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Anthocyanins, Flavonols, and Free Radical Scavenging Activity of Chinese Bayberry (*Myrica rubra*) Extracts and Their Color Properties and Stability

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Characterization of anthocyanins and flavonols and radical scavenging activity assays of extracts from four Chinese bayberry (*Myrica rubra*) varieties with different fruit colors were carried out. One dominant anthocyanin and three major flavonols were isolated by HPLC, and cyanidin-3-*O*-glucoside and two of three flavonols, myricetin and quercetin-3-*O*-rutinoside, were identified by cochromatography with authentic standards. Both DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ cation assays indicated that the black varieties (Biji and Hunan) demonstrated much higher radical scavenging activities than the pink (Fenhong) and yellow (Shuijing) varieties, which may be attributed to much higher levels of anthocyanins, flavonoids, and total phenolics in the black varieties. Biji and Hunan had 6.49 and 6.52 mM Trolox equivalent antioxidant capacity (TEAC) per 100 g of fresh weight, whereas the pink (Fenhong) and yellow (Shuijing) bayberries had 1.32 and 1.31 mM TEAC/100 g. Different fruit color was reflected by the surface color and pigment extract color. Color stability of the pigment was dependent on pH, and the pigment was more stable at low pH (pH ∼1.5). The lightness (L^*) increased while the chroma (C value) decreased with increase of pH until pH 5, but higher pH caused a small decrease for L^* and an increase for C.

KEYWORDS: Chinese bayberry; *Myrica rubra*; flavonoids; anthocyanins; flavonols; antioxidant; radical scavenging activity; color; stability

INTRODUCTION

Chinese bayberry (*Myrica rubra* Seib & Zucc.) is one of six *Myrica* species native to China, belonging to the genus *Myrica* in the family Myricaceae (*1*). As a subtropical and tropical tree, Chinese bayberry is grown commercially for fruit in eastern and southern China (*2*). Chinese bayberry is noted for its attractive colorful fruits and appealing flavors and is praised as a "precious southern Yangtze fruit of early summer" (*1*−*3*).

The color of Chinese bayberry is due to the presence of anthocyanins, which is one of the most broadly distributed pigment groups in the plant world and the largest group of water-soluble pigments. Anthocyanins provide colors ranging from salmon-pink through red and from violet to nearly black in a variety of plant sources. Considerable research is currently directed toward the identification and characterization of pigments as colorants from plant sources, such as vegetables, fruits, flowers, cereal grains, and other plant tissues (*4*−*6*). Among them, various berries such as raspberry, blueberry, bilberry, black currant, cowberry, chokeberry, cranberry, and elderberry represent popular sources for anthocyanin isolation, identification, and food application, although they are from different genera and families (*5*, *7*−*10*).

Pigments from natural sources are safe and have attractive bright colors. There are challenges in the food industry to replace synthetic dyes by natural pigments. For food applications, stability of anthocyanins is of great concern because they are usually less stable and more sensitive to changes in pH compared to synthetic colorants. Consequently, many studies on the stability of anthocyanins have been published (*6*−*8*, *11*−*13*). Factors affecting the color and stability of anthocyanins include structure and concentration, pH, light, copigments, self-association, metallic ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products, proteins, sulfur dioxide, and the temperature and time of processing and storage conditions (*13*−*16*).

Besides their food colorant roles, anthocyanins are also important as antioxidant (*17*, *18*), which have roles in promoting good health and reducing the risk of chronic diseases. The

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potential antioxidant and antiradical properties of anthocyanins have been reported for fruits of *Viburnum dilatatu* (*19*), various berries (*9*, *10*, *20*, *21*), sweetpotato (*17*), etc. Anthocyanins were also found to inhibit oxidation of low-density lipoprotein and liposomes (*22*), to prevent the risk of several chronic diseases (*23*), and to possess better anti-inflammatory activities than aspirin (*24*).

Anthocyanins are one type of flavonoids. Other flavonoids include flavonols, flavones, isoflavones, flavanones, flavanols, and chalcones (*25*). Characterization of anthocyanins and other flavonoids from Chinese bayberry has been little studied apart from the primary reports of Ye et al. (*26*), who identified only one anthocyanin component from one variety. It is well-known that anthocyanin contents as well as antioxidant activity differ in different species and genotypes (*9*). Interspecific variation in anthocyanin and antioxidant capacity was reported among blueberry genotypes (*10*). However, there have been no reports on other flavonoids identified from Chinese bayberry and the antioxidant activity of Chinese bayberry to date.

The objectives of this study were to characterize anthocyanin and flavonol components from the extracts of four Chinese bayberry varieties, assay radical scavenging activity of Chinese bayberry extracts, and study their color properties and color stability under different pH value.

MATERIALS AND METHODS

Materials. Four varieties of Chinese bayberry (*Myrica rubra*) with different fruit colors, that is, Biji (black), Hunan (black), Fenhong (pink), and Shuijing (yellowish), were employed for this study. The fruits of each variety were harvested on June 26, 2004, in Yuyao county of Zhejiang province, a major production region in eastern China; they were directly shipped to the laboratory and stored at −20 °C. 2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and potassium persulfate were purchased from Sigma/Aldrich (St. Louis, MO), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) was obtained from Fluka Chemie AG (Buchs, Switzerland), and HPLC grade organic reagents and formic acid were from BDH (Dorset, U.K.). Authentic standards of five anthocyanins were obtained: cyanidin-3-*O*-glucoside, cyanidin-3-*O*-galactoside, and pelargonidin-3-*O*-glucoside from Extrasynthèse, Genay, France, and myricetin and rutin (quercetin-3-*O*-rutinoside) from Sigma.

Extraction. Fresh pulps (10 g) of each genotype were extracted with 30 mL of methanol containing 1% HCl at room temperature for 24 h. The procedure was repeated twice. The methanolic extracts were filtered by a Millipore filter (Millipore Corp., Bedford, MA) with a 0.2-μM nylon membrane under vacuum at 23 °C and centrifuged at ∼20000*g* for 8 min. The supernatants were directly used for the identification of anthocyanins and flavonoids by HPLC and for the determination of color properties by colorimeter. For the study on the effect of pH on pigment stability, 100 g pulps of Biji genotype were extracted with methanol, and the extracts were freeze-dried in a Heto FD3 freeze-dryer (Heto-Holten A/S, Denmark) for 24 h and stored at 4 °C until use.

Color of Fruit Surface and Pigment Extracts. After the surface of the fruit had been sliced, the fruit surface color was measured on the smooth surface by a colorimeter (Chroma Meter CR-301, Minolta Co., Osaka, Japan), standardized with calibration plate sets CR-A47 and a white plate. The color of the pigment extracts was measured with a liquid cup in the same instrument. Color parameters were expressed as tristimulus parameters, that is, L^*, a^*, b^*, C, and $H°$. Negative a^* indicates green and higher positive a^* indicates red color. Higher positive b^* indicates more yellow color. The chroma (C) value indicates color intensity or saturation, calculated as $C = (a^{*2} + b^{*2})^{1/2}$. Hue angle was calculated as $H° = \tan^{-1}(b^*/a^*)$, where 0° or 360° = red-purple, 90° = yellow, 180° = green, and 270° = blue (*27*).

Effect of pH. The effect of pH on pigment stability was measured by a Spectronic Genesys-5 spectrophotometer (Milton Roy, New York). UV−vis absorbance spectra of the samples in 1.0 cm path length quartz cuvettes were recorded from 200 to 700 nm. Partially purified dried pigment powders were dissolved in distilled water. An aliquot of 35 g of solution was used to adjust the pH to different values by different concentration of NaOH and HCl with final increase of weight of <0.8 g. The solution samples were kept at 24 °C in the dark for 24 h prior to determining their spectroscopic properties and color parameters in triplicate.

HPLC. HPLC analysis was carried out by means of a Hewlett-Packard 1100 series HPLC system equipped with an HP 1100 diode array detector (DAD) and HPLC 2D ChemStation software. The chromatographic column was a 250 × 4 mm i.d., 5 μm, Nucleosil 100-C_{18} column with a 4 × 4 mm i.d., 5 μm, Nucleosil 5 C_{18} guard column (Agilent Technologies). The mobile phase consisted of 5% aqueous formic acid (A) and HPLC grade methanol (B). The flow rate was 1 mL/min; a stepwise linear gradient was programmed at 86:14 (A/B, v/v) for 1 min, then changed to 40:60 over 39 min, followed by 100% B over 2 min. The injection volume was 20 μL, and multiwavelength detections (280, 370, and 520 nm) were set.

Total Flavonoids. Total flavonoid content was determined by a colorimetric method (*18*, *28*) with minor modification. Aliquots (0.5 mL) of appropriately diluted or standard solutions were pipetted into 15-mL polypropylene conical tubes containing 2 mL of double-distilled H_2O and mixed with 0.15 mL of 5% $NaNO_2$. After 5 min, 0.15 mL of 10% $AlCl_3 \cdot 6H_2O$ solution was added; the mixture was allowed to stand for another 5 min, and then 1 mL of 1 M NaOH was added. The reaction solution was well mixed and kept for 15 min, and the absorbance was determined at 415 nm. Total flavonoid content was calculated using the standard rutin curve and expressed as milligrams of rutin per 100 g of fresh weight (FW).

Total Phenolics. Total phenolic content was estimated by using the Folin−Ciocalteu colorimetric methods with a little modification (*29*). Briefly, the 5× dilutions of the extracts were oxidized with 0.5 mL of 0.5 N Folin−Ciocalteu reagent, and then the reaction was neutralized with saturated sodium carbonate (75 g/L). The absorbance of the resulting blue color was measured at 760 nm with a Spectronic Genesys 5 spectrophotometer (Milton Roy) after incubation for 2 h at 23 °C. Quantification was done on the basis of a standard curve of gallic acid. Results were expressed as milligrams of gallic acid equivalent (mg of GAE) per 100 g of FW.

Radical DPPH Scavenging Activity. The free radical scavenging activity of Chinese bayberry extracts was measured according to the DPPH method reported by Brand-Williams (*30*) with modifications (*31*). DPPH$^\bullet$ solution (60 μM, 3.9 mL) was added to 0.1 mL of the extract at 5× dilution. The reaction for scavenging DPPH$^\bullet$ radicals was in 15 mL polypropylene tubes (Becton Dickinson) at room temperature (23 °C). The decrease in absorbance of DPPH$^\bullet$ at 515 nm was measured at different time intervals by a Spectronic Genesys 5 spectrophotometer (Milton Roy) until 6 h. Ethanol (80%) was used as a blank solution, and DPPH$^\bullet$ solution without test samples (3.9 mL of DPPH$^\bullet$ + 0.1 mL of 80% ethanol) served as the control. Trolox (0.5 mM) served as a reference antioxidant. All tests were performed in triplicate. Antioxidant activity was expressed with inhibition (percent) of DPPH$^\bullet$ absorbance = $(A_{control} - A_{test}) \times 100/A_{control}$ (*31*, *32*).

Radical Cation ABTS$^{\bullet+}$ Scavenging Activity. The total antioxidant capacity of Chinese bayberry extracts was carried out using a Spectronic Genesys 5 spectrophotometer according to the improved ABTS$^{\bullet+}$ method as described by Re et al. (*33*) and modified by Cai et al. (*29*). ABTS$^{\bullet+}$ cation solution (3.9 mL, absorbance of 0.700) was added to 0.1 mL of the pigment extracts and mixed thoroughly. The reaction mixture was kept at room temperature for 6 min, and the absorbance was immediately recorded at 734 nm. Trolox standard solution in 80% ethanol was prepared and assayed under the same conditions. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, mM Trolox equivalents per 100 g of FW of the fruit).

RESULTS

Identification and Quantification. Flavonoids, including anthocyanins, are important phenolic compounds in fruits and vegetables. Identification of most flavonoids including anthocyanins can be ascertained by cochromatography with authentic standards and by comparison with literature data (*25*, *29*). In

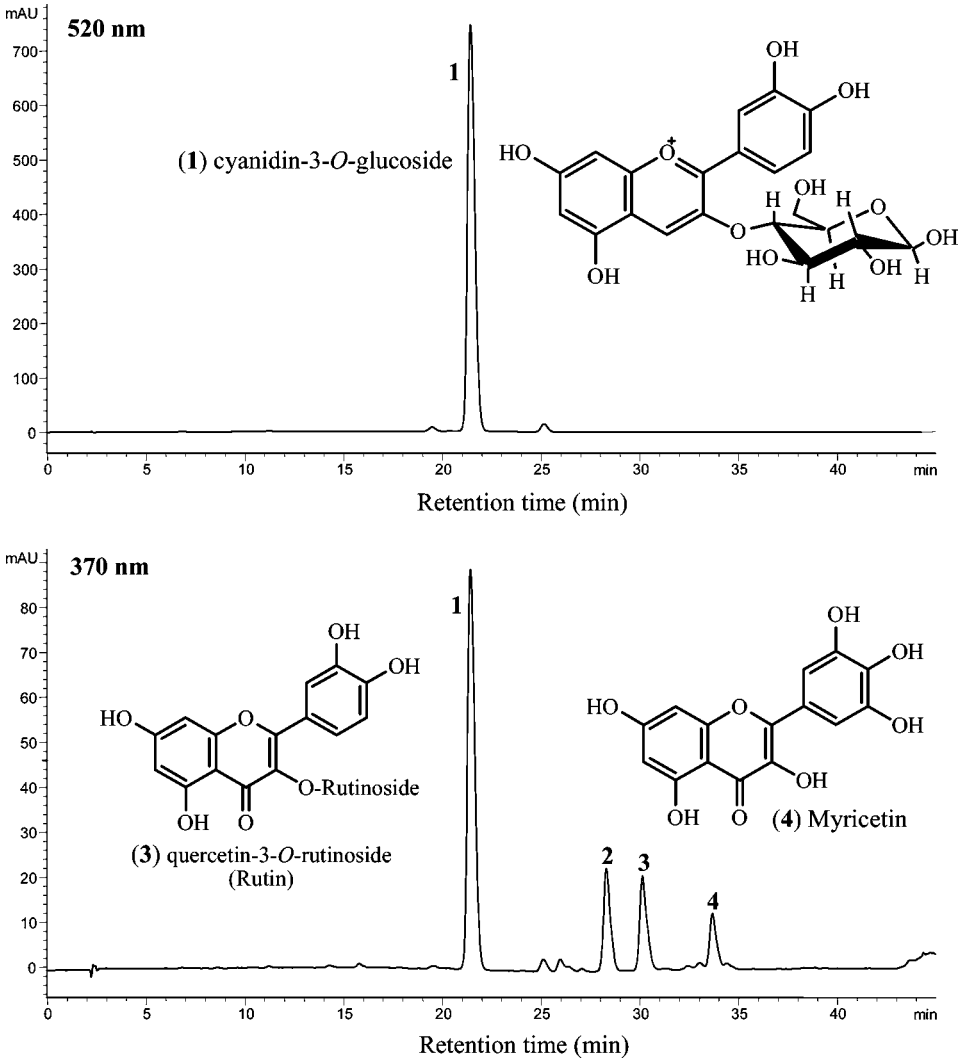

Figure 1. HPLC profiles at 520 and 370 nm and chemical structures of anthocyanin (peak 1, cyanidin-3-*O*-glucoside) and flavonols (peak 3, rutin; peak 4, myricetin) isolated from Chinese bayberry (Hunan).

the present study, the methanolic extracts of four Chinese bayberry genotypes were analyzed by HPLC. Typical HPLC profiles (**Figure 1**) of the extracts display one major peak (1) at a detection wavelength of 520 nm (anthocyanins) and four major peaks (1−4) at 370 nm (flavonol-type of flavonoids). The chemical structures of the anthocyanin and flavonols components identified are also shown in **Figure 1**.

The major peak (1) at 520 nm was readily identified by cochromatography with the standard of cyanidin-3-*O*-glucose. Through calculation of the peak area percentage from HPLC, cyanidin-3-*O*-glucose accounted for ~95% of total anthocyanins in the tested Chinese bayberry extracts, and therefore it is the dominant anthocyanin component in Chinese bayberry. Of four peaks at 370 nm, peaks 2−4 were identified as flavonols according to their UV−vis spectroscopic data and peak shapes by HPLC-DAD. By comparison with the relevant standards, peak 3 and 4 were identified as quercetin-3-*O*-rutinoside (rutin) and myricetin, respectively. Because of the very high concentration of cyanidin-3-*O*-glucose in the Chinese bayberry extracts and common anthocyanins with additional absorption in the wavelength of UV region, peak 1 was certain to be due to cyanidin-3-*O*-glucose at 370 nm. However, peak 2 was not identified.

The contents of anthocyanins, flavonoids, and total phenolics of differently colored fruits in four Chinese bayberry genotypes are listed in **Table 1**. Quantitative HPLC revealed that there was significant variation in anthocyanin contents in the Chinese bayberry extracts, ranging from 0 (Shuijing) to 79.2 mg/100 g of FW. Biji and Hunan contained much higher levels of anthocyanins than Fenghong and Shuijing. Also, there was variation in the total flavonoid contents among four genotypes, ranging from 28.9 to 122.3 mg/100 g of FW, but this variation was much less than that of the anthocyanin contents among the four genotypes.

Table 1. Contents of Anthocyanins, Flavonoids, and Total Phenolics and Total Equivalent Antioxidant Capacity (TEAC) of Chinese Bayberry Extracts (Mean ± SD)

variety	anthocyanins[a] (mg/100 g)	flavonoids[b] (mg/100 g)	total phenolics[c] (mg of GAE)	TEAC[d] (mM Trolox/ 100 g)
Biji	79.2 ± 0.2	122.3 ± 6.3	253.9 ± 3.3	6.49 ± 0.01
Hunan	60.3 ± 0.3	121.7 ± 5.2	281.5 ± 0.6	6.52 ± 0.01
Fenhong	2.9 ± 0.4	31.9 ± 5.6	98.0 ± 0.5	1.32 ± 0.00
Shuijing	0.0 ± 0.0	28.9 ± 3.3	94.3 ± 1.5	1.31 ± 0.00

[a] Anthocyanin contents are expressed as millgrams of cyanidin-3-*O*-galactoside per 100 g of fresh weight. [b] Flavonoid contents are expressed as milligrams of rutin per 100 g of fresh weight. [c] mg of GAE = milligrams of gallic acid equivalent per 100 g of fresh weight. [d] TEAC = Trolox equivalent antioxidant capacity. Data are expressed as millimoles of Trolox equivalent per 100 g of fresh weight.

Table 2. Color Properties of Chinese Bayberry Fruits and Their Pigment Extracts (Mean ± SD)

	fruit surface					pigment extracts				
variety	*L**	*a**	*b**	*C*	*H*°	*L**	*a**	*b**	*C*	*H*°
Biji	24.4 ± 3.1	12.4 ± 1.9	−1.55 ± 0.6	12.5 ± 1.8	352.4 ± 3.8	44.9 ± 0.03	80.1 ± 0.04	77.4 ± 0.07	111.4 ± 0.08	44.0 ± 0.02
Hunan	29.6 ± 1.7	12.1 ± 1.5	−0.84 ± 0.4	12.2 ± 1.5	355.8 ± 2.6	40.9 ± 0.15	71.7 ± 0.30	70.3 ± 0.23	100.3 ± 0.36	44.4 ± 0.07
Fenhong	45.5 ± 2.3	16.3 ± 2.6	4.11 ± 0.9	16.9 ± 2.4	14.7 ± 5.4	75.7 ± 0.12	31.9 ± 0.01	10.4 ± 0.01	33.5 ± 0.01	18.1 ± 0.01
Shuijing	56.7 ± 1.4	0.5 ± 0.5	8.0 ± 1.7	8.1 ± 1.7	86.3 ± 3.9	91.6 ± 0.08	0.3 ± 0.01	9.3 ± 0.06	9.3 ± 0.06	88.3 ± 0.06

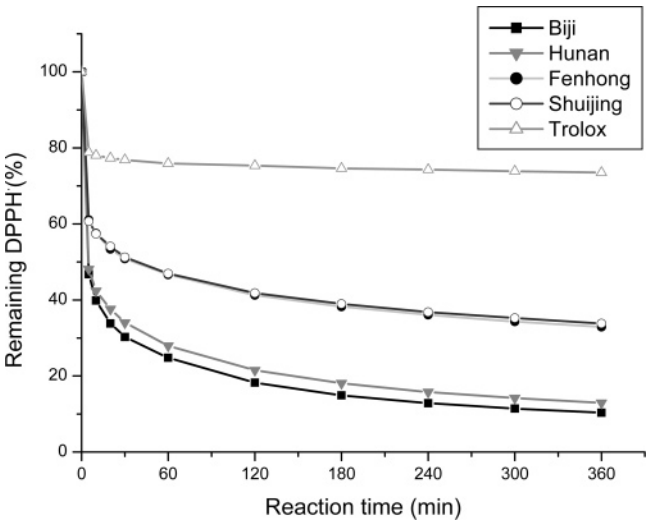

Figure 2. Antioxidant reaction kinetics (free radical DPPH scavenging activity) of four Chinese bayberry varieties and a referent antioxidant, Trolox (0.5 mM).

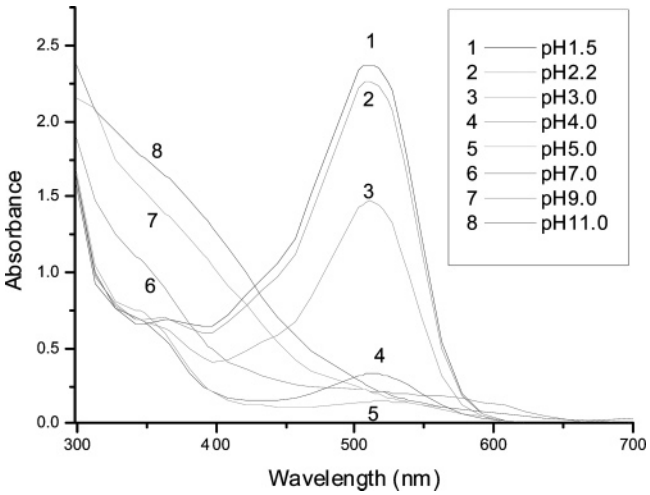

Figure 3. UV–vis spectra of Chinese bayberry (Biji) pigment extract at different pH values after 24 h of storage at 24 °C.

In addition to anthocyanins and flavonols, Chinese bayberry extracts actually contained a small amount of other phenolics. Total phenolic contents of the four samples were examined in this study as gallic acid equivalents. The total phenolic content in Hunan fruit (281.5 mg of GAE/100 g) was a little higher than that of Biji (253.9 mg of GAE/100 g), but it was much higher than that of Fenhong (98.0 mg of GAE/100 g) or Shuijing (94.3 mg of GAE/100 g) (**Table 1**).

Radical Scavenging Activity. The free radical scavenging activity of four Chinese bayberry extracts diluted five times was measured in the DPPH$^{\bullet}$ system. Antioxidant reaction kinetics against DPPH$^{\bullet}$ was established under the same reaction conditions (**Figure 2**). The lower remaining DPPH$^{\bullet}$ percentage indicates the high radical scavenging activity. After 6 h of reaction, the remaining DPPH$^{\bullet}$ of Biji and Hunan was <20%, whereas the remaining DPPH$^{\bullet}$ of Fenghong and Shuijing was <50%. This indicated that the black Chinese bayberry (Biji and Hunan) with a high level of phenolics (especially anthocyanins) exhibited potent radical scavenging activity, and the pink Fenhong and yellow Shuijing with low levels of phenolics (especially anthocyanins) had lower activity. However, the radical scavenging activities of all four extracts were much higher than that of the reference antioxidant, Trolox (0.5 mM) (**Figure 2**). Radical scavenging activity was also assayed by the ABTS$^{\bullet+}$ cation method in this study. The four Chinese bayberry extracts differed obviously in their radical scavenging activities (Trolox equivalent antioxidant capacity, TEAC) (**Table 1**). The black bayberry (Biji and Hunan) extracts had 6.49 and 6.52 mM TEAC per 100 g of FW, whereas the pink (Fenhong) and yellow (Shuijing) bayberries had 1.32 and 1.31 mM TEAC/100 g, respectively.

Appearance and Color Properties. The Chinese bayberry fruits were round in shape, with longitudinal length to transverse length ratios of ∼0.94 (Supplementary Table 1). The fruit sizes of the four genotypes differed significantly. Biji had the smallest fruits, with 2.60 cm in longitudinal length and 2.78 cm in transverse length. Shuijing had the largest fruit size (2.94 and 3.20 cm, respectively). Similarly, Shuijing fruit had an average weight of 15.8 g, whereas Biji fruits were only 10.2 g. For the color of the fruit surface, Biji and Hunan were black or purple, with lightness values (*L**) of 24.4 and 29.6 and hue angles of 352 and 356 (**Table 2**), respectively, indicating they were the typical purple color. Fenhong was pink as denoted from its Chinese name with an *H*° of 14.7, indicating redness. Shuijing was a typical yellow (*H*° = 86); it was the brightest among all varieties, but the color was lowest in purity with a *C* value of 8 (**Table 2**). Also, it was observed that the pigment extracts of same fresh weight of fruits for each variety were different in color (**Table 2**). However, they were much brighter (larger *L**) and more vivid (larger *C*) than the fruit surface color. The extracts of Biji and Hunan were red in color, with *H*° values of 44 and 45, respectively. Fenghong was pink with an *H*° value of 18, whereas the extract of Shuijing was still a typical yellow color with an *H*° value of 88.

Color Stability under Different pH Values. When pigment extracts (with "as-is" pH ∼3.8) were adjusted to different pH values, the color of the solution differed significantly (Supplementary Table 2; **Figure 3**). The brightness of the solution at pH 1.5 and 2.2 was ∼68 and increased with increasing pH to the highest value of 91 at pH 5; it then showed a small change at higher pH. The solution at pH 1.5 had the highest *C** value, but it decreased gradually until pH 5 and then increased with pH (Supplementary Table 2). Similarly, the hue angles of the solutions at pH 1.5 and 2.2 were 50 and 49, indicating a dark red color. The hue angle decreased gradually to ∼20 at pH 5 (pink), but it increased sharply to 62 at pH 7 and to 92 at pH 9 and 11 (yellow) (Supplementary Table 2). The dramatic

changes suggested the color was not stable at pH >5. The peak in the spectrophotometer was at 511 nm between pH 1.5 and 5, but the peak decreased dramatically and disappeared at pH >5 (**Figure 3**). These results indicated the pigment extracts of Chinese bayberry were most stable at very low pH, around pH 1.5.

DISCUSSION

As an attractive fruit, Chinese bayberry has wide diversity in fruit color, from yellowish to black or purple, representing different anthocyanin contents. Chinese bayberry is sometimes called red bayberry, but for yellowish varieties, this name is not appropriate. Because anthocyanins are not detectable in the yellowish fruit (**Table 1**), the variety might be an albino mutant of another colored bayberry. The yellowish fruit may result from lack of expression of anthocyanin-synthesizing genes.

Although genetic diversity in fruit color exists in Chinese bayberry, only one anthocyanin component (cyanidin-3-*O*-glucoside) was isolated and identified by HPLC in the present study, which accounted for 95% of the total anthocyanins. Ye et al. (*26*) also identified the major fraction as cyanidin-3-glucoside using paper chromatography. The results indicated that the difference in fruit color may result from the differential expression of anthocyanin-synthesizing genes, with highest expression in the black Chinese bayberry.

In addition to anthocyanins, other flavonoids are also widely distributed in fruits and vegetables. In the present study, we found the occurrence of flavonols in the four Chinese bayberry genotypes. This is the first report identifying rutin and myricetin in Chinese bayberry fruit extracts. Myricetin has already been identified from the bark of *Myrica rubra* (*34*) and other edible berries (*35*). However, another flavonol compound (peak 2 in **Figure 1**) is not yet definitively identified. According to spectroscopic data and retention time obtained in this study and literature data (*25*), peak 2 might be a glucoside of myricetin (i.e., myricitrin, myricetin-3-*O*-rhamnoside). Myricetin (peak 4) has a longer retention time than its glucoside, myricitrin. Only a few flavonol glucosides (e.g., myricitrin) have a shorter retention time than rutin (peak 3).

Previous studies have shown that flavonoids, including anthocyanins, have antioxidant or antiradical activities (*24*, *36*, *37*), which contribute to the explanation of the protective effect of vegetable-rich diets against coronary diseases. The antioxidant activity of plant extracts was significantly positively correlated with their phenolic content (*29*). Phenolic compounds mainly include flavonoids (e.g., flavonols, flavones, isoflavones, flavanones, flavanols, chalcones), phenolic acids, quinones, tannins, etc. (*25*). The results in this study showed that anthocyanin (cyanidin-3-*O*-glucoside) and flavonols (rutin and myricetin) are major phenolic components in the Chinese bayberry. Both $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ cation assays indicated that the black Chinese bayberry (Biji and Hunan) had higher radical scavenging activities than the pink and yellowish varieties (Fenghong and Shuijing) (**Table 1**; **Figure 2**), which can be attributed to a higher level of anthocyanins, flavonoids, and total phenolics in the black varieties. Although the pink and yellowish varieties contained fewer anthocyanins, they still had higher radical scavenging activities than Trolox (0.5 mM) (**Table 1**; **Figure 2**), because they had other flavonoids, such as myricetin and rutin. Both myricetin and rutin demonstrated stronger antioxidant activity than most flavonoids (*36*).

All anthocyanins have stability problems, but are most stable at low pH. The present study indicated that Chinese bayberry anthocyanin is most stable at pH ~1.5. Wheat pigments containing cyanidin-3-*O*-glucoside were reported to be thermally most stable at pH 1 (*4*). Cabrita et al. (*38*) reported that the absorptivities are highest at pH 1 for all anthocynidin 3-glucosides and decrease toward pH 5. The trend of color change at various pH values in the present study is very similar to the result of Torskangerpoll and Andersen (*39*). Our present study showed that all color parameters (L, a^*, b^*, C, $H°$) significantly changed above pH 4 (Supplementary Table 2), and the peaks above pH 4 at 515 nm also declined markedly (**Figure 3**), suggesting the anthocyanin pigments of Chinese bayberry was highly unstable above pH 4. However, when anthocyanins are formulated in food systems, the stability of cyanidin-3-*O*-glucoside is expected to increase because some food ingredients will improve the stability. For example, in a model beverage system, the cyanidin-3-*O*-glucoside had a stability similar to that of pigments of *Zebrina pendula*, the major components of which are tricaffeoylcyanidin-3,7,3′-triglucoside and caffeoylferuloylcyanidin-3,7,3′-triglucoside (*12*). Whether the other compounds, such as rutin or myricetin, in the bayberry fruit extracts will contribute to stabilizing the color under different pH values is unknown. The stability of anthocyanins is also dependent on their molecular structure. Earlier studies showed that the pigments from sweet potato (*11*), morning glory (*12*), and *Tradescanina paccida* (*16*) were much more stable than the cyanidin-3-*O*-glucoside and encoyanin. These studies also suggest that acylation in the anthocyanin molecules confers stability because deacylated pigments were found to be less stable (*11*, *12*). Inami et al. (*8*) further indicated that acylation improves both heat and light stabilities, whereas glucosidation stablizes anthocyanins only in the presence of light. However, acylated anthocyanins in the Chinese bayberry extracts were not found in the present study.

Suitable consumption of fruits and vegetables may significantly reduce the incidence of many chronic diseases, such as cardiovascular disorders, cancer, and other degenerative diseases caused by oxidative stress (*40*). The Chinese bayberry is either eaten fresh or traditionally processed into juice, canned fruit, jam, wine, and confections (*1*−*3*). The black Chinese bayberry varieties contained high levels of anthocyanins and flavonols and demonstrated strong antioxidant activity. The Chinese bayberry is not only a precious southern Yangtze fruit of early summer but also a potential source of potent natural antioxidants.

Supporting Information Available: Tables of physical traits and changes in color parameters of Chinese bayberries. This material is available free of charge via the Internet at http://pubs.acs.org.

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