

Phytochemical analysis of *Aronia melanocarpa* and $\times$ *Sorbaronia fallax* fruit

V. M. Mezhen'skyj*, L. M. Shevchuk* **, S. P. Kovalchuk*,
O. S. Havryliuk*, L. M. Levchuk**, S. M. Babenko**, Y. Y. Vint'skov'ska**

*National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

**Institute of Horticulture of the National Academy of Agrarian Sciences, Kyiv Region, Ukraine

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National University of Life
and Environmental Sciences
of Ukraine, Heroiv Oborony
st., 15, Kyiv, 03041, Ukraine.
Tel.: +38-050-297-27-39.
E-mail: mezh1956@ukr.net

Institute of Horticulture
of the National Academy
of Agrarian Sciences,
Sadova st., 23, Novosil'skyi,
Kyiv Region, 03027, Ukraine.
Tel.: +38-050-383-10-81.
E-mail:
l.shevchuk_2021@ukr.net

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Aronia melanocarpa (Michx.) Elliott, $\times$ *Sorbaronia fallax* (C. K. Schneid.) C. K. Schneid. nothosubsp. *fallax*, and $\times$ *Sorbaronia fallax* nothosubsp. *mitschurinii* (A. Skvortsov & Maitul.) A. Stalažs, belonging to the Rosaceae family, are grown as fruit plants. They are known to horticulturists as garden chokeberry or black chokeberry. The fruit of these species is the richest source of biophenols in the plant kingdom and suitable raw material for the production of functional foods with high nutraceutical value. The work aimed to examine the basic and polyphenolic composition of fruit chokeberry and rowan-chokeberry hybrids of different taxonomic groups. Experimental plants were grown at the orchard of the National University of Life and Environmental Sciences of Ukraine in the Kyiv region. Fruit of 10 cultivars developed in Belorussia, the Czech Republic, Finland, Russia, Ukraine, and the USA were collected during 2020 and 2021. The fruit was analyzed for dry matter, soluble solids, sugars, pectin substances, organic titratable acidity, ascorbic acid, and biophenols. Chokeberry fruit contains on average 24.4–29.2% dry matter, 16.0–21.1% soluble solids, 6.5–8.9% sugars, 0.40–0.80% pectin, and 0.95–2.15% organic acids on raw material. Bioactive components of chokeberry fruit are ascorbic acid (24.7–45.9 mg/100 g), total polyphenols (998–4840 mg/100 g), including anthocyanidins (9–217 mg/100 g), flavonoids (23–1422 mg/100 g), and chalcones (9–59 mg/100 g). If the group $\times$ *S. fallax* nothosubsp. *mitschurinii* cultivars is morphologically constant and like *A. melanocarpa* simple in leaf shape, then the group $\times$ *S. fallax* nothosubsp. *fallax* cultivars was different in the morphology of leaves from lobate to pinnate and fruit color from purple to black in the study. Chokeberry fruit composition varied between each year and especially between taxonomic groups and cultivars. Fruit harvested in 2021 had the lowest dry matter, soluble solid, sugars, and ascorbic acid in comparison with 2020. In contrast, titrated acidity was consistent between years. The fruit of garden chokeberries is a good source of ascorbic acid. $\times$ *S. fallax* cultivars with the exception 'Titan' are richer on ascorbic acid. The fruit of *A. melanocarpa* 'Dwarf' has the highest content of total polyphenols, flavonoids, and chalcones. There are significant differences between $\times$ *S. mitschurinii* cultivars in biochemical content. Purple-fruited 'Titan' has the lowest content of all biologically active substances, than other cultivars belonging to the same $\times$ *S. fallax* taxonomic group. The black-fruited $\times$ *S. fallax* nothosubsp. *fallax* genotypes, including the first chokeberry cultivar 'Veslava' of Ukrainian breeding, often have a high or the highest content of ascorbic acid, total polyphenols, anthocyanidins, flavonoids, and chalcones, which is valuable for garden chokeberry breeding.

Keywords: intergeneric hybrids; chokeberry; ascorbic acid; polyphenols; anthocyanidins; flavonoids; chalcones.

Introduction

The natural range of *Aronia melanocarpa* (Michx.) Elliott is in the eastern part of Northern America. It was introduced into Europe and used by Ivan Michurin (1932) to develop a new fruit crop named by him "black rowan". This name is explained by the fact that in those times chokeberry was included in the genus *Sorbus*. Later, when chokeberry was separated from *Sorbus*, horticulturists began to use the same names "black rowan" and "black chokeberry" for the designation of Michurin's plants, believing them to be *A. melanocarpa*, since the common name chokeberry was used for replacing genus name *Aronia*. Among horticulturists, this practice exists to this day (Denev et al., 2018; Sidor & Gramza-Michałowska, 2019; Tasinov et al., 2022).

In the 1980s it was found that Michurin's chokeberry is morphologically and cytologically different from wild *A. melanocarpa* (Fig. 1a, 1b). Therefore, a new species name *A. mitschurinii* A. Skvortsov & Maitul. was proposed for it (Skvortsov & Maitulina, 1982) and it was theorized that this taxon resulted from the hybridization and polyploidization of *A. melanocarpa* (Skvortsov et al., 1983). After some time, it was found that Michurin's chokeberry is an allopolyploid with *A. melanocarpa* as one of

the parents (Persson Hovmalm et al., 2004). Since the hybrid *A. $\times$ prunifolia* (Marshall) Rehder originated from a cross *A. melanocarpa* with another *Aronia* species, this name also began to be applied to Michurin's chokeberry (Pirc, 2015; Evarte-Bunrdee et al., 2022). However, molecular data analysis show that the other parent for crossing with *A. melanocarpa* is not chokeberry, but rowan (Jeppsson, 2000). Notwithstanding, other analyses did not reveal evidence of *A. mitschurinii* hybrid and higher-resolution markers are needed to resolve species boundaries and putative hybridization events (Shipunov et al., 2019).

Intergeneric hybrids between *Aronia* and *Sorbus* belong to $\times$ *Sorbaronia*, and the nothospecies name $\times$ *S. fallax* is used for the designation of hybrids between *A. melanocarpa* and *S. aucuparia* L. (Schneider, 1906a, 1906b). It is assumed that these two species are the parents of Michurin's chokeberry, therefore the botanical name for it should be $\times$ *S. fallax* according to the rules of the International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) (Turland et al., 2018). Therefore *A. mitschurinii* is only a synonym of $\times$ *S. fallax* (Govaerts et al., 2021).

Plants of $\times$ *S. fallax* has lobate or pinnate leaves, whereas Michurin's chokeberry has entire leaves (Fig. 1b, 1c, 1d, 1e). That's why some scientists use the name *A. mitschurinii*, because the leaves of Michurin's plant

are like the leaves of other *Aronia* species. Generally, they decided to maintain *A. mitschurinii* in *Aronia* because of its similarity to other *Aronia* species (Leonard et al., 2013). Some scientists don't use either the name $\times S. fallax$ or *A. mitschurinii* for Michurin's chokeberry because they consider it should be treated as an apomictic microspecies of cultigenous origin that is taxonomically distinct from the first nothospecies and genetically distinct from the second species (Sennikov & Phipps, 2013).

Since both names *A. mitschurinii* and $\times S. mitschurinii$ are synonymous with $\times S. fallax$, then to distinguish the morphologically different Michurin's chokeberry it is proposed to raise it from synonyms to nothosubspecies status – $\times S. fallax$ nothosubsp. *mitschurinii* A. Stalažs (Stalažs & Bädere, 2023).

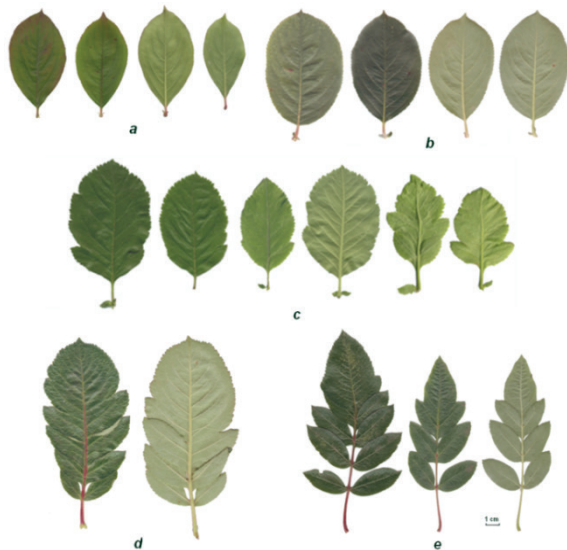


Fig. 1. Leaves of *A. melanocarpa* ‘Dwarf’ (a), $\times S. fallax$ nothosubsp. *mitschurinii* ‘Ahrostantsiia’ (b), $\times S. fallax$ nothosubsp. *fallax* ‘Vseslava’ (c), $\times S. fallax$ nothosubsp. *fallax* ‘Ruslana’ (d), $\times S. fallax$ nothosubsp. *fallax* ‘Titan’ (e) according to Mezhenksyj & Mezhenksa (2023)

We agree with the proposal of Stalažs & Bädere (2023), therefore in this paper, we will use the name *A. melanocarpa* for the cultivar of this North American species, $\times S. fallax$ nothosubsp. *mitschurinii* for Michurin's chokeberry cultivars, and $\times S. fallax$ nothosubsp. *fallax* for other garden chokeberry cultivars, morphologically different from Michurin's chokeberry. We have repeatedly observed that plants morphologically similar to $\times S. fallax$ nothosubsp. *mitschurinii* occur in the seed offspring of $\times S. fallax$ nothosubsp. *fallax*.

The development of the plant industry, including horticulture, particularly depends on the introduction of new plants. Blueberry, honeysuckle, kiwifruit, sea buckthorn, Japanese quince, chokeberry, and other fruit plants were introduced during the last century (Mezhenksyj, 2021; Mezhenksyj & Mezhenksa, 2023). Their fruit contains components, named “nutraceuticals”, such as biophenolics as well as ascorbic acid, and others. Consumption of foods rich in nutrients, including biologically active foods with an increased content of antioxidants, is not only the trend of today but also a common health concern (Shevchuk et al., 2022). It is generally accepted that fruits are an important part of the diet and are consumed as part of the general movement towards a healthier lifestyle. The fruit of

garden chokeberry is high in sugar and ascorbic acid (Oszmiański & Sapis, 1988; Tanaka & Tanaka, 2001). It has a very high content of polyphenols and the largest amount of substances coloring fruits (Jeppsson, 1999; Ochmian et al., 2012). The antioxidant content of chokeberry fruit is significantly higher than that of other fruits known and natural antioxidants have been praised for their positive effects on health (Hwang et al., 2014; Jurikova et al., 2017; Sidor & Gramza-Michałowska, 2019).

That is why chokeberry fruits and their processing products are increasingly used in the modern food system and as a natural food colorant. The importance of garden chokeberry culture is emphasized by its distribution in many countries of the world. If half a century ago it was grown on the territory of the Soviet Union, where it originated, now commercial plantations are also widespread in Northern Europe (Jeppsson, 1999, 2000; Jeppsson & Johansson, 2000), Central Europe (Oszmiański & Wojdyło, 2005; Jurikova et al., 2017; Trenka et al., 2020), Southeast Europe (Ochmian et al., 2012; Tasinov et al., 2022), and Eastern Europe (Mezhenksyj, 2019; Mezhenksyj & Mezhenksa, 2023), Asia Minor (Poyraz Engin & Mert) and East Asia (Tanaka & Tanaka, 2001; Hwang et al., 2014; Yang et al., 2019). The new fruit crop has become interesting in North America, where the natural range of its wild parents is located (Brand et al., 2019, 2022; Green et al., 2023).

Chokeberry plants are very hardy and relatively simple to grow. It is grown for larger edible fruits and ornamental value (Green et al., 2023; Mezhenksyj & Mezhenksa, 2023). Chokeberry is suitable for both organic and conventional cultivations (Trenka et al., 2020).

Chokeberry fruit has been traditionally used for jellies, jams, and functional beverages, but has wider use as a natural food colorant, rich source of antioxidants, and dietary fiber and has medicinal properties (Kulling & Rawel, 2008; Denev et al., 2018). Nevertheless, garden chokeberry cultivars have not been studied in Ukraine and they are less known for their contents of nutraceuticals such as polyphenols, anthocyanins, and ascorbic acid. ‘Vseslava’ is the first cultivar of Ukrainian breeding, included in the State Register of Plant Varieties in 2020 (Mezhenksyj & Mezhenksa, 2023).

Material and methods

Plant Materials. Fruit of 10 cultivars including 1 cultivar of *A. melanocarpa*, 5 cultivars of $\times S. fallax$ nothosubsp. *mitschurinii*, and 4 cultivars of $\times S. fallax$ nothosubsp. *fallax* was used in the study (Table 1, Fig. 2). The fruits were collected at the orchard of the Educational, Research and Productive Laboratory “Genetic Resources, Introduction and Breeding of Non-traditional Fruit and Ornamental Crops”, located at the Agronomic Research Station of the National University of Life and Environmental Sciences of Ukraine (Pshenychne, Bila Tserkva District, Kyiv Region). Fruits were harvested in the middle of August in two consecutive years, 2020 and 2021, when pomes were assessed to be visually ripe based on their black color at the initial stage of maturation. The difference between taxa and cultivars in terms of the fruit size and infructescence size as well as the number of fruits in the infructescence is demonstrated in Figure 2.

Dry matter. The sample drying method was used to determine the content of dry matter. For this purpose, sliced fruit particles of 3–4 g were put in a box with previously prepared weighed river sand. Drying to a constant weight of the prepared sample was carried out in a drying cabinet SNOL 58/350 (Ukraine, 2016) at a temperature of 98–100 °C, the data were expressed as a percentage.

Table 1
Taxa and cultivars used in this study

Taxon	Cultivar	Origin of cultivar	Origin of accession
<i>Aronia melanocarpa</i>	Dwarf	USA	Nursery "One Green World", Oregon, USA
$\times Sorbaronia fallax$ nothosubsp. <i>mitschurinii</i>	Nero	Czech Republic	"One Green World" Nursery, Oregon, USA
$\times Sorbaronia fallax$ nothosubsp. <i>mitschurinii</i>	Viking	Finland	"One Green World" Nursery, Oregon, USA
$\times Sorbaronia fallax$ nothosubsp. <i>mitschurinii</i>	Nadzeya	Belorussia	Institute of Fruit Growing, Samokhvalovich, Belorussia
$\times Sorbaronia fallax$ nothosubsp. <i>mitschurinii</i>	Venisa	Belorussia	Institute of Fruit Growing, Samokhvalovich, Belorussia
$\times Sorbaronia fallax$ nothosubsp. <i>mitschurinii</i>	Ahrostantsiia	Ukraine	National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine
$\times Sorbaronia fallax$ nothosubsp. <i>fallax</i>	Vseslava	Ukraine	Volodymyr Mezhenksyj, Bakhmut, Ukraine
$\times Sorbaronia fallax$ nothosubsp. <i>fallax</i>	Ruslana	Ukraine	Volodymyr Mezhenksyj, Bakhmut, Ukraine
$\times Sorbaronia fallax$ nothosubsp. <i>fallax</i>	Ruslana 2	Ukraine	Volodymyr Mezhenksyj, Bakhmut, Ukraine
$\times Sorbaronia fallax$ nothosubsp. <i>fallax</i>	Titan	Russia	All-Russian Research Institute of Genetics and Breeding of Fruit Plants, Michurinsk, Russia

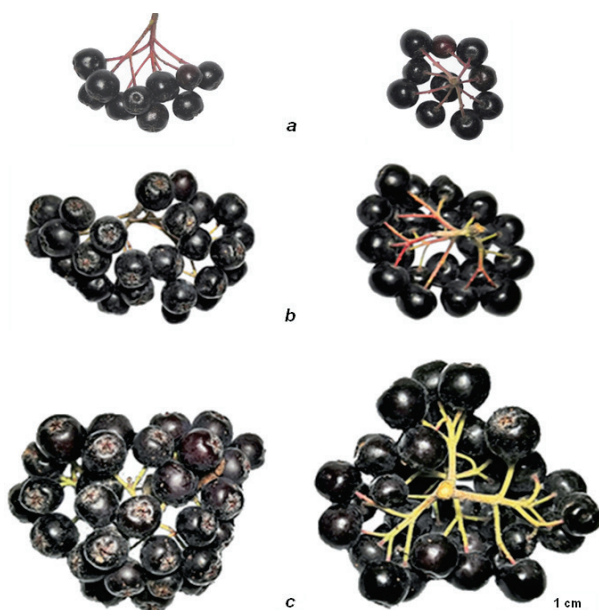


Fig. 2. Infructescences of *A. melanocarpa* 'Dwarf' (a), *×S. fallax* nothosubsp. *mitschurinii* 'Ahrostantsia' (b), *×S. fallax* nothosubsp. *fallax* 'Vseslava' (c); a is original photos, b, and c according to Mezhen'skyj & Mezhen'ska (2023)

Soluble solids were determined using an ATAGO PAL-1 portable refractometer (China, 2019). To prepare an analytical sample, fruits in the amount of 15–20 pieces were crushed using a homogenizer, and then a drop of juice was squeezed through the tissue on the refractometer glass, while recording the data, the error on the temperature was taken into account. The data were denoted as a percentage per raw mass.

Organic titrated acids. For the extraction of acids, 25 g of the crushed sample was transferred without loss, by washing with hot distilled water, with a volume of not more than 150 mL, into a volumetric flask with a capacity of 250 mL. The flask was held in a water bath for 30 min at a temperature of 80 °C and cooled. The content of the flask was adjusted to the mark with distilled water and filtered through a filter into a conical flask with a capacity of 250 mL. 20 mL of the extract was pipetted into a 250 mL conical flask, 3–4 drops of phenolphthalein were added and 0.1 N sodium hydroxide was titrated until a pink coloration corresponding to pH 7.0 appeared. The content of titrated acids in the sample was calculated according to the formula, using a titer index of 0.1 N sodium hydroxide and a conversion factor to citric acid (Kondratenko et al., 2008). The data were denoted as a percentage per raw mass.

Sugars. Extraction of sugars from blue honeysuckle was carried out with hot distilled water. The extract obtained was purified from proteins and pigments obtained by precipitation of the latter with acetic lead. Sucrose was subjected to hydrolysis of glucose and fructose when heated in the presence of 10% hydrochloric acid. The hydrolysis products were oxidized with Fehling's solution. The optical density of the resulting solutions was determined on a ULAB 102UV (China, 2020) spectrophotometer at a wavelength of 640 nm. The content of sugars in the sample was calculated by the formula, using the indicator of the graduated graph. Standard solutions of glucose with different concentrations were used to construct a graduated curve of the dependence of optical density (unit of optical density) on the glucose concentration (mg/mL) (Kondratenko et al., 2008). The data were denoted as a percentage per raw mass.

Ascorbic acid. For the extraction of ascorbic acid, the sample was mashed in a porcelain mortar with the addition of broken glass and a mixture of 2% oxalic and 1% hydrochloric acids (80+20, vol+vol), transferred to a volumetric flask with a capacity of 100 mL. The content of the flask was adjusted to the mark with a mixture of 2% oxalic acid and 1% hydrochloric acid (80+20, vol+vol) and filtered. The resulting extract was titrated with a solution of 2,6-dichlorophenolindophenol (Tilman's paint). The content of ascorbic acid in the sample was calculated by the formula using the Tilman's paint titer (Kondratenko et al., 2008). The data was expressed as 1 mg/100 g of raw mass.

Pectin substances. To determine pectin content, the sample was purified from sugars and pigments with the ethyl alcohol. The extraction of soluble pectin was carried out with water, and of protopectin – with 1 N sulfuric acid, hydrolysis of the latter to galacturonic acid – with heating. The 0.2%-carbazole solution was added to the extraction of the soluble pectin and protopectin acidified with the concentrated sulfuric acid to form a colored complex. The optical density of the attained solutions was determined using the ULAB 102UV (China, 2020) spectrophotometer at the wavelength of 535 nm. The content of soluble pectin and protopectin in the sample was calculated utilizing the coefficient of the recapitulation for apple pectin and indices of the calibration graph. The standard solutions of the galacturonic acid with different concentrations were applied for the formation of the calibration graph of the optical density (unit of the optical density) dependence on the mentioned acid concentration (μg/mL) (Kondratenko et al., 2008).

Total phenolic content. For the extraction of total phenolic content, the sample was mashed in a porcelain mortar with a small amount of ethyl alcohol and filtered using a vacuum on the Büchner funnel through a blue-ribbon paper filter into a Bunsen flask. The residue on the filter was washed with small amounts of ethyl alcohol until the sample was completely discolored. The volume of used alcohol (mL) was traced. 7.9 mL of distilled water, 0.1 mL of extract, and 1 mL of Folin-Denis reagent were added to the tube, stirred, and after 3 minutes 1 mL of saturated sodium carbonate solution was added and stirred again. For an hour, the optical density of the contents of the tubes was recorded on a ULAB 102UV (China, 2020) spectrophotometer at a wavelength of 640 nm. As a control, a mixture prepared as follows was used: 8 ml of distilled water, and 1 mL of Folin-Denis reagent were poured into a tube, and stirred, after 3 minutes 1 mL of saturated sodium carbonate solution was added and stirred again. At least three parallel measurements were carried out and the average value of the optical density index was found. The total phenolic content in the sample was calculated according to the formula, using the indicators of the graduated graph. Standard solutions of chlorogenic acid with different concentrations were used to construct a graduated graph of the dependence of optical density (unit of optical density) on the concentration of chlorogenic acid (μg/mL). The data were expressed as mg/100 g of raw mass. Anthocyanin and chalcone content was determined by the spectrophotometric method using ULAB 102UV (China, 2020) spectrophotometer. The absorption of extracts was determined at wavelengths of 530 and 364 nm, respectively, using an alcohol extract from plant homogenate acidified with 3.5% hydrochloric acid. The data was expressed as mg/100 g of raw mass.

Flavonoid content was determined by absorption spectrophotometry by measuring the absorption of a flavonoid complex with a 3% aluminum chloride solution. A ULAB 102UV (China, 2020) spectrophotometer with a wavelength of 410 nm was used for the measurement. The data was expressed as 1 mg/100 g of raw mass.

Statistical analysis of the study data was performed using Statistica 13.1 software (StatSoft, Inc., USA). Results are expressed as means ± standard deviation of three analytical replicates in each year. The differences between cultivars were determined using Tukey's test, where the differences were considered significant at $P < 0.05$ (accounting for Bonferroni's correction).

Results

Our research shows significant both annual and varietal differences in garden chokeberry fruit in dry matter, titrated acidity, sugars, pectin, ascorbic acid, and the total content of polyphenols and their components such as anthocyanidins, flavonoids, and chalcones (Table 2–5).

The average dry matter and soluble solids in chokeberry fruit lie between 23.2–29.9% and 16.0–20.2%, respectively. The 'Nero' and 'Nadzeja' fruit were the juiciest, and 'Dwarf' and 'Nero' fruit consisted of the biggest amount of soluble solids. Simultaneously, 'Dwarf' fruit is not significantly different juiciest 'Nero' and 'Nadzeja' ones. Most garden chokeberry cultivars are similar to each other in soluble solids in fruit.

Garden chokeberry fruit was more sugary at the beginning of fruit ripening when it acquired its typical color in 2020. Then 'Viking' fruit accumulated up to 10.0% sugars. Most garden chokeberry cultivars are

similar to each other in mean sugars in fruit. Although fruit had the highest amount of sugars under our condition in 2020, the amount of pectin in fruit was greater in 2021.

Table 2

Dry matter and soluble solids' content of garden chokeberry fruit, 2020–2021 ($\bar{x} \pm \text{SD}$, $n = 3$)

Cultivar	Dry matter, %		Soluble solids, %	
	2020	2021	2020	2021
Dwarf	31.48 $\pm$ 0.50 ^b	26.36 $\pm$ 0.06 ^c	24.51 $\pm$ 0.46 ^a	17.67 $\pm$ 0.35 ^b
Nero	32.97 $\pm$ 0.45 ^a	26.91 $\pm$ 0.07 ^b	23.21 $\pm$ 0.40 ^b	18.97 $\pm$ 0.35 ^a
Viking	26.59 $\pm$ 0.36 ^d	22.22 $\pm$ 0.09 ^e	22.54 $\pm$ 0.51 ^c	17.77 $\pm$ 0.32 ^b
Nadzeya	32.52 $\pm$ 0.55 ^a	27.34 $\pm$ 0.09 ^a	23.58 $\pm$ 0.35 ^b	16.33 $\pm$ 0.35 ^c
Venisa	26.49 $\pm$ 0.50 ^d	23.95 $\pm$ 0.15 ^c	22.51 $\pm$ 0.56 ^c	16.60 $\pm$ 0.36 ^c
Ahrostantsiia	25.34 $\pm$ 0.35 ^e	21.16 $\pm$ 0.22 ^b	20.41 $\pm$ 0.53 ^d	16.41 $\pm$ 0.10 ^e
Vseslava	27.80 $\pm$ 0.26 ^c	19.22 $\pm$ 0.09 ^k	22.37 $\pm$ 0.47 ^e	14.63 $\pm$ 0.08 ^d
Ruslana	27.40 $\pm$ 0.53 ^c	19.94 $\pm$ 0.06 ^j	20.11 $\pm$ 0.85 ^d	14.80 $\pm$ 0.10 ^d
Ruslana 2	27.95 $\pm$ 0.33 ^c	23.42 $\pm$ 0.09 ^f	22.41 $\pm$ 0.53 ^c	17.83 $\pm$ 0.07 ^b
Titan	24.66 $\pm$ 0.35 ^e	25.58 $\pm$ 0.42 ^d	18.31 $\pm$ 0.36 ^c	13.73 $\pm$ 0.25 ^e

Note: different letters indicate values that are significantly different within one column according to results of the Tukey test ($P < 0.05$).

Table 3

Total sugars and pectin substances content of garden chokeberry fruit, 2020–2021 ($\bar{x} \pm \text{SD}$, $n = 3$)

Cultivar	Total sugars, %		Pectin substances, %	
	2020	2021	2020	2021
Dwarf	8.36 $\pm$ 0.06 ^c	4.71 $\pm$ 0.30 ^f	0.345 $\pm$ 0.051 ^c	0.968 $\pm$ 0.025 ^a
Nero	9.19 $\pm$ 0.11 ^b	7.45 $\pm$ 0.35 ^a	0.647 $\pm$ 0.007 ^a	0.951 $\pm$ 0.020 ^a
Viking	9.97 $\pm$ 0.19 ^a	7.90 $\pm$ 0.50 ^a	0.267 $\pm$ 0.040 ^d	0.564 $\pm$ 0.025 ^c
Nadzeya	7.66 $\pm$ 0.08 ^c	7.74 $\pm$ 1.38 ^a	0.258 $\pm$ 0.045 ^d	0.537 $\pm$ 0.035 ^c
Venisa	9.86 $\pm$ 0.13 ^a	6.39 $\pm$ 0.30 ^b	0.428 $\pm$ 0.030 ^b	0.487 $\pm$ 0.025 ^d
Ahrostantsiia	8.60 $\pm$ 0.31	8.02 $\pm$ 0.88 ^a	0.647 $\pm$ 0.050 ^a	0.171 $\pm$ 0.020 ^f
Vseslava	9.36 $\pm$ 0.06 ^b	6.01 $\pm$ 0.23 ^{bc}	0.482 $\pm$ 0.020 ^b	0.692 $\pm$ 0.045 ^b
Ruslana	9.49 $\pm$ 0.10 ^b	5.32 $\pm$ 0.35 ^c	0.667 $\pm$ 0.042 ^a	0.660 $\pm$ 0.020 ^b
Ruslana 2	9.66 $\pm$ 0.15 ^{ab}	7.34 $\pm$ 0.33 ^{ab}	0.678 $\pm$ 0.065 ^a	0.581 $\pm$ 0.020 ^c
Titan	8.04 $\pm$ 0.15 ^d	4.93 $\pm$ 0.40 ^f	0.609 $\pm$ 0.070 ^a	0.391 $\pm$ 0.020 ^e

Note: see Table 2.

Table 4

Titrateable acidity and ascorbic acid content of garden chokeberry fruit, 2020–2021 ($\bar{x} \pm \text{SD}$, $n = 3$)

Cultivar	Titrateable acidity, %		Ascorbic acid, %	
	2020	2021	2020	2021
Dwarf	1.120 $\pm$ 0.085 ^c	0.780 $\pm$ 0.040 ^e	43.0 $\pm$ 2.0 ^{cd}	26.4 $\pm$ 2.1 ^d
Nero	1.087 $\pm$ 0.090 ^c	1.247 $\pm$ 0.050 ^f	38.1 $\pm$ 2.0 ^e	33.3 $\pm$ 4.7 ^c
Viking	1.433 $\pm$ 0.070 ^d	1.487 $\pm$ 0.035 ^{de}	57.1 $\pm$ 2.0 ^a	30.9 $\pm$ 3.0 ^f
Nadzeya	1.430 $\pm$ 0.075 ^d	1.297 $\pm$ 0.055 ^f	45.3 $\pm$ 3.0 ^e	38.7 $\pm$ 1.5 ^b
Venisa	1.420 $\pm$ 0.072 ^d	1.550 $\pm$ 0.050 ^d	51.2 $\pm$ 2.0 ^b	40.6 $\pm$ 2.2 ^{ab}
Ahrostantsiia	1.800 $\pm$ 0.100 ^c	1.530 $\pm$ 0.082 ^d	48.3 $\pm$ 2.5 ^{bc}	43.0 $\pm$ 0.9 ^a
Vseslava	1.357 $\pm$ 0.060 ^d	1.407 $\pm$ 0.090 ^e	40.9 $\pm$ 2.0 ^{de}	35.2 $\pm$ 1.6 ^{bc}
Ruslana	1.700 $\pm$ 0.056 ^c	1.717 $\pm$ 0.050 ^b	44.2 $\pm$ 3.0 ^d	39.1 $\pm$ 0.8 ^{ab}
Ruslana 2	2.263 $\pm$ 0.071 ^a	2.033 $\pm$ 0.042 ^a	35.7 $\pm$ 1.5 ^c	41.9 $\pm$ 2.7 ^{ab}
Titan	2.027 $\pm$ 0.155 ^b	1.560 $\pm$ 0.053 ^d	25.4 $\pm$ 2.5 ^f	23.9 $\pm$ 2.1 ^d

Note: see Table 2.

The index of titrated acidity, in contrast to the content of sugars and pectin in fruit, is much more stable over the years. 'Ruslana' is distinguished by the highest average content of organic acids in the fruit 2.15%, and 'Dwarf' is distinguished by the lowest average content of organic acids in the fruit, only 0.95%. All garden chokeberry cultivars are high in C-vitamins with the richest average content of 38.8–45.9 mg/100 g in the fruit of 'Ruslana 2', 'Ruslana', 'Nadzeya', 'Viking', 'Ahrostantsiia', and 'Venisa'. 'Viking' fruit has up maximum of ascorbic acid to 57.1 mg/100 g in 2020.

Significant differences between garden chokeberry cultivars exist in the content of total polyphenols. 'Dwarf' fruit has the highest content of total polyphenols, flavonoids, and chalcones, and 'Titan' fruit has the lowest content of all phenol compounds. Other cultivars in the content of biophenols occupy an intermediate position between these two cultivars, differing among themselves, and 'Venisa' is ahead of all cultivars in terms of anthocyanidins' quantity in fruit.

Table 5

Polyphenols' content of garden chokeberry fruit, 2020 ($\bar{x} \pm \text{SD}$, $n = 3$)

Cultivar	Total polyphenols, mg/100 g	Anthocyanidins, mg/100 g	Flavonoids, mg/100 g	Chalcones, mg/100 g
Dwarf	4840 $\pm$ 20 ^a	201.93 $\pm$ 7.00 ^b	1422.37 $\pm$ 7.50 ^a	58.70 $\pm$ 2.52 ^a
Nero	3148 $\pm$ 41 ^b	200.90 $\pm$ 6.00 ^b	790.37 $\pm$ 5.50 ^d	52.43 $\pm$ 2.50 ^b
Viking	2769 $\pm$ 49 ^d	199.47 $\pm$ 5.05 ^b	969.90 $\pm$ 8.53 ^b	41.77 $\pm$ 1.97 ^c
Nadzeya	3063 $\pm$ 61 ^c	181.43 $\pm$ 6.50	823.63 $\pm$ 7.50 ^c	43.33 $\pm$ 3.79 ^c
Venisa	2525 $\pm$ 37 ^c	217.33 $\pm$ 1.15 ^a	825.40 $\pm$ 5.50 ^c	55.53 $\pm$ 4.50 ^{ab}
Ahrostantsiia	2317 $\pm$ 35 ^f	194.00 $\pm$ 7.55 ^b	642.67 $\pm$ 10.41 ^e	35.33 $\pm$ 2.08 ^d
Vseslava	2266 $\pm$ 34 ^f	198.67 $\pm$ 6.51 ^b	970.23 $\pm$ 15.01 ^b	40.00 $\pm$ 3.00 ^e
Ruslana	1794 $\pm$ 95 ^g	57.00 $\pm$ 2.00 ^d	282.07 $\pm$ 8.00 ^g	17.47 $\pm$ 2.50 ^f
Ruslana 2	3063 $\pm$ 61 ^c	16.33 $\pm$ 1.53 ^e	485.10 $\pm$ 10.00 ^f	28.33 $\pm$ 1.53 ^c
Titan	988 $\pm$ 20 ^h	9.40 $\pm$ 0.53 ^e	22.67 $\pm$ 3.06 ^h	8.00 $\pm$ 1.00 ^g

Note: see Table 2.

Discussion

Although the phenotypical variation in *×S. fallax* nothosubsp. *Mitschurinii* cultivars is very limited (Skvortsov et al., 1993; Jeppsson, 1999), there are significant differences in the chemical composition of their fruits (Kulling & Rawel, 2008; Ochmian et al., 2012). Fruit moisture content, total phenolic content, and total anthocyanin content depend on the taxonomic group of chokeberries. The *×S. fallax* nothosubsp. *mitschurinii* group showed the smallest year-to-year change for biochemical parameters. There may be a couple of reasons for this, including their being much more genetically homogeneous than wild *Aronia* species (Brand et al., 2017, 2022). Our data show significant differences between Michurin's chokeberry cultivars in terms of biochemical composition. As Jeppsson (2000) pointed out, the biochemical data varies from year to year and is influenced by seasonal differences in rainfall and temperature. Besides, there are differences in ripeness at harvest, even when berries appear fully ripe.

The content of dry matter and soluble solids is strongly influenced by the year's conditions. Thus, in 2021, the fruits were juicier, the average dry matter content was 23.6%, while in 2020 it was 28.3%. In general, the fruits of 'Nadzeya', 'Nero', and 'Dwarf' contained less water (Table 2). The dry matter in chokeberry fruit lies between 15.3–28.8% (Kulling & Rawel, 2008; Ochmian et al., 2012; Poyraz Engin & Mert, 2020), which is close to our data as well as the content of soluble solids (table 2). According to Ochmian et al. (2012), content of soluble solids is 14.2–18.7°Brix. Soluble solids values of chokeberry fruit did not change significantly over the harvest period, ranging from 17.3% to 19.4% (Poyraz Engin & Mert, 2020).

The average sugar content is 9.0% in 2020, and 6.6% in 2021 under our conditions. It is less in comparison with the data of other researchers 9.6–13.8% (Ochmian et al., 2012) or 19.3–24.3% (Poyraz Engin & Mert, 2020). According to Tanaka & Tanaka (2001), the sugar content in chokeberry fruit is 8.9–11.2 Brix%. We confirm the data of Ochmian et al. (2012) and Poyraz Engin & Mert (2020) that the majority of the sugar component is reducing sugar.

Literature sources state that chokeberry fruits are moderately rich in pectin, with their content in fresh fruits ranging from 0.30–0.75% (Kulling & Rawel, 2008), and 0.35–0.50% (Trenka et al., 2020). In our study, the mean pectin content ranged from 0.40–0.80%.

Total acidity in the Michurin's chokeberry cultivars is 0.8–1.8% (Jeppsson & Johansson, 2000; Tanaka & Tanaka, 2001; Kulling & Rawel, 2008; Ochmian et al., 2012) and higher than for black chokeberry (Tanaka & Tanaka, 2001). Variation in fruit quality among similar cultivars is very limited, whereas variation in acid content may increase by 110% from a poor year to a good year (Jeppsson, 2000). The titrateable acidity is 1.3%, and the differences between cultivars and between maturity stages were negligible (Yang et al., 2019). Jeppsson & Johansson (2000) detected a negative correlation of variation among sampling dates in total acidity with variation in fruit weight, suggesting that dilution by water uptake affects total acidity. *A. melanocarpa* 'Dwarf' with the smallest fruit has the lowest organic acid content. In general, titrateable acidity fluctuated little over the years, unlike other indicators of biochemical composition.

The fruit of garden chokeberries is a good source of ascorbic acid, containing an average 24.5–45.9 mg/100 g (Table 4). By comparison, blueberries contain 17.3–20.9 mg/100 g of this vitamin (Shevchuk et al., 2021). However, Tanaka & Tanaka (2001) reported a lower content of ascorbic acid (13.7 mg/100 g) in chokeberry fruit, while according to other investigations the chokeberry fruit contained this vitamin at the level of 65.2 mg/100 g (Denev et al., 2018).

Polyphenols are the main substances that determine the greatest value of garden chokeberry fruit. In particular, the fruit of popular blueberry contains 482 mg/100 g of it (Shevchuk et al., 2021), and blue honeysuckle 848 mg/100 g in raw fruit (Shevchuk et al., 2022). Some factors must be taken into account, in particular, the content of total polyphenols and particular phenolic compounds of different berries crops depending on genetic factors and growing conditions. The different analysis methods must be taken into account too (Jurikova et al., 2017). A review of studies on the biochemical composition of chokeberry fruits indicates large differences in the amount of total polyphenols and their components determined by different authors (Denev et al., 2012). Weather conditions significantly affect the accumulation of polyphenols. In experiments by Brand et al. (2017) polyphenol content for the same genotypes varied significantly between years. Ochmian et al. (2012) reported 2340 mg/100 g total polyphenols for *A. melanocarpa* cultivar, and 1845–2185 mg/100 g for *×S. fallax* nothosubsp. *mitschurinii* cultivars, at which ‘Nero’ has higher polyphenol content than ‘Viking’. According to Poyraz Engin & Mert (2020) total phenol content for ‘Nero’ is 1890 mg/100 g in mid August against 1927 mg/100 g for ‘Viking’. In our study, the difference between these cultivars was even greater, 3148 mg/100 g against 2769 mg/100 g, respectively (Table 5).

It is known that polyphenols *A. melanocarpa* and *×S. fallax* nothosubsp. *mitschurinii* consist of anthocyanins, proanthocyanins, flavonols, chlorogenic acid, and neochlorogenic acid (Oszmianski & Wojdylo, 2005; Ochmian et al., 2012; Taheri et al., 2013; Brand et al., 2017). It can be assumed that the *×S. fallax* nothosubsp. *fallax* fruits have the same composition of polyphenols. A comparison of anthocyanin content in different berry crops showed that among black currant, red currant, gooseberries, strawberry, blackberry, and red raspberry, chokeberry has the highest anthocyanidin concentration (Jurikova et al., 2017; Yang et al., 2019). The content of anthocyanidin in chokeberry fruits is directly related to the color of fruits in different *Aronia* species. Black-fruited *A. melanocarpa* had higher content than purple-fruited *A. ×prunifolia*, and red-fruited *A. arbutifolia* had the lowest anthocyanidin content (Taheri et al., 2013). Genetic studies have shown all varieties of *×S. fallax* nothosubsp. *mitschurinii* to be closely related clones (Persson Hovmalm et al., 2004), but they are significantly different from each other in biophenol levels. The vast majority of *Aronia* accessions showed very similar anthocyanin form composition, but there were a few exceptions (Brand et al., 2017). According to both studies by Ochmian et al. (2012) and by us, anthocyanin content of ‘Nero’ and ‘Viking’ fruits are similar. However, another study showed anthocyanin content of ‘Nero’ is higher than that of ‘Viking’ with an increase in ‘Nero’ observed 15 days earlier than that in ‘Viking’ (Poyraz Engin & Mert, 2020). The anthocyanin content of chokeberry fruit tended to increase with maturation, reaching in ripe ‘Viking’ fruits 225 mg/100 g (Yang et al., 2019).

As can be seen in Table 5 *A. melanocarpa* ‘Dwarf’ fruit has the highest content of chalcones and flavonoids, 59.1 and 1422.1 mg/100g, respectively, among evaluated cultivars. Its fruit contains also the highest total polyphenols, up to 4840 mg/100g in 2020. The fruit of ‘Ruslana’ and especially ‘Titan’ have a much lower content of total polyphenols as well as anthocyanidins, flavonoids, and chalcones compared to other cultivars. Both these cultivars belong to *×S. fallax* nothosubsp. *fallax*, which according to the morphology of the leaves, inflorescences, and infructescences are more like *Sorbus*, whereas Michurin's chokeberry cultivars are morphologically and biochemically close to black chokeberry. The lowest content all classes of biophenol was in purple-colored fruit of *×S. fallax* nothosubsp. *fallax* ‘Titan’. Therefore, there is an order of total polyphenol, flavonoid, and chalcone content *A. melanocarpa* > *×S. fallax* nothosubsp. *mitschurinii* > *×S. fallax* nothosubsp. *fallax*. Nevertheless, there is a significant difference in the content of biophenols between the three closely related black-fruited *×S. fallax* nothosubsp. *fallax* cultivars: ‘Vseslava’,

‘Ruslana’, and ‘Ruslana 2’, and the last two cultivars are seedlings of ‘Vseslava’. At the same time, the very high content of total polyphenols in ‘Ruslana 2’ fruit, which is higher in many *×S. fallax* nothosubsp. *mitschurinii* cultivars, seems surprising. Fruit of *×S. fallax* nothosubsp. *fallax* ‘Vseslava’ accumulates the same total polyphenols as *×S. fallax* nothosubsp. *mitschurinii* ‘Ahrostantsiia’. Therefore, the selection of genotypes with a high level of biophenols is also possible in *×S. fallax* nothosubsp. *fallax*. According to Yang et al. (2019) polyphenols and flavonoids are comparatively more abundant in the red tip stage of chokeberry fruits as opposed to anthocyanidin content, which increases as the fruits ripen. The flavonoid content of Michurin's chokeberry fruit decreased significantly as the ripening process progressed. The total flavonoid content of ‘Viking’ fruit was highest at the red tip stage at 3176 mg/100 g with a threefold decrease in their quantity in ripe fruits. This is consistent with our data, which indicates a flavonoid content of 970 mg/100 g in ripe ‘Viking’ fruit. It is well known that polyphenolic compounds are the main substances, responsible for the antioxidant activity of plant materials, and there is a good correlation between their content and antioxidant properties of plant foods (Denev et al., 2012; Green et al., 2023). In our study, anthocyanins have a moderate correlation with total polyphenols 0.51, and a very strong correlation with both flavonoid and chalcone content, 0.81 and 0.87, respectively. Similarly, flavonoid content has a moderate correlation with total polyphenols 0.55, and a very strong correlation with chalcone content, 0.90. The correlation between chalcone and total polyphenols is strong at 0.72.

Conclusion

The current study presents data on the chemical composition of fruit of 10 garden chokeberry cultivars grown under the climatic conditions of Kyiv region of Ukraine. ‘Dwarf’ belongs to true North American *A. melanocarpa*, ‘Nero’, ‘Viking’, ‘Nadzeya’, ‘Venisa’, and ‘Ahrostantsiia’ selected in Belorussia, Czech Republic, Finland, and Ukraine, are typical Michurin's chokeberry, or *×S. fallax* nothosubsp. *mitschurinii*, ‘Vseslava’ and its progeny ‘Ruslana’, ‘Ruslana 2’ developed in Ukraine together with ‘Titan’ of Russian breeding are morphologically different from Michurin's chokeberry, belong to *×S. fallax* nothosubsp. *fallax*. We demonstrated that cultivars and taxonomic groups were often different from each other in both the content and the composition of dry matter, soluble solids, organic acids, ascorbic acid, sugars, and biophenolic compounds. The chemical content is dependent on genotype and climatic conditions.

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