Extraction of Antioxidants from *Aronia mitschurinii* Juice Using Macroporous Resins

Breann V. Green, Travis W. Ford, Heather Goldsborrough, Mohamed Abdelmotalab, Andrew G. Ristvey, Deborah G. Sauder, and Victoria V. Volkis*

ABSTRACT: Antioxidants play a vital role in the human body by defending cells from damage caused by free radicals, highly reactive products of oxidation reactions. A major source of antioxidants is fruits and vegetables. *Aronia mitschurinii*, a breed created at the end of the 19th century by crossbreeding wild *Aronia melanocarpa* and Russian Mountain Ash, produces fruits with one of the highest known content of hydrophilic antioxidants. Aronia fruit contains a potent blend of anthocyanins, polyphenols, and flavonoids. The most popular way of consuming the fruit is through juicing. Yet, due to very high concentrations of tannins in the juice, very few food-related applications have been developed. Resin extraction of antioxidants provides an alternative for utilizing valuable phytochemicals from crops for applications in the food industry as nutraceutical supplements and more. To increase the market value of the plant, it is important to determine what resins can extract the optimum concentration of antioxidants from aronia juice, pulp, and whole berries. We have shown that macroporous resins such as Amberlite XAD 1180N, Amberlite XAD 7HP, Amberlite XAD 761, and Amberlite FPX66, which have been reported to be effective in extracting the anthocyanins and polyphenols from other fruit juices, skins of red grapes, and the wild breed, are also effective for use in juice, pulp, and whole fruits of *Aronia mitschurinii*. However, the extremely high content of antioxidants presents a challenge to obtaining high recovery; a notable change in the juice/resin ratio is required to obtain a higher recovery value. Our results showed that Amberlite FPX66 was the best at extracting anthocyanins, polyphenols, and flavonoids from aronia juice. A separate experiment conducted to determine how to optimize the efficiency of FPX66 extraction revealed that increasing the resin/juice ratio increased the percent recovery of anthocyanins from aronia juice. Moreover, we have compared recovery between juice, pulp, and whole aronia berries and batch versus column extraction.

INTRODUCTION

*Aronia* (*Aronia melanocarpa*), commonly known as black chokeberry, is a shrub in the *Rosaceae* family (rose family) that produces fruit with one of the highest contents of hydrophilic antioxidants of any fresh fruit. Aronia is an Eastern North American native plant. In the 19th century, a Russian plant breeder, Ivan Mitchurin, crossed *A. melanocarpa* with *S. aucuparia* (European Mountain Ash), producing an intergeneric hybrid named *Aronia mitschurinii*. Like its parent species, *A. mitschurinii* is a deciduous fruiting shrub, but it is hardy, high yielding, and easy to grow, requiring very little care and having only a few potential pests. While the true origins of the Viking and Nero cultivars are still being determined, it is clear that they are not identical to the wild *A. melanocarpa*.

Aronia fruit contains a potent mix of antioxidants including anthocyanins, tannins, polyphenols, flavonoids, and carotenoids. Most notably, aronia is very high in anthocyanin content. Preliminary data show that fruits from the cultivated varieties of aronia, Viking, and Nero are significantly higher in antioxidant content than the wild *A. melanocarpa*. Aronia juice is sweet, having a soluble sugar content between 15 and 20% Brix, however, the whole fruit and the pulp (pomace) are not very palatable because the solids contain bitter-tasting tannins. For this reason, the juice is highly marketable for wine and jams, but the solids left over after juicing are sold at a very low price, often for use in animal feed or thrown away by farmers. A large portion of valuable anthocyanins remain unutilized in the pulp (the average ratio we have measured in our lab was 58%:42% for anthocyanins in juice and pulp, respectively, when pulp was extracted in water/ethanol.

Received: May 5, 2022  
Accepted: August 10, 2022  
Published: August 18, 2022
These antioxidants can be easily extracted.\textsuperscript{14} Development of improved methods for isolating antioxidants from both the juice and pulp of aronia berries stand to greatly increase the total market value of the \textit{A. mitchurinii} (henceforth aronia) fruit to improve the profit margins of growers.

Recently published studies have proven the effectiveness of macroporous resins in the extraction of anthocyanins from the fruit, first from water extracts of \textit{A. melanocarpa} pulp\textsuperscript{1,2} and second from acidified water extracts of grape pumice.\textsuperscript{13} Extraction by resin solves a problem by using less palatable parts of fruits to concentrate antioxidants in powdered form to be used as food supplements. These supplements can be used in pills, as natural colorants, and in many other applications. Some of these applications are technical, such as anti fouling protection of surfaces.

The study involving \textit{A. melanocarpa} looked at the effectiveness of only 3 resins, Amberlite XAD7HP, XAD16, and XAD1180, and reported measurements for polyphenol and anthocyanin concentrations in extracts, where both groups of antioxidants were isolated as one fraction. The study conducted with grape pomace was more comprehensive in that it compared two more resins in addition to those of the \textit{A. melanocarpa} study: FPX66 and XAD761. Only the data relevant to anthocyanin concentration and typification were presented. Neither study looked at extraction from juice, which, in the case of aronia, contains just slightly higher concentrations of antioxidants than pulp does. The antioxidant content results of \textit{A. melanocarpa} have never been compared with those cultivars of aronia, where the total content of antioxidants is significantly lower as compared to aronia juice.

To fill gaps within the literature, information regarding the effectiveness of four resins: XAD1180N, XAD7HP, XAD761, and FPX66 from aronia juice is presented in this paper. Procedural comparisons are made from both studies mentioned above, as well as the conclusions made regarding the most effective resin. These comparisons will provide information about utilizing the best methods to increase the extraction of antioxidants and increase the potential profitability of aronia growers, especially small farm operators in rural areas of the US.

## MATERIALS AND METHODS

### Aronia mitchurinii Samples. The aronia fruit samples were obtained from the University of Maryland’s Wye Research and Education Center (WyeREC) in Queenstown, MD. The soil was a Mattapex-Butlertown silt loam with a 0–2 percent slope and a pH of 6.1. The soil was maintained with sod for longer than a ten-year period before planting. Soil tests revealed adequate amounts of all nutrients except for potassium, which was applied with the nitrogen. Fruit samples were harvested manually from established plants six years old or older undergoing nitrogen rate studies.

Once harvested, fruit samples were maintained in a $-25 \, ^\circ C$ freezer prior to juicing. The samples were defrosted for about 3 min before juicing by placing the sample in water at room temperature. For juicing samples, a mortar and pestle were used to grind the aronia fruit; then, a vacuum filtration apparatus was used for rapid filtration of the juice from the pulp. Then the juice sample was stored in Eppendorf vials at $-25 \, ^\circ C$ for further analysis and resin extraction. Juice samples were never stored in the freezer for longer than one week. Before resin extraction procedures, juices were defrosted in water, in the same manner as fruit samples.

### General Procedures. Chemical reagents used included aluminum chloride (99% extra pure, anhydrous, granules), ethyl alcohol (99% ACS spectroscopic grade), and quercetin hydrate (95%), which were purchased from Thermo Fisher Scientific. Potassium acetate (certified ACS Crystalline) and sodium carbonate anhydrous (HPLC grade powder) were purchased from Fisher Scientific. Folin and Ciocalteu’s Phenol reagent and gallic acid monohydrate (ACS reagent grade) were purchased from MP Biomedicals (Santa Ana, CA). Sodium acetate (Sigma Ultra minimum 99.0%) was purchased from Sigma-Alrich. Amberlite resins XAD1180 N, XAD7HP, XAD761, and FPX66 were purchased from Sigma-Alrich. Conc. HCl, Conc. NaOH, pure ethanol, and 95% ethanol were purchased from Fisher Scientific. Distilled water was used for all procedures. UV/Vis determination of total concentrations of anthocyanins, flavonoids, and polyphenols was performed on a Spectronic 10 Genesis spectrophotometer, as described in the procedures below.

### Resin Activation. Four resins, described in Table 1 were purchased from DOW Chemicals Company via Sigma-Alrich as a vendor. The resins were used to extract anthocyanins, polyphenols, and flavonoids. Portions of each of the four resins mentioned above were soaked overnight in two bed volumes of 100% ethanol. To remove any impurities, each portion was cleaned twice by stirring in two bed volumes of 5% NaOH aq. solution for 1 h. This was followed by two soaks in 5% HCl using the same procedure. Resins were separated from each solution via vacuum filtration through Whatman #5 filter paper. After cleaning, each portion was rinsed with distilled water, while on the filter after the last vacuum filtration, until the resulting filtrate had a neutral pH. Resins were stored wet in airtight containers in the refrigerator until used for the extraction. This procedure was performed one resin at a time, right before the resin extraction experiment for this resin was conducted. The weight of resin used is that of the vacuum filtered dried resin, which was stored in the dry container.

### Resin Extraction Method. 0.1 g of each resin and 3 mL of juice were placed in 10 mL beakers. A blank was prepared containing only the juice. Samples were placed in room temperature water baths and stirred for 24 h. The juice was vacuum filtered out and stored in sealed vials in the freezer. Resins were then rinsed with 10 mL of distilled water before recombining with 5 mL of 95% acidified ethanol (5% formic acid) and set to stir for another 24 h. Anthocyanins, flavonoids, and polyphenols were extracted from the resins by rinsing with 10 mL of distilled water before recombining with 5 mL of 95% acidified ethanol (5% formic acid) and then for another 24 h set to stir at room temperature. The resulting desorbate from each extraction was removed by gravity filtration and stored in the
freezer for phytochemical analysis. This extraction was repeated with each type of resin three times, and all measurements were done in triplicates.

Maximizing Efficiency of Extraction with Resin FPX66. Using only the resin FPX66, three 25 mL beakers were set up as follows: (#1) 0.25 g resin and 6 mL juice; (#2) 0.125 g resin, 3 mL of juice diluted, and 3 mL of distilled water; and (#3) 3 mL juice as a control. The further extraction was performed as described previously in the "Resin Extraction Method" section and compared to the results of the original extraction procedure using FPX66.

Measuring Total Anthocyanins Content. Measurement and calculation of anthocyanin pigment concentration were performed based on the procedure outlined by Lee et al. After defrosting in room temperature water, samples were vortexed. A portion of each sample was diluted 2000 times into 0.025 M aqueous KCl and into 0.4 M aqueous sodium acetate. The UV/Vis absorbance of each dilution was read at 520 and 700 nm using a spectrophotometer. Anthocyanin pigment concentration (APC) was calculated as cyanidin-3-glucoside equivalents in mg/L (and later converted into mg/g of juice) using the equation

\[
APC = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}
\]

where \(A\) = absorbance = (A520−A700 nm) at pH 1.0−(A520−A700 nm) at pH 4.5); \(MW\) (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); \(DF\) = dilution factor (200×); \(l\) = pathlength in cm; \(\varepsilon\) = 26,900 L/mole/cm, the molar extinction coefficient for cyd-3-glu; and \(10^3\) = factor for conversion from g to mg.

Measuring Total Flavonoids. Flavonoid content was measured using a method based on those published by Wiosky and Salatino as well as Chang et al. Standards of 0, 30, 60, 90, 120, and 150 \(\mu\)g/mL were made from a dilution of 0.005 M quercetin stock solution in 95% ethanol. Samples were prepared for measurement by the creation of a solution with the following ratios by volume: 1% sample, 39% acidified ethanol, 2% \(\text{AlCl}_3\), 2% potassium acetate, and 56% distilled water. Components were added and mixed, one by one, in order. The solution was incubated for 5 min at room temperature following both the addition of \(\text{AlCl}_3\) and the addition of potassium acetate. The final solution was incubated as before for 20 min. The absorbance of both standards and samples was measured at 405 nm using a PerkinElmer Victor 3 1420 Multilabel Counter. Readings of standards were used to make a calibration curve for the calculation of sample values. Flavonoid concentration was expressed as \(\mu\)g Quercetin equivalents/mL. This concentration was converted to mg Quercetin Equivalents based on sample volume.

Totals of Polyphenols Measurement. Total polyphenol content was measured using a method published by Singleton and Rossi. Standards of 0, 30, 60, 90, 120, and 150 \(\mu\)g/mL were prepared from a gallic acid stock solution in 80% ethanol and 20% deionized water (DI). Experimental samples were prepared for measurement by mixing 198 \(\mu\)L distilled water with 2 \(\mu\)L Aronia juice and adding 200 \(\mu\)L of each of the gallic acid standards, in turn. 1250 \(\mu\)L of Folin’s Reagent was added to each sample. Samples were incubated for 5 min at room temp. Then, 1500 \(\mu\)L of 7% (w/v) \(\text{NaCO}_3(\text{aq})\) was added, and the resulting solutions were incubated at 40 °C in an oven for 15 min. Analysis was conducted in triplicate. Before measuring absorbance, all samples were cooled in a refrigerator for 5 min. The spectrophotometer was used to measure absorbance at 750 nm. The standards were used to make a calibration curve for the determination of experimental concentrations. All polyphenol concentrations are expressed as \(\mu\)g gallic acid equivalents/mL of juice.

Phase Contrast Microscopy. An image was obtained for each resin while it is dry, then activated, and finally saturated in juice. An Olympus CX41 Phase Contrast & Darkfield Microscope at 4× magnification was used.

Calculations. The adsorption ratio, desorption ratio, and total recovery were calculated as follows

\[
\text{adsorption ratio} = \frac{\text{mass of control adsorption} - \text{mass of absorbate absorbance}}{\text{mass of control adsorbate absorbance}}
\]

\[
\text{desorption ratio} = \frac{\text{mass of desorbate}}{\text{mass of control} - \text{mass of adsorbate}}
\]

\[
\text{total recovery} = \frac{\text{mass of desorbate}}{\text{mass of control}}
\]

Statistics. Each extraction was repeated three times, and all measurements of antioxidant concentrations were done in triplicate for each of the extractions. The data then was analyzed statistically for mean ± standard error, t-test, and one way analysis of variance (ANOVA) within the 95% confidence limit. The values of \(p < 0.05\) were considered significant.

Adsorption Kinetics. The concentration of flavonoids, anthocyanins, and polyphenols in batch tests was monitored to evaluate the time for adsorption equilibrium. Adsorption was performed by adding 1.7 g of resin FPX66 (dry weight) to 50 mL of aronia juice in a flask at 25 °C. The mixture was stirred at 150 rpm in an orbital shaker for 5 h. Samples of the juice (1 mL each) were taken at 0, 20, 40, 60, 90, 120, 180, 240, and 300 min. The samples were analyzed for total flavonoids, anthocyanins, and polyphenols. This batch test was performed using methods described by Padilla de la Rosa et al.

Column Test. A fixed bed with 1.7 g of resin was used to evaluate the dynamic adsorption and desorption of polyphenols, anthocyanins, and flavonoids from aronia juice. The temperature and weight of resin were selected from the batch test. Ascending flow was used for the adsorption and desorption phases of the test. The purification operation was performed in cycles comprising the following steps by Padilla de la Rosa. First wash: The column was washed with 1 L of DI. Adsorption: 1 L of diluted aronia juice was passed through the column. Samples at 25, 50, and 100 mL were collected at the exit of the column until the whole volume had been treated. Second wash: The column was washed with 250 mL of DI to remove any remaining aronia juice. Desorption: 1 L of ethanol 96% was passed through the column to recover the adsorbed flavonoids, anthocyanins, and polyphenols. Samples at 50 and 100 mL were collected until there was no volume of ethanol left in the column.

RESULTS AND DISCUSSION

Resin extraction is a powerful technique for the isolation of antioxidants from fruits. It is adaptable, comparatively simple, and substitutes for more energy-intensive procedures. Adsorption to resins is powered by attractive forces between the adsorbent and the adsorbate. Depending on the nature between the adsorbent and the adsorbate, it can be classified as chemical adsorption or physical adsorption. Resin extraction is an extremely powerful tool when phytochemically valuable plant components are extracted and utilized in applications outside of food production. Around half of the total antioxidant content of
this fruit remains in the pulp and is typically lost in traditional processing by farmers. Resin extraction and isolation of antioxidants from aronia produces a product that can be directly utilized as components of health supplements, energy drinks, or nonedible products such as antifouling materials.

**Resins.** Resin adsorption and desorption performance is related to its chemical structure, pore size, and pore shape. For hydrophilic antioxidants, such as anthocyanins, polyphenols, and flavonoids, the adsorption on macroporous resins is a physical process mediated through hydrophilicity and Van der Waals force. Surface area is one of the key factors that affect resin adsorption capacity.

Four different macroporous resins including XAD 1180N, XAD 7HP, XAD 761, and FPX66 with different polarity and pore size were selected in this study, as shown in Table 1.

Resins were tested and analyzed for adsorption, desorption, and recovery value of anthocyanins, polyphenols, and flavonoids from aronia juice. Resins were activated before usage, as described in Materials and Methods.

Amberlite XAD 1180N is a nonionic, hydrophobic, cross-linked polymer with a macroporous structure and a high surface area. It has a macroporous cross-linked matrix, and it comes in a form of spheres. It is typically used for recovery and purification of antibiotics, water-soluble steroids, enzymes and proteins, recovery of plant extracts, enzyme immobilization, and separation of nonpolar organic solutes from polar solvents.

Amberlite XAD 7HP and Amberlite XAD 761 polymeric adsorbents are macroporous, nonionic, aliphatic acrylic resins with a high surface area. Due to its aliphatic nature, AmberLite XAD7HP can adsorb nonpolar compounds from aqueous systems and can also adsorb polar compounds from nonpolar solvents. The two resins are only different by the pore size, as it is shown in Table 1.

Amberlite FPX66 polymeric adsorbent is a uniform particle size, macroporous, weak base anion resin that is functionalized with terminal amines. It is used for a wide variety of food processing applications to both recover high-value materials and to purify and decolorize food and food additive streams. AmberLite FPX66 resin has excellent physical resistance and thermal stability making it ideal for use in static and column-based systems over a large number of process cycles.

Below in Table 2, surface images of each resin—dry, activated, and after being spun in juice are presented. As can be seen, resins change their mechanical structure significantly after being stirred with juice, and often become powder-like, which prevents their reuse after juice recovery.

We assume that the mechanism of sorption is via the formation of strong hydrogen bonds between phenolic groups of antioxidants and resins. Additionally, for the FPXN66 resin (which has demonstrated the best sorption properties with aronia in our experiments below), acid/base reactions between phenolic groups of antioxidants and terminal amino groups of the resin are possible. To confirm this, we have treated aronia juice with an excessive amount of sodium hydroxide, followed by restoring the original pH of aronia juice to be around pH = 3.5. The chemical reaction of anthocyanins, for example, being a di-acid, with sodium hydroxide is described by Dangles and Fenger. The reaction converts into salts all phenolic groups preventing them from forming hydrogen bonds and/or participating in acid/base reactions. The sample after this treatment was exposed to the resin, following the standard batch procedure that was also used for all sorption experiments below but did not show any sorption or recovery.

### Table 2. Microscopic Characterization of Resins During the Sorption Process

| Resin    | Dry                | Activated | After Stirring with Juice |
|----------|--------------------|-----------|---------------------------|
| FPXN66   | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |
| XAD1180N | ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) |
| XAD7HP   | ![Image](image7.png) | ![Image](image8.png) | ![Image](image9.png) |
| XAD761   | ![Image](image10.png) | ![Image](image11.png) | ![Image](image12.png) |

*Resins XAD 1180N, XAD 7HP, XAD 761, and FPX66 at 4× magnification. Each image is a square with a side length of 500 μm.

**Batch Experiments with Resins.** Typically, aronia berries are juiced after harvesting. Pulp, which is unpalatable is often treated as a waste, while the juice is used for food applications. However, a typical farmer will harvest more aronia than is needed to process food-related products, and many farmers have juice left. Therefore it was important to develop a resin extraction procedure for the juice first.

The pH differential method was used to analyze anthocyanins in aronia juice as cyanidin-3-glucoside (cyd-3-glu) equivalent in mg/L. Figure 1 shows absorption ratios, desorption ratios, and total recovery for anthocyanins from the aronia juice. The anthocyanin extraction results are shown in Figure 2.
four resins. Results showed that FPX 66 has the highest recovery value (40%) among the four resins studied.

The same four resins were used to evaluate the flavonoid content in aronia juice. The adsorption ratios, desorption ratios, and recovery values for flavonoids are shown in Figure 2. Flavonoid content was measured as quercetin equivalents in mg/L using Chang’s method. Results show that macroporous resin FPX66 has the highest recovery value of 45.5% for extracting flavonoids among the four resins investigated. This same resin recovered the highest amount of anthocyanins compared to other resins (Figure 1) and, therefore, can be used for the recovery of all antioxidants together.

The four resins tested for anthocyanin and flavonoid extractions were then tested to isolate polyphenols from aronia juice. Woisky’s method for the determination of polyphenol concentrations was used. Polyphenol concentrations are reported as gallic acid equivalent in mg/L. Adsorption ratios, desorption ratios, and % recovery values are shown in Figure 3. The macroporous resin FPX66 had the highest recovery value (33%) for extracting polyphenols among the four resins investigated. Polyphenol’s recovery was less efficient as compared to flavonoids and anthocyanins recovery, but FPX66 showed the highest recovery value of the four resins studied.

Influence of Sorption Conditions on Total Recovery. For the extraction of anthocyanins, the XAD 761 and FPX66 resins give equally high recovery values of 40.0%. For the extraction of flavonoids, the FPX66 resin gives the highest recovery value of 45.5%. For the extraction of polyphenols, the FPX66 resin gives the highest recovery value of 33.0%.

The FPX66 resin has demonstrated that it is the best of the four resins considered in this study in allowing the recovery of antioxidant components from aronia juice. Some procedure optimization experiments to maximize the recovery have been performed with this resin, as shown in Table 4. This table presents data from a single extraction experiment with altered resin, juice, and volume ratios using FPX66 only. Due to the extremely high concentration of anthocyanins in A. mitschurinii juice, as compared to muscadine grape and A. melanocarpa pomace extracts, it was hypothesized that changing ratios between the resin and juice bed volume might improve extraction efficiency. To investigate this potential, we have performed resin extractions of anthocyanins with the best FPX66 resin at different bed volumes and dilutions of juice, as presented in Table 3.

Table 3. Comparison of Modified Extraction Procedures for the FPX66 Resin and Anthocyanins Extraction

| effect | mass of resin (g) | V of Juice, mL | V of DI water, mL | % adsorbed (%) | % desorbed (%) | total % recovery (%) |
|--------|------------------|----------------|-------------------|----------------|-----------------|---------------------|
| original ratio | 0.1 | 5.0 | | 78 | 47 | 39 |
| less resin/diluted juice | 0.05 | 1:1 | | 51 | 43 | 39 |
| less resin/full-strength juice | 0.05 | 1:0 | | 49 | 42 | 28 |
| more resin/diluted juice | 0.25 | 1:1 | | 90 | 55 | 49 |
| more resin/full-strength juice | 0.25 | 1:0 | | 81 | 76 | 62 |
| twice more resin/full-strength juice | 0.40 | 1:00 | | 79 | 69 | 76 |

Figure 2. Four types of resins adsorption, desorption, and recovery values for flavonoids.

Figure 3. Four types of resins adsorption, desorption, and recovery values for polyphenols.
Although *A. mitchurinii* has the advantage of much higher content of all phenolic antioxidants, as compared to the wild breed and most other fruits, for whom resin extraction process was reported in the literature, it also presents a challenge for recovery in the resin extraction process. Thus, results in Table 3 show that increasing the resin to juice ratio, relative to the original procedure that is like the one used for the wild aronia breed, improved the adsorption ratio over the original procedure, even when the juice was diluted. The desorption ratio increases when the desorbate to juice volume ratio increases. A total anthocyanin recovery ratio of 62−76% was obtained using full-strength juice at a higher resin-to-juice ratio. This recovery is compatible with what is described in the literature for wild aronia and grape skins. Sandhu and Gu found that the adsorption/desorption characteristics of anthocyanins in *Vitis rotundifolia* (Muscadine) juice pomace had a total recovery of 70% using the FPX66 resin with 70% ethanol. D’Allessandro et al. found that the adsorption/desorption characteristic of *A. melanocarpa* berries had a total recovery of 82% for polyphenols and 92% for anthocyanins. They performed this recovery with the XAD7HP resin using 70% ethanol. Our best extraction conditions yielded 62−76% recovery.

We have also compared the resin extraction process from the aronia juice with resin extraction using an extract of aronia pulp and another extract from whole berries. The results are presented in Table 4. For this experiment, the aronia juice was used as is, while pulp and whole berries were first extracted in the mixture of ethanol and water at 50:50% for 48 h and filtered using vacuum filtration.

As can be concluded from Table 4, the juice and extract from pulp result in approximately the same values of sorption and desorption, whereas the whole berries result in slightly lower adsorption.

**Influence of Sorption Time on the Recovery of Antioxidants.** To determine how fast the resin reaches the equilibrium in the sorption process, we conducted experiments, in which the batch method was used, yet small samples of juice were taken from the batch for the analysis of total anthocyanins, polyphenols, and flavonoids as a function of time, while the juice was stirred with the resin, and antioxidants were adsorbed from the juice. We used the FPX66 resin, which has shown the best recovery results for all types of antioxidants in aronia juice. The results are presented in Figures 4−6, respectively, for the total anthocyanins, polyphenols, and flavonoids.

We have noticed a fast decrease in the concentration of anthocyanins during the first hour and a half of the experiment (Figure 4) before it reaches equilibrium after about 2 h.

The concentration of polyphenols decreases during the first 2 h of the experiment and then stayed approximately the same, reaching equilibrium (Figure 5). For flavonoids (Figure 6) noticeable decrease in concentration continued during the first hour and a half, and the equilibrium was reached after 2 h. Therefore, 2 h would be the optimal sorption time for all antioxidants with the FPX66 resin and aronia juice.

**Resin Extraction Using the Column.** Column resin adsorption is a technique alternative to the batch method and more acceptable for the technology transfer of the process to an industrial scale. To estimate the potential of industrialization for resin antioxidants extraction from aronia, we have performed the experiment with the FPX66 resin (the best in batch experiments above) at the same mass of resin and bed volume ratio of resin to juice, as in the previously described batch experiment, using 1.7 g of resin. A fixed bed with resin was used to evaluate the dynamic adsorption and desorption of polyphenols, anthocyanins, and flavonoids from aronia juice. Ascending flow was used for the adsorption and desorption phases of the test. The purification operation was performed in cycles, as described by Padilla de la Rosa. In this experiment, activated wet FPX66 resin was placed in the column, followed by passing the volume of juice through the same column and then recovering the adsorbed antioxidants. The results are presented in Figure 7.

Total recovery of anthocyanins and flavonoids was 72−75%, which is compatible with the best results in our batch experiments, where recovery was in the range of 62−76%.

### Table 4. Extracts of Aronia Juice, Whole Fruit, and Pulp and Their Adsorption and Desorption Values Using FPX66

| extract sample preparation               | adsorption (%) | desorption (%) |
|-----------------------------------------|----------------|----------------|
| juice extract (5 mL juice and 50 mL of 1:1 ethanol and DI water, 48 h) | 90             | 67             |
| whole fruit (5 g fruit and 50 mL of 1:1 ethanol and DI water, 48 h) | 79             | 65             |
| pulp (5 mg pulp and 50 mL of 1:1 ethanol and DI water, 48 h) | 83             | 64             |

![Figure 4. Kinetics of anthocyanin adsorption.](https://doi.org/10.1021/acsomega.2c02785)
recovery of polyphenols in the column method was slightly lower, 58%.

Additionally, the dependences of the concentration of total anthocyanins, total flavonoids, and total polyphenols during the desorption process from the column as a function of the total volume of eluent are presented in Figures 8–10, respectively, averaged after running the column experiment three times.

As can be observed from Figures 8–10, 400 mL of eluent is enough to desorb most anthocyanins, while a volume of 800–900 mL is required to fully desorb flavonoids and polyphenols.
CONCLUSIONS

Four microporous resins XAD1180N, XAD7HP, XAD761, and FPX66 have been tested for the extraction of phenolic antioxidants from juice, pulp, and whole berries of *A. mitchurinii*. All resins were capable of sorption and recovery of antioxidants, whereas the FPX66 resulted in the highest recovery for anthocyanins, polyphenols, and flavonoids. Since *A. mitchurinii* has a much higher content of antioxidants, as compared to previously reported *A. melanocarpa* and grape skins, significant modifications in the resin to juice/extract bed volume ratios were needed to reach a total recovery of 62−76%.

Resins are capable of sorption of antioxidants due to strong hydrogen bonds formed between the resin and phenolic groups of antioxidants. The sorption process with vigorous stirring causes the resin to lose its granular structure and become a powder. This prevents resins from being reused after the recovery of antioxidants.

For aronia juice and pulp extract, total recovery is approximately the same, whereas the recovery from the extract of whole berries is slightly lower.

Recovery using the column method is compatible with the one in the batch method. The recovery in the column experiment is slightly higher for anthocyanins, as compared to flavonoids and simple phenolics. Moreover, we have shown that most of the antioxidants can be recovered from experiments where sorption lasts for 120 min.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c02785.

Column Adsorption and desorption of anthocyanins, flavonoids, and polyphenols; batch kinetic experiments’ raw data; Anthocyanin varying treatments for FPX66 experimentation raw data; and raw data or all sorption and desorption batch experiments (PDF).

AUTHOR INFORMATION

Corresponding Author

Victoria V. Volkis — Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, Maryland 21853, United States; orcid.org/0000-0001-8415-0134; Phone: 1-443-736-0898; Email: vvolkis@umes.edu

Authors

Breann V. Green — Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, Maryland 21853, United States

Travis W. Ford — Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, Maryland 21853, United States

Heather Goldsborrough — Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, Maryland 21853, United States

Mohamed Abdelmotallah — Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, Maryland 21853, United States

Andrew G. Ristvey — University of Maryland Extension, Wye Research & Education Center, Queenstown, Maryland 21658-0169, United States

Deborah G. Sauder — Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, Maryland 21853, United States

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c02785

Funding

This work is supported by the AFRI-EWD-REEU program, grant no. 2020-69018-30655, from the U.S. Department of Agriculture; the National Institute of Food and Agriculture; the USDA-NIFA Evans Allen formula grant at the University of Maryland Eastern shore; and the Department of NAVY MSI grant and distinguished faculty fellowship no. N0014-21-1-2756. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and should not be construed to represent any official USDA or U.S. Government determination or policy.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

V.V. would like to thank undergraduate researchers Blessing Aroh, Ezra Cable, Eseoreghene Mowoe, Adaobi S. Egwuagu, Jasmine Turner, and Bokary Sylla for their help and contributions. M.A. would like to thank the Department of...
Natural Sciences at the University of Maryland Eastern Shore for teaching assistantship support during his graduate study.

### ABBREVIATIONS

| Abbreviation | Meaning |
|--------------|---------|
| UV/Vis | UV/visible spectrophotometry |
| mL | milliliters |
| M | gram |
| DF | dilution factor |
| C3GE | cyanidine-3-glucoside equivalents |
| GAE | gallic acid equivalents |
| QE | quercetin equivalent |
| DI water | de-ionized water |
| °C | degrees Celsius |
| Aq. | aqueous |
| nm | nano-meter |

### REFERENCES

1. Valcheva-Kuzmanova, S.; Gadjeva, V.; Ivanova, D.; Belcheva, A. Antioxidant Activity of Aronia Melanocarpa Fruit Juice in Vitro. *Acta Aliment.* 2007, 36, 425–428.
2. Gasiorowska, K.; Szybaa, K.; Brokosa, B.; Kozłaczynska, B.; Jankowiak-Włodarczyk, M. Antimutagenic Activity of Anthocyanins Isolated from Aronia Melanocarpa Fruits. *Cancer Lett.* 1997, 119, 37–46.
3. Erichsen-Brown, C. *Medicinal and Other Uses of North American Plants: a Historical Survey with Special Reference to the Eastern Indian Tribes*; Dover Publications: New York, NY, 1989; p 163.
4. Leonard, P. J. Aronia Mitschurinii: Solving a Horticultural Enigma. *Arnoldia* 2010, 61, 144–158.
5. Kask, K. Large-fruited Black Chokeberry (Aronia Melanocarpa). *Fruit Var.* J. 1987, 41, 47.
6. Brand, M. Aronia: Native Shrubs with Untapped Potential. *Arnoldia* 2010, 67, 14–24.
7. Strik, B.; Finn, C.; Wrolstad, R. Performance of Chokeberry (Aronia Melanocarpa) in Oregon, USA. *Acta Hort.* 2003, 626, 439–443.
8. Skvortsov, A. K.; Maitul, Y. K. Rosaceae Aronia Mitschurinii. *Byull. Gl. Bot.* Sada 1982, 126, 40.
9. Kmpuse, S.; Kríma, Z.; Kmpuss, K.; Krásnova, I. Nutritional Value of Minor Fruits in Latvia. *Acta Hort.* 2010, 877, 1221–1228.
10. Taheri, R.; Connolly, B. A.; Brand, M. H.; Bolling, B. W. Underutilized Chokeberry (Aronia Melanocarpa, Aronia Arbutilofila, Aronia Prunifolia) Accessions are Rich Sources of Anthocyanins, Flavonoids, Hydroxycinnamic Acids, and Proanthocyanidins. *J. Agric. Food Chem.* 2013, 61, 8581–8588.
11. Aroh, B. The Effect of In-field Plant Nutrient Fertility on the Antioxidant Capacity of Aronia Mitschurinii Grown in Maryland. M.Sc.; University of Maryland Eastern Shore. 2015.
12. Atanassova, M.; Christova-Bagdassarian, V. Determination of Tannins Content by Titrimetric Method for Comparison of Different Plant Species. *J. Univ. Chem. Technol. Metall.* 2009, 44, 413–415.
13. Grudzava, A. E.; Grishatova, N. V.; Timofeeva, E. A.; Krylova, E. A.; Redzhepova, G. R. Method for Preparing Biologically Active Feed Additive from Natural Materials, 2004, RU 2232514 C2.
14. D’Alessandro, L. G.; Vauchel, P.; Przybyski, R.; Chataigne, G.; Nikov, I.; Dimitrov, K. Integrated Process Extraction-Adsorption for Selective Recovery of Antioxidant Phenolics from Aronia melanocarpa Berries. *J. Sep. Purif. Technol.* 2013, 120, 92–101.
15. Sandhu, A. K.; Gu, L. Adsorption/Desorption Characteristics and Separation of Anthocyanins from Muscadine (Vitis rotundifolia) Juice Pomace by Use of Macroporous Adsorbent Resins. *J. Agric. Food Chem.* 2013, 61, 1441–1448.
16. Lee, J.; Durst, R. W.; Wrolstad, R. E. Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *J. AOAC Int.* 2005, 88, 1269–1278.
17. Woisky, R. G.; Salatino, A. Analysis of Propolis: Some Parameters and Procedures Chemical Quality Control. *J. Apic. Res.* 1998, 37, 99–105.
18. Chang, C.-C.; Yang, M. H.; Wen, H. M.; Chern, J. C. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. *J. Food Drug Anal.* 2002, 10, 178–182.
19. Singleton, V. L.; Rossi, J. A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* 1965, 16, 144–158.
20. Padilla de la Rosa, J.; Ruiz-Palomin, P.; Arriola-Guevara, E.; García-Fajardo, J.; Sandoval, G.; Guatemala-Morales, G. A green process for the extraction and purification of hesperidin from Mexican lime peel (citrus aurantifolia swingle) that is extendible to the citrus genus. *Processes* 2018, 6, 266.
21. DuPont product description. *DuPont AmberLite XAD1180N Polymeric Adsorbent Product Data Sheet* and 5df37f295d73f6.pdf, 2021 (prep-hplc.com).
22. DuPont product description. *DuPont AmberLite XAD7HP Polymeric Adsorbent Product Data Sheet*, 2020.
23. DuPont product description. *DuPont AmberLite FPX66 Polymeric Adsorbent Product Data Sheet*, 2021.
24. Dangles, O.; Fenger, J.-A. The Chemical Reactivity of Anthocyanins and Its Consequences in Food Science and Nutrition. *Molecules* 2018, 23, 1970–1993.