Potential of sweet cherry (Prunus avium L.) by-products: bioactive compounds and antioxidant activity of leaves and petioles

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Abstract
In recent years many researchers have taken into account other parts of plants than commonly edible ones because of their beneficial chemical composition. The objective of the study was to determine the content of bioactive compounds, including HPLC analysis of polyphenols, and antioxidant activity of leaves, petioles, and fruit of selected cultivars of the sweet cherry. Cultivars Kordia, Regina, Vega, Hedelfińska and Vanda from Sandomierz (Poland), Kordia, Regina, and Summit from Szczodrkowice (Poland) as well as sweet cherries imported from Spain and Hungary (only petioles and fruit) were analyzed. Statistically significant effect of cultivar and part of the plant on the bioactive compounds content and antioxidant activity was found. The leaves and petioles had a higher concentration of dietary fiber, vitamin C, carotenoids and polyphenols as well as an antioxidant activity than the fruit. The fruit was characterized by the presence of total anthocyanins. In the studied samples, the following polyphenols were identified: coffee acid, chlorogenic acid, p-coumaric acid, and myricetin. Additionally, the ferulic acid was detected in leaves. Due to the high antioxidants level, the leaves and petioles can be a potential source to produce functional food. Further studies are needed to prove processability and usefulness of this plant material in the food industry.

Keywords Sweet cherry · Petioles · Leaves · Polyphenols · Antioxidant activity · Chlorogenic acid

Introduction
Polyphenols are a large group of secondary metabolites of plants with a wide spectrum of biological activities. They are a subject of many studies because of their possible beneficial effects on human health. They exhibit antioxidant activity. Polyphenols are capable of scavenging free radicals, chelating of metal ions (irons, copper, etc.) as well as inhibiting the enzymes which are involved in the reactive oxygen species production [1, 2]. Anti-inflammatory mechanisms of polyphenols are based on inhibition of pro-inflammatory enzymes and induction of the nitric oxide synthase (iNOS), inhibition of the nuclear factor-kappa B (NF-κB) and the activating protein-1 (AP-1), activation of phase-II antioxidant detoxifying enzymes, mitogen activated protein kinase (MAPK) and protein kinase-C [3]. Numerous in vitro and in vivo studies indicated the anticancer activity of polyphenolic compounds. They are considered to be chemopreventive agents based on their inhibitory effects on tumor initiation, promotion, and progression [4]. The oxidative stress and chronic inflammation are closely linked to carcinogenesis process and therefore antioxidant and anti-inflammatory activities of polyphenols may potentially protect and fight against cancer [5]. Because of above-mentioned biological activities, polyphenols consumption can result in a reduced risk of a number of chronic diseases, including cardiovascular disease, obesity, diabetes, cancer, and neurodegenerative disorders [6].

Commonly consumed fruits and vegetables are main sources of polyphenols in a diet [7]. However, other parts of plants (i.e., leaves) were used in traditional medicine as a treatment of cold, inflammations and diabetes [8]. Recently, many researchers have taken into account shrubs and fruit trees leaves because of their phytochemical composition. Teleszko and Wojdyło [9], Tabart et al.
leaves, and comparing composition with fruit.

The antioxidant activity of sweet cherry petioles and compounds, especially polyphenols, as well as examination of the bioactive compounds in the petioles and leaves of sweet cherry (Prunus avium L.) have been already published. It may be caused by the fact that these parts of sweet cherry are not popular and they do not have commercial applications [15]. The objective of this study was determination and identification of bioactive compounds, especially polyphenols, as well as examination of the antioxidant activity of sweet cherry petioles and leaves, and comparing composition with fruit.

**Plant material**

The following parts of sweet cherry (Prunus avium L.) were analyzed: leaves, petioles and fruit. Samples were collected in June and July 2016. The studies included the cultivars Kordia, Regina, Vega, Hedelfińska and Vanda from Sandomierz (Poland), cultivars Kordia, Regina and Summit from Szczodrkowice (Poland) as well as sweet cherries (the cultivars are unknown) which were imported from Spain and Hungary (only petioles and fruit). All parts of Polish sweet cherry were randomly picked at commercial maturity of fruit. Immediately after collection, they were transported to the laboratory. The fresh material was used to determine the content of vitamin C as well as to prepare the alcoholic extracts. From the remaining material, three samples were taken for each cultivar. All sample included 50 randomly selected leaves, petioles, and fruits. The samples were fragmented (additionally fruits were pitted) and frozen on plastic plates at −80 °C. Then the leaves, petioles and fruit were lyophilized using freeze dryer Christ Alpha 1–4 (Osterode am Harz, Germany). The lyophilized samples were stored in tightly closed plastic bags at room temperature until analysis.

**Materials and methods**

**Reagents**

All of the chemicals used in the study were of analytical grade. Potassium persulfate, hydrochloric acid, sodium hydroxide, ascorbic acid, ferric chloride hexahydrate, oxalic acid, acetic acid, sulfuric acid, sodium sulfide, mercury chloride, acetone and n-hexane were obtained from Chempur (Piekary Śląskie, Poland), methanol (also for HPLC) from POCh (Katowice, Poland), chlorogenic acid, Folin–Ciocalteu reagent, ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)], DPPH (2,2-diphenyl-1-picrylhydrazyl) and TPTZ [2,4,6-tris(2-pyridyl)-s-triazine] from Sigma-Aldrich (Saint Louis, MO, USA).

According to the available literature, little data on content and identification of bioactive compounds in the petioles and leaves of sweet cherry (Prunus avium L.) have been already published. It may be caused by the fact that these parts of sweet cherry are not popular and they do not have commercial applications [15]. The objective of this study was determination and identification of the bioactive compounds, especially polyphenols, as well as examination of the antioxidant activity of sweet cherry petioles and leaves, and comparing composition with fruit.

**Determination of selected bioactive compounds**

The content of vitamin C was determined in fresh leaves, petioles and fruit and was defined as a sum of ascorbic and dehydroascorbic acids by Tillmans method, as modified by Pijanowski [16]. This assay is based on reduction of dehydroascorbic acid to ascorbic acid using sodium sulfide, and precipitation of sodium sulfate excess by mercury chloride. The last step is determination of total ascorbic acid by 2,6-dichlorophenolindophenol titration. The level of total dietary fiber was assessed in lyophilized material using commercially available kit (cat no. K-TDFR-100A, Megazyme International Ireland, Wicklow, Ireland). The concentration of total carotenoids was measured spectrophotometrically, according to the PN-EN 12136:2000 [17], by extracting these compounds from the lyophilized samples using acetone–hexane mixture (4:6 v/v), and measuring the absorbance at 450 nm (UV-1800, Rayleigh, Beijing Beifen-Ruili Analytical Instrument Co., Ltd., Beijing, China).

To determine the total polyphenols and anthocyanins contents as well as antioxidant activity, the extracts were prepared. One gram of fresh fruit and 0.5 g of fresh leaves and petioles were added to 80 ml of 70% methanol acidified HCl, shaken in a water bath shaker (Elpin Plus, type 357, Lubawa, Poland) at a room temperature for 2 h and centrifuged at 1500 rpm for 15 min. Supernatants were...
decanted and stored at –20 °C until further use. The level of total polyphenols was determined by spectrophotometric method using the Folin–Ciocalteu reagent as previously reported [18]. The obtained results were expressed as mg chlorogenic acid (CGA) per 100 g of sample. The content of total anthocyanins was also studied spectrophotometrically by the pH differential method, using potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M) [19]. The absorbance was measured at 510 nm and 700 nm (spectrophotometer UV-1800, Rayleigh, Beijing Beifen-Ruili Analytical Instrument Co., Ltd., Beijing, China).

HPLC analysis of polyphenols

HPLC analysis of polyphenols was conducted on leaves, petioles and fruits of four cultivars, which were characterized by the highest content of total polyphenols measured using the Folin–Ciocalteu reagent, i.e., Kordia, Regina, Vega and Hedelfińska from Sandomierz (Poland). To identify the polyphenolic compounds with HPLC the extracts were prepared according to Klimczak et al. [20] with some modifications. Lyophilized leaves, petioles, and fruits of sweet cherry were mixed with 1% ascorbic acid dissolved in HPLC grade methanol (w/v) using vortex (Labnet, Edison, NJ, USA). The samples were sonicated for 15 min at 20 °C with the ultrasonic bath. Next 2M NaOH was added. The specimens were mixed using vortex and held in darkness for 4 h at 20–22 °C. Then, the samples were neutralized to pH 2.1–2.6 using 2M HCl and transferred quantitatively to the volumetric flask using 1% ascorbic acid dissolved in HPLC methanol (w/v). Prepared samples were centrifuged at 18,000 rpm for 20 min (MPW—260R centrifuge, Warsaw, Poland) and filtered through the 153 PTFE-L filter with a pore diameter of 0.22 μm. The samples were stored at 4 °C until further use.

The HPLC analysis of polyphenols was conducted using HPLC DionexUltiMate 3000 system (Thermo Scientific, Germering, Germany), DAD detector (Thermo Scientific, Germering, Germany) and column 5C18—MS-II 250×4.6 mm ID, 5 μm (NacalaiTesque, INC, Kyoto, Japan). The mobile phase was a mixture of two eluents: 2% water solution of acetic acid (v/v) and 100% methanol. The flow rate of the mobile phase was 1 ml/min. The analysis took 50 min with the following conditions: 10 min 70%; 25 min 50%; 35 min 30%; 40 min 95%; 50 min 95% until the end of the analysis, with the following wavelengths: 254 nm, 280 nm, 320 nm, and 360 nm. The HPLC grade standard compounds were used for quantification.

Determination of antioxidant activity

Antioxidant activity was estimated using the ABTS [2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)], DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) assays according to the procedure reported by Dziadek et al. [21]. The results were calculated based on the calibration curve and expressed as micromoles of Trolox equivalent per gram of sample (TEAC).

Statistical analysis

The determination of bioactive compounds and antioxidant activity were carried out in triplicate as well as HPLC analysis was performed in quadruplicate. Results were expressed as the mean ± standard deviation (SD). Statistical analysis was carried out using Statistica software v. 13.1 (Tulsa, OK, USA). Multiple-way analysis of variance (MANOVA) was conducted. Two factors have been taken into account: cultivar and part of sweet cherry. The exception was a statistical analysis of total anthocyanins content in the fruit of sweet cherry and polyphenolic compounds level (measured by HPLC) in individual samples, which were analyzed by one-way ANOVA. Significant differences in the mean values were compared using Duncan’s test with α = 0.05.

Results

The leaves of sweet cherry were significantly the richest source of total dietary fiber, vitamin C and total carotenoids in comparison to petioles and fruit. Albeit in cultivars Kordia and Regina from Szczodrkowice, the significant differences in concentration of total dietary fiber between leaves and petioles were not found (Table 1). Moreover, in almost all of the studied petioles the largest amount of total polyphenols, in comparison to other parts of sweet cherry, was found. The fruit was characterized by the presence of anthocyanins, which were found only in this part of sweet cherry, as well as by the lowest content of dietary fiber, vitamin C, total carotenoids and total polyphenols.

The leaves of cultivars Regina, Vega, and Hedelfińska from Sandomierz as well as Kordia and Summit from Szczodrkowice had statistically significant the largest amount of total dietary fiber (Table 1). Among the petioles, the highest content of fiber was measured in those collected in Hungary, whereas among the fruit, in cultivar Hedelfińska.

The highest concentration of vitamin C was determined in leaves of cultivars Hedelfińska and Regina from Szczodrkowice compared to other ones (Table 1). Among the tested petioles, cultivars Hedelfińska, Summit and Kordia from Szczodrkowice were the richest in this compound. The obtained results indicated that the cultivar did not have a significant effect on vitamin C level in fruits of sweet cherry.

Among the leaves, the significantly largest amount of total carotenoids was found in cultivar Kordia from Szczodrkowice, among the petioles in cultivar Regina from
Sandomierz as well as among the fruit, in sweet cherry imported from Hungary, respectively (Table 1).

Among both leaves and petioles of sweet cherry, the highest content of total polyphenolic compounds was measured in the cultivar Kordia collected in Sandomierz, whereas among fruit, in the cultivars Kordia, Regina and Vanda from Sandomierz.

The significantly highest level of total anthocyanins was assessed in the fruit of cultivar Kordia harvested in Szczodrkowice and the lowest in cultivar Vega, respectively (Table 1).

### Table 1 Dietary fiber and selected bioactive compounds content in leaves, petioles and fruit of sweet cherry

| Place of harvesting | Cultivar | Part of sweet cherry | Total dietary fiber (g/100 g DW) | Vitamin C (mg/100 g DW) | Total carotenoids (mg/100 g DW) | Total polyphenols (mg/100 g DW) | Total anthocyanins (mg/100 g DW) |
|---------------------|----------|----------------------|-------------------------------|------------------------|-------------------------------|-------------------------------|-------------------------------|
| Sandomierz (Poland) | Kordia   | Leaves               | 42.60 ± 0.43 hi               | 328.21 ± 17.19 g       | 38.19 ± 0.21 t                | 15318.42 ± 166.53 l           | nd                            |
|                     |          | Petioles             | 40.67 ± 0.44 efg              | 109.00 ± 4.40 de       | 16.36 ± 0.02 o                | 3881.03 ± 435.16 t            | nd                            |
|                     |          | Fruit                | 5.21 ± 0.18 a                 | 39.59 ± 2.67 abc       | 1.54 ± 0.00 c                 | 3736.08 ± 145.04 de           | 505.70 ± 3.48 h               |
| Regina              | Leaves   |                      | 45.42 ± 1.39 k                | 475.74 ± 20.39 i       | 28.72 ± 0.12 r                | 9957.37 ± 588.97 j            | nd                            |
|                     | Petioles  |                      | 43.64 ± 0.60 ij               | 69.99 ± 3.41 abcd      | 18.32 ± 0.02 p                | 29997.32 ± 134.58 r           | nd                            |
|                     | Fruit    |                      | 5.88 ± 0.31 a                 | 35.33 ± 2.17 abc       | 1.41 ± 0.02 c                 | 4054.32 ± 223.34 e            | 252.12 ± 4.81 d               |
| Vega                | Leaves   |                      | 46.21 ± 1.15 k                | 324.57 ± 34.00 g       | 37.65 ± 0.23 s                | 14547.95 ± 316.52 k           | nd                            |
|                     | Petioles  |                      | 38.82 ± 0.25 d                | 81.29 ± 3.28 cd        | 8.24 ± 0.02 i                 | 24250.55 ± 14.33 o            | nd                            |
|                     | Fruit    |                      | 8.28 ± 0.34 b                 | 27.75 ± 2.07 a         | 2.22 ± 0.00 d                 | 29133.18 ± 155.24 c           | nd                            |
| Hedelfińska         | Leaves   |                      | 45.01 ± 0.70 jk               | 623.95 ± 40.11 k       | 40.82 ± 0.20 w                | 9454.94 ± 156.49 i            | nd                            |
|                     | Petioles  |                      | 41.74 ± 1.13 fghi             | 147.93 ± 4.65 ef       | 6.21 ± 0.03 h                 | 32309.37 ± 256.13 s           | nd                            |
|                     | Fruit    |                      | 10.23 ± 0.24 c                | 36.95 ± 3.07 abc       | 3.42 ± 0.05 f                 | 28777.20 ± 50.23 c            | 427.22 ± 2.81 f               |
| Vanda               | Leaves   |                      | 43.33 ± 1.56 hij              | 429.79 ± 30.91 h       | 44.76 ± 0.00 y                | 74264.45 ± 268.38 g           | nd                            |
|                     | Petioles  |                      | 40.06 ± 1.06 def              | 70.65 ± 3.45 abcd      | 3.39 ± 0.15 x                 | 22265.90 ± 53.93 m            | nd                            |
| Szczodrkowice (Poland) | Kordia   | Leaves               | 41.41 ± 1.39 jk               | 521.19 ± 19.92 j       | 54.90 ± 0.12 z                | 6011.69 ± 228.90 f            | nd                            |
|                     | Petioles  |                      | 43.33 ± 0.66 hij              | 123.02 ± 4.46 ef       | 13.43 ± 0.05 m                | 6074.64 ± 57.15 f             | nd                            |
|                     | Fruit    |                      | 5.18 ± 0.35 a                 | 42.63 ± 1.28 abc       | 1.18 ± 0.05 e                 | 2934.68 ± 37.06 c             | 683.80 ± 3.11 i               |
| Regina              | Leaves   |                      | 39.92 ± 0.06 de               | 641.32 ± 41.23 k       | 40.52 ± 0.22 u                | 7229.50 ± 105.77 g            | nd                            |
|                     | Petioles  |                      | 39.31 ± 0.96 de               | 61.34 ± 3.21 abc       | 13.23 ± 0.04 m                | 3347.26 ± 57.73 cd            | nd                            |
|                     | Fruit    |                      | 5.57 ± 0.46 a                 | 37.64 ± 2.80 abc       | 3.23 ± 0.01 ef                | 1604.17 ± 22.03 a             | 182.96 ± 4.93 c               |
| Summit              | Leaves   |                      | 45.68 ± 1.16 k                | 542.11 ± 32.39 j       | 41.86 ± 0.00 x                | 8994.22 ± 129.58 h            | nd                            |
|                     | Petioles  |                      | 42.34 ± 1.73 ghi              | 162.50 ± 48.75 f       | 12.71 ± 0.04 l                | 22879.88 ± 303.92 n           | nd                            |
| Spain Unknown       | Petioles  |                      | 43.37 ± 0.96 hij              | 78.29 ± 6.27 cd        | 9.13 ± 0.04 j                 | 29780.17 ± 424.80 r           | 516.57 ± 0.00 b               |
| Hungary Unknown     | Petioles  |                      | 48.24 ± 0.39 l                | 37.70 ± 3.77 bc        | 10.90 ± 0.25 k                | 25875.39 ± 124.55 p           | nd                            |
|                     | Fruit    |                      | 5.52 ± 0.12 a                 | 33.15 ± 0.00 abc       | 4.44 ± 0.07 g                 | 2312.32 ± 53.61 b             | 287.34 ± 4.50 e               |

Results are expressed as mean ± SD (n = 3)
Mean values with different letters (a–z) within the each column are statistically different (p < 0.05)

DW dry matter, nd not determined

In studied leaves, petioles and fruit the following polyphenolic compounds were found: coffee acid, chlorogenic acid, p-coumaric acid, and myricetin. Additionally, in leaves of sweet cherry, the ferulic acid was identified (Table 2).

The results obtained from HPLC analysis showed that leaves were significantly the richest source of polyphenols, while fruit the poorest ones. Among the leaves, cultivar Kordia was characterized by the highest content of coffee acid, chlorogenic acid, and p-coumaric acid, whereas cultivar Regina was the richest in ferulic acid and myricetin in comparison to other cultivars. Among the petioles, statistically

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significant the largest amount of coffee acid and chlorogenic acid were determined in cultivar Kordia, p-coumaric in cultivar Regina, while myricetin in cultivars Kordia, Regina and Vega. Among the fruit, the highest level of coffee acid and chlorogenic acid were detected in cultivar Kordia as well as p-coumaric in cultivar Vega, compared to cultivars Kordia and Regina. Statistically significant differences in concentration of myricetin in fruit among cultivars were not found.

In leaves, petioles, and fruit of sweet cherry, the dominant polyphenolic compound was a chlorogenic acid which was confirmed in all of the studied cultivars.

According to three used assays: ABTS, DPPH and FRAP, the petioles exhibited statistically significant the highest antioxidant activity in comparison to other parts of sweet cherry (Table 3). The exception was the cultivar Regina collected in Szczodrkowice in which the leaves were characterized by higher antioxidant capacity than the petioles (ABTS and FRAP tests). Significantly the lowest radical scavenging activity had fruit of sweet cherry, what was confirmed by all of the applied assays and for all of the examined cultivars. The highest antioxidant activity was found in petioles imported from Spain (ABTS and DPPH tests) and in petioles of cultivar Kordia collected in Sandomierz (DPPH and FRAP assays). Among the leaves, cultivar Kordia from Sandomierz was characterized by the highest antioxidant capacity in comparison to other ones, what was confirmed by ABTS, DPPH, and FRAP methods. Statistically significant effect of the cultivar on the antioxidant activity of fruit was not found only in ABTS assay. DPPH and FRAP tests indicated that the cultivar Vanda and the cultivar Kordia from Sandomierz, respectively, showed significantly highest radical scavenging activity among fruit.

Table 2
Concentration of polyphenolic compounds in leaves, petioles and fruit of sweet cherry

| Cultivar | Part of sweet cherry | Coffee acid (mg/100 g DW) | Chlorogenic acid (mg/100 g DW) | p-Coumaric acid (mg/100 g DW) | Ferulic acid (mg/100 g DW) | Myricetin (mg/100 g DW) |
|----------|---------------------|--------------------------|-------------------------------|-------------------------------|--------------------------|-------------------------|
| Kordia   | Leaves              | 465.65 ± 2.76 j C        | 2295.94 ± 147.79 g D          | 165.04 ± 3.06 k B            | 33.06 ± 2.20 a A         | 399.16 ± 7.84 e C       |
|          | Petioles            | 93.30 ± 1.16 f B         | 473.07 ± 31.05 d C            | 59.14 ± 2.14 f A             | nd                       | 60.95 ± 3.06 c A        |
|          | Fruit               | 57.54 ± 1.39 d B         | 305.15 ± 13.44 bc C           | 12.32 ± 0.30 a A             | nd                       | 2.79 ± 0.51 a A         |
| Regina   | Leaves              | 330.30 ± 9.32 h B        | 1960.22 ± 158.19 f D          | 51.47 ± 2.87 e A             | 97.24 ± 7.95 c A         | 451.19 ± 20.47 f C      |
|          | Petioles            | 68.31 ± 1.77 c B         | 339.54 ± 6.48 c D             | 77.68 ± 0.35 i C             | nd                       | 57.24 ± 1.44 c A        |
|          | Fruit               | 24.84 ± 0.49 a B         | 130.80 ± 1.85 a D             | 30.62 ± 0.91 b C             | nd                       | 2.64 ± 0.13 a A         |
| Vega     | Leaves              | 369.60 ± 5.69 i D        | 1684.23 ± 54.82 e E           | 91.26 ± 3.83 j B             | 39.43 ± 1.54 a A         | 215.56 ± 18.04 d C      |
|          | Petioles            | 61.49 ± 0.80 d A         | 397.23 ± 8.84 cd C            | 70.02 ± 2.01 h B             | nd                       | 56.69 ± 2.75 c A        |
|          | Fruit               | 47.80 ± 0.82 c B         | 230.17 ± 11.03 ab C           | 48.58 ± 3.60 d B             | nd                       | 10.08 ± 0.90 a A        |
| Hedelfińska | Leaves              | 262.94 ± 6.50 g B       | 1617.48 ± 52.41 e C           | 63.48 ± 2.77 g A             | 67.52 ± 1.68 b A         | 227.02 ± 11.68 d B      |
|          | Petioles            | 58.03 ± 1.55 d B         | 402.11 ± 17.86 cd C           | 43.63 ± 2.18 c AB            | nd                       | 30.78 ± 1.91 b A        |
|          | Fruit               | 39.85 ± 1.03 b B         | 200.11 ± 6.68 a C             | 45.05 ± 2.83 cd B            | nd                       | 3.87 ± 0.20 a A         |

Results are expressed as mean ± SD (n=4)
Mean values with different letters (a–k) within the each column are statistically different (p<0.05). Mean values with different letters (A–E) within the each row are statistically different (p<0.05)
DW dry matter, nd not determined

Discussion

The obtained results and our previous study [21] indicated that leaves and petioles of sweet cherry contained a larger amount of total dietary fiber, vitamin C, carotenoids and polyphenols (excluding anthocyanins) as well as they were characterized by higher antioxidant activity than fruit. To our best knowledge, other authors have not analyzed and compared the content of bioactive compounds in leaves, petioles, and fruit of sweet cherry. However, in the literature, the research, focused on the concentration of polyphenols in fruit and leaves of other plants, showed similar results [22, 23]. Oszmiański et al. [24] found that the leaves of cranberry, crabapple, murta (Chilean berry) and red-jambo contained higher amounts of polyphenolic compounds than fruits of these plants. In this study, we have demonstrated that the level of total dietary fiber in the leaves, petioles, and fruit was in the range of 39.92–46.21 g/100 g DW, 38.82–48.24 g/100 g DW, and 5.18–10.23 g/100 g DW, respectively. In our previous research individual parts of sweet cherry exhibited a similar concentration of fiber [21]. The data presented by the US Department of Agriculture [25] and the Victorian Cherry Association [26] also indicated its similar values in sweet cherry fruit.

Schmitz-Eiberger and Blanke [27] and Gündoğdu and Bilge [28] reported a similar level of vitamin C in sweet
cherry fruit as was shown in present work; whereas lower concentration of vitamin C was shown by Leong et al. [29] in the fruit of cultivars Lapins, Staccato, Stella and Sweetheart collected in Alexandra, Central Otago, New Zealand. These differences might have occurred due to various tested cultivars and place of cultivation, because of different climate in comparison with Poland [30]. The leaves of cultivars Kordia and Regina harvested in Szczodrkowice were characterized by the significantly higher content of vitamin C than these cultivars collected in Sandomierz. These differences were probably caused by the various growth conditions in Szczodrkowice and Sandomierz. The literature data showed that climatic conditions, including temperature, amount and intensity of light, cultural practices, including fertilizer and irrigation, as well as soil factors have a huge influence on vitamin C content in sweet cherry [30]. The obtained results showed that petioles and fruit imported from Spain and Hungary had lower concentration of vitamin C in comparison with most cultivars from Poland. Sweet cherries from Poland were transported to the laboratory immediately after having been picked (at commercial maturity) and analyzed, whereas the ones from Spain and Hungary were collected, stored and transported. Wani et al. [30] as well as Lee and Kader [31] reported that vitamin C tends to decrease during storage, what might explain those differences. Additionally, commercially available fruit are usually collected at a premature state what might also have affected vitamin C content [30].

The obtained results showed that the sweet cherry leaves had 28.72–54.90 mg/100 g DW, petioles had 6.21–18.32 g/100 g DW and fruit had 0.52–4.44 g/100 g DW of total carotenoids, respectively. In our previous study, the leaves and fruit were characterized by similar content of Table 3 Antioxidant activity of leaves, petioles and fruit of sweet cherry measured by ABTS, DPPH and FRAP assays

| Place of harvesting | Cultivar | Part of sweet cherry | ABTS (µmol Trolox/g DW) | DPPH (µmol Trolox/g DW) | FRAP (µmol Trolox/g DW) |
|---------------------|----------|----------------------|-------------------------|-------------------------|-------------------------|
| Sandomierz (Poland) | Kordia   | Leaves               | 1702.93 ± 110.25 g      | 1754.72 ± 48.05 g       | 1939.41 ± 103.50 j      |
|                     |          | Petioles             | 6002.77 ± 72.21 k       | 3212.03 ± 90.07 lm      | 4189.11 ± 194.03 p      |
|                     |          | Fruit                | 318.91 ± 3.29 a         | 174.14 ± 4.11 ab        | 345.58 ± 9.21 bc        |
| Regina              | Leaves   | 1295.63 ± 68.12 ef   | 1688.22 ± 28.32 g       | 1397.76 ± 36.61 i       |
|                     | Petioles  | 4414.69 ± 47.56 j    | 2932.91 ± 201.69 k      | 3209.63 ± 51.12 n       |
|                     | Fruit    | 202.22 ± 3.28 a      | 122.22 ± 4.77 ab        | 202.24 ± 3.52 ab        |
| Vega                | Leaves   | 1160.25 ± 86.66 de   | 1089.15 ± 77.73 e       | 1202.27 ± 25.76 h       |
|                     | Petioles  | 4153.78 ± 45.58 i    | 2425.01 ± 125.08 i      | 2334.84 ± 97.98 k       |
|                     | Fruit    | 241.16 ± 1.96 a      | 117.49 ± 1.84 ab        | 196.43 ± 1.05 ab        |
| Hedelfińska         | Leaves   | 990.09 ± 52.54 cd    | 608.72 ± 3.45 cd        | 840.58 ± 14.86 ef       |
|                     | Petioles  | 7807.09 ± 103.02 n   | 2687.29 ± 96.38 j       | 3503.71 ± 41.52 o       |
|                     | Fruit    | 345.35 ± 4.79 a      | 169.52 ± 7.97 ab        | 298.70 ± 7.73 bc        |
| Vanda               | Leaves   | 791.86 ± 147.95 bc   | 1300.92 ± 42.59 f       | 1011.99 ± 0.00 g        |
|                     | Petioles  | 3226.67 ± 47.49 h    | 1965.51 ± 23.70 h       | 2425.67 ± 132.72 kl     |
|                     | Fruit    | 364.53 ± 3.07 a      | 288.11 ± 6.37 b         | 309.16 ± 3.30 bc        |
| Szczodrkowice (Poland) | Kordia   | Leaves               | 680.95 ± 28.60 b        | 473.77 ± 6.49 c         |
|                     | Petioles  | 1588.41 ± 48.46 g    | 1705.61 ± 45.34 g       | 1132.66 ± 26.05 h       |
|                     | Fruit    | 306.00 ± 5.30 a      | 147.92 ± 1.47 ab        | 294.24 ± 6.33 bc        |
| Regina              | Leaves   | 880.85 ± 8.41 bc     | 560.25 ± 6.99 cd        | 732.87 ± 39.17 e        |
|                     | Petioles  | 820.49 ± 100.98 bc   | 1089.84 ± 45.80 e       | 523.27 ± 0.00 d         |
|                     | Fruit    | 180.99 ± 2.80 a      | 104.79 ± 0.87 a         | 181.55 ± 1.51 ab        |
| Summit              | Leaves   | 1374.60 ± 8.83 f     | 712.96 ± 22.03 d        | 899.36 ± 9.49 fg        |
|                     | Petioles  | 6300.68 ± 77.33 l    | 3087.32 ± 144.68 l      | 3212.71 ± 96.96 n       |
|                     | Fruit    | 172.10 ± 7.01 a      | 107.90 ± 1.75 a         | 147.50 ± 1.51 a         |
| Spain               | Unknown  | Petioles             | 8057.36 ± 328.10 o      | 3278.67 ± 192.60 m      |
|                     |          | Fruit                | 298.24 ± 9.54 a         | 151.90 ± 6.49 ab        |
| Hungary             | Unknown  | Petioles             | 7399.14 ± 205.98 m      | 2719.64 ± 79.06 j       |
|                     |          | Fruit                | 255.82 ± 0.85 a         | 139.55 ± 3.19 ab        |

Results are expressed as mean ± SD (n = 3)

Mean values with different letters (a–p) within the each column are statistically different (p < 0.05)

DW dry matter
these compounds, however the petioles by a higher concentration of total carotenoids [21]. Giménez et al. [32] found also similar level of carotenoids in the fruit of cultivar Early Lory from Spain. Gonçalves et al. [33] reported the larger content of these compounds in sweet cherry leaves of three cultivars, which was in the range of 124.7–148.3 mg/100 g DW, comprising the results for cultivar Summit analyzed also in this study. Another method of carotenoids determination, including using a different extraction solvent, may be the reason for the differences described above. Additionally, the level of carotenoids in the fruits and vegetables, including sweet cherry, depends on factors such as cultivar, weather conditions (temperature and precipitations), maturation stage as well as harvesting and post-harvesting conditions [32, 34–37].

In the available literature, little data on the concentration of polyphenolic compounds in other parts of sweet cherry than fruit have been presented. The leaves which were analyzed by Gonçalves et al. [33] had 3930–8890 mg/100 g DW of polyphenolic compounds. Their results showed that leaves of cultivar Summit were characterized by a lower content of total polyphenols compared to leaves of this cultivar, which were analyzed in our previous study. The petioles of sweet cherry, characterized by Prvučević et al. [38], contained a lower level of polyphenols. The content of these compounds in the fruit of sweet cherry was in the range of 1744.62–4045.32 mg/100 g DW. Other authors who studied the level of polyphenols in the fruit of various cultivars reported lower values [27, 39, 40]. Hayaloglu and Demir [41] and Serra et al. [42] who have also analyzed fruit of cultivar Summit, reported lower content of these compounds in comparison with our results. The leaves, petioles and fruit of cultivars Kordia and Regina harvested in Garlica Murowana (near Krakow, Poland) in 2016, assessed in our previous study [21], had higher concentration of polyphenolic compounds in comparison to sweet cherry from Szczodrkowice as well as lower concentration compared to the ones collected in Sandomierz, in all tested parts of sweet cherry. The level of total anthocyanins in the fruit was in the range of 120.17 mg/100 g DW–683.80 mg/100 g DW. Serradilla et al. [43] for cultivar Ambrunés from Spain and Ballistreri et al. [44] for 19 cultivars from Italy reported similar results. However, Usenik et al. [40] and Hayaloglu and Demir [41] showed lower concentrations of total anthocyanins in many cultivars, including cultivar Summit, in comparison to those analyzed in the present study. The differences in the content of polyphenols, including anthocyanins, might have resulted from various environmental conditions during the growing of sweet cherry. Literature data showed that the presence of these secondary metabolites, like other bioactive compounds, is related to temperature, illumination, precipitation, humidity, and soils [45].

To our best knowledge in the available literature, no data on content of individual polyphenols in leaves and petioles have been presented. Jakobek et al. [46], who studied the cultivar Lapins cultivated on six different vegetative rootstocks, reported that in fruit the main phenolic acid was chlorogenic acid, what was consistent with our results. Mahmood et al. [47], who analyzed polyphenol profile of sweet cherry at different maturity stages, showed that fully ripened fruit had a lower content of p-coumaric acid (the exception was cultivar Kordia) and chlorogenic acid as well as higher concentration of myricetin in comparison to our results. Sotelo et al. [48] found a smaller amount of chlorogenic acid and myricetin in the fruit of cultivar Stella from Alexandria (New Zealand). Cultivars Kordia and Regina from Croatia, which were studied by Milinović et al. [49], as well as cultivars Van, Noir De Guben, Larian and 0-900 Ziraat from Turkey, which were analyzed by Kelebek and Selli [50], were characterized by lower level of chlorogenic acid. To our best knowledge no data about the content of coffee acid in sweet cherry fruit have been presented. Phenolic acids and myricetin, detected in leaves, petioles and fruit, are known from their antioxidant, anti-inflammatory, and possible anticarcinogenic activities [51].

The results of tests carried out using Folin–Ciocalteu reagent showed that the richest source of polyphenolic compounds were petioles, however the results of HPLC analysis indicated the leaves of sweet cherry. These differences may be due to the fact that Folin–Ciocalteu reagent reacts not only with polyphenols, but also with other reducing compounds, i.e., proteins, carbohydrates, unsaturated fatty acids, and vitamins [52]. In the literature, fewer studies which characterized and compared the chemical compositions of sweet cherry petioles and leaves have been presented, thus it is difficult to discuss these results. Our previous study showed that the petioles were characterized by the highest antioxidant activity in comparison to other parts of sweet cherry what was also confirmed with ABTS, DPPH and FRAP methods [21]. Other authors, who analyzed the antioxidant activity of sweet cherry fruit, reported different results in comparison to results presented in obtained in study. De Souza et al. [53] in fruit from Brazil and Serradilla et al. [43] in cultivar Ambrunés from Spain obtained lower values of antioxidant activity in ABTS test. Twelve cultivars of sweet cherry collected in Turkey, studied by Hayaloglu and Demir [41] were characterized by lower antioxidant capacity, which was confirmed by ABTS, DPPH and FRAP methods. Kelebek and Selli [50], who used the ABTS and DPPH assays, have also found lower radical scavenging activity in four cultivars. These differences may result from various cultivars which were cultivated in different countries, as well as from some modifications and adaptations of ABTS, DPPH and FRAP methods.
The obtained results of antioxidant activity, measured with the ABTS, DPPH, FRAP methods differed slightly. All of the three used assays based on a single electron transfer reaction and spectrophotometrically measurement of a color change in the solution [54]. However, these tests differ from each other. The ABTS and DPPH methods are based on the reduction of different radicals by antioxidant compounds, ABTS+ and DPPH, respectively, whereas the FRAP test is based on the reduction of ferric iron (Fe3+) to ferrous iron (Fe2+) by antioxidants [55]. Differences in antioxidant activity of leaves, petioles and fruit might have resulted from the fact that the studied samples had various chemical composition and antioxidants content. Literature data indicated that different kinds of antioxidants can react in a different manner with radicals and chemicals used during tests [41, 56, 57].

**Conclusion**

The content of bioactive compounds and antioxidant activity of sweet cherry depended on the cultivar and part of the plant. The leaves and petioles were characterized by a higher concentration of total dietary fiber, vitamin C, total carotenoids and total polyphenols in comparison to fruit (except for anthocyanins which were detected only in fruit). The identified phenolic acids and myricetin are known as a strong antioxidant and anti-inflammatory agents. Due to high antioxidants level, the leaves and petioles can be a potential source for production of new functional food such as drinks and food additives in the form of lyophilized powders or extracts. They can be helpful in a treatment of the non-communicable diseases such as cardiovascular diseases, diabetes, obesity as well as some types of cancer. Further studies are needed to prove processability and usefulness of this plant material in the food industry.

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