Optimization of extraction of polyphenols from chestnut shell by response surface methodology

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Abstract. Chestnut shell is one of the main by-products in the chestnut processing, which contains abundant polyphenols, so it can be used as a source of polyphenols. In this study, using ethanol as the extraction solvent, the optimum extraction conditions of polyphenols from chestnut shell were determined by single factor experiment and response surface experiment: liquid-solid ratio of 39:1, extraction temperature of 74°C, extraction time of 91 min, ethanol concentration of 65%. Under these conditions, the extraction rate (ER) of polyphenols from chestnut shell was 6.09%. The results can assist in better exploitation of chestnut shell, which can not only reduce resource waste and environmental pollution, but also obtain economic benefits.

1. Introduction
Chestnut, a species of the Fagaceae family, is widely distributed in Asia, Americas and Europe [1] The main economic varieties are Castanea mollissima BL., Castanea sativa Mill., Castanea dentata Marsh. and Castanea crenata Sieb. & Zucc. China has a long history of chestnut cultivation, which is widely distributed in the Yangtze River Valley and North China, among which Shandong, Henan, Hubei, Hebei, Anhui, Zhejiang and Guangxi are the main production areas of chestnut. In addition to starch, protein and fat, chestnut kernel also contains a variety of vitamins and minerals, and rich in unsaturated fatty acids and flavonoids, with a variety of health care effects. At present, chestnut kernel has been processed into chestnut flour, chestnut candied fruit, chestnut wine, chestnut bread and other food with chestnut flavor [2].

Chestnut shell is composed of inner shell and outer shell, accounting for about 10%~15% of the total weight of chestnut [3]. The main components of chestnut shell are lignin (41.7%, dry weight) and carbohydrates (41.6%, dry weight), among which cellulose is the main carbohydrate (28.4%, dry weight), followed by xylan (7.9%), galactose (2.8%), arabinose (2.2%) and cellobiose (0.3%). The chestnut shell also contains polyphenols, which account for 2.7%~5.2% of the dry weight [4].

Chestnut shell, as one of the main by-products in the chestnut processing, was usually burned or discarded, which not only leaded to environmental pollution, but also caused waste of resources. Extracting polyphenols from chestnut shell can not only solve the problem that chestnut shell is not used effectively, but also obtains the economic benefit. In this study, the extraction conditions of polyphenols from chestnut shell were optimized by response surface methodology (RSM), considering four independent variables, that is, liquid-solid ratio, extraction temperature, extraction time, and ethanol concentration.
2. Materials and methods

2.1. Materials and reagents
Chestnut shell is provided by Shandong Lyzhirun Food Co., Ltd. Chestnut variety: Jinhua. Gallic acid was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Folin–Ciocalteu reagent was purchased from Beijing Solaiiboao Technology Co., Ltd. (Beijing, China). Other chemical reagents were analytical grade and purchased from local suppliers.

2.2. Extraction process
The chestnut shell was dried in an air-circulating oven at 45°C for about 72 h, and then it was milled into powders of 40 mesh. For each extraction, chestnut shell powders were weighed accurately and mixed with proper volume of ethanol solution (assigned according to the experiment design). Subsequently, the mixture was extracted with desired temperature and time as per experiment design. After extraction, such a mixture was cooled to room temperature, and then were centrifuged for 10 min at 4,000 rpm. The supernatants were transferred to 50 mL volumetric flask and diluted with deionized water to volume.

2.3. Determination of total phenolic content
The total phenolics content was determined using the Folin–Ciocalteu colorimetric method. 0.1 mL extract solutions were added into the colorimetrical cylinder, and diluted with deionized water to 10 mL. After the addition of 0.5 mL Folin-Ciocalteu reagent, the mixture was mixed with 9.5 mL of 7.5% (w/v) Na2CO3, and then kept for 30 min in room temperature. The absorbance of mixture was measured at 760 nm using a spectrophotometer. The total phenolic content was calculated as gallic acid equivalents from the calibration curve of gallic acid standard solutions.

2.4. Single factor experiment design
The range of the four factors affecting the ER of polyphenols from chestnut shell, including liquid-solid ratio (10:1, 20:1, 30:1, 40:1, 50:1, 60:1, v/w), extraction temperature (30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C), extraction time (15 min, 30 min, 45 min, 60 min, 75 min, 90 min, 105 min, 120 min), ethanol concentration (0, 15%, 30%, 45%, 60%, 75%, 90%, 100%) were determined through single factor experiments.

2.5. Box-Behnken design (BBD)
Based on the result of single factor experiment, the optimal extraction conditions of polyphenols from chestnut shell were determined by BBD.

2.6. Statistical analysis
SPSS18.0 and Design Expert 8.0.6 were used to analyzed the experimental data. The differences between the means obtained from triplicate experiments were assessed by Duncan's multiple comparisons, and considered to be significant for \( p < 0.05 \).
3. Results and discussion

3.1 Single Factor Analysis

![Figure 1](image.png)

Figure 1 Effect of the four factors on the extraction rate (ER) of polyphenols from chestnut shell: (a) liquid-solid ratio, (b) extraction temperature, (c) extraction time, (d) ethanol concentration.

As the figure 1a shown that, when the liquid-solid ratio was less than 40:1 (v: w), the ER of polyphenols from chestnut shell increased with the increasing of liquid-solid ratio. The increasing of the liquid-solid ratio could increase the concentration gradient between solid and liquid, which was beneficial to the diffusion of compounds [5]. When the liquid-solid ratio reached 40:1 (v: w), there was no significant difference in the ER of polyphenols from chestnut shell with the increasing of the liquid-solid ratio. In view of saving solvent, the liquid-solid ratio of 40:1 was selected for subsequent tests.

The effect of extraction temperature on the ER of polyphenols from chestnut shell was shown in figure 1b. When the temperature was lower than 70℃, the ER of polyphenols from chestnut shell gradually increased with the increasing of the extraction temperature. When the extraction temperature was higher than 70℃, the ER of polyphenols decreased, which was due to the degradation of some polyphenols by high temperature [6]. Therefore, the extraction temperature of 70℃ was selected for subsequent experiments as the center point.

From figure 1c, we could see that with the prolonging of time, the ER of polyphenols from chestnut shell was increased, and the maximum ER was obtained at 90 min. However, further increased of extraction time decreased the ER of polyphenols. Therefore, the extraction time of 90 min was considered as the center point for subsequent experiments.

As depicted in figure 1d, with the increasing of ethanol concentration from 0 to 100%, the ER of polyphenols from chestnut shell increased till peak value (5.19%) at 75%, and then, declined sharply. These results showed that solvent polarity plays an important role in the extraction of polyphenols [7]. Under the conditions of this experiment, the polyphenols from chestnut shell had the highest solubility in 75% ethanol solution. Thus, the ethanol concentration of 75% was selected for the following experiments as the center point.
3.2 Response surface experiment

3.2.1. Response surface experimental design and results
With the ER of polyphenols from chestnut shell as the response value, liquid-solid ratio, extraction temperature, extraction time and ethanol concentration as independent variables, the Box-Behnken experiment was designed using Design Expert. The coding level of experimental factors was shown in Table 1. The response surface test design and results were shown in Table 2. Using Design Expert to carry out regression fitting analysis, the quadratic multiple regression equation between the liquid-solid ratio, extraction temperature, extraction time, ethanol concentration and the ER of polyphenols from chestnut shell could be obtained: Y=0.533-0.096A+0.76B+0.047C-1.83D+0.0061AB-0.29AC+0.11AD+0.082BC-0.12BD-0.028CD-0.94A^2-1.16B^2-0.94C^2-1.38D^2.

Table 1 The coded value of factors levels

| Factors                        | Code | Level |
|--------------------------------|------|-------|
| Liquid-solid ratio A           | 30:1 | 40:1  | 50:1 |
| Extraction temperature B (℃)  | 60   | 70    | 80   |
| Extraction time C (min)        | 75   | 90    | 105  |
| Ethanol concentration D (%)    | 60   | 75    | 90   |

Table 2 Response surface experimental design and results

| Run | A       | B       | C       | D       | Extraction rate (%) |
|-----|---------|---------|---------|---------|---------------------|
| 1   | 1(50:1)| 0(70)   | 0(90)   | 1(90)   | 1.17                |
| 2   | 0(40:1)| 1(80)   | 0(90)   | 1(90)   | 1.54                |
| 3   | 1(50:1)| 0(70)   | 0(90)   | -1(60)  | 4.68                |
| 4   | -1(30:1)| 0(70)   | -1(75)  | 0(75)   | 3.15                |
| 5   | 0(40:1)| -1(60)  | 1(105)  | 0(75)   | 2.38                |
| 6   | 0(40:1)| -1(80)  | 1(105)  | 0(75)   | 4.25                |
| 7   | -1(30:1)| 0(70)   | 0(90)   | 0(75)   | 2.54                |
| 8   | 0(40:1)| 0(70)   | -1(75)  | -1(60)  | 4.89                |
| 9   | -1(30:1)| 0(70)   | 0(90)   | 1(90)   | 1.14                |
| 10  | 0(40:1)| 1(80)   | -1(75)  | 0(75)   | 3.93                |
| 11  | 0(40:1)| -1(60)  | -1(75)  | 0(75)   | 2.39                |
| 12  | 0(40:1)| 0(70)   | 1(105)  | -1(60)  | 4.88                |
| 13  | 0(40:1)| 0(70)   | 0(90)   | 0(75)   | 5.33                |
| 14  | 0(40:1)| -1(60)  | 0(90)   | 1(90)   | 0.50                |
| 15  | 1(50:1)| 0(70)   | 1(105)  | 0(75)   | 3.12                |
| 16  | 1(50:1)| -1(60)  | 0(90)   | 0(75)   | 2.37                |
| 17  | -1(30:1)| 0(70)   | 0(90)   | -1(60)  | 5.11                |
| 18  | -1(30:1)| 1(80)   | 0(90)   | 0(75)   | 4.12                |
| 19  | 0(40:1)| 0(70)   | 0(90)   | 0(75)   | 5.35                |
| 20  | -1(30:1)| 0(70)   | 1(105)  | 0(75)   | 3.93                |
| 21  | 0(40:1)| 0(70)   | 0(90)   | 0(75)   | 5.24                |
| 22  | 0(40:1)| -1(60)  | 0(90)   | -1(60)  | 3.78                |
| 23  | 0(40:1)| 0(70)   | -1(75)  | 1(90)   | 1.23                |
| 24  | 0(40:1)| 0(70)   | 1(105)  | 1(90)   | 1.12                |
| 25  | 1(50:1)| 0(70)   | -1(75)  | 0(75)   | 3.52                |
| 26  | 0(40:1)| 0(70)   | 0(90)   | 0(75)   | 5.39                |
| 27  | 0(40:1)| 0(70)   | 0(90)   | 0(75)   | 5.36                |
| Run | A      | B  | C    | D   | Extraction rate (%) |
|-----|--------|----|------|-----|---------------------|
| 28  | 1(50:1)| 1(80)| 0(90)| 0(75)| 3.98                |
| 29  | 0(40:1)| 1(80)| 0(90)| -1(60)| 5.30                |

Note: A, Liquid-solid ratio; B, Extraction temperature(℃); C, Extraction time (min); D, Ethanol concentration (%).

3.2.2. Regression equation analysis of variance (ANOVA)

As shown in Table 3, the p value (p<0.0001) of the model, indicated that the model was significant. The lack of fit was not significant with p value of 0.0709 (p>0.05) for the response model [8]. The coefficient of determination (R²=0.9974) and adjustive coefficient of determination (R²_adj = 0.9947) indicated the adequacy for the prediction of the experimental results and a high degree of fit [9].

| Source  | df | Sun of squares | Mean square | F value | p value |
|---------|----|----------------|-------------|---------|---------|
| Model   | 14 | 68.97          | 4.93        | 379.32  | <0.0001 |
| A       | 1  | 0.11           | 0.11        | 8.53    | 0.0112  |
| B       | 1  | 7              | 7           | 538.63  | <0.0001 |
| C       | 1  | 0.026          | 0.026       | 2.01    | 0.1785  |
| D       | 1  | 40.14          | 40.14       | 3090.73 | <0.0001 |
| AB      | 1  | 0.0001         | 0.0001      | 0.011   | 0.9164  |
| AC      | 1  | 0.35           | 0.35        | 26.74   | 0.0001  |
| AD      | 1  | 0.051          | 0.051       | 3.91    | 0.0682  |
| BC      | 1  | 0.027          | 0.027       | 2.06    | 0.1731  |
| BD      | 1  | 0.057          | 0.057       | 4.37    | 0.0552  |
| CD      | 1  | 0.0031         | 0.0031      | 0.24    | 0.6344  |
| A²      | 1  | 5.72           | 5.72        | 440.13  | <0.0001 |
| B²      | 1  | 8.68           | 8.68        | 668.36  | <0.0001 |
| C²      | 1  | 5.79           | 5.79        | 445.89  | <0.0001 |
| D²      | 1  | 12.28          | 12.28       | 945.63  | <0.0001 |
| Residual| 14 | 0.18           | 0.013       |         |         |
| Lack of Fit | 10 | 0.17           | 0.017       | 4.85    | 0.0709  |
| Pure Error | 4  | 0.014          | 0.0035      |         |         |
| Cor. total | 28 | 69.15          |             |         |         |

R² = 0.9974  R²_adj = 0.9947  R²_pred = 0.9857

3.2.3. Response surface analysis

Figure 2a-f clearly reflected the effect of independent factors on the ER of polyphenols from chestnut shell. The degree of interaction between the two factors can be judged by the shape of the contour plot. The trend of response surface can reflect the influence of two independent factors on the ER of polyphenols from chestnut shell. The interaction of liquid-solid ratio and extraction temperature had a significant effect on the ER of polyphenols from chestnut shell.
3.2.4. Optimal conditions and model validation

The optimal extraction conditions predicted by the regression model were as follows: liquid-solid ratio 38.99:1, extraction temperature 73.67℃, extraction time 90.98 min, ethanol concentration 64.73%, and the theoretical ER of polyphenols from chestnut shell was 6.11%. In order to facilitate the verification test, the above conditions were corrected as: liquid-solid ratio 39:1, extraction temperature 74℃, extraction time 91 min, ethanol concentration 65%. Under these conditions, the ER of polyphenols from chestnut shell was 6.09%. There was no difference between the experimental value and the predicted value, indicating that the mathematical model can well reflect the relationship between the independent factors and the ER of polyphenols from chestnut shell.

4. Conclusion

In this study, response surface methodology was used to determine the optimal extraction conditions of polyphenols from chestnut shell, including liquid-solid ratio, extraction temperature, extraction time, ethanol concentration. Result showed that the highest ER of polyphenols from chestnut shell (6.09%) was obtained under the conditions: liquid-solid ratio 39:1, extraction temperature 74℃, extraction time 91 min, ethanol concentration 65%. The results can provide a theoretical basis for the reuse of chestnut shell.

Acknowledgments

This study was supported by the Forestry Science and Technology Innovation Project of Shandong Province (LYCX04-2018-21).

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