Optimization on the Extraction of Polysaccharides from Fructus Corni Using Uniform Design

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Abstract

Uniform Design was used to optimize the effects of processing parameters for extraction of polysaccharides from Fructus Corni. Four independent variables including extraction temperature (℃), extraction time (min), liquid ratio (times) and pH value were studied. The optimal conditions were determined and tri-dimensional response surfaces were plotted according to the try grid method. The results indicated that liquid ratio (times) and pH value variables markedly affect the polysaccharides value. Relevant parameters of the extract of the highest concentration of polysaccharide are as follows that the liquid ratio is 1:33 with the pH value of 8.5. Under optimized conditions the experimental yield was in agreement with the predicted value.

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Keywords: Fructus Corni; Polysaccharides; Extraction; Uniform Design

Uniform design is a multi-factor optimization experimental design method, which was created by famous Chinese mathematicians Fang Kaitai and Wang Yuan [1]. Through this design method, the test points can be fully "dispersed uniformly" within the experimental range and each point is of better representativeness. Hence, the number of test points can be reduced greatly and also the factor level can be adjusted properly, preventing high-grade level and low-grade level from meeting. Thus, it is a powerful tool to select optimization conditions when seeking the best experimental conditions, the best proportion, etc [2]. In recent years, it has been applied widely in the optimization of extraction technologies. Fructus Corni is mainly distributed in Zhejiang, Henan, Anhui, etc. Japanese scholars used to research Fructus Corni [3-5]. Organic chemicals in Fresh fructus corni mainly include reducing sugar, polysaccharide, organic acids, phenols, glycoside, iridoids, saponins, tannin, etc. Also, its pulp and core contain over 10 amino acids and rich vitamin B, vitamin C and over 20 mineral substances. Polysaccharides are indispensable substances for life metabolism, and can adjust the immune function, improve the organism
immunity, and have the effects of anti-aging, anti-tumor and anti-AIDS, etc. Also, there have been some reports on the extraction of fructus corni polysaccharides, but the extraction rate was not ideal. To extract polysaccharides more effectively from fructus corni, the authors analyze effects of extraction temperature, time, solid-liquid ratio and pH value on the polysaccharides yield and make a uniform design for the fructus corni polysaccharides extraction \cite{6} to select the best extraction process conditions at a lower cost.

1. Materials and methods

1.1 Materials and instruments

Dried fructus corni is made in Anhui; D (+)-glucose and phenol (Fluka split packaging) are imported by Shanghai HUASHUO Fine Chemicals Co., LTD; and others are the domestic analytical reagents. TU-190 ultraviolet and visible spectrophotometer is from Beijing PERSEE; TDL80-2B centrifuge, thermostat water bath and precision acidity meter are from Shanghai TIANDA Instrument Co., LTD; and others are electronic scale, miniature turbine mixer and all-powerful pulverizer, etc.

1.2 Extraction of fructus corni polysaccharide

1.2.1 Technological process

The technological process is shown as below:

Weigh dried Fructus Corni sifted with a 100-mesh sieve → water extract adjust solid-liquid ratio → adjust pH → set temperature → set time → extract → centrifuge → add supernatant \cite{7}

1.2.2 Extracting method by using uniform design to optimize fructus corni polysaccharides

Grind dried Fructus Corni and sift with a 100-mesh sieve to gain the powder. Add eightfold-volume 95% ethanol to reflux for 8 hours to defat. Cool and filter it, and take residues and dry them. Set aside. Weigh a certain amount of pre-processed Fructus Corni powder, and then select the extraction temperature, time, solid-liquid ratio and pH value. Conduct the four-factor and ten-level uniform design experiment by using the contents of Fructus Corni as investigation indexes to optimize the extraction conditions of the Fructus Corni polysaccharides.

1.2.3 Analytical method

(1) Content determination of fructus corni polysaccharides: Use phenol-sulfuric acid method \cite{9} and take glucose as standard substance to absorb a certain volume of polysaccharide extract, and determine the light-sucking value based on the standard curve method, and calculate the relative content of polysaccharide based on standard curve.

(2) Determination of the Maximum Absorption Wavelength: Prepare different-concentration glucose standard solution and polysaccharide extract and then operate based on the making methods of standard curve, and scan 400 nm-500nm wavelengths to absorb spectrums. The result is that standard substances and sample substances are similar in terms of absorbing spectrums and have the maximum absorption at 489nm; therefore, 489nm is selected as measurement wavelength.

(3) Establishment of standard curve: Accurately absorb 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml, 0.6ml, 0.7ml, 0.8ml and 0.9ml glucose standard fluids (concentration: 0.114 mg/ml) into test tube slowly and add distilled water until it is 1ml, add 1ml 5% phenol (saved in refrigerator to avoid light), add 5ml 98% concentrated sulfuric acid and stir uniformly, and set aside at room temperature quietly for 30 min. Make a blank control with distilled water, and use TU-190 ultraviolet and visible spectrophotometer to measure the absorbance at 489nm. The result is shown in Table 1.

Table 1: Standard Polysaccharide Concentration (SPC)—Absorbance
Make a regression processing on the above data and gain the regression equation $C = 0.0975A + 0.0011$, and related coefficient $r$ is 0.9995.

### 1.3.4 Determination of polysaccharide content of sample solution

Accurately absorb 0.1ml extract after dilution and add distilled water until it is 1ml based on standard curve method (the same operation method).

## 2. Result and analysis

### 2.1 Single-factor experiment

(1) Effects of extraction temperature on extraction process: Separately weigh N samples of the processed dried Fructus Corni powder. Each sample weighs 10g. Add 200ml distilled water to each sample and stir them respectively for 90 min at different temperatures. Centrifuge the mixture at 4000r/min for 20min, and measure the sugar concentration. The result is shown in Figure 1.

![Fig.1 Effects of Extraction Temperature on Extraction Rate of Polysaccharides](image)

From figure 1, it is known that the change of polysaccharides concentration is not significant under the different temperature extractions.

(2) Effects of extraction time on extraction process: Separately weigh processed dried Fructus Corni powder (10g) and add 200ml distilled water, and stir them for 150 min and extract the mixture at 80°C at different times. Centrifuge the mixture at 3500r/min for 20min, and measure the sugar concentration. The result is as shown in Figure 2.

![Fig.2: Effects of Extraction Time on Extraction Rate of Polysaccharides](image)

From figure 2, it is known that the change of polysaccharides concentration is not significant under the extraction of different times.

(3) Effects of different water content on extraction process: Separately weigh processed dried Fructus Corni powder (10g) and add different amounts of distilled water, stir at 80°C for 150min and extract the mixture. Centrifuge the mixture at 3500r/min for 20min, and measure the sugar concentration. The result is shown in Figure 3.

![Fig.3: Effects of Different Solid-liquid Ratio on Extraction Rate of Polysaccharides](image)
From figure 3, it is known that the polysaccharide concentration in extraction increases when different water content is extracted, but it changes little when water content reaches a certain extent.

(4) Effects of pH Value on Extraction Process: Separately weigh processed dried Fructus Corni powder (10g) and add 200ml distilled water, and stir them for 150 min and extract the mixture at 80°C at different times. Centrifuge the mixture at 3500r/min for 20min, and measure the sugar concentration. The result is shown in Figure 4.

![Fig.4: Effects of pH Value on Extraction Rate of Polysaccharides](image)

From figure 4, it is known that different pH values have effects on polysaccharide concentration of extract, and the polysaccharide of fructus corni is easier to be extracted under the condition of neutral to alkali.

2.2 Uniform experimental design

(1) Uniform design experimental plan: In the process of extracting fructus corni polysaccharides, the investigated factors mainly include extraction temperature, time, water amount and pH value. There are temperature X1 (55°C-100°C), time X2 (30min-200min), water amount X3 (6-33 times) and pH value (4-8.5). According to the values of all factors and the experimental accuracy requirements, all factors are designed in a level table (see Table 2). Then, based on the U10*(108) in the uniform design table, the experimental plan can be designed (see Table 3).

Table 2: the Uniform Design Factor Levels

<table>
<thead>
<tr>
<th>Factor</th>
<th>Levels*</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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</thead>
<tbody>
<tr>
<td>X1(°C)</td>
<td></td>
<td>55</td>
<td>60</td>
<td>65</td>
<td>70</td>
<td>75</td>
<td>80</td>
<td>85</td>
<td>90</td>
<td>95</td>
<td>100</td>
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<tr>
<td>X2(min)</td>
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<td>50</td>
<td>70</td>
<td>90</td>
<td>110</td>
<td>130</td>
<td>150</td>
<td>170</td>
<td>180</td>
<td>200</td>
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<tr>
<td>X3(times)</td>
<td></td>
<td>6</td>
<td>9</td>
<td>12</td>
<td>15</td>
<td>18</td>
<td>21</td>
<td>24</td>
<td>27</td>
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<td>33</td>
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<tr>
<td>X4(pH)</td>
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<td>5.5</td>
<td>6</td>
<td>6.5</td>
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<td>7.5</td>
<td>8</td>
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Table 3: the Experimental Plan U10*(108)

<table>
<thead>
<tr>
<th>Level</th>
<th>X1(°C)</th>
<th>X2(min)</th>
<th>X3(times)</th>
<th>X4(pH)</th>
<th>Polysaccharide concentration (mg/ml)</th>
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<tbody>
<tr>
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<td>55</td>
<td>70</td>
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<tr>
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<td>100</td>
<td>170</td>
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<td>6.5</td>
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</tr>
</tbody>
</table>

(2) Analysis on results of uniform design experiment: Wang Yufang’s uniform design software 3.0 can be used for multivariate gradual regression analysis based on the data in Table 3, and ultimately the regression equation and corresponding parameters can be gained.

Regression equation: \[ y = b(0) + b(1) \times X(3) + b(2) \times X(4) \]
Regression coefficient: $b(i): b(0) = 4.858; b(1) = 0.1257; b(2) = 0.3591$

Standard regression coefficient: $B(i): B(1) = 0.8144; B(2) = 0.3879; \text{multiple correlation coefficient: } R = 0.9696; \text{determination coefficient: } R^2 = 0.9402; \text{correction determination coefficient: } R^2a = 0.9327$

Regression equation significant inspection: sample capacity $N=10; \text{significance level } \alpha = 0.05; \text{check value } F_t = 55.01; \text{critical value } F(0.05, 2, 7) = 4.737, F_t > F(0.05, 2, 7); \text{Regression equation is significant.}$

Residual standard deviation: $s = 0.3887.$

The contribution of elements of all equations to regression (per partial regression square and descending order): $U (1) = 11.26, U (1)/U = 67.73%; U (2) = 2.554, U (2)/U = 15.37%.$

From the standard regression coefficients of all factors, the factor exerting the biggest effect on polysaccharide concentration is solid-liquid ratio within the above levels and follows pHs, but the extraction temperature and time have insignificant effects on the concentration. Thus, when the dried fructus corni powder is under the 100-mesh sieve condition, the extraction temperature and time have insignificant effects on extraction rate of polysaccharides. To increase the extract concentration and maximize the extraction rate, it is better to only select the proper solid-liquid ratio and pH, but not to raise temperature and time. The experimental optimization method is the grid trial method. The best extraction condition is $X_3 = 33$ and $X_4 = 8.5; \text{the pre-test polysaccharide concentration } Y \text{ is } 12.06 (+ / – 0.9190 \text{ mg/ml}).$

(3) Three-dimensional graphic surface figure can be drawn in the experimental range based on the solid-liquid ratio (Figure 5).

![Fig. 5: 3D Graphic Surface for the Effects of X4 and X3](image)

From figure 5, it can be seen that the factor influencing the polysaccharide concentration most is the solid-liquid ratio ($X_3$) in the curve, and follows pH ($X_4$). Figure-5 shows that the results of the 3-D graphic surfaces for polysaccharide extraction are consistent with the above analysis.

(4) Verifying the experiment: To verify the reliability of optimization results, $X_1=80^\circ \text{C}, X_2=60\text{min}, X_3=33$ and $X_4=8.5$ are used to verify the experiment (operate it in parallel for four times). The average polysaccharide concentration is 12.68 mg/ml and is close to the predicted value. Thus, this uniform design is of significance.

3. Conclusion

Through the above uniform design, the optimization condition to extract polysaccharose from fructus corni is that the solid-liquid ratio is 1:33 and pH is 8.5; and extraction temperature and time have insignificant influences on polysaccharides concentration within the experimental range. The fructus corni in this experiment was made in Anhui. Regretfully, due to the limited conditions, the other conditions of polysaccharides extraction, the polysaccharides purification and properties analysis were not studied further.

References


