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How To Improve Bayberry (*Myrica rubra* Sieb. et Zucc.) Juice Color Quality: Effect of Juice Processing on Bayberry Anthocyanins and Polyphenolics

ZHONGXIANG FANG,[†] MIN ZHANG,*[,†] YUNFEI SUN,[§] AND JINGCAI SUN[§]

Key Laboratory of Food Science and Safety, Ministry of Education, Southern Yangtze University, Wuxi 214036, China, and Food Research Institute of Haitong Food Group Corporation Limited, Cixi 315300, China

Fresh bayberries (*Myrica rubra* Sieb. et Zucc.) were processed into juice at an industrialized scale with four treatments: control, SO_2 addition, pasteurization of the crushed pulp, and blanching before fruit crushing. Changes in anthocyanin pigments and polyphenolics (hydrobenzoic acids and flavonol glycosides) were monitored during processing. Centrifuged juice yield ranged from 73 to 78% (w/w), but only 12−27% of the anthocyanins and 20−32% of the polyphenolics were recovered in the ultrahigh-temperature (UHT) juices. Fifty-two to 58% of anthocyanins and 30−35% of polyphenolics were present in the centrifuged cakes. The initial processing steps of blanching, crushing, pasteurization, and depectinization caused a great loss of total and individual polyphenolics. Total monomeric anthocyanins were significantly higher ($p < 0.05$) in pasteurization- and blanching-treated samples than those in the SO_2 treated samples, whereas those in the SO_2-treated sample were significantly higher ($p < 0.05$) than those in the control juice. Overall polyphenolic levels were significantly higher ($p < 0.05$) in pasteurization- and blanching-treated samples than in the SO_2-treated and control samples after each processing step. Important changes occurred in the polyphenolic profile with a 50−150% of hydroxybenzoic acids increase due to the free gallic acid from the flavonol glycoside-gallates during the initial processing steps. Flavonol deoxyhexosides were more stable than the flavonol hexosides during bayberry juice processing.

KEYWORDS: Bayberry; *Myrica rubra* Sieb. et Zucc.; polyphenolics; anthocyanins; SO_2; pulp pasteurization; blanching; juice processing

INTRODUCTION

Bayberry (*Myrica rubra* Sieb. et Zucc.) is one of six *Myrica* species native to China, the family of Myricaceae (*1*). The wild-growing history for this plant goes back about 7000 years, and the cultivation history goes back more than 2000 years (*2*). Bayberry fruits have a special sweet, sour taste and exquisite flavor, with an attractive purple, red, or dark red color. Except for being the food of the local people, traditionally, bayberry fruits have been used for the treatment of various gastric intestinal problems such as diarrhea and gastroenteritis (*2*).

Anthocyanins and polyphenolics are organic micronutrients of fruits and vegetables and play an important role in the quality of food for their contribution to the nutritional, organoleptic, and commercial properties (*3*). The antioxidant capacity of fruits and vegetables, which benefits human health, is highly correlated to their anthocyanin and total phenolic contents (*4*−*7*). The major anthocyanin in bayberry fruits has been identified as cyanidin 3-glucoside, which represents >95% of the total pigments (*8*, *9*). Our previous work showed that polyphenolics in bayberry fruits are classified into two groups: one is hydroxybenzoic acids (gallic acid and protocatechuic acid), and the other is flavonol glycosides, including quercetin, myricetin, and kaempferol glycosides (*10*). The strong antioxidant activities of bayberry fruits (*8*), juice (*11*), and jam (*12*) have also been shown to correlate with their anthocyanin and polyphenolic contents.

Color instability and flavonoid degradation in bayberry juice are of great interest to food scientists and industrialists (*13*−*15*). Studies on blueberry indicated that the native enzyme polyphenol oxidase (PPO) caused significant degradation of anthocyanins and polyphenolics when the fruit was processed (*16*, *17*). The degradation mechanism of cyanidin 3-glucoside was proposed as being that native PPO oxidizes polyphenolics (such as chlorogenic acid) to quinones, which subsequently react with cyanidin 3-glucoside to produce brown pigments (*18*, *19*). The major anthocyanin in bayberry is also cyanidin 3-glucoside (*8*, *9*). We presumed that the main anthocyanin degradation

* Address correspondence to this author at Professor of School of Food Science and Technology, Southern Yangtze University, 214036 Wuxi, Jiangsu Province, China (e-mail min@sytu.edu.cn; telephone +86-510-5815590; fax +86-510-5807976).
[†] Southern Yangtze University.
[§] Haitong Food Group Corp. Ltd.

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mechanism in bayberry and its products undergo the same coupled enzymatic oxidation reactions.

Blanching and pasteurization have been shown to inhibit native enzyme activity and increase anthocyanin extraction (*16*, *17*, *20*, *21*). Sulfur dioxide (SO_2) addition was also effective in inhibiting PPO activity (*17*, *22*, *23*). These treatments all enhanced the color stability of the anthocyanin-containing products. No work has been done to determine the anthocyanin and polyphenolic changes resulting from bayberry juice processing. The objective of this study was to determine the effectiveness of blanching, pasteurization after fruit crushing, and sulfur dioxide addition on bayberry juice color quality. The research was carried out on an industrial scale because sufficient laboratory studies and pilot trials have been performed.

MATERIALS AND METHODS

Chemicals and Solvents. Pure standards of gallic acid, protocatechuic acid, quercetin, quercetin 3-glucoside, and cyanidin 3-glucoside were purchased from Sigma (St. Louis, MO), Fluka (Buchs, Switzerland), and Extrasynthèse (Genay, France). Standards were dissolved in methanol. Working solutions were prepared daily by appropriate dilution with methanol to make the concentration from 1.2 to 120.0 mg/L. Acetonitrile and methanol (HPLC grade), ethyl acetate, acetic acid, formic acid, and hydrochloric acid (analytical grade) were purchased from Shanghai Chemical Reagent Co., Shanghai, China. All solution preparations were made using distilled−deionized water.

Fruits. The major bayberry cultivar used in industries, Biqi, was harvested on June 23, 2005, in Cixi, Zhejiang Province, China, and transported to the factory in 2 h. The ripe fruits have a pH of 3.15 and soluble solids of 9.5, and the titratable acidity is 0.86 g/100 g of fresh weight (FW; expressed as citric acid).

Juice Processing. Bayberry juice was produced replicatively on an industrial scale at Haitong Food Group Co. Ltd., Zhejiang Province, China. The procedure for juice processing is shown in **Figure 1**. There were four groups: no treatment, SO_2 addition, pasteurization after crushing, and blanching before crushing. Control fruits were washed, crushed, depectinized, centrifuged, fined, filtered, and sterilized. The SO_2 treatment differed from the control by the addition of potassium metabisulfite ($K_2S_2O_5$) to the crushed pulp (free SO_2 = 50 mg/L). The pasteurization treatment differed from the control in that the crushed pulp was pasteurized at 90 °C for 1 min in a tubular heater (model BRO-5, Jinron Co., Zhangjiagang, Jiangsu Province, China) and then cooled to 35 °C. The blanching treatment differed from the control by heating the fruits at 90 °C for 2 min; the fruits were then immediately cooled to 40 °C before crushing. Bayberry fruits were processed into juice within 3 h of arrival, and ∼2 tons of bayberry fruits were used for each trial. The fresh berries were crushed with a horizontal crusher (model DJ-25, Ningbo Weiai Beverage Machine Works, Zhejiang Province, China), with the sieve pore of 1 mm. Pectinase (0.1 mL/kg, type Klerzyme 150, DSM Food Specialties, Allonne, France) was then added, and the juice was treated for 1 h at room temperature (30−32 °C, negative alcohol precipitation test was used as an indication of depectinization). Then the depectinized pulp was centrifuged with a decanter centrifuge (model LW420×1680-NC, Nanjing Lüzhou Machine Works, Jiangsu Province, China). For fining, a 50 g/L gelatin (type A, 175 bloom, Shanghai Chemical Reagent Co.) solution, which was prepared in hot water (60 °C) just prior to use, was added to the depectinized juice at 0.2 g/L with good agitation for 10 min and a 50 g/L bentonite (Shanghai Chemical Reagent Co.) suspension, which was also prepared in 60 °C water, was then added at 0.2 g/L. The mixture was well agitated for 10 min to allow flocculation to take place. After 1 h at room temperature, the supernatant was taken out, 0.2 g/100 mL diatomaceous earth was added, and the mixture was then filtered through a diatomaceous earth filter (model GZL-60, Ningbo Weiai Beverage Machine Works). The clarified juice was then subjected to sterilization at 120 °C for 3 s with an ultrahigh-temperature unit (model UHT-4, Ningbo Weiai Beverage Machine Works) and packaged in sterilized high-density polyethylene containers.

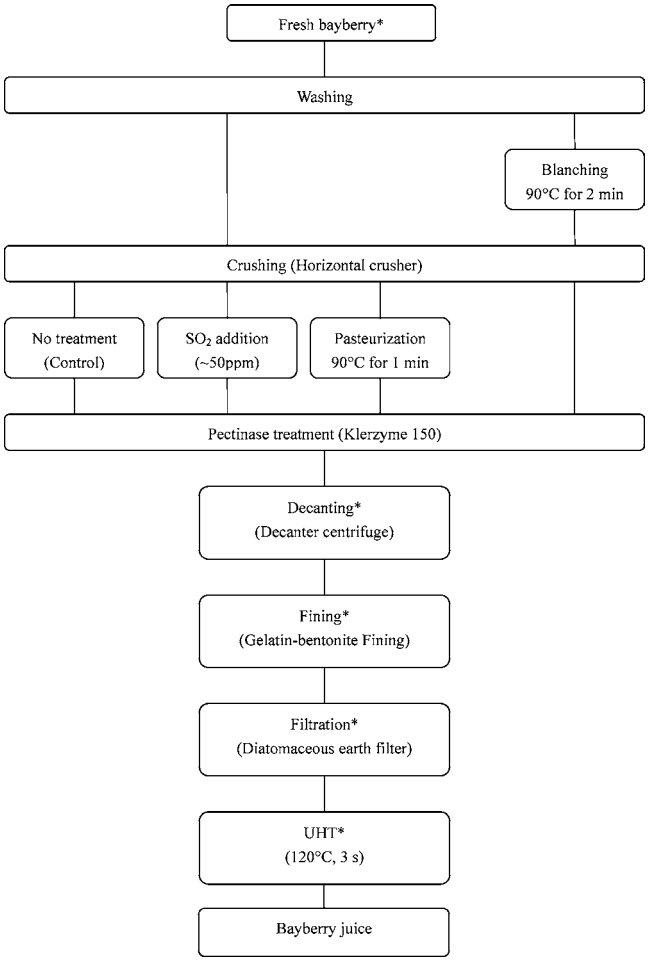

Figure 1. Flow chart for bayberry juice processing (*, samples taken for analysis).

Anthocyanin and Polyphenolic Extraction. Bayberry and presscake sample extraction were performed following the modified procedure described by Pazmiño-Durán et al. (*24*). Twenty grams of sample was blended with 100 mL of 0.15% HCl in methanol (v/v) and stored overnight at 5 °C. The samples were filtered on a Büchner funnel, and the filter cake residue was re-extracted with 0.15% HCl in methanol until a clear solution was obtained. Filtrates were combined and concentrated to ∼20 mL in a rotavapor at 35 °C under reduced pressure. The aqueous extract was made up to a known volume with distilled water.

Total Monomeric Anthocyanin Content, Percent Polymeric Color, and Browning Index. Total monomeric anthocyanin (ACN) contents of bayberry and juice were determined using the pH differential methods (*25*). Absorbance was measured at 510 and 700 nm. ACN was calculated as cyanidin 3-glucoside using an extinction coefficient of 26900 L cm^{-1} mg^{-1} and a molecular mass of 449.2 g mol^{-1}. Percent polymeric color contents of these samples were determined according to the bisulfite bleaching method (*25*). Absorbance was measured at 420, 510, and 700 nm. The browning index was calculated as the absorbance at 420 nm of the undiluted bisulfite bleached samples. A Vis-723 UV−visible spectrophotometer (Shanghai Precise Instrument Corp. Ltd., Shanghai, China) and 1 cm path length disposable cells were used for all measurements.

Analysis of Total Polyphenols. For total polyphenol assay of the bayberry fruits, 10 fruits were randomly selected from 500 g of fruits. Two slices of flesh from each fruit were cut symmetrically and pooled, and 5 g was weighed. The samples were pasted with a mortar and pestle in 50 mL of 4% acetic acid in acetonitrile, and the final volume was made up to 100 mL with the same solution (*26*). Contents were shaken at 200 rpm for 1 h at 30 °C in a water bath shaker. The extract was centrifuged at 10000*g* for 10 min, and the supernatant was used for

analysis. The bayberry juice was used for total polyphenol assay without any pretreatment.

Total polyphenols were estimated colorimetrically using the modified Folin−Ciocalteu method reported by Sellappan et al. (*26*). An aliquot of 0.2 mL of supernatant (or juice) was added to 0.8 mL of water, 5 mL of 0.2 N Folin−Ciocalteu reagent, and 4 mL of saturated sodium carbonate solution (75 g/L) and mixed in a screw-cap test tube. The absorbance was measured at 765 nm with a Vis-723 UV−visible spectrophotometer after incubation for 2 h at room temperature. Quantification was based on the standard curve established with 10, 20, 30, 40, and 50 mg/100 mL of gallic acid, and the results were expressed as gallic acid equivalents (GAE) in milligrams per 100 g of FW. The results were the average of triplicate analyses.

Anthocyanin and Polyphenolic Sample Preparation for HPLC-DAD-ESIMS Analysis. For HPLC-DAD-ESIMS analysis, anthocyanin and polyphenolic purifications were performed as described by Lee et al. (*17*). The aqueous extract or the bayberry juice was passed through a C-18 Sep-Pak cartridge (Waters Associates, Milford, MA), which had been previously rinsed with methanol, and activated with deionized water.

Anthocyanins were adsorbed onto the cartridge while other flavonoids, sugars, and acids were removed by rinsing with excessive acidified water (0.01% HCl). Anthocyanins were collected with acidified methanol (0.01% HCl) and filtered through a 0.45 μm Millipore membrane filter (type HV, Millipore Corp., Bedford, MA) before injection.

The polyphenolic fraction was obtained by eluting 2 mL of ethyl acetate through the cartridge after the aqueous extract or juice was adsorbed. Ethyl acetate was removed with a rotovapor at 40 °C, and the residue was redissolved in 2 mL of methanol and filtered through a 0.45 μm Millipore membrane filter (type HA, Millipore Corp.) before injection. Duplicate injections were performed, and average peak areas were used for the measurement.

HPLC-DAD-ESIMS Analyses. HPLC-DAD-ESIMS analyses was done on a Waters platform ZMD 4000 system, composed of a Micromass ZMD mass spectrometer, a Waters 2690 HPLC, and a Waters 996 photodiode array detector (Waters Corp.). Data were collected and processed via a personal computer running MassLynx software version 3.1 (Micromass, a diversion of Waters Corp., Beverly, MA).

For anthocyanin analysis, 5 μL aliquots of purified bayberry extract were separated by an ODS-Hypersil column (250 × 4 mm, 5 μm particle size, Agilent, Palo Alto, CA). Solvent A was 5% formic acid and 95% water (v/v), and solvent B was 5% formic acid, 45% water, and 50% methanol (v/v/v). The elution profile consisted of a linear gradient from 0 to 100% B for 40 min, from 100% to 0% B for 10 min, and washing (100% methanol) and re-equilibration of the column for 10 min with the flow rate of 0.3 mL min^{-1}.

For the polyphenolic analysis, bayberry extract in a 10 μL aliquot was separated by a Purospher STAR C-18 column (250 × 4.6 mm, 5 μm particle size, Merck KGaA, Darmstadt, Germany). Solvent A was 0.1% formic acid in water (v/v), and solvent B was 80% acetonitrile in water (v/v). The elution profile consisted of a linear gradient from 0 to 66% B for 40 min, washing (100% methanol) for 5 min, and re-equilibration of the column for 10 min using solvent A with the flow rate of 1.0 mL min^{-1}.

UV−visible absorption spectra were recorded on-line during HPLC analysis. Spectral measurements were made over the range of 200−600 nm. Anthocyanins were detected at 520 nm, whereas phenolic acids were detected at 280 nm and flavonols at 360 nm.

Mass parameters were optimized by the compounds of cyanidin 3-glucoside for anthocyanins and quercitin 3-glucoside for polyphenolics, respectively. Mass spectra were achieved by electrospray ionization in positive mode (ES^+) for anthocyanins with the following ion optics: capillary, 3.87 kV; cone, 30 V; and extractor, 7 V. Mass spectra of polyphenolics were obtained in negative mode (ES^-) with the capillary at 3.88 kV, the cone at 25 V, and the extractor at 5 V. The source block temperature was 120 °C, and the desolvation temperature was 300 °C. The electrospray probe flow was adjusted to 70 mL min^{-1}. Continuous mass spectra were recorded over the range of *m*/*z* 100−800 with a scan time 1 s and an interscan delay of 0.1 s.

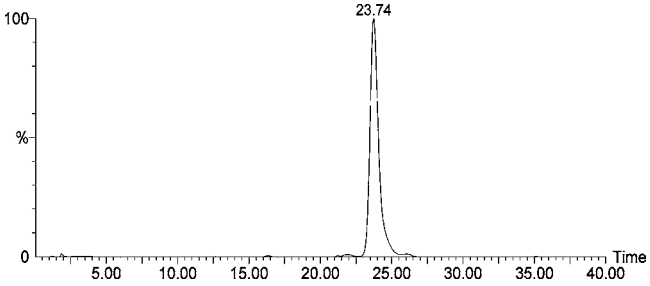

Figure 2. HPLC chromatogram of bayberry (*M. rubra* Sieb. et. Zucc.) fruit anthocyanin detected at 520 nm. The peak eluting at 23.74 min was identified as cyanidin 3-glucoside.

Identification and Quatification of Polyphenols. The anthocyanins and polyphenolics were identified by their UV−visible spectra and mass spectra and, when available, by chromatographic comparisons with standards. Contents of cyanidin 3-glucoside, gallic acid, protocatechuic acid, and quercitin were calculated with the regression equations from the standard curves. All flavonol glycosides were quantified as quercetin 3-glucoside because insufficient standards were available. Concentrations were expressed as milligrams per 100 g of FW or milligrams per 100 mL of juice. Repeatability of the analysis was ±5%.

Color Measurements. Color coordinates (*L*, *a*, *b*) measurements were made with an SC-80C colorimeter (Kanguan Instruments Co. Ltd., Beijing, China). The colorimeter was set to measure total transmittance with illuminant C and a 10° observation angle. Samples were placed in a 1 cm path length cell, and Hunter CIE *L* (lightness), *a* (redness), *b* (yellowness), and hue angle [h = arctan (b/a), color itself] were recorded. Data are reported as the means of three determinations.

Statistical Analysis. The significant differences among control and treatments were determined at the 95% level using the Tukey test of means. SPSS 10.0 was used as the statistic software.

Sensory Evaluation. The color and flavor of the juices after processing (final products) were immediately evaluated by a 11-member panel of Haitong Food Group Co. Ltd., all of whom were experienced in the sensory evaluation of fruit juices and wines. Overall color, clarity, and flavor were scored on a 1−5 hedonic scale. A score of 1 was represented very poor, 2 was poor, 3 was fair, 4 was good, and 5 was excellent for the characteristic evaluated. The data were subjected to two-way analysis of variance (ANOVA) to test the significance at the 5% level.

RESULTS AND DISCUSSION

Characterization of Bayberry Anthocyanins. The total monomeric anthocyanin content of Biqi bayberry was 125.2 mg/100 g of FW, determined by the pH differential method as cyanidin 3-glucoside. Compared with some other berries, the anthocyanin content in Biqi bayberry is comparable to that of blackberries, with 83−326 mg/100 g of berries (*27*), but is higher than those in 18 strawberry selections (14.8−41.8 mg/100 g) as reported by Wrolstad et al. (*28*). The aqueous extract had 2.58% polymeric color and a 0.32 browning index.

Very interestingly, the Biqi bayberry fruits showed only one major anthocyanin in the HPLC chromatogram detected at 520 nm (**Figure 2**). This peak eluted at 23.74 min under the analytical conditions and contained 97% of the total peak area. The mass spectrum of this compound showed a molecular ion (M^+) at *m*/*z* 449.2 and a fragment ion at *m*/*z* 287.1. Compared with the standard, this pigment was unambiguously identified as cyanidin 3-glucoside. The total anthocyanin content was 79.71 mg/100 g of FW as determined by the HPLC method, which was lower (63−65% of the pH differential method) than that of the pH differential method. Lee et al. (*17*) explained that the differences were from the different solvent systems and analytical methods. Furthermore, polymeric pigments might have also contributed to the pH differential results. The high purity of

Table 1. Changes in Total Monomeric Anthocyanin Content (ACN), Percent Polymeric Color (PC), and Browning Index (BI) during Bayberry Juice Processing[a]

	fruit			centrifuged cake			centrifuged juice			fined juice			filtered juice			UHT juice		
	ACN[b]	% PC	BI	ACN	% PC	BI	ACN	% PC	BI	ACN	% PC	BI	ACN	% PC	BI	ACN	% PC	BI
C[c]	125.2	3.58	0.32	291.8a			24.5a	60.65a	9.32a	22.1a	12.12a	2.12a	21.8a	10.23a	0.53a	20.1a	11.45a	0.58a
S				290.1a			37.9b	41.24b	5.41b	35.3b	8.13b	1.03b	33.6b	4.78b	0.28b	32.1b	4.92b	0.35b
P				260.6b			47.3c	41.66b	5.58b	44.0c	7.05b	1.05b	41.3c	4.58b	0.31b	41.3c	4.85b	0.38b
B				258.7b			50.5c	40.15b	5.48b	48.1c	7.09b	1.09b	46.5c	5.07b	0.40b	45.2c	5.39b	0.48b

[a] Different letters indicate significant difference ($p < 0.05$) in the same column. [b] Total monomeric anthocyanin content is expressed as cyanidin 3-glucoside and reported as mg/100 g of sample. [c] C, control; S, SO_2 addition; P, pasteurization after fruit crushing; B, blanching treatment.

cyanidin 3-glucoside in bayberry makes the following HPLC-DAD-ESIMS analysis for anthocyanins at every juice processing step unnecessary, because almost no other individual anthocyanins may exist or change.

Changes in Total Monomeric Anthocyanin Content during Juice Processing. The starting material of bayberry fruit had 125.2 mg of anthocyanin/100 g, determined by the pH differential method. After crushing, depectinization, and centrifugation (also pasteurization and blanching for the heat-treated samples), lots of anthocyanins were lost (**Table 1**), which agrees with the results of blueberry juice processing reported by Skrede et al. (*16*) and Lee et al. (*17*). The centrifuged juice yields ranged from 73 to 78% (w/w), but <31% (24.5−50.5 mg/100 g) of the bayberry anthocyanins were present in the centrifuged juice. The control juice had the lowest anthocyanin recovery, with significantly less anthocyanins than the SO_2-treated juice ($p < 0.05$). In this study, the two heat-treated (blanching and pasteurization) juices also had significantly more anthocyanins than the SO_2-treated juice ($p < 0.05$). The reason may be that the heat treatment not only inhibited the activity of PPO but also caused sample tissue breakdown and increased cell permeation, which led to more anthocyanins being extracted. The SO_2 treatment inhibited the PPO activity and reduced the anthocyanin degradation, but no damage was caused to the bayberry tissues and it cannot effectively increase the anthocyanin extraction. However, this result is not the case in the research of Lee et al. (*17*), who reported that there were no significant differences between SO_2 addition and heat treatment in anthocyanins recovery.

Some anthocyanins were probably deposited in the flocculation step, and ∼5−10% of anthocyanins were lost during the gelatin−bentonite fining operation. The gelatin−bentonite fining method is widely used in juice clarification to remove haze-active polyphenols and proteins (*29*−*31*). Anthocyanins, as one class of polyphenols, are also partially removed in this procedure. Bakker et al. (*32*) also reported considerable anthocyanin losses in the clarification step during strawberry juice processing. There were about 2−5 and 1−6% anthocyanin losses after diatomaceous earth filtration and ultrahigh-temperature (UHT) operation, respectively.

After UHT, the SO_2-, pasteurization-, and blanching-treated juices had 1.6−2.2 times the anthocyanin contents (32.1, 40.3, and 45.2 mg/100 g, respectively) of the control juice (20.1 mg/100 g), which indicated these treatments decreased the anthocyanin degradation or increased the anthocyanin extraction during processing. There were substantial anthocyanin losses (73−88%) in the processing procedures, considering the anthocyanins existed in the centrifuged cake; ∼21−32% of the initial bayberry anthocyanins degraded. Lee et al. reported 25−35% of anthocyanin degradation in blueberry juice processing (*17*), and Skrede et al. demonstrated that the degradation is mainly due to native enzymes present in the fruits and not due to the processing enzymes used (*16*).

It is noteworthy that the centrifuged cake residues contained a substantial amount of anthocyanins, 258.7−291.8 mg/100 g of cake, which corresponded to 64.7−73.0 mg/100 g of bayberry fruit (calculated as 25% cake yield) or 52−58% of the fresh fruit anthocyanins. Control centrifuged cake contained the highest amount of anthocyanins (291.8 mg/100 g of cake), whereas blanched cake had the lowest (258.7 mg/100 g of cake). As discussed earlier, heat treatment damaged the sample tissues and increased the anthocyanin extraction, but still a number of anthocyanins remained in the centrifuged cakes. The results agree with the study of Lee et al. (*17*), who demonstrated that the heat-treated presscakes of blueberry juice had the least anthocyanin and suggested that the actual anthocyanin content of the presscake could have been higher, because native or processing enzymes may have degraded anthocyanins prior to analysis. The high anthocyanin content in pressed cakes makes them rich resources of natural pigments and nutraceuticals (antioxidants).

Changes in Percent of Polymeric Color and Browning Index during Juice Processing. Percent of polymeric color is a measure of the pigments' resistance to bleaching by bisulfite and reflects the degree of anthocyanin polymerization, whereas browning index expresses the browning occurring in the products (*25*). Changes in percent of polymeric color and browning index had the same general trend during bayberry juice processing (**Table 1**). The fresh fruit had 3.58% polymeric color, and the browning index was 0.32. After crushing, depectinization, and centrifugation, juices were visually brown and very turbid. The centrifuged juices all had very high percentages of polymeric color (40.15−60.65%) and browning indices (5.48−9.32); the control juice had the highest, which was significantly higher than that of the SO_2- and heat-treated juices ($p < 0.05$). The polymeric color and browning index were mainly due to enzymatic depectinization. Very interestingly, the fining and filter steps reduced the percent of polymeric color and browning index significantly ($p < 0.01$). Most of the polymeric color (83−89%) and browning pigments (92−94%) were precipitated or settled in the gelatin−bentonite flocculation and removed during the fining and filter procedures. Rommel et al. (*33*) also observed polymeric color losses through fining during red raspberry juice and wine processing. The UHT operation slightly (2−8%) increased the polymeric color and browning values for the control and treated juices.

Changes in Color Measurements during Juice Processing. Hunter *L* and *b* values of all the trials increased during bayberry juice processing, especially in the fining steps (**Table 2**). During red raspberry juice and wine processing, fining combined with depectinization and pasteurization significantly increased Hunter *L* and *b* values (*33*). Pilando et al. (*34*) observed a high negative correlation of both values with total anthocyanins, and they

Table 2. Changes in Color Measurements during Bayberry Juice Processing

	centrifuged juice				fined juice				filtered juice				UHT juice			
	L	*a*	*b*	*h*	*L*	*a*	*b*	*h*	*L*	*a*	*b*	*h*	*L*	*a*	*b*	*h*
C[a]	22.04[b]	20.81	20.59	44.69	31.22	19.85	21.00	46.61	32.96	17.58	21.35	50.53	33.07	16.73	21.64	52.29
S	10.34	24.52	16.46	33.87	19.44	31.06	21.63	34.85	19.65	30.73	21.97	35.56	20.04	30.28	22.06	36.07
P	9.33	27.24	18.73	34.51	17.92	38.47	27.63	35.69	19.33	37.88	27.79	36.26	19.68	37.36	28.27	37.11
B	9.32	27.35	19.32	35.24	17.52	39.12	28.27	35.85	18.99	38.28	28.64	36.80	19.64	37.55	29.04	37.72

[a] C, control; S, SO_2 addition; P, pasteurization after fruit crushing; B, blanching treatment. [b] Except of Hunter *b* values, Hunter *L*, *a*, and *h* values of the control juice are significantly higher or lower ($p < 0.05$) than the SO_2, pasteurization, and blanching treated juices within the same column.

suggested that the increased values of Hunter *L* and *b* resulted from anthocyanin loss. In our study, a reversed correlation ($R^2 = 0.905$ and 0.893 for Hunter *L* and *b*) of both values with total anthocyanin also occurred (see **Tables 1** and **2**). **Table 2** also showed that the SO_2- and heat-treated samples had significantly ($p < 0.05$) lower *L* values (darker) than the control juice. Although the heat-treated juices were darker than the SO_2-treated juices, no significant differences existed between them for *L* values. The hue angles (*h*) increased slowly for all samples during juice processing, which makes the sample color change from red to relatively more yellow. The overall color of the heat- and SO_2-treated samples was visually redder than that of the control juices.

Hunter *a* values had a change pattern almost opposite to those of Hunter *L* and *b* values and hue angles. Hunter *a* values decreased throughout the bayberry juice processing, except the fining step increased *a* values of the treated juices (**Table 2**). The Hunter *a* value had a positive correlation ($R^2 = 0.914$) to total anthocyanin content, which implied that the *a* value is mainly due to the total anthocyanin content. As expected, the control juice had a significantly lower ($p < 0.05$) *a* value than those of the treated juices. The reason the centrifuged juices of the SO_2- and heat-treated samples had relatively higher anthocyanin contents but lower *a* values than those of the fined juices is not very clear. We assumed that the very turbidly centrifuged juices disturbed the analysis of the colorimeter because the instrument will only be effective in determination when the liquid samples are relatively transparent (introduction of the SC-80C colorimeter, Kanguan Instruments Co. Ltd., Beijing, China). The results suggested that the clarity of the liquid samples interferes the color measurements.

Characterization of Bayberry Polyphenolics. From a comparison of their UV−visible spectra, mass spectra, and HPLC retention times with those of the available standards, polyphenolics in the ethyl acetate fraction of the Biqi bayberry fruit and juices can be classified into two groups: one is hydroxybenzoic acids, and the other is flavonols (**Figure 3** and **Table 3**), which is in accordance with the results of Fang et al. (*10*).

In the fruit, hydroxybenzoic acids including gallic acid (8.6 mg/100 g) and protocatechuic acid (4.2 mg/100 g) were identified. Flavonol glycosides in bayberry were identified or tentatively identified as myricetin deoxyhexoside, quercetin 3-glucoside, quercetin hexoside-gallate, kaempferol hexoside, quercetin deoxyhexoside, and myricetin deoxyhexoside-gallate (total = 159.6 mg/100 g), respectively (**Figure 3** and **Table 3**). No free flavonol aglycons were detected. The flavonol analyses were in good agreement with the research of Amakura et al. (*12*), who reported that myricetin, quercetin, and kaempferol are present in an unknown cultivar bayberry fruit, and suggested that most flavonols in plants exist as glycoside forms. Myricetin and quercetin 3-*O*-rutinosides were recently reported in the bayberry acidic methanol extractions (*8*). Amakura et al. (*35*) detected ellagic acid in bayberry fruits, but this was not found in the present study materials.

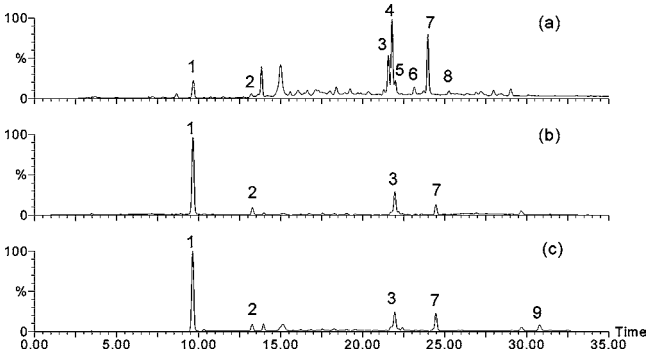

Figure 3. HPLC chromatogram of the ethyl acetate fraction from *Biqi* bayberry fruit and centrifuged juices detected at 280 nm: (**a**) bayberry fruit; (**b**) bayberry juice, control; (**c**) bayberry juice, blanching. Peak numbers refer to **Table 3**.

Table 3. Identification of Phenolic Compounds in Ethyl Acetate Fractions from Bayberry Fruits and Juices by Using Their Spectral Characteristics, Negative Ions in HPLC-DAD-ESIMS, and Respective Standards

peak	HPLC t_R (min)	HPLC-DAD (nm)	mol wt	HPLC-ESIMS (*m*/*z*)	tentative identification
1	9.68	219, 269	170	169	gallic acid (std[a])
2	13.24	257, 291	154	153	protocatechuic acid (std)
3	21.56	261, 300sh,[b] 349	464	463, 317	myricetin deoxyhexoside
4	21.78	254, 300sh, 354	464	463, 301	quercetin 3-glucoside (std)
5	21.99	265, 354	616	616, 463, 301	quercetin hexoside-gallate
6	23.14	265, 290sh, 346	448	447, 285	kaempferol hexoside
7	23.98	255, 264sh, 348	448	447, 301	quercetin deoxyhexoside
8	25.26	265, 295sh, 349	616	616, 463, 317	myricetin deoxyhexoside-gallate
9	30.78	255, 300sh, 371	302	301	quercetin (std)

[a] Identified with standard compounds. [b] Shoulder peaks in the UV spectra.

The total polyphenolics in Biqi bayberry was 172.4 mg/100 g (HPLC method), which was >10 times higher than those of the results of Fang et al. (*10*), although the bayberries were from the same orchard. In the present study, the researchers used fresh bayberry produced in 2005, which was very dry during the fruit-growing season. In our previous study, the fruits produced in 2004 had been stored frozen (−20 °C) for 6 months before analysis (*10*). It is well-known that polyphenolic contents in plants may differ in different growing seasons and that phenolic compounds may degrade even at very low temperature storage. However, the total phenolic content in Biqi bayberry was 360.3 mg/100 g as determined by using the Folin−Ciocalteu method, which was in good agreement with the results of Fang et al. (*10*). The substantial differences from the two analytical methods are mainly due to the different extraction methods. In the HPLC method, anthocyanins were not calculated into the contents of total phenolics. Furthermore, the Folin−Ciocalteu method tends

Table 4. Changes of Polyphenolics (Milligrams per 100 g of Fresh Weight) in Bayberry Fruit and Juices (Values Are Averages of Duplicate Analyses) during Processing

		centrifuged cake				centrifuged juice				fined juice				filtered juice				UHT juice			
	fruit	C[a]	S	P	B	C	S	P	B	C	S	P	B	C	S	P	B	C	S	P	B
hydroxybenzoic acids	12.8	17.9	16.8	15.9	15.4	19.2[b]	20.8	31.6	32.4	16.5	17.6	26.7	28.1	15.2	15.8	24.8	25.9	14.5	15.3	22.3	22.7
total flavonols[c]	159.6	223.4	217.7	193.6	191.4	40.6	46.7	64.7	66.2	33.2	35.7	55.5	56.4	33.1	36.5	53.4	54.6	32.0	35.1	49.1	49.7
total phenolics[d]	172.4	241.3	235.6	209.5	206.8	59.8	67.5	96.3	98.6	49.7	53.3	82.2	85.5	48.3	52.3	78.2	80.5	46.5	50.4	71.4	73.4
total phenolics[e]	360.3	403.5	397.2	378.3	365.8	101.3	112.4	160.2	165.7	88.3	96.2	140.1	148.3	80.5	87.4	132.3	139.7	76.9	82.6	120.8	125.6

[a] C, control; S, SO_2 addition; P, pasteurization after fruit crushing; B, blanching treatment. [b] Values of every index of the control and SO_2-treated juices are significantly lower ($p < 0.05$) than those of pasteurization- and blanching-treated juices within the same processing unit. [c] Total flavonols include flavonols and flavonol glycosides. [d] Sum of the individual polyphenolics in ethyl acetate fractions and determined by the HPLC-DAD method. [e] Estimated by the Folin-Ciocalteu method.

to overestimate total phenol contents due to interference of reducing substances (*36*).

Changes in Polyphenolics during Juice Processing. For convenience, changes of the individual phenolic compounds were calculated as hydroxybenzoic acids and total flavonols during juice processing (**Table 4**). The starting material of fresh bayberry had 172.4 mg/100 g of polyphenolics. Amounts of 57−74% of the berry polyphenolics lost during crushing, depectinization, and centrifugation (also pasteurization and blanching for the heat-treated samples). The phenolic contents of samples taken at each step of pasteurization and blanching treatments were significantly higher than the control and SO_2-treated samples ($p < 0.05$), but this was not the case in blueberry juice processing (*17*). Lee et al. (*17*) reported that 60−65% of the blueberry phenolic compounds were lost during the initial steps of processing, but no significant differences were observed between the heat and SO_2 treatments and the control. Compared to heat treatments, SO_2 treatment was not very effective in retarding polyphenolic degradation, although the polyphenolic contents in SO_2-treated samples were higher than that of the control (**Table 4**). A possible explanation is that the SO_2 dosage was relative low (free $SO_2 = 50$ mg/L in this study) and the PPO activity was not efficiently inhibited. In other studies, 100 mg/L SO_2 was used in blueberry juice processing (*17*) and 140 mg/L SO_2 was used in strawberry juice processing (*37*). When the SO_2 dosage was increased to 100 mg/L, SO_2 residuals in the final bayberry juices reached 13.7 mg/L (data not shown), which exceeds the state standard of 10 mg/L (GB2760-1996, China National Standard, 1996). Pasteurization after fruit crushing and blanching treatment effectively inhibited the native PPO activity, but 57−58% of the polyphenolics were also lost in these two treated and centrifuged juices. The centrifuged cakes held 30−35% of the fresh berry polyphenolics, which is less than the proportion of anthocyanins (52−58%) left in the centrifuged cakes but higher than that in the blueberry presscakes (15−20%) (*16*). Amounts of 13−17% polyphenolics were lost through fining processing, and 3−9% polyphenolics were lost during diatomaceous earth filter operation. Amounts of 20−32% of total polyphenolics remained in the UHT juices as calculated by the HPLC method, but only 16−26% of total polyphenolics remained as deteremined by using the Folin−Ciocalteu method.

Although there was only 12.8 mg/100 g of hydroxybenzoic acids in Biqi bayberry fruit, after the primary steps of processing (crushing, depectinization, centrifugation, and also pasteurization and blanching for the heat-treated samples), substantial hydroxybenzoic acids (50−150%) were increased in the centrifuged juices (**Table 4**). The HPLC-DAD-ESIMS analysis revealed that probably myricetin deoxyhexoside-gallate and quercetin hexoside-gallate existed in the fruit (**Figure 3** and **Table 3**). After the initial processing steps, the peaks of these two flavonol glycoside gallates disappeared from the HPLC chromatogram, and the peak height and areas of the gallic acid increased significantly (**Figure 3**), which implied that gallic acid was freed from the flavonol glycoside gallates and caused the content of the total hydroxybenzoic acids to increase. Correspondingly, this phenomenon confirmed the existence of flavonol glycoside gallates in bayberry fruit. Compared to the centrifuged juices, fining (12−15%), filtering (7−9%), and UHT (5−12%) also caused the content of total hydroxybenzoic acids to decrease, although their levels were still higher than those of the starting material of bayberry fruit (**Table 4**). The contents of hydroxybenzoic acids in pasteurization- and blanching-treated juices were significantly higher ($p < 0.05$) than the control and SO_2-treated samples within the same operation unit.

Table 5. Sensory Evaluation Scores of Differently Treated Juices[a]

	overall color	flavor	clarity
C[b]	3.0a	3.75a	3.57
S	3.77b	3.65a	3.60
P	3.92b	3.52a	3.62
B	3.87b	3.08b	3.59

[a] Different letters indicate significant difference ($p < 0.05$) in the same column. [b] C, control; S, SO_2 addition; P, pasteurization after fruit crushing; B, blanching treatment.

There were four flavonol glycosides and two flavonol glycoside-gallates identified or tentatively identified in bayberry fruit (**Figure 3** and **Table 3**), and the total flavonols were 159.6 mg/100 g of berry (**Table 4**). After the initial processing steps, striking changes were observed in the HPLC chromatogram with only myricetin deoxyhexoside and quercetin deoxyhexoside remaining in the centrifuged juices (**Figure 3**), which implied that flavonol deoxyhexosides were more stable than the flavonol hexosides or flavonol glycoside-gallates during bayberry juice processing. The appearance of quercetin in blanching-treated juice indicated that flavonol glycosides were hydrolyzed through the operation of blanching, crushing, depectinization, and centrifugation. Amakura et al. (*12*) reported that myricetin, quercetin, and kaempferol are presented in an unknown cultivar bayberry jam, but myricetin and kaempferol were not found in the studied bayberry juices. Even quercetin was not detected in the control juice, suggesting that more severe degradation occurred during the control juice processing. Amounts of 19−31% of the fresh bayberries total flavonols were extracted into the centrifuged juices, which were relatively lower than the pressed blueberry juice (38−48%) (*17*). The following processing steps of fining (15−18%), filtering (∼3%), and UHT (3−9%) also caused the total flavonols to decrease (**Table 4**).

Sensory Evaluation. Analysis of variance of the sensory data (**Table 5**) showed that the score of the overall color of the SO_2- and heat-treated juices was significantly higher than that of the control juice, which indicated the treated samples are more

attractive in overall color appearance. As previously described, the pasteurization- and blanching-treated juices had no statistical differences in the above analytical indexes, but the flavor score of the blanching-treated juice was significantly different from those of the other three juices (**Table 5**), which had a light cooked flavor and in which the special bayberry flavor was weakest. The blanching treatment was carried out on hot water blanching equipment at 90 °C for 2 min. Aromatic materials may have been volatilized or flavor materials lost in the hot water. Pasteurization of the crushed pulp in an airtight tubular heater at 90 °C for 1 min effectively reduced the aromatic and flavor materials loss, so the flavor score of the pasteurization-treated juice was not significantly different from that of the non-heat-treated juices (juices C and S, **Table 5**), which indicated that, with a view to the juice flavor, pasteurization of the crushed pulp was superior to blanching treatment in bayberry juice processing. The scores of the clarity of the four trial juices had no significant differences ($p > 0.05$) among them.

The results of this investigation indicated that, among the four pretreatments studied, pasteurization of the crushed pulp was the best in improving the bayberry juice color quality and provided a natural bayberry flavor, although blanching treatment and SO_2 addition were also beneficial to the juice color quality.

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NOTE ADDED AFTER ASAP PUBLICATION

The original posting of December 14, 2005, contained an error in the caption to Figure 3. This has been corrected as of December 14, 2005.

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