

Article

# Analysis of Selected Properties of Fruits of Black Chokeberry (*Aronia melanocarpa* L.) from Organic and Conventional Cultivation

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Abstract: Chokeberry fruits can be treated as very rich sources of bioactive compounds and, therefore, have a very high biological value. The purpose of the study was to compare selected chemical and physical properties of chokeberry fruits, both from organic and conventional cultivations located near Cracow. Chemical composition of the fruit, content of the antioxidant activity, bioactive compounds, and ultra-weak luminescence were analyzed. It was proved that chokeberry fruits are rich in bioactive compounds and that ecological crops produce fruits with a higher level of such compounds. Chokeberry fruits from organic farms were proven to have a higher content of bioactive ingredients and antioxidant activity than in traditionally grown fruits. The total amount of sugars was ambiguous because both the highest and the lowest values were determined in fruits from traditional cultivation. Photon emissivity determined on the basis of ultra-weak luminescence was higher in fruits from organic cultivations. A very high correlation was also found between the photon emissivity and the content of polyphenols as well as the antioxidant activity.

Keywords: antioxidant activity; polyphenols; sugar; ultra-weak luminescence; black chokeberry

## 1. Introduction

Black chokeberry (Aronia melanocarpa L.) is a good source of selected bioactive compounds, indicating high nutritional and biological value [1,2]. Black chokeberry is a good source of dietary fiber, vitamins (provitamin A, E, B1, B2, B6, P, PP), β-carotene, minerals (Mn, Fe, B, Mo, Cu, Mg, J, Ca), and sugar such as sorbitol, fructose, glucose, organic acids such as malic, quinic, and citric [3]. Phenolic acids, such as neochlorogenic acid, chlorogenic acid, and polyphenolic compounds, such as flavonoids (flavan-3-ols, anthocyanins, flavonols) are the most important compound groups in analyzed fruit [4]. Chokeberry is a fruit with a very high level of proanthocyanidins and anthocyanins. Both of them are very rarely found in fruits [5]. Red grapes are the exception, their skin is rich in anthocyanins and the seeds in proanthocyanidins. The polyphenols in chokeberry strongly impact its exceptionally high antioxidant capacity [5]. The berries of chokeberry have one of the highest in vitro antioxidant activities that is connected with the presence of bioactive components, mainly polyphenols. They indicate antioxidant properties, reduce the content of LDL cholesterol and triglycerides [6], and reduce the risk of metabolic diseases. Chokeberry is used in the treatment of cardiovascular diseases and for lowering blood glucose levels and blood pressure due to its high biological value since it has a very high antioxidant capacity compared to other fruits, such as blue honeysuckle, blueberry, raspberry, and elderberry. Black chokeberry has anti-inflammatory, gastroprotective, antidiabetic,



and hepatoprotective properties [7]. However, the bitter and tart taste of chokeberry is caused by a large amount of polyphenols, especially strongly polymerized proanthocyanins. Procyanidin oligomers indicate a high affinity for proteins, which causes their denaturation and that affects the feeling of tartness, choking, and dry mouth [8].

Chokeberry fruits are used to produce juices, jams, preserves, jellies, syrups, and marmalades, pasteurized fruits for producing yogurts and desserts, and food coloring due to the high content of anthocyanins [3]. Valuable chokeberry-based preserves include liquid products, such as juices, nectars, drinks, wines, and liqueurs.

For over a dozen years, interest in food that brings not only health benefits but also environmental benefits related to its production has increased. The answer to this demand is an ecological system of food management, production, and processing. Organic farming is a management system that involves the least possible interference with the natural environment because this system does not allow using agricultural chemicals, i.e., synthetic fertilizers and plant protection products (pesticides). Instead, natural fertilizers (manure, compost, green fertilizers) and natural methods of plant protection (plant extracts) are widely used. This system causes plants to significantly change their pathway of synthesis of biologically active compounds in order to fight diseases and pests on their own. In the case of fruits, studies on the impact of the ecological farming system are still ambiguous. Works conducted by Kazimierczak et al. [9], Cayuela et al. [10], Wojdyło et al. [11] on strawberries, plums, or currants can serve as an example.

Ultra-weak luminescence measurement method has been used in biomedical research for many years [12,13], while to define the so-called "naturalness" of food products and their low level of technological processing are still entering research methods [14–18]. Combining this method with other methods of quality assessment makes a specific group of physical and chemical measurements even more precise and meeting social expectations.

Those studies analyzed the chemical composition (ash, dry matter, pectin, acidity, sugars—glucose, fructose, and sorbitol), the composition of polyphenolic compounds (UPLC-PDA-MS/MS), and antioxidant activity (radical scavenging spectrophotometric assay (ABTS), ferric reducing antioxidant potential (FRAP)), as well as ultra-weak luminescence.

The aim of the studies was to compare the chemical composition, content of bioactive compounds and the antioxidant activity, and ultra-weak luminescence of fruits from organic farming with conventional crops located in the vicinity of the Cracow agglomeration.

## 2. Materials and Methods

#### 2.1. Reagent and Standard

The reagents were acetonitrile, formic acid, methanol, ABTS, trolox, TPTZ, (+)-catechin, (–)-epicatechin, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, di- caffeic quinic acid, p-coumaric acid, procyanidins B2, caffeic acid, cyanidin 3-O-glucoside, and cyanidin-O-galactoside.

## 2.2. Plant Materials

Fruits chokeberry v. Galicjanka, cultivated in various conditions, 3 were from organic farming and 3 from conventional crops. All crops were located in the vicinity of Cracow (Gdów, C 1e; Lipnica Murowana, C 2e; Zbierzów Bocheński, C 3e—Ecological farms and Dobczyce, C 4c; Królówka, C 5c; Okulice, C 6c,—Conventional farms). The fruit was harvested in the first half of September 2019.

## 2.3. Dry Matter, Ash Content, Titratable Acidity, Pectin

Each measurement was done three times and results were expressed as a percent. Dry matter and fruit ash content were determined by the standard method [19]. Titratable acidity was determined according to Polish norms [20]. Pectin was determined by the method of Morris [21].

Samples for the analysis of antioxidant activity ABTS and FRAP were prepared as follows. Approximately about 0.5 g of each chokeberry fruit was weighed into a test tube for antioxidant property analysis. A total of 15 mL of 80% aqueous ethanol was added, and the suspension was stirred. The sample was sonicated and left at 4 °C for 24 h. After this time, the extract was centrifuged, and the supernatants were recovered.

ABTS<sup>+</sup> radical scavenging activity of the sample was performed with the help of the method proposed by Re et al. [22] and previously described [23]. All investigations were done in triplicate. The results were expressed in  $\mu$ mol Trolox/100 g FM.

FRAP (Fe<sup>3+</sup>) to the ferrous ion (Fe<sup>2+</sup>). The reducing potential of the sample was determined using the method proposed by Benzie and Strain [24]. All determinations were done three times and the results were expressed in  $\mu$ mol Trolox/100 g FM.

#### 2.5. Ultra-Weak Luminescence

The test method consists of measuring the photons resulting from the secondary emission from the analyzed biological material. A prototype equipment was used to measure the photon emission level as an ultra-weak luminescence. It was determined by the measurement of fluorescence decay using the time-correlated method counting single photons using the TCSPC technique (Time-Correlated Single Photon Counting). The device consists of, among others, from 8 photomultipliers placed above the measuring chamber. The measuring system enables automatic recording of test results and the LabView-based application software enables quick adjustment of the measurement process characteristics to the specificity of biological material. The fruit after picking was kept at 18–20 °C for 48 h and at  $4 \degree C (\pm 0.1 \degree C)$  for the next 48 h. Before the measurement itself, the fruits were kept in the dark for about 1 h until they reached 18 °C (±0.1 °C). Samples were excited by exposure to standard light with an intensity of 300 lx (wavelength 555 nm) for 600 s at the distance of 12 cm between the samples and the light source. The weight of a single sample was 5 g ( $\pm 0.1$  g) of intact fruits with a temperature of 18 °C (±0.1 °C) and relative humidity less than 40%. Prepared samples were irradiated in the aforementioned conditions and then each of them was placed in the measuring chamber in a manner enabling standardization of the measurement. Before the measurements, the intensity of background photon emission was measured, the value of which for all laboratory measurements ranged from 50 to 90 units. Background measurements according to an internal procedure were always performed after 10 appropriate measurements on a given day and at the beginning of the following day. The results of the photon emissivity given in the paper are reduced by the background emissivity. After commenced measurement system, it was stabilized for the first 120 s to prevent disturbances resulting from temporary destabilization of standard conditions. The initial phase was followed by the main (measuring) phase with a time interval of 600 s, and the sampling frequency was 1 Hz [14]. The result was provided in conventional photon emission units depending not only on the actual photon emissivity from the analyzed samples but also on the device and measurement methodology. Obtained results enable a relative analysis of the photon emissivity under identical experimental conditions.

## 2.6. Analysis of Sugars with HPLC-ELSD Method

The extract for sugar analysis was prepared as described by Kolniak-Ostek [25]. Chromatographic analysis was carried out with L-7455 liquid chromatography. Calibration curves (R2 = 0.9999) were created for glucose, fructose, and sorbitol. All determinations were done three times and results are expressed as g/100 g of fresh mass (FM).

The extract of polyphenol analysis was prepared by Kolniak-Ostek [25]. Samples (10  $\mu$ L) were eluted according to the linear gradient described previously by Kolniak-Ostek [24]. The conditions of the mass spectrometer were: A source block temperature of 130 °C, desolvation temperature of 350 °C, capillary voltage of 2.5 kV, cone voltage of 30 V, and a desolvation gas (nitrogen) flow rate of 300 L/h. All determinations were done in triplicate, and the results were expressed as mg/100 g FM.

## 2.8. Statistical Analysis

All obtained data were analyzed with Statistica 13.0 (StatSoft, Cracow, Poland). They were calculated as means  $\pm$  SD. One-way analysis of variance was conducted, and significant differences ( $p \le 0.05$ ) between the mean values were determined by Duncan's multiple range test.

## 3. Results and Discussion

Chokeberry fruits marked as C1e, C2e, or C3e came from organic farming and C4c, C5c, and C6c from conventional cultivation. The dry weight of the tested chokeberries ranged from 23.8% to 26.5%, depending on location and method of cultivation, on average 24.8% (Table 1). On the basis of the study of Białek [26], dry matter content in chokeberry fruit varied in the range of 17–29%, depending on the variety. In this study, the range is smaller, which may result from studying one variety, and the differences result only from weather and soil conditions. Similarly, slight differences in the ash content were found (Table 1), which was affected by the same factors as in the case of dry matter. In the fruit studied by Nawirska-Olszańska et al. [27], the ash content in fresh chokeberry fruits was higher (by 2.93%), which may result from testing another variety.

| Variant of Chokeberry | Dry Matter                   | Ash                          | Pectins                      | Titratable Acidity           |  |
|-----------------------|------------------------------|------------------------------|------------------------------|------------------------------|--|
|                       | %                            |                              |                              |                              |  |
| C 1e                  | $24.2 \pm 1.24$ <sup>c</sup> | $0.82 \pm 0.01$ <sup>b</sup> | $0.47 \pm 0.01$ <sup>c</sup> | $1.29 \pm 0.12^{a}$          |  |
| C 2e                  | 26.1 ± 1.21 <sup>b</sup>     | $0.81 \pm 0.01$ <sup>b</sup> | $0.54\pm0.01$ $^{\rm a}$     | $1.21 \pm 0.11$ <sup>b</sup> |  |
| C 3e                  | 24.2 ± 1.14 <sup>c</sup>     | $0.97 \pm 0.02$ <sup>a</sup> | $0.50 \pm 0.01$ <sup>b</sup> | $1.22 \pm 0.11$ <sup>b</sup> |  |
| C 4c                  | $24.0 \pm 1.11$ <sup>c</sup> | $0.61 \pm 0.01$ <sup>d</sup> | $0.49 \pm 0.02^{\text{ b}}$  | $0.99 \pm 0.09$ <sup>c</sup> |  |
| C 5c                  | 23.8 ± 0.99 <sup>d</sup>     | $0.79 \pm 0.04 {\rm ~b,c}$   | $0.46 \pm 0.01$ <sup>d</sup> | $0.93 \pm 0.09$ <sup>c</sup> |  |
| C 6c                  | $26.5 \pm 1.01^{a}$          | $0.73 \pm 0.02$ <sup>c</sup> | $0.35 \pm 0.01 \ ^{\rm e}$   | $1.01 \pm 0.09$ <sup>c</sup> |  |

Table 1. Content of dry matter, ash, pectins, and acidity of chokeberry fruit.

Mean values with different letters (a–e) within the same row were statistically different (p = 0.05), the same letters form one homogeneous group. Values expressed as mean ± standard deviation.

Chokeberry fruits are among the fruits moderately rich in pectin, literature sources state that their content in fresh fruits ranges from 0.30–0.75% [3]; in this study, the pectin content ranged from 0.35–0.54%, on average—0.47%. The highest number of pectins was found in fruits no. 2, obtained from organic farming. More pectin was found in fruits from organic farming than in the case of traditional cultivation; however, sample C4 (conventional) did not differ statistically from the ecological trials.

The acidity of fresh chokeberry fruit ranged from 0.93 to 1.29%. In the study of Skupień and Oszmiański [28], the acidity of chokeberry fruit was much lower (0.493%). That may be due to the testing of another variety and the lower ripeness of the tested fruit. In the event of fruits from organic farming, acidity was above 1.2%, while in fruits from conventional cultivation, it fluctuated at the level of 1%.

#### 3.1. Antioxidant Activity and Ultra-Weak Luminescence

Antioxidant activity was marked as ABTS and FRAP, and the results were indicated in Table 2. Both indicators were statistically higher in fruits from organic farming than traditional cultivations. C 1e (ABTS—126.58  $\mu$ mol/100 g and FRAP—95.53  $\mu$ mol/100 g) had the highest antioxidant activity. The lowest antioxidant activity was specific for C 6c (92.19  $\mu$ mol/100 g and 54.99  $\mu$ mol/100 g), constituting one homogeneous group with C 4c (92.37  $\mu$ mol/100 g and 55.05  $\mu$ mol/100 g). Chokeberry fruits are included in the group of fruits with high antioxidant activity [29,30], which was confirmed by the results herein.

| Variant of Chakeberry | ABTS                           | FRAP                          | Photon Emission               |
|-----------------------|--------------------------------|-------------------------------|-------------------------------|
| variant of Chokeberry | µmole/100 g                    | μmole/100 g                   | -                             |
| C 1e                  | $126.58 \pm 2.00^{a}$          | 95.53 ± 1.10 <sup>a</sup>     | $416.3 \pm 2.84$ <sup>a</sup> |
| C 2e                  | $109.85 \pm 8.08$ <sup>b</sup> | $89.12 \pm 2.04$ <sup>b</sup> | $402.8 \pm 3.11$ <sup>b</sup> |
| C 3e                  | 102.79 ± 3.69 <sup>c</sup>     | 78.99 ± 2.11 <sup>c</sup>     | 398.7 ± 3.00 <sup>c</sup>     |
| C 4c                  | 92.37 ± 2.10 <sup>e</sup>      | $55.05 \pm 2.08$ <sup>e</sup> | $352.2 \pm 2.45$ <sup>e</sup> |
| C 5c                  | 95.83 ± 1.54 <sup>d</sup>      | 62.19 ± 1.37 <sup>d</sup>     | 367.2 ± 2.78 <sup>d</sup>     |
| C 6c                  | 92.19 ± 3.69 <sup>e</sup>      | 54.99 ± 2.22 <sup>e</sup>     | 349.9 ± 2.29 <sup>e</sup>     |

Table 2. Antioxidant activity and ultra-weak luminescence black chokeberry fruits.

ABTS—Radical scavenging spectrophotometric assay; FRAP—Ferric reducing antioxidant potential; mean values with different letters (a–e) within the same row were statistically different (p = 0.05), the same letters form one homogeneous group. Values expressed as mean ± standard deviation.

Ultra-weak luminescence is a relatively new method of food study. There are more and more reports on the correlation between the high content of bioactive compounds and the number of emitted photons. Probably, the greater number of emitted photons is related to the pro-health values of food [14–16]. The number of photons emitted by fresh chokeberry fruits has not been studied so far. This research confirms a very high correlation between the photon emissivity and the biological activity of the analyzed products. Organic chokeberry fruits were described with significantly higher photon emissivity as well as the values of antioxidant activity (expressed as ABTS and FRAP) compared to fruits from traditional cultivation. This differentiation was bigger in the case of organic fruit (3 homogeneous groups within each discriminant) than in the case of traditional cultivation (2 groups). The highest number of emitted photons was determined in ecological chokeberry no. 1 (416.3). In the same chokeberry sample, the highest values of ABTS, FRAP (Table 2), and total fructose (Table 3) and polyphenols (Table 5) were also found.

| Variant of Chokeberry   | Fructose                     | Sorbitol                     | Glucose                      | Sum  |  |  |
|-------------------------|------------------------------|------------------------------|------------------------------|------|--|--|
| variant of Chokeberry - |                              |                              |                              |      |  |  |
| C 1e                    | $1.54 \pm 0.04$ <sup>a</sup> | $1.97 \pm 0.03$ f            | $2.82 \pm 0.01$ <sup>c</sup> | 6.33 |  |  |
| C 2e                    | $1.41 \pm 0.03$ <sup>b</sup> | $3.16 \pm 0.01^{a}$          | $3.36 \pm 0.03$ <sup>b</sup> | 7.93 |  |  |
| C 3e                    | $0.85 \pm 0.01$ f            | $2.20 \pm 0.02$ <sup>d</sup> | $2.69 \pm 0.02$ <sup>d</sup> | 5.75 |  |  |
| C 4c                    | $1.16 \pm 0.01 \ ^{\rm d}$   | $2.69 \pm 0.01$ <sup>c</sup> | $1.87 \pm 0.02^{\text{ e}}$  | 5.73 |  |  |
| C 5c                    | $1.01 \pm 0.01 \ ^{e}$       | $2.08 \pm 0.01 \ ^{e}$       | $1.92 \pm 0.01 \ ^{e}$       | 5.01 |  |  |
| C 6c                    | $1.30 \pm 0.04$ <sup>c</sup> | $2.90 \pm 0.02$ <sup>b</sup> | $3.76 \pm 0.04$ <sup>a</sup> | 7.96 |  |  |

Table 3. Sugar content in black chokeberry fruits.

Mean values with different letters (a–e) within the same row were statistically different (p = 0.05), the same letters form one homogeneous group.

## 3.2. Identification of Sugars

Sugar content in fruits depends on the species and variety of the tested samples, but it also depends on the weather conditions and the degree of fruit ripeness. For this reason, different total sugar contents may be determined in fruits of the same species and variety. Depending on maturity degree, there might also be sugars contained.

Three sugars were determined in the tested samples—fructose, sorbitol, and glucose (Table 3). Studies by other authors confirm the presence of fructose, glucose, and sorbitol in fresh chokeberry fruits [27,31]. In these studies, the main sugar in chokeberry fruit is glucose and there are slightly

less sorbitol and the least fructose were determined. In studies by other authors [3], sorbitol was not found in fresh fruit, but it was present in chokeberry juice. Studies of Denev et al. [31] also indicated fructose, glucose, and sorbitol sucrose. In the above studies, the highest concentration was sorbitol (6.55–12.99 g/100 g FW), while the content of fructose and glucose was at a similar level (glucose 1.53–3.02 g/100 g FW) and fructose 2.2–369 g/100 g FW). A similar analogy was obtained in the studies by Nawirska-Olszańska et al. [27]. In the cited studies, the content of total sugars was significantly higher (10.26–19.70 g/100 g FW) than in the study below (5.01–7.96 g/100 g FW). This may be due to the selected different varieties and locations of cultivation. Studies were conducted on fruit from Bulgaria, which is characterized by greater sunlight than the regions of Cracow in Poland.

## 3.3. Identification of Polyphenolic Compounds

Chokeberry are fruits rich in various polyphenol fractions [3,29,32]. Table 4 indicates the qualitative identification of polyphenolic compounds found in chokeberry fruit. Compounds identification was performed by LC-PDA-ESI-MS/MS and quantitative analysis by UPLC-MS/MS. The extracts from black chokeberry fruits contained selected 27 compounds, including 11 flavonols, 7 anthocyanins, 5 phenolic acids, 3 flavan-3-ols, and 1 flavanone. Identification of polyphenolic compounds was conducted on a comparison of their MS, MS/MS data, retention times, UV spectra of standards, and data available in the literature [27–30,33]. Compounds identified in the negative ion mode belonged to the group of flavonols, hydroxycinnamic acids, flavanone, and flavan-3-ols. However, positive ionization was used to identify anthocyanins.

| Nr | Compounds                               | Rt (min) | λ <sub>max</sub> (nm) | MS     | MS-MS       |
|----|---|----------|-----------------------|--------|-------------|
| 1  | Cyanidin-3-hexoside-(epi)catechine      | 2.54     | 520                   | 737+   | 575/423/287 |
| 2  | Neochlorogenic acid                     | 2.57     | 323                   | 353    | 191         |
| 3  | Cyanidin-3-pentoside-(epi)catechine     | 2.98     | 520                   | 707+   | 557/329/287 |
| 4  | (+) Catechin                            | 3.03     | 280                   | 289    |             |
| 5  | Cyanidin-3-hexoside-(epi)cat-(epi)cat   | 3.15     | 520                   | 1025 + | 575/409/287 |
| 6  | 3-O-p-Coumaroylquinic acid              | 3.30     | 310                   | 337    | 191         |
| 7  | Cyanidin-3-O-galctoside                 | 3.51     | 516                   | 449+   | 287         |
| 8  | Chlorogenic acid                        | 3.62     | 323                   | 353    | 191         |
| 9  | Cryptochlorogenic acid                  | 3.71     | 323                   | 353    | 191         |
| 10 | Cyanidin-3-O-glucoside                  | 3.81     | 517                   | 449+   | 287         |
| 11 | Cyanidin-3-O-arabinoside                | 4.03     | 515                   | 419+   | 287+        |
| 12 | Procyanidin B2                          | 4.20     | 280                   | 577    | 289         |
| 13 | Cyanidin-3-O-xyloside                   | 4.68     | 515                   | 419+   | 287+        |
| 14 | (–) Epicatechin                         | 4.88     | 280                   | 289    |             |
| 15 | Quercetin-dihexoside                    | 5.23     | 352                   | 625    | 445/301     |
| 16 | Quercetin-dihexoside                    | 5.29     | 352                   | 625    | 445/301     |
| 17 | Quercetin-3-O-vicianoside               | 5.52     | 353                   | 595    | 432/301     |
| 18 | Quercetin-3-robinobioside               | 5.87     | 353                   | 609    | 463/301     |
| 19 | Quercetin-3-O-rutinoside                | 6.02     | 353                   | 609    | 463/301     |
| 20 | Quercetin-3-O-galctoside                | 6.09     | 352                   | 463    | 301         |
| 21 | Quercetin-3-O-glucoside                 | 6.22     | 352                   | 463    | 301         |
| 22 | Eriodictyol-glucuronide                 | 6.28     | 280                   | 463    | 287         |
| 23 | Isorhamnetin pentosylhexoside           | 6.41     | 352                   | 609    | 315         |
| 24 | Quercetin-O-deoxyhexose-O-deoxyhexoside | 6.76     | 352                   | 593    | 433/301     |
| 25 | Isorhamnetin rhamnosylhexosideisomer    | 6.71     | 352                   | 623    | 463/315     |
| 26 | Isorhamnetin rhamnosylhexosideisomer    | 6.88     | 352                   | 623    | 421/315     |
| 27 | Di-caffeic quinic acid                  | 6.93     | 323                   | 515    | 353/191     |

Table 4. Groups of polyphenolic compounds identified by LC-PDA-ESI-MS/MS in chokeberry fruits.

## 3.4. Comparison of Phenolic Compounds Found in Black Chokeberry

Results of the analyses of the content of polyphenolic compounds in tested samples are shown in Table 5. Cultivation conditions affected the content of individual polyphenol fractions. Organic chokeberry fruits contained more polyphenols in total. The most polyphenols in chokeberry from cultivation 1 were 2598.72 mg/100 g FW, while the lowest in fruit from cultivation 6—2293.76 mg/100 g

FW. There were statistical differences in the total polyphenol content between the places of cultivation. However, for all cultivation sites, the same dependence of the content of individual fractions can be established, but the major polyphenolic compounds were found to be anthocyanins > procyanidin polymers >> phenolic acids  $\geq$  flavonols > flavan-3-ols > flavanone. The content of polyphenols in fruits was affected by weather conditions. Although plantations were located in one area, there were slight local differences in the number of rainy and sunny days. That translated into differences in their content. However, it can be seen that the conditions of organic farming favored the formation of polyphenolic compounds.

| Numbers                 | C 1e                         | C 2e                        | C 3e                         | C 4c                         | C 5c                             | C 6c                             |
|-------------------------|------------------------------|-----------------------------|------------------------------|------------------------------|----------------------------------|----------------------------------|
| 1                       | $4.43 \pm 0.09$              | $4.33 \pm 0.07$             | $3.23 \pm 0.07$              | $2.87\pm0.08$                | $2.16 \pm 0.07$                  | $2.04 \pm 0.06$                  |
| 2                       | $174.35 \pm 1.38$            | $161.53 \pm 1.75$           | $141.99 \pm 1.51$            | $128.49 \pm 1.14$            | $91.56 \pm 0.89$                 | $98.81 \pm 0.53$                 |
| 3                       | $1.30 \pm 0.05$              | $1.22 \pm 0.04$             | $1.26 \pm 0.02$              | $1.14 \pm 0.04$              | $1.09 \pm 0.03$                  | $1.06 \pm 0.03$                  |
| 4                       | $18.27\pm0.03$               | $17.81 \pm 0.70$            | $18.21\pm0.10$               | $16.18\pm0.90$               | $16.66\pm0.68$                   | $16.70 \pm 0.59$                 |
| 5                       | $10.23 \pm 0.09$             | $9.61 \pm 0.07$             | $9.97 \pm 0.08$              | $8.74 \pm 0.07$              | $8.77 \pm 0.05$                  | $8.98 \pm 0.05$                  |
| 6                       | $6.70\pm0.06$                | $5.96 \pm 0.05$             | $6.12\pm0.05$                | $5.32 \pm 0.08$              | $5.31 \pm 0.06$                  | $4.81\pm0.03$                    |
| 7                       | $661.70 \pm 4.48$            | $621.34 \pm 2.22$           | $666.44 \pm 2.18$            | $651.55 \pm 4.24$            | $652.15 \pm 4.43$                | $626.4 \pm 4.13$                 |
| 8                       | $92.69 \pm 0.46$             | $88.17 \pm 1.17$            | $84.26 \pm 2.02$             | $74.74 \pm 4.42$             | $77.51 \pm 3.64$                 | $76.25 \pm 3.73$                 |
| 9                       | $5.60 \pm 0.25$              | $4.57\pm0.20$               | $4.82 \pm 0.22$              | $3.66 \pm 0.28$              | $4.21\pm0.22$                    | $3.91 \pm 0.01$                  |
| 10                      | $22.06 \pm 1.01$             | $25.01 \pm 0.62$            | $19.99 \pm 0.69$             | $19.99 \pm 0.34$             | $19.71 \pm 0.15$                 | $22.07 \pm 1.09$                 |
| 11                      | $316.02 \pm 2.28$            | $285.62 \pm 9.02$           | $235.42 \pm 1.33$            | $254.90 \pm 1.68$            | $248.72 \pm 1.02$                | $246.79 \pm 2.21$                |
| 12                      | $4.13\pm0.19$                | $3.40\pm0.18$               | $3.64 \pm 0.09$              | $3.19 \pm 0.04$              | $3.29\pm0.07$                    | $3.16\pm0.12$                    |
| 13                      | $29.41 \pm 0.26$             | $26.86 \pm 0.82$            | $28.81 \pm 0.43$             | $23.35 \pm 0.41$             | $22.32 \pm 0.13$                 | $24.14 \pm 0.42$                 |
| 14                      | $160.13 \pm 1.19$            | $166.19 \pm 1.16$           | $165.89 \pm 1.63$            | $155.28 \pm 1.98$            | $153.58 \pm 1.65$                | $154.53 \pm 1.85$                |
| 15                      | $3.35 \pm 0.14$              | $3.19 \pm 0.07$             | $3.28 \pm 0.04$              | $2.15 \pm 0.04$              | $2.89 \pm 0.01$                  | $3.09 \pm 0.15$                  |
| 17                      | $4.80\pm0.20$                | $4.41 \pm 0.10$             | $4.99\pm0.09$                | $3.50 \pm 0.05$              | $3.45\pm0.02$                    | $3.32 \pm 0.22$                  |
| 18                      | $2.52\pm0.12$                | $2.60 \pm 0.13$             | $2.56 \pm 0.09$              | $1.95 \pm 0.09$              | $1.94\pm0.04$                    | $2.45 \pm 0.03$                  |
| 19                      | $10.68 \pm 0.10$             | $10.74 \pm 0.11$            | $9.99 \pm 0.05$              | $8.98 \pm 0.08$              | $9.29 \pm 0.03$                  | $9.31 \pm 0.21$                  |
| 20                      | $32.43 \pm 0.47$             | $38.97 \pm 0.24$            | $36.42 \pm 0.18$             | $31.46 \pm 0.14$             | $31.77 \pm 0.05$                 | $32.11 \pm 0.50$                 |
| 21                      | $27.24 \pm 0.21$             | $26.75 \pm 0.16$            | $26.97 \pm 0.18$             | $23.54 \pm 0.11$             | $22.98 \pm 0.05$                 | $23.27 \pm 0.11$                 |
| 22                      | $44.40 \pm 0.39$             | $47.61 \pm 0.28$            | $45.33 \pm 0.12$             | $38.97 \pm 0.24$             | $39.24 \pm 0.15$                 | $41.36 \pm 0.39$                 |
| 23                      | $6.20\pm0.06$                | $6.81 \pm 0.05$             | $6.47\pm0.02$                | $5.81 \pm 0.01$              | $6.30\pm0.02$                    | $6.33 \pm 0.02$                  |
| 25                      | $3.16 \pm 0.04$              | $3.18 \pm 0.03$             | $3.09 \pm 0.01$              | $3.16 \pm 0.01$              | $2.89 \pm 0.01$                  | $2.95 \pm 0.01$                  |
| 27                      | $2.35\pm0.02$                | $2.29 \pm 0.02$             | $2.15\pm0.02$                | $2.33 \pm 0.01$              | $2.00\pm0.01$                    | $2.08\pm0.02$                    |
| Procyanidin<br>polymers | 954.57 ± 6.45                | 921.73 ± 1.79               | 946.09 ± 2.16                | 871.07 ± 4.13                | 872.27 ± 4.21                    | 877.84 ± 3.78                    |
| Total                   | 2598.72 ± 17.86 <sup>a</sup> | 2489.9 ± 15.78 <sup>b</sup> | 2477.39 ± 11.56 <sup>b</sup> | 2342.32 ± 15.87 <sup>c</sup> | $2302.06 \pm 13.85$ <sup>d</sup> | $2293.76 \pm 12.74$ <sup>d</sup> |

Table 5. Comparison of phenolic compounds detected in black chokeberry fruits [mg/100g FW].

Mean values with different letters (a–d) within the same row were statistically different (p = 0.05), the same letters form one homogeneous group.

The main group of polyphenolic compounds identified in chokeberry powders was anthocyanins (seven cyanidins derivatives), which accounted for approximately 40% of total polyphenols (Table 5). The dominant compound was cyanidin-3-*O*-galctoside. The total amount of anthocyanins in the tested samples ranged from 931.48 to 1045.15 mg/100g FW.

The second group of compounds in terms of the amount in the tested chokeberry fruits were procyanidin polymers and they constituted ~35% of all polyphenolic compounds. They are responsible for the tart chokeberry taste, but they are also an important group of healthy compounds. Procyanidin oligomers have a high affinity for proteins by binding to proteins, which caused shearing. That effect is particularly felt when consuming chokeberry fruit. These compounds, reacting with the proteins of the mouth's taste buds located on the tongue are binding, which affects the feeling of dryness and tartness in the mouth. However, these compounds are interesting due to their strong antioxidant activity and beneficial effects on health because they indicate antitumor activities and antiproliferative effects [8].

The next group of compounds was phenolic acids (Table 5). On the basis of ultra-efficient liquid chromatography techniques, 5 phenolic acids were identified. Dominating compounds were chlorogenic acid and its determined content in the tested fruits ranged from 91.56 to 174.35 mg/100 g FW. Oszmiański and Lachowicz obtained similar dependencies in their study on various products from chokeberry fruit [30].

Flavonols were another group of polyphenolic compounds found in chokeberry fruits. Eleven flavonols have been identified, including eight quercetin derivatives and three isoramnetin derivatives. A significant amount of flavonols has been designated as quercetin glycosides.

Another group of compounds found in chokeberry and constituting ~7.0% of the total content of polyphenolic compounds was flavan-3-ols. Three compounds have been identified, two monomers ((+)-catechins and (–)-epicatechins) and a dimer—procyanidin B2 (Tables 4 and 5).

The antioxidant activity of *A. melanocarpa* fruit is mostly caused by the polyphenolic compounds. Results of the correlation analysis presented in Table 6 indicate a correlation between the antioxidant activity marked as ABTS and FRAP, and total polyphenols and ultra-weak luminescence was very high. On the other hand, the correlation of total sugars with other indices was very low. Results indicate that the sugar content in the fruit does not significantly affect their antioxidant activity. Moreover, the total sugar content does not affect the number of photons emitted; the correlation of 0.04 is even minimal. It is not clear at the moment why FRAP has a higher correlation with ultra-weak luminescence (0.99) compared to ABTS (0.91).

**Table 6.** Correlation between antioxidant activity and polyphenol content, ultra-weak luminescence, and sugars.

|                         | ABTS | FRAP | Polyphenolic | Ultra-Weak Luminescence | Sugars |
|-------------------------|------|------|--------------|-------------------------|--------|
| ABTS                    |      | 0.94 | 0.94         | 0.91                    | 0.11   |
| FRAP                    |      |      | 0.96         | 0.99                    | 0.17   |
| Polyphenolic            |      |      |              | 0.96                    | 0.10   |
| Ultra-weak luminescence |      |      |              |                         | 0.04   |
| Sugars                  |      |      |              |                         |        |

## 4. Conclusions

Conducted studies confirmed the information that chokeberry fruits are rich in bioactive compounds and that ecological crops produce fruits with better health properties. The cultivation conditions of chokeberry fruits had the greatest influence on their chemical composition. In the event of organic farms, despite slight differences among themselves, higher content of bioactive ingredients and antioxidant activity was demonstrated than in those grown in a conventional manner. The total amount of sugars was ambiguous, both the highest and the lowest were determined in fruits from traditional cultivation. The determination of ultra-weak luminescence was higher in fruits from organic farming and correlated well with both the polyphenol content and antioxidant properties. It is planned to continue research on ultra-weak luminescence. Information on this method of rapidly determining the properties of plant raw materials may be used in other studies.

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