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Chemical Composition of Clarified Bayberry (*Myrica rubra* Sieb. et Zucc.) Juice Sediment

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Clarified bayberry juice turned hazy upon storage at 25 °C for 6 months, and the chemical composition of centrifugally separated sediment was analyzed. Bayberry juice haze was mainly protein−tannin haze. The lyophilized sediment contained 20.4 ± 4.3% of protein, 70.2 ± 2.6% of total polyphenols, 7.2% of monosaccharides, and 6.7 ± 0.6% of ash. Amino acid analyses and molecular weight distribution estimation indicated that bayberry proteins were haze-active proteins with a molecular weight less than 8 kDa. Gallic acid, quercetin hexoside, quercetin deoxyhexoside, and quercetin were found in the methanol-dissolved sample, while gallic acid, protocatechuic acid, cyanidin, ellagic acid, and quercetin were detected in the acid-hydrolyzed sample. Ellagic acid was the dominant individual phenolic (9.9 ± 0.19 g/100 g dry weight, 55.3% of the total amount) in the sediment. Monosaccharides of rhamnose, arabinose, mannose, glucose, and galactose in the sediment were most probably the glycoside moieties of the anthocyanins, flavonols, and elllagitannins. Metal ions of calcium, magnesium, potassium, iron, and copper also indicated the heterogeneous characteristics of the sediment.

KEYWORDS: Bayberry juice; ellagic acid; protein−tannin haze; sediment

INTRODUCTION

Bayberry (*Myrica rubra* Sieb. et Zucc.) is a fascinating berry fruit because of its unique sweet sour taste and exquisite flavor. The fruits of most cultivars ripen in the hot and rainy season of May to July and can only be stored fresh with an attractive dark red color for 3 days at 20−22 °C and 9−12 days at 0−2 °C (*1*). Bayberry fruits often are processed into juice and juice concentrate to allow a longer consumption time. However, during storage and commercial circulation, haze and sediment are readily formed in the clarified juice, which is considered a quality defect and limits the utilization and consumption of the product.

Chemical composition of post-bottling haze and sediment in fruit juices was first reported about a century ago (*2*). In 1908, Von Kelhofer reported that the sediment in fermented pear juice contained protein, pectin, and oxidized tannins (*2*). Further, subsequent works have indicated that clarified fruit juice sediments are heterogeneous complexes of protein, polyphenols, cell wall fragments, nucleii, and other cellular constituents (*3*−*8*). Ellagic acid has been observed in almost every sediment from berry juices or wines and is believed to result from ellagitannin hydrolysis (*9*−*14*). Anthocyanins are also present in sediments from blackberry juices (*14*) and red wine (*15*).

There are generally four types of chemical hazes in fruit juices: starch, gums and cell wall materials, protein−polyphenol (or protein−tannin), and miscellaneous haze particles (filter media such as bentonite and diatomaceous earth) (*16*). The protein−polyphenol haze formation is significantly affected by the ratio of haze-active protein to haze-active polyphenol, and the largest amount occurs when the numbers of polyphenol binding ends and protein binding sites are nearly equal (*17*−*19*). The interactions between proteins and polyphenols appear to be mainly hydrophobic with some contribution from hydrogen bonding (*7*, *19*, *20*). Other investigations suggested that interactions between polyphenols and proline-containing peptides might involve formation of π-bonded complexes in which the rings of the two compounds overlap (*21*, *22*). In addition, the polyphenols can polymerize and grow large enough to produce haze and sediment without protein intervention (*17*).

To our knowledge, no literature has been reported on the formation and composition of haze and sediment in bayberry juices. The objective of the present work was to determine the chemical composition and identify the precursors of the sediment, which may lead to industrial processes for remediation or prevention of this quality defect in bayberry juices.

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MATERIALS AND METHODS

Chemicals and Solvents. Pure standards of gallic acid, protocatechuic acid, ellagic acid, quercetin, quercetin 3-glucoside, and cyanidin 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, methanol, and trifluoroacetic acid (HPLC grade), acetic acid, dimethyl formamide, and hydrochloric acid (analytical grade) were purchased from Shanghai Chemical Reagent Co., Shanghai, China. All solution preparations were made using distilled−deionized water.

Bayberry Juice. Bayberry juice was processed in the factory of Haitong Food Group Corp. according to the procedure described by Fang et al. (*23*). Briefly, the fresh bayberries of *Biqi* cultivar were crushed with a horizontal crusher and pasteurized at 90 °C for 1 min in a tubular heater to inhibit the polyphenoloxidase activity. The pulp was cooled to 35 °C for depectinization and then centrifuged with a decanter centrifuge. After being fined with 0.2 g/L of gelatin and 0.2 g/L of bentonite, the juice was filtered through a diatomaceous earth filter. Subsequently, the clarified juice was sterilized at 120 °C for 3 s with an ultrahigh-temperature unit and packaged in high-density polyethylene containers. The bayberry juice was stored at 25 °C for 6 months until analysis.

Tannin and Protein−Tannin Haze Test. To identify whether the haze was of tannin and protein−tannin type, we followed the method of Van Buren (*7*). The procedure was as follows: 3 vol of dimethyl formamide was added to 1 vol of hazy juice, the solution was mixed well, and the clarity of the mixture was observed after 5 min. A control was prepared by adding 3 vol of distilled water to 1 vol of the hazy juice. The treated sample was compared with the control. Hazes consisting largely of tannins or protein−tannin complexes greatly diminish when suspended in the 75% dimethyl formamide, while starch, dextrin, microbiological, and inorganic hazes should be unaffected under this condition (*7*).

Sediment Preparation. The bayberry juice stored for 6 months was centrifuged (26000*g*, 30 min, 4 °C) to separate the juice and sediment. The sediment was then thoroughly mixed with deionized water and centrifuged (10000*g*, 10 min, 4 °C) again. The supernatant was discarded. This step was repeated until the supernatant solution contained no color or no further visual dilution in color was observed (*14*). Then the washed sediment was lyophilized, weighed, and kept in a tight desiccator until analysis.

Protein and Amino Acid Analyses. Total protein of the juice sediment was estimated using the Kjeldahl nitrogen analyses of the AOAC method 928.08 (*24*).

For amino acid analyses, 50 mg of dried sediments was hydrolyzed with 10 mL of 6 M HCl at 110 C for 24 h. The amino acid composition was determined on a HP1100 HPLC system (Agilent Corp., USA) equipped with an ODS-Hypersil C-18 column (250 × 4 mm, 5 μm particle size, Agilent Corp., USA), following the method described by Cohe et al. (*25*).

Analysis of Total Polyphenols. Fifteen milligrams of sediment were dissolved in 100 mL of 4% acetic acid in acetonitrile, and the solution was shaken at 200 rpm for 1 h at 30 °C in a water bath shaker (*26*). The extract was centrifuged at 10000*g* for 10 min, and the supernatant was used for total polyphenol analysis.

Total polyphenols were estimated colorimetrically using the modified Folin−Ciocalteu method as described previously (*23*). The results were the average of triplicate analyses and were expressed as gallic acid equivalents (GAE) in g/100 g of dry weight (g/100 g dw).

Sample Preparation for HPLC−DAD−ESIMS Analyses. HPLC−DAD−ESIMS analyses were applied to determine the individual phenolic compounds in the sediment. Samples were prepared in two methods: (1) Methanol dissolution: fifteen milligrams of sediment was dissolved in 15 mL of methanol, and the sample was sonicated with an ultrasonic crusher (model JY98-3D, Xingzhi Biotech Corp. Ningbo, China) for 5 min; (2) acid hydrolysis: fifteen milliliters of 2 M HCl were added to 1 mg of lyophilized sediment in a screw-cap test tube, and the sample was flushed with nitrogen and capped (*27*). The sediment was hydrolyzed for 45 min at 100 °C and then cooled in an ice bath. The hydrolysate was purified by using a C-18 Sep-Pak cartridge (Waters Associates, Milford, MA) and redissolved in methanol. The methanol-dissolved and the acid-hydrolyzed solutions were filtered through 0.45 μm Millipore membrane filters (type HV, Millipore Corp., Bedford, MA) before being injected onto the HPLD−DAD−ESIMS system.

HPLC−DAD−ESIMS Analyses. HPLC−DAD−ESIMS analyses were performed 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., Milford, MA). Data were collected and processed via a personal computer running MassLynx software version 4.0 (Micromass, a diversion of Waters Corp., Beverly, MA).

The methanol-dissolved and acid-hydrolyzed solutions were separated by a SunFire C-18 column (150 × 2.1 mm, 5 μm, Waters Corp. USA). Solvent A was 1% acetic acid and 99% water (v/v), and solvent B was 100% methanol. The elution profile consisted of a linear gradient from 20 to 100% B for 30 min, washing, and re-equilibration of the column for 5 min with a flow rate of 0.3 mL min^{-1}. An aliquot of 10 μL of methanol-dissolved solution and 5 μL of hydrolyzed solution was injected onto the HPLC system.

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, flavonols and ellagic acid were detected at 360 nm, whereas phenolic acids were detected at 280 nm.

The compound of ellagic acid was used for mass spectra parameters optimization. Mass spectra were achieved by electrospray ionization in positive mode (ES^+) for anthocyanins and negative mode (ES^-) for other phenolics in the different channels. The following ion optics were used: capillary, 4.02 kV; cone, 38 V; and extractor, 5 V. The source block temperature was 100 °C, and the desolvation temperature was 250 °C. The electrospray probe flow was adjusted to 70 mL min^{-1}. Continuous mass spectra were recorded over the range of *m*/*z* 150−950 with a scan time of 1 s and an interscan delay of 0.1 s.

Identification and Quatification of Polyphenols. The phenolic compounds were identified by their UV−visible spectra and mass spectra, and when available, by chromatographic comparison with standards. Contents of gallic acid, protocatechuic acid, cyanidin, ellagic 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 g/100 g dw. Repeatability of the analysis was ±5%.

Monosaccharide Analyses. Fifty milligrams of sediment was hydrolyzed with 5 mL of 2 M trifluoroacetic acid at 120 °C for 75 min (*28*). The alditol acetate derivatives (*29*) of the hydrolyzed monosaccharides were determined on a Shimadzu 14A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a DB-1701 quartz capillary column (30 m × 0.53 mm, 1.0 μm; Agilent Corp. USA). The injection volume was 1 μL, and the split ratio was 30:1. The flow rates of the carrier gas (N_2), flame gas (H_2), combustion-supporting gas (air), and make up gas (N_2) were 3, 45, 400, and 30 mL/min, respectively. The temperatures of the injector and the detector were 260 °C. The oven was held at 185 °C for 3 min, programmed from 185−240 °C at 3 °C/min, and kept at the ultimate temperature for 20 min.

Rhamnose, arabinose, xylose, mannose, glucose, and galactose were used as external standard monosaccharides, and inositol was added as internal standard.

Ash and Mineral Analyses. Sediment ash was analyzed by using the AOAC method 938.08 (*24*). Ashed samples were dissolved in 2 mL of concentrated acid (HCl/HNO_3; 1:1), then diluted with distilled water. The diluted solution was analyzed for calcium, magnesium, potassium, iron, and copper contents with a Varian SpectrAA220 atomic absorption spectrophotometer (Varian Corp., USA).

Molecular Weight Estimation. To estimate the molecular weight (MW) of protein, 25 mg of sediment was dissolved in 4 mL of 0.1 M NaOH (*4*, *30*). The solution was adjusted to pH 7.0 with concentrated HCl and centrifuged to remove insoluble materials. The sample was

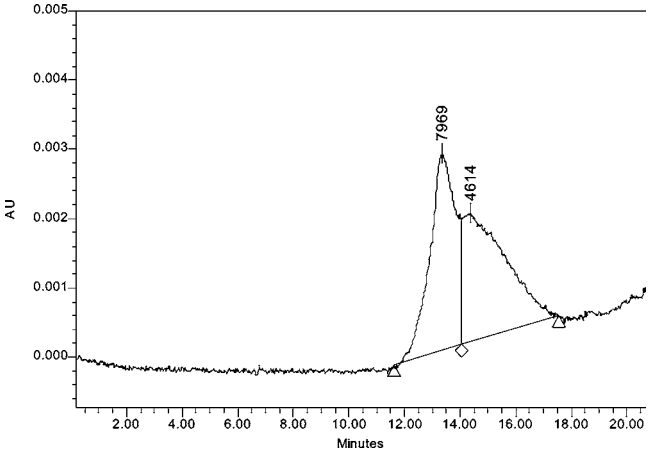

Figure 1. Molecular weight distribution of proteins of bayberry juice sediment dissolved in 0.1 M NaOH solution. Proteins with a MW of 7969 and 4614 were 49 and 51% of the total protein peak areas, respectively.

then analyzed by a Waters 600 HPLC (Waters Corp. USA) equipped with a TSKgel G2000 SWXL column (300 × 7.8 mm, Tosoh Corp. Japan). The mobile phase was acetonitrile/water/trifluoroacetic acid (45:55:0.1, v/v/v) with a flow rate of 0.5 mL/min. The UV detector was selected at 220 nm, and the column temperature was 30 °C. The MWs were estimated from a calibration equation obtained from standard peptides or proteins of glycine−glycine−glycine (MW 189), glycine−glycine−tyrosine−arginine (MW 451), bacteriocin (MW 1450), insulin (MW 5800), and cytochrome C (MW 12500).

To estimate the MW of carbohydrate, 25 mg of sediment was resuspended in 0.1 M $NaNO_3$ solution and incubated at 50 °C for 1 h (*31*). The solution was centrifuged and then analyzed by the Waters 600 HPLC. The column was Ultrahydrogel linear (300 × 7.8 mm × 2, Waters Corp. Japan), and the mobile phase was 0.1 M $NaNO_3$ with a flow rate of 0.9 mL/min. The elution was monitored using a 2410 differential refractometer, and the column temperature was 45 °C. The MWs were estimated from a calibration equation obtained from standard carbohydrates of maltopentaose (MW 828), dextran T-5 (MW 4600), dextran T-10 (MW 10000), dextran T-70 (MW 70000), and dextran T-190 (MW 188000).

RESULTS AND DISCUSSION

Characterization of Sediment. After being stored at 25 °C for 6 months, bayberry juice was visually very hazy, and sediment was deposited at the bottom of almost every container. The tannin and protein−tannin tests indicated that the juice consisted largely of tannins or protein−tannin complexes. The centrifugally separated sediment was thoroughly washed with deionized water to eliminate all water-soluble components. From visual observations, the color of the lyophilized sediment was dark red, similar to the blackberry juice sediment of purple black (*14*). The sediment was found to be 119 ± 14 mg/L of starting of hazy juice, which was higher than the values of 0.3−1.7 mg/L (*32*) and 9−70 mg/L (*4*) in apple juices, and of 100 mg/Kg in blackberry juice (*14*).

Protein and Amino Acid in Sediment. The nitrogen analyses estimated 20.4 ± 4.3% of protein present in the lyophilized sediment, which was in the range values for apple juice haze of 11.4−29.0% (% N × 6.25) (*32*) and 7.2−31.9% (% N × 6.25) (*4*) but higher than the values of of 6.69 ± 2.21% in blackberry juice sediment (*14*).

In our preliminary study, we performed SDS−PAGE analysis to estimate the MWs of the sediment proteins, but no protein band appeared in the MW range of 9250−66200; thus, the protein or peptide MWs ranging from 189 to 12500 were used in the MW distribution analysis. **Figure 1** showed that the two biggest MW proteins in bayberry juice sediment were 7969 and 4614 Da, respectively. These two proteins accounted for 49 and 51% of the total protein peak areas. The results suggested that the MWs of haze-forming proteins in bayberry juice were quite small, and maybe they were peptides. However, SDS−PAGE analyses of oxidized apple juice estimated that the MWs of sediment-forming proteins range from less than 14 to greater than 116 kDa (*30*), and Hsu et al. determined that proteins in the range of 21−31 kDa were related to haze formation in apple juice stored for 3 months (*33*). The results indicated that the MWs of haze-forming proteins originating from different materials had substantial variabilities.

Table 1. Amino Acid Composition in Bayberry Juice Sediment

amino acid	amount (g/100 g)	relative amount (%)
aspartic acid	0.2 ± 0.02[a]	1.4
glutamic acid	2.1 ± 0.01	14.7
serine	1.2 ± 0.02	8.4
histidine	0.1 ± 0.01	0.7
glycine	1.4 ± 0.01	9.8
threonine	0.6 ± 0.02	4.2
alanine	1.4 ± 0.03	9.8
arginine	0.5 ± 0.01	3.5
tyrosine	1.0 ± 0.03	6.9
cystine–s	0.9 ± 0.02	6.3
valine	0.8 ± 0.02	5.6
methionine	0.1 ± 0.01	0.7
phenylalanine	0.8 ± 0.02	5.6
isoleucine	0.8 ± 0.01	5.6
leucine	1.5 ± 0.03	10.5
lysine	0.6 ± 0.01	4.2
proline	0.3 ± 0.01	2.1
total	**14.3**	**100**

[a] Data are expressed as means ± standard deviations ($n = 3$).

Amino acid composition of bayberry juice sediment is shown in **Table 1**. Glutamic acid, alanine, and leucine were the major amino acids in the sediment, which were also the major ones in the bayberry fruits (data not shown; see Supporting Information), implying that the sediment proteins originated from the fruits.

Model system results showed that proline was apparently required for peptide to demonstrate haze-forming activity (*19*, *20*) and a significant amount of proline (ranging from 4.6 to 15.9%) was found in apple juice sediment (*4*). However, the proline concentration was only 0.3 ± 0.01 g/100 g in the bayberry juice sediment, accounting for only 2.1% of the total amino acids (**Table 1**). Although the relative amount of proline was quite low, 20.4 ± 4.3% of protein in the sediment indicated the bayberry juice proteins were active proteins in haze formation. This phenomenon could be explained by the fact that protein with proline but not free amino acids have the binding activity with polyphenol molecules in haze formation (*18*, *19*). The glycine content was relatively high (9.8%) in the sediment, which is also active in reacting with polyphenols (*34*).

Phenolic Compounds in Sediment. The content of total polyphenols estimated by the modified Folin−Ciocalteu method was 70.2 ± 2.6 g/100 g dw, which was in the range of leucoanthocyanin contents (45.7−75.8%) in apple juice sediment (*32*). Individual phenolic compounds in bayberry juice sediment analyzed by the HPLC−DAD−ESIMS method are shown in **Figure 2**, **Table 2**, and **Table 3**. Gallic acid (1.1 ± 0.06 g/100 g dw), quercetin hexoside (most probably galactoside because it eluted earlier than the standard of quercetin 3-glucoside, 1.2 ± 0.08 g/100 g dw), quercetin deoxyhexoside (most probably rhamnoside, 1.2 ± 0.15 g/100 g dw), and quercetin (0.3 ±

Table 2. Identification of Phenolic Compounds in the Sediments from Bayberry Juice by Using Their Spectral Characteristics, Positive Ions, Negative Ions in Hplc–DAD–ESIMS, and Respective Standards[a]

peaks no.	HPLC t_R (min)	HPLC–DAD (nm)	molecular weight	HPLC–ESIMS (*m/z*)	tentative identification
1	1.99	221, 269	170	169	gallic acid (std[b])
2	2.98	259, 293	154	153	protocatechuic acid (std)
3	6.99	269, 518	287	287	cyanidin (std)
4	11.45	255, 355	464	463, 301	quercetin hexoside
5	11.98	252, 364	302	301	ellagic acid (std)
6	13.25	255, 349	448	447, 301	quercetin deoxyhexoside
7	15.83	255, 371	302	301	quercetin (std)

[a] Positive ions were only used for anthocyanin (peak 3) identification. [b] std: identified with standard compounds.

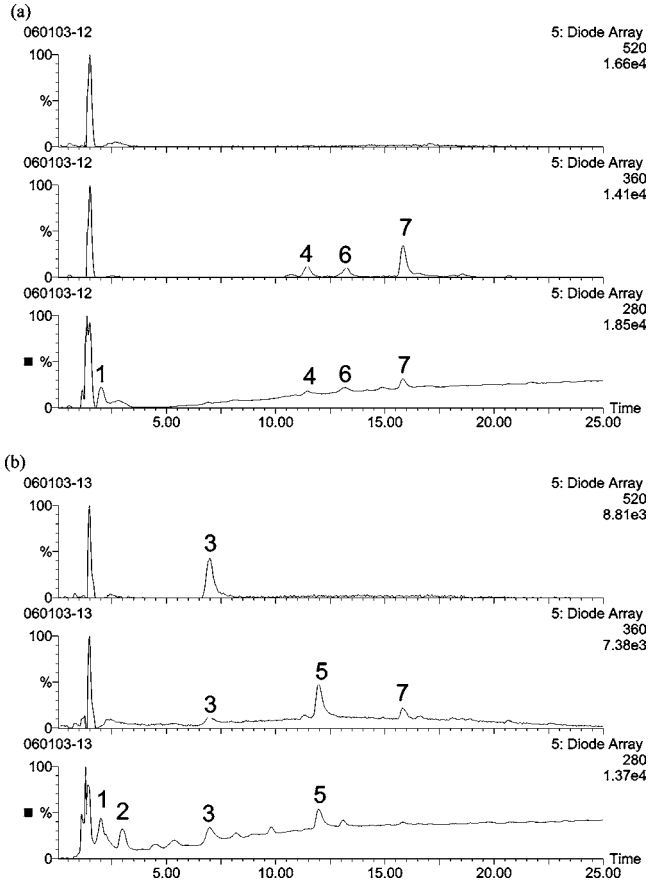

Figure 2. HPLC chromatogram of the phenolic compounds in the bayberry juice sediments detected at 520, 360, and 280 nm. (a) Methanol-dissolved sample; injection volume was 10 μL; (b) acid-hydrolyzed sample; injection volume was 5 μL. Peak numbers refer to **Table 2**.

0.05 g/100 g dw) were present in the methanol-dissolved sample (total of 3.8 g/100 g dw), while gallic acid (2.1 ± 0.05 g/100 g dw), protocatechuic acid (1.2 ± 0.07 g/100 g dw), cyanidin (2.9 ± 0.07 g/100 g dw), ellagic acid (9.9 ± 0.19 g/100 g dw), and quercetin (1.8 ± 0.11 g/100 g dw) were identified in the acid-hydrolyzed sample. The sum of the identified individual phenolics in the acid-hydrolyzed sample was 17.9 g/100 g dw, accounting for 25.5% of the total polyphenols of the Folin–Ciocalteu method. The fact suggested that most of the phenolics in the sediment may consist of complicatedly conjugated forms of polyphenols or unidentified compounds. Similar results were obtained in the sediments of blackberry juice (10.8% of identified individual phenolics) (*14*) and muscadine juice and wine (<12% of identified individual phenolics) (*13*).

In our previous study, gallic acid, protocatechuic acid, quercetin hexoside, quercetin deoxyhexoside, quercetin, and cyanidin 3-glucoside were identified or tentatively identified in the bayberry fruits (*35*) or juice (*23*). The results indicated that the phenolic compounds in bayberry juice could precipitate proteins or self-polymerize to form haze and sediment during juice storage.

Table 3. Phenolic Contents (g/100 g dw) in Bayberry Juice Sediment

phenolic compounds	methanol-dissolved sample	acid-hydrolyzed sample
gallic acid	1.1 ± 0.06[b]	2.1 ± 0.05
protocatechuic acid	ND[c]	1.2 ± 0.09
cyanidin	ND	2.9 ± 0.07
quercetin 3-hexoside[a]	1.2 ± 0.08	ND
ellagic acid	ND	9.9 ± 0.19
quercetin deoxyhexoside	1.2 ± 0.15	ND
quercetin	0.3 ± 0.05	1.8 ± 0.11
total	**3.8**	**17.9**

[a] All flavonol glycosides are calculated as quercetin 3-glucoside. [b] Data are expressed as means ± standard deviations ($n = 2$). [c] ND, not detected.

It is well-known that the solubility of free ellagic acid in solution is low, and ellagitannins are difficult to determined (*13*). Ellagic acid was not detected in the previously studied material of bayberry fruits (*35*) and juice (*23*). This compound was even not detectable in the methanol solution of the sediment (**Figure 2**). Surprisingly, it appeared as the dominant phenolic compound (9.9 ± 0.19 g/100 g dw, 55.3% of the total amounts) in the acid-hydrolyzed sample (**Figure 2** and **Table 3**). The fact suggested that the ellagic acid existed as the ellagitannin forms in the bayberry fruits, juice, and sediment. It may bind to some insoluble compounds in the sediment and be released by acid hydrolysis. Ellagic acid also has been demonstrated as the major phenolic compound in sediments of muscadine juice (*10*–*13*), blackberry juice (*14*), loganberry wine (*9*), and muscadine wine (*13*, *36*), and its concentration in sediments is significantly affected by the processing methodologies (*9*–*14*).

Anthocyanins were observed in blackberry juice sediment (*14*) and red wine deposits (*15*). Cyanidin 3-glucoside was readily detected in bayberry fruits (*37*) and juice (*23*) but was not detected in the methanol solution of the sediment. However, after acid hydrolysis at 100 °C for 45 min (*27*), cyanidin appeared in the HPLC chromatogram (**Figure 2** and **Table 2**). This fact implied that cyanidin or cyanidin 3-glucoside may bind to proteins and other polyphenolics and/or self-polymerize during storage to produce compact haze particles; thus, it was not dissolved in methanol solution but only released as aglycon form after acid hydrolysis. Because procyanidins were not detected in the bayberry fruits (*35*, *37*), juice (*23*), and the present study material, the possibility of cyanidin from procyanidin hydrolysis was reasonably low. In other research, procyanidins were thought to be the precursors of fruit juice sediments (*3*, *4*, *38*) or bottled red wine deposits (*15*).

Table 4. Monosaccharide Composition in Bayberry Juice Sediment

monosaccharides	amount (g/100 g)	relative amount (%)
rhamnose	0.6 ± 0.05[a]	8.8
arabinose	1.2 ± 0.03	16.5
mannose	0.6 ± 0.02	7.9
glucose	3.4 ± 0.07	47.6
galactose	1.4 ± 0.06	19.2
total	**7.2**	**100**

[a] Data are expressed as means ± standard deviations ($n = 3$).

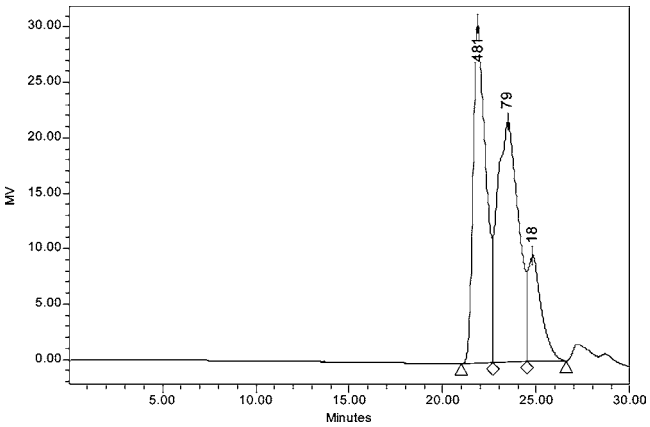

Figure 3. Molecular weight distribution of carbohydrates of bayberry juice sediment dissolved in 0.1 M $NaNO_3$ solution.

Although phenolic acid of protocatechuic acid was not detected in the methanol-dissolved sample, it appeared in the acid-hydrolyzed ones, indicating that it may associate with other compounds in the sediment to form methanol-undissolved complexes. Gallic acid was detected in the methanol-dissolved and acid-hydrolyzed samples, with the latter having a higher concentration, suggesting that methanol-undissolved complexes were also formed by some of this compound and liberated by the acid hydrolysis.

Flavonol and flavonol glycosides (quercetin and quercetin glycosides, **Figure 2** and **Table 2**) were first detected in the fruit juice sediment. That they were readily detected in the methanol solution suggested that their bondages to the haze particles were not as strong as that of anthocyanin or that their polymerization was not as complex as that of anthocyanin in the sediment.

A study of the ability of various polyphenols to bind β-glucosidase led to the conclusion that an *o*-dihydroxybenzene group is needed for each attachment to the protein (*39*). Moreover, the precipitating ability of polyphenols increases as the number of *o*-diphenol groups in the molecule increases (*17*, *18*). All the phenolic compounds (phenolic acids, cyanidin, ellagic acid, flavonols) identified in bayberry juice sediment have at least one *o*-diphenol group, implying that they have the protein-binding activities.

Monosaccharides in Sediment. Analysis of the carbohydrate liberated by trifluroacetic acid hydrolysis showed that bayberry juice sediment contained rhamnose (0.6 ± 0.05 g/100 g), arabinose (1.2 ± 0.03 g/100 g), mannose (0.6 ± 0.02 g/100 g), glucose (3.4 ± 0.07 g/100 g), and galactose (1.4 ± 0.06 g/100 g) (**Table 4**). Glucose was the major sugar (47.2%) of the total identified monosaccharides. No carbohydrate with a MW above 481 Da appeared in the MW distribution analyses (**Figure 3**) implied that these monosaccharides were most probably the glycoside moieties of the anthocyanin, flavonols, and ellagitannins. Although galacturonic acid analyses were not performed in the present study, the possibility of the arabinogalactans of pectin from the bayberry fruit cell wall was quite low because the MWs of these compounds are commonly known to be very high. Waters et al. also demonstrated that the glucose found in red wine deposits was the moiety of anthocyanin units in the polymer (*15*).

Table 5. Mineral Contents in Bayberry Juice Sediment

minerals	amount (μg/g)	relative amount (%)
Ca	2500 ± 12[a]	53.5
Mg	178 ± 9	3.8
K	1053 ± 17	22.5
Fe	796 ± 5	17.0
Cu	150 ± 6	3.2
total	**4677**	**100**

[a] Data are expressed as means ± standard deviations ($n = 3$).

Ash and Minerals in Sediment. There was 6.7 ± 0.6% of ash in the bayberry juice sediment, which was in the range of apple juice sediments of 1.80−11.38% (*4*). The mineral contents of Ca, Mg, K, Fe, and Cu analyzed by atomic absorption spectrophotometric method are shown in **Table 5**. Calcium (53.5%) and potassium (22.5%) were the major minerals while magnesium (3.8%) and copper (3.2%) were the minor minerals of the analyzed metals. Because stainless steel and nonmetal equipment was used in juice processing practice, and these minerals are all present in the bayberry fruits (*40*), we deduced that they were from the processing material of bayberries. The role of metal ions in haze formation, for example, high copper levels in apple juice, may increase the amount of protein−tannin haze that develops during storage (*41*).

The results of the research indicated that bayberry juice haze was mainly protein−tannin haze and the sediment obviously had heterogeneous characteristics. The sediment was composed of 20.4 ± 4.3% of protein, 70.2 ± 2.6% of polyphenols, 7.2% of monosaccharides (after acid hydrolysis), and 6.7 ± 0.6% of ash. Because the monosaccharides were considered as the glycoside moieties of the phenolic compounds, they were not calculated in the total recovery. Therefore, the sum of the protein, total polyphenols, and ash accounted for 97.3% of the original weight of the sediment, which indicated that most chemical information of the bayberry juice sediment was obtained.

On the basis of this knowledge, to avoid sediment formation in bayberry juice could be a dilemma. To avoid sediment formation, polyphenols especially ellagic acid must be completely eliminated via thermal processing or hydrolysis (*12*, *14*). In the present study, anthocyanins, flavonols, and phenolic acids should also be eliminated, but the color or flavor quality of the bayberry juice will decline, which consequently would affect the product's commercial values. Furthermore, the strong antioxidant activities of bayberry fruits (*37*), juice (*42*), and jam (*43*) have been shown to correlate with their anthocyanin and polyphenolic contents. Ellagic acid and ellagitannins also have important biological functions and can scavenge both superoxide and peroxyl radicals in solution (*44*). Eliminating these compounds will certainly decrease the health beneficial properties of the bayberry juice. Our recommendation was to eliminate the proteins as most as possible by optimizing the usage of protein binding agents such as bentonite during juice processing. However, completely eliminating of proteins seems impossible and haze or sediment formation appears inevitable. Moreover, polyphenols can polymerize and grow large enough to produce

haze and sediment even without protein intervention (*17*). Maybe it is the responsibility of food scientists, food technologists, nutritionists, and food industrialists to inform our consumers that a few protein−tannin hazes or sediments in fruit juices are not a quality defect but rather are health beneficial compounds and can be taken safely.

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Supporting Information Available: Comparison of amino acid composition in bayberry juice sediment and fruits (Table S1) and the chemical composition of bayberry juice sediment with other fruit juice or wine sediments (Table S2). UV spectra and and ESIMS+ fragmentation patterns of peaks 3, 5, and 7 (Figures S1−3). Molecular weight distribution of proteins of bayberry fruits extracted with pH 7.0 phosphate buffers (Figure 4). This material is available free of charge via the Internet at http://pubs.acs.org.

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