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Extraction of polysaccharides from *Phellinus nigricans* mycelia and their antioxidant activities *in vitro*

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ABSTRACT

In this study, response surface methodology was employed to optimize the extraction of polysaccharides from *Phellinus nigricans* mycelia. A central composite design was adopted to determine optimum parameters (extraction time, extraction temperature, extraction frequency, and ratio of water to raw material) that could yield a maximum polysaccharide. Results revealed the following optimum extraction conditions: extraction time, 2.8 h; ratio of water to raw material, 28; extraction frequency, 5; and extraction temperature, 95 °C. Under optimized conditions, the experimental yield of *P. nigricans* mycelia polysaccharides was 15.33 ± 0.21%, which is consistent with the predicted yield. The antioxidant activity assay *in vitro* showed that the polysaccharides exhibited a high scavenging activity against superoxide anion, hydroxyl, and 1,1-diphenyl-2-picrylhydrazyl radicals. These polysaccharides also exhibited a strong reducing power. Thus, these polysaccharides can be used as natural antioxidants in functional foods or medicine.

1. Introduction

Studies have revealed that polysaccharides, particularly mushroom polysaccharides, have complex and multiple biological activities (Borchers, Stern, Hackman, Keen, & Gershwin, 1999; Ooi & Liu, 1991). Polysaccharides are slightly toxic; hence, further studies on polysaccharides have been conducted. Various kinds of mushroom polysaccharides have been isolated and found to have a wide range of bioactivities, such as antitumor, antioxidant, anticancer, and immunological properties (Chen et al., 2010; Li et al., 2008; Sun et al., 2008; Yan et al., 2011; Zhang, Cui, Cheung, & Wang, 2007).

Phellinus nigricans (Fr.) P. Karst is a perennial wood-decaying fungus. Given the specificity of physiological and ecological constraints and complexity as well as external environmental conditions, the formation of the fruiting bodies of *P. nigricans* rarely occurs in nature. In other studies, polysaccharides have been isolated from other *Phellinus* species. For instance, a polysaccharide exhibiting significant anti-tumor activities has been isolated from *P. igniarius* (Chen et al., 2011). Polysaccharides from *P. gilvus* can inhibit respiratory system inflammation and treat diabetes mellitus (Bae, Jang, Yim, & Jin, 2005). In Japan and South Korea, *Phellinus* polysaccharide products have been used in anti-tumor activity and immunity (Hata, 1997).

Artificial propagation and submerged fermentation can be performed to compensate some drawbacks when available fungal resources become limited in nature. Artificial cultivation is difficult and needs a long training period, whereas liquid fermentation is a fast and efficient way to obtain *P. nigricans* mycelia for polysaccharide extraction. In this study, *P. nigricans* mycelia, which were harvested by liquid fermentation, were used to produce polysaccharides. A central composite design (CCD) was used to optimize polysaccharide production of *P. nigricans* mycelia (PNMP). The antioxidant activities of the polysaccharides were then evaluated for further applications in food and pharmaceutical industries.

2. Materials and methods

2.1. Chemical reagents

Ascorbic acid, pyrogallic acid, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Unless otherwise stated, all of the chemicals used were of analytical grade.

2.2. Organism and extraction preparation

P. nigricans was initially grown on potato dextrose agar medium in a Petri dish for 6 d–7 d. A portion of the agar plate culture (10 mm^2) was sliced with a sterilized cutter and then inoculated in a 500 mL flask with 200 mL of culture medium (pH 7.2) containing 30 g/L corn powder, 20 g/L bran, 1.5 g/L $MgSO_4$, 1.0 g/L K_2HPO_4,

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Table 1
Experimental domain of central composite design (CCD).

Variables	levels				
	−2	−1	0	1	2
Extraction time (X_1) (h)	1.5	2.0	2.5	3.0	3.5
Ratio of water to raw material (X_2) (n)	15	20	25	30	35
Extraction frequency (X_3) (n)	2	3	4	5	6
Extraction temperature (X_4) (°C)	80	85	90	95	100

1.0 g/L KH_2PO_4, 210 μg/L thiamine, and 250 μg/L riboflavin. The culture medium was then incubated in a rotary shaker (160 rpm) at 28 °C for 7 d. Afterward, the original block of inoculation in the liquid medium was discarded, and *P. nigricans* mycelia were obtained by centrifugation (3000 × *g*, 15 min) after cultivation.

Cultured mycelia were washed twice with distilled water and then defatted with ethanol. The defatted sample was dried in vacuum for 16 h at 60 °C to obtain a constant weight and homogenized in a blender for the next extraction.

2.3. Extraction of PNMP

Each of the dried, pretreated sample (10 g) was extracted with deionized water at a specified temperature, extraction time, ratio of water to raw material, and extraction frequency. The mixture was centrifuged (2000 × *g*, 15 min) and the supernatant was separated from the insoluble residue with a four-layer filter cloth. The extract was precipitated by adding ethanol to a final concentration of 75% (v/v) and then incubated overnight. The polysaccharide precipitate was collected by centrifugation (2000 × *g*, 15 min) and lyophilized to obtain PNMP. The polysaccharide content was determined using the phenol-sulfuric acid method with D-glucose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1958).

2.4. Experimental design and statistical analysis

A CCD with four independent variables (X_1, extraction time; X_2, ratio of water to raw material; X_3, extraction frequency; and X_4, extraction temperature) at five levels was performed. For statistical calculation, the variables were coded according to Eq. (1):

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

where x_i is a coded value of the independent variable, X_i is the actual value of the independent variable, X_0 is the actual value of X_i on the center point, and ΔX_i is the value of step change. The range of the independent variables and their levels is presented in Table 1. The independent variables and their ranges were chosen based on the preliminary experimental results.

The design consisted of 31 experimental points (16 factorial points, 8 axial points, and 7 center points). All of the design points were performed in triplicate in a randomized order. The response value in each trial was the average of duplicates.

The CCD data were analyzed by multiple regressions to fit the following quadratic polynomial model:

$$Y_k = \beta_{k_0} + \sum_{i=1}^{4} \beta_{k_i} x_i + \sum_{i=1}^{4} \beta_{k_{ii}} x_i^2 + \sum_{i<j=2}^{4} \beta_{k_{ij}} x_i x_j \quad (2)$$

where Y_k is the response function and β_{k_0} is an intercept. β_{k_i}, $\beta_{k_{ii}}$, and $\beta_{k_{ij}}$ are the coefficients of the linear, quadratic, and interactive terms, respectively. x_i and x_j represent the coded independent variables, respectively. The fitted polynomial equation was expressed as surface and contour plots to visualize the relationship between the response level and the experimental level of each factor. This equation was also used to determine the optimum conditions. The regression coefficients of the individual linear, quadratic, and interaction terms were determined based on variable analysis results. The regression coefficients were then used to calculate statistically and generate three-dimensional (3-D) surface plots and contour plots from the fitted polynomial equation. Design Expert software (Version 8.0.6) was used to analyze the experimental data. P value < 0.05 was considered statistically significant.

2.5. Antioxidant activity test in vitro

Antioxidant activity was determined according to the scavenging activities of superoxide anion, hydroxyl, DPPH radicals and to the reducing power.

2.5.1. Superoxide radical scavenging assay

Approximately 4.5 mL of 50 mM Tris–HCl buffer (pH 8.2) was mixed with 4.2 mL of deionized water and then incubated at 25 °C for 20 min. After incubation, 1 mL of PNMP solution and 0.4 mL of pyrogallic acid were added and then shaken quickly. After incubation at 25 °C for 5 min, 8 mM HCl was added to terminate the reaction. The absorbance was determined at 320 nm and ascorbic acid was used as a positive control. The superoxide radical scavenging capability was calculated by the following formula:

$$\text{Scavenging rate } (\%) = \left[\frac{(A_c - A_s)}{A_c}\right] \times 100 \quad (3)$$

where A_c and A_s are the absorbances of the blank sample and PNMP/ascorbic acid, respectively.

2.5.2. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was determined according to Winterbourn and Sutton (1984). The reaction mixture contained 1 mL of 0.15 M phosphate buffer (pH 7.4), 1 mL of 40 μg/mL safranin, 1 mL of 0.945 mM EDTA-Fe(II), 1 mL of 3% (v/v) H_2O_2, and 0.5 mL of PNMP solution. After incubation at 37 °C for 30 min, the absorbance was determined at 560 nm and ascorbic acid was used as a positive control. EC_{50} (mg/L) of PNMP or ascorbic acid was the effective concentration at which 50% of the hydroxyl radicals were scavenged. The hydroxyl radical scavenging activity was expressed as follows:

$$\text{Scavenging rate } (\%) = \left[\frac{(A_c - A_s)}{A_c}\right] \times 100 \quad (4)$$

where A_c and A_s are the absorbances of the blank sample and PNMP/ascorbic acid, respectively.

2.5.3. DPPH scavenging assay

DPPH radical scavenging activity was determined according to Shimada, Fujikawa, and Yahara (1992) with slight modifications. In brief, the reaction mixture contained 2 mL of DPPH (0.1 μM in 95% ethanol) and 2 mL of PNMP solution. The mixture was incubated at 25 °C for 15 min and the absorbance of the mixture was determined at 517 nm and ascorbic acid was used as a positive control. EC_{50} (mg/L) of PNMP was the effective concentration at which 50% of the DPPH radicals were scavenged. The antioxidant activity of PNMP was evaluated according to the following formula:

$$\text{Scavenging rate } (\%) = \left(\frac{1 - A_s}{A_c}\right) \times 100 \quad (5)$$

where A_s and A_c are the absorbances of PNMP/ascorbic acid and DPPH solution, respectively.

2.5.4. Determination of the reducing power of PNMP

The reducing power of PNMP was evaluated according to the method of Deng et al. (2011). The reaction mixtures contained

Table 2
Response surface central composite design and results for extraction yield of PNMP.

No.	X_1, Extraction time (h)	X_2, Ratio of water to raw material	X_3, Extraction frequency	X_4, Extraction temperature (°C)	Extraction yield of PNMPs (%)
1	−1 (2.0)	−1 (20)	−1 (3)	−1 (80)	10.62
2	1 (3.0)	−1 (20)	−1 (3)	−1 (80)	11.54
3	−1 (2.0)	1 (30)	−1 (3)	−1 (80)	11.7
4	1 (3.0)	1 (30)	−1 (3)	−1 (80)	12.52
5	−1 (2.0)	−1 (20)	1 (5)	−1 (80)	13.11
6	1 (3.0)	−1 (20)	1 (5)	−1 (80)	12.95
7	−1 (2.0)	1 (30)	1 (5)	−1 (80)	12.57
8	1 (3.0)	1 (30)	1 (5)	−1 (80)	12.89
9	−−1 (2.0)	−1 (20)	−1 (3)	1 (95)	12.52
10	1 (3.0)	−1 (20)	−1 (3)	1 (95)	12.54
11	−1 (2.0)	1 (30)	−1 (3)	1 (95)	12.33
12	1 (3.0)	1 (30)	−1 (3)	1 (95)	12.97
13	−1 (2.0)	−1 (20)	1 (5)	1 (95)	13.51
14	1 (3.0)	−1 (20)	1 (5)	1 (95)	15.38
15	−1 (2.0)	1 (30)	1 (5)	1 (95)	15.67
16	1 (3.0)	1 (30)	1 (5)	1 (95)	15.74
17	−2 (1.5)	0 (25)	0 (4)	0 (90)	11.42
18	2 (3.5)	0 (20)	0 (4)	0 (90)	14.42
19	0 (3.0)	−2 (15)	0 (4)	0 (90)	11.86
20	0 (3.0)	2 (35)	0 (4)	0 (90)	14.86
21	0 (3.0)	0 (25)	−2 (2)	0 (90)	12.33
22	0 (3.0)	0 (25)	2 (6)	0 (90)	12.78
23	0 (3.0)	0 (25)	0 (4)	−2 (80)	14.52
24	0 (3.0)	0 (25)	0 (4)	2 (100)	14.25
25	0 (3.0)	0 (25)	0 (4)	0 (90)	14.52
26	0 (3.0)	0 (25)	0 (4)	0 (90)	14.52
27	0 (3.0)	0 (25)	0 (4)	0 (90)	14.52
28	0 (3.0)	0 (25)	0 (4)	0 (90)	14.52
29	0 (3.0)	0 (25)	0 (4)	0 (90)	14.52
30	0 (3.0)	0 (25)	0 (4)	0 (90)	14.52
31	0 (3.0)	0 (25)	0 (4)	0 (90)	14.52

2.5 mL of phosphate buffer (pH 6.6, 0.2 M), 2.5 mL of potassium ferricyanide (1%, w/v), and a PNMP solution. After incubation at 50 °C for 20 min, 2.5 mL of trichloroacetic acid (10%, w/v) was added to the mixture to end the reaction. The mixture was then centrifuged (1200 × *g*, 10 min). Approximately 2.5 mL of the supernatant was collected and mixed with 2.5 mL of deionized water and 0.5 mL of $FeCl_3$ (0.1%, w/v). After incubation at room temperature for 15 min, the absorbance was obtained at 700 nm and ascorbic acid was used as a positive control.

3. Results and discussion

3.1. Statistical analysis and model fitting

The design matrix and corresponding results of the RSM experiments were considered to determine the effects of the four independent variables, including X_1, X_2, X_3, and X_4 (Table 2). The PNMP yield ranged from 10.62% to 15.74%. The maximum PNMP yield was recorded under the following experimental conditions: extraction time, 3.0 h; ratio of water to raw material, 30; extraction frequency, 5; and extraction temperature, 95 °C. The experimental data were subjected to multiple regression analysis. The results revealed that the response and test variables were related by the following second-order polynomial equation ($R^2 = 0.92$):

$$Y = 14.52 + 0.4375X_1 + 0.4258X_2 + 0.6658X_3 + 0.5092X_4 - 0.05X_1X_2 - 0.0188X_1X_3 + 0.0438X_1X_4 - 0.0238X_2X_3 + 0.0813X_2X_4 + 0.3X_3X_4 - 0.445X_1^2 - 0.335X_2^2 - 0.5363X_3^2 - 0.0788X_4^2 \quad (6)$$

The fitting statistics of the extraction yield *Y* for the selected quadratic predictive model is shown in Table 3. In Table 3, the model could be used to predict within the range of the experimental variables. The regression coefficients in Eq. (6) are listed in Table 4. *P* values were used to determine the significance of each coefficient, which indicated the pattern of the interactions between variables. $P < 0.05$ indicated a more significant coefficient (Guo, Zou, & Sun, 2010). Table 4 shows that the linear coefficients (X_1, X_2, X_3, and X_4) and quadratic term coefficients (X_1^2 and X_3^2) were significant with low *P* values ($P < 0.05$). The other coefficients were not significant ($P > 0.05$).

Table 3
Analysis of variance for the fitted quadratic polynomial model.

Source	SS	DF	MS	*F*-value	Prob > *F*
Model	41.7452	14	2.98180	3.55	0.009
Residual	13.4490	16	0.84056		
Lack of fit	13.4490	10	1.34490		
Pure error	0.0000	6	0.00000		
Cor. total	55.1942	30			

Table 4
Regression coefficients of the predicted quadratic polynomial model.

Parameter	Estimate	Standard error	*t* Ratio	*P*-value
X_1	0.4375	0.1871	2.338	0.033
X_2	0.4258	0.1871	2.275	0.037
X_3	0.6658	0.1871	3.558	0.003
X_4	0.5092	0.1871	2.721	0.015
$X_1 X_1$	−0.4450	0.1714	−2.596	0.020
$X_2 X_2$	−0.3350	0.1714	−1.954	0.068
$X_3 X_3$	−0.5362	0.1714	−3.128	0.006
$X_4 X_4$	−0.0788	0.1714	−0.459	0.652
$X_1 X_2$	−0.0500	0.2292	−0.218	0.830
$X_1 X_3$	−0.0187	0.2292	−0.082	0.936
$X_1 X_4$	0.0438	0.2292	0.191	0.851
$X_2 X_3$	−0.0237	0.2292	−0.104	0.919
$X_2 X_4$	0.0813	0.2292	0.354	0.728
$X_3 X_4$	0.3000	0.2292	1.309	0.209

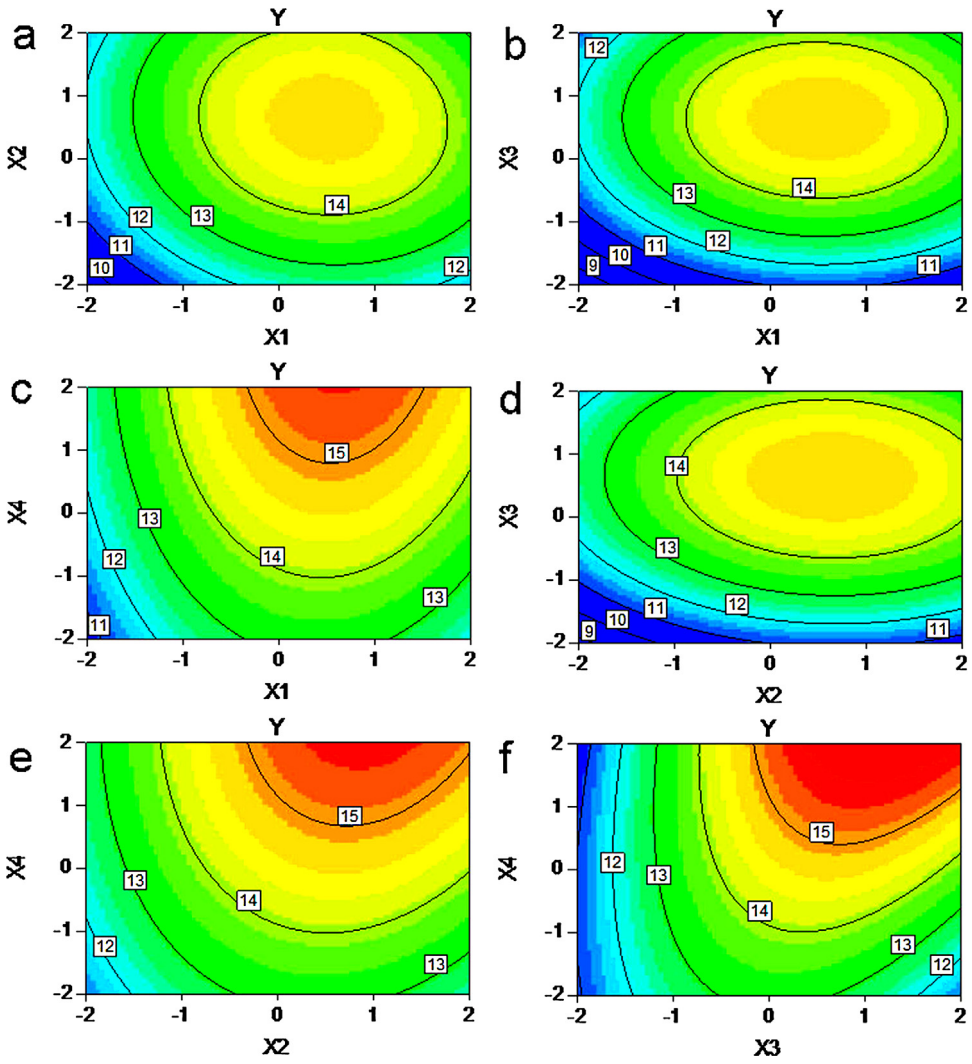

Fig. 1. Contour plots (2-D) showing the effects of variables (X_1: extraction time; X_2: ratio of water to raw material; X_3: extraction frequency; X_4: extraction temperature) on the response Y (yield of PNMP).

3.2. Optimization of PNMP extraction conditions

The full model was created as a 3-D response surface with contour plots to predict the relationships between independent and dependent variables. The 3-D response surface and contour plots were created using the Design Expert software (Version 8.0.6). The PNMP yield affected by extraction time (X_1), ratio of water to raw material (X_2), extraction frequency (X_3), and extraction temperature (X_4) are shown in Figs. 1 and 2.

In the response surface and contour plots, the PNMP yield was obtained along with two continuous variables as the other two variables were fixed at 0 (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 were obtained when perfect interaction existed between the independent variables. The independent variables and the maximum predicted values from the figures corresponded to the optimum values of the dependent variables (responses) obtained using the equations (6). Figs. 1a and 2a show the contour and 3-D response surface plots based on the independent variables (extraction time and ratio of water to raw material). The other two independent variables (extraction frequency and extraction temperature) were maintained at 0. The yield increased as extraction time was extended from 1.5 h to 2.8 h. Afterward, the yield slightly decreased. The yield also increased as the ratio of water to raw material increased from 15 to 28; at a ratio ranging from 28 to 35, the yield decreased. Figs. 1b and 2b show the PNMP yield at varying extraction times and extraction frequencies at a fixed ratio of water to raw material (0) and extraction temperature (0). The maximum PNMP yield was obtained when the extraction time and the ratio of water to raw material were 2.8 h and 5, respectively. The PNMP yield affected by the extraction time and extraction temperature is shown in Figs. 1c and 2c, whereas the two variables (ratio of water to raw material and extraction frequency) were fixed at 0. The maximum PNMP yield was obtained when the extraction time and the extraction temperature was 2.8 h and 95 °C, respectively. The contour surface and 3-D response plots at different ratios of water to raw material and various extraction frequencies as extraction time and extraction temperature were fixed at 0 are shown in Figs. 1d and 2d. The PNMP yield increased as the ratio of water to raw material increased from 15 to 28. As the ratio of water to raw material further increased, the yield was maintained at a constant level. At an extraction frequency of 5, the PNMP yield reached the maximum value but did not further increase beyond this level. The contour and 3-D response surface plots (Figs. 1e and 2e) show the PNMP yield with different ratios of water to raw material and extraction temperatures as extraction time and extraction frequency were maintained at 0. This finding

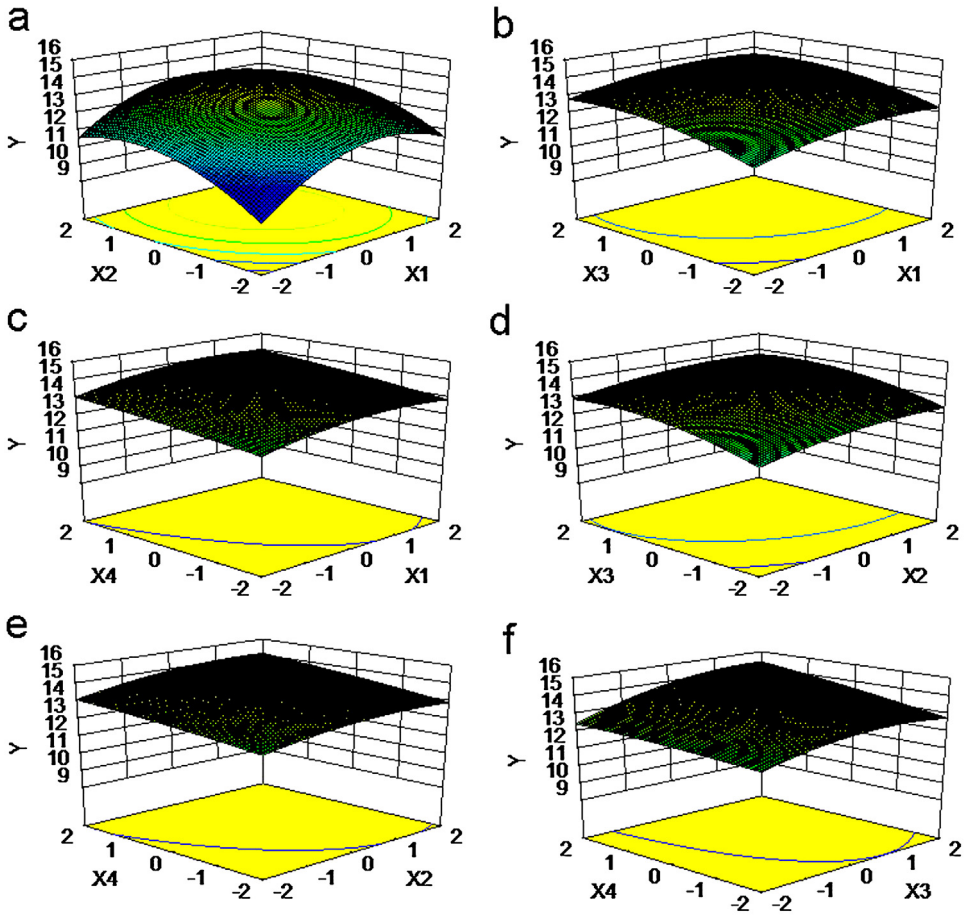

Fig. 2. Response surface plots (3-D) showing the effects of variables (X_1: extraction time; X_2: ratio of water to raw material; X_3: extraction frequency; X_4: extraction temperature) on the response Y (yield of PNMP).

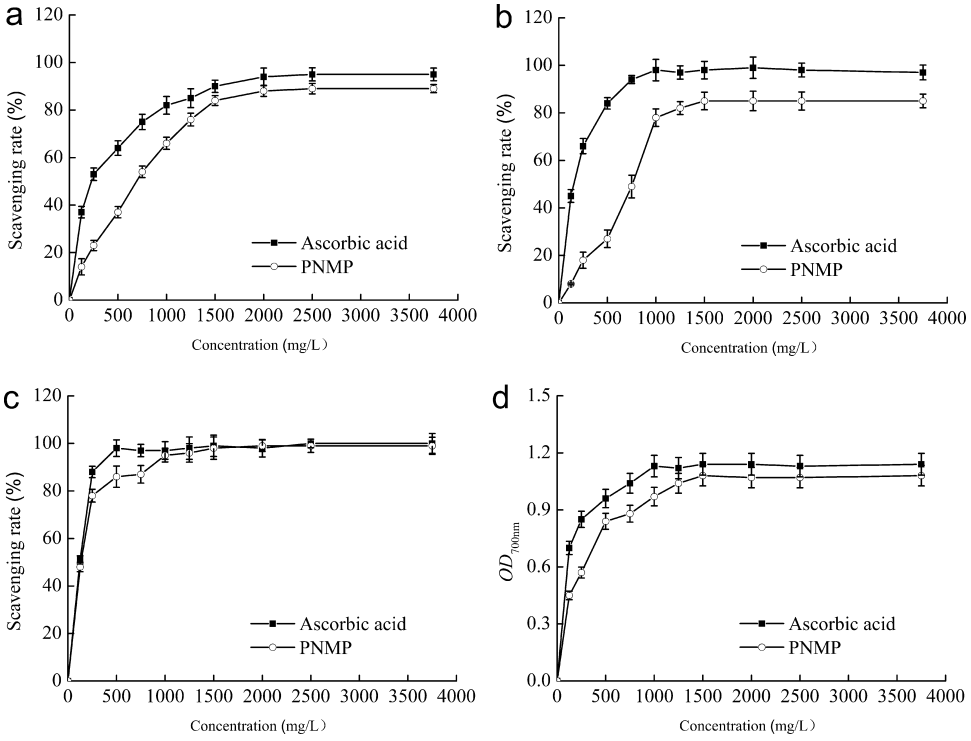

Fig. 3. Antioxidant activity assay of PNMP and ascorbic acid. (a) Scavenging effects on superoxide anion radicals. (b) Scavenging effects on hydroxyl radicals. (c) Scavenging effects on DPPH. (d) Reducing power of PNMP and ascorbic acid.

indicated that the maximum PNMP yield was achieved at ratio of water to raw material and extraction frequency of 28 and 95 °C, respectively. Figs. 1f and 2f show the contour and 3-D response surface plots at varied extraction frequencies and extraction temperatures as the extraction time and the ratio of water to raw material were fixed at 0. This finding indicated that the maximum PNMP yield was obtained at extraction frequency and extraction temperature of 5 and 95 °C, respectively.

Figs. 1 and 2 show the following optimal extraction conditions for PNMP: extraction time, 2.8 h; ratio of water to raw material, 28; extraction frequency, 5; and extraction temperature, 95 °C.

3.3. Verification of the predictive model

The suitability of the model equations for predicting the optimum response values was determined under the optimized conditions. This set of conditions was considered as optimum by RSM and used to validate experimentally and predict the values of the responses by using the model equation. The mean value of 15.33 ± 0.21 ($n = 3$) obtained from the real experiments validated the RSM model compared with the predicted value (15.41), indicating that the model could be used for extraction.

3.4. Antioxidant activity assay

Fig. 3a shows that the scavenging rates of PNMP and ascorbic acid against superoxide radicals were directly proportional to their concentrations. The scavenging rates of PNMP were lower than those of ascorbic acid within the test dosage range. EC_{50} of PNMP (728.2 ± 3.5 mg/L) was higher than that of ascorbic acid (227.3 ± 4.8 mg/L).

Fig. 3b shows that the hydroxyl radical scavenging activity of PNMP was concentration dependent in lower concentration (0–1000 mg/L), and the maximum scavenging rate was approximately 80% at PNMP > 1000 mg/L. EC_{50} of the scavenging activity of PNMP against hydroxyl radicals was 754.2 ± 4.7 mg/L, which was significantly different from that of ascorbic acid (137.6 ± 2.8 mg/L).

The scavenging effects of PNMP on DPPH radical were determined (Fig. 3c). Both PNMP and ascorbic acid showed evident scavenging activities on DPPH radical in a concentration-dependent manner at a relatively low concentration range of 0 mg/L to 250 mg/L. PNMP and ascorbic acid exhibited their maximum scavenging activities at a relatively high concentration range of 250 mg/L to 3750 mg/L. EC_{50} of the scavenging activity of PNMP against DPPH radical was 128 ± 24 mg/L, which did not significantly differ from the scavenging effect of ascorbic acid (122.2 ± 4.2 mg/L).

The reducing capacity of a compound may be used as a significant indicator of a potential antioxidant activity (Kallithraka, Bakker, & Clifford, 2001). The reducing powers of PNMP and ascorbic acid were also investigated and the results are shown in Fig. 3d. Although the reducing capacity of PNMP was slightly lower than that of ascorbic acid, the results showed that PNMP had potential antioxidant properties.

4. Conclusion

In the present study, polysaccharide production of *P. nigricans* mycelia was optimized using a CCD. The effect of the four independent variables (extraction time; ratio of water to raw material; extraction frequency; and extraction temperature) was determined effectively. The optimal experimental PNMP yield of $15.33 \pm 0.21\%$ was obtained at an extraction time of 2.8 h, a ratio of water to raw material of 28, an extraction frequency of 5, and an extraction temperature of 95 °C. Under these optimized conditions, the experimental yield of PNMP is consistent with the predicted yield of 15.41%.

Our study also revealed that PNMP exhibited positive radical scavenging activities against superoxide anion, hydroxyl, and DPPH radicals. PNMP also showed strong reducing power *in vitro*. These results indicated that PNMP could be used as a potential antioxidant. This study provided important information that can be used as a guide for large-scale polysaccharide production from *P. nigricans*. Polysaccharide products can then be used as a functional food or an antioxidant.

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References

Bae, J. S., Jang, K. H., Yim, H., & Jin, H. K. (2005). Polysaccharides isolated from *Phellinus gilvus* inhibit melanoma growth in mice. *Cancer Letters*, *218*, 43–52.

Borchers, A. T., Stern, J. S., Hackman, R. M., Keen, C. L., & Gershwin, M. E. (1999). Mushrooms, tumors and immunity. *Proceedings of the Society for Experimental Biology and Medicine*, *221*, 281–293.

Chen, L., Pan, J., Li, X., Zhou, Y., Meng, Q., & Wang, Q. (2011). Endo-polysaccharide of *Phellinus igniarius* exhibited anti-tumor effect through enhancement of cell mediated immunity. *International Immunopharmacology*, *11*, 255–259.

Chen, X. P., Wang, W. X., Li, S. B., Xue, J. L., Fan, L. J., & Sheng, Z. J. (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.

Deng, P., Zhang, G., Zhou, B., Lin, R., Jia, L., Fan, K., et al. (2011). Extraction and in vitro antioxidant activity of intracellular polysaccharide by *Pholiota adiposa* SX-02. *Journal of Bioscience and Bioengineering*, *111*, 50–54.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1958). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

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.

Hata, T. (1997). *Recent advances in medical and veterinary mycology*. Baltimore: University Park Press., 299.

Kallithraka, S., Bakker, J., & Clifford, M. N. (2001). Correlatlons between saliva protein composition and some T-I arameters of astringency. *Food Quality and Preference*, *12*, 145–152.

Li, X., Jiao, L., Zhang, X., Tian, W., Chen, S., & Zhang, L. (2008). Anti-tumor and immunomodulating activities of proteoglycans from mycelium of Phellinus nigricans and culture medium. *International Immunopharmacology*, *8*, 909–915.

Ooi, V. E. C., & Liu, F. (1991). A review of pharmacological activities of mushroom polysaccharides. *International Journal of Medicinal Mushrooms*, *1*, 195–206.

Shimada, K., Fujikawa, K., & Yahara, K. T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agriculture and Food Chemistry*, *40*, 945–948.

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 mycelia of *Polyporus albicans* (Imaz.) Teng. *Bioresource Technology*, *99*, 900–904.

Winterbourn, C. C., & Sutton, H. C. (1984). Hydroxyl radical production from hydrogen peroxide and enzymatically generated paraquat radicals: Catalytic requirements and oxygen dependence. *Archives of Biochemistry and Biophysics*, *235*, 116–126.

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, M., Cui, S. W. M., Cheung, P. C. K., & Wang, Q. (2007). Antitumor polysaccharides from mushroom: A review on their isolation process, structural characteristics and antitumor activity. *Trends in Food Science and Technology*, *18*, 4–19.