Carbohydrate Polymers 90 (2012) 284–288

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Optimization of total polysaccharide extraction from *Dioscorea nipponica Makino* using response surface methodology and uniform design

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ARTICLE INFO

Article history:
Received 31 March 2012
Received in revised form 28 April 2012
Accepted 14 May 2012
Available online 20 May 2012

Keywords:
Dioscorea nipponica Makino
Extraction
Uniform design
Response surface methodology

ABSTRACT

On the base of single factor investigation, effects of extraction temperature, extraction time and ratio of water to material as well as their interactions on the yield of total polysaccharide from *Dioscorea nipponica Makino* were studied by uniform design and response surface methodology, respectively. The optimal process conditions were obtained by response surface methodology as follows: ratio of liquid to solid 33:1, extracting duration 134 min, and extracting temperature 95 °C, under optimized conditions, and the experimental yield 3.82% agreed closely with the predicted yield 3.9%. The tri-dimensional response surfaces were plotted by design-expert, and it was indicated that both extraction temperature and extraction time had interaction effects on the response value and there were no interaction effects of extraction time and liquid:solid ratio. Therefore, the application of response surface methodology in the extraction of total polysaccharide from *D. nipponica Makino* is more significant than uniform design.

Dioscorea nipponica Makino is well known as a traditional edible and medicinal plant in oriental countries. The crude water extracts of *D. nipponica Makino* had been reported to possess antitumor activities (Liu, Chen, & Wang, 2004). Our research result also shows that water extract and polysaccharides from *D. nipponica Makino* had higher antioxidant activities than vitamin C in vitro (Luo, 2008; Wang & Luo, 2007). The cultures of *D. nipponica Makino* or their extracts processed in health care had been put into production on a large scale. However, there has not been much study on the extraction optimization of total polysaccharide from *D. nipponica Makino* which may account for its biology activities and medical effects. In order to exploit this resource, total polysaccharide extraction from *D. nipponica Makino* was carried out. It will be of scientific significance in researching and developing rare medical plant and be useful for searching new natural activities medicine.

The experimental technique of uniform design is a method established together by Fang Kaitai & academician Wang Yuan (Fang, 2001). It applies the experiments with many factors and many levels and is based on orthogonal test (Zeng, 1994). Like orthogonal design, uniform design offers lots of experimental tables for users to conveniently utilize. Uniform design tables of form $U_n(n^s)$ is purposely chosen to mimic the tables of orthogonal designs, $L_n(q^s)$, except that the number of levels equals the number of experiments (Wang, Luo, & Cai, 2007). Uniform design allows the largest possible amount of levels for each factor, and the number of levels can be equal to the number of experiment runs (Xia, Gong, &Wang, 2005). In general, uniform design is preferred since it reduces the number of experiments significantly to evaluate multiple parameters.

Response surface methodology is a collection of statistical and mathematical techniques that has been successfully used for developing, improving and optimizing biochemical processes (Chandrika & Fereidoon, 2005; Lee, Yusof, Hamid, & Baharin, 2006). When many factors and interactions affect desired response, response surface methodology (RSM) is an effective tool for optimizing the process (Box & Wilson, 1951). Response surface methodology is preferred since it can determine the effect of factors on characteristic properties, the best optimal conditions of process, and parameters interactions (Cai, Gu, & Tang, 2007). Therefore, it is less laborious and more informational than other approaches (Wang et al., 2007a,b).

The objective of this study was to optimize extraction conditions of total polysaccharide from *D. nipponica Makino* by hot water extraction. Response surface methodology and uniform design were used to study the effects of liquid: solid ratio, water extraction temperature, and water extraction time for the yield of total polysaccharide from *D. nipponica Makino*. Then, the best extraction conditions of total polysaccharide from *D. nipponica Makino* were obtained by contrasting response surface methodology and uniform design.

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http://dx.doi.org/10.1016/j.carbpol.2012.05.036

Table 1
Uniform design with the observed responses.

Run	Coded variable levels			Observed (*Y*)
	A[a]	*B*[b]	*C*[c]	
1	3 (30:1)	5 (85)	7 (210)	1.58
2	6 (45:1)	2 (70)	6 (190)	2.69
3	1 (20:1)	7 (95)	5 (170)	2.80
4	4 (35:1)	4 (80)	4 (150)	3.81
5	7 (50:1)	1 (65)	3 (130)	2.09
6	2 (25:1)	6 (90)	2 (110)	2.30
7	5 (40:1)	3 (75)	1 (90)	1.17

[a] Liquid:solid ratio (v/w).
[b] Extraction temperature (°C).
[c] Extraction time (min).

1. Materials and methods

1.1. Materials

Dried *D. nipponica Makino* was purchased from a local shop (Xiamen, Fujian Province, China).

Chemicals: Glucose ($C_6H_{12}O_6$) (AR class) from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences; all other chemicals used were of analytical grade. *Apparatus*: 722 spectrometry from Shanghai No. 3 Instrument Company; High Speed Tabletop Centrifuge from Shanghai Anting Scientific Instrument Co., Ltd.

1.2. Extraction of total polysaccharide from D. nipponica Makino

3 g of the samples was used for each treatment. *D. nipponica Makino* juice was extracted by heating *D. nipponica Makino* pieces in water bath at selected temperature for various periods of time. The supernatant was collected by the centrifuge for the determination of total polysaccharide yield.

1.3. Determination of total polysaccharide yield

Total glucose concentration was measured by phenol–vitriol method. The percentage total polysaccharide yield (%, w/w) is calculated as the total polysaccharide content of extraction divided by dried sample weight.

1.4. Experimental design

At first, the effect of changing a single factor on the yield of total polysaccharide was studied. Namely, we studied the variable condition of a factor when the others were invariable.

Uniform design was applied to determine the optimum condition of hot water extraction of total polysaccharide from dried *D. nipponica Makino*. The investigated levels of each factor were selected depending on the above experiment results of the single factor. The combination effect of independent variables A (liquid:solid ratio, ml/g), B (water extraction temperature, °C) and C (water extraction time, min) at seven variation levels in the extraction process, is shown in Table 1.

On the other hand, the optimum condition of hot water extraction of total polysaccharide from dried *D. nipponica Makino* was studied by using response surface methodology. In this experiment, three same factors as those used in uniform design which were X_1 (liquid:solid ratio, ml/g), X_2 (water extraction temperature, °C), and X_3 (water extraction time, min), were studied, but there were only three variation levels in the extraction process according the Box–Behnken design (Box & Behnken, 1960; Wang et al., 2007a,b) for every factor, which were X_1 (20:1, 35:1, 50:1), X_2 (65 °C, 80 °C, 95 °C), and X_3 (90 min, 150 min, 210 min). The complete design consisted of 17 experimental points including five replications of the center points, which is shown in Table 3.

Table 2
Significance of regression coefficient for the yield.

Variables	Standard	Computed *t*-value	Significance level *p*-value
B	−0.8898	1.9501	0.1905
C	0.9875	6.2548	0.0246
A × A	−0.9489	3.0083	0.0950
C × *C*	−0.9886	6.5528	0.0225
B × *C*	−0.6563	0.8698	0.4761

1.5. Statistical analysis

For uniform design and subsequent analysis, the software named as Data Processing System (DPS Version7.05, Refine Information Tech. Co., China) was used to generate statistical analysis and regression model (Tang & Feng, 1997). A total of seven combinations were chosen in random order according to DPS software configuration for three factors. The coded and actual values are also shown in Table 1. The significance of each coefficient was determined using the Student's *t*-value and *p*-value, and the result is shown in Table 2.

On the base of single factor investigation, effects of extraction temperature, extraction time and ratio of water to material on yield were studied, the experiments procedure was designed by Box–Behnken design, and the predictive polynomial quadratic equation model and interactions among three factors were developed by Design-Expert (Version 8.0, Stat-Ease Inc., Minneapolis, MN, USA) analysis. The significance of all terms in the polynomial was judged statistically by computing the *F*-value at a probability (p) of 0.001, 0.01 or 0.05. Experimental data were fitted to the Design-Expert, the tri-dimensional response surfaces and contour plots were also generated. These response surfaces and contour plots could visualize the relationship between the response and experimental levels of each factor and to deduce parameters interactions (Triveni, Shamala, & Rastogi, 2001).

The behavior of the surface was investigated for the response function (Y) using the regression equation (Lee, Yusof, Hamid, & Baharin, 2006). Responses were monitored and results were compared with model predictions. The optimum condition was verified by conducting experiments under these conditions.

Table 3
Box–Behnken design with the observed responses for yield.

Run	Coded variable levels			Observed (*Y*)
	X_1[a]	X_2[b]	X_3[c]	
1	−1 (20:1)	−1 (65 °C)	0 (150 min)	1.88
2	1 (50:1)	−1 (65 °C)	0 (150 min)	3.09
3	−1 (20:1)	1 (95 °C)	0 (150 min)	3.34
4	1 (50:1)	1 (95 °C)	0 (150 min)	3.27
5	−1 (20:1)	0 (80 °C)	−1 (90 min)	2.15
6	1 (50:1)	0 (80 °C)	−1 (90 min)	2.33
7	−1 (20:1)	0 (80 °C)	1 (210 min)	2.90
8	1 (50:1)	0 (80 °C)	1 (210 min)	2.99
9	0 (35:1)	−1 (65 °C)	−1 (90 min)	1.94
10	0 (35:1)	1 (95 °C)	−1 (90 min)	3.73
11	0 (35:1)	−1 (65 °C)	1 (210 min)	2.98
12	0 (35:1)	1 (95 °C)	1 (210 min)	3.32
13	0 (35:1)	0 (80 °C)	0 (150 min)	3.41
14	0 (35:1)	0 (80 °C)	0 (150 min)	3.56
15	0 (35:1)	0 (80 °C)	0 (150 min)	3.41
16	0 (35:1)	0 (80 °C)	0 (150 min)	3.51
17	0 (35:1)	0 (80 °C)	0 (150 min)	3.54

[a] Liquid:solid ratio (v/w).
[b] Extraction temperature (°C).
[c] Extraction time (min).

2. Results and discussion

2.1. Single factor results

2.1.1. The effect of liquid:solid ratio on the yields

This experiment adopted 5:1, 15:1, 25:1, 35:1 and 45:1 liquid:solid ratio to study the effect of different liquid:solid ratio on yields in hot water extraction. In these extractions, other experimental conditions were as follows: water extraction temperature, 80 °C; water extraction time, 120 min. The result shows that the yield of total polysaccharide increased with elevating liquid:solid ratio, and the yield reached the highest when liquid:solid ratio increased to 35:1. According to the results, it is better that liquid:solid ratio was chose between 20:1 and 50:1 in latter experiments.

2.1.2. The effect of water extraction temperature on the yields

The effect of water extraction temperature on yields had been studied in this work when different water extraction temperature (50, 60, 70, 80, 90 and 100 °C) was set under the reaction conditions as follows: liquid:solid ratio 35:1; water extraction time 120 min. The result implied the yield of total polysaccharide was always enhanced before the temperature 90 °C, and then it reached the highest and was not changed when the temperature was added.

2.1.3. The effect of extraction time on the yields

This experiment in turn adopted 60, 90, 120, 150, and 180 min water extraction to investigate the effect of water extraction time on yields. Other experimental conditions were as follows: liquid:solid ratio 35:1; water extraction temperature 90 °C. It could be concluded that the yield gradually increased with changed water extraction time, and the yield reached the highest when extraction time was 150 min.

So in the RSM and uniform design experiment, we adopted liquid:solid ratio of 20:1–50:1, water extraction temperature of 65–95 °C, and water extraction time of 90–210 min for further study objects in the extraction process of total polysaccharide from *D. nipponica Makino*.

2.2. Data analysis of uniform design

A regression analysis (in Table 2) was carried out to fit mathematical models to the experimental data aiming at an optimal region for the studied. The following regression equation, which is an empirical relationship between the yield and the test variable in coded unit as given in Eq. (1), can describe the predicted model.

$$Y = 15.5723 - 0.2597B + 0.2155C - 0.0045A \times A - 0.0006C \times C - 0.0003B \times C \quad (1)$$

The multiple coefficients of correlation $R = 0.9912$, the coefficient of determination $R^2 = 0.9824$, and $F = 11.155$, suggested a good fit. Thus, the response was sufficiently explained by the model (Little & Hills, 1978; Mendenhall, 1975).

The significance of each coefficient was determined using the Student's *t*-test and *p*-value in Table 2. It can be seen that the variable with the largest effect was the terms of water extraction time.

2.3. Results of RSM

2.3.1. Fitting the model

A Design-Expert Software was used to generate the statistical analysis and regression model. The results of regression analysis were summarized, and regression equation was given in Eq. (2).

Table 4
Test results of significance for regression coefficient.

Variables	Sum of squares	df	Mean square	*F*-value	*p*-Value
X_1	0.25	1	0.25	7.86	0.0264
X_2	1.78	1	1.78	56.22	0.0001
X_3	0.52	1	0.52	16.46	0.0048
X_1X_2	0.41	1	0.41	12.96	0.0087
X_1X_3	0.002	1	0.002	0.064	0.8074
X_2X_3	0.53	1	0.53	16.63	0.0047
X_1^2	1.03	1	1.03	32.71	0.0007
X_2^2	0.038	1	0.038	1.22	0.3067
X_3^2	0.67	1	0.67	21.11	0.0025

$$Y = -16.9246 + 0.2834X_1 + 0.2095X_2 + 0.0705X_3 - 0.0014X_1X_2 - 0.00003X_1X_3 - 0.0004X_2X_3 - 0.0022X_1^2 - 0.0004X_2^2 - 0.0001X_3^2 \quad (2)$$

The statistical analysis indicates the proposed model was adequate, possessing significant *p*-value ($p = 0.0004 < 0.01$) and with very satisfactory values of the R^2 ($R^2 = 0.9609$) for the response.

The significance of each coefficient was determined using the Student's *F*-test and *p*-value in Table 4. The corresponding variables will be more significant if the absolute *F*-value becomes larger and the *p*-value becomes smaller (Amin & Anggoro, 2004). It can be seen that significant terms were the linear terms of water extraction temperature (X_2), water extraction time (X_3) and liquid:solid ratio (X_1), and the quadratic term of liquid:solid ratio (X_1^2) and water extraction time (X_3^2), followed by the interaction effects of water extraction temperature (X_2) and water extraction time (X_3), and the interaction effects of liquid:solid ratio (X_1) and water extraction temperature (X_2).

Lack of Fit was 13.04, the difference of value between Adj *R*-Squared (0.9095) and Pred *R*-Squared (0.4195) was long-range, suggesting the regression equation (2) could be more reasonable by the optimization. Some insignificant terms, such as X_2^2 and X_1X_3, were neglected, and the predicted model was refitted. The predicted model can be described by the following Eq. (3) in terms of coded values:

$$Y_N = -14.1759 + 0.2813X_1 + 0.1416X_2 + 0.0701X_3 - 0.0014X_1X_2 - 0.0004X_2X_3 - 0.0022X_1^2 - 0.0001X_3^2 \quad (3)$$

Analysis of variance for the optimum model ($p < 0.0001$) was given by Design-Expert. The coefficient of determination (R^2) of the optimum model was 0.9532 which was changed little in contrast to Eq. (2), Lack of Fit was reduce to 9.4, and the value of Adj *R*-Squared (0.9167) was closer to the value of Pred *R*-Squared (0.6949), suggesting the optimum model was more accurate. Thus, the response was more sufficiently explained by the optimum model.

2.3.2. Analysis of response surface

Response surface was used to illustrate the effects of liquid:solid ratio, extraction temperature and extraction time on the response. The regression model equation (2) allowed the prediction of the effects of the three parameters on the yield. The relationship between independent and dependent variables is illustrated in tri-dimensional representation of the response surfaces, and the results are shown in Figs. 1–3.

The normal plot of residuals and the map of residuals vs. predicted were given by Design-Expert, the former was linear and the latter ruleless, which shows that the model was credible.

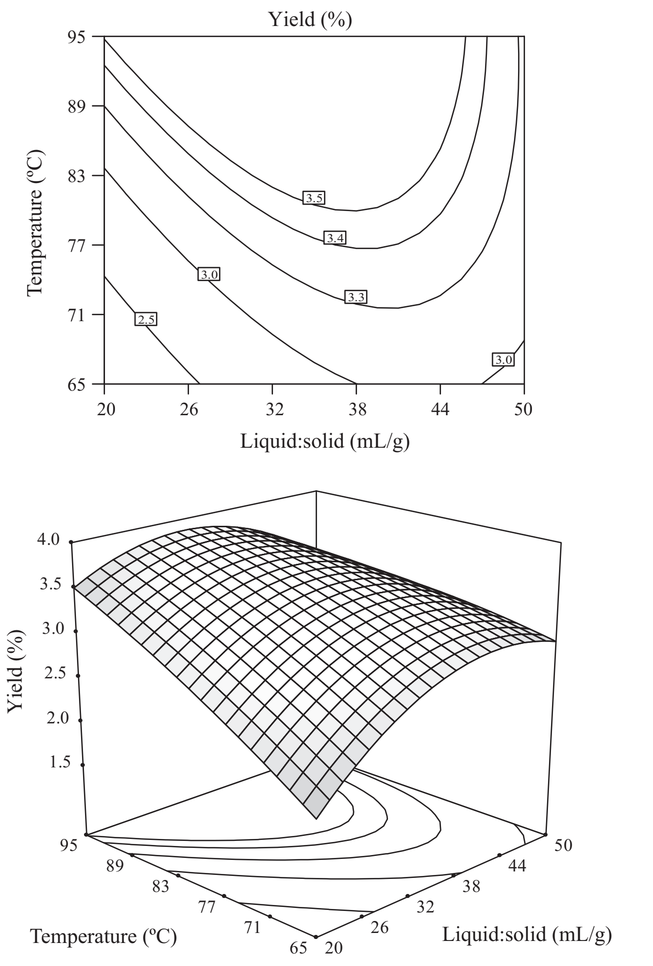

Fig. 1. 3D graphic surface and contour plots for the effects of extraction temperature and liquid:solid ratio.

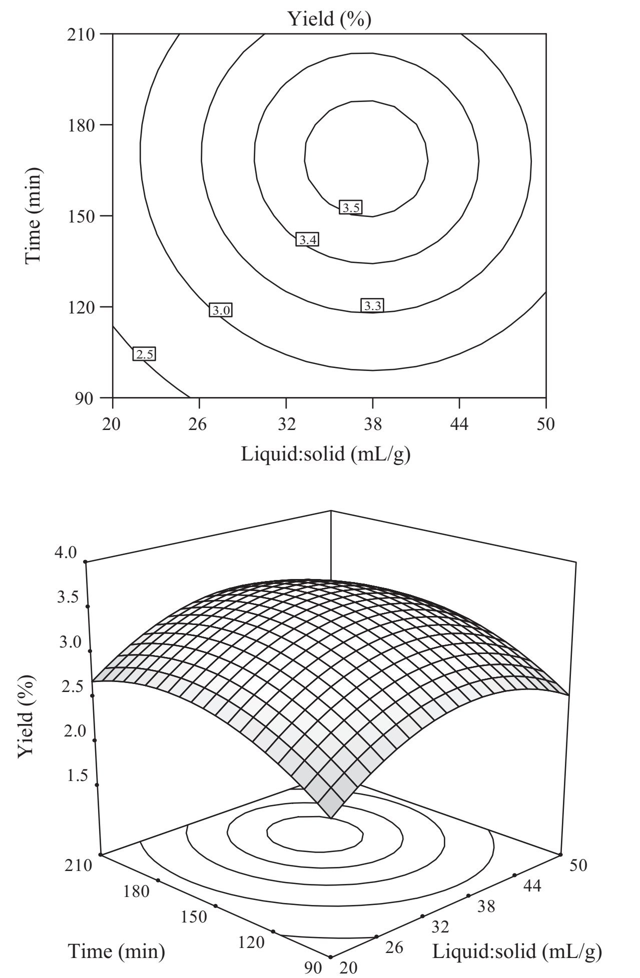

Fig. 2. 3D graphic surface and contour plots for the effects of extraction time and liquid:solid ratio.

The interaction effects of liquid:solid ratio and extraction temperature (X_1X_2) are shown in Fig. 1, which indicated there was the strong interaction between this two factors (Guo, Li, Guo, & Cheng, 2006). At smaller liquid:solid ratio, the higher yield could be obtained with the more increasing extraction temperature. However, the yield was increased little with enhancive extraction temperature when the value of liquid:solid ratio was added to 38:1. It was indicated that the greater yield could be obtained when the lower liquid:solid ratio and the higher extraction temperature were selected.

Fig. 2 depicts 3D graphic surface and contour plot of the effects of the two variables, namely, the interaction effect of extraction time and liquid:solid ratio (X_3X_1) on the yield, the smooth surface and rounded contour plot indicated there were no interaction effects between extraction time (X_3) and liquid:solid ratio (X_1) (Hu, Wang, Wang, & Wu, 2008).

The tortuose surface and oval contour plot in Fig. 3 shows the dramatically complex interaction effects of X_2X_3, indicating that extraction time (X_3) demonstrated a linear increase on the yield when extraction temperature was below 75 °C, but quadratic reduce when extraction temperature was above 75 °C. Then the less water extraction time and the higher extraction temperature could bring the greater total polysaccharide yield, and long extraction time was not advantage to the high yield.

The results above accorded with the analysis of Table 4. Overall, these analysis based 3D graphic surfaces also consisted with results of single factor experiments.

2.4. Verification experiments

Then optimum conditions and the prediction were obtained by DPS in uniform design experiment and by Design-Expert in response surface methodology experiment, respectively. Optimum conditions and predicting optimum response values are given in Table 5. The results summarizes that the calculated yield (3.9%), predicted in optimum conditions (X_1 = 33:1, X_2 = 95 °C, X_3 = 134 min) by the method of RSM, was in agreement with the three experimental mean values of 3.82%. However, the predication from uniform design was not reasonable. In this study, the application of RSM in optimizing extraction conditions of total polysaccharide from *D. nipponica Makino*, improving yields of total polysaccharide, was more appropriate than uniform design.

Table 5
Predicted and experimental yield at optimum conditions.

Method	Optimum conditions		Predicted yield	Experimental yield[a]
RSM	Liquid:solid ratio (v/w)	33:1	3.9%	3.82%
	Extraction temperature (°C)	95		
	Extraction time (min)	134		
Uniform design	Liquid:solid ratio (v/w)	20:1	12.21%	3.65%
	Extraction temperature (°C)	65		
	Extraction time (min)	157		

[a] Mean standard deviation of triplicate determinations.

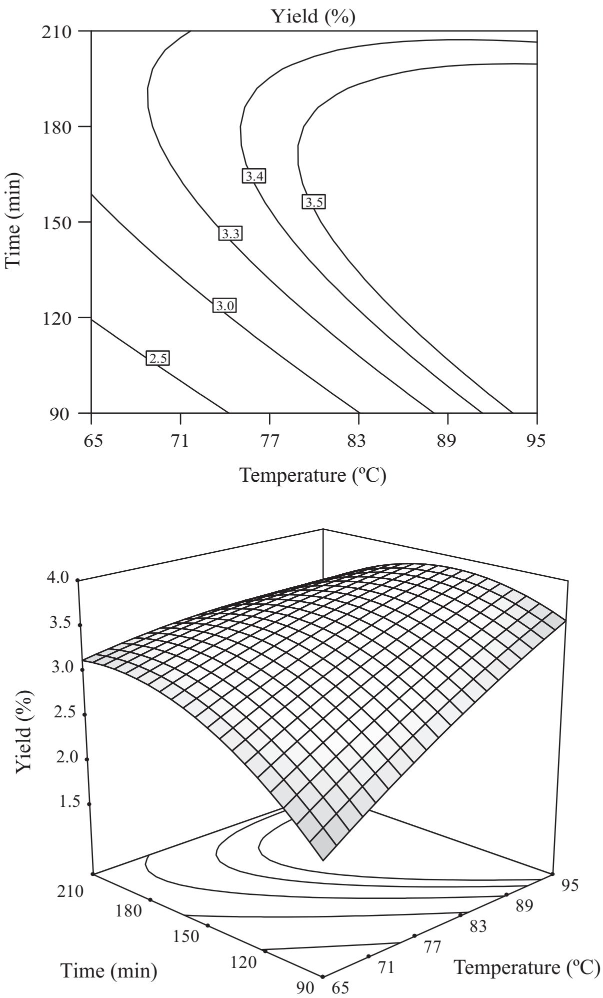

Fig. 3. 3D graphic surface and contour plots for the effects of extraction time and extraction temperature.

3. Conclusion

Response surface methodology was more effective for estimating the effect of three independent variables (liquid:solid ratio, extraction time and extraction temperature) for the yield in the extraction process of total polysaccharide from *D. nipponica Makino*, when we compared response surface methodology with uniform design. Both extraction temperature and extraction time (X_2X_3) had interaction effects on the response value, and short extraction time and high extraction temperature were advantaged, so we should pay attention to the combined choice for extraction temperature and extraction time in the extraction process of total polysaccharide from *D. nipponica Makino*. The optimal predicted total polysaccharide yield of 3.9% was obtained by the method response surface methodology when the optimum conditions for the yield was liquid:solid ratio of 33:1, extraction temperature of 95 °C, and extraction time of 134 min. Under optimized conditions the experimental yield 3.82% agreed closely with the predicted yield.

Acknowledgments

We gratefully acknowledge the financial support received by the High Level Scientific Research Foundation 11BS428 of Huaqiao University and the Fundamental Research Funds for the Central Universities 10QZR14 in PR China.

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