Study on the Extraction of Total Flavonoids from Chinese Kale Using Response Surface Methodology

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Abstract. The extraction process of total flavonoids in Chinese kale was studied. The mathematical models of extraction rate of total flavonoids related to ultrasonic time, ultrasonic temperature, liquid-to-material ratio and ethanol concentration were established by response surface methodology. Through the mathematical model, ultrasound assisted method is the optimum conditions to extract the total flavonoids from Chinese kale. The results showed that the optimum conditions for the extraction was: ultrasonic time 29 min, ultrasonic temperature 71 ℃, liquid-to-material ratio 27 mL/g and ethanol concentration 77%. Based on these conditions, the extraction rate of total flavonoids in Chinese kale was 18.47 mg/g, with a relative error of 0.75% compared with the predicted value. The mathematical model had a high fitting degree and a better predictability about the extraction rate of total flavonoids in Chinese kale. The obtained conditions were reliable and could be used to optimize the extraction process of total flavonoids in Chinese kale.

1. Introduction
Chinese kale (Brassica alboglabra LH Bailey), also known as green leaf cabbage or white-flowered Chinese kale. It is one of China's specialty vegetables [1]. Chinese kale has high nutritional value, its vitamin C content far exceeds that of vegetables such as spinach and amaranth [2]. Kale is also rich in minerals, glucosinolates, flavonoids and other natural effective ingredients. Among them, flavonoids are a class of natural substances with two benzene ring structures [3], which can anti-inflammatory, anti-oxidant, anti-bacterial, and improve human immunity [4]. Ultrasonic-assisted extraction refers to the continuous oscillation under the action of ultrasonic cavitation, which destroys the cell membrane structure, promotes the swelling of cells, improves the permeability of the cell wall, and promotes the dissolution rate and efficiency of ingredients, thereby can increase the extraction rate [5]. In this paper, ultrasonic was used during the extraction process of total flavonoids in Chinese kale. The effects of ultrasonic time, ultrasonic temperature, liquid-solid ratio and ethanol concentration were investigated
by single factor experiment and the response surface method, to get the optimal process, which provided a theoretical basis for the development and utilization of Chinese kale.

2. Materials and methods

2.1. Materials and Apparatus
Fresh Chinese kale was cleaned, chopped, dried and crushed for use. All the reagents used were analytical grade. Electronic Balance (TP-114, Denver Instruments (Beijing)), Ultrasonic Cleaner (KQ-100DE, Kunshan Ultrasonic Instrument), UV/Visible Spectrophotometer (UV-200, Shanghai Mepuda), electric heating constant temperature blast dryer (DHG-9030A, Shanghai Jinghong) were used.

2.2. Methods

2.2.1. Determination of total flavonoids. 0.1000 g of the rutin standard was dissolved in 75% ethanol, then was diluted to 1.0 g/L as standard solution. Take 0, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mL of the solution, respectively. Then 0.3 mL of 5% NaNO₂ solution, 0.3 mL of 10% Al(NO₃)₃ solution and 2.0 mL of 4% NaOH solution were added and diluted with 75% ethanol to 10 mL. The absorbance was tested at the wavelength of 510 nm. Take rutin as standard sample, using Al(NO₃)₃-NaNO₂ colorimetry, the regression equation of absorbance (A) was: \[ Y = 11.281X - 0.0166, \] \[ R^2 = 0.9996. \]

2.2.2. Extraction process of total flavonoids in Chinese kale. 1.0000 g of kale powder was weighed and 75% ethanol was added. According to the set process, ultrasonic extraction was carried out for a period of time, then the upper liquid was diluted to certain times for test, and the total flavonoid extraction rate was calculated by \[ r = \frac{C \times N \times V}{M \times 10^3}. \] (r: Extraction process of total flavonoids in Chinese kale; C: total flavonoid concentration (g/L) of the liquid to be measured; N: the diluted multiply; V: volume of the crudely extraction (mL); M: weight of the kale(g))

2.2.3. Single factor experiment. The effects of ultrasonic times (15, 20, 25, 30, 35 min), ultrasonic temperatures (50, 65, 70, 75, 80°C), liquid-solid ratios (15, 20, 25, 30, 35 mL/g) and different ethanol concentrations (65, 70, 75, 80, 85%) on the extraction rate of total flavonoid were examined, respectively.

2.2.4. Response face experiment. After a single-factor test, the basic process conditions were obtained, and the Box-Behnken method was used, with the four factors (ultrasonic time, ultrasonic temperature, liquid-solid ratio and ethanol concentration) as the variables and the total flavonoid extraction rate as the response value for experimental were designed by Design Expert 8.0b.

3. Results

3.1. Single factor test results
The effects of ultrasonic time (15, 20, 25, 30, 35 min), ultrasonic temperature (60, 65, 70, 75, 80°C), liquid-solid ratio (15, 20, 25, 30, 35 mL/g), and ethanol concentration (65, 70, 75, 80, 85 %), on the extraction rate of total flavonoid were examined, respectively. Results show that the optimum conditions were: ultrasonic time 30 min (Figure 1), ultrasonic temperature 70°C (Figure 2), the liquid-solid ratio 25 mL/g (Figure 3), and the ethanol concentration 75% (Figure 4).
3.2. Response surface test

3.2.1. Response surface experimental parameters and results. According to response surface methodology, the results of the single-factor test were considered and analyzed, the four factors of ultrasonic time (A), ultrasonic temperature (B), liquid-solid ratio (C) and ethanol concentration (D) were selected as response variables, and the total flavonoid extraction rate was tested with the response value. Results are listed in Table 1.

Table 1. Parameters and results of response surface test.

| NO. | A  | B  | C  | D  | Rate (%) | NO. | A  | B  | C  | D  | Rate (%) |
|-----|----|----|----|----|----------|-----|----|----|----|----|----------|
| 1   | 0  | 0  | -1 | -1 | 15.27    | 16  | 0  | 0  | 1  | -1 | 14.97    |
| 2   | 0  | -1 | 1  | 0  | 16.27    | 17  | 1  | 0  | -1 | 0  | 16.93    |
| 3   | -1 | 0  | 0  | -1 | 15.83    | 18  | 0  | 1  | 0  | -1 | 15.75    |
| 4   | 1  | 1  | 0  | 0  | 17.74    | 19  | 0  | -1 | -1 | 0  | 16.82    |
The dense arrangement of high lines on the response surface (i.e., solid ratio extraction rate, while the change of ultrasonic time has less significant effect. However, both factors are slightly significant.) have a smaller effect on the response value is less obvious.

It can be learned from Figure 6, the liquid-solid ratio is steeper than the ultrasonic time, indicating that the liquid-solid ratio has a greater effect on the total flavonoid extraction rate. According to the contour map, the interaction between the extraction temperature and the extraction time is not significant.

The results of Table 1 were analyzed by the response surface methodology, the regression equation of the total flavonoid extraction rate (Y) of kale was obtained: $Y = 18.39 - 0.14A + 0.18B + 0.70D + 0.45AB - 0.21AC - 0.18AD + 0.44BC - 0.04BD + 0.64CD - 0.68A^2 - 0.77B^2 - 0.87C^2 - 1.39D^2$.

3.2.2. Effects of any two-factor interaction on flavonoid extraction rates. By comparing any two influence factors and the response surface diagram, the interaction relationship of any factors on the extraction rate was obtained. In the center of the oval, the extraction rates were the highest, which decreased from center to around. The dense arrangement of high lines on the response surface (i.e., steep slopes) indicates high sensitive to the change of response values, while loose surface (i.e., slopes are relatively flat) have a smaller effect on the response [6].

According to the surface slope of Figure 5, the slope of ultrasonic temperature is steeper, indicating that ultrasonic temperature has a greater effect on the total flavonoid extraction rate, while the change of ultrasonic time has less significant effect. However, both surfaces are relatively flat, indicating that the impact on the response value is less obvious.

From Figure 6, the liquid-solid ratio is steeper than the ultrasonic time, indicating that the liquid-solid ratio has a greater effect on the total flavonoid extraction rate. According to the contour map, the interaction between the extraction temperature and the extraction time is not significant. It can be learned from Figure 7 that ethanol concentration was more sensitive on the total flavonoid extraction rate than ultrasonic time. According to the contour, the interaction correlation between the two factors is not obvious.

When the ultrasonic temperature and liquid-solid ratio are fixed, the extraction rate shows a tendency to rise and then fall. The slope formed by ultrasonic temperature is relatively flat, indicating that the liquid-solid ratio has a greater impact than the ultrasonic temperature. The interaction between the two factors is slightly significant (Figure 8).

When the ultrasonic temperature is fixed, the extraction rate shows a tendency to rise and then fall, and the surface of the ethanol concentration is steeper, indicating that the ethanol concentration has a greater effect on the rate of flavonoids. According to contour, the two factors are slightly related (Figure 9).

As can be seen from Figure 10, the surface of ethanol concentration is steeper, indicating that the ethanol concentration has a stronger effect on the total flavonoid extraction rate, while the liquid-solid ratio has less significant effect. When the liquid-solid ratio is fixed, the extraction rate increases and decreases with the ethanol concentration. It can be known from the figure that the interaction between the two factors is strong, the influence is significant.
3.2.3. Optimal conditions. From the result analysed by Design Expert 8.0b, the optimal extraction conditions for total flavonoids in kale was: ultrasonic time 29.23 min, ultrasonic temperature 70.88 ℃, liquid-solid ratio 26.98 mL/g, ethanol concentration 76.76%. Under the optimal conditions, the predicted extraction rate of total flavonoids is 18.61 mg/g. The result was corrected to ultrasound time 29 min, ultrasonic temperature of 71 ℃, liquid-solid ratio of 27 mL/g and ethanol concentration of 77%. According to verification experiments, the total extraction rate of total flavonoids of kale was
18.47 mg/g, with a relative error of 0.75%. The model was of high fit and it can predict the total flavonoid extraction rate in kale with reliable result.

4. Conclusions
After a single-factor test, the response surface experiment was designed with four influence factors: ultrasonic time, ultrasonic temperature, liquid-solid ratio and ethanol concentration as variables. The data were fitted with Design Expert 8.0b software to establish a mathematical model of total flavonoid extraction. According to the regression equation of the mathematical model and its contour map, the correlation of four factors was compared. The optimal parameters for extracting was: ultrasonic time 29 min, ultrasonic temperature of 71°C, liquid-solid ratio of 27 mL/g and ethanol concentration of 77%. Under this conditions, the extraction rate of total flavonoid in kale was 18.47 mg/g, and the relative error to the estimated value was 0.75%, which showed that the mathematical model had a higher establishment fit, and the total flavonoid extraction rate in kale could be well predicted, and the process conditions were reliable.

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