Carbohydrate Polymers 82 (2010) 209–214

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Extraction optimization of antioxidant polysaccharides from the fruiting bodies of *Chroogomphis rutilus* (Schaeff.: Fr.) O.K. Miller by Box-Behnken statistical design

Yong-Xu Sun [a,*], Ji-Cheng Liu [a,*], John F. Kennedy [b]

[a] *Department of Medicinal Chemistry and Biomacromolecules, Qiqihar Medical University, Qiqihar 161042, China*
[b] *Chembiotech Laboratories, Institute of Advanced Science and Technology, 5 The Croft, Buntsford Drive, Stoke Heath, Bromsgrove, Worcs B60 4JE, UK*

ARTICLE INFO

Article history:
Received 13 April 2010
Received in revised form 23 April 2010
Accepted 27 April 2010
Available online 2 May 2010

Keywords:
Chroogomphis rutilus
Polysaccharides
Extraction
Optimization
Response surface methodology

ABSTRACT

Response surface methodology (RSM) was used to optimize the extraction conditions of polysaccharides (CRP) from the fruiting bodies of *Chroogomphis rutilus* with a Box-Behnken design (BBD). Three independent variables such as extraction temperature (°C), extraction time (h) and ratio of water to raw material were investigated. The experimental data obtained were fitted to a second-order polynomial equation using multiple regression analysis. The 3-D response surface plot and the contour plot derived from the mathematical models were applied to determine the optimal conditions. The optimum extraction conditions were as follows: extraction temperature 96.6 °C, extraction time 2.7 h and ratio of water to raw material 22. Under these conditions, the experimental value of 57.7 ± 0.77 was well in close agreement with value predicted by the model. Preliminary in vitro antioxidant activity test showed CRP could scavenge hydroxyl radicals in a dose-dependent manner.

1. Introduction

Nowadays more and more attention was cast on polysaccharide by biochemical and nutritional researchers due to their various biological activities used in health-care food or medicine, especially antioxidant, immunostimulatory, and antitumor effects (Li, Chen, Wang, Tian, & Zhang, 2009; Qiao et al., 2009; Sun & Liu, 2009; Sun et al., 2009; Yuan, Zhang, Fan, & Yang, 2008). These polysaccharides are often identified as biological response modifiers (BRMs), and much of them are derived from the mushrooms. However maybe only 10% of mushrooms (approximately 14,000 named species) are known. Mushrooms comprise a vast and yet largely untapped source of powerful new pharmaceutical products. In particular, and most importantly for modern medicine, they represent an unlimited source of polysaccharides with antitumor and immunostimulating properties (Sun et al., 2008).

Optimizing refers to improving the performance of a system, a process, or a product in order to obtain the maximum benefit from it. When many factors and interactions affect desired response, response surface methodology (RSM) is an effective tool for optimizing the process, which was originally developed by Box and Wilson in the 50s (Box & Wilson, 1951). RSM is a collection of statistical and mathematical techniques that are based on the fit of empirical models to the experimental data obtained in relation to experimental design (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). Today, application of response surface methodology is popular to optimize technical parameter principally because of its advantages to other approaches required optimizing a process, such as a decrease of time and expenses as well as a saving in the consumption of reagents and materials. Usually, it applies an experimental design such as three level factorial, Box-Behnken (BBD), central composite (CCD), and Doehlert designs (DDD) to fit a second-order polynomial by a least squares technique, and to evaluate the quality of the fitted model and its accuracy to make previsions in relation to the experimental data obtained (Liu, Miao, Wen, & Sun, 2009).

C. rutilus is a traditional Chinese medicinal and edible fungus distributed in the Northeast Provinces of China, which is a Gomphidius fungus belonging to the Basidiomycotina. In our previous research, we successfully characterized the structural features of one water-soluble polysaccharide from the fruiting bodies of *C. rutilus* (Sun, Li, Yang, Liu, & Kennedy, 2010). However, up to now, no detailed investigation has been conducted on optimization of polysaccharide extraction from the fruiting bodies of *C. rutilus*. Therefore, the purpose of the present study was to employ a BBD (3 factors and 3 levels) to optimize the effects of extraction temperature, extraction time and ratio of water to raw material on the purity of CRP from the fruiting bodies of *C. rutilus*. Furthermore on basis of hydroxyl

* Corresponding authors. Tel.: +86 452 6731303; fax: +86 452 6727648.
E-mail address: yongxusun1978@yahoo.com.cn (Y.-X. Sun).

0144-8617/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.04.076

radical scavenging assay, we preliminarily evaluated its antioxidant activity for seeking high quality biological functional principle used in food and pharmaceutical industry.

2. Materials and methods

2.1. Materials

The fruiting bodies of *C. rutilus* were purchased from Heilongjiang Tianjin Fungi Co., Ltd. Phenol was from Beijing Dingguo Biotechnology Co., Ltd. D-Glucose was form Amresco Inc. Deoxyribose, trichloride ferric, ethylene diamine tetraacetic acid (EDTA), H_2O_2, ascorbate acid, and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade.

2.2. Extraction and purity determination of CRP

The fruiting bodies of *C. rutilus* (2000 g) were ground in a blender to obtain a fine powder. Each dried pretreated sample (20 g) was extracted by water in a designed temperature, extraction time, water to raw material ratio and extraction number. The water extraction solutions were separated form insoluble residue by centrifugation (2000 × *g* for 10 min, at 20 °C), and then precipitated by the addition of dehydrated alcohol to a final concentration of 80% (v/v). The precipitates (CRP) collected by centrifugation (2000 × *g* for 10 min, at 20 °C) were washed by dehydrated alcohol for three times and dried under reduced pressure. The sugar content was measured by phenol-sulfuric method using D-glucose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The purity (%) of CRP is calculated as the sugar content of extraction/dried crude polysaccharide weight.

2.3. Experimental design and statistical analysis

On the basis of single-factor experiment for the polysaccharides production, proper ranges of extraction temperature, extraction time, ratio of water to raw material and extraction number were preliminarily determined. A BBD with three independent variables (X1, extraction temperature; X2, extraction time; X3, ratio of water to raw material) at three levels was performed. Based on the investigations on single-factor experiment, the variables considered were extraction temperature, extraction time and ratio of water to raw material in this experimental design. For statistical calculation, the variables were coded according to

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

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

Independent variables	Factor level		
	−1	0	1
X1: extraction temperature (°C)	90	95	100
X2: extraction time (h)	2	2.5	3
X3: ratio of water to raw material	16	19	22

where x_i was a coded value of the variable; X_i was the actual value of variable; X_0 was the actual value of the X_i on the center point; and ΔX was the step change value. The range of independent variables and their levels is presented in Table 1, which was based on the results of preliminary experiments. The purity of CRP was the dependent variables. As seen from Table 2, the whole design consisted of 15 experimental points carried out in random order. Three replicates (treatment 13–15) at the center of the design were used to allow for estimation of a pure error sum of squares. The response value in each trial was average of duplicates.

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

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

Y represented the response function. A_0 was constant. A_i, A_{ii} and A_{ij} were the coefficients of the linear, quadratic and interactive terms, respectively. And accordingly X_i and X_j represented the coded independent variables. According to the analysis of variance, the regression coefficients of individual linear, quadratic and interaction terms were determined. In order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions, the regression coefficients were then used to make statistical calculation to generate 3-D surface plots and contour plots from the fitted polynomial equation (Lu et al., 2008). Design-Expert (Version 7.0) software package was used to analyze the experimental data. The *P*-values of less than 0.05 were considered to be statistically significant.

2.4. Hydroxyl radical assay

Assessment of the scavenging ability of CRP on hydroxyl radicals was performed by the method previously described by Halliwell, Gutteridge, & Aruoma (1987), with a minor modification. Reaction mixtures in a final volume of 1.0 ml contained deoxyribose (60 mM), phosphate buffer (pH 7.4, 20 mM), ferric trichloride (100 μM), EDTA (100 μM), H_2O_2 (1 mM), ascorbic acid (100 μM)

Table 2
Box-Behnken design matrix of three variables and the experimentally observed responses.

No.	X1/extraction temperature (°C)	X2/extraction time (h)	X3/ratio of water to raw material	Polysaccharides purity (%)
1	−1 (90)	−1 (2)	0 (19)	42.1
2	−1 (90)	1 (3)	0 (19)	44.5
3	1 (100)	−1 (2)	0 (19)	41.4
4	1 (100)	1 (3)	0 (19)	46.2
5	0 (95)	−1 (2)	−1 (16)	42.5
6	0 (95)	−1 (2)	1 (22)	46.5
7	0 (95)	1 (3)	−1 (16)	43.4
8	0 (95)	1 (3)	1 (22)	55.6
9	−1 (90)	0 (2.5)	−1 (16)	44.9
10	1 (100)	0 (2.5)	−1 (16)	43.1
11	−1 (90)	0 (2.5)	1 (22)	48.5
12	1 (100)	0 (2.5)	1 (22)	54.3
13	0 (95)	0 (2.5)	0 (19)	53.3
14	0 (95)	0 (2.5)	0 (19)	53.3
15	0 (95)	0 (2.5)	0 (19)	53.3

and different concentrations of CRP (0, 0.25, 0.5, 1, 2, or 4 mg/ml). Solutions of ferric trichloride and ascorbic acid were made immediately before use. The reaction solution was incubated for 1 h at 37 °C, and then 1 ml of 1% TBA and 1 ml of 20% (v/v) HCl were added to the mixture. The mixture was boiled for 15 min and cooled on ice. Deionized water and ascorbic acid served as blank and positive control, respectively. The absorbance of the resulting mixture was measured at 532 nm. The scavenging activity of hydroxyl radical (%) was calculated according to the following equation:

$$\text{Scavenging effect } (\%) = \frac{A_{532(\text{blank})} - A_{532(\text{sample})}}{A_{532(\text{blank})}} \times 100$$

Where $A_{532(\text{blank})}$ was the absorbance of the control (deionized water, instead of sample); $A_{532(\text{sample})}$ was the absorbance of the test sample mixed with reaction solution.

3. Results and discussion

3.1. The effect of different extraction temperature on the purity of CRP

Different extraction temperature was set at 70, 75, 80, 85, 90, 95 and 100 °C to investigate the influence of extraction temperature on the purity of CRP when the other reaction conditions were set as follows: extraction time 2.5 h, ratio of water to raw material 19 and extraction number 3. Fig. 1A indicates that the purity of CRP increased with the increasing extraction temperature and reached the peak value around 95 °C. And then there was no increase when extraction temperature continued to rise. Therefore, 90–100 °C was considered to be optimal extraction temperature in this experiment.

3.2. The effect of different extraction time on the purity of CRP

The purity (%) of CRP affected by different extraction time (0.5, 1, 1.5, 2, 2.5, 3, and 3.5 h) is seen in Fig. 1B, when the other three factors (extraction temperature, ratio of water to raw material and extraction number) were fixed at 95 °C, 19 and 3 times, respectively. The purity of CRP reached a maximum percentage of 53.1 ± 1.03 when the extraction time was 2.5 h. After this point, the purity of CRP started to maintain a descending dynamic equilibrium with increasing the extraction time. This situation maybe due to the polysaccharide hydrolyses under some temperature and long extraction time. Therefore, extraction time range of 2–3 h was adopted in the present work.

3.3. The effect of different ratio of water to raw material on the purity of CRP

The purity of CRP affected by different ratio of water to raw material (10, 13, 16, 19, 22, 25 and 28) is seen in Fig. 1C, when the other three factors (extraction temperature, extraction time and extraction number) were fixed at 95 °C, 2.5 h and 3 times, respectively. The result implied that the purity of CRP was enhanced to the critical value (53.2 ± 0.53%) at the ratio of 19, and then it maintained a mild slope when the ratio of water to raw material increasing. Herein the ratio of water to raw material range of 16–22 was investigated in the present work.

3.4. The effect of different extraction number on the purity of CRP

The purity of CRP affected by different extraction number (1–7 times) is seen in Fig. 1D, when other three factors (extraction temperature, ratio of water to raw material and extraction time) were fixed at 0 level, respectively. The results showed that the purity of CRP had obvious increased accompanying the increase of extracting times, but there was no significant increase after 2 times. In order to save the production cost and time for industrialization, 2 times were sufficient for the extraction of polysaccharides.

Therefore 2 times were chosen as the extracting number through all the extraction optimization experiments.

3.5. Optimization of the procedure

3.5.1. Statistical analysis and the model fitting

RSM optimization is more advantageous than the traditional single parameter optimization in that it saves time, space and raw material. There were a total of 15 runs for optimizing the three individual parameters in the BBD. Table 2 shows the

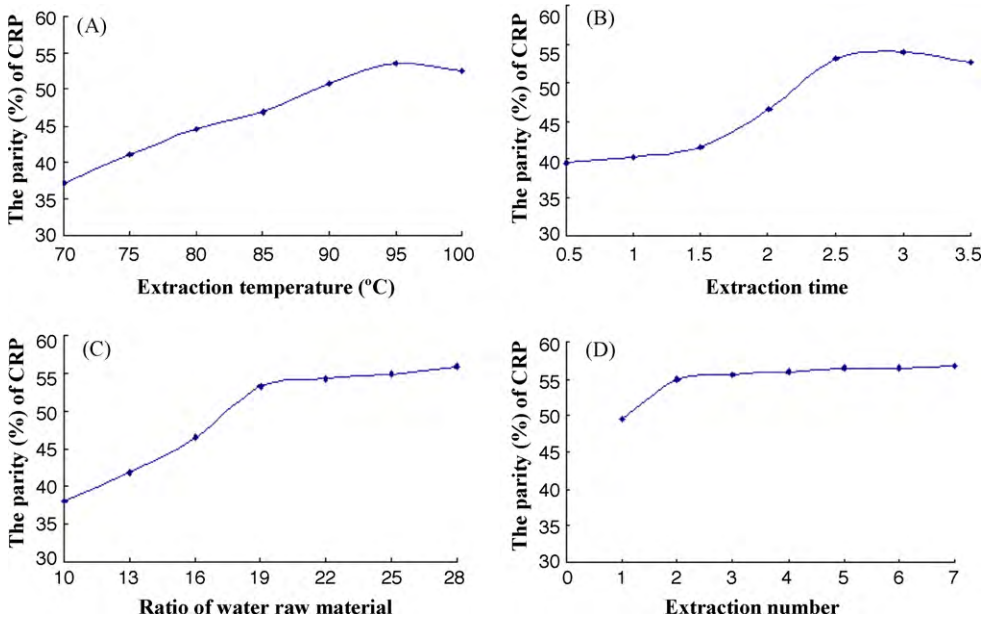

Fig. 1. Effect of different extraction parameters on the purity of CRP (extraction temperature, °C; extraction time, h; ratio of water to raw material; extraction number).

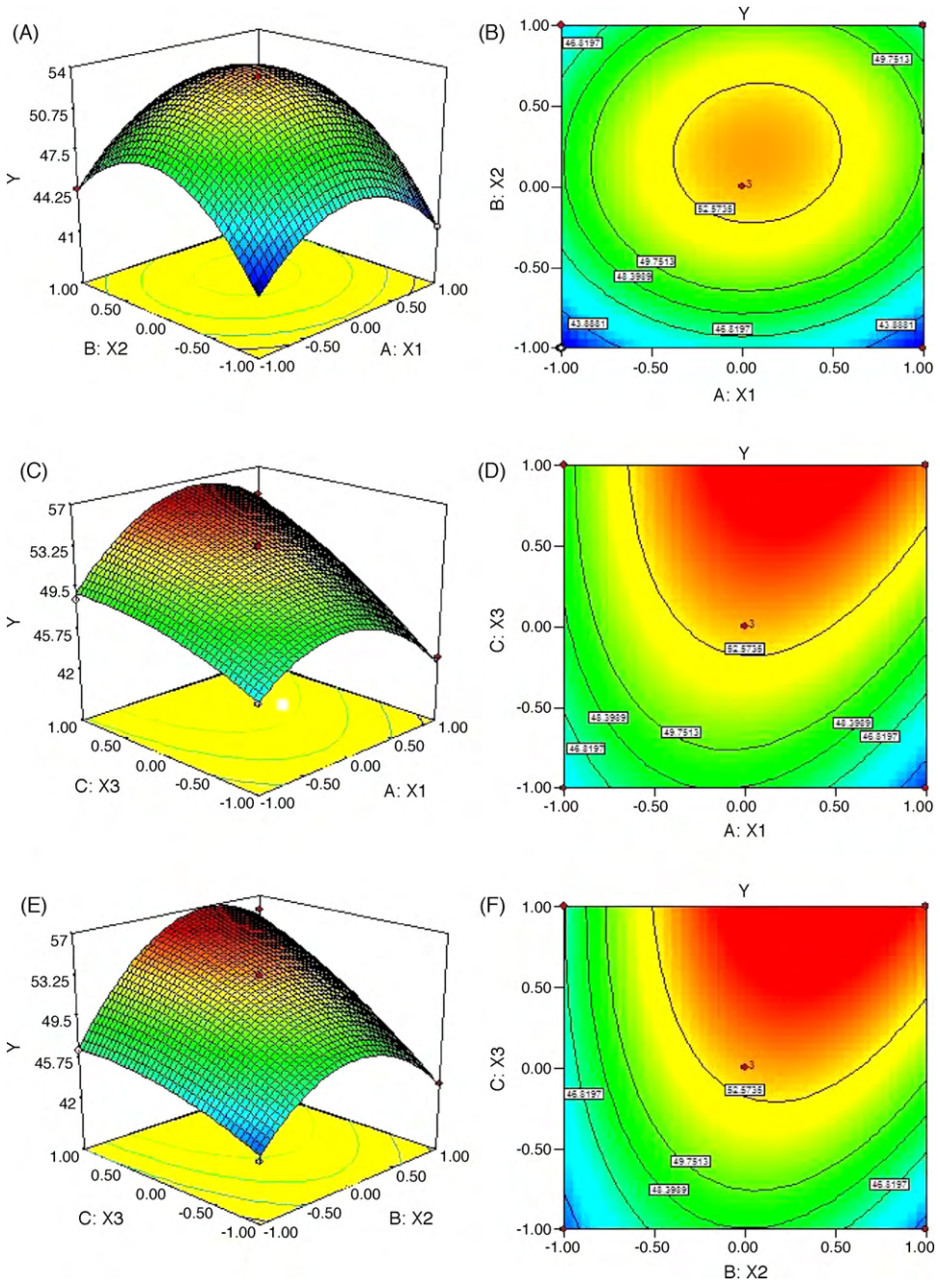

Fig. 2. Response surface plots (3-D) and contour plots (2-D) showing the effects of variables (X1: extraction temperature; X2: extraction time; X3: ratio of water to raw material) on the response Y.

experimental conditions and the results of purity of CRP according to the factorial design. Maximum purity of CRP (55.6%) was recorded under the experimental conditions of extraction temperature 95 °C, extraction time 3 h and ratio of water to raw material 22. 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:

$$Y = 53.3 + 0.625 \times X1 + 2.15 \times X2 + 3.875 \times X3 - 4.525 \times X1 \times X1 + 0.6 \times X1 \times X2 + 1.9 \times X1 \times X3 - 5.225 \times X2 \times X2 + 2.05 \times X2 \times X3 - 1.075 \times X3 \times X3 \quad (3)$$

The results of the analysis of variance, goodness-of-fit and the adequacy of the models are summarized in Table 3. The determination coefficient (R^2 = .9935) was showed by ANOVA of the quadratic regression model, indicating that only 0.65% of the total variations was not explained by the model. The value of the adjusted determination coefficient (Adj R^2 = .9817) also confirmed that the model was highly significant. At the same time, a very low value

Table 3
Fit statistics for Y.

	Master model	Predictive model
Mean	47.53	47.53
R-square	99.35%	99.35%
Adjusted *R*-square	98.17%	98.17%
Coefficient of variation	1.442486	1.442486

Table 4
Regression coefficients of the predicted quadratic polynomial model.

Parameter	Estimate	Standard error	*t* ratio	*P*-value
X1	0.625	0.2424	2.5786	0.0495
X2	2.15	0.2424	8.8702	0.0003
X3	3.875	0.2424	15.9870	0.0001
X1 × X1	−4.525	0.3568	−12.6829	0.0001
X1 × X2	0.6	0.3428	1.7504	0.1405
X1 × X3	1.9	0.3428	5.5429	0.0026
X2 × X2	−5.225	0.3568	−14.6449	0.0001
X2 × X3	2.05	0.3428	5.9805	0.0019
X3 × X3	−1.075	0.3568	−3.0131	0.0297

1.44 of coefficient of the variation (C.V.) 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) are listed in Table 4. The *P*-value was 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 the value of *P*, the more significant the corresponding coefficient. It can be seen from this table that the linear coefficients (X1, X2, X3), quadratic term coefficients ($X1^2$, $X2^2$, $X3^2$) and cross product coefficients (X1 × X3, X2 × X3) 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 the 3-D response surface plot and the contour plot to predict the relationships between the independent and dependent variables.

3.5.2. Optimization of extraction conditions of CRP

The graphical representations of the regression Eq. (3), called the response surface and the contour plots, were obtained using Design-Expert 7.0, and the results of purity of CRP affected by extraction temperature, extraction time and ratio of water to raw material are presented in Fig. 2. RSM played a key role in identifying the optimum values of the independent variables efficiently, under which dependent variable could reach the maximum response. In the 3-D response surface plot and contour plot, the purity of CRP was obtained along with two continuous variables, while the other variable was fixed constant at their respective 0 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 obtained by the equations.

The purity of CRP affected by different extraction temperature and extraction time is seen in Fig. 2A and B, when the ratio of water to raw material was fixed at 0 level. It can be seen that maximum purity of CRP can be achieved when extraction temperature and extraction time were 96.6 °C and 2.7 h, respectively. In Fig. 2C and D, when the 3-D response surface plot and the contour plot were developed for the purity of CRP with varying extraction temperature and ratio of water to raw material at fixed extraction time (0 level), the purity of CRP increased with the increasing ratio of water to raw material, and reached the peak value rapidly at extraction temperature 96.6 °C, then dropped from 96.6 to 100 °C. The 3-D response surface plot and the contour plot based on independent variables extraction time and ratio of water to raw material are shown in Fig. 2E and F, while extraction temperature was kept at 0 level. An increase in the purity of CRP could be significantly achieved with the increases of ratio of water to raw material. It was obvious that the purity of CRP was increased with the increasing extraction time from 2 to 2.7 h, meaning that further increases of extraction time would not increase the purity of CRP any longer.

According to Fig. 2, and the above single parameter study, it can be concluded that optimal extraction condition of CRP from the fruiting bodies of *C. rutilus* were extraction temperature 96.6 °C, extraction time 2.7 h and ratio of water to raw material 22. Among the three extraction parameters studied, ratio of water to raw material was the most significant factor to affect the purity of CRP, followed by extraction time and extraction temperature 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. 2).

3.5.3. Verification of predictive model

The suitability of the model equations for predicting optimum response values was tested under the conditions: extraction temperature 96.6 °C, extraction time 2.7 h and ratio of water to raw material 22. 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 57.7 ± 0.77 ($N = 3$), obtained from real experiments, demonstrated the validation of the RSM model, indicating that the model was adequate for the extraction process (Table 5).

3.6. Scavenging activity of CRP on hydroxyl radical

Generation of reactive oxygen species (ROS) beyond the body's antioxidant capacity gives rise to oxidative stress. Accumulating evidence strongly suggests that such stress is an important causative factor of aging, brain dysfunction, liver diseases, cardiovascular disorders, and carcinogenesis (Zha et al., 2009). Among the reactive oxygen species, Hydroxyl radical is the most active free radical that attacks all the biological molecules by setting off free radical chain reactions (Barry & Susanna, 1993).

Hydroxyl radicals, generated by reaction of an iron–EDTA complex with H_2O_2 in the presence of ascorbic acid, attack deoxyribose to yield a chromogen upon heating with TBA of low pH, Additional hydroxyl radical scavengers compete with deoxyribose for the produced hydroxyl radicals and diminish chromogen formation (Luo & Fang, 2008). The above model was used to measure inhibitory activities of all fractions on hydroxyl radicals. As shown in Fig. 3, CRP was found to have a higher scavenging effect on hydroxyl radicals at concentrations from 0.25 to 4 mg/ml, and even exceed the potency of ascorbate acid. The scavenging effects of CRP increased in a dose-dependent manner. This result proved that CRP had a significant effect on scavenging of hydroxyl radicals.

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

Optimum condition			Purity of CRP (%)	
Extraction temperature	Extraction time	Ratio of water to raw material	Experimental[a]	Predicted
96.6 °C	2.7 h	22	57.7 ± 0.77	57.4

[a] Mean ± SD ($n = 3$).

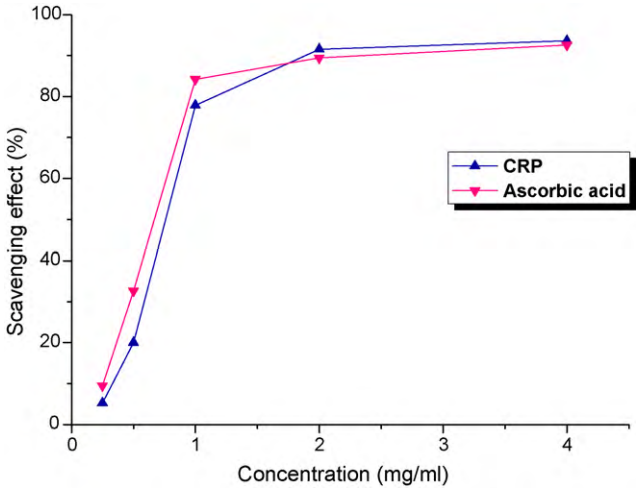

Fig. 3. Hydroxyl radical scavenging activity of CRP and ascorbic acid. Values are means ± SD of three determinations.

4. Conclusion

The extraction conditions have significant effects on the purity of CRP. Using the surface and contour plots in RSM was effective for estimating the effect of three independent variables (extraction temperature, extraction time and ratio of water to raw material). The optimum set of the independent variables was obtained graphically in order to obtain the desired levels of crude polysaccharides extraction. The optimal experimental purity of 57.7 ± 0.77% was obtained when the optimum conditions of CRP extraction were extraction temperature 96.6 °C, extraction time 2.7 h and ratio of water to raw material 22. Under these optimized conditions the experimental purity of CRP agreed closely with the predicted yield. Based on the hydroxyl radical scavenging assay, we preliminarily identified CRP had antioxidant potential for use in medicine or health-care food.

Acknowledgement

This work was financially supported by the Natural Science Foundation for Young Scientists of Heilongjiang Province, China (grant no. QC2009C106).

References

Atkinson, A. C., & Donev, A. N. (1992). *Optimum experimental designs*. Oxford: Oxford University Press., pp. 132–189.

Barry, H., & Susanna, C. (1993). Lipid peroxidation: Its mechanism, measurement and significance. *The American Journal of Clinical Nutrition*, *57*, 715–725.

Batistuti, J. P., Barros, R. M. C., & Areas, J. A. G. (1991). Optimization of extrusion cooking process for chickpea (*Cicer arietinum*, L.) defatted flour by response surface methodology. *Journal of Food Science*, *56*, 1695–1698.

Box, G. E. P., & Wilson, K. B. (1951). On the experimental attainment of optimum conditions. *Journal of the Royal Statistical Society Series A (General)*, *13*, 1–45.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Halliwell, B., Gutteridge, J. M. C., & Aruoma, O. I. (1987). The deoxyribose method: A simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry*, *165*, 215–219.

Ibanoglu, S., & Ainsworth, P. (2004). Effect of canning on the starch gelatinization and protein in vitro digestibility of tarhana, a wheat flour-based mixture. *Journal of Food Process Engineering*, *64*, 243–247.

Li, R., Chen, W. C., Wang, W. P., Tian, W. Y., & Zhang, X. G. (2009). Extraction, characterization of *Astragalus* polysaccharides and its immune modulating activities in rats with gastric cancer. *Carbohydrate Polymers*, *78*, 738–742.

Liu, J. C., Miao, S., Wen, X. C., & Sun, Y. X. (2009). Optimization of polysaccharides (ABP) extraction from the fruiting bodies of *Agaricus blazei* Murill using response surface methodology (RSM). *Carbohydrate Polymers*, *78*, 704–709.

Lu, C. H., Engelmann, N. J., Lila, M. A., & Edward, J. W., Jr. (2008). Optimization of lycopene extraction from tomato cell suspension culture by response surface methodology. *Journal of Agricultural and Food Chemistry*, *56*, 7710–7714.

Luo, D. H., & Fang, B. S. (2008). Structural identification of ginseng polysaccharides and testing of their antioxidant activities. *Carbohydrate Polymers*, *72*, 376–381.

Muralidhar, R. V., Chirumamil, R. R., Marchant, R., & Nigam, P. (2001). A response surface approach for the comparison of lipase production by *Candida cylindracea* using two different carbon sources. *Biochemical Engineering Journal*, *9*, 17–23.

Qiao, D. L., Ke, C. L., Hu, B., Lou, J. G., Ye, H., Sun, Y., et al. (2009). Antioxidant activities of polysaccharides from *Hyriopsis cumingii*. *Carbohydrate Polymers*, *78*, 199–204.

Shieh, C. J., Koehler, P. E., & Akoh, C. C. (1996). Optimization of sucrose polyester synthesis using response surface methodology. *Journal of Food Science*, *61*, 97–100.

Sun, Y. X., Li, X., Yang, J. F., Liu, J. C., & Kennedy, J. F. (2010). Water-soluble polysaccharide from the fruiting bodies of *Chroogomphis rutilus* (Schaeff.: Fr.) O.K. Miller: Isolation, structural features and its scavenging effect on hydroxyl radical. *Carbohydrate Polymers*, *80*, 720–724.

Sun, Y. X., Li, Y. J., Li, M. Q., Tong, H. B., Yang, X. D., & Liu, J. C. (2009). Optimization of extraction technology of the *Anemone raddeana* polysaccharides (ARP) by orthogonal test design and evaluation of its anti-tumor activity. *Carbohydrate Polymers*, *75*, 575–579.

Sun, Y. X., & Liu, J. C. (2009). Structural characterization of a water-soluble polysaccharide from the roots of *Codonopsis pilosula* and its immunity activity. *International Journal of Biological Macromolecules*, *43*, 279–282.

Sun, Y. X., Wang, S. S., Li, T. B., Li, X., Jiao, L. L., & Zhang, L. P. (2008). Purification, structure and immunobiological activity of a new water-soluble polysaccharide from the mycelium of *Polyporus albicans* (Imaz.) Teng. *Bioresource Technology*, *99*, 900–904.

Varnalis, A. I., Brennan, J. G., MacDougall, D. B., & Gilmour, S. G. (2004). Optimisation of high temperature puffing potato cubes using response surface methodology. *Journal of Food Process Engineering*, *61*, 153–163.

Vega, P. J., Balaban, M. O., Sims, C. A., O'Keefe, S. F., & Cornell, J. A. (1996). Supercritical carbon dioxide extraction efficiency for carotenes from carrots by RSM. *Journal of Food Science*, *61*, 757–765.

Yuan, J. F., Zhang, Z. Q., Fan, Z. C., & Yang, J. X. (2008). Antioxidant effects and cytotoxicity of three purified polysaccharides from *Ligusticum chuanxiong* Hort. *Carbohydrate Polymers*, *74*, 822–827.

Zha, X. Q., Wang, J. H., Yang, X. F., Liang, H., Zhao, L. L., Bao, S. H., et al. (2009). Antioxidant properties of polysaccharide fractions with different molecular mass extracted with hot-water from rice bran. *Carbohydrate Polymers*, *78*, 570–575.