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Research Article

The Response Surface BBD Method was Used to Optimize the Ultrasonic-Assisted Extraction of Anthocyanins from the Fruits of *Eleutherococcus brachypus* and Its Storage Stability and Antioxidant Properties

Xiaonan Zhang,[1,2] **Manmin Zhang**,[1] **Muxiang Lan**,[1,3] **Heng Xu**,[1] **Xiongjun Liu**,[1,2] **Xiaocheng Zhuang**,[1] **and Tianqi Guo**[1,2]

[1]*Jiaying University, Meizhou 514015, China*
[2]*Guangdong Provincial Key Laboratory of Conservation and Precision Utilization of Characteristic Agricultural Resources in Mountainous Areas, Meizhou 514015, China*
[3]*Agricultural Science Department, Faculty of Technical and Vocational, Universiti Pendidikan Sultan Idris, Tanjong Malim 35900, Malaysia*

Correspondence should be addressed to Xiaonan Zhang; xiaonanzhang@jyu.edu.cn, Xiongjun Liu; 609449126@qq.com, and Tianqi Guo; 202001172@jyu.edu.cn

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Peroxidation during organism growth and development might have catastrophic implications. It is critical to further investigate the antioxidant potential of plant anthocyanins. In this study, anthocyanins from *Eleutherococcus brachypus* fruits (EBF) were extracted by an ultrasonic-assisted method. The anthocyanins were then tested for stability under various storage conditions. Based on single-factor combination with response surface optimization, the best ethanol concentration for anthocyanin extraction was 75%, the ideal ultrasonic irradiation power was 160 W, the liquid-to-solid ratio was 10.18 mL/g, and the maximum anthocyanin yield was 1.86 mg/g. Anthocyanins are readily degraded by bright light and remain stable under acidic storage conditions (pH 3.0) and at temperatures below 60°C. The inhibition rates of anthocyanins against ABTS and DPPH radicals were 54.59% and 48.70%, respectively, using vitamin C (Vc) as a positive control. The data cited above make it clear that anthocyanins may act as natural antioxidants. In addition, this research provides a sound theoretical foundation for the creation of natural green antioxidants.

1. Introduction

The cells of the organism will create highly active harmful chemicals during regular metabolic processes, such as hydrogen peroxide, superoxide ions, and hydroxyl ions (OH−, H_2O_2) [1]. The metabolism of an organism's DNA, proteins, and enzymes can be easily impacted by these free bases. To stop peroxidation and free radical damage, antioxidant usage is crucial. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are examples of antioxidants made chemically [2, 3]. However, they have serious adverse effects, which has greatly increased interest in natural antioxidants like anthocyanin. The potential of anthocyanins to act as antioxidants is crucial in the fields of medicine, cosmetics, food, and other things [4].

Eleutherococcus brachypus fruit (EBF), belonging to the *Araliaceae*, is native to central China and distributed in Gansu and Shanxi provinces. EBF can be used as a natural food. The fresh EBF is oval, has a petal-shaped ribbed surface, and is dark purple.

There are many kinds of bioactive substances in EBF that have good free radical scavenging activity [5], so it is widely welcomed and sold as a tea drink. The content of

anthocyanin in EBF was higher, and it had significant antioxidative, antitumor, antiulcer, and anti-inflammatory characteristics [6]. Moreover, the antioxidant activity of anthocyanins in EBF is also an important index [7].

Anthocyanins are flavonoid compounds formed by a variety of water-soluble natural pigments, which are usually found in tea [8], fruit wine [9], plant seeds [10], and vegetables [11]. The colors of food are often made up of anthocyanin [12], which provides protection against a variety of degenerative diseases and constitutes an important antioxidant defense system in humans [13].

However, a number of variables, including pH, ultraviolet irradiation, storage temperature [14], chemical structure, enzymes, proteins, and metal ions [15], have an impact on anthocyanin stability. Due to its lower stability, natural anthocyanin has challenges in food processing, storage, and marketing. Anthocyanins are potential dietary antioxidants and colorants due to their low toxicity and biological properties [16]. The low chemical and thermal durability of anthocyanins limits their economic use. This approach has a number of advantages for anthocyanin stability. Natural anthocyanin antioxidants and pigments (including polymers, phenolic chemicals, and carbohydrates) create non-covalent complexes that regulate and stabilize color in a range of plants, fruits, and foods produced from them, including wine, jams, and other preserves [17]. Ultrasonic technology is a developed high-frequency vibration technology, and ultrasonic wave cavitation can enhance the rapid motion of particles in solution. When an ultrasonic wave penetrates a saturated solution, the formation and destruction of cavitation bubbles raises the temperature, generates pressure in the cavitation bubble, promotes effective collision of molecules, local saturation is too high, and millions of tiny bubbles in the liquid form, grow, and break, resulting in pressure. Many plant components have been extracted using ultrasonic-assisted extraction, such as fresh juice [18], mangosteen bark polysaccharide [19], anthocyanins from blueberry [20], and anthocyanins from blackcurrant [21]. In addition, Mohammadabadi et al. used response surface optimization ultrasound to assist in the extraction of active ingredients from eggplant peel [22].

The goal of this research was to separate anthocyanins; the response surface BBD optimization method was used to optimize and improve the results of ultrasonic treatment and analyze the extracted anthocyanins using physical and chemical techniques. For deep-processing plants, combining anthocyanin antioxidant capacity under different treatment settings is a great idea.

2. Materials and Methods

2.1. Materials. Prof. Yuangang Zu of Northeast Forestry University confirmed the EBF, which was then acquired from the medical supplies market in Harbin Province. To obtain dry raw materials, we spread the EBF for seven days in a well-ventilated area away from light and kept it at 4°C. Anhydrous ethanol, glacial acetic acid, and hydrochloric acid were purchased from Tianli Chemical Reagent Co., Ltd. (Tianjin, China) and Tianjin Dongda Chemical Co., Ltd. (Tianjin, China). Vitamin C (Vc), potassium chloride, sodium acetate, citric acid, and sodium citrate were purchased from Jinbei Fine Chemical Co., Ltd. (Tianjin, China).

2.2. Methods. The EBF was dried in an electric blast drying oven (101-0A, Shaoxing China) at 40°C. After grinding, a unit with a screen diameter of 250 μm was utilized for filtration, and finally, a consistent powder was produced. Referring to Saeeduddin et al.'s research methods [23], the first step involved placing the materials into a 150 mL triangle in a bottle (the triangular bottle's outer layer was wrapped in a jacketed reactor, and the temperature was kept constant at room temperature by flowing cold water in the outside jacket, which was utilized to keep the extraction temperature constant). A water bath-type ultrasound vibrating device (ultrasonic oscillating machine, F-040SD, Shenzhen, China) has a continuous ultrasonic radiation power range of 0–240 W. The capacity is 10 L, the frequency is 40 kHz, the heating power is 450 W, and the external dimensions are $328 \times 268 \times 277$ mm. The inner slot size is $300 \times 240 \times 150$ mm, with an ultrasound irradiation power range of 80–240 W, a liquid–solid ratio of 5–25 (mL/g), an ethanol concentration of 45–85%, and an extraction time range of 10–50 minutes.

The extraction rate was optimized by an orthogonal test after the yield of the optimum conditions was determined. The UV absorbance of the anthocyanin extract (Ultraviolet spectrophotometer, 723 N, Shanghai, China) was determined under different conditions of temperature, light, and pH, and its stability was discussed.

2.3. Experimental Optimization. To ascertain the regression coefficients and the statistical significance of the model variables, we employed response surface analysis. The findings showed that the model's predictions for the yield changes of anthocyanins achieved by ultrasonic-assisted extraction were significantly accurate. Notably, RSM can offer a general optimal design for the response variables under consideration by fitting the regression model to the experimental data. The following describes the general polynomial model explaining the change in the response variable:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3. \quad (1)$$

In equation (1), Y_i is the response variable, calculated by the model, and β_0 is a constant. Among them, the regression coefficient of the β_1–β_3 main variable effect, β_{11}, β_{22}, and β_{33} are quadratic effects, β_{12}, β_{13}, and β_{23} are interactive effects, and X_1–X_3 are independent variables.

2.4. Experimental Optimization Process. To extract anthocyanin, a single-factor approach with four variables was first used: liquid-to-solid ratio (A), ethanol concentration (B), ultrasonic irradiation duration (C), and ultrasonic irradiation intensity (D). Given that the influence of experimental factors on yield (X1: liquid-to-solid ratio-mL/g, X2: ethanol concentration-%, X3: ultrasonic irradiation power-W) is a curve relationship, the response surface BBD technique is utilized to find the suitable process conditions and levels. The ideal level in the single-factor experiment was used as the 0 level of the response surface design, and the experimental scheme was created. According to the Box–Behnken design principle of the response surface, the quadratic regression equation was derived, and the best process parameters were determined.

The response surface was adjusted for the aforementioned data, the anthocyanin yield was employed as an indication, the three key factors were screened, and the ideal process conditions were discovered and discussed.

2.5. Determination of Anthocyanin Content in EBF. The content of total anthocyanins in the EBF was calculated [5, 24]. A pipette was used to absorb 2 mL of the sample, and the sample was diluted to 20 mL with a buffer solution of pH 1.0 (0.2 mol/L KCl: 0.2 mol/L HCl = 25 : 67, V/V) and pH 4.5 (0.2 mol/L NaAc.3H_2O: 0.2 mol/L HAc = 1 : 1, V/V). The absorbency with 2 mL ethanol and 18 mL of buffer as a blank sample was measured at wavelengths of 510 nm and 700 nm. The content of total anthocyanin in EBF (calculated by cyanidin 3-glucoside) is as follows:

$$\begin{aligned} A &= (A510 - A700)_{\text{pHl.0}} - (A510 - A700)_{\text{pH4.5}}, \\ \text{Yield}_{A(\text{mg}/g)} &= \frac{(A \times 449.2 \times V)}{(26900 \times 10 \times m)} \times 100, \end{aligned} \tag{2}$$

where $\text{Yield}_{A(\text{mg/g})}$ is the yield of anthocyanins, m is the sample quantity (g), V is the total volume of the extract (mL), A is the absorbance value, 26900 is the molar extinction coefficient, and 449.2 is the molar mass.

2.6. The Stability of Anthocyanin in EBF. The anthocyanin solution of EBF was diluted with citric acid-sodium citrate buffer (the pH range was 1–9). Weighing 50 mL of anthocyanin in a 100 mL conical flask, the samples were irradiated by ultraviolet radiation a (UVA: the range of wavelength was 315–400 nm) and ultraviolet radiation *b* (UVB: the range of wavelength was 280–315 nm) to verify the stability of anthocyanin at different wavelengths. Sampling was carried out in the irradiation time range of 0–64 h. Then, the absorption value was measured at 510 nm by an ultraviolet spectrophotometer, and the data were recorded.

2.7. The Stability of Anthocyanin in EBF. A total of 50 mL of anthocyanin solution from a 100 mL conical flask was taken, and the pH was adjusted to 5.0 with citric acid-sodium citrate buffer. After treating the anthocyanin solution at 20, 40, 60, 80, and 100°C for 1–4 hours, samples were obtained at various temperatures and time intervals, and the anthocyanin concentration was measured at 510 nm. To confirm the stability of anthocyanins at various wavelengths, the samples were exposed to ultraviolet a (UVA; wavelength range: 315–400 nm) and ultraviolet *b* (UVB; wavelength range: 280–315 nm). Samples were taken between 0 and 64 hours after radiation exposure. Then, using a UV spectrophotometer, the absorption value was determined to be 510 nm. It was established that anthocyanins are stable in the pH range of 1.0–9.0. The pH was set to 5.0, and the anthocyanin temperature was maintained at 40°C. Infrared UVA radiation was applied to the samples for 24 hours to measure their light absorption value at 510 nm.

2.8. Antioxidant Capacity. The scavenging ability of DPPH (1,1-diphenyl-2-picrylhydrazyl) was tested according to Zhang et al. [25, 26]. DPPH was accurately weighed at 25.76 mg, poured into a volumetric flask, and filled with 50% ethanol to 100 mL, obtaining a DPPH reserve solution of 0.65 mmol^{-1}, which was stored away from light for later use. Anthocyanin samples were designed, and Vc was used as a positive control. The phosphate buffer solution (pH 6.9) was used to set up 5 concentration gradient samples (0.0625, 0.125, 0.25, 0.5, and 1 mg/mL). The samples were tested three times at 517 nm UV spectral. The DPPH radical-scavenging rate was calculated using the following formula:

$$\frac{(A_0 - A_t)}{A_0} \times 100\%, \tag{3}$$

where A_0 is the initial absorbance and A_t is the absorbance at 60 min.

According to Yu et al. [27], ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) was dissolved in 50 mL of phosphate buffer to prepare a reserve solution of 7 mmol/L. The ABTS solution was then diluted with 2.45 mmol/L potassium persulfate, potassium hypersulfate, and PBS. The absorbance of the above-mentioned diluted solution was measured at 734 nm using Vc as a positive control. After reaction at room temperature for 5 min, the absorbance was measured at 734 nm with a UV photometer, and parallel experiments were carried out 3 times with PBS as a blank control. The ABTS radical scavenging rate was calculated using the following formula:

$$\left(\frac{1 - A_s}{A_t}\right) \times 100\%, \tag{4}$$

where A_s is the absorbance of the samples and A_t is the absorbance of the blank control.

3. Results and Discussion

3.1. Effect of Different Factors on the Yield of Anthocyanin. We optimized the experimental parameters in the extraction range of ultrasonic irradiation power of 80–240 W, liquid-solid ratio of 5–25 (mL/g), ethanol concentration of 45–85%, and extraction time of 10–50 min in order to extract anthocyanins using a water bath ultrasonic vibration device. The yield of anthocyanin rose in step with the increase in the liquid-to-solid ratio, reaching its peak (1.35 mg/g) at a liquid-to-solid ratio of 15 mL/g (Figure 1(a)). The ideal ratio of liquid-to-solid was 15 mL/g, but when the liquid-to-solid ratio increased, the yield tended to diminish. This may be because an excessive liquid-to-solid ratio reduced the extraction efficiency. Ethanol had the maximum anthocyanin yield of 75%, indicating that the extraction solution had achieved the ideal equilibrium phase (Figure 1(b)). The yield of anthocyanin increased in the extract time of 10–30 min, and the yield was the highest in the extract time of 40 min (Figure 1(c)). The anthocyanin yield decreased within 50 min because the cell wall structure of EBF would be damaged for a long ultrasound time, leading to a decrease in anthocyanin yield [28]; therefore, the extraction time of 40 min was chosen as the best time in this experiment. The yield was the highest at an ultrasonic irradiation power of 200 W (Figure 1(d)).

3.2. Response Surface Optimization of the Yield of Anthocyanin. For the experimental design, which was optimized by Design Expert 10, we employed response surface BBD for the three parameters of liquid-to-solid ratio, ethanol content (%), and ultrasonic irradiation power (W). The fitted regression model $p < 0.0001$ indicates that the optimization result of the equation is highly significant.

The table displays the experimental data as well as the outcomes of the predictions. We used response surface analysis to fit the data in Table 1 to obtain the following regression equation:

$$\begin{aligned} Y = {} & 0.66 - 0.23X_1 - 0.3038X_2 - 0.1088X_3 \\ & + 0.0625X_1X_2 + 0.0275X_1X_3 - 0.02X_2X_3 \\ & + 0.0975X_1^2 + 0.48X_2^2 + 0.295X_3^2. \end{aligned} \quad (5)$$

In addition, the correlation coefficient R^2 of the equation was 0.9796 (Table 1), so the equation can be used to model the relationship between the index values and the factors.

With increasing ultrasonic irradiation power (200–240 W) and ethanol concentration (80–85%), the anthocyanin yield gradually increased. The response surface optimization process revealed that an excessive ratio of liquid-to-solid would not effectively increase the anthocyanin yield but would instead result in solvent wastage (Figure 2).

As a result of the aforementioned analysis, the anthocyanin yield was determined to be dependent on the independent variables. Water is helpful for the swelling of plant materials, increasing the contact surface area between the solvent and the plant substrate, and ultimately improving the extraction rate during the extraction process [29]. Ultrasonic power can change the diffusion coefficient during the extraction process, and the extraction rate can be improved by adding the right amount of water to the ethanol. According to Vinatoru et al. [30], the extraction rate is often increased when plant stromal cells are destroyed by ultrasonication, releasing the cell contents into the extraction media.

Following the study above, the ideal ethanol concentration for the extraction of anthocyanins was 75%, the ideal ultrasonic irradiation power was 160 W, and the ideal ratio of liquid-to-solid was 10.18 mL/g. The maximum production of anthocyanin was 1.86 mg/g, which was approximately the expected result when compared to the 1.84 mg/g predicted by the model equation.

3.3. The Stability of Anthocyanin. The effects of temperature, light, and pH on the stability of anthocyanin were analyzed using controlling variables, and the best preservation conditions were obtained.

3.3.1. Effect of Temperature on the Stability of Anthocyanin. At 20°C, the absorbance value of anthocyanins varied from 0.52 to 0.49, with no significant change, as shown in Figure 3(a). However, the absorbance at 40 and 60°C decreased slowly 2 h before (from 0.52 to 0.44), after which there was no significant change with time. With increasing temperature, the overall absorbance of anthocyanins decreased within 50 min, among which the decreasing speed was faster for samples at 80°C and 100°C (from 0.52 to 0.31), which may be due to the high-temperature environment, leading to the accelerated reaction of anthocyanin molecules until rapid decomposition, reflecting a decrease in the light absorption value [31]. We used an accelerated storage stability experiment, which involved examining anthocyanin stability at various temperatures (20, 40, 60, 80, and 100°C) while maintaining the same time conditions. At higher temperatures, anthocyanins underwent modifications that were visible [32].

Anthocyanins disintegrate quickly at high temperatures. Upon heating, the chemical link between anthocyanin binding systems is altered, and the discoloration of all the samples was caused by the breakdown of chalcone glycoside produced by heating. Chalcone glycosides were further broken down into the derivatives hydroxy-benzoic acid and hydroxy-benzaldehyde [33]. At 60, 80, and 100°C, the degradation rate constant k of the four systems (control system and binding system) rose, and the $t1/2$ value fell in accordance. This accelerates anthocyanin degradation, which is consistent with our work [32].

As a result, anthocyanin stability at various temperatures is also exhibited laterally. Therefore, anthocyanin was stable when stored below 60°C [24]. The main issue with using anthocyanins as a natural food coloring is that they are not very stable.

According to Figure 3(b), the first-order reaction kinetic model, the kinetic parameters in the degradation of anthocyanins, the reaction rate constant k, the half-life $t_{1/2}$, and

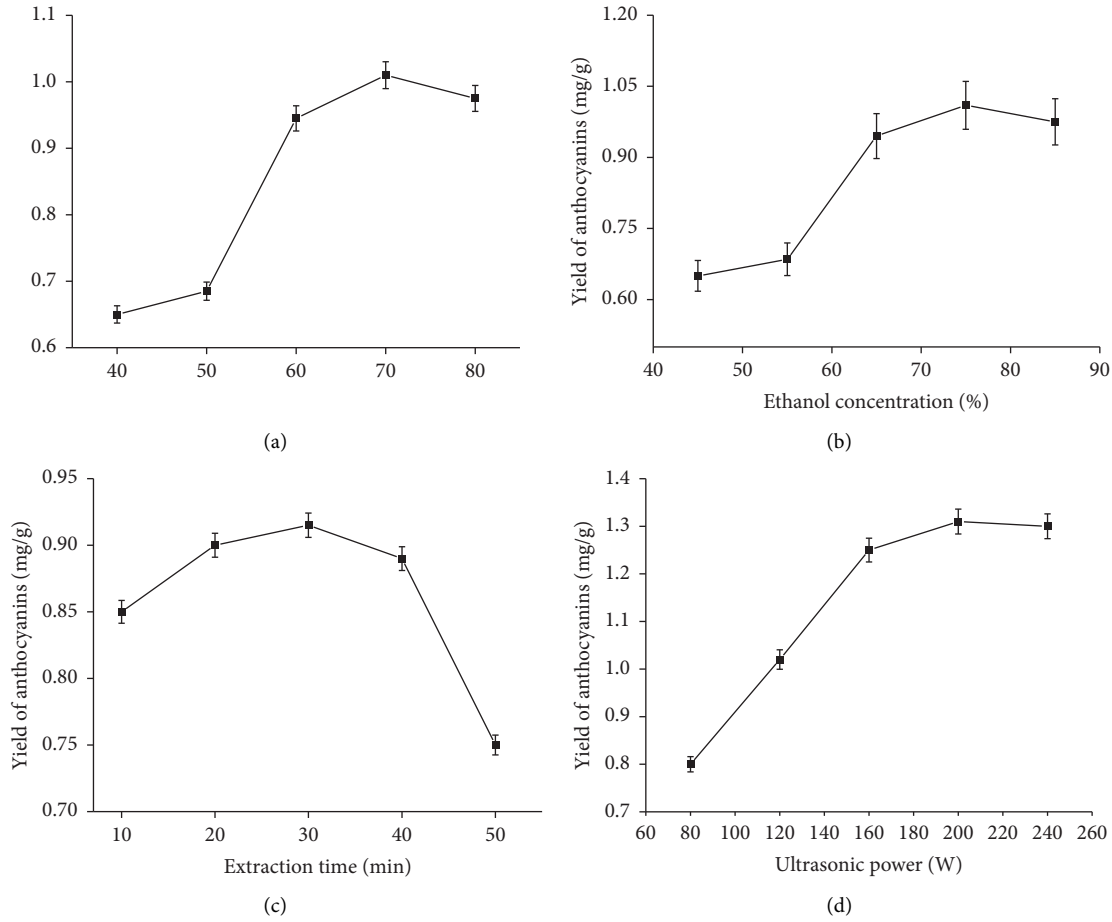

Figure 1: Effects of different extraction factors on the yield of anthocyanins; (a) liquid-to-solid ratio, (b) ethanol concentration; (c) extraction time; and (d) ultrasonic irradiation power.

their correlation coefficients R^2 are shown in the table on the left; a linear fit to lnk and $1/T$ was performed according to the values of k at different temperatures (as shown on the right), and then the Arrhenius equation $k = k_0 \exp(-\mathrm{Ea/RT})$, where R is the gas constant 8.314×10^{-3}, the activation energy Ea can be obtained as 26.74 kJ/mol.

3.3.2. Effect of Light Irradiation on the Stability of Anthocyanin. The absorbance of anthocyanin decreased slowly with time under UVA ultraviolet irradiation (0.00475 – 0.00455), while the absorbance decreased significantly with time under ultraviolet UVB (0.00475 – 0.00355) ultraviolet light (Figure 4). These results indicated that anthocyanin is not stable in UVB ultraviolet light. However, it is stable in UVA ultraviolet light. Therefore, we should preserve EFB anthocyanins away from light.

The anthocyanin retention rate was 67.37% without exposure when Sendri et al. exposed red cabbage anthocyanins to 120 days of light, and they concluded that during this process [31]. In addition, on days 60 and 120, the retention rate of anthocyanins produced by microcapsules exposed to sunshine fell to 74.36% and 67.37%, respectively. According to the aforementioned study findings, sunlight exposure directly affects the loss of anthocyanins, with UV light in sunlight serving as the primary culprit. In addition, oxygen in the sample, direct oxidation, or the activity of oxidase may all hasten the loss of anthocyanins [34], which finally results in the function of oxidase. Eventually, some anthocyanins break down [35]. The storage stability of anthocyanins after embedding is then further investigated using anthocyanins that have been microencapsulated.

3.3.3. Effect of pH on the Stability of Anthocyanins. At pH ranges of 1.0 to 3.0, the maximum absorption wavelength of anthocyanin was not considerably altered. At pH 5.0, the maximum absorption wavelength vanished. At pH values between 7.0 and 9.0, the maximum absorption wavelength is transversely displaced. With a drop in pH, the absorption value of anthocyanin increased under acidic environments. With time, the absorbance at pH 1.0 did not change considerably; pH 3.0 showed a moderate decline; and pH 5.0–7.0 showed a large decline (Figure 5).

The effects of pH at 1, 3, 5, 7, and 9 on anthocyanins were studied. The UV absorption value of anthocyanins at pH 9.0 showed the widest floating range (from 0.23 to 0.61) as time increased. The shift in anthocyanins becomes less noticeable as the pH decreases. When the pH is 1, for example, the light

Table 1: Box–Behnken design optimization yield of anthocyanins (mg/g) and variance.

No	BBD experiments						ANOVA					
	X_1	X_2	X_3	Y	Actual value	Predicted value	Source	Sum of squares	Degree of freedom	Mean square	F value	P value
1	15	80	200	0.66	0.66 ± 0.06	0.66	*Model*	2.760	9	0.307	86.39	<0.0001***
2	15	75	240	1.61	1.61 ± 0.03	1.65	X_1	0.423	1	0.423	119.09	<0.0001***
3	20	85	200	0.80	0.80 ± 0.01	0.77	X_2	0.738	1	0.738	207.71	<0.0001***
4	10	75	200	1.80	1.80 ± 0.11	1.83	X_3	0.095	1	0.095	26.62	0.0013
5	10	80	160	1.40	1.40 ± 0.04	1.42	X_1 X_2	0.016	1	0.016	4.40	0.0742
6	15	80	200	0.66	0.66 ± 0.01	0.66	X_1 X_3	0.003	1	0.003	0.85	0.3869
7	15	80	200	0.64	0.64 ± 0.01	0.66	X_2X_3	0.002	1	0.002	0.45	0.5237
8	20	75	200	1.27	1.27 ± 0.03	1.25	X_1^2	0.040	1	0.040	11.26	0.0121
9	10	80	240	1.22	1.22 ± 0.05	1.15	X_2^2	0.970	1	0.970	272.99	<0.0001***
10	15	80	200	0.65	0.65 ± 0.07	0.66	X_3^2	0.366	1	0.366	103.11	<0.0001***
11	10	85	200	1.08	1.08 ± 0.02	1.10	*Residual*	0.025	7	0.004		
12	15	85	160	1.30	1.30 ± 0.07	1.26	*Lack of fit*	0.024	3	0.008	22.36	0.0058
13	15	80	200	0.69	0.69 ± 0.01	0.66	*Pure error*	0.001	4	0.001		
14	15	75	160	1.88	1.88 ± 0.15	1.83	*Cor total*	2.790	16			
15	15	85	240	0.95	0.95 ± 0.02	1.00	Credibility analysis of the regression equations					
16	20	80	240	0.76	0.76 ± 0.06	0.74	*Std.dev*	*Mean*	*C.V.%*	R^2	*Adjusted* R^2	*Predicted* R^2	*Adeq precision*
17	20	80	160	0.83	0.83 ± 0.10	0.90	0.0596	1.07	5.57	0.9911	0.9796	0.8645	25.6725

X1, ratio of liquid-to-solid (mL/g); X2, ethanol concentration (%); X3, ultrasonic irradiation power (W). *Y*, yield of anthocyanins (mg/g). ***, extremely significant.

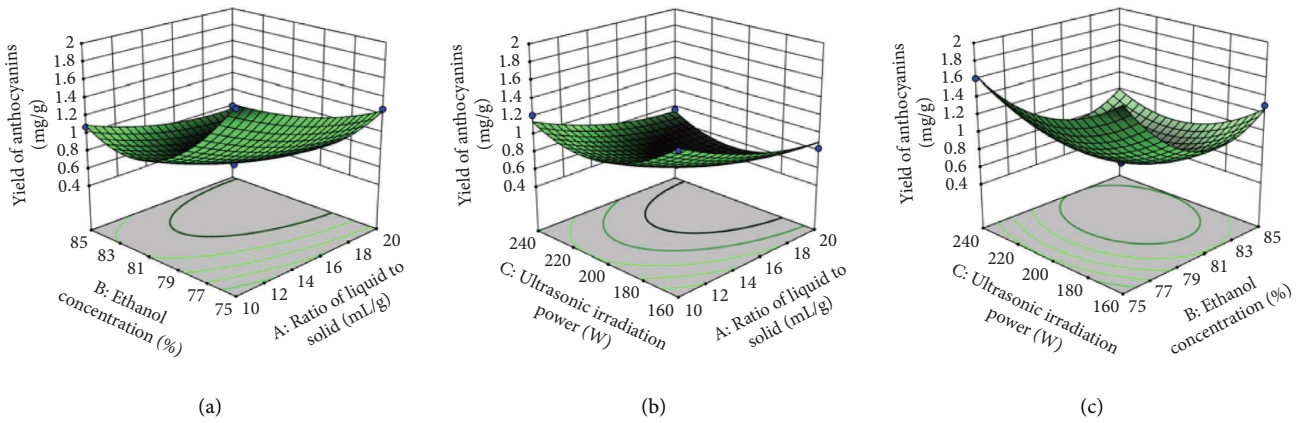

Figure 2: Response surface optimization of yield of anthocyanin. (a) Ethanol concentration and liquid-to-material ratio; (b) ultrasonic irradiation power and liquid-to-solid ratio; and (c) ultrasonic irradiation power and ethanol concentration.

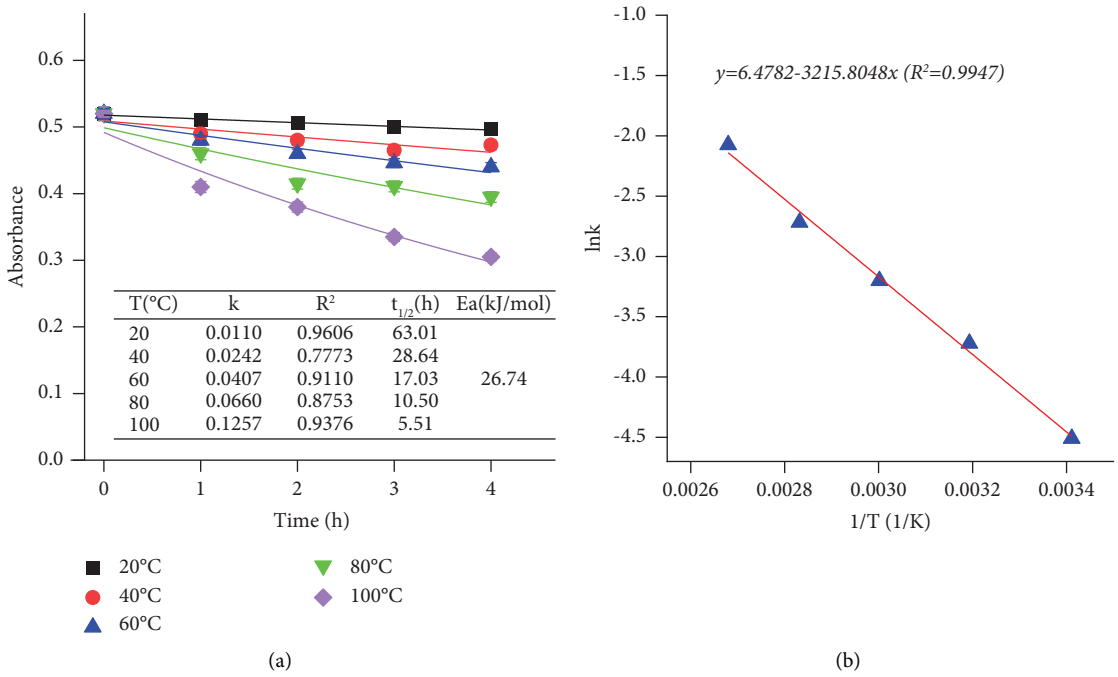

Figure 3: Effect of temperature on the stability of anthocyanins.

absorption value decreases from 0.78 to 0.765, a difference of only 0.015, and the absorbance decreases by 2%. According to the aforementioned findings, the gradient-lowering tendency in the influence of pH on anthocyanins may be useful in enhancing the stability of other unstable chemicals under varied oxidation situations [36].

Gamage and Choo's investigation of the anthocyanin stability of anthocyanins from black goji berries in an acidic pH range (3.0–6.0) [37]. The findings suggested that anthocyanins were well maintained under acidic settings since the retention rate of anthocyanins at pH 3.0 was approximately 70% after 30 days of storage, which was greater than the storage effect at other pH levels.

3.4. Antioxidant Activity of Anthocyanin. The antioxidant properties of anthocyanin in foods could help to prevent various oxidative stress-related diseases, such as cardiovascular disease [38], a degenerative disorder of the central nervous system [39], and diabetes [40]. The antioxidant activity was assessed by using ABTS and DPPH to determine the free-radical scavenging capacity of the anthocyanins in EBF. The scavenging effect of anthocyanin and Vc on free radicals was positively correlated with the increase in concentration (as shown in Figure 6). The anthocyanin of EBF had stronger antioxidant activity, and the inhibition rates of ABTS and DPPH free radicals were 54.59% and 48.70% at an anthocyanin concentration of 1000 μg/mL,

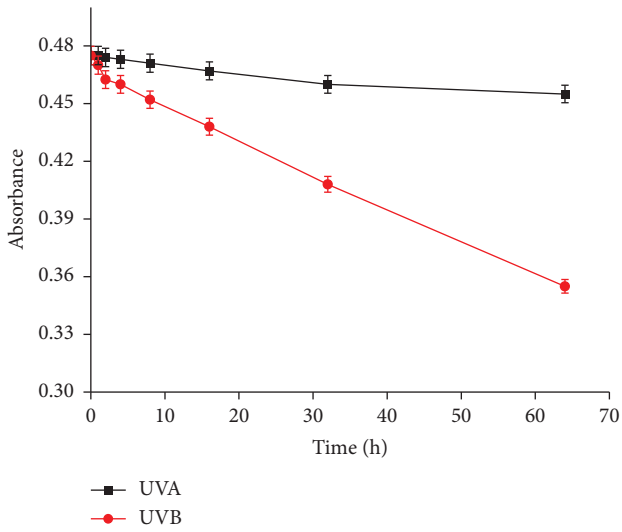

Figure 4: Effect of ultraviolet light irradiation on the stability of anthocyanins.

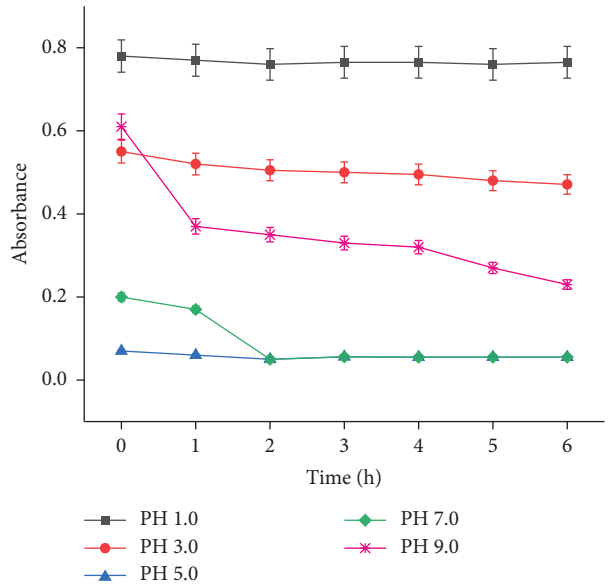

Figure 5: Effect of pH on the stability of anthocyanins.

respectively, which were higher than those in the Vc positive control (Figure 6). The IC50 values for DPPH were 737.56 ± 0.05 and $64.09 \pm 0.03\,\mu g/mL$, respectively; the IC50 values for EBF and Vc against ABTS were 454.67 ± 0.02 and $23.25 \pm 0.03\,\mu g/mL$, respectively. At low concentrations, Vc exhibited high free-radical scavenging activity, and the free-radical scavenging rates of the samples increased in a dose-dependent manner. As anticipated by past investigations, anthocyanin had essentially comparable anti-free-radical activity against both varieties. Therefore, it is theorized that employing certain anthocyanin concentrations when preparing food may be advantageous to organisms.

3.5. Discussion. The experimental results of anthocyanin extraction using the aforementioned ultrasonic power show a positive association between the yield of anthocyanins and various ultrasonic powers. It is clear that, under the same other circumstances, the main cause of these occurrences may be that, as a mechanical wave, ultrasonic waves cause significant cavitation effects in liquid media. In the ultrasonic process, mechanical forces and increased cavitation combine to create explosive forces and pressures inside the solution. This causes additional cavitation bubbles to burst, which raises temperatures and pressures and causes more cell damage in

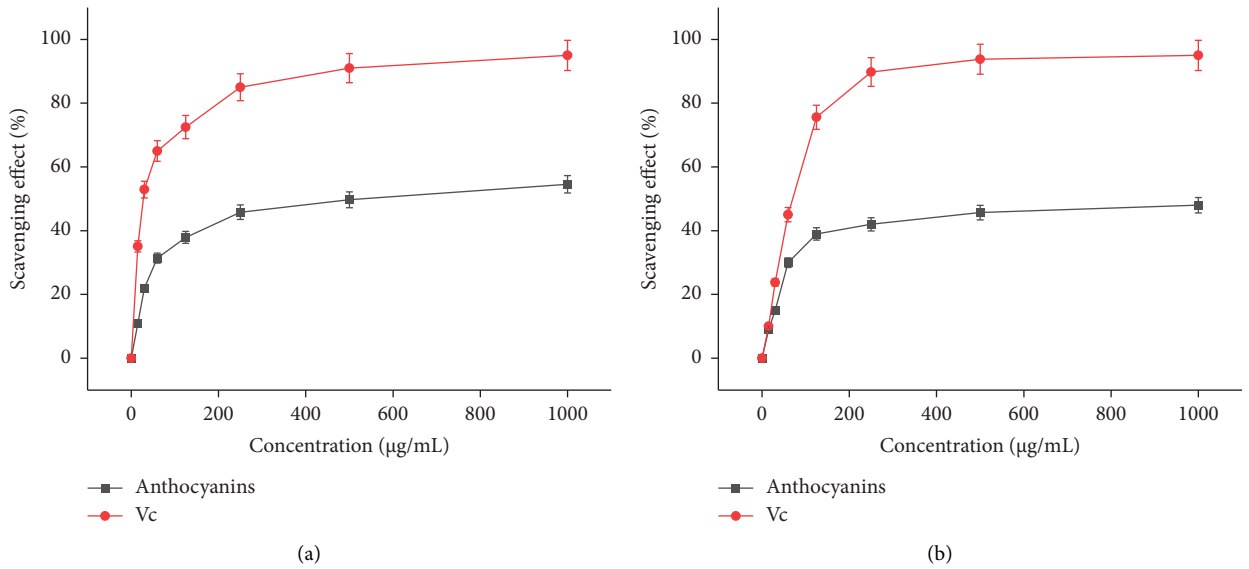

Figure 6: Antioxidant capacity of anthocyanins against (a) ABTS and (b) DPPH.

Acanthus acanthus fruit [41]. The cavitation bubble is unable to collapse in time because its movement frequency is unpredictable. UAE is a quicker, less time-consuming, and more effective extraction technique.

A frequent mechanism for anthocyanin degradation is the combination of cellular and environmental variables, such as light [35], temperature [42], pH [36], and oxygen [31].

They are readily biodegraded, lose their bioactivity, and lose their color during food processing as a result of their instability, which restricts their use and results in financial losses. As a result, it is becoming more crucial to determine how to increase the storage stability of anthocyanins in complex food and beverage systems. The formation of stable structures through the modification of structural elements, including copigmentation, acylation, and biosynthesis, may increase the stability of anthocyanins. In addition, it has been demonstrated that encapsulation methods, including microencapsulation, liposomes, and nanoparticles, work well to lessen instability.

Anthocyanins' rates of thermal, oxidative, and photodegradation were lowered to a larger extent when the material was preheated at 50°C for 15 min. According to Attaribo et al. [43], silkworm pupae protein warmed at 80°C had the best protective impact on anthocyanin stability and had the highest binding affinity toward cyanidin-3-O-glucoside. Different food processing procedures and outside influences generally have a substantial impact on the color and chemical stability of anthocyanins. In any event, pigmentation in the food sector is heavily constrained by process variables, including pH and heat treatment. Therefore, it is important to properly plan and choose pigment molecules to suit food and associated processing circumstances. By adjusting for environmental variables, packaging is essential to preserving and enhancing the shelf life and stability of anthocyanins.

4. Conclusion

Analyzing the impact of various extraction conditions on the yield of anthocyanin from EBF allowed for the identification of the ideal extraction parameters. According to a single-factor combination and response surface optimization, the ideal ethanol concentration for anthocyanin extraction was found to be 75%. Anthocyanins were prepared by water bath-type ultrasound vibrating device (ultrasonic oscillating machine, F-040SD, Shenzhen, China) under the condition of 40 kHz. When the ultrasonic irradiation power was 160 W, the highest anthocyanin yield was 1.86 mg/g. The stability of anthocyanins is closely related to different intensities of light, pH, and temperature. The inhibition rates of anthocyanins against ABTS and DPPH radicals, using vitamin C (Vc) as a positive control, were 54.59% and 48.70%, respectively. The findings mentioned above show that anthocyanins have unique qualities and may be useful as natural antioxidants.

Data Availability

Data used in this study are available upon reasonable request to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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