Extraction of Anthocyanin from Eggplant Peel by Ultrasonic Assisted Method and Application in Cosmetics

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Abstract: The anthocyanin of eggplant skin was extracted by ultrasonic assisted method. Single factor experiment and orthogonal optimization experiment were used to optimize the extraction factors of anthocyanin from eggplant peel. The results showed that the optimum extraction of anthocyanin from eggplant peel was obtained by orthogonal experiment under the condition of feed-liquid ratio of 13:15, time of 40 min, and temperature of 50°C. The anthocyanin is stable by treating 1 min after 100°C.

1 Introduction

Anthocyanin (Anthocyanin), also known as anthocyanins, are water-soluble flavonoids found in the epidermis of flowers, fruits and stems and leaves of plants and in the cortical cells of the following table, especially in dark red, blue or purple fruits. The color of anthocyanin can change with the acidity and alkalinity of cell fluid, the acidity of cell fluid is red, and the alkalinity of cell fluid is blue. Anthocyanin has the ability of anti-oxidation, anti-mutation and so on, which plays an important role in human physiological function [1-3]. Therefore, it is of great significance to reduce the cost of anthocyanin extraction. Although the synthetic pigment has bright color, good stability and low price, natural anthocyanins have been paid more and more attention in food, medicine and cosmetics because of its toxicity [4-5].

Eggplant (*Solano melongena* L.) is an annual herb. The shape of the fruit is long or round. The color is mostly purple or purple black, less white, red and so on. Eggplant tastes delicious, and it is nutritious and popular. Eggplant peel is rich in anthocyanins and has certain development value as natural food pigment.

At present, anthocyanin extraction methods include ultrasonic extraction, microwave extraction, organic solvent extraction and so on. Because organic solvents are mostly toxic reagents, they are volatile and have low extraction rate, and they are faced with organic solvent residues, which have a bad effect on human health. Therefore, the selection of non-toxic organic solvent for eggplant skin anthocyanin extraction has positive practical significance.

2 Materials and methods

2.1 Materials

Eggplant, distilled water, anhydrous ethanol, glucose, phosphate buffer, UV-Vis spectrophotometer, (7600CRT, Shanghai jinghua technology Co.,Ltd) ultrasonic cleaner (Sinobakr,) traditional Chinese medicine pulverizer(DF-50-A,dade), constant temperature water tank(BHS-1,LAB FISH), electronic balance(shanghai jinghua technology Co., Ltd), electrothermalconstant temperature blast dryer, Abbe refractometer.(DigPol-R200,Shanghai jiahang technology Co.,Ltd)

2.2 Extraction methods

2.2.1 Pretreatment

The eggplant was deviated, separated pericarp and pulp. Then the eggplant peel was washed and dried, and cut into small pieces in electrothermal blast dryer. The small pieces of eggplant peel were baked to constant weight at 60°C, and crushed to powder. Finally the powder of eggplant peel was sealed storage and set aside.

2.2.2 Anthocyanins

In this experiment, ultrasonic assisted ethanol extraction method can accelerate the destruction of plant cell wall, improve the extraction rate of intracellular substances and shorten the extraction time [6].

A 25 g pretreated powder was added to a round bottom flask containing a certain amount of anhydrous ethanol, and then rocked and heated for a certain time. The

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ultrasonic extracted extract was transferred to the centrifuge tube and the supernatant was centrifuged and separated. Since the extraction rate of anthocyanin is positively correlated with the absorbance at wavelength 525 nm, the extraction rate of anthocyanin can be indirectly reflected by the determination of absorbance at 525 nm.

2.2.3 Process flow

eggplant peel → dry → smash → sieving → ultrasonic extraction → centrifuge → keep liquid supernatant → take samples of the liquid supernatant and measure absorbance → calculate the extraction rate

2.3 Single-factor experiment

2.3.1 Time on extraction rate of anthocyanins

The extraction rate of anthocyanin in different ultrasonic extraction time was determined at a wavelength of 525 nm, at a solid-liquid ratio of 1:15 and an extraction temperature of 50°C, with the same concentration of glucose aqueous solution as a blank control, and 10 min were selected as the unit time interval of 10-50 min. The results were determined three times parallelly.

2.3.2 Temperature on extraction rate of anthocyanins

The extraction rate of anthocyanin in different ultrasonic extraction temperature was determined at a wavelength of 525 nm, at a solid-liquid ratio of 1:15 and the highest extraction rate of time, with the same concentration of glucose aqueous solution was added as blank control. And 10°C was selected as the unit time interval of 20-60°C. The results were determined three times parallelly.

2.3.3 Solid-liquid ratio on extraction rate of anthocyanins

The solid-liquid ratio refers to the ratio of eggplant powder to anhydrous ethanol. At the wavelength of 525 nm, the extraction temperature of 50°C, and the extraction time of 40 min, the extraction rate of anthocyanins at different solid-liquid ratios was determined. The solid-liquid ratios were 1:5, 1:7.5, 1:10, 1:12.5, 1:15, 1:17.5, 1:20, 1:22.5, 1:25. The results were determined three times parallelly.

2.4 Thermal stability of anthocyanins

Thermal stability of anthocyanins was evaluated by absorbance. The absorbance values were obtained by measuring the equivalent anthocyanin extract which were dissolved by phosphoric acid buffer solution. In the temperature range from 40°C to 100°C, taking 10 as the unit interval, six temperatures of 50°C, 60°C, 70°C, 80°C, 90°C and 100°C were selected and kept in the constant temperature water bath for 30 min, 25 min, 20 min, 15 min, 10 min and 5 min respectively. The absorbance values were determined three times parallelly.

2.5 Orthogonal experiment

The orthogonal experimental factors were ultrasonic extraction temperature, ultrasonic extraction time and solid-liquid ratio. They are shown in Table 1.

Table1. Positive experimental factors

Level determination Experimental factors	(A) Time of ultrasonic extraction	(B) Ultrasonic extraction temperature	(C) Solid-liquid ratio
1	20	30	1:12.5
2	30	40	1:15
3	40	50	1:17.5

3 Experimental Results and Analysis

3.1 Single-factor experiment

3.1.1 Time on extraction rate of anthocyanins

The extraction time-absorbance curve is shown in figure 1(a) under the condition of the ethanol as extractant, the solid-liquid ratio of 1:15, the extraction temperature of 50°C, and 10 min was selected as the unit time interval of 10-50 min. The extraction rate gradually increased before the extraction time of 40 min and its maximum value appeared at 40 min. Then, the extraction rate tended to be constant after 40 min. The reason was that almost all anthocyanins in eggplant peel were extracted more than 40 min of extraction time, and the extraction rate remained unchanged. The extraction effect is the best at 40 min because short time will cause inadequate extraction, and long time will cause energy waste.

Fig 1. Effects of different factor on extraction rate of anthocyanins **(a)** different time **(b)** different temperature

3.1.2 Temperature on extraction rate of anthocyanins

The extraction temperature-absorbance curve is shown in figure 1(b) under the condition of the ethanol as extractant, the solid-liquid ratio of 1:15, and the extraction time of 40 min. According to the experimental data, the absorbance of the extract increases first and then decreases with the increase of temperature. The absorbance is the largest at 50°C. It is speculated that if the temperature continues to rise, the absorbance will decrease. Therefore, increasing the temperature in a certain temperature range can increase the extraction rate of anthocyanin, but once it exceeds this range, the extraction rate will be significantly reduced. Therefore, the extraction rate of anthocyanin was the highest at 50°C.

3.1.3 Solid-liquid ratio on extraction rate of anthocyanins

The solid-liquid ratio-absorbance curve is shown in figure 2 under the condition of the ethanol as extractant, the extraction temperature of 50°C, and the extraction time of 40 min. It can be seen from figure 2 that when the solid-liquid ratio is below 1:15, the extraction rates increase and the absorbance of anthocyanins increases gradually. With the slow increase of the extraction solvent, anthocyanin and the extraction solvent can be fully contacted, increasing the amount of anthocyanin extraction, so the extraction rate increases. When the solid-liquid ratio exceeds 1:15, the absorbance decreases. So the best solid-liquid ratio is 1:15.

Fig 2. Effects of different solid-liquid ratio on extraction rate of anthocyanins

3.2 Anthocyanin thermal stability test

Anthocyanin solution of pH=4 was prepared by citric acid-disodium hydrogen phosphate to examine the thermal stability of anthocyanin. They were heated in a water bath of 65°C for 30 min, 75°C for 15 min, 85°C for 10 min, 95°C for 5 min, and 100°C for 1min, respectively. The absorbance was measured at 525 nm. The results are shown in Table 2. According to the table, the absorbance of anthocyanin at 100°C is still larger than that at other temperatures, and the anthocyanin is stable at high temperature. So the anthocyanin can be stable in the production process of related products.

Table2. Thermal stability test results of anthocyanin

Temperature/°C	Time/ min	Absorbance values
65	30	0.66
75	15	0.52
85	10	0.20
95	5	0.47
100	1	0.73

3.3 Orthogonal test

Through the analysis of orthogonal software design, the

orthogonal result can be obtained for different ultrasonic treatment time, ultrasonic treatment temperature and solid-liquid ratio, as shown in Table 3. Table 3 shows that the influence of various factors on anthocyanin extraction is $B > C > A$. That is ultrasonic temperature > solid-liquid ratio > ultrasonic time. The optimal combination of extraction condition is $A_3B_3C_2$ that is solid-liquid ratio of 1:15, ultrasonic time of 40 min, and ultrasonic temperature of 50°C.

Table3. Orthogonal experimental results

Experimental serial number	Factors			Absorbance/Ann 525
	(A) Ultrasonic time	(B) Ultrasonic temperature	(C) Solid-liquid ratio	
1	1	1	1	0.233
2	2	1	2	0.269
3	3	1	3	0.310
4	1	2	2	0.245
5	2	2	3	0.455
6	3	2	1	0.332
7	1	3	3	0.200
8	2	3	1	0.306
9	3	3	2	0.397
k1	0.226	0.272	0.290	$A_3B_3C_2$
k2	0.343	0.259	0.304	
k3	0.346	0.301	0.221	

4 Application of Anthocyanins in Cosmetics

Studies have shown that anthocyanins have antioxidant, anti-mutation, and ascorbic acid-related physiological functions. They are a very healthy natural pigment of protecting the skin on the lips, resisting free radicals, reducing UV damage and melanin precipitation, preventing dark around the mouth, and maintaining proper elasticity and tension[7]. The principle that anthocyanins can show different colors in different acid and alkali environments which can be used to add them to cosmetics. In addition, anthocyanins can play a related physiological role to achieve the purpose of environmental protection and health.

5 Conclusions

Single factor experiment showed that ultrasonic time was a significant factor in the extraction of anthocyanin from eggplant peel. Through orthogonal experiment, the anthocyanin extracted from eggplant peel was the best

under the condition of feed-liquid ratio of 1:15, time of 40 min, and temperature of 50°C. The thermal stability of anthocyanin was good after 100°C for 1 min treatment. It shows that high temperature short-time treatment has little effect on the stability of anthocyanin. Anthocyanins of physiological functions can be used to add to cosmetics.

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