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Application of response surface methodology for optimization of polysaccharides production parameters from the roots of *Codonopsis pilosula* by a central composite design

Yongxu Sun [a,*], Jicheng Liu [a,*], John F. Kennedy [b]

[a] Department of Medicinal Chemistry and Biomacromolecules, Qiqihar Medical University, Qiqihar 161042, China
[b] Birmingham Carbohydrate and Protein Technology Group, School of Chemistry, University of Birmingham, Birmingham B15 2TT, UK

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ABSTRACT

Response surface methodology (RSM) was used to optimize the extraction conditions of polysaccharides from the roots of *Codonopsis pilosula*. A central composite design (CCD) was used for experimental design and analysis of the results to obtain the optimal processing parameters. Four independent variables such as extraction temperature (°C), ratio of water to raw material, extraction time (h), and number of extraction (n) were investigated. The experimental data obtained were fitted to a second-order polynomial equation using multiple regression analysis and also analyzed by appropriate statistical methods. The 3-D response surface and the contour plots derived from the mathematical models were applied to determine the optimal conditions. The optimum extraction conditions were as follows: extraction temperature 94 °C, ratio of water to raw material 9, extraction time 2.5 h, and number of extraction 5. Under these conditions, the experimental percentage value was 15.2 ± 0.28, which is well in close agreement with value predicted by the model.

1. Introduction

Today, more and more attentions have been placed on herbs due to their potential use in a wide variety of industries including cosmetics, pharmaceutical, food industries, etc. in many countries. Radix Codonopsis is a traditional Chinese medicine herb distributed in the Northeast Provinces of China, belonging to the family Campanulaceae, and is sometimes used as a substitute of the much more costly *Panax ginseng* for medication or medicated diet. It has been used commonly in China folk for strengthen the middle warmer, invigorate the spleen and nourish lung. Its main constituents include sterol, triterpenes, glycoside, alkaloid, polysaccharide and so on (Liu, 1983; Nörr & Wagner, 1994; Sun & Liu, 2008; Wang, Ng, Yeung, & Xu, 1996; Wong, Chiang, & Chang, 1983). The modern pharmacology research indicated that it has the functions of antitumor, antimicrobial, antioxidant, and improving cellular immunity (Liu, Cai, & Shao, 1988; Luo et al., 2007; Shan, Yoshida, Sugiura, & Yamashita, 1999; Zneg, Li, & Zhang, 1992). In our previous research, one water-soluble polysaccharide was identified from the roots of *C. pilosula* and it possesses potent stimulating effect on murine lymphocyte proliferation induced by concanavalin A or lipopolysaccharide (Sun & Liu, 2008). However, so far there is not any information published about the optimization of extraction conditions for polysaccharides from the roots of *C. pilosula*.

When many factors and interactions affect desired production process response, response surface methodology (RSM) is an effective tool for optimizing the process, which was originally described by Box and Wilson (1951). RSM is a collection of statistical and mathematical techniques that has been successfully used to determine the effects of several variables and optimize processes (Atkinson & Donev, 1992). RSM has been successfully applied for optimizing conditions in food and pharmaceutical research (Batistuti, Barros, & Areas, 1991; Ibanoglu & Ainsworth, 2004; Shieh, Koehler, & Akoh, 1996; Varnalis, Brennan, MacDougall, & Gilmour, 2004; Vega, Balaban, Sims, O'Keefe, & Cornell, 1996). The main advantage of RSM is to reduce number of experimental trials needed to evaluate multiple variables and their interactions. Therefore, it is less laborious and time-consuming than other approaches required optimizing a process (Giovanni, 1983). Usually, it applies an experimental design such as central composite design (CCD) to fit a second-order polynomial by a least squares technique. An equation is used to describe how the test variables affect the response and determine the interrelationship among the variables.

The purpose of the present study was to optimize the process for production of polysaccharides from the roots of *C. pilosula* using response surface methodology (RSM). Employing a CCD (4 factors and 5 levels) to study the effects of extraction temperature, ratio

* Corresponding authors. Tel.: +86 452 6731303; fax: +86 452 6727648.
E-mail addresses: yongxusun1978@yahoo.com.cn (Y. Sun), jichengliu@yahoo.cn (J. Liu).

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of water to raw material, extraction time, and number of extraction on the extraction yield of CPP.

2. Materials and methods

2.1. Materials

Radix Codonopsis were purchased from drugstore in Changchun, and identified by Professor Ming-quan Li at Changchun University of Chinese Medicine according to China Pharmacopoeia (CP). All reagents were of analytical grade.

2.2. Preparation of polysaccharides and determination of polysaccharides yield

The roots of *C. Pilosulae* (1000 g) were ground in a blender to obtain a fine powder (Particle diameter size: 400–500 μm) and then were extracted for three times with 80% EtOH at 60 °C and 2 h each time to defat and remove some colored materials, oligosaccharides, and some small molecule materials under reflux in the apparatus, Soxhlet's. The pretreated samples were separated from the organic solvent by centrifugation (2000 *g* for 10 min). Each dried pretreated sample (20 g) was extracted by water in a designed temperature, water to raw material ratio, time, and number. The water extraction solutions were separated form insoluble residue through the nylon cloth (Pore diameter: 38 μm), concentrated and then precipitated by the addition of ethanol to a final concentration of 80% (v/v) to obtain crude polysaccharides (CPP). The content of the polysaccharides was measured by phenol-sulfuric method (Sun et al., 2008). The percentage polysaccharides extraction yield (%) is calculated as the polysaccharides content of extraction divided by dried sample weight (20 g).

2.3. Experimental design and statistical analysis

After determining the preliminary range of the extraction variables through single-factor test, a CCD with four independent variables ($X1$, extraction temperature; $X2$, ratio of water to raw material; $X3$, extraction time; $X4$, number of extraction) at five levels was performed (Box & Behnken, 1960). For statistical calculation, the variables were coded according to

$$\chi_i = (X_i - X_0)/\Delta X_i \tag{1}$$

where χ_i is a coded value of the variable; X_i the actual value of variable; X_0 the actual value of the X_i on the center point; and ΔX_i the step change value. The range of independent variables and their levels are presented in Table 1, which was based on the results of preliminary experiments. The extraction yield of CPP was the dependent variables. As seen from Table 2, the complete design consisted of 31 experimental points (16 factorial points, 8 axial points and 7 center points), and the experiment was carried out in a random order.

Data from CCD were analyzed by multiple regressions to fit the following quadratic polynomial model.

$$Y = \beta_{k0} + \sum_{i=1}^{4}\beta_{ki}\chi_i + \sum_{i=1}^{4}\beta_{kii}\chi_i^2 + \sum_{i<j=2}^{4}\beta k_{ij}\chi_i\chi_j \tag{2}$$

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

Independent variables	Factor level				
	−2	−1	0	1	2
Extraction temperature (°C)	80	85	90	95	100
Ratio of water to raw material	5	6	7	8	9
Extraction time (h)	1	1.5	2	2.5	3
Number of extraction	2	3	4	5	6

Y represent the response function. β_{k0} is an intercept. Where β_{ki}, β_{kii} and β_{kij} are the coefficients of the linear, quadratic and interactive terms, respectively. And accordingly χ_i and χ_j represent the coded independent variables, respectively. The fitted polynomial equation is expressed as surface and contour plots in order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions (Lu, Engelmann, Lila, & Erdman, 2008). The analysis of variance tables were generated, and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The regression coefficients were then used to make statistical calculation to generate dimensional and contour maps from the regression models. SAS (Version 8.0, USA) software package was used to analyze the experimental data. P-values of less than 0.05 were considered to be statistically significant.

3. Results and discussion

3.1. Statistical analysis and the model fitting

Response surface optimization is more advantageous than the traditional single parameter optimization in that it saves time, space and raw material. There were a total of 31 runs for optimizing the four individual parameters in the current CCD. Table 2 shows the experimental conditions and the results of extraction yield of CPP according to the factorial design. Maximum extraction yield of CPP (14.69%) was recorded under the experimental conditions of extraction temperature 90 °C, ratio of water to raw material 7, extraction time 3 h, and number of extraction 4. By applying multiple regression analysis on the experimental data, the response variable and the test variables were related by the following second-order polynomial equation:

$$\begin{aligned}Y = {}& 12.07 + 0.5925 * X1 + 1.514167 * X2 + 0.529167 * X3 \\ & + 1.071667 * X4 - 0.4725 * X1 * X1 - 0.05875 * X1 * X2 \\ & + 0.0575 * X1 * X3 + 0.255 * X1 * X4 - 0.145 * X2 * X2 \\ & + 0.0125 * X2 * X3 - 0.4025 * X2 * X4 - 0.31625 * X3 * X3 \\ & + 0.00625 * X3 * X4 - 0.3025 * X4 * X4\end{aligned} \tag{3}$$

The results of the analysis of variance, goodness-of-fit and the adequacy of the models were summarized in Table 3. The determination coefficient (R^2 = .9660) was showed by ANOVA of the quadratic regression model, indicating that only 3.4% of the total variations were not explained by the model. The value of the adjusted determination coefficient (Adjusted R^2 = .9363) also confirmed that the model was highly significant. At the same time, a very low value 4.45 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 4. The P-values were used as a tool to check the significance of each coefficient, which in turn may indicate the pattern of the interactions between the variables. The smaller was the value of P, the more significant was the corresponding coefficient. It can be seen from this table that the linear coefficients ($X1$, $X2$, $X3$, $X4$), a quadratic term coefficient ($X1^2$, $X3^2$, $X4^2$) and cross product coefficients ($X2 * X4$) 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.

3.2. Optimization of extraction conditions of CPP

The graphical representations of the regression Eq. (3), called the response surfaces and the contour plots were obtained using

Table 2
Response surface central composite design (uncoded) and results for extraction yield of CPP.

No.	X1, Extraction temperature (°C)	X2, Ratio of water to raw material	X3, Extraction time (h)	X4, Number of extraction	Extraction yield (%)
1	−1(85)	−1(6)	−1(1.5)	−1(3)	7.03
2	−1(85)	−1(6)	−1(1.5)	1(5)	8.98
3	−1(85)	−1(6)	1(2.5)	−1(3)	7.24
4	−1(85)	−1(6)	1(2.5)	1(5)	10.46
5	−1(85)	1(8)	−1(1.5)	−1(3)	10.67
6	−1(85)	1(8)	−1(1.5)	1(5)	11.89
7	−1(85)	1(8)	1(2.5)	−1(3)	11.96
8	−1(85)	1(8)	1(2.5)	1(5)	12.35
9	1(95)	−1(6)	−1(1.5)	−1(3)	7.48
10	1(95)	−1(6)	−1(1.5)	1(5)	10.68
11	1(95)	−1(6)	1(2.5)	−1(3)	8.3
12	1(95)	−1(6)	1(2.5)	1(5)	11.97
13	1(95)	1(8)	−1(1.5)	−1(3)	10.9
14	1(95)	1(8)	−1(1.5)	1(5)	13.3
15	1(95)	1(8)	1(2.5)	−1(3)	12.43
16	1(95)	1(8)	1(2.5)	1(5)	14.02
17	−2(80)	0(7)	0(2)	0(4)	9.21
18	2(100)	0(7)	0(2)	0(4)	12.07
19	0(90)	−2(5)	0(2)	0(4)	9.21
20	0(90)	2(9)	0(2)	0(4)	14.69
21	0(90)	0(7)	−2(1)	0(4)	10.04
22	0(90)	0(7)	2(3)	0(4)	12.49
23	0(90)	0(5)	0(2)	−2(2)	9.3
24	0(90)	0(9)	0(2)	2(6)	13.34
25	0(90)	0(7)	0(2)	0(4)	12.07
26	0(90)	0(7)	0(2)	0(4)	12.07
27	0(90)	0(7)	0(2)	0(4)	12.07
28	0(90)	0(7)	0(2)	0(4)	12.07
29	0(90)	0(7)	0(2)	0(4)	12.07
30	0(90)	0(7)	0(2)	0(4)	12.07
31	0(90)	0(7)	0(2)	0(4)	12.07

Table 3
Fit statistics for *Y*.

	Master model	Predictive model
RMSE	0.495039	0.495039
R^2	96.60%	96.60%
Adjusted R^2	93.63%	93.63%
Coefficient of variation	4.454637	4.454637

Table 4
Regression coefficients of the predicted quadratic polynomial model.

Parameter	Estimate	Standard error	*t* Ratio	*P*-value
X1	0.5925	0.10105	5.863463	0.0001
X2	1.514167	0.10105	14.9844	0.0001
X3	0.529167	0.10105	5.236707	0.0001
X4	1.071667	0.10105	10.60536	0.0001
X1 ∗ *X1*	−0.4725	0.092574	−5.10402	0.000106
X1 ∗ *X2*	−0.05875	0.12376	−0.47471	0.641409
X1 ∗ *X3*	0.0575	0.12376	0.464609	0.648471
X1 ∗ *X4*	0.255	0.12376	2.060442	0.056003
X2 ∗ *X2*	−0.145	0.092574	−1.56631	0.136837
X2 ∗ *X3*	0.0125	0.12376	0.101002	0.920804
X2 ∗ *X4*	−0.4025	0.12376	−3.25227	0.004997
X3 ∗ *X3*	−0.31625	0.092574	−3.41619	0.003537
X3 ∗ *X4*	0.00625	0.12376	0.050501	0.960348
X4 ∗ *X4*	−0.3025	0.092574	−3.26766	0.004838

SAS version 8.0, and the results of extraction yield of CPP affected by extraction temperature, ratio of water to raw material, extraction time, and number of extraction were presented in Figs. 1 and 2. Response surface methodology plays a key role in identifying the optimum values of the independent variables efficiently, under which dependent variable could arrive the maximum response. In the response surface plot and contour plot, the extraction yield of CPP was obtained along with two continuous variables, while the other two variables were fixed constant at their respective zero level (center value of the testing ranges). In the two figures, the maximum predicted value indicated by the surface was confined in the smallest ellipse in the contour diagram. Elliptical contours are obtained when there is a perfect interaction between the independent variables (Muralidhar, Chirumamil, Marchant, & Nigam, 2001). The independent variables and maximum predicted values from the figures corresponded with the optimum values of the dependent variables (responses) obtained by the equations.

In Figs. 1a and 2a, when the 3-D response surface plot and the contour plot were developed for the extraction yield of CPP with varying extraction temperature and ratio of water to raw material at fixed extraction time (0 level) and number of extraction (0 level), the extraction yield of CPP increased with the increasing ratio of water to raw material, and increased rapidly with increase of extraction temperature from 90 to 94 °C, then dropped from 94 to 100 °C. The 3-D response surface plot and the contour plot in Figs. 1b and 2b, which give the extraction yield of CPP as a function of extraction temperature and time at fixed ratio of water to raw material (0 level) and number of extraction (0 level), indicated that the maximum extraction yield of CPP can be achieved when extraction temperature and time at the threshold level of 94 °C and 2.5 h, respectively. The extraction yield of CPP affected by different extraction temperature and number of extraction was seen in Figs. 1c and 2c, when other two variables (ratio of water to raw material and extraction time) were fixed at 0 level. It can be seen that the extraction yield of CPP increased with the increasing number of extraction and reached the maximum value when extraction temperature at 94 °C, but beyond this level, extraction yield of CPP decreased with increasing extraction temperature. The Figs. 1d and 2d showed the 3-D response surface plot and the contour plot at varying ratio of water to raw material and extraction time at fixed extraction temperature (0 level) and

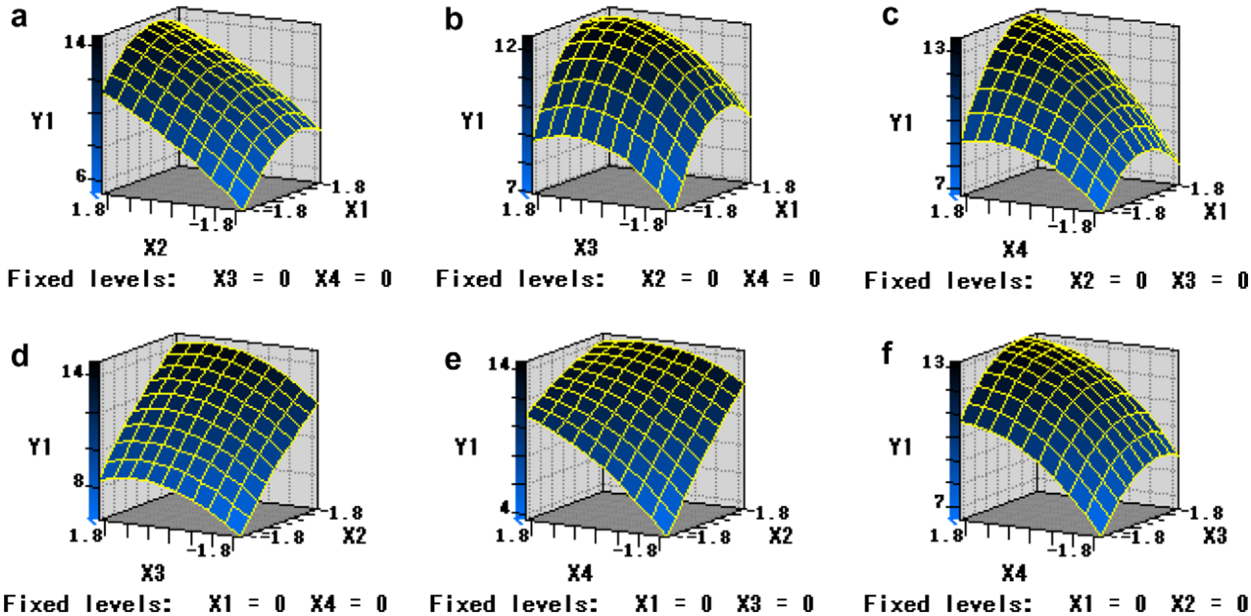

Fig. 1. Response surface plots (3-D) showing the effects of variables (*X*1: extraction temperature, °C; *X*2: ratio of water to raw material; *X*3: extraction time, h; *X*4: number of extraction, *n*) on the response *Y*1.

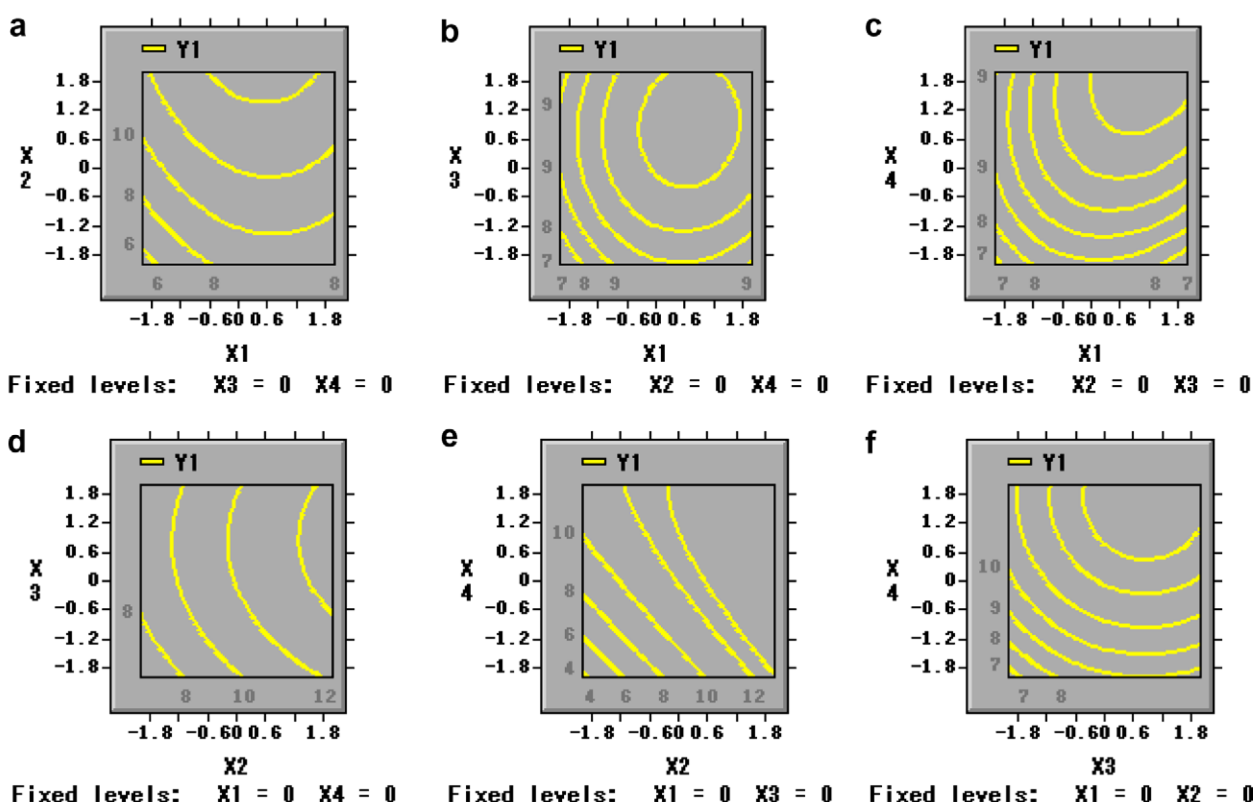

Fig. 2. Contour plots (2-D) showing the effects of variables (*X*1: extraction temperature, °C; *X*2: ratio of water to raw material; *X*3: extraction time, h; *X*4: number of extraction, *n*) on the response *Y*1.

number of extraction (0 level). As in the case of the extraction yield of CPP, ratio of water to raw material had a positive impact on the extraction yield of CPP. There was an increase in the extraction yield of CPP with increase in the ratio of water to raw material. However, the extraction yield of CPP was found to increase rapidly with increase of extraction time from 1 h to 2.5 h, but beyond 2.5 h, the yield decreased with increasing extraction time. The 3-D response surface plot and the contour plot based on independent variables ratio of water to raw material and number of extraction were shown in Figs. 1e and 2e, while the other two independent variables, extraction temperature and extraction time were kept at a zero level. An increase in the extraction yield of CPP could be evidently achieved with the increases of ratio of water to raw material or number of extraction. It was obvious that the

Table 5
Predicted and experimental values of the responses at optimum conditions.

Optimum condition				Extraction yield of CPP (%)	
Extraction temperature	Ratio of water to raw material	Extraction time	Number of extraction	Experimental[a]	Predicted
94 °C	9	2.5 h	5	15.2 ± 0.28	15.1

[a] Mean ± standard deviation (n = 3).

extraction yield of CPP was directly proportional to ratio of water to raw material and number of extraction in certain range of variables. The Figs. 1f and 2f showed the 3-D response surface plot and the contour plot at varying extraction time and number of extraction at fixed extraction temperature (0 level) and ratio of water to raw material (0 level). From two figures, we can concluded that the extraction yield of CPP increase with increase in number of extraction, and extraction yield of CPP was found to increase rapidly with increase of extraction time from1 to 2.5 h, but beyond 2.5 h, extraction yield of CPP decreased with increasing extraction time.

As shown in Figs. 1 and 2, it can be concluded that optimal extraction condition of CPP from the roots of *C. pilosula* were extraction temperature 94 °C, ratio of water to raw material 9, extraction time 2.5 h, and number of extraction 5. Among the four extraction parameters studied, ratio of water to raw material was the most significant factor to affect the extraction yield of CPP, followed by number of extraction, extraction temperature, and extraction time according to the regression coefficients significance of the quadratic polynomial model (Table 4) and gradient of slope in the 3-D response surface plot (Fig. 1).

3.3. Verification of predictive model

The suitability of the model equations for predicting optimum response values was tested under the conditions: extraction temperature 94 °C, ratio of water to raw material 9, extraction time 2.5 h, and number of extraction 5. This set of conditions was determined to be optimum by the RSM optimization approach and was also used to validate experimentally and predict the values of the responses using the model equation. A mean value of 15.2 ± 0.28 (N = 3), obtained from real experiments, demonstrated the validation of the RSM model, indicating that the model was adequate for the extraction process (Table 5).

4. Conclusion

The extraction conditions have significant effects on the purity of CPP. Using the contour and surface plots in RSM was effective for estimating the effect of four independent variables (extraction temperature, °C; ratio of water to raw material; extraction time, h; and number of extraction). The optimum set of the independent variables was obtained graphically in order to obtain the desired levels of crude polysaccharides extraction. The optimal experimental extraction yield of 15.2 ± 0.28% was obtained when the optimum conditions of POP extraction was extraction temperature 94 °C, ratio of water to raw material 9, extraction time 2.5 h, and number of extraction 5. Under these optimized conditions the experimental purity of CPP agreed closely with the predicted yield of 15.1%.

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