

## Research Article

### Study on the Process of Extracting Natural Anthocyanin from Globe Amaranth Flower by Microbial Method

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**Abstract:** Anthocyanin of globe amaranth flower could be implied in inflorescence or whole body. Anthocyanins have physiological functions such as scavenging free radicals, antitumor, beauty, has a broad application prospect in the field of food and medicine. Microbial wall-breaking method is used to extract gomphrena anthocyanins, studied the temperature, pH value, extraction time and solid-liquid ratio on the microbial degradation effect and using the orthogonal experiment method to establish the optimum extraction conditions. The experimental results show that the optimized extraction conditions of gomphrena anthocyanins is temperature 30°C, pH 4.0, extraction time of 72 h and solid-liquid ratio 1:12.

**Keywords:** Anthocyanin, globe amaranth flower, microorganism wall-broken, stability

#### INTRODUCTION

Anthocyanins are a kind of widespread in the world, the largest number of natural edible pigment, rich in resources. This kind of pigment not only there is no security issues and has a lipid-lowering flavonoids substances, such as anti-aging health care function (Zhao *et al.*, 2000). Around the world, the study of anthocyanins increasingly extensive and in-depth, especially in the 21<sup>st</sup> century, the development of functional anthocyanins has become the focus of attention and research. The British Long Ashton research center anthocyanins stability method of long engaged in research, in the simulation of natural biological conditions stable, anthocyanins natural analogue synthesis, chemical modification of natural anthocyanins derivatives synthesis of many important results are obtained, the results a lot of realized industrialization production (Assen, 2002).

The anthocyanins of extraction and purification methods of many domestic and foreign scholars have done a lot of research. Application of cellulase to extract the anthocyanins of red flowers, compared with the traditional water extraction technology, can greatly improve the extraction efficiency, extraction of active ingredients mild conditions, the physical and chemical properties stable, etc., (Jiaying, 2001). Solution of sugar as its purification process is difficult to get rid of the impurities. Therefore, in order to improve the products of natural pigment, must carry on the purification of the crude extract, main methods of adsorption desorption method, membrane separation, enzymatic etc., (Wang, 2000). Nyman and Kumpulainen (2001) used Droplets Countercurrent Chromatography method (DCCC) and purification of anthocyanins.

The current international market growing demand for natural anthocyanins, but has higher requirement for the quality of the product, the extraction process of pure pigment product, because of its low natural pigment, high content of impurities, has not accepted by the international market. Thus find high extraction yield and quality of technology means has become a focus topic.

#### MATERIALS AND METHODS

**Preparation of medium:** Plate medium (PDA medium): potato 200 g; Sugar (or glucose) 20 g; AGAR 15~20 g; 1000 mL water. Fermentation medium: potatoes peeled, sliced boiled; Add sugar 20 g, filling water to 1000 mL.

**Strain activation and cultivation:**

**Strains activation:** Move three kind of mold are in PDA medium slant to the fresh, at 28°C constant temperature incubator in 3 days or so and then through 3~4 times.

**Expand training:** Will the activation switch strains in PDA medium slant, at 28°C constant temperature incubator in 3 days.

**Cellulase-producing strain cell wall extraction process:**

**The technological process:** Globe amaranth flower petals to drying, crushing to join the microbiological culture media, the sterilization to vaccination, microbial degradation to the suction filter, column chromatography to vacuum distillation, anthocyanin's solution.

Table 1: Test level of form factors

Level A	(pH)	B (temperature/°C)	C (solid- liquid ratio)	D (time/h)
1	5.0	30	1:12	72
2	4.5	27	1:10	48
3	4.0	24	1:8	24

**Microorganisms to break the wall:** Dried globe amaranth flower petals were pulverized and passed through a 40 mesh sieve; respectively according to take 2 g pollen in the three test tubes, to join the PDA medium, sterilized under the condition of 120°C for 20 min. Then, in the sterile work bench to vaccinate cellulose enzyme bacteria in 28°C constant temperature incubator in 3 days.

After completion of training, on the cultures of the suction filter, filter residue, then through low speed centrifuge separation, abandon precipitation, anthocyanins solution part.

**Gomphrena anthocyanins visible absorption spectrum:** According to the process of 2.3.3 method to extract gomphrena anthocyanins. To obtain a certain amount of anthocyanins concentrate, dilute in a ratio of 1:4 mixed with distilled water, using 722 spectrophotometer in nm difference measure its absorbance values.

**Determination of color value:** Experiment using the natural anthocyanins as evaluation index, at lambda Max measured absorbance value, according to the following formula for natural pigment:

$$\text{Color value} = A_{\lambda} \times N$$

where,

$A_{\lambda}$  : Absorbance values of gomphrena anthocyanin at the wavelength of maximum time

N : Diluted multiples

**Strains screening:** Three parts of amaranth powder 2 g were weighed, according to the proportion of solid liquid ratio 1:10 immerse pollen in microbial culture medium, the medium pH value to 5.0, working in the aseptic stage respectively three kinds of cellulose enzyme bacteria: *Aspergillus niger*, *Trichoderma viride* Kang Shimu mildew, green; Then at 28°C constant temperature incubator in 3 days. For one thousand days after the raw red anthocyanins, dilute in a ratio of 1:4 mixed with distilled water, in the globe amaranth flower anthocyanins lambda Max measure its absorbance value and calculate the natural pigment.

**The determination of microbial degradation:**

**The effect of temperature on microbial method extracting effect:** Globe amaranth flower powder 2 g was weighed, according to 2.3 process for processing, in the pH value of 5, solid-liquid ratio of 1:10,

temperature of 20, 25, 30, 35 and 40°C, respectively condition, in constant temperature incubator in the degradation of 72 h. After 1000 days raw red anthocyanins, dilute in a ratio of 1:4 mixed with distilled water, 530 nm to measure its absorbance value and calculate the natural pigment.

**The effect of pH on microbial method extracting effect:** Globe amaranth flower powder 2 g was weighed, according to the technological process of microbial wall-breaking method for processing, the solid-liquid ratio of 1:10, 28°C, the temperature of pH were 1.0, 3.0, 5.0, 7.0, 9.0, 11.0 and 13.0, respectively and under the condition of the constant temperature incubator in the degradation of 72 h, 1000 days after the raw red anthocyanins, dilute in a ratio of 1:4 mixed with distilled water, 530 nm to measure its absorbance value and calculate the natural pigment.

**The effect of solid-liquid ratio on the extraction of microbial method:** Globe amaranth flower powder 2 g was weighed, according to 2.3.3 method for processing, in the pH value of 5, solid-liquid ratio is 1:6 respectively, 1:8, 1:10, 1:12, 1:14 conditions, at 28°C constant temperature incubator in the degradation of 72 h. Determination of 530 nm anthocyanins obtained by the thick liquid absorbance value and calculates color value.

**The effect of microbial treatment time on the extraction:** Globe amaranth flower powder 2 g was weighed, according to 2.3.3 method for processing, in the pH value of 5, under the condition of solid-liquid ratio of 1:10, at 28°C constant temperature incubator degradation in the 12, 24, 48, 72 and 96 h, respectively. The absorbance value measured at 530 nm and calculates color value.

**The optimized experiment of anthocyanin extraction conditions:** According to the results of single factor experiment with orthogonal experiment, the condition of cellulose degradation bacteria were optimized. On the basis of single factor experiment, 4 factors 3 levels orthogonal experiment design, using  $L_9$  ( $3^4$ ) orthogonal test table, with anthocyanins natural as examining index. Factor design is shown in Table 1.

**Anthocyanins purification:** Using adsorption chromatography technology to red, isolation and purification of anthocyanins in one thousand days with silica gel chromatography medium, choose a diameter of 1.5 cm, column chromatography column length is 22.5 cm, the original two 1.64l can hydrolyze 530 nm thick liquid and then purified by the rotary evaporation apparatus for purification after the globe amaranth flower anthocyanins for enrichment. According to the proportion of 1:4 dilution with distilled water and its absorbance value is determined at 530 nm.

## RESULTS AND DISCUSSION

**Gomphrena anthocyanins visible absorption spectrum:** Spectrophotometer is used to test in 370~700 nm wavelength absorbance values, drawing gomphrena anthocyanins characteristic absorption spectrum. The results are shown in Fig. 1.

Table 2: The effect of different strains on extraction

Strains	<i>Aspergillus niger</i>	green <i>Trichoderma viride</i>	<i>Trichoderma koningii</i>
Color value	1.634	1.557	1.601

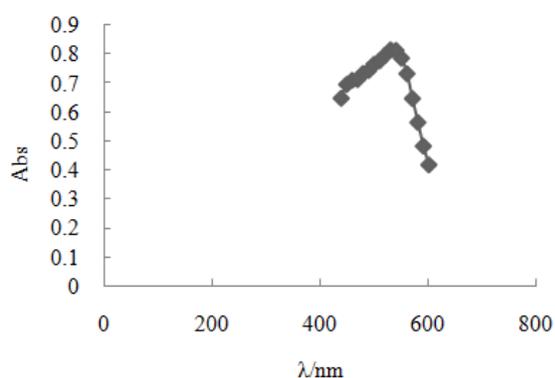


Fig. 1: Gomphrena anthocyanins visible absorption spectrum

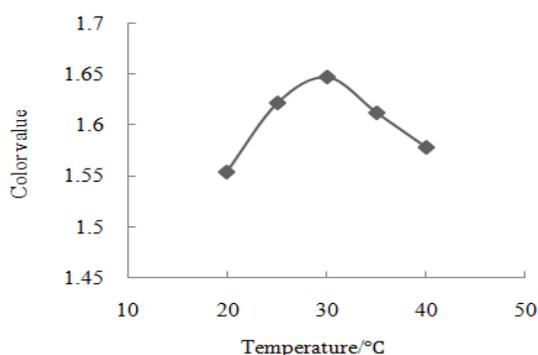


Fig. 2: The relationship between temperature and color value

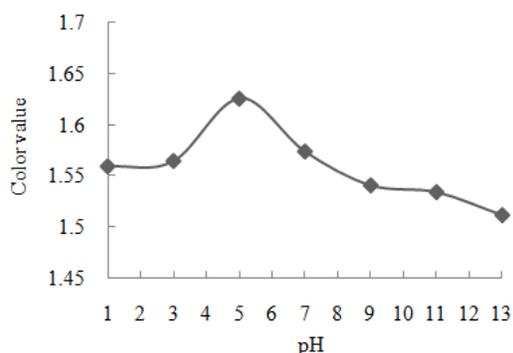


Fig. 3: Relationship between pH and color price

As can be seen from Fig. 1, amaranth anthocyanins in the visible range of maximum absorbance at 530 nm.

**The effect of single factor on microbial degradation:**  
**The effect of different strains on extraction:** Gomphrena pollen was degraded respectively by three cellulose-producing strains of *Aspergillus niger*, *Trichoderma koningii* and Green *Trichoderma viride*, with its color value measured at 530 nm index, its extraction is shown in Table 2.

From the above table it can be seen when selecting *Aspergillus niger* amaranth dry powder processing, extraction of anthocyanins better than the other two mold. Cellulose there are high-energy hydrogen and therefore very difficult to hydrolysis (Joseph, 2001). Many microbes can degrade cellulose, particularly focusing on mold (Xu, 2006). However, these strains are produced there is low activity, the role of a narrow pH range and other shortcomings. Derived from the table of *Aspergillus niger* degrading activity than the other two, so you can use as test species *Aspergillus niger*.

**The effect of temperature on microbial extraction:** By the determination of *Aspergillus niger* to red petals processing 72 h, one thousand days when the pH value of 5, solid-liquid ratio of 1:10, anthocyanins of natural change with the change in temperature. The results are shown in Fig. 2.

As can be seen from Fig. 2, when the temperature is low, the color value decreases as the temperature increases the color value also will increase; but when the temperature reaches a certain value, color value began to decline, when the temperature is 25~30°C, the color value gradually increased; when the temperature is 35°C, the color value began to decline. This is mainly due to the presence of four kinds of anthocyanins balance under different temperature conditions, namely quinoid alkali, salt ions, false bases and chalcone (Ren *et al.*, 2005). Under normal conditions mainly the presence of cations, which was red. As the temperature increases, the balance toward colorless false base and direction chalcone conducted while causing lower colored type compound. It can be concluded that the temperature is 30°C, the extraction of anthocyanins best.

**The effect of pH on microbial extraction:** It was determined that when the solid-liquid ratio of 1:10, the temperature is at 28°C, *Aspergillus niger* petal of amaranth processing 72 h, anthocyanin color value with changes in pH change, the results shown in Fig. 3.

As it can be seen from Fig. 3, the degradation process of the color value of 5 from the rising value of pH 3 to 9 with the rise of the color value of pH, pH value decreases rapidly from 5 to pH. In order to determine the effect of pH on the degradation in the pH

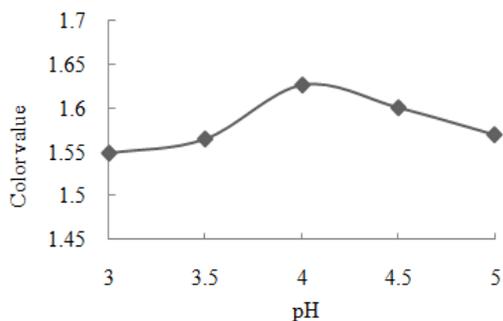


Fig. 4: Relationship between pH and color price

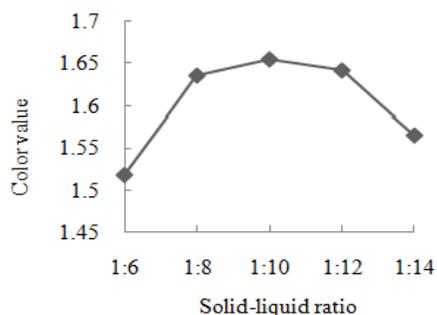


Fig. 5: Relationship between solid-liquid ratio and color price

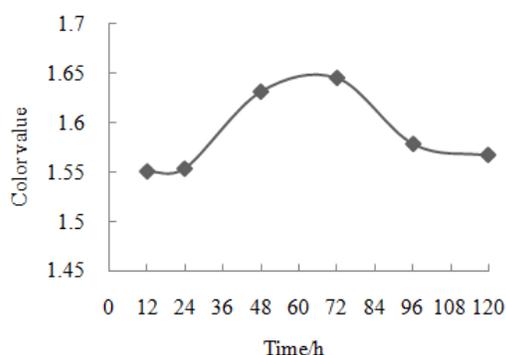


Fig. 6: Relationship between processing time and color price

Table 3: Orthogonal experiment and result analysis

Test No.	A	B	C	D	Color value
1	1	1	1	1	1.546
2	1	2	2	2	1.315
3	1	3	3	3	1.301
4	2	1	2	3	1.470
5	2	2	3	1	1.354
6	2	3	1	2	1.320
7	3	1	3	2	1.687
8	3	2	1	3	1.604
9	3	3	2	1	1.672
K <sub>1</sub>	4.162	4.703	4.470	4.572	
K <sub>2</sub>	4.144	4.273	4.457	4.322	
K <sub>3</sub>	4.963	4.293	4.342	4.375	
k <sub>1</sub>	1.387	1.568	1.490	1.524	
k <sub>2</sub>	1.381	1.424	1.486	1.441	
k <sub>3</sub>	1.654	1.431	1.447	1.458	
R	0.273	0.144	0.043	0.083	

3 to pH values between 5 to continue with *Aspergillus niger* anthocyanins processing. Test results shown in Fig. 4.

Under acid condition, natural pigment of anthocyanins is higher, with the increase of pH value, natural gradually reduced. When the pH value of 4.0~4.0, suitable for the growth of *Aspergillus niger*, is helpful for the extraction of anthocyanins, as shown in Fig. 4. This is mainly because of the pigment under different pH, there is also the above four kinds of balance, with the increase of pH, anthocyanins from violet quinoid alkali towards red cation, until it became colorless false alkali and chalcone ketones, when reaches a certain value, with the increase of pH value of natural decline (Criston, 2002). Therefore, when the pH value of 4.0, extract the best effect.

**The effect of solid-liquid ratio on microbial extraction:** It was determined that when the *Aspergillus niger* petal of amaranth processing 72 h, pH value of 5, the temperature is 28°C, anthocyanin color value with changes in the solid-liquid ratio varies. Solid-liquid ratio on the extraction of visible Fig. 5.

As can be seen from Fig. 5, the solid-liquid ratio was 1:12 when the highest price of anthocyanin color, high moisture content affect anthocyanin extract is mainly due to the solid-liquid ratio increases, the density of microbial reduction small concentrations of cellulase is also reduced, leading to reduced enzyme activity; on the other hand, the solid-liquid ratio is too high ventilation conditions affect matrix, cannot provide enough oxygen, microbial growth is limited (Cui and Sun, 2004). If the solid-liquid ratio is too low, then a thousand days safflower powder was completely imbibed, microbial growth will be limited. Then select the solid-liquid ratio was 1:12, the extraction of anthocyanins best.

**The effect of microbial processing time on extraction:** When the solid-liquid ratio was 1:10, the temperature is 28°C, pH value 5, *Aspergillus niger* petal of amaranth processing time is different, the extraction effect is different. Experimental results are shown in Fig. 6.

As can be seen from Fig. 6, with the increase in processing time, anthocyanin color price increases. But after a certain time, the color value began to decline. This is mainly due to the anthocyanin feature easily oxidized, when the anthocyanin from the plant, the longer the exposure, the more susceptible to oxidative degradation (Wu, 2003). Thus *Aspergillus niger* petal of amaranth optimal treatment time was 72 h.

**Orthogonal design results:** Amaranth dried powder 180 g was divided into nine parts, according to Table 3, respectively, at different temperatures and pH values', at different times and in different conditions of solid-

liquid ratio, extraction methods for microbiological broken concrete steps as above the extraction process to operate, anthocyanin color value is measured according to the above method.

Orthogonal experimental results are shown in Table 3, the test results are statistically processing.

By the R value can be seen in Table 3, the factors influencing the extraction effect of sorts for A>D>B>C, after operation can be concluded that the level of each factor and K<sub>1</sub>, K<sub>2</sub>, K<sub>3</sub>. Comprehensive factors K value and intuitive comparison, the optimum process conditions for A<sub>3</sub>B<sub>1</sub>D<sub>1</sub>C<sub>1</sub> in theory. The impact on the extraction yield of the smallest C, so the extraction of the solid-liquid ratio used minimal impact. Because there is no interaction and visual analysis by judge the A<sub>3</sub>B<sub>1</sub>D<sub>1</sub>C<sub>1</sub> level of good combination is better than other combination. A<sub>3</sub>B<sub>1</sub>D<sub>1</sub>C<sub>1</sub> group cooperation validation test results prove that this combination is better than other combination treatment. As a result, gomphrena anthocyanins the best extraction technology conditions for temperature 30°C, pH value of 4.0 72 h, extraction time and solid-liquid ratio 1:12.

**Anthocyanins purification experiment results:** By adsorption chromatography on gomphrena anthocyanins were isolated crude liquid hydrolysis original color value 1.641 at 530 nm, then purified by a rotary evaporator to Gomphrena anthocyanins purified and concentrated. Purified anthocyanin color value 1.679.

## CONCLUSION

In amaranth as raw materials extraction amaranth anthocyanins broken by microbial method and study its stability, we can draw the following conclusions: Amaranth anthocyanins in the visible range of the strongest absorption peak at 530 nm; When *Aspergillus niger* degradation amaranth anthocyanins, the color value can reach 1.634, with better degradation effect compared with Green *Trichoderma viride* and *Trichoderma koningii*; Through the process of the degradation of gomphrena pollen by mold, temperature,

time, pH and other conditions of amaranth extract anthocyanins have a significant impact. Optimum extraction conditions gomphrena anthocyanin is pH value 4.0, temperature is 30°C, time 72 h, the solid-liquid ratio 1:12.

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