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Research Article

Comparative analysis of bioactive compounds identified in the fruits of three chokeberry (*Aronia melanocarpa*) cultivars in different cultivation systems

Anca Maria CHIOREAN[1,2], Zsolt JAKAB- ILYEFALVI[1], Smaranda Doina ROȘU-MAREȘ[1], Georgeta Maria GUZU[1], Claudiu MOLDOVAN[1], Ioan ZAGRAI[1], Mirela Irina CORDEA[2], Floricuța RANGA[3], Loredana-Florina LEOPOLD[3,4], Florina SCURTU[4]*

[1]*Fruit Research and Development Station Bistrita, Drumul Dumitrei Nou, no. 3, Bistrita, Romania; anca-maria.chiorean@usamvcluj.ro; zsolt.jakab@yahoo.com; rmsd_smery@yahoo.com; georgeta.guzu13@gmail.com; clau92moldovan@gmail.com; izagrai@yahoo.com*
[2]*University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Horticulture and Bussines in Rural Development, Romania; mcordea@usamvcluj.ro*
[3]*University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Food Science and Technology, 3-5 Mănăștur Street, 400372, Cluj-Napoca, Romania; ranga.floricuta@usamvcluj.ro; loredana.leopold@usamvcluj.ro*
[4]*University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Life Science Institute, 3-5 Mănăștur Street, 400372, Cluj-Napoca, Romania; florina.scurtu@usamvcluj.ro (*corresponding author)*

Abstract

Aronia melanocarpa (Michx.) Elliot, commonly known as chokeberry, has become visible due to its rich polyphenol content and its exceptional antioxidant and anti-inflammatory properties. The present study aimed to investigate how different cultivation systems can influence the bioactive compounds in three chokeberry cultivars, 'Nero', 'Viking' and 'Melrom', grown in the Northern Transylvania, Romania. The results showed that each variety exhibits distinct characteristics: 'Nero' excelled in ascorbic acids and amount of flavonols, particularly quercetin derivatives; 'Melrom' had the highest overall sorbitol content and flavonols level, and also a significantly content of hydroxybenzoic acid, while the 'Viking' cultivar presented an unusual profile with high levels of cyanidin and quercetin but a significant deficiency in quercetin-rutinoside. Compared with conventional and control, the ecological treatments significantly enhanced total phenolic content in 'Viking' and 'Melrom' cultivars. The variations in chokeberry composition can also be attributed to genetic factors, fertilizers, pedoclimatic conditions and harvest time. Understanding these factors is essential for optimizing cultivation practices and enhancing the nutritional value of chokeberry fruits.

Keywords: antioxidants; ascorbic acid; carbohydrates; fertilizers; polyphenols

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Introduction

Aronia melanocarpa (Michx.) Elliot is a deciduous fruiting shrub native to North America, belongs to the Rosaceae family, Aronia genus. It is one of the fruits known for its powerful antioxidant properties and was introduced to Europe in the early 20th century. It comprises three species distinguished by their fruit color: black chokeberries (*A. melanocarpa*), red chokeberries *(A. arbutifolia*), and purple chokeberries (*A. prunifolia*) (Negreanu-Pirjol *et al*., 2023). *A. melanocarpa* is primarily cultivated for its fruit, with notable cultivars including 'Nero' (Czech Republic), 'Viking' (Finland), 'Aron' (Denmark), 'Hugin' (Sweden), 'Rubina' (Russia), and 'Melrom' (Romania) (Sasmaz *et al*., 2024).

Responsible for antioxidant activity is a variety of compounds such as polyphenols, organic acids, proteins, vitamins, and carotenoids, among which polyphenols predominate, playing a key role in their health benefits (Kasprzak-Drozd *et al*., 2021). The predominant polyphenols in aronia berries are tannins (procyanidins), anthocyanins, phenolic acids (chlorogenic and neochlorogenic acids), and flavonols (quercitin glycosides) (Meng *et al*., 2019). Of these, chlorogenic acid and procyanidins are responsible for the astringent, bitter, and sour tastes while the dark blue color of chokeberry fruit is caused by the high concentration of anthocyanins. Previous studies indicate polyphenols and flavonols have the potential to reduce the activity of free radicals and inhibit the progression of diverse cancer types, including leukemia, breast cancer, colon cancer, cancer stem cells, anti-obesity, anti-diabetic, and anti-inflammatory cardio-vascular protective effects (Skupień and Oszmianski, 2007; Lee *et al.*, 2014; Veberic *et al.*, 2015; Yang *et al*., 2019).

A series of factors, such as growth area, harvest date, cultivar, fertilizations, and harvest time can influence their chemical composition (Skender *et al*., 2014; Djuric *et al*., 2015; Wójtowicz *et al.*, 2023). Some reports indicate that additional fertilization can increase the yield and promote plant growth but may lead to a reduction in pigment content and total acidity (Jeppesson, 2000), or a decrease in sucrose content in treated aronia.

Still, it can lead to a decrease in chlorogenic and neochlorogenic acids, pigment content, and total acidity, a lower degree of polymerization of procyanidins compared to the control fruits (Skupień *et al.*, 2008). Research consistently demonstrates the wide range of benefits, including nutritional, pharmacological, antioxidant, and protective effects against oxidative stress (Zhang *et al.*, 2021; Banach *et al*., 2020; Catană *et al.*, 2018; Sidor *et al.* 2019).

Considering the established role of fertilizers in enhancing fruit yield and quality, this study aimed to investigate the effects of different cultivation systems ecologic versus conventional, with respect to fertilization and phytosanitary management on the polyphenol profile, ascorbic acid, and carbohydrate content of three *Aronia melanocarpa* cultivars.

Materials and Methods

Plant material and experimental design

Three chokeberry cultivars 'Viking,' 'Melrom,' and 'Nero' were evaluated within an experimental field trial in 2022, under pedoclimatic conditions at Fruit Research and Development Station Bistrita (FRDS Bistrita) in the Northern Transylvania, Romania. The 'Melrom' and 'Nero' chokeberry cultivars were planted in 2020 on a molic eutricambosoil, and 'Viking' two years later in 2022, using two-year plants transferred from pots. The experimental design was organized into randomized blocks, with each variant replicated three times, seven plants per replicate. Three treatment variants were included in the study: conventional (T1), ecological (T2), and control (T3).

The analyses were carried out after two years of treatment in the case of 'Melrom' and 'Nero' and after one vegetative treatment period in the case of 'Viking'. They were focused on the contents of phenolic compounds, ascorbic acid, and carbohydrates of the chokeberry fruits.

Characterization of treatment variants

For the established treatment options, conventional chemical and ecological products were used.

1. Conventional (T1) variant included products based on copper and acetamiprid such as Toil conc. 0.5%, Merpan 80 WDG conc. 0.15%, Champ 77WG conc. 0.2%, Mospilan 20 SG/SP conc. 0.02%, and Score 250 EC conc. 0.03%.

Soil fertilizer NPK 16-16-16, 150 g/m2 (N, P, and K), and foliar fertilizer Agroleaf Total Power 20+20+20+TE in a concentration of 0.03% were used. Agroleaf Power fertilizer contained: N- 20 %, P_2O_5 ~ 20 %, K_2O ~ 20 %, S ~1 %, Fe cca. 0.14 %, Mn ~ 0.07 %, B ~ 0.03 %, Cu ~ 0.07 %, Mo ~ 0.001 %, Zn ~ 0.070 %. Agroleaf Power Total is a complete foliar fertilizer that provides plants with all the necessary elements that will help prevent and correct the deficiencies of all macro and microelements. The product is very well absorbed in the leaves and can be mixed with a very wide range of pesticides, as well as applied at the same time. It is especially necessary in unfavorable and stressful conditions, when absorption by the roots is deficient (cold, too much water, too high pH).

2. Ecologic (T2) variant included products based on copper, paraffinic oil, and natural plant extract such as: Ovipron top 0.2%, Champ 77WG 0.2%, Mimox 0.3%, Laser 240 SC 0.6 l/ha, Prev-Am 0.4%, Wetcit conc. 0.25%, and both soil and foliar fertilizers were applied such as Biohumussol 50 mL / m^2 and Cropmax 0.03%, respectively.

Biohumussol is a 100% organic and biologically active fertilizer produced by the processing of organic matter from Californian red worms. It contains: macro and micro nutrients, humic substances and phytohormones, enzymes, vitamins, soil antibiotics, useful bacteria and microorganism, and a higher concentration of nitrogen, phosphorus, potassium, calcium. Cropmax is a fertilizer supplement, 100 % organic, made from vegetal raw material. Its activity is based on the combination of trace elements, amino acids, vitamins, polysaccharides and plant hormones (growth promotors). Cropmax contains: nitrogen (0.2%), phosphorus (0.4%), potassium (0.02%), iron (220 mg/L), zinc (49 mg/L), manganese (54 mg/L), copper (35 mg/L), boron (70 mg/L), Ca, Mo, Co, Ni (10 mg/L), 17 amino acids, plants vitamins: ascorbic acid, vitamin E, carotenoids.

3. The control (T3) variant is where no fertilizer or other chemicals was applied.

To ensure uniform application of the treatments and foliar fertilizers, the atomizer and electric sprayer was used. The fertilizers were applied twice, in different phenophases: first one before flowering stage (BBCH 59) and the second at onset of fruit stage (BBCH 69/71).

Experimental procedures

Determining soluble vitamins

To extract soluble vitamins, 0.5 g of milled chokeberries was mixed with 3 mL aqueous solution containing 3% metaphosphoric acid and 8% acetic acid under shaking for 30 s at the Heidolph Reax top vortex; the mixture was sonicated for 15 min in the Elmasonic E 15 H sonication bath, and centrifugated at 10000 rpm for 10 min at 4 °C in the Eppendorf AG 5804 centrifuge. The supernatant was filtered through a 0.45 μm Chromafil Xtra PA-45/13 nylon filter prior to injection into HPLC system.

Analysis were performed on a Agilent 1200 HPLC system, equipped with solvent degasser, quaternary pumps, a diode array detector (DAD), and an automatic injector (Agilent Technologies, Santa Clara, CA, USA) coupled to single-quadrupole mass detection (MS) Agilent 6110 (Agilent Technologies, CA, USA), on a Eclipse XDB C-18 column (150 × 4.6 mm), 5 μm (Agilent Technologies, CA, USA) using a mobile phase consisting in a mixture of water: acetonitrile (95:5, v:v) with 1% formic acid, run at 0.5 mL/min. Chromatograms were recorded at 240 nm, using Sigma standards for identification and quantification.

For the MS detection, the positive ESI ionization mode was used, with 3000 V capillary voltage, 7 L/min nitrogen flow, full scan, m/z 100-600, 350 °C. L-ascorbic acid standard were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Determining carbohydrate composition

Glucides were extracted from 0.5 g of milled chokeberries mixed with 4 mL double distilled water, followed by shaking for 30 s; then the mixture was sonicated for 30 min and centrifuged at 10000 rpm, 24 °C, for 10 min. 20 µL of the supernatant, filtered through a 0.45 µm nylon filter was analysed by high-performance liquid chromatography.

Analysis was carried out using an Agilent 1200 HPLC system with refractive index detector (RID) (Agilent Technologies, CA, USA). It was used a Polaris Hi-Plex H column (300 × 7.7 mm, Agilent Techonologies, CA, USA) and a mobile phase consisting in 5 mM H_2SO_4 (Chempur, Poland) with a flow rate of 0.6 mL/min. Glucose, fructose and sorbitol standards were obtained from Sigma-Aldrich (Germany).

Determining phenolic compounds

For the determining of phenolic compounds, the 0.5 g of milled chokeberries were extracted with 5 mL of methanol + 1% HCl under by vortexing for 1 min, sonicated for 15 min and centrifugation at 10000 rpm, 10 min at 20 °C. The supernatant was collected and the steps was repetead 7 times (until the complete decoloration of the sample). The filtered samples were analysed by HPLC Agilent 1200 HPLC system coupled to single-quadrupole mass detection (MS) Agilent 6110 (Agilent Technologies, CA, USA), on a Kinetex XB C18 column (4.6 × 150 mm, 5 µm, Phenomenex, USA) at 25 °C. The mobile phase consisting was 0.1 % acetic acid in distilled water and acetonitrile + acetic acid with a of 0.5 mL/min flow-rate.

For the MS detection, the positive ESI ionization mode was used, with 3000 V capillary voltage, 7 L/min nitrogen flow, full scan, m/z 100-600, 350 °C. The runs were monitored at 280 nm, 340 nm and 520 nm, respectively. Chlorogenic acid (>98% HPLC), gallic acid (>99% HPLC), rutin and cyanidin standards were obtained from Sigma-Aldrich (USA). Data acquisition and interpretation were performed using the Agilent ChemStation software.

Statistical analysis

The data were analyzed using XLSTAT by Addinsoft software (version 2019.3.2), which runs on the MS Office Excel Professional Plus 2019 platform. Then, using XLSTAT software, analysis of variance (ANOVA, Fisher, 1992) was performed. Subsequently, Tukey's (HSD) Multiple Range test was applied to analyse the differences between the different variants (at a significance level of $p < 0.0001$).

Results

Soluble vitamins

The results indicate that the level of ascorbic acid (AA) in the analyzed samples, ranged from approximately 53 µg/g in the 'Viking' cultivar to 79 µg/g in the 'Melrom' cultivar and 90 µg/g in the 'Nero' cultivar. The ecologic (T2) and conventional variant (T1) of the 'Nero' cultivar exhibits the highest quantities of water-soluble vitamins (ascorbic acids), approximately 1.8 times more than the 'Viking' cultivar and 1.2 times more than the 'Melrom' (Figure 1).

The ecologic variant (T2) of the 'Melrom' cultivar exhibited ascorbic acid levels closely comparable to those of the 'Nero' control variant (T3), indicating a high degree of similarity. Notably, both the ecological (T2) and conventional variant (T3) of the 'Nero' cultivar demonstrated the highest ascorbic acid content across all cultivars.

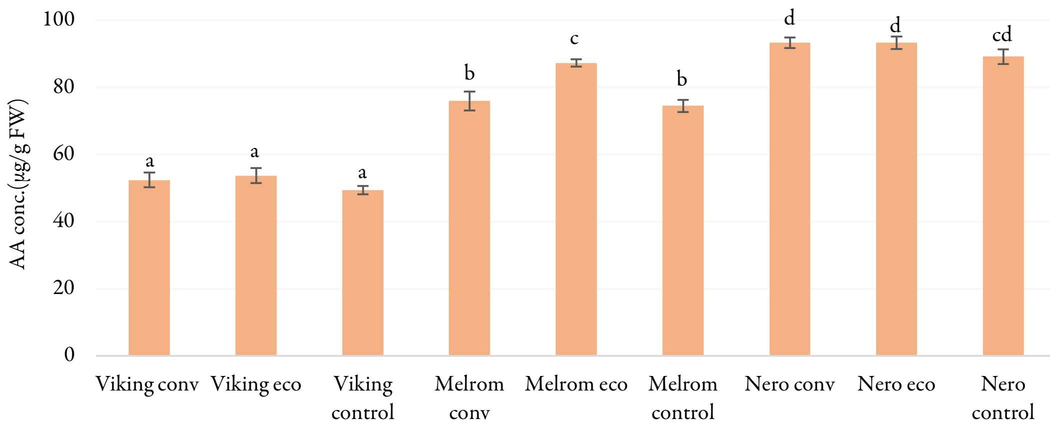

Figure 1. Ascorbic acid (AA) in aronia samples (µg/g FW) depending on treatment variant to 'Viking' 'Melrom' and 'Nero' aronia cultivars (each value represents the average of three determination results)
*Different letters between cultivars denote significant differences (Tukey test, $p < 0.0001$)

However, in terms of dehydroascorbic acid (DHAA), 'Nero' significantly surpassed both 'Viking' and 'Melrom'. As illustrated in Figure 2, 'Nero' ecologic (T2) and control variant (T3) exhibited 1.5 times greater DHAA levels comparing to other cultivars, demonstrating the highest concentration. Furthermore, the ecologic 'Nero' variant also contained the highest total amount of ascorbic acids among all cultivars and variants analyzed, solidifying its position as a superior source of ascorbic acids components.

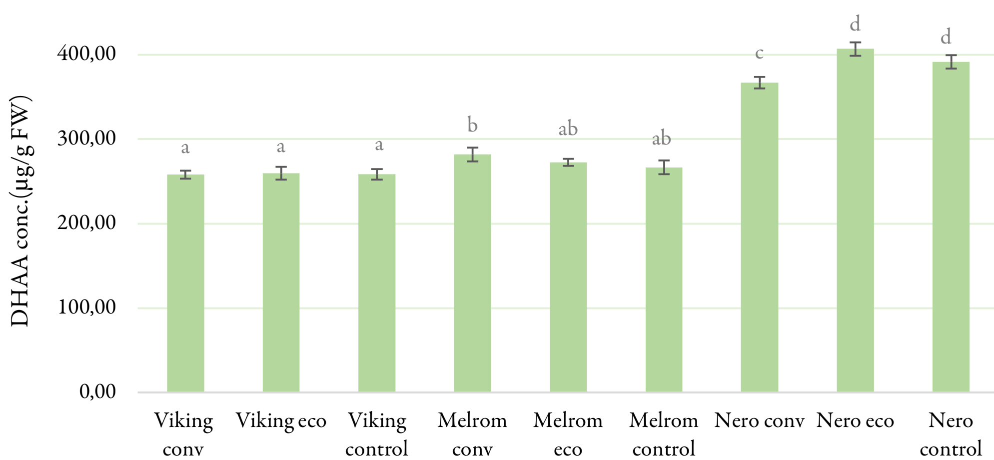

Figure 2. Dehydroascorbic acid (DHAA) in aronia samples (µg/g FW) depending on treatment variant to 'Viking' 'Melrom' and 'Nero' aronia cultivars (each value represents the average of three determination results)
*Different letters between cultivars denote significant differences (Tukey test, $p < 0.0001$)

Carbohydrate composition

Among the three types of carbohydrates, sorbitol predominates in each cultivar, for approximately 60%, followed by glucose and fructose respectively (Figure 3). The total carbohydrates content in the analyzed samples ranged from approximately 163 mg/g in the 'Melrom' cultivar, 135 mg/g in the 'Nero' cultivar and 115 mg/g in the 'Viking' cultivar.

The 'Melrom' cultivar had the significantly higher quantities of carbohydrates: approximately 1.2 times higher than 'Nero' and 1.5 times higher than 'Viking' cultivar. In this case, as well, the ecological variant (T2) stands out from the others, having the highest amounts of sorbitol among the three chokeberry cultivars studied (Figure 3).

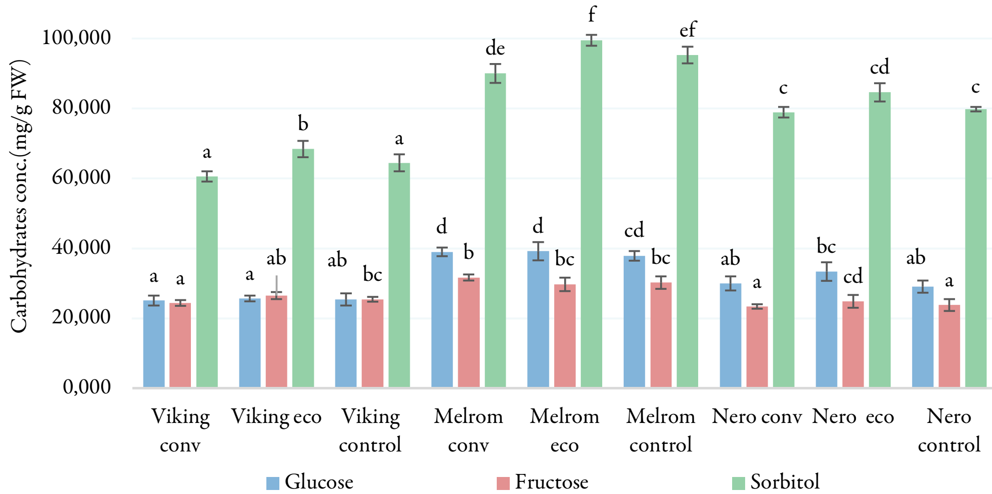

Figure 3. Carbohydrates content in aronia samples (µg/g FW) depending on treatment variant to 'Viking' 'Melrom' and 'Nero' aronia cultivars (each value represents the average of three determination results)
*Different letters between cultivars denote significant differences (Tukey test, $p < 0.0001$)

Phenolic compounds

Further, an analysis of phenolic compounds in aronia was conducted across the three cultivars. Overall, anthocyanins predominate with a percentage of 43%, followed by hydroxycinamic acid at 40%, and finally flavonols and hydroxybenzoic acid with a percentage below 15%. However, the total of phenolic acid compounds is higher or close to that of anthocyanins. The highest concentration of phenolic compounds is found in the ecological variant (T2) of the 'Melrom', followed closely by the control variant (T3) of the same cultivar.

Anthocyanins

Four types of anthocyanins were isolated and identified, three represented by glycosylated forms of cyanidin. Comparing the experimental variants between cultivars, it was observed that the 'Melrom' control variant (T3) has singnificant higher level of cyanidin-glucoside (2,986.5 µg/g), followed by the 'Nero' control variant (T3) (2,659.9 µg/g), while the other values were similar.

Regarding cyanidin-arabinoside and cyanidin-xyloside, the 'Melrom' cultivar (the ecologic (T2) and control variant (T3) had the highest concentration compared to the 'Nero' and 'Viking' cultivars. Additionally, the 'Melrom' and 'Nero' showed low and very low values of cyanidins, indicating that this compound is likely present in its derivatives mentioned above. However, the highest overall anthocyanin content is found in the eco variant of the 'Viking' cultivar and the control variant of the 'Melrom', with similar values (Table 1).

Table 1. The total quantity of anthocyanin compounds (μg/g) identified in aronia extracts

Cultivar/ Treatment variant	Content, as mean ± S.E. (in μg/g)				Total anthocyanins
	Cyanidin-glucoside	Cyanidin-arabinoside	Cyanidin-xyloside	Cyanidin	
‘Viking’ T1	2,244.0 ± 16.40 b	439.3 ±13.20 a	92.2 ± 7.31 a	1007.8 ± 21.30 e	3,783.3 ± 58.20 de
‘Viking’ T2	2,387.7 ± 21.63 c	620.3 ±15.50 c	112.0 ± 13.80 a	876.4 ± 19.60 d	3,996.4 ± 75.40 f
‘Viking’ T3	2,408.1 ± 27.10 c	646.7 ±12.50 cd	112.2 ± 12.70 a	683.7 ± 16.40 c	3,850.7 ± 69.00 e
‘Melrom’ T1	2,108.2 ± 17.00 a	5,26.9 ± 13.71 b	183.3 ±8.65 bc	0.00 ± 0.0 a	2,818.4 ± 44.40 a
‘Melrom’ T2	2,589.3 ± 23.50 d	811.8 ± 19.20 f	200.8 ± 12.15 c	0.00 ± 0.0 a	3,601.9 ± 56.30 c
‘Melrom’ T3	2,986.5 ± 28.90 f	812.2 ± 18.60 f	198.2 ± 13.40 c	0.00 ± 0.0 a	3,996.9 ± 60.60 f
‘Nero’ T1	2,143.4 ± 18.20 a	765.7 ± 17.40 e	171.7 ±23.42 bc	52.7 ± 10.91 b	3,133.5 ± 57.70 b
‘Nero’ T2	2,290.2 ± 21.10 b	669.2 ± 13.70 d	159.8 ± 12.30 b	67.2 ± 9.71 b	3,186.4 ± 58.00b
‘Nero’ T3	2,659.9 ± 19.70 e	756.3 ± 15.40 e	203.7 ± 18.40 c	62.7 ± 10.10 b	3,682.6 ± 63.60 cd

*Notes (legend): Anthocyanins concentrations in aronia samples (μg/g)) depending on treatment variant to ‘Viking’ ‘Melrom’ and ‘Nero’ aronia cultivars (each value represents the average of three determination results)
*Different letters between cultivars denote significant differences (Tukey test, $p < 0.0001$)

Hydroxycinnamic acid

The ‘Viking’ cultivar had the lowest total hydroxycinnamic acid content, whereas the ‘Melrom’ and 'Nero' cultivars exhibited the highest levels, regardless of cultivation system. Among the three detected and presented forms, feruloyl quinic acid isomers (especially 5-feruloylquinic acid) predominate, followed by caffeic acid-glucoside. The ecological variant of the ‘Nero’ is notable for its superior concentration, predominantly caffeic acid-glucoside, and 5-feruloylquinic acid, followed by the conventional variant of the ‘Nero’ and then the ecological variant of the ‘Melrom’ which has a slightly higher concentration of 3-feruloylquinic acid compared to the ones aforementioned variants (Table 2).

Table 2. The quantity of hydroxycinnamic acids identified in aronia extracts

Cultivar/Treatment variant	Content in μg/g			Total hydroxycinamic acids
	Caffeic acid-glucoside	3-Feruloylquinic acid	5-Feruloylquinic acid	
‘Viking’ T1	313.6 ± 16.00 a	166.6 ± 12.90 a	914.9 ± 17.40 a	1,395.1 ± 46.30 a
‘Viking’ T2	752.1 ±1 8.616 e	380.9 ± 39,274 b	1,851.1 ± 16.50 e	2,984.1 ± 54.70 c
‘Viking’ T3	923.4 ± 18.80 b	447.6 ± 16.80 c	1,313.7 ± 14.60 b	2,684.7 ± 50.20 b
‘Melrom’ T1	1,262.9 ± 31.532 e	516.9 ± 19.400 d	1,831.1 ± 19,20 e	3,610.9 ± 70.06 e
‘Melrom’ T2	1,260.6 ± 24.302 f	640.5 ± 11.90 f	1,995.9 ± 19,50 f	3,897 ± 55.70 e
“Melrom’ T3	1,057.4 ± 18.60 c	571.5 ± 13.10 e	1,689.1 ± 16.20 c	3,318 ± 47.90 d
‘Nero’ T1	1,200.0 ± 24.243 g	585.7 ± 17.40 e	2,205.7 ± 21.50 g	3,991.4 ± 63.11 f
‘Nero’ T2	1227.8 ± 12.40 h	587.6 ± 18.40 e	2,388.6 ± 25.60 h	4,204 ± 58.40 g
‘Nero’ T3	1,189.0 ± 19.309 d	582.1 ± 10.81 e	1,767.5 ± 17.20 d	3,538.6 ± 47.31 e

*Notes (legend): Hydroxycinnamic acids concentration in aronia samples (μg/g) depending on treatment variant to ‘Viking’ ‘Melrom’ and ‘Nero’ aronia cultivars (each value represents the average of three determination results)
*Different letters between cultivars denote significant differences (Tukey test, $p < 0.0001$)

Flavonols

The highest quantity of flavonols is found in the control variant(T3) of the ‘Nero’ cultivar (1273 μg/g), which is distinguished by having the highest amounts of quercitin derivatives, followed by the ecological variant (T2) of the ‘Melrom’ (1234.9 μg/g). An interesting aspect is observed in the ‘Viking’ cultivar, which contains high quantities of cyanidins and quercitin, but very low quantities of quercitin-rutinoside (Table 3). This unique composition suggests that the genetic and environmental factors had an influence the plants metabolic pathways, leading to variations on phelonics content.

Table 3. The flavonols content (μg/g) identified in aronia extracts

Cultivar/Treatment variant	Content, as mean ± S.E. (in μg/g)			Total flavonols
	Quercetin-rutinoside	Quercetin-glucoside	Quercetin	
'Viking' T1	77.5 ± 9.20 a	140.7 ± 10.40 a	349.3 ± 12.25 cd	567.5 ± 31.85 a
'Viking' T2	70.2 ± 7.40 a	132.3 ± 10.80 a	600.9 ± 28.40 f	803.4 ± 46.60 b
'Viking' T3	69.2 ± 7.50 a	156.0 ± 12.50 a	539.1 ± 22.50 e	764.3 ± 42,50 bc
'Melrom' T1	414.3 ± 13.60 bc	297.9 ± 16.40 c	165.6 ± 14.40 a	877.8 ± 44.40 c
'Melrom' T2	5,06.3 ± 19.60 d	435.6 ± 18.30 d	293.0 ± 17.50 b	1234.9 ± 55,40 f
"Melrom' T3	400.5 ± 14.60 b	251.4 ± 12.30 b	304.0 ± 17.90 bc	955.9 ± 44.80 d
'Nero' T1	395.3 ± 12.10 b	448.5 ± 15.90 d	364.7 ± 11.705 d	1,208.5 ± 39.70 f
'Nero' T2	396.0 ± 13.60 b	333.4 ± 12.00 c	334.7 ± 13.25 bcd	1,064.1 ± 38.85 e
'Nero' T3	447.9 ± 13.60 b	441.1 ± 11.50 d	384.0 ± 15.152 d	1,273.0 ± 44.95 f

*Notes (legend): Flavolons concentration in aronia samples (μg/g) depending on treatment variant to 'Viking' 'Melrom' and 'Nero' aronia cultivars (each value represents the average of three determination results)
*Different letters between cultivars denote significant differences (Tukey test, $p < 0.0001$)

<u>Hydroxybenzoic acid</u>

In terms of hydroxybenzoic acid concentration, the 'Melrom' cultivar, particularly its ecological (T2) and control variant (T3), significantly outperformed 'Viking' and 'Nero', with values approximately two to three times higher (Table 4).

Table 4. The total quantity of hydroxybenzoic acid (μg/g) identified in aronia extracts, depending on the treatment variant and cultivar

Cultivar/ Treatment variant	Content, as mean ± S.E. (in μg/g)
	Hydroxybenzoic acid
'Viking' T1	80.4 ± 11.101 a
'Viking' T2	272.9 ± 15.450 b
'Viking' T3	327.8 ± 17.950 c
'Melrom' T1	471.8 ± 19.400 d
'Melrom' T2	856.2 ± 16.605 e
"Melrom' T3	876.5 ± 18.80 e
'Nero' T1	314.9 ± 11.953 bc
'Nero' T2	433.3 ± 12.353 d
'Nero' T3	339.0 ± 13.710 c

*Notes (legend): Hydroxybenzoic acids content in aronia samples (μg/g FW) depending on treatment variant to 'Viking', 'Melrom' and 'Nero' aronia cultivars (each value represents the average of three determination results)
*Different letters between cultivars denote significant differences (Tukey test, $p < 0.0001$)

The ecologic variant demonstrated significant performance in both 'Viking' and 'Melrom' cultivars regarding the total phenolics (μg/g FW) concentration compared to conventional and control variants. In contrast the 'Nero' cultivar showed no differences in values across all treatment variants, including conventional and control (Figure 4).

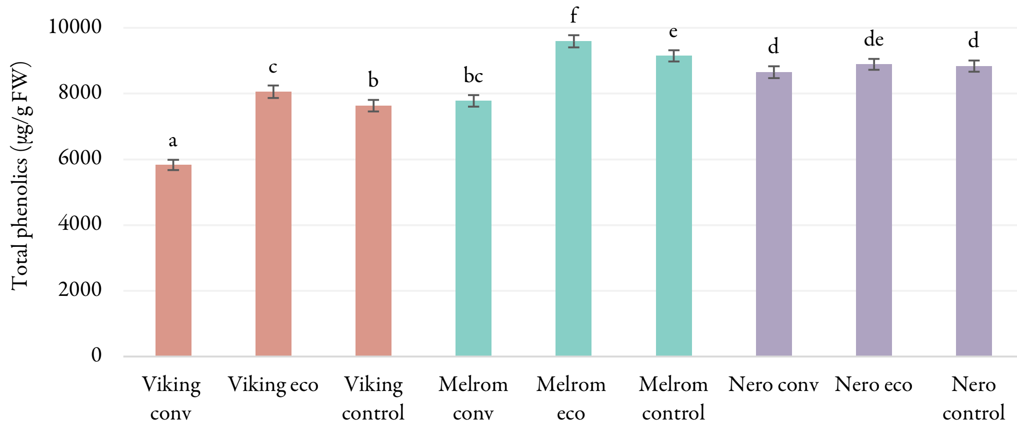

Figure 4. Total quantity (µg/g FW) of phenolics identified in aronia samples depending on treatment variant to 'Viking' 'Melrom' and 'Nero' aronia cultivars (each value represents the average of three determination results)

*Different letters between cultivars denote significant differences (Tukey test, $p < 0.0001$)

To understand the relationship between the cultivars and chemical composition, Principal Component Analysis (PCA) was performed (Figure 4). The resulting biplot clearly separated antocyanins (A conv., A eco., A control) and hydroxycinamic acids (HC conv, HC eco, HC control) in the second and the third quadrants from carbohydrates (G conv, G eco, G control), ascorbic acids (AA conv, AA eco, AA control) in the first and fourth quadrants (Figure 5).

The PCA analysis, examining the association between cultivars and chemical composition revealed a total variability by two components (F1 and F2) captured 99.49%, which means a significant 92.93% of the variance was explained by the first component (X-axis). Additionally, the PCA reveals a positive correlation between 'Nero' and 'Melrom' cultivars and their hydroxycinamic acids concentration, while 'Vilking' cultivar had a positive correlation with its anthocyanin concentration. The other observations are more similar to each other and less influenced by these specific variables (Figure 5).

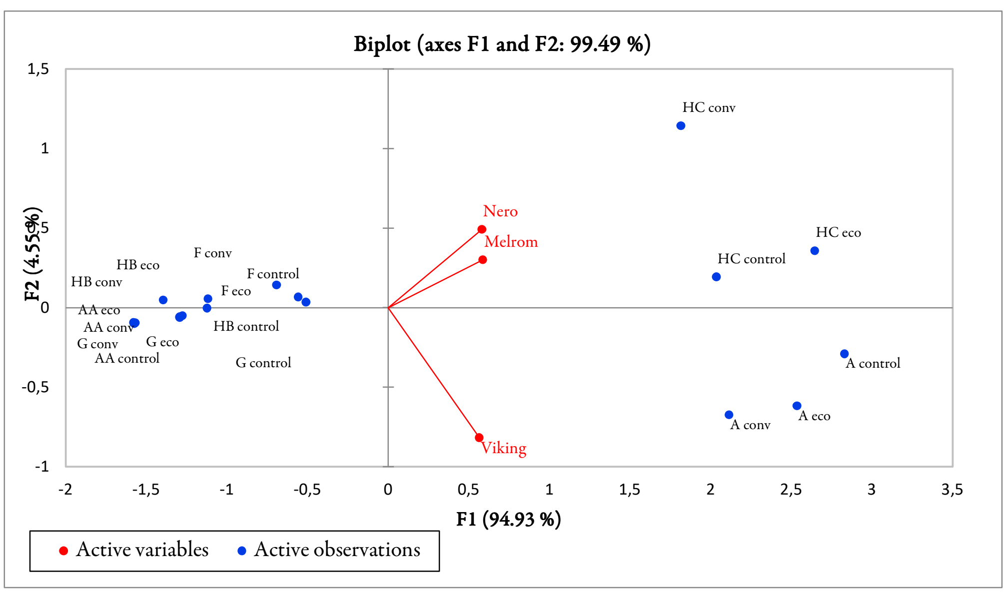

Figure 5. Principal Components Analysis (PCA) of the cultivars regarding chemical compounds

Discussion

Aronia berries have gained significant attention due to their potent antioxidant and health-promoting properties. These fruits are widely used in various food products, including juices, jams, and dietary supplements (Yu *et al.*, 2021). Their rich content of phenolic compounds, particularly anthocyanins, contributes to their numerous health benefits. In this study, we compared the antioxidant compounds and carbohydrate content of 'Viking', 'Nero', and 'Melrom' aronia cultivars treated with different combinations of foliar and organic fertilizers.

While the 'Melrom' cultivar, particularly the ecological variant, exhibited the highest levels of phenolic compounds and carbohydrates, the 'Nero' cultivar was found to contain the highest quantities of soluble vitamins.

Aronia fruits are renowned for their exceptional polyphenol content. Various studies have reported polyphenol concentrations in aronia fruit ranging from 1079 to 2996 mg GAE/100 g FW (Veberic *et al.*, 2015), 819 to 1330 mg GAE/100 g FW (Ochmian *et al.*, 2009), and 778 to 1285 g GAE/kg (Gao *et al*., 2024). However, Öztürk *et al.*, (2023) reported lower polyphenol levels, determined by HPLC-DAD, of 647.5 ± 27.8 mg/100 g FW for the 'Viking' cultivar and 641.7 ± 34.4 mg/100 g FW for the 'Nero' cultivar.

The findings of Jakobek *et al.* (2012), who reported that anthocyanins constitute approximately 41% of all polyphenols in aronia fruits are in agreement with the present research. Deineka *et al.* (2020) identified cyanidin-3-glucoside, cyanidin-3-arabinoside, and cyanidin-3-xyloside as the main anthocyanins, which is similar to the results obtained in this phase of the study. In contrast, Ochmian *et al.* (2009) reported that over 50% of the anthocyanins in the 'Nero', 'Viking', 'Galicjanka' and 'Hugin' cultivars consist of cyanidin-3-O-galactoside. Sidor and Gramza (2019) and Öztürk *et al.* (2023) reports that 'Nero' and 'Viking' cultivars possess similar cyanidin derivative concentrations.

Denev *et al.* (2012) reported a wide range of total anthocyanin content in fresh Aronia berries (357-1790 mg/100 g FW, expressed as CGE) (McDougall *et al.*, 2016). A concentration of 447 mg/100 g FW (CGE) was reported for the 'Nero' variety, while the 'Viking' cultivar showed a concentration of 227 mg/100 g FW (CGE) (Viskelis *et al*., 2014; Rugina *et al.*, 2012). However, another study showed values of 282 mg/100 g FW for cyanidin-galactoside and 149 mg/100 g FW for cyanidin-arabinoside in the 'Nero' cultivar (Wangensteen *et al*., 2014). Veberic *et al.* (2015) observed that dark-colored fruits had higher anthocyanin content, but the composition of anthocyanins remained consistent between cultivated and wild-growing species.

Flavonols, though a minor component of chokeberry phenolics (1.3-1.5%), are dominated by quercetin, comprising approximately 93% of the total flavonol content (around 71 mg/kg). Quercetin derivatives, such as quercetin-3-O-rutinoside, quercetin-3-O-glucoside, and quercetin-3-O-galactoside, are present in smaller quantities (71.0 mg/100 g FW), while quercetin-3-O-vicianoside and quercetin-3-O-robinobioside have been detected in trace amounts (Gao *et al.*, 2024; Tian *et al.*, 2017; Gironés-Vilaplana *et al.*, 2012).

The research made by Tirla *et al.* (2023) identified quercetin and two of its derivatives: quercetin-rutinoside and quercetin-glucoside, with the latter being the most abundant, that provide evidence supporting the findings of this research.

Additionally, a study on pomace from 'Nero' and 'Melrom' cultivars revealed higher flavonoid levels in 'Nero' pomace (Enescu Mazilu *et al.*, 2022). For two of the three cultivars analyzed ('Nero' and 'Melrom'), quercetin derivatives were more abundant than quercetin itself. Other flavonols, such as kaempferol and myricetin, were present in significantly lower quantities.

Phenolic acids constituted approximately 7.5% of the total polyphenols in chokeberry. Chlorogenic acid (61-193 mg/100 g FW) and neochlorogenic acid (85-123 mg/100 g FW) were the predominant compounds (McDougall *et al*., 2016; Šnebergrová *et al*., 2014). Smaller amounts of caffeic acid (0.13 mg/100 g FW) and cryptochlorogenic acid were also detected, along with trace levels of vanillic, ferulic, syringic, and gallic acids

(Dudonné *et al*., 2015). In Romanian aronia berries, Tirla *et al.* (2023) identified three hydroxybenzoic acids and four hydroxycinnamic acids, including 5-caffeoylquinic acid, caffeic acid, 3-feruloylquinic acid, and 5-feruloylquinic acid, that align with those from this research.

Assessing biochemical changes during the harvest season for the 'Melrom' and 'Nero' varieties in Arges County, Romania (Enescu-Mazilu *et al.*, 2022) the 'Nero' cultivar was found to have the highest content of vitamin C which is consistent with the results presented in this study. Skrede *et al*. (2012) reported ascorbic acid values in chokeberry ranging from 3.5 to 7.2 mg/100 g, while Calalb *et al.* (2014) obtained values of ascorbic acid ranging between 64.4 mg/100 g- 74.5 mg/100 g, results similar to those reported by us for the three cultivars. Skender *et al.* (2017) reports the highest sugar content for the experimental site treated with mineral fertilizers, the highest vitamin C contents was found in the fruits from the control site, and the highest fruit anthocyanin contents was found at the experimental site treated with the combination of mineral and organic fertilizers.

According to Green *et al.* (2023) study, *Aronia mitschurinii* yields increased with organic nitrogen fertilization, it superior to conventional nitrogen. However, an application of organic nitrogen yielded the best results, demonstrating the interplay of source and rate. The reducing sugar content of fresh aronia fruits was ranging from 6.2 to 20.9 g/100 g FW (Bursać *et al*., 2016; Kinga *et al*., 2020) similar to ours. Fructose, glucose, and sorbitol, as well as smaller amounts of sucrose, were identified in chokeberry berries. Among these, sorbitol was detected as the main sugar in all chokeberry fruits (Mikulic-Petkovsek *et al*., 2012). In agreement with our results, Denev *et al.* (2012) reported for several aronia fruits of the 'Nero' cultivar that sorbitol was the main sugar, with values of 6.6 to 13.0 g/100 g. Additionally, Yang *et al.* (2019) analyzed three varieties of aronia, including 'Viking', and concluded that sorbitol was the highest in all cultivars.

Conclusions

The study indicated a trend towards higher total phenolic content in the ecologic variant across all cultivars, when compared to conventional and control variants. Regarding ascorbic acids content, 'Nero' displayed the highest levels across cultivars, while the carbohydrate content, 'Melrom' ecologic variant exhibited the highest overall levels, reaching around 163 mg/g, 1.2 times higher than 'Nero' (135 mg/g) and 1.5 times higher than 'Viking' (115 mg/g). 'Melrom' distinguished itself with higher levels of cyanidin-arabinoside and cyanidin-xyloside, the ecologic variant of 'Viking' and the control variant of 'Melrom' exhibited the highest overall anthocyanin content. The control variant of 'Nero' had the highest flavonol content, followed by the ecologic variant of 'Melrom'.

Notably, the 'Viking' cultivar demonstrated a distinct phenolic profile, characterized by high levels of cyanidin and quercitin alongside very low levels of quercitin-rutinoside, potentially offering unique health benefits. The 'Melrom' cultivar, particularly its ecologic and control variants, had as a rich source of hydroxybenzoic acid among the analyzed chokeberry cultivars. The PCA analysis also revealed distinct cultivar-specific correlations: 'Nero' and 'Melrom' cultivars were positively associated with hydroxycinnamic acid concentration, while 'Viking' displayed a positive correlation with anthocyanin concentration.

These results suggest that the synthesis and accumulation of certain chemical compounds can be influenced by a number of factors such as growth conditions, genetic factors and cultivation systems. Sustainable practices can enhance the cultivation of the fruits with high levels of essential vitamins and antioxidants. Continuous monitoring of chemical content of aronia over several years is necessary to draw generally valid conclusions and identify the factors that most strongly influence the quality of aronia berries.

Authors' Contributions

All authors helped to conceive, create, study design, and analyze the data. CAM conceived and designed the study, analyzed the data, and wrote the paper, SF performed the chemical analysis, supervised the concept, designed the study and wrote the paper, LL performed the chemical analysis, reviewed, and edited the manuscript, RF performed HPLC analysis, JIZs, GGM, MC, RMSD contributed to the refinement of the ideas and assisted with the writing, CMI and ZI reviewed and edited the paper. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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