Evaluation of the antioxidant activity of blueberry ethanol extracts under microwave extraction

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Abstract: Blueberry extract contains diversiform monomers of anthocyanins, whose antioxidant activity determines the functionality of anthocyanidins and the efficacy of extraction technology. The evaluation of the antioxidant activity of anthocyanidins of blueberry ethanol extracts (BEE) was investigated by considering the monomers' identification and thermal degradation in microwave-assisted extraction (MAE). Thirteen monomers of anthocyanins were identified from the extracts of blueberry under MAE, where the highest content was for cyanidin glycosides, and the lowest content was for the malvidin glycosides. The onset temperatures of degradation for anthocyanidins monomers in BEE were 41.07 ℃-48.50 ℃, and the glycoside contents of delphinidin, cyanidin, petunidin, peonidin, peonidin, and malvidin, in turn, decreased obviously. The degradation of anthocyanidins with extraction temperature improved, other than weakened, its antioxidant activities in BEE under MAE. The research results may provide valuable guidance for the antioxidant application of extracts from blueberries.
Keywords: blueberry, anthocyanins, microwave-assisted extraction, extracts, monomer, antioxidant activity
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1 Introduction

The blueberry fruit (*Vaccinium corymbosum* L.) is widely cultivated in the world, whose nutritional value and health function have been highly approved due to its high antioxidant activity as a rich source of anthocyanidins[1]. Anthocyanidins have potential health benefits from their particular chemical structure and colorant properties as potent molecules in the treatment of diabetic retinopathy[2,3], and cardiovascular risk factors[4]. Antioxidant activities of anthocyanidins present strongly scavenging free radicals and reducing oxidative stress with the curative function of antimicrobial, antiviral, and anti-inflammatory[5-7]. The high yield and strong antioxidant activity of anthocyanidins extracted from blueberry are desirable targets for the economic improvement and application of the blueberry processing industry[8]. Microwave-assisted extraction (MAE) is a promising extraction method for the extraction of bioactive compounds from plant-origin materials with the advantages of high yield, great efficiency, and low solvent consumption[9], and has been widely applied to extract bioactive components, such as the thymol from the seed of *Trachelospermum ammi*[10], and total phenols from black tea powder[11]. In the MAE, the dissipation of microwave energy inside extracts generates microwave volumetric heating to improve the extraction efficiency[12]. However, the generation of intense microwave volumetric heating in MAE may simultaneously cause the acquirement of anthocyanidins accompanied by its degradation in blueberry ethanol extracts (BEE)[13].

Existing studies focus on the thermal degradation properties and antioxidant activity of anthocyanidins[6]. The degradation of anthocyanin, as a non-spontaneous endothermic reaction[14], depends on the species of its monomers in extracts[15] and critical temperature in MAE[16]. The correlation of degradation of anthocyanins with its antioxidant in MAE, as a valuable research issue, determines the capability of antioxidant activity of anthocyanidins in BEE for origin-plant material[17]. However, the correlation may have either positive or negative trends controlled by influencing factors including the ingredients of raw material, extraction temperature, and degradation products[18]. Microwave extraction may improve the bioactivity of extracted components due to the generation of polyphenols substance and rich anthocyanidins in extracts with pronounced cellular antioxidant activity against peroxyl radical damage[19] to improve the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity from the degradation of anthocyanidins at extraction temperature of 65 ℃[20,21]. However, previous researches indicate that the thermal degradation of anthocyanin in microwave extraction weakens the antioxidant activity of extracts[22]. The extracts of blueberry powder undergoing microwave extraction are a mixture of multicomponent, which contain diversified monomers with variable ratios and different degradation products of anthocyanidins, which are separated for the purification of monomers in anthocyanidins extracts. The extracts are a key intermediate product linking the feasibility of microwave extraction conditions

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to the yield and antioxidant activity of anthocyanidins. In a qualitative analysis of anthocyanidin monomers in extracts, evaluation of antioxidant activity contributes to the selection of anthocyanidins purification and optimization of extraction parameters in MAE.

The antioxidant capacity of ethanol extracts from blueberry powder is complex and uncertain due to the thermal degradation of anthocyanins. However, ethanol extracts from blueberry powder, as the mixture of anthocyanin monomers have variable monomer contents of anthocyanins with uncertain antioxidant capability. To the general knowledge, little information has been published about the changes in antioxidant capacity of extracts considering its degradation components from blueberry in MAE. Therefore, the objectives of this study were 1) to identify the monomers of anthocyanidins from blueberry extracts under different extraction temperatures, 2) to analyze the degradation kinetics of monomers in extracts with the extraction temperatures in MAE; 3) to evaluate the antioxidant capacity of ethanol extracts describing thermal degradation in MAE.

2 Materials and methods

2.1 Preparation of blueberry powder

Fresh wild blueberries were collected from the Xiangfang farm of Harbin in China. Blueberry was selected considering the uniform size and similar maturity, and stored in a 4 ℃ refrigerator (BC/BD-829HN, Haier, Qingdao, China) till further experiments. The cooling stored blueberry were taken out from the refrigerator and then smashed by a fruit pulping machine (Philips-30, Zhuhai, China) to prepare the blueberry purees. The fresh blueberry purees were quickly frozen in a refrigerator. The frozen blueberry purees with a mass of 1000 g were dehydrated by using a freezing-vacuum dryer (TD-50, Shanghai Pudong freeze drying equipment Co., Ltd., Shanghai, China). Considering the energy consumption and dehydration efficiency, the cold trap temperature of the freezing-vacuum dryer was set at −18°C and working duration of 48 h. The blueberry powders were prepared by using a plant grinder and screening over 40 meshes. The blueberry powders were sealed in the aluminum bags with air-evacuated and stored at −20°C for further experiments.

2.2 Chemical reagents used in experiments of anthocyanidins in MAE

Chemical reagents for extraction and purification of anthocyanidins were concentrated hydrochloric acid (Tianjin Fuyu Fine Chemical Co., Ltd, Tianjin, China), vanillin (Shanghai Aladdin Biochemical Technology Co., Ltd, Shanghai, China), anhydrous ethanol (Hubei Xinrunde Chemical Co., Ltd, China), proanthocyanidin standard (Shanghai Yuanye Biotechnology Co., Ltd, Shanghai, China), methanol (Liaoyang Herun Chemical Co., Ltd, Liaoyang, Liaoning, China), deionized water (Shenyang Shengyida Water Treatment Engineering Co., Ltd, Shenyang, China), purified water (Hangzhou Wahaha Group Co., Ltd, Hangzhou, China), and distilled water (Northeast Agricultural University Water Factory, Harbin, China).

Chemical reagents for the identification of monomers of anthocyanidins were the standard samples of anthocyanins monomers, which included delphinidin-3-glucoside, delphinidin-3-galactoside, delphinidin-3-arabinoside, cyanidin-3-glucoside, cyanidin-3-galactoside, cyanidin-3-arabinoside, petunidin-3-glucoside, petunidin-3-galactoside, petunidin-3-arabinoside, peonidin-3-glucoside, peonidin-3-galactoside, peonidin-3-arabinoside, and malvidin-3-galactoside were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.3 Extraction experiment procedures

A multimode microwave extraction system (Ethos-1, Milestone Inc., USA) with a frequency of (2.45±0.05) GHz and rated power of 1600 W was used to perform the MAE experiments. The extraction system consists of a microwave cavity and a control pad. According to the previous method[23], the lyophilized blueberry powders (2.0000 g±0.0005 g) were dissolved with 60% ethanol of 60 mL. The mixtures of ethanol and blueberry powders were placed in closed vessels, then fixed in the chamber in the microwave extraction system. Extraction temperatures were set by using a control pad embodied in the extraction system. The setting temperatures were achieved in the pre-heating duration of 2 min. In MAE of blueberry powder, the onset of anthocyanin degradation at a temperature of 41.2 ℃[13], however, anthocyanin yield increases with extraction temperatures. To evaluate the antioxidant activity of blueberry extracts from monomers identification to thermal degradation of anthocyanins in MAE, the extraction temperatures were selected as the variable in the range of 30 ℃-70 ℃ to cover the acquirement and degradation of anthocyanin. The extracts were separated by using a centrifuge (International Equipment Co., MA, USA) at 10 000 g for 15 min at 4 ℃ and filtrated using filter paper (Whatman No.1). The filter residue was extracted twice times under the same conditions. Collected filtrate and supernatant were fully mixed to obtain the crude blueberry extract, then evaporated by using a rotary evaporator (RE-52AA, Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China) to remove the ethanol at a temperature lower than 40 ℃ for 2 h. The evaporated filtrate was dehydrated by using the freeze-drying method to obtain the crude anthocyanidins extract powders for further experiments.

2.4 Determination of extraction rate and degradation rate of blueberry anthocyanins

The total anthocyanidin contents in extracts were determined by using the pH differential method[24]. The absorbance of anthocyanidins was measured at 510 nm and 700 nm, respectively. The anthocyanidin contents were calculated by using Equation (1).

$$c = \frac{[(A_{510} - A_{700})_{\text{pH1.0}} - (A_{510} - A_{700})_{\text{pH4.5}}] \times M_\omega \times \text{DF} \times 1000}{\varepsilon \times L} \quad (1)$$

where, M_ω is the molecular weight of anthocyanidins, 449.2 g/mol; DF is the dilution factor; ε is the molar extinction coefficient, 26 900 L/cm·mol; L is the path length of the cuvette, 1 cm.

Accurately weighing (2.0000±0.0005) g blueberry powder was put into the extraction container. Based on the results of previous research[16], add the ethanol solution with the solid-liquid ratio of 1:30 g/mL and volume concentration of 56% into the extraction vial to build the extraction system, then place the extraction container in the center of the microwave workstation and connect the optical fiber temperature sensor. On the control panel, the microwave power was set as 800-1100 W at the interval of 100 W with an extraction time of 20 s during the extraction process, and the temperature changes of the extraction solution in real-time were measured till the end of the extraction. The extracts were put into a centrifuge at 4000 r/min for 15 min to separate the filter residue from the supernatant. The filter residue was washed by using 56% ethanol to colorless. The filtrate and supernatant were diluted, respectively, by using 56% ethanol solution in a 100 mL brown volumetric flask for further analysis.

The extraction rate of anthocyanins in blueberries was determined by using the low concentration vanillin hydrochloric acid method. The standard solution of proanthocyanidins was

prepared with a concentration of 1.2 mg/mL, took 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL, respectively, to dilute to 10 mL with ethanol. 1 mL prepared solution (another 1 mL methanol solution as the blank solution) was added by a 5 mL chromogenic agent (mass fraction 1% vanillin solution and volume fraction 8% hydrochloric acid solution 1:1), then shaken well and kept away from light. The colorimetric tube was taken out to measure the absorbance value at the wavelength of 500 nm, and draw the standard curve. Taking the absorbance value A as the ordinate and the concentration C as the abscissa, the standard curve equation was obtained as shown in Equation (2):

$$A = 1.612C - 0.0485 \quad (2)$$

where, A is the absorbance value of extracts; C is anthocyanin concentration, mg/mL.

2 mL blueberry extracts were put into a 20 mL colorimetric tube to dilute 1 mL of the supernatant 10 times, then took 2 mL of the diluted supernatant into a 20 mL colorimetric tube; 10 mL chromogenic solution was kept in a water bath with a constant temperature of 30 ℃ for 30 min. The absorbance values of the supernatant and filtrate at 500 nm wavelength were measured and then substituted into Equation (2) to calculate the anthocyanin content in the supernatant and filtrate, respectively. The anthocyanin extraction rate, residue rate, and degradation rate in extracts under MAE were calculated by using Equations (3)-(5), respectively.

$$Y_1 = \frac{C_1 \times V_1 \times n}{W} \times 100\% \quad (3)$$

$$Y_2 = \frac{C_2 \times V_2 \times n}{W} \times 100\% \quad (4)$$

$$Y_3 = 1 - Y_1 - Y_2 \quad (5)$$

where, Y_1, Y_2, and Y_3 are the extraction rate, residue rate, and degradation rate of anthocyanin in blueberry extracts under MAE, respectively, %; C_1 and C_2 are the anthocyanin concentration in extracts and residue liquid, respectively, mg/mL; V_1 and V_2 is the constant volume of extracts and residue liquid, respectively, mL; n is the dilution multiple; W is the total content of anthocyanins, mg.

2.5 Identification of monomers of anthocyanidins by using HPLC-DAD-ESI-MS/MS

The anthocyanidins of raw blueberry powder and its extracts were identified by using HPLC-DAD-ESI-MS/MS[15]. Each sample was dissolved in HPLC-grade methanol and filtered through a 0.45 μm filter membrane (Fisher Scientific, Pittsburgh, PA, USA) for further analysis. A liquid chromatography system (HPLC with 1100 Series, Agilent Technologies Inc., USA) equipped with a diode array detector (DAD) and Zorbax Eclipse XDB-C_{18} column (4.6 mm×150.0 mm, 5 μm, Agilent) was used to measure the anthocyanidins content. The operation parameters of mass spectrometry detection and MS/MS were determined as listed in Table 1[24], where anhydrous ethanol is the mobile phase and formic acid solution with a 3% volume fraction as mobile phase B. In this measurement, the run conditions of HPLC-DAD-ESI-MS/MS were at a temperature of 35 ℃, a flowing mass of 10 μL, a flow speed of 0.6 mL/min, and the detected wavelength of 520 nm.

Table 1 Mobile phase composition for gradient elution

Time /min	Mobile phase A/%	Mobile phase B/%	Time /min	Mobile phase A/%	Mobile phase B/%
0	5	95	55	15	85
20	5	95	60	25	75
30	8	92	70	60	40
45	15	85	80	5	95

2.6 Measurement of antioxidant capacity of anthocyanidins from blueberry ethanol extracts in MAE

2.6.1 DPPH radical-scavenging capacity assay

DPPH radical scavenging capacity was determined according to the modified method[25]. Aliquots (2.0 mL) with a concentration of 10 μg/mL of crude extracts under different temperatures (30 ℃-70 ℃) by microwave treatment dissolved in distilled water were added to 2.0 mL of 0.2 mmol/L DPPH radical that was dissolved in 95% ethanol. The mixtures were then shaken by a mixer. The reaction mixtures were kept in a 30 ℃ water bath for 30 min away from the light. The absorbance of the resulting solution was measured by a UV/Vis spectrometer (Lambda 35, Perkinelmer, Singapore) at 517 nm wavelength, and expressed as the content (μg/mL) of 50% inhibition (IC_{50}). The scavenging capacity was calculated by using Equation (6).

$$SC = \frac{(A_1 - A_2)}{A_3} \times 100\% \quad (6)$$

where, SC is the scavenging capacity of blueberry extracts, %; A_1 is the absorbance of 2 mL of sample solution+DPPH solution; A_2 is the absorbance of 2 mL sample solution + 2 mL ethanol with 95% concentration; A_3 is the absorbance of 2 mL ethanol + DPPH· solution with 95% concentration.

2.6.2 $ABTS^+$ radical cation-scavenging capacity assay

$ABTS^+$ radical cation-scavenging capacity of anthocyanidins was measured as described by Zhang et al.[26], with a slight modification. Crude extracts of anthocyanidins from MAE were adjusted by adding distilled water to a concentration of 30 μg/mL. 40 μL crude extracts of anthocyanidins with the above concentration were placed into 4 mL diluted $ABTS^+$ solution, then were shaken for 30 min away from sunshine. An equivalent volume of distilled water, instead of the sample, was used as the blank. The absorbance of the resultant solution was measured at the wavelength of 734 nm. The degree of $ABTS^+$ radical-scavenging capacity of anthocyanidins was calculated based on the Trolox standard curve, which was presented in mg Trolox equivalent to antioxidant capacity (TEAC)/mg anthocyanidins.

2.6.3 Reducing power assay

The reducing power of anthocyanidins was determined according to a slight modification method[27]. The crude anthocyanidins extract powders samples under temperatures of 30 ℃-70 ℃ in microwave extraction were dissolved in distilled water to achieve concentrations of 10 μg/mL for further measurement. 1 mL sample solution was mixed with 2.5 mL sodium phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL potassium ferricyanide with concentration of 1% (w/v). The mixture was incubated at 50 ℃ in a dark environment for 20 min. Then, 2.5 mL trichloroacetic acid with a concentration of 10% was added to the mixture. The mixture liquid was centrifuged by using a high-speed centrifuge at 1.0000 g at 4^{o}C for 15 min. The supernatant of 2.5 mL was collected and then mixed with 2.5 mL distilled water and 0.4 mL ferric chloride with a concentration of 0.1% (w/v) in a test tube. Distilled water was selected as the blank sample. The absorbance of the reaction mixture at the wavelength of 700 nm indicates a reducing power assay.

2.6.4 α-glucosidase inhibition assays in vitro

The α-glucosidase inhibition assays were carried out according to the method of slight modification[28]. 40 μL samples of different concentrations were added into 20 μL yeast α-glucosidase solution with 0.25 unit/mL (in 0.1 mol/L sodium phosphate buffer at pH 6.9) and incubated at 37 ℃ for 15 min in 96-well plate.

Then, 40 μL of *p* NPG with 0.5 mmol/L in PBS was added to each well and incubated at 37 ℃ for 20 min. Acarbose was selected as positive control and PBS as blank control. Three independent experiments were performed. The inhibition capacity was expressed as the percentage inhibition of enzyme activity shown in Equation (7).

$$\mathrm{IC}=\frac{I_1-I_2}{I_3-I_4}\times 100\% \tag{7}$$

where, IC is the inhibition capacity of blueberry extracts, %; I_1 is the absorbance of the samples; I_2 is the absorbance of PBS that replaces the enzyme; I_3 is the absorbance of PBS that replaces the samples; I_4 is the absorbance of PBS that replaces the samples and the enzyme.

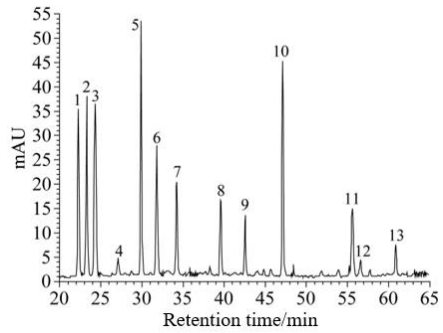
2.7 Statistical analysis of experimental data

All experiments were conducted in triplicate, and the average value was reported. Analysis of variance (ANOVA) for each set of data was performed by SAS software (8.0, SAS Institute Inc., NC, USA). Sigmaplot software (12.0, Systat Software Inc., USA) was employed to fit the regression equations. The coefficients of Equation (8) with extraction temperature were obtained by using the nonlinear fitting method.

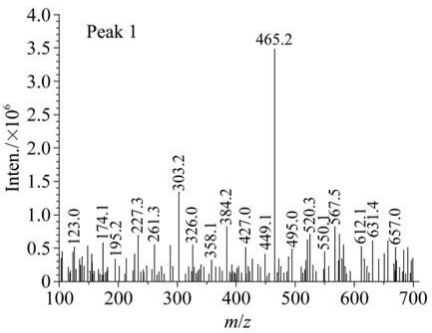
3 Results and discussions

3.1 Determination of monomers of anthocyanidins from blueberry extracts in MAE

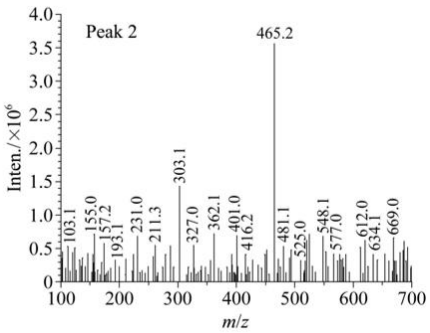
The anthocyanidins extracted from blueberry powder are mixtures, where the monomer content and antioxidant activities are uncertain and variable. To distinct, the changes of anthocyanidins monomers with extraction temperatures, the monomers of anthocyanidins in BEE under selected temperatures in MAE were determined according to the molecular ion, fragmentation information[29]. The monomers of the anthocyanidins in MAE extracts from blueberry powder were isolated by using HPLC–DAD–MS/MS are shown in Figure 1 and Table 2. The fragment (M^+-162) represents the loss of a glucose or galactose moiety, and the fragment (M^+-132) represents the Arabin moiety[30], as the mixture of arabinogalactan oligosaccharides, polysaccharides, and proteoglycans.



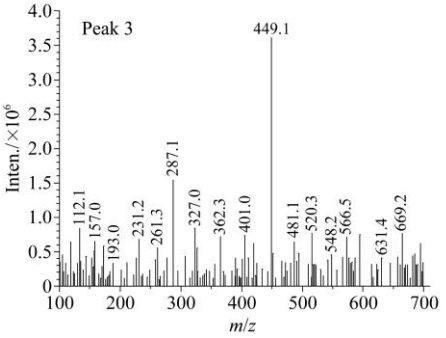
a. HPLC chromatogram for total anthocyanidins from blueberry extracts



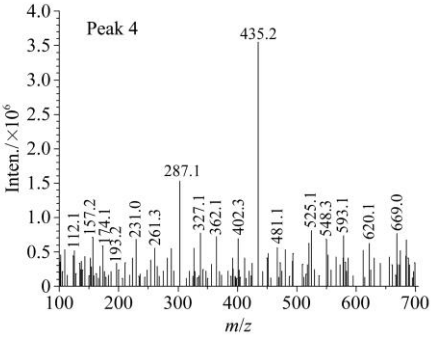
b. Mass spectrogram for 1#monomers (shown in Figure 1a) of anthocyanidins in blueberry extracts



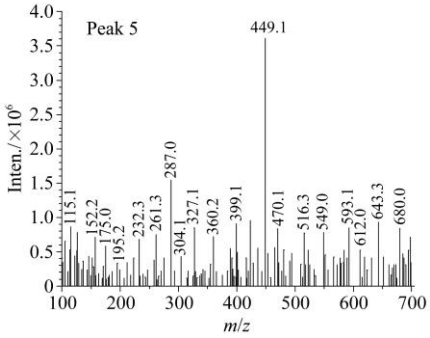
c. Mass spectrogram for 2#monomers (shown in Figure 1a) of anthocyanidins in blueberry extracts



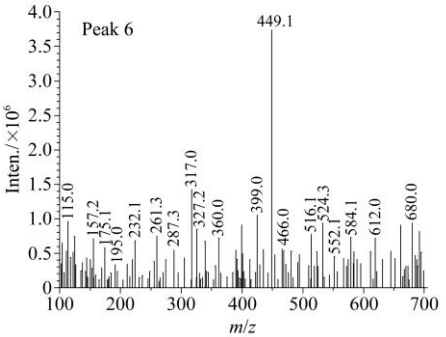
d. Mass spectrogram for 3#monomers (shown in Figure 1a) of anthocyanidins in blueberry extracts



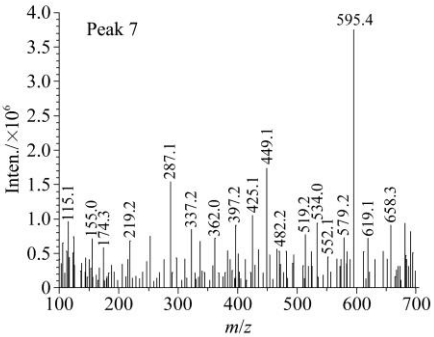
e. Mass spectrogram for 4#monomers (shown in Figure 1a) of anthocyanidins in blueberry extracts



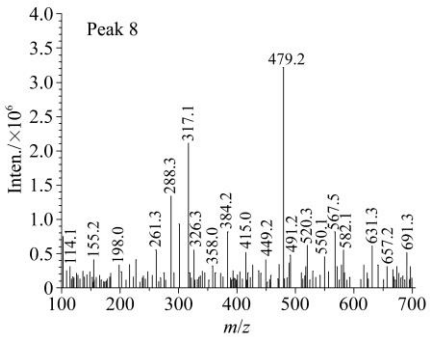
f. Mass spectrogram for 5#monomers (shown in Figure 1a) of anthocyanidins in blueberry extracts



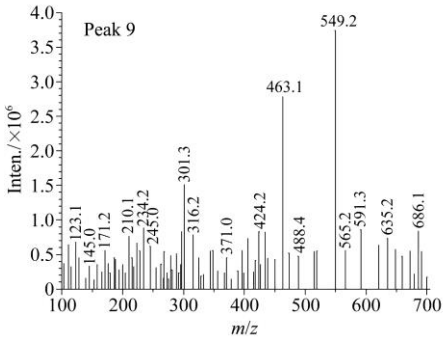
g. Mass spectrogram for 6#monomers (shown in Figure 1a) of anthocyanidins in blueberry extracts



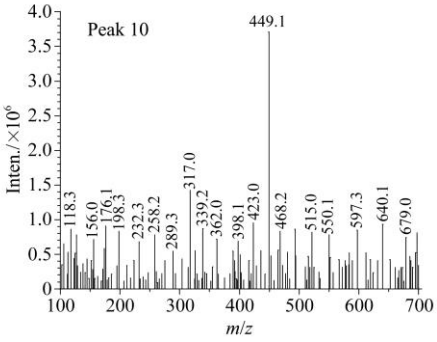
h. Mass spectrogram for 7#monomers (shown in Figure 1a) of anthocyanidins in blueberry extracts



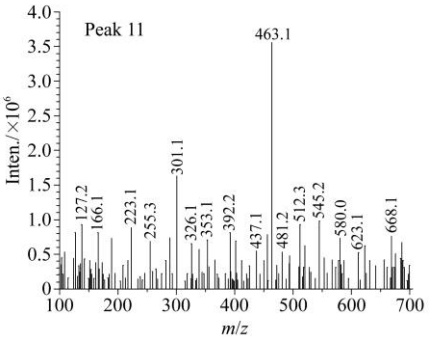
i. Mass spectrogram for 8#monomers (shown in Figure 1a) of anthocyanidins in blueberry extracts



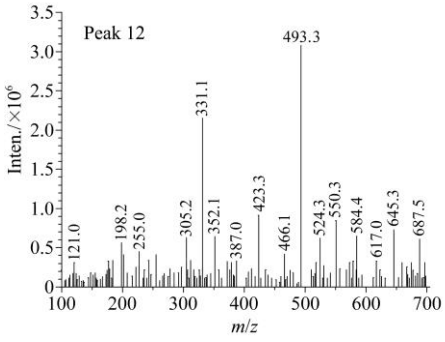
j. Mass spectrogram for 9#monomers (shown in Figure 1a) of anthocyanidins in blueberry extracts



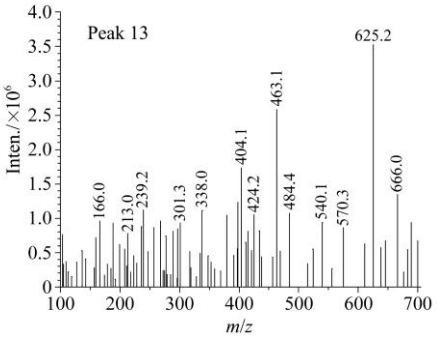
k. Mass spectrogram for 10#monomers (shown in Figure 1a) of anthocyanidins in blueberry extracts



l. Mass spectrogram for 11#monomers (shown in Figure 1a) of anthocyanidins in blueberry extracts



m. Mass spectrogram for 12#monomers (shown in Figure 1a) of anthocyanidins in blueberry extracts



n. Mass spectrogram for 13#monomers (shown in Figure 1a) of anthocyanidins in blueberry extracts

Note: In mass spectrometry, *m*/*z* refers to the ratio of the mass number of charged particles to the charge number, where the mass number *m* is in atomic mass (AU) and the charge number *z* is in proton charge (*E*).

Figure 1 HPLC chromatogram for the total content of anthocyanidins and mass spectrogram for the identification of monomers of anthocyanidins in blueberry ethanol extracts

Table 2 Identification of anthocyanins in blueberry ethanol extracts under MAE by using UPLC–DAD–MS/MS

Peak in Figure 1	Retention time/min	Molecular ion M^+ (*m*/*z*)	Fragment ion (*m*/*z*)	Lost fragment (*m*/*z*)	Monomers of anthocyanin	References resource
Peak 1	22.35	465.0	303.1	162	Delphinidin-3-glucoside	[31]
Peak 2	23.30	465.1	303.1	162	Delphinidin-3-galactoside	[32]
Peak 3	24.34	449.1	287.1	162	Cyanidin-3- galactoside	[33]
Peak 4	29.90	435.1	303.1	132	Delphinidin-3-arabinose	[32]
Peak 5	31.81	449.1	287.0	162	Cyanidin-3- glucoside	[34]
Peak 6	32.41	449.1	317.0	162	Petunidin-3- galactoside	[35]
Peak 7	34.26	419.1	287.1	132	Cyanidin-3- arabinose	[34]
Peak 8	39.56	479.1	317.1	162	Petunidin-3- glucoside	[36]
Peak 9	42.07	363.1	301.1	162	Peonidin-3- galactoside	[31]
Peak 10	47.17	449.1	317.0	132	Petunidin-3- arabinose	[31]
Peak 11	55.65	463.1	301.1	162	Peonidin-3- glucoside	[34]
Peak 12	56.61	493.1	331.1	163	Malvidin-3- galactoside	[37]
Peak 13	60.91	433.1	301.1	132	Peonidin-3- arabinose	[36]

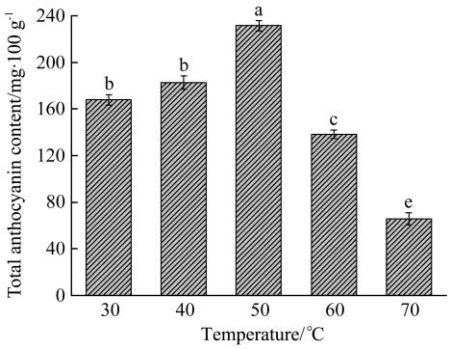
Note: In mass spectrometry, *m*/*z* refers to the ratio of the mass number of charged particles to the charge number, where the mass number *m* is in atomic mass (AU) and the charge number *z* is in proton charge (*E*).

Figure 1a presents the characteristic curves of UV spectral from HPLC chromatogram for total monomers of anthocyanins in ethanol extracts under MAE as the basis for identifying anthocyanin monomer species (shown in Table 2). According to the identification of anthocyanins monomers from related references (listed in Table 2) in the retention time shown in Figure 1a and the characteristic protonated molecular ion M^+ and the corresponding fragment shown in Fig 1b-1n, thirteen anthocyanins are identified shown in Table 2 as Delphinidin-3-glucoside, Delphinidin-3-galactoside, Cyanidin-3-galactoside, Delphinidin-3-arabinose, Cyanidin-3-glucoside, Petunidin-3-galactoside, Cyanidin-3-arabinose, Petunidin-3-glucoside, Peonidin-3-galactoside, Petunidin-3-arabinose, Peonidin-3-glucoside, Malvidin-3-galactoside, Peonidin-3-arabinose.

3.2 Effects of extraction temperatures on the content of anthocyanidins in MAE

The changes in the total content of anthocyanidins extracted from blueberry extracts with extraction temperature are shown in Figure 2. The total content of anthocyanidins increases significantly to (231.56±4.41) mg/100g with an extraction temperature of 50 ℃, followed by an obviously decrease under the extraction temperature of 50 ℃-70 ℃ (p<0.05). In MAE, the improvement of rising temperature on the extraction of anthocyanidins in BEE is attributed to 1) the cell wall broken caused by the generation of microwave volumetric heating to promote anthocyanidins release[13]; 2) the enhancement of intercellular diffusion[38]; 3) high solubility in extract solvent[39]. With the yield of anthocyanidins from blueberry powder,

excessively high extraction temperature also results in its degradation in MAE[40]. The net anthocyanidins content in extracts declines under the extraction temperature exceeding 50 ℃ due to the greater degradation of anthocyanidins than that of output from blueberry powder in MAE[16]. The extracts of anthocyanidins are a complex system consisting of monomers and degradation substances of anthocyanidins.



Note: In MAE, ethanol concentration of 60%, solid-to-liquid ratio of 1:30, and extraction time of 4 min. Columns marked with different letters mean significant difference (p<0.05).

Figure 2 Effects of temperature on the total content of anthocyanidins from blueberry powder in MAE

The model of anthocyanidins content in BEE as the function of temperature may characterize the effects of temperatures on the monomers of anthocyanidins in MAE. According to the results shown in Figure 2, a peak equation with four parameters shown in Equation (8) is selected to characterize the changes of anthocyanidin content of blueberry powder with the extraction temperatures, which may explain quantitatively the extraction kinetics of extraction temperature for anthocyanidins in MAE.

$$y = y_0 + a\exp\left[-0.5\left(\frac{T-T_1}{k}\right)^2\right] \quad (8)$$

where, y is the special monomer content of anthocyanidins, mg/100 g; y_0 is the initial monomer content in extractants, mg/100 g; a is the maximum anthocyanidins content (y peak value); T is extraction temperature, ℃; T_1 is defined as critical extraction temperature, ℃; indicating the degradation rate of monomer higher than its acquisition rate in extracts[16]; k is the kinetic coefficient of extraction, which characterized the driving force of temperature on the yield of anthocyanidins in MAE.

The changes in the monomers of anthocyanidins tend to increase then decrease with extraction temperature in BEE as shown in Table 3. The HPLC peak area of each monomer of anthocyanidins from blueberry powder under different temperatures is introduced into the corresponding standard equations to calculate the content of each monomer of anthocyanidins shown in Table 3. The changes in the monomers of anthocyanidins are in accordance with that of the total anthocyanidins content. The glycoside contents of delphinidin, cyanidin, petunidin, peonidin, peonidin, and malvidin increase remarkably, and then significantly decrease with the extraction temperature (p<0.05). The contents of monomers increase with the extraction temperature of 30 ℃-50 ℃ as shown in Table 3. These results indicated that the cyanidin glycosides of blueberry were the highest, and the content of malvidin glycosides was the lowest in blueberry ethanol extracts. The glycoside contents of delphinidin, cyanidin, petunidin, peonidin, peonidin, and malvidin obviously decrease under an extraction temperature higher than 50 ℃. The total content of anthocyanidins measured by using HPLC chromatogram is shown in Figure 1a, where the monomers species of anthocyanins are identified by mass spectrogram for the identification of monomers of anthocyanidins at Figure 1b-1n in BEE in MAE. The results indicate thirteen monomers of anthocyanidins in BEE at temperatures of 30 ℃, 40 ℃, and 50 ℃, and no Peonidin-3-glucoside and Malvidin-3-galactoside at temperatures of 60 ℃ and 70 ℃, respectively, in MAE.

Table 3 Changes in anthocyanidins monomers and contents and regression coefficients of Equation (8) in MAE

Item	Extraction temperature/ ℃					Regression coefficients of Equation (8)					
	30	40	50	60	70	y_0	a	T_1/ ℃	k	R^2	SEE
Tan	167.35±4.58^b	181.95±5.90^b	231.02±4.58^a	138.14±3.36^c	65.40±5.40^e	−166.49	379.70	44.44	25.67	0.89	0.13
Del-3-Glu	23.22±0.34^c	26.76±0.72^b	30.54±0.42^a	21.08±0.22^d	10.45±0.62^e	−48.58	78.06	44.65	33.88	0.96	9.18
Del-3-Gal	4.51±0.27^b	5.44±0.38^a	5.66±0.30^a	3.11±0.23^c	2.34±0.18^d	1.82	4.03	42.57	12.91	0.95	0.44
Cya-3-Gal	6.23±0.17^c	7.32±0.31^b	8.17±0.35^a	5.29±0.29^d	2.11±0.12^e	−12.05	20.04	44.05	31.07	0.97	0.63
Del-3-Ara	21.24±0.36^c	24.22±0.14^b	26.46±0.41^a	23.46±0.38^b	8.46±0.30^d	0	27.56	45.25	18.58	0.85	27.20
Cya-3-Glu	18.36±0.32^c	21.29±0.29^b	24.55±0.39^a	20.21±0.12^d	8.97±0.17^e	0	24.60	45.93	18.61	0.91	12.20
Pet-3-Gal	16.18±0.17^c	20.08±0.23^b	23.14±0.28^a	20.19±0.55^b	8.39±0.27^d	0	23.52	47.01	17.84	0.90	12.5
Cya-3-Ara	4.38±0.27^b	4.76±0.21^b	6.16±0.20^a	2.40±0.31^c	1.84±0.14^c	1.08	4.67	43.12	13.25	0.80	2.53
Pet-3-Glu	14.29±0.30^c	18.15±0.15^b	18.97±0.17^a	7.77±0.28^d	6.07±0.22^e	4.50	15.47	42.36	11.82	0.93	10.30
Peo-3-Gal	24.44±0.38^b	25.35±0.26^b	28.64±0.32^a	22.36±0.42^c	10.25±0.33^d	−17.72	24.28	42.37	34.67	0.96	1.13
Pet-3-Ara	15.59±0.37^c	19.26±0.27^b	21.46±0.34^a	9.33±0.23^d	8.08±0.23^e	6.57	15.16	43.03	11.35	0.89	15.80
Peo-3-Glu	16.25±0.16^c	19.24±0.26^b	22.26±0.33^a	0^d	0^d	−3.62	26.72	41.07	12.54	0.85	75.20
Mal-3-Gal	5.27±0.25^c	5.85±0.23^b	6.71±0.24^a	3.25±0.15^d	0^e	−17.72	24.28	42.37	34.67	0.96	1.14
Peo-3-Ara	1.26±0.17^b	1.71±0.30^{ab}	2.36±0.27^a	1.45±0.25^b	1.09±0.12^b	1.15	1.24	48.50	6.86	0.99	0.01

Note: The abbreviation of anthocyanin and monomers: Total anthocyanidins as Tan, Delphinidin-3-glucoside as Del-3-Glu, Delphinidin-3-galactoside as Del-3-Gal, Cyanidin-3-galactoside as Cya-3-Gal, Delphinidin-3-arabinose as Del-3-Ara, Cyanidin-3-glucoside as Cya-3-Glu, Petunidin-3-galactoside as Pet-3-Gal, Cyanidin-3-arabinose as Cya-3-Ara, Petunidin-3-glucoside as Pet-3-Glu, Peonidin-3-galactoside as Peo-3-Gal, Petunidin-3-arabinose as Pet-3-Ara, Peonidin-3-glucoside as Peo-3-Glu, Malvidin-3-galactoside as Mal-3-Gal, Peonidin-3- arabinose as Peo-3-Ara. The unit of contents of anthocyanidin monomers is mg/100 g. Data marked with different letters (a, b, c, d, and e) means significant differences (p<0.05). R^2 indicates the coefficient of determination and SEE indicated the standard error of estimate of fitted results.

Degradation kinetics of monomers provide the degradation properties and changes of anthocyanidins content. The coefficients of Equation (8) with extraction temperature in Table 3 were fitted by using the nonlinear regression method. This model indicated anthocyanidins content in BEE increase with the extraction temperature until the highest level at the temperature of 44.42 ℃, defined as critical temperature, followed by the decreasing trends due to the domination of degradation of

anthocyanidins in extracts. Higher critical temperatures (T_1) can reasonably infer the greater thermal stability of monomers of anthocyanidins. As listed in Table 3, their critical temperatures were in a descending order as Peonidin-3-arabinose, Petunidin-3-galactoside, Cyanidin-3-glucoside, Delphinidin-3-arabinose, Delphinidin-3-glucoside, Cyanidin-3-galactoside, Cyanidin-3-arabinose, Petunidin-3-arabinose, Delphinidin-3-galactoside, Peonidin-3-galactoside (Malvidin-3-galactoside), Petunidin-3-glucoside, Peonidin-3-glucoside. The critical temperature of monomers of anthocyanidins in BEE is in a range of 41.07 ℃-48.50 ℃ in MAE.

According to kinetic coefficient *k* listed in Table 3, Peonidin-3-galactoside (Malvidin-3-galactoside) has the highest degradation kinetic, followed by Delphinidin-3-glucoside, Cyanidin-3-galactoside, Cyanidin-3-glucoside, Delphinidin-3-arabinose, Petunidin-3-galactoside, Cyanidin-3-arabinose, Delphinidin-3-galactoside, Peonidin-3-glucoside, Petunidin-3-glucoside, Petunidin-3-arabinose, and Peonidin-3-arabinose is the least. The higher degradation kinetic coefficient indicates the greater degradation rate of monomer in MAE.

3.4 Effects of extraction temperature on the antioxidant capacities of anthocyanidins from blueberry powder in MAE

The IC_{50} of anthocyanidins extracts in terms of IC_{50}/DPPH, IC_{50}/TEAC, IC_{50}/reducing power, IC_{50}/α-glucosidase inhibition decrease with the increase of extraction temperature as listed in Table 4, which indicates the rising antioxidant activities of anthocyanidins in MAE. The antioxidant capacity of Cyanidin increases with the amount of glucoside chloride inside the structure[41]. The double bond of phenol ring, hydroxyl side chain, and glycosylation in the structure of anthocyanin are conducive to the activity of free radical scavengers[2]. The thermal degradation of anthocyanidins may produce aldehyde derivatives containing benzene ring, monohydroxybenzaldehyde, parahydroxyben-zaldehyde, and trihydroxybenzaldehyde, which have a stimulating effect on the cell to cause liver injury[42]. Extraction temperatures of 30 ℃-70 ℃ in MAE improve the DPPH and $ABTS^+$ radical-scavenging capacities of the anthocyanidins extracts in Table 4, which determines in vitro the free radical capacity[43]. As shown in Table 4, the DPPH radical-scavenging capacity in BEE increases with extraction temperature. The reduction of IC50/reducing power listed in Table 4 signifies the reducing powers of the anthocyanidins in BEE remarkably increase with the extraction temperature of 30 ℃-70 ℃ (p<0.05) due to the enhancement of electron-donating activity in antioxidative action of phenolics[44]. α-Glucosidase inhibitor has the activity of inhibition of α-glucosidase in polysaccharide decomposition into glucose[45]. The inhibitory activity against α-Glucosidase of anthocyanidins in BEE increases with the extraction temperature in Table 4.

Table 4 Indexes of antioxidant activities of anthocyanidins extracted from blueberry with the extraction temperature

Temperature / ℃	A	B	C	D
	IC_{50}/DPPH /μg mL^{-1}	IC_{50}/TEAC /μg mL^{-1}	IC_{50}/reducing power /μg mL^{-1}	IC_{50}/α-glucosidase inhibition /μg mL^{-1}
30	9.00±0.50^{ab}	168.90±3.44^{b}	61.93±1.46^{a}	79.15±2.77^{b}
40	8.40±0.53^{b}	151.23±4.55^{c}	51.07±3.72^{b}	61.20±2.31^{c}
50	7.27±0.31^{c}	128.80±4.35^{d}	46.30±3.16b^{c}	52.94±3.35^{d}
60	6.27±0.31c^{d}	108.83±4.51^{e}	40.67±2.52^{cd}	50.50±2.54^{de}
70	6.00±0.30^{d}	80.47±4.08^{f}	34.57±2.50^{de}	45.07±1.01^{e}

Note: The temperature shown in the first column presented the microwave extraction temperature for the anthocyanidins for the evaluation of antioxidant activities. Data in the same column with different letters are significantly different (p<0.05), and in column A, D-The antioxidant activity was calculated as the concentration of the test sample needed to decrease the absorbance at 517 nm and 405 nm by 50%, respectively; Column B-The antioxidant activity was evaluated as the concentration of the test sample required to decrease the absorbance at 734 nm by 50%; Column C-The antioxidant activity was evaluated as the concentration of the test sample needed to raise the absorbance at 700 nm to 50%.

In order to elucidate the improvement kinetics of antioxidant activity for four evaluation indexes, an exponential decay equation with three parameters was selected to characterize the changes in the antioxidant activities of blueberry anthocyanidins with extraction temperatures in Table 4. The regression coefficients of Equation (9) fitted by using the nonlinear regression method are shown in Table 5.

$$AC = AC_0 + a\exp(-kT) \quad (9)$$

where, *AC* is the antioxidant activities of anthocyanidins, the corresponding unit shown in Table 5; AC_0 is the initial antioxidant activities of anthocyanidins, the same unit as *AC*; *T* is the temperature of the extract, ℃; *a* is the coefficient; *k* is the kinetic coefficient characterizing the changes of antioxidant capacity.

Table 5 Regression coefficients for Equation (9) describing the antioxidant activities of anthocyanidins from blueberry extracts

Parameters in Equation (9) and statical analysis results	IC_{50}/DPPH /μg mL^{-1}	IC_{50}/TEAC /μg mL^{-1}	IC_{50}/reducing power /μg mL^{-1}	IC_{50}/α-glucosidase inhibition /μg mL^{-1}
AC_0	0.4444	0	16.0770	44.0050
a	12.3441	296.3829	86.3865	263.9064
k	0.0118	0.0175	0.0215	0.0674
R^2/SEE	0.9757/0.2877	0.9677/0.6703	0.9899/1.4824	0.9921/1.6736

Note: AC_0 is the initial antioxidant activities of anthocyanidins; *a* is the coefficient; *k* is the kinetic coefficient characterizing the changes in antioxidant capacity. All the tests were performed in triplicate (n=3) and all the data represent the means±standard deviation (n>3). R^2 indicates the coefficient of determination and SEE indicates the Standard Error of Estimate of fitted results.

In Table 5, the statistical analysis results indicate that the model has high reliability with a high determination coefficient R^2 and low SEE. Equation (9) presents that IC50 of antioxidant capacities of anthocyanidins extracts from blueberry tend to decrease trends with temperatures. These results indicate the improvement of antioxidant capacities of anthocyanidins extracts with extraction temperature, although the degradation of anthocyanins is caused by rising temperature.

General viewpoints deem that the degradation of anthocyanin weakens its antioxidant capability[2]. However, the antioxidant activity of anthocyanidins in extracts increases with extraction temperature until the critical temperature shown in Table 3 due to the higher acquisition of anthocyanidins than its degradation in MAE. The anthocyanidins content in BEE declines in the condition of the degradation of anthocyanidins exceeding its acquirement from blueberry in MAE. The rising antioxidant capacity of anthocyanidins instead of decreasing one in BEE with the increase of extraction temperatures under MAE is attributed as 1) rising temperature indicates sufficient thermal accumulation in extracts breaking the bond between the sugars moieties and the

3-hydroxyl position of the anthocyanidins to produce deglycosylation reaction, resulting in the deglycosylation of anthocyanidins to generate intermediate compounds as delphinidin, cyaniding, petunidin, peonidin, malvidin with strongly antioxidant capacity[46,47]; 2) in MAE, the mechanism of thermal degradation of anthcoyaninidins of blueberry caused by the hydrolysis of C4-position of benzene ring or ring-opening reation of sugar adical, followed by the generation of chalcone or α-dione, then final substances as cyanidin→2.4.6 trihydroxybenzaldehyde at A-ring+ 3.4 dihydroxybenzoic acid at B-ring, delphinidin→2.4.6 trihydroxybenzaldehyde at A-ring+3,4,5-trihydroxybenzoic acid at B-ring, petunidin→2.4.6 trihydroxybenzaldehyde at A-ring+benzoic acid at B-ring, malvidin→2.4.6 trihydroxybenzaldehyde at A-ring+3,5-dimethoxybenzoic acid[48,49], where the accumulation of antioxidant capacity of these substances[50,51] enhances the total antioxidant activities of extracts[52] as listed in Table 4; 3) thermal decomposition of anthocyanidins leads to the formation of polyphonic decomposition products[47], which dominates antioxidant activity of extracts in BEE under MAE[53].

Therefore, the antioxidant activities in BEE increase in DPPH and $ABTS^+$ radical scavenging capacity, reducing power and α-Glucosidase inhibition with extraction temperature in MAE due to the high anthocyanidins in extraction temperature below onset temperatures and accumulation of antioxidant activities of degradation substances of anthocyanidins from blueberry in extraction temperature beyond onset level. Compared to conventional extraction of thermal convection, for example, hot flux extraction, MAE has outstanding features in high great extraction kinetic[13] due to the interior generation of microwave volumetric heating, other than the inward transfer of heat[16]. The volumetric heating results in high acquirement from blueberry powder and obvious degradation in extracts for anthocyanins exceeding critical temperature[12]. Uniformity of temperature distribution in extracts, in cold and hot spots, severely influences the degradation of anthocyanins[16]. However, the degradation of anthocyanins in extracts does not weaken their antioxidant activity.

4 Conclusions

The content of total and each species of anthocyanidins initially significantly increases, and then decreases with the increase of extraction temperature. Thirteen monomers of anthocyanidins in BEE were identified. The critical temperature of each species of anthocyanidins from the blueberry powder was in the range of 42.57 ℃-48.50 ℃ in MAE. Peonidin-3-arabinose has great stability, and Peonidin-3-galactoside and Malvidin-3-galactoside are sensitive to degradation in ethanol extracts of anthocyanidins from blueberry powder in MAE. Both anthocyanidin content and its degradation in MAE improve the antioxidant capacities in terms of DPPH and $ABTS^+$ radical scavenging capacity, reducing power, and α-Glucosidase inhibition in ethanol extracts from blueberry powder. The research results indicate the degradation of anthocyanidins may enhance the antioxidant capacity in BEE under MAE, which provide a valuable route for the application of ethanol extracts from blueberry powder.

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[References]

[1] Zia M P, Alibas I. Influence of the drying methods on color, vitamin C, anthocyanin, phenolic compounds, antioxidant activity, and in vitro bioaccessibility of blueberry fruits. Food Bioscience, 2021; 42: 101179. doi: 10.1016/j.fbio.2021.101179.

[2] Rojas-Ocampo E, Torrejon-Valqui L, Munoz-Astecker L D, Medina-Mendoza M, Mori-Mestanza D, Castro-Alayo E M. Antioxidant capacity, total phenolic content and phenolic compounds of pulp and bagasse of four Peruvian berries. Heliyon, 2021; 7(8): e07787. doi: 10.1016/j.heliyon.2021.e07787.

[3] Nabavi S F, Habtemariam S, Dagila M, Shafighi N. Anthocyanidins as a potential therapy for diabetic retinopathy. Current Medicinal Chemistry, 2015; 22(1): 51–58.

[4] Kruger M J, Davies N, Myburgh K H, Lecour S. Proanthocyanidins, anthocyanidins and cardiovascular diseases. Food Research International, 2014; 59: 41–52.

[5] T. Irrazabal, B. K. Thakur, M. Kang, et al. Limiting oxidative DNA damage reduces microbe-induced colitis-associated colorectal cancer. Nature Communications, 2020; 11: 1802–1815.

[6] Burgos G, Amoros W, Munoa L, Sosa P, Cayhualla, Cinthia S, et al. Total phenolic, total anthocyanin and phenolic acid concentrations and antioxidant activity of purple-fleshed potatoes as affected by boiling. Journal of Food Composition and Analysis, 2013; 30(1): 6–12.

[7] Heinonen J, Farahmandazad H, Vuorinen A, Kallio H, Yang B R, Sainio T. Extraction and purification of anthocyanidins from purple-fleshed potato. Food and Bioproducts Processing, 2016; 99: 136–146.

[8] Silva S, Costa E M, Calhau C, Morais R M, Pintado M E. Anthocyanin extraction from plant tissues: A review. Critical Reviews in Food Science and Nutrition, 2017; 57: 3072–3083.

[9] Rodriguez-Gonzalez I, Ortega-Toro R, Diaz-Moreno A C. Influence of microwave- and ultrasound-assisted extraction on bioactive compounds from pollen. Contemporary Engineering Sciences, 2018; 11: 1669–1676.

[10] J.G. Gujar, S.J. Wagh, V.G. Gaikar. Experimental and modeling studies on microwave-assisted extraction of thymol from seeds of *Trachyspermum ammi* (TA). Separation and Purification Technology, 2010; 70: 257–264.

[11] Spigno G, De Faveri D M. Microwave-assisted extraction of tea phenols: A phenomenological study. Journal of Food Engineering, 2009; 93: 210–217.

[12] Liu C H, Xue H K, Shen L Y, Liu C, Zheng X Z, Shi J, et al. Improvement of anthocyanins rate of blueberry powder under variable power of microwave extraction. Separation and Purification Technology, 2019; 226: 286–298.

[13] Xue H K, Xu H, Wang X R, Shen L Y, Liu H, Liu C, et al. Effects of microwave power on extraction kinetic of anthocyanin from blueberry powder considering absorption of microwave energy. Journal of Food Quality, 2018; Article ID 9680184. doi: 10.1155/2018/9680184.

[14] Jiang T, Mao Y, Sui L S, Yang N, Li S Y, Zhu Z Z, et al. Degradation of anthocyanins and polymeric color formation during heat treatment of purple sweet potato extract at different pH. Food Chemistry, 2019; 274: 460–470.

[15] Li D N, Meng X J, Li B. Profiling of anthocyanidins from blueberries produced in China using HPLC-DAD-MS and exploratory analysis by principal component analysis. Journal of Food Composition and Analysis, 2016; 47: 1–7.

[16] Sun Y, Xue H K, Liu C H, Liu C, Su X L, Zheng X Z. Comparison of microwave assisted extraction with hot reflux extraction in acquirement and degradation of anthocyanin from powdered blueberry. Int J Agric & Biol Eng, 2016; 9(6): 186–199.

[17] Wu H Y, Yang K M, Chiang P Y. Roselle anthocyanins: Antioxidant properties and stability to heat and pH. Molecules, 2018; 23(6): 1357. doi: 10.3390/molecules23061357.

[18] Kim A N, Lee K Y, Kim B G, Cha S W, Jeong E J, Kerr W L, et al. Thermal processing under oxygen-free condition of blueberry puree: Effect on anthocyanin, ascorbic acid, antioxidant activity, and enzyme activities. Food Chemistry, 2021; 342: 128345. doi: 10.1016/j.ffodchem.2020.128345.

[19] Alvarez A, Poejo J, Matias A A, Duarte C M M, Cocero M J, Mato R B, et al. Microwave pretreatment to improve extraction efficiency and polyphenol extract richness from grape pomace. Effect on antioxidant bioactivity. Food and Bioproducts Processing, 2017; 106: 162–170.

[20] Qiu G, Wang D F, Song X Y, Deng Y, Zhao Y Y. Degradation kinetics and antioxidant capacity of anthocyanidins in air-impingement jet dried purple potato slices. Food Research International, 2018; 105: 121–128.

[21] Piovesan N, Viera V B, Mello R O, Santos R C V, Vaucher R, Dressler V L, et al. Microwave-assisted extraction of bioactive compounds from blueberry (*Vaccinium ashei* Reade) and their antioxidant and antimicrobial capacity. International Food Research Journal, 2017; 24: 2526–2533.

[22] Peron D V, Fraga S, Antelo F. Thermal degradation kinetics of anthocyanins extracted from juçara (*Euterpe edulis* Martius) and "Italia" grapes (*Vitis vinifera* L.), and the effect of heating on the antioxidant capacity. Food Chemistry, 2017; 232: 836–840.

[23] Zheng X Z, Xu X W, Liu C H, Sun Y, Lin Z, Liu H J. Extraction characteristics and optimal parameters of anthocyanin from blueberry powder under microwave-assisted extraction conditions. Separation and Purification Technology, 2013; 104: 17–25.

[24] Wang E L, Yin Y G, Xu C N, Liu J B. Isolation of high-purity anthocyanin mixtures and monomers from blueberries using combined chromatographic techniques. Journal of Chromatography A, 2014; 1327: 39–48.

[25] Cui C, Zhang S M, You L J, Ren J Y, Luo W, Chen W F, et al. Antioxidant capacity of anthocyanidins from Rhodomyrtus tomentosa (Ait.) and identification of the major anthocyanidins. Food Chemistry, 2013; 139: 1–8.

[26] Zhang L H, Fu Q J, Zhang Y H. Composition of anthocyanidins in pomegranate flowers and their antioxidant activity. Food Chemistry, 2011; 127(4): 1444–1449.

[27] Li D N, Li B, Ma Y, Sun X Y, Lin Y, Meng X J, et al. Polyphenols, anthocyanidins, and flavonoids contents and the antioxidant capacity of various cultivars of highbush and half-high blueberries. Journal of Food Composition and Analysis, 2017; 62: 84–93.

[28] Mojica L, Meyer A, Berhow M A, Mejia E G. Bean cultivars (*Phaseolus vulgaris* L.) have similar high antioxidant capacity, in vitro inhibition of α-amylase and *α*-glucosidase while diverse phenolic composition and concentration. Food Research International, 2015; 69: 38–48.

[29] He B, Zhang L L, Yue X Y, Liang J, Jiang J, Gao X L, et al. Optimization of ultrasound-assisted extraction of phenolic compounds and anthocyanidins from blueberry (*Vaccinium ashei*) wine pomace. Food Chemistry, 2016; 204: 70–76.

[30] Barnes J S, Nguyen H P, Shen S J, Schug K A. General method for extraction of blueberry anthocyanidins and identification using high performance liquid chromatography-electrospray ionization-ion trap-time of flight-mass spectrometry. Journal of Chromatography A, 2009; 1216(23): 4728–4735.

[31] Sun L Q, Ding X P, Qi J, Yu H, He S A, Zhang J, et al. Antioxidant anthocyanins screening through spectrum-effect relationships and DPPH-HPLC-DAD analysis on nine cultivars of introduced rabbiteye blueberry in China. Food Chemistry, 2012; 132(2): 759–765.

[32] You Q, Wang B W, Chen F, Huang Z L, Wang X, Luo P J G. Comparison of anthocyanins and phenolics in organically and conventionally grown blueberries in selected cultivars. Food Chemistry, 2011; 125(1): 201–208.

[33] Pertuzatti P B, Barcia M T, Rebello L P G, Gomez-Alonso S, Duarte R M , Duarte M C T, et al. Antimicrobial activity and differentiation of anthocyanin profiles of rabbiteye and highbush blueberries using HPLC-DAD-ESI-MS^n and multivariate analysis. Journal of Functional Foods, 2016; 26: 506–516.

[34] Yang Z D, Zhai W W. Optimization of microwave-assisted extraction of anthocyanins from purple corn (*Zea mays* L.) cob and identification with HPLC-MS. Innovative Food Science & Emerging Technologies, 2010; 11(3): 470–476.

[35] Ferreira L F, Minuzzi N M, Rodrigues R F, Pauletto R, Rodrigues E, Emanuelli T, et al. Citric acid water-based solution for blueberry bagasse anthocyanins recovery: Optimization and comparisons with microwave-assisted extraction (MAE). LWT, 2020; 133: 110064. doi: 10.1016/j.lwt.2020.110064.

[36] Müller D, Schantz M, Richling E. High performance liquid chromatography analysis of anthocyanidins in bilberries (*Vaccinium myrtillus* L.), blueberries (*Vaccinium corymbosum* L.), and corresponding juices. Journal of Food Science, 2012; 77(4): C340–C345.

[37] Liu Y X, Song X, Han Y, Zhou F, Zhang D, Ji B P, et al. Identification of anthocyanin components of wild Chinese blueberries and amelioration of light-induced retinal damage in pigmented rabbit using whole berries. Journal of Agricultural and Food Chemistry, 2011; 59(1): 356–363.

[38] Chan C H, Lim J J, Yusoff R, Ngoh G C. A generalized energy-based kinetic model for microwave-assisted extraction of bioactive compounds from plants. Separation and Purification Technology, 2015; 143: 152–160.

[39] Hayat K, Hussain S, Abbas S, Farooq U, Ding B M, Xia S Q, et al. Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity in vitro. Separation and Purification Technology, 2009; 70: 63–70.

[40] Farhat A, Fabiano-Tixier A S, Maataoui M E, Maingonnat J F, Romdhane M, Chemat F. Microwave steam diffusion for extraction of essential oil from orange peel: Kinetic data, extracts global yield and mechanism. Food Chemistry, 2011; 125(1): 255–261.

[41] Seeram N P, Momin R A, Nair M G, Bourquin L D. Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. Phytomedicine, 2001; 8(5): 362–369.

[42] Li H Y, Deng Z Y, Zhu H H, Hu C L, Liu R H, Young J C, et al. Highly pigmented vegetables: Anthocyanin compositions and their role in antioxidant activities. Food Research International, 2012; 46: 250–259.

[43] Coklar H, Akbulut M. Anthocyanidins and phenolic compounds of *Mahonia aquifolium* berries and their contributions to antioxidant activity. Journal of Functional Foods, 2017; 35: 166–174.

[44] Leopoldini M, Russo N, Toscano M. The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chemistry, 2011; 125(2): 288–306.

[45] Johnson M H, de Mejia E G, Fan J F, Lila M A. Anthocyanidins and proanthocyanidins from blueberry-blackberry fermented beverages inhibit markers of inflammation in macrophages and carbohydrate-utilizing enzymes in vitro. Molecular Nutrition & Food Research, 2013; 57(7): 1182–1197.

[46] Castaneda-Ovando A, Pacheco-Hernandez M L, Paez-Hernandez M E< Rodriguez J A, Galan-Vidal C A. Chemical studies of anthocyanins: A review. Food Chemistry, 2009; 113(4): 859–871.

[47] Patras A, Brunton N P, O'Donnell C, Tiwari B K. Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. Trends in Food Science & Technology, 2010; 21(1): 3–11.

[48] Furtado P, Figueiredo P, das Neves H C, Pina F. Photochemical and thermal degradation of anthocyanidins. Journal of Photochemistry and Photobiology A: Chemistry, 1993; 75(2): 113–118.

[49] Song H G, Liu M Y, Dong N, Zhang Z P, Zhao G H. Research progress on degradation mechanisms of anthocyanins in foods. Food Science, 2011; 32(13): 355–359. (in Chinese)

[50] Hayat K, Zhang X M, Farooq U, Abbas S, Xia S Q, Jia C S, et al. Effect of microwave treatment on phenolic content and antioxidant activity of citrus mandarin pomace. Food Chemistry, 2010; 123(2): 423–429.

[51] Liu C Y, Tang J, Zhao L Y. Research progress on the stability and function of anthocyanins. Hans Journal of Food and Nutrition Science, 2018; 7(1): 53–63. (in Chinese)

[52] Peng B, Li H Y, Deng Z Y. Degradation of anthocyanidins in foods during heating process and its mechanism. Journal of Food Safety and Quality, 2016; 7(10): 3851–3858. (in Chinese)

[53] Yang W, Kortesniemi M, Ma X Y, Zheng J, Yang B R. Enzymatic acylation of blackcurrant (*Ribes nigrum*) anthocyanins and evaluation of lipophilic properties and antioxidant capacity of derivatives. Food Chemistry, 2019; 281: 189–196.