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Comparative Analysis of Polyphenolic Content and Antioxidant Capacity in By-Products of Different Berry Species and Cultivars

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Sažetak

Listovi i komina jagodastog voća, koji se često smatraju otpadom nakon prerade, sadrže značajnu količinu bioaktivnih molekula. Ovim istraživanjem ispitan je sadržaj polifenola i antioksidacijski kapacitet u listovima i komini različitih vrsta i sorti jagodastog voća. Analizirani su uzorci pet sorti borovnica, tri sorte crnog ribiza, dvije sorte crvenog ribiza i dvije sorte aronije kako bi se usporedio ukupni sadržaj polifenola i antocijana te njihova antioksidacijska svojstva korištenjem ABTS, DPPH i FRAP metoda. Rezultati su pokazali značajne razlike u sadržaju bioaktivnih spojeva i antioksidacijskom kapacitetu među različitim sortama. Općenito, sadržaj polifenola kretao se od 48,55 do 145,70 mg/g suhe tvari u listovima i od 7,25 do 91,20 mg/g suhe tvari u komini. Sadržaj antocijana u komini bio je između 0,06 i 10,21 mg/g suhe tvari, a svi uzorci pokazali su visok antioksidacijski kapacitet. Listovi i komina borovnica, posebno sorta Aurora, imali su najviše koncentracije polifenola i antocijana te najvišu vrijednost antioksidacijskog kapaciteta među svim analiziranim uzorcima. Kod crnog ribiza, listovi sorte Titania imali su najviše vrijednosti ukupnih polifenola i antioksidacijskog kapaciteta, dok je kod komine to bila sorta Gofert. Kod crvenog ribiza, sorta Rotet istaknula se po sadržaju analiziranih bioaktivnih molekula i u listovima i u komini. Kod aronije, sorta Viking se istaknula bioaktivnim potencijalom listova, dok je kod komine to bila sorta Nero. Ove spoznaje potvrđuju potencijal listova i komine bobičastog voća, koji se često odbacuju kao nusproizvodi, kao vrijednih izvora antioksidansa za razvoj funkcionalnih proizvoda.

Abstract

Berry leaves and pomace, which are often considered waste after processing, contain a significant amount of bioactive molecules. This study investigated the polyphenolic content and antioxidant capacity in leaves and pomace of various berry species and cultivars. Samples of five blueberry, three blackcurrant, two redcurrant and two chokeberry cultivars were analyzed to compare the total polyphenol and anthocyanin content and their antioxidant properties using ABTS, DPPH and FRAP assays. The results showed significant differences in the content of bioactive compounds and antioxidant capacity of the different berry cultivars. In general, polyphenol content ranged from 48.55-145.70 mg/g dw in leaves and 7.25-91.20 mg/g dw in pomace. Anthocyanin content in pomace was between 0.06-10.21 mg/g dw, and all samples showed a high antioxidant capacity. Blueberry leaves and pomace, particularly cv. Aurora, showed the highest polyphenol and anthocyanin concentrations and the strongest antioxidant capacity among all analyzed samples. In blackcurrants, the cv. Titania had the highest values of polyphenols and antioxidant capacity in the leaves, while in the pomace it was cv. Gofert. When analyzing the redcurrants, cv. Rotet stood out in bioactive molecules in both leaves and pomace. For chokeberries, cv. Viking stood out for the bioactive potential of its leaves and cv. Nero for the pomace. These findings highlight the potential of berry leaves and pomace, commonly discarded as by-products, as valuable sources of antioxidants for the development of functional products.

Keywords: berries, leaves, pomace, polyphenols, anthocyanins

Introduction

Berries belong to a group of plants whose fruits represent a great source of nutrients, antioxidants, vitamins and minerals. Berries such as blueberries, currants and chokeberries are known for their high content of bioactive compounds, especially polyphenols, which are responsible for their strong antioxidant activity and their positive effects on human health (Manganaris et al., 2014). Although the berries have often been studied for their health benefits, less attention has been paid to plant residues such as leaves and pomace. These plant by-products, which contain even greater amounts of polyphenols than the berries themselves, may have antioxidant, antimicrobial and other effects. In the production of berries, the pomace, which consists of the skin, seeds and stalks, makes up a considerable proportion of the total weight of the fruit after processing, around 20-30% (Struck et al., 2016). The leaves of the berries are often neglected in comparison to the pomace, but they have a rich nutritional and medicinal value that is lately being researched more intensively (Ferlemi and Lamari, 2016). As pomace and leaves are usually considered a by-product of berry processing, research into their chemical composition and potential use represents an important step towards a more sustainable utilization of resources in the food and pharmaceutical industries.

Different types of berries contain different proportions of bioactive molecules and antioxidant capacity. Struck et al. (2016) and Ferlemi and Lamari (2016) stated that chokeberry fruit and pomace contained the highest levels of polyphenols and anthocyanins among the studied berries. According to the literature (Tian et al., 2018), berry leaves contain more polyphenols and have up to 20-fold higher antioxidant capacity compared to berries and pomace. In the research of Oszmiański et al. (2011) it was concluded that the leaves of different berry fruits differ according to their polyphenolic profile, and blueberry leaves contained the highest amount of polyphenols. Within a specific berry variety, there are also numerous cultivars that differ from each other in terms of the proportion of bioactive molecules and antioxidant capacity (Li et al., 2017).

Therefore, the aim of this work is to investigate the polyphenol content and antioxidant capacity of leaves and pomace of different berry species

(blueberry, blackcurrant, redcurrant and black chokeberry) focusing on the comparison between different cultivars.

2. Materials and methods

2.1. Chemicals and Reagents

The chemicals used for the extraction were ethanol (96 %) purchased from Lach-Ner (Neratovice, Czech Republic) and formic acid (≥95%) purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals used in reactions were gallic acid (>97.5%), 6-hydro-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox solution, 97%), 2,4,6-tripyridyl-S-triazine (TPTZ, ≥98%), diammonium salt of 2,2′-azino-bis(3-ethylbenzthiazolin-6-yl) sulfonic acid (ABTS), 2,2-azobis (2-methylpropionamidine) dihydrochloride (AAPH, 97%) as well as DPPH (2,2-diphenyl-1-picrylhydrazyl), obtained from Sigma-Aldrich (St. Louis, MO, USA). The potassium persulfate ($K_2S_2O_8$, >98%) was purchased from Scharlau (Regensburg, Germany), Folin-Ciocalteu reagent from Merck KgaA (Darmstadt, Germany) while iron (III) chloride hexahydrate ($FeCl_3 \times 6H_2O$) was obtained from Gram-mol (Zagreb, Croatia). Methanol (p.a.) and sodium acetate trihydrate ($CH_3COONa \times 3H_2O$) were obtained from Kemika (Zagreb, Croatia). Also, sodium carbonate (p.a.) was obtained from Lach-Ner (Neratovice, Czech Republic) and hydrochloric acid (37%) from Carlo Erba (Emmendingen, Germany).

2.2. Plant material

The samples of plant leaves and berries (blueberry, blackcurrant, redcurrant and black chokeberry) of different cultivars (Tables 1 and 2) were collected in June and July, 2024 in Donja Zelina orchard, Center for fruit growing and vegetable growing of the Croatian Agency for Agriculture and Food (Croatia). After harvesting, plant leaves were air-dried and vacuum-sealed in polyamide/polyethylene bags, while berries were immediately processed into juice (VerVita HU-100, Dong Ah Ind. Co., Ltd., Korea) and obtained pomace was freeze-dried (CHRIST Alpha 1-4 LSCplus, Osterode am Harz, Germany) during 24 h/-55 °C, and afterwards vacuum packaged in polyamide/polyethylene bags. All samples were stored at -18 °C till analysis. Prior extraction, an electric grinder (Waring WSG30, Sprzęt Laboratoryjny i Medyczny Labpartner KBS, Warszawa, Poland) was used to grind the dried samples of pomace and leaves.

2.3. Moisture content determination

The moisture content was determined using a moisture analyzer (OHAUS – MB23, Ohaus, New Jersey, SAD). To determine the dry mass of leaves and pomace of each variety, 3 g of the ground sample was properly distributed over the entire surface of the analysis container and the measurement was started. The measurement was continued until the sample reached a constant mass. Leaf dry matter ranged from 87.15-95.83%, while for the pomace it was between 94.99 and 99.11%.

2.4. Extraction

In this study, pressurized liquid extraction (PLE) was used for the polyphenol extraction from berry leaves while berry pomace polyphenols were extracted using microwave-assisted extraction (MAE). Both extraction procedures were carried out under predetermined optimal conditions using an optimal technique selected for each material (Elez Garofulić et al., 2024).

2.4.1. Pressurized liquid extraction

PLE of polyphenolic compounds from blueberries, blackcurrants, redcurrants and black chokeberry leaves was performed according to the previously described method (Balbino et al., 2021; Repajić et al., 2021) with slight modifications using the ASE Dionex 350® instrument (Thermo Fisher Scientific, Sunnyvale, CA, USA). The extraction conditions were: for blueberry temperature of 125 °C, static extraction time of 10 min and sample:solvent (SS) ratio of 1:40 g/mL, and for blackcurrant, redcurrant and black chokeberry temperature of 150 °C, static extraction time of 5 min and SS of 1:30 g/mL, respectively. An aqueous ethanol solution (30%, v/v) was used as the extraction solvent. Procedure involved filling the stainless-steel cells with 2 g of diatomaceous earth mixed with the specified amount of sample (according to the SS ratio) and the extraction was carried out with fixed parameters of pressure (10.34 MPa), number of cycles (3), nitrogen flush (30 s) and flush volume (30%). The obtained extracts were filtered, transferred to 50 mL volumetric flasks, filled to the mark with solvent and stored at -18 °C until analysis.

2.4.2. Microwave assisted extraction

Extraction of polyphenolic compounds from blueberry, blackcurrant, redcurrant, and black chokeberry pomace was performed according to the previously described method (Elez Garofulić et al., 2024) in a microwave reactor (Ethos Easy, Milestone, Sorisole, Italy). In brief, an appropriate amount of the sample was placed in the extraction vessel and 20 mL of an acidified aqueous ethanol solution (1% formic acid in 50% ethanol, v/v) was added. The extraction conditions were: temperature 80 °C, irradiation time 5 min and SS ratio 1:40 g/mL for blueberries; temperature 60 °C, irradiation time 5 min and SS ratio 1:80 g/mL for black- and redcurrants; and temperature 80 °C, irradiation time 10 min and SS ratio 1:40 g/mL for black chokeberry pomace. The general parameters were preheating time (3 min), microwave power (400 W), stirring level (50%) and cooling time after extraction (1 min). The extracts were filtered, transferred to 25 mL volumetric flasks and filled with the solvent to the mark and afterwards stored at -18 °C until further analysis.

2.5. Total polyphenolic content determination

The total polyphenolic content (TPC) of berry leaves and pomace was determined using a modified spectrophotometric method described by Repajić et al. (2021). The method was carried out as follows: 100 µL of extract (or pure solvent as blank) was mixed with 2 mL of distilled water and 200 µL of Folin–Ciocalteu reagent. After 3 min, 1 mL sodium carbonate solution (20%, w/v) was added and the reaction mixture was thermostated at 50 °C. After 25 min, the absorbance was measured at 765 nm. TPC of the samples was expressed as mg gallic acid equivalents (GAE) per g leaf or pomace dry weight (dw).

2.6. Total monomeric anthocyanins content determination

The content of monomeric anthocyanins (TMAC) in the berry pomace was determined using the spectrophotometric method previously described by Lee et al. (2001). The reaction was carried out in two test tubes: 1 mL of extract with 4 mL of buffer pH 1.0 was added to the first test tube, and 1 mL of extract with 4 mL of buffer pH 4.5 was added to the second test tube for each sample. After 20 min at room temperature, the absorbance was measured at 520 nm and 700 nm for each sample, using the corresponding buffers as blanks. The results were expressed as mg cyanidin-3-glucoside equivalents (C3GE) per g leaf or pomace dw.

2.7. Antioxidant capacity determination

The antioxidant capacity of the extracts obtained was measured by DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate), ABTS (reduction of radical cation) and FRAP (ferric reducing antioxidant power) assays. For analysis by DPPH assay, 0.75 mL of the berry leaf or pomace extract was mixed with 1.5 mL of a 0.2 mM DPPH solution and the mixture was kept in the dark for 20 min, after which the absorbance at 517 nm was measured. The antioxidant capacity by ABTS was carried out in test tubes in which 40 µL of the sample and 4 mL of 1% ABTS•+ were mixed, and after 1 min the absorbance was measured at 734 nm. Finally, FRAP was performed by mixing 80 µL of the extract, 240 µL of distilled water and 2080 µL of the previously prepared FRAP reagent (0.01 M TPTZ solution in 0.04 M hydrochloric acid, 20 mM FeCl3 6H2O aqueous solution and 0.3 M and sodium acetate buffer, pH 3.6 in the ratio of 1:1:10, v/v/v). The mixture was shaken and thermostated at 37 ºC for 5 min. The absorbance was measured at 593 nm. In brief, all reactions were performed with the appropriate reagents, the color change was measured in comparison to the blank sample and the results were expressed as µmol Trolox equivalents (TE) per g leaf or pomace dw. The methods were previously described by Braca et al. (2001), Miller and Rice-Evans (1997) and Benzie and Strain, (1996) for DPPH, ABTS and FRAP assays, respectively.

2.8. Statistical analysis

All analysis were performed in duplicate and values obtained are given as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for testing the influence of species and cultivars on TPC, TMAC and antioxidant capacity of samples and significant differences between samples were observed with Tukey's HSD test. The significance level for all tests was established at $p \leq 0.05$. Statistica ver. 10.0 software (Statsoft Inc., Tulsa, USA) was used for statistical analysis.

3. Results and discussion

Berry leaves and pomace are an excellent source of bioactive molecules, especially polyphenols. Although polyphenols contribute to antioxidant capacity, these types of extracts represent a complex medium, usually containing of various molecules that express their antioxidant activity through different mechanisms of action, so it is desirable to test antioxidant capacity using several different methods. Therefore, this study compared the TPC and antioxidant capacity (using ABTS, DPPH and FRAP) in extracts from leaves and pomace of different berry species and cultivars. In addition, pomace samples were also analyzed for TMA.

3.1. Differences between berry leaves

According to the research results, the leaves of five blueberry cultivars (Draper, Blue Crop, Brigitta Blue, Chandler, Aurora) were statistically ($p < 0.01$) different from each other in terms of TPC and antioxidant capacity tested by the ABTS and FRAP methods, while the difference in antioxidant capacity tested by the DPPH method was not statistically significant ($p = 0.50$) (Table 1). The cv. Blue Crop contained the lowest amount of polyphenols, while the cv. Aurora had the highest level of polyphenols. According to a study by Deng et al. (2014) the proportion of polyphenols in blueberry leaves was between 67.15-349.17 mg/g dw, which is slightly higher than obtained in this study. They investigated how both the choice of solvent and the ripening stage affected the polyphenol content and found that each factor had a significant influence on the amount of polyphenols. In addition, a different extraction technique was used in their study, namely maceration. As for the antioxidant capacity measured by the ABTS and FRAP methods, the cv. Aurora showed the highest values, while the other cultivars were characterized with lower values. In general, different values obtained when measuring antioxidant capacity can be attributed to the different principles on which each of the

Table 1. Total polyphenolic content and antioxidant capacity (determined by ABTS, DPPH and FRAP) of different cultivars of blueberries, blackcurrants, redcurrants and black chokeberry leaves

LEAVES	Cultivar	TPC (mg GAE/g dw)	ABTS (µmol TE/g dw)	DPPH (µmol TE/g dw)	FRAP (µmol TE/g dw)
		$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
		$p < 0.01$	$p < 0.01$	$p = 0.50$	$p < 0.01$
BLUEBERRY	DRAPER	$137.54 \pm 2.53^{b,c}$	1043.80 ± 10.70^{a}	693.09 ± 6.11^{a}	1059.15 ± 11.76^{b}
	BLUE CROP	124.30 ± 4.01^{a}	1030.27 ± 19.82^{a}	681.29 ± 1.14^{a}	948.56 ± 9.38^{a}
	BRIGITTA BLUE	$130.64 \pm 1.91^{a,b}$	1051.49 ± 4.56^{a}	686.94 ± 21.29^{a}	$1012.50 \pm 14.04^{a,b}$
	CHANDLER	$127.09 \pm 3.53^{a,b}$	1055.79 ± 22.63^{a}	686.99 ± 4.90^{a}	956.72 ± 2.32^{a}
	AURORA	145.70 ± 3.39^{eD}	1148.33 ± 4.56^{bD}	699.88 ± 0.76^{aD}	1434.45 ± 44.46^{cC}
		$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
BLACKCURRANT	GOFERT	48.55 ± 0.96^{a}	575.99 ± 2.24^{b}	430.17 ± 3.91^{b}	441.37 ± 15.48^{a}
	TITANIA	63.05 ± 0.14^{cB}	642.19 ± 2.37^{cB}	361.31 ± 17.22^{aB}	678.20 ± 5.48^{cB}
	TIESEL	59.26 ± 0.14^{b}	536.52 ± 12.14^{a}	368.22 ± 0.61^{a}	499.37 ± 3.74^{b}
		$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
REDCURRANT	JUNIFER	52.43 ± 1.24^{a}	526.89 ± 17.15^{a}	325.20 ± 5.14^{a}	603.16 ± 1.76^{a}
	ROTET	82.02 ± 0.84^{bC}	978.85 ± 4.51^{bC}	455.84 ± 0.56^{bC}	762.29 ± 5.21^{bB}
		$p = 0.02$	$p < 0.01$	$p = 0.79$	$p < 0.01$
BLACK CHOKEBERRY	NERO	49.44 ± 0.40^{a}	414.48 ± 10.39^{b}	244.30 ± 3.46^{a}	389.28 ± 10.66^{a}
	VIKING	52.89 ± 0.63^{bA}	341.69 ± 3.31^{aA}	247.25 ± 13.24^{aA}	561.94 ± 16.98^{bA}

TPC-total polyphenolic content, dw-dry weight. Results are expressed as mean ± SD. Different lowercase letters assigned to the values indicate a statistically significant difference between cultivars of a particular berry species ($p \leq 0.05$), while different uppercase letters assigned to the most significant values indicate a statistically significant difference between berry species ($p \leq 0.05$).

above methods is based, as well as the way in which radicals react with polyphenols and other antioxidants (Moharram and Youssef, 2014).

The results for blackcurrant leaves showed that cultivar type had a statistically significant influence ($p < 0.01$) on the TPC and antioxidant capacity. Of the 3 cultivars analyzed (Gofert, Titania, Tiesel), the cv. Titania contained the highest amount of polyphenols and showed the highest antioxidant capacity according to the ABTS and FRAP methods, while the cv. Gofert showed the highest value of antioxidant capacity according to the DPPH method. Nour et al. (2014) recorded a lower proportion of total polyphenols (39.96 mg/g dw) as well as a range of antioxidant capacity measured by the DPPH method (329.7 μmol/g dw) in blackcurrant leaves. The variation was probably due to the use of different cultivars of blackcurrant leaves, differences in harvest time, the choice of extraction technique, and the type of solvent used.

TPC and antioxidant capacity of redcurrant leaves were also significantly influenced ($p < 0.01$) by the cultivar type. The cv. Rotet showed a higher proportion of polyphenols and a higher antioxidant capacity compared to the cv. Junifer (Table 1). These results differ from those of in the study by Kendir and Köroğlu (2015) who found higher levels of polyphenols (25.63-483.75 mg/g dw) in redcurrant leaves depending on the solvent used.

The results for black chokeberry leaves showed that the content of polyphenols ($p = 0.02$) and antioxidant capacity measured by ABTS and FRAP methods were significantly different ($p < 0.01$) between the cv. Nero and Viking, while there was no statistically significant difference ($p = 0.79$) between these cultivars in antioxidant capacity measured by DPPH. As it is shown in Table 1, the cv. Viking had the higher proportion of polyphenols and antioxidant capacity tested by FRAP, while the antioxidant capacity tested by ABTS was higher in the cv. Nero. In the study by Saracila et al. (2024) a slightly higher value of total polyphenols in chokeberry leaves (61.06 mg/g dw) was recorded than in cv. Viking examined in this study.

To summarize obtained results for berry leaves, blueberry cv. Aurora, blackcurrant cv. Titania, redcurrant cv. Rotet and black chokeberry cv. Viking stood out as cultivars characterized with the highest bioactive potential in terms of polyphenols content and antioxidant capacity. In order to determine the berry species that is the richest source of natural antioxidants among ones tested in this study, further comparisons were conducted between these cultivars (and also species). Obtained results showed that the highest values of the analyzed parameters were found in blueberry leaves (cv. Aurora). It was followed by redcurrant leaves (cv. Rotet), then blackcurrant leaves (cv. Titania). The black chokeberry leaves (cv. Viking) had the lowest amount of polyphenols and antioxidant activity. These results demonstrated that the content of polyphenols and antioxidant capacity in berry leaves greatly depend on the genetics of the plant, as it was concluded by Tian et al. (2018) who examined the differences between various varieties of berry leaves and observed a different polyphenolic profile and antioxidant capacity of the tested varieties.

3.2. Differences between berry pomace

The TPC and TMAC as well as the antioxidant capacity of the berry pomace varied within the cultivars of the same species, but also between the different berry species. Based on the results for blueberry pomace (Table 2), there was a statistically significant difference ($p < 0.01$) between the cultivars in all analyzed parameters. The cv. Aurora showed the highest values in all assays performed, indicating the highest antioxidant capacity, which correlates with the highest TPC determined in this sample. Tagliani et al. (2019) reported that the TMAC in blueberry pomace was 1.26 mg/g dw, what is 8.1-fold lower than in the cv. Aurora examined in this study. However, cv. Aurora showed the highest TMAC

Table 2. Total polyphenolic and anthocyanin content and antioxidant capacity (determined by ABTS, DPPH and FRAP) of different cultivars of blueberries, black and red currants and black chokeberry pomace.

POMACE	Cultivar	TPC (mg GAE/g dw)	TMAC (mg C3GE/g dw)	ABTS (μmol TE/g dw)	DPPH (μmol TE/g dw)	FRAP (μmol TE/g dw)
		$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
		$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
BLUEBERRY	DRAPER	41.22 ± 0.58^{a}	4.78 ± 0.06^{c}	254.23 ± 8.67^{a}	376.60 ± 2.53^{a}	$440.18 \pm 4.45^{a,b}$
	BLUE CROP	39.27 ± 1.58^{a}	4.58 ± 0.12^{b}	307.98 ± 13.08^{b}	378.87 ± 1.45^{a}	501.00 ± 24.59^{b}
	BRIGITTA BLUE	37.84 ± 0.58^{a}	4.09 ± 0.19^{a}	$346.34 \pm 11.63^{b,c}$	382.59 ± 12.73^{a}	389.18 ± 4.48^{a}
	CHANDLER	70.42 ± 0.49^{b}	7.72 ± 0.15^{d}	514.28 ± 11.39^{d}	488.11 ± 3.56^{b}	681.54 ± 43.81^{c}
	AURORA	91.20 ± 2.62^{cC}	10.21 ± 0.11^{eD}	712.71 ± 4.30^{eD}	560.56 ± 3.94^{cB}	1087.44 ± 22.03^{dC}
		$p < 0.01$	$p < 0.01$	$p < 0.01$	$p = 0.49$	$p < 0.01$
BLACK CURRANT	GOFERT	50.20 ± 0.17^{cB}	4.74 ± 0.08^{cC}	535.39 ± 26.43^{cC}	652.73 ± 13.22^{aC}	667.77 ± 13.56^{cB}
	TITANIA	34.15 ± 0.34^{a}	3.43 ± 0.01^{a}	362.77 ± 8.96^{a}	623.02 ± 51.51^{a}	406.07 ± 22.97^{a}
	TIESEL	39.05 ± 0.98^{b}	4.12 ± 0.00^{b}	269.71 ± 8.55^{b}	612.89 ± 2.14^{a}	483.78 ± 6.68^{b}
		$p < 0.01$	$p = 0.27$	$p < 0.01$	$p = 0.18$	$p = 0.02$
RED CURRANT	JUNIFER	7.25 ± 0.66^{a}	0.06 ± 0.00^{a}	132.62 ± 11.58^{a}	483.11 ± 28.22^{a}	143.26 ± 21.93^{a}
	ROTET	17.51 ± 0.49^{bA}	0.06 ± 0.00^{aA}	281.77 ± 8.55^{bA}	414.28 ± 39.19^{aA}	248.06 ± 0.00^{bA}
		$p < 0.01$	$p < 0.01$	$p < 0.01$	$p = 0.02$	$p = 0.03$
BLACK CHOKEBERRY	NERO	53.56 ± 0.41^{bB}	2.44 ± 0.01^{bB}	467.69 ± 11.46^{bB}	527.98 ± 12.18^{bB}	252.52 ± 13.23^{aA}
	VIKING	46.20 ± 0.25^{a}	1.95 ± 0.04^{a}	235.26 ± 17.39^{a}	451.97 ± 7.97^{a}	362.57 ± 22.29^{b}

TPC-total polyphenolic content, TMAC-total monomeric anthocyanins content, dw- dry weight. Results are expressed as mean ± SD. Different lowercase letters assigned to the values indicate a statistically significant difference between cultivars of a particular berry species ($p \leq 0.05$), while different uppercase letters assigned to the most significant values indicate a statistically significant difference between berry species ($p \leq 0.05$).

among the blueberry cultivars studied, while cv. Brigitta Blue was characterized with the lowest content of both TPC and TMAC and expectedly the lowest antioxidant capacity. Reque et al. (2014) reported an ABTS value of blueberry pomace of 122.56 μmol/g dw, which is 5.8- and 2.1-fold lower than the results for blueberry pomace of cv. Aurora and Draper obtained in this study.

In contrast to blueberry, blackcurrant pomace samples were not significantly different in antioxidant capacity between cultivars when using the DPPH method ($p = 0.49$), while a significant difference was found for the other antioxidant activity methods used (ABTS, $p < 0.01$, FRAP, $p < 0.01$). The TPC and TMAC were significantly different ($p < 0.01$) within the cultivars with the cv. Gofert having the highest and the cv. Titania the lowest values. The TPC and TMAC in blackcurrant pomace was previously reported to be 26.2 and 3.68 mg/g dw (Blejan et al., 2023), which is 1.9- and 1.3-fold lower than results obtained in this study for the cv. Gofert, respectively. In addition, the TPC of different blackcurrant pomace extracts was reported as 20.04 mg/g dw for acetone (Puganen et al., 2018) and 22.41 mg/g dw for methanol:water:formic acid (50:48:2, v/v/v) extracts obtained by sonication (Sójka and Król, 2009). In comparison, results in this study are 2.5- and 2.2-fold higher (Table 2) for the pomace of cv. Gofert, and these differences could be due to the choice of extraction method, as MAE was used in this study, or the solvent type as well as the cultivar of species studied.

The redcurrant cultivars differed in terms of TPC and antioxidant capacity tested by ABTS ($p < 0.01$) and FRAP ($p = 0.02$) methods, while the difference between cultivars in terms of TMAC ($p = 0.27$) and antioxidant capacity by DPPH method ($p = 0.18$) was not detected. Puganen et al. (2018) reported that the TPC in the methanolic extract of redcurrant pomace was 34.46 mg/g dw, which is 2-fold higher than results obtained in this study.

Black chokeberry pomace samples also showed statistically significant differences between cultivars in terms of TPC, TMAC ($p < 0.01$) and all antioxidant capacity methods used (ABTS, DPPH and FRAP with $p < 0.01$, $p = 0.02$ and $p = 0.03$, respectively). The highest TPC for black chokeberry pomace was detected in cv. Nero (Table 2), while Oszmiański and Wojdylo (2005) and Kapci et al. (2013) reported slightly higher values, 105.83 and 99.93 mg/g dw, respectively. The reason for the discrepancy between the results could lie in the different cultivars and the harvest season.

As it is shown in Table 2, the pomace with the highest TPC and the highest antioxidant capacity were ones from cv. Aurora (blueberry), Gofert (blackcurrant), Rotet (redcurrant) and Nero (chokeberry). Significant differences ($p < 0.01$) were found when comparing the species in terms of TPC, TMAC and antioxidant capacity. The results showed that the pomace of the cv. Aurora (blueberry) had the highest value of TPC and TMAC as well as the highest antioxidant capacity determined by ABTS and FRAP, while the pomace of the cv. Gofert (blackcurrant) showed the highest antioxidant capacity when using the DPPH method. The redcurrant pomace of the cv. Rotet showed the lowest content of polyphenols and anthocyanins as well as the lowest antioxidant capacity compared to the other species tested (for all methods).

Conclusions

According to the research results, when comparing leaves and pomace, the leaves contain more polyphenols and promote higher antioxidant capacity. However, pomace represents an excellent source of anthocyanins. Among the analyzed berry leaves samples, blueberry leaves (cv. Aurora), emerged as the ones containing the highest amount of polyphenols and the highest antioxidant capacity, while black chokeberry leaves (cv. Viking) had the lowest values of polyphenols and antioxidant capacity. Among the berry pomace samples, the blueberry pomace (cv. Aurora) also contained the highest levels of polyphenols, anthocyanins and the highest value of antioxidant capacity, while the lowest level of bioactive molecules and antioxidant capacity were found in the redcurrant pomace (cv. Rotet). The chemical diversity and the amount of bioactive molecules in berries depend on the genetic variability of the cultivar. This study demonstrated that due to the richness of bioactive molecules which possess great antioxidant activity, berry by-products (leaves and pomace), although often considered as a waste after processing, represent an excellent basis for further use. Particular niche that can surely expand and profit taking into account these findings is certainly the development of new functional products.

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References

Balbino S., Repajić M., Obranović M., Medved A.M., Tonković P., Dragović-Uzelac V. (2021) Characterization of lipid fraction of Apiaceae family seed spices: Impact of species and extraction method. Journal of Applied Research on Medicinal and Aromatic Plants, 25, 100326.

Blejan A.M., Nour V., Păcularu-Burada B., Popescu S.M. (2023) Wild bilberry, blackcurrant, and blackberry by-products as a source of nutritional and bioactive compounds. International Journal of Food Properties, 26 (1), 1579–1595.

Braca A., De Tommasi N., Di Bari L., Pizza C., Politi M., Morelli I. (2001) Antioxidant principles from Bauhinia tarapotensis. Journal of Natural Products, 64 (7), 892–895.

Deng Y., Yang G., Yue J., Qian B., Liu Z., Wang D., Zhong Y., Zhao Y. (2014) Influences of ripening stages and extracting solvents on the polyphenolic compounds, antimicrobial and antioxidant activities of blueberry leaf extracts. Food Control, 38, 184–191.

Elez Garofulić I., Repajić M., Cegledi E., Dobroslavić E., Dobrinčić A., Zorić Z., Pedisić S., Franković T., Breški M., Dragović-Uzelac V. (2024) Green approach to enhance the recovery of polyphenols from blackcurrant and bilberry leaves: Evaluation of microwave-assisted and pressurized liquid extraction. Molecules, 29 (6), 1351.

Ferlemi A.V., Lamari F.N. (2016) Berry leaves: An alternative source of bioactive natural products of nutritional and medicinal value. Antioxidants, 5 (2).

Kapci B., Neradova E., Čižkova H., Voldrich M., Rajchl A., Capanoglu E. (2013) Investigating the antioxidant potential of chokeberry (Aronia melanocarpa) products. Journal of Food & Nutrition Research, 52, 219–229.

Kendir G., Köroğlu A. (2015) In vitro antioxidant effect of the leaf and branch extracts of Ribes L. species in Turkey. International Journal of Pharmaceutical Sciences Research, 2 (1).

Lee J., Durst R.W., Wrolstad R.E. et al. (2001) Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. Journal of AOAC International, 88 (5), 1269–1278.

Li D., Li B., Ma Y., Sun X., Lin Y., Meng X. (2017) Polyphenols, anthocyanins, and flavonoids contents and the antioxidant capacity of various cultivars of highbush and half-high blueberries. Journal of Food Composition and Analysis, 62, 84–93.

Manganaris G.A., Goulas V., Vicente A.R., Terry L.A. (2014) Berry antioxidants: Small fruits providing large benefits. Journal of the Science of Food and Agriculture, 94 (5), 825–833.

Moharram H.A., Youssef M.M. (2014) Methods for determining the antioxidant activity: A review. Alexandria Journal of Food Science and Technology, 11 (1).

Nour V., Trandafir I., Cosmulescu S. (2014) Antioxidant capacity, phenolic compounds and minerals content of blackcurrant (Ribes nigrum L.) leaves as influenced by harvesting date and extraction method. Industrial Crops and Products, 53, 133–139.

Oszmiański J., Wojdyło A. (2005) Aronia melanocarpa phenolics and their antioxidant activity. European Food Research and Technology, 221 (6), 809–813.

Oszmiański J., Wojdyło A., Gorzelany J., Kapusta I. (2011) Identification and characterization of low molecular weight polyphenols in berry leaf extracts by HPLC-DAD and LC-ESI/MS. Journal of Agricultural and Food Chemistry, 59 (24), 12830–12835.

Puganen A., Kallio H.P., Schaich K.M., Suomela J.P., Yang B. (2018) Red/green currant and sea buckthorn berry press residues as potential sources of antioxidants for food use. Journal of Agricultural and Food Chemistry, 66 (13), 3426–3434.

Repajić M., Cegledi E., Zorić Z., Pedisić S., Garofulić I.E., Radman S., Palčić I., Dragović-Uzelac V. (2021) Bioactive compounds in wild nettle (Urtica dioica L.) leaves and stalks: Polyphenols and pigments upon seasonal and habitat variations. Foods, 10 (1).

Reque P.M., Steffens R.S., Silva A.M., Jablonski A., Flôres S.H., Rios A.O., Jong E.V. (2014) Characterization of blueberry fruits (Vaccinium spp.) and derived products. Food Science and Technology, 34 (4), 773–779.

Saracila M., Untea A.E., Oancea A.G., Varzaru I., Vlaicu P.A. (2024) Comparative analysis of black chokeberry (Aronia melanocarpa L.) fruit, leaves, and pomace for their phytochemical composition, antioxidant potential, and polyphenol bioaccessibility. Foods, 13 (12).

Sójka M., Król B. (2009) Composition of industrial seedless black currant pomace. European Food Research and Technology, 228 (4), 597–605.
Struck S., Plaza M., Turner C., Rohm H. (2016) Berry pomace – A review of processing and chemical analysis of its polyphenols. International Journal of Food Science and Technology, 51 (6), 1305–1318.

Tagliani C., Perez C., Curutchet A., Arcia P., Cozzano S. (2019) Blueberry pomace, valorization of an industry by-product source of fibre with antioxidant capacity. Food Science and Technology, 39 (3), 644–651.

Tian Y., Puganen A., Alakomi H.L., Uusitupa A., Saarela M., Yang B. (2018) Antioxidative and antibacterial activities of aqueous ethanol extracts of berries, leaves, and branches of berry plants. Food Research International, 106, 291–303.