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Response surface methodology for optimization of the ultrasonic extraction of polysaccharides from *Codonopsis pilosula* Nannf.var.*modesta* L.T.Shen

Yuanfeng Zou, Xingfu Chen*, Wenyu Yang, Sha Liu

College of Agronomy, Sichuan Agricultural University, Ya'an 625014, PR China

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ABSTRACT

The best ultrasonic extractions of polysaccharides with water from *Codonopsis pilosula* were investigated based on a Box–Behnken design.

Three independent and main variables, including extraction time (min), ratio of water to raw material and ultrasonic power (W), which were of significance for the yields of polysaccharides were studied and the Box–Behnken design was based on the results of a single-factors test.

The experimental data were fitted to a second-order polynomial equation using multiple regression analysis and also examined using the appropriate statistical methods. The best extraction conditions are as follows: extraction time 44 min, ratio of water to raw material 56 and ultrasonic power 320 W. Under the optimization conditions, the experimental yield was 36.264%, which was well matched with the predictive yield.

1. Introduction

Radix Codononpsis is one of the traditional Chinese herbal medicines (TCM), which was the root of *Codonopsis pilosula* (Franch.) Nannf., *C. pilosula* Nannf.var.*modesta* L.T.Shen and *C. tangshen* Oliv. It was used in TCM to lower blood pressure and increase white blood cell counts, cure appetite loss, strengthen the immunize system (China Pharmacopoeia Committee, 2010). *Radix Codononpsis* was utilized primarily as a substitution for ginseng (*Panax ginseng*). Recently, several investigators reported that the polysaccharides extracted from *Radix Codonopsis* had several bioactivities such as the immune function and improving the compensatory hematopoiesis of spleen (Yang, Li, Liu, & Xian, 2005; Zhang, Zhu, Hu, Lai, & Mo, 2003) and anti-active oxygen free radicals (Li & Yang, 2001). Meanwhile, reports about the reflux extraction, purification and structural elucidation of polysaccharides from *Radix Codonopsis* appeared too (Han, Cheng, & Chen, 2005; Sun & Liu, 2008; Zhang, Zhang, Yang, & Liang, 2010). Hot-water reflux extraction technology is the main extraction method of polysaccharides from *Radix Codononpsis* in recent researches, which is a classical extraction method of polysaccharides. It usually requires long extraction time, high temperature, but the extraction efficiency is low (Sun, Liu, & Kennedy, 2010). Therefore, it is essential and desirable to find an economical and high efficient extraction method of polysaccharides from *Radix Codonopsis*.

Recently, ultrasonic extraction was tested as an alternative method for isolating polysaccharides from different plant materials (Hromadkova and Ebringerova, 2003; Wang, Cheng, Mao, Fan, & Wu, 2009). It is reported that extraction efficiency is greatly enhanced by ultrasonic treatments. The ability of ultrasonic treatment to improve the recovery of polysaccharides is mainly attributed to its facilitation of mass transfer during immiscible phases through agitation, especially at low frequency (Vinatoru et al., 1997). Compared with classical methods, the ultrasonic treatment can reduce extraction time and energy, and enhance the extraction quotiety.

Response surface methodology (RSM) is an effective statistical technique for optimizing complex processes. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions. Therefore, it is less laborious and time-consuming than other approaches required to optimize a process (Zhong & Wang, 2010). It was widely used in optimizing the polysaccharide extraction process variables (Gan, Abdul Manaf, & Latiff, 2010; Guo, Zou, & Sun, 2010; Zhu, Heo, & Row, 2010). Box–Behnken design (BBD) is a type of response surface design. It is an independent quadratic design in that it does not contain an embedded factorial or fractional factorial design. In this design the treatment combinations are at the midpoints of edges of the process space and at the center. These designs are rotatable (or near rotatable) and require 3 levels of each factor. It is more efficient and easier to arrange and interpret experiments in comparison with others. It is widely used in many researches (Sun, Li, Yan, & Liu, 2010; Zhao, Wang, & Lu, 2009).

Even the ultrasonic treatment has so many advantages, there is no information published about the optimization of ultrasonic

* Corresponding author. Tel.: +86 835 2882612; fax: +86 835 2882612.
E-mail address: chenxf64@sohu.com (X. Chen).

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extraction conditions for polysaccharides from *C. pilosula*. So, in the present experiments, we optimized the extraction parameters of polysaccharides from *C. pilosula* by employing a Box–Behnken design. We wish to optimize the ultrasonic extraction conditions through a BBD (3 factors and 3 levels), to study the effects of extraction time, ratio of water to raw material and ultrasonic power on the yield of polysaccharides.

2. Materials and methods

2.1. Materials and equipments

The roots of *C. pilosula* were collected from Jiuzhaigou County, Sichuan Province, China, and identified by Xingfu Chen professor of Sichuan Agriculture University. Ethanol, phenol, sulphuric acid and glucose were obtained from the Chengdu Kelong Chemical Factory (Chengdu, China). KQ-400KDZ ultrasonic generator (Kunshan Ultrasonic Instruments Co., LTD, Jiangsu, China) was used for extraction. All other chemicals were of analytical grade.

2.2. Extraction methods

C. pilosula (2.0 g) was ultrasonically extracted with 80 mL of 80% ethanol for 30 min. Then, the filtered residues without ethanol were ultrasonically extracted with distilled water for different extraction times, twice. Finally, mixed the filter liquor of two extraction times together, added distilled water to 250 mL, prepared as sample liquor.

2.3. Determination of polysaccharides yield

The total sugar content of polysaccharide was quantified by the phenol–sulphuric acid method (Masuko et al., 2005), glucose was used as standard, and the results were then expressed as glucose equivalents (Hou & Chen, 2008).

2.4. Design of statistical experiments

After determining the preliminary range of extraction variables through single-factor test, a three-level-three-factor, Box–Behnken factorial design (BBD) was adopted in this optimization study. Extraction time (X_1), ratio of water to raw material (X_2), ultrasonic power (X_3) were the independent variables selected to be optimized for the extraction of polysaccharides. The range of independent variables and their levels were presented in Table 1. Extraction yield (Y) was taken 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.

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

Independent variables	Levels −1	0	1
Extraction time (X_1) (min)	30	40	50
Ratio of water to raw material (X_2)	30	40	50
Ultrasonic power (X_3) (W)	280	320	360

The variables were coded according to the equation

$$Xi = \frac{X_i - X_0}{\Delta X} \quad (1)$$

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:

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

where Y is the dependent variable, 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 by using SAS JMP Software (version 8.0) to estimate the response of the independent variables. Subsequently, three additional confirmation experiments were conducted to verify the validity of the statistical experimental strategies.

3. Results and discussion

3.1. Effect of extraction time on extraction yield of polysaccharides

The effect of extraction time on extraction yield of polysaccharides from *C. pilosula* was shown in Fig. 1. Firstly, the extraction time was set at 10, 20, 30, 40, 50 and 60 min while other extraction parameters were given as the followings: water volume 80 mL, ultrasonic power 360 W. It could be found that the extraction yield increased as extraction time ascended from 10 to 40 min, peaked at 40 min, and then no longer increased when the extraction time exceeded 40 min (see Fig. 1a).

Table 2
Box–Behnken experimental design and results for extraction yield.

No.	X_1 (extraction time, min)	X_2 (ratio of water to raw material)	X_3 (ultrasonic power, W)	Extraction yield (%)
1	1(50)	1(50)	0(320)	32.50
2	0(40)	−1(30)	1(360)	28.64
3	−1(30)	−1(30)	0(320)	18.30
4	1(50)	0(40)	1(360)	35.24
5	1(50)	−1(30)	0(320)	26.50
6	0(40)	1(50)	1(360)	34.20
7	−1(30)	1(50)	0(320)	26.45
8	0(40)	1(50)	−1(280)	31.40
9	0(40)	0(40)	0(320)	32.16
10	0(40)	0(40)	0(320)	32.22
11	1(50)	0(40)	−1(280)	25.46
12	−1(30)	0(40)	1(360)	21.35
13	0(40)	0(40)	0(320)	32.15
14	−1(30)	0(40)	−1(280)	16.85
15	0(40)	−1(30)	−1(280)	22.36

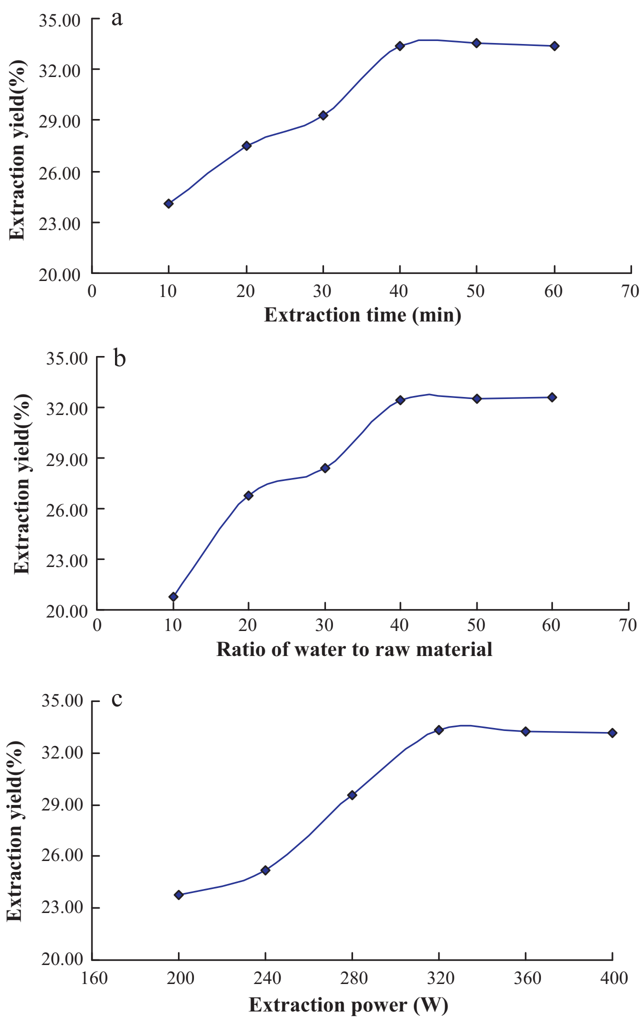

Fig. 1. Effect of different extraction parameters on extraction yield of polysaccharides from *C. pilosula* (extraction time, min; ratio of water to raw material; ultrasonic power, W).

3.2. Effect of ratio of water to raw material on yield of polysaccharides

Different ratio of water to raw material could significantly affect the extraction yield. If ratio of water to raw material is too small, polysaccharides in raw material cannot be completely extracted up. If the ratio of water to raw material is too high, it will cause high process cost (Govender et al., 2005). In this study, the effect of ratio of water to raw material on extraction yield of polysaccharides from *C. pilosula* was investigated, and the results were listed in Fig. 1b. The ratio of water to raw material was set at 10, 20, 30, 40, 50 and 60 while other extraction parameters were given as the followings: ultrasonic power 360 W, extraction time 30 min. It could be founded that the extraction yield of polysaccharides from *C. pilosula* continued to increase evidently with the increasing ratio of water to raw material. But the extraction yield of polysaccharides from *C. pilosula* started to increase slowly after the ratio of water to raw material exceeded 40 (Fig. 1b).

3.3. Effect of ultrasonic power on yield of polysaccharides

In this experiment, the efficiencies of different ultrasonic power on the yield of polysaccharides from *C. pilosula* were investigated, and the results were listed in Fig. 1c. Firstly, the other extraction conditions such as water volume, extraction time, were fixed at 80 mL, 30 min respectively and only ultrasonic power was changed. As shown in Fig. 1c, the extraction yield of polysaccharides from *C. pilosula* continued to increase with the increasing of ultrasonic power and reached the peak value (33.30%) when ultrasonic power was 320 W. The extraction yield of polysaccharides from *C. pilosula* no longer increased when the ultrasonic power exceeded 320 W.

Table 3
Regression coefficients of the predicted quadratic polynomial model.

Parameter	Estimate	Standard error	*t* ratio	*P*-value Prob. > *F*
X_1	4.59375	0.545802	8.42	0.0004[a]
X_2	3.59375	0.545802	6.58	0.0012[a]
X_3	2.92	0.545802	5.35	0.0031[a]
$X_1{}^*X_2$	−0.5375	0.771881	−0.70	0.5172
$X_1{}^*X_3$	1.32	0.771881	1.71	0.1479
$X_2{}^*X_3$	−0.87	0.771881	−1.13	0.3109
$X_1{}^*X_1$	−5.332083	0.803399	−6.64	0.0012[a]
$X_2{}^*X_2$	−0.907083	0.803399	−1.13	0.3101
$X_3{}^*X_3$	−2.119583	0.803399	−2.64	0.0461[a]

[a] Means significance (values of "Prob. > *F*" <0.0500).

3.4. Optimization of the extraction parameters of polysaccharides

3.4.1. Statistical analysis and the model fitting

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 were summarized. The percentage yield ranged from 16.85% to 35.24%. The maximum yield of polysaccharides (35.24%) was recorded extraction time 50 min, ratio of water to raw material 40 and ultrasonic power 360 W. The application of RSM suggested, 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:

$$Y = 32.1776 + 4.5938X1 + 3.5938X2 + 2.92X3 - 0.5375X1X2 + 1.32X1X3 - 0.87X2X3 - 5.3221X_1^2 - 0.9071X_2^2 - 2.1196X_3^2 \quad (3)$$

The determination coefficient ($R^2 = 0.9751$) was showed by ANOVA of the quadratic regression model, indicating that only 2.49% of the total variations were not explained by the model. The value of the adjusted determination coefficient (adjusted $R^2 = 0.9304$) also confirmed that the model was highly significant. At the same time, a very low value 5.57 of coefficient of the variation (CV) clearly indicated a very high degree of precision and a good deal of reliability of the experimental values. The model was found to be adequate for prediction within the range of experimental variables. The regression coefficient values of Eq. (3) were listed in Table 3. The *P*-values were used as a tool to check the significance of each coefficient, which in turn might indicate the pattern of the interactions between the variables. The smaller the value of *P* was, the more significant the corresponding coefficient was. It can be seen from this table that the linear coefficients (X_1, X_2, X_3), a quadratic term coefficient (X_1^2, X_3^2) were significant, with very small *P*-values ($P < 0.05$). The other term coefficients were not significant ($P > 0.05$). The full model filled Eq. (3) was made three-dimensional and contour plots to predict the relationships between the independent variables and the dependent variables.

3.4.2. Optimization of extraction conditions

Response surfaces were plotted by using SAS JMP (version 8.0) software to study the effects of parameters and their interac-

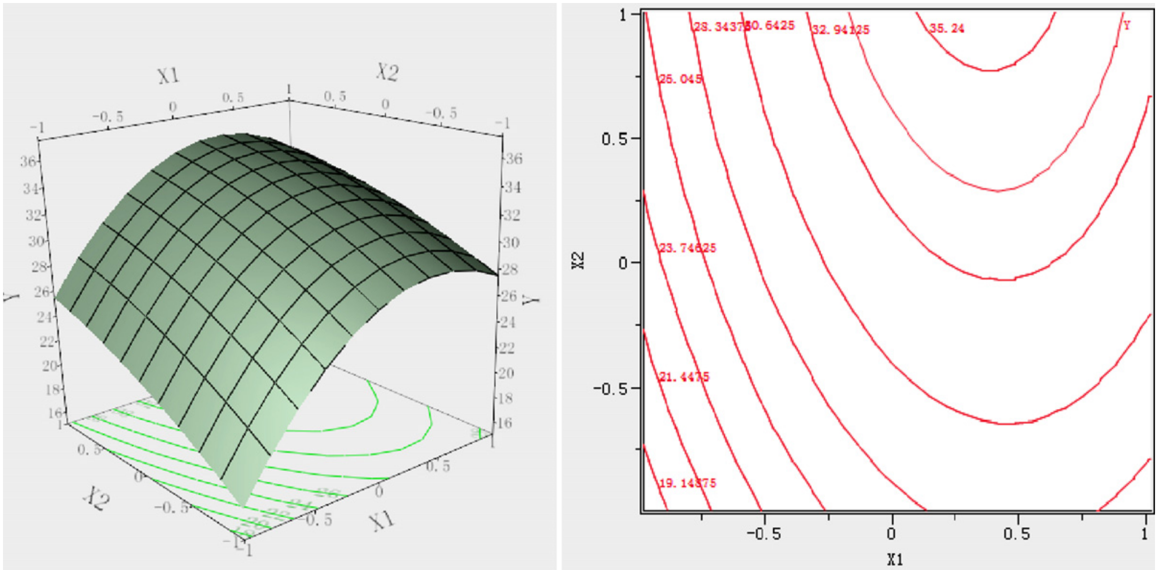

Fig. 2. Contour plot and response surface plot of $Y = f_1\ (X_1, X_2)$.

tions on polysaccharides yield. Three-dimensional response surface plots and two-dimensional contour plots, as presented in Figs. 2–4, were very useful to judge 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. The 3-D plot and the contour plot in Fig. 2, which gives the ultrasonic power (0 level), shows that extraction yield of polysaccharides increased with increasing of ratio of water to raw material, extraction time from 30 to 44.1 min, but beyond 44.1 min, extraction yield of polysaccharides decreased with increasing extraction time. Fig. 3 shows the 3-D plot and the contour plot at varying extraction time and ultrasonic power at fixed ratio of water to raw material (0 level). From Fig. 3, it can be seen that maximum extraction yield of polysaccharides can be achieved when extraction time and ultrasonic power were 44.1 min and 339.2 W, respectively. The extraction yield of polysaccharides increased evidently with increasing of extraction time from 30 to 44.1 min, but beyond 44.1 min, extraction yield of polysaccharides decreased as extraction time ascended. The extraction yield of polysaccharides increased evidently with increasing of extraction time from 320 to 339.2 W, but beyond 339.2 W, the extraction yield of polysaccharides descended with increasing ultrasonic power. The 3-D plot and the contour plot based on independent variables ratio of water to raw material and ultrasonic power were shown in Fig. 4, while the extraction time was kept at a zero level. An increase in the extraction yield of polysaccharides could be significantly achieved with the increases of ratio of water to raw material. It was obvious that the extraction yield of polysaccharides was increased with the increasing ultrasonic power from 320 to 339.2 W, meaning that further increases of ultrasonic power would not increase the extraction yield of polysaccharides.

According to Figs. 2–4, and above single parameter study, it can be concluded that optimal extraction condition of polysaccharides from *C. pilosula* were extraction time 44.1 min, ratio of water to raw material 56, ultrasonic power 339.2 W. Among the three extraction parameters studied, the extraction time was the most

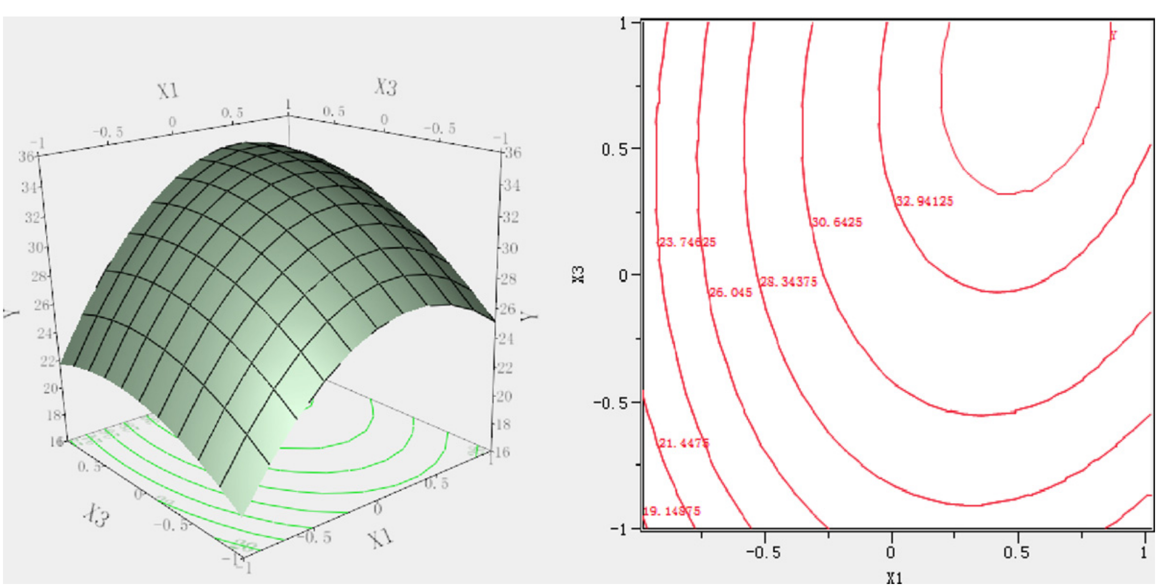

Fig. 3. Contour plot and response surface plot of $Y = f_2\ (X_1, X_3)$.

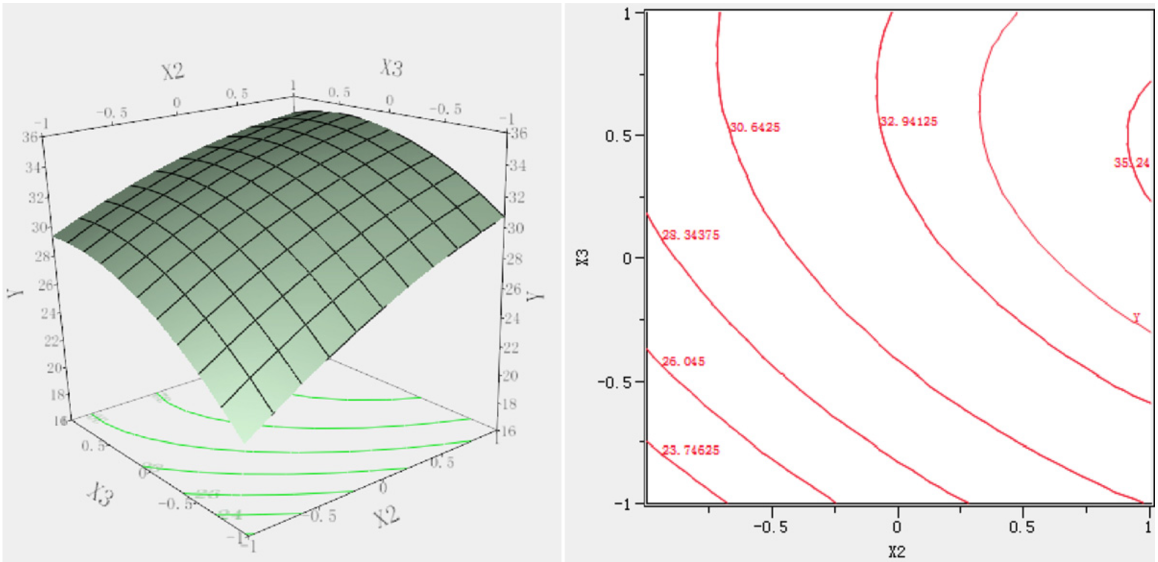

Fig. 4. Contour plot and response surface plot of $Y = f_3(X_2, X_3)$.

Table 4
Predicted and experimental values of the responses at optimum and modified conditions.

	Extraction time (min)	Ratio of water to material	Ultrasonic power (W)	Yield of polysaccharides
Optimum conditions	44.1	56.3	339.2	36.745 (predicted)
Modified conditions	44	56	320	36.264 ± 0.067% (actual)

significant factor to affect the extraction yield of polysaccharides from *C. pilosula*, followed by ultrasonic power and ratio of water to raw material according to the regression coefficients significance of the quadratic polynomial model (Table 3) and gradient of slope in the 3-D response surface plot (Figs. 2–4).

3.5. Verification of the models

The suitability of the model equation for predicting the optimum response values was tested by using the selected optimal conditions. The maximum predicted yield and experimental yield of *C. pilosula* polysaccharides were given in Table 4. Additional experiments by using the predicted optimum conditions for polysaccharides extraction were carried out: extraction time of 44.1 min, ratio of water to material 56.3 mL/g, ultrasonic power of 339.2 W, and the model predicted a maximum response of 36.745%. To ensure the predicted result was not biased toward the practical value, experiment rechecking was performed by using these modified optimal conditions: extraction time of 44 min, ratio of water to material 56 mL/g, ultrasonic power of 320 W. A mean value of 36.264 ± 0.067% ($N = 3$) was gained, obtained from real experiments, demonstrated the validation of the RSM model. The results of analysis confirmed that the response model was adequate for reflecting the expected optimization (Table 4), and the model of Eq. (3) was satisfactory and accurate.

4. Conclusions

Ultrasonic technology was performed for the polysaccharides extraction from *C. pilosula* in order to increase the yield extraction. Based on the single-factor experiments, Response surface methodology (RSM) was used to estimate and optimize the experimental variables: extraction time (min), ratio of water to raw material and ultrasonic power (W). All the independent variables, quadratic of extraction time and ultrasonic power had highly significant effects on the response values. The optimal extraction conditions for the polysaccharides were determined as follows: extraction time of 44 min, ratio of water to material 56, ultrasonic power of 320 W. Under these conditions, the experimental yield of polysaccharides was 36.264 ± 0.067%, which was closed with the predicted yield value.

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