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Improved Stability of Chokeberry Juice Anthocyanins by β-Cyclodextrin Addition and Refrigeration

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ABSTRACT: Chokeberry anthocyanins are susceptible to degradation during processing and storage of processed products. This study determined the effects of three pH levels (2.8, 3.2, and 3.6) and four β-cyclodextrin (BCD) concentrations (0, 0.5, 1, and 3%) alone and in combination on the stability of chokeberry juice anthocyanins before and after pasteurization and over 8 months of storage at 4 and 25 °C. Lowering the pH from 3.6 to 2.8 in the absence of BCD provided marginal protection against anthocyanin losses during processing and storage. Addition of 3% BCD at the natural chokeberry pH of 3.6 resulted in excellent protection of anthocyanins, with 81 and 95% retentions after 8 months of storage at 25 and 4 °C, respectively. The protective effect of BCD was lessened with concentrations <3% and reduction in pH, indicating changes in anthocyanin structure play an important role in BCD stabilization of anthocyanins.

KEYWORDS: *anthocyanins, β-cylodextrin, chokeberries, juice, processing, storage*

INTRODUCTION

Chokeberries (*Aronia melancarpa*) have recently received much attention as a functional food due to their abundant levels of polyphenols. The dark purple berries contain exceptionally high levels of anthocyanins and procyanidins compared to other berries.[1−3] The anthocyanin composition of chokeberries has been well characterized, with eight compounds identified, but two (galactoside and arabinoside) of the four cyanidin glycosides (glucoside, xyloside, galactoside, and arabinoside) predominate.[2] The berries have a complex procyanidin profile ranging from monomers to decamers and also contain exceptionally high levels of polymers.[2] The berries are also reported to contain appreciable levels of quercetin derivatives, chlorogenic acid, and neochlorogenic acid.[1,4] Due to an abundance of polyphenols, chokeberries have demonstrated potent antioxidant capacity in both in vitro assays[2,5−7] and animal models.[8] Additionally, chokeberry and/or chokeberry extracts are reported to possess antidiabetic, antiproliferative, and antimutagenic properties as well as cardio-, hepato-, and chemoprotective effects.[3,9−11]

Chokeberries are commonly processed into various forms including juices, nectars, wines, and liqueurs, and processing and storage of chokeberry nectars and purees have been shown to cause major losses of anthocyanins.[12,13] Processing of other anthocyanin-rich berries such as blueberries,[14] blackberries,[15] and black raspberries[16] also results in significant losses of anthocyanins, and further losses of anthocyanins occur in juices during storage. These changes are accompanied by increased polymeric color values, indicating the formation of anthocyanin−tannin polymers.[14−16] Due to the abundant levels of anthocyanins and procyanidins in chokeberry, we suspect that similar losses of anthocyanins occur during juice processing and storage, and methods are needed to prevent these losses. Encapsulation of anthocyanins with cyclodextrins is a potential treatment that could ameliorate anthocyanin losses. Anthocyanins can form inclusion complexes with cyclodextrin molecules, which may protect anthocyanins from hydration and polymerization reactions.[17] β-Cyclodextrin (BCD) treatment has been shown to prevent anthocyanin losses during storage of tart cherry juice[18] and an anthocyanin-rich extract obtained from *Hibiscus sabdariffa*.[19] Encapsulation of anthocyanins may also prove useful in improving their bioavailability in the gastrointestinal tract, which may improve health benefits.[20]

The objective of this study was to evaluate the individual and combined effects of BCD treatment and pH modification on the stability of anthocyanins in chokeberry juice stored at 4 and 25 °C.

MATERIALS AND METHODS

Chemicals. Chokeberry concentrate (65 °B) was obtained from Maes' Health and Wellness (Omaha, NE, USA). BCD was purchased from Cyclodextrin Technologies Development, Inc. (High Springs, FL, USA), and the mixture of 3-*O*-β-glucoside standards of delphinidin, cyanidin, petunidin, peonidin, pelargonidin, and malvidin was obtained from Polyphenols Laboratories (Sandnes, Norway).

Juice Preparation. Chokeberry concentrate was diluted with deionized water to 12 °Brix. The single-strength juice received four BCD and three pH treatments in a completely randomized design (CRD) as shown in Table 1. The BCD treatments of 0.5, 1, and 3% correspond to 3, 6, and 18 times the levels of total anthocyanins found in the nonpasteurized chokeberry juice.

Following addition of BCD, juices were acidified to pH 3.2 and 2.8 by dropwise addition of a saturated (73%) citric acid solution. After pH adjustment, the pH 3.2 and 3.6 juices were adjusted with deionized water to the same volume of the pH 2.8 juice. Juices were dispensed into 6 oz glass bottles and pasteurized by heating in a steam box (American Sterilizer Co., Erie, PA, USA) until the juice temperature monitored using a thermocouple reached 90 °C (~90 s). The bottle caps were tightened and the juices allowed to cool overnight. Samples of each juice treatment were stored in the dark at 4 and 25 °C.

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Table 1. Completely Randomized Design Used for Juice Processing

BCD concn (%)	pH	storage temp (°C)
0	2.8	4
0.5	2.8	4
1	2.8	4
3	2.8	4
0	3.2	4
0.5	3.2	4
1	3.2	4
3	3.2	4
0	3.6[a]	4
0.5	3.6	4
1	3.6	4
3	3.6	4
0	2.8	25
0.5	2.8	25
1	2.8	25
3	2.8	25
0	3.2	25
0.5	3.2	25
1	3.2	25
3	3.2	25
0	3.6[a]	25
0.5	3.6	25
1	3.6	25
3	3.6	25

[a]Natural pH of chokeberry juice.

Sampling of juices (three bottles/treatment) was performed before and after pasteurization and after 2, 4, 6, and 8 months of storage.

HPLC and HPLC-MS Analysis of Anthocyanins. Juices were filtered through 0.45 μm filters, and anthocyanins were separated on a 250 × 4.6 mm Symmetry C_{18} column (Waters Corp., Milford, MA, USA) using the method of Cho et al.[21] A linear gradient of 5% formic acid (A) and methanol (B) from 2 to 60% B for 60 min at 1 mL/min was used to separate anthocyanins. The peaks were monitored at 510 nm using a Waters model 996 photodiode array detector. Individual cyanidin monoglycosides were quantified as cyanidin 3-glucoside equivalents, and pelargonidin 3-arabinoside was quantified as pelargonidin 3-glucoside equivalents using external calibration curves of authentic standards ranging from 5 to 125 μg/mL. Total anthocyanins were calculated as the sum of individual anthocyanin monoglycosides, with results expressed as milligrams per 100 mL juice. The identification of anthocyanins was performed by HPLC-MS using identical conditions described above with the HPLC system interfaced to a Bruker Esquire LC/MS ion trap mass spectrometer (Billerica, MA, USA). Mass spectral analysis was conducted in positive ion electrospray mode with conditions previously described in Cho et al.[21]

Polymeric Color Analysis. Percent polymeric color (PC) of juices was determined using the spectrophotometric assay described by Giusti and Wrolstad.[22] Absorbance values of two matching samples, one treated with water (control) and the other with potassium metabisulfite, were recorded at 420, 520, and 700 nm. Potassium metabisulfite was used to bleach the monomeric anthocyanins present in the juices, whereas polymeric anthocyanins are resistant to bleaching and remain colored. The ratio of the absorbance value of the potassium metabisulfite bleached sample to the control sample (nonbleached) × 100 reflects percent polymeric color.

Statistical Analysis. All statistical analysis was performed in the Fit Model platform of JMP (JMP Pro ver. 0, SAS Institute, Cary, NC, USA). The statistical model for all responses for the pasteurization data set involved the basic 3 × 4 × 2 factorial treatment design (i.e., 3 pH levels (2.8, 3.2, and 3.6) and 4 BCD concentrations (0, 0.5, 1, and 3%), pasteurized and not pasteurized) in a CRD design with three replications for each of the 24 treatment combinations.

The statistical model for all responses for the storage study involved the basic 3 × 4 × 4 × 2 factorial treatment design in a CRD design with three replications for each of the 96 treatment combinations. Again, we started with the basic 3 × 4 as before (3 pH levels (2.8, 3.2, and 3.6) and 4 BCD concentrations (0, 0.5, 1, and 3%)), but this time each sample replicate was analyzed after storage in each of the (4 × 2) 8 factorial storage treatments conditions corresponding to storage times (2, 4, 6, 8 months) and 2 storage temperatures (25 and 4 °C). Tukey's HSD multiple comparisons were utilized to report mean differences among interactions, and simple and main effects were appropriate using $\alpha = 0.05$ significance level in all cases.

JMP Graph Builder was utilized to draw the graphs of Figures 2 and 3 to show and compare the linear main effects in terms of the BCD

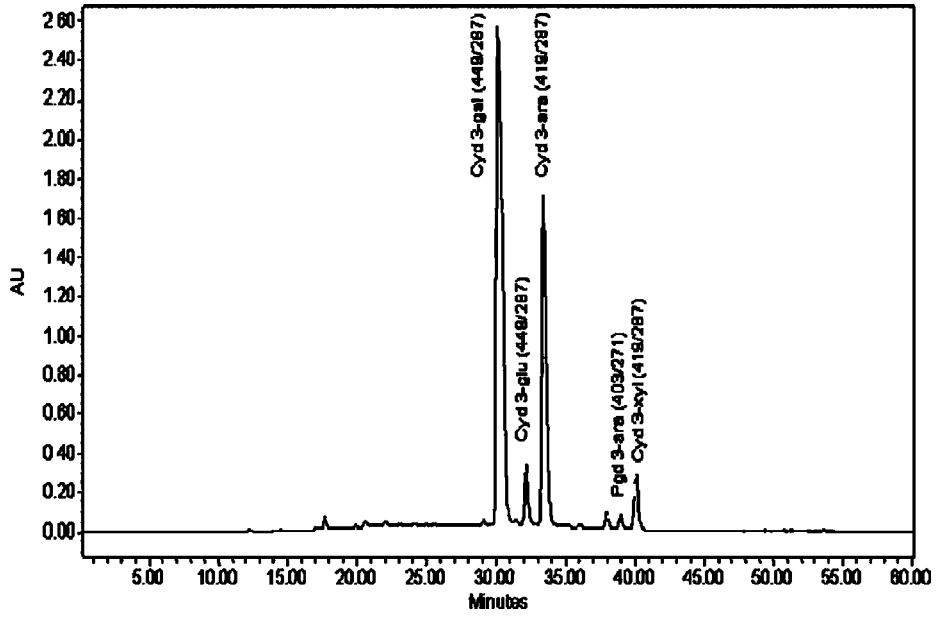

Figure 1. Typical HPLC chromatogram (Abs 520 nm) of chokeberry juice anthocyanins.

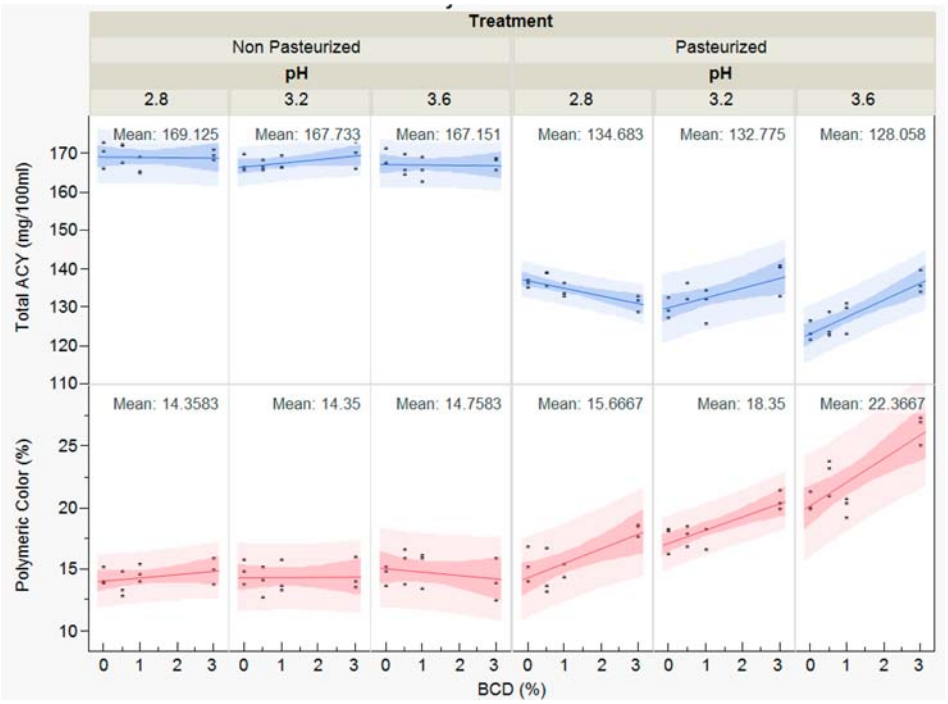

Figure 2. Total anthocyanin (ACY) content (mg/100 mL) and percent polymeric color of chokeberry juice before and after pasteurization as affected by pH and BCD. Dark narrow band represents 95% confidence interval for the fit, and light wide band represents 95% prediction interval. HSD values (p = 0.05) for total anthocyanins and percent polymeric color were 8.80 and 3.67, respectively.

effects and its interactions for the first data set and in terms of the storage linear effects and its interactions with the other major factors in Figure 2. Both 95% confidence about the fit and the prediction (narrower and wider confidence bands, respectively) are shown in the figures to add comparisons and interpretations.

RESULTS AND DISCUSSION

The ANOVA indicated highly significant four-factor interaction among storage temperature, storage time, BCD, and pH for all responses.

Anthocyanin Composition of Chokeberry Juice. A typical HPLC chromatogram of chokeberry juice is presented in Figure 1. Five peaks were identified by HPLC-MS (peak 1, Cyd 3-gal (m/z 449/287); peak 2, Cyd 3-glu (m/z 449/287); peak 3, Cyd-3 ara (m/z 419/287); peak 4, Pgd 3-ara (m/z 403/271); and peak 5, Cyd 3-xyl (m/z 419/287)), confirming the identification of major anthocyanins in fresh chokeberries,[2,9] juice,[23] and concentrate.[1] Cyd 3-gal and Cyd 3-ara were the major anthocyanins in the nonpasteurized juice, accounting for 59 and 28%, respectively, of total anthocyanins, whereas Cyd 3-glu, Cyd 3-xyl, and Pgd 3-ara each accounted for about 7% of total anthocyanins, and an unidentified anthocyanin accounted for <2%.

BCD and pH Effects on Anthocyanins and Percent Polymeric Color of Nonpasteurized and Pasteurized Chokeberry Juice. The effects of BCD and pH on total anthocyanin content and percent PC of nonpasteurized and pasteurized chokeberry juice are shown in Figure 2, and the effects of these variables on individual anthocyanins are presented in Table 2. BCD treatment and pH modification had no effect on total anthocyanin content or percent PC of nonpasteurized juices. As expected, pasteurization resulted in significant losses of anthocyanins (ranging from 19 to 27%) and increased percent PC values (ranging from 13.6 to 26.4), but the losses were influenced by BCD, pH, and the BCD × pH interaction. Anthocyanin losses were generally ameliorated with increasing concentration of BCD and lowering of pH, but the protective effect of BCD at 3% was observed only at pH values of 3.2 and 3.6. With no or <3% BCD concentration, acidifying juices from the natural level of 3.6 to 3.2 and 2.8 ameliorated anthocyanin losses. Similar trends were generally observed for the two major anthocyanins in chokeberry Cyd 3-gal and Cyd 3-ara, as well as the less abundant anthocyanins Cyd 3-glu, Cyd 3-xyl, and Pgd 3-ara. In terms of stability of individual anthocyanins in response to pasteurization, Cyd hexosides (galactoside and glucoside) were better retained than Cyd pentosides (arabinoside and xyloside) as well as Pgd 3-ara. These results are consistent with previous studies reporting anthocyanin hexosides to be more stable than pentosides.[13,24]

Percent polymeric color values increased with increase in both BCD concentration and pH, with the highest value observed in juices treated with 3% BCD. Percent PC values indicate the formation of anthocyanin−procyanidin polymers that are resistant to bleaching in the presence of potassium bisulfite. The inverse relationship between total anthocyanins and percent PC values in response to pH was evident with lowering of pH and BCD concentration to 1% or less, but juices containing 3% BCD had higher percent PC values than all other juices regardless of pH, while retaining high levels of anthocyanins. We suspect that the high concentration of BCD prevented bleaching of monomeric anthocyanins in the polymeric color assay either by encapsulating the pyran ring, and thus blocking attachment of procyanidins, or by acting as a strong copigment.

BCD and pH Effects on Anthocyanins and Percent Polymeric Color of Pasteurized Chokeberry Juice during Storage at 25 and 4 °C. The effects of BCD and pH on total anthocyanin content and percent PC of pasteurized chokeberry juice stored at 4 °C over 8 months are shown in Figure 3, and the effects of these variables on individual anthocyanins are presented in Table 3. As expected, refrigerated storage resulted in much greater retention of anthocyanins than storage at ambient

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Table 2. Anthocyanin Content (Milligrams per 100 mL) of Chokeberry Juice before and after Pasteurization As Affected by pH and BCD

pH	% BCD	Cyd 3-gal	Cyd 3-glu	Cyd 3-ara	UI[a]	Cyd 3-xyl	Pgd 3-ara
				Nonpasteurized			
2.8	0	98.5	7.0	48.6	1.6	7.3	6.8
	0.5	99.4	7.0	48.9	1.5	7.1	6.7
	1	97.1	6.5	47.3	1.6	7.1	6.8
	3	99.2	7.3	48.3	1.5	6.9	6.4
3.2	0	96.6	6.9	48.0	1.6	7.2	6.9
	0.5	97.5	6.6	47.5	1.5	6.9	6.6
	1	99.8	6.3	46.6	1.5	6.8	6.4
	3	99.2	7.9	47.8	1.5	7.1	6.2
3.6	0	98.1	6.9	48.4	1.6	7.1	6.7
	0.5	97.0	6.8	47.7	1.5	6.9	6.6
	1	96.8	6.9	47.1	1.5	6.9	6.5
	3	96.9	7.7	47.8	1.5	7.1	6.5
				Pasteurized			
2.8	0	82.0	5.7	36.3	1.2	5.5	5.3
	0.5	83.9	5.4	36.5	1.2	5.4	5.2
	1	82.4	5.2	35.0	1.1	5.3	5.1
	3	81.9	4.7	33.3	1.1	5.0	4.9
3.2	0	80.0	5.3	33.5	1.1	4.8	4.7
	0.5	81.6	5.5	35.0	1.2	5.2	4.8
	1	79.8	4.9	34.5	1.2	5.4	4.6
	3	83.8	5.7	36.7	1.2	5.4	5.0
3.6	0	76.2	5.1	32.1	1.2	4.5	4.3
	0.5	77.1	4.8	32.1	1.2	4.8	4.7
	1	79.4	4.6	32.7	1.1	5.3	4.7
	3	83.4	5.1	36.3	1.2	5.7	4.5
HSD[b]		5.69	1.19	2.93	0.16	0.67	0.59

[a]Unidentified anthocyanin. [b]HSD, Tukey's honestly significant difference test (p = 0.05).

temperature. Juices stored at 4 °C had, on average, 21, 25, 29, and 47% higher levels of total anthocyanins than juices stored at 25 °C after 2, 4, 6, and 8 months of storage, respectively. Total anthocyanins were affected by pH, BCD, and the pH × BCD interaction at both storage temperatures. For juices stored at 4 °C with no addition of BCD, lowering the pH from 3.6 to 2.8 resulted in greater retention of anthocyanins, with juices acidified to pH 2.8 containing 19 and 29% higher levels of anthocyanins than juices with natural pH of 3.6 after 6 and 8 months of storage. The greater stability of anthocyanins at pH 2.8 is most likely the result of a shift in anthocyanin structure toward the red flavylium cation,[25] which has a pK of 2.60,[26] and is consistent with several studies reporting improved stability of anthocyanins by acidification.[27,28] The protection of BCD against anthocyanin degradation in samples stored at 4 °C was concentration- and pH-dependent and was more pronounced late during storage. BCD regardless of concentration afforded no protection in samples acidified to pH 2.8. However, 3% BCD treatment showed moderate protection against anthocyanin degradation in juices acidified to pH 3.2 and excellent protection against degradation in juices with natural pH of 3.6. After 8 months of storage, juices containing 3% BCD at the natural pH of 3.6 contained 49% more anthocyanins than pH 3.6 juices that received no BCD. Remarkably, the juices treated with 3% BCD at pH 3.6 retained 95% of total anthocyanins from 2 to 8 months of storage, compared to 63% retention for the corresponding juices with no BCD addition. The protective

Table 3. Anthocyanin Content (Milligrams per 100 mL) of Chokeberry Juice over 8 Months of Storage at 4 °C As Affected by pH and BCD

storage time (months)	pH	%BCD	Cyd 3-gal	Cyd 3-glu	Cyd 3-ara	UI[a]	Cyd 3-xyl	Pgd 3-ara
2	2.8	0	90.6	5.3	36.1	1.4	0.8	5.6
		0.5	90.1	6.8	36.7	2	1.2	5.3
		1	87.6	6.7	35.9	1.7	1	5.5
		3	86.7	7.3	35.2	1.7	1.1	5.2
	3.2	0	87.9	5.3	35.7	1.5	0.7	5.5
		0.5	91.6	7	37.4	1.9	1.2	5.5
		1	86.5	6.6	35.5	1.9	1.2	5.2
		3	88.6	7.8	36.4	1.8	1.2	5.4
	3.6	0	87.1	6.9	36.1	2.1	1.3	5.1
		0.5	79.2	6.2	32	1.8	1.1	4.7
		1	80.6	6.6	32.7	1.8	1.2	4.8
		3	86.9	7.7	36.2	1.7	1.1	5.3
4	2.8	0	79.5	6.4	34.6	1.7	1	5.1
		0.5	83.9	6.3	34.9	1.6	1	5.1
		1	83.4	5.9	34.1	1.5	0.9	5
		3	77.6	6.4	32	1.5	0.9	4.8
	3.2	0	79.7	5.8	32.7	1.6	1	4.7
		0.5	74.1	5.4	30.8	1.5	0.9	4.6
		1	80.1	6.3	33.9	1.6	1.1	5
		3	85.7	7.1	36.1	1.5	1	5.4
	3.6	0	77.5	5.6	31.6	1.6	1	4.4
		0.5	73.6	5.7	29.8	1.5	0.9	4.4
		1	72.8	6.1	30.5	1.5	1	4.6
		3	86.1	7.1	36.7	1.5	1	5.4
6	2.8	0	80.2	6.1	32.5	1.5	0.9	4.8
		0.5	80.5	6.1	33.1	1.7	1.1	4.9
		1	79.8	5.3	31.7	1.6	1	4.7
		3	79	5.7	30.7	1.3	0.8	4.5
	3.2	0	74.7	5.2	29.6	1.6	1	4.3
		0.5	74.7	5.1	29.3	1.4	0.9	4.1
		1	77.3	5.4	31.3	1.4	0.9	4.6
		3	87.7	6.4	34.8	1.6	1	5.1
	3.6	0	68	5	26.4	1.7	1	3.8
		0.5	72	5.4	28.7	1.5	1	4.2
		1	72.1	5.2	27.9	1.5	0.8	4
		3	84.5	6.6	34.7	1.5	1	5.2
8	2.8	0	72.1	5.5	28.8	1.5	0.9	4.3
		0.5	75.4	5.3	28	1.5	1	4.2
		1	68.1	5.4	27.6	1.5	1	4.2
		3	75.2	5.8	27.7	1.4	0.9	4.3
	3.2	0	62.8	4.9	25.4	1.5	1	3.8
		0.5	66.3	4.9	23.9	1.5	1	3.6
		1	67.4	5.6	28	1.6	1.1	4.3
		3	75	6.4	31.4	1.5	1	4.8
	3.6	0	55.5	4.3	21.9	1.5	1	3.2
		0.5	58.9	4.9	23	1.5	1	3.6
		1	63.3	5.4	24.9	1.5	0.9	3.8
		3	81.8	6.8	34	1.5	1	5.1
HSD[b]			8.04	1.49	3.32	0.36	0.28	0.58

[a]Unidentified anthocyanin. [b]HSD, Tukey's honestly significant difference test (p = 0.05).

effect of 3% BCD at pH 3.6 was evident for all anthocyanins, except the unidentified anthocyanin and Cyd 3-xyl, which were

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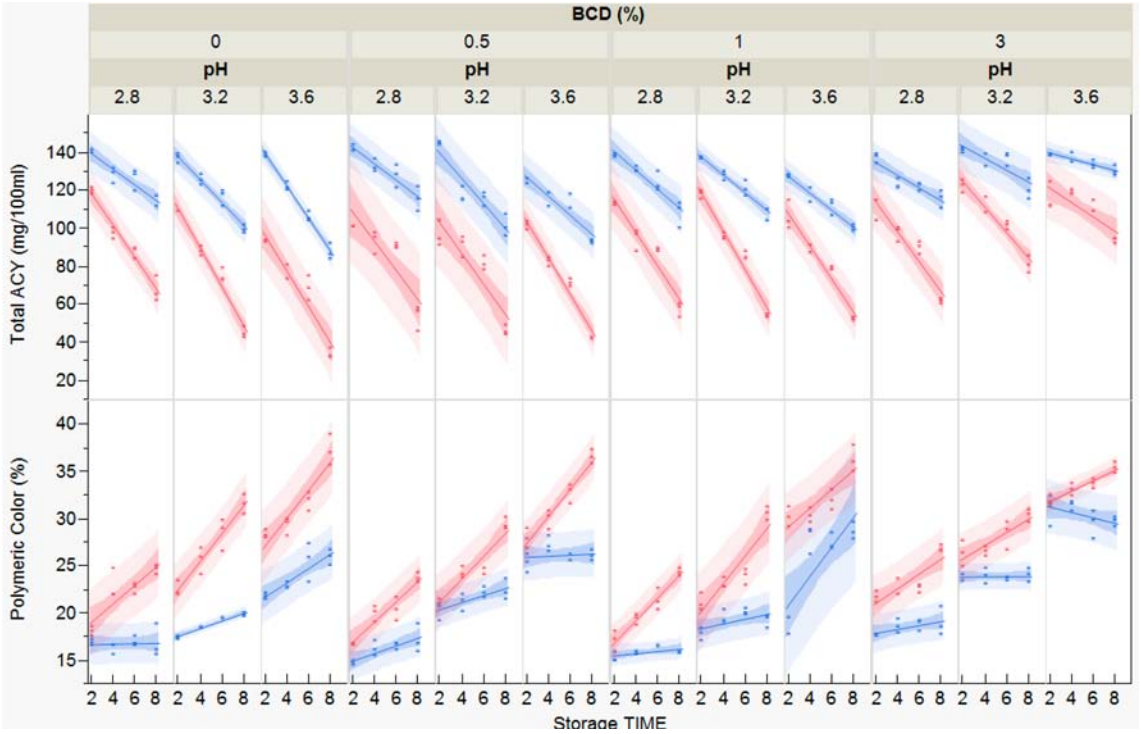

Figure 3. Total anthocyanin (ACY) content (mg/100 mL) and percent polymeric color of chokeberry juice over 8 months of storage at 4 °C (blue) and 25 °C (red) as affected by pH and BCD. Dark narrow band represents 95% confidence interval for the fit, and light wide band represents 95% prediction interval.

present at low levels. Levels of Cyd 3-gal, Cyd 3-ara, and Pgd 3-ara were well retained, 94, 94, and 96%, respectively, over 8 months of storage, compared with corresponding pH 3.6 juices with no BCD addition (64, 61, and 63% retentions, respectively).

Percent PC values of juices stored at 4 °C generally increased over storage and were influenced by pH, BCD, and the pH × BCD interaction, with increasing pH and BCD concentrations resulting in higher values. Consistent with changes observed after pasteurization, juices treated with 3% BCD had the highest percent PC values, especially in samples with pH values of 3.6 and 3.2, and percent PC values in these treatments changed little during storage. Because these treatments resulted in greater retention of anthocyanins over storage, it appeared that the 3% BCD treatment prevented bleaching of monomeric anthocyanins in the presence of potassium metabisulfite, resulting in elevated percent PC values.

The effects of BCD and pH on total anthocyanin content and percent PC of pasteurized chokeberry juice stored at 25 °C over 8 months are shown in Figure 3, and the effects of these variables on individual anthocyanins are presented in Table 4. Similar trends in anthocyanin retention as those obtained during storage at 4 °C were observed, but the effects were more pronounced due to greater anthocyanin degradation at ambient storage temperature. For juices receiving no BCD, lowering the pH from 3.6 to 2.8 resulted in greater retention of anthocyanins, with juices acidified to pH 2.8 containing 29 and 28% higher levels of anthocyanins than juices with natural pH of 3.6 after 4 and 6 months of storage and 50 and 101% higher levels of anthocyanins than juices with pH of 3.2 and 3.6 after 8 months of storage. The protective effect of 3% BCD against anthocyanin degradation was pH-dependent, with no protection at pH 2.8, moderate protection at pH 3.2, and excellent protection at pH 3.6. Juices treated with 3% BCD at pH 3.2 contained 29, 35, and 81% more anthocyanins than corresponding pH 3.2 juices containing no BCD after 4, 6, and 8 months of storage, respectively, and juices treated with 3% BCD at natural pH contained 24, 57, 62, and 178% more anthocyanins than corresponding pH 3.6 juices containing no BCD after 2, 4, 6, and 8 months of storage, respectively. The juices treated with 3% BCD at natural pH of 3.6 retained 81% of total anthocyanins from 2 to 8 months of storage, compared to 36% retention for pH 3.6 juices with no BCD addition. Similar to results obtained with 4 °C storage, the protective effect of 3% BCD at pH 3.6 was evident for all anthocyanins, excluding the minor anthocyanins Cyd 3-xyl and unidentified compound. Levels of Cyd 3-gal, Cyd 3-ara, Cyd 3-glu, and Pgd 3-ara were well retained at 80, 76, 111, and 68%, respectively, over 8 months of storage, compared with pH 3.6 juices with no BCD addition having 36, 32, 37, and 33% retentions, respectively.

Percent PC values increased in all juice samples from 2 to 8 months of storage, and similar to juices stored at 4 °C, the values were affected by pH and BCD concentration. Increasing pH level led to increasing percent PC values, and BCD treatment at the 3% level also resulted in higher percent PC values. The percent PC values of juices treated with 3% BCD were initially higher than those of other treatments, but they did not increase as readily over 8 months of storage. This was especially evident for pH 3.6 juices treated with 3% BCD, for which percent PC values increased only from 32 to 35 from 2 to 8 months. In contrast, percent PC values increased from 28.3 to 37.1 from 2 to 8 months in corresponding pH 3.6 juices receiving no BCD. Hence, although BCD treatment at 3% results in high initial percent PC values, the treatment appears to retard formation of polymeric pigments during storage.

The protection of BCD against anthocyanin degradation is consistent with previous studies in which BCD addition to tart cherry juice improved anthocyanin retention after 12 weeks of

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Table 4. Anthocyanin Content (Milligrams per 100 mL) of Chokeberry Juice over 8 Months of Storage at 25 °C As Affected by pH and BCD

storage time (months)	pH	%BCD	Cyd 3-Gal	Cyd 3-Glu	Cyd 3-Ara	UI[a]	Cyd 3-xyl	Pgd 3-Ara
2	2.8	0	76.2	5.7	30.6	1.6	1	4.5
		0.5	64.5	4.9	25.3	1.7	1	3.7
		1	69.6	5	27.7	1.4	1	5.2
		3	71.4	5.7	27.7	1.3	0.8	5.5
	3.2	0	69.6	5.3	27.5	1.7	1	4
		0.5	66.7	5.3	19.2	1.2	0.9	4.8
		1	71.5	5.3	29.4	1.5	0.9	5.4
		3	77	5.3	31.9	1.4	0.9	6.1
	3.6	0	59.5	4.6	22.9	1.7	1	3.3
		0.5	61.8	5	24.6	1.4	0.9	4.8
		1	64.5	5	26	1.4	0.9	5.1
		3	73.9	5.2	30	1.2	0.8	5.6
4	2.8	0	62.8	4.7	24.1	1.3	0.8	3.6
		0.5	60.3	4.3	22.7	1.4	0.8	3.4
		1	60.8	4.5	23.4	1.3	0.8	3.4
		3	62.6	5	24	1.3	0.7	3.7
	3.2	0	56.8	4.1	21.5	1.4	0.9	3.1
		0.5	58.2	4.4	22.9	1.2	0.8	3.4
		1	61.2	4.8	23.8	1.4	0.8	3.5
		3	71.1	6.5	28.9	1.3	0.9	4.4
	3.6	0	48.7	3.6	18.2	1.5	0.9	2.6
		0.5	53.2	4	20.2	1.3	0.8	3
		1	56.5	4.8	21.8	1.3	0.8	3.3
		3	74.4	6.9	30.6	1.3	0.8	4.7
6	2.8	0	57.1	4.1	21	1.3	0.7	3
		0.5	58.5	4.4	22	1.5	0.9	3.3
		1	57.1	4.8	21.5	1.4	0.8	3.3
		3	57.9	4.9	21.6	1.3	0.7	3.3
	3.2	0	48.9	3.7	17.6	1.5	0.8	2.6
		0.5	52.7	3.9	19.4	1.5	0.9	2.9
		1	54.8	4.4	20.7	1.5	0.9	3.1
		3	65.4	5.1	25	1.3	0.7	3.9
	3.6	0	43.1	3.5	16.9	1.5	0.9	2.4
		0.5	45.8	3.8	16.9	1.4	0.8	2.5
		1	50.5	4.4	18.9	1.4	0.8	2.9
		3	70.4	5.7	28	1.3	0.8	4.3
8	2.8	0	42.5	3.6	16.9	1.2	0.6	2.6
		0.5	34.7	2.8	12	1.3	0.7	1.9
		1	37.3	3.2	13	1.2	0.5	2.1
		3	39.5	4	14	1.1	0.4	2.4
	3.2	0	29.2	2.2	9.9	1.3	0.5	1.6
		0.5	29.7	2.5	10.2	1.3	0.6	1.7
		1	34.4	3.2	12.1	1.3	0.8	2.1
		3	51.6	5.2	19.3	1.2	0.5	3.3
	3.6	0	21.4	1.7	7.3	1.4	0.8	1.1
		0.5	27	2.4	9.2	1.3	0.7	1.5
		1	33.3	3.4	11.9	1.3	0.6	2
		3	59.3	5.8	22.9	1.1	0.7	3.8
HSD[b]			8.3	1.27	4.55	0.34	0.65	0.77

[a]Unidentified anthocyanin. [b]HSD, Tukey's honestly significant difference test (p = 0.05).

storage[18] and improved the thermal stability of an anthocyanin-rich extract isolated from *H. sabdariffa* L.[19] However, the protective effect of BCD was most pronounced at the highest concentration tested (3%) and influenced significantly by pH, with no protection observed at pH 2.8, moderate protection at 3.2, and excellent protection at 3.6. The greater effect at pH 3.6 may be due to a shift of anthocyanin structure from flavylium cation to hemiacetal and chalcone colorless forms.[29,30] It has been proposed that the flavylium cation is a poor candidate for inclusion in the cyclodextrin cavity due to its polarity and planar structure, whereas the neutral and more flexible colorless forms can more readily form inclusion complexes.[29,30] However, greater inclusion of anthocyanin colorless forms into the cyclodextrin cavity is reported to result in rapid loss of color, the so-called "anti-co-pigment effect".[29,30] In our study we did not observe any fading of color in BCD-treated juices over storage, but we did observe rapid color fading upon addition of 3% BCD to a 150 μg/mL solution of cyanidin 3-glucoside standard (data not shown). The lack of anti-co-pigment effect in juices may be due to the presence of other copigments present in chokeberries such as chlorogenic acid, flavonols, or procyanidins, which helped stabilize the color. Despite improved stability of anthocyanins as a result of BCD treatment, several studies report that only ring B of anthocyanins can fit within the BCD cavity.[19,31] Using NMR, Mourtzinos et al.[19] demonstrated that BCD interacts externally with anthocyanins and suggested that steric hindrance may play an important role in preventing hydration of the anthocyanin molecule. BCD may also have conferred stability of anthocyanins to nucelophilic attack of water at C2 of the pyrilium ring via a stacking process involving hydrophobic interactions.[32] It is possible that the protection of BCD against anthocyanin degradation at pH 3.6 is due to the combination of partial inclusion of the anthocyanin molecule as well as several external associations including hydrogen bonding, hydrophobic interactions, and steric phenomena. Further studies are needed to identify the exact mechanism(s) responsible for improved stability of anthocyanins in the presence of BCD.

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Notes

The authors declare no competing financial interest.

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