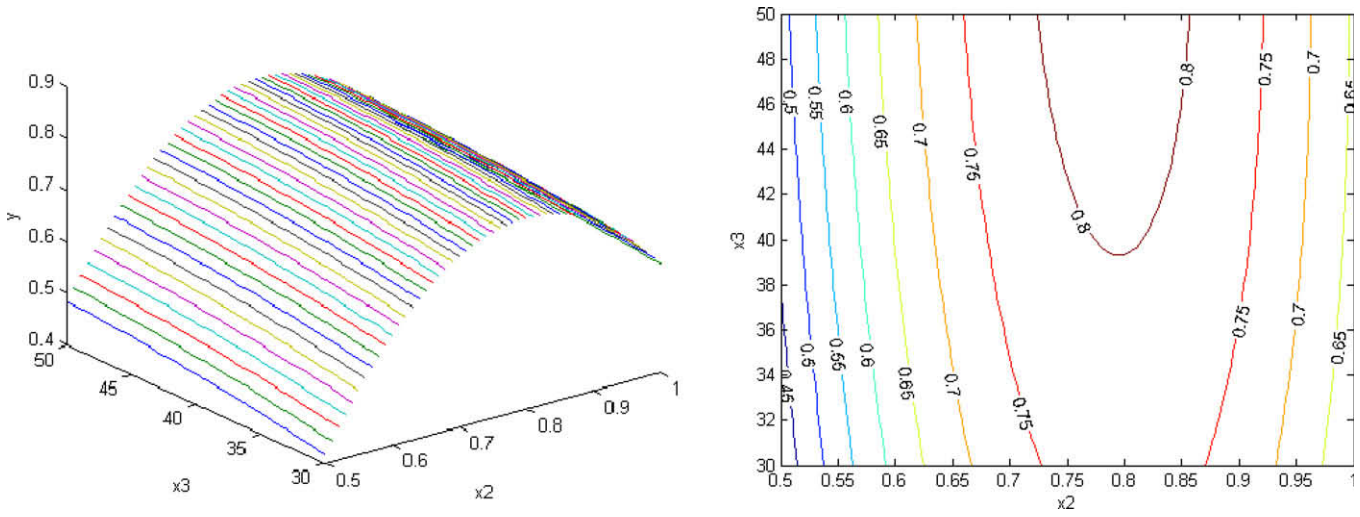

Fig. 6. Contour plot and response surface plot of $Y = f_3(X_2, X_3)$.

very useful to see interaction effects of the factors on the responses. These types of plots show effects of two factors on the response at a time. In all the presented figures, the other one factor was kept at level zero.

As expected, a greater increase in polysaccharides yield resulted when the extraction concentration of NaOH (X_2) was increased in the range from 0.25 to 0.75 mol/L, then the concentration of NaOH curve started to go down at 0.75 mol/L, which may indicate that a concentration of NaOH of 0.75 mol/L (X_2) is required to achieve maximum increase (Fig. 4). Likewise, an increase in polysaccharides yield resulted when the extraction time (X_1) was increased in the range from 1 to 3 min. The time curve did not level off at 3 min, which may indicate that a slightly more time is required to achieve maximum increase (Fig. 4).

As in the case of polysaccharides extract, extraction time (X_1) and ratio of aqueous NaOH to raw material (X_3) used both had a positive impact on the polysaccharides production. There was a linear increase in the yield of polysaccharides with increase in the extraction time (X_1) and ratio of aqueous NaOH to raw material (X_3) (Fig. 5). The contours were slightly inclined to the horizontal showing that there was a significant interaction between the two parameters. Thus, it may be said that a higher level of extraction time (X_1) and ratio of aqueous NaOH to raw material (X_3) is required to achieve maximum increase of polysaccharides yield (Fig. 5).

The 3D response surface based on independent variables extraction concentration of NaOH (X_2) and ratio of aqueous NaOH to raw material (X_3) was shown in Fig. 6, while the extraction time (X_1) was kept at a zero level. An increase in yield was observed with increase in concentration of NaOH (X_2) from 0.5 to 0.75 mol/L, which was in good agreement to the above mention single-factor test (Fig. 2). A single parameter study would overlook this entity. An interaction of extraction concentration of NaOH (X_2) and ratio of aqueous NaOH to raw material (X_3) was no obvious as time was a factor that influenced the yield of polysaccharides. It was obvious that the yield of polysaccharides was increase with the increase in extraction ratio of aqueous NaOH to raw material (X_3) from 30 to 50, meaning that a larger extraction ratio of aqueous NaOH to raw material (X_3) is required to achieve maximum increase of the yield of polysaccharides (Fig. 6).

3.5. Validation of the models

In order to validate the adequacy of the model equations (Eq. (3)), a verification experiment was carried out under the optimal conditions (within the experimental range): extraction time 2.44 min, extraction concentration of NaOH 0.789 mol/L, ratio of aqueous NaOH to raw material 53.0. Under the optimal conditions, the model predicted a maximum response of 82.3 (%). To ensure the predicted result was not biased toward the practical value, experimental rechecking was performed using this deduced optimal condition. A mean value of 81.4 ± 1.09 (%) ($N = 3$), obtained from real experiments, demonstrated the validation of the RSM model. The good correlation between these results confirmed that the response model was adequate for reflecting the expected optimization. The results of analysis indicated that the two groups of experimental values were in good agreement with the predicted one, and also suggested that the model of Eq. (3) are satisfactory and accurate.

4. Conclusion

The performance of the ultrasonic-assisted extraction of polysaccharides from *P. cocos* using aqueous NaOH was studied with a statistical method based on the response surface methodology in order to identify and quantify the variables which may maximize the yield of polysaccharides. The three variables chosen, namely extraction time, extraction concentration of NaOH, and ratio of aqueous NaOH to raw material all have influence on the yield of polysaccharides using the extraction method. The optimal conditions obtained by RSM for production of polysaccharides include the following parameters: extraction time 2.44 min, concentration of NaOH 0.789 mol/L, and ratio of aqueous NaOH to raw material 53.0.

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