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Effect of Fining and Filtration on the Haze Formation in Bayberry (*Myrica rubra* Sieb. et Zucc.) Juice

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Bayberry juice was fined with the methods of xanthan/chitosan (XC) or gelatin/bentonite (GB), and then filtered with diatomaceous earth filtration (DF) or ultrafiltration (UF, MWCO 100 kDa). Their effects on juice haze formation were investigated. The XC fining method was more effective than the GB method in removal of the total monomeric anthocyanin, total phenolics, and protein, with less haze formed in the XC fined juice. The DF reduced 2−5% of the total phenolics and 21−23% of protein, while UF reduced 19−24% of the total phenolics and 34−38% of protein, respectively. The results showed that fining and then UF can reduce but cannot eliminate haze formation in bayberry juice. The storage temperature was a critical factor affecting haze formation, and the juice was more stable when stored at lower temperature (4 °C).

KEYWORDS: Bayberry juice; fining; filtration; haze formation

INTRODUCTION

The long historical Chinese bayberry (*Myrica rubra* Sieb. et Zucc.) fruits have an attractive red color, special sweet sour taste, and exquisite flavor, and are praised as the "precious southern Yangtze fruits of early summer" (*1*, *2*). The strong antioxidant activities of bayberry fruits (*3*), juice (*4*), and jam (*5*) highly correlate with their anthocyanin and total phenolic contents. The major anthocyanin in bayberries is cyanidin 3-glucoside (*3*, *6*, *7*), while other phenolics include hydroxybenzoic acids and flavonol glycosides (*3*, *8*). Unfortunately, the fruits decay easily and can only be stored with commercial values for 3 days at 20−22 °C and 9−12 days at 0−2 °C (*9*). Bayberry juice can not only preserve the most merits of the fruits, but also reach a longer consumption. However, post-bottling haze formation in clarified bayberry juice is a big challenge facing both food technologists and industrialists.

Bayberry juice haze was the main type of protein−tannin haze (*10*). The lyophilized sediment contained about 20.38% of protein, 70.24% of polyphenols, 7.2% of monosaccharides (considered as the glycoside moieties of the polyphenols), and 6.65% of ash (*10*). Further, most of the identified haze precursors were native components of bayberry fruits or juice, which implied that to remove all of these haze-forming compounds from the juice in common practices is difficult, and thus to avoid haze formation seems impossible (*10*). However, careful investigation of the processing techniques, especially clarification and filtration, may provide useful information on how to better deal with this problem.

Clarification or fining is an important step in the processing of clarified fruit juices. The common used fining agents include bentonite, gelatin, silica sol, polyvinyl pyrrolidone (PVPP), or a combination of these compounds (*11*−*13*). Recently, chitosan (deacetylated chitin, poly-β-(1−4)*N*-acetyl-glucosamine) was also applied in fruit juice (*14*−*16*) and white wine (*17*) clarification. Although no literature has referred to use of xanthan in juice clarification, it was very effective in flocculation of food dispersions (*18*). Our preliminary experiments indicated that the combination of chitosan and xanthan had amazing effects on bayberry juice clarification.

It has been demonstrated that ultrafiltration can remove proteins, soluble starch, dextrins, gums, and polymerized tannins from juice, resulting in a product with little tendency to become turbid (*19*). The purpose of this work was to compare the effects of the conventional fining method of gelatin/bentonite with xanthan/chitosan on the haze formation of bayberry juices. The effects of diatomaceous earth filtration and ultrafiltration on the bayberry juice haze formation were also investigated.

MATERIALS AND METHODS

Chemicals and Solvents. Pure standards of gallic acid, protocatechuic acid, quercetin, and quercetin 3-glucoside were purchased from Sigma (St. Louis, MO) and Fluka (Buchs, Switzerland). 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), gelatin (type A, 175 bloom), bentonite, and diatomaceous earth (chemical grade) were purchased from Shanghai Chemical Reagent Co., Shanghai, China. Xanthan (type BP9270, 1200−1400 mPa s) was purchased from Shandong Deosen Corp. (Shandong Province, China), and chitosan (80 mesh, 200 mPa s, deacetyl ≥ 85%) was from Kehai Chitosan Corp.

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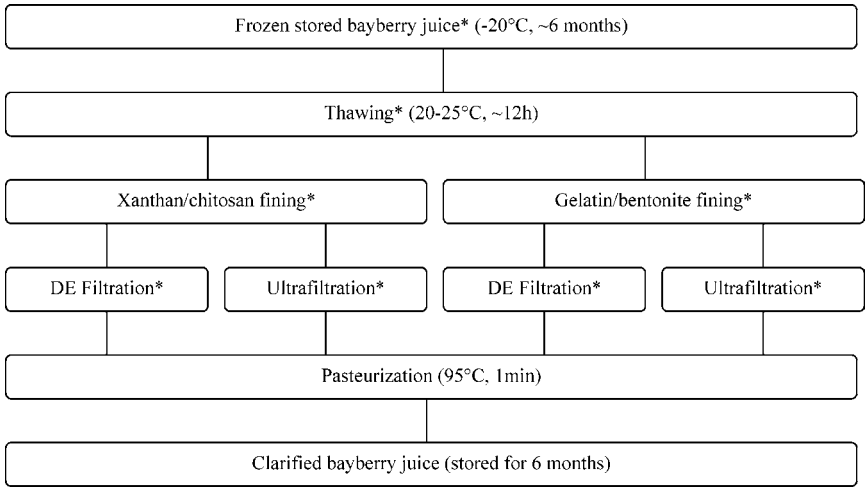

Figure 1. Flow chart for clarified bayberry juice processing (*samples taken for analysis). DE: diatomaceous earth.

(Weifan, Shandong Province, China), respectively. All solution preparations were made using distilled deionized water.

Raw Bayberry Juice. The raw bayberry juice was processed in the factory of Haitong Food Group Corp. according to the procedure described by Fang et al. (*6*). 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 to remove the juice cake. The juice was packaged in laminated foil bags with 2 kg/bag and frozen stored at −20 °C for 6 months until the following process.

Juice Fining and Filtration. The raw bayberry juice was fined and filtered following the procedure shown in **Figure 1**. The frozen stored (FS) juice was thawed at 20−25 °C for 10−12 h and fined via two methods:

(1) Xanthan/Chitosan (XC) Fining. Xanthan and chitosan were dissolved in water overnight to make the concentration of 10 g/L. Five milliliters of acetic acid was added to 1 L of chitosan solution. The xanthan solution was heated to 60 °C and added at 0.3 g/L to the 4 kg juice, and then agitated well. Flocculation was formed immediately at the surface of the juice when the chitosan was added to the juice at 0.1 g/L with agitation. The flocculation was easily taken out, and the clarified juice of XC fining was coded as XC juice.

(2) Gelatin/Bentonite (GB) Fining. Gelatin and bentonite were prepared in hot water (60 °C) with the concentration of 50 g/L just prior to use. Gelatin was added to the juice at 0.2 g/L with good agitation for 10 min, and bentonite suspension was also added at 0.2 g/L. The mixture was well agitated for 10 min. After 1 h at room temperature to allow flocculation to take place, the supernatant was taken out, and the clarified juice of GB fining was coded as GB juice.

The clarified juice was divided into two parts with 2 kg each and filtered following two methods:

(1) Diatomaceous Earth Filtration (DF). Two grams of diatomaceous earth was added to 1 L of juice, and the mixture was then filtered on a Büchner funnel through 2 layers of Whatman No. 1 filter paper. The XC fined and diatomaceous earth filtered juice was coded as XCF juice, and the GB fined and diatomaceous earth filtered juice was coded as GBF juice, respectively.

(2) Ultrafiltration (UF). Two kilograms of juice was ultrafiltered using a tubular polyacrylonitrile (PAN) membrane with the molecular weight cut-off (MWCO) of 100 kDa (laboratory ultrafiltration unite, Wuxi Ultrafiltration Equipment Co., Wuxi, Jiangsu Province, China). Juice temperature was maintained at 25 °C, and the transmembrane pressure was 300 kPa. The volumetric concentration factor (VCF) is defined as the initial volume divided by the retentate volume at any time. The retentate volume was the difference between the initial and permeate volume. The retentate was recycled back to the feed tank until the VCF was equal to 4. The XC fined and ultrafiltered juice was coded as XCU juice, and the GB fined and ultrafiltered juice was coded as GBU juice, respectively.

The fined and filtered juices were then bottled in 80 mL glass jars and subjected to pasteurization at 95 °C for 1 min in a hot water bath. The juices were cooled to room temperature and stored at 4 and 25 °C in the dark for 6 months to investigated the haze stability. The experiments were conducted in triplicates.

pH and Soluble Solids. The pH was measured using a Toledo 320S pH-meter (Mettler-Toledo Instruments (Shanghai) Ltd., China) calibrated with pH 4 and 7 buffers. The soluble solids were measured using a Spoif refractometer (Shanghai Precise Instrument Corp. Ltd., Shanghai, China), and the results were reported as Brix degrees.

Analysis of Total Monomeric Anthocyanin Content. Total monomeric anthocyanin (ACN) contents of bayberry juices were determined using the pH differential method (*20*). Absorbance was measured at 510 and 700 nm. ACN was calculated as cyanidin 3-glucoside using an extinction coefficient of 26 900 L cm^{-1} mg^{-1} and a molecular mass of 449.2 g mol^{-1}.

Analysis of Total Phenolics. Total phenolics were estimated colorimetrically using the modified Folin−Ciocalteau method reported by Sellappan et al. (*21*). An aliquot of 0.2 mL of juice was added to 0.8 mL of water, 5 mL of 0.2 N Folin−Ciocalteau reagent, and 4 mL of saturated sodium carbonate solution (75 g/L), and then mixed in a screw-up test tube. The absorbance was measured at 765 nm with a 752 UV−visible spectrophotometer (Shanghai Precise Instrument Corp. Ltd., Shanghai, China) after incubation for 2 h at room temperature. Quantification was based on the standard curve established with 100, 200, 300, 400, and 500 mg/L of gallic acid, and the results were expressed as gallic acid equivalents in milligrams per liter of juice (mg GAE/L). The results were the average of triplicate analyses.

Protein Determination. Protein concentrations of the juices were determined using the dye-binding method of Bradford (*22*) with bovine albumin as the standard, measuring optical density (OD) at 595 nm.

Extraction of Phenolic Compounds. To analyze the non-anthocyanin individual phenolics, the extraction procedure was a modification described by Määttä et al. (*23*). Five milliliters of bayberry juice was extracted three times using 10 mL of ethyl acetate with intermittent mixing in a separating funnel. Organic phases were collected, pooled, and evaporated to dryness with a rotavapor under reduced pressure. The dry residue was redissolved in 5 mL of methanol for analysis by HPLC-DAD-ESIMS. All samples were filtered through a 0.45 μm cellulose acetate filter (Millipore Corp., Bedford, MA). Duplicate injections were performed, and average peak areas were used for the measurement.

HPLC-DAD-ESIMS Analyses. HPLC-DAD-ESIMS analyses were 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., 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).

Table 1. Effects of Fining and Filtration on the Physiochemical Characteristics of Bayberry Juices[a]

juices	FS[b]	XC	GB	XCF	GBF	XCU	GBU
pH	3.28	3.34	3.36	3.34	3.33	3.37	3.36
soluble solids (Brix)	9.0a	7.9 b	8.1 b	7.9 b	8.0 b	7.8 b	8.0 b
ACN[c] (mg/L)	329.6 a	292.9 b	296.6 b	288.6 b	290.4 b	204.1 c	222.8 c
hydroxybenzoic acids (mg/L)	106.1 a	81.1 b	90.2 c	79.9 b	89.2 c	79.5 b	88.7 c
total flavonol[d] (mg/L)	341.1 a	273 b	298.5 c	269.2 b	289.7 c	267 b	287.9 c
total phenolics[e] (mg/L)	447.2 a	354.1 b	388.7 c	348.1 b	378.9 c	346.5 b	375.6 c
total phenolics[f] (mg/L)	1050.4 a	781.5 b	905.2 c	768.3 b	860.9 c	634.6 d	689.4 e
protein (mg/100 mL)	60.6 a	32.8 b	34.3 b	25.7 c	26.4 c	20.4 d	21.8 d
turbidity (NTU)	1010 a	69.3 b	71.3 b	8.1 c	8.9 c	2.7 d	2.6 d

[a] Results were means of triplicate analysis, and different letters in the same line indicate significant differences ($p \leq 0.05$). [b] FS, freeze stored juice after thawing; XC, xanthan/chitosan fined juice; GB, gelatin/bentonite fined juice; XCF, xanthan/chitosan fined/diatomaceous earth filtered juice; GBF, gelatin/bentonite fined/diatomaceous earth filtered juice; XCU, xanthan/chitosan fined/ultrafiltered juice; GBU, gelatin/bentonite fined/ultrafiltered juice. [c] ACN, total monomeric anthocyanin content. [d] Total flavonol includes flavonol and flavonol glycosides. [e] Sum of the individual polyphenolics determined by the HPLC-DAD method. [f] Estimated by the Folin–Ciocalteu method.

Bayberry juice 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.

UV−visible absorption spectra were recorded online during HPLC analysis. Spectral measurements were made over the range of 200−600 nm. Phenolic acids were detected at 280 nm, and flavonols were detected at 360 nm.

Mass spectra were achieved by electrospray ionization in negative mode. The following ion optics was used: capillary 3.88 kV, cone 25 V, and extractor 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. Continuous mass spectra were recorded over the range *m*/*z* 100−800 with scan time 1 s and interscan delay 0.1 s.

Identification and Quatification of Polyphenols. The individual phenolics in bayberry juice extracts were identified by their UV−visible spectra, mass spectra, and, where available, chromatographic comparisons with standards. Contents of gallic acid, protocatechuic acid, and quercetin were calculated with the regression equations from the standard curves. All flavonol glycosides were quantified as quercetin 3-glucoside because sufficient standards were not available. Concentrations were expressed as mg/L of juice. Repeatability of the analysis was ±5%.

Turbidity Measurements. Samples of 50 mL were placed in a 50-mL cell holder for turbidity measurements. Haze sediments in the juices were resuspended prior to measuring sample turbidity by gently rocking the sample holder immediately before measurements were taken (*24*). Turbidity was measured as Nephelometric Turbidity Unit (NTU) using an STZ-A24 turbidimeter (Wuxi Guangming Turbidimeter Co., Jiangsu Province, China).

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

RESULTS AND DISCUSSION

Changes of pH and Soluble Solids. The pH of the thawed FS bayberry juice was 3.28. After the juice was fined with XC and GB, the pH was increased to 3.34 and 3.36 (**Table 1**), but no statistical differences ($p > 0.05$) were observed. To the contrary, soluble solids of the juices decreased significantly after the two fining methods, with the XC method decreasing more. The facts implied that some soluble components were flocculated and then removed by the fining operations. In the processing of red raspberry juice and wine, fining (with betonite suspension, gelatin solution, or silica solution suspension) combined with depectinizing also significantly reduced soluble solids (*25*).

Neither the DF nor the PAN membrane UF has significant effects on the changes of pH and soluble solids in bayberry juices (**Table 1**). de Bruijin et al. (*26*) compared the conventional filtered (fining with gelatin, bentonite, and activated carbon) and ultrafiltered (MWCO, 50kDa) apple juices, and no differences were observed of the pHs and soluble solids when the pressure of ultrafiltration was 400 kPa.

Changes of Total Monomeric Anthocyanin Contents. Because many analytical methods have identified that cyanidin 3-glucoside is the only major anthocyanin in bayberry fruits and juice (*3*, *6*, *7*), to monitor the individual anthocyanin changes during juice processing seems unnecessary. However, the contents of ACN were determined by the pH differential method (*20*).

The ACN in the thawed bayberry juice was 329.6 mg/L (**Table 1**). After XC and GB fining, ∼10−11% of anthocyanin was lost. Although the XC fining method induced more anthocyanin reduction, no statistical differences were observed ($p > 0.05$). In our previously industrial scale study, about 5−10% of anthocyanins was lost during the bayberry juice GB fining operation (*6*). Rommel et al. (*25*) observed ∼13.7% of anthocyanin decreases when raspberry juice was pasteurized and fined.

There was 1.5−2% of anthocyanin loss during DF, but 25−30% of anthocyanin was lost after the juice was ultrafiltered with PAN membrane of the MWCO 100 kDa (**Table 1**), indicating UF caused substantial anthocyanin losses. However, the anthocyanin contents of strawberry juices were essentially the same for conventional filtration (a pad lined with a 0.5 cm layer of Hyflow Supercell) as UF (MWCO of 10 kDa) (*27*). The differences might come from the UF operation in the present study. The laboratory UF unit was performed in the open air, and lots of oxygen was induced to the juice through the recycling of the retentate, which might cause anthocyanin degradation, thus lowering the anthocyanin contents in the UF juice.

Changes of Polyphenolics. From a comparison of their UV−visible spectra, mass spectra, and HPLC retention times to those of the available standards, polyphenolics in the bayberry juices can be classified into two groups (**Figure 2**, **Tables 1** and **2**): one is hydroxybenzoic acids (peaks 1 and 2, gallic acid and protocatechuic acid), and the other is flavonols (peaks 3−5, myricetin deoxyhexoside, quercetin deoxyhexoside, and quercetin), which is in accordance with our previous study of bayberry juice (*6*). Because the polyphenolic profiles in the HPLC chromatograms for all treated juices were similar, only one HPLC chromatogram of the FS juice was presented (**Figure**

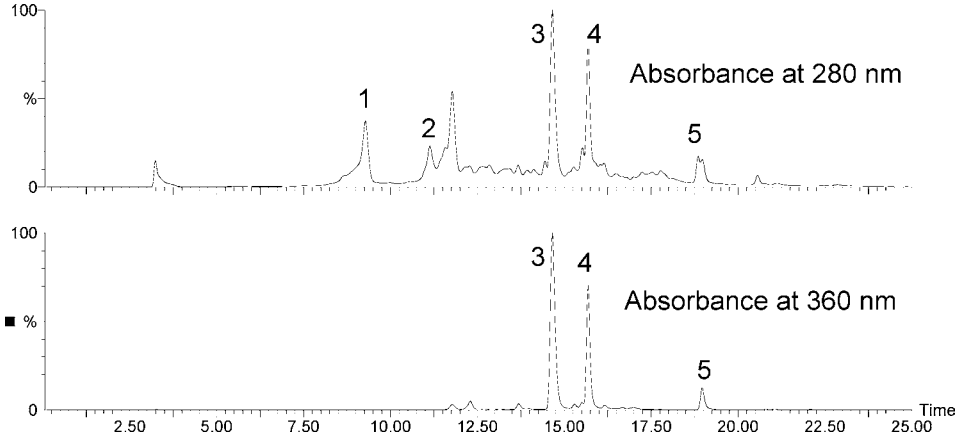

Figure 2. HPLC chromatogram of the polyphenolics from the thawed bayberry juice detected at 280 and 360 nm. Peak numbers refer to **Table 2**.

Table 2. Identification of Phenolic Compounds in Bayberry Juices

	HPLC t_R (min)	HPLC-DAD (nm)	molecular weight	HPLC-ESIMS (*m/z*)	tentative identification
1	9.45	227, 271	170	169	gallic acid (std[a])
2	11.51	260, 294	154	153	protocatechuic acid (std)
3	14.66	256, 352	464	463, 317	myricetin deoxyhexoside
4	15.69	255, 349	448	447, 301	quercetin deoxyhexoside
5	18.95	255, 371	302	301	quercetin (std)

[a] std: identified with standards.

2). However, the contents of the polyphenolics were different in the various fined and filtered juices, which were presented in **Table 1**.

About 24% of hydroxybenzoic acids, 20% of total flavonols, and 21% of the sum of these two classes of polyphenolics were lost during XC fining, while 15% of hydroxybenzoic acids, 12% of total flavonols, and 13% of the sum of these two classes of polyphenolics were lost during GB fining (**Table 1**), indicating XC fining can remove more phenolics from the bayberry juices ($p \leq 0.05$). DF (1−3%) and UF (2−4%) also lowered these contents, but no significant differences ($p > 0.05$) were observed between the two methods. This phenomenon could be explained in that the molecular weights of the hydroxybenzoic acids and flavonols are small (less than 500 Da). They can easily pass through the UF membrane of the MWCO 100 kDa; thus their contents in the DF and UF juices had no statistical differences.

Total phenolics in the juices estimated by the Folin−Ciocalteu method were obviously higher than the sum of the hydroxybenzoic acids and total flavonols (**Table 1**). The substantial differences are understandable because the sum of individual phenolics is not fully comparable to the total polyphenols of Folin−Ciocalteau. The former is specific and the latter unspecific. Furthermore, the Folin−Ciocalteu method tends to overestimate total phenol contents due to interference of reducing substances (*28*).

The DF reduced 2−5% of the total phenolics in bayberry juice, but UF reduced 19−24% (**Table 1**), which suggested UF is more effective in removal of total phenolics. In the study of Nagel and Schobinger (*29*), only 2.1% of phenolics was removed through the UF membrane of 50 kDa. The results implied that most of the phenolics in bayberry juice existed as conjugated forms or they associated with other compounds to form larger molecular weight compounds, and then were retained by the membrane of 100 kDa.

Changes of Protein Contents. The thawed bayberry juice contained 60.6 mg/100 mL of soluble protein (**Table 1**). XC and GB fining decreased 46% and 43% of proteins in bayberry juices, indicating the two fining methods are very effective in juice protein removing. Further, the DF reduced 21−23% of protein, while UF reduced 34−38%, suggesting the latter removed more proteins from the juices. In the processing of apple juice, UF with the membrane of MWCO 20 or 100 kDa had no significant effects on the protein and polyphenol contents, and the optimized GB method was more effective in protein and polyphenol removal (*30*). The molecular weight of proteins in bayberry juice sediment was less than 81 kDa (*10*), so they might easily pass through the membrane MWCO of 100 kDa, and their contents in UF juices should be similar to those of DF juices. The evident lower protein contents in UF juice implied that most of the proteins in bayberry juice could not exist as free form. They were most probably associated with other compounds (such as polyphenols, sugars) to form larger molecular weight compounds and be retained by the membrane.

Changes of Turbidity. From visual observation, the thawed bayberry juice was very hazy, and the turbidity was 1010 NTU (**Table 1**). After being fined by the XC and GB method, the turbidity was decreased to 71.3 and 69.3 NTU, suggesting the methods were very effective in bayberry juice clarification. The DF also lowered the turbidity, but the UF reached a significant level in decreased turbidity, indicating the latter can get a clearer bayberry juice.

The turbidities of the DF bayberry juices were 8.1−8.9, and those of the UF juices were 2.6−2.7 (**Table 1**), which were higher than or approximate to the turbidity of apple juice (<3) stored at 37 °C for 24 weeks (*31*). However, these bayberry juices were very clear from a visual observation. It was probably the red color that interfered with our visual perception, because perception of haze in red solution samples was more difficult than the other color or uncolored samples (*32*).

The XC fining method was more effective than the conventional GB method in removal of ACN and protein from bayberry juices, and the removal of all phenolic contents even had statistical differences from the GB method (**Table 1**). Because the bayberry juice haze was mainly the type of protein−polyphenol haze (*10*), it was expected that bayberry juices fined with the XC method will have lower turbidities than those of the GB fined juices, and the results were as expected (**Table 1**). Another advantage of the XC fining method is it is very convenient in fining operation (see section of Juice Fining and Filtration), and the time needed for flocculation is only 5−10 min. The GB method needs at least 1 h for flocculation, and Zhong (*33*) demonstrated that 5 h was needed for bayberry juice clarification. The results suggested that the XC fining method is an effective, convenient, and time-saving fining method, and might be useful in other juice clarification.

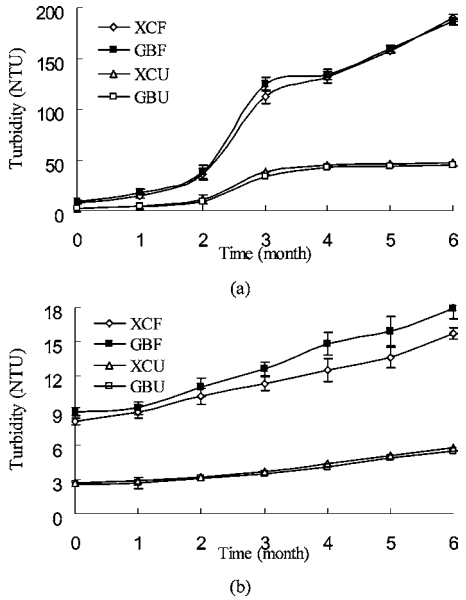

Figure 3. Changes of turbidity (**a**, 25 °C; **b**, 4 °C) during bayberry juice storage. XCF, xanthan/chitosan fined/diatomaceous earth filtered juice; GBF, gelatin/bentonite fined/diatomaceous earth filtered juice; XCU, xanthan/chitosan fined/ultrafiltered juice; GBU, gelatin/bentonite fined/ultrafiltered juice.

It is commonly known that gelatin can combine polyphenols in solution, and bentonite is very effective in removing haze-active protein (*34*, *35*); thus the GB fining method is widely used in fruit juice clarification (*24*, *34*, *35*).

However, the mechanism of XC fining method on juice clarification is not very clear. In the study of Koczo et al. (*18*), when stable food dispersion was added with 0.1% xanthan and 0.05% guar gums at ambient temperature, the food dispersion separated into a cream phase above an aqueous phase. A sharp border formed between the cream and the lower phase, with hardly any particles in the lower phase. Although the combined use of xanthan and chitosan in juice clarification has not been reported, chitosan alone had been successfully used in fruit juice clarification (*14*−*16*). Chitosan carrying a strong positive charge had been shown to be effective in aiding the separation of colloidal and dispersed particles from food processing wastes (*36*). It can also bind pectin to form a gel network (*37*). The authors demonstrated that chitosan acts as a cross-linker of concentrated pectin solutions, and the effectiveness depends mainly on charge density. We proposed that chitosan cross-links xanthan in juices to form a pectin−chitosan-like gel network, and the haze particles were also cross-linked into the network. After the gel was taken out, the juice was clarified. When we added 0.3 g/L of xanthan and then 0.1 g/L of chitosan to the distilled deionized water, a gel was also floated on the surface of the water, which indicated that xanthan and chitosan can form a gel structure in solution, and its formation was not affected by the particles in the solutions.

Changes of Turbidity during Bayberry Juice Storage. When stored at 25 °C, the DF juices showed two haze developing phases: a lag phase and a growth phase (**Figure 3a**). In the lag phase (0−2 months), turbidities increased very slowly. However, after 2 months of storage, haze development proceeded at a very fast rate, and the haze growth phase appeared (∼2−6 months). There was a similar haze-developing pattern for packaged beer (*38*). In addition, haze formation for grape juice and cranberry juice stored at 37 °C also had the two developing phases (*35*). However, apple juices stored at 37 °C had a terminal phase, at which a maximum amount of haze was formed and the turbidity stabilized (*24*). We did not store bayberry juice at 37 °C for turbidity determination, because in our preliminary study, when bayberry juice was stored at 37 °C for 1 week, the browning pigment developed very fast and the juice color turned almost dark, which showed the juice had no commercial value.

Table 3. Linear Equations and R^2 Values for Bayberry Juice Turbidity Increase Rates when Stored at 4 and 25 °C for 6 months

	4 °C		25 °C	
	Log(NTU/NTU$_0$)	R^2	Log(NTU/NTU$_0$)	R^2
XCF[a]	$0.0479t - 0.0021$	0.9976	$0.2416t + 0.118$	0.9041
GBF	$0.055t - 0.0179$	0.9833	$0.2275t + 0.1532$	0.8890
XCU	$0.0582t - 0.0232$	0.9855	$0.2254t + 0.1425$	0.8566
GBU	$0.0579t - 0.0264$	0.9810	$0.2246t + 0.1246$	0.8648

[a] XCF, xanthan/chitosan fined/diatomaceous earth filtered juice; GBF, gelatin/bentonite fined/diatomaceous earth filtered juice; XCU, xanthan/chitosan fined/ultrafiltered juice; GBU, gelatin/bentonite fined/ultrafiltered juice; NTU, turbidity (NTU) of juices at any storage time; NTU$_0$, initial turbidity (NTU); *t*, storage time (month).

There are two possible explanations for this behavior (*24*, *35*). During the lag phase of haze development, perhaps small protein−polyphenol complexes are formed that are initially soluble and therefore do not contribute to turbidity. Alternatively, some other chemical reactions of polyphenols, such as oxidation or polymerization, may have to take place to make them haze active. Once formed, interactions between these active precursors result in rapid formation of haze during the second phase (growth phase) (*24*). When there are no more active haze precursors or binding sites for cross-linking (*34*, *35*), haze development proceeds into the third phase, terminal phase. It might be the storage time is not long enough that the DF bayberry juices showed no terminal phase characteristics when stored at 25 °C for 6 months.

However, the turbidity increase pattern for UF juices stored at 25 °C was not the same as that of DF juices (**Figure 3a**). The turbidity development curve was flat for the first 2 months, increased very fast for 2−4 months, and was flat again for 4−6 months, which showed the UF juices had the terminal phase of haze development. The fact could be explained in that the contents of precursors for haze formation in UF juices, and the contents of polyphenols and proteins, are lower than those in the DF juices (**Table 1**). After 4 months of storage, these low contents of haze active compounds might all bind each other, and thus the turbidity did not increase for the following storage months (4−6 months).

It was obvious that the fined and ultrafiltered bayberry juices can reduce but cannot eliminate haze formation during storage. From visual observation, the ultrafiltered juices were also very hazy (44.5−47.5 NTU) after 6 months of storage; thus we think preventing haze formation in bayberry juices seems impossible (*10*). Nagel et al. reported that UF with MWCO of 50 kDa can still form protein−tannin haze in apple and pear juices (*29*). Juices that passed through a membrane with a pore size of 18 kDa did not have lower tannin or animo acid concentrations than juices not subjected to UF (*39*). Studies suggested that ultrafiltered juice might be more susceptible to post-bottling haze than traditionally clarified juice, as potential haze precursors may not be eliminated to the same extent (*40*). Gao et al. (*41*) demonstrated that, when ultrafiltered with a MWCO of 10 kDa, apple juice can remain stable for at least 6 months at 20 °C. However, membranes with MWCO of 50 kDa or larger are

suggested to be used in industry to obtain economic flow rates (*42*). In another investigation of apple juice, the use of fining with GB before UF using ZrO_2 membranes of 20−100 kDa resulted in a clear and stable apple juice (*43*). In the present study, fining and UF only reduced haze formation. Haze still formed during the commercial storage time, which implied bayberry juice is more difficult to stabilize than the apple juice.

When stored at 4 °C, the turbidities of DF and UF juices did not show two-phase or three-phase characteristics in haze formation. They all increased flatly and slowly, and only showed the lag phase characteristics (**Figure 3b**). After 6 months of storage, the turbidities of treated juices increased ∼2-fold. However, the turbidities of DF and UF juices increased 21−24 and 9−13 times when stored at 25 °C (**Figure 3a**), which suggested that storage temperature is a critical factor affecting bayberry juice haze formation. The higher is the temperature, the more and faster the haze formed in the juices. There were almost no differences in haze development between the 4 and 25 °C stored apple juices, but the turbidity increased significantly faster when stored at 37 °C (*24*).

As measured by the increase in turbidity, the overall rate of haze formation for all treated juices can be described by the first-order reaction kinetics. By calculation of the linear equations obtained from the increase rate of turbidity, the R^2 values were all over 0.85 (**Table 3**), which indicated that the first-order model is suitable for the description of the rate of haze formation for bayberry juices stored at 4 and 25 °C. The rate of haze formation for apple juice also fit the first-order reaction kinetics (*24*).

In conclusion, fining with XC or GB, and then UF with the MWCO of 100 kDa, was an effective methodology for reducing haze formation in bayberry juice. The XC fining method was more effective than the GB method in removal of haze precursors and had the advantages of convenience and time-saving in operation. However, turbidity increased during 6 months of storage in either treated juices, and the rate of turbidity fit the first-order kinetics. Bayberry juice was more stable when stored at lower temperature (4 °C).

Supporting Information Available: HPLC chromatograms of phenolics from bayberry juice and photograph of chitosan/xanthan gel on bayberry juice. This material is available free of charge via the Internet at http://pubs.acs.org.

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