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Optimization of extraction process of crude polysaccharides from wild edible BaChu mushroom by response surface methodology

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Abstract

Polysaccharides production from wild edible BaChu mushroom was carried out using boiling water decoction. Response surface methodology (RSM), based on a five level, four variable central composite rotatable design (CCRD), was employed to obtain the best possible combination of extraction temperature, extraction time, particle size and ratio of water to mushroom for maximum polysaccharides production. The optimum extraction conditions were as follows: extraction temperature 94 °C, extraction time 10 h, particle size 33 and ratio of water to mushroom 6. Under these conditions, the experimental yield was 8.73%, which is well matched with the predictive yield.

Keywords: BaChu mushroom; Extraction; Optimization experiment; Response surface methodology; Temperature

1. Introduction

In Asia, mushrooms have long been used as traditional foods and medicines (Wong, Wong, Chiu, & Cheung, 2007; Zhang, Cheung, Chiu, Wong, & Ooi, 2006). Edible mushrooms contain an abundance of resources that possess a multitude of biological activities. They produce various classes of secondary metabolites with interesting biological activities and, thus, have the potential to be used as valuable chemical resources for drug discovery (Carbonero et al., 2006). The natural products extracted from edible mushrooms exhibit lower toxicity and fewer side effects than chemical drugs; therefore, mushrooms represent a potential valuable resource for natural drugs (Chihara, 1992).

Wild edible BaChu mushrooms grow in northern edge of Tarium basin in Xinjiang Province, China. It is named because it is produced in BaChu county in Xinjiang Province, China. BaChu mushrooms belonging to the genera *helvella*, was reported to possess antitumour, antioxidant, cholesterol reducing, and immunomodulating activities (Zhu et al., 1998) and have been used as traditional medicines for the treatment of gastrointestinal cancer, cerebral arteriosclerosis, cardiovascular disease, tuberculosis, liver or heart diseases, fester, bellyache, hypercholesterolemia, hyperlipidemia, stomach ailments, and diabetes (Zhang & Xiao, 1993). Recently, BaChu mushrooms are reported to efficiently enhance engulfing ability of leucocytes, lymphocytes conversion ratio and antibody titer (Meng, Zhang, & Hu, 2005). In this region, the climate is mild and rainy. The seasons are normally high day–night temperature difference. The climate during the year, especially, in spring and autumn, is ideal for wild mushroom growth (Chrysayi-Tokousbalides, Kastanias, Philippoussis, & Diamantopoulou, 2007; Özçelik & Pekşen, 2007). Mushroom polysaccharides exist as a structural component of fungal cell wall. Fungal cell wall is composed of two major types of polysaccharides: one is a rigid fibrillar of chitin (or cellulose), the other one is a matrix-like β-glucan, α-glucan and glycoproteins (Ruiz-Herrera, 1956). Polysaccharides, especially β-glucan, are considered to be responsible for their biological activity and there are many reports in the

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literature on the isolation and biological activity of polysaccharides derived from medicinal mushrooms (Gonzaga, Ricardo, Heatley, & Soares, 2005; Lim et al., 2005).

The objective of this present study was to optimize the process for production of the polysaccharides from wild edible BaChu mushroom, using response surface methodology (RSM), employing a five-level, four-variable central composite rotatable design (CCRD).

2. Materials and methods

2.1. Materials

The edible wild BaChu mushrooms were collected from BaChu county in northern edge of Tarium basin in Xinjiang Province, China.

All other chemicals were of analytical grade.

2.2. Extraction of crude polysaccharides

Dried BaChu mushroom (20 g) was ground in a high speed disintegrator (Model SF-2000, Chinese Traditional Medicine Machine Works, Shanghai, China) to obtain a fine powder, then were extracted in a Soxhlet apparatus with aether (20–40 °C), and pretreated with 80% aether twice to remove some colored materials, oligosaccharides and some small molecule materials. The organic solvent was volatilized and pretreated dry powder was obtained, as described previously (Yang, Qu, & Cheng, 2004; Zykwinska, Rondeau-Mouro, Garnier, Thibault, & Ralet, 2006). The pretreated dry powder (20.0 g) was extracted with deionized water (water–mushroom (ml/g) ranging from 1:1 to 6:1) at pH 6.5–7.5 (adjusting the suspension pH by 0.1 mol/l NaOH or HCl), while the temperature of the water bath ranged from 20 to 90 °C and was kept steady (within ±1.0 °C). The water–mushroom slurry in a 2.0 l stainless steel boiler in the water bath was stirred with an electric mixing paddle for a given time (extraction time ranging from 2 to 10 h) during the entire extraction process. The mixture was centrifuged (2000*g*, 20 min), then the supernatant was separated from insoluble residue with nylon cloth (Pore diameter: 38 μm). The extracts were then defatted by the method of sevag (Sevag, Lackman, & Smolens, 1938), precipitated by the addition of ethanol to a final concentration of 75% (v/v), and the precipitates were collected by centrifugation (2000*g*, 20 min), then solubilized in deionized water and lyophilized to get the crude polysaccharides.

2.2.1. Preparation of standard curve

Preparation of standard curve: 0.1, 0.2, 0.4, 0.8 and 1.0 ml glucose standard solution were put into separate tubes, and diluted with water till 1.0 ml for a final concentration of 20, 40, 60, 80, 100 and 120 ppm. Then 1.0 ml 6% phenol and 5 ml dense H_2SO_4 were added into each tube. After fully mixing for 20 min, OD value of each tube was examined in 620 nm with 1 ml distilled water as contrast.

Table 1
OD value of glucose solution of various concentration

Concentration (ppm)	20	40	60	80	100	120
Average OD value	0.057	0.104	0.147	0.196	0.245	0.292

Table 2
OD value and corresponding content of polysaccharides solution of BaChu mushroom and sample extract

	Polysaccharides sample	Sample extract
Average OD value	0.132	0.115
Corresponding content (ppm)	52.71	45.63

The regression equation between microgram (pg) value of glucose and OD value were obtained as $y = 0.0024X + 0.0055$, ($r = 0.9994$) (Tables 1 and 2).

2.2.2. Conversion factor measurement

Fifty milligrams polysaccharide of BaChu mushroom was weighed and dissolved in a capacity bottle with a small amount of water first and then to the final scale of 100 ml. Exactly 100 μl of the stored solution was measured with the OD value. According to the standard curve, the concentration of glucose from the regression equation was obtained, and the conversion factor could be calculated with the formula of $f = m/c$, where m in μg is weight of polysaccharide, c in (μg ml^{-1}) is concentration of glucose in the polysaccharide solution, D is a dilution factor of the polysaccharide. The content (c%) of polysaccharide could be calculated with the formula of $c\% = f \times \mathrm{cm}/w \times 100\%$, where w is weight of mushroom sample, cm is corresponding concentration of polysaccharide sample solution, f is a conversion factor of the polysaccharide. The measurement result was $f = 0.9486$ (Table 3).

2.2.3. Extraction and measurement of polysaccharides of mushroom sample

About 0.5 g dry BaChu mushroom powder were weighed and then put into 50 ml distilled water and extracted for 8 h in a 30 °C warm water box according to Section 2.2. 0.5 ml of this extraction was taken out and mixed with 0.5 ml 6% phenol and 2.5 ml dense H_2SO_4. OD value of the mixture was examined at the 620 nm with 722 spectrophotometer at room temperature. Then content of sample extract was calculated by regress equation (Table 3).

2.3. Design of statistical experiments

After determining the primential range of the extraction variables through single-factor test, experiments were

Table 3
Content and conversion factor of polysaccharides of BaChu mushroom

	Conversion factor	Extraction yield of polysaccharides (%)
Calculation value	0.9486	8.7

Table 4
Coded and uncoded values of the experimental variables

	−α (−2)	−1	0	1	α (2)
Extraction temperature (°C)	80	85	90	95	100
Extraction time (h)	6	7	8	9	10
Particle size (mesh)	20	25	30	35	40
Ratio of water to mushroom	2	3	4	5	6

designed to find the interaction of four variables, i.e., extraction temperature, extraction time, particle size and ratio of water to mushroom. Table 4 represents the coded and non-coded values of the experimental variables. Design of experiment along with the extraction yield is given in Table 5. The experiments were carried out in duplicate that was necessary to estimate the variability of measurements. The yields are reported as mean of the duplicates. The relationship of the independent variables and the response was calculated by the second-order polynomial equation. SAS (Version 8.0, USA) software package was used to estimate the response of dependent variables and optimized conditions (Tables 6 and 7).

The variables were coded according to the equation

$$\mathrm{xi} = (X_i - X_0)/\Delta X \quad (1)$$

where xi is the (dimensionless) coded value of the variable X_i, X_0 is the value of X_i at the centre point, and ΔX is the step change. Table 5 shows the actual design of experiments. The behavior of the system was explained by the following second degree polynomial equation:

$$Y = \sum A_0 + \sum_{i=1}^{4} A_i X_i + \sum_{i=1}^{4} A_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} A_{ij} X_i X_j \quad (2)$$

3. Results and discussion

3.1. Effect of different temperature on extraction yield of polysaccharides

The increase of the polysaccharides diffusion coefficient and the enhanced solubility of the polysaccharides in the extracting solvent at higher temperatures caused the increase of the polysaccharides mass going out from the mushroom particles into the solution (Li, Cui, & Kakuda, 2006). The extraction coefficient increased with increasing the extraction temperature due to the increase of the polysaccharides solubility (Braga, Moreschi, & Meireles, 2006). To study effect of different temperature on extraction yield of polysaccharides, extraction process was carried out using the different extraction temperature of 60, 65, 70, 75, 80, 85, 90, 95 and 100 °C when other extraction condition was as following: extraction time 8 h, particle size 30 mesh and ratio of water to mushroom 4. The extraction yield of polysaccharides had been increasing when extraction temperature increased from 60 to 95 °C. As shown in Fig. 1, the maximum yield (8.7%) of polysaccharides was observed when extraction temperature was 95 °C. This

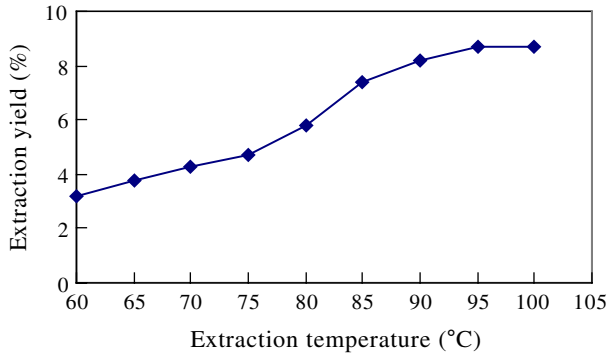

Fig. 1. Effect of different temperature on extraction yield of polysaccharides.

tendence is in agreement with reports of other authors in extracting polysaccharides (Ray, 2006; Vinogradov, Brade, Brade, & Holst, 2003). Although the extraction yield of polysaccharides was also high at 100 °C, increasing temperature will bring about the increase in cost for extraction process from the industrialisation point of view. Therefore, extraction temperature range of 90–100 °C was considered to be optimal in the present experiment.

3.2. Effect of different time on extraction yield of polysaccharides

Extraction time is another factor that would influence the extraction efficiency and selectivity of the fluid. A longer extraction time also presents a positive effect on the yield of polysaccharides. It was reported that a long extraction time favors the production of polysaccharides (Liu, Wei, Guo, & Kennedy, 2006; Ros et al., 2004). The effect of different time on extraction yield of polysaccharides is shown in Fig. 2. Extraction was carried out at different time conditions while other extraction parameters were same to ones described in Section 3.1. When extraction time varied from 2 to 7 h, the variance of extraction yield was relatively rapid, and polysaccharides production reached a maximum at 7–8 h, and then no longer changed as the extraction proceeded. This indicated that extraction time of 7 h was sufficient to obtain the polysaccharides pro-

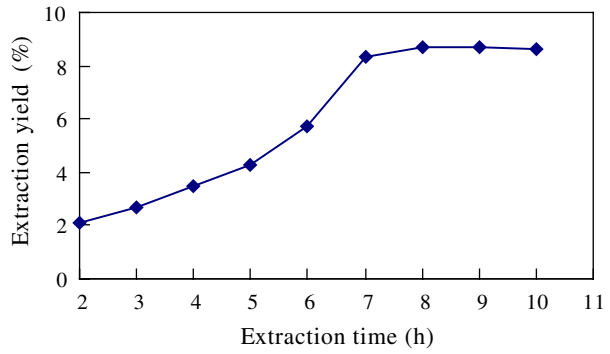

Fig. 2. Effect of different time on extraction yield of polysaccharides.

duction. Thus, extraction of 7–8 h was favorable for producing the polysaccharides.

3.3. Effect of different particle sizes on extraction yield of polysaccharides

Particle sizes were set at 10, 15, 20, 25, 30, 35 and 40 mesh to examine effect of different particle sizes on extraction yield of polysaccharides when other extraction parameters were same to ones described in Section 3.1. As seen from Fig. 3, we can find that the extraction yields of the polysaccharides significantly increased as particle sizes of the mushroom sample increased from 10 to 30 meshs. The maximum yield (8.9%) was also was achieved when particle size was 30 mesh. Small size of the raw material would lead to high yield of the polysaccharides. The yields decreased when the particle size increased from 30 to 40 mesh. This may be assumed that particle size range of 25–35 is proper in this experiment. The reason for this was that a smaller amount of the polysaccharides could be transported from the interior of smaller mushroom particles to the bulk of liquid extract than from the larger ones, due to a smaller resistance and short path to the polysaccharides diffusion (Biais, Le Bail, Robert, Pontoire, & Buléon, 2006; Sun & Tomkinso, 2002). However, when the particle size continually reduced, mass transfer resistance started to become large. The greater mass transfer resistance between solid-phase and liquid-phase may become a large bottle-neck to raise extraction yield of the polysaccharides (Di, Chan, Leung, & Huie, 2003; Shatalov & Pereira, 2007).

3.4. Effect of different ratio of water to mushroom on extraction yield of polysaccharides

The effect of different ratio of water to mushroom on extraction yield of polysaccharides is shown in Fig. 4. Extraction was carried out at different ratio of water to mushroom (1–6) conditions while other extraction parameters were same to ones described in Section 3.1. The extraction yields of the polysaccharides significantly increased from 1.5 to 8.8 as the ratio of water to mushroom

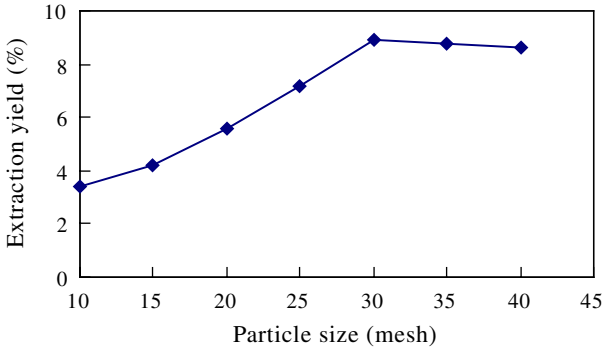

Fig. 3. Effect of different particle sizes on extraction yield of polysaccharides.

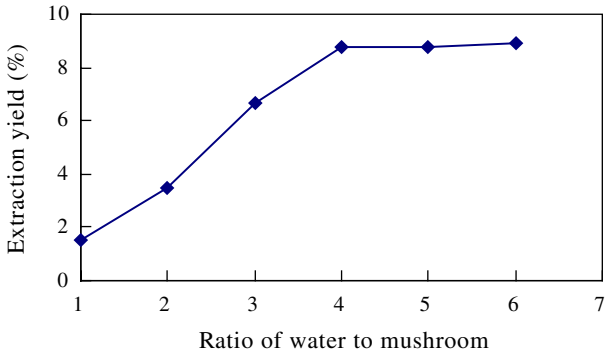

Fig. 4. Effect of different ratio of water to mushroom on extraction yield of polysaccharides.

increased from 1 to 4 shown in Fig. 4, due to the increase of the driving force for the mass transfer of the polysaccharides (Bendahou, Dufresne, Kaddami, & Habibi, 2007). However, when the ratio continued to increase, the extraction yields no longer changed.

3.5. Optimization of extraction conditions of polysaccharides

3.5.1. Predictive model of response

The value of responses (extraction yield of polysaccharides) at different experimental combination for coded variables is given in Table 5. The percentage yield ranged from 1.4% to 9.3%. The maximum value was found at the extraction temperature 95 °C, extraction time 9 h, particle size 35 mesh and ratio of water to mushroom 5. The application of RSM offers, based on parameter estimates, an empirical relationship between the response variable (extraction yield of polysaccharides) and the test variables under consideration. By applying multiple regression analysis on the experimental data, the response variable and the test variables are related by the following second-order polynomial equation:

$$\begin{aligned} Y = {} & 7.7 + 0.575 * X1 + 1.583333 * X2 + 0.566667 \\ & * X3 + 1.183333 * X4 - 0.383333 * X1 * X1 \\ & - 0.05 * X1 * X2 - 0.05 * X1 * X3 + 0.0625 * X1 \\ & * X4 - 0.545833 * X2 * X2 + 0.05 * X2 * X3 \\ & - 0.4375 * X2 * X4 - 0.370833 * X3 * X3 \\ & + 0.0875 * X3 * X4 - 0.483333 * X4 * X4 \end{aligned} \quad (3)$$

A summary of the analysis of fit Statistics of extraction yield (Y) for the selected quadratic predictive model is shown in Table 6. The correlation measure for testing the goodness of fit of the regression equation is the adjusted determination coefficient (R^2_{Adj}). The value of R^2_{Adj} (0.9554) for Eq. (3) is reasonably close to 1, and indicates a high degree of correlation between the observed and predicted values. A very low value of coefficient of the variation (C.V.) (9.77%) clearly indicated a very high degree of precision and a good deal of reliability of the experimental

Table 5
Response surface central composite design and experimental polysaccharides yield

Run	$X1$/Extraction temperature (°C)	$X2$/Extraction time (h)	$X3$/Particle size (mush)	$X4$/Ratio of water to mushroom	Polysaccharides experimental yield (%l)
1	−1 (85)	−1 (7)	−1 (25)	−1 (3)	1.4
2	−1 (85)	−1 (7)	−1 (25)	1 (5)	3.9
3	−1 (85)	−1 (7)	1 (35)	−1 (3)	1.9
4	−1 (85)	−1 (7)	1 (35)	1 (5)	5.9
5	−1 (85)	1 (9)	−1 (25)	−1 (3)	5.3
6	−1 (85)	1 (9)	−1 (25)	1 (5)	6.4
7	−1 (85)	1 (9)	1 (35)	−1 (3)	6.5
8	−1 (85)	1 (9)	1 (35)	1 (5)	8.6
9	1 (95)	−1 (7)	−1 (25)	−1 (3)	2.1
10	1 (95)	−1 (7)	−1 (25)	1 (5)	6.0
11	1 (95)	−1 (7)	1 (35)	−1 (3)	3.7
12	1 (95)	−1 (7)	1 (35)	1 (5)	7.0
13	1 (95)	1 (9)	−1 (25)	−1 (3)	6.3
14	1 (95)	1 (9)	−1 (25)	1 (5)	8.3
15	1 (95)	1 (9)	1 (35)	−1 (3)	7.8
16	1 (95)	1 (9)	1 (35)	1 (5)	9.3
17	−2 (80)	0 (8)	0 (30)	0 (4)	5.9
18	2 (100)	0 (8)	0 (30)	0 (4)	7.5
19	0 (90)	−2 (6)	0 (30)	0 (4)	3.2
20	0 (90)	2 (10)	0 (30)	0 (4)	8.9
21	0 (90)	0 (8)	−2 (20)	0 (4)	6.1
22	0 (90)	0 (8)	2 (40)	0 (4)	7.4
23	0 (90)	0 (8)	0 (30)	−2 (2)	4.3
24	0 (90)	0 (8)	0 (30)	2 (6)	8.3
25	0 (90)	0 (8)	0 (30)	0 (4)	7.7
26	0 (90)	0 (8)	0 (30)	0 (4)	7.7
27	0 (90)	0 (8)	0 (30)	0 (4)	7.7
28	0 (90)	0 (8)	0 (30)	0 (4)	7.7
29	0 (90)	0 (8)	0 (30)	0 (4)	7.7
30	0 (90)	0 (8)	0 (30)	0 (4)	7.7
31	0 (90)	0 (8)	0 (30)	0 (4)	7.7

Table 6
Fit statistics of Y

	Master model	Predictive model
RMSE	0.617497	0.617497
R-square	95.54%	95.54%
Adjusted R-square	91.64%	91.64%
Coefficient of variation	9.771514	9.771514

values. Statistical testing of the model was performed in the form of analysis of ANOVA, which is required to test the significance and adequacy of the model. F-values for the lack of fit were nonsignificant ($P > 0.05$) thereby confirming the validity of the models. The model is found to be adequate for prediction within the range of experimental variables. The coefficient values of Eq. (3) were calculated and tested for their significance using SAS v8.0 and are listed in Table 7. The P values are 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 is the value of P, the more significant is the corresponding coefficient. It can be seen from this table that the linear coefficients ($X1, X2, X3, X4$), a quadratic term coefficient (X^2, X^3, X^4) and cross product coefficients ($X2 * X4$) were significant, with very small P values

Table 7
Test of significance for regression coefficients

Effect	Estimate	Stand error	t ratio	P value
$X1$	0.575	0.12605	4.5618	0.0003
$X2$	1.5833	0.12605	12.562	<0.0001
$X3$	0.56667	0.12605	4.4957	0.0004
$X4$	1.1833	0.12605	9.3881	<0.0001
$X1 * X1$	−0.38333	0.11547	−3.3197	0.0043
$X1 * X2$	−0.05	0.15437	−0.32389	0.7502
$X1 * X3$	−0.05	0.15437	−0.32389	0.7502
$X1 * X4$	0.0625	0.15437	0.40486	0.6909
$X2 * X2$	−0.54583	0.11547	−4.7269	0.0002
$X2 * X3$	0.05	0.15437	0.32389	0.7502
$X2 * X4$	−0.4375	0.15437	−2.834	0.0120
$X3 * X3$	−0.37083	0.11547	−3.2114	0.0054
$X3 * X4$	0.0875	0.15437	0.5668	0.5787
$X4 * X4$	−0.48333	0.11547	−4.1856	0.0007

($P < 0.01$). The other term coefficients ($X1 * X2, X1 * X3, X1 * X4, X2 * X3, X3 * X4$) are not significant ($P > 0.05$).

3.5.2. Response surface plot and contour plot showing effects of extraction variables on yield of polysaccharides

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

obtained using SAS version 8.0 and are presented in Figs. 5 and 6. The 3-D response surface plot in Fig. 5a and the contour plot in Fig. 6a, which gives the extraction yield of polysaccharides as a function of extraction temperature and time at fixed particle size (0 level) and ratio of water to mushroom (0 level), shows that extraction yield of polysaccharides increase with increase in extraction time, and extraction yield of polysaccharides is found to increase rapidly with increase of extraction temperature from 90 to 94 °C, but beyond 94 °C, extraction yield of polysaccharides decreases with increasing extraction temperature. Figs. 5b and 6b show the 3-D response surface plot and the contour plot at varying extraction temperature and particle size at fixed extraction time (0 level) and ratio of water to mushroom (0 level). From Figs. 5b and 6b, it can be seen that maximum extraction yield of polysaccharides can be achieved when extraction temperature and particle size are 94 °C and 33, respectively. Figs. 5c and 6c show the 3-D response surface plot and the contour plot at varying extraction temperature and ratio of water to mushroom at fixed extraction time (0 level) and particle size (0 level). From Figs. 5c and 6c, it can be seen that extraction yield of polysaccharides increase with increase in ratio of water to mushroom, and extraction yield of polysaccharides is found to increase rapidly with increase of extraction temperature from 90 to 94 °C, but beyond 94 °C, extraction yield of polysaccharides decreases with increasing extraction temperature. Figs. 5d and 6d show the 3-D response surface plot and the contour plot at varying extraction time and particle size at fixed extraction temperature (0 level) and ratio of water to mushroom (0 level). From Figs. 5d and 6d, it can be seen that extraction yield of polysaccharides increase with increase in extraction time, and extraction yield of polysaccharides is found to increase rapidly with increase of particle size from 30 to 33, but beyond 33, extraction yield of polysaccharides decreases with increasing particle size. Figs. 5e and 6e show the 3-D response surface plot and the contour plot at varying extraction time and ratio of water to mushroom at fixed extraction temperature (0 level) and particle size (0 level). From Figs. 5e and 6e, it can be seen that extraction yield of polysaccharides increase evidently with increasing extraction time and ratio of water to mushroom. The results suggest that extraction yield of polysaccharides was directly proportional to extraction time and ratio of water to mushroom within given range of variables employed in the matrix. Figs. 5f and 6f show the 3-D response surface plot and the contour plot at varying particle size and ratio of water to mushroom at fixed extraction time (0 level) and extraction temperature (0 level). From Figs. 5f and 6f, it can be seen that extraction yield of polysaccharides increase with increase in ratio of water to mushroom, and extraction yield of polysaccharides is found to increase rapidly with increase of particle size from

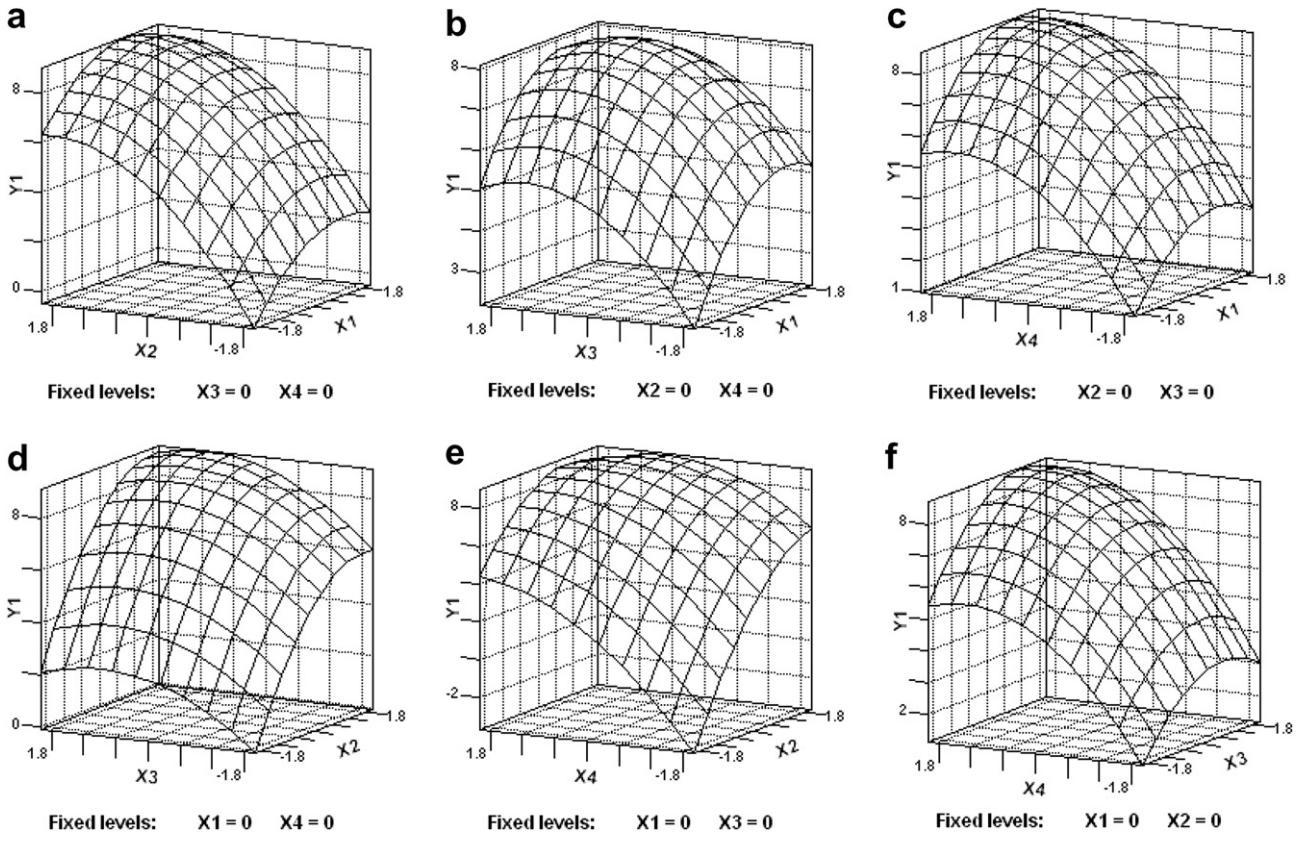

Fig. 5. Response surface (3D) showing the effect of the extraction temperature, extraction time, particle size and ratio of water to mushroom on the response Y1.

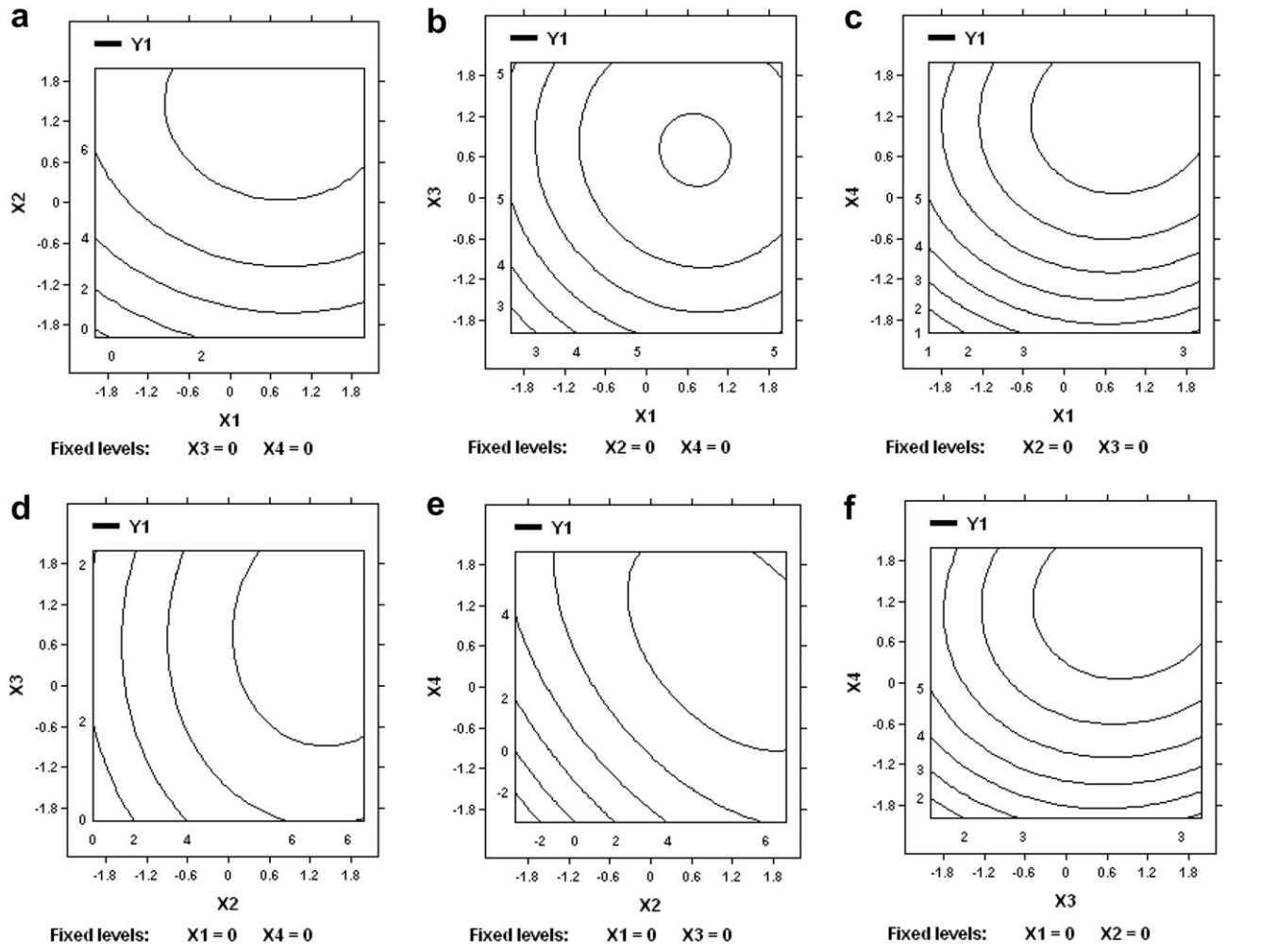

Fig. 6. Contour plots showing the effect of the extraction temperature, extraction time, particle size and ratio of water to mushroom on the response Y1.

30 to 33, but beyond 33, extraction yield of polysaccharides decreases with increasing extraction temperature. From Figs 5 and 6, it can be concluded that optimal extraction condition of polysaccharides from BaChu mushroom are extraction temperature 94 °C, extraction time 10 h, particle size 33 and ratio of water to mushroom 6. Among the four extraction parameters studied, extraction time is the most significant factor to affect yield of polysaccharides, followed by ratio of water to mushroom according to gradient of slope in the 3-D response surface plot (Fig. 5).

3.5.3. Verification of predictive model

To ensure the predicted result was not biased toward the practical value, experimental rechecking was performed using this deduced optimal condition. A mean value of 8.73 ± 0.72 ($N = 3$), obtained from real experiments, demonstrated the validation of the RSM model. The good correlation between these results confirmed that the response model was adequate for reflecting the expected optimization (Table 8).

4. Conclusion

The performance of the extraction of polysaccharides from wild edible BaChu mushroom was studied with a statistical method based on the response surface methodology in order to identify and quantify the variables which may maximize the yield of polysaccharides. The four variables chosen, namely extraction temperature, extraction time, particle size and ratio of water to mushroom all have a

Table 8
Optimum conditions, predicted and experimental value of response at that condition

Optimum condition				Extraction yield of polysaccharides	
Extraction temperature	Extraction time	Particle size	Ratio of water to mushroom	Experimental[a]	Predicted
94 °C	10 h	33	6	8.73 ± 0.72	8.79

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

positive influence on the yield of oil using the extraction method. The optimal conditions obtained by RSM for production of polysaccharides include the following parameters: extraction temperature 94 °C, extraction time 10 h, particle size 33 and ratio of water to mushroom 6.

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