JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Changes in Chokeberry (*Aronia melanocarpa* L.) Polyphenols during Juice Processing and Storage

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Supporting Information

ABSTRACT: Chokeberries are an excellent source of polyphenols, but their fate during juice processing and storage is unknown. The stability of anthocyanins, total proanthocyanidins, hydroxycinnamic acids, and flavonols at various stages of juice processing and over 6 months of storage at 25 °C was determined. Flavonols, total proanthocyanidins, and hydroxycinnamic acids were retained in the juice to a greater extent than anthocyanins, with losses mostly due to removal of seeds and skins following pressing. Anthocyanins were extensively degraded by thermal treatments during which time levels of protocatechuic acid and phloroglucinaldehyde increased, and additional losses occurred following pressing. Flavonols, total proanthocyanidins, and hydroxycinnamic acids were well retained in juices stored for 6 months at 25 °C, whereas anthocyanins declined linearly. Anthocyanin losses during storage were paralleled by increased polymeric color values, indicating that the small amounts of anthocyanins remaining were present in large part in polymeric forms.

KEYWORDS: *anthocyanins, chokeberries, flavonols, hydroxycinnamic acids, juice, processing, proanthocyanidins, storage*

INTRODUCTION

Chokeberries (*Aronia melanocarpa*) are gaining popularity as a result of their exceptional content of polyphenols. The berries are exceptionally rich in anthocyanins, which are responsible for the deep purple color. Chokeberry anthocyanins consist mainly of cyanidin glycosides (glucoside, xyloside, galactoside, and arabinoside), but the galactoside and arabinoside generally account for >75% of total anthocyanins.[1] Chokeberries are also rich in proanthocyanidins (PACs), which impart a very astringent taste. The proanthocyanidin profile of the berries is complex, ranging from monomers to decamers and to exceptionally high levels of polymers.[1] The berries also contain high levels of hydroxycinnamic acids (chlorogenic acid and neochlorogenic acid) and considerable levels of flavonols, mostly quercetin derivatives.[2−5] As a result of their exceptional polyphenol composition, chokeberries are thought to play an important role in protection against many chronic diseases. There is accumulating evidence that chokeberries or polyphenolic-rich extracts from chokeberries possess cardioprotective, hepatoprotective, antidiabetic, and anticarcinogenic effects.[6−10]

Due to their astringent taste, chokeberries are commonly processed and consumed in various shelf-stable forms including juices, nectars, wines, and liqueurs. Unfortunately, chokeberry processing has been shown to result in marked losses of anthocyanins.[11−13] Several studies have reported on the concentration of polyphenols in chokeberries, pomace, and juice, but results are expressed on a dry weight basis and do not provide any indications of actual concentrations in the three fractions as weights of the berries, juice, and pomace were not measured or taken into account.[3,13]

In addition to anthocyanin losses during juice processing, further extensive losses of anthocyanins during storage have been reported in other anthocyanin-rich berries.[14−16] Anthocyanin losses in these studies were paralleled by increased polymeric color values indicating anthocyanin−tannin polymers were formed during storage; these polymers are resistant to bleaching in the presence of potassium metabisulfite. Because chokeberries are particularly rich in polyphenols, we suspect that similar losses of anthocyanins and possibly other polyphenols occur during juice processing and storage. The objective of this study was to determine the fate of chokeberry anthocyanins, total PACs, hydroxycinnamic acids, and flavonols during various stages of juice processing and throughout 6 months of storage at ambient temperature.

MATERIALS AND METHODS

Frozen Berries. Frozen chokeberries (*A. melanocarpa* cv. Viking) were obtained from Mae's Health and Wellness (Omaha, NE, USA). The berries grown outside Davenport, NE, USA, were harvested at commercial ripeness and frozen within 4 hours after harvest. They were then transported overnight under refrigeration to the University of Arkansas, Food Science Department. Upon receipt, the berries were stored at −20 °C for <2 weeks prior to processing.

Chemicals and Reagents. A mixture of 3-*O*-*β*-glucoside standards of delphinidin, cyanidin, petunidin, peonidin, pelargonidin, and malvidin was obtained from Polyphenols Laboratories (Sandnes, Norway). HPLC grade methanol, acetone, formic acid, and acetic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Chlorogenic acid, rutin, protocatechuic acid, phloroglucinaldehyde, (+)-catechin, 4-dimethylaminocinnamaldehyde, and quercetin were also obtained from Sigma-Aldrich.

Juice Processing. Chokeberries were processed into nonclarified juice as previously described.[14] The juice processing scheme with five sampling points indicated is shown in Figure 1. Frozen berries were

Special Issue: 2013 Berry Health Benefits Symposium

Received: September 24, 2013
Revised: November 22, 2013
Accepted: November 26, 2013
Published: November 26, 2013

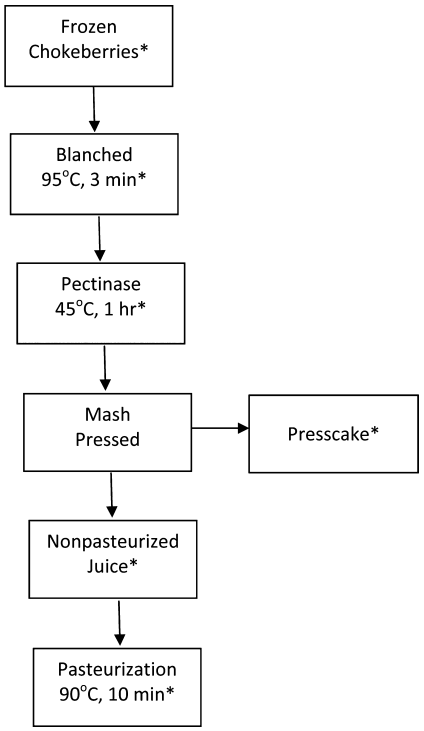

Figure 1. Flowchart of chokeberry juice processing with sampling points indicated by asterisks.

simultaneously heated and mixed with a Mixco Batch mixer (Avon, NY, USA) in a large steam kettle until the berry mash reached a temperature of 95 °C. After 3 min of incubation, the mash was allowed to cool to 40 °C prior to depectinization. Pectinex Smash XXL (Novozyme, Bagsvared, Denmark) was added at a concentration of 0.0827 mL/kg, and the mash was incubated for 1 h at 40 °C. Complete depectinization was verified by the negative alcohol precipitation test where no flocculation of pectin was observed following the addition of 2 volumes of 96% ethanol (v/v) to 1 volume of juice. The mash was then pressed in a 25 L Enrossi bladder press (Enoagricol Rossi, s.r.l., Calzolaro, Italy) operated at 20 psi to separate the juice from the presscake. The nonclarified juice was dispensed into 6 oz glass bottles and heated in a steam box (American Sterilizer Co., Erie, PA, USA) until the juice temperature monitored using a thermocouple reached 90 °C. The bottle caps were tightened, and the juices were allowed to cool overnight. Samples of each juice treatment were stored in the dark at 25 °C. Five samples were taken at each sampling point during the juicing process, and five bottles of juice were sampled after 1, 2, 3, 4, 5, and 6 months of storage at 25 °C.

Extraction of Polyphenols. For isolation of anthocyanins, hydroxycinnamic acids, and flavonols, five samples each of frozen and blanched berries, enzyme-treated mash, and presscake were homogenized with 20 mL of methanol/water/acetic acid (60:37:3, v/v/v) using a EuroTurrax T18 tissuemizer (Tekmar-Dohrman Corp., Mason, OH, USA) and then filtered through MiraCloth (Calbiochem, La Jolla, CA, USA). The residue was collected, the extraction repeated two more times, and the volume of the extracts adjusted to 100 mL with extraction solvent. The same protocol was used to isolate PACs except acetone/water/acetic acid (70:29.5:0.5, v/v/v) was used as extraction solvent. Juice samples were analyzed directly and required no extraction.

HPLC Analysis of Anthocyanins and Hydroxycinnamic Acids. Methanol/water/acetic acid extracts (8 mL) were dried using a SpeedVac concentrator (ThermoSavant, Holbrook, NY, USA) and resuspended in 1 mL of 3% formic acid in water. The reconstituted samples were passed through 0.45 μm PTFE syringe filters prior to HPLC analysis. Anthocyanin monoglycosides and hydroxycinnamic acids were separated by reverse phase HPLC on a Symmetry C_{18} column (Waters Corp., Milford, MA, USA) according to the method described by Cho et al.[17] Anthocyanins were monitored at 520 nm and hydroxycinnamic acids at 320 nm. Anthocyanin glycosides were quantified as cyanidin 3-glucoside equivalents and hydroxycinnamic acids as chlorogenic acid equivalents using external calibration curves of authentic standards. Protocatechuic acid and phloroglucinaldehyde were monitored at 280 nm and quantified using external calibration curves of authentic standards.

HPLC and HPLC-MS Analysis of Flavonols. Methanol/water/acetic acid extracts extracts (8 mL) were dried using a SpeedVac concentrator and resuspended in 1 mL of 50% methanol in water. The reconstituted samples were passed through 0.45 μm PTFE syringe filters prior to HPLC analysis. Individual flavonols were separated by reverse phase HPLC on an Aqua C_{18} column (Phenomenex, Torrance, CA, USA) according to the method described by Cho et al.[18] Flavonol peaks were monitored at 360 nm and quantified as rutin equivalents using external calibration curves of an authentic standard. Quercetin was quantified using an external calibration curve of an authentic standard. The identification of flavonols was performed by HPLC-MS using the HPLC conditions described above, except the HPLC system was interfaced to a Bruker Esquire LC-MS ion trap mass spectrometer (Billerica, MA, USA). Mass spectral analysis was conducted in negative ion electrospray mode using conditions previously described in Cho et al.[18]

Percent Polymeric Color Analysis. Percent polymeric color of methanol/water/acetic acid extracts from samples taken throughout juice processing and juices was determined using the spectrophotometric assay of Giusti and Wrolstad.[19] 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 resistant to bleaching remain colored. The ratio of the absorbance value of the potassium metabisulfite bleached sample to the control sample (nonbleached) × 100 reflects percent polymeric color.

Total Proanthocyanidin Analysis. Total PACs were measured by the 4-dimethylaminocinnamaldehyde (DMAC) assay of Prior et al.[20] The absorbance (640 nm) of samples in 96-well plates was measured every min for 30 min on a BioTek Synergy HT (Winooski, VT, USA) plate reader. Catechin was used as standard with results expressed as milligrams of catechin equivalents per 100 g berry fresh weight.

Calculations. To account for dilution and concentration effects, anthocyanin, flavonol, proanthocyanidin, hydroxycinnamic acid, and phenolic acid concentrations were converted to original berry weight to allow comparison of all samples on an equivalent basis. The following equation was used:

$$C_{berry} = C_{product} \times R$$

C_{berry} = concentration on original berry weight basis, R = mass of the product divided by mass of original berry, and $C_{product}$ = concentration in the product.

Statistical Analysis. The effects of processing and storage on anthocyanin, hydroxycinnamic acid, flavonol, total proanthocyanidin, and phenolic acid concentrations and percent polymeric color were determined by one-way analysis of variance (ANOVA) using JMP 8.0 (Cary, NC, USA). Differences between means (n = 5) were determined by Student's t test (α = 0.05).

RESULTS AND DISCUSSION

Polyphenol Composition of Chokeberries and Juice. Cyanidin 3-galactoside (cyd 3-gal), cyanidin 3-glucoside (cyd 3-glu), cyanidin 3-arabinoside (cyd 3-ara), and cyanidin 3-xyloside (cyd 3-xyl) were identified and quantified by HPLC (Supporting Information Figure S1), and the results are presented in Table 1. These anthocyanins were previously identified in chokeberry juice by HPLC-MS using identical HPLC conditions described above.[21] Cyd 3-gal and cyd 3-ara

Table 1. Concentrations of Anthocyanins, Hydroxycinnamic Acids, and Total Proanthocyanidins (PACs) and Percent Polymeric Color throughout Chokeberry Juice Processing[a]

	anthocyanins (mg/100 g berry FW)					hydroxycinnamic acids (mg/100 g berry FW)				
processing step	cyanidin 3-galactoside	cyanidin 3-glucoside	cyanidin 3-arabinoside	cyanidin 3-xyloside	total	neochlorogenic acid	chlorogenic acid	total	% polymeric color	total PACs (mg/100 g berry FW)
frozen	424.7 ± 9.6 a	19.8 a	154.7 a	20.1 ± 0.9 a	619.2 ± 16.5 a	46.2 ± 1.0 a	70.2 ± 1.9 b	116.4 ± 2.7 b	16.2 b	845.2 ± 25.6 b
blanched	205.5 ± 5.5 b (48%)	10.9 ± 0.3 b (55%)	57.5 ± 1.2 b (37%)	7.3 ± 0.2 b (36%)	281.2 ± 7.2 b (45%)	46.8 ± 1.1 a (101%)	80.2 ± 1.6 a (114%)	127.0 ± 2.6 a (109%)	7.4 e	868.6 ± 7.4 b (103%)
enzyme	190.7 ± 0.8 c (45%)	10.5 ± 0.1 b (53%)	54.2 ± 0.5 b (35%)	6.9 ± 0.0 b (35%)	262.4 ± 1.4 b (42%)	45.1 ± 0.2 a (98%)	77.2 ± 0.4 a (110%)	122.2 ± 0.5 a (105%)	10.1 de	940.7 ± 29.1 a (111%)
juice, NP[b]	77.1 ± 0.7 e (18%)	4.6 ± 0.0 d (23%)	20.4 ± 0.2 d (13%)	2.7 ± 0.0 c (13%)	104.8 ± 1.0 d (17%)	30.1 ± 0.0 b (65%)	47.7 ± 0.1 c (68%)	77.9 ± 0.2 c (67%)	13.4 bc	392.6 ± 8.6 e (62%)
presscake	99.8 ± 1.8 d (23%)	5.4 ± 0.1 c (27%)	29.3 ± 0.6 c (19%)	3.7 ± 0.1 c (18%)	138.3 ± 2.6 c (22%)	14.9 ± 0.1 d (32%)	28.8 ± 0.3 d (41%)	43.7 ± 0.3 d (38%)	12.2 cd	524.2 ± 13.4 c (46%)
juice, P[b]	32.4 ± 0.6 f (8%)	2.3 ± 0.0 e (12%)	6.3 ± 0.1 e (4%)	0.9 ± 0.0 d (4%)	42.0 ± 0.8 e (7%)	27.9 ± 0.7 c (61%)	45.9 ± 1.2 c (65%)	73.9 ± 1.9 c (63%)	29.4 a	464.8 ± 20.8 d (55%)

[a]Mean values (n = 5) within a column with different letters are significantly different ($p \leq 0.05$). Values in parentheses represent percent retention compared with frozen berries. [b]NP, nonpasteurized; P, pasteurized.

were the major anthocyanins in the chokeberry samples and juices, confirming previous results.[1−4] Two hydroxycinnamic acids, neochlorogenic acid (NCA) and chlorogenic acid (CA), were present in the berries and juices (Supporting Information Figure S2 and Table 1). The high concentrations of NCA and CA in chokeberry samples and juices confirm previous findings.[3,4] Eight flavonols were identified by HPLC and HPLC-MS (Supporting Information Figure S3) and included the following derivatives of quercetin: two dihexosides (m/z 625/301), vicianoside (arabinosyl-glucoside) (m/z 595/301), robinobioside (rhamnosyl-galactoside) (m/z 609/301), rutinoside (rhamnosyl-glucoside) (m/z 609/301), galactoside (m/z 463/301), glucoside (m/z 463/301), and aglycone (m/z 301). All of these compounds have previously been identified in chokeberries.[4,5] The quantities of individual flavonols in samples taken throughout juice processing are presented in Table 2. The relative abundance of quercetin derivatives in frozen berries was similar to findings of Mikulic-Petkovsek,[5] who reported that galactoside was the predominant quercetin derivative followed by glucoside, rutinoside, vicianoside, robinobioside, and dihexosides. The amount of total flavonols in frozen chokeberries in our study, 33.9 mg/100 g FW, falls within the range of values (26−71 mg/100 g FW) previously reported for fresh chokeberries.[4,5]

Processing Changes in Chokeberry Polyphenols and Percent Polymeric Color. *Anthocyanins.* Anthocyanins were readily degraded during the two thermal treatments, blanching and pasteurization, as well as physical removal of the presscake (Table 1). Cyd pentosides (ara and xyl) were more susceptible to degradation during blanching (63 and 64% losses, respectively) than cyd hexosides (gal and glu, 52 and 45% losses, respectively). A similar trend was observed in response to pasteurization with cyd 3-ara and cyd 3-xyl, each incurring 69% losses, whereas cyd 3-gal and cyd 3-glu showed 58 and 50% losses, respectively, compared with levels found in nonpasteurized juice. Our results are consistent with previous studies reporting anthocyanin hexosides to be more stable than pentosides.[12,21,22] Following pasteurization, the juices contained only 8, 12, 4, 4, and 7% of the levels of cyd 3-gal, cyd 3-glu, cyd 3-ara, cyd 3-xyl, and total anthocyanins found in frozen chokeberries. A major loss of anthocyanins also occurred during the pressing operation when presscake containing seeds and skins was removed from the mash. The presscake contained 52, 51, 54, and 54% of the levels of cyd 3-gal, cyd 3-glu, cyd 3ara, and cyd 3-xyl found in the enzyme-treated mash, respectively, with most of the balance transferred to the juice. These results indicate that chokeberry presscake is an excellent source of anthocyanins and may be useful as a substrate for recovering natural pigments. The anthocyanin losses observed in response to thermal treatments were much higher than those previously reported for blueberry,[14] blackberry,[15] and black raspberry[19] juices processed under similar conditions. The extensive anthocyanin losses observed in this study could be due to several factors. It is clear that thermal treatments had a detrimental effect on anthocyanins. We observed significant increases in two HPLC peaks following blanching that were identified as protocatechuic acid (PCA) and phloroglucinaldehyde (PGA) (Figure 2), and the concentrations of these two compounds increased upon thermal treatments (Figure 3). Levels of PCA and PGA increased by 343 and 400% after blanching, by 30 and 24% from blanched berries to enzyme treated mash, and by 29 and 7% from nonpasteurized to pasteurized juice. The formation of PCA and PGA in response

Table 2. Concentrations of Flavonols throughout Chokeberry Juice Processing[a]

	flavonols (mg/100 g berry FW)								
processing step	quercetin 3-dihexoside	quercetin 3-dihexoside	quercetin 3-vicianoside	quercetin 3-robinobioside	quercetin 3-rutinoside	quercetin 3-galactoside	quercetin 3-glucoside	quercetin	total
frozen	3.0 ± 0.2 b	1.4 ± 0.1 b	4.0 ± 0.2 b	3.5 ± 0.2 a	3.9 ± 0.1 b	10.6 ± 0.5 a	7.6 ± 0.3 a	0.74 ± 0.0 a	34.7 ± 1.4 a
blanched	3.6 ± 0.1 a (120%)	1.6 ± 0.0 a (114%)	4.5 ± 0.1 a (115%)	3.1 ± 0.1 b (89%)	4.1 ± 0.1 ab (105%)	10.2 ± 0.2 a (96%)	7.2 ± 0.1 a (95%)	0.66 ± 0.0 b (89%)	35.0 ± 0.6 a (101%)
enzyme	4.1 ± 0.2 a (137%)	1.7 ± 0.0 a (121%)	4.8 ± 0.0 a (123%)	3.3 ± 0.0 ab (94%)	4.3 ± 0.0 a (110%)	10.6 ± 0.1 a (100%)	7.5 ± 0.1 a (99%)	0.71 ± 0.0 a (96%)	36.9 ± 0.2 a (106%)
juice, NP[b]	2.2 ± 0.0 c (73%)	1.0 ± 0.0 c (71%)	3.1 ± 0.0 c (79%)	2.1 ± 0.0 c (60%)	2.8 ± 0.0 c (72%)	5.7 ± 0.0 b (54%)	4.2 ± 0.0 b (55%)	0.19 ± 0.0 d (26%)	21.3 ± 0.0 b (61%)
presscake	1.6 ± 0.0 d (53%)	0.7 ± 0.0 d (50%)	1.9 ± 0.0 e (49%)	1.4 ± 0.0 e (40%)	1.7 ± 0.0 e (44%)	5.2 ± 0.1 bc (49%)	3.6 ± 0.0 c (47%)	0.66 ± 0.0 b (89%)	16.7 ± 0.2 c (48%)
juice, P[b]	2.3 ± 0.3 c (77%)	0.9 ± 0.1 cd (64%)	2.6 ± 0.2 d (67%)	1.7 ± 0.2 d (49%)	2.3 ± 0.2 d (59%)	4.5 ± 0.4 c (42%)	3.5 ± 0.3 c (46%)	0.31 ± 0.0 c (42%)	18.2 ± 1.8 c (52%)

[a]Mean values (n = 5) within a column with different letters are significantly different ($p \leq 0.05$). Values in parentheses represent percent retention compared with frozen berries. [b]NP, nonpasteurized; P, pasteurized.

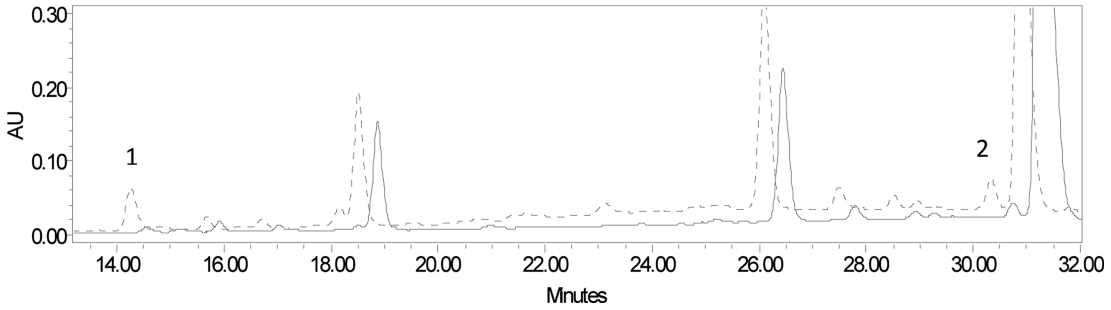

Figure 2. HPLC chromatogram (260 nm) of frozen (solid line) and blanched (dashed line) chokeberry extracts. Peaks: 1, protocatechuic acid; 2, phloroglucinaldehyde.

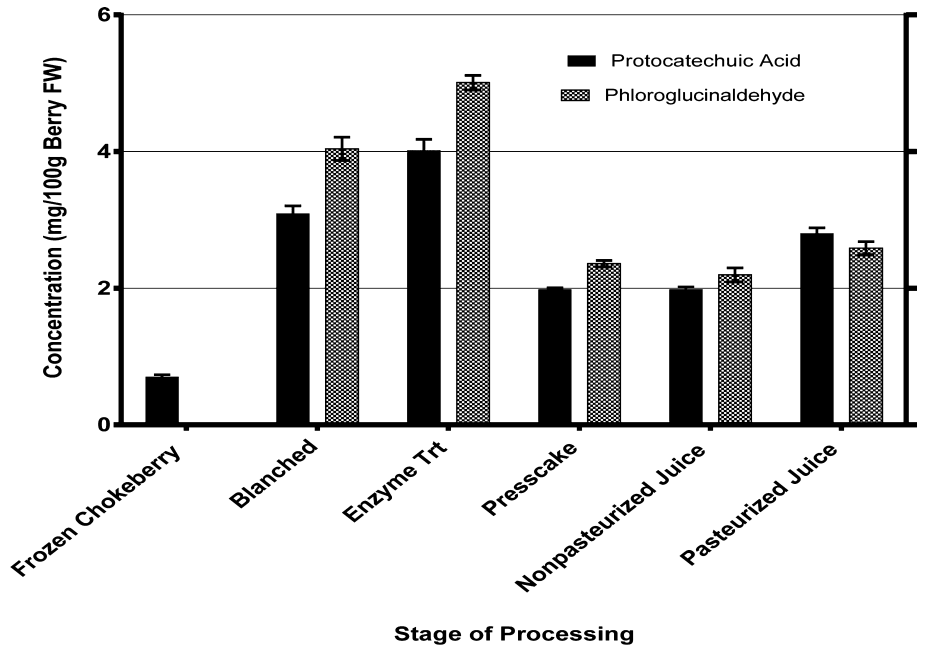

Figure 3. Concentrations of protocatechuic acid and phloroglucinaldehyde throughout chokeberry juice processing. Bars represent standard errors of the mean (n = 5). Bars with different letters are significantly different ($p \leq 0.05$).

to thermal treatments is consistent with the scheme of cyanidin 3-glycoside degradation proposed by Sadilova et al.[23] According to Sadilova et al.,[23] the first step of pH-dependent thermal degradation of cyanidin at pH 3.5, which is similar to the pH of chokeberries used in this study, involves opening of the pyrilium ring and chalcone glycoside formation. The next step involves deglycosylation to yield chalcone, which degrades quickly in the presence of heat to protocatechuic acid, 4-hydroxybenzoic acid, and phloroglucinaldehyde. We did not detect 4-hydroxybenzoic acid in our chromatograms, which is consistent with results reported for heated elderberry extracts that are also rich in cyd glycosides.[23] However, the increases in

dx.doi.org/10.1021/jf404281n | J. Agric. Food Chem. 2014, 62, 4018−4025

Table 3. Concentrations of Anthocyanins, Hydroxycinnamic Acids, and Total Proanthocyanidins (PACs) and Percent Polymeric Color throughout Chokeberry Juice Storage at 25 °C[a]

storage time (months)	anthocyanins (mg/100 g berry FW)					hydroxycinnamic acids (mg/100 g berry FW)			% polymeric color	total PACs (mg/100 g berry FW)
	cyanidin 3-galactoside	cyanidin 3-glucoside	cyanidin 3-arabinoside	cyanidin 3-xyloside	total	neochlorogenic acid	chlorogenic acid	total		
1	22.2 ± 0.8 a (69%)	1.6 ± 0.1 a (70%)	4.1 ± 0.21 a (65%)	0.58 ± 0.0 a (64%)	28.6 ± 1.1 a (68%)	27.5 ± 1.1 a (99%)	45.2 ± 1.7 a (98%)	72.7 ± 2.8 a (98%)	34.8 d	447.8 ± 8.7 ab (97%)
2	18.1 ± 0.3 b (56%)	1.3 ± 0.0 b (57%)	3.3 ± 0.1 b (52%)	0.51 ± 0.0 b (57%)	23.3 ± 0.4 b (55%)	27.9 ± 0.6 a (100%)	46.3 ± 1.2 a (101%)	74.3 ± 1.8 a (101%)	34.4 d	450.8 ± 10.1 a (96%)
3	15.1 ± 0.1 c (47%)	1.1 ± 0.0 c (48%)	2.7 ± 0.0 c (43%)	0.43 ± 0.0 c (48%)	19.3 ± 0.2 c (46%)	28.3 ± 0.2 a (101%)	47.0 ± 0.5 a (102%)	75.3 ± 0.7 a (102%)	37.5 c	425.7 ± 14.8 abc (92%)
4	10.0 ± 0.6 d (31%)	0.7 ± 0.0 d (30%)	1.8 ± 0.1 d (29%)	0.30 ± 0.0 d (33%)	12.8 ± 0.8 d (30%)	22.9 ± 1.2 b (82%)	37.7 ± 2.0 b (82%)	60.6 ± 3.2 b (82%)	40.4 b	413.2 ± 13.8 bc (89%)
5	5.5 ± 1.2 e (17%)	0.5 ± 0.1 e (22%)	1.0 ± 0.2 e (16%)	0.22 ± 0.0 e (24%)	7.3 ± 0.4 e (17%)	18.9 ± 2.6 c (68%)	31.2 ± 4.4 bc (68%)	50.1 ± 7.0 bc (68%)	39.9 b	409.9 ± 12.8 c (88%)
6	5.6 ± 0.3 e (17%)	0.4 ± 0.0 e (17%)	1.0 ± 0.0 e (16%)	0.21 ± 0.0 e (23%)	7.2 ± 1.5 e (17%)	18.4 ± 1.1 c (66%)	30.4 ± 1.8 c (66%)	48.9 ± 2.9c (66%)	44.5 a	406.0 ± 9.2 c (87%)

[a]Mean values (n = 5) within a column with different letters are significantly different ($p \leq 0.05$). Values in parentheses represent percent retention compared with pasteurized juice.

cyd degradation products PCA and PGA during processing accounted for only about 3% of the losses in total anthocyanins that occurred during processing. The presence of ascorbic acid and pro-oxidant metals in the chokeberries may have played a role in anthocyanin degradation.[24] Enzymes such as polyphenol oxidase and peroxidase are also reported to cause degradation of anthocyanins,[25−27] but the frozen chokeberries used in this study were immediately blanched to inactivate enzymes. The extensive loss of anthocyanins during blanching was most likely due to the long time it took the 23 kg batch of frozen chokeberries to heat to the blanching temperature of 95 °C and then cool to 40 °C. In an additional experiment involving a 5.4 kg batch of chokeberries, in which it took 12 min for the frozen berries to heat to 95 °C and 3 min to cool to 40 °C, we observed a 25% loss of anthocyanins. Because chokeberry anthocyanins appear to be very heat labile, use of a blanching treatment prior to depectinization may be counterproductive, especially if the berries contain low polyphenol oxidase activity.

Percent Polymeric Color. Percent polymeric color values decreased in response to blanching and enzyme treatments, which may indicate cleavage of some anthocyanin−tannin linkages (Table 1). The major change in polymeric color occurred when juice was pasteurized, from 13.4% in non-pasteurized juice to 29.4% in pasteurized juice. According to Kunsagi-Mate et al.[28] the formation of anthocyanin−tannin polymers is slow at room temperature due to the high activation energy of the reaction, but is accelerated at elevated temperatures. Hence, the increased polymeric color values observed following pasteurization may reflect higher levels of anthocyanin−tannin polymers, which may account for some of the losses of monomeric anthocyanins.

Hydroxycinnamic Acids. The level of NCA did not change in response to blanching, whereas the level of CA increased by 14%, indicating CA was not completely extracted from frozen berries (Table 1). Significant losses of HCAs occurred during the pressing operation. The presscake contained 33 and 37% of the levels of NCA and CA found in enzyme-treated mash, respectively, whereas the NP juice contained 67 and 62% of NCA and CA, respectively. Levels of NCA and CA were minimally affected by pasteurization with CA and total HCAs showing no losses and NCA showing a 7% loss compared with the value for NP juice. Following pasteurization, the juices contained 60, 65, and 63% of the levels of NCA, CA, and total HCAs found in frozen chokeberries, respectively.

Total Proanthocyanidins. The levels of total PACs were stable upon blanching, but increased by 11% following the enzyme treatment (Table 1). Disruption of cell wall polysaccharides presumably allowed for enhanced extraction of the PACs following enzyme treatment. The major loss of total PACs occurred during the pressing operation. The presscake contained 60% of the levels of total PACs found in enzyme-treated mash, whereas the NP juice contained 46%. Surprisingly, PACs increased 17% in response to pasteurization. This may be due to disruption of cell wall polysaccharide−proanthocyanidin complexes, which allowed more PACs to react with the DMAC reagent. It is also possible that pasteurization resulted in depolymerization of large molecular weight PACs to monomers, which are reported to react more readily with the DMAC reagent than polymers.[20]

Flavonols. Levels of quercetin dihexosides, vicianoside, robinobioside, and rutinoside showed modest increases (11−20%) in response to either blanching or enzyme treatment, compared with values for frozen berries, whereas levels of the

Table 4. Concentrations of Flavonols throughout Chokeberry Juice Storage at 25 °C[a]

	flavonols (mg/100 g berry FW)								
storage time (months)	quercetin 3-dihexoside	quercetin 3-dihexoside	quercetin 3-vicianoside	quercetin 3-robinobioside	quercetin 3-rutinoside	quercetin 3-galactoside	quercetin 3-glucoside	quercetin	total
1	2.3 ± 0.2 a (100%)	0.9 ± 0.0 a (100%)	2.9 ± 0.1 a (112%)	2.0 ± 0.1 a (118%)	2.7 ± 0.1 a (117%)	5.0 ± 0.2 a (111%)	3.9 ± 0.2 a (111%)	0.35 ± 0.0 b (113%)	19.7 ± 0.9 ab (108%)
2	2.4 ± 0.1 a (104%)	0.9 ± 0.0 a (100%)	3.1 ± 0.0 a (119%)	2.0 ± 0.0 a (118%)	2.8 ± 0.0 a (122%)	5.2 ± 0.0 a (116%)	4.0 ± 0.0 a (114%)	0.39 ± 0.0 ab (126%)	20.9 ± 0.2 a (115%)
3	2.3 ± 0.1 a (100%)	1.0 ± 0.0 a (111%)	3.2 ± 0.0 a (123%)	2.1 ± 0.0 a (124%)	2.9 ± 0.0 a (126%)	5.3 ± 0.0 a (118%)	4.1 ± 0.0 a (117%)	0.40 ± 0.0 a (129%)	21.3 ± 0.2 a (117%)
4	2.1 ± 0.1 a (91%)	1.0 ± 0.0 a (111%)	3.1 ± 0.0 a (119%)	2.1 ± 0.0 a (124%)	2.9 ± 0.0 a (126%)	5.2 ± 0.0 a (116%)	4.1 ± 0.0 a (117%)	0.40 ± 0.0 a (129%)	20.9 ± 0.1 a (115%)
5	1.6 ± 0.2 b (70%)	0.8 ± 0.1 b (89%)	2.5 ± 0.3 b (96%)	1.7 ± 0.2 b (100%)	2.4 ± 0.3 b (104%)	4.2 ± 0.5 b (93%)	3.3 ± 0.3 b (94%)	0.35 ± 0.0 b (113%)	16.7 ± 1.8 b (92%)
6	1.5 ± 0.2 b (65%)	0.8 ± 0.1 b (89%)	2.5 ± 0.3 b (96%)	1.6 ± 0.2 c (94%)	2.4 ± 0.3 b (104%)	4.1 ± 0.4 b (91%)	3.2 ± 0.3 b (91%)	0.35 ± 0.0 b (113%)	16.5 ± 1.8 b (91%)

[a]Mean values (n = 5) within a column with different letters are significantly different ($p \leq 0.05$). Values in parentheses represent percent retention compared with pasteurized juice.

two major quercetin conjugates, galactoside and glucoside, and total flavonols were stable (Table 2). Significant losses of flavonol glycosides occurred during the pressing operation, with 39−49% of the compounds being retained in the presscake, whereas 54−64% were expressed into the juice. The less polar quercetin hexosides (galactoside and glucoside) were retained to a greater extent in the presscake (48 and 49%) than the more polar quercetin diglycosides (39 and 41%). Additionally, the majority of the nonpolar quercetin aglycone was retained in the presscake, whereas only 27% was expressed into the juice. Pasteurization did not affect levels of the two quercetin hexosides, but other derivatives decreased by 16−21% in response to pasteurization. Although present at low level, the concentration of quercetin aglycone increased by 63% in response to pasteurization, indicating that some sugar conjugates were cleaved from the quercetin glycosides in response to thermal treatment. Overall, total flavonol levels decreased by 15% in response to pasteurization.

Storage Changes in Chokeberry Polyphenols and Percent Polymeric Color. *Anthocyanins.* Levels of cyd 3-gal, cyd 3-glu, cyd 3-ara, cyd 3-xyl, and total anthocyanins in juices declined in a linear fashion over 1−5 months of storage at 25 °C, but levels appeared to stabilize from 5 to 6 months (Table 3). After 6 months of storage, the juices lost 75, 75, 76, 64, and 75% of the levels of cyd 3-gal, cyd 3-glu, cyd 3-ara, cyd 3-xyl, and total anthocyanins, respectively, found in juices after 1 month of storage. These results are consistent with other studies reporting marked losses of anthocyanins during ambient-temperature storage of chokeberry juice,[21,29] nectars, and purees.[11] The type of sugar attached had little effect on anthocyanin stability during long-term storage, which contrasts with results observed in response to thermal treatments during processing, where hexosides showed greater retention than pentosides (Table 1). Consistent with our findings, Hellstrom et al.[29] reported no differences in stability of chokeberry anthocyanin glycosides stored at 4, 9, and 21 °C over 12 weeks of storage. The mechanisms responsible for anthocyanin degradation during storage have not been well elucidated, but may be associated with water nucleophilic attack at the 2-position of the anthocyanin nucleus, resulting in decolorization,[30] or the formation of anthocyanin−tannin polymers.[31] Levels of PCA and PGA were stable for up to 3 months of storage and then declined about 30% from 3 to 6 months of storage (data not shown), indicating that anthocyanins were not degraded into phenolic acids during ambient temperature storage.

Percent Polymeric Color. Percent polymeric color values of the juices increased (from 34.8 to 44.5%) from 1 to 6 months of storage, and these values were inversely correlated with anthocyanins (r = −0.81) (Table 3). These changes indicate that some losses of anthocyanins throughout storage may be due to the formation of anthocyanin−epicatechin polymers that are resistant to bleaching by potassium metabisulfite in the polymeric color assay. However, it is also possible that the anthocyanin−epicatechin polymers formed in the juices during processing were more resistant to degradation during storage. The anthocyanin−epicatechin polymers appeared to play an important role in color stability as the juices retained a dark purple color despite marked losses of monomeric anthocyanins.

Hydroxycinnamic Acids. In contrast to anthocyanins, levels of NCA, CA, and total HCAs were stable from 1 to 3 months of storage (Table 3), but levels declined by about 30% from 3 to 6 months of storage. Levels of chlorogenic acid in blueberry and chokeberry juices were also found to be relatively stable over long-term storage at ambient temperature.[29,32]

Total Proanthocyanidins. Levels of total PACs were stable (>90% retention) from 1 to 6 months of storage (Table 3). The stability of PACs over storage contrasts with the marked decline in anthocyanins and increase in percent polymeric color values observed from 1 to 6 months of storage. These results indicate that PACs did not react with anthocyanins to any great extent to form polymeric pigments throughout storage. This finding supports the hypothesis that polymeric pigments formed during processing are more resistant to degradation than monomeric anthocyanins and play an important role in color stability.

Flavonols. Quercetin glycosides and aglycone were stable from 1 to 4 months of storage at 25 °C, but levels decreased by 17−29% from 4 to 6 months (Table 4). Total flavonol levels decreased by 21% from 4 to 6 months of storage at 25 °C. Levels of quercetin followed the same trend as quercetin glycosides, indicating that losses of glycosides from 4 to 6 months of storage were not due to cleavage of sugar moieties. The losses of flavonols late during storage may be due to physical binding to insoluble solids, resulting in precipitation, or oxidation to quinones.

In summary, juice processing had a much greater effect on polyphenol losses in chokeberries than did storage of juices at 25 °C. Anthocyanins were more susceptible to losses during

processing than flavonols, total PACs, and hydroxycinnamic acids as a result of thermal degradation, evident by increased levels of protocatechuic acid and phloroglucinaldehyde, and polymerization, evident by increased polymeric color values. The juice pressing step resulted in losses of all polyphenols due to physical removal of skins, but anthocyanins and total PACs were retained in the presscake to a greater extent than hydroxycinnamic acids and flavonols. Flavonols, total PACs, and hydroxycinnamic acids were well retained over 6 months of storage at 25 °C compared with anthocyanins, which degraded in a linear manner up to 5 months. Losses of anthocyanins during storage were accompanied by increased polymeric color values, indicating that the small amounts of anthocyanins remaining after long-term storage were present largely in polymeric forms. The polymeric forms of anthocyanins appear to be more stable than monomeric anthocyanins and likely play an important role in color stability. Novel, cost-effective treatments are needed to stabilize anthocyanins during chokeberry juice processing and storage.

■ ASSOCIATED CONTENT

Supporting Information

HPLC chromatograms used to identify and quantify anthocyanins (Figure S1), hydroxycinnamic acids (Figure S2), and flavonols (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Mae's Health and Wellness for providing the frozen chokeberries used in this study.

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