Ultrasonic extraction of flavonoid from walnut green Husk

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Abstract. In this experiment, ethanol was used to extract flavonoids from walnut green husk by ultrasonic assisted extracting method. The extracting time, ethanol concentration and extraction temperature were first studied by a single factor test, and then an orthogonal test was designed to study the effect of different conditions on the extracting amount of flavonoids from walnut green husk. The results showed that the order of three factors affected the extraction amount of flavonoids was: extraction temperature > ethanol concentration > extracting time; the optimal extraction conditions were: extracting time 55 min, ethanol concentration 35% and extraction temperature 55 ℃, under these conditions, the amount of flavone was 56.8486 mg/g.

1. Introduction
Walnut green husk is one of the main by-products of walnut, medically called Qinglong coat[1]. Green husk is rich in polyphenols, flavonoids, quinones, diarylheptanes and other chemical components, and has high medicinal value and bacteriostatic effect [2-4]. In addition, walnut green husk can also be used for the extraction of natural dye, for the dying of silk and cotton fabrics, and also for hair dyeing, which is safer and more reliable [5-6]. However, in real production, a large amount of walnut green husk can not be used effectively, which not only causes the waste of resources, but also causes pollution to the environment[7].

Ultrasonic assisted extraction is one of the commonly used methods to extract active components in plants. It can use ultrasonic waves to destroy plant cells and make the chemical components in plants dissolve and be easier to extract[8-9].

In this paper, the amount of flavonoids extracted from walnut green husk was determined by ultrasonic assisted extraction and UV spectrophotometry[10] and which provided a certain reference for the extraction of flavonoids from walnut green husk.

2. Materials and methods

2.1. Samples and chemicals
Fresh walnut green husks were collected from Luancheng District, Shijiazhuang in Hebei Province. Absolute ethanol, sodium hydroxide, aluminium nitrate and sodium nitrite were analytical grade.

2.2. Making of flavonoid standard curve

Absorbance was determined at 510 nm using spectrophotometry with rutin as the standard. The standard curve was drawn by NaNO₂-Al(NO₃)₃ colorimetric method to determine the brass content in the sample. The accurate removal of 0.2 mg/mL melim standard solution 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 mL in 10 mL test tubes was mixed with 0.3 mL of 5% NaNO₂ solution respectively and placed for 6 min, then added 0.3 mL 10% Al(NO₃)₃ solution for 6 min, then added 4 mL 4% NaOH solution, then used 60% ethanol to 10 mL for 15 min after mixing and determined at 510 nm in each test tube. The standard curve of flavonoid was drawn with the concentration c(mg/mL) of melim standard solution as the horizontal coordinate and the absorption value A as the vertical coordinate, and the regression equation was established.

2.3. Effect of extracting time on the content of flavonoids

Accurately weighed 10 g of fresh walnut green husk, added 30 mL of 50% ethanol, and sonicated at 60 ℃ for 40 min, 50 min, 60 min, 70 min, and 80 min, respectively. After filtration, diluted to 50 mL with ethanol solution of corresponding concentration, then diluted 10 times, took 1 mL for color development, and measured the absorbance at 510 nm. Finally, the absorbance was brought into the standard curve to calculate the extraction amount of flavonoids.

2.4. Effect of ethanol concentration on the content of flavonoids

Accurately weighed 10 g of fresh walnut green husk, added 30 mL of 30%, 40%, 50%, 60%, and 70% ethanol respectively, and sonicated at 60 ℃ for 60 min. The remaining steps are the same as 2.3.

2.5. Effect of extraction temperature on the content of flavonoids

Accurately weighed 10 g of fresh walnut green husk, added 30 mL of 50% ethanol, and sonicated for 60 min at 40 °C, 50 °C, 60 °C, 70 °C, and 80 °C respectively. The remaining steps are the same as 2.3.

2.6. Orthogonal Test

On the basis of single factor test, three groups were selected under each factor, and four factors and three horizontal orthogonal tests were set up. Accurately weighed 1 g walnut green husk in triangle bottle with adding ethanol for ultrasonic extraction and filtration and rotary evaporation, then moved to the capacity bottle with distilled water to 50 mL, diluted 10 times, and measured at 510 nm, the absorbance value was brought into the flavonoid standard curve to calculate the amount of flavonoid extraction.

| Level | A. Extracting time(min) | B. Ethanol Concentration(%) | C. Temperature(℃) |
|-------|-------------------------|-----------------------------|-------------------|
| 1     | 45                      | 45                          | 45                |
| 2     | 50                      | 40                          | 50                |
| 3     | 55                      | 35                          | 55                |

3. Results and discussion

3.1. Making of flavonoid standard curve
3.2. Effect of extraction time on the content of flavonoids

The effect of extraction time on the amount of flavonoid extraction was shown in Figure 2. When the extracting time was 30 to 70 min, the content of flavonoids increased first and then decreased. It was the largest at 50 min. It might be due to the long time ultrasonic treatment, which affected the stability of flavonoids.

3.3. Effect of ethanol concentration on the content of flavonoid

The effect of ethanol concentration on the amount of flavonoid extraction was shown in figure 3. It can be seen from the figure that when the concentration of ethanol was between 30% and 40%, the amount of extraction increased with the increase of concentration, the maximum amount of extraction was at 40%, and the amount of extraction decreased gradually with the increase of ethanol concentration at 40% and 70%. It might be because when the concentration of ethanol was too high, the dissolution of other substances in walnut green husk increased, causing interference to flavonoids,
which led to the decrease of extracting amount[11].

3.4. Effect of Extraction Temperature on the content of flavonoids

Figure 4. Effect of Extraction Temperature on the content of flavonoids

The effect of extraction temperature on the amount of flavonoid extraction was shown in figure 4. From the figure, it could be seen that in the range of 30-70℃, the extraction amount of flavonoids shown the trend of first rose and then decreased, reached the maximum at 50 ℃. It might be that the temperature was too high, which affected the stability of flavonoids[12].

3.5. Orthogonal test results

Table 2. Orthogonal test results

| Test Number | A  | B  | C  | Content of Flavonoids mg/g |
|-------------|----|----|----|---------------------------|
| 1           | 1  | 1  | 1  | 41.2976                   |
| 2           | 1  | 2  | 3  | 56.1186                   |
| 3           | 1  | 3  | 2  | 46.6667                   |
| 4           | 2  | 1  | 2  | 49.6249                   |
| 5           | 2  | 2  | 1  | 43.7025                   |
| 6           | 2  | 3  | 3  | 50.4139                   |
| 7           | 3  | 1  | 3  | 56.8486                   |
| 8           | 3  | 2  | 2  | 47.5615                   |
| 9           | 3  | 3  | 1  | 41.4094                   |

K1 144.0829 147.7711 126.4095  
K2 143.7413 147.3826 143.8531  
K3 145.8195 138.49 163.3811  
k1 48.02763333 49.2570333 42.1365  
k2 47.91376667 49.12753333 47.9510333  
k3 48.6065 46.16333333 54.46036667  
R 0.57886667 3.0937 12.32386667  

The results of the orthogonal test were shown in table 2. According to the range size in table 2, the order of influence of three single factors on the amount of flavonoid extraction was extraction temperature (℃) > ethanol concentration (%) > extraction time (min). When the extracting condition was A3B1C3, the extraction time was 55 min, the ethanol concentration was 45%, and the extraction
temperature was 55℃, the maximum amount of flavonoids extracted was 56.8486 mg/g. Then, the same method was used to do the verification test. The flavonoid extraction amount was 58.4732 mg/g, 63.4329 mg/g, 55.4385 mg/g. The test results were credible.

4. Conclusion
On the basis of single factor experiment, orthogonal experiment was designed. According to the results of orthogonal experiment, the order of influence of three single factors on flavonoid extraction was: extraction temperature (℃) > ethanol concentration (%) > extraction time (min), the optimum extracting conditions of flavonoids were as follows: extraction time was 55 min, ethanol concentration was 45%, extraction temperature was 55℃, and the maximum amount of flavonoids extracted was 56.8486 mg/g.

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