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# Optimization of enzyme assisted extraction of polysaccharides from *Tricholoma matsutake* by response surface methodology

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ABSTRACT

Polysaccharides production from the fruiting body of *Tricholoma matsutake* was carried out using enzyme assisted extraction methodology. Response surface methodology (RSM), based on a five level, four variable central composite design (CCD), was employed to obtain the best possible combination of extraction temperature ($X_1$: 54–66 °C), extraction pH ($X_2$: 3.6–4.4), extraction time ($X_3$: 2.4–3.6 h), and complex enzyme (the ratio of papain:pectinase:cellulase was 1:1:1) amount ($X_4$: 1.6–2.4%) for maximum polysaccharides production. The experimental data obtained were fitted to a second-order polynomial equation using multiple regression analysis and also were analyzed by analysis of variance (ANOVA). The 3-D response surface plot and the contour plot derived from the mathematical models were applied to determine the optimal conditions. The optimum extraction conditions were: extraction temperature of 61.8 °C, extraction pH of 4.14, extraction time of 3.2 h, and complex enzyme amount of 2.1% (w/v). Under these conditions, the experimental yield of polysaccharides was 7.53 ± 0.26%, which is well in close agreement with the value predicted by the model.



## 1. Introduction

*Tricholoma matsutake* is a kind of fungi belonging to Subgenus *Tricholoma* and it is an ectomycorrhizal symbiotic mushroom. Production of the fruiting body is limited, and cultivating the fruiting bodies from mycelia in artificial culture medium has proven to be very difficult (Kim, Lee, Cho, Kim, & Hong, 2010). Domestication and cultivation of the *T. matsutake* was studied by our group for many years, and, fruiting body of the *T. matsutake* was successfully cultivated, we applied one patent and were granted by state intellectual property office of the P.R.C (notice of authorization number: CN100490630C).

The fruiting body of *T. matsutake* is one of the most important edible fungus, which is not only very delicious and nutrimental, but also rich in polysaccharides. Polysaccharides, made up of many monosaccharides joined together by glycosidic bonds, have multiple immunostimulatory activities (Byeon et al., 2009; Hoshi, Iijima, Ishihara, Yasuhara, & Matsunaga, 2008; Ishihara, Iijima, Yagi, & Matsunaga, 2003; Ishihara, Iijima, Yagi, Hoshi, & Matsunaga, 2004; Young et al., 2008), potent anti-cancer bioactivities (Ding et al., 2010), antioxidation (Kim et al., 2010), anti-mutagenic and hematopoietic activities (Bohn & BeMiller, 1995). It has been used for the prevention and treatment of diseases for hundreds years in Asian countries, such as China, Japan, and Korea (Gong, Su, Chen, Wang, & Cao, 2002; Gurein et al., 2003; Hur, Park, Kang, & Joo, 2004).

The extraction methods of polysaccharides commonly including traditional water extraction (TWE), Soxhlet extraction, and methods assisted by ultrasonic wave and microwave to improve the extraction efficacy (Hou & Chen, 2008; Hou, Zhang, Xiong, Li, & Yang, 2008; Liang, 2008; Wang, Zhou, & Wen, 2006; Zhao, Dong, Chen, & Hu, 2010), however, it is usually associated with longer extraction time and higher temperature but lower extraction efficiency. Enzyme assisted extraction is undoubtedly an emerging technology in the food industry since it offers many advantages such as high extraction yield, lower investment costs and energy requirements, high reproducibility at shorter times, simplified manipulation (Nyam, Tan, Lai, Long, & Che Man, 2009; Rosenthal, Pyle, & Niranjan, 1996) compared to conventional extraction method. Thus, enzyme assisted extraction may be an effective and advisable technique for the extraction of polysaccharides. To the best of our knowledge, there is hardly any report that enzyme assisted extraction has been applied to extract polysaccharides from *T. matsutake*.

Response surface methodology (RSM) is an effective statistical technique for optimizing complex processes because it allows more efficient and easier arrangement and interpretation of experiments compared to other methods (Box & Behnken, 1960; Gan & Latiff, 2011; Gan, Manaf, & Latiff, 2010). In addition, it is less laborious and time-consuming than other approaches that applied to optimize a process. It is widely used in optimizing the extraction process

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variables of bioactive compounds, such as polysaccharides, Silybin (Liu, Du, Yuan, & Zhu, 2009; Ye & Jiang, 2011; Zhong & Wang, 2010).

In our previous paper, polysaccharides from fruiting body of the *T. matsutake* which was cultivated by our study group was extracted by TWE (Yin, You, & Jiang, 2009). On the basis of this work, the present study was to optimize the process for the extraction of polysaccharides from the fruiting body of the *T. matsutake* using response surface methodology, employing a five-level, four-variable central composite rotatable design to study the effects of extraction time, extraction temperature, extraction pH, and the complex enzyme amount on the yield of polysaccharides from the fruiting body of the *T. matsutake*.

## 2. Materials and methods

### 2.1. Materials

The fruiting bodies of the *T. matsutake* were cultivated by our laboratory. Samples were dried at 60 °C for 12 h and grinded by a grinder and were sieved through a 60 mesh sieve. All other reagents were of analytical grade. Papain (6000 U/mg), cellulase (15,000 U/g) were obtained from Sinopharm Chemical Reagent Co., Ltd. Pectinase (20,000 U/g) were acquired from TianJin LiHua Enzyme Co., Ltd.

### 2.2. Complex enzyme extraction and determination of polysaccharides yield

Ten grams of dried sample was defatted in a Soxhlet apparatus with petroleum ether (boiling point: 60–90 °C) and then pretreated with 80% ethanol twice to remove some colored materials, monosaccharides, oligosaccharides, and some small molecule materials. The pretreated samples were separated from the organic solvent by centrifugation (2000 × *g* for 10 min).

Five grams of the pretreated dried powder was immersed in 200 ml citric acid–sodium hydroxide–chlorhydric acid buffer in a 1000 ml beaker and the sample was then extracted with complex enzyme (the ratio of papain:pectinase:cellulase was 1:1:1, the content of each enzyme in the buffer ranging from 0.5 to 2.5% (w/v)) at pH 2.5–6 for different hours (extraction time ranging from 1 to 5 h), while the temperature of the water bath was kept steady at a given temperature (extraction temperature ranging from 30 to 80 °C) during the entire extraction process.

The suspension was centrifuged (5000 × *g*, 10 min) and the insoluble residue was treated again for 2 times as mentioned above. The supernatant was incorporated and concentrated to one-fifth of the initial volume using a rotary evaporator at 50 °C under vacuum. The supernatant was precipitated by the addition of anhydrous ethanol to a final concentration of 80% (v/v) and the precipitates as crude extract were collected by centrifugation (5000 × *g*, 10 min). After being washed three times with anhydrous ethanol, the precipitate was air-dried at 50 °C until its weight was constant. The content of the polysaccharides was measured by phenol–sulfuric method (Masuko et al., 2005).

The polysaccharides yield (%) is calculated as follows:

$$\text{polysaccharides yield (\%)} = \frac{\text{the polysaccharides content of extraction (g)}}{\text{weight of } T.\ matsutake \text{ powder (g)}} \times 100 \quad (1)$$

### 2.3. Experimental design and statistical analysis

The extraction parameters were optimized by RSM. A five level, four variable central composite design (CCD) was applied to determine the best combination of extraction variables for the yields of *T. matsutake* polysaccharides. The range and center point values of four independent variables (Table 1) were based on the results of preliminary experiments. The CCD in the experimental design consists of twenty-four factorial points and five replicates of the central point (Table 2), the experiment was carried out in a standard order. The behavior of the system was explained by the following second degree polynomial equation:

$$y = \beta_{k0} + \sum_{i=1}^{4} \beta_{ki} X_i + \sum_{i=1}^{4} \beta_{kii} X_i^2 + \sum_{i<j=2}^{4} \beta_{kij} X_i X_j \quad (2)$$

$y$ is the response function, $\beta_{k0}$ is an intercept, $\beta_{ki}$, $\beta_{kii}$ and $\beta_{kij}$ are the coefficients of the linear, quadratic and interactive terms, respectively. And accordingly $X_i$ and $X_j$ represent the coded independent variables. The fitted polynomial equation is expressed as surface and contour plots in order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions (Lu, Engelmann, Lila, & Erdman, 2008). According to the analysis of variance, the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The regression coefficients were then used to make statistical calculation to generate dimensional and contour maps from the regression models. Statistica (Version 8.0, USA) software package was used to analyze the experimental data. *p*-Values of less than 0.05 were considered to be statistically significant.

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

| Independent variables | Factor level | | | | |
|---|---|---|---|---|---|
| | −2 | −1 | 0 | 1 | 2 |
| Extraction temperature (°C) | 54 | 57 | 60 | 63 | 66 |
| Extraction pH | 3.6 | 3.8 | 4.0 | 4.2 | 4.4 |
| Extraction time (h) | 2.4 | 2.7 | 3.0 | 3.3 | 3.6 |
| Complex enzyme amount (%) | 1.6 | 1.8 | 2.0 | 2.2 | 2.4 |

## 3. Results and discussion

### 3.1. Effect of different complex enzyme amount on the polysaccharides yield

Complex enzyme amount is an important factor that could influence the extraction efficiency. The polysaccharides yield affected by complex enzyme amount was shown in Fig. 1a. Different complex enzyme amount was set at 0.5%, 1%, 1.5%, 2%, 2.5%, respectively, while other extraction parameters were: extraction temperature 40 °C, extraction pH 5.0, and extraction time 3 h. Fig. 1a indicated that the polysaccharides yield increases with the increasing of the complex enzyme amount and reached the peak value about 6.3% when the complex enzyme amount is 2–2.5%, and no longer changed as the extraction proceeded. This indicated that the complex enzyme amount of 2% was sufficient to obtain good polysaccharides yield. Thus, 2% was considered to be optimal complex enzyme amount in this experiment.

### 3.2. Effect of different enzyme action temperatures on the polysaccharides yield

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 yield increased with the increasing of the extraction temperature due to the increase of the polysaccharides solubility at higher temperature (Braga, Moreschi, & Meireles, 2006). However, different enzymes have their own

**Table 2**
Response surface central composite design and experimental polysaccharides yield.

| Run | $X_1$/extraction temperature (°C) | $X_2$/extraction pH | $X_3$/extraction time (h) | $X_4$/complex enzyme amount (%) | Polysaccharides experimental yield (%) |
|---|---|---|---|---|---|
| 1 | 57.000 | 3.800 | 2.700 | 1.800 | 1.24 |
| 2 | 57.000 | 3.800 | 2.700 | 2.200 | 3.25 |
| 3 | 57.000 | 3.800 | 3.300 | 1.800 | 1.49 |
| 4 | 57.000 | 3.800 | 3.300 | 2.200 | 5.02 |
| 5 | 57.000 | 4.200 | 2.700 | 1.800 | 4.48 |
| 6 | 57.000 | 4.200 | 2.700 | 2.200 | 5.26 |
| 7 | 57.000 | 4.200 | 3.300 | 1.800 | 5.35 |
| 8 | 57.000 | 4.200 | 3.300 | 2.200 | 6.56 |
| 9 | 63.000 | 3.800 | 2.700 | 1.800 | 1.86 |
| 10 | 63.000 | 3.800 | 2.700 | 2.200 | 3.62 |
| 11 | 63.000 | 3.800 | 3.300 | 1.800 | 3.27 |
| 12 | 63.000 | 3.800 | 3.300 | 2.200 | 6.20 |
| 13 | 63.000 | 4.200 | 2.700 | 1.800 | 5.57 |
| 14 | 63.000 | 4.200 | 2.700 | 2.200 | 6.36 |
| 15 | 63.000 | 4.200 | 3.300 | 1.800 | 6.88 |
| 16 | 63.000 | 4.200 | 3.300 | 2.200 | 6.69 |
| 17 | 54.000 | 4.000 | 3.000 | 2.000 | 5.22 |
| 18 | 66.000 | 4.000 | 3.000 | 2.000 | 6.63 |
| 19 | 60.000 | 3.600 | 3.000 | 2.000 | 2.83 |
| 20 | 60.000 | 4.400 | 3.000 | 2.000 | 6.53 |
| 21 | 60.000 | 4.000 | 2.400 | 2.000 | 5.40 |
| 22 | 60.000 | 4.000 | 3.600 | 2.000 | 6.15 |
| 23 | 60.000 | 4.000 | 3.000 | 1.600 | 3.81 |
| 24 | 60.000 | 4.000 | 3.000 | 2.400 | 6.51 |
| 25 | 60.000 | 4.000 | 3.000 | 2.000 | 6.81 |
| 26 | 60.000 | 4.000 | 3.000 | 2.000 | 6.82 |
| 27 | 60.000 | 4.000 | 3.000 | 2.000 | 6.82 |
| 28 | 60.000 | 4.000 | 3.000 | 2.000 | 6.81 |
| 29 | 60.000 | 4.000 | 3.000 | 2.000 | 6.81 |

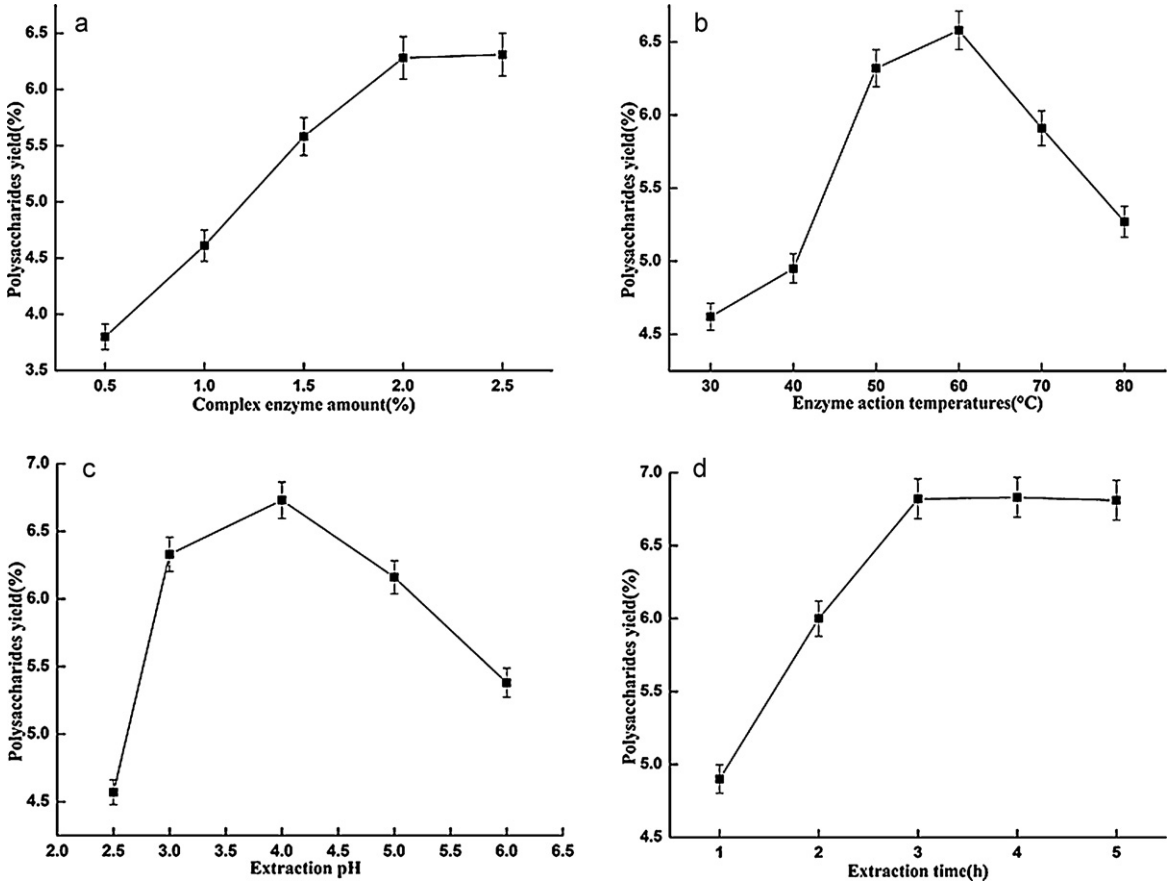


**Fig. 1.** Effects of different (a) complex enzyme amount, (b) enzyme action temperatures, (c) pH, and (d) extraction time on extraction yield of polysaccharides.

appropriate optimal effect temperatures. In order to study the effect of different temperature on the yield of polysaccharides, extraction process was carried out by using different extraction temperatures of 30, 40, 50, 60, 70 and 80 °C while the other extraction conditions were: complex enzyme amount 2%, extraction pH 5.0, and extraction time 3 h. The extraction yield of polysaccharides increased when extraction temperature increased from 30 to 60 °C. As shown in Fig. 1b, the maximum yield (6.58%) of polysaccharides was observed when extraction temperature was 60 °C. When extraction temperature varied from 60 to 80 °C, the polysaccharides yield was rapidly decreased, thus extraction temperature of 60 °C was considered to be optimal in the present experiment. The possible reason for this result may be the complex effect of the following two aspect: firstly, higher temperature can enhance the mass transfer and accelerate the extraction speed; secondly, the complex enzymes have a suitable effect temperatures, when the temperature was under the suitable effect temperatures, the enzyme activity increased with the increase of temperature, when the temperature was higher than the suitable effect temperatures, the enzyme activity decreased with the increase of temperature. This tendency is in agreement with reports of other authors' in extracting polysaccharides (Zou & Guo, 2010).

### 3.3. Effect of different pH on extraction yield of polysaccharides

pH value can affect enzyme activity, different enzymes have their own optimal pH. This might due to the changing of pH affects the spatial structure of enzyme, thus altered the enzyme conformation, enzymatic activity. Extraction operation was carried out at different pH conditions while other extraction variables were set as follow: complex enzyme amount 2%, and extraction time 3 h, extraction temperature 60 °C. The effect of different pH on the extraction yield of polysaccharides is shown in Fig. 1c. The extraction yield of polysaccharides continued to increase with the increase of pH value (2.5–4) and reached the peak value (6.73%) at pH value 4. However, the extraction yield of polysaccharides no longer increased when the pH value exceeded 4. The possible reason for this phenomenon may be that the appropriate pH of the complex enzyme was in the range of 2.5–4, and when the pH value exceeded 4 the activity of the complex enzyme was decreased (Li, Zhang, Xin, Yu, & Liu, 2010; Liu, Liu, Dai, & Hu, 2010; Xiao, 2005).

### 3.4. Effect of different extraction time on extraction yield of polysaccharides

A longer extraction time also presents a positive effect on the yield of polysaccharides. It has been reported that a longer extraction time favors the production of polysaccharides (Liu, Wei, Guo, & Kennedy, 2006; Ros et al., 2004). The extraction yield of polysaccharides affected by different extraction time is shown in Fig. 1d, when the other three factors (complex enzyme amount, and extraction pH, extraction temperature) were fixed at 2%, 4.0 and 60 °C, respectively. It showed that the extraction yield increased as the extraction time ascended from 1 to 3 h, the maximum yield of polysaccharides (6.82%) was observed when the extraction time was 3 h, after this point, the extraction yield of polysaccharides started to maintain a dynamic equilibrium with the increasing of the extraction time, and no longer increased when the extraction time exceeded 3 h (Fig. 1d). This phenomenon maybe due to partial of the polysaccharide was hydrolyzed under some temperature and long extraction time (Liu, Miao, Wen, & Sun, 2009). Therefore, extraction time of 3 h was adopted in the present work.

### 3.5. Statistical analysis and the model fitting

Response surface optimization is more advantageous than the traditional single parameter optimization in that it saves time, space and raw material. There were a total of 29 runs for optimizing the four individual parameters in the CCD, the experimental conditions and the yield of polysaccharides according to the factorial design was shown in Table 2. Results also showed that the yield of polysaccharides ranged from 1.24 to 6.88%. The maximum extract value (7.65%) was found in conditions of $X_1 = 61.84$ °C, $X_2 = 4.14$, $X_3 = 3.2$ h and $X_4 = 2.08$%. The results were fitted with a second order polynomial equation. The values of regression coefficients were calculated, the response variable and the test variables are related by the following second-order polynomial equation:

$$\begin{aligned} Y = {} & 6.814 + 0.885X_1 + 2.38333X_2 + 0.94333X_3 + 1.518333X_4 \\ & - 0.7165833X_1^2 - 1.339083X_2^2 - 0.7915833X_3^2 - 1.0990833X_4^2 \\ & - 0.0125X_1X_2 + 0.18X_1X_3 - .28X_1X_4 - 0.27500X_2X_3 \\ & - 0.95500X_2X_4 + 0.26750X_3X_4 \end{aligned} \quad (3)$$

The statistical significance of the regression model was checked by *F*-test and *p*-value, and the analysis of variance (ANOVA) for the response surface quadratic model was shown in Table 3. The determination coefficient ($R^2 = 0.92139$), showed by ANOVA of the quadratic regression model, indicating that the model was adequate for prediction within the range of experimental variables. The *p*-values were used as a tool to check the significance of each coefficient, and the smaller the *p*-value was, the more significant the corresponding coefficient was (Guo, Zou, & Sun, 2010).

In this table the linear coefficients ($X_1$, $X_2$, $X_3$, $X_4$), a quadratic term coefficient ($X_1^2, X_2^2, X_3^2, X_4^2$) and the interaction coefficient ($X_2X_4$) were found significant ($p < 0.02$). The other term coefficients ($X_1 \times X_2$, $X_1 \times X_3$, $X_1 \times X_4$, $X_2 \times X_3$, $X_3 \times X_4$) were not significant ($p > 0.05$). The full model filled Eq. (3) was made three dimensional and contour plots to predict the relationships between the independent variables and the dependent variables.

### 3.6. Optimization of extraction conditions of polysaccharides

Response surfaces were plotted by using Statistica (version 8.0) software to study the effects of parameters and their interactions on polysaccharides yield. The results of extraction yield of polysaccharides affected by extraction temperature, pH, time and complex enzyme amount are presented in Figs. 2 and 3. These types of plots show effects of two factors on the response at a time and the other factor was kept at level zero.

**Table 3**
Estimated regression model of relationship between response variables ($Y$) and independent variables ($X_1, X_2, X_3, X_4$).

| Factor | SS | df | MS | *F* | *p* |
|---|---|---|---|---|---|
| $X_1$ | 4.69935 | 1 | 4.69935 | 9.55311 | 0.007977 |
| $X_1^2$ | 3.33076 | 1 | 3.33076 | 6.77096 | 0.020892 |
| $X_2$ | 34.08167 | 1 | 34.08167 | 69.28321 | 0.000001 |
| $X_2^2$ | 11.63121 | 1 | 11.63121 | 23.64460 | 0.000251 |
| $X_3$ | 5.33927 | 1 | 5.33927 | 10.85397 | 0.005320 |
| $X_3^2$ | 4.06446 | 1 | 4.06446 | 8.26247 | 0.012245 |
| $X_4$ | 13.83202 | 1 | 13.83202 | 28.11854 | 0.000112 |
| $X_4^2$ | 7.83557 | 1 | 7.83557 | 15.92861 | 0.001339 |
| $X_1 \times X_2$ | 0.00063 | 1 | 0.00063 | 0.00127 | 0.972069 |
| $X_1 \times X_3$ | 0.12960 | 1 | 0.12960 | 0.26346 | 0.615758 |
| $X_1 \times X_4$ | 0.31360 | 1 | 0.31360 | 0.63750 | 0.437951 |
| $X_2 \times X_3$ | 0.30250 | 1 | 0.30250 | 0.61494 | 0.446000 |
| $X_2 \times X_4$ | 3.64810 | 1 | 3.64810 | 7.41607 | 0.016488 |
| $X_3 \times X_4$ | 0.28623 | 1 | 0.28623 | 0.58185 | 0.458247 |

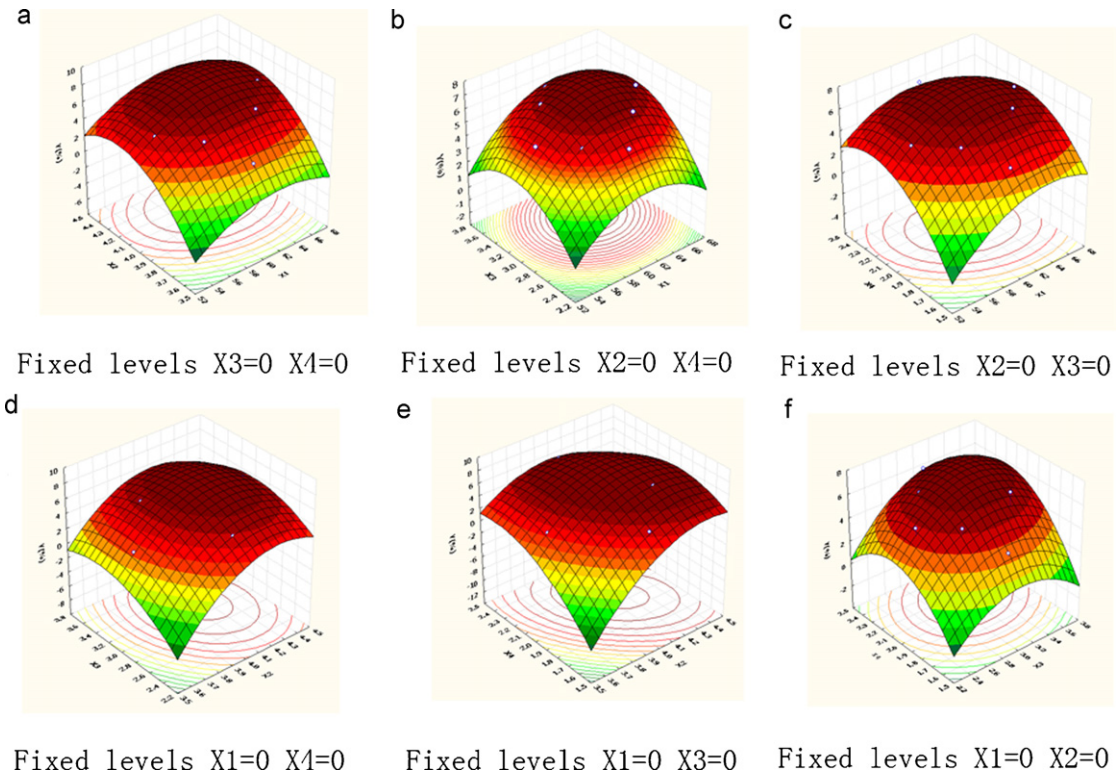


**Fig. 2.** Response surface (3-D) showing the effect of the complex enzyme amount, enzyme action temperatures, pH, and extraction time on the response *Y*.

The maximum value predicted by the surface was confined in the smallest ellipse in the contour diagram. Elliptical contours were obtained when there was a perfect interaction between the independent variables.

The 3-D response surface plot and the contour plot in Figs. 2 and 3a, which give the extraction yield of polysaccharides as a function of extraction temperature and pH at fixed extraction time (3 h) and complex enzyme amount (2%), indicated that the extraction yield of polysaccharides increased with the increasing of the extraction temperature from 52 to 62 °C, but beyond 62 °C, the extraction yield of polysaccharides decreased gradually with the increase of the extraction temperature, and the extraction yield of polysaccharides was found to increase rapidly with the increase of extraction pH from 3.5 to 4.18, then decreased rapidly from 4.18 to 4.5.

Figs. 2b and 3b show the 3-D response surface plot and the contour plot at varying extraction time and a number of extractions at fixed extraction pH 4 and complex enzyme amount 2%. And

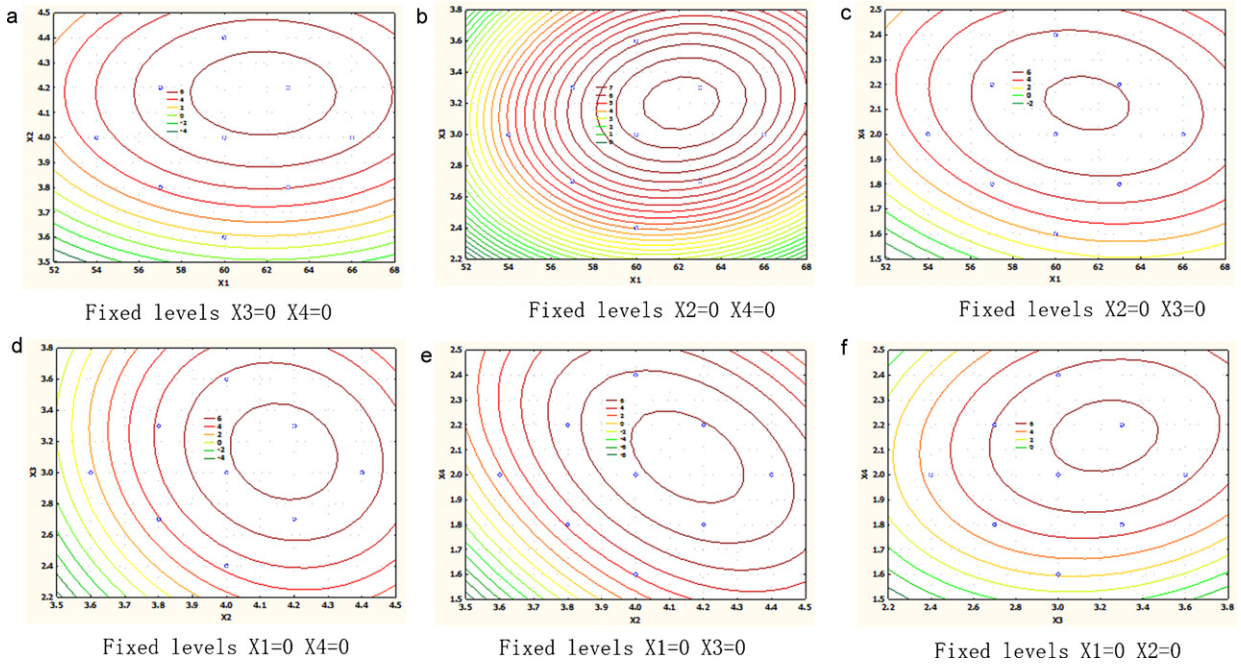


**Fig. 3.** Contour plots showing the effect of the complex enzyme amount, enzyme action temperatures, pH, and extraction time on the response *Y*.

**Table 4**
Predicted and experimental values of the responses at optimum conditions.

| | Extraction temperature (°C) | Extraction pH | Extraction time (h) | Complex enzyme amount (%) | Yield of polysaccharides (%) |
|---|---|---|---|---|---|
| Optimum conditions | 61.84 | 4.14 | 3.19 | 2.08 | 7.65 (predicted) |
| Modified conditions | 61.8 | 4.14 | 3.2 | 2.1 | 7.53 ± 0.26[a] (actual) |

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

the extraction yield of polysaccharides increased rapidly within the extraction time from 2.2 to 3.14 h, but when beyond 3.14 h, the extraction yield of polysaccharides reached the plateau region where the yield was maximized and did not increase any more, and the yield increased rapidly with the increase of the extraction temperature from 52 to 62 °C, then dropped slightly from 62 to 68 °C.

Figs. 2c and 3c showed the 3-D response surface plot and the contour plot at varying extraction temperature and complex enzyme amount at fixed extraction pH 4 and extraction time 3 h. It can be seen that maximum extraction yield of polysaccharides could be achieved when the extraction temperature and complex enzyme amount were 62 °C and 2.02%, respectively.

In Figs. 2d and 3d, when the 3-D response surface plot and the contour plot were developed for the extraction yield of polysaccharides with varying extraction pH and extraction time at fixed extraction temperature 60 °C and complex enzyme amount 2%. It indicated that the maximum extraction yield of polysaccharides can be achieved when extraction pH and extraction time were at the threshold level of 4.02 and 3.2 h, respectively.

In Figs. 2e and 3e, when the 3-D response surface plot and the contour plot were developed for the extraction yield of polysaccharides with varying extraction pH and complex enzyme amount at fixed extraction temperature 60 °C and extraction time 3 h. It indicated that the maximum extraction yield of polysaccharides can be achieved when extraction pH and complex enzyme usage at the threshold level of 4.2 and 1.88%, respectively.

The 3-D response surface plot and the contour plot based on independent variables extraction time and complex enzyme amount are shown in Figs. 2f and 3f, while the other two independent variables, extraction temperature and extraction pH were kept at 60 °C and 4, respectively. It can be seen that the yield of polysaccharides increased with the increase of complex enzyme amount from 1.5 to 2.13%, then dropped slightly from 2.13 to 2.5%, and the yield of polysaccharides increased rapidly with the increase of the extraction time from 3.0 h to 3.2 h, but when beyond 3.2 h, the yield of polysaccharides did not further increase.

From Figs. 2 and 3, it can be concluded that the optimal extraction conditions for polysaccharides from *T. matsutake* are extraction temperature of 61.8 °C, pH of 4.14, extraction time of 3.2 h, and complex enzyme amount 2.08%. Among the four extraction parameters that have been studied, extraction pH was the most significant factor that affects the yield of polysaccharides, followed by the complex enzyme amount, extraction time and extraction temperature according to the regression coefficients significance of the quadratic polynomial model (Table 3) and gradient of slope in the 3-D response surface plot (Fig. 2).

### 3.7. Verification of predictive model

The suitability of the model equation for predicting the optimum response values was tested by using the selected optimal conditions. The maximum predicted yield and experimental yield of *T. matsutake* polysaccharides were given in Table 4. To ensure the predicted result was not biased toward the practical value, experiment rechecking was performed by using these modified optimal conditions: extraction temperature of 61.8 °C, extraction pH of 4.14, extraction time of 3.2 h and complex enzyme amount of 2.1% (w/v). These set of conditions were determined to be optimum by the RSM optimization approach and were also validated experimentally and predict the values of the responses using the model equation. A mean value of 7.53 ± 0.26% ($N$ = 3), obtained from real experiments, demonstrated the validation of the RSM model, indicating that the model was adequate for the extraction process (Table 4).

## 4. Conclusion

Enzyme assisted extraction of polysaccharides of technology was performed for the polysaccharides extraction from *T. matsutake* in order to increase the polysaccharides extraction yield. Based on the single-factor experiments, RSM was used to estimate and optimize the experimental variables: extraction temperature (°C), extraction pH, extraction time (h), and complex enzyme amount (%). All the independent variables, quadratic of all the independent variables had highly significant effects on the response values ($p$ < 0.021). The optimal extraction conditions for the polysaccharides were as follows: extraction temperature of 61.8 °C, extraction pH 4.14, extraction time 3.2 h, and complex enzyme amount 2.1%. Under these conditions, the experimental yield of polysaccharides was 7.53 ± 0.26%, which was close with the predicted yield value (Table 4).

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