Study on Extraction Process of Polyphenols from Actinidia

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Abstract. To study the optimum extraction conditions of polyphenols in actinidia. The extraction rate of polyphenols was used as the inspection index. Single factor test and orthogonal experiment were used to study the effect of the type and volume fraction of organic solvents, extraction temperature, extraction time and solid-liquid ratio on the extraction rate of polyphenols from kiwifruit. [Result] The optimum extraction process was as follows: methanol volume fraction was 60%, extraction time was 30 min, extraction temperature was 60 °C, solid-liquid ratio was 1:3, the extraction rate of polyphenols in kiwifruit was 35.7%. The process is stable and feasible, which can provide basis for the development and application of polyphenols in kiwifruit.

1. Introduction

Actinidia chinensis Planch is a kind of fruit with oval shape, long hairy appearance, green pulp and black seed[1]. Soft texture, taste sweet and sour. Most of the plant polyphenols are retained between the fleshy parts of the skin and skin of some plants and can reach up to 20%[2,3]. Plant polyphenols have a strong antioxidant effect, and have a good application in medicine and anti-UV industry[4]; promote skin metabolism, can make cosmetics and skin care products. Relevant data point out that polyphenols can reduce the probability of gene mutations, thereby reducing the possibility of cancer[5-7]. At present, the research and development of kiwifruit resources at home and abroad are mostly limited to the study of vitamin C[8], and there are few reports on polyphenols, which are important functional ingredients. The current research on polyphenols in kiwifruit has become a hot research area in the pharmaceutical industry[9,10].

In this paper, the extraction process of polyphenols in kiwifruit was studied. The optimum extraction parameters were determined by orthogonal experiments, and the stability of the process was verified. The results provided a basis for the development and application of industrial production.

2. Materials and Methods

2.1. Materials

2.1.1. Experimental materials and main reagents
Kiwifruit was purchased from Jinzhai County, Liu'an City, Anhui Province; Gallic acid, standard reference substance, Shanghai Ronghe Pharmaceutical Technology Co., Ltd.; Methanol, ethanol, acetone, Folin phenol reagent and sodium carbonate, etc. Commercially available analytical grade; experimental water is distilled water.
2.1.2. The main instrument
ZNHW-JI Electronic Energy-saving Temperature Controller, Gongyi City Yuhua Instrument Co., Ltd.; Electronic Analytical Balance (AUY 120), Shimadzu Corporation, Japan; HH-2 Digital Thermostat Water Bath, Jintan Jieerer Electric Co., Ltd.; KQ5200B ultrasonic cleaner, Kunshan Ultrasonic Instrument Co., Ltd.; UV-Vis spectrophotometer (UV-1800PC), Shanghai Customs Puda Instrument Co., Ltd.

2.2. Methods

2.2.1. Determination of polyphenols in kiwifruit
The polyphenols were obtained using a solution extraction method that controlled a single variable. At the characteristic absorption wavelength, the absorbance was measured and the extraction rate of polyphenols was calculated according to the regression equation of the standard curve.

2.2.1.1. Determination of Characteristic Absorption Wavelength
Gallic acid was used as the standard reference substance, and a full wavelength scan was performed between 200 and 800 nm using a UV-Vis spectrophotometer to measure the maximum absorption wavelength. Figure 1 shows that there is a characteristic absorption peak at 715 nm.

![UV absorption spectra of polyphenol reference](image)

Figure 1. UV absorption spectra of polyphenol reference

2.2.1.2. Standard Curve Drawing
Prepare a series of concentrations of Gallic acid standard solution, respectively, take 2mL in stoppered test tube, with a UV spectrophotometer at 715nm to measure the absorbance of a series of concentration gradient solution, record the value, draw a standard curve, as shown in Figure 2, where Standard curve linear regression equation: \( y = 12.8872x + 0.4433 \), Linear regression coefficients: \( r = 0.9992 \).
2.2.1.3. Calculation of polyphenol extraction rate of kiwifruit
Polyphenol extraction rate = polyphenols / total polyphenols × 100%.

Polyphenol formula: \[ W = C \times \frac{V}{M} \]

Note: \( W \) - polyphenol content, \( C \) - Gallic acid equivalent, that is calculated according to the standard curve, \( V \) - extracted solution volume, \( M \) - extracted volume.

2.2.1.4. Extraction of polyphenols from kiwifruit
The graduated cylinder measures 5 ml of juice and is placed in a volumetric flask. Additional 5 mL of 40% methanol solution was added and leached for 45 min at 60°C. Take 10mL of the extract to centrifuge. The supernatant was taken and the absorbance was measured at 715 nm. The values were recorded and the standard curve was used to calculate the Gallic acid equivalent value. Then take the above supernatant, perform the above operation, and repeatedly extract until the absorbance value is close to zero, as shown in Figure 3. The value of several determinations calculated according to the formula is the total polyphenol content of kiwifruit. Five times of leaching, the total polyphenol content of kiwifruit was 512.88 mg/L.

2.2.2. Extraction method of kiwifruit polyphenols

2.2.2.1. Single-factor experiment
2.2.2.1.1. Effect of the type and volume fraction of organic solvents on the polyphenol extraction rate of kiwifruit
At an extraction temperature of 50°C, an extraction time of 60 min, and a constant solid-liquid ratio of 1:4, the effects of methanol, ethanol, and acetone solutions on the extraction rate of polyphenols by volume fractions of 20%, 40%, 60%, 80%, and 100% was tested respectively.

2.2.2.1.2. Effect of extraction time on polyphenol extraction rate of kiwifruit
Under constant conditions of extraction temperature 50 °C, solid-to-liquid ratio of 1:4 and 40% methanol solution, the effect of extraction time at 15 min, 30 min, 45 min, 60 min, and 75 min on the extraction rate of polyphenols was tested.

2.2.2.1.3. Effect of extraction temperature on polyphenol extraction rate of kiwifruit
The effects of extraction temperature at 40°C, 50°C, 60°C, 70°C, and 80°C on the extraction rate of polyphenols were studied under constant conditions of 30min extraction time and a 1:4 and 40% methanol solution.

2.2.2.1.4. Effect of liquid-to-liquid ratio on extraction rate of polyphenols in kiwifruit
At a constant extraction temperature of 50°C, extraction time of 30 min, feed solution and 40% methanol solution, the effect of extraction rate at the ratios of solid to liquid 1:1, 1:2, 1:3, 1:4 and 1:5 were tested respectively.

2.2.2.2. Orthogonal experiments
The extraction rate of kiwifruit polyphenols was used as the evaluation index. The volume fraction of methanol (A), leaching time (B), extraction temperature (C) and material-liquid ratio (D) were selected as reference factors. According to the results of single factor experiments, select the range of reaction conditions, design the orthogonal experiment scheme L₉ (3⁴), and visually analyze the results and analysis of variance.

| Level | Methanol volume fraction%/ (A) | Extraction time/min (B) | Extraction temperature/°C (C) | Solid material ratio g/ml (D) |
|-------|------------------------------|-------------------------|-----------------------------|-----------------------------|
| 1     | 20                           | 15                      | 50                          | 1:3                         |
| 2     | 40                           | 30                      | 60                          | 1:4                         |
| 3     | 60                           | 45                      | 70                          | 1:5                         |

2.2.2.3. Verification Experiment
In order to ensure the stability of the experiment, 3 sets of verification experiments were performed on the best extraction process.

3. Results and Analysis

3.1. Single factor experiment

3.1.1. Effect of the type and volume fraction of organic solvents on the extraction rate of polyphenols from kiwifruit
From Figure 4, it can be seen that the type and volume fraction of organic solvents have a great influence on the extraction rate of polyphenols from kiwifruit, and 80% acetone solution and kiwifruit have higher extraction rates of polyphenols. However, acetone can increase the dissolution of some substances such as proteins and polysaccharides. The measurement data is inaccurate, and the amount of acetone inhalation is too high, which can easily lead to anesthesia. Therefore, 40% methanol is the best solvent for extraction.

3.1.2. Effect of extraction time on polyphenol extraction rate of kiwifruit

From Figure 5, it can be seen that when the time is 30 minutes, the polyphenol extraction rate is the highest. Over time, the extraction rate of polyphenols in kiwifruit gradually became stable and even declined.
3.1.3. Effect of extraction temperature on polyphenol extraction rate of kiwifruit

Figure 6. Effect of extraction temperature on polyphenol extraction of kiwifruit

From Figure 6, the extraction rate of polyphenols in Kiwifruit is the highest when the extraction temperature is 60°C.

3.1.4. Effect of solid-liquid ratio on extraction rate of polyphenols from kiwifruit

Figure 7. Effect of solid-liquid ratio on the extraction of polyphenols from kiwifruit

It can be seen from Figure 7 that when the ratio of solid to liquid is 1:3, the extraction rate of polyphenols from kiwifruit is the highest.

3.2. Orthogonal experiments

3.2.1. Intuitive Analysis

According to the orthogonal experiment design, 9 sets of experimental data were obtained, and the extraction rate of kiwifruit polyphenols was used as a test index for data processing. The results are shown in Table 2.

| Experiment number | A | B | C | D | Extraction rate of kiwifruit polyphenols (%) |
|------------------|---|---|---|---|--------------------------------------------|
| 1                | 1 | 1 | 1 | 1 | 22.3                                       |
| 2                | 1 | 2 | 2 | 1 | 34.7                                       |
| 3                | 1 | 3 | 3 | 1 | 20.9                                       |
According to the orthogonal experimental values, the average value $k_i$ of the experimental index is calculated within the extent of each factor, and the optimal extraction scheme is determined according to the size of $k_i$. The above effect curve can be viewed directly under this factor. The best level, taking the volume fraction A of methanol as an example, the experimental index value $k_3 > k_2 > k_1$, so the optimal level of this factor is $A_3$, that is, the volume fraction of methanol is 60%, and can also be obtained according to the same method. The best conditions for the other three factors were $B_2C_2D_2$, in other words, extraction time 30 min, extraction temperature 60°C, and solid-liquid ratio 1:3. Then according to the experimental index value to calculate the magnitude of change R, the formula is $R = \text{MAX} (k_i) - \text{MIN} (k_i)$, to determine the order of influence between the various components, it can be inferred that the primary and secondary relationship between the extraction rate of polyphenols in Jinzhai kiwifruit is affected by: $A$ (volume fraction of methanol)$> B$ (extraction time)$> C$ (extraction temperature)$> D$ (liquid-to-liquid ratio).

### 3.2.2. Analysis of Variance

Using the intuitive analysis of orthogonal experiments as a reference to analyze the variance, calculate the minimum mean square error $S$ of each error source as the error estimate to calculate the $F$ value, and compare it with the $F$ critical value to judge the significance of each influencing factor. From Table 3, the methanol volume fraction has a highly significant effect on the extraction rate of polyphenols in kiwifruit. The extraction time has a significant effect on the extraction rate of polyphenols in kiwifruit.

| Factors               | Square deviation sum | Q | Degree of freedom | $F$ ratio | $F$ critical value |
|-----------------------|----------------------|---|-------------------|-----------|-------------------|
| Methanol volume fraction A | 163.307              | 2 |                   | 1.774     | 4.460             |
| Time B                | 110.580              | 2 |                   | 1.201     | 4.460             |
| Temperature C         | 46.747               | 2 |                   | 0.508     | 4.460             |
| Material-liquid ratio D | 47.527              | 2 |                   | 0.516     | 4.460             |
| Error                 | 368.16               | 8 |                   |           |                   |

### 3.3. Verification experiment

In order to ensure the stability of the experiment, three groups of verification experiments were performed on the best extraction process. The results are shown in Table 4.

| Experiment number | 1    | 2    | 3    | Average | RSD  |
|-------------------|------|------|------|---------|------|
| Polyphenol extraction rate% | 36.8 | 35.6 | 34.7 | 35.7    | 0.18 |
From the experimental results, we can see that the extracting method of polyphenols in Jinzhai kiwifruit is practically stable under solvent extraction conditions, and the average extraction rate of polyphenols is 35.7%.

4. In conclusion

The effects of various factors on the polyphenols in kiwifruit are as follows: the volume fraction of methanol > extraction time > extraction temperature > ratio of solid to liquid, and the volume fraction of methanol has the most influence on the extraction rate of polyphenols from Kiwifruit. Significantly, the best extraction process is A_3B_2C_2D_2. This conclusion is inconsistent with one-factor experiments, namely the volume fraction of methanol. As the volume fraction of methanol increases and the concentration increases, dissolved polyphenols also increase, so the extraction rate of polyphenols increases. Therefore, the optimum extraction process of polyphenols in kiwifruit: methanol volume fraction 60%, extraction time 30 min, extraction temperature 60 °C, solid-liquid ratio 1:3, the average extraction rate of polyphenols in kiwifruit can reach 35.7%.

In this paper, organic solvents were used to extract polyphenols, and methanol was used instead of traditional acetone extraction. It had the advantages of easily accessible materials, greatly reduced environmental damage to the body, and a good extraction effect. The extraction rate of polysaccharide under traditional conditions can only reach about 28%. By changing the extraction conditions, the extraction rate of polyphenols is greatly improved. After many experiments, the extraction process of this method is stable and operable, and it is a modern industrialized production. Provides the basis.

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