

Optimization for Extraction Process of Total Flavonoid from Chenopodium quinoa Willd Seeds by Orthogonal Test

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Abstract: To optimize the extraction conditions of total flavonoid from quinoa seeds and further provide technical reference for the development of quinoa. In this experiment, based on the four factors of ethanol volume fraction, extraction temperature, ultrasonic power and ultrasonic time, orthogonal test was designed to determine the total flavonoid content of quinoa. The results showed the affecting order of the four factors on the extraction amount of total flavonoid from quinoa: ultrasonic time < extraction temperature < ultrasonic power < ethanol volume fraction. The optimal extraction process is ethanol volume fraction 90%, extraction temperature 60°C, ultrasonic power 350 w and ultrasonic time 10 min. The total flavonoid content of quinoa seeds was 1.184 mg/g.

Chenopodium quinoa Willd originated from the Andean mountains, and was widely introduced to the world. Quinoa contains many kinds of amino acids and flavonoid which can't be produced by human body, which are deeply loved by vegetarians. Quinoa is not only listed as the top ten nutritious and healthy food, but also an ideal "space food"[1-2]. Flavonoid are a natural organic compound, which can enhance the immune function of human body and play an important role in the treatment of cardiovascular diseases [3-5].

The research on the extraction technology of flavonoid is mainly concentrated on some medicinal plants and common vegetables. However, there are also some reports on the content of total flavonoid in quinoa. Sun Xueting et al. studied the flavonoid extraction of Zhejiang Nongda quinoa in 2015. The results showed that under the best extraction conditions the total flavonoid content of quinoa obtained was 2.64 mg/g [6]. Liang Bin et al. found that the best extraction conditions of quinoa flavonoid were: microwave time 125S, microwave power 260W, material liquid ratio (g / ml) 1:50, and the total flavonoid content was 6.5mg/g [7].

However, there is no relevant research on quinoa in Qinghai Tibet Plateau. In this experiment, quinoa varieties (NEGRA COLLANA) in Qinghai Tibet Plateau are selected as materials, and the best technological conditions of total flavonoid in quinoa seeds are obtained by orthogonal test, which provides reference for the selection of high-quality quinoa varieties in Qinghai Tibet Plateau and the further development and deep processing of quinoa.

1. Materials and Methods

1.1 Experimental Materials

The quinoa seeds (NEGRA COLLANA) were collected from "SongPan quinoa planting base" in Qinghai Tibet Plateau.

1.2 Experimental Methods

1.2.1 Quinoa sample preparation

The quinoa seeds are cleaned and leachate, and then placed in the electrothermal constant temperature dryness box (to 50 degrees centigrade) and dried to the constant weight, and then mashed

by the tissue mashed homogenizer. Select 60 target sieves to get very fine powder samples and store them in refrigerator.

1.2 .2 Drawing of standard curve

Referring to Yin P et al. [8], the regression equation and the standard curve are drawn as shown in Figure 1.

1.2 .3 Single factor test

The quinoa sample of 1g were accurately measured, and the absorbable value was measured under different ethanol volume fraction(40%, 50%, 60%, 70%, 80%, 90%), different extraction temperature(30°C, 40°C, 50°C, 60°C, 70°C, 80°C), ultrasonic power(150 w, 200 w, 250 w, 300 w, 350 w, 400 w) and different ultrasonic time(5min, 10 min, 15 min, 20 min, 25 min, 30 min). And the total flavonoid content was calculated by Yin P et al. [8].

1.2.4 Orthogonal test

Take the total flavone content in the extracted solution as the inspection index, analyze and select the factor levels that are meaningful for the extraction of total flavone content of quinoa, and design the four factor four level orthogonal test according to the orthogonal table L16 (45) to optimize the extraction process.

1.2.5 Verification test

According to the best extraction conditions, the validation test was carried out, and the total flavone content was determined. Parallel test 3 times.

1.3 Data analysis

The test data were analyzed and sorted out by Microsoft Excel 2010.

2. Result

2.1 The standard curve

The regression equation of rutin standard curve is $y = 9.9457x + 0.0122$, $R^2 = 0.9971$. It can be seen from this that when the concentration range of rutin is 0-0.6 mg / ml, the absorption value shows a good linear relationship (as shown in Figure 1), which will also be an important basis for calculating the total flavone content.

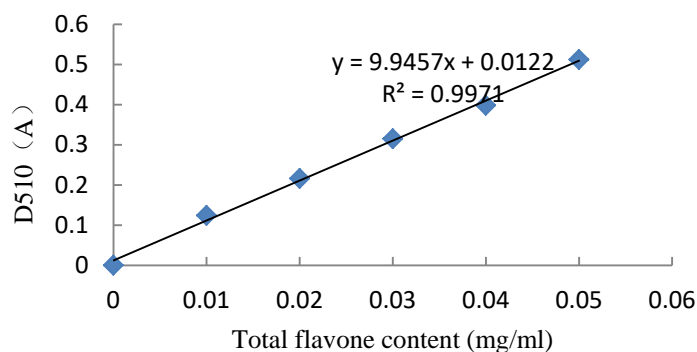


Fig.1 The standard curve of rutin

2.2 Single factor test result

2.2.1 Effect of ethanol volume fraction on the total flavonoid content of quinoa

With the increase of ethanol volume fraction, the total flavonoid content of quinoa increased first and then decreased. When the volume fraction of ethanol is 80%, the extraction amount of total flavonoid of quinoa is the largest, reaching 0.481 mg / g (as shown in Figure 2). Therefore, the ethanol volume fraction of 60%, 70%, 80% and 90% were selected in the orthogonal test.

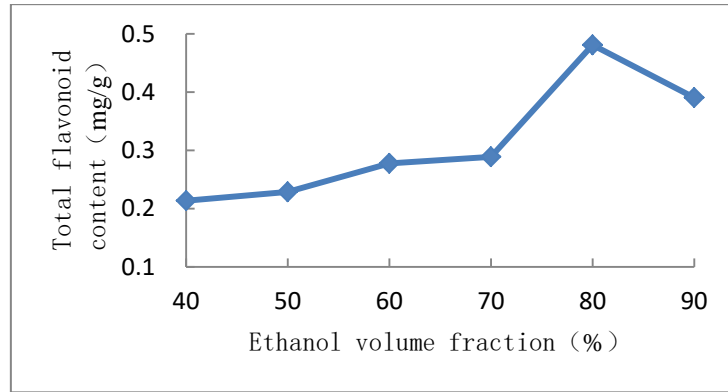


Fig.2 Effect of ethanol volume fraction on the total flavonoid content of quinoa seeds

2.2.2 Effect of extraction temperature on the total flavonoid content of quinoa

As shown in Fig. 3, when the extraction temperature is 80 °C, the total flavonoid extraction amount of resveratrol reaches the maximum. Before that, with the increasing of extraction temperature, the total flavonoid content of quinoa increased. Therefore, the extraction temperature 50 °C, 60 °C, 70 °C and 80 °C were selected in the orthogonal test.

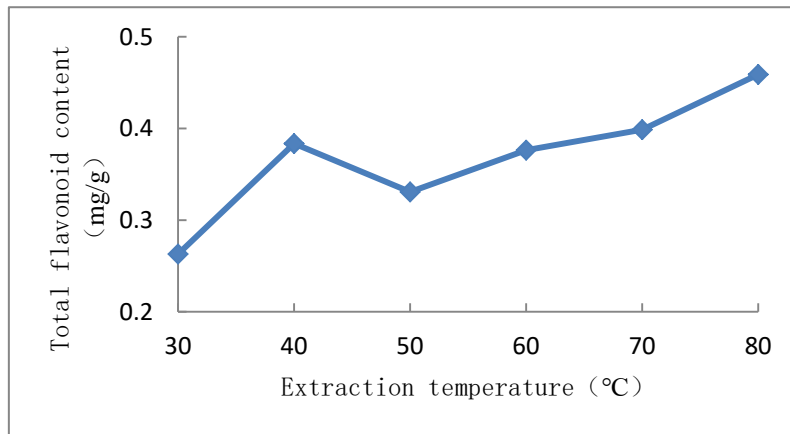


Fig.3 Effect of extraction temperature on the total flavonoid content of quinoa seeds

2.2.3 Effect of ultrasonic power on the total flavonoid content of quinoa

As shown in Figure 4, with the increase of ultrasonic power, the total flavonoid extraction of quinoa increased first and then decreased. When the ultrasonic power was 300 W, the extraction amount of total flavonoid was the largest, reaching 0.418 mg / g. Therefore, the ultrasonic power levels of 200 W, 250 W, 300 W and 350 W were selected in the orthogonal test.

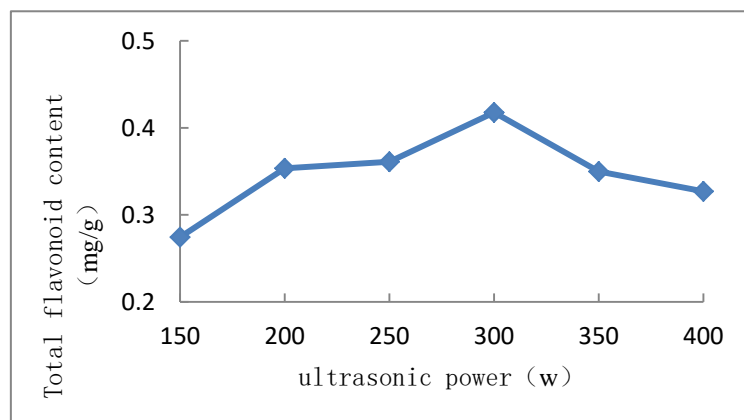


Fig.4 Effect of ultrasonic power on the total flavonoid content of quinoa seeds

2.2.4 Effect of ultrasonic time on the total flavonoid content of quinoa

As shown in Fig. 5, the change trend of total flavonoid extraction amount of quinoa in the period of 15-30 min is gentle. When the ultrasonic time was 10 minutes, the content of total flavonoid in quinoa was the highest. Therefore, the 5 min, 10 min, 15 min and 20 min were chosen in the orthogonal test.

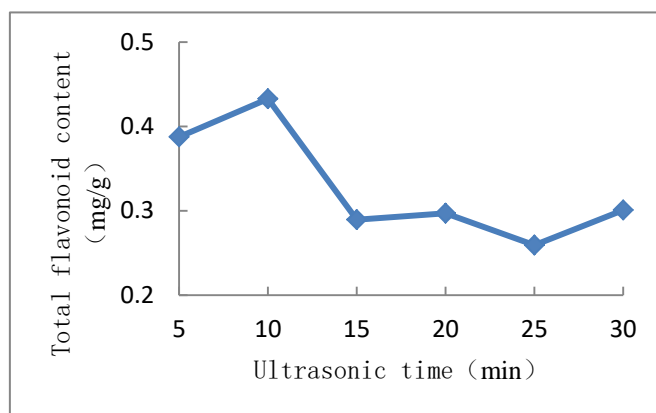


Fig.5 Effect of ultrasonic time on the total flavonoid content of quinoa seeds

2.3 Orthogonal test results

2.3.1 Setting of experimental conditions

According to the single factor experiment results, orthogonal test was conducted by selecting the most significant levels of total flavonoid extraction (Table 1).

Table 1 Factor and level of orthogonal test

| Level | A. Ethanol volume fraction(%) | Extraction temperature (°C) | ultrasonic power (W) | ultrasonic time (min) |
|-------|-------------------------------|-----------------------------|----------------------|-----------------------|
| 1 | 60 | 50 | 200 | 5 |
| 2 | 70 | 60 | 250 | 10 |
| 3 | 80 | 70 | 300 | 15 |
| 4 | 90 | 80 | 350 | 20 |

2.3.2 Orthogonal test results analysis

It can be seen from table 2 that the order of factors affecting the total flavonoid content of quinoa is from low to high: ultrasonic time < extraction temperature < ultrasonic power < ethanol volume fraction. The results showed that the best extraction parameters were: ultrasonic time 5 minutes, ethanol volume fraction 90%, extraction temperature 60 °C, ultrasonic power 300 W. According to the data range analysis, the best process parameters of total flavonoid were: ultrasonic time 10 minutes, ethanol volume fraction 90%, extraction temperature 60 °C, ultrasonic power 350 W. Because the best extraction conditions of visual analysis and range analysis of data are different, it is necessary to carry out validation test to determine the best extraction conditions of total flavonoid of quinoa.

Tab.2 Results analysis of orthogonal test

| Test number | Factor | | | | Total Flavonoid content (mg/g) |
|-------------|---------------------------|--------------------------------|----------------------|-----------------------|--------------------------------|
| | Ethanol concentration (%) | Total Flavonoid content (mg/g) | Extraction power (w) | Extraction time (min) | |
| 1 | 1 | 1 | 1 | 1 | 0.587 |
| 2 | 1 | 2 | 2 | 2 | 0.647 |
| 3 | 1 | 3 | 3 | 3 | 0.602 |
| 4 | 1 | 4 | 4 | 4 | 0.689 |
| 5 | 2 | 1 | 2 | 3 | 0.824 |

| | | | | | |
|----|-------|-------|-------|-------|-------|
| 6 | 2 | 2 | 1 | 4 | 0.838 |
| 7 | 2 | 3 | 4 | 1 | 0.862 |
| 8 | 2 | 4 | 3 | 2 | 0.911 |
| 9 | 3 | 1 | 3 | 4 | 0.959 |
| 10 | 3 | 2 | 4 | 3 | 1.034 |
| 11 | 3 | 3 | 1 | 2 | 0.941 |
| 12 | 3 | 4 | 2 | 1 | 0.907 |
| 13 | 4 | 1 | 4 | 2 | 1.132 |
| 14 | 4 | 2 | 3 | 1 | 1.162 |
| 15 | 4 | 3 | 2 | 4 | 0.948 |
| 16 | 4 | 4 | 1 | 3 | 0.993 |
| K1 | 0.631 | 0.876 | 0.840 | 0.880 | |
| K2 | 0.859 | 0.920 | 0.832 | 0.908 | |
| K3 | 0.960 | 0.838 | 0.908 | 0.863 | |
| K4 | 1.059 | 0.875 | 0.929 | 0.859 | |
| R | 0.427 | 0.082 | 0.097 | 0.049 | |

2.4 Verification test result

According to the results of three parallel experiments, the average content of total flavonoid in quinoa is 1.184 mg / g. Compared with the total flavonoid in each combination in the orthogonal experiment, we can see that the content of total flavonoid in this condition is the highest. Therefore, the best extraction technology of total flavonoid: ethanol volume fraction 90%, extraction temperature 60 °C, ultrasonic power 350 W and ultrasonic time 10 min. The average content of total flavonoid in quinoa is 1.184 mg / g.

Table 3 Test result of verification test

| Times / time | 1 | 2 | 3 |
|--------------------------------|-------|-------|-------|
| Total Flavonoid content (mg/g) | 1.185 | 1.193 | 1.174 |
| Average value (mg/g) | 1.184 | | |

3. Discussion

In the single factor experiment, when the ethanol volume fraction is 80%, the total flavonoid content of quinoa is the highest. However, in the ethanol volume fraction of 80% - 90%, the total flavonoid content of quinoa is reduced. The reason may be that as the ethanol volume fraction increases to a certain extent, some impurities in the cell dissolve out of the cell, which makes the binding rate of flavonoid and ethanol decrease, thus the total flavonoid extraction amount decreases. With the increase of extraction temperature, the total flavonoid extraction of quinoa showed an overall upward trend. The main reason may be that the increase of temperature provides energy to overcome the intermolecular force, enhances the movement state of molecules, and enhances the ability of ethanol to penetrate into cells, thus increasing the total flavonoid extraction amount [9-10]. The absolute ethanol is volatile, and the boiling point of ethanol extractant is less than 80 °C. When the extraction temperature reaches 80 °C, the ethanol extractant is boiling, and the high extraction temperature will lead to the biological activity of substances, so the extraction temperature of quinoa should not exceed 80 °C. With the increase of ultrasonic power, the content of total flavonoid first decreased and then increased. The reason may be that the ultrasonic effect increases the damage to the cells, which makes the flavonoid in the cell membrane dissolve out of the cell membrane and mix with ethanol to a great extent, and improves the extraction amount of flavonoid. After 10 minutes of ultrasound time, the amount of flavonoid extracted decreased. The reason may be that the ultrasound time is too long and other substances in the cell flow out along the broken cell membrane and into the ethanol solution, resulting in the decrease of the amount of flavonoid. In this experiment, the total flavonoid of the quinoa from Songpan planting base of Qinghai Tibet Plateau is 1.184 mg/g under the optimum extraction conditions, which is lower than that of sun Xueting and Liang Bin [6-7]. It may

be the reason for the difference of total flavonoid content in different quinoa varieties and different habitats [6].

4. Conclusion

The single factor experiment was used to study the influence of each factor on the total flavonoid extraction of quinoa, and then four factors and four levels of orthogonal experiment were used to get the order of the four factors: ultrasonic time < extraction temperature < ultrasonic power < ethanol volume fraction. The best extraction technology is 90% ethanol, 60 °C, 350 W ultrasonic power and 10 min ultrasonic time. The total flavonoid content of quinoa is 1.184 mg / g. It provides a reference for the selection of quinoa species and the further processing, development and utilization in other aspects.

Acknowledgements

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