Extraction of anthocyanin from purple-fleshed potato through ultrasound-assisted extraction

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Abstract. In this study, the ultrasound-assisted extraction was utilized to extract anthocyanin from purple-fleshed potato, and the extraction process was optimized in the selection of various conditions of extracting agent, solid-liquid ratio, ultrasound time. In addition, single factor experiment and orthogonal experiment were carried out, which determined that the highest extraction efficiency of anthocyanin from purple-fleshed potato was achieved at the purple-fleshed potato-to-extracting agent ratio of 1:50 (g/mL), the ratio of 0.2% hydrochloric acid extracting agent to 50% ethanol at 2:3, and the ultrasound time of 15 min.

1. Introduction
Purple-fleshed potato is the typical example of the potato resource. In recent years, the net production of potato rises at a rapid rate, and the potato mass production is estimated to be about 30 million tons in China in 2018. Purple-fleshed potato contains abundant nutrient elements, including anthocyanin. Anthocyanin is a kind of flavonoid belonging to the natural water-soluble pigment, which is extensively distributed in plants [1]. Natural anthocyanin is the product condensed from catechin and epicatechin, which is mainly constituted by the cyanidin and peonidin anthocyanin, with poor stability and easy oxidation [2]. Apart from the pigment function, anthocyanin can also prevent cardiovascular disease (CVD) and neurogenic disease, which is mostly related to its oxidative function. Besides, anthocyanin is mostly applied in the fields of medicine, food and cosmetics, and it is usually extracted by microwave extraction, chemical extraction, and ultrasound extraction [3]. Compared with traditional plants, purple-fleshed potato has high yield, strong stability to light and heat, as well as edibility, and extraction of anthocyanin from purple-fleshed potato is of wide application value [4]. This study adopted the ultrasound-assisted extraction method to strengthen and break the purple-fleshed potato cells under certain conditions using the ultrasonic cavitation, destroy its cell wall, release its cytoplasm, and increase the anthocyanin extraction efficiency from purple-fleshed potato. Moreover, to obtain the best ratio of anthocyanin extraction from purple-fleshed potato, first of all, single factor experiment was conducted to determine the optimal ethanol extraction concentration[5]; and then the best extracting agent, solid-liquid ratio, and ultrasound time were determined; finally, the three-factor three-level orthogonal experiment was performed to determine the most suitable conditions for anthocyanin extraction from purple-fleshed potato, thus optimizing the anthocyanin extraction process from purple-fleshed potato.
2. Materials and methods

2.1. Pretreatment

The fresh purple-fleshed potato was washed with tap water, then with deionized water for 5 times, drained off, sliced into 2 mm sections, put into the oven and dried to 40 °C until constant weight, ground into powders, and filtered with the 100-mesh sieve to obtain the dry purple-fleshed potato powders, which were sealed for subsequent use[6].

2.2 Standard curve plotting

The pipette was used to transfer the cyanidin standard solution at different volumes, which were then diluted with 0.2% hydrochloric acid to 10 mL, respectively, to prepare into standard solutions with different concentrations[7]. Then, 1.2 mL cyanidin standard solution was put into the 10 mL volumetric flask, 0.5 mL of the 10% Na2SO3 solution was added and diluted with 0.2% hydrochloric solution as the reference solution, and the absorbance value was determined at 526 nm. Later, the standard curve was plotted, with cyanidin concentration as the x-coordinate, while absorbance value as the y-coordinate.

2.3 Sample determination

2.5 mL supernatant was added into the 25 mL volumetric flask and diluted with 0.2% hydrochloric solution by 10 folds to the calibration tail. Later, 2.5 mL sample supernatant was transferred using another test tube into the 25 mL volumetric flask, and 2.0 mL of 10% Na2SO3 solution was added for blank experiment, and the sample absorbance value was detected[8]. Afterwards, the anthocyanin extraction rate from purple-fleshed potato was calculated according to the cyanidin standard curve. The extraction rate=extracted anthocyanin mass (g)/ purple-fleshed potato mass (g) *100%.

2.4 Anthocyanin extraction

The dry purple-fleshed potato powders were weighed, mixed with the extracting agent, and crushed using the ultrasonic cell crusher. After crushing, the samples were centrifuged for 5 min at 8000 g, and the supernatant was collected[9].

2.5 Extraction single-factor experiment

2.5.1 Ethanol content

2.0 g dry purple-fleshed potato powders were weighed and placed into the 100 mL beaker, and then 20 mL of 25%, 50%, 75% and 95% ethanol solutions were added, followed by 30 min of crushing using the ultrasonic cell crusher.

2.5.2 Extracting agent

2.0 g dry purple-fleshed potato powders were weighed and placed into the 100 mL beaker, then different extracting agents were added, respectively, and the samples were crushed with the ultrasonic cell crusher for 30 min.

2.5.3 Solid-liquid ratio

2.0 g dry purple-fleshed potato powders were weighed, then extracting agents at corresponding volumes based on certain solid-liquid ratio were added (0.2% hydrochloric acid:50% ethanol=2:3), and the samples were crushed with the ultrasonic cell crusher for 30 min.

2.5.4 Ultrasound time

1.0 g dry purple-fleshed potato powders were weighed, then 50 mL extracting agent was added at the solid-liquid ratio of 1:50 (0.2% hydrochloric acid:50% ethanol=2:3), and the samples were placed into the ultrasonic cell crusher for different time periods.

2.6 Orthogonal experiment

Based on the results of single factor experiment, the optimal conditions of various factors in anthocyanin extraction from purple-fleshed potato were selected to design the three-factor three-level experiment. Finally, the sizes of various influencing factors and the optimal extraction conditions were obtained[10].
3 Results and discussion

3.1 Standard curve plotting

The purity of the standard cyanidin was ≥98% (calculated at the cyanidin purity of 98%).

The standard curve is shown in Fig.1. The regression equation was shown below.

\[ A = 0.0040 + 0.0230 \times C, \quad R^2 = 0.9999 \]

Where A is absorbance, C represents the mass concentration of cyanidin μg/mL, and \( R^2 \) is the correlation coefficient.

![Figure 1. The standard curve of cyanidin](image)

3.2 Single factor experiment

3.2.1 Effect of different ethanol concentrations on anthocyanin extraction

Ethanol was used as the extracting agent to extract anthocyanin from purple-fleshed potato, when the ethanol concentration was >50%, increasing the ethanol concentration was to the disadvantage of anthocyanin extraction from purple-fleshed potato, and the maximal absorbance value was measured at the ethanol concentration of 50% (Table 1).

| No. | Ethanol concentration(%) | Solid-liquid ratio (g/mL) | Ultrasound Time(min) | Purple-fleshed potato (g) | Absorbance value |
|-----|--------------------------|---------------------------|----------------------|---------------------------|------------------|
| 1   | 25                       | 1 : 25                    | 30                   | 2.00                      | 0.12             |
| 2   | 50                       | 1 : 25                    | 30                   | 2.00                      | 0.13             |
| 3   | 75                       | 1 : 25                    | 30                   | 2.00                      | 0.09             |
| 4   | 95                       | 1 : 25                    | 30                   | 2.00                      | 0.03             |

3.2.2 Effect of different extracting agents on anthocyanin extraction from purple-fleshed potato

Different extracting agents led to slightly different anthocyanin extraction efficiencies. In this study, the citric acid-acidified ethanol extracting agent was compared with the hydrochloric acid-acidified ethanol extracting agent, and the results suggested that, the highest extraction efficiency was achieved when 0.2% hydrochloric acid and 50% ethanol were used as the extracting agents, which was about 0.38%. Therefore, 0.2% hydrochloric acid and 50% ethanol at the volume ratio of 2:3 were used as the extracting agents (Table 2).
Table 2. Absorbance values of anthocyanin extraction from purple-fleshed potato using different extracting agents

| No. | 0.2 % HCl (mL) | 5% Citric acid (mL) | 50% Ethanol (mL) | Solid-liquid ratio (g/mL) | Ultrasound Time (min) | Purple-fleshed potato (g) | Absorbance value |
|-----|----------------|---------------------|-----------------|--------------------------|------------------------|---------------------------|-----------------|
| 1   | 25             | -                   | 25              | 1 : 25                   | 30                     | 2.00                      | 0.34            |
| 2   | -              | 25                  | 25              | 1 : 25                   | 30                     | 2.00                      | 0.31            |
| 3   | 20             | -                   | 30              | 1 : 25                   | 30                     | 2.00                      | 0.36            |
| 4   | -              | 20                  | 30              | 1 : 25                   | 30                     | 2.00                      | 0.31            |
| 5   | 10             | -                   | 40              | 1 : 25                   | 30                     | 2.00                      | 0.35            |
| 6   | -              | 10                  | 40              | 1 : 25                   | 30                     | 2.00                      | 0.31            |

3.2.3 Effect of different solid-liquid ratios on anthocyanin extraction
The anthocyanin extraction rate was relatively high when the solid-liquid ratio was 1:50 (Table 3.3).

Table 3. Anthocyanin extraction rates from purple-fleshed potato at different solid-liquid ratios

| No. | Solid-liquid ratio (g/mL) | Solvent 0.2 % HCl / 50% Ethanol (mL) | Ultrasound Time (min) | Purple-fleshed potato (g) | Absorbance | Extraction rate (%) |
|-----|---------------------------|--------------------------------------|------------------------|---------------------------|------------|--------------------|
| 1   | 1:25                      | 20 : 30                              | 30                     | 2.001                     | 0.315      | 0.3379             |
| 2   | 1:50                      | 40 : 60                              | 30                     | 2.0006                    | 0.171      | 0.3629             |
| 3   | 0.09375                   | 60 : 90                              | 30                     | 2                         | 0.107      | 0.3359             |
| 4   | 0.1111111111             | 80 : 120                             | 30                     | 2                         | 0.074      | 0.3043             |

3.2.4 Effect of different ultrasound time periods on anthocyanin extraction
Using the ultrasound-assisted extraction method, the anthocyanin extraction rate from purple-fleshed potato was relatively high under the ultrasound time of 15 min. When the ultrasound time was too short, the anthocyanin was incompletely extracted, which resulted in the low extraction rate. When the ultrasound time was over 15 min, the anthocyanin extraction rate from purple-fleshed potato gradually decreased, which might be because that a large amount of heat was released due to the ultrasonic effect, so that part of anthocyanin was inactivated.

Table 4. Anthocyanin extraction rates from purple-fleshed potato under different ultrasound time periods

| No. | Ultrasound Time (min) | Solvent 0.2 % HCl / 50% Ethanol (mL) | Solid-liquid ratio (g/mL) | Purple-fleshed potato (g) | Absorbance | Extraction rate (%) |
|-----|-----------------------|--------------------------------------|--------------------------|---------------------------|------------|--------------------|
| 1   | 0                     | 20 : 30                              | 1 : 50                   | 1.00                      | 0.14       | 0.29               |
| 2   | 5                     | 20 : 30                              | 1 : 50                   | 1.00                      | 0.15       | 0.32               |
| 3   | 15                    | 20 : 30                              | 1 : 50                   | 1.00                      | 0.17       | 0.36               |
| 4   | 30                    | 20 : 30                              | 1 : 50                   | 1.00                      | 0.16       | 0.34               |
| 5   | 45                    | 20 : 30                              | 1 : 50                   | 1.00                      | 0.16       | 0.33               |
| 6   | 60                    | 20 : 30                              | 1 : 50                   | 1.00                      | 0.15       | 0.33               |
3.3 Orthogonal experiment

During the anthocyanin extraction process, the solid-liquid ratio had the greatest influence on the anthocyanin extraction rate, followed by acid-ethanol ratio, while ultrasound time had the lowest influence. The optimal processing conditions were determined by the range analysis of orthogonal experimental results to be $A_3B_2C_2$, in other words, the ratio of dry purple-fleshed potato powder to extracting agent was 1:50 (g/mL), the ratio of 0.2% hydrochloric acid to 50% ethanol was 2:3, and the ultrasound time was 15 min.

Table 5. Orthogonal experimental results for anthocyanin extraction from purple-fleshed potato

| No. | A          | B          | C           | Blank column | Extraction rate (%) |
|-----|------------|------------|-------------|--------------|---------------------|
|     | solid-liquid ratio (g/mL) | Acid to alcohol ratio (v/v) | Ultrasound Time (min) |              |                     |
| 1   | 1 : 40     | 1 : 2      | 10          | 1            | 0.39                |
| 2   | 1 : 40     | 2 : 3      | 15          | 2            | 0.39                |
| 3   | 1 : 40     | 5 : 6      | 20          | 3            | 0.38                |
| 4   | 1 : 50     | 1 : 2      | 15          | 3            | 0.42                |
| 5   | 1 : 50     | 2 : 3      | 20          | 1            | 0.41                |
| 6   | 1 : 50     | 5 : 6      | 10          | 2            | 0.39                |
| 7   | 1 : 60     | 1 : 2      | 20          | 2            | 0.44                |
| 8   | 1 : 60     | 2 : 3      | 10          | 3            | 0.45                |
| 9   | 1 : 60     | 5 : 6      | 15          | 1            | 0.43                |
| K1  | 1.16       | 1.24       | 1.22        | 1.22         |                     |
| K2  | 1.22       | 1.25       | 1.24        | 1.22         |                     |
| K3  | 1.31       | 1.20       | 1.23        | 1.24         |                     |
| ’K1 | 0.39       | 0.41       | 0.41        | 0.41         |                     |
| ’K2 | 0.41       | 0.42       | 0.41        | 0.41         |                     |
| ’K3 | 0.44       | 0.40       | 0.41        | 0.41         |                     |
| R   | 0.14       | 0.05       | 0.01        | 0.01         |                     |

Influence size order: A>B>C

The optimum conditions: $A_3B_2C_2$

4. Conclusion

We discovered from this study that, the extraction efficiency of anthocyanin from purple-fleshed potato was greatly improved through ultrasound-assisted extraction under certain conditions, which exhibits great advantages. However, some issues should still be managed when ultrasound-assisted extraction is adopted to extract anthocyanin from purple-fleshed potato. For instance, we found that an excessively long ultrasound extraction time reduced the extraction efficiency of anthocyanin, which might be because that a large amount of heat produced during the ultrasound process destroyed the anthocyanin structure and inactivated it. At the same time, ultrasound is mainly applied in the small laboratory, which is restricted in large-scale industrial production. Therefore, more efforts should be made on this basis to develop an efficient and energy-saving ultrasound extraction method that is more suitable for large-scale industrial production.

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