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Optimization of enzyme-assisted extraction and characterization of polysaccharides from *Hericium erinaceus*

Yang Zhu[a,1], Qian Li[b,1], Guanghua Mao[c], Ye Zou[c], Weiwei Feng[c], Daheng Zheng[c], Wei Wang[b], Lulu Zhou[b], Tianxiu Zhang[a], Jun Yang[b], Liuqing Yang[a,*], Xiangyang Wu[d,**]

[a] School of Chemistry and Chemical Engineering, Jiangsu University, Xuefu Rd. 301, Zhenjiang 212013, China
[b] School of Pharmacy, Jiangsu University, Xuefu Rd. 301, Zhenjiang 212013, Jiangsu, China
[c] School of Food and Biological Engineering, Jiangsu University, Xuefu Rd. 301, Zhenjiang 212013, China
[d] School of the Environment, Jiangsu University, Xuefu Rd. 301, Zhenjiang 212013, Jiangsu, China

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ABSTRACT

The enzyme-assisted extraction (EAE) of polysaccharides from the fruits of *Hericium erinaceus* was studied. In this study, response surface methodology and the Box–Behnken design based on single-factor and orthogonal experiments were applied to optimize the EAE conditions. The optimal extraction conditions were as follows: a pH of 5.71, a temperature of 52.03 °C and a time of 33.79 min. The optimal extraction conditions resulted in the highest *H. erinaceus* polysaccharides (HEP) yield, with a value 13.46 ± 0.37%, which represented an increase of 67.72% compared to hot water extraction (HWE). The polysaccharides were characterized by FT-IR, SEM, CD, AFM, and GC. The results showed that HEP was composed of mannose, glucose, xylose, and galactose in a molar ratio of 15.16:5.55:4.21:1. The functional groups of the *H. erinaceus* polysaccharides extracted by HWE and EAE were fundamentally identical but had apparent conformational changes.

1. Introduction

Hericium erinaceus belongs to the Aphyllophorales, Hydnaecae, and Hericium families and is a well-known edible and medicinal mushroom in East Asia. The bodies of *H. erinaceus* have attracted much attention due to their health effects when used as a home remedy for gastric and duodenal ulcers as well as some other diseases (Jia, Liu, Dong & Fang, 2004). This fungus contains valuable constituents, including polysaccharides, lectins, proteins, lipids, hericenone, erinacol, erinacine, and terpenoids, and these constituents possess various biological activities such as antimicrobial effects (Kim, Pyun, Ko, & Park, 2000), antitumor activities (Kim, Kang, Kim, Nam, & Friedman, 2011; Lee & Hong, 2010), antioxidant properties (Malinowska et al., 2009; Zhang et al., 2012), cytotoxic effect (Kawagishi, Ando, & Mizuno, 1990), and hypolipidemic effects (Wang, Hu, Wang, Chen, & Chia, 2005). Moreover, this fungus can promote neurogrowth factor synthesis (Kawagishi et al., 1994).

Hot water extraction (HWE) is the traditional extraction method for polysaccharides, and it has been widely investigated (Samavati, 2013; Yin & Dang, 2008; Zha et al., 2009). Enzyme-assisted extraction (EAE) possesses the advantage of being environmentally friendly, highly efficient, and easily operated owing to the relatively mild reaction conditions. Thus, it has been represented as an alternative method of natural product extraction (Li, Smith, & Hossain, 2006; Li et al., 2012; Zhang, Jia, Liu, Wu, & Ran, 2011). Mushroom polysaccharides exist as a structural component of fungal cell walls. The fungal cell wall is composed of two major types of polysaccharide: one is a rigid fibrillar structure composed of chitin (or cellulose), and the other one is a matrix-like structure composed of β-glucan, α-glucan and glycoproteins (Ruiz-Herrera, 1992). Enzymes can effectively catalyze the degradation of cell walls, favoring the release of bioactive components contained inside the cells. Hence, the degradation of cell wall polysaccharides is of major importance in many applications. Response surface methodology (RSM) is a collection of statistical and mathematical techniques that have been successfully used for developing, improving, and optimizing biochemical processes in recent years (Gan & Latiff, 2011; Sui et al., 2013; Zhu, Nie, Liang, & Wang, 2013). RSM is based on the fit of empirical models to the experimental data obtained in relation to experimental design. Employing RSM could lead to simplifying the complexity of the experimental trials needed to evaluate multiple variables and their interactions.

* Corresponding author. Tel.: +86 511 88791800; fax: +86 511 88791800.
** Corresponding author. Tel.: +86 511 88791200; fax: +86 511 88791200.
E-mail addresses: yangliuqing@ujs.edu.cn (L. Yang), wuxy@ujs.edu.cn (X. Wu).
[1] Contributed to this article equally and are co-first authors.

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Therefore, RSM is more labor and time-saving than other methods required to optimize a process (Wu, Cui, Tang, & Gu, 2007). The Box–Behnken design (BBD), a type of RSM, only has three levels and requires fewer experiments. The BBD is more efficient and make it easier to arrange and interpret experiments in comparison with others methods, and it is widely used by many researchers (Chen et al., 2010; Han, Jiang & Zhang, 2011; Luo & Chen, 2010).

The objective of this study was to investigate the extraction process of *H. erinaceus* polysaccharides (HEP) with complex enzymes (cellulase, pectinases, and trypsinase) and to further optimize the extraction conditions for HEP by RSM. In addition, the preliminary characterization was estimated by FT-IR, SEM, CD, AFM, and GC. To date, little research has been reported on this subject.

2. Materials and methods

2.1. Materials

Fruits of *H. erinaceus* identified by Dr.Guanghua Mao were provided by FangGe Pharmaceutical Co., Ltd. in Zhejiang Province. All solvents and chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Methods

2.2.1. Enzyme-assisted extraction procedure

Dried fruits of *H. erinaceous* samples (10.0 g) were ground and extracted with distilled water (solid:liquid ratio of 1:30, w/v) in a round bottom flask with a designated pH (pH values of the mixtures were adjusted with 0.1 M HCl), temperature, time, and enzyme concentration (cellulose:pectinase:trypsin the ratio of 2:2:1). After the enzymolysis process, samples were rapidly heated at 100 °C in boiling water with stirring for 3 h. The mixture was centrifuged (4000 r/min for 10 min) and filtered, and the insoluble residue was treated again as mentioned above. The supernatant was incorporated and concentrated using a rotary evaporator at 65 °C under vacuum. The extract was precipitated by the addition of 95% (v/v) ethanol to a final concentration of 80% (v/v) and incubated for 12 h at 4 °C. The polysaccharide precipitate was collected by centrifugation (4000 r/min for 10 min) and lyophilized to obtain crude polysaccharides. The HEP yield (%) resulting from in the extraction was calculated by the following equation:

$$\text{HEP yield}\,(\%) = \frac{\text{weight of dried crude HEP}\,(\text{g})}{\text{weight of sample}\,(\text{g})} \times 100\% \quad (1)$$

The polysaccharide content was measured by the phenol-sulfuric acid method. Hot water extraction (HWE) of HEP without enzymatic pretreatment was performed as a control experiment. The HEP were re-dissolved and subjected to the trichloroacetic acid method for the removal of proteins. The obtained polysaccharides were further characterized.

2.2.2. Single factor experimental design

The effects of pH, temperature, time, and enzyme concentration were first studied by a single-factor design as follows: one factor was changed while the other factors were kept constant in each experiment. The effect of each factor was evaluated by determining the yield of HEP.

2.2.3. Orthogonal test design of complex enzyme-assisted extraction

A preliminary study was performed to investigate the experimental factors and to narrow the corresponding ranges before application of the statistical design. An orthogonal L_9 (3^4) test design in the extraction mode was used to optimize the extraction conditions. Table 1 shows that the extraction experiment was performed with 4 factors and 3 levels and the range of each factor was based on the results of the single-factor experiments. Each set of experiments was conducted in triplicate. The HEP yield (%) was the dependent variable and was obtained following the method in Section 2.2.1.

Table 1
Results and analysis of orthogonal experiment.

No.	A	B	C	D	Polysaccharide yield (%)
1	4.0	40	2.0	30	11.78
2	4.0	50	2.5	40	12.29
3	4.0	60	3.0	50	11.34
4	5.0	40	2.5	50	12.03
5	5.0	50	3.0	30	12.52
6	5.0	60	2.0	40	11.97
7	6.0	40	3.0	40	13.29
8	6.0	50	2.0	50	13.03
9	6.0	60	2.5	30	12.76
K_1	11.803	12.367	12.260	12.353	
K_2	12.173	12.613	12.360	12.517	
K_3	13.027	12.023	12.393	12.133	
R	1.224	0.590	0.123	0.384	

R refers to the result of extreme analysis.

2.2.4. Box–Behnken design and statistical analysis

After the preliminary study, Box–Behnken design (BBD) with three independent variables was used for the optimization. The parameters including pH, temperature, and pretreatment time were chosen as key variables based on the results of preliminary experiments and designated as X_1, X_2, and X_3, respectively. The complete quadratic equation used was as follows:

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

where Y is the response variable; 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 ($i \neq j$). The variables were coded according to the equation:

$$x_i = \frac{X_i - X_0}{\Delta X} \quad (3)$$

where x_i is the coded value of the independent variable, X_i is the real value of the independent variable, X_0 is the real value of an independent variable at the center point, and ΔX is the step change value. Five replicates at the center of design were used to estimate a pure error sum of squares.

2.3. Preliminary characterization of HEP

2.3.1. Infrared spectra analysis

The IR spectra of the polysaccharides were determined using a Fourier transform IR spectrophotometer (FT-IR) (Nicolet Avatar-370, USA). The polysaccharide extracts were ground with KBr powder and then pressed into pellets for FT-IR measurement in the frequency range of 400–4000 cm^{-1}.

2.3.2. Circular dichroism (CD) spectroscopy analysis

The samples were dissolved in deionized water at 0.05 mg/mL and the solution was kept at 45 °C for 2 h. Circular dichroism was performed in a Jasco spectrophotometer using a 3 mL rectangular quartz cuvette (Jasco) with a 1 cm path length. Spectra were recorded at 25 °C with a JASCO J-815 spectropolarimeter in the wavelength (λ) range of 190–250 nm with the following setup: bandwidth of 2.5 nm; time constant of 2 s; scan rate of 50 nm/min.

2.3.3. Scanning electron microscopy (SEM) analysis

To evaluate the effect of enzyme-assisted extraction of HEP on the microstructure of the materials, SEM was used to reveal the microstructure of the material (Zhao et al., 2011). Three sample particles (A, untreated *H. erinaceus* powders; B, remaining solids collected and air-dried after HWE; and C, remaining solids obtained after the procedures of EAE under the optimum condition for HEP extraction.) were fixed on the silicon wafer. The shape and surface characteristics were observed and recorded using a scanning electron microscope (SEM; JSM-7001F, JEOL, Ltd. Japan).

2.3.4. Atomic force microscopy (AFM) analysis

The sample was dissolved in deionized water at 1 mg/mL, and the solution was kept at 45 °C for 2 h. The solution (5 μL) was dropped on to a freshly cleaved mica surface and allowed to dry at room temperature. Atomic force microscopy was performed in the tapping-mode using a MFP-3D instrument (Asylum Research, USA).

2.3.5. Monosaccharide composition analysis

The monosaccharide composition of the sample was analyzed by GC after transesterification. Briefly, approximately 5 mg of freeze-dried HEP was dissolved in an ampoule containing 4 mL of 2 M trifluoroacetic acid (TFA). The sample was hydrolyzed at 100 °C for 6 h. After hydrolysis, the resulting solution was concentrated in a vacuum, and the excess acid was removed by repeated co-distillations with methanol. Neutral monosaccharides were reduced to alditol using 25 mg of sodium borohydride with 3 mL of distilled water at room temperature for 12 h. Acetic acid was added to the solution to decompose excess sodium borohydride until bubble formation stopped. A stream of nitrogen gas was used to dry the solution and 3 mL of methanol was added to remove the borohydrate. The procedure was repeated four times. The hydrolyzed products were acetylated with 5 mg of hydroxylamine hydrochloride and myo-inositol (internal standard) in 0.5 mL of pyridine for 30 min at 90 °C, and the mixture was then cooled to room temperature. Acetic anhydride (0.5 mL) was then added to the mixture was incubated for additional 30 min at 90 °C. The hydrolysate was then converted into the corresponding alditol acetates and analyzed with GC using a Shimadzu 2010 instrument equipped with a HP-5MS column (0.25 mm × 30 m × 0.25 μm) and a flame-ionization detector. The initial column temperature was held at 130 °C for 5 min, increased to 240 °C at 4 °C/min and held at 240 °C for 5 min. Nitrogen gas (N_2) was used as the carrier gas with a flow rate of 1.0 mL/min. Alditol acetates were used as standards (glucose, mannose, rhamnose, galactose, xylose, and arabinose) and they were processed using the same approach as described above for the sample.

3. Results and discussion

3.1. Single factor experimental analysis of EAE

3.1.1. Effect of different pH on extraction yield of polysaccharides

The pH value can affect enzyme activity because different enzymes have their own optimal pH, which might be because a change in pH affects the spatial structure of enzymes. Thus, the enzyme conformation and enzymatic activity can be altered by changes in pH (Yin, You, & Jiang, 2011). The extraction procedure was performed at different pH conditions with the following fixed extraction variables: an enzyme concentration of 3%, a pretreatment time of 50 min, and an extraction temperature of 50 °C. The effect of different pH values on the extraction yield of polysaccharides is shown in Fig. 1A. The yield of total polysaccharide increased with elevating pH levels, and the highest yield (13.13%) was reached when the pH was increased to 5.0. For future experiments, pH values between 4.0 and 6.0 were used according to these results.

3.1.2. Effect of different enzyme action temperatures on the polysaccharides yield

Different enzymes have their own appropriate optimal effect temperatures. To study the effect of different temperatures on the yield of polysaccharides, the extraction process was performed using different extraction temperatures of 30, 40, 50, 60 and 70 °C while the other extraction factors (pH, enzyme concentration, and time) were fixed (5.0, 3%, and 50 min, respectively). The polysaccharide extraction yield increased when the extraction temperature increased from 30 to 50 °C. As shown in Fig. 1B, the maximum yield (13.17%) of polysaccharides was observed when the extraction temperature was 50 °C. When the extraction temperature varied from 60 to 70 °C, the polysaccharide yield decreased. Thus, the extraction temperature of 50 °C was considered to be optimal in the present experiment.

3.1.3. Effect of different enzyme concentration on the polysaccharides yield

The enzyme concentration was not constant during the extraction stages. The enzyme concentrations of 1.5%, 2.0%, 2.5%, 3.0% and 3.5% were used to examine the influence of enzyme concentration on the extraction of HEP when the other reaction conditions were fixed: (pH of 5.0, enzyme action temperature of 50 °C, and enzymatic time 50 min). Fig. 1C shows the effects of enzyme concentration on the extraction yield of HEP. The yield of HEP increased from 11.93% to 13.18% as the enzyme concentration increased from 1.5% to 2.5%. Taking into consideration that there were no significant differences among the 2.5%, 3.0% and 3.5% concentrations ($P > 0.05$), 2.5% was selected as the center point for further experiments.

3.1.4. Effect of different enzymatic action time on extraction yield of polysaccharides

The effect of time on HEP yield of is shown in Fig. 1D when the other three factors (pH, enzyme action temperature, and enzyme concentration) were fixed at 5.0, 50 °C, and 2.5%, respectively. The HEP yield of greatly increased with increasing time and the HEP yield reached a maximum value (13.01%) when the sample was extracted for 40 min.

According to the single-parameter study, the following conditions were used for latter experiments: a pH of 3.0–6.0, an enzyme action temperature of 40–60 °C, an enzyme concentration of 2.0%-3.0%, and an enzymatic pretreatment time of 30–50 min.

3.2. Orthogonal analysis of complex enzyme-assisted extraction

The results of the orthogonal test and extreme difference analysis are shown in Table 1. According to the *R* values, the influence of enzyme-assisted extraction factors on the extraction yield was as follow: A > B > D > C (pH > temperature > time > enzyme concentration). The extraction temperature was the most important determinant of the yield. Thus, the optimal condition was the combination of $A_3B_1C_3D_2$ (1–3 are the levels). The maximum extraction yield of 13.31 ± 0.22% was achieved at a pH of 6.0, a temperature of 40 °C, an enzyme concentration of 3.0% and a time of 40 min. The influence of the enzyme concentration was not important for the extraction yield. Therefore, the enzyme concentration was kept at 3.0% (w/w) in the subsequent Box–Behnken design experiments to further investigate the effect of the independent factors (A, B, and D) and their mutual interactions.

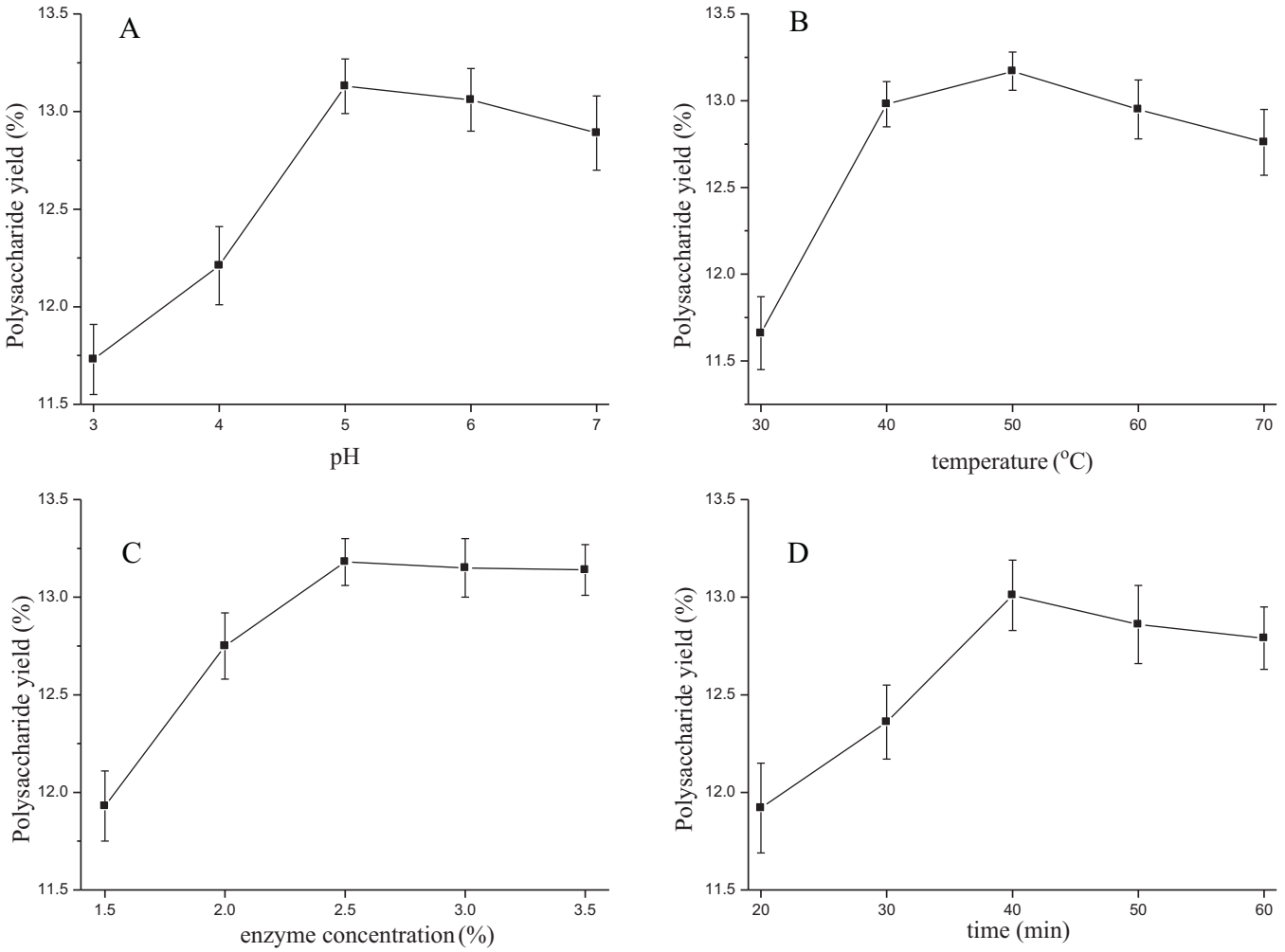

Fig. 1. Effect of different extraction parameters ((A) pH; (B) temperature, °C; (C) enzyme concentration, %, and (D) time, min) on yield of polysaccharides. The effects of pH, temperature, time, and enzyme concentration were first studied by a single-factor design as follows: one factor was changed while the other factors were kept constant in each experiment. The effect of each factor was evaluated by determining the yield of HEP.

3.3. Optimization of the procedure by RSM

3.3.1. Model fitting and statistical analysis

Based on the results of the single-factor experiments and orthogonal test, a 17-run BBD with three factors and three levels was utilized to determine the optimal levels for pH, temperature, and time. The extraction yields of HEP under different conditions are shown in Table 2, and the data were analyzed by Design-Expert (Version 8.06) software. As a result, the second-order polynomial equation that revealed the relationship between the HEP yield and the test variables was obtained as follows:

$$Y = 13.20 + 0.40X_1 + 0.30X_2 - 0.27X_3 + 0.077X_1X_2 - 0.26X_1X_3 - 0.010X_1X_3 - 0.41{X_1}^2 - 0.88{X_2}^2 - 0.37{X_3}^2 \quad (4)$$

Table 2
Box–Behnken design matrix (in coded level of three variables) and response values for the yield of HEP.

Number	X_1 (pH)	X_2 (temperature, °C)	X_3 (time, min)	Polysaccharide yield (%)
1	−1 (4.0)	−1 (40)	0 (40)	11.25
2	1 (6.0)	−1 (40)	0 (40)	11.92
3	−1 (4.0)	1 (60)	0 (40)	11.76
4	1 (6.0)	1 (60)	0 (40)	12.74
5	−1 (4.0)	0 (50)	−1 (30)	12.06
6	1 (6.0)	0 (50)	−1 (30)	13.36
7	−1 (4.0)	0 (50)	1 (50)	12.02
8	1 (6.0)	0 (50)	1 (50)	12.27
9	0 (5.0)	−1 (40)	−1 (30)	11.94
10	0 (5.0)	1 (60)	−1 (30)	12.48
11	0 (5.0)	−1 (40)	1 (50)	11.46
12	0 (5.0)	1 (60)	1 (50)	11.96
13	0 (5.0)	0 (50)	0 (40)	13.21
14	0 (5.0)	0 (50)	0 (40)	13.25
15	0 (5.0)	0 (50)	0 (40)	13.19
16	0 (5.0)	0 (50)	0 (40)	13.23
17	0 (5.0)	0 (50)	0 (40)	13.13

The analysis of variance, goodness-of-fit and the adequacy of the models are summarized in Table 2. The determination coefficient ($R^2 = 0.9971$) resulting from the ANOVA of the quadratic regression model indicated that only 0.29% of the total variation was not explained by the model. The value of the adjusted determination coefficient (${R_{Adj}}^2 = 0.9934$) also confirmed that the model was highly significant. At the same time, the low coefficient variation (C.V.) value of 0.45 clearly indicated a high degree of precision and good 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. (4) are listed in Table 3. 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. Smaller the

Table 3
Analysis of the variance (ANOVA) for the second-order polynomial model.

Source	Sum of squares	*df*	Mean square	*F*-value	Prob > *F*
Model	7.76	9	0.86	270.09	<0.0001
X_1	1.28	1	1.28	400.81	<0.0001
X_2	0.70	1	0.70	219.85	<0.0001
X_3	0.57	1	0.57	177.58	<0.0001
X_1X_2	0.024	1	0.024	7.52	0.0288
X_1X_3	0.28	1	0.28	86.31	<0.0001
X_2X_3	4.000E−004	1	4.000E−004	0.13	0.7338
X_1^2	0.70	1	0.70	220.01	<0.0001
X_2^2	3.23	1	3.23	1011.74	<0.0001
X_3^2	0.56	1	0.56	176.61	<0.0001
Residual	0.022	7	3.194E−003		
Lack of fit	0.014	3	4.625E−003	2.18	0.2328
Pure error	8.480E−003	4	2.120E−003		
Cor Total	7.79	16			
	$R^2 = 0.9971$	$R_{Adj}^2 = 0.9934$	C.V.% = 0.45		

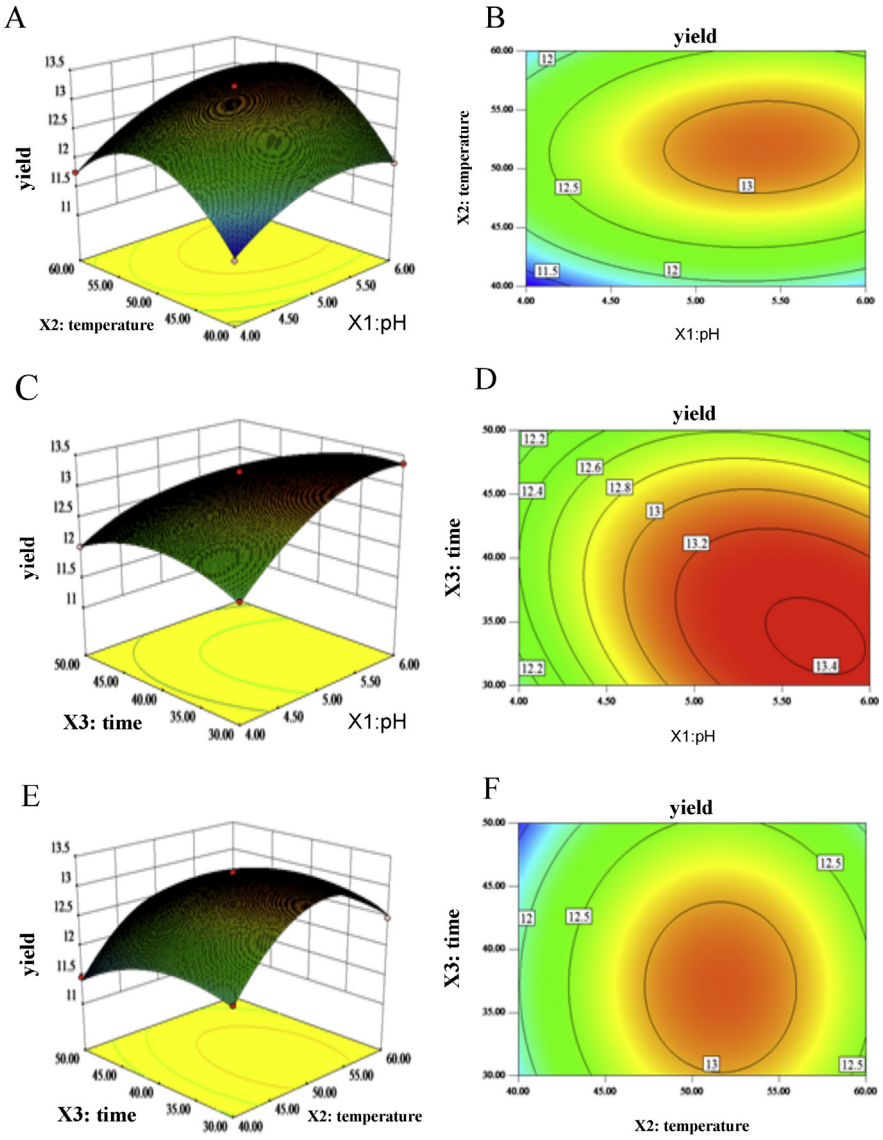

Fig. 2. Response surface plots (A, C, and E) and contour plots (B, D, and F) showing the interactive effects of pH (X_1), temperature (X_2), and time (X_3) on the extraction yield of HEP. Based on the results of the single-factor experiments and orthogonal test, a 17-run BBD with three factors and three levels was utilized to determine the optimal levels for pH, temperature, and time. The extraction yields of HEP under different conditions. The data were analyzed by Design-Expert (Version 8.06) software.

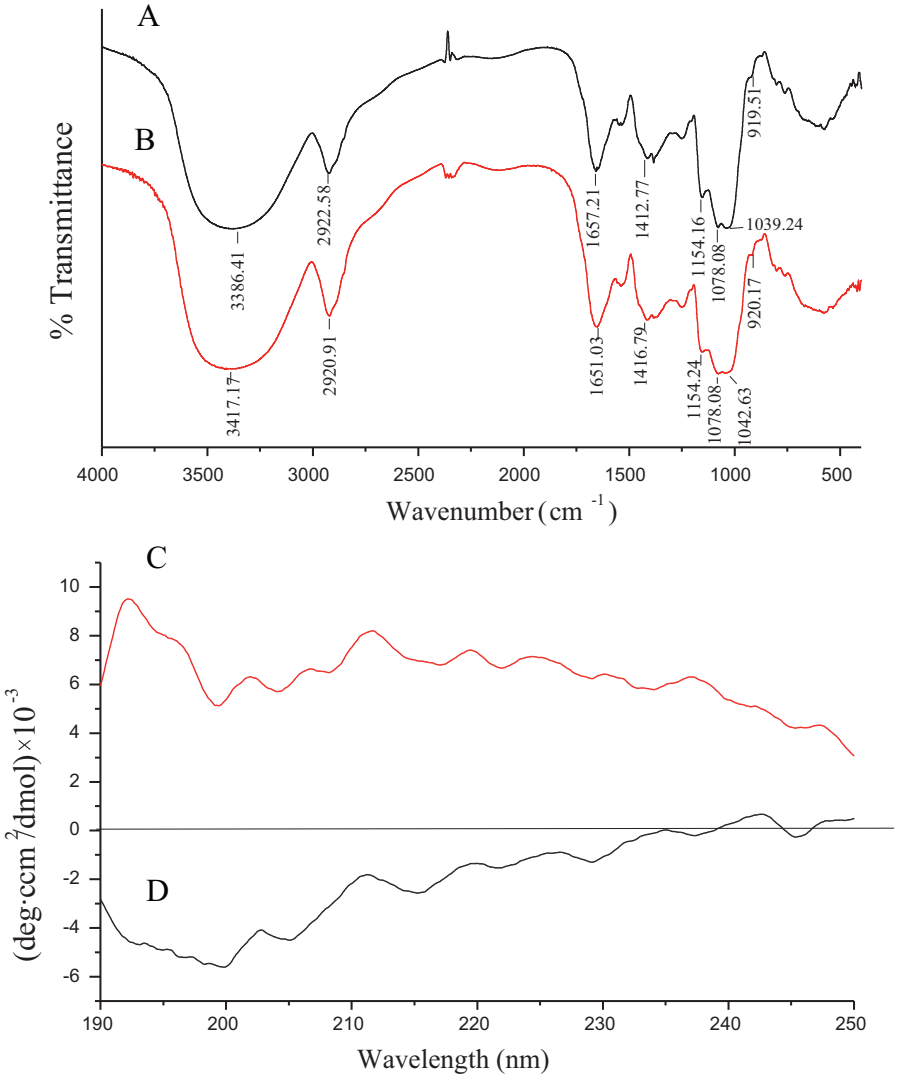

Fig. 3. FT-IR and CD spectra of the polysaccharides extracted from *Hericium erinaceus*. ((A, D) extracted by hot water method; (B, C) extracted by enzyme-assisted method). FT-IR spectrum of the HEP were recorded with a NEXUS 670 FT-IR spectrophotometer between 400 and 4000 cm^{-1} using the KBr-disk method. Circular dichroism was performed in a Jasco Spectrophotometer using a 3 ml rectangular quartz cuvette (Jasco) with a 1 cm path length.

P-values represented more significant corresponding coefficients. Table 3 shows that the linear coefficients (X_1, X_2, and X_3), quadratic term coefficients (X_1^2, X_2^2, and X_3^2) and cross product coefficients (X_1X_2 and X_1X_3) were significant, with small *P*-values ($P < 0.05$). The other terms' coefficients were not significant ($P > 0.05$). The full model resulting from Eq. (4) was used to generate the 3D response surface plot and contour plot to predict the relationships between the independent and dependent variables.

3.3.2. Optimization of the procedure

The 3D response surface and 2D contour plots were provided as graphical representations of the regression equation (Fig. 2). These provided a method to visualize the relationship between responses and experimental levels of each variable as well as the type of interactions between the two test variables. The shapes of the contour plots (either circular or elliptical) indicated whether the mutual interactions between the variables were significant. A circular contour plot indicated that the interactions between the corresponding variables were negligible and an elliptical contour plot indicated that the interactions between the corresponding variables were significant (Yu & Chao, 2013). The relationship between the independent and dependent variables was illustrated in a tri-dimensional representation of the response surfaces and two-dimensional contour plots generated by the model for yield of polysaccharides (two variables were depicted in one tri-dimensional surface plots, and the other variable was fixed at level zero). The yield of polysaccharides was sensitive to minor alterations of the test variables (pH, extraction temperature, and time). Fig. 2A and B shows the effect of pH (X_1), temperature (X_2) and their reciprocal interaction on extraction yield when the time was fixed at level 0. The pH (X_1) and temperature (X_2) demonstrated quadratic effects on the extraction yield. When the pH (X_1) was kept at a lower level, the yield increased at first and then decreased with the increase of temperature (X_2). Likewise, Fig. 2C and D shows that the pH (X_1) and time (X_3) demonstrated quadratic effects on the extraction yields when the extraction temperature was fixed at level 0. The elliptical contour plot shown in Fig. 2D indicates that the mutual interactions between the pH and time were significant. Fig. 2E and F shows that when the pH (X_1) was fixed at level 0, the variations of yields were insignificant with increased temperature. The pretreatment time (X_3) demonstrated quadratic effects on the response.

3.3.3. Verification of predictive model

The suitability of the model equations for predicting optimum response values was tested under the following conditions: a pH

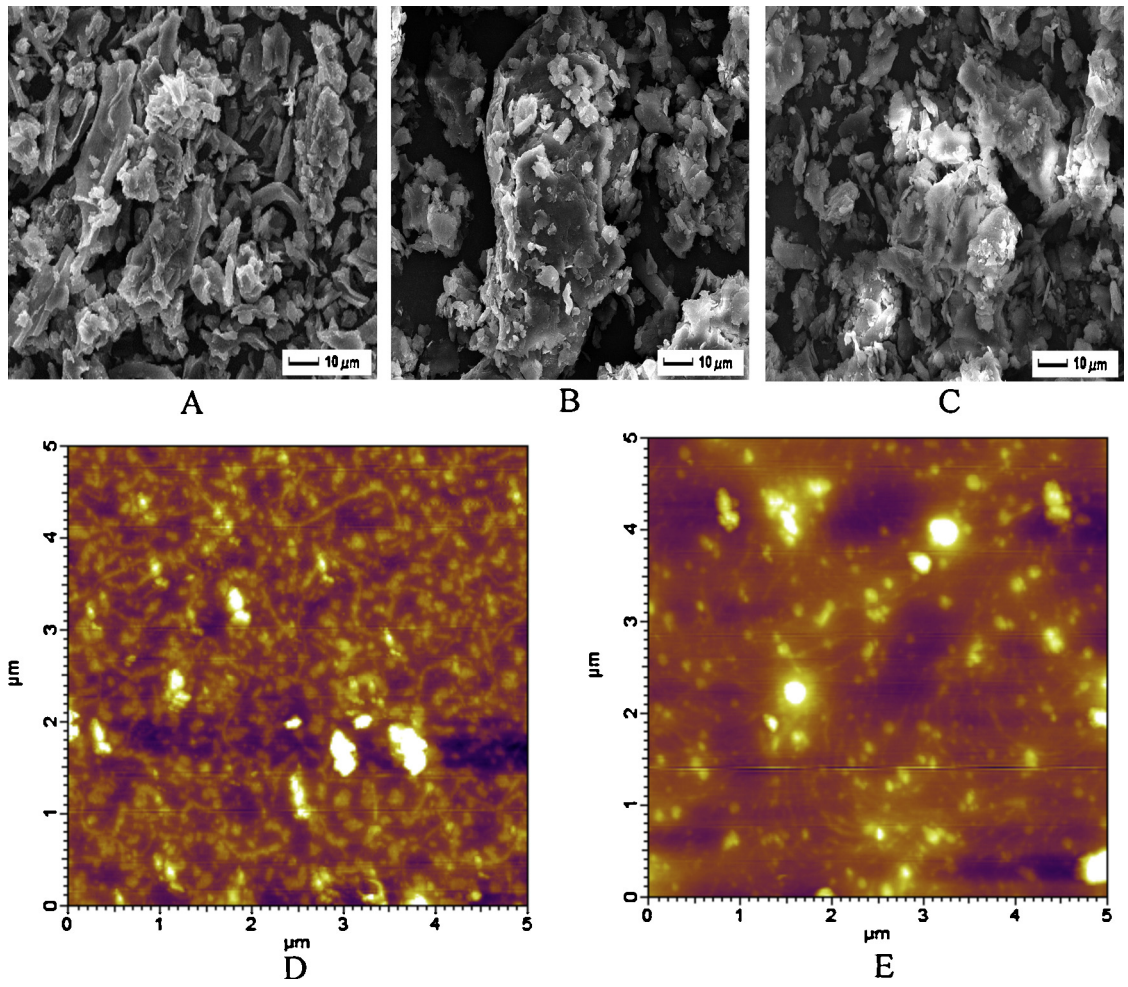

Fig. 4. Scanning electron microscope (SEM) photographs of the raw material (A) and residues after extraction by HWE (B) and EAE (C); the AFM images of HEP by HWE (D) and EAE (E) were obtained with a MFP-3D atomic force microscope (Asylum Research, American) and collected by tapping mode. The shape and the surface characters were observed and recorded on the scanning electron microscope (SEM, JSM-7001F, JEOL, Ltd, Japanese). The atomic force microscopy was operated in the tapping-mode using a MFP-3D instrument (Asylum Research, American).

of 5.71, a temperature of 52.03 °C, and a time of 33.79 min. This set of conditions was determined to be optimum by the RSM optimization approach, and it was also used to validate experimentally and predict the values of the responses using the model equation. A mean value of 13.46 ± 0.37% ($N = 3$) obtained from actual experiments, demonstrated the validation of the RSM model, thereby indicating that the model was adequate for the extraction process.

3.4. Comparison of HWE and EAE

In the control experiment, the HEP yields obtained by HWE and EAE were 8.03 ± 0.26% and 13.46 ± 0.37%, respectively. Thus, the application of EAE positively affected the HEP yield. Under the optimal conditions of EAE, the HEP yield increased by 67.72% compared to that of HWE. In addition, the content of polysaccharides was 44.19 ± 1.64% resulting from EAE, which was higher than that resulting from HWE (40.09 ± 1.59%). Comparisons of the structure and conformation between the two methods need to be studied further to promote the commercial application of EAE for use in the field of bioactive compounds.

3.5. Preliminary characterization of HEP

3.5.1. Infrared spectroscopy (IR) analysis

The FT-IR spectra of carbohydrates are used for determination of their structural features. The infrared spectra (Fig. 3) showed strong and wide stretching peak around 3386.41 (Fig. 3A) and 3417.17 cm^{-1} (Fig. 4B) for O—H stretching vibrations as well as a weak absorption peak at 2835–2940 cm^{-1} for C—H stretching vibrations. The signals at 1651.03 and 1416.79 cm^{-1} were attributed to asymmetric and symmetric stretching of the carboxylate anion group (C=O). Each particular polysaccharide has a specific band in the 1000–1200 cm^{-1} region. This region is dominated by ring vibrations overlapped with stretching vibrations of (C—OH) side groups and the (C—O—C) glycosidic band vibration. The absorptions at 1154.24, 1078.08, and 1042.63 cm^{-1} indicated a pyranose form of sugar. Absorbance at 920.17 cm^{-1} suggested a β-type linkage in the molecular structure of fungal polysaccharides (Wu, Zhu, Zhang, Yang, & Zhou, 2012). From the spectra, there was no marked difference between HEP extracted by hot water and enzyme-assisted methods.

3.5.2. Circular dichroism (CD) spectroscopy analysis

The CD spectra of the polysaccharides using HWE and EAE in the range of 190–250 nm are shown in Fig. 3. It should be emphasized that the two polysaccharides showed highly different CD behavior depending upon (i) the local ring geometry around the carboxyl chromophore, and (ii) the effect of the neighboring group. In particular, the polysaccharides resulting from EAE were positive throughout the considered range, but the polysaccharides resulting from HWE were negative below 238 nm.

3.5.3. Scanning electron microscopy (SEM) analysis

The scanning electron microscope (SEM) date showed that there was a remarkable the material structure change after HWE and EAE. Fig. 4A shows the SEM photographs of the raw *Hericium erinaceus*. It was clear that the raw material showed powder residues (Fig. 4A), and the residues had more ruptured structures after extraction by HWE (Fig. 4B) and EAE (Fig. 4C). Moreover, the residues resulting from HWE and EAE had large lamellar on their surface. As compared to HWE, a significant impact on material structure was observed after EAE. The reason for this impact may be that the cell wall degrading enzymes (i.e., cellulose) can weaken or break down the cell wall, rendering the intracellular materials more accessible for extraction, which contributed to the higher extraction efficiency of polysaccharides.

3.5.4. Atomic force microscopy (AFM) analysis

The HEP morphology was observed by AFM to obtain direct chain conformation of the polysaccharides. Fig. 4D and F shows the plane of the HWE and EAE AFM images of the polysaccharides in water, respectively. The height of a single polysaccharide chain was approximately 0.1 to 1 nm, which indicated that the HEP structures were branched and entangled with each other. As compared to HWE, the large molecular aggregation was reduced with EAE and the polysaccharide chain was extended with EAE, increasing the flexibility of the molecule. This change was caused by the chair-boat transition of glucose.

3.5.5. Gas chromatography (GC) analysis

The monosaccharide composition of HEP was analyzed by GC. By comparing the retention time with standards, the monosaccharide composition was identified. Composition analysis indicated that the HEP resulting from EAE and HWE was composed of mannose, glucose, xylose, and galactose in molar ratios of 15.16:5.55:4.21:1 and 11.99:6.48:2.6:1, respectively. The HEP monosaccharide compositions were not markedly different between the HEPs extracted by hot water and enzyme-assisted methods, but the molar ratios were different.

4. Conclusion

The optimal conditions for polysaccharide extraction were as follows: a pH of 5.71, a temperature of 52.03 °C, and a time of 33.79 min. Under the optimal conditions, an actual experimental yield of 13.46 ± 0.37% was obtained. As compared to HWE, EAE provided higher extraction yields and contents of polysaccharides. The preliminary characterization showed that the functional groups of polysaccharides from *H. erinaceus* extracted by HWE and EAE were fundamentally identical but had apparent conformational changes. The extraction information for *H. erinaceus* polysaccharides obtained in this work should also be helpful when studying other species. Further studies on the precise chemical structures and biological functions of HEP are currently in progress.

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