Ultrasonic-assisted extraction of carotenoids from navel orange in southern Jiangxi

Suo Guanwen¹, Zhou Chunli*, Su Wei¹, Yan Qiao¹

¹School of Life Science, Jiangxi Science &Technology Normal University (Nanchang 330013

* Corresponding author: zhoucl6212@126.com

Abstract. In this study, single factor experiment and orthogonal experiment were used to study the extraction effect of carotenoids from navel orange in southern Jiangxi Province assisted by ultrasonic technology. The experimental results showed that when hexane: ethanol=1:1 as the extraction agent, liquid to solid ratio of 1:30, extraction time of 8 min, ultrasonic power of 250 W, extraction temperature of 25℃, extraction times of 4 times, the best extraction effect of carotenoids from Gannan navel orange was obtained.

1. Introduction

Gannan navel orange is a specialty of Ganzhou City, Jiangxi Province, with an annual output of more than one million tons. It is one of the pillar industries in Ganzhou City. Gannan navel orange fruit is large and round, usually weighing up to 50 grams each, with a bright orange skin and a sweet aroma. The fruit of Gannan navel orange is large and round, usually weighing up to 50 grams each. The skin color of the fruit is bright orange and it emits a sweet aroma. After peeling the skin, you can see the round and full fruit. It tastes sweet, sweet but not greasy, and rich in juice. In addition, Gannan navel orange is rich in carotenoids with stable yield and economical and convenient way to obtain them. Therefore, it is of certain value to study the extraction of carotenoids from them.

The yellow, orange and red fruits and vegetables such as orange, tangerine, mango, carrot, pumpkin is rich in carotenoids [1]. Carotenoids are a kind of important natural pigments, which is the main source of vitamin A in the body [2]. Meanwhile, they also have the functions of anti-oxidation, immune regulation, anti-cancer and anti-aging [3]. The traditional extraction methods include Soxhlet extraction and ordinary distillation. At present, the popular extraction methods include microwave extraction, supercritical extraction, microwave co-extraction, pressurized solvent extraction, ultrasonic-assisted extraction. The content of carotenoids in navel orange in southern Jiangxi is relatively rich, but in the processing of fruits and vegetables, the research on the extraction of carotenoids in navel orange in southern Jiangxi with the aid of ultrasonic technology is limited. In order to make full use of this resource, the extraction technology of carotenoids in navel orange in southern Jiangxi was studied in this paper. So as to provide a theoretical basis for the abundant navel orange resource utilization in southern Jiangxi in China, in order to promote the development of southern Jiangxi.
2. Materials and methods

2.1. Materials and instruments

2.1.1. Materials and reagents
Anhydrous ethanol, n-hexane, Gannan navel orange (Ganzhou City, Jiangxi Province)

2.1.2. Test instrument
KQ-300DE type CNC ultrasonic instrument, Kunshan Ultrasonic Instrument Co., Ltd.
Midea Juicer MJ-WJE2802D, Guangdong Midea Household Appliance Manufacturing Co., Ltd.
D series electronic balance, China Jiangsu Changshu Changqing Instrument Factory;
Table Top Centrifuge TGL-16G, Shanghai Anting Scientific Instrument Factory;
722 visible light spectrophotometer, Shanghai Youke Instrument Co., Ltd.;

2.2. Test methods

2.2.1. Operation process
1) Sample preparation
orange was peeled and juiced, and a certain amount of orange juice was put into a centrifuge tube for use.
2) Extractant: hexane: ethanol =1:1
3) Ultrasonic-assisted carotenoid extraction
   Extraction process reference Nie et al [4], slightly modified according to the test design; In the preparation of good than orange juice to add a certain amount of material liquid extraction agent, set up the ultrasonic power and ultrasonic time, centrifuge tube to be included in the ultrasonic apparatus for ultrasonic assisted extraction, 500 g centrifuge for 10 min, select the upper extract, each corresponding to extract constant volume after volume from dilute to 10 ml volumetric flask, brown repeated three times and averaged. Then we used a 722 visible light photometer to detect the absorbance value of the extract at the maximum absorption wavelength of 445 nm [5]. All test procedures must be kept out of the light, otherwise it will cause carotenoid decomposition and increase the test error.

2.2.2. Calculation of carotenoids
The calculation formula is as follows:
\[ M = \frac{A_{445} \times V}{2500 \times m} \times 10 \]
M: total carotenoid content (mg/g); 2500: carotenoid empirical absorption coefficient; V: Total volume (ml); M: mass of orange juice (g).

2.2.3. Test process

2.2.3.1. Single factor test
(1) Influence of different ratio of material to liquid on extraction effect
1g of orange juice was accurately weighed, and the ultrasonic conditions were fixed (40 kHz, 150 W, 25 °C, 6 min). The extraction times were once, the extraction agent was hexane: ethanol =1:1, and the solid-liquid ratios were set at 1:10, 1:20, 1:30, 1:40 and 1:50(g/mL).
(2) Influence of different ultrasonic power on extraction effect
1g of orange juice was accurately weighed, the ultrasonic conditions were fixed (40 kHz, 25°C, 6 min), the extraction times were once, the extraction agent was n-hexane: ethanol =1:1, the solid-liquid ratio was 1:40 (g/mL), and the ultrasonic power was set at 150, 200, 250, 300, 350(W).
(3) Influence of different ultrasonic time on extraction effect
1g of orange juice was accurately weighed, and the ultrasonic conditions were fixed (40 kHz, 150 W,
25 °C). The extraction times were once, the extraction agent was hexane: ethanol = 1:1, the solid-liquid ratio was 1:40 (g/mL), and the ultrasonic extraction time was set at 4, 6, 8, 10, 12 (min).

(4) Influence of different extraction times on extraction effect
1 g of orange juice was accurately weighed and the ultrasonic conditions were fixed (40 kHz, 150 W, 25 °C, 6 min). The extraction times were once. The extraction agent was hexane: ethanol = 1:1, and the solid-liquid ratio was 1:40 (g/mL).

2.2.3.2. Orthogonal test of the optimal extraction process by ultrasonic-assisted method
On the basis of single factor experiment, the influence of interaction among these four factors is further studied. Orthogonal tests with 4 factors and 3 levels were designed: liquid-solid ratio was 1:20, 1:30, 1:40 (g/mL), ultrasonic power was 200, 250, 300 (W), ultrasonic extraction time was 6, 8, 10 (min), extraction times were 2, 3, 4 (times), respectively. The optimal extraction process of carotenoids from navel orange was determined by ultrasonic-assisted extraction.

3. Results and discussion
3.1. Influence of different liquid-solid ratios on the extraction of carotenoids from navel orange
As can be seen from Figure 1, when the solid-liquid ratio is 1:30, the extraction effect of carotenoids is the best. The reason may be that when the extraction agent is too small, the carotenoids in Gannan navel orange cannot be fully extracted, and some of the carotenoids in Gannan navel orange still remain in the cells. However, when the extractant was increased to a certain amount, the extraction amount of carotenoids did not increase significantly, possibly because the carotenoids in navel orange in southern Jiangxi had been fully extracted at this time [6]. Therefore, based on the above analysis, it could be concluded that the optimal solid-liquid ratio was 1:30 (g/mL), and the best extraction effect of carotenoids was achieved at this time.

![Figure 1. Effects of different liquid-solid ratios on the extraction of carotenoids from navel orange in southern Jiangxi](image)

3.2. Effects of different ultrasonic power on the extraction of carotenoids from navel orange
As can be seen from Figure 2, when the ultrasonic power is at 250 W, the extraction effect of carotenoids in navel orange in southern Jiangxi is the best. This may be because, when the ultrasonic power is too low, the cavitation effect in the ultrasonic wave does not play a full role, leading to the insufficient dissolution of carotenoids into the extractant, thus making the extraction effect unsatisfactory [7]. However, high ultrasonic power will lead to severe cavitation effect, which may decompose the carotenoids in Gannan navel orange. Therefore, in conclusion, the optimal ultrasonic power is 250 W, which has the best effect on carotenoid extraction and can fully extract carotenoids from navel orange in southern Jiangxi.
3.3. Effects of different ultrasonic extraction time on carotenoid extraction from navel orange

As can be seen from Figure 3, when the ultrasonic extraction time was 8 min, the extraction effect of carotenoids in navel orange in southern Jiangxi was the best. This may be because, when the ultrasonic extraction time is too short, the carotenoids in Gannan navel orange cannot dissolve in the extraction agent, while when the extraction time is too long, the extraction agent is organic solvent and easy to volatilize, which leads to the unsatisfactory extraction effect [8].

According to the comprehensive analysis, the optimal ultrasonic extraction time was 8 min, and the best effect of extracting carotenoids from navel orange in southern Jiangxi was achieved at this time.

3.4. Effects of different extraction times on the extraction of carotenoids from Gannan navel orange

As can be seen from Figure 4, when the number of extractions is 4, the extraction effect of carotenoids in Gannan navel orange is relatively good and the extraction is sufficient. The reason may be that the carotenoids in Gannan navel orange cannot be completely dissolved in the extractant when the extraction times are too small, so the extraction effect of carotenoids is poor. When the extraction times are 4 times, the extraction effect is the best, and the carotenoids are fully extracted at this time. Therefore, the most appropriate number of extractions is 4.
Figure 4. Effects of different extraction times on the extraction of carotenoids from navel orange

3.5. Influence of various factors on the extraction of carotenoids in orthogonal test

Table 1 Influence of various factors on extraction of carotenoids in orthogonal test

| combination | A  | B  | C  | D  | M   |
|------------|----|----|----|----|-----|
| 1          | 1:20| 6  | 200| 2  | 1.004|
| 2          | 1:20| 8  | 250| 3  | 1.260|
| 3          | 1:20| 10 | 300| 4  | 1.408|
| 4          | 1:30| 6  | 250| 4  | 1.520|
| 5          | 1:30| 8  | 300| 2  | 1.106|
| 6          | 1:30| 10 | 200| 3  | 1.141|
| 7          | 1:40| 6  | 300| 3  | 1.227|
| 8          | 1:40| 8  | 250| 4  | 1.519|
| 9          | 1:40| 10 | 200| 2  | 1.189|
| K_1        | 1.224| 1.250| 1.221| 1.100 |
| K_2        | 1.256| 1.295| 1.323| 1.209 |
| K_3        | 1.312| 1.246| 1.247| 1.482 |
| R          | 0.088| 0.049| 0.102| 0.382 |

Captions: A Solid liquid ratio (g/ml); B Extraction time (min); C Ultrasonic power (W); D Number of extraction (times); M (total carotenoid content)

According to the orthogonal test results in Table 1, the influences of the four factors on the extraction of carotenoids are D (extraction times) > C (ultrasonic power) > A (solid-liquid ratio) > B (extraction time). When the solid-liquid ratio was set as 1:30 (g/mL), the extraction time was set as 8 min, the ultrasonic power was set as 250 W, and the extraction times were set as 4 times, the best extraction effect of carotenoids from Gannan navel orange was obtained.

Table 2 Results of ANOVA

| Factor | SS  | df  | F-ratio | F value |
|--------|-----|-----|---------|---------|
| A      | 0.012| 2   | 0.180   | 4.460   |
| B      | 0.004| 2   | 0.060   | 4.460   |
| C      | 0.017| 2   | 0.256   | 4.460   |
| D      | 0.233| 2   | 3.504   | 4.460   |
| Error  | 0.27 | 8   |         |         |

Captions: A Solid liquid ratio (g/ml); B Extraction time (min); C Ultrasonic power (W); D Number of extraction (times)
of extraction (times); M (total carotenoid content)

In order to fully discuss the relationship between the above 4 factors and their influence on the auxiliary extraction of carotenoids from navel orange in southern Jiangxi, the orthogonal test data were analyzed by means of variance analysis method, so as to find out the variation source of the leading role of the above 4 factors. The results of ANOVA are shown in Table 2.

According to the above test results and comprehensive analysis, the optimal process for ultrasonic-assisted extraction of carotenoids from Gannan navel orange was as follows: liquid-solid ratio was set at 1:30 g/mol, extraction time was set at 8 min, ultrasonic power was set at 250 W, and extraction times were set at 4 times.

### 4. Conclusion

In this experiment, four single factor experiments including liquid-solid ratio, ultrasonic power, ultrasonic extraction time and extraction times were designed to study the effects of the above factors on ultrasonic-assisted extraction of carotenoids. On this basis, orthogonal tests with 4 factors and 3 levels were designed to determine the relatively optimal extraction process of carotenoids: liquid to solid ratio was set as 1:30 g/mol, extraction time was set as 8 min, ultrasonic power was set as 250 W, and extraction times were set as 4 times.

Ultrasonic assisted extraction of carotenoids disadvantages clearly, is currently on large-scale industrial use have no economy, and safety remains to be proven, at the same time also has the problem of large noise, but it also has a short time, high efficiency, the advantages of convenient, so further research is needed in future trials.

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