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Optimization of extraction process of *Glycyrrhiza glabra* polysaccharides by response surface methodology

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ABSTRACT

Experimental design was used to investigate the effect of three parameters (extraction time, extraction number and ratio of water to raw material) on polysaccharides yields. The ranges of the factors investigated were 3.5–4.5 h for extraction time (X_1), 4–6 for extraction number (X_2), and 25–35 for ratio of water to raw material (X_3). The statistical analysis of the experiment indicated that extraction time and ratio of water to raw material had significant effect on *Glycyrrhiza glabra* polysaccharides yields. The central composite design showed that polynomial regression models were in good agreement with the experimental results with the coefficients of determination of 0.924 for *Glycyrrhiza glabra* polysaccharides yield. The optimal condition for *Glycyrrhiza glabra* polysaccharides yield within the experimental range of the variables studied was at 4.3 h, 6, and 35. At this condition, the predicted yield of polysaccharides extracted was 3.6%.

1. Introduction

Glycyrrhiza glabra (*Licorice*) is a ligneous perennial shrub growing in Mediterranean region and Asia. It is found in dry grassy plains, and sunny mountainsides from much of northern China, especially the Asian steppes to the west. Most of the supply comes from northwest China (Asada, Li, & Yoshikawa, 2000). *Glycyrrhiza glabra* (*Licorice*) is a well-known Chinese herb used in food and medicinal remedies for thousands of years. This herb has long been valued as a demulcent (soothing, coating agent), to relieve respiratory ailments (such as allergies, bronchitis, cold, sore throats and tuberculosis), stomach burn including heart burn from reflux or any other cause, gastritis, inflammatory disorders, skin diseases and liver problems (Xu et al., 2002). *Glycyrrhiza glabra* contains a variety of substances. The medicinal and pharmacological uses of *Glycyrrhiza glabra* have been described in several studies (Arase et al., 1997; Davis & Morris, 1991; Takahara & Watanabe, 1994). *Glycyrrhiza glabra* polysaccharide (GAP), one of the main active ingredients of *Glycyrrhiza glabra* is attributed to many healing properties of the herb. Recently, it has been reported that GAP has many functions such as immunity regulation (Yang & Yu, 1990), phagocytosis (Nose et al., 1998), anti-complement (Takada, Tomoda, & Shimizu, 1992), anti-virus (Wang et al., 2000), anti-tumor (Wang, Xie, Shi, & Zhang, 2003), and it has low cellular toxicity (Wang et al., 2000). Zhou, Qi, Wang, Zhu, and Yu (1999) reported that *Glycyrrhiza glabra* polysaccharides were composed of rhamnose, glucose, arabinose and galactose. Of all monosaccharide composition, glucose was identified as the largest chemical component in the polysaccharides.

The objective of this study was to investigate the significant variables (extraction time, extraction number and ratio of water to raw material), and further to optimize the levels of the extraction variables, for *Glycyrrhiza glabra* polysaccharides production by employing response surface methodology (RSM). This study is a component of a broader investigation into the influences of natural herbs such as *Angelica sinensis* and *Atractylodes macrocephala Koidz*, as determined using statistical methodologies.

2. Materials and methods

2.1. Materials and equipments

Glycyrrhiza glabra was purchased from local herbs market and then ground to pass through 1 mm screen and stored overnight at 4 °C in refrigerator.

Acetone and tetrachloromethcme were purchased from Guangzhou Reagent Co. (Guangzhou, China). Ethanol was purchased from NanJing jiancheng Reagent Co. (NanJing, China). All other chemicals used in the experiment were of analytical grade.

A self-made vessel (1000 ml) was used for the polysaccharides extraction. The extraction procedure was carried out in the water bath (HH-2 Guohua Wiring Company, Shanghai, China).

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2.2. Preparation of Glycyrrhiza glabra polysaccharides

The procedure for *Glycyrrhiza glabra* polysaccharide extracts was carried out consulting the scientific literature on this subject (Cai, Gu, & Tang, 2008).

Around 15 g of those powder material with distilled water were put in a vessel, heated and kept boiling for some time and stirred regularly. Then the extraction (liquid fraction) and the residue were collected separately; the residue was also re-extracted twice more. The extracted liquid fraction was collected and enriched, extracted with 90% ethanol for 12 h. The precipitate was collected by centrifugation (3000 r/min, 20 min) and, respectively, washed twice with acetone and tetrachloromethcme to remove lipid, and then vacuum-dried at 40 °C to obtain the desired polysaccharides. The crude polysaccharides was dissolved in distilled water again and then clarified by centrifugation (10,000*g*, 10 min) and filtration, and dialyzed against 0.01 M sodium acetate buffer (pH 5.0) at 4 °C until the dialyzate was free of sugars. The dialyzed malt extract was then filtered to remove any precipitated material.

2.3. Experimental design

The extraction parameters were optimized using RSM. The central composite design (CCD) was employed in this regard. The range and center point values of three independent variables presented in Table 1 were based on the results of preliminary experiments. CCD in the experimental design consists of eight factorial points, six axial points and six replicates of the central point (Table 2). Extraction time (X_1), extraction number (X_2) and ratio of water to raw material (X_3) were chosen for independent variables. Yield of polysaccharides was selected as the response for the combination of the independent variables given in Table 2. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

The variables were coded according to the equation

$$x = (X_i - X_0)/\geq X \quad (1)$$

The behavior of the system was explained by the following quadratic equation:

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

where Y is the dependent variable (yield of polysaccharides in real value), A_0 is constant, and A_i, A_{ii} and A_{ij} are coefficients estimated by the model. X_i, X_j are levels of the independent variables. They represent the linear, quadratic and cross-product effects of the X_1 X_2 and X_3 factors on the response, respectively. The model evaluated the effect of each independent variable to a response. Analysis of the experimental design and calculation of predicted data were carried out using SAS Software (version 7.0, USA) to estimate the response of the independent variables. Subsequently, three additional confirmation experiments were conducted to verify the validity of the statistical experimental strategies.

Table 1
Independent variables and their levels used for central composite rotatable design

Variables	Level		
	−1	0	1
Extraction time (X_1)	3.5	4	4.5
Number of extraction (X_2)	4	5	6
Ratio of water to raw material (X_3)	25	30	35

Table 2
Central composite arrangement for independent variables and their response

No.	X_1 (Extraction time, h)	X_2 (number of extractions)	X_3 (Ratio of water to raw material)	Extraction yield (%)
1	−1	−1	−1	0.6
2	−1	−1	1	1.7
3	−1	1	−1	1.1
4	−1	1	1	3.1
5	1	−1	−1	1.5
6	1	−1	1	3.4
7	1	1	−1	2.8
8	1	1	1	3.9
9	−1.68179	0	0	2.1
10	−1.68179	0	0	2.1
11	0	−1.68179	0	2.9
12	0	−1.68179	0	2.9
13	0	0	−1.68179	2.5
14	0	0	−1.68179	2.5
15	0	0	0	3.1
16	0	0	0	3.1
17	0	0	0	3.1
18	0	0	0	3.1
19	0	0	0	3.1
20	0	0	0	3.1

3. Result and discussion

3.1. Effect of different time on extraction yield of ***Glycyrrhiza glabra*** 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 (Hou & Chen, 2008). The effect of different time on extraction yield of polysaccharides is shown in Fig. 1. 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 5 h, the variance of extraction yield was relatively rapid, and polysaccharides production reached a maximum at 4 h, and then no longer changed as the extraction proceeded. This indicated that 5 h was sufficient to obtain the polysaccharides production. Thus, 5 h was favorable for producing the polysaccharides.

3.2. Effect of number of extraction on extraction yield of Glycyrrhiza glabra polysaccharides

The effect of number of extraction on extraction yield of polysaccharides is shown in Fig. 2. Extraction was carried out at different number of extraction (1–7) conditions while other extraction

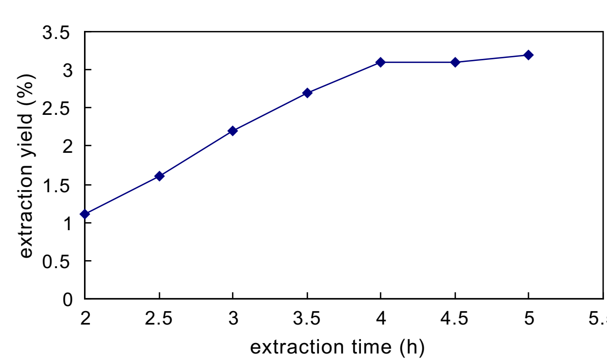

Fig. 1. Effect of extraction time on extraction yield.

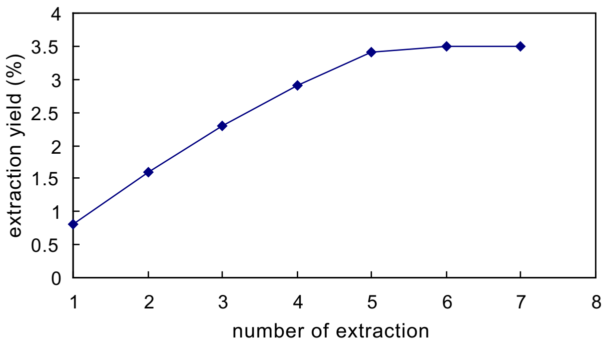

Fig. 2. Effect of number of extraction on extraction yield.

parameters were same to ones described in Section 3.1. The extraction yields of the polysaccharides significantly increased from 0.8% to 3.4% as the ratio of water to raw material increased from 1 to 5. The highest extraction yield was observed when number of extraction was 5.

3.3. Effect of different ratio of water to raw material on extraction yield of *Glycyrrhiza glabra* polysaccharides

The effect of different ratio of water to raw material on extraction yield of polysaccharides is shown in Fig. 3. Extraction was carried out at different ratio of water to raw material (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 1.2% to 3.4% as the ratio of water to mushroom increased from 10 to 40 shown in Fig. 3, 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.

3.4. Modeling of extraction of *Glycyrrhiza glabra* polysaccharides

Table 2 shows the process variables and experimental data. The results of the analysis of variance, goodness-of-fit and the adequacy of the models are summarized. The percentage yield ranged from 0.6% to 3.9%. The maximum value was found at the extraction time 4.5 h, extraction number 6 and ratio of water to raw material 35. 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:

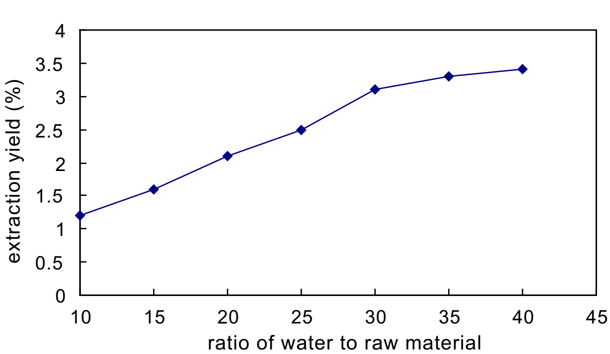

Fig. 3. Effect of ratio of water to raw material on extraction yield.

$$Y = 3.106495 + 0.37344 * X_1 + 0.270927 * X_2 + 0.446663 * X_3 - 0.395992 * X_1 * X_1 - 0.0125 * X_1 * X_2 - 0.0125 * X_1 * X_3 - 0.113149 * X_2 * X_2 + 0.0125 * X_2 * X_3 - 0.254571 * X_3 * X_3 \quad (3)$$

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} (0.96) 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.) (6.09) clearly indicated a very high degree of precision and a good deal of reliability of the experimental 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. The data showed a good fit with the Eq. (3), which were statistically acceptable at $P < 0.05$ level and adequate with satisfactory R^2 value (R^2 for yield were 97.14%). 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.

3.5. Optimum extraction conditions for the maximum *Glycyrrhiza glabra* polysaccharides yield

The effect of component variables on the yield of *Glycyrrhiza glabra* polysaccharides is presented. By analyzing the effects of extraction conditions on the yield of polysaccharides, the yield changed substantially with extraction time and extraction number. Contour plot for yield as a function of extraction time and extraction number was analysed. It suggested that optimum conditions of extraction time and extraction number for yield were 4.3 h, and 6, respectively. The yield of polysaccharides was found to be more dependent on extraction time rather than extraction number. This result was in agreement with Li et al.'s work (2006) and who examined the effects of extraction time and extraction number on the yield and composition of *Glycyrrhiza glabra* polysaccharides. They reported that longer extraction time increased the polysaccharides yield.

By analyzing the effects of extraction conditions on the yield of polysaccharides, the yield changed substantially with extraction time and ratio of water to raw material. Contour plot for yield as a function of extraction time and ratio of water to raw material was analysed. It suggested that optimum conditions of extraction time and ratio of water to raw material for yield were 4.3 h, and 35, respectively.

By analyzing the effects of extraction conditions on the yield of polysaccharides, the yield changed substantially with number of extraction and ratio of water to raw material. Contour plot for yield as a function of number of extraction and ratio of water to raw material was analysed. Higher ratio of water to raw material and longer number of extraction resulted higher yield of polysaccharides in the studied experimental range. The yield of polysaccharides was found to be most dependent on ratio of water to raw material rather than number of extraction. It suggested that optimum conditions of number of extraction and ratio of water to raw material for yield were 6 and 35, respectively. The steady increase in the yield of polysaccharides with increasing ratio of water to raw material could be due to the addition of increasing amounts of solvent molecules to the blend which may affect the extent of polysaccharides gelatinization and thus the rheological properties of the raw material. The polysaccharides in raw material may bind water more tightly during extraction. This binding may increased the yield of polysaccharides when more water was added (Liu & Wang, 2007).

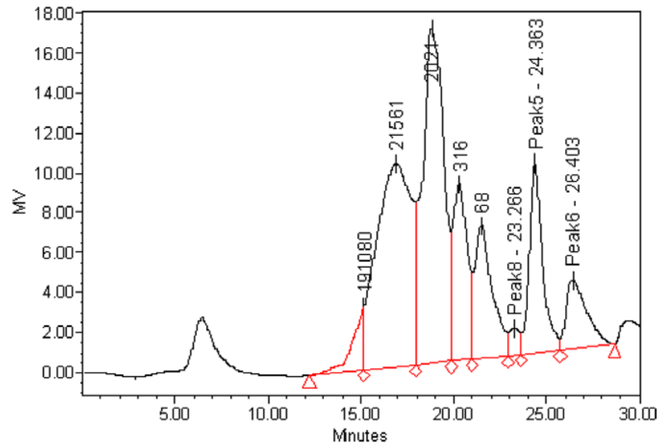

Fig. 4. HPLC of *Glycyrrhiza glabra* polysaccharides.

3.6. 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 3.5 ± 0.13 ($N = 4$), 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. Weight-average molecular weights (M_w) of six separated fractions of *Glycyrrhiza glabra* polysaccharides were 506336 (retention Time, 15.150), 41238 (retention Time, 16.895), 2111 (retention Time, 18.788), 296 (retention Time, 20.272), 59 (retention Time, 21.497) and 11 (retention Time, 23.266), respectively. Number average molecular weights (M_n) of six separated fractions of *Glycyrrhiza glabra* polysaccharides were 328707 (retention Time, 15.150), 18712 (retention Time, 16.895), 1484 (retention Time, 18.788), 254 (retention Time, 20.272), 43 (retention Time, 21.497) and 11 (retention Time, 23.266), respectively (Fig. 4).

4. Conclusion

The performance of the extraction of polysaccharides from *Glycyrrhiza glabra* 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 three variables chosen, namely extraction time, extraction number, and ratio of water to raw material all have a positive influence on the yield of polysaccharides using the extraction method. The optimal conditions obtained by RSM for production of polysaccharides include the following parameters: extraction time 4.3 h, extraction temperature 6, and ratio of water to raw material 35.

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