Asian Journal of Chemistry; *Vol. 24, No. 5 (2012), 2290-2294*

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Optimization of the Extraction of the *Magnolia officinalis* Polysaccharides Using Response Surface Methodology

M.B. LAN[1,2], J. GUO[1], H.L. ZHAO[1] and H.H. YUAN[1,*]

[1]Shanghai Key Laboratory of Functional Materials Chemistry, Research Center of Analysis and Test, East China University of Science and Technology, Shanghai 200237, P.R. China
[2]Key Laboratory for Ultrafine Materials of Ministry of Education, East China University of Science and Technology, Shanghai 200237, P.R. China

*Corresponding author: Fax: +86 21 64250624; Tel: +86 21 64252055; E-mail: yuanhuihui@ecust.edu.cn

(*Received*: 7 June 2011; *Accepted*: 21 December 2011) AJC-10883

Magnolia officinalis is an important Chinese traditional herb in Chinese medicinal soups that have been used for centuries to treat thrombotic stroke, gastrointestinal complaints, anxiety and nervous disturbance. The *Magnolia officinalis* polysaccharides are the main active ingredients in the decoction. The objective of this study is to investigate the yield of polysaccharides from *Magnolia officinalis* under different extraction temperatures, extraction times and water/material ratios by using the response surface methodology (RSE), with a Box-Benhken design (3 factors and 3 levels). The optimal water extraction conditions for polysaccharides are as follows: an extraction temperature of 93.7 ºC, an extraction time of 3.25 h and a water/material ratio 26.2:1. Under these conditions, the yield of polysaccharides reached a peak value of 5.29 ± 0.03 % (w/w).

Key Words: *Magnolia officinalis* polysaccharides, Extraction, Optimization, Response surface methodology.

INTRODUCTION

Plant polysaccharides have been shown to possess a variety of biological activities, such as anticancer[1], antiaging[2] and strong antioxidant activity for scavenging free radicals[3]. *Magnolia officinalis* is an important traditional herb in Chinese medicinal soups used to treat thrombotic stroke, gastrointestinal complaints, anxiety and nervous disturbance. In recent years, it was found that the polysaccharides of *Magnolia officinalis* extracted by water were the main effective ingredients, at least for some bioactivities[4]. However, no research has been reported on the optimization of extraction conditions of polysaccharides from *Magnolia officinalis*.

Response surface methodology (RSM) has been introduced into many fields, including food processing and biotechnology application[5,6] to determine the optimal values for condition variables that influence any complex or multistep process[7]. Indeed, RSM can reduce costs by minimizing manpower, time and raw materials. It employs an experimental design such as Box-Behnken design (BBD) to develop a second order polynomial that can express the effects of variables and their interrelationships. Recently, RSM has been applied to optimize extraction conditions of polysaccharides[8,9].

The aim of the study is to optimize the hot water extraction conditions of polysaccharides from *Magnolia officinalis* using RSM. The influences of extraction temperature, extraction time, water/material ratio and extraction steps on the water extraction process were investigated individually by single-factor experiments. Then RSM was employed to optimize the extraction conditions and obtain the maximum yield of polysaccharides.

EXPERIMENTAL

Magnolia officinalis (family magonoliaceae), originated from Sichuan, China and was purchased from a local herb market (Shanghai, China). Double distilled water was used as the solvent.

All reagents were of analytical grade. The absorption spectra were measured at room temperature with an UV-2102-PC Spectrophotometer (Shanghai UNICO Instrument Inc, China).

Extraction: The *Magnolia officinalis* was ground to a fine powder and incubated for 2 days in 95 % EtOH at room temperature to remove EtOH-soluble dyes, oligosaccharides and other small molecules. Approximately 1 g of the pretreated dried sample was extracted in a constant temperature water bath under designed extraction conditions. The water-soluble extracts were passed through filter paper and concentrated to a volume of about 10 mL by evaporation under vacuum below 50 ºC. The concentrate was then mixed with four times (v/v) anhydrous acohol and cooled at 4 ºC overnight. The precipitate was collected by centrifugation (8000 rp/m, 20 min), washed twice with anhydrous alcohol and dissolved in double distilled water. The solution was transferred to volumetric flasks (250 mL) to a final volume of 250 mL.

Experimental design: The Box-Behnken design was used to optimize the conditions equired for optimal polysaccharides yield and to assess both the independent effects and interactions between variables[10]. After determining the preliminary range of the extraction variables through single-factor experiments, the RSM experiments were designed to test three independent variables (X_1, extraction temperature; X_2, extraction time; X_3, ratio of water/material;) and three levels.

The variables were coded according to the equation:

$$X_i = \frac{(x_i - x_0)}{\Delta x_i} \quad (1)$$

where X_i is a coded value of the variable x_i; x_i is the actual value of the variable; x_0 is the actual value of x_i at the centre point and Δx_i is the step change value. Table-1 presents the ranges and levels of independent variables. There were a total 15 runs for optimizing the three individual parameters in Box-Behnken design. The experimental conditions and the yield of polysaccharides are shown in Table-2.

TABLE-1
INDEPENDENT VARIABLES AND THEIR LEVELS USED IN THE RESPONSE SURFACE DESIGN

	Symbol		Factor levels		
Independent variables	Actual	Coded	-1	0	1
Extraction temperature (°)	x_1	X_1	80	90	100
Extraction time (h)	x_2	X_2	2.5	3	3.5
Ratio of water to raw material	x_3	X_3	20	25	30

TABLE-2
CENTRAL COMPOSITE DESIGN AND EXPERIMENTAL POLYSACCHARIDES YIELD

Run	Coded variables			Actual variables			Extraction yield (%)
	X_1	X_2	X_3	x_1	x_2	x_3	
1	-1	-1	0	80	2.5	25	3.43
2	-1	0	-1	80	3	20	3.28
3	-1	0	1	80	3	30	3.76
4	-1	1	0	80	3.5	25	3.58
5	0	-1	-1	90	2.5	20	3.93
6	0	-1	1	90	2.5	30	3.98
7	0	1	-1	90	3.5	20	4.15
8	0	1	1	90	3.5	30	4.45
9	1	-1	0	100	2.5	25	4.38
10	1	0	-1	100	3	20	4.04
11	1	0	1	100	3	30	4.40
12	1	1	0	100	3.5	25	5.07
13	0	0	0	90	3	25	5.12
14	0	0	0	90	3	25	5.08
15	0	0	0	90	3	25	5.19

Analysis method: The concentration of polysaccharides was determined by the anthrone/sulfuric acid method with some modifications[11]. D-Glucose was used as standard and the optical density valued was measured at 580 nm. The result was calculated as extraction yield (% w/w)
= [mass of polysaccharides (in extraction solution)/mass of material (sample)] × 100 (2)

Data analysis: Through the response surface regression procedure, the experimental data were fitted to the following second-order polynomial equation

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

In eqn. 3, Y is the yield of polysaccharides in real values, A_0 is a constant, A_i, A_{ii} and A_{ij} are coefficients of the linear, quadratic and interactive terms, respectively and X_i and X_j are the independent variables. The polynomial equation established response surfaces and contour plots that predicted the relationship between the response and experimental levels of each factor and the optimal extraction conditions[12]. The experimental data were analyzed with SAS Software (version 8.0, USA).

RESULTS AND DISCUSSION

Effect of extraction temperature: Extraction temperature was an important factor determining the yield of polysaccharides. The experiments were carried out at temperatures of 60, 70, 80, 90 and 100 ºC. The other conditions were a one-step extraction, an extraction time of 1.5 h and a water/material ratio of 25:1. Fig. 1 illustrates the effect of extraction temperature on the yield of polysaccharides. The yield gradually increased above 60 ºC and reached a maximum near 90 ºC. The yield of polysaccharides did not increase further at 100 ºC. The result indicated that high temperature was advantageous for the extraction of polysaccharides, with increasing temperature, the solubility of the analytes was enhanced and the surface tension and solvent viscosity decreased, thus improving sample wetting and matrix penetration. However, temperatures above 90 ºC did not further improve the yield of polysaccharides, consistent with other reports[13,14]. It is suggested that an extraction temperature range of 80-100 ºC was favorable for extraction of polysaccharides.

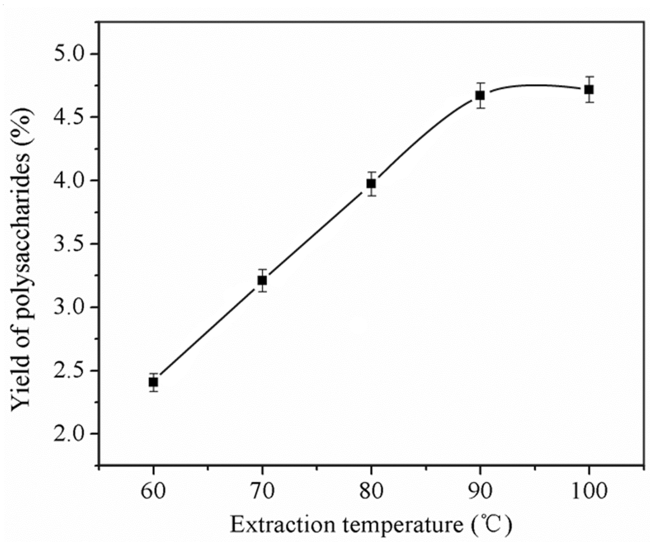


Fig. 1. Effect of the extraction temperature on polysaccharides yield (%) (n = 3)

Effect of extraction time: A long extraction time was also advantageous to yield of polysaccharides[14,15]. Hence, test extraction times were set at 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 h. The other conditions were a one-step extraction, an extraction temperature of 90 ºC and a ratio of water/material of 25:1. Fig. 2 illustrates the yield as extraction times were extended from 1.5-3.0 h. The yield increased over 1.5-3.0 h and reached a peak value at 3 h. Subsequently, the yield of polysaccharides decreased as the extraction time was extended to 4 h, but the yield maintained a dynamic equilibrium from 4-6 h, possibly due to polysaccharides hydrolysis at high temperatures. A similar trend was reported previously[16]. Therefore, a range of 2.5-3.5 h for extraction was considered to be optimal.

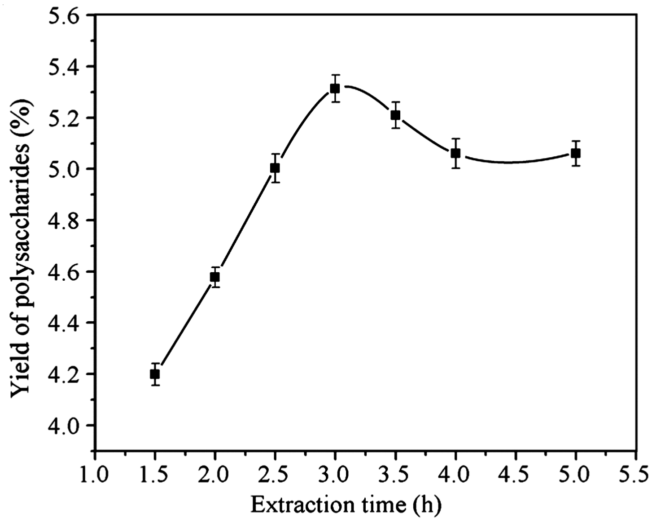


Fig. 2. Effect of the extraction time on polysaccharides yield (%) (n = 3)

Effect of water/material ratio: The water/material ratio was the third important parameter that impacted extraction yield of polysaccharides. The effect of this parameter was studied at water/material ratios of 15:1, 20:1, 25:1, 30:1, 35:1 and 40:1. The other conditions were a one-step extraction, an extraction temperature of 90 ºC and an extraction time of 1.5 h. As shown in Fig. 3, the polysaccharides yield increased from 3.75-4.19 % as the water/material ratio increased from 15:1 to 25:1, but did not increase further over the range of 25:1 to 40:1. Therefore, a ratio range of 20:1-30:1 was adopted in the experiment.

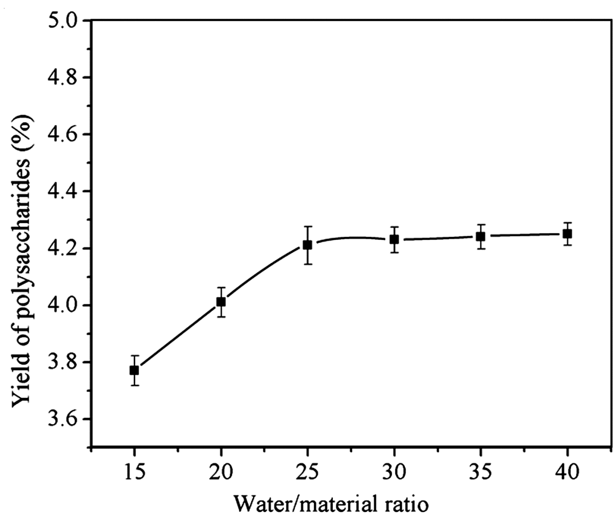


Fig. 3. Effect of the ratio of water to raw material on the polysaccharides yield (%) (n = 3)

Effect of extraction steps: To investigate the effect of the number of extraction steps, a mixture consisting of 1 g of powdered material and 25 mL of double distilled water was extracted at 90 ºC for 1.5 h. The residue was re-extracted three times using fresh distilled water under the same conditions. As Fig. 4 illustrates, the yield of polysaccharides increased with an increasing number of extraction steps. The yield of polysaccharides obtained from the first, second, third and fourth extraction steps were 4.23, 4.47, 4.58 and 4.65 %, respectively but more extractions did not lead to any significant improvement in yield. Considering energy and time constraints, one-step extraction was optimal for the extraction process.

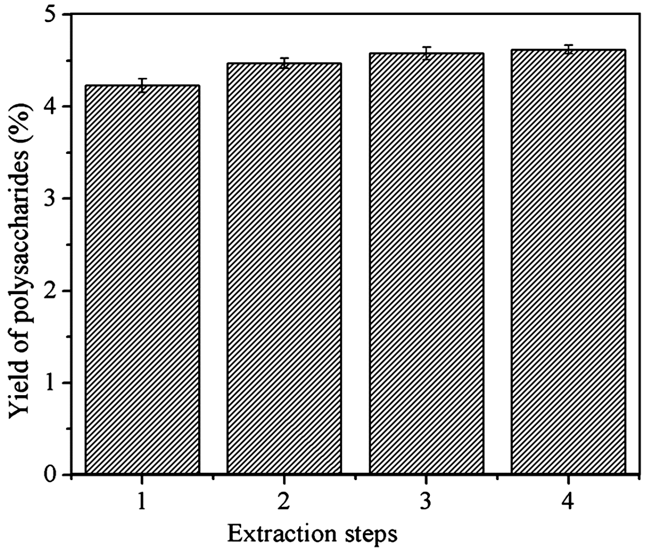


Fig. 4. Effect of the number of extraction steps on polysaccharides yield (%) (n = 3)

Response analysis

Fitting the model: RSM was used for simultaneous analysis of the effects of three extraction parameter conditions (temperature, time and water/material ratio). The experimental data of the three factors and three levels are listed in Table-1. Table-2 shows the experimental design matrix and the yield of polysaccharides obtained in each run. By applying multiple regression analysis on the experimental data, the response variables and the test variables were related by the following second-order polynomial equation:

$$Y = 5.132758 + 0.481322 * X_1 + 0.191092 * X_2 + 0.149425 * X_3 - 0.63841 * X_1 * X_1 + 0.135057 * X_1 * X_2 - 0.028736 * X_1 * X_3 - 0.379389 * X_2 * X_2 + 0.063218 * X_2 * X_3 - 0.624042 * X_3 * X_3 \quad (4)$$

The coefficients for each parameter and parameter estimates are listed in Table-3. The significance of each coefficient is determined by *P* value and *P* values are also shown for interactions between the variables. In Table-3, it can be seen that the first-order main effect of extraction temperature (X_1), the second-order main effect of temperature ($X_1 * X_1$) and the second-order main effect of water/material ratio ($X_3 * X_3$) were highly significant (P = 0.0008, 0.0011 and 0.0014) which indicated that the extraction temperature and water/material ratio directly affected the yield of polysaccharides.

TABLE-3
TEST OF SIGNIFICANCE FOR REGRESSION COEFFICIENTS

Effect	DF	Estimate	Standard error	*t*-Value	*p*-Value
Intercept	1	5.1328	0.1091	47.05	<0.0001
X_1	1	0.4813	0.066	7.21	0.0008
X_2	1	0.1911	0.066	2.86	0.0354
X_3	1	0.1494	0.066	2.24	0.0755
X_1X_1	1	-0.6384	0.098	-6.49	0.0013
X_2X_1	1	0.1351	0.094	1.43	0.2122
X_2X_2	1	-0.1351	0.098	-3.86	0.0188
X_3X_1	1	-0.0287	0.094	-0.30	0.7732
X_3X_2	1	0.0632	0.094	0.67	0.5330
X_3X_3	1	-0.6240	0.098	-6.35	0.0014

In Table-4, the model *F* value of 29.98 and the value of '*Prob* < *F*' indicated that the model was significant. The *P* values of the linear and the quadratic were 0.0027 and 0.0014 both significant. The experimental data were analyzed using the SAS package for analysis of variance (ANOVA). The value of the determination coefficient (R^2 = 0.9684) meant a high degree of association between the obtained and predicted value and that only 3.16 % of the total variations was not explained by the model. The coefficient of variation (CV) is the ratio of the standard error to the observed mean value and used as a measure of reproducibility of the models. A very low coefficient of the variation (CV = 4.44 %) indicated a very high degree of precision and reliability of the experimental values.

TABLE-4
ANALYSIS OF VARIANCE FOR REGRESSION MODEL OF POLYSACCHARIDES YIELD

Regression	DF	Sum of squares	R^2	F-Value	Pr < F
Linear	3	2.3241	0.4120	21.70	0.0027
Quadratic	3	3.0457	0.5400	28.44	0.0014
Cross product	3	0.0922	0.0164	0.86	0.5185
Total model	3	5.4621	0.9684	17.00	0.0031

The graphical representations of regression eqn. 4, called the response surfaces and the contour plots were obtained using SAS version 8.0. Figs. 5-7 illustrate the effects of parameters and their interaction effects on the yield of polysaccharides. The maximum yield is illustrated by the response surfaces and confined to the smallest ellipse in the three contour plots. The ellipse contour indicated a significant interaction between the independent variables, as shown in previous reports[17].

Optimization of extraction conditions: Fig. 5 illustrates the effects of the extraction temperature and extraction time on polysaccharides yield. Quadratic effects of temperature and time could be observed. The yield of polysaccharides increased rapidly with increasing temperature and extraction time and the peak yield was achieved when the temperature and time were 94 ºC and 3.3 h, respectively but decreased with further increase of temperature and extraction time.

Fig. 6 reveals the quadratic effects of temperature and water/material ratio on yield. The yield increased with increasing water/material ratio and reached a maximum value at 26:1, beyond which, yield decreased.

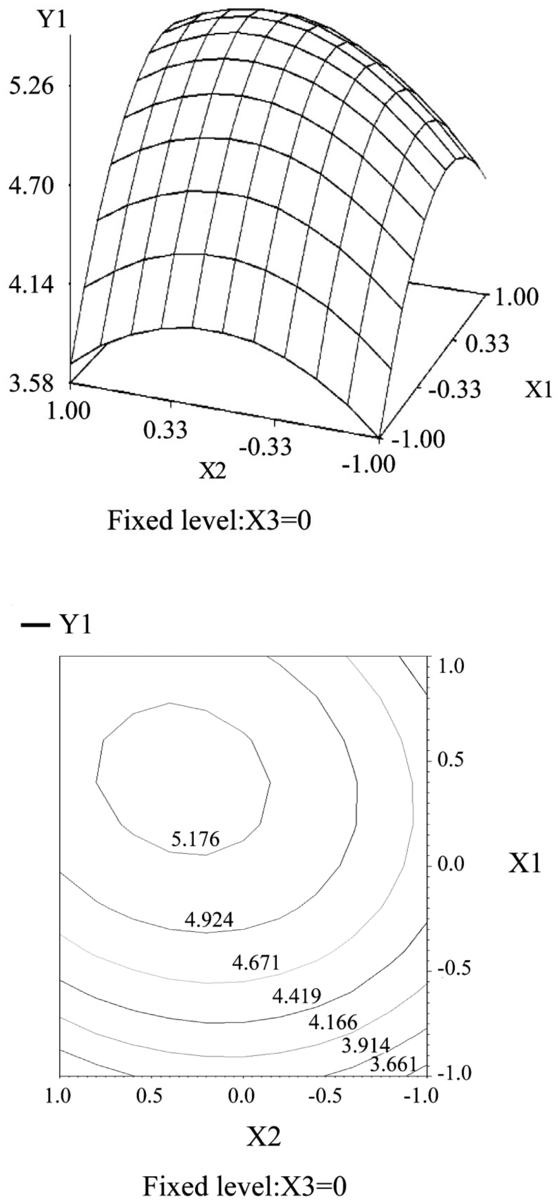
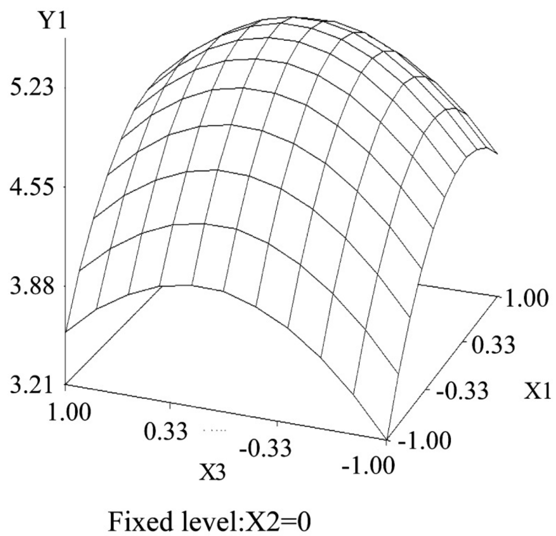


Fig. 5. Response surface (above) and contour (below) plots for effects of extraction temperature and extraction time on polysaccharides yield (%)



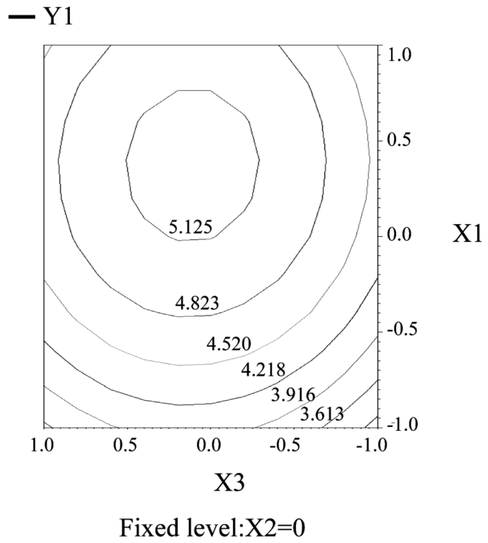


Fig. 6. Response surface (above) and contour (below) plots for effects of extraction temperature and water/material ratio on polysaccharides yield (%)

A similar effect is illustrated in Fig. 7. The yield increased as the extraction time increased from 2.0-3.3 h, but then fell from 3.3-4.0 h. The yield also rose with the increase of water/material ratio from 15:1-26:1, while further increase reduced polysaccharides yield.

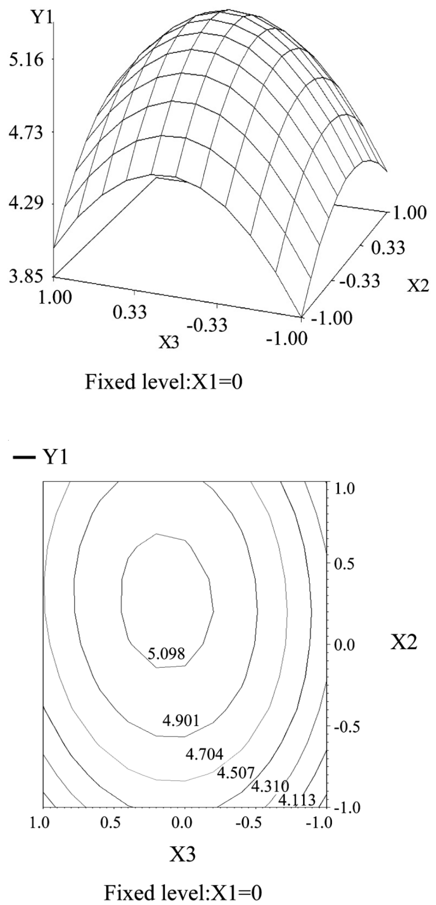


Fig. 7. Response surface (above) and contour (below) plots for effects of extraction time and water/material ratio on polysaccharides yield (%)

From Figs. 5-7, it can be seen that the quadratic effects of two variables were the main influence on the yield of polysaccharides. According to the gradient of slope in the 3-D response surface plot, extraction temperature was the most significant factor impacting yield, followed by water/material ratio.

The optimal values were acquired by solving the regression equation. These optimal values were: $X_1 = 0.376$, $X_2 = 0.252$, $X_3 = 0.119$, with $Y = 5.13 \pm 0.04$ %. It was concluded that the optimal extraction conditions of polysaccharides from *Magnolia officinalis* were an extraction temperature of 93.7 ºC, an extraction time of 3.25 h and a water/material ratio of 26.2:1.

To evaluate RSM model reliability, verification tests were performed under the optimal conditions. A mean yield of 5.29 ± 0.03 % (n = 3) was obtained from real experiments while the predicted yield was 5.27 %, demonstrating that the RSM model was effective and adequate to predict the optimal conditions for the extraction process.

Conclusion

A high polysaccharides yield from *Magnolia officinalis* was obtained under the optimal conditions derived by using the RSM model. Under the optimal conditions of an extraction temperature of 93.7 ºC, an extraction time of 3.25 h and a water/material ratio of 26.2:1, the maximum yield was 5.29 % and the yield obtained from the verification test was well matched with the predicted yield. The three independent variables, extraction temperature, extraction time and water/material ratio all impacted on the yield and their interactions were significant.

ACKNOWLEDGEMENTS

This study was supported by the Science and Technology Commission of Shanghai Municipality (STCSM, No. 08DZ1974000 and No. 10dz2220500).

REFERENCES

1. B.J. Shi, X.H. Nie, L.Z. Chen, Y.L. Liu and W.Y. Tao, *Carbohydr. Polym.*, **68**, 687 (2007).
2. X.M. Li, Y.L. Ma and X.J. Liu, *J. Ethnopharmacol.*, **111**, 504 (2007).
3. Z. Wang and D. Luo, *Carbohydr. Polym.*, **68**, 54 (2007).
4. Y. Guo, L. Kong, Y. Wang and Z. Huang, *Phytother. Res.*, **18**, 204 (2004).
5. E. Amonsou, E. Sakyi-Dawson, F. Saalia, S. Sefa-Dedeh and L.D. Abbey, *Food Sci. Technol. Int.*, **16**, 351 (2010).
6. D. Justine, A. Marion, D.M. Claire, B. Gilles, J. Pascal and C. Marie Elise, *J. Sci. Food Agric.*, **89**, 1599 (2009).
7. A.C. Atkinson, A. Donev and R. Tobias, In ed.: A.C. Atkinson, Optimum Experimental Designs, with SAS, Oxford University Press, USA, Ch. 3, p. 27 (2007).
8. D. Qiao, B. Hu, D. Gan, Y. Sun, H. Ye and X. Zeng, *Carbohydr. Polym.*, **76**, 422 (2009).
9. H. Zhao, J. Wang and Z. Lu, *Carbohydr. Polym.*, **77**, 677 (2009).
10. G.E.P. Box and D.W. Behnken, *Technometrics*, **2**, 455 (1960).
11. L.H. Koehler, *Anal. Chem.*, **24**, 1576 (1952).
12. A.I. Khuri and J.A. Cornell, In ed.: M. Dekker, Response Surfaces: Designs and Analyses, CRC Press, Ch. 6, p. 139 (1996).
13. G. Yin and Y. Dang, *Carbohydr. Polym.*, **74**, 603 (2008).
14. J.M. Ros, J. Laencina, P. Hellín, M.J. Jordan, R. Vila and K. Rumpunen, *LWT-Food Sci. Technol.*, **37**, 301 (2004).
15. Z.D. Liu, G.H. Wei, Y.C. Guo and J.F. Kennedy, *Carbohydr. Polym.*, **64**, 548 (2006).
16. J. Liu, S. Miao, X. Wen and Y. Sun, *Carbohydr. Polym.*, **78**, 704 (2009).
17. R.V. Muralidhar, R.R. Chirumamila, R. Marchant and P. Nigam, *Biochem. Eng. J.*, **9**, 17 (2001).