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Harvest date affects aronia juice polyphenols, sugars, and antioxidant activity, but not anthocyanin stability

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ABSTRACT

The goal of this work was to characterize how the date of harvest of 'Viking' aronia berry impacts juice pigmentation, sugars, and antioxidant activity. Aronia juice anthocyanins doubled at the fifth week of the harvest, and then decreased. Juice hydroxycinnamic acids decreased 33% from the first week, while proanthocyanidins increased 64%. Juice fructose and glucose plateaued at the fourth week, but sorbitol increased 40% to the seventh harvest week. Aronia juice pigment density increased due to anthocyanin concentration, and polyphenol copigmentation did not significantly affect juice pigmentation. Anthocyanin stability at pH 4.5 was similar between weeks. However, addition of quercetin, sorbitol, and chlorogenic acid to aronia anthocyanins inhibited pH-induced loss of color. Sorbitol and citric acid may be partially responsible for weekly variation in antioxidant activity, as addition of these agents inhibited DPPH scavenging 13–30%. Thus, aronia polyphenol and non-polyphenol components contribute to its colorant and antioxidant functionality.

1. Introduction

Anthocyanin-rich berries are an important food and ingredient source. Their demand is driven by taste, the apparent health-promoting activities of polyphenols and fiber, and also by their functionality as colorants, antioxidants, and flavors. Providing standardized berry ingredients and foods to meet formulation demands is challenging because of the diverse polyphenol composition of berries and natural variability in phytochemicals arising from horticultural practices and climate. While the effect of the ripening process on berry polyphenol content has been established for North American strawberries, blueberries, raspberries, and

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others, less is known about the impact of ripening on other berries, such as aronia berries (Wang & Lin, 2000).

While aronia berries are primarily cultivated in Poland and other European countries, demand and production of aronia berries are increasing in North America (Brand, 2010). Aronia berries are consumed fresh, juiced, or processed further for jams, juice blends, extracts, or food colorants. Cultivated aronia berries are primarily *Aronia mitschurinii* 'Viking', but *Aronia melanocarpa*, *Aronia arbutifolia*, and *Aronia prunifolia* berries are native to North America (Brand, 2010). Aronia berries are rich in cyanidin anthocyanins, chlorogenic acids, and proanthocyanidins; and also contain quercetin flavonols (Taheri, Connolly, Brand, & Bolling, 2013). These polyphenols contribute to the high *in vitro* antioxidant activity of aronia extracts (Jakobek, Šeruga, & Krivak, 2011; Oszmiański & Wojdylo, 2005). Likewise, they apparently contribute to anti-inflammatory activity, modulation of antioxidant enzymes, and also modulate lipid metabolism (Kim, Park, Wegner, Bolling, & Lee, 2013; Martin et al., 2014). Aronia berries are unique among other berries in that they maintain apparent ripeness for up to 7 weeks on the plant, whereas other berries have shorter, highly-defined peak ripeness. Thus, data about the extent that aronia polyphenols and other components such as sugars and acids, vary during harvest are expected to improve horticultural practices and formulation with aronia ingredients and juices.

The objectives of the present study were to (1) characterize the change in 'Viking' aronia polyphenols, sugars, and acids during a 7 week harvest period; (2) determine the potential impact of these changes on aronia pigmentation and antioxidant activity; and (3) develop strategies to enhance the use of aronia as a natural colorant.

2. Materials and methods

2.1. Chemicals and reagents

Dimethyl sulfoxide (DMSO), ellagic acid, trans-stilbene, β-cyclodextrin, quercetin, chlorogenic acid, trans-ferulic acid, reduced L-glutathione, polydatin, and caffeic acid were from Sigma–Aldrich (St. Louis, MO, USA). Ascorbic acid was from Acros Organics (Geel, Belgium). Citric acid and LC–MS grade solvents were from Fisher (Fairlawn, NJ, USA). (+)-Catechin monohydrate was from Enzo Life Sciences (Farmingdale, NY, USA). Daidzein and genistein were from LC laboratories (Woburn, MA, USA). Aronia extract was a commercial hydroethanolic spray dried powder of aronia, obtained from Artemis International (Fort Wayne, IN, USA).

2.2. Aronia cultivation, harvest, and juice preparation

A. mitschurinii 'Viking' plants were grown in Storrs, CT and maintained by Dr. Brand. Aronia plants were 3 y old and conventionally cultivated in a plot of ~600 plants. Prior to harvest, 10 blocks of 5 plants each were randomized. Beginning at the first signs of ripening, 5 blocks were harvested weekly beginning on Aug 1, 2012 and ending on Sept 12, 2012, representing berries from 50 plants at each week (wk). Berries prior to Aug 1, 2012 were unripe and unsuitable for juicing. Within a wk after the last harvest date, the majority of berries were shriveled on the plant. Immediately after harvest, the aronia berries were manually removed from stems, washed in cold water, surface dried for ~2 h at ambient temperature, and stored in a −20 °C freezer until juicing. Within 1 month of the last harvest, ~1 kg from each of the 5 blocks were thawed, macerated, and pressed using an apple cider press, creating a composite juice sample for each wk. Juice aliquots were frozen within 1 h of processing and stored at −80 °C until analysis.

2.3. Quantification of polyphenols

Aronia juice polyphenols were quantified by UHPLC–UV–MS analysis as previously described with slight modifications (Taheri et al., 2013). Briefly, a Shimadzu Nexera UHPLC was equipped with binary pumps, an autosampler set to 4 °C, column oven, diode array detector, and LCMS 2020 single quadrupole mass spectrometer operating in DUIS mode with detector settings as previously described (Taheri et al., 2013). The aronia juice was thawed, centrifuged, diluted 10-fold with 30% methanol in water, and injected in 1 μL volumes onto a Kinetex PFP 2.1 × 50 mm, 1.7 μm, 100A column (Phenomenex Inc., Torrance, CA). A gradient of 0.5% formic acid in water (A) and 100% methanol (B) was used at a total flow rate of 0.2 mL/min to resolve aronia polyphenols. The gradient consisted of 70% A in 30% B from 0 to 6 min, then a linear gradient to 55% B at 12 min, then descending to 30% B at 14 min, holding 30% B to 16 min to allow for column equilibration. Cyanidin-3-galactoside (Cy3Gal) + cyanidin-3-glucoside (Cy3Glu), cyanidin-3-arabinoside (Cy3A), and cyanidin-3-xyloside (Cy3X), were identified by MS analysis and quantified at 520 nm using Cy3Gal as a standard. Anthocyanin content was expressed as Cy3Gal equivalents. The limit of detection (LOD) for anthocyanins was 1.2 ng on column (OC) and the limit of quantitation (LOQ) was 1.4 ng OC. Chlorogenic acid (Cga), neochlorogenic acid (nCga), quercetin-3-galactoside (Q3Gal), quercetin-3-glucoside (Q3Glu), and quercetin-3-rutinoside (Q3R) were identified and quantified by MS of polyphenol $[M-H]^-$ in negative operating mode as previously described (Taheri et al., 2013). LOD and LOQ were determined in serially diluted standard solutions, with the LOQ for Q3Gal, Q3Glu, and rutin were 0.5, 0.5, and 0.4 ng OC, respectively. LOD for flavonols was 0.4 ng OC. LOD for hydroxycinnamic acids was 0.5 ng OC. LOQ for Cga and nCga were 0.7 and 0.6 ng OC respectively. Representative chromatograms of aronia juice polyphenols are available in the Supplementary data.

The Folin–Ciocalteu assay was used to determine total phenol content of aronia juice based on a method by Singleton, Orthofer, and Lamuela-Ravent (1999), and as previously described (Bolling, Chen, & Chen, 2013). Aronia juice was diluted 10-fold in ultrapure water and total phenol was quantitated as gallic acid equivalents. Aronia juice proanthocyanidins were determined by reaction with 4-(dimethylamino)cinnamaldehyde (DMAC) using a method previously validated for cranberry analysis (Prior et al., 2010). Aronia juice was diluted 100-fold with acetone/water/acetic acid solution (70/29.5/0.5) and quantitated in a microplate reader at 15 min at 640 nm as (+)-catechin equivalents.

2.4. Analysis of °Brix, acidity, and sugars

°Brix of aronia juice was determined using a handheld refractometer. Acid content was determined by titration of juice with a standardized base, using citric acid equivalents based on AOAC Official Method 942.15. pH values were determined using an Acumet AB15 pH meter (Fisher Scientific, Pittsburgh, PA, USA). Sugar and sugar alcohol were analyzed by a commercial laboratory using high performance anion exchange chromatography with pulsed amperometric detection and GC methods (Covance Laboratories, Madison, WI, USA).

2.5. Analysis of aronia juice and extract pigmentation

Spectrophotometry was performed in quartz cuvettes using a Beckman Coulter DU800 spectrophotometer (Indianapolis, IN, USA). Polymeric anthocyanins were determined in juice as previously described (Wrolstad, 1993). Briefly, aronia juice was diluted with a 20% potassium metabisulphite solution until its absorbance

was less than 1 at 420 nm and 520 nm. The percent polymeric color of juice was determined by the following equations:

$$\text{Color density} = [(\text{Abs}_{520\text{nm}} - \text{Abs}_{700\text{nm}}) + (\text{Abs}_{420\text{nm}} - \text{Abs}_{700\text{nm}})] \times \text{DF without potassium metabisulphite.} \quad (1)$$

$$\text{Polymeric color} = [(\text{Abs}_{520\text{nm}} - \text{Abs}_{700\text{nm}}) + (\text{Abs}_{420\text{nm}} - \text{Abs}_{700\text{nm}})] \times \text{DF with potassium metabisulphite.} \quad (2)$$

$$\%\text{Polymeric color} = (\text{polymeric color/color density}) \times 100 \quad (3)$$

For spectral analysis of pigmentation, aronia juice was diluted in buffers at pH 1.0, 3.5, 4.5, 7.0, and 8.1 as previously described (Fossen, Cabrita, & Andersen, 1998). Loss of aronia pigmentation was modeled at pH 4.5 using an anthocyanin-rich aronia extract. Briefly, 30 mg aronia extract was diluted with 20 mL 50% acidic methanol (0.05% formic acid in 50:50 methanol:water). Additives were prepared in DMSO at 2–8 mM, and 5 μL of DMSO was added to 5 μL of the aronia extract solution in wells of a 96-well microtiter plate. Pigmentation loss was initiated by the addition of 250 μL of pH 4.5 buffer. Thus, final assay conditions were 28.8 μg aronia extract/mL with 40–120 μM of additives. Controls contained equivalent acidified methanol and DMSO without further additives and were run concurrently, in parallel. The absorbance of control and additives were monitored at 540 nm in 15 s increments with 2 s mixing between reads for 5 min using a Molecular Devices SpectraMax M2 spectrophotometer (Sunnyvale, CA, USA) set to "Kinetic Mode."

Pigmentation loss was modeled by non-linear regression using GraphPad Prism 5 (La Jolla, CA, USA). Absorbance values were normalized to a blank well reading, and subsequently modeled by one decay phase (Eq. (4)):

$$Y = (Y_0 - \text{plateau}) \times e^{(-K \times X)} + \text{plateau} \quad (4)$$

where X is time, Y represents the absorbance at 540 nm, Y_0 represents the absorbance at 540 nm at time 0, the plateau represents the absorbance at infinite time, and K represents the rate constant.

2.6. Determination of antioxidant activity

Antioxidant activity of aronia juice was determined by the FRAP (Ferric Reducing Antioxidant Power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays, as previously described with slight modification (Bolling et al., 2013). For FRAP analysis, aronia juice was diluted 100-fold in water. FRAP values were expressed as Trolox

Table 1
Variability of antioxidant activity, acids, and sugars of aronia juice by harvest week.

Determination	Harvest week							ANOVA *P*-value
	1	2	3	4	5	6	7	
pH[A]	3.45 ± 0.07	3.40 ± 0.14	3.30 ± 0.00	3.40 ± 0.00	3.15 ± 0.07	3.40 ± 0.14	3.40 ± 0.28	0.4387
Acidity (%)[A]	1.15 ± 0.01^{f}	1.12 ± 0.01^{d}	1.14 ± 0.01^{e}	1.22 ± 0.00^{g}	1.03 ± 0.00^{c}	0.85 ± 0.00^{a}	0.91 ± 0.00^{b}	<0.0001
Brix (°)[A]	10.5 ± 0.0^{b}	7.8 ± 0.6^{a}	11.2 ± 0.3^{b}	12.7 ± 0.6^{c}	11.0 ± 0.5^{b}	13.3 ± 0.3^{cd}	14.3 ± 0.3^{d}	<0.0001
Brix:acid	9.1	7.0	9.8	10.4	10.6	15.6	15.7	ND[C]
Fructose (g/kg)[B]	22.0	24.0	26.0	31.0	31.0	31.0	32.0	ND
Glucose (g/kg)[B]	22.0	25.0	28.0	33.0	33.0	33.0	34.0	ND
Sorbitol (g/kg)[B]	27.4	32.4	37.0	43.4	45.3	44.6	48.1	ND
FRAP (mg TE/mL)[A]	6.79 ± 0.34^{a}	8.46 ± 0.67^{b}	8.19 ± 0.06^{ab}	9.93 ± 0.28^{cd}	9.18 ± 0.11^{bcd}	8.70 ± 0.00^{bc}	10.41 ± 0.51^{d}	0.0003
DPPH IC_{50} (% juice)[A]	0.21 ± 0.02^{ab}	0.27 ± 0.01^{b}	0.76 ± 0.05^{c}	0.18 ± 0.00^{a}	0.15 ± 0.00^{a}	0.20 ± 0.00^{ab}	0.13 ± 0.00^{a}	<0.0001

[A] Data are mean ± standard deviation of at least duplicate determinations of a composite juice sample prepared for each harvest week. Values within rows bearing different letters are statistically significant by ANOVA and Tukey's multiple comparison test ($P < 0.05$).
[B] Data are a single determination of a composite juice sample by a commercial laboratory, as described in materials and methods.
[C] ND: not determined.

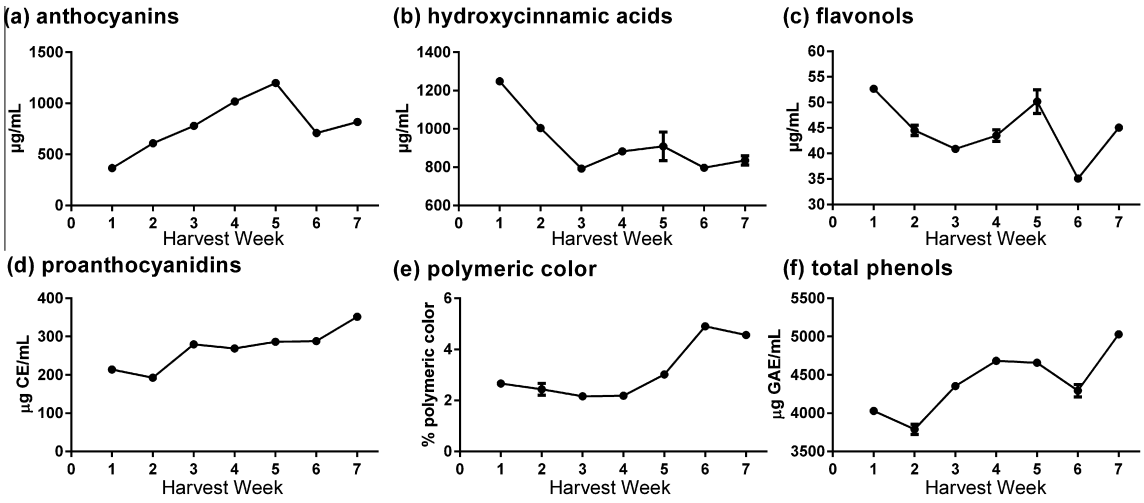

Fig. 1. Time of harvest differentially affects aronia juice polyphenols and antioxidant activity; (a) anthocyanins by UHPLC–UV–MS analysis, (b) hydroxycinnamic acids by UHPLC–MS analysis, (c) flavonols by UHPLC–MS analysis, (d) proanthocyanidins by DMAC method, as catechin equivalent (CE), (e) polymeric color (%), and (f) total phenols by Folin–Ciocalteu method as gallic acid equivalents (GAE). Data are mean ± standard deviation of duplicate determinations of juice and by one way ANOVA of weekly changes, $P < 0.0001$ for anthocyanin, hydroxycinnamic acid, flavonol, DMAC, polymeric color, and total phenol values.

equivalents. For determination of DPPH IC_{50} values, aronia juice was diluted in methanol. A 0.04 mg DPPH/mL methanol solution was made and calibrated with methanol until the solution had an absorbance of ~1.3 at 517 nm. The aronia juice sample (100 μL) was mixed with the DPPH solution (900 μL) in a cuvette, incubated in the dark for 30 min, and read at 517 nm. IC_{50} values were determined by interpolation of non-linear regression curves response fitted in GraphPad Prism 5.0 (Eq (5)):

$$Y = 100/\left(1 + 10^{((\log IC_{50} - X) \times \text{HillSlope})}\right) \quad (5)$$

where IC_{50} is inhibition of 50% of the DPPH solution, and HillSlope is the steepness of curve. Inhibition of DPPH was calculated as Eq. (6):

$$\%\text{DPPH inhibition} = (1 - \text{Abs}_{\text{sample}}/\text{Abs}_{\text{DPPH}}) \times 100\% \quad (6)$$

where $\text{Abs}_{\text{sample}}$ is the absorbance of juice samples incubated with DPPH at 30 min incubation, and Abs_{DPPH} is the absorbance of a solution containing DPPH and methanol as a blank.

2.7. Data and statistical analysis

Data were usually collected in at least duplicate independent samples, with degrees of replication as indicated in Tables and Figures. Significance of weekly changes or additive effects on DPPH inhibition were determined by one-way ANOVA, with $P < 0.05$ considered significant. Differences between one-phase decay kinetic parameters were determined by the extra sum-of-squares F tests using GraphPad Prism, where $P < 0.05$ was considered significantly different. Pearson correlations were determined using GraphPad Prism, where $P < 0.05$ was considered significant.

3. Results & discussion

It is widely known that the acid, sugar, and polyphenol content of berries changes through the ripening process. However, the extent that these changes impact pigment stability and antioxidant activity is not clear. Aronia is unique among berries in that its berries ripen and are apparently harvestable for more than 1 month, which allows for a considerable variation in the composition of berries (Jeppsson & Johansson, 2000). Aronia cultivation is increasing in the USA, and it is used for colorants, juices, and as a source of nutraceuticals for functional food products. Thus, the primary objectives of the present study were to characterize the extent that aronia composition changed during its harvest period and to further identify how its composition affects antioxidant activity and pigmentation.

3.1. Aronia juice acidity and sugars differentially change during harvest

Aronia juice pH was not significantly different between harvest wk (Table 1). In contrast, acidity decreased from 1.22% at wk 4 to 0.81 at wk 7. Brix values of aronia juice increased from 10.5 ± 0 to 14.33 ± 0.3 °Brix between wk 1 and wk 7, representing a 36% increase over the harvest period (Table 1). The increased Brix at wk 6 and 7 appeared to be due to increased sorbitol. Fructose and glucose content increased 40–50% from wk 1, but plateaued at wk 4, whereas sorbitol content continued to increase by 43% through wk 7 (Table 1). At wk 7, sorbitol represented 42% of the total sugar content of juice. Accordingly, Brix:acid ratio increased from 10.4 at wk 4, to 15.6–15.7 at wk 6 and wk 7. Other sugar alcohols were <0.5 g/kg and other sugars were <1 g/kg (data not shown).

Aronia acidity and Brix values are comparable to ranges observed in many fruits (Sadler and Murphy, 2003). Aronia has a greater concentration of sugars than most sweet commercial

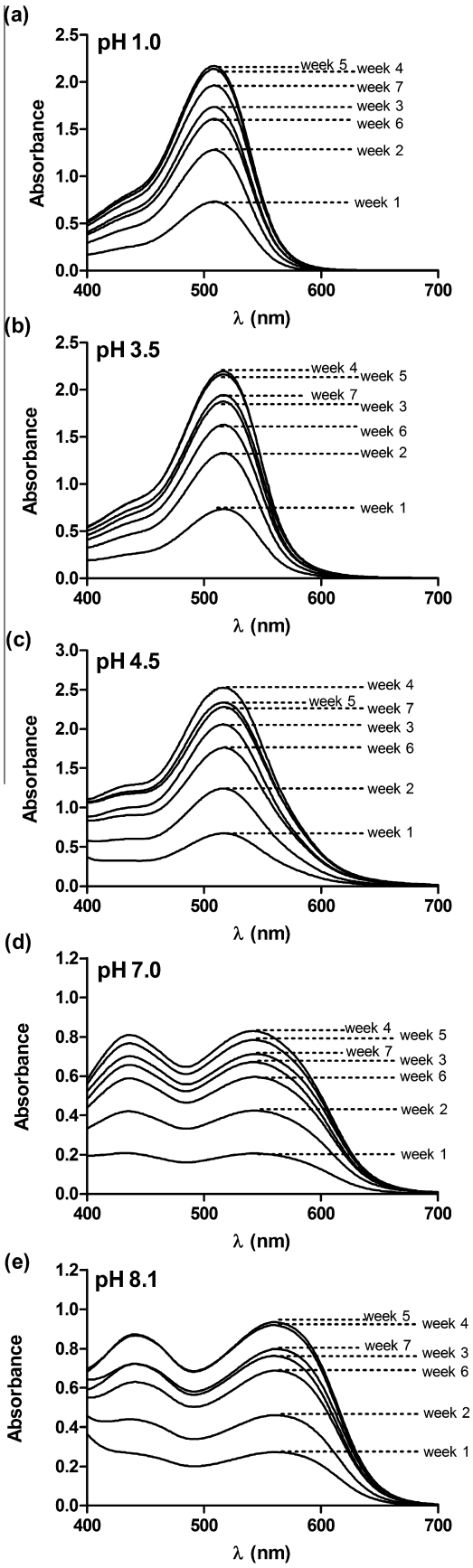

Fig. 2. Impact of harvest week on aronia juice absorbance at (a) pH 1.0, (b) pH 3.5, (c) pH 4.5, (d) pH 7.0, and (e) pH 8.1. Juice was diluted in buffers as described in materials and methods at DF of 32 (a), 16 (b), 4 (c), 32 (d), or 32 (e) and spectra recorded at ~5 min of dilution.

Table 2
Differential effects of phytochemicals and carbohydrates on the copigmentation and loss of color from an anthocyanin-rich aronia extract at pH 4.5.

Additive	[ ]	ΔY_0 (Abs 540 nm)	Δ plateau (Abs 540 nm)	ΔK (s^{-1})	Δ half-life (s)	*P*-value vs control
Ascorbic acid	80 μM	−0.026	−0.002	−0.00003	+0.04	<0.0001
Caffeic acid	80 μM	−0.025	−0.006	+0.00054	−0.63	<0.0001
(+)-Catechin	80 μM	−0.003	+0.002	−0.00122	+1.76	<0.0001
Chlorogenic acid	80 μM	+0.057	+0.002	−0.00065	+0.87	<0.0001
Citric acid	40 μM	−0.003	−0.001	+0.00008	−0.12	0.2431
Daidzein	80 μM	−0.038	+0.005	−0.00093	−1.21	<0.0001
Ellagic acid	40 μM	+0.023	+0.010	+0.00035	−0.51	<0.0001
trans-Ferulic acid	80 μM	+0.015	+0.001	+0.00002	−0.03	<0.0001
Genistein	80 μM	−0.020	+0.011	−0.00024	+0.30	<0.0001
Reduced L-glutathione	80 μM	−0.017	−0.004	−0.00034	+0.49	0.0002
Polydatin	80 μM	+0.026	+0.015	−0.00009	+0.15	<0.0001
trans-Stilbene	40 μM	−0.056	−0.010	−0.00017	+0.2	<0.0001
Quercetin	40 μM	+0.055	+0.027	−0.0007	+0.97	<0.0001
Quercetin	80 μM	+0.115	+0.051	−0.00337	+4.86	<0.0001
Quercetin	160 μM	+0.098	+0.091	−0.00485	+8.13	<0.0001
β-cyclodextrin	40 μM	−0.000	+0.009	−0.00156	+2.06	<0.0001
Fructose	30% w/v	+0.091	+0.034	+0.00064	−0.89	0.0002
Glucose	30% w/v	+0.060	−0.041	+0.00136	−1.73	<0.0001
Sorbitol	30% w/v	+0.116	−0.040	−0.00042	+0.51	<0.0001
Sucrose	30% w/v	+0.044	−0.047	+0.00096	−1.34	<0.0001

Data are the difference of kinetic parameters of one phase decay model of aronia extract from the aronia extract with additives, at an absorbance of 517 nm at pH 4.5 [e.g. $\Delta Y_0 = Y_{0\ \text{aronia}} - Y_{0\ \text{aronia+additive}}$]. Data were simultaneous determinations of additives and a control solution without additives in quadruplicate. Final aronia concentration was 28.8 μg/mL. Statistical analysis is the sum-of-squares *F* test between the additive and control, with $P < 0.05$ considered significant.

berries including strawberries, blueberries, blackberries, currants, and raspberries (Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, & Veberic, 2012). Unlike most berries, aronia contains significant levels of sorbitol (Mikulic-Petkovsek et al., 2012). A previous study reported 13–19 °Brix values in 'Nero' aronia berries over three seasons, but the stage of ripeness at harvest was not determined (Strik, Finn, & Wrolstad, 2003). Reduced acidity did not significantly change pH, which indicates juices had adequate buffering capacity despite lower acid content.

3.2. Changes in polyphenol content by aronia harvest date

Aronia juice anthocyanins and hydroxycinnamic acids experienced the greatest changes by harvest date among polyphenols. Initial total anthocyanin content of aronia juice was 366 ± 3 μg/mL at wk 1 and peaked at 1200 ± 39 μg/mL at wk 5, and then decreased to 817 ± 13 μg/mL at wk 7 (Fig. 1). The anthocyanin profile of 'Viking' juice at wk 1 was 75.4% Cy3Gal + Cy3Glu and 24.6% Cy3A. Cy3X was not detected until wk 2. By wk 5, Cy3X increased to 3.5% of total anthocyanins. Thus, despite changes in total anthocyanin content, the profile remained stable between weeks. Swedish aronia berry anthocyanin content also peaked in early September (Jeppsson & Johansson, 2000). In contrast, strawberry and lowbush blueberry anthocyanins increased throughout ripening and did not decrease prior to senescence (Kalt & McDonald, 1996; Wang & Lin, 2000).

The total hydroxycinnamic acid content of aronia juice gradually decreased from 1,248 ± 0 to 835 ± 24 μg/mL between wk 1 and wk 7, a 33% decrease (Fig. 1). The proportion of Cga to nCga remained constant (data not shown), and was similar to extracts of whole berries (Taheri et al., 2013). Similarly, cinnimates appear to decrease in other berries during ripening. Blueberry cinnamic acid derivatives decreased during ripening, however chlorogenic acid values did not change between the slightly unripe and overripe stages (Herrmann, 1989). Likewise, apple hydroxycinnamic acids decreased up to 58% during ripening (del Campo, Berregi, Iturriza, & Santos, 2006).

Flavonol content of aronia juice was 52.65 ± 0.15 μg/mL at wk 1, with no apparent trend by harvest date (Fig. 1). The order of abundance for flavonols was Q3Gal > Q3Glu > Q3R at wk 1, but was Q3Glu > Q3Gal > Q3R at wk 2 due to reduced Q3Gal. Q3Glu remained the predominant flavonol in wk 3 to 7. 'Stevens' and 'Ben Lear' cranberry total flavonols were also stable during fruit development, although less-abundant methoxyquercetin glycoside increased with fruit development (Vvedenskaya & Vorsa, 2004).

Aronia juice proanthocyanidins increased from 214 to 352 μg catechin equivalents (CE)/mL between wk 1 and 7, but decreased at wk 2 (Fig. 1). In contrast, a prior study reported that blueberry proanthocyanidins decreased by ~40% at later stages of ripeness, but mean degree of polymerization increased from ~8.2 to 8.5 units (Zifkin et al., 2012). Ripening studies from several grape cultivars suggest proanthocyanidin development occurs at different rates in the seeds and pulp (Bautista-Ortin et al., 2012). Aronia seeds contain proanthocyanidins, but its distribution in seed, pulp, and skin are yet to be determined (Esatbeyoglu & Winterhalter, 2010). Aronia seeds were not disrupted or recovered by the methods used for juice making in the present study. Therefore, changes in aronia juice proanthocyanidins likely reflect pulp and skin differences.

Aronia juice total phenols increased from 4.03 to 5.03 mg gallic acid equivalents (GAE)/mL between wk 1 and wk 7, a 24% increase (Fig. 1). Unexpectedly, total phenol peaked earlier than anthocyanin content, and its peak at wk 4 was not attributable to any particular polyphenol class. However, weekly total phenol values were significantly correlated to proanthocyanidin values, ($r = 0.91$, $P = 0.004$), but not anthocyanins ($P = 0.094$) or sum of polyphenols ($P = 0.133$). Juice matrix, dilution, or polyphenol synergy can increase total phenols values (Bolling et al., 2013). Alternatively, less abundant and undetermined aronia polyphenols, such jaboticabin, prunin, hyperin, or isorhamnetin 3-*O*-rutinoside, may contribute to the peak aronia juice total phenol at wk 4 (Li et al., 2012). Ripening differentially affects berry total phenols. For example, strawberry juice total phenols changed little during advanced ripening (Wang & Lin, 2000), while blueberry total phenols increased or decreased during advanced ripeness (Castrejón, Eichholz, Rohn, Kroh, & Huyskens-Keil, 2008).

3.3. Pigmentation of aronia juice by harvest week

We hypothesized that the phytochemical changes would impact aronia pigmentation by harvest wk. Indeed, aronia juice visible spectra absorbance values varied in the visible region at

all pH values examined (Fig. 2). Absorbance maxima were observed at wk 4 and 5, which corresponded to peak anthocyanin content (Fig. 1). Previous reports indicated that aronia polyphenols copigment with hydroxycinnamic acids (Wilska-Jeszka & Korzuchowska, 1996). Aronia and strawberry juices experience 3–4 nm bathychromic shifts with the addition of 10- to 25-fold more chlorogenic acid than anthocyanins. Thus, because juice polyphenol content changed significantly during harvest, we expected that we would also observe bathochromic shifts in spectra maxima. In contrast, λ maxima remained stable at all pH values (Fig. 2). The only visible change was a lack of the ~430 nm shoulder peak in wk 1 at pH 4.5 (Fig. 2c). This difference is likely attributed to low cyanidin content, rather than flavonoids, as juice flavonoids were not largely decreased at wk 1. We further evaluated the rate of pigmentation loss at pH 4.5, but found no differences by harvest wk (data not shown). Thus, main changes to aronia pigmentation during harvest wk appear related to pigment density, rather than changes in λ maxima or stability.

3.4. Impact of additives on aronia pigment stability

Polyphenols and other constituents are known to copigment or affect the color stability of anthocyanin-rich juices (Malien-Aubert, Dangles, & Amiot, 2001). The relative stability of aronia polyphenol pigmentation led us to evaluate if polyphenols or other constituents could improve aronia extract copigmentation and stability. We utilized a commercially available aronia polyphenol extract and monitored the loss of pigmentation at 540 nm with pH-jump experiments at pH 4.5. This pH was chosen to evaluate the transition from the anthocyanin flavylium cation to its colorless hemiketal and chalcone (Pina, 2014).

Changes to the model Y_0 values indicate the initial copigmentation of additives (Table 2). Initial Y_0 values were ~1.0 at (Fig. 3). ΔY_0 ranged from −0.056 to 0.115, so equal or less than ~10% of the initial conditions. Thus, these additives had very little effect on copigmentation at the experimental conditions. In solutions of aronia juice and chlorogenic acid, a 50:1 ratio of chlorogenic acid to anthocyanin produced a 6 nm bathochromic shift, while a 10:1 ratio shifted less than 2 nm (Wilska-Jeszka & Korzuchowska, 1996). In comparison, 80 μM chlorogenic acid in our experiment was at a >1000:1 weight ratio of chlorogenic acid to total aronia anthocyanins to saturate copigmentation. Therefore, the flavonoids and other polyphenols present in the aronia extract were sufficient to saturate anthocyanin copigmentation prior to the addition of chlorogenic acid or other additives.

More apparent effects were observed in kinetics of pigmentation loss at pH 4.5, indicated by ΔK and Δ half-life values (Table 2). The polyphenols quercetin, chlorogenic acid, genistein, polydatin, and catechin inhibited loss of aronia pigmentation, with 160 μM quercetin increasing half-life by 8.13 s. Quercetin also increased Δ plateau values by up to Abs 0.091 at 160 μM. This suggests a strong interaction of quercetin and aronia anthocyanins at pH 4.5. Further work is warranted to determine the nature and stability of this interaction. In contrast, the addition of daidzein, ellagic acid, ferulic acid, and caffeic acid reduced aronia pigmentation half-life at pH 4.5. Similarly, sugars did not affect loss of color to the same extent, as sorbitol increased aronia pigmentation, but fructose, glucose, and sucrose increased loss of color at pH 4.5. This is corroborated by a prior study that demonstrated sorbitol stabilized black currant and elderberry anthocyanins (Hubbermann, Heins, Stöckmann, & Schwarz, 2005). Likewise, β-cyclodextrin increased aronia pigment half-life by 2.06 s. The ability of β-cyclodextrin to stabilize aronia juice anthocyanins has been previously described (Howard, Brownmiller, Prior, & Mauromoustakos, 2013). The addition of up to 3% β-cyclodextrin to aronia juice prevented anthocyanin loss during long-term storage and was more effective at pH 3.6 than 2.8 (Howard et al., 2013).

3.5. Aronia juice antioxidant activity and influence of additives

Aronia juice FRAP values increased from 6.79 mg/mL Trolox equivalents (TE)/mL at wk 1–10.4 mg/mL at wk 7, representing a 53% increase (Table 1). In contrast, DPPH potency decreased from

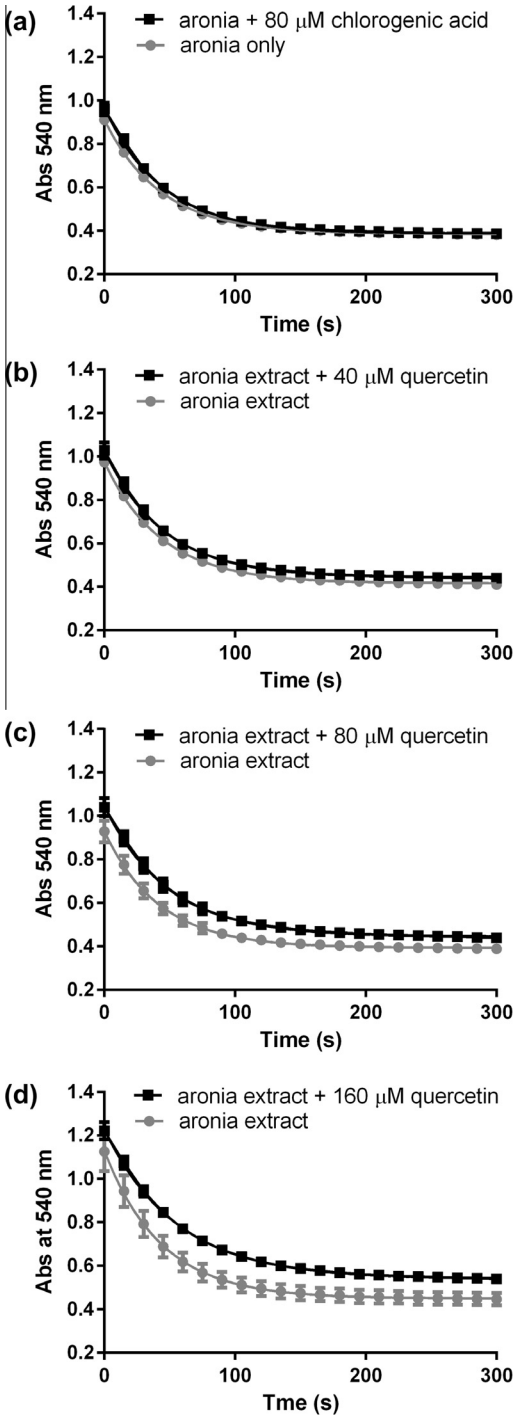

Fig. 3. Representative one-phase decay models of aronia extract pigmentation at pH 4.5 with (a) 80 μM chlorogenic acid, (b) 40 μM quercetin, (c) 80 μM quercetin, and (d) 160 μM quercetin. Kinetic parameters are listed in Table 2.

wk 1 to 3, then increased to an IC_{50} value of 0.13% juice at wk 7 (Table 1). Therefore, maximum polyphenol content and antioxidant activity of aronia juice was observed at wk 7 of harvest. The degrees of changes in antioxidant activity appeared to be in range with the changes in antioxidant activity of other berries during ripening. In blackberries, ORAC values increased from 43.0 μmol TE/g FW to 62.7 μmol TE/g FW in 'Marion' berries, and from 46.1 μmol TE/g FW to 64.4 μmol/g FW in 'Evergreen' berries during ripening (Wang & Lin, 2000).

The FRAP assay determines the ability to reduce Fe(III) to Fe(II) at pH 3.6, whereas the DPPH assay determines reduction of the stable DPPH radical in methanol (Huang, Ou, & Prior, 2005). FRAP and DPPH values were not correlated in aronia juice samples ($P = 0.470$). FRAP exhibited a weak correlation with juice proanthocyanidins ($P = 0.069$) and anthocyanins ($P = 0.071$). FRAP values were significantly correlated with juice total phenol values ($r = 0.802$, $P = 0.030$), suggesting that aronia matrix or polyphenol interactions were similar in these assays.

In contrast, DPPH IC_{50} values of aronia juices were not correlated with polyphenols. We sought to define the contribution of sugars and organic acids to the DPPH antioxidant activity of aronia juices, since prior work suggests these components interact with fruit juice polyphenols (Bolling et al., 2013). Addition of up to 30% sucrose, glucose, and fructose did not change the DPPH scavenging of aronia extract (Fig. 4a, data not shown). In contrast, 5–30% sorbitol, the principal sugar in aronia, decreased DPPH inhibition of aronia polyphenols by ~20% (Fig. 4b). Similarly, 0.1–1% citric acid reduced aronia extract DPPH inhibition by 13 to 26% (Fig. 4c). The interactions of polyphenols with citric acid and sorbitol may warrant further study. Interestingly, sorbitol, increased polyphenol half-life, while fructose, glucose, and sucrose reduced half-life in pH-jump experiments (Table 2). In contrast, citric acid did not have a significant interaction with aronia pigments (Table 2). A possible explanation for citric acid interacting with DPPH scavenging is that pH affects some, but not all antioxidants in the DPPH assay (Sharma & Bhat, 2009). Thus, both phenolic and non-phenolic constituents of aronia berries modulate its antioxidant activity. The contribution of antioxidant activity to aronia juice stability and quality remains to be established.

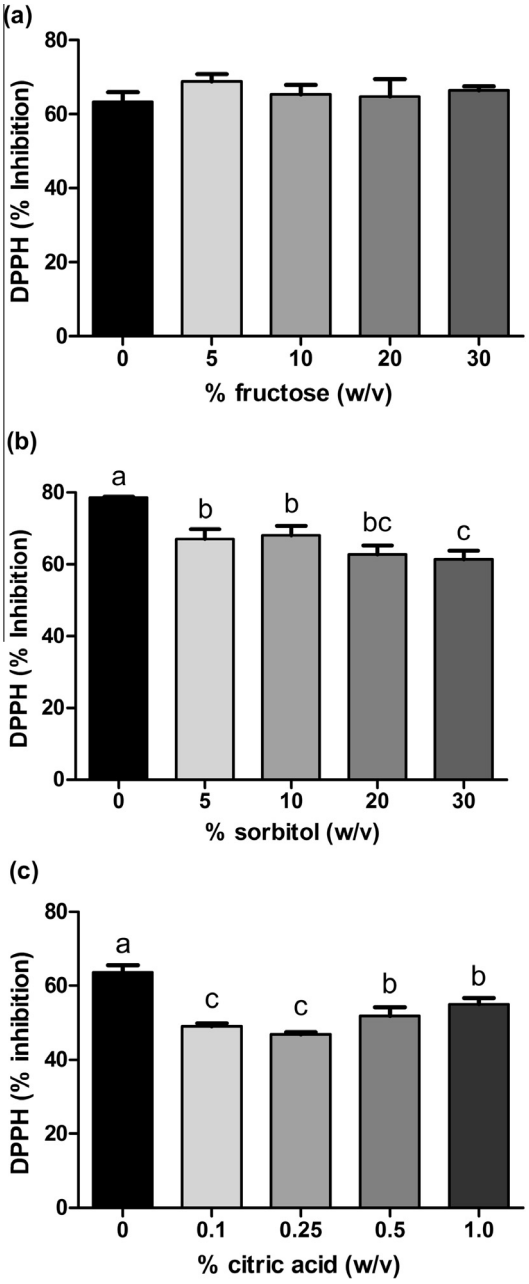

Fig. 4. The impact of added (a) fructose, (b) sorbitol, and (c) citric acid on the DPPH radical inhibition by polyphenol-rich aronia extracts. Data are mean ± standard deviation of triplicate determinations, where ANOVA analysis by % concentration was $P = 0.2578$ for fructose, $P = 0.0003$ for sorbitol, and $P < 0.0001$ for citric acid. Bars bearing different letters are significantly different by Tukey's multiple comparison test where $P < 0.05$.

4. Conclusions

Aronia berry juice polyphenols, sugars, and antioxidant activity substantially change during the seasonal harvest period, with earlier juice having a greater chlorogenic acid to anthocyanin ratio and less sugar compared to later juice. A discernable peak in juice anthocyanin content was observed at wk 5. Variation in anthocyanin content rather than polyphenol copigmentation appeared to modulate juice color. While a number of endogenous aronia polyphenols and other constituents were found to influence aronia copigmentation and antioxidant quality, the changes throughout harvest had minimal impact on these characteristics. Post-harvest addition of some constituents may be warranted to improve pigmentation stability, but further work is necessary to extend the findings of this work to model beverages or other systems employing berry-based colorants.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.04.106.

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