Optimization of The Extraction Process of Polysaccharide from Schisandra Chinensis (Turcz.) Baill

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Abstract. Based on Box-Behnken response surface method, it utilizes ultrasound-microwave synergistic effect to extract polysaccharide of Schisandra chinensis by taking the polysaccharide extraction ratio of fructus schisandra. On the basis of designing single factor experiment, it selects the extraction temperature, microwave power, ultrasonic wave power, feed liquid ratio and reaction time as the review factors and further determines the optimal extraction process by the response surface method: the extraction temperature is 65°C; the microwave power is 550W; the ultrasonic wave power is 60W; the reaction time is 15min; the feed liquid ratio is 1:25; under such condition, the polysaccharide extraction ratio is 13.23%.

Schisandra chinensis (Turcz.) Baill is the dried fruit of Schisandraceae, with an application history of over 2,000 years, first recorded in the Shennong Herbal Medicine [1]. Polysaccharides are one of the most important active ingredients of Schisandra chinensis (Turcz.) Baill, widely used in clinical medicine, and their main effects are in immune regulation, antiviral and anticancer, hypoglycemic and cosmetology [2].

Currently, the reflux method is the main method for polysaccharides extraction from Schisandra chinensis (Turcz.) Baill. However, this method has disadvantages such as low polysaccharide extraction rate, long extraction time, and excessive extraction usage of solvent. In recent years, microwave assisted method [3] and flash method [4], ultrasonic assisted method [5], etc. have been widely used in the extraction of natural organic products, as far as we know, ultrasonic-microwave collaborative extraction technology is seldomly reported in the extraction of natural organic products, so in the experiment, ultrasound - microwave synergy extraction technology is applied to extract polysaccharides from Schisandra chinensis (Turcz.) Baill.

1. Materials and Methods

1.1. Materials and Instruments
The Schisandra chinensis medicine is produced in the Changbai Mountain area and is purchased in Jilin City, Jilin Province. The raw material used in the experiment is identified as the mature dried fruit of Schisandra chinensis (Turcz.) Baill.
B-491 rotary evaporator (BUCHI Labortechnik AG CH-9230 Flawil 1/Switzerland), High-speed desktop refrigerated centrifuge (Shanghai Anting Scientific Instrument Factory), UV-2450 UV-visible spectrophotometer (Shimadzu Corporation, Japan), XH-300UL Computer Microwave Ultrasound Ultraviolet Light Combination Catalytic Synthesizer (Beijing XiangHu Science and Technology Development Co., Ltd.), XS-10 500g multifunctional crusher (Shanghai Shenzhao Technology Co., Ltd.), SHB-Ⅲ circulation water type multipurpose vacuum pump (Zhengzhou Greatwall Scientific Industrial and Trade Co., Ltd.), and all the reagents are analytical reagents.

1.2. Experimental methods and procedures

1.2.1. Pretreatment of raw materials
The dried Schisandra was crushed and passed through a 60-mesh sieve. The sieved experimental raw material was placed in a cable extractor and petroleum ether was selected and applied for heat extraction for 2 hours to remove fat. After the completion of the experiment, 80% volume of ethanol was added to reflux for a period of time to remove small organic molecule substances such as glucose and dry it to constant weight to obtain pretreated raw materials for reserve.

1.2.2. Extraction of polysaccharides from Schisandra chinensis
Accurately weigh 1.00000g of pretreated schisandra powder in a three-necked flask, add the solvent according to a certain solid-liquid ratio, set the microwave power, ultrasonic power, reaction time, reaction temperature, and apply the XH-300UL computer microwave ultrasonic ultraviolet light combination catalyzed synthesizer to extract Schisandra chinensis polysaccharides. After completion, the extract was transferred to a centrifuge tube. The centrifuge operation was repeated three times. The experimental supernatant was collected and evaporated to 1/4 of the original volume using a rotary evaporator. The Sevag method was used (V chloroform: V n-butanol = 3:1) to remove protein, and then active carbon was added to remove pigment. Anhydrous ethanol was added to the solution until the volume fraction reached 80%, and the mixture was allowed to stand overnight, suction filtration, and dried to obtain the product.

1.2.3. Analysis of extraction ratio of polysaccharide from Schisandra chinensis and determination of conversion factor
Based on the previous experiments [6], extraction rate of Schisandra chinensis polysaccharide was applied by the author as the study index, glucose solution as the reference substance, the phenol-sulfuric acid method was applied to calibrate the polysaccharides of Schisandra chinensis. The abscissa was glucose concentration μg/ml, and the ordinate is the absorbance A, and its standard curve is determined as Y=0.0099X-0.017 (R²=0.9997). At the same time, the conversion factor of polysaccharides and glucose of Schisandra chinensis was measured, f=1.76, and calculated using the following equation.

Polysaccharide extraction rate (%) = \( \frac{\text{Polysaccharide mass (g)}}{\text{Herbal mass (g)}} \times 100\% \)

2. Single-factor experiments and results analysis

2.1. Single factor experiments and results analysis of polysaccharide extracted by ultrasonic-microwave synergistic effect

2.1.1. Effects and selection range of ultrasonic power
The results of the extraction efficiency of polysaccharides from Schisandra chinensis by ultrasonic power are shown in Figure 1.
Figure 1 shows that the change of ultrasonic power increases the extraction rate of polysaccharides first and then decreases the extraction rate, and the extraction rate of polysaccharides significantly decreases when the ultrasonic power is greater than 60W. The reason may be that the glycosidic bonds of polysaccharides of Schisandra chinensis have broken and destroyed the macromolecular chains of polysaccharides when the ultrasonic power is too large. Select the process optimization experiment in the range of 50W to 70W.

2.1.2. Influence and selection range of extraction temperature

The extraction rate of polysaccharides from Schisandra chinensis affected by the extraction temperature is shown as in Figure 2.
As can be seen in Figure 2.2, the extraction rate of polysaccharides increases sharply in the range of 55°C to 65°C. This is because the higher temperature accelerates the Brownian movement of the molecule, and it is also advantageous to destroy the tissue structure of the Schisandra cell. However, when the temperature is too high, the polysaccharide molecules can be easily decomposed to form small molecular substances, so that the polysaccharide content is reduced. Therefore, the process optimization experiment is performed within the range of 55°C to 75°C.

2.1.3. Influence and selection range of extraction time

The results of the extraction rate of polysaccharides from Schisandra chinensis affected by the extraction time are shown in Figure 3.

![Extraction rate of polysaccharide by extraction time](image)

**Fig 3 Extraction rate of schisandra polysaccharide by extraction time**

Figure 3 shows that with the extension of extraction time, the polysaccharide extraction rate increases first and then decreases slightly. The synergetic effect of ultrasound and microwave accelerates the destruction of the cell wall and other tissue structures, which results in rapid dissolution of polysaccharides. In summary, the range of 10 min to 25 min is selected to perform process optimization experiments.

2.1.4. Influence and selection range of solid-liquid ratio

The results of the extraction rate of polysaccharide from Schisandra chinensis by the solid-liquid ratio are shown in Figure 4.
Figure 4 shows that the extraction rate of polysaccharides of Schisandra chinensis decreases when the solvent is too much. The reason may be that the increase of the solvent amount enhances the extraction rate of impurities and inhibits the dissolution of polysaccharides. Therefore, the ratios of 1:15, 1:25, and 1:35 are applied to perform process optimization experiments.

2.1.5. The influence of microwave power and the determination of power

The results of the extraction of polysaccharides from Schisandra chinensis by microwave power are shown in Figure 5.
Figure 5 shows that after the power exceeds 550W, the polysaccharide extraction rate decreases with the increase of microwave power. It may be that high-power is favorable for the decomposition of polysaccharides to form small-molecule substances, so that the extraction rate of polysaccharides is decreased. Therefore, the condition with a microwave power of 550W is applied to perform process optimization experiments in this research.

3. Optimization experiment and result analysis of response surface

3.1. Determination of response surface experimental scheme
Response surface experimental factors design on the Box-Behnken software is applied to optimize the single factor experiment in the early stage, with the experimental scheme shown as in Table 1.

| Level | Extraction temperature/℃ | Ultrasonic power/W | Solid-liquid ratio/g · ml | Extraction time/min |
|-------|---------------------------|--------------------|--------------------------|---------------------|
| -1    | 55                        | 50                 | 1:15                     | 10                  |
| 0     | 65                        | 60                 | 1:25                     | 15                  |
| 1     | 75                        | 70                 | 1:35                     | 25                  |

3.2. Design and result analysis of response surface experiment
Response surface experiments were performed with the experimental data in Table 1. The results are shown in Table 2.

| Test number | A extraction temperature / ℃ | B Ultrasonic power/W | C Solid-liquid ratio / g · ml | D extraction time/min | Polysaccharide extraction rate/% |
|-------------|------------------------------|----------------------|------------------------------|----------------------|--------------------------------|
| 1           | -1                           | 0                    | 0                            | -1                   | 9.58                            |
| 2           | 1                            | 0                    | 1                            | 0                    | 9.65                            |
| 3           | 0                            | 0                    | 0                            | 0                    | 13.23                           |
| 4           | -1                           | 1                    | 0                            | 0                    | 9.17                            |
| 5           | 0                            | -1                   | 1                            | 0                    | 10.29                           |
| 6           | -1                           | 0                    | -1                           | 0                    | 9.23                            |
| 7           | 1                            | -1                   | 0                            | 1                    | 10.81                           |
| 8           | 0                            | 0                    | 0                            | 0                    | 13.23                           |
| 9           | 0                            | 0                    | 0                            | 0                    | 13.23                           |
| 10          | 0                            | -1                   | 0                            | -1                   | 9.36                            |
| 11          | 1                            | 0                    | -1                           | 0                    | 10.15                           |
| 12          | 0                            | 1                    | 0                            | -1                   | 10.24                           |
| 13          | 0                            | 0                    | 0                            | 0                    | 13.23                           |
| 14          | 0                            | 1                    | -1                           | 0                    | 10.44                           |
| 15          | -1                           | 0                    | 0                            | 1                    | 10.31                           |
| 16          | 1                            | 0                    | 0                            | 1                    | 9.87                            |
| 17          | 0                            | 1                    | 1                            | 0                    | 9.62                            |
| 18          | 0                            | -1                   | 0                            | 1                    | 10.29                           |
| 19          | 0                            | 0                    | -1                           | 1                    | 11.16                           |
| 20          | 0                            | 0                    | 0                            | 0                    | 13.23                           |
| 21          | -1                           | 0                    | 1                            | 0                    | 9.93                            |
| 22          | 0                            | 0                    | -1                           | -1                   | 10.39                           |
| 23          | 0                            | -1                   | -1                           | 0                    | 9.39                            |
| 24          | 0                            | 1                    | 0                            | 1                    | 9.83                            |
| 25          | 1                            | 1                    | 0                            | 0                    | 8.91                            |
| 26          | 0                            | 0                    | 0                            | 0                    | 13.23                           |
| 27          | 0                            | 0                    | 1                            | -1                   | 11.36                           |
| 28          | 1                            | 0                    | 0                            | -1                   | 10.31                           |
| 29          | -1                           | -1                   | 0                            | 0                    | 8.32                            |
The Box-Behnken software was applied to perform multivariate regression fitting for the experimental data. The quadratic regression model for the four factors of ultrasonic power $A$, extraction temperature $B$, extraction time $C$, and solid-liquid ratio $D$ of polysaccharide extraction rate $Y$ from Schisandra was obtained as follows: 

$$
Y = +13.23 + 0.13A + 0.11B + 0.075C + 0.086D - 0.29AB - 0.30AC - 0.29BD - 0.43BC - 0.33BD - 0.33CD - 2.19A^2 - 2.14B^2 - 1.22C^2 - 1.09D^2
$$

Where, the correction coefficient of the model is $R^2_{pred}=0.9842$, the correlation coefficient of the second-order polynomial is $R^2=0.9973$, and $R^2_{Adj}=0.9945$, which indicates that the software model can explain the change of the response value of 98.42%, that is, the degree of equation fitting for this experiment is favorable. $R^2_{pred}$ and $R^2_{Adj}$ are reasonable, so this model can be used for the design and guidance of the response surface.

3.3. Variance analysis of the response surface experiment

A variance analysis was performed on the experimental model. The results of the analysis are shown in Table 3.

| source                  | Sum of squares | df  | Mean Square | F Value | p-value Prob>F | Significance |
|-------------------------|----------------|-----|-------------|---------|----------------|--------------|
| Model                   | 57.11          | 14  | 4.08        | 364.38  | <0.0001        | ***          |
| A-extraction temperature| 0.20           | 1   | 0.20        | 18.12   | 0.0008         | ***          |
| B-ultrasonic power      | 0.15           | 1   | 0.15        | 13.57   | 0.0025         | **           |
| C-Solid-liquid ratio    | 0.067          | 1   | 0.067       | 6.03    | 0.0277         | **           |
| D-extraction time       | 0.088          | 1   | 0.088       | 7.90    | 0.0139         | **           |
| AB                      | 0.33           | 1   | 0.30        | 29.53   | <0.0001        | ***          |
| AC                      | 0.36           | 1   | 0.36        | 32.16   | <0.0001        | ***          |
| AD                      | 0.34           | 1   | 0.34        | 30.57   | <0.0001        | ***          |
| BC                      | 0.74           | 1   | 0.74        | 66.07   | <0.0001        | ***          |
| BD                      | 0.45           | 1   | 0.45        | 40.10   | <0.0001        | ***          |
| CD                      | 0.44           | 1   | 0.44        | 38.91   | <0.0001        | ***          |
| A^2                     | 31.23          | 1   | 31.23       | 2789.58 | <0.0001        | ***          |
| B^2                     | 29.72          | 1   | 29.72       | 2654.58 | <0.0001        | ***          |
| C^2                     | 9.68           | 1   | 9.68        | 864.78  | <0.0001        | ***          |
| D^2                     | 7.64           | 1   | 7.64        | 682.64  | <0.0001        | ***          |
| Residual                | 0.16           | 14  | 0.011       |         |                |              |
| Lack of Fit             | 0.16           | 10  | 0.016       |         |                |              |
| Pure Error              | 0.000          | 4   | 0.000       |         |                |              |
| Cor Total               | 57.26          | 28  |             |         |                |              |
| R^2                     | 0.9973         | 0.9945 | 0.9842        |         |                |              |

Note: *** stands for extremely significant difference (P<0.001); ** stands for highly significant difference (P<0.05)

From Table 3, it can be concluded that the model reaches a very significant level of difference, that is, P<0.0001 and the error is not significant, indicating that the experimental error is small and the experimental results are favorable. Through the variance analysis, it can be concluded that the highly significant difference items are B, C and D. The highly significant difference items are A, AB, AC, AD, BC, BD, CD, A2, B2, C2, and D2. From the P value and F value, we can see that the order of the factors affecting polysaccharide extraction efficiency of Schisandra chinensis is: extraction
temperature B>ultrasonic power A>extraction time D>solid-liquid ratio C. Combined with Table 3 and 3.2, the best extraction process can be obtained as follows: at a constant microwave power of 550 W, the extraction temperature of Schisandra chinensis polysaccharide is 55° C, the ultrasonic power for extraction 60 W, the extraction time 15 min, and the solid-liquid ratio 1:25. At this point, the polysaccharide extraction rate reaches a maximum of 13.23%.

3.4. Analysis and results of response surface
The response surface analysis is shown in Figure 6-11.

Fig. 6 Interaction influences between reaction temperature and ultrasonic wave power on polysaccharide extraction ratio of Schisandra chinensis.

Fig. 7 Interaction influences between reaction temperature and solid-liquid ratio on polysaccharide extraction ratio of Schisandra chinensis

Fig. 8 Interaction influences of reaction temperature and time on polysaccharide extraction ratio of Schisandra chinensis
From Fig. 6-11, the curve of Fig. 6 shows the strongest variation and the most significant curve bending. The extraction temperature shows the most significant effect on the extraction rate of polysaccharides from Schisandra chinensis, followed by the ultrasonic power, the extraction time, and the solid-liquid ratio. The results of the analysis results are consistent with results by variance analysis.
4. **Experimental verification and conclusion**

On the basis of single factor tests, the optimized conditions were obtained through Box-Behnken analysis: extraction temperature 65°C, microwave power 550W, ultrasonic power 60W, reaction time 15 min, solid-liquid ratio 1:25, polysaccharide extraction rate 13.23%. The difference between the actual measurement result and the prediction result is small and the result is reliable.

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