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Nutritional, phytochemical, and bioactive prospects of black chokeberry (*Aronia melanocarpa)* and saskatoon berry (*Amelanchier ovalis*) grown in the Republic of Kazakhstan

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ABSTRACT

Berries are consumed worldwide, but recently, they have attracted more attention due to their valuable phytochemical profiles and diverse applications. This study comprehensively characterized the saskatoon berry (*Amelanchier ovalis*) and black chokeberry (*Aronia melanocarpa*) from Kazakhstan, documenting their nutritional, chemical, and bioactive profiles. Nutritional analysis showed proteins at 2.52±0.2 g/100 g DW in *A. melanocarpa* and 4.22±0.1 g/100 g DW in *A. ovalis*. Sixteen phenolic compounds were identified in *A. melanocarpa* and twenty-one in *A. ovalis*, with chlorogenic acid, quercetin, and cyanidin derivatives predomination. Sugars, organic acids, and tocopherols were also abundant. Both berries exhibited potent antioxidant activity (EC_{50}: 0.35 ±0.02 mg/mL for *A. ovalis*, 3.9 ± 0.1 mg/mL for *A. melanocarpa* through TBARS assay) and antimicrobial properties against foodborne strains. These findings highlight these species' phytochemical and nutrient richness, emphasizing the value of Kazakhstan-originating berries.

1. Introduction

Saskatoon berry is a wild fruit from Rosaceae family widely spread worldwide, with about 30 species identified (Lachowicz et al., 2017). The most common cultivated species grown as a fruit crop is the Canadian saskatoon berry *Amelanchier canadensis. Amelanchier ovalis* is another saskatoon species, which is a frost-resistant plant composed of oval or round-leaved berries; the shrub can reach 0.5–3.0 m in height, with large flowers that are replaced by red berries that gradually darken. These are small bluish–black or reddish-purple fruits with a bluish bloom and sweet taste (Zhakupova, 2018). Traditionally, people use fresh berries and their juice for stomach disorders, as an astringent, fixing and anti-inflammatory agent, to help in reducing the risk of peptic ulcer disease, avitaminosis, and for the general strengthening of the human organism (Lachowicz et al., 2019a; Lachowicz et al., 2019c).

Black chokeberry (*Aronia melanocarpa*), another wild-growing berry from Rosaceae family, is well-known and widely consumed fruit. It is a shrub 0.5—2 m high whose fruits grow in clusters, reaching 6–8 mm in diameter, differing in the black and bluish color of berries (Zhakupova, 2018). The great bioactive potential of this type of berry was reported in many studies, and its beneficial properties for human health were verified at the cardiovascular, antibacterial, immunomodulatory and anticancer levels (Gajić et al., 2022). In some countries, it is traditionally used as a natural antihypertensive and anti-atherosclerotic drug (Zhang et al., 2021).

Both chokeberry and saskatoon berries are widespread in different countries and continents, such as North America, Canada, Europe, Poland, and Russian Federation. In the Republic of Kazakhstan, they occur predominantly in the northern part of the country due to their adaptability to the region's climate and soil conditions (Zhakupova et al., 2023). For instance, they can tolerate frosts as low as −30 °C and frosts during flowering as severe as −70 °C. Currently, these berries are

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well-known among the local population, but their use remains limited to homemade culinary applications and candy production (Zhakupova et al. 2023). Nevertheless, to broaden their use and explore novel applications, it is essential to investigate their nutritional and phytochemical profiles, as well as their potential biological activities.

Previous studies on chokeberry and saskatoon berries have demonstrated that they are rich in bioactive compounds, to which their beneficial biological effects may be attributed (Lachowicz et al., 2017; Lavola et al., 2012). Their dark color is attributed to the high concentration of anthocyanins, including cyanidin 3-glucoside, 3-galactoside, and 3-xyloside. Quercetin derivatives, such as quercetin 3-robinobioside, 3-glucoside, and 3-vicianoside, are the predominant flavonoids. Additionally, chlorogenic acid and other caffeic acid derivatives have been identified as primary phenolic acids in the composition of both saskatoon and chokeberry fruits (**n**Lachowicz et al., 2017, Lavola et al., 2012). This unique profile sets them apart from other commonly consumed berries, such as blueberries, cranberries, and raspberries, which are generally less rich in chlorogenic acids and quercetin derivatives, particularly the specific compounds found in chokeberry and saskatoon berries (quercetin 3-robinobioside, 3-vicianoside) (Golovinskaia, Wang, 2021).

Despite these existing studies on the composition of saskatoon and chokeberry fruits, berries from Kazakhstan have been largely overlooked. Notably, to our knowledge, the composition and potential bioactivities of *A. ovalis* grown in Kazakhstan remains unaddressed. Given that the chemical and phytochemical profiles of plants, fruits, and vegetables can vary significantly based on species and growing conditions—and, consequently, the potential biological activities associated with these compounds— the present study is aimed at the investigation of nutritional, chemical and bioactive profiles of the species of *Amelanchier ovalis* and *Aronia melanocarpa* grown in Republic of Kazakhstan. Research on these species is essential for providing insights that could support their valorization and promote the development of novel products and ingredients for the food and nutraceutical sectors, thereby fostering local development.

2. Materials and methods

2.1. Reagents and samples

LC-MS grade acetonitrile and formic acid were purchased from Fischer Scientific (Lisbon, Portugal). Chlorogenic acid, *p*-coumaric acid, quercetin-3-*O*-glucoside, rutin, caffeic acid, -(-) epicatechin, luteolin-7-*O*-glucoside, protocatechuic acid were obtained from Sigma Aldrich (St. Louis, MO, USA). Cyanidin-3-glucoside was obtained from Extrasyntèse (Genay, France). N-hexane 95 %, and ethyl acetate 99.8 % were of HPLC grade from Fisher Scientific (Lisbon, Portugal). Fatty acid methyl ester (FAME) reference standard mixture 37 (standard 47,885-U) was purchased from Sigma (St Louis, MO, USA), as also were other individual fatty acid isomers, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), l-ascorbic acid, tocopherol, sugar, and organic acid standards. Racemic tocol, 50 mg mL−1, was purchased from Matreya (Pleasant Gap, PA, USA). Dulbecco's modified Eagle's medium, Hank's balanced salt solution (HBSS), fetal bovine serum (FBS), l-glutamine, trypsin-EDTA, penicillin/streptomycin solution (100 U mL−1 and 100 mg mL−1, respectively) were purchased from Gibco Invitrogen Life Technologies (Paisley, UK). Sulforhodamine B, trypan blue, trichloroacetic acid (TCA), and Tris were purchased from Sigma Chemical Co. (Saint Louis, MO, USA). Ethanol and all other chemicals and solvents were of analytical grade and purchased from scientific retailers. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

In the Akmola region, the Republic of Kazakhstan, saskatoon berries (*Amelanchier ovalis*) and black chokeberry (*Aronia melanocarpa*) were collected in August and September 2022. Six kilograms of each berry in a ripe stage were collected. The samples were cleaned to discard foreign materials, and the whole berries were lyophilized, grounded in a Foss Knifetec™ 1095 mill at a controlled temperature (20 °C), and stored at −18 °C protected from light until further analysis.

2.2. Proximate composition

The studied fruits' proximate composition (protein, fat, ash, and carbohydrates) was assessed using (AOAC, 2016). Specifically, the macro-Kjeldahl assay was used to measure proteins by determining the mass fraction of nitrogen and multiplying it by 6.25 ($N \times 6.25$). The distillation and titration were performed in an automatic unit (model Pro-Nitro-A, JP Selecta, Barcelona, Spain). The Soxhlet extraction method was used to determine the fat content, which was extracted with petroleum ether in a cyclic mode for seven hours. A muffle furnace was used to determine the ash content, where the samples were treated at 550 ± 10 °C for six h. Total carbohydrates were calculated by difference using the equation: total carbohydrates (g/100 g) = 100 − (g fat + *g* ash + *g* proteins). Total energetic value was calculated according to the formula: energy (kcal/100 g dry weight (DW)) = 4 × (g protein + *g* carbohydrates) + 9 × (g fat).

2.3. Free sugars composition

Free sugars were evaluated by high-performance liquid chromatography (HPLC, Knauer, Smartline System 1000, Berlin, Germany) connected with a refractive index detector (Smartline System 1000). Melezitose (Sigma-Aldrich, St Louis, MO, USA) was used as an internal standard (Liberal et al., 2016). The results were managed in Clarity software (Data Apex, Prague, Czech Republic) and expressed in g per 100 g of DW.

2.4. Organic acids

Organic acids analysis was performed by ultra-fast liquid chromatography coupled with a photodiode array detector (UFLC-PDA; Shimadzu Corporation, Kyoto, Japan), as described by the authors (Liberal et al., 2016). Approximately 1.5 g of each sample was extracted in 25 mL of metaphosphoric acid, stirred (25 °C at 60 g) for 25 min, and filtered through the Whatman no.4 paper. The assays were carried out in a C18 SphereClone (Phenomenex, Alcobendas, Spain) reverse phase column (5 μm, 250 × 4.6 mm id) thermostated at 35 °C using 3.6 mM sulfuric acid (0.02 %) solution as an eluent at a flow rate of 0.8 mL/min. Identification was accomplished by comparing the chromatograms obtained for the analyzed samples with those obtained using commercial standards. The quantification of the compounds was completed by relating the peak areas, recorded at 215 nm, with the calibration curves obtained with commercial standards for each compound. The results were expressed in g per 100 g of DW.

2.5. Tocopherols

Tocopherols were determined in the HPLC system (Smartline System 1000, Knauer, Berlin, Germany) coupled to a fluorescence detector (FP-2020, Jasco, Easton, USA) programmed for excitation at 290 nm and emission at 330 nm and using tocol as an internal standard (Matreya, Pleasant Gap, PA, USA). This method was previously described by Liberal et al. (2016). Data were recorded and processed using Clarity 2.4 software (Data Apex, Prague, Czech Republic), and the results were given as mg per 100 g DW.

2.6. Fatty acids

Fatty acid methyl esters (FAME) were investigated after transesterification of the lipid fraction, obtained through Soxhlet extraction, as previously described by Liberal et al. (2016), and determined by gas-liquid chromatography with flame ionization detection, using a

YOUNG IN Chromass 6500 GC System instrument equipped with a split/splitless injector, a flame ionization detector (FID), and a Zebron-Fame column. Fatty acid identification and quantification were performed by comparing the relative retention times of FAME peaks from samples with commercial standards (standard mixture 47,885-U, Sigma, St. Louis, MO, USA), the results being recorded and processed using the Software Clarity DataApex 4.0 software (Prague, Czech Republic) and expressed in relative percentage of each fatty acid.

2.7. Phenolic composition

2.7.1. Extraction of anthocyanins and non-anthocyanin phenolic compounds

Non-anthocyanin phenolic compounds were extracted with hydroethanolic solutions (80:20 v/v) using 3 g of each lyophilized sample under magnetic agitation for one h. The residue obtained after filtration (filter paper Whatman N.° 4) was re-extracted as described above. The combined filtrates were then rota-evaporated (rotary evaporator Büchi R-210, Flawil, Switzerland) under pressure at 40 °C to eliminate the organic phase and subsequently purified according to da Silveira et al. (2019). After solvent evaporation, the aqueous phase was partitioned three times with 10 mL of ethyl acetate. The upper phase (ethyl acetate free of anthocyanins) was collected, combined, dried, and re-suspended in 1 mL of ethanol: water (20:80 v/v). The samples were passed through a 0.22 μm nylon filter and injected into the LC/MS system.

Anthocyanins were also extracted twice (1h + 1 h) using 1 g of the lyophilized samples with ethanol: water solution (80:20 *v/v*) with 30 μL of trifluoracetic acid (TFA). After filtering the supernatant, ethanol was evaporated, and the remaining water phase was freeze-dried. Then, 10 mg of the extract was re-suspended in 1 mL of ethanol:water:0.5 % TFA (20:80 v/v), filtered (0.22 μm nylon filters), and injected into the LC/MS system.

2.7.2. Equipment

Phenolic compounds were analyzed by high-performance liquid chromatography coupled to a diode detector and mass spectrometer (UHPLC-DAD-ESI-MS^n) (Dionex Ultimate 3000 UHPLC, Thermo Scientific, San Jose, CA, USA) operating under the conditions proposed by Liberal et al. (2016). The compound identification was carried out by comparing the obtained retention times, UV–Vis, and mass spectra with the available standards. When standards were unavailable, the compounds were tentatively identified based on the fragmentation pattern and data from the literature. The identified compounds were quantified using the calibration curves in the range of 2.5–800 μg/mL obtained from caffeic acid, luteolin-6-*C*-glucoside, protocatechuic acid, chlorogenic acid, *p*-coumaric acid, rutin and quercetin-3-*O*-glycoside standards (Extrasynthese, Genay, France). The results were expressed in mg per g of extract.

2.8. Bioactive properties

2.8.1. Antioxidant activity

The lyophilized hydroethanolic extracts were used for the thiobarbituric acid reactive substances (TBARS) assay, which were dissolved in distilled water and diluted from 0.625 to 10 mg/mL. The decrease in TBARS was assessed by lipid peroxidation inhibition in porcine (*Sus scrofa*) brain homogenates, and the malondialdehyde–thiobarbituric acid (MDA-TBA) complex color strength was evaluated by its absorbance at 532 nm. The inhibition ratio (%) was determined using the equation: $[(A - B)/A] \times 100$ %, where A and B are the absorbance of the control and the sample solutions, respectively. The results are given as EC_{50} values (mg/mL; sample concentration providing 50 % of antioxidant activity). Trolox was used as the positive control.

2.8.2. Antimicrobial activity

The antimicrobial activity of the hydroethanolic extracts was evaluated according to previously described methods (Fernandes et al., 2021). The extracts were redissolved in 5 % dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Saint Louis, MO, USA) to a final 10mg/mL concentration and further diluted. The antibacterial activity was tested against clinical and food-isolated Gram-negative and Gram-positive bacteria. The minimal extract concentrations that completely inhibited bacterial growth (MICs) were determined by a colorimetric microbial viability assay, with minimal bactericidal concentration (MBC) also being calculated. Streptomycin, ampicillin, imipenem, and vancomycin (Sigma-Aldrich, St. Louis, MO, USA) were used as positive controls, and 5 % DMSO was used as a negative control.

2.9. Statistical analysis

The results were presented as means±standard deviation (except for antibacterial activity) and compared through paired *t*-tests at a 95 % confidence level. Samples were considered statistically different when $p < 0.05$.

3. Results and discussion

3.1. Nutritional value and chemical composition

The results attained for the nutritional value and chemical composition of black chokeberry (*Aronia melanocarpa*) and saskatoon berry (*Amelanchier ovalis*) grown in the Republic of Kazakhstan are shown in Table 1. As can be seen, there are proteins (4.22±0.1 g/100 g_{DW} in *A. ovalis* and 2.5 ± 0.2 in *A. melanocarpa*) and lipids that represented less than 2 % of the composition.

The nutritional composition of *A. melanocarpa* was also studied by Witczak et al. (2021), with slightly different results, namely, regarding proteins (6.4 ± 0.3 g/100 g dw), lipids (4.49±0.01 g/100 g dw) and ashes (1.95±0,01 g/100 g dw). These differences may be due to the different origins of *A. melanocarpa* (Germany and Republic of Kazakhstan) and, therefore, also to the different edaphoclimatic conditions to which they were subjected during their development.

As regards *A. ovalis* grown in Kazakhstan, to the best of our knowledge, this is the first report regarding its nutritional composition. However, other species of the genus *Amelanchier* were evaluated, with (Rop et al., 2012), for example, reporting protein concentrations ranging from 6.19 to 11.79 g/kg_{fw} in different cultivars of *Amelanchier alnifolia*. The scarcity of studies regarding the nutritional value of both species under study may be related to the fact that they are particularly and mostly rich in bioactive compounds of high relevance for human health and well-being, and, therefore, the majority of studies were focused on the evaluation of this type of compounds. Regarding the chemical composition of *A. melanocarpa* and *A. ovalis*, the analysis of free sugars allowed the identification of fructose, glucose, and trehalose (Table 1), with glucose standing out as the primary sugar in both species (10.3 and 9.4 g/100 g DW, respectively). In *A. ovalis*, higher fructose concentrations were detected (7.23±0.08 g/100 g DW) than in *A. melanocarpa* (3.7 ± 0.5 g/100 g DW). (Witczak et al., 2021) identified fructose as the primary sugar in *A. melanocarpa* (7.49±0.06 g/100 g DW), followed by similar amounts of glucose (6.3 ± 1.2 g/100 g DW). Overall, *A. ovalis* has been neglected and underutilized compared to other species of the same genus, mostly *A. alnifolia*, which is part of the traditional native American diet and medicine (Donno et al., 2018). Lachowicz et al. (2019b) identified glucose as the significant sugar present in the fruit of different selected genotypes of *A. alnifolia* grown in Poland and Canada, representing 37–50 % of the total sugar content, which agrees with our results. These authors also identified fructose (32–41 %) and sorbitol (8–31 %).

Table 1
Proximate, chemical composition, and antioxidant activity of *A. melanocarpa* and *A. ovalis* (mean ± SD, $n = 2$).

	Aronia melanocarpa	*Amelanchier ovalis*
Proximate Composition		
Lipids (g/100 g DW)	1.25±0.01[a]	1.15±0.07[a]
Proteins (g/100 g DW)	2.5 ± 0.2[b]	4.2 ± 0.1[a]
Ash (g/100 g DW)	0.002±0.001[a]	0.003±0.00 [a]
Moisture, % fw	52.28+0.01[a]	51.13+0.01[a]
Carbohydrates (g/100 g DW)	43.97±0.1[a]	43.51±0.01[a]
Energy (Kcal/100 g DW)	197.10±0.05	201.24±0.35
Sugars (g/100 g DW)		
Frutose	3.70±0.50[b]	7.23±0.08[a]
Glucose	10.30±0.80 [a]	9.40±0.20[a]
Trealose	0.60±0.50[a]	0.31±0.01[b]
Total sugars	14.50±1.30	17.00±0.20
Organic Acids (g/100 g DW)		
Oxalic	0.31±0.03[a]	0.37±0.02 [a]
Quinic	2.40±0.50[a]	0.60±0.20[b]
Malic	4.80±0.30[a]	3.50±0.20[b]
Shikimic	0.06±0.01[a]	0.02±0.01[b]
Citric	0.44±0.04[a]	0.21±0.03[b]
Succinic	0.56±0.07	nd
Total organic acids	8.50±1.90	4.70±1.50
Tocopherols (mg/100 g DW)		
α-tocopherol	5.62±2.27[a]	3.30±0.34[a]
β-tocopherol	0.30±0.15[a]	0.47±0.01[a]
Total tocopherols	5.9 ± 2.10	3.80±0.30
Fatty acid	%	%
C8:0	0.02±0.01	nd
C10:0	0.06±0.01	nd
C12:0	0.32±0.01	0.22±0.01
C14:0	0.37±0.01	0.42±0.01
C15:0	0.16±0.01	0.15±0.01
C16:0	8.08±0.02	9.23±0.03
C16:1	0.14±0.01	0.33±0.02
C17:0	0.2 ± 0.1	0.20±0.01
C18:0	2.0 ± 0.1	1.99±0.02
C18:1n9c	15.82±0.06	20.5 ± 0.1
C18:2n6c	54.2 ± 0.1	43.6 ± 0.1
C18:3n3	1.73±0.09	3.45±0.08
C20:0	1.04±0.04	3.03±0.08
C20:1	0.22±0.02	0.82±0.05
C20:2	0.27±0.01	nd
C21:0	0.2 ± 0.1	0.45±0.01
C22:0	0.8 ± 0.1	4.77±0.07
C23:0	0.14±0.01	0.70±0.01
C24:0	14.25±0.01	10.2 ± 0.1
SFA	**27.6 ± 0.1**	**31.3 ± 0.1**
MUFA	**16.2 ± 0.1**	**21.7 ± 0.1**
PUFA	**56.2 ± 0.1**	**47.0 ± 0.1**
Antioxidant Activity		
TBARS (EC_{50} - mg/mL)	3.86±0.12	0.35±0.02

DW – dry weight; fw-fresh weight; nd – non detected. C16:0 – Palmitic acid; C18:1n9c – Oleic acid; C18:2n6c – Linoleic acid; C24:0- Lignoceric acid; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; Only the fatty acids with abundance higher than 5 % were presented in the table; the difference to 100 % corresponds to other eleven, eighteen, and sixteen, respectively, less abundant fatty acids. Results of the antioxidant activity are expressed in EC50 values: sample concentration providing 50 % of the antioxidant activity.

As for organic acids (Table 1), oxalic, quinic, malic, shikimic, and citric acids were determined in both fruit species under investigation, with succinic acid only detected in *A. melanocarpa*. Malic acid was the one present in higher amounts in both species (4.8 and 3.5 g/100 g DW in *A. melanocarpa* and *A. ovalis*, respectively), followed by quinic acid, although this being present in much lower concentrations in the last (0.6 ± 0.2 g/100 g DW) when compared to *A. melanocarpa* (2.4 ± 0.5 g/100 g DW).

(Zhang et al., 2021) also identified malic (2.39 g/L) and quinic (2.97 to 3.53 g/L) acids in *A. melanocarpa* fresh pressed juice, these being also identified by Denev et al. (2018) in the same fruit native from Bulgaria, as well succinic acid, and it is in part in agreement with the results.

As for saskatoon berries (*A. alnifolia*), other authors (Sabina Lachowicz, Jan Oszmiański, et al. 2019) were also able to identify malic (3.46 mg/kg), quinic (1.37 mg/kg), and succinic (0.30 mg/kg) acids. The organic acids profile in plants depends on several factors, namely seasonal regimes, species, varietal characteristics, and growing region, among others; these can modulate the accumulation of different types of organic acids in plants and their concentrations.

Regarding tocopherols (vitamin E) composition (Table 1), in both fruit species α- and β- tocopherols were identified, with the first being present in higher concentrations (5.62±2.27 mg/100 g DW in *A. melanocarpa* and 3.3 ± 0.34 mg/100 g DW in *A. ovalis*). The identical tocopherol isoforms were also identified by Lachowicz et al. (2017) in *A. alnifolia* Nutt and δ- and γ-tocopherol isoforms. As for *A. melanocarpa*, Lee and colleagues were able to identify α-, β-, γ-, and δ-tocopherol isoforms, as well as γ-tocotrienol, with α-tocopherol being present in higher amounts, although lower than in this investigation (1.35–1.47 mg/100 g).

Finally, the study of the fatty acids profile (Table 1) showed the prevalence of polyunsaturated fatty acids (PUFA) in both fruit species, this being majorly due to the high percentage of linoleic acid (C18:2n6c). Monounsaturated fatty acids (MUFA) are also present in high amounts in *A. melanocarpa* and *A. ovalis* (27.6 ± 0.1 and 31.3 ± 0.1 %, respectively), mainly due to the presence of oleic acid (C18:1n9c). (Jurendić & Ščetar, 2021) we also identified linoleic acid as the primary fatty acid in *A. melanocarpa*, with PUFA representing 73.6 % of the total fatty acids. To our knowledge, this is the first report on the fatty acids profile in *A. ovalis*.

3.2. Phenolic composition

Anthocyanin and non-anthocyanin phenolic compounds of *A. melanocarpa* and *A. ovalis* were investigated, and the results are presented in Tables 2 and 3. The anthocyanin profile of *A. melanocarpa* (Fig. 2, Table 2) reveals the presence of cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, and cyanidin-3-*O*-xyloside compounds. The *m/z* 449 (peaks 1 and 2) gave characteristic fragments in MS^2 of *m/z* 287, indicating cyanidin as the aglycone molecule and the neutral loss of 162 Da, typical of a hexose moiety. M^+ at *m/z* 419 also gave fragment ions at *m/z* 287 (cyanidin) and neutral loss of 132 Da, suggesting a pentose group's presence (Oszmiański & Lachowicz, 2016). Previously, it was reported that sugar moieties in *A. melanocarpa* anthocyanins are glucose, galactose, xylose, and arabinose (Meng et al., 2019; Zhang et al., 2021). Thus, the final assignment of the compounds' identity was based on the elution order of these sugars bonded to cyanidins (Meng et al., 2019; Wu & Prior, 2005).

The same anthocyanin profile (Fig. 4, Table 3) was detected in *A. ovalis*, with cyanidin derivatives predomination. This is in agreement with other studies that investigated the phenolic composition of saskatoon berries (Lachowicz et al., 2019; Sabina Lachowicz, Rafał Wiśniewski, et al. 2019). Moreover, the fact that both species belong to the Rosaceae family likely contributes to their similar phytochemical profiles.

Both fruit species were revealed to be rich in non-anthocyanin phenolic compounds, with sixteen compounds tentatively identified in *A. melanocarpa* and twenty-one in *A. ovalis,* which presented slight differences in total phenolics concentration (59.88±0.88 mg/g extract for *A. melanocarpa* and 55.97±0.62 mg/g extract for *A. ovalis*).

Concerning *A. melanocarpa* (Fig. 1, Table 2), chlorogenic acid and 3-caffeoylquinic acid were the predominant phenolic compounds, with the latter compound showing the lower concentration (26.15±0.38 and 15.95±0.18mg/g extract, respectively). These compounds presented a deprotonated molecule $[M\text{-}H]^-$ at *m/z* 353 and characteristic fragments in MS^2 at *m/z* 191 and 179 (Clifford et al., 2003). They were differentiated due to their distinct chromatographic behavior (elution order) and fragmentation pattern, which differ in the intensity of the generated ions in MS^2 (Clifford et al., 2003). These results corroborate previous

Table 2
Chromatographic (retention time) and spectral (UV, MS and MS/MS) data, tentative identification and concentration of phenolic compounds in *A. melanocarpa*.

Peak	Rt (min)	Assignment	λ max(nm)	$[M-H]^-/M^+$	MS^2	MS^3	(mg/g extract)
Aronia melanocarpa							
Anthocyanins							
1	12.59	Cyanidin 3-*O*-galactoside	280, 515	449	287	–	2.07±0.01
2	14.21	Cyanidin 3-*O*-glucoside	281, 516	449	287	–	0.01±0.01
3	16.02	Cyanidin 3-*O*-arabinoside	280, 515	419	287	–	0.66±0.001
4	21.56	Cyanidin 3-*O*-xyloside	280, 516	419	287	–	tr
	Non-anthocyanin phenolic compounds						
1	4.89	3-Caffeoylquinic acid	327	353	191, 179	–	15.94±0.18
2	5.79	Protocatechuic acid	260, 293	153		–	3.99±0.07
3	6.46	3-*p*-coumaroylquinic acid	310	337	163, 173, 191, 119	–	tr
4	6.73	4-Caffeoylquinic acid	–	353	173, 179, 191	–	1.88±0.10
5	7.42	Chlorogenic acid	327	353	191, 179	–	26.15±0.38
6	14	Quercetin-*O*-dihexoside	–	625	301, 445, 463, 505, 427	–	0.36±0.01
7	14.26	Quercetin-*O*-dihexoside	–	625	301, 445, 463, 505, 427	–	0.30±0.01
8	16.18	Quercetin 3-*O*-vicianoside	–	595	301	–	0.44±0.01
9	16.5	Eriodictyol 7-*O*-glucuronide	–	463	287	–	3.90±0.01
10	17.58	Quercetin 3-*O*-robinobioside	–	609	301	–	0.53±0.01
11	17.85	Rutin	266, 354	609	301	–	0.57±0.01
12	18.73	Quercetin-*O*-galactoside	256, 355	463	301	–	2.43±0.02
13	19.12	Quercetin 3-*O*-glucoside	256, 354	463	301	–	1.76±0.01
14	19.57	Caffeic acid derivative	327	381	179, 161, 191	135	1.76±0.01
15	21.55	Isorhamnetin-*O*-rutinoside	–	623	315	300	0.50±0.01
16	22.13	Isorhamnetin-*O*-rutinoside isomer	–	623	315	300	0.33±0.01
						Total phenolic acids	48.76±0.82
						Total flavonoids	11.12±0.06
						Total phenolics	59.88±0.88

Table 3
Chromatographic (retention time) and spectral (UV, MS and MS/MS) data, tentative identification and concentration of phenolic compounds in *A. ovalis*.

Peak	Rt (min)	Assignment	λ max(nm)	$[M-H]^-/M^+$	MS^2	MS^3	(mg/g extract)
Amelanchier ovalis							
Anthocyanins							
1	12.59	Cyanidin 3-*O*-galactoside	281, 515	449	287		1.50±0.001
2	14.25	Cyanidin 3-*O*-glucoside	281, 516	449	287		1.23±0.005
3	16.04	Cyanidin 3-*O*-arabinoside	281, 516	419	287		0.24±0.012
4	21.56	Cyanidin 3-*O*-xyloside	280, 516	419	287		0.07±0.002
	Non-anthocyanin phenolic compounds						
1	4.89	3-Caffeoylquinic acid	327	353	191, 179		10.14±0.12
2	5.79	Protocatechuic acid	260, 293	153			2.40±0.02
3	6.46	3-*p*-coumaroylquinic acid	310	337	163, 173, 191, 119		tr
4	6.73	4-Caffeoylquinic acid	320	353	173, 179, 191		1.50±0.02
5	7.42	Chlorogenic acid	327	353	191, 179		27.08±0.54
6	9.11	Caffeoyl threonic acid	327	295	135, 179		0.66±0.03
7	9.85	Epicatechin	–	289	179, 245		Traces
8	10.54	Caffeoyl malic acid	327	295	179	135	1.42±0.06
9	15.50	Coumaroyl malic acid	318	279	163	117	0.77±0.01
10	16.18	Quercetin 3-*O*-vicianoside	267, 341	595	301		0.48±0.01
11	17.61	Quercetin 3-*O*-robinobioside	–	609	301		0.70±0.01
12	17.85	Rutin	266, 354	609	301		tr
13	18.73	Quercetin 3-*O*-galactoside	256, 355	463	301		4.96±0.01
14	19.07	Quercetin 3-*O*-glucoside	256, 354	463	301		0.78±0.01
15	19.57	Caffeic acid derivative	327	381	179, 161, 191, 135		0.63±0.10
16	20.39	Quercetin-*O*-malonylhexoside	–	549	505, 435	301, 463	0.42±0.00
17	20.7	Quercetin-*O*-arabinoside	–	433	301	151, 179, 257	0.87±0.02
18	20.96	3,5-dicaffeoylquinic acid	–	515	353	191, 179	2.01±0.08
19	21.54	Quercetin-*O*-xyloside	–	433	301	151, 179, 257	0.46±0.01
20	22.71	Quercetin-*O*-deoxyhexoside		447	301	179, 255, 151	0.38±0.01
21	23.41	4,5-Dicaffeoylquinic acid		515	353	173, 179, 191, 135	0.31±0.00
						Total phenolic acids	**46.92±0.58**
						Total Flavonoids	**9.05±0.04**
						Total Phenolics	**55.97±0.62**

findings for this sample, with caffeoylquinic acid derivatives predominating in juices of *A. melanocarpa* (Oszmiański & Lachowicz, 2016).

In this species, most of the tentatively identified compounds belong to flavonoids. Among them, peaks 6 and 7 were assigned as quercetin-*O*-dihexoside due to their $[M-H]^-$ at *m/z* 625 and MS^2 data at *m/z* 463 and 301 $[M-H-162–162]^-$. Additionally, quercetin-*O*-galactoside and quercetin 3-*O*-glucoside (peaks 12 and 13, Table 2) were assigned using comparison with authentic standards, elution order (Slimestad et al., 2005), deprotonated molecule at *m/z* 463, and MS^2 fragments at *m/z* 301 (Oszmiański & Lachowicz, 2016; Slimestad et al., 2005).

Peak 10 (Table 2) had deprotonated molecule at *m/z* 609 and ions at *m/z* 301 due to the neutral loss of 308 Da Similarly, peak 8 showed $[M-H]^-$ at *m/z* 595 and fragment ions at *m/z* 301 following the neutral loss of 294 Da, which could be attributed to the simultaneous loss of pentose

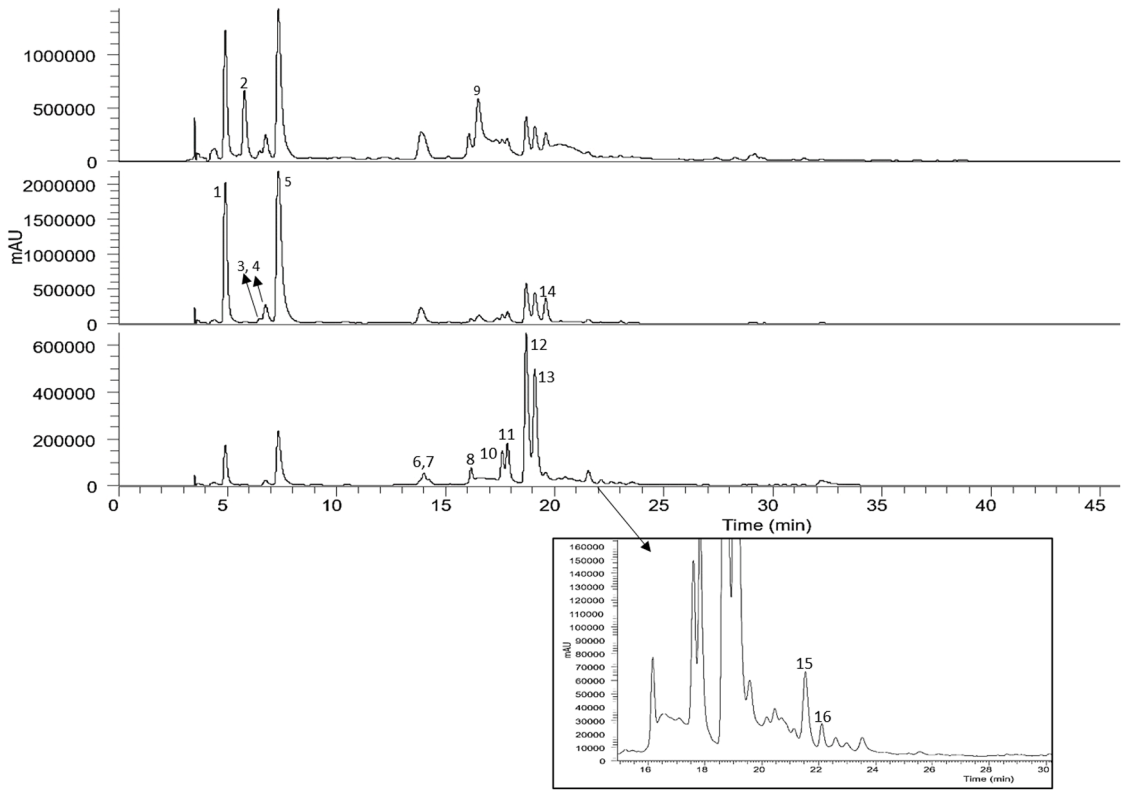

Fig. 1. Chromatogram for non-anthocyanin phenolic compounds (a: 280 nm, b: 330 nm, c: 370 nm) in *A. melanocarpa*. Peak 1: 3-Caffeoylquinic acid; Peak 2: Protocatechuic acid; Peak 3: 3-*p*-coumaroylquinic acid; Peak 4: 4-Caffeoylquinic acid; Peak 5: Chlorogenic acid; Peak 6: Quercetin-*O*-dihexoside; Peak 7: Quercetin-*O*-dihexoside; Peak 8: Quercetin 3-*O*-vicianoside; Peak 9: Eriodictyol 7-*O*-glucuronide; Peak 10: Quercetin 3-*O*-robinobioside; Peak 11: Rutin; Peak 12: Quercetin-*O*-galactoside; Peak 13: Quercetin 3-*O*-glucoside; Peak 14: Caffeic acid derivative; Peak 15: Isorhamnetin-*O*-rutinoside; Peak 16: Isorhamnetin-*O*-rutinoside isomer. For chromatographic conditions, see the text.

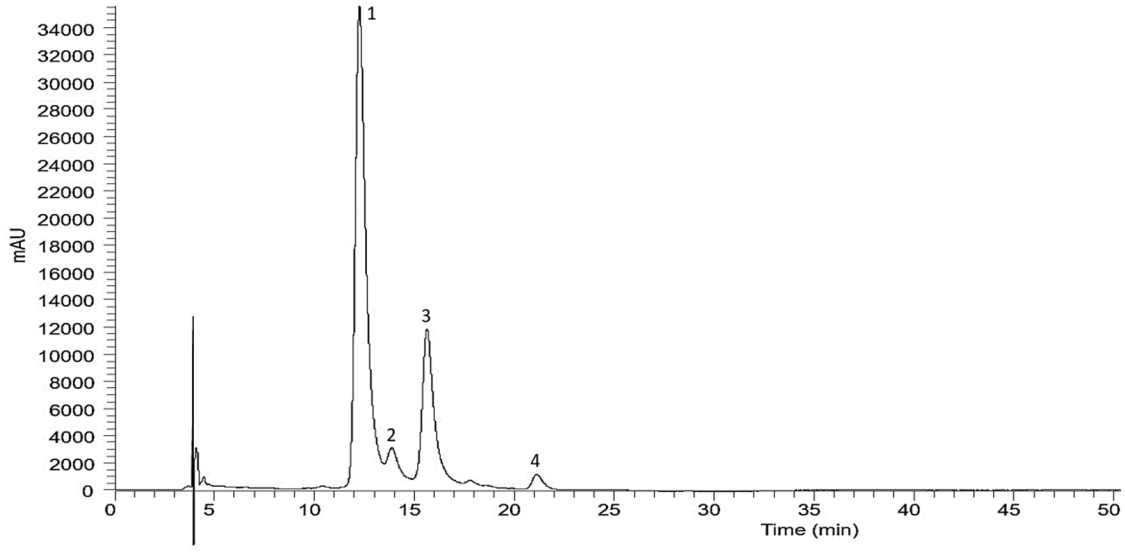

Fig. 2. Chromatogram for anthocyanins (520 nm) in A. melanocarpa. Peak 1: Cyanidin 3-O-galactoside; Peak 2: Cyanidin 3-O-glucoside; Peak 3: Cyanidin 3-O-arabinoside; Peak 4: Cyanidin 3-O-xyloside. For chromatographic conditions, see the text.

and hexose moieties $[M\text{-}H\text{-}162\text{–}132]^{-}$. Previous studies on *A. melanocarpa* assigned these compounds as quercetin 3-*O*-robinobioside and quercetin 3-*O*-vicianoside, respectively (Oszmiański & Lachowicz, 2016; Slimestad et al., 2005). Rutin (peak 11, Table 2) was distinguished from peak 10 due to its chromatographic behavior (retention time), which was compared with authentic standards.

Quercetin-*O*-galactoside, quercetin 3-*O*-glucoside, and eriodictyol 7-*O*-glucuronide (peak 9, Table 2) were the most abundant flavonoids in *A. melanocarpa*, as it was reported previously (Oszmiański & Lachowicz, 2016; Oszmiański & Wojdylo, 2005). The identity of peak 9 was assigned based on the deprotonated molecule at *m/z* 463, which gave *m/z* 287 as the base peak in MS^2 (Meng et al., 2019; Oszmiański &

Wojdylo, 2005). Eriodictyol 7-*O*-glucuronide was also found in various *A. melanocarpa* products, such as juice and dry powders of pomace and whole fruits (Oszmiański & Lachowicz, 2016).

Finally, isorhamnetin derivates (peaks 15 and 16, Table 2) were identified due to their deprotonated molecule at *m/z* 623 and fragment ions at *m/z* 315 (MS^2) and 300 (MS^3), indicating the neutral loss of 308 Da, typical of rhamnose and hexose moieties.

Table 3 and Fig. 3 presents the phenolic composition of *A. ovalis*, which, overall, displays a profile similar to *A. melanocarpa*. These similarities can likely be attributed, at least in part, to the fact that both species belong to the Rosaceae family. 3-caffeoylquinic acid and chlorogenic acid were also identified as the major compounds in the extract (10.14±0.12 and 27.08±0.54 mg/g extract, respectively). These compounds were also identified in *A. alnifolia* L. cultivars by other authors (Lachowicz et al., 2019; Sabina Lachowicz, Rafał Wiśniewski, et al. 2019), but in lower concentrations (10.73 mg/g dry matter). Other caffeoylquinic acid derivatives were also detected in *A. ovalis,* such as 4-caffeoylquinic acid (peak 4), 3,5-dicaffeoylquinic acid (peak 18, Table 3) and 4,5-dicaffeoylquinic acid (peak 21, Table 3). They were identified based on their chromatographic behavior (elution order) and fragmentation pattern, as detailed by (S. Lachowicz et al., 2019).

Caffeic acid derivatives were assigned in peaks 6 and 8 (Table 3). Compound 6 showed deprotonated molecules at *m/z* 295 and fragment ions at *m/z* 179 and 135, characteristic of caffeic acid groups. This

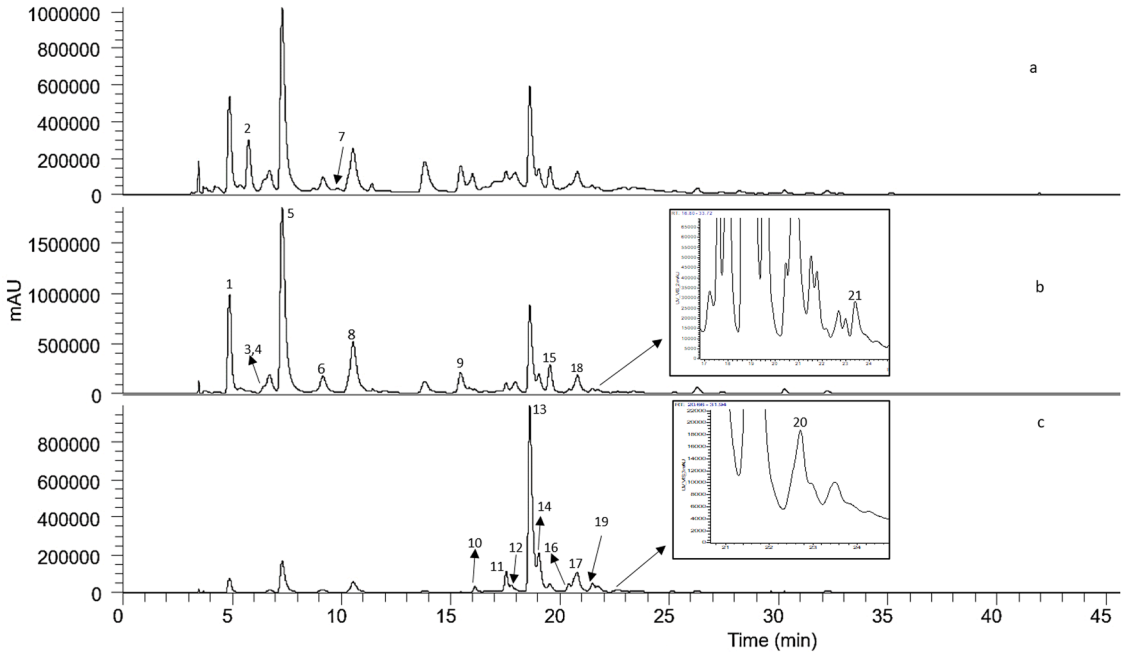

Fig. 3. Chromatogram for phenolic compounds (a: 280 nm, b: 330 nm,c: 370 nm) in *A. ovalis.* Peak 1: 3-Caffeoylquinic acid; Peak 2: Protocatechuic acid; Peak 3: 3-*p*-coumaroylquinic acid; Peak 4: 4-Caffeoylquinic acid; Peak 5: Chlorogenic acid; Peak 6: Caffeoyl threonic acid; Peak 7: Epicatechin; Peak 8: Caffeoyl malic acid; Peak 9: Coumaroyl malic acid; Peak 10: Quercetin 3-*O*-vicianoside; Peak 11: Quercetin 3-*O*-robinobioside; Peak 12: Rutin; Peak 13: Quercetin 3-*O*-galactoside; Peak 14: Quercetin 3-*O*-glucoside; Peak 15: Caffeic acid derivative; Peak 16: Quercetin-*O*-malonylhexoside; Peak 17: Quercetin-*O*-arabinoside; Peak 18: 3,5-dicaffeoylquinic acid; Peak 19: Quercetin-*O*-xyloside; Peak 20: Quercetin-*O*-deoxyhexoside; Peak 21: 4,5-Dicaffeoylquinic acid. For chromatographic conditions, see the text.

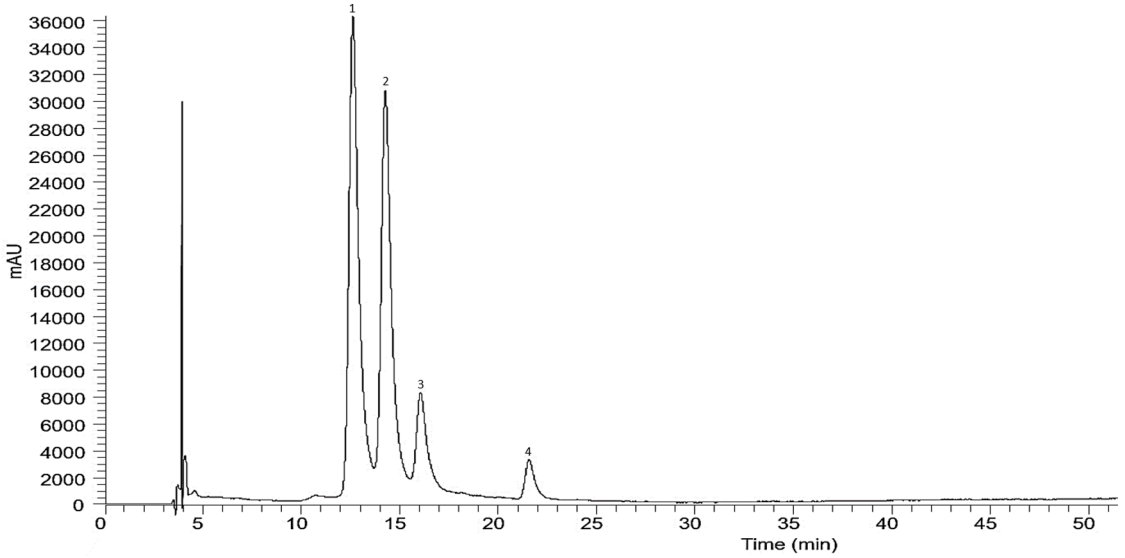

Fig. 4. Chromatogram for anthocyanins (520 nm) of *A. ovalis*. Peak 1: Cyanidin 3-*O*-galactoside; Peak 2: Cyanidin 3-*O*-glucoside; Peak 3: Cyanidin 3-*O*-arabinoside; Peak 4: Cyanidin 3-*O*-xyloside. For chromatographic conditions, see the text.

compound was tentatively identified as caffeoyl chronic acid, and it was previously described by (Grzegorczyk-Karolak et al., 2020) in shoots of *Salvia bulleyana* plants (Grzegorczyk-Karolak et al., 2020). Peak 8 presented deprotonated molecule at *m/z* 295, giving a neutral loss of 116 Da, attributed to a malic acid moiety, to yield *m/z* 179 and 135 in MS^2 and MS^3. Therefore, it was assigned as caffeoyl malic acid. Malic acid groups were also assigned bonded to *p*-coumaric acid, giving coumaroyl malic acid (peak 9, Table 3), with $[M-H]^-$ at *m/z* 279 generating *m/z* 163 and 119 in MS^2 and MS^3. To our knowledge, this is the first time these compounds have been reported in *A. ovalis*.

As in *A. melanocarpa*, quercetin derivatives were also identified in *A. ovalis*, with many present in both species. However, in this sample, quercetin derivatives comprised all the assigned flavonoid compounds (Table 3). Quercetin 3-*O*-galactoside was the primary flavonoid compound, which aligns with former studies on *A. alnifolia* (Lavola et al., 2012; Sabina Lachowicz, Rafał Wiśniewski, et al. 2019). Quercetin 3-*O*-vicianoside, quercetin 3-*O*-robinobioside, quercetin 3-*O*-glucoside and rutin were also assigned in this sample.

Differently from *A. melanocarpa, A. ovalis* showed quercetin-*O*-malonylhexoside (peak 16), which gave $[M-H]^-$ at *m/z* 549 and fragments ions at *m/z* 505, 463 and 301. Additionally, peaks 17 and 19 (Table 3) showed deprotonated molecules at *m/z* 433 and *m/z* 301 as the main fragments in MS^2 ($[M-H-132]^-$), and they were identified as quercetin-*O*-arabinoside and quercetin-*O*-xyloside, respectively. Moreover, peak 20 had deprotonated molecules at *m/z* 447, yielding *m/z* 301 due to the neutral loss of 146 Da, which could correspond to a deoxyhexose moiety. Based on this data, compound 20 was assigned as quercetin-*O*-deoxyhexoside. These quercetin derivatives were previously described in saskatoon berries (Lavola et al., 2012; Sabina Lachowicz, Rafał Wiśniewski, et al. 2019), aligning with our findings. Mass spectra of predominant compounds in both samples are provided in *Supplementary Material*.

3.3. Bioactive properties

3.3.1. Antioxidant activity

Table 1 shows the antioxidant activity of the two species studied through TBARS assay. *A. ovalis* exhibited a more potent antioxidant activity compared to *A. melanocarpa*, with a lower concentration of extract required to inhibit 50 % of lipid peroxidation (0.35±0.02 mg/mL *vs* 3.86±0.12 mg/mL). The TBARS assay evaluates the capacity of antioxidant compounds or extracts to inhibit the oxidation of a lipid substrate, which in this study was porcine brain homogenates (Ghani et al., 2017). This method measures the neutralization of peroxyl radicals and other reactive species involved in lipid oxidation, such as alkyl and alkoxyl radicals. Therefore, the results indicate that *A. ovalis* extracts exhibited superior efficacy in counteracting lipid oxidation compared to *A. melanocarpa* through distinct pathways. Lipid oxidation of cell membrane components, such as unsaturated fatty acids and phospholipids, is linked to the development of chronic diseases, including atherosclerosis. The ability to scavenge free radicals is a key mechanism by which antioxidants from these species may exert protective effects in the body.

The data suggest that while *A. ovalis* and *A. melanocarpa* contained similar levels of total individual phenolic compounds (Tables 2 and 3), differences in their phenolic profiles, the number of identified compounds (25 in *A. ovalis* and 20 in *A. melanocarpa*), and the concentration of specific phenolics may have contributed to the observed outcomes. These variations could enhance antioxidant activity through the stronger activity of certain compounds or potential synergistic effects.

The antioxidant efficacy of phenolic compounds is highly dependent on their chemical structure, particularly the number and position of phenolic hydroxyl groups (Corrigan et al., 2023). For flavonoids such as quercetin, ortho-hydroxylation in the B-ring increases antioxidant potential by stabilizing the phenoxyl radical formed when electrons or hydrogen atoms are transferred to free radicals (Rice-Evans et al., 1996. In this context, quercetin derivatives were present in greater diversity and total concentration in *A. ovalis* compared to *A. melanocarpa*

Table 4
Antibacterial activity (minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) mg/mL) of A. melanocarpa and A. ovalis hydroethanolic extracts.

Positive Control										
	Aronia melanocarpa		*Amelanchier ovalis*		Streptomycin 1mg/mL		Methicillin 1mg/mL		Ampicillin 10mg/mL	
Food bacteria	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria										
Enterobacter Cloacae	10	>10	10	>10	0.007	0.007	n.t.	n.t	0.15	0.15
Escherichia coli	10	>10	>10	>10	0.01	0.01	n.t.	n.t.	0.15	0.15
Pseudomonas aeruginosa	10	>10	10	>10	0.06	0.06	n.t.	n.t.	0.63	0.63
Salmonella enterica	5	>10	5	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Yersinia enterocolitica	5	>10	10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Gram-positive bacteria										
Bacillus cereus	5	>10	5	>10	0.007	0.007	n.t.	n.t.	n.t.	n.t.
Listeria monocytogenes	2.5	>10	5	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Staphylococcus aureus	1.25	>10	2.5	>10	0.007	0.007	0.007	0.007	0.15	0.15

Positive Control										
					Ampicillin (10mg/mL)		Imipenem (1mg/mL)		Vancomycin (1mg/mL)	
Clinical bacteria	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria										
E. coli	5	>10	10	>10	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
Klebsiella pneumoniae	>10	>10	>10	>10	10	>10	<0.0078	<0.0078	n.t.	n.t.
Morganella morganii	5	>10	10	>10	>10	>10	<0.0078	<0.0078	n.t.	n.t.
Proteus mirabilis	10	>10	10	>10	<015	<0.15	<0.0078	<0.0078	n.t.	n.t.
Pseudomonas aeruginosa	5	>10	10	>10	>10	>10	0.5	1	n.t.	n.t.
Gram-positive bacteria										
Enterococcus faecalis	5	>10	10	>10	<0.15	<0.15	n.t.	n.t.	<0.0078	<0.0078
Listeria monocytogenes	5	>10	5	>10	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
MRSA	1.25	>10	5	>10	<0.15	<0.15	n.t.	n.t.	0.25	0.5

(Tables 2 and 3). Regarding phenolic acids, it has been shown that hydroxycinnamic acid derivatives exhibit greater antioxidant activity than hydroxybenzoic acid derivatives (Chen et al., 2020), with the antioxidant capacity improving as the number of hydroxyl groups increases (up to four). Hydroxycinnamic acid derivatives were also found in greater abundance and diversity in *A. ovalis*, with caffeic acid derivatives, including caffeoyl and dicaffeoylquinic acids, as well as caffeoyl malic acid, identified in *A. ovalis* but not in *A. melanocarpa*. These compositional differences may have contributed to the superior antioxidant activity observed in *A. ovalis* compared to *A. melanocarpa,* although further studies are necessary to ascertain the specific contribution of each compound. The antioxidant capacity of *A. ovalis* was also studied by other authors (Asyakina et al., 2022), who attribute this bioactivity to the rich phenolic profile of both fruit species.

3.3.2. Antimicrobial activity

The antimicrobial activity of the hydroethanolic extracts from *A. melanocarpa* and *A. ovalis* against foodborne and clinical bacteria is detailed in Table 4. Overall, *A. melanocarpa* exhibited superior performance compared to *A. ovalis* against both types of bacteria, displaying lower MIC values. Specifically, *A. melanocarpa* demonstrated stronger antimicrobial efficacy against Gram-positive bacteria in comparison to Gram-negative strains, with its most notable activity observed against *S. aureus* and *Methicillin-resistant Staphylococcus aureus* (MRSA) (MIC=1.25 mg/mL), followed by L. *monocytogenes* (MIC=2.5 mg/mL). Although *A. ovalis* exhibited notable activity against these bacteria, its MIC values were slightly higher at 2.5 mg/mL and 5 mg/mL, respectively. Despite these promising findings, all MIC values remained below those of the positive controls utilized.

The antimicrobial activity of berry fruits was extensively documented, revealing distinct susceptibility patterns of various bacterial species to different berry extracts (Jeyaraj et al., 2023). Anthocyanins and other non-anthocyanin phenolic compounds present in these matrices were consistently associated with the observed antimicrobial effects against species included in this and other studies (Denev et al., 2019; Jeyaraj et al., 2023; Nikolajeva, 2013; Pertuzatti et al., 2016; Sabina Lachowicz, Rafał Wiśniewski, et al. 2019; Sun et al., 2018).

Aronia and *Amelenchier* species (Nikolajeva, 2013) demonstrated antibacterial activity against Gram-positive bacteria, particularly *Staphylococcus aureus*, with extracts of *A. melanocarpa* exhibiting lower efficacy. Conversely, (Denev et al., 2019) found no antimicrobial activity in crude extracts of *A. melanocarpa* against various foodborne pathogens such as *S. aureus, E. coli, Salmonella*, and L. *monocytogenes*. In a separate investigation, (Sabina Lachowicz, Rafał Wiśniewski, et al. 2019) observed the antimicrobial effects of *A. alnifolia* solely against Enterococcus. The discrepancies compared to the samples analyzed in the present study may originate, for example, from differences in extraction techniques and chemical compositions of the samples. As for *A. ovalis*, to the best of our knowledge, this is the first report regarding the antimicrobial activity of its fruit extracts.

Despite exhibiting different levels of antimicrobial activity, both *A. melanocarpa* and *A. ovalis* contain similar total concentrations of phenolic compounds. Interestingly, *A. ovalis* presented many compounds, which did not translate into higher antimicrobial properties. Thus, the variations observed in the antimicrobial activity of the studied samples could be attributed to the distinct profiles and concentrations of specific individual phenolics in these samples and to synergistic effects between these compounds. For instance, *A. melanocarpa* exhibited higher levels of cyanidin 3-*O*-galactoside, 3-caffeoylquinic acid, protocatechuic acid, and eriodictyol 7-*O*-glucuronide than *A. ovalis* ($p < 0.05$). These molecules were reported to be effective against both Gram-positive and Gram-negative bacteria such as *S. aureus*, L. *monocytogenes, Y. enterocolitica*, and *E. coli*, respectively (Chen et al., 2022; Jeyaraj et al., 2023; Lou et al., 2011; Sun et al., 2018; Wu et al., 2022). In a study conducted by (Denev et al., 2019), purified extracts of *A. melanocarpa* enriched with derivatives of caffeoylquinic acids (chlorogenic acid and 3-caffeoylquinic acid) exhibited antimicrobial solid activities, with MIC values ranging from 2.5 to 5 mg/mL for strains of *S. aureus* and 0.63 mg/L for *Proteus vulgaris*. The antimicrobial mechanism of action of these compounds involves various pathways, including damage to bacterial cell membranes leading to increased permeability, inhibition of nucleic acid and protein synthesis, and mitochondrial dysfunction (Chen et al., 2022; Jeyaraj et al., 2023; Lou et al., 2011; Sun et al., 2018; Wu et al., 2022).

4. Conclusions

In the present investigation, the full screening of *A. melanocarpa* and *A. ovalis* were accomplished, namely, regarding their nutritional, chemical and bioactive assets. Our results suggest that both species are rich sources of compounds of interest, including organic acids and sugars. Also, a rich phenolic profile was tentatively identified in both species, these being very similar, with chlorgenic acid being the major phenolic found in both fruits hydroethanolic extracts, as well as quercetin and cyanidin (anthocyanins) derivatives, these being an essential part of the biodynamic nature of these berry fruits. In this field, the analysis of the antioxidant and antibacterial assets of *A. melanocarpa* and *A. ovalis* hydroethanolic extracts showed satisfactory results, these being able to inhibit lipid peroxidation of porcine brain tissues (TBARS) and the growth of bacterial strains, such as *S. aureus, MRSA,* L. *monocytogenes*, and other in higher concentrations.

In sum, the attained results contributed to the full knowledge of these less-known berry fruits grown in the Republic of Kazakhstan, allowing the assessment of their particular nutritional, chemical, and bioactive assets, thus encouraging their inclusion in the diet. Future studies should focus on the application of these berries and their extracts in food formulations. Furthermore, research on the bioaccessibility and bioavailability of the identified compounds should be encouraged, along with investigations into the biological properties of digested samples.

Ethical statement - Studies in humans and animals

This study did not apply to any human or animal studies, thus, ethics approval was not required for this research.

CRediT authorship contribution statement

Assem T. Sagandyk: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Ângela Liberal:** Data curation, Writing – review & editing, Formal analysis. **Tayse F.F. da Silveira:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Maria José Alves:** Formal analysis, Writing – review & editing. **Isabel C.F.R. Ferreira:** Writing – review & editing, Methodology. **Gulmira N. Zhakupova:** Writing – review & editing, Conceptualization, Funding acquisition, Investigation, Supervision. **Kadyrzhan Makangali:** Writing – review & editing, Supervision, Funding acquisition, Investigation, Conceptualization. **Tamara Ch. Tultabayeva:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **Lillian Barros:** Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2024.100564.

Data availability

Data will be made available on request.

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