2021

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Journal of Agriculture and Food Research 6 (2021) 100248

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Time of harvest affects United States-grown *Aronia mitschurinii* berry polyphenols, °Brix, and acidity

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ARTICLE INFO

Keywords:
Aronia berry
Polyphenols
Ripening
Quality
Anthocyanin
Proanthocyanidin

ABSTRACT

The goal of this study was to determine how the date of harvest impacts the quality characteristics of *Aronia mitschurinii* (A. K. Skvortsov and Maitul.) 'Viking' and 'Galicjanka' berries. Aronia berries were collected from farms in the Midwestern and Northeastern United States over seven weeks of harvest during 2018, 2019 and 2020. The berries were analyzed for total phenol, anthocyanins, proanthocyanins, sugar, and acid. Aronia berry composition modestly deviated between each year of the study. Berries harvested in 2018 had the highest total phenols and proanthocyanidins, both increasing in content from weeks 1–5 from 15.90 ± 3.15–19.65 mg gallic acid equivalents/g fw, a 24% increase, and 2.22 ± 0.40–2.94 mg (+)-catechin equivalents/g fw, a 32% increase, respectively. Berries harvested in 2019 had the lowest total phenol and proanthocyanidin levels and had increasing anthocyanins until week 4. In 2020, aronia berry proanthocyanidins differed from those in 2018 by having 38% lower levels after the 4th week. Across years, berries had increasing °Brix, °Brix: acid, and pH throughout the seven weeks of harvest. Additionally, all years had slight, but statistically insignificant decreases in acidity over the harvest period. Moreover, analysis from berries collected in 2019 suggests no significant difference in quality factors between Viking and Galicjanka aronia cultivars. In conclusion, aronia berry total phenols, proanthocyanidins, pH, and berry size can be significantly affected by the growing year and time of harvest. Acidity was impacted more by growing year than harvest week. In contrast, anthocyanins and °Brix were consistent between years, but influenced considerably by the week of harvest.

1. Introduction

The consumption of berries has increased in the United States [1]. For example, the growth in per capita consumption for blueberries from 2017 to 2019 was over 510% [2]. The increase in berry consumption correlates with consumers' knowledge of the health benefits they may receive when adding them to their diet. The recognition of the health benefits of berries has led to increasing interest in underutilized berries with bioactive, including aronia berry, elderberry, bilberry, and goji berry.

Aronia berries grown for fruit production and human consumption are *Aronia mitschurinii* (A. K. Skvortsov and Maitul.), a Eurasian domesticated taxon resulting from hybridization between *Aronia melanocarpa* (Michx.) Elliott and *Sorbus aucuparia* L. [3–6]. The polyphenol profile of aronia berry is well-established and it contains cyanidin glycosides as the main anthocyanins, highly-polymerized B-type proanthocyanidins, chlorogenic acids, and quercetin glycosides as reviewed elsewhere [7]. Aronia berries contain high levels of anthocyanin and proanthocyanidin polyphenols that may help reduce the risk of cardiovascular disease, gastrointestinal disease, diabetes, and cancer [8]. However, aronia berries are astringent and bitter; therefore, the appeal of berries to consumers is mainly for the bioactive compounds and the health benefits they may receive upon consumption [1,9]. Berry quality and polyphenol content are known to be affected by genotypes (species and varieties), climate (temperature, humidity, rain), year, location, and soil [8]. A prior study reported aronia berry anthocyanin content increased over 100% between two different years and can decrease by 50% when exposed to low soil mineral content [10].

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https://doi.org/10.1016/j.jafr.2021.100248
Received 6 October 2021; Received in revised form 29 November 2021; Accepted 29 November 2021
Available online 1 December 2021

Beyond these latent factors, harvest time impacts the quality and bioactive contents of aronia berries. Contrary to most fruit, aronia berries appear ripe for about 4–6 weeks; due to this extended time resembling ripeness, it is difficult to optimize the harvest time of aronia berries. In addition, the extended harvest period may increase the variability of aronia berry polyphenols, sugars, and acids [11]. Despite several studies on harvest time and the quality of aronia berries, there is a need to define the quality of U.S. aronia berries more rigorously. Previous studies vary on recommendations for harvesting aronia berries with the best quality and most polyphenols. Yang et al. [12] concluded that immature aronia berries should be harvested for use in dietary supplements because of their higher polyphenol content than mature berries. In contrast, prior studies concluded that the best time to harvest is when anthocyanins and soluble solids are highest for improved flavor and appeal [9,13]. Additionally, studies have reported different trends in aronia berry pH as berries mature. For example, berries from Germany, Turkey, and Japan have distinct pH values by harvest week [11,14,15]. However, Bolling et al. [13] reported no significant difference in U.S. aronia berry juice pH during different harvest times within a single year.

Previous studies on aronia berry quality by harvest date have limitations in the number of plants studied, locations, or length of study. Therefore, the objective of the study was to determine the quality traits of aronia berries from different farms within the primary U. S. horticultural zones over three years to understand how harvest date affects polyphenols, total soluble solids, pH, and titratable acidity. The results from this study can help growers define benchmarks of aronia berry quality. Also, these data can help improve cultivation practices to maximize aronia berry quality and inform berry processors seeking to develop high quality ingredients, juices, and foods from U.S.-grown aronia berries.

2. Materials and methods

2.1. Reagents

(+)-Catechin hydrate (98% purity) was from Cayman Chemical (Ann Arbor, MI, U.S.A.). 4- (Dimethylamino) cinnamaldehyde, Folin & Ciocalteu's phenol reagent, formic acid (reagent grade ≥95% pure)), sodium bicarbonate (BioReagent), were from Sigma-Aldrich (St. Louis, MO, U.S.A). Hydrochloric acid (ACS reagent grade), potassium acid phthalate, potassium chloride (ACS reagent grade) were from Thermo Fisher Scientific (Waltham, MA, U.S.A). Ethanol (anhydrous, USP standard) was from Decon Labs (King of Prussia, PA, U.S.A.). Gallic acid monohydrate (ACS reagent grade) was from Acros Organic morris plains NJ, USA. Sodium Hydroxide (1.0007 N) was from La-Mar-Ka (Baton Rouge, LA. U.S.A). Ultrapure water was filtered at >18.1 M Ω·cm using a Barnstead water filtration system (Thermo Fisher Scientific).

2.2. Collection of aronia berry samples

Participating farms received kits with instructions to randomize plants for the collection of berry samples. First, the farmers assigned numbers to 35 aronia plants in their plantings. Each farm had a unique random sequence provided by the investigators with instructions to collect six fruit clusters berries from each of five randomized plants over seven weeks. Week 1 was defined as the timepoint when 95% of the aronia berries contained purple/black pigmentation. The farmers were instructed not to pick berries that appeared inedible and to remove stems and leaves before placing the berries in a Ziploc bag and storing in a freezer. These steps were repeated with new plants each week for a total of 7 weeks. Once all weeks were collected, the frozen berries were sent to Madison, WI in insulated containers with ice packs. Aronia cultivars were self-identified by participating farmers. Participating farms reported the berry cultivar collected and Dr. Mark Brand from the University of Connecticut confirmed the cultivar identity using AFLP analysis on DNA isolated from young shoot tips of aronia plants.

Aronia berries were collected from different farms from 2018 to 2020 to analyze how aronia berry quality changed during 7 weeks of harvest. In this study, a total of 10 farms participated, with n = 6 farms in 2018 and 2019, and n = 4 farms in 2020 (Table 1). Berries from n = 2 farms were collected over all 3 years. Some farms could not participate the entirety of the study due to poor growing seasons and other circumstances.

2.3. Extraction of polyphenols for spectrophotometric analysis

High-throughput spectrophotometric assays were used to assess aronia berry polyphenols because of the large number of samples in the present study. Furthermore, these techniques capture the principle phenolics present in aronia berry and are appropriate for within-plant comparisons [16]. Aronia berries were submerged in liquid nitrogen and homogenized to a powder by a blender. In a 25 mL centrifuge tube, 0.10 g of berry powder was mixed with 10 mL of 70:30 acetone/water (v/v). Tubes were placed on a rocker for 24 h at ambient temperature in darkness. Afterwards, samples were centrifuged at 2465 ×g for 15 min. Supernatants were collected and used to determine total polyphenols, anthocyanins, and proanthocyanidins as described below using a SpectraMax Plus Microplate Reader (Molecular Devices, Sunnyvale, CA, USA) with the software SoftMax Pro 5.4.4.

2.4. Total phenols

Total polyphenols were analyzed from a modified method using Folin–Ciocalteu's reagent [17]. Gallic acid was used for the calibration curve ranging from 16.125 to 2000 μg gallic acid/mL solution. In a 96-well plate (Thermo Scientific, Waltham, MA, U.S.A) 10 μL of extract, water, or standards were pipetted in triplicate. Then, 173 μL of ultrapure water and 15 μL Folin-Ciocalteu reagent were added, mixed, and set aside for 5 min at ambient temperature. After this, 45 μL of 20% Na_2CO_3 solution and 57 μL water were pipetted into the mixture. The microplate was placed in darkness for 1 h at ambient temperature. Finally, samples were analyzed for absorbance at 765 nm by microplate spectrophotometry.

2.5. Monomeric anthocyanins by pH differential method

The total monomeric anthocyanins were measured by the AOAC pH differential method [18]. In a 96-well plate (Thermo Scientific), 20 μL of aronia extract was diluted by a factor of 16 for two different dilutions: at pH 1 buffer (potassium chloride, 0.025 M) and pH 4.5 buffer (sodium acetate, 0.4 M). Each sample and blanks were prepared in triplicates. After 20 min at ambient temperature, absorbance was measured at 520 nm and 700 nm. Values were reported as cyanidin-3-glucoside equivalents.

Table 1
Overview of participating farm locations and aronia berry cultivars.

Aronia cultivar	City	State	Participating years
Galicjanka	Madrid	IA	2018, 2019, 2020
Viking	Hinesburg	VT	2018,2019, 2020
Galicjanka	Canton	IA	2018, 2019
Viking	Monroe	IA	2019, 2020
Viking	Plattsmouth	NE	2018
Viking	Marydel	MD	2018
Viking	Ocean View	NJ	2018
Viking	Storrs	CT	2019
Viking	Madrid	IA	2020

2.6. Proanthocyanidins analysis by reaction with 4-dimethylaminocinnamaldehyde (DMAC)

Total proanthocyanidins were determined using the DMAC method from a modified version of methods reported Prior et al. [19]. (+)-Catechin was used for the standard calibration curve ranging from 1.56 to 100 (+)-catechin μg/mL in the dilution solution (91% ethanol: water; 80:20, (v/v)). Extracts were diluted 10-fold with the dilution solution before analysis. In a microplate, 70 μL of extractions, blanks, and standards were pipetted in triplicates. Then, 210 μL of the DMAC reagent (25 mg of DMAC and 25 mL of HCl (12.5%), ultrapure water (12.5%), and 91% ethanol (75%) (v/v/v)) was pipetted into the wells. After, the plate was quickly added to the spectrophotometer with absorbance set at 640 nm. The plate was read every minute for 30 min. The peak absorbance value was collected and used for determination of proanthocyanidins as (+)-catechin equivalents.

2.7. Aronia juice for °Brix, pH, and titratable acidity

Aronia berry juice from 45 g of aronia berries were used per sample. If samples were frozen, they were first thawed. The berries were hand pressed in a stainless-steel juicer to yield about 22 mL of aronia berry juice. The juice was placed in a 50 mL centrifuge tube and centrifuged at 2465 ×g for 10 min. The juice was decanted into another centrifuge tube and vortexed for 10 s. The juice was used for pH, titratable acidity, °Brix, and °Brix: acid.

2.8. pH of aronia juice

The pH of aronia juice was measured using Seven Compact pH/Ion meter S220 (Mettler Toledo, Columbus, OH, U.S.A.).

2.9. Titratable acidity

Freshly prepared aronia juice from thawed berries was titrated with 0.1 N sodium hydroxide to determine the titratable acidity in aronia berries, using a modified method from Nielsen [20]. A 0.1 N sodium hydroxide solution was prepared and standardized using Potassium acid phthalate (KHP). Then, 10 mL of juice was pipetted into a clean beaker and the initial pH of the juice was recorded before titrating. Next, the sodium hydroxide solution was slowly added to the juice until 7.0 pH (12–19 mL). Duplicates were measured for each sample, the average volume to reach 7.0 pH was recorded and used to calculate the titratable acidity of the aronia juice on the basis of citric acid equivalents.

The equivalence point for aronia berry juice is around the pH of 7.0 (Fig. 1). If we titrated to 8.2, the number of acid equivalents would not equal the number of base equivalents, thus not achieving a neutralized acid [20].

2.10. Brix analysis

°Brix was determined from the juice of 45 g of aronia berry, using an Abbe refractometer (Thermo-Spectronic, U.S.A). Samples were measured in duplicate, and the average °Brix was recorded.

2.11. Berry size classification

Berries from 2018 were hand-counted to determine cup equivalents. In subsequent years, one cup of aronia berries was poured onto a mat and spread out so none of the berries touched. A digital camera mounted above the berries was used to photograph the berries. The images of the berries were imported to ImageJ software (National Institutes of Health) which was used to count the number of berries per cup.

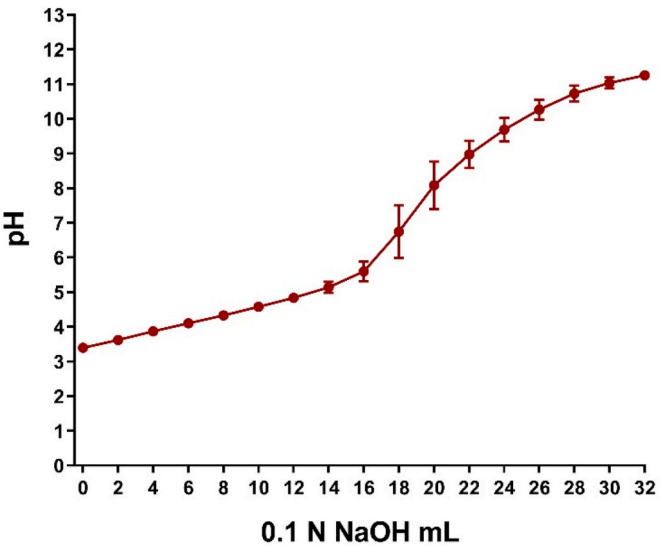

Fig. 1. Aronia juice titration curve. Data are means ± standard deviation of n = 4 aliquots aronia plants.

2.12. Statistical analysis

The samples representing different plants were pooled on an equivalent mass basis to create a composite sample for each week of harvest for each farm. Composite samples were analyzed in triplicate and the data were expressed as the means ± standard deviations of different farms (2018 n = 6, 2019 n = 6, and 2020 n = 4). Results were analyzed using a linear mixed model across time for each year (2018, 2019, and 2020), with significance as $P < 0.05$. For statistical analysis between years, the n = 2 farms that provided samples over all three years were analyzed using a linear mixed model with a split-plot design, considering significant difference as $P < 0.05$. Years were considered as the whole plot treatment, and weeks were the subplot treatment, with the assumption that week 1 of 2018, 2019, and 2020 were all the same week of harvest. Tukey's multiple comparisons were performed if the linear mixed model determined the data were significantly different. Data were analyzed using Rstudio (Rstudio, Boston, MA, U.S.A) and SAS (SAS Institute, Cary, NC, U.S.A) software, using total phenols, monomeric anthocyanins, proanthocyanidins, pH, titratable acidity, °Brix, and °Brix: acid as variables. Our description focuses on the full data set because the trends and significance were similar to the berries from the n = 2 farms that provided samples each year. Figures from these two farms and for each year are presented in the supplementary data (Figs. S1 and S2).

3. Results and discussion

In the present study, United States-produced aronia berry was collected from four to six farms over seven weeks for a three-year period. Frozen berry quality was assessed based on polyphenol content (total polyphenol, anthocyanin, and proanthocyanidins), acidity, pH, °Brix, and berry size. The resulting data were analyzed for differences between the weeks and years of harvest and lastly as differences between 'Galicjanka' and 'Viking' aronia berries.

3.1. Total phenols by year and week

Aronia berry total phenols were significantly different between study years (Table 2, Fig. S1). In contrast to other years, berries from 2018 were different between harvest week and farms. In 2018, berry total phenols increased weekly until week 5. At the week 5 plateau, there were 23.6% more total phenols than in week 1 berries. After week 5, the

Table 2
Statistical analysis of aronia berry total phenols, anthocyanins, and proanthocyanins by harvest week across three years.[†]

Polyphenol (content)	Harvest year	Harvest week						
		1	2	3	4	5	6	7
Total phenols (mg/g fw)	2018*	15.9 ± 3.2^{c}	$17.0 \pm 3.4^{b,c}$	$18.5 \pm 2.9^{a,b}$	$17.8 \pm 4.6^{a,b}$	19.7 ± 3.7^{a}	19.4 ± 4.0^{a}	19.5 ± 3.6^{a}
	2019	7.51 ± 2.03	7.78 ± 0.63	8.38 ± 1.27	8.13 ± 1.92	8.06 ± 0.89	7.96 ± 2.81	7.96 ± 2.02
	2020	14 ± 0.0	13.1 ± 3.0	17.0 ± 4.8	16.1 ± 3.0	16.9 ± 3.4	20.2 ± 1.9	17.7 ± 3.1
Anthocyanins (mg/g fw)	2018*	1.78 ± 0.43^{c}	2.09 ± 0.59^{bc}	2.48 ± 0.41^{a}	2.59 ± 0.52^{a}	2.45 ± 0.45^{a}	2.05 ± 0.34^{a}	$2.31 \pm 0.51^{a,b}$
	2019	1.87 ± 1.15	1.97 ± 0.77	2.53 ± 0.42	2.45 ± 0.40	2.70 ± 0.30	2.65 ± 0.27	2.95 ± 0.24
	2020	$1.64 \pm 0.0^{b,c}$	1.62 ± 0.98^{c}	$2.86 \pm 0.95^{a,b}$	$2.79 \pm 0.55^{b,c}$	$3.26 \pm 0.32^{a,b}$	3.76 ± 1.21^{a}	3.90 ± 0.91^{a}
Proanthocyanins (mg/g fw)	2018*	2.22 ± 0.40^{c}	$2.35 \pm 0.52^{b,c}$	$2.61 \pm 0.51^{a,b,c}$	$2.40 \pm 0.53^{b,c}$	2.94 ± 0.85^{a}	$2.68 \pm 0.50^{a,b}$	$2.40 \pm 0.57^{b,c}$
	2019*	0.55 ± 0.16	0.61 ± 0.18	0.66 ± 0.22	0.56 ± 0.24	0.67 ± 0.15	0.65 ± 0.27	0.69 ± 0.16
	2020	2.09 ± 0.0	1.32 ± 0.50	1.32 ± 0.15	1.29 ± 0.52	1.41 ± 0.32	1.70 ± 0.32	1.69 ± 0.28

[†]Total phenols assessed by by Folin–Ciocalteu method as gallic acid equivalents; anthocyanins by pH differential method as cyandin-3-glucoside equivalents; proanthocyanins by 4-dimethylaminocinnamaldehyde (DMAC) method as (+)-catechin equivalents; Data are means ± standard deviations of farms for each week. Year 2018 with n = 6 farms, 2019 with n = 6 farms, and 2020 with n = 4 farms. Values within the rows contains the same letters are not significantly different by linear mixed model across time per year and Tukey's multiple comparison test ($p < 0.05$). Years with (*) indicates farms in that year are significantly different by linear mixed model across time per year and Tukey's multiple comparison test ($p < 0.05$). Abbreviation: fw-fresh weight.

total phenolic content fluctuated but there was no significant change at subsequent time points. Aronia berries collected in 2019 contained less total phenols than those collected in 2018 and 2020. The maximum total phenols concentration in 2019 was 8.38 mg/g fw at 6 weeks, compared to 19.7 mg/g fw in 2018 and 20.1 mg/g fw in 2020. In berries collected in 2020, there was no significant differences in total phenols between harvest weeks.

Engin [11] and Bolling et al. [13] found similar results in total phenol content. Conversely, past studies have seen the total phenol content decrease during maturation of aronia berries [12,21]. The different patterns and concentrations of polyphenols seen in former studies may again be associated with geographical location, weather, soil, cultivar, and harvest time. Tolić et al. [22] determined that climate conditions including rain, sun, and temperature can positively or negatively impact the composition of aronia berries over a three-year period. Furthermore, Gralec et al. [21] reported aronia berry polyphenol content was significantly lower in 2016 compared to 2012 and 2013 due to cold weather damage to plants.

3.2. Anthocyanins by year and week

Aronia berry anthocyanin content was not statistically different between harvest years (Table 2, Fig. S1). However, each year, the aronia berry anthocyanins concentration increased by harvest week. In 2018, aronia berry anthocyanins were different by farms and weeks. In this year, berry anthocyanins had increased 46% from week 1 at week 4 and then remained at ~2.6 mg/g fw with a minor reduction at week 6. In 2019, anthocyanins increased throughout the 7 weeks of harvest, but these differences were not statistically significant. In 2020, berries collected at different weeks were significantly different, with a steady increase throughout the 7 weeks of harvest. By week 7 there was a 140% increase in anthocyanins from 1.6 to 3.9 mg anthocyanins/g fw.

Earlier findings also reported aronia berry anthocyanin variability [11–13,21,23]. The differences in anthocyanin content between berries produced by different farms is possibly due to the different geological areas and cultivation practices. Hwang and Thi [24] studied the impact of different growing locations on physicochemical components in aronia berries. They concluded polyphenol content, including anthocyanins, can be impacted by geographic location. Furthermore, aronia berries grow in open fields, thus causing them to experience different pedo-climatic environments when grown in different locations [25].

3.3. Proanthocyanidins by year and week

Aronia berry proanthocyanidins were also significantly different between years (Table 2, Fig. S1). Across harvest week, proanthocyanidin content of aronia berries increased in 2018, slightly decreased in 2020, and had almost no change in 2019. In 2018, the proanthocyanidin content peaked at 5 weeks with a 32% increase compared to week 1. After week 5, the content decreased, but this did not reach statistical significance (Table 2, Fig. S1). Farms were significantly different in 2018 and 2019, but there was no substantial change in weeks during 2019. The proanthocyanidin content during 2019 was also low compared to 2018 and 2020 and never went over 1 mg/g fw. In contrast to 2018, 2020 aronia berry proanthocyanidins decreased to week 4 and then slightly increased to week 7. There was no significant change in berry proanthocyanidins by week in 2019. Gralec et al. [21] reported decreasing proanthocyanidins and differences between harvest seasons during the harvest window. However, Engin [11] and Bolling et al. [13] reported increases in aronia berry proanthocyanidins by harvest week, with 11% and 24% increases during maturation, respectively.

3.4. pH and acidity by year and week

The pH of aronia berries in all three years follows almost the same trend; they slightly increase throughout the 7 weeks of harvest (Table 3). During 2019, pH peaked at week 5 and decreased during week 7, containing the lowest pH value at week 7 compared to 2018 and 2020. In 2020, week 1 had the lowest pH value of the 3 years, with a pH of 3.09. There was no significant change in the weeks during 2020; however, by week 7 there was about an 11% increase in pH.

The aronia berries' acidity was significantly different in years: 2018, 2019, and 2020 (Table 3). In 2018, aronia berry titratable acidity was comparatively higher than in 2019 and 2020. During the 7 weeks of harvest, the titratable acidity continually lessened till week 6, reducing acidity by 19% compared to week 1. At week 7 there was an increase compared to week 6, however, there was no significant difference (Table 3). In 2019 and 2020 there was no statistical difference in acidity during the 7 weeks of harvest. Nevertheless, there was a slight decrease in acidity till week 6 in 2019 and 2020 years. The aronia berries harvested in 2020 had the lowest amount of acidity in comparison to prior years.

Harvest year may be a possible reason for the acidity difference in the 3 years, but also plant age could have caused the statistical difference in acidity. As seen in strawberries and raspberries, the age of the plant can affect the acid content as well as sugar and yield [26,27]. Both studies concluded weather was not the determining factor of variation. Previous studies have also seen age can affect other physicochemical components and organoleptic quality in berries, including aronia berries [23,27,28].

Our data may differ from other titratable acidity reports that use pH 8.2 as an endpoint. The U.S.A. (AOAC) and Europe (OIV) methods use two different titration indicators to identify the endpoint, phenolphthalein and NaOH with bromothymol blue, respectively [29]. As a result of using separate indicators, the terminal pH of these methods are

Table 3
Statistical analysis of aronia berry pH, titratable acidity, °Brix, and °Brix:acid by harvest week, across harvest years.[1]

Category	Year of Harvest	Harvest week						
		1	2	3	4	5	6	7
pH	2018*	3.27 ± 0.15^{e}	$3.34 \pm 0.18^{d,e}$	$3.35 \pm 0.17^{c,d}$	$3.46 \pm 0.24^{b,c}$	3.52 ± 0.23^{a}	$3.53 \pm 0.20^{a,b}$	3.52 ± 0.21^{a}
Acidity (%)	2019*	3.20 ± 0.11^{c}	$3.28 \pm 0.12^{b,c}$	$3.30 \pm 0.13^{a,b,c}$	$3.31 \pm 0.11^{a,b,c}$	$3.38 \pm 0.14^{a,b}$	3.37 ± 0.14^{a}	$3.33 \pm 0.19^{a,b}$
	2020*	3.09 ± 0.0	3.29 ± 0.01	3.26 ± 0.12	3.30 ± 0.17	3.33 ± 0.17	3.39 ± 0.30	3.42 ± 0.31
	2018*	1.75 ± 0.56^{a}	1.75 ± 0.56^{a}	1.63 ± 0.54^{a}	$1.60 \pm 0.67^{a,b}$	1.48 ± 0.62^{b}	1.41 ± 0.52^{b}	1.59 ± 0.56^{b}
	2019	1.20 ± 0.09	1.17 ± 0.09	1.21 ± 0.10	1.24 ± 0.17	1.46 ± 0.14	1.04 ± 0.16	1.14 ± 0.12
	2020	1.06 ± 0.0	1.06 ± 0.11	1.08 ± 0.11	1.02 ± 0.10	1.01 ± 0.11	0.97 ± 0.20	1.04 ± 0.25
°Brix (°)	2018*	13.8 ± 1.5^{c}	16.7 ± 1.9^{b}	$16.6 \pm 2.1^{a,b}$	17.4 ± 3.0^{a}	$16.6 \pm 3.0^{a,b}$	17.6 ± 3.0^{a}	17.1 ± 2.6^{a}
	2019	14.1 ± 3.9	13.3 ± 1.5	15.5 ± 1.3	15.4 ± 2.4	16.3 ± 1.0	16.7 ± 2.3	16.8 ± 2.8
	2020*	13.00 ± 0.0^{b}	14.25 ± 1.26^{b}	15.50 ± 0.58^{b}	16.75 ± 0.50^{b}	17.00 ± 1.83^{b}	15.00 ± 4.24^{b}	21.00 ± 3.61^{a}
°Brix: Acid	2018*	8.7 ± 3.1^{c}	9.8 ± 3.6^{c}	$11.2 \pm 4.2^{b,c}$	$13.0 \pm 6.4^{a,b}$	$13.1 \pm 6.0^{a,b}$	14.2 ± 6.3^{a}	$12.7\ 0.7^{a}$
	2019	11.8 ± 3.0^{c}	11.4 ± 1.3^{c}	$12.9 \pm 1.3^{b,c}$	$12.5 \pm 2.2^{b,c}$	$14.8 \pm 1.8^{a,b}$	16.04 ± 1.23^{a}	$14.9 \pm 3.2^{a,b}$
	2020	12.2 ± 0.0	13.0 ± 1.8	14.5 ± 2.0	16.7 ± 2.0	17.3 ± 3.1	16.4 ± 7.8	21.3 ± 8.3

[1]Data are mean ± Standard deviation of farms for each week. Year 2018 n = 6 farms, 2019 n = 6 farms, 2020 n = 4 farms. Values within the rows contains the same letters are not significantly different by linear mixed model across time per year and Tukey's multiple comparison test ($p < 0.05$). Years with a * means farms in that year are significantly different by mixed model across time per year and Tukey's multiple comparison test ($p < 0.05$).

different, Europe ending at pH 7 and U.S.A. ending at 8.2. Both methods have been reported in literature. Most organic acids, including tartaric, malic, and acidic, are fully titrated at pH 7. The conjugate base may linger, creating an equivalence point at a pH slightly higher than 7 [20]. However, the primary organic acids found in aronia berries are malic acid (pKa 3.40), quinic acid (pKa 3.46), and citric acid (pKa 2.79), containing a pKa lower than ascorbic acid (pKa 4.10), causing the equivalence to be 7.0 [7,20].

3.5. °Brix and °Brix:acid by year and week

The °Brix of aronia berry juice had a similar trend between years, with °Brix concentration increasing during the harvest period (Table 3, Fig. S2). In 2018, aronia berry juice °Brix increased until week 4, then decreased slightly, but peaked at week 6 having a 28% increase in °Brix compared to week 1. In 2020, the juice °Brix values were the highest of the 3 years. In this year, °Brix decreased at week 6, but week 7 juice had the highest °Brix value of the harvest period with a 49% increase from week 1 (Table 3). In 2019, harvest week did not significantly affect aronia berry juice °Brix.

The °Brix content of aronia berries in this study was from 13 to 21 and matches prior reports. Tolić et al. [30] measured the °Brix content of 11 different aronia juices, ending with a range of 13.3–21.0 °Brix with an average of 15.54 °Brix. They also concluded the wide range of °Brix values in the aronia berry juice was due to the geographical location, weather, and crop period. A different study assessed aronia berries from three different years, resulting in a higher °Brix range, 18.2 to 25.6 °Brix [22]. Lastly, Taskin [31] found aronia puree had lower °Brix values than juice, with 13.2 ± 0.1 °Brix. The increase in °Brix concentration was similar to that reported by Bolling et al. [13] where °Brix increased 36% over the 7 weeks in aronia berry juices. Additionally, past studies of aronia berries or elderberries reported °Brix varied slightly between harvest year [22,32]. Tolić et al. [22] concluded that berry juice °Brix depends on multiple different factors including environment, weather, variety, and harvest time.

In contrast to other berries, aronia berry has significant levels of sorbitol. Sorbitol is synthesized in the leaves and then sent through the phloem to reach the aronia berry tissue [33]. During maturation of most berries, sorbitol is converted into starch, glucose, fructose, and malic acid. Most of the sorbitol in aronia berries does not convert into other compounds, thus causing sorbitol to be the most abundant sugar in aronia berries [34]. Since aronia berries have low sucrose levels, it could lead to errors in estimating soluble solids as °Brix, since °Brix is the refractive index of dissolved sucrose in a solution [35]. Furthermore, glucose and fructose have different refractive indexes, causing minor differences in °Brix values between solutions [35]. However, a 70% sorbitol solution has about the same refractive index (1.46) as a 70% sucrose solution [36,37]. In a study with apple maturation, Aprea et al. [38] found sorbitol strongly correlated ($r = 0.635$; $p < 0.001$) with the soluble solid concentration, while the sucrose concentration did not correlate. Guan et al. [39] obtained similar results finding a significant correlation between sorbitol and soluble solids content of apples.

Nevertheless, °Brix may not be a reliable indicator to determine the sweetness of aronia berries because of the high concentration of polyphenols [40]. Studies with strawberries and blueberries showed a low correlation between sensory sweetness and the total soluble solids [41,42]. The low correlation is due to the polyphenols attributing to the refractive index. The refractive index for 100 mg of anthocyanins per 1 mL is 1.33 equaling a 0.63 °Brix [43]. Additionally, Kader et al. [38] found that phenolic compounds can change fruit juices' soluble solid concentration up to 32%. Therefore, the °Brix value for juices that contain high pigmentation may not be a reliable indicator for the sweetness of the fruit [40].

°Brix: acid had the same trend as the °Brix value for the aronia berry juice during harvest. In 2018 and 2019, the °Brix: acid ratio of juices were the highest at week 6, increasing 64% and 36%, respectively. There was no significant change in the harvest weeks of 2020.

3.6. Berry size by year and week

Aronia berries were mainly between 130 and 190 berries per cup, resulting in the same size as a medium-sized blueberry (Fig. 2) [44]. Aronia berry size was not significantly different between weeks of harvest throughout the study (Fig. 2). In blueberries, Zorenc et al. [45] reported weight decreased with harvest time. Zorenc et al. [45] also

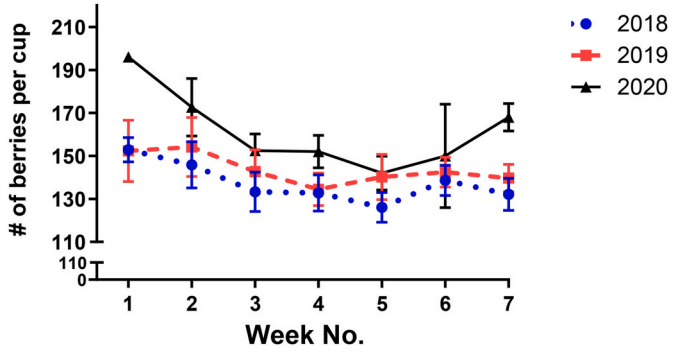

Fig. 2. Aronia berry size at time of harvest. Data are means ± standard deviations of farms for each week. Data are n = 6 farms in 2018 and 2019, and n = 4 farms in 2020. Weeks were not significantly different by a linear mixed model across time per year ($p < 0.05$).

concluded a possible correlation between high temperature and large amounts of sunlight, causing the berries to have lower amounts of water. In this study, the average aronia berry in 2019 was 126 berries per cup in week 5, but shrinks in berry size during weeks 6 through 7. In 2020, aronia berry slightly increased in size until week 5 and slowly decreased in size for the last 2 weeks. None of the weeks from 2020 were less than 142 berries per cup. Large berries are considered more favorable to producers and consumers due to their appearance [45]. The smaller size of aronia berries from 2020 may be due to the poor weather condition that year. Some growers who participated in 2018 and 2019 could not participate in 2020 because of poor aronia berry harvests. Furthermore, cultivation practices, including water management, mulching, and fertilization, impact berries' size and weight [25].

3.7. Differences in quality factors between aronia berry cultivars

In 2019, sufficient berries were available to compare differences between two cultivars. The results indicated there was no significant difference between the cultivars in anthocyanins, proanthocyanidins, total phenols, °Brix: acid, titratable acid, and pH (Figs. 3 and 4).

Former studies reported differences between aronia berry cultivars.

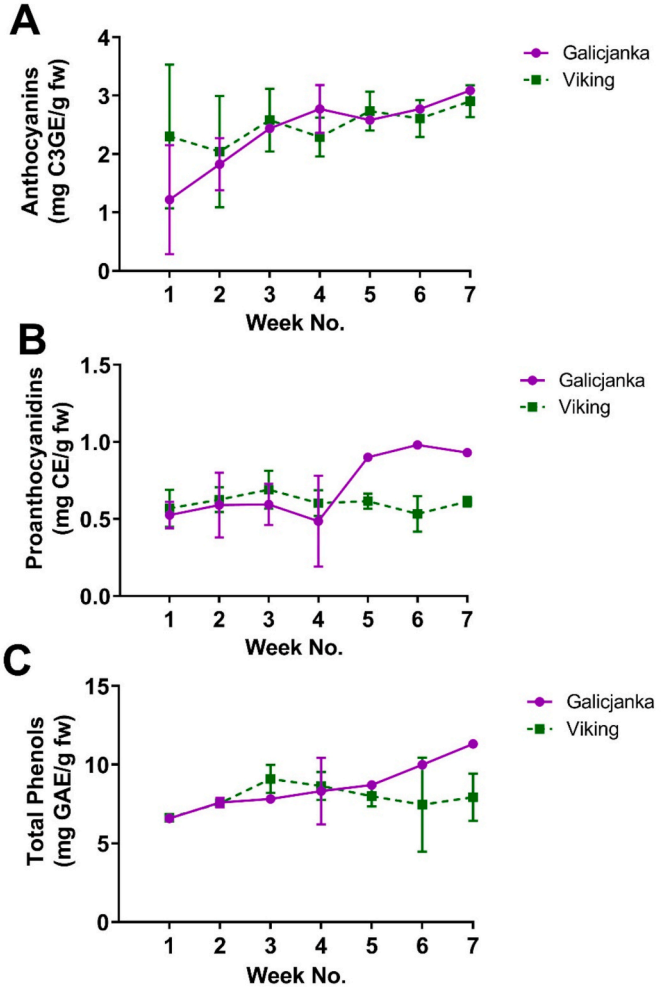

Fig. 3. 'Galicjanka' or 'Viking' aronia berry total phenols, anthocyanins, and proanthocyanins by harvest time and aronia berry. Aronia berry was extracted and then extracts analyzed for A) anthocyanins by pH differential method as cyandin-3-glucoside equivalents; (B) proanthocyanins by 4-dimethylaminocinnamaldehyde (DMAC) method as catechin equivalents (CE); (C) Total phenols by Folin–Ciocalteu method as gallic acid equivalents; Data are means ± standard deviation of cultivar in 2019, with triplicates from each plant; Galicjanka n = 2 and Viking n = 4.

Jakobek et al. [46] reported Galicjanka aronia berry had lower amounts of polyphenols than Viking. However, Jakobek et al. [46] sampled only one period of harvest time in a season instead of across multiple weeks. Ochmain et al. [47], found a significant differences in °Brix for Galicjanka and Viking, resulting in 16.6 and 14.2 °Brix respectively. Similar to our results, Ochmain et al. [47] did not find a significant difference in total phenolic compounds in the two cultivars.

The berries analyzed in the present study were *Aronia mitschurinii* (A. K. Skvortsov and Maitul.) [3–6]. These large-fruited aronia berries are very often incorrectly referred to as *A. melanocarpa*. Nearly all the research reports on aronia berry biochemical properties in the scientific literature (including studies using the cultivars 'Aron', 'Galicjanka', 'Mackenzie', 'Nero', and 'Viking') incorrectly identify the study plants as *A. melanocarpa*, when they are instead *A. mitschurinii* [3,48]. Therefore, findings reported in this study can be directly compared to literature reporting on *A. melanocarpa*. *A. mitschurinii* is a tetraploid species and produces seed asexually through diplosporous apomixis [49]. Therefore, *A. mitschurinii* seedlings are nearly genetically identical to the maternal parent. Since new cultivars of *A. mitschurinii* have been derived as seedlings of a primary *A. mitschurinii* genotype, all existing cultivars are essentially identical to each other and would be expected to express identical phenotypes, fruiting and performance.

4. Conclusions

The present study indicates that harvest time and harvest year impacts the polyphenol, sugar, and acid content in U.S.-grown aronia berries. Moreover, in 2018, the production location, weather, and cultivation practices had a statistical impact on the polyphenols, sugar, and acid. Anthocyanins and total phenols increase in concentration as the aronia berry matures, while proanthocyanidins vary in how they accumulate during maturation, depending on the year. °Brix, °Brix:acid and pH show a general increase in aronia berries as harvest weeks increased. Aronia berries' sizes did not significantly change and were mainly medium-sized. Furthermore, Viking and Galicjanka had no statistical difference in polyphenol content or other quality factors. Aronia berries with the highest quality were harvested at week 5, giving more elevated sugar and polyphenol content. However, other studies are needed to determine the post-harvest factors that impact aronia quality factors.

CRediT author statement

ESK: Methodology, Investigation, Formal analysis, Writing - Original draft; JC: Methodology, Investigation; VL: Methodology, Investigation; XJ: Methodology, Investigation; AKM: Investigation; MKW: Methodology, Investigation; SG: Formal analysis; MHB: Conceptualization, Funding acquisition; CX: Conceptualization, Funding acquisition; BWB: Conceptualization, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Funding

Funded by USDA Specialty Crops Multistate Project Award AM170200XXXXG008, managed by Nebraska Department of Agriculture under project # 18-13-352 to CX.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

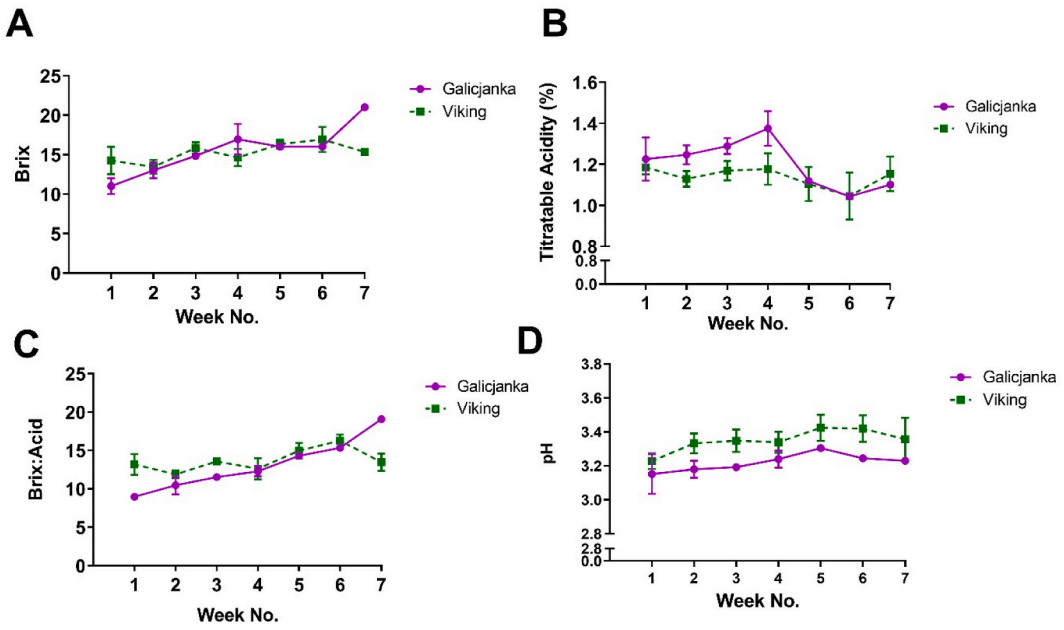

Fig. 4. 'Galicjanka' and 'Viking' aronia berry °Brix, titratable acidity, °Brix:acid, and pH by harvest week. Aronia berries were juiced and analyzed for (A) °Brix; (B) °Brix: acid; (C) titratable acidity (%); (D) pH; Data are means ± standard deviations of cultivar in 2019, with triplicates from each plant; Galicjanka n = 2 and Viking n = 4.

org/10.1016/j.jafr.2021.100248.

References

[1] B.O. Hoke, M. Campbell, M. Brand, T. Hau, Impact of information on northeastern U.S. consumer willingness to pay for aronia berries, Hortscience 52 (2017) 395–400, https://doi.org/10.21273/hortsci11376-16.

[2] USDA, USDA Economic Research Service, 2015. https://www.ers.usda.gov/. (Accessed 7 August 2021).

[3] M.H. Brand, S.G. Obae, J.D. Mahoney, B.A. Connolly Ploidy, Genetic diversity and speciation of the genus Aronia, Sci. Hortic. 291 (2022) 110604, https://doi.org/10.1016/j.scienta.2021.110604.

[4] P.J. Leonard, M.H. Brand, B.A. Connolly, S.G. Obae, Investigation of the origin of *Aronia mitschurinii* using amplified fragment length polymorphism analysis, Hortscience 48 (2013) 520–524, https://doi.org/10.21273/HORTSCI.48.5.520.

[5] A.K. Skvortsov, Y.K. Maitulina, On distinctions of cultivated black-fruited aronia from its wild ancestors (in Russian), Bull. Central Bot. Garden., AN SSSR 126 (1982) 35–40.

[6] A.K. Skvortsov, Y.K. Maitulina, Y.N. Gorbunov, Cultivated black-fruited aronia: place, time and probable mechanism of formation (in Russian), Bull. MOIP, Otd. Biol. 88 (1983) 88–96.

[7] E.S. King, B.W. Bolling, Composition, polyphenol bioavailability, and health benefits of aronia berry: a review, J. Food Bioact. 11 (2020) 13–30, https://doi.org/10.31665/jfb.2020.11235.

[8] L. Burdejova, B. Tobolkova, M. Polovka, Effects of different factors on concentration of functional components of aronia and Saskatoon berries, Plant Foods Hum. Nutr. 75 (2019) 83–88, https://doi.org/10.1007/s11130-019-00780-4.

[9] V.B. Duffy, S. Rawal, J. Park, M.H. Brand, M. Sharafi, B.W. Bolling, Characterizing and improving the sensory and hedonic responses to polyphenol-rich aronia berry juice, Appetite 107 (2016) 116–125, https://doi.org/10.1016/j.appet.2016.07.026.

[10] N. Jeppsson, R. Johansson, Changes in fruit quality in black chokeberry (*Aronia melanocarpa*) during maturation, J. Hortic. Sci. Biotechnol. 75 (2000) 340–345, https://doi.org/10.1080/14620316.2000.11511247.

[11] S. Engin, C. Mert, The effects of harvest time on the physicochemical components of aronia berry, Turk. J. Agric. For. 44 (2020) 361–370, https://doi.org/10.3906/tar-1903-130.

[12] H. Yang, Y.J. Kim, Y. Shin, Influence of ripening stage and cultivar on physicochemical properties and antioxidant compositions of aronia grown in South Korea, Foods 8 (2019) 598, https://doi.org/10.3390/foods8120598.

[13] B.W. Bolling, R. Taheri, R. Pei, S. Kranz, M. Yu, S.N. Durocher, M.H. Brand, Harvest date affects aronia juice polyphenols, sugars, and antioxidant activity, but not anthocyanin stability, Food Chem. 187 (2015) 189–196, https://doi.org/10.1016/j.foodchem.2015.04.106.

[14] A.W. Strigl, E. Leitner, W. Pfannhauser, Qualitative and quantitative analyse der anthocyane in schwarzen apfelbeeren (*Aronia melanocarpa* Michx. Ell.) mittels TLC, HPLC und UV/VIS-spektrometrie, Zeitschrift für Lebensmittel-Untersuchung Und -Forschung 201 (1995) 266–268, https://doi.org/10.1007/bf01193001.

[15] T. Tanaka, A. Tanaka, Chemical components and characteristics of black chokeberry, Nippon, Shokuhin Kagaku Kogaku Kaishi 48 (2001) 606–610, https://doi.org/10.3136/nskkk.48.606.

[16] R. Taheri, B.A. Connolly, M.H. Brand, B.W. Bolling, Underutilized chokeberry (*Aronia melanocarpa, Aronia arbutifolia, Aronia prunifolia*) accessions are rich sources of anthocyaniins, flavonoids, hydroxycinnamic acids, and proanthocyanidins, J. Agric. Food Chem. 61 (2013) 8581–8588, https://doi.org/10.1021/jf402449q.

[17] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Methods Enzymol. 299 (1999) 152–178, https://doi.org/10.1016/s0076-6879(99)99017-1.

[18] J. Lee, R.W. Durst, R.E. Wrolstad, T. Eisele, M.M. Giusti, J. Hach, H. Hofsommer, S. Koswig, D.A. Krueger, S. Kupina, S.K. Martin, B.K. Martinsen, T.C. Miller, F. Paquette, A. Ryabkova, G. Skrede, U. Trenn, J.D. Wightman, 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. 88 (2005) 1269–1278, https://doi.org/10.1093/jaoac/88.5.1269.

[19] R.L. Prior, E. Fan, H. Ji, A. Howell, C. Nio, M.J. Payne, J. Reed, Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders, J. Sci. Food Agric. 90 (2010) 1473–1478, https://doi.org/10.1002/jsfa.3966.

[20] S.S. Nielsen, Food Analysis Laboratory Manual, third ed., Kluwer Academic/Plenum Publishers, Ohio, 2003.

[21] M. Gralec, I. Wawer, K. Zawada, *Aronia melanocarpa* berries: phenolics composition and antioxidant properties changes during fruit development and ripening, Emir. J. Food Agric. (2019) 214–221, https://doi.org/10.9755/ejfa.2019.v31.i3.1921.

[22] M.-T. Tolić, I. Krbavčić, P. Vujević, B. Milinović, I. Jurčević, N. Vahčić, Effects of weather conditions on phenolic content and antioxidant capacity in juice of chokeberries (Aronia melanocarpa L.), Pol. J. Food Nutr. Sci. 67 (2017) 67–74, https://doi.org/10.1515/pjfns-2016-0009.

[23] J. Andrzejewska, K. Sadowska, L. Klóska, L. Rogowski, The effect of plant age and harvest time on the content of chosen components and antioxidative potential of black chokeberry fruit, Acta Sci. Pol. Hortorum Cultus. 14 (2015) 105–114, https://doi.org/10.3390/molecules21081098.

[24] E.S. Hwang, N.D. Thi, Effects of different growing regions on quality characteristics, bioactive compound contents, and antioxidant activity of aronia (*Aronia melanocarpa*) in Korea, Prev. Nutr. Food Sci. 21 (2016) 255–262, https://doi.org/10.3746/pnf.2016.21.3.255.

[25] L.D. Vittori, L. Mazzoni, M. Battino, Pre-harvest factors influencing the quality of berries, Sci. Hortic. 233 (2018) 310–322, https://doi.org/10.1016/j.scienta.2018.01.058.

[26] S. Conti, G. Villari, S. Faugno, G. Melchionna, S. Somma, G. Caruso, Effects of organic vs. conventional farming system on yield and quality of strawberry grown as an annual or biennial crop in southern Italy, Sci. Hortic. 180 (2014) 63–71, https://doi.org/10.1016/j.scienta.2014.10.015.

[27] E. Krüger, H. Dietrich, E. Schöpplein, S. Rasim, P. Kürbel, Cultivar, storage conditions and ripening effects on physical and chemical qualities of red raspberry fruit, Postharvest Biol. Technol. 60 (2011) 31–37, https://doi.org/10.1016/j.postharvbio.2010.12.001.

[28] S.Y. Wang, H.-S. Lin, Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage, J. Agric. Food Chem. 48 (2000) 140–146, https://doi.org/10.1021/jf9908345.
[29] J. Darias-Martín, A. Socas-Hernández, C. Díaz-Romero, E. Díaz-Díaz, Comparative study of methods for determination of titrable acidity in wine, J. Food Compos. Anal. 16 (2003) 555–562, https://doi.org/10.1016/s0889-1575(03)00032-2.
[30] M.-T. Tolić, I.L. Jurčević, I.P. Krbavčić, K. Marković, N. Vahčić, Phenolic content, antioxidant capacity and quality of chokeberry (*Aronia melanocarpa*) products, Food Technol. Biotechnol. 53 (2015) 171–179, https://doi.org/10.17113/ftb.53.02.15.3833.
[31] O. Taskin, Evaluation of freeze drying for whole, half cut and puree black chokeberry (*Aronia melanocarpa* L.), Heat Mass Tran. 56 (2020) 2503–2513, https://doi.org/10.1007/s00231-020-02867-0.
[32] S.S. Ferreira, P. Silva, A.M. Silva, F.M. Nunes, Effect of harvesting year and elderberry cultivar on the chemical composition and potential bioactivity: a three-year study, Food Chem. 302 (2020) 125366, https://doi.org/10.1016/j.foodchem.2019.125366.
[33] S. Yamaki, Metabolism and accumulation of sugars translocated to fruit and their regulation, J. Jpn. Soc. Hortic. Sci. 79 (2010) 1–15, https://doi.org/10.2503/jjshs1.79.1.
[34] A. Sidor, A. Drożdżyńska, A. Gramza-Michałowska, Black chokeberry (*Aronia melanocarpa*) and its products as potential health-promoting factors - an overview, Trends Food Sci. Technol. 89 (2019) 45–60, https://doi.org/10.1016/j.tifs.2019.05.006.
[35] J.A. Considine, E. Frankish, Essential analyses, in: J.A. Considine, E. Frankish (Eds.), A Complete Guide to Quality in Small-Scale Wine Making, Elsevier Inc., Massachusetts, 2014, pp. 137–154.
[36] P. Zaca-Morán, J.P. Padilla-Martínez, J.M. Pérez-Corte, J.A. Dávila-Pintle, J. G. Ortega-Mendoza, Etched optical fiber for measuring concentration and refractive index of sucrose solutions by evanescent waves, Laser Phys. 28 (2018) 116002, https://doi.org/10.1088/1555-6611/aad846.
[37] Pubchem, The PubChem Project, 2018. https://pubchem.ncbi.nlm.nih.gov/. (Accessed 7 August 2021).
[38] E. Aprea, M. Charles, I. Endrizzi, M. Laura Corollaro, E. Betta, F. Biasioli, F. Gasperi, Sweet taste in apple: the role of sorbitol, individual sugars, organic acids and volatile compounds, Sci. Rep. 7 (2017) 44950, https://doi.org/10.1038/srep44950.
[39] Y. Guan, C. Peace, D. Rudell, S. Verma, K. Evans, QTLs detected for individual sugars and soluble solids content in apple, Mol. Breed. 35 (2015) 135, https://doi.org/10.1007/s11032-015-0334-1.
[40] L.S. Magwaza, U.L. Opara, Analytical methods for determination of sugars and sweetness of horticultural products—a review, Sci. Hortic. 184 (2015) 179–192, https://doi.org/10.1016/j.scienta.2015.01.001.
[41] R. Saftner, J. Polashock, M. Ehlenfeldt, B. Vinyard, Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars, Postharvest Biol. Technol. 49 (2018) 19–26, https://doi.org/10.1016/j.postharvbio.2008.01.008.
[42] A. Kader, B. Hess-Pierce, E. Almenar, Relative contribution of fruit constituents to soluble solids content measured by refractometer, Hortscience 38 (2003) 833.
[43] L.I.L. Favaro, V.M. Balcão, L.K.H. Rocha, E.C. Silva, J.M. Oliveira Jr., M.M.D. C. Vila, M. Tubino, Physicochemical characterization of a crude anthocyanin extract from the fruits of jussara (Euterpe edulis Martius): potential for food and pharmaceutical applications, J. Braz. Chem. Soc. 29 (2018) 2072–2088, https://doi.org/10.21577/0103-5053.20180082.
[44] USDA, United States Standards for Grades of Blueberries, 1995. https://www.ams.usda.gov/sites/default/files/media/Blueberries_Standard%5B1%5D.pdf. (Accessed 10 August 2021).
[45] Z. Zorenc, R. Veberic, F. Stampar, D. Koron, M. Mikulic-Petkovsek, Changes in berry quality of northern highbush blueberry (*Vaccinium corymbosum* L.) during the harvest season, Turk. J. Agric. For. 40 (2016) 855–864, https://doi.org/10.3906/tar-1607-57.
[46] L. Jakobek, M. Drenjančević, V. Jukić, M. Šeruga, Phenolic acids, flavonols, anthocyanins and antiradical activity of "Nero", "Viking", "Galicianka" and wild chokeberries, Sci. Hortic. 147 (2012) 56–63, https://doi.org/10.1016/j.scienta.2012.09.006.
[47] I.D. Ochmian, J. Grajkowski, M. Smolik, Comparison of some morphological features, quality and chemical content of four cultivars of chokeberry fruits (*Aronia melanocarpa*), Not. Bot. Horti Agrobot. Cluj-Napoca 40 (2012) 253, https://doi.org/10.15835/nbha401718.
[48] M.H. Brand, B.A. Connolly, L.H. Lavine, J.T. Richards, S.M. Shine, L.E. Spencer, Anthocyanins, total phenolics, ORAC and moisture content of wild and cultivated dark-fruited *Aronia* species, Sci. Hortic. 224 (2017) 332–342, https://doi.org/10.1016/j.scienta.2017.06.021.
[49] J.D. Mahoney, T.M. Hau, B.A. Connolly, M.H. Brand, Sexual and apomictic seed reproduction in Aronia species with different ploidy levels, Hortscience 54 (2019) 642–646, https://doi.org/10.21273/HORTSCI13772-18.