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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