Study on Extraction Process of Anthocyanins from Mulberry

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Abstract: Anthocyanin was extracted from mulberry with Wenshan tea mulberry as raw material by ultrasonic extraction. The effects of extraction time, solid-liquid ratio, ethanol volume fraction and hydrochloric acid volume fraction on the extraction rate of anthocyanins of mulberry were investigated by single factor and orthogonal experiments. The experimental results show that the extraction time is 15 minutes, the ratio of material to liquid is 1: 5 (g / ml), the volume fraction of ethanol is 70%, and the volume fraction of HCl is 0.5%. Under these conditions, the extraction content of anthocyanins was 2.43 mg / g, which provided a reference basis for the large-scale development and utilization of anthocyanins from mulberry.

1. Introduction

Mulberry (Mulberry) can also be called Mulberry, which belongs to the Morusae Morus plant of the family Mulaceae. It is the mature fruit of the deciduous tree of the perennial Mulberry family. - 2]. Mulberry juice is rich in water-soluble anthocyanins such as mulberry redpigment. Mulberry red pigment is a good natural pigment resource. Its chemical structure is anthocyanins containing anthocyanins. Its basic structure is 2 Polyphenols formed by the bonding of polyhydroxy or polymethoxy substituents of phenylbenzofuran with sugars, due to the different anthocyanin hydroxyl groups and methoxy ligands, the types of anthocyanins also differ, among which the main ingredient is cornflower pigments [3-5]. Anthocyanins have the functions of anti-oxidation, anti-virus, scavenging free radicals, etc., and have prevention, improvement and health functions for cardiovascular disease, liver function, dementia, etc. [6-8]. And cosmetics have a wide range of development prospects and application value.

There are many reports on the research on the extraction process of anthocyanins. The research methods mainly focus on traditional solvent extraction, enzymatic extraction, ultrasonic extraction, etc. [9-10]. Among these several extraction methods, the ultrasonic extraction method has the advantages of simple experimental equipment, convenient operation, high yield, convenient adjustment of experimental conditions, short extraction time, and no heating required [11]. The content of anthocyanins in mulberry under different ultrasonic extraction conditions was measured using the pH-differential method of ultraviolet-visible spectrum [12]. The orthogonal design of the anthocyanins extraction process conditions was used to determine the optimal mulberry anthocyanins. Extraction process. It provides basis for large-scale development and utilization of anthocyanin compound mulberry red pigment and industrial production.

2. Experimental Part

Mulberry (from Chaan Village, Wenshan City), cyanidin-3-O-glucoside standard (Shanghai Jinsui Biological Co., Ltd.), absolute ethanol, concentrated hydrochloric acid, sodium hydroxide, potassium chloride, glacial acetic acid, Anhydrous sodium acetate (both analytical grade).

pH = 1 ± 0.1 buffer solution preparation: accurately weigh 1.49g of potassium chloride reagent, make up to 100ml with distilled water, shake well and set aside. Measure 1.62ml of concentrated hydrochloric acid solution, make up to 100ml with distilled water, shake well and set aside. The prepared potassium chloride solution and the hydrochloric acid solution were mixed at a ratio of 1: 1, and the pH value of the prepared solution was measured with a precision pH meter to 1.08.

pH = 4.5 buffer solution preparation: accurately weigh 1.64g of anhydrous sodium acetate reagent, make up to 100ml with distilled water, shake well and set aside. Measure 1.14ml of glacial acetic acid, make up to 100ml with distilled water, shake well and set aside. The prepared sodium acetate solution and the glacial acetic acid solution were mixed at a ratio of 1: 1, and the pH value of the prepared solution was measured with a precision pH meter to 4.48 [13].

SK2200HP Ultrasonic Cleaner (Shanghai Kedao Ultrasonic Instrument Co., Ltd.);

pHS-3C Precision pH Meter (Shanghai Precision Instrument Co., Ltd.);

DHG-9076 electric heating constant temperature blast drying oven (Shanghai Yuejin Medical Optical Instrument Factory);

Uv-2550 UV spectrophotometer (Shimadzu Corporation, Japan);

XS225A electronic analytical balance (Prises International (Shanghai) Co., Ltd.);

Juicer (Jiuyang Co., Ltd.);

800 centrifuge (Jiangsu Dadi Automation Instrument Factory);

Put fresh 1000g mulberry banned into a juicer to squeeze the pulp, put it in a glass container and put it in the refrigerator to keep it fresh. Fresh mulberries must not be washed with tap water, as it will not affect the determination of anthocyanins.

Using an electronic analytical balance, accurately weigh 2g of mulberry fruit pulp into a 100ml stoppered triangle bottle, and add the ratio of material to liquid 1: 5 to a 0.1% HCl 80% ethanol solution (at this time, the pH value of the HCl ethanol solution was measured to be 2.08). Mix well and extract mulberry pigment at 40 ° C under ultrasonic cleaning power of 40%. Centrifuge for 10 min in a 2000 r / min centrifuge, and dilute the obtained extract to 10 ml with distilled water for later use.

Take 0.5ml of fruit pulp extract and 25mg of cyanidin-3-o glucoside standard, and use 0.1% hydrochloric acid 80% ethanol extractant to make a volume into a 10ml volumetric flask, shake well and set aside. Then, the dilution solution was used as a blank, and the two solutions were scanned at a wavelength of 200 to 800 nm for full-band absorption spectrum to determine the maximum absorption wavelength.

Take 0.5ml of mulberry anthocyanin extract from section 2.3.2, dilute to 10ml with buffer solutions of pH 1.08 and 4.48, mix well, and then use a 1cm cuvette with 0.1% HCl80% ethanol solution as a blank control. The absorption value was measured at the maximum absorption wavelength and 700nm wavelength, and the anthocyanin content was calculated by substituting into the formula (1) and (2) [14].

A total = (Amax-A700) pH = 1.08- (Amax-A700) pH = 4.48 (1)

Anthocyanin content (mg / 100g) = A × M × f × V / (ε × b × m) × 100 (2)

Among them: Amax is the absorbance of the extract at the maximum absorption wavelength; A700 extract is at the absorbance wavelength of 700 nm; M relative molecular weight of cyanidin-3-o glucoside (449.2); f The amount of extraction solution used is different Multiples of the pH buffer solution after dilution; V Total volume of the extraction solution mL; ε Centaurin 3-o glucoside extinction coefficient (26900L · cm-1 · mg-1); m Mulberry fruit pulp sampling amount g ; Anthocyanin content mg / 100g is expressed by the equivalent of cyanidin-3-o glucoside.

An electronic analytical balance was used to accurately weigh 2 g of mulberry fruit pulp into a 100 ml stoppered triangular flask, and the extraction solution containing 0.1% HCl 80% ethanol solution was added at a ratio of 1: 5. At a temperature of 40 ° C, the ultrasonic cleaning power was 40%. Extraction of mulberry pigment under the conditions of. The extraction time was 5min, 10min, 15min, 20min, 25min. After ultrasonic extraction, the extract was centrifuged with a 2000r / min centrifuge for 10min, and the obtained extract was made up to 10ml with distilled water. The content of anthocyanins under different extraction conditions was analyzed by pH differential method.

Accurately weigh 2g mulberry fruit pulp in a 100ml stoppered triangle bottle, and add 1: 5%, 1:10, 1:15, 1:20, 1:25 proportions containing 0.1% HCl 80% ethanol solution for extraction according to the ratio of material to liquid. The extraction of mulberry pigment was carried out at the temperature of 40 ° C and the ultrasonic cleaning power of 40% for 10 min. After ultrasonic

extraction, the extract was centrifuged at 2000 r / min centrifuge for 10 minutes, and the obtained extract was made to 10 ml with distilled water. The content of anthocyanins under different material-liquid ratio conditions was analyzed by pH differential method.

Accurately weigh 2g mulberry fruit pulp in a 100ml stoppered triangular bottle, and add volume fractions of 0.1%, 0.2%, 0.3%, 0.4%, 0.5% concentrated hydrochloric acid 80% ethanol solution according to the ratio of material to liquid respectively 1: 5. The extract was subjected to mulberry pigment extraction at a temperature of 40 ° C and an ultrasonic cleaning power of 40% for 10 min. After ultrasonic extraction, the extract was centrifuged at 2000 r / min centrifuge for 10 minutes, and the obtained extract was made to 10 ml with distilled water. The content of anthocyanins under different concentrated hydrochloric acid volume fraction conditions was analyzed by pH differential method.

Accurately weigh 2g mulberry fruit pulp in a 100ml stoppered triangle bottle, and add a volume fraction of 0.1% concentrated hydrochloric acid ethanol at a ratio of 1: 5 to the volume ratio of 30%, 40%, 50%, and 60%. 70%, 80% solution extraction solution, temperature 40 ° C, ultrasonic cleaning power of 40% for 10 minutes. After ultrasonic extraction, the extract was centrifuged at 2000 r / min centrifuge for 10 minutes, and the obtained extract was made to 10 ml with distilled water. The content of anthocyanins under different ethanol volume fraction conditions was analyzed by pH differential method.

Based on the results of the above single-factor tests, the optimal conditions for each factor were obtained. A four-factor three-level test was drawn up. An orthogonal test for extracting anthocyanins from mulberry was made according to the orthogonal test design table.

3. Results and Discussion

The mulberry extract solution was scanned with a 25 mg cyanidin-3-o glucoside standard in the 200-800 nm band, and the maximum absorption wavelength of the extract and the standard was found to be 534 nm.
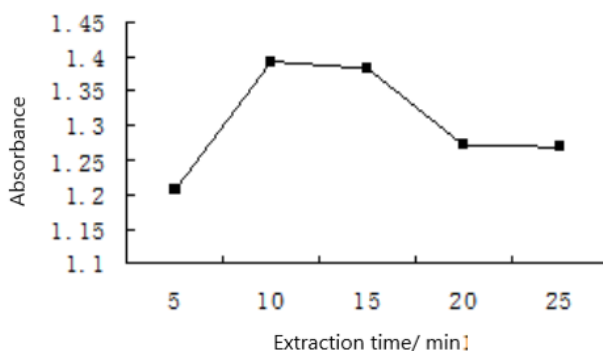

Fig.1 Effect of Extraction Time on Absorbance of Anthocyanins of Mulberry

It can be seen from Figure 1 that the extraction time has a significant effect on the absorbance of mulberry anthocyanins. As the extraction time increases, the absorbance of the extract solution increases. When the extraction time was 10 min, the absorbance of anthocyanins was at the maximum, indicating that the extraction effect was the best at this time. However, after the extraction time is longer than 15 minutes, the absorbance begins to decrease. The reason may be that the structure of anthocyanins is destroyed as the ultrasonic extraction time increases, which affects the extraction effect of anthocyanins. So the extraction time of 10min is beneficial to the extraction effect of anthocyanins.
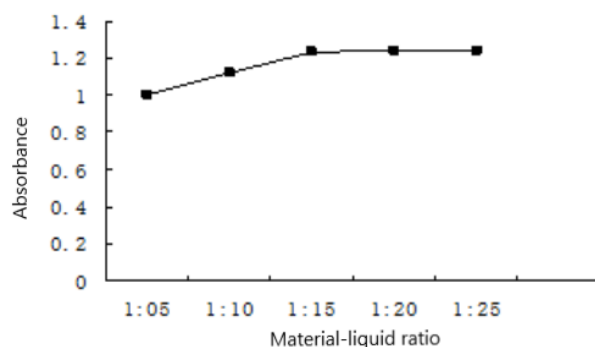
Fig.2 Effect of Material-Liquid Ratio on Absorbance of Anthocyanins of Mulberry

It can be seen from Fig.2 that as the ratio of material to liquid decreases, the absorbance of mulberry anthocyanins increases. When the material-liquid ratio is lower than 1:15, the anthocyanin absorbance value tends to decrease, and the change is not large, which indicates that if the material-liquid ratio is increased, the effect of anthocyanin extraction efficiency is not significant. Therefore, when the ratio of material to liquid is 1:15, the extraction of anthocyanins is most suitable.
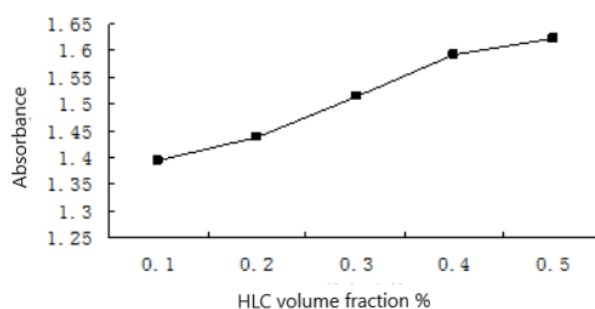

Fig.3 Effect of Hcl Volume Fraction Ratio on Anthocyanin Absorbance of Mulberry

The different volume fraction of HCl affects the pH value of the extractant. As the volume fraction of HCl increases, the pH value gradually decreases (wherein the pH is less than 2.0), and the absorbance of anthocyanins gradually increases, and the extraction effect also increases Improve accordingly. The increase in absorbance of HCl volume fraction greater than 0.4% is not very high. Because the acidity is too strong to cause anthocyanins, the extraction of anthocyanins from mulberry is optimal when the volume fraction of HCl is 0.4%.
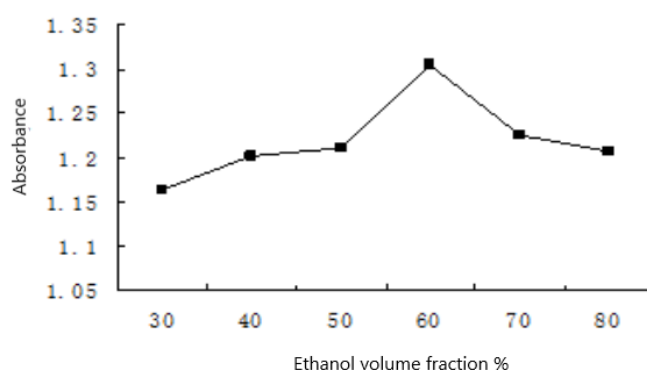

Fig.4 Effect of Ethanol Volume Fraction Ratio on Anthocyanin Absorbance of Mulberry

Because of its low cost and low toxicity, ethanol is often used for the extraction of polyphenols, and its concentration has a great influence on the extraction effect of anthocyanins. It can be seen from Figure 3-4 that the absorbance value of anthocyanins of mulberry gradually increases with the increase of the volume fraction of ethanol. When the volume fraction of ethanol is 60%, the absorbance value of anthocyanins is the largest. As the volume fraction of ethanol increases, the

absorbance value decreases. Therefore, when the volume fraction is 60%, the extraction of mulberry anthocyanins is the best.

Based on the results of the above single-factor test, a four-factor three-level test was developed, and an orthogonal test for the extraction of anthocyanins from mulberry was performed according to the orthogonal test design table (Table1).

Table 1 Single Factor Level Table

Level	A Extra Time /min	B M-l ratio	C HCl Volume frac/%	D Ethanol volume frac /%
1	5	1:5	0.3%	50%
2	10	1:10	0.4%	60%
3	15	1:15	0.5%	70%

Table 2 Orthogonal Test Results and Analysis

No.	A		B	C	D	Anthocyanin extraction rate mg/g
1	1		1	1	1	1.33
2	1		2	2	2	1.31
3	1		3	3	3	1.36
4	2		1	2	3	1.45
5	2		2	3	1	1.34
6	2		3	1	2	1.37
7	3		1	3	2	1.62
8	3		2	1	3	1.53
9	3		3	2	1	1.50
K_1	4.00		4.40	4.23	4.17	
K_2	4.16		4.18	4.26	4.30	
K_3	4.65		4.23	4.32	4.34	
k_1	1.33		1.47	1.41	1.39	
k_2	1.39		1.39	1.42	1.43	
k_3	1.55		1.41	1.44	1.45	
R	0.22		0.08	0.03	0.06	
Major order		A>B>D>C				
Excellent level	A3		B1	C3	D3	
Excellent combination		$A_3B_1C_3D_3$				

According to the results of the single-factor test, determine the extraction time, material-liquid ratio, HCl volume fraction, and ethanol volume fraction as the factors to be examined. Calculate by pH difference method and use the anthocyanin extraction rate as an index. Select the L9 (34) orthogonal table, Perform a four-factor three-level orthogonal test. The orthogonal test results are shown in Table 2.

As can be seen from Table 2, the major and minor effects of each factor on the extraction rate of anthocyanins from fruit mulberry are A> B> D> C. The optimal combination for extracting anthocyanins from fruit mulberry by ultrasonic instrument is A3B1C3D3, that is, the extraction time is 15min, the ratio of material to liquid is 1: 5, the volume fraction of HCl is 0.5%, and the volume fraction of ethanol is 70%.

4. Conclusion

The best extraction results obtained in single-factor experiments and orthogonal experiments are: the extraction time is 15min, the material-liquid ratio is 1: 5, the volume fraction of HCl is 0.5%, and the volume fraction of ethanol is 70%. The absorbance measured by the photometer was brought into the formula (1) of the pH differential method to obtain a total of 1.458, and the content of anthocyanins was calculated from formula (2) to be 2.43 mg / g. The optimization of the extraction process provided the basis for the large-scale development and utilization of anthocyanins of mulberry.

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