Study on Optimization of extraction process of *Lycium barbarum* L. polyphenols by Response Surface Methodology

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Abstract. Extracting the polyphenol from the fruit of the *Lycium barbarum* L. by using a solvent method. On the basis of single factor test, the content of chlorogenic acid in polyphenol was the main factor, and the content of chlorogenic acid in polyphenol was the response value. The extraction conditions were optimized by three factors and three levels Box-Behnken test. The results showed that the relative effects of each factor on the extraction rate of *Lycium barbarum* L. polyphenols were as follows: extraction solvent, solid-liquid ratio and extraction times. Through the regression model analysis predicted by response surface method, the optimum extraction conditions of *Lycium barbarum* L. polyphenols can be predicted as follows: 72% methanol, the ratio of material to liquid is 1:19, the extraction times is 2.15 times, and the content of chlorogenic acid in Lycium barbarum is 0.0842 g/100g. In order to investigate the consistency between the test results and the real situation, the verification test was carried out. The extraction process was set to 2 times, and the content of chlorogenic acid was 0.0837 g/100g. The predicted process conditions by regression equation are in good agreement with the actual situation, which proves that the model is effective and the process is stable and feasible.

1. Introduction

*Lycium barbarum* L., selected in the ‘Chinese Pharmacopoeia 2010’, is a Chinese medicinal material that has the functions of nourishing the liver, nourishing the kidney and lungs. The main active ingredients are polysaccharides, polyphenols, flavonoids, carotenoids and trace elements[1-3]. Due to the anti-oxidation and anti-aging properties of polyphenols, its extraction process is now increasingly concerned by researchers. Among the polyphenols, chlorogenic acid has high antibacterial and antiviral pharmacological properties due to its high content, which has also attracted the attention of researchers[4].

The response surface analysis method uses reasonable experimental design methods and obtains certain data through experiments, uses multiple quadratic regression equations to fit the functional relationship between factors and response values, and seeks the optimal process parameters through analysis of regression equations. A statistical method for solving multivariate problems[5-7]. In the extraction process of *lycium barbarum* polyphenols, different liquid-to-material ratios, extraction time, and ethanol concentration were used as single-factor tests to investigate the effect of different extraction conditions on the extraction efficiency of polyphenols. On this basis, a response surface test was
designed to the best extraction process is optimized to determine the best extraction process for *Lycium barbarum* L. polyphenols.

2. Materials and Methods

2.1 Materials.

The *Lycium barbarum* L. fruit was from Zhongning, Ningxia. Methanol, anhydrous ethanol, methanol, acetonitrile and formic acid are all chromatographically pure; chlorogenic acid and caffeic acid standards (Sigma company, American).

High performance liquid chromatograph (U.S. Waters) - UV detector (2487); T10basic high speed homogenizer (Germany); Accuracy 0.01 mg electronic balance (Swiss mettler-tolido group); UV-2000 ultraviolet spectrophotometer (Unico instrument co., LTD); Td-401 centrifuge (Shanghai anting scientific instrument factory); HF-5B ultrasonic circulation extractor (Ningbo Xinzhi biotechnology company); MS3 vortex mixer (German Digital company), KQ-200VDB dual-frequency numerical control ultrasonic cleaner, B-260 rotary evaporator (Shanghai Yarong chemical instrument factory).

2.2 Methods.

2.2.1 Test sample preparation.

Place the *Lycium barbarum* L. fruit in a 55°C oven, dry to constant weight, crush it through a 100 mesh sieve, and place it in a desiccator for use.

2.2.2 Preparation of standard samples.

Dissolve the purchased chlorogenic acid standard with methanol, accurately weigh 10 mg, dissolve it in a 10 mL volumetric flask, prepare a 1000 mg/L standard mother liquor, and store in -20°C refrigerator.

2.2.3 Standard curve drawing of chlorogenic acid.

Take 0.2, 0.4, 0.5, 0.6, 0.8, 1.0, and 1.5 mL of chlorogenic acid 1000 mg/L standard solution respectively, place in a 10 mL volumetric flask, and add methanol to a solution volume of 10 mL. After thoroughly mixing, pour into the solution In the phase loading sample bottle, the standard working curve is plotted with the peak area versus concentration in the sample bottle.

2.2.4 Determination method of polyphenol content in *Lycium barbarum* L. by high performance liquid chromatography.

HPLC detection conditions: water e2695 HPLC detector-UV detector; Agilent ZORBAX SB-C18 (4.6 × 250mm, 5 μm) chromatographic column; column temperature 30°C; injection 10 μL; detection wavelength 360nm; mobile phase A 1% formic acid, B acetonitrile, gradient elution.

2.2.5 Single factor experiment of polyphenol extraction from *Lycium barbarum* L.

The ratio of methanol to water, material-liquid ratio, and number of extractions are the key factors influencing the extraction of medlar polyphenols, and the extraction content of chlorogenic acid is used as the evaluation index of extraction efficiency of medlar polyphenols.

a) Accurately weigh 10g (4 parts) of *Lycium barbarum* L. samples, and extract once under the conditions of extraction time of 2h and material-liquid ratio of 1:10, and investigate methanol solutions of different concentrations: 20% methanol, 50% methanol, 70% methanol, 100% methanol, the extraction efficiency of chlorogenic acid in *Lycium barbarum* L. extract.

b) Accurately weigh 10g (4 servings) of *Lycium barbarum* L., and extract once under the condition that the extraction time is 2h, and the extraction solvent is not 70% methanol. Under different conditions of solid-liquid ratio: 1: 5, 1:10, 1: 20, 1:30, the extraction efficiency of chlorogenic acid in wolfberry extract.
c) Accurately weigh 10g (4 parts) of *Lycium barbarum* L., under the condition that the extraction time is 2h, 70% methanol is extracted once, and the ratio of material to liquid is 1:10. The extraction efficiency of chlorogenic acid in *Lycium barbarum* L. extract.

### 2.2.6 Corresponding surface method to optimize extraction efficiency

In order to optimize the process conditions for extracting polyphenols from *Lycium barbarum*, based on the concentration of chlorogenic acid, according to the Box-Behnken test design principle, based on the single-factor test, a total of 17 test points, 12 analysis points, and 5 zero Estimation error: Weigh 10g of *Lycium barbarum* L. samples each time, and select three factors: extract concentration A, material-to-liquid ratio B, and number of extractions C for response surface analysis to determine the main factors of polyphenol extraction efficiency.

### 3. Results and discussion

#### 3.1 Method for determination of polyphenols in *Lycium barbarum* L.

Draw the chlorogenic acid standard curve under the condition of 360nm detection wavelength, as shown in Figure 1. The relationship between chlorogenic acid concentration (x) and peak area (y) is: 
\[
y = 16368x - 101212, \quad R^2 = 0.9993.
\]
That is, the peak area of chlorogenic acid content within 20-150 mg / L has a good linear relationship with the concentration.

![Figure 1. Standard curve of chlorogenic acid in Lycium barbarum L.](image)

#### 3.2 Single factor experiment of extracting polyphenols from *Lycium barbarum* L.

Taking the ratio of methanol to water, the ratio of material to liquid and the number of extractions as the influencing factors, the extraction content of chlorogenic acid and caffeic acid was used as the evaluation index.

#### 3.2.1 Effect of different extraction solvents on the concentration of *Lycium barbarum* polyphenols.

The extraction time is 2h, and the material-liquid ratio is 1:10. The extraction is 20% methanol, 50% methanol, 70% methanol, and 100% methanol.

| Serial number | Extraction reagent | Material-liquid ratio | Number of extractions | Extract time | Chlorogenic acid content (g/100g) |
|---------------|--------------------|-----------------------|-----------------------|--------------|----------------------------------|
| 1             | 20% Methanol       | 1:10                  | 1                     | 2h           | 0.0620                           |
| 2             | 50% Methanol       | 1:10                  | 1                     | 2h           | 0.0654                           |
| 3             | 70% Methanol       | 1:10                  | 1                     | 2h           | 0.0761                           |
| 4             | 100% Methanol      | 1:10                  | 1                     | 2h           | 0.0604                           |
When the methanol concentration is gradually increased, the chlorogenic acid content is also increased. After the methanol concentration is greater than 70%, the chlorogenic acid concentration is reduced. Adding water in the solvent appropriately to increase the polarity of the extraction solvent is beneficial to the extraction of polyphenols.

3.2.2 Effect of material-liquid ratio on the concentration of Lycium barbarum polyphenols.
The extraction time is 2h, 70% methanol is extracted once, and the material-liquid ratio is 1: 5, 1:10, 1:20, 1:30.

| Seria number | Material-liquid ratio | Number of extractions | Extract time | Chlorogenic acid content (g/100g) |
|--------------|-----------------------|-----------------------|--------------|---------------------------------|
| 1            | 1: 5                  | 1                     | 2h           | 0.0467                          |
| 2            | 1: 10                 | 1                     | 2h           | 0.0761                          |
| 3            | 1: 20                 | 1                     | 2h           | 0.0830                          |
| 4            | 1: 30                 | 1                     | 2h           | 0.0727                          |

With the increase of the material-liquid ratio, the extraction efficiency of polyphenols increased. When the material-liquid ratio reached 1:20, the concentration of chlorogenic acid reached the maximum; when the material-liquid ratio continued to increase to 1:30, chlorogenic acid The content is reduced, this may be because the liquid ratio in the solution is increased, and it is no longer conducive to the dissolution of chlorogenic acid, so the concentration is lower than that when the material-liquid ratio is 1:20 [8].

3.2.3 Number of extractions
The extraction time is 2h, 70% methanol is extracted once, and the material-liquid ratio is 1:10. The extraction is performed once, twice, three times, and four times.

| Seria number | Extraction reagent | Material-liquid ratio | Number of extractions | Extract time | Chlorogenic acid content (g/100g) |
|--------------|-------------------|-----------------------|-----------------------|--------------|----------------------------------|
| 1            | 70% Methanol      | 1: 10                 | 1                     | 2h           | 0.0761                           |
| 2            | 70% Methanol      | 1: 10                 | 2                     | 2h           | 0.0782                           |
| 3            | 70% Methanol      | 1: 10                 | 3                     | 2h           | 0.0792                           |
| 4            | 70% Methanol      | 1: 10                 | 4                     | 2h           | 0.0785                           |

When the number of extractions reaches 3, the extraction concentration reaches the maximum, and there is no significant difference in the content of chlorogenic acid between the 2nd extraction and the 3rd extraction.

3.3 Response surface method to optimize experimental design
In order to optimize the process conditions for extracting polyphenols from Lycium barbarum, based on the concentration of chlorogenic acid, according to the Box-Behnken test design principle, based on the single-factor test, a total of 17 test points, 12 analysis points, and 5 zero Estimation error: Weigh 10g of Lycium barbarum L. samples each time, and select three factors: extract concentration A, material-to-liquid ratio B, and number of extractions C for response surface analysis to determine the main factors of polyphenol extraction efficiency. The factors and levels of response surface analysis are shown in Table 4, and the analysis results are shown in Table 5.
Table 4. Response surface analysis factors and levels

| Factor               | Level                  |
|----------------------|------------------------|
| A Extract concentration | 50% Methanol, 70% Methanol, 100% Methanol |
| B Material-liquid ratio | 1:10, 1:20, 1:30       |
| C Number of extractions | 1, 2, 3               |

Table 5. Response surface analysis test results

| Seria number | A | B | C | Chlorogenic acid content (g/100g) |
|--------------|---|---|---|-----------------------------------|
| 1            | -1 | -1 | 0 | 0.0732                           |
| 2            | 1  | -1 | 0 | 0.0624                           |
| 3            | -1 | 1  | 0 | 0.0618                           |
| 4            | 1  | 1  | 0 | 0.0700                           |
| 5            | -1 | 0  | -1| 0.0813                           |
| 6            | 1  | 0  | -1| 0.0802                           |
| 7            | -1 | 0  | 1 | 0.0731                           |
| 8            | 1  | 0  | 1 | 0.0659                           |
| 9            | 0  | -1 | -1| 0.0761                           |
| 10           | 0  | 1  | -1| 0.0727                           |
| 11           | 0  | -1 | 1 | 0.0792                           |
| 12           | 0  | 1  | 1 | 0.0806                           |
| 13           | 0  | 0  | 0 | 0.0798                           |
| 14           | 0  | 0  | 0 | 0.0790                           |
| 15           | 0  | 0  | 0 | 0.0796                           |
| 16           | 0  | 0  | 0 | 0.0800                           |
| 17           | 0  | 0  | 0 | 0.0807                           |

Using the design-expert software to perform multiple regression fitting on the data in Table 5. The quadratic polynomial regression model equations for the extraction solvent, material-liquid ratio and extraction times of *Lycium barbarum* L. polyphenol are obtained: Y = 0.07982 - 0.00244A + 2.5 × 10^-5B + 3.87 × 10^-4C + 4.75 × 10^-3AB - 0.00367AC - 0.0003BC - 5.67 × 10^-3A^2 - 7.30 × 10^-3B^2 - 3.13 × 10^-3C^2

The coefficient of determination R^2 = 0.8598 (greater than 0.8), the coefficient of variation CV is 4.92%, indicating that the model has a variation of 14.02%. It can be used to analyze the extraction process of chlorogenic acid in *Lycium barbarum* L.

![Figure 2. Effect of ratio of material to liquid and extraction concentration on extraction amount of *Lycium barbarum* L. polyphenols](image1.png)

![Figure 3. The effect of the concentration of the extract and the number of times of extraction on the extraction of the polyphenol from *Lycium barbarum* L.](image2.png)

![Figure 4. Effect of the ratio of liquid and the number of times of extraction on the extraction of polyphenol from *Lycium barbarum* L.](image3.png)
Figure 2 is a graph of the response curve of the ratio of material to liquid and the concentration of chlorogenic acid when the number of extractions is two. It can be seen from the three-dimensional graph that the concentration of chlorogenic acid is the highest in the extract concentration of 60% to 85% and the material-liquid ratio is 1:16-1:20, and the slope of the response surface changes significantly, indicating that the methanol extract The concentration and material-liquid ratio have a significant effect on the chlorogenic acid content. When the extraction concentration is less than 65%, the chlorogenic acid concentration gradually increases with the increase of the material-liquid ratio. When the material-liquid ratio exceeds this value, the chlorogenic acid concentration shows a downward trend, indicating that the concentration of the extraction solution in the extraction process It has a dominant effect and has a significant effect on the concentration of polyphenols.

Figure 3 shows the response curve of the extraction concentration and the number of extractions to the chlorogenic acid content when the material-liquid ratio is 1:20. It can be seen from the three-dimensional graph that the methanol concentration of the extract is between 60% and 80%, the number of extractions is between 2 and 4 times, and the slope of the response surface is gentle, indicating that the concentration of the extract and the number of extractions affect the chlorogenic acid content. Not significant, which is consistent with the results in Table 3.

Figure 4 shows the response curve of the ratio of material to liquid and the number of extractions on the content of chlorogenic acid when the concentration of the extract is 70%. It can be seen from the three-dimensional graph that when the ratio of material to liquid is 1:20-1:25 and the concentration of methanol in the extraction liquid is between 50% and 70%, the slope of the response surface is gentle. When the ratio of material to liquid exceeds a certain ratio, there are many medlars The concentration of phenol decreases slightly with the increase of the material-liquid ratio. This is because the increase of the material-liquid ratio decreases the viscosity of the solution and reduces the diffusion resistance of polyphenols, which is conducive to the dissolution of polyphenols, thereby gradually increasing the polyphenol concentration. If the feed-liquid ratio is too large, it will cause the loss of polyphenols in the subsequent extraction process, resulting in a decrease in the concentration of polyphenols. When the extraction concentration is constant, the concentration of Lycium barbarum polyphenols increases slowly as the number of extractions increases, indicating that the number of extractions is not the dominant factor in the extraction of polyphenols.

According to the regression model analysis predicted by the response surface method, the optimal extraction process conditions for the medlar polyphenols can be predicted to be: 72% methanol, the material-liquid ratio is 1:19, the extraction times are 2.15 times, the medlar chlorogenic acid content It is 0.0842g / 100g.

In order to check the consistency of the test results with the real situation, a verification test was carried out. Taking into account the convenience of the actual operation, the extraction process was set to 2 times, and the chlorogenic acid content was 0.0837 g / 100g, which was obtained through the prediction of the regression equation. The process conditions are consistent with the actual.

4. Conclusion

4.1 This experiment uses response surface analysis to optimize the extraction experiment of medlar polyphenols. Through the Box-Behnken model, a regression analysis method for the interaction between three single factors is designed. Using this method can reduce the number of trials, To get a higher precision regression equation, thereby greatly improving the experimental efficiency [9].

4.2 The experimental results fully verify the correctness of the model, indicating that the response surface method is effective in optimizing the extraction process of Lycium barbarum polysaccharides. In this experiment, methanol water extraction method was used, and the three-factor three-level response surface analysis method was used to optimize the experiment. A single factor investigation was conducted on the concentration, material-liquid ratio and number of extractions of medlar polyphenol extraction. Response surface experiments were carried out to determine the best extraction conditions of Lycium barbarum L. polyphenols, and the content was determined by high performance liquid chromatography. The optimal process conditions for the extraction of crude polyphenols from
Lycium barbarum L. are as follows: methanol with a concentration of 72% is extracted, the ratio of material to liquid is 1:19, and the number of extractions is 2 times.

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