Article

Potentially Bioactive Compounds and Sensory Compounds in By-Products of Several Cultivars of Blackberry (Rubus fruticosus L.)

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Abstract: This study aimed to determine the amounts of phenols, antioxidant activity, and sensory compounds in three commercial cultivars of blackberries popular in Lithuania: ‘Polar’, ‘Brzezina’, and ‘Orkan’. Blackberry pomace was analyzed by the spectrophotometric method for total phenolic content, total flavonoid content and radical-scavenging capacity using the DPPH• and ABTS•+ assays. The phenolic profiles, organic acids, and sugars were analyzed by HPLC. The Heracles II electronic nose, which is based on ultrafast gas chromatography, was used for the quantification of volatile organic compounds. The results show that the total phenolic content of blackberry pomace varied from 2380.60 to 2088.00 mg 100 g−1 and that the total flavonoid content varied from 161.29 to 148.10 mg 100 g−1, depending on the cultivar. A total of 14 polyphenols were also identified, with epigallocatechin and anthocyanin cyanidin-3-O-glucoside being quantified in the highest concentrations (7.28 to 9.72 and 6.19 to 9.79 mg g−1, respectively) and being the predominant phenolic compounds in the blackberry-pomace samples. The odor profiles of blackberry pomace from different cultivars varied. The main volatile organic compounds found in all blackberry pomace were 1-Nonanol and cis-3-Hexen-1-ol, are associated with herbaceous and citrusy aromas. All these results show the potential of using blackberry pomace to enrich food products with bioactive phytochemicals.

Keywords: antioxidant activity; blackberries; byproducts; phenolic profile; organic acids; sugars

1. Introduction

The term “berry” or “red fruit” refers to small fruits that grow on bushes and can be sweet or bitter, with juicy flesh and an intense red-to-purple/blue color. The most common berries worldwide are cranberries, blackberries, blueberries, raspberries, and strawberries [1]. Global production of blackberries, which is difficult to estimate accurately, reached approximately 300,000 tons in 2017 [2]. The fragility and high respiration rate of berries after harvest contribute significantly to their nutritional and microbiological deterioration, resulting in a limited shelf-life and reduced quality and health benefits [3]; therefore, a significant part of the harvest is processed. Large quantities of byproducts containing bioactive phytochemicals are discarded after the berries have been processed into juices, concentrates, jams, and jellies. These byproducts are referred to as marc, residue, or waste, but are most commonly referred to as pomace [4]. The increasing interest in R. fruticosus fruits, seeds, and pomace is largely due to their rich content of lipophilic and hydrophilic compounds [5].
Blackberry seeds contain oil, which is composed of fatty acids, phytosterols, and other important lipophilic compounds such as vitamins (especially E and A) [4], sterols and lipids [6,7]. Many of these compounds exhibit antioxidant properties. From a technological point of view, they can serve as natural preservatives, emulsifiers, thickeners, bulking agents, or colorants. This is significant because they have the potential to compete with synthetic additives [8].

Blackberries pomace represents 20% of the total fruit. Parts such as pulp and skin are rich in polyphenolic antioxidants such as flavonoids, monomeric anthocyanins, glycosides, terpenes, phenolic acids, vitamins, and pigments, which have pharmacological activities such as antioxidant, anti-carcinogenic, anti-inflammatory, antimicrobial, anti-diabetic, anti-diarrheal, antiviral, and UV-protective properties. Tannins—mainly ellagitannins—contribute to their high antioxidant activity and reduce the risk of cancer and other pathologies [8–12]. Nevertheless, the berry pomace left over after industrial processing is an excellent source not only of phenolic antioxidants but also of dietary fiber [13].

In addition to compounds with bioactive potential, blackberry pomace contains compounds that contribute to its properties. Organic acids, sugars, and volatile organic compounds are the main components forming the sensory profile.

Although numerous scientific studies have described the content of sugar [14,15], organic acids [14,16,17], and VOCs in blackberries [4,18–20], there is a lack of information on these compounds in blackberry pomace. This may be important if the pomace is to be used directly in food products, for the production of extracts, or for the separation of individual compounds. It is obvious that components of blackberry byproducts may offer new opportunities for value-added utilization of agricultural raw materials and byproducts from their processing [4,21]; however, the concentration of bioactive and nutritional compounds in blackberries and their pomace depends on pre- and post-harvest factors, with genetic factors also playing a role.

From an economic perspective, it is beneficial to the agricultural and food industry to recover byproducts for use as raw material for processing into new food products and medicines [21,22]. Therefore, the valorization process should be optimized for each material to ensure efficient management of agro-food byproducts on an industrial scale [23,24].

Consequently, the objective of the research was to investigate the bioactive and sensory compounds, as well as the level of antioxidant activity, in pomace from three blackberry cultivars that are commercially cultivated in Lithuania.

2. Materials and Methods

2.1. Chemicals and Reference Substances

The ethanol of agricultural origin used for extractions was analytical-grade ethanol from MV group (MV group, Kaunas, Lithuania). Demineralized water was obtained from a Milli-Q system (Millipore, Burlington, MA, USA). Gallic acid (97%), Folin–Ciocalteu reagent, (3,4,5-trihydroxy benzoic acid, 99%), 2,2-diphenyl-1-picrylhydrazyl hydrate free radical (DPPH•, 95%), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS•+), 6-hydroxyl-2,5,7,8-tetramethylethrom-2-carboxylic acid (Trolox, ≥97%), methanol (99.9% HPLC grade), aluminum chloride, diphenyl, cyanopropylphenyl, n-butane, and n-hexadecane were purchased from Sigma–Aldrich (Sigma–Aldrich, Steinheim, Germany). Sodium carbonate, potassium chloride, and sodium acetate, all ≥99.5% pure, were purchased from Enola (Enola, Riga, Latvia). Standards for organic acids and sugars (dehydroascorbic acid, citric acid, l-ascorbic acid, malic acid, propionic acid, butyric acid, glucose, and fructose) were purchased from Sigma–Aldrich (Sigma–Aldrich, Steinheim, Germany) and Merck Company (Merck Company, Poznan, Poland). Polyphenol standards (epigallocatechin, catechin, chlorogenic acid, epigallocatechin gallate, p-coumaric, quercetin-3-O-rutinoside, kaempferol-3-O-glucoside, myricetin, quercetin, kaempferol, quercetin-3-O-glucoside, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, and ellagic acid) were purchased from Sigma–Aldrich Company (Sigma–Aldrich, Poznan, Poland). Sulfuric acid 98% was purchased from Merck Company (Merck, Poznan, Poland). Solvents (acetonitrile, acetone,
hydrochloric acid, acetic acid, and formic acid) were LiChrosolv HPLC grade and were purchased from Merck KGaA (Merck KGaA, Darmstadt, Germany).

2.2. Blackberry Pomace

Blackberries were obtained from a farmer in the Joniškis region (56.30219045284591, 23.603429519328024) in Lithuania, and the juice was extracted using a commercial slow-speed juicer (Stollar, Riga, Latvia). The pomace was freeze-dried in a lyophilizator at −55 °C for 48 h. Freeze-dried blackberry pomace was ground to a flour consistency (using a 0.2 mm particle-size sieve) with a food mill (Model Retsch ZM200, Retsch GmbH, Haan, Germany) and stored in hermetically sealed bags at −38 °C in a freezer until the analyses.

2.3. Analysis of Total Polyphenolic Compounds (TPC)

The total polyphenol content of blackberry pomace was determined according to the Folin–Ciocalteu method, using gallic acid (GA) as a standard, according to the method of Bobinaite et al. [25]. Briefly, 1 g of freeze-dried blackberry pomace powder was extracted with 40 mL of aqueous ethanol (70%). The extracts were homogenized for 60 s using a homogenizer (model VDI 25 s40, Retsch GmbH, Haan, Germany) and left in the dark for 24 h, then filtered using Whatman paper (retention 8–12 µm). The reagent was prepared by diluting a stock solution with pure distilled water (1/10, v/v). The sample (1.0 mL) was added to the test cuvette, following which 5.0 mL of Folin–Ciocalteu’s reagent and 4.0 mL of Na₂CO₃ (7.5%) were added and the sample was then left at ambient temperature for 1 h. The absorbance of all samples was measured at 765 nm using a Spectro UVD-3200 (Spectro UV-VIS Double Beam PC, Labomed, Los Angeles, CA, USA) spectrophotometer. The total concentration of phenolic compounds was determined from the gallic-acid calibration curve and expressed in mg 100 g⁻¹ of dry sample, as described by Urbonavičienė, et al. [26].

2.4. Determination of Total Flavonoid Content (TFC)

The aluminum chloride colorimetric method was used to determine the total flavonoid content of the sample. Briefly, 0.5 g of pomace was extracted with 10 mL EtOH (75%) for 1 h in an automatic shaker (Heidolph Vibramax 100, 31 W, Retsch GmbH, Haan, Germany) (1200 rpm), and the extract was then filtered through Whatman paper (retention 8–12 µm). For the sample mixtures, 10 mL of aluminum chloride solution (2% m/v), 2 mL of ethanol (96%) and 1 mL of 1 M sodium acetate were added to 1 mL of blackberry-pomace extract, and the sample was then incubated for 40 min at room temperature. Absorbance was then measured at a wavelength of λ = 420 nm on a Varian Cary 50 UV spectrophotometer and a Spectro UVD-3200 (Spectro UV-VIS Double Beam PC, Labomed, Los Angeles, CA, USA) spectrophotometer. Results were expressed as mg quercetin 100 g⁻¹ dry weight. Measurements were performed in triplicate for each sample.

2.5. Assay for Antioxidant Activity ABTS** Discoloration Method

The TEAC (Trolox equivalent antioxidant capacity) assay measured the antioxidant activity of blackberry pomace using the ABTS** discoloration method. The fractions were extracted sequentially from 0.1 g of each sample with methanol and acetone (1:1, v/v), respectively, in an ultrasonic bath for 1 h, then filtered through Whatman paper (retention 8–12 µm). Next, 20 µL was mixed with 2.0 mL of ABTS** solution and the decrease in absorbance was measured at 734 nm in a Spectro UVD-3200 (Spectro UV-VIS Double Beam PC, Labomed, Los Angeles, CA, USA) spectrophotometer. The antioxidant activity of the samples was calculated from the Trolox concentrations at 734 nm, with the inhibition time fixed at 30 min. Antioxidant activities were expressed as µmol Trolox equivalent (TE)/g dry weight of sample, as described by Urbonavičienė, et al. [26].

2.6. Analysis of DPPH* Radical-Scavenging Activity (DPPH* -RSA)

The radical-scavenging activity (RSA) was determined per the method reported by Urbonavičienė, et al. [26]. The method is based on the yellow discoloration of stable DPPH*
(2,2, diphenyl-picryl-hydrazyl) radical, which is otherwise strongly colored red-violet, by antioxidant substances. Ethanolic DPPH• solution (2 mL) was mixed with 20 µL of the prepared extract (by the same procedure as the ABTS•+ discoloration method). Absorbance was measured at 515 nm after 30 min using a Spectro UVD-3200 spectrometer (Spectro UV-VIS Double Beam PC, Labomed, Los Angeles, CA, USA). DPPH• radical-scavenging activity was expressed as Trolox equivalents (µmol TE/g dry weight of sample).

2.7. Determination of Polyphenolic Compounds by HPLC

Identification and quantification of polyphenolic compounds were performed by HPLC [28]. First, 100 mg of freeze-dried pomace powder was weighed into plastic test tubes and extracted with 80% methanol. After extraction, the samples were centrifuged (10 min, 6000 rpm, temp. 0 °C). Then, 100 µL of the supernatant was injected into a Fusion RP-80 column (250 × 4.6 mm, Phenomenex, Warsaw, Poland). The HPLC setup included two pumps (LC-20AD), an autosampler (SIL-20AC), a controller unit (CMB-20A), a UV-Vis detector (SPD-20AV), and a column oven (CTO-20AC; Shimadzu, USA Manufacturing Inc., Canby, OR, USA). The analysis parameters were as follows: column temperature 30 °C; flow rate of 1 mL min⁻¹; gradient phase (acetonitrile and water with phosphoric acid pH 3.0). The time phases were as follows: -1.00–22.99 min, phase A 95% and phase B 5%; 23.00–27.99 min, phase A 50% and phase B 50%; 28.00–28.99 min, phase A 80% and phase B 20%; 29.00–38.00 min, phase A 50% and phase B 50%; finally, 39.00–42.00 min, phase A 95% and phase B 5%; analysis time: 42 min. The detection wavelength was 360 nm for flavonoids and 250 nm for phenolic acids. Polyphenols were identified on the basis of retention time and external standards, and concentrations were expressed as mg g⁻¹ dry weight.

2.8. Anthocyanins Determination by HPLC

The first step of anthocyanin analysis was the same as that for polyphenols, as anthocyanins are a fraction of polyphenols. Identification and quantification of anthocyanins were performed by HPLC [28]. The second step of anthocyanin extraction was as follows: 2.5 mL of centrifuged supernatant was mixed with 2.5 mL of 10 mol hydrochloric acid and 5 mL of pure methanol (100%) in a test tube. The samples were mixed up-and-down several times and placed in a refrigerator for 10 min (5 °C). Then, 100 µL of the supernatant was injected into a Fusion RP-80 column (250 × 4.6 mm, Phenomenex, Warsaw, Poland). The HPLC setup included two pumps (LC-20AD), an autosampler (SIL-20AC), a controller unit (CMB-20A), a UV-Vis detector (SPD-20AV), and a column oven (CTO-20AC; Shimadzu, USA Manufacturing Inc., Canby, OR, USA). The analysis parameters were as follows: flow rate of 1 mL min⁻¹; an isocratic phase (5% acetic acid, acetonitrile, methanol (70:10:20)) at a flow rate of 0.5 mL min⁻¹; analysis time 18 min; and analysis temperature 30 °C. The detection wavelength for anthocyanins was 530 nm. Anthocyanins were identified on the basis of retention time and external standards, and concentrations were expressed as mg g⁻¹ dry weight [29].

2.9. Organic Acids and Sugars Determination by HPLC

The content of organic acids and sugars was determined by HPLC [30]. Briefly, 100 mg of sample was weighed into plastic test tubes with 5 mL of deionized water. The samples were extracted in an ultrasonic bath (30 °C, 25 min, 5.5 kHz), then centrifuged for 10 min at 6000 rpm. Then, 1.0 mL of each sample was filtered through 0.22 µm PES syringe filters for HPLC vials. Standard curves were prepared and identified in the chromatogram on the basis of the retention time of the standard compounds. The parameters for the HPLC analysis were as follows: isocratic phase with 10 Mm sulfuric acid and flow rate 0.5 mL/min; injection volume of 25 µL; column used, Aminex HPX-87H (300 × 7.8 mm, 9 µm, 8% cross linkage, pH 1–3); analysis time, 1 h 15 min; detection, 210 nm; and analysis temperature, 40 °C. An SPD-20AV detector was used for organic acids, and an RID/SPD-M20A detector was used for sugars. Organic acids and sugars were expressed in mg g⁻¹ dry weight.
2.10. Analysis of Volatile Compounds

VOCs were analyzed with the Heracles II electronic nose gas chromatograph (Alpha M.O.S., Toulouse, France). The volatile compounds of blackberry pomace were analyzed according to the method of Wojtasik-Kalinowska et al. [31]. Briefly, 3 g of blackberry pomace was placed in a glass vial (20 mL) and capped with a Teflon-faced silicone-rubber cap. All samples were placed in the Heracles II electronic nose automated sampler. Each sample was incubated at 40 °C for 20 min under agitation at 500 rpm. The carrier gas—hydrogen—circulated at a flow rate of 1 mL min⁻¹. The accumulated gas in the headspace was then injected into a GC with 10 m length, 0.18 mm internal diameter, and two columns with different polarities (non-polar MXT-5 (5% diphenyl) and semi-polar MXT-1701 (14% cyanopropylphenyl)), with two flame ionization detectors (FID). The analysis parameters were as follows: injected volume, 2500 µL; injector temperature, 200 °C; temperature of the two flame ionization detectors, 260 °C. The injection was carried out in three replicates. The method was calibrated with an alkane solution (n-butane to n-hexadecane) to convert the retention time into Kovats indices and to identify the volatile compounds using the AroChemBase.

2.11. Statistical Analysis

Data are expressed as means ± standard deviations for at least three independent measurements. Statistical analyses were performed using one-way analysis of variance (ANOVA). Fisher’s test was applied to assess significant differences (p < 0.05) among the samples. In order to evaluate the influence of cultivar on the aroma profile of blackberry pomace, a principal component analysis (PCA) was performed using the Alpha M.O.S. Heracles II device (Alpha MOS, Toulouse, France).

3. Results

3.1. Total Polyphenol Levels, Flavonoid Concentrations, and Antioxidant Activities (DPPH• and ABTS•⁺) in Blackberry Pomace

Table 1 summarizes the total amounts of polyphenols and flavonoids, as well as the level of antioxidant activity for the pomace from each blackberry cultivar.

<table>
<thead>
<tr>
<th>Blackberry Cultivar</th>
<th>TPC mg 100 g⁻¹ DW</th>
<th>TFC mg 100 g⁻¹ DW</th>
<th>DPPH• µmol TE/g</th>
<th>ABTS•⁺ µmol TE/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Polar’</td>
<td>2380.60 ± 15.10 a</td>
<td>161.29 ± 1.80 a</td>
<td>223.96 ± 5.30 ab</td>
<td>343.78 ± 11.20 c</td>
</tr>
<tr>
<td>‘Orkan’</td>
<td>2347.20 ± 19.20 b</td>
<td>154.25 ± 4.30 b</td>
<td>227.36 ± 9.10 a</td>
<td>373.44 ± 6.30 b</td>
</tr>
<tr>
<td>‘Brezezina’</td>
<td>2088.00 ± 10.30 c</td>
<td>148.10 ± 0.90 c</td>
<td>220.59 ± 7.40 b</td>
<td>390.81 ± 9.30 a</td>
</tr>
</tbody>
</table>

* Values expressed as mean ± standard deviation (SD). In each column, different letters indicate statistically significant differences (p < 0.05).

The research results indicate that the total polyphenol content in blackberry pomace varied by 292.60 mg 100 g⁻¹, depending on the cultivar, while the total flavonoid content varied by 13.19 mg 100 g⁻¹ DW. These data suggest that, under the same pre- and post-harvest conditions, blackberry genetic factors had a significant influence on the polyphenol content of the pomace. According to Albert et al. [27], the total levels of phenolic compounds in blackberry pomace can vary depending on cultivar, climatic conditions, soil fertility, and harvest time. The amounts of polyphenols and flavonoids obtained in this study are consistent with the findings of other researchers. According to Anna Maria Blejan et al. [32], the total phenol content of blackberry pomace was 14.4 mg GAE g⁻¹ DW, while the flavonoid content was 8.55 mg QE g⁻¹ DW. Miodrag Jazic et al. [33] reported that blackberry pomace from the Chester Thornless cultivar contained 35.40 mg GAE g⁻¹ fresh pomace DW of total phenols and 5.66 mg Q equivalent g⁻¹ fresh pomace DW of total flavonoid, while pomace from the Čačak Thornless cultivar contained 26.30 mg GAE g⁻¹ and 3.32 mg Q equivalent g⁻¹ fresh pomace DW. However, wild blackberry pomace contained high levels...
of total phenols, the content of which varied from 48.28 to 50.16 mg GAE g$^{-1}$ fresh pomace DW, and the content of total flavonoids varied from 7.45 to 7.73 mg Q equivalent g$^{-1}$ fresh pomace DW.

Meanwhile, Paun et al. [34] reported that blackberries contain 2583 mg 100 g$^{-1}$ of total polyphenols, while Albert et al. [27] found 2143 mg 100 g$^{-1}$. According to Pinto et al. [9], the total phenol content of ‘Loch Ness’ cultivar blackberries was 2165 mg 100 g$^{-1}$ and that of ‘Chester Thornless’ blackberries was 1828 mg 100 g$^{-1}$. Albert et al. [27] found that the total flavonoid content in blackberries was very similar to our results for pomace, at 163.01 mg 100 g$^{-1}$ DW. However, the concentration of phenols in pomace is quite similar to their concentration in the berries.

According to Zafra-Rojas et al. [35], blackberry pomace has high biological potential due to its high total polyphenol and flavonoid content, making it suitable for use as a value-added functional food. Due to the complexity of bioactive compounds in food, it is common to use a mix of analytical methods to measure the antioxidant capacity of food samples by several methods [36]. Antioxidant activity may depend on the extraction solvent, the hydrophilicity of the compounds, the sample, and the type of phenolic compounds, with different phenolic compounds responding differently in these assays [37].

Phenolic compounds are among the most easily reactive with DPPH• [38]. However, the ABTS$^{•+}$ assay is more sensitive for identifying antioxidant activity due to its faster reaction kinetics and greater response to antioxidants [39]. Therefore, the antioxidant capacity of pomace from three different blackberry cultivars was determined using both the DPPH$^{•}$ and ABTS$^{•+}$ assays. The antioxidant activity determined by the ABTS$^{•+}$ assay ranged from 343.78 µmol TE/g to 390.81 µmol TE/g, which was significantly higher than that determined using the DPPH$^{•}$ assay. The pomace of the ‘Orkan’ cultivar showed the highest radical-scavenging activity, at 227.36 ± 5.3 µmol TE/g, whereas the ‘Brrezina’ cultivar showed the lowest activity, at 220.59 ± 7.4 µmol TE/g according to the DPPH$^{•}$ assay. The pomace of the ‘Brrezina’ cultivar has the lowest TPC and TFC contents, at 2088.0 and 148.10 mg 100 g$^{-1}$ DW, respectively. This suggests that the lower polyphenol content in this cultivar results in lower antioxidant activity in the DPPH$^{•}$ but the highest activity in the ABTS$^{•+}$ assay. According to Van Hoed et al. [6], the presence of lipids in the seeds may also affect the overall antioxidant activity of the blackberry pomace, which consists of both seeds and skins. In Mexican blackberry pomace, the DPPH$^{•}$ antioxidant activity was 136.56 ± 53.2 µmol TE/g [34]. However, results can vary widely; for instance, Kalusevic et al. [40] found that the antioxidant activity of blackberry pomace, as assessed by the DPPH$^{•}$ radical-scavenging activity assay, was 10.9 µmol TE/g. Paun et al. [34] reported similar antioxidant-capacity values for ABTS$^{•+}$ in blackberries, ranging from 264.27 to 189.30 µmol TE/g. Pinto et al. [9] reported ABTS$^{•+}$ values of 222 µmol TE/g for the cultivar ‘Loch Ness’ and 92 µmol TE/g for the cultivar ‘Chester Thornless’. Polyphenols are generally considered to be excellent antioxidants and effective DPPH$^{•}$ and ABTS$^{•+}$ quenchers [4].

### 3.2. Phenolics Profile in Blackberry Pomace

The content of individual groups of phenolic compounds in blackberry pomace from different cultivars varies significantly. According to our findings, anthocyanins represent the main group of phenolic compounds in blackberry pomace, while flavonoids represent the second-most-abundant group (Figure 1).

These findings are in the line with those of Gil-Martínez et al. [41], wherein the predominant anthocyanin in fresh blackberries was cyanidin-3-O-glucoside, which was present in 1635.15 µg g$^{-1}$ DW. Statistically significant amounts of anthocyanins were highest in the ‘Orkan’ cultivar pomace, and the lowest amounts were present in the ‘Brrezina’ cultivar pomace. Studies by other researchers have demonstrated that blackberry waste is an important sources of natural colorants and nutraceuticals due to its high anthocyanin content [22]. Regarding flavonoids, the highest amount was detected in ‘Brrezina’ and ‘Polar’, while the lowest was detected in ‘Orkan’. The highest content of hydroxybenzoic
acids was detected in blackberry pomace from the ‘Polar’ cultivar, while the lowest content was found in ‘Brzezina’; only the amount of hydroxycinnamic acids in blackberry pomace was not statistically different among different cultivars. Hydroxycinnamic acid compounds occur as esters with hydroxycarboxylic acids or glucose, while hydroxybenzoic acid compounds occur most commonly as glucosides [42]. The results show that blackberry pomace, regardless of cultivar, contains lower amounts of phenolic acids than of other phenol groups. According to other researchers, blackberries are a rich source of polyphenols, containing 7–64 mg of phenolic acids per 100 g–1 of dry fruits [4,43].

![Figure 1. Groups of decomposed phenolic compounds in pomace from different blackberry cultivars, mg g–1 DW. Different letters indicate statistically significant differences (p < 0.05).](image)

Fourteen polyphenols were identified in samples of blackberry pomace from three different cultivars (Table 2). Cultivar had a significant effect on the phenol content of blackberry pomace. Among the cultivars, ‘Brzezina’ pomace exhibited the highest content of polyphenols, specifically of kaempferol-3-O-glucoside, myricetin, and ellagic acid, with concentrations of 0.26, 0.02, and 0.30 mg g–1, respectively. The anthocyanins cyanidin 3-O-glucoside and cyanidin-3-O-rutinoside and other compounds such as catechin, chlorogenic acid, p-coumaric acid, quercetin-3-O-rutinoside, kaempferol, and quercetin-3-O-glucoside were found in higher concentrations in the pomace of cultivar ‘Orkan’ than in the pomace of other cultivars, with values of 9.79, 8.23, 0.48, 0.30, 1.17, 0.30, 0.05, and 0.03 mg g–1, respectively. The pomace of the blackberry cultivar ‘Polar’ showed the highest content of epigallocatechin gallate. It also had the highest overall total phenolic content (TPC) and the highest total flavonoid content (TFC), with values of 2380.6 and 161.29 mg 100 g–1, respectively (Tables 1 and 2). Epigallocatechin and p-coumaric acid were quantified in the highest concentrations, with that of epigallocatechin ranging from 9.72 mg g–1 in ‘Orkan’ cultivar pomace to 7.28 mg g–1 in ‘Polar’ pomace and that of p-coumaric acid ranging from 1.17 mg g–1 in ‘Polar’ cultivar pomace to 1.05 mg g–1 in ‘Orkan’ pomace. These were the predominant phenolic compounds found in the blackberry pomace samples. According to numerous researchers, the main phenolic acid in blackberry pomace is p-coumaric acid, which has the potential to protect against free-radical damage [44,45]. According to Blejan et al. [32], the major phenolic compounds in blackberry pomace were epigallocatechin (22.70 mg g–1), chlorogenic acid (1.89 mg g–1), and catechin (0.97 mg g–1). Catechins are flavonoids belonging to the flavan-3-ols group and include (–)-epigallocatechin gallate, (–)-epicatechin gallate, (+)-epicatechin, and (–)-epicatechin. Epigallocatechin gallate, an ester of epigallocatechin and gallic acid, is the main biologically active compound in green tea. It is classified as having very strong antioxidant activity and versatile cellular and molecular health-promoting effects [46]. According to Masek et al. [47], epigallocatechin gallate with pyrogallol-type groups has a
higher antioxidant-scavenging capacity than epigallocatechin with catechol-type groups. In addition to its well-known antioxidant properties, epigallocatechin gallate exhibits pro-oxidant effects that may be either beneficial or harmful to human health, depending on the specific biological context [48]. The amount of quercetin was consistent across all cultivar pomaces and amounted to 0.01 mg g\(^{-1}\).

Table 2. Phenolic profiles of blackberry pomace, mg g\(^{-1}\) dw.

<table>
<thead>
<tr>
<th>Polyphenols/Cultivar</th>
<th>'Polar'</th>
<th>'Orkan'</th>
<th>'Brzezina'</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flavonoids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epigallocatechin</td>
<td>9.72 ± 0.40 a</td>
<td>7.28 ± 0.58 b</td>
<td>9.60 ± 0.24 a</td>
</tr>
<tr>
<td>Catechin</td>
<td>0.33 ± 0.01 c</td>
<td>0.48 ± 0.04 a</td>
<td>0.42 ± 0.01 b</td>
</tr>
<tr>
<td>Epigallocatechin gallate</td>
<td>0.13 ± 0.02 a</td>
<td>0.12 ± 0.02 a</td>
<td>0.13 ± 0.00 a</td>
</tr>
<tr>
<td>Quercetin-3-O-rutinoside</td>
<td>0.26 ± 0.01 b</td>
<td>0.30 ± 0.01 a</td>
<td>0.25 ± 0.01 b</td>
</tr>
<tr>
<td>Kaempferol-3-O-glucoside</td>
<td>0.10 ± 0.01 b</td>
<td>0.04 ± 0.01 c</td>
<td>0.26 ± 0.01 a</td>
</tr>
<tr>
<td>Myricetin</td>
<td>0.01 ± 0.00 b</td>
<td>0.01 ± 0.00 b</td>
<td>0.02 ± 0.00 a</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.01 ± 0.00 a</td>
<td>0.01 ± 0.00 a</td>
<td>0.01 ± 0.00 a</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>0.03 ± 0.00 b</td>
<td>0.05 ± 0.01 a</td>
<td>0.05 ± 0.00 a</td>
</tr>
<tr>
<td>Quercetin-3-O-glucoside</td>
<td>0.02 ± 0.00 b</td>
<td>0.03 ± 0.00 a</td>
<td>0.02 ± 0.00 b</td>
</tr>
<tr>
<td><strong>Phenolic acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>0.27 ± 0.01 b</td>
<td>0.30 ± 0.01 a</td>
<td>0.26 ± 0.01 b</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>0.28 ± 0.01 b</td>
<td>0.16 ± 0.00 c</td>
<td>0.30 ± 0.00 a</td>
</tr>
<tr>
<td>p-coumaric acid</td>
<td>1.05 ± 0.04 a</td>
<td>1.17 ± 1.14 a</td>
<td>1.16 ± 0.02 a</td>
</tr>
<tr>
<td><strong>Anthocyanins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyanidin-3-O-glucoside</td>
<td>8.93 ± 0.02 b</td>
<td>9.79 ± 0.05 a</td>
<td>6.19 ± 0.02 c</td>
</tr>
<tr>
<td>Cyanidin-3-O-rutinoside</td>
<td>7.65 ± 0.03 b</td>
<td>8.23 ± 0.03 a</td>
<td>5.33 ± 0.08 c</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard deviation (SD). In each row, different letters indicate statistically significant differences (\(p < 0.05\)).

Schulz et al. [49] found that quercetin is one of the major compounds in blackberries (\(R. \text{ fruticosus}\)). In our study, the myricetin content ranged from 0.01 mg g\(^{-1}\) in the pomace of the cultivars ‘Orkan’ and ‘Polar’ to 0.02 mg g\(^{-1}\) in the pomace of the cultivar ‘Brzezina’. According to Jacques et al. [30], gallic acid, (-)-epicatechin, ferulic acid, and quercetin are the main phenolic compounds found in mature blackberry fruit. According to Akin et al. [14], blackberries contain 0.082 mg g\(^{-1}\) of catechin, 0.141 mg g\(^{-1}\) of quercetin, 2.324 mg g\(^{-1}\) chlorogenic acid, and 0.098 mg g\(^{-1}\) \(p\)-coumaric acid. Li et al. [51] identified the two major anthocyanins in blackberries as cyanidin-3-O-glucoside and cyanidin-3-O-sophoroside. In our study, we also found that cyanidin-3-O-glucoside was the predominant anthocyanin, with its content ranging from 9.79 to 6.19 mg g\(^{-1}\) depending on the cultivar (Table 3). Other researchers have identified two major anthocyanins in blackberries: cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside [52]. This is in agreement with our findings. According to Vega et al. [53], blackberries contain cyanidin-3-O-glucoside, cyanidin-3-O-arabinoside, and cyanidin-3-O-galactoside, while malvidin-3-O-glucoside, pelargonidin-3-O-glucoside, cyanidin-3-O-xylloside, cyanidin-3-O-rutinoside, and cyanidin-3-O-malonylg glucoside are present in smaller amounts [53]. According to Petruskevicius et al. [54], cyanidin-3-O-glucoside accounts for 90.72% of the total anthocyanin content in blackberries. Other researchers found that cyanidin-3-O-glucoside accounted for 92.76% of the total anthocyanins in blackberries [55]. Anthocyanins are the main compounds responsible for the color of blackberry pomace. Previous research shows that the pomace of the ‘Orkan’ cultivar was the lightest (\(L^* = 33.87\)) and the reddest (\(a^* = 28.96\)) and had the highest chroma (\(C^*\)) value—30.49 [56]—which could be due to higher concentrations of the individual anthocyanins cyanidin-3-O-glucoside (9.79 mg g\(^{-1}\)), and cyanidin-3-O-rutinoside (8.23 mg g\(^{-1}\)) in the pomace of this cultivar. By contrast, the lowest content of individual anthocyanins was found in the ‘Brzezina’ cultivar pomace: cyanidin-3-O-glucoside (6.19 mg g\(^{-1}\)), and cyanidin-3-O-rutinoside (5.33 mg g\(^{-1}\)) ; additionally, the chroma (\(C^*\)) value was the lowest—26.95 [56]. According to Jara-Palacios et al. [10], higher \(C^*\) values depend on higher concentrations of anthocyanins.
Table 3. Content of organic acids and sugars in blackberry pomace, mg g\(^{-1}\) dw.

<table>
<thead>
<tr>
<th></th>
<th>‘Polar’</th>
<th>‘Orkan’</th>
<th>‘Brzezina’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehydroascorbic acid</td>
<td>107.92 ± 3.15 c</td>
<td>174.58 ± 0.63 a</td>
<td>118.34 ± 0.91 b</td>
</tr>
<tr>
<td>L-ascorbic acid</td>
<td>2.38 ± 0.12 c</td>
<td>4.80 ± 0.05 a</td>
<td>3.40 ± 0.02 b</td>
</tr>
<tr>
<td>Citric acid</td>
<td>33.66 ± 1.14 c</td>
<td>47.98 ± 0.08 b</td>
<td>30.14 ± 0.17 a</td>
</tr>
<tr>
<td>Malic acid</td>
<td>17.94 ± 0.68 c</td>
<td>28.99 ± 0.27 b</td>
<td>13.28 ± 1.42 b</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>8.89 ± 0.32 c</td>
<td>16.96 ± 0.98 a</td>
<td>0.38 ± 0.05 b</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>0.17 ± 0.04 c</td>
<td>0.52 ± 0.03 a</td>
<td></td>
</tr>
<tr>
<td>Sugars</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>84.57 ± 1.04 c</td>
<td>126.31 ± 0.84 b</td>
<td>147.66 ± 0.51 a</td>
</tr>
<tr>
<td>Fructose</td>
<td>77.13 ± 0.83 c</td>
<td>117.78 ± 2.53 b</td>
<td>145.23 ± 1.37 a</td>
</tr>
</tbody>
</table>

Different letters indicate statistically significant differences (\(p < 0.05\)).

3.3. Organic Acids and Sugars in Blackberry Pomace

Organic acids and sugars are the main components that determine the taste of fruits and berries [57]. This is crucial not only for the berries themselves, but also for extracts and pomace, as these compounds influence the potential for agro-food-waste utilization. The content of these compounds in blackberries varies with cultivar [16], as well as with climatic conditions and agricultural practices. The highest total amount of organic acids was detected in ‘Orkan’ cultivar pomace with 273.83 mg g\(^{-1}\), while the highest amount of sugars was found in ‘Brzezina’ cultivar pomace, at 292.89 mg g\(^{-1}\) (Table 3).

According to Zafra-Rojas et al. [35], blackberry residues contain 0.59 mg g\(^{-1}\) oxalic acid, 57.06 mg g\(^{-1}\) malic acid, 1.25 mg g\(^{-1}\) citric acid, 2.30 mg g\(^{-1}\) fumaric acid, and 0.06 mg g\(^{-1}\) ascorbic acid, which differs from our findings. We detected lower quantities of malic acid but a higher amount of ascorbic acid. According to Akin et al. [14], wild blackberries contain 9.10 mg g\(^{-1}\) of malic acid. In our research, malic acid concentrations varied from 30.14 mg g\(^{-1}\) in cultivar ‘Brzezina’ to 17.94 mg g\(^{-1}\) in cultivar ‘Polar’, while L-ascorbic acid concentrations varied from 4.80 mg g\(^{-1}\) in cultivar ‘Orkan’ to 2.38 mg g\(^{-1}\) in cultivar ‘Polar’ pomace. Environmental factors and cultivation practices may influence the amounts of these organic acids in berries [58]. According to Akamatsu et al. [59], water-deficit stress can increase the concentrations of citric and malic acids in the fruits and berries. The total amount of vitamin C, considered as the sum of the concentrations of ascorbic acid and dehydroascorbic acid, was higher in the pomace of ‘Orkan’ cultivar, reaching 179.38 mg g\(^{-1}\). Ascorbic acid is the major biologically active form of vitamin C and can be reversibly oxidized into dehydroascorbic acid [60]. It has been found that ascorbic acid is present in foods in much higher proportions than is dehydroascorbic acid; however, there have been data presented showing that up to 43% of the total amount of vitamin C is composed of dehydroascorbic acid [61–63]. According to the results, dehydroascorbic acid was detected as the main organic acid in blackberry pomaces, with concentrations ranging from 174.58 mg g\(^{-1}\) in pomace from the ‘Orkan’ cultivar to 107.92 mg g\(^{-1}\) in pomace from the ‘Polar’ cultivar. This form of vitamin C is therefore predominant in blackberry pomace, regardless of cultivar. Other researchers [17] have not considered both ascorbic acid and dehydroascorbic acid contents when reporting the vitamin C content of blackberry pomace.

The sugar profiles of fruits and berries consist mainly of fructose, glucose, and sucrose [64]. Our research results show that the glucose content in blackberry pomace varied from 147.66 mg g\(^{-1}\) dw in the ‘Brzezina’ cultivar to 84.57 mg g\(^{-1}\) dw in the ‘Polar’ cultivar blackberry pomace. Similarly, the fructose content varied from 145.23 mg g\(^{-1}\) dw in the ‘Brzezina’ cultivar pomace to 77.13 mg g\(^{-1}\) dw in the ‘Polar’ cultivar pomace. Based on previous research on blackberry pomace [56], a relationship between total phenols and sugar content can be observed. The pomace of cultivar ‘Brzezina’ has the lowest TPC, but the highest sugar content, while pomace of cultivar ‘Polar’ has the highest amount of TPC and the lowest sugar content. According to Granucci et al. [65], apple, orange, and carrot pomace can be broadly described as high in reducing sugar, but low in phenolic...
compounds. According to Benvenutti et al. [66], sugars in apple pomace can influence the bioavailability and stability of phenolic compounds.

The sugar content in blackberry berries, and pomace varies. According to Milivojevic et al. [15], blackberry cultivars ‘Thornfree’ and ‘Čačanska bezstra’ contain 67.7 and 66.8 mg g\(^{-1}\) (fresh weight) of glucose and 88.1 and 86.0 (fresh weight) of fructose, respectively. Akin et al. [14] reported that the fructose content of wild blackberries was 161.8 mg g\(^{-1}\), while the glucose content was 85.07 mg g\(^{-1}\). However, the pomace of cultivated blackberries contains more glucose (Table 3).

3.4. VOCs Analysis

The volatile compounds responsible for fruit flavor are biosynthesized by metabolic pathways during ripening. Their production is influenced by genetic makeup; harvest post-harvest, and storage conditions; and cultivation factors such as climate, soil and fertilization. These compounds depend on many factors related to the species, cultivar, and type of technological treatment [18,19]. The aroma of blackberries is one of the most important characteristics. The volatile organic compounds identified in blackberry pomace are important flavor components, including alcohols (alcoholic, floral, fruity, green), aldehydes (green, fruity, vegetal), ketones (floral, fruity), esters (floral, fruity, sweet), and terpenoids (citrus, pine, terpene-like). Blackberry flavor is mainly formed during a short ripening period [19,67]. According to Padilla-Jimenez et al. [20], the compound cis-3-hexen-1-ol in blackberries is associated with the herbaceous and citrusy aroma, while ethyl hexanoate and 2-Heptanol are associated with a bitter, fruity aroma. However, 2-Heptanol was found only in the pomace of the ‘Brzezina’ cultivar (Table 4). Wajs-Bonikowska et al. [4] also determined that the most important flavor-forming compound in blackberries is 2-Heptanol, which is associated with fruity–herbaceous and flowery–spicy scents. These researchers findings are consistent with ours, as we also identified the same compounds in blackberry pomace, including cis-3-Hexen-1-ol, heptanal, decanal, and 1-Hexanol. Iso-amyl acetate was determined in pomace from the ‘Orkan’ and ‘Polar’ cultivars. According to the researchers, this compound is an oily liquid and is found exclusively in the blackberry seeds [20].

Table 4. Tentative list of volatile organic compounds in blackberry pomace.
According to Turemis et al. [18], the most abundant aromatic compound found in fresh blackberries was 5-hydroxyethylfurfural (C₅H₈O₅), which accounted for 79.7–96.1% of the total aroma profile depending on the cultivar. In our samples, we found this same compound, which is also called maltol; it is naturally produced in sugar-containing foods [18].

Differences in the volatile composition of blackberry pomace samples were observed (Figure 2). The ‘Orkan’ cultivar was situated at the negative PC2 values (green color), ‘Brzezina’ samples were situated at the positive PC2 and negative PC1 values (blue color), whereas ‘Polar’ cultivar samples were situated at the positive and negative PC1 values (red color).

![Figure 2. Principal component analysis (PCA) for organic volatile compounds in blackberry pomace. Circled in red, ‘Polar’ cultivar samples (4,5,6); circled in green, ‘Orkan’ cultivar samples (7,8,9); circled in blue, ‘Brzezina’ cultivar samples (1,2,3).](image)

4. Conclusions

Cultivar had a significant effect on the total phenol and total flavonoid contents and the antioxidant activity of blackberry pomace. The data reveals that all cultivars of blackberry pomace contain high levels of bioactive compounds, particularly of anthocyanins and flavonoids, which have potent antioxidant properties. The main significant differences in phenolic content among blackberry pomace from different cultivars were observed between the anthocyanin and flavonoid groups. Epigallocatechin was found to be the predominant polyphenol with strong antioxidant activity in blackberry pomace. The cultivars ‘Polar’ and ‘Orkan’, which were characterized by elevated anthocyanin pigment levels, have potential applications as natural food colorants, providing a sustainable alternative to synthetic dyes. Fructose and glucose were the two most abundant sugars, while dehydroascorbic acid, citric acid, and L-ascorbic acid were the most abundant organic acids in blackberry pomace. The highest amounts of these compounds were found in pomace of the ‘Orkan’ and ‘Brzezina’ cultivars. All VOCs detected in the pomace were typical blackberry-like compounds, which is important, as there is a lack of literature on which VOCs remain in the pomace. These results confirm that blackberry pomace is a promising source of high-quality bioactive compounds with demonstrated radical-scavenging capabilities.

Author Contributions: Conceptualization, Ž.T. and I.Č.; methodology, Ž.T., E.H. and J.V.; software, I.Č.; validation, I.Č. and E.H.; formal analysis, I.Č. and M.K.; investigation, I.Č. and P.V.; writing—
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