Carbohydrate Polymers 87 (2012) 614–619

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Optimization of ultrasonic-assisted extraction of water-soluble polysaccharides from *Boletus edulis* mycelia using response surface methodology

Wei Chen[a,b], Wei-Ping Wang[a,b,*], Hua-Shan Zhang[a,b], Qin Huang[a,b]

[a] College of Bioengineering, Hubei University of Technology, Wuhan 430068, China
[b] Key Laboratory of Fermentation Engineering (Ministry of Education), Hubei University of Technology, Wuhan 430068, China

ARTICLE INFO

Article history:
Received 27 May 2011
Received in revised form 3 August 2011
Accepted 12 August 2011
Available online 19 August 2011

Keywords:
Ultrasonic extraction
Response surface methodology
Boletus edulis
Polysaccharides
Optimization of extraction

ABSTRACT

An ultrasound-assisted procedure for the extraction of water-soluble polysaccharides from the submerged-cultured mycelia of *Boletus edulis* was investigated using response surface methodology (RSM). Three independent variables were ratio of dried mycelia to water (X_1: 1:40–1:60), extraction time (X_2: 6–10 min), and ultrasonic temperature (X_3: 50–70 °C). The statistical analysis indicated the independent variables (X_2, X_3), the quadratic terms (X_2^2 and X_3^2) and the interaction between X_3 and X_1 had significant effects on the yield of polysaccharides ($p < .05$). The optimized conditions were 56 °C, 1:55 of ratio of dried mycelia to water, and a time of contact of 8.4 min. Under these conditions, the experimental yield of polysaccharides was 15.48%, which was well matched with the predictive yield of 15.53%.

1. Introduction

Boletus edulis is one of the most well-known edible mushroom collected especially in the Northern Hemisphere across Europe, Asia and North America. Polysaccharides extracted from *B. edulis* have been reported to have many biological functions such as anticancer, antioxidant and anti-inflammatory effects (Cengiz, Bektas, & Mustafa, 2008; Dentinger, Ammirati, & Both, 2010). However, fruit bodies of *B. edulis* are precious due to their rareness and difficulty in cultivation (Salerni & Perini, 2004). *B. edulis* is a mycorrhizal fungus. Its mycelia but not fruit bodies can be easily cultivated. So *B. edulis* can be obtained in the form of mycelia from submerged culture and bioactive polysaccharides can be extracted from *B. edulis* mycelia. A literature survey indicated that there was no investigation on the extraction of water-soluble polysaccharides from *B. edulis* mycelia. Heating or boiling was conventionally used to extract water-soluble polysaccharides. However, during the extraction, many bioactive compounds due to ionization, hydrolysis and oxidation are easily lost. Recently, various novel extraction techniques have been developed for the extraction of bioactive compounds such as ultrasound-assisted extraction, microwave-assisted extraction and supercritical fluid extraction (Wang & Weller, 2006). Among these, ultrasound-assisted extraction is one of the most inexpensive, simple and efficient techniques (Chen et al., 2010; Huang, Xue, Niu, Jia, & Wang, 2009; Yan et al., 2011; Zhang, Yang, Zhao, & Wang, 2009; Zhong & Wang, 2010), which can increase the yield of extracted components, reduce extraction time and make higher processing throughput. It is very useful for the extraction of thermolabile and unstable compounds, presumably by avoiding degradation reactions (Vilkhu, Mawson, Simons, & Bates, 2008). In this study, we investigated the ultrasound-assisted extraction condition of polysaccharides from submerged-cultured *B. edulis* mycelia.

To improve the yield of polysaccharides, response surface methodology (RSM) was designed to systematically analyze the effects of extraction parameters on the yields of polysaccharides from *B. edulis* mycelia and their interactions. RSM is an effective statistical technique for optimizing complex processes, which is widely used in optimizing the extraction process variables (Ebru & Ozgul, 2010; Guan & Yao, 2008; Guo, Zou, & Sun, 2010; Hou & Chen, 2008; Pompeu, Silva, & Rogez, 2009; Silva, Rogez, & Larondelle, 2007). The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions, which is more efficient and easier to arrange and interpret experiments in comparison with others. Box–Behnken design (BBD) (Ferreira, Bruns, Ferreira, & Matos, 2007), one of RSM, based on a 3 levels and 3 variables central composite, was employed to obtain the best possible combination of extraction temperature, extraction time and ratio of water to dried mycelia for maximum polysaccharides production.

* Corresponding author at: College of Bioengineering, Hubei University of Technology, China. Tel.: +86 27 87210399; fax: +86 27 88032320.
E-mail addresses: wang1wei1ping1@163.com, wangwp@21cn.com (W.-P. Wang).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.08.029

Table 1
Factors and levels for RSM, and Box–Behnken experimental design with the independent variables.

Run	Coded and uncoded variable levels			Yield of polysaccharide (%)	
	X_1/ratio of dried mycelia to water (g/mL)	X_2/extraction time (min)	X_3/ultrasonic temperature (°C)	Actual values	Predicted values
1	−1 (1:40)	−1 (6)	0 (60)	14.27	14.28
2	−1 (1:40)	0 (8)	−1 (50)	14.31	14.45
3	−1 (1:40)	1 (10)	0 (60)	14.40	14.28
4	−1 (1:40)	0 (8)	1 (70)	13.43	13.41
5	1 (1:60)	1 (10)	0 (60)	14.77	14.77
6	1 (1:60)	0 (8)	−1 (50)	15.05	15.07
7	1 (1:60)	−1 (6)	0 (60)	13.77	13.90
8	1 (1:60)	0 (8)	1 (70)	13.04	12.90
9	0 (1:50)	1 (10)	−1 (50)	14.36	14.34
10	0 (1:50)	1 (10)	1 (70)	12.58	12.73
11	0 (1:50)	−1 (6)	−1 (50)	14.05	13.90
12	0 (1:50)	−1 (6)	1 (70)	12.28	12.30
13	0 (1:50)	0 (8)	0 (60)	15.29	15.34
14	0 (1:50)	0 (8)	0 (60)	15.50	15.34
15	0 (1:50)	0 (8)	0 (60)	15.23	15.34

2. Materials and methods

2.1. Microorganism and culture conditions

B. edulis ACCC50559 was from Agricultural Culture Collection of China (ACCC). Agar slants containing potato–dextrose–agar were inoculated with mycelia and incubated at 25 °C for 6 days and then used as inoculums for seed culture. The seed culture was grown in 250 mL baffled flasks on a rotary shaker for 60 h at natural pH, 25 °C and 120 rpm with a medium (g/L) containing: glucose 20, potato 200 (put 200 g potato into the water, boiled for 30 min, and filtration, metered volume to 1 L), $MgSO_4{\cdot}7H_2O$ 0.5, KH_2PO_4 1.0, $ZnSO_4{\cdot}7H_2O$ 0.1, yeast extract 14, peptone 6. Fermentation was carried out in the medium of following composition (g/L): potato 100 (put 100 g potato into the water, boiled for 30 min, and filtration, metered volume to 1 L), sucrose 20, peptone 6, $MgSO_4{\cdot}7H_2O$ 2, KH_2PO_4 3, $CaCO_3$ 2. All media were sterilized at 115 °C for 30 min. The fermentation cultivation was inoculated at 10% (v/v) of the above seed culture medium and kept at 28 °C and 200 rpm in 250 mL baffled flasks on a rotary shaker for 5 days.

2.2. Extraction of crude polysaccharides from B. edulis mycelia with ultrasound-assisted treatment

B. edulis mycelia from submerged culture were washed by distilled water. The mycelia were dried at 30 °C. Then, it was grinded and stored in desiccators at room temperature (15–20 °C) until used. The process of polysaccharides extraction from *B. edulis* mycelia by ultrasound-assisted treatment was performed in an ultrasonic processor (SY-360, Shanghai Ninson Inc., Shanghai, China). One-tenth of dried mycelia powders were extracted with distilled water in a 50 mL centrifuge tube. The centrifuge tube was held in the ultrasonic processor and exposed to extract polysaccharides for different time at varied ultrasonic temperatures in different ratios of dried mycelia to water.

2.3. Determination of the yield of polysaccharides from B. edulis mycelia

After ultrasonic treatment, the extracted slurry was centrifuged at 7000 rpm for 10 min to collect the supernatant and the polysaccharides were determined by phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.4. Experimental design and statistical analyses

Single-factor-test was employed to determine the preliminary range of the extraction variables including X_1 (ratio of dried mycelia to water), X_2 (extraction time) and X_3 (ultrasonic temperature). Then, a three-level-three-factor BBD was employed to determine the best combination of extraction variables for the yields of *B. edulis* mycelia water-soluble polysaccharides. Table 1 represents the coded and non-coded values of the experimental variables and 15 experimental points. Three replicates (13–15) were used to evaluate the pure error. Experimental data shown that response variables were fitted to a quadratic polynomial model. The general form of the quadratic polynomial model was as follows:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} X_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij} X_i X_j \qquad (1)$$

where Y is the measured response associated with each factor lever combination; β_0, β_i, β_{ii} and β_{ij} are the regression coefficients for intercept, linearity, square and interaction, respectively; X_i and X_j are the independent variables. Design Expert software (Trial Version 7.1.6.) was used to estimate the response of each set of experimental design and optimized conditions. The fitness of the quadratic polynomial model was inspected by the regression coefficient R^2. F-value and p-value were used to check the significances of the regression coefficient.

3. Results and discussion

3.1. Selection of ratio of dried mycelia to water for extraction yield of polysaccharides

Preliminary studies were performed in order to determine the required ratio of dried mycelia to water for the extraction yields of polysaccharides from *B. edulis*. Extraction was carried out at different ratios of dried mycelia to water (1:20, 1:30, 1:40, 1:50 and 1:60), while other extraction parameters were as following: extraction time 2 min, ultrasonic temperature 40 °C. The results showed that the extraction of the polysaccharides was dependent on the solid-to-liquid ratio (Fig. 1(A)). The yield of polysaccharides increased with the increase of the solid-to-liquid ratio. A plateau in the mass transfer was reached at the solid-to-liquid ratio of 1:50. The maximum yield (14.1%) was also achieved. Thus, ratio of dried mycelia to water 1:40–1:60 was favorable for producing polysaccharides.

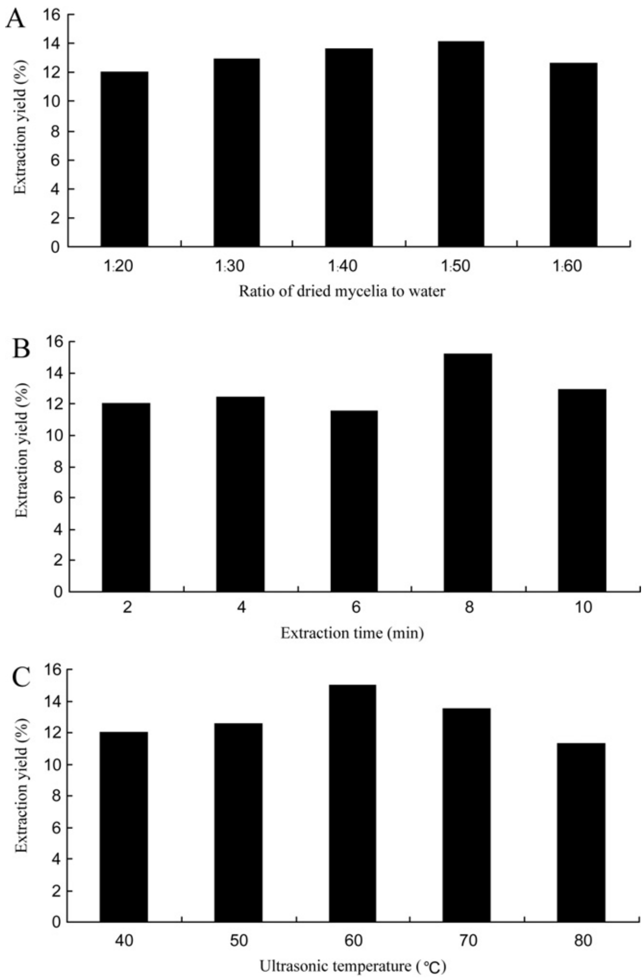

Fig. 1. Effect of different ratio of dried mycelia to water (A), extraction time (B) and ultrasonic temperature (C) on extraction yield of polysaccharides.

3.2. Selection of time on extraction yield of polysaccharides

Extraction time is another factor that would influence the extraction efficiency. Extraction process was carried out using the time of 2, 4, 6, 8 and 10 min, when other extraction parameters were as following: ultrasonic temperature 40 °C, ratio of dried mycelia to water 1:20. The effect of different time on extraction yield of polysaccharides was shown in Fig. 1(B). When extraction time varied from 6 to 10 min, the variance of extraction yield was relatively rapid and polysaccharides production reached a maximum at 8 min (15.22%), and then decreased as the extraction proceeded. This indicated that extraction time of 6–10 min was sufficient to obtain polysaccharides, which was less than the conventional heating or boiling extraction time (Guo et al., 2010; Hou & Chen, 2008).

3.3. Selection of temperature on extraction yield of polysaccharides

To study the effect of different temperature on extraction yield of polysaccharides, extraction process was carried out using the different extraction temperature of 40, 50, 60, 70, 80 °C, when other extraction condition was as following: extraction time 2 min, ratio of dried mycelia to water 1:20. The extraction yield of polysaccharides had been increasing when ultrasonic temperature increased from 40 to 60 °C. As shown in Fig. 1(C), the maximum yield (15.01%) of polysaccharides was observed when extraction temperature was 60 °C, and then decreased as the extraction proceeded. Therefore, extraction temperature range of 50–70 °C was considered to be optimal in the present experiment. Because conventional heating extraction temperature was more than 70 °C (Guo et al., 2010; Hou & Chen, 2008), ultrasound-assisted extraction was lower.

Table 2
Analysis of variance for the fitted quadratic polynomial model of extraction of polysaccharides.

Source	SS[a]	DF[b]	MS[c]	*F*-value	*p*-Value
Model	13.34	9	1.48	47.12	0.0003
Residual	0.16	5	0.031		
Lack of fit	0.12	3	0.039	1.94	0.3578
Pure error	0.040	2	0.020		
Cor. total	13.50	14			

$R^2 = 0.9883$; $R^2_{adj} = 0.9674$; C.V.% = 1.25.
[a] Sums of squares.
[b] Degree freedom.
[c] Mean square.

3.4. Optimization of extraction conditions of polysaccharides

3.4.1. Predicted model and statistical analysis

The design matrix and the corresponding results of RSM experiments to determine the effects of the three independent variables including ratio of dried mycelia to water (X_1), extraction time (X_2) and ultrasonic temperature (X_3) were shown in Table 1. Through multiple regression analysis on the experimental data, the model for the predicted response Y could be expressed by the following quadratic polynomial equation (in the form of coded values):

$$Y = 15.34 + 0.027X_1 + 0.22X_2 - 0.81X_3 - 0.2X_1^2 - 0.84X_2^2 - 1.18X_3^2 + 0.22X_1X_2 - 0.28X_1X_3 - 2.5 \times 10^{-3}X_2X_3 \quad (2)$$

where Y is the yield of polysaccharides, X_1, X_2 and X_3 are the coded variables for the ratio of dried mycelia to water, extraction time and ultrasonic temperature, respectively.

Statistical testing of the model was performed in the form of analysis of variance (ANOVA). The ANOVA for the fitted quadratic polynomial model of extraction of polysaccharides were shown in Table 2. The quadratic regression model showed the value of the determination coefficient (R^2) was 0.9883, which implied that 98.83% of the variations could be explained by the fitted model. For a good statistical model, R^2_{adj} should be close to R^2 .As shown in Table 2, R^2_{adj} was 0.9674, which implied that only less 4.0% of the total variations were not explained by the model. It also indicated that a high degree of correlation between the observed and predicted values. A relatively low value of C.V. (coefficient of variation) (1.25%) indicated a better reliability of the experiments values. The corresponding variables would be more significant if the *F*-value becomes greater and the *p*-value becomes smaller (Atkinson and Donev, 1992). Values of *p*-value less than 0.05 showed model terms were significant. SO the *F*-value ($F = 47.12$) and *p*-value ($p = 0003$) implied this model was significant. Significance of the model was also judged by lack-of-fit test. As shown in Table 2, *F*-value and *p*-value of the lack of fit were 1.94 and 0.3578, respectively, which implied that it was not significant and a 35.78% chance could occur due to noise. The significance of each coefficient was determined using *F*-value and *p*-value. The results were given in Table 3. It could be seen that two independent variables (X_2, X_3) and two quadratic terms (X_2^2 and X_3^2) significantly affected the yield of polysaccharides, and the interaction between X_1 and X_3 was significant too ($p < .05$). Results also showed that the independent variable X_3 was the most significant factor on the experimental yield of polysaccharides.

Table 3
Estimated regression model of relationship between response variables (yield of polysaccharides) and independent variables (X_1, X_2, X_3).

Variables	DF[a]	SS[b]	MS[c]	*F*-value	*p*-Value
X_1	1	6.05×10^{-3}	6.05×10^{-3}	0.19	0.6793
X_2	1	0.38	0.38	12.03	0.0179
X_3	1	5.18	5.18	164.84	<0.0001
X_1^2	1	0.15	0.15	4.64	0.0839
X_2^2	1	2.60	2.60	82.59	0.0003
X_3^2	1	5.17	5.17	164.51	<0.0001
X_1X_2	1	0.19	0.19	6.02	0.0577
X_1X_3	1	0.32	0.32	10.15	0.0244
X_2X_3	1	2.50×10^{-5}	2.50×10^{-5}	7.949×10^{-4}	0.9786

[a] Degree freedom.
[b] Sums of squares.
[c] Mean square.

3.4.2. Analysis of response surface

The relationship between independent and dependent variables was illustrated by the three-dimensional representation of the response surfaces and the two-dimensional contours generated by the model (seen in Figs. 2–4). Different shapes of the contour plots indicated different interactions between the variables, an elliptical contour plot indicated the interactions between the variables were significant while a circular contour plot means otherwise. In these three variables (ratio of dried mycelia to water, extraction time and ultrasonic temperature), when two variables within the experimental range were depicted in three-dimensional surface plots, the third variable was kept constant at zero level. As shown in Fig. 2, when ultrasonic temperature (X_3) was fixed at 0 level, extraction time (X_2) demonstrated quadratic effects on the extraction yields. When ratio of dried mycelia to water kept at lower level, the yield increased at first and then decreased with the increase of extraction time (X_2). As shown in Fig. 3, when extraction time (X_2) was fixed at 0 level, ultrasonic temperature (X_3) displayed a quadratic effect on the response yield. The elliptical contour plot shown in Fig. 3 indicated the mutual interactions between ratio of dried mycelia to water and extraction temperature were significant. The results of Fig. 4 showed that when ratio of dried mycelia to water (X_1) was fixed at 0 level, the ultrasonic temperature (X_3) was increased with increases in extraction time (X_2). And then, the ultrasonic temperature (X_3) was decreased with further increase in extraction time. Extraction time and ultrasonic temperature demonstrated quadratic effects on the response.

3.5. Optimization of extracting parameters and validation of the model

Through these three-dimensional plots and their respective contour plots, the suitability of the model equation for predicting the optimum response values were tested using the selected optimal conditions. The results (Table 4) showed that the optimized

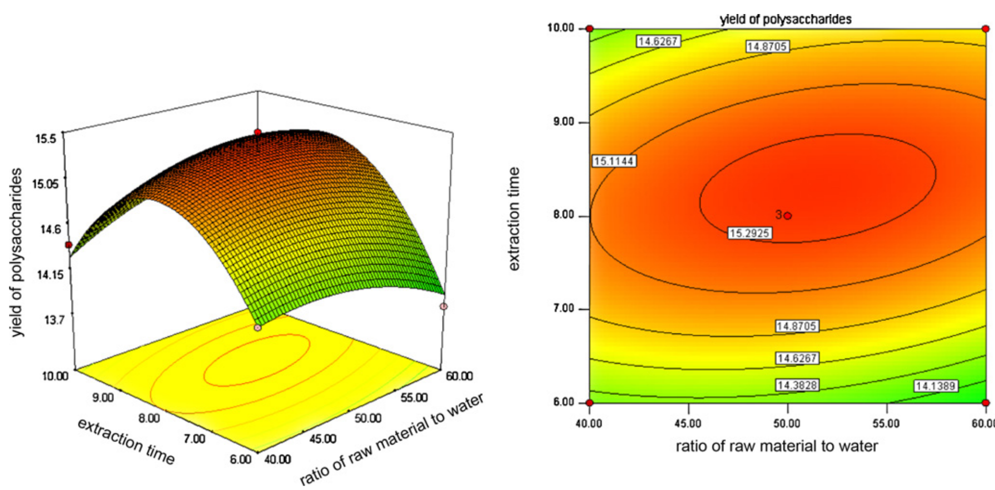

Fig. 2. Response surface plot and contour plot of ratio of raw material to water and extraction time, and their mutual interactions on the yield of polysaccharides.

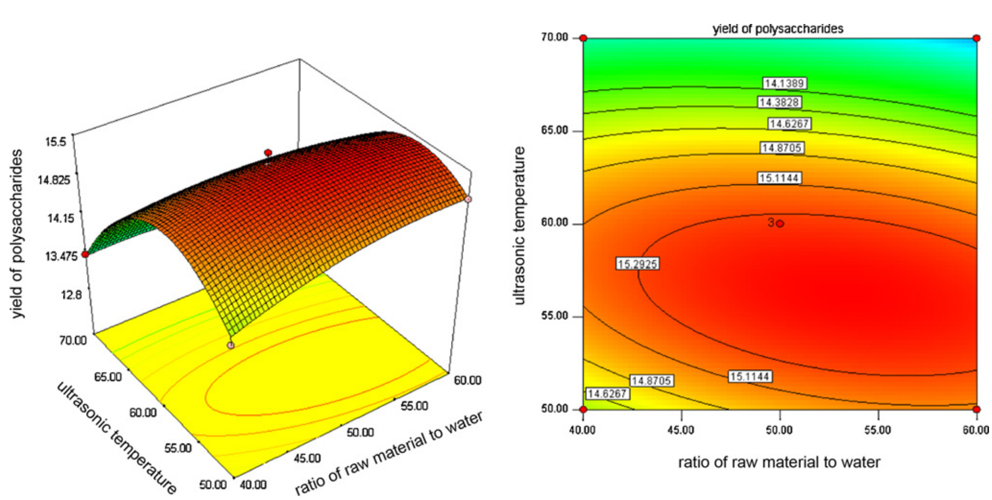

Fig. 3. Response surface plot and contour plot of ratio of raw material to water and ultrasonic temperature, and their mutual interactions on the yield of polysaccharides.

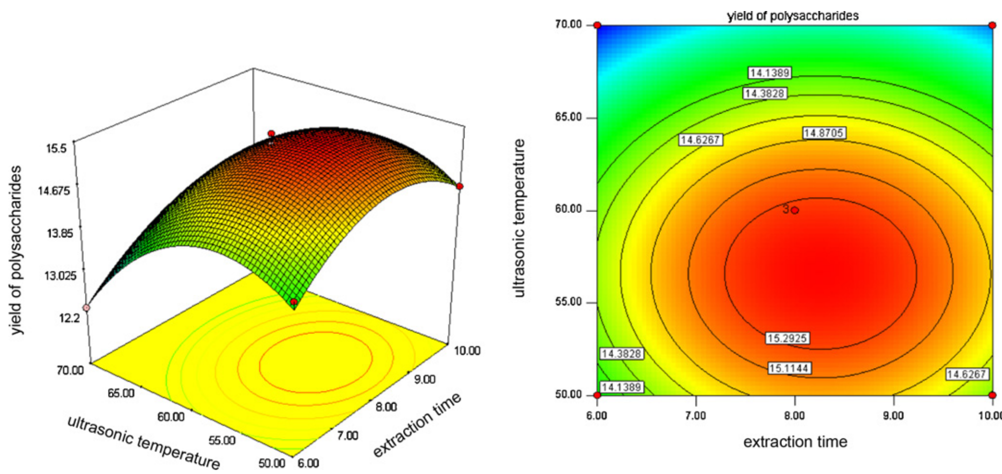

Fig. 4. Response surface plot and contour plot of extraction time and ultrasonic temperature, and their mutual interactions on the yield of polysaccharides.

Table 4
Optimum conditions, and the predicted and experimental value of response at the optimum conditions.

	Ultrasonic temperature (°C)	Extraction time (min)	Ratio of raw material to water	Yield of polysaccharide (%)
Optimum conditions (predicted)	56.06	8.38	1:54.53	15.53
Modified conditions (actual)	56	8.4	1:55	15.48

conditions were ultrasonic temperature of 56.06 °C, extraction time of 8.38 min, and ratio of dried mycelia to water 1:54.53. Under the conditions, the extraction yield of polysaccharides was 15.53%. However, considering the operability in actual production, the optimal conditions can be modified as follows: ultrasonic temperature of 56 °C, extraction time of 8.4 min, and ratio of dried mycelia to water 1:55. Under the modified conditions, the experimental yield of polysaccharides was 15.48% (N = 3), which was close to the predicted value. Generally, the extraction yields of polysaccharides from fungal mycelia or mushrooms were below 10%. Hou & Chen (2008) investigated that the extraction efficiency of polysaccharides from wild edible BaChu mushroom was 8.75%. Yan et al. (2011) reported the extraction yields of polysaccharides from *Tremella mesenterica* was 8.26%. Guo et al. (2010) found that the extraction yields of polysaccharides from *Phellinus igniarius* was 5.04%. So the extraction efficiency of 15.48% was very high.

4. Conclusion

In this study, we had investigated an ultrasonic-assisted method to extract polysaccharides from the *B. edulis* mycelia using RSM. The results showed that the independent variables (ultrasonic temperature and extraction time), and quadratic terms of ultrasonic temperature and extraction time, and the interaction effects between ultrasonic temperature and ratio of dried mycelia to water had significant effects on the yield of polysaccharides. Ultrasonic temperature was the most significant factor on the experimental yield of polysaccharides. A second-order polynomial model was employed to optimize polysaccharides extraction from *B. edulis* mycelia by ultrasonic technology. The optimal extraction conditions for the polysaccharides were as follows: ultrasonic temperature 56 °C, extraction time of 8.4 min, and ratio of dried mycelia to water 1:55. Under these conditions, the experimental yield of polysaccharides was 15.48%, which was agreed closely with the predicted yield value of 15.53%. The study provided a new and efficient method for the extraction of water-soluble polysaccharides from *B. edulis* mycelia. Further studies on the chemical structures and the bioactive function of polysaccharides from *B. edulis* mycelia are under process.

Acknowledgement

We gratefully acknowledge the financial support received from the Doctoral Scientific Research Foundation of Hubei University of Technology (Contract No. BSQD0911).

References

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

Cengiz, S., Bektas, T., & Mustafa, Y. (2008). Evaluation of the antioxidant activity of four edible mushrooms from the Central Anatolia, Eskisehir – Turkey: *Lactarius deterrimus*, *Suillus collitinus*, *Boletus edulis*, *Xerocomus chrysenteron*. *Bioresource Technology*, *99*, 6651–6655.

Chen, X. P., Wang, W. X., Li, S. B., Xue, J. L., Fan, L. J., Sheng, Z. J., et al. (2010). Optimization of ultrasound-assisted extraction of Lingzhi polysaccharides using response surface methodology and its inhibitory effect on cervical cancer cells. *Carbohydrate Polymers*, *80*, 944–948.

Dentinger, B. T. M., Ammirati, J. F., & Both, E. E. (2010). Molecular phylogenetics of porcini mushrooms (Boletus section). *Molecular Phylogenetics and Evolution*, *57*, 1276–1292.

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.

Ebru, F. D., & Ozgul, E. (2010). Response surface methodology for protein extraction optimization of red pepper seed (*Capsicum frutescens*). *Food Science and Technology*, *43*, 226–231.

Ferreira, S. L. C., Bruns, R. E., Ferreira, H. S., & Matos, G. D. (2007). Box–Behnken design: An alternative for the optimization of analytical methods. *Analytica Chemical Acta*, *597*, 179–186.

Guan, X., & Yao, H. Y. (2008). Optimization of viscozyme L-assisted extraction of oat bran protein using response surface methodology. *Food Chemistry*, *106*, 345–351.

Guo, X., Zou, X., & Sun, M. (2010). Optimization of extraction process by response surface methodology and preliminary characterization of polysaccharides from *Phellinus igniarius*. *Carbohydrate Polymers*, *80*, 344–349.

Hou, X. J., & Chen, W. (2008). Optimization of extraction process of crude polysaccharides from wild edible BaChu mushroom by response surface methodology. *Carbohydrate Polymers*, *72*, 67–74.

Huang, W., Xue, A., Niu, H., Jia, Z., & Wang, J. W. (2009). Optimised ultrasonic-assisted extraction of flavonoids from *Folium eucommiae* and evaluation of antioxidant activity in multi-test systems in vitro. *Food Chemistry*, *114*, 1147–1154.

Pompeu, D. R., Silva, E. M., & Rogez, H. (2009). Optimisation of the solvent extraction of phenolic antioxidants from fruits of *Euterpe oleracea* using response surface methodology. *Bioresource Technology*, *100*, 6076–6082.

Salerni, E., & Perini, C. (2004). Experimental study for increasing productivity of *Boletus edulis* s.l. in Italy. *Forest Ecology and Management*, *201*, 161–170.

Silva, E. M., Rogez, H., & Larondelle, Y. (2007). Optimization of extraction of phenolics from *Inga edulis* leaves using response surface methodology. *Separation and Purification Technology*, *55*, 318–387.

Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry – A review. *Innovative Food Science and Emerging Technologies*, *9*, 161–169.

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science and Technology*, *17*, 300–312.

Yan, Y. L., Yu, C. H., Chen, J., Li, X. X., Wang, W., & Li, S. Q. (2011). Ultrasonic-assisted extraction optimized by response surface methodology, chemical composition and antioxidant activity of polysaccharides from *Tremella mesenterica*. *Carbohydrate Polymers*, *83*, 217–224.

Zhang, H. F., Yang, X. H., Zhao, L. D., & Wang, Y. (2009). Ultrasonic-assisted extraction of epimedin C from fresh leaves of Epimedium and extraction mechanism. *Innovative Food Science and Emerging Technologies*, *10*, 54–60.

Zhong, K., & Wang, Q. (2010). Optimization of ultrasonic extraction of polysaccharides from dried longan pulp using response surface methodology. *Carbohydrate Polymers*, *80*, 19–25.