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Optimization of polysaccharides extraction from *Gynostemma pentaphyllum Makino* using Uniform Design

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Abstract

Uniform Design was used to optimize the effects of processing parameters of extraction from *Gynostemma pentaphyllum Makino* on the yield of polysaccharides. Four independent variables such as extraction time (min), solid:liquid ratio (g/ml), immersing time (min), and extraction temperature (°C) were study. The optimal conditions were determined and Three-dimensional response surfaces were plotted from the mathematical models. The *t*-value and *p*-value indicated that both immersing time and water extraction time (X_2X_4) had interaction effects on the response value, followed by the linear terms of water extraction time (X_4), and the interaction effects of immersing time and water extraction temperature(X_2X_3). Considering the efficiency, the optimum conditions of polysaccharides extraction were liquid:solid ratio of 1:67, immersing time of 10 min, water extraction temperature of 95 °C, and water extraction time of 15 min. Under optimized conditions, the experimental yield 11.29% agreed closely with the predicted yield. Therefore, application of Uniform Design in the extraction of polysaccharides from *Gynostemma pentaphyllum Makino* dramatically reduced extraction time.

Keywords: *Gynostemma pentaphyllum Makino*; Polysaccharide; Extraction; Uniform Design

1. Introduction

It is well accepted that carbohydrates can serve as structural components and energy source of the cell. More interestingly, their highly complex structure allows very specific interactions so that these biomolecules are involved in a variety of molecular recognition processes in intercellular communication and signal transduction such as cell adhesion, differentiation, development, regulation, etc. (Varki, 1993). For these reasons, great interest arose on polysaccharides-based pharmaceuticals and on the development of the analysis and extraction of polysaccharides.

Gynostemma pentaphyllum Makino is a well known edible and medicinal plant in oriental countries (Hu, Chen, & Xie, 1996; Tsunematu, Shigenobu, & Kazuko, 1986). Recently, *Gynostemma pentaphyllum Makino* has attracted great attention owing to its anti-tumor activities (Zhou, Liang, & Hu, 2001), anti-gastric ulcer effect (Rujjanawate, Kanjanapothi, & Amornlerdpison, 2004), immunomodulatory effect (Qian, Wang, & Tang, 1998), anti-oxidant properties (Cai, Zhang, & Wang, 2005), and treating hyperlipidemia (Birgitte, Per, & Zhao, 1995). The cultures of *Gynostemma pentaphyllum Makino* or their extracts processed in health care have been put into production on a large scale. However, there has not been much study on the extraction optimization of polysaccharides from *Gynostemma pentaphyllum Makino*.

The experimental technique of Uniform Design is a new method established together by Fang Kaitai and 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

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experiments. 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). Uniform Design can determine the effect of factors on characteristic properties and the optimal conditions of factors. Orthogonal arrays and analysis of variance are used as the tools of analysis. When many factors and interactions affect desired response, Uniform Design is an effective tool for optimizing the process (Fang, Wang, & Hall, 1993). Uniform Design is a collection of statistical and mathematical techniques that has been successfully used for developing, improving and optimizing processes (Qiu, Zhang, Li, Li, & Zhang, 2005; Yang, Qu, & Cheng, 2004; Zhang, Li, & Li, 2005). In general, Uniform Design is preferred since it reduces the number of experiments significantly to evaluate multiple parameters and their interactions. Therefore, it is less laborious and time-consuming than other approaches required to optimize a process.

In this study, we report on the optimization of extraction of polysaccharide from *Gynostemma pentaphyllum Makino* by hot water extraction. Uniform Design was used to study the effects of solid:liquid ratio, immersing time, water extraction temperature, and water extraction time for the yield of polysaccharides from *Gynostemma pentaphyllum Makino.*

2. Materials and methods

2.1. Materials

Dried *Gynostemma pentaphyllum Makino* was purchased from a local shop (Quanzhou, Fujian Province, China).

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. Seven hundred and twenty-two spectrometry was from Shanghai No. 3 Instrument Company; High Speed Tabletop Centrifuge was from Shanghai Anting Scientific Instrument Company.

2.2. Extraction of polysaccharides from Gynostemma pentaphyllum Makino

The *Gynostemma pentaphyllum Makino* was extracted with 95% ethanol at 50 °C for 6 h, dried, and then extracted with distilled water. Three grams of the samples was used for each treatment. *Gynostemma pentaphyllum Makino* juice was extracted by immersing the *Gynostemma pentaphyllum Makino* pieces in water at a selected ratio and time, then heating in water bath at selected temperature for various periods of time. The supernatant was collected for the determination of polysaccharides yield.

2.3. Determining content of glucose

Total glucose concentration was measured by phenol–vitriol method. One milliliter of the supernatant for each treatment was accurately taken and filled into a 10 ml cuvette. Then 0.5 ml, 6% phenol was added into the cuvette and shaken-up. Then 2.5 ml vitriol was filled into the mixture in cuvette, shaken-up and stand for 30 min at room temperature. At last, absorbance values were recorded by a 722 spectrophotometer at the wavelength of 490 nm. At the same time, the wash solution was measured as blank control in an identical way.

2.4. Determination of polysaccharides yield

The percentage polysaccharides yield (%) is calculated as the polysaccharides content of extraction divided by dried sample weight.

2.5. Research of extraction parameters of polysaccharides

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

2.6. Experimental design and statistical analysis

Uniform Design was applied to determine the optimum condition of hot water extraction of polysaccharides from dried *Gynostemma pentaphyllum Makino*. For Uniform Design and subsequent analysis, the software named as Data Processing System (DPS Version 3.0) was used to generate the experimental designs, statistical analysis and regression model (Tang & Feng, 1997). The investigated levels of each factor were selected depending on the above experiment results of the single factor. The combination effect of independent variables X_1(solid:liquid ratio, g/ml), X_2 (immersing time, min), X_3 (water extraction temperature, °C), and X_4 (water extraction time, min) at 13 variation levels (Table 1) in the extraction process, is showed in Table 2. A total of thirteen combinations were chosen in random order according to DPS software configuration for four factors. The coded and actual values are also shown in Table 2. The responses functions (Y) were polysaccharides yield. These values were related to the coded variables by a second-order polynomial Eq. (1) below:

$$Y = \beta_0 + \sum_{i-1}^{m} \beta_i X_i + \sum_{i-1}^{m} \beta_{ii} X_i^2 + \sum_{i<j} \beta_{ij} X_i X_j + E \qquad (1)$$

Where Y is the predicted response, β_0, β_i, β_{ii} and β_{ij} are the regression coefficients, and X_i and X_j are the independent variables.

The significance of each coefficient was determined using the student on t-value and p-value. 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

Table 1
Independent variables of the process and their corresponding levels

Independent variables	Levels												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Solid:liquid ratio (g/ml)	1:10	1:15	1:20	1:25	1:30	1:35	1:40	1:45	1:50	1:55	1:60	1:65	1:70
Immersing time (min)	0	10	20	30	40	50	60	70	80	90	100	110	120
Water extraction temperature (°C)	35	40	45	50	55	60	65	70	75	80	85	90	95
Water extraction time (min)	15	30	45	60	75	90	105	120	135	150	165	180	195

Table 2
Uniform Design with the observed responses and predicted values

Treat	Variable levels				Experimental *Ye*	Predicted *Y*	*Ye*−*Y*
	X_1	X_2	X_3	X_4			
1	1	5	9	11	7.13000	7.36332	−0.23332
2	2	10	4	8	5.30000	5.69461	−0.39461
3	3	1	13	5	4.89000	4.79510	0.09490
4	4	6	8	2	6.89000	6.33304	0.55696
5	5	11	3	13	5.05000	4.51014	0.53986
6	6	2	12	10	7.49000	7.21565	0.27435
7	7	7	7	7	6.99000	7.06128	−0.07128
8	8	12	2	4	6.31000	6.60065	−0.29065
9	9	3	11	1	5.67000	6.17014	−0.50014
10	10	8	6	12	5.20000	5.56554	−0.36554
11	11	13	1	9	3.39000	3.41260	−0.02260
12	12	4	10	6	6.55000	6.58711	−0.03711
13	13	9	5	3	8.09000	7.64082	0.44918

conditions. In order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions, the fitted polynomial equation was expressed as surface and contour plots (Floros & Chinnan, 1988; Triveni, Shamala, & Rastogi, 2001).

3. Results and discussion

3.1. Single factor results

3.1.1. The effect of the immersing time on the polysaccharides yields

In this study, polysaccharides were extracted in solid:liquid ratio 1:30 and different immersing times (10, 30, 60, 90, 120, and 150 min). Fig. 1 shows the variability of polysaccharides yields was trivial with the change of immersing time.

3.1.2. The effect of the solid:liquid ratio on the polysaccharides yields

This experiment adopted 1:10, 1:20, 1:30, 1:40, and 1:50 solid:liquid ratio to study the effect of different solid:liquid ratio on polysaccharides yields in hot water extraction. In these extractions, other experimental conditions were as follows: water extraction temperature, 80 °C; water extraction time, 120 min. Fig. 2 shows the yield of polysaccharides increased with elevating solid:liquid ratio.

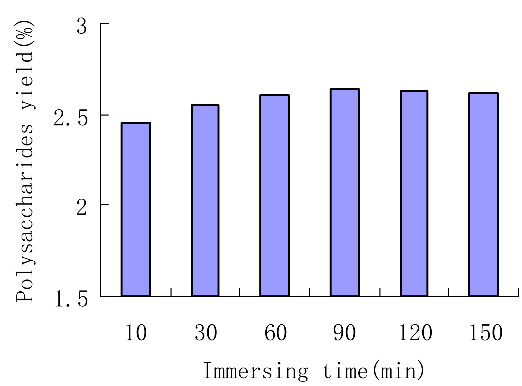

Fig. 1. Effect of immersing time on the polysaccharides yield in water extraction.

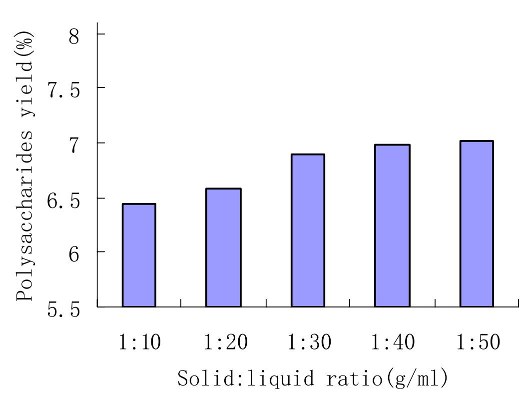

Fig. 2. Effect of solid:liquid ratio on the polysaccharides yield in water extraction (80 °C, 2 h).

3.1.3. The effect of the water extraction temperature on the polysaccharides yields

The effect of water extraction temperature on polysaccharides yields had been studied in this work when different water extraction temperature (30, 40, 50, 60, 70, 80, 90, and 100 °C) was set under the reaction conditions as follows: solid:liquid ratio 1:35; water extraction time 120 min. The results are demonstrated in Fig. 3.The result implied the yield of polysaccharides was always enhanced before the temperature 80 °C, and then it reduced when the temperature was added in 90 °C.

3.1.4. The effect of the extraction time on the polysaccharides yields

This experiment in turn adopted 30, 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: solid:liquid ratio 1:35; water extraction temperature 80 °C. It could be seen in Fig. 4 that the yield of polysaccharides gradually reduced with changed water extraction time.

So in the Uniform Design experiment, we adopted solid:liquid ratio of 1:10–1:70, immersing time of 0–120 min, water extraction temperature of 35–95 °C, and water extraction time of 15–195 min for farther study objects in the hot water extraction.

3.2. Data analysis of Uniform Design

A regression analysis (in Table 3) 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. (2), can describe the predicted model.

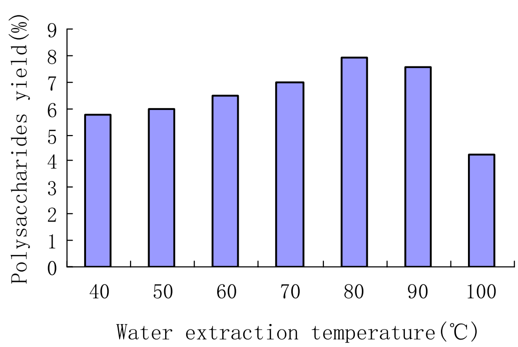

Fig. 3. Effect of water extraction temperature on the polysaccharides yield (1:35, 2 h).

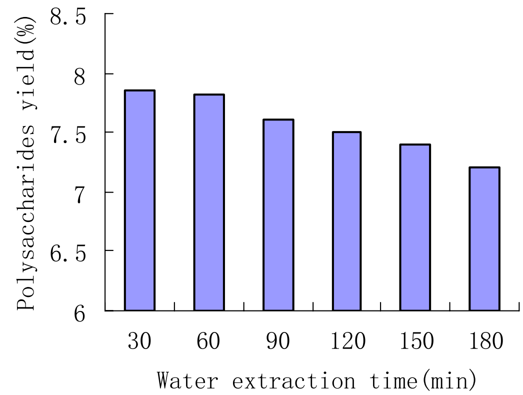

Fig. 4. Effect of water extraction time on the polysaccharides yield (1:35, 80 °C).

$$\begin{aligned} Y = {} & 4.37243730 + 0.5891985101X1 \\ & - 0.30156065499X3 + 0.6125130254X4 \\ & - 0.021596663038X1 * X1 \\ & - 0.03892431823X1 * X4 + 0.04462723203X2 \\ & * X3 - 0.05782499153X2 * X4 \end{aligned} \tag{2}$$

The multiple coefficients of correlation $R = 0.96018$ indicated a close agreement between experimental and predicted values of the polysaccharides yield. The significance of each coefficient was determined using the Student t-test and p-value in Table 3. The corresponding variables will be more significant if the absolute t-value becomes larger and the p-value becomes smaller (Amin & Anggoro, 2004; Atkinson & Donev, 1992). It can be seen that the variable with the largest effect was the interaction effects of immersing time and water extraction time(X_2X_4), the linear terms of water extraction time (X_4), followed by the interaction effects of immersing time and water extraction temperature(X_2X_3).

The coefficient of determination (R^2) of the predicted model was 0.92194, suggesting a good fit, and the predicted model seemed to reasonably represent the observed values.

Table 3
Significance of regression coefficient for the yield of polysaccharides

Variables	Standard error	Computed t-value	Significance level p-value
X_1 (solid:liquid ratio)	0.69671	2.17172	0.07289
X_3 (water extraction temperature)	−0.79796	2.96041	0.02527
X_4 (water extraction time)	0.89200	4.41237	0.00451
X_1 X_1	−0.55070	1.47527	0.19059
X_1 X_4	−0.76446	2.65159	0.03795
X_2 X_3	0.83780	3.43125	0.01395
X_2 X_4	−0.89249	4.42438	0.00445

$R =$ 0.96018; $R^2 = 0.92194$.

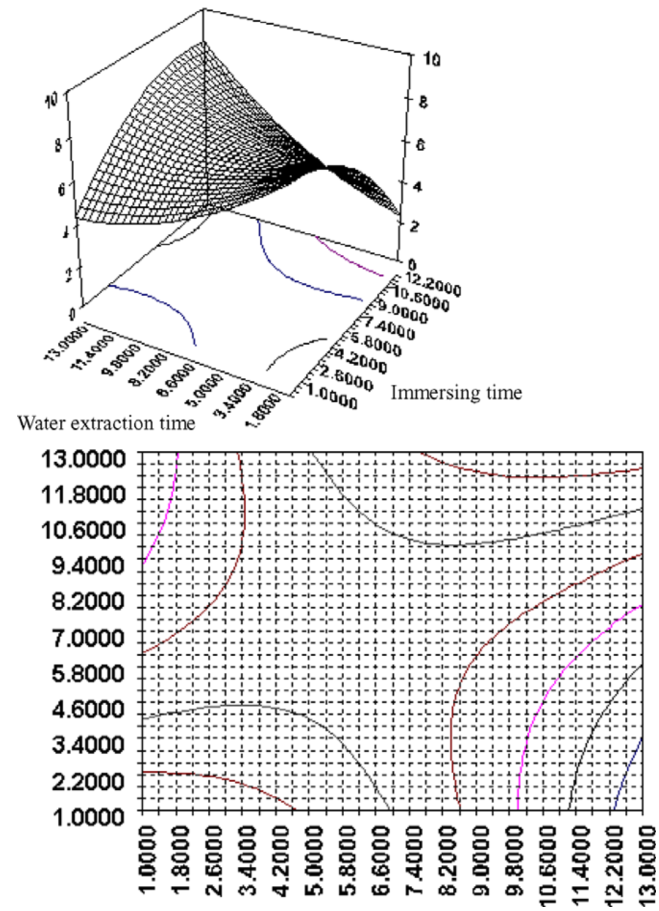

Fig. 5. Three-dimensional graphic surface and contour plots for the effects of X_2 and X_4.

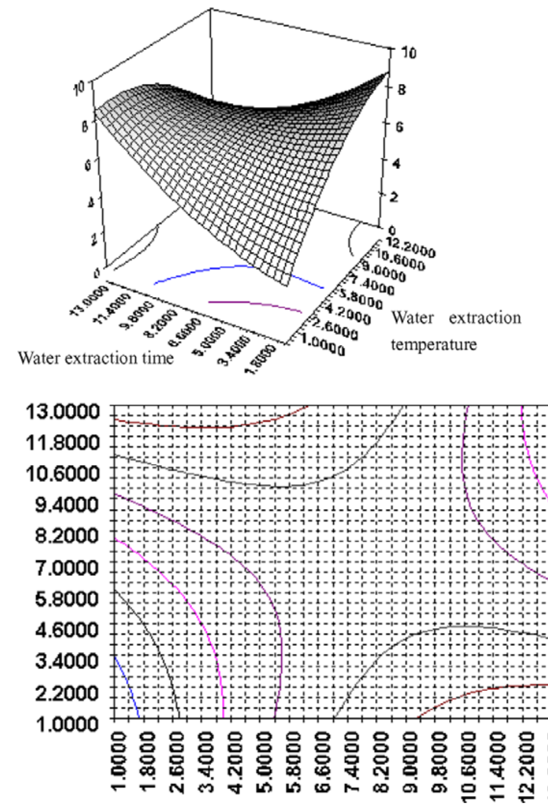

Fig. 6. Three-dimensional graphic surface and contour plots for the effects of X_3 and X_4.

Thus, the response was sufficiently explained by the model (Little & Hills, 1978; Mendenhall, 1975).

The regression model Eq. (2) allowed the prediction of the effects of the four parameters on the polysaccharides yield. The relationship between independent and dependent variables is illustrated in Three-dimensional representation of the response surfaces generated by the model (Figs. 5–7). Two variables within experimental range were depicted in one Three-dimensional surface plots while the two other variables kept constant.

The interaction effects of immersing time and water extraction time (X_2X_4) was showed at Fig. 5, which indicated there was the cooperation between this two factors. In addition, the water extraction time (X_4) also demonstrated a linear increase on the yield of polysaccharides in Fig. 6 when extraction temperature was below 60 °C, but a linear reduce when extraction temperature was above 80 °C, which accorded with the result of Table 3 and Fig. 4, and then the less water extraction time and the less immersing time could bring the greater polysaccharides yield.

Fig. 7 depicts 3D graphic surfaces and contour plots of the effects of the two variables, namely, the interaction effects of immersing time and water extraction temperature(X_2X_3) on the yield of polysaccharides, the tortuose surfaces showed the dramatically complex interaction effects of X_2X_3. At short immersing time (below 50 min), high yield of polysaccharides was obtained with increasing water extraction temperature. It was indicated that the greater yield could be obtained when the lower immersing time and the higher extraction temperature were selected.

In addition, the effect of water extraction temperature (X_3) on the yield is showed in Fig. 6, the water extraction temperature displayed a linear effect on the yield of polysaccharides in the 3D graphic surface. The results above accorded with the analysis of Table 3. It may be because that the cells of plant expand and the polysaccharides are easy to be separated out in higher temperature.

Overall, these analysis based 3D graphic surfaces were accorded with results of single factor experiments showed Figs. 1–4.

Then the prediction was obtained by DPS. Although the prediction showed that the more immersing time and the lower solid:liquid ratio were, the higher yield of polysaccharides, a low solid:liquid ratio 1:67 and a less immersing time 10 min were selected in this study. It is considered the effect for yield of polysaccharides in independent variable of immersing time was trivial, and longer immersing time will lead lower efficiency. So, based on the results in Fig. 5–7, a optimum condition was found as follow: solid: liquid ratio of 1:67,

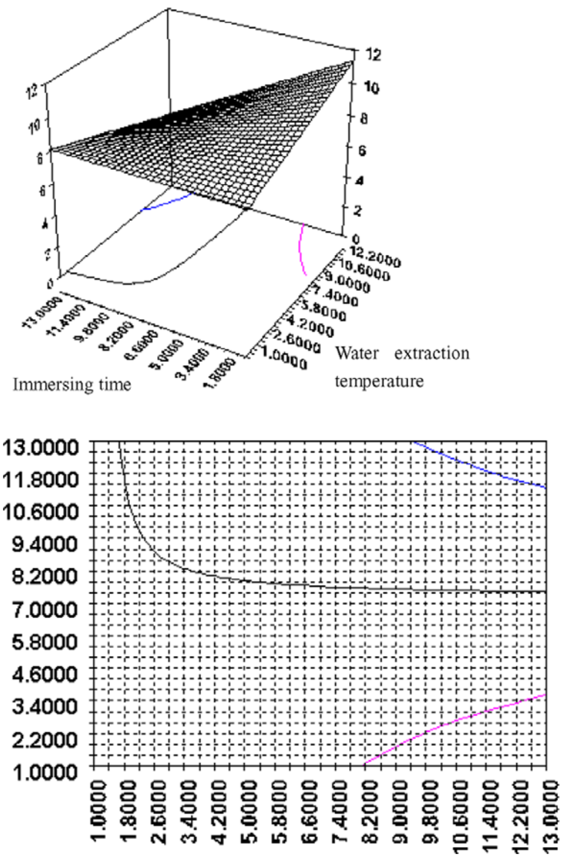

Fig. 7. Three-dimensional graphic surface and contour plots for the effects of X_3 and X_2.

immersing time of 10 min, water extraction temperature of 95 °C, and water extraction time of 15 min, then the highest polysaccharides yield of 11.36% predicted by the contour plots could be obtained. Maybe, a more liquid volume can rapidly dissolve more polysaccharides from sample cells in the same high temperature than little volume, the dissolved polysaccharides reached to be saturated in a definite extraction time, and then the polysaccharide was obtained the highest yield.

The calculated yield was in agreement with the four experimental mean values of 11.29%. In this study, application of Uniform Design in the extraction from *Gynostemma pentaphyllum Makino* improved yields of polysaccharides and was less extraction time than conventional heating (Luo & Wang, 2005).

4. Conclusion

The different conditions (solid:liquid ratio, immersing time, extraction time and temperature) for hot water extraction of polysaccharides from *Gynostemma pentaphyllum Makino* showed that all these variables markedly affect the polysaccharides yield. Uniform Design was effective for estimating the effect of four independent variables. Both immersing time and water extraction time (X_2X_4) had interaction effects on the response value, followed by the linear terms of water extraction time (X_4), and the interaction effects of immersing time and water extraction temperature(X_2X_3). The optimal predicted polysaccharides yield of 11.29% was obtained when the optimum conditions for the yield was liquid:solid ratio of 1:67, immersing time of 10 min, water extraction temperature of 95 °C, and water extraction time of 15 min. Under optimized conditions the experimental yield agreed closely with the predicted yield.

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