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# Selected Properties of Juices from Black Chokeberry (*Aronia melanocarpa* L.) Fruits Preserved Using the PEF Method

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**Abstract:** Black chokeberry (*Aronia melanocarpa* L.) fruit and processed berries have very high biological value because they are a rich source of bioactive compounds. The method of black chokeberry juice preservation is of key importance. One of the more recent ways in which semiliquid and liquid products can be preserved is the pulsed electric field (PEF) method. The aim of our study was to compare chosen physical and chemical properties of fresh and PEF-preserved chokeberry fruit juices derived from farms located in the vicinity of Cracow (Kraków). The analysis focused on the physical properties and chemical composition of black chokeberry juices, their bioactive compound content, and antioxidant activity. After using the PEF method, there was an increase in dry weight, sugars, and polyphenols, which could be caused by the slight evaporation of water during the process. During this process, antioxidant activity decreased, and ultra-weak luminescence slightly increased. The process of preserving juices using the PEF method is a good way to preserve the bioactive properties of the obtained aronia juices.

**Keywords:** ultra-weak luminescence; antioxidant activity; polyphenols; sugars; pulsed electric field

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## 1. Introduction

*Aronia melanocarpa* L., which belongs to the Rosaceae family, Pomoideae subfamily, is a species of North American flora. In Russia, it is known as "black rowanberry," in America as "black chokeberry" or "gueles noires," and in Germany as the "Schwarze Apfelbeere". It is best known and used as a fruiting and decorative shrub. Chokeberry is robust and resilient to diseases, fungi, pesticides, herbicides, artificial fertilizers, and industrial and traffic pollution—it does not accumulate heavy metals. These specific properties are due to its high content of polyphenolic compounds, mainly the procyanidins' capacity to react with proteins. Because of their high content of these substances, when compared with other berries, chokeberry fruits show an exceptionally high shelf life and resistance to microorganisms and other pathogens [1].

Black chokeberry fruit and processed products are valued as a very good source of bioactive compounds, with extremely high biological and nutritional value [2,3]. This high biological value of fruit and juice is caused by a high content of phenolic compounds: phenolic acids (chlorogenic, neochlorogenic) and flavonoids (flavonols, anthocyanins, flavan-3-ols) [4]. Chokeberries are extraordinarily rich in anthocyanins and procyanidins. It is very rare to find large amounts of both of these compounds in fruit. Exceptions include red wine grapes, with skin rich in anthocyanins and seeds with high contents of procyanidins. Polyphenol content in chokeberries has a strong influence on the fruit's

exceptionally high antioxidant capacity [4,5]. The antioxidant properties of chokeberry give a number of prohealth properties, reduce LDL cholesterol and triglycerides in blood [6], and, as a consequence, reduce the risk of metabolic diseases. Aronia juice can be used in cardiovascular diseases for prevention and treatment because they reduce blood pressure and the level of glucose in blood [7]. Chokeberry juice is called a natural antibiotic and is recommended for use in arteriosclerosis and achlorhydria. Moreover, it is used for children and postoperative patients to strengthen their health and as a vitamin source of use in the case of cold and fever. Tannic substances disinfect the digestive tract and prevent the excessive development of harmful bacteria in the intestines [8].

The astringent and bitter taste of chokeberry fruit and juice is due to the high content of polyphenols, especially procyanidins, presenting a high degree of polymerization. Procyanidin oligomers are connected to proteins, causing their denaturation, which contributes to the astringent, choking, and dry mouth sensation [9]. Due to such characteristics, chokeberry juice is not very popular for direct consumption but constitutes an excellent addition to other juices, increasing both their nutritional value and organoleptic qualities, especially color.

Methods of food preservation are a very important factor in maintaining foods' bioactive and antioxidant properties. One of the most important modern food preservation methods involves the use of a pulsed electric field (PEF), which can be used for the preservation of liquid and semiliquid food products and is also part of the so-called hurdle technology [10]. The general idea behind PEF-based food preservation consists of the inactivation of microorganisms present in the fluid by applying high-voltage pulses. The medium under preservation is placed between two electrodes, with flow-through operation also possible. PEF is effective against vegetative forms of microorganisms but not very effective or ineffective against their dormant forms [11,12].

The ultra-weak luminescence (UWL) measurement method has been used in biomedical research for many years [13,14], but it has been a relatively new method for determining whether food products are "natural" and unprocessed [12,15–19]. The use of this method in conjunction with other quality assessment methods makes it possible to measure specific physical and chemical properties with high precision, thus meeting public expectations.

The aim of our study was to compare the chemical composition, content of bioactive compounds, and antioxidant activity of fresh and preserved chokeberry fruit juices using the PEF method.

This study involved the analysis of physical properties and chemical composition (dry matter, ash, pectin, acidity, level of sugars such as fructose, sorbitol, and glucose), the composition of polyphenolic compounds (UPLC-PDA-MS/MS), antioxidant activity (FRAP, ABTS), and ultra-weak luminescence.

## 2. Materials and Methods

### *2.1. Reagent and Standard*

Acetonitrile, formic acid, methanol, ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), Trolox (6-hydroxy-2,5,7,8-tetramethylchro man-2-carboxylic acid), and TPTZ (2,4,6-tri(2-pyridyl)-s-triazine were purchased from Sigma-Aldrich (Steinheim, Germany). (+)-catechin, (-)-epicatechin, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, di-caffeic quinic acid, p-coumaric acid, procyanidins $B_2$, caffeic acid, cyanidin 3-O-glucoside, and cyanidin-O-galactoside were purchased from Extrasynthese (Lyon, France).

### *2.2. Plant Materials*

All crops of black chokeberry of the 'Galicjanka' variety were sourced from farms located in the Cracow area (Gdów, 1; Lipnica Murowana, 2; Zbierzów Bocheński, 3; Dobczyce, 4; Królówka, 5; Okulice, 6). The fruits were cultivated in various conditions and harvested in the first decade of September 2020.

The juice was obtained by pressing 2 kg of fresh fruit in a slow-running juicer. The juice was then divided into two parts. One of them was used to determine luminescence,

with the remaining juice frozen for further analysis. The other portion of the juice was subjected to pulsed electric field (PEF) treatment and then luminescence analysis, with the remaining juice frozen for subsequent analysis.

### 2.3. Pulsed Electric Field (PEF)

The control sample was fresh chokeberry juices not treated with PEF. Preliminary tests demonstrated that chokeberry juices were preserved using a pulsed electric field with an electric field intensity of 20 kV/cm at 300 pulses with a 15 s interval between pulses. The pulse shape was sinusoidal with a width of 0.17 µs [20].

### 2.4. Dry Matter, Ash Content, Titratable Acidity, and Pectin

Dry matter content was determined by using a moisture analyzer (MB 25, Ohaus, Parsippany, NJ, USA). Approximately 1.5 g of chokeberry juice was dried at 105 °C [21]. Measurements were carried out in triplicate and are expressed as %.

Black chokeberry juice ash content was determined using the Association of Official Analytical Chemists [21] method 930.09. Measurements were carried out in triplicate and are expressed as %.

Titratable acidity of samples was determined by using a titrator (TitroLine 6000), in line with the Polish norm [22]. Black chokeberry juice was transferred to a volumetric flask (100 mL), which was then filled with water. Prepared samples were boiled and filtered after cooling down, and about 10 mL of obtained filtrate was titrated with NaOH (0.1 mol/L) up to pH 8.1. The measurements were carried out in triplicate and expressed as % (in g of malic acid/100 mL).

Pectin content was determined using the method by Morris [23], with approx. 10 g of sample juice, with the addition of 50 mL of distilled water, which was then boiled for 30 min and filtered. The resulting filtrate was transferred to a 50 mL volumetric flask that was then filled with distilled water up to the marking. A total of 10 mL of the solution was mixed with 50 mL of acetone and kept for 1 h. It was then filtered through a dried and weighed filter and then dried at 75 °C for 4 h, then weighed. Pectin content was calculated from the difference between the weight of the filter with the sediment after drying and the weight of the dried filter, considering the amount of pectin solution for determination and the sample weight. Result was expressed as %.

### 2.5. Analysis of Antioxidant Activity

Samples for the analysis of antioxidant activity (ABTS) and the ferric-reducing ability of juices (FRAP) were prepared as follows. Black chokeberry juice (5 mL) was centrifuged at 20,878× *g* for 10 min.

$ABTS^+$ radical scavenging spectrophotometric assay. The $ABTS^+$ radical scavenging activity of the sample was measured using the method developed by Re et al. [24] and described elsewhere [25], using a Shimadzu UV-2401 PC spectrophotometer (Kyoto, Japan). Determination of all characteristics was carried out in triplicate. The results were expressed as µmol Trolox per 100 mL of juices.

FRAP reduces ferric iron ($Fe3^+$) to ferrous iron ($Fe2^+$). The reducing potential of the sample was determined using the FRAP Assay proposed by Benzie and Strain [26] as a measure of antioxidant power. An antioxidant reduces the ferric iron ($Fe^{+3}$) to the ferrous iron ($Fe^{+2}$); the latter forms a blue complex ($Fe^{+2}$/TPTZ), which increases the absorbance at 593 nm. A standard curve was prepared using different concentrations of Trolox. The results were corrected for dilution and expressed as µmol Trolox per 100 mL of juices. Determination of all characteristics was carried out in triplicate using a Shimadzu UV-2401 PC spectrophotometer.

### 2.6. Ultra-Weak Luminescence

This method consists of measuring photon emissions from the biological material sample. The measuring system has automatic recording of test results, and LabView-

based software enables quick adjustment of the measurement process characteristics to the specific characteristics of the biological material. Before the photon emission measurement, the samples were exposed to 300 lx standard light (555 nm wavelength) for 600 s. The mass of every sample was 5 g (±0.1 g). The samples were then placed in the measuring chamber in line with the standard procedure. The measuring system was first stabilized for 120 s to prevent interferences resulting from a momentary destabilization of standard conditions. The preliminary phase was followed by the main (measurement) phase of the experiment, which lasted 600 s, with a 1 Hz frequency of photon emission capture [15]. The result is expressed as conventional photon emission units, accounting not only for the actual photon emission from the samples but also for the device and measurement methodology. The results enabled relative analysis of photon emission under identical experimental conditions.

### 2.7. Analysis of Sugars Using HPLC-ELSD Method

The extract for sugar analysis was prepared as described by Kolniak-Ostek [27]. Chromatographic analysis was carried out using an L-7455 liquid chromatograph (Merck-Hitachi, Tokyo, Japan) fitted with an evaporative light scattering detector (PL-ELS 1000; Polymer Laboratories Ltd., Church Stretton, UK) and an L-7100 quaternary pump (Merck-Hitachi) equipped with a D-7000 HSM Multisolvent Delivery System (Merck-Hitachi), an L-7200 autosampler (Merck-Hitachi), and a Prevail Carbohydrate ES HPLC Column-W (250 × 4.6 mm, 5 µm; Alltech Inc., Nicholasville, KY, USA). Calibration curves ($R^2$ = 0.9999) were created for glucose, fructose, sorbitol, and sucrose. Determination of all characteristics was carried out in triplicate. Results are expressed as g/100 mL of juices.

### 2.8. Identification and Quantification of Polyphenols Using the UPLC-PDA-MS Method

The extract for polyphenol analysis was prepared as described by Kolniak-Ostek [27]. Analysis of polyphenols was carried out using an ACQUITY Ultra Performance LC system (UPLC) equipped with a binary solvent manager (Waters Corp., Milford, MA, USA), a UPLC BEH C18 column (1.7 µm, 2.1 mm 50 mm, Waters Corp., Milford, MA, USA), and a Q-Tof Micro mass spectrometer (Waters, Manchester, UK), with an ESI source operating in negative and positive modes. The mobile phase consisted of aqueous 0.1% formic acid (A) and 100% acetonitrile (B). Samples (10 µL) were eluted according to the linear gradient described previously by Kolniak-Ostek [27]. The conditions of the mass spectrometer were: 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.

The compounds were monitored at 280 nm (flavan-3-ols and hydroquinones), 320 nm (phenolic acids), 340 nm (flavones), 360 nm (flavonol glycosides), and 520 nm (anthocyanins). All experiments were carried out in triplicate. The results were corrected for dilution and expressed in mg/L of juices.

### 2.9. Statistical Analysis

Statistical analysis was performed using Statistica v. 13.0 (StatSoft, Kraków, Poland) and converted in Excel 2007 to means ± SD. Analysis of variance was made using the ANOVA method. Significant differences ($p \leq 0.05$) between the mean values were determined using Duncan's multiple range test.

## 3. Results and Discussion

Chokeberry juices are marked as 1–6; the letter J stands for fresh juice, and PEF stands for juice subjected to pulsed electric field treatment.

The dry matter in the chokeberries juice ranged from 23.2% to 28.2%, depending on the place for taking the sample (Table 1). Research by Białek [28] demonstrated that the dry matter content of chokeberry fruit ranged from 17–29%, depending on the place of cultivation. In our study, the differences in dry matter content were smaller, which may be because our research was focused on one cultivar and the differences were only due

to microclimatic and soil conditions. The dry matter content in PEF-treated juices was higher than in fresh juices. This may have been caused by the evaporation of water during the process. Similar small differences were found in the ash content (Table 1). In the fruit studied by Nawirska-Olszańska et al. [29], the ash content in fresh chokeberry fruits was higher (on average by 2.93%), which may be due to another cultivar and different place and season of cultivation of the samples used in the research. In a study by Trenek et al. [4], the mentioned characteristics were similar due to the origin of the fruit from the same cultivated area.

**Table 1.** Content of dry matter, ash, pectins, and acidity of chokeberry fresh juice and juice treated using PEF.

| | Dry Matter | Ash | Pectins | Titratable Acidity * |
|---|---|---|---|---|
| | | % | | |
| J 1 | 24.9 ± 1.23 $^{e}$ | 0.83 ± 0.01 $^{b}$ | 0.57 ± 0.02 $^{c}$ | 1.31 ± 0.12 $^{a}$ |
| J 2 | 27.6 ± 1.29 $^{c}$ | 0.80 ± 0.01 $^{b,c}$ | 0.59 ± 0.01 $^{c}$ | 1.24 ± 0.11 $^{b}$ |
| J 3 | 24.8 ± 1.22 $^{e}$ | 0.96 ± 0.02 $^{a}$ | 0.53 ± 0.01 $^{d}$ | 1.22 ± 0.11 $^{b}$ |
| J 4 | 23.2 ± 1.11 $^{e,f}$ | 0.61 ± 0.01 $^{d}$ | 0.48 ± 0.02 $^{f}$ | 0.98 ± 0.07 $^{c,d}$ |
| J 5 | 23.4 ± 0.99 $^{e,f}$ | 0.74 ± 0.03 $^{c}$ | 0.47 ± 0.02 $^{f}$ | 1.23 ± 0.09 $^{b}$ |
| J 6 | 28.2 ± 1.32 $^{b}$ | 0.73 ± 0.02 $^{c}$ | 0.38 ± 0.01 $^{d}$ | 1.01 ± 0.03 $^{c}$ |
| PEF 1 | 26.7 ± 1.12 $^{c,d}$ | 0.61 ± 0.01 $^{d}$ | 0.82 ± 0.01 $^{b}$ | 1.36 ± 0.12 $^{a}$ |
| PEF 2 | 29.1 ± 0.98 $^{a}$ | 0.74 ± 0.02 $^{c}$ | 0.89 ± 0.02 $^{a}$ | 1.26 ± 0.09 $^{b}$ |
| PEF 3 | 27.3 ± 1.05 $^{c}$ | 0.83 ± 0.01 $^{b}$ | 0.58 ± 0.01 $^{c}$ | 1.21 ± 0.08 $^{b}$ |
| PEF 4 | 25.9 ± 1.32 $^{d}$ | 0.63 ± 0.01 $^{d}$ | 0.50 ± 0.01 $^{e}$ | 1.04 ± 0.04 $^{c}$ |
| PEF 5 | 26.0 ± 0.8 $^{d}$ | 0.75 ± 0.01 $^{c}$ | 0.81 ± 0.02 $^{b}$ | 1.32 ± 0.07 $^{a}$ |
| PEF 6 | 30.0 ± 1.24 $^{a}$ | 0.75 ± 0.03 $^{c}$ | 0.61 ± 0.01 $^{c}$ | 1.06 ± 0.03 $^{c}$ |

* Of malic acid; mean values with different letters (a–f) within same row were statistically different ($p$ = 0.05); values expressed as mean ± standard deviation.

Chokeberry fruits belong to a group of fruits with an average pectin content. The literature on the subject indicates that the pectin content in fresh fruit ranges from 0.30 to 0.75% [30], while in the present study, the pectin content in chokeberry juice ranged from 0.38 to 0.89%. The highest pectin content was present in juice from fruit number 2. Juices treated using PEF had a higher content of pectins.

Research by Trenka et al. [4] showed that the fruit acidity of chokeberries of the 'Galicjanka' cultivar from organic and conventional farms ranged from 0.93 to 1.29%. Results in Table 1 show that the difference in acidity between fresh juice and PEF juice was not statistically significant, except for J 5 and PEF 5. Both types of juice, therefore, had slightly higher acidity than fruit.

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

Antioxidant activity was tested as FRAP and ABTS, and the results are presented in Table 2. Both values were higher in fresh juices than in PEF. The highest antioxidant activity was detected in fresh juice from chokeberry 1 (ABTS—112.58 µmole/100 mL and FRAP—91.12 µmole/100 mL). The lowest antioxidant activity was detected in juice treated using PEF chokeberry 6 (62.00 µmole/100 mL and 43.13 µmole/100 mL), forming one homogeneous group with chokeberry juice 4 (87.79 µmole/100 mL and 54.98 µmole/100 mL). Chokeberry fruit has high antioxidant activity [31,32], which has also been confirmed by results obtained by Trenka et al. [4]. PEF treatment decreased the antioxidant activity of all the juices studied, and these differences were statistically significant. This phenomenon is not fully understood. It seems that it may be related to complex interactions of various chemical compounds in juices. To date, we do not have a definitive explanation for this unsuitable link.

The PEF treatment did not affect photon emission in a statistically significant way, which should be regarded as a potentially favorable phenomenon suggesting the neutrality of the PEF technology with respect to photon emission. The reasons for higher or lower photon emission in food treated using various technologies are currently difficult to ex-

plain and assess unambiguously. This phenomenon may be related to both the so-called spontaneous and induced emission by electrons in an excited state returning to a state with lower energy, which is accompanied by simultaneous photon emission. Ultra-weak luminescence is a new technique for studying food properties. An increasing number of studies report a simple correlation between the high content of bioactive compounds and the number of emitted photons. A higher number of photons is probably associated with the health-promoting properties of foodstuffs [15,16,18]. The number of photons emitted by chokeberry fruit juices has not been studied before, and neither has the effect of PEF treatment on luminescence. The present study shows a very high correlation between photon emission and the biological activity of juices. This correlation applied to both fresh juices and juices treated using PEF. The highest number of emitted photons was determined in chokeberry 1 (416.3). The same chokeberry sample was found to have the highest values of ABTS and FRAP (Table 2) and the highest content of fructose, glucose (Table 3), and total polyphenols (Table 4).

**Table 2.** Antioxidant activity and ultra-weak luminescence in black chokeberry juice.

| | ABTS | FRAP | Ultra-Weak Luminescence |
|---|---|---|---|
| | µmole/100 mL | | - |
| J 1 | 112.58 ± 2.12 $^{a}$ | 91.12 ± 1.02 $^{a}$ | 414.6 ± 32.85 $^{a}$ |
| J 2 | 108.85 ± 3.00 $^{b}$ | 86.44 ± 2.19 $^{b}$ | 400.2 ± 13.18 $^{b}$ |
| J 3 | 99.72 ± 3.17 $^{c}$ | 77.21 ± 2.89 $^{d}$ | 395.4 ± 16.91 $^{c}$ |
| J 4 | 87.79 ± 2.34 $^{e}$ | 54.98 ± 3.01 $^{g}$ | 349.8 ± 26.36 $^{f}$ |
| J 5 | 90.36 ± 1.22 $^{d}$ | 60.24 ± 2.79 $^{f}$ | 364.9 ± 29.78 $^{d}$ |
| J 6 | 86.43 ± 2.12 $^{e}$ | 52.27 ± 1.68 $^{h}$ | 346.4 ± 25.29 $^{g}$ |
| PEF 1 | 109.44 ± 5.24 $^{b}$ | 82.58 ± 2.12 $^{c}$ | 418.9 ± 28.34 $^{a}$ |
| PEF 2 | 95.83 ± 1.54 $^{c}$ | 76.92 ± 2.84 $^{d}$ | 404.9 ± 24.11 $^{b}$ |
| PEF 3 | 82.14 ± 3.69 $^{f}$ | 72.99 ± 2.11 $^{e}$ | 400.7 ± 18.09 $^{c}$ |
| PEF 4 | 72.84 ± 3.39 $^{h}$ | 49.95 ± 2.97 $^{i}$ | 352.2 ± 28.45 $^{e}$ |
| PEF 5 | 79.99 ± 2.82 $^{g}$ | 42.99 ± 1.37 $^{j}$ | 369.2 ± 22.32 $^{d}$ |
| PEF 6 | 62.00 ± 2.43 $^{i}$ | 43.13 ± 2.78 $^{j}$ | 353.9 ± 25.36 $^{e}$ |

ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid; FRAP, ferric reducing antioxidant potential; mean values with different letters (a–j) within same row were statistically different ($p$ = 0.05); values expressed as mean ± standard deviation.

**Table 3.** Sugar content in black chokeberry juice.

| | Fructose | Sorbitol | Glucose | Sucrose | Total |
|---|---|---|---|---|---|
| | g/100 mL | | | | |
| J 1 | 1.54 ± 0.04 $^{d}$ | 1.97 ± 0.03 $^{j}$ | 2.82 ± 0.01 $^{e}$ | 0.00 | 6.33 |
| J 2 | 1.41 ± 0.03 $^{e,f}$ | 3.16 ± 0.01 $^{d}$ | 3.36 ± 0.03 $^{c}$ | 0.00 | 7.93 |
| J 3 | 1.30 ± 0.04 $^{g}$ | 2.90 ± 0.02 $^{e}$ | 3.76 ± 0.04 $^{a}$ | 0.00 | 7.96 |
| J 4 | 1.16 ± 0.01 $^{h}$ | 2.69 ± 0.01 $^{f}$ | 1.87 ± 0.02 $^{g}$ | 0.00 | 5.73 |
| J 5 | 1.01 ± 0.01 $^{i}$ | 2.08 ± 0.01 $^{i}$ | 1.92 ± 0.01 $^{g}$ | 0.00 | 5.01 |
| J 6 | 0.85 ± 0.01 $^{j}$ | 2.20 ± 0.02 $^{d}$ | 2.69 ± 0.02 $^{d}$ | 0.00 | 5.75 |
| PEF 1 | 1.73 ± 0.03 $^{b}$ | 3.65 ± 0.03 $^{c}$ | 3.47 ± 0.02 $^{b}$ | 0.07 ± 0.00 $^{b}$ | 8.92 |
| PEF 2 | 1.63 ± 0.02 $^{c}$ | 4.05 ± 0.04 $^{a}$ | 3.47 ± 0.02 $^{b}$ | 0.10 ± 0.00 $^{a}$ | 9.25 |
| PEF 3 | 2.26 ± 0.02 $^{a}$ | 3.89 ± 0.04 $^{b}$ | 3.51 ± 0.03 $^{b}$ | 0.05 ± 0.00 $^{c}$ | 9.71 |
| PEF 4 | 0.96 ± 0.02 $^{i,j}$ | 2.45 ± 0.02 $^{h}$ | 3.17 ± 0.01 $^{d}$ | 0.00 | 6.59 |
| PEF 5 | 1.18 ± 0.01 $^{h}$ | 2.51 ± 0.01 $^{g}$ | 2.62 ± 0.01 $^{f}$ | 0.00 | 6.30 |
| PEF 6 | 1.46 ± 0.04 $^{e}$ | 2.42 ± 0.02 $^{h}$ | 2.96 ± 0.02 $^{e}$ | 0.00 | 6.85 |

Mean values with different letters (a–j) within same row were statistically different ($p$ = 0.05); values expressed as mean ± standard deviation.

**Table 4.** Comparison of phenolic compounds detected in black chokeberry juice (mg/100 mL).

| Compounds | Juice | | | | | | PEF | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **J 1** | **J 2** | **J 3** | **J 4** | **J 5** | **J 6** | **PEF 1** | **PEF 2** | **PEF 3** | **PEF 4** | **PEF 5** | **PEF 6** |
| | | | | | | **mg/100 mL** | | | | | | |
| | | | | | | Anthocyanins | | | | | | |
| Cyanidin-3-hexoside-(epi)catechine | 3.96 ± 0.29 | 4.17 ± 0.11 | 3.07 ± 0.18 | 2.08 ± 0.15 | 1.97 ± 0.12 | 1.92 ± 0.05 | 4.02 ± 0.41 | 4.24 ± 0.19 | 3.12 ± 0.26 | 2.46 ± 0.17 | 2.06 ± 0.12 | 1.99 ± 0.08 |
| Cyanidin-3-pentoside-(epi)catechine | 1.12 ± 0.09 | 1.18 ± 0.06 | 1.19 ± 0.11 | 1.07 ± 0.04 | 0.98 ± 0.01 | 0.95 ± 0.01 | 1.27 ± 0.03 | 1.24 ± 0.11 | 1.25 ± 0.12 | 1.13 ± 0.08 | 1.06 ± 0.01 | 1.03 ± 0.04 |
| Cyanidin-3-hexoside-(epi)cat-(epi)cat | 9.78 ± 0.79 | 8.95 ± 0.51 | 9.22 ± 0.39 | 8.26 ± 0.43 | 8.14 ± 0.56 | 8.26 ± 0.37 | 9.95 ± 0.92 | 9.13 ± 0.87 | 9.68 ± 0.66 | 8.46 ± 0.83 | 8.28 ± 0.76 | 8.45 ± 0.39 |
| Cyanidin-3-O-galctoside | 606.36 ± 52.09 | 595.22 ± 48.11 | 597.64 ± 47.35 | 577.59 ± 38.39 | 562.98 ± 51.12 | 543.48 ± 49.27 | 616.32 ± 54.26 | 601.67 ± 49.39 | 597.64 ± 44.73 | 586.21 ± 52.39 | 563.31 ± 48.33 | 549.98 ± 53.78 |
| Cyanidin-3-O-glucoside | 21.54 ± 2.09 | 24.98 ± 2.11 | 18.12 ± 1.39 | 19.24 ± 1.34 | 19.53 ± 1.22 | 21.79 ± 1.33 | 21.72 ± 1.34 | 25.04 ± 2.18 | 18.33 ± 1.63 | 19.56 ± 1.66 | 19.68 ± 1.28 | 21.98 ± 1.99 |
| Cyanidin-3-O-arabinoside | 283.99 ± 21.85 | 266.04 ± 19.15 | 228.41 ± 18.62 | 252.98 ± 22.32 | 239.28 ± 21.53 | 238.27 ± 20.69 | 298.14 ± 22.85 | 277.93 ± 21.87 | 232.48 ± 21.45 | 253.46 ± 22.22 | 242.36 ± 22.36 | 240.64 ± 21.84 |
| Cyanidin-3-O-xyloside | 28.99 ± 2.64 | 26.33 ± 2.11 | 28.42 ± 2.39 | 23.02 ± 1.93 | 21.78 ± 1.97 | 23.88 ± 1.14 | 29.02 ± 2.39 | 26.35 ± 2.45 | 28.48 ± 2.32 | 23.11 ± 2.12 | 21.81 ± 1.99 | 23.92 ± 2.34 |
| | | | | | | Phenolic acid | | | | | | |
| Neochlorogenic acid | 159.38 ± 12.97 | 158.39 ± 9.89 | 138.49 ± 10.14 | 124.32 ± 12.08 | 88.29 ± 6.14 | 97.62 ± 3.76 | 166.24 ± 0.02 | 150.77 ± 0.02 | 139.92 ± 0.02 | 125.37 ± 0.02 | 89.86 ± 0.02 | 98.46 ± 0.02 |
| 3-O-p-Coumaroylquinic acid | 6.15 ± 0.32 | 5.74 ± 0.47 | 5.86 ± 0.39 | 5.04 ± 0.36 | 5.08 ± 0.28 | 4.43 ± 0.17 | 6.32 ± 0.54 | 5.84 ± 0.42 | 5.98 ± 0.45 | 5.11 ± 0.37 | 5.12 ± 0.44 | 4.68 ± 0.39 |
| Chlorogenic acid | 91.75 ± 6.77 | 87.09 ± 5.98 | 83.97 ± 7.31 | 73.98 ± 4.35 | 77.23 ± 6.39 | 75.89 ± 3.79 | 91.92 ± 8.31 | 87.29 ± 7.98 | 84.06 ± 7.86 | 74.02 ± 7.12 | 77.48 ± 7.31 | 75.97 ± 7.22 |
| Cryptochlorogenic acid | 5.24 ± 0.44 | 4.51 ± 0.18 | 4.74 ± 0.39 | 3.28 ± 0.26 | 4.03 ± 0.13 | 3.26 ± 0.08 | 5.36 ± 0.54 | 4.54 ± 0.44 | 4.78 ± 0.46 | 3.32 ± 0.24 | 4.12 ± 0.38 | 3.48 ± 0.26 |
| Di- caffeic quinic acid | 1.93 ± 0.09 | 1.75 ± 0.16 | 2.02 ± 0.19 | 2.03 ± 0.17 | 1.92 ± 0.12 | 1.67 ± 0.13 | 2.06 ± 0.19 | 1.83 ± 0.13 | 2.1 ± 0.17 | 2.13 ± 0.16 | 1.98 ± 0.12 | 1.74 ± 0.11 |
| | | | | | | Flavanols | | | | | | |
| Quercetin-dihexoside | 2.76 ± 0.16 | 3.06 ± 0.24 | 3.11 ± 0.28 | 1.96 ± 0.14 | 2.39 ± 0.15 | 2.46 ± 0.22 | 2.81 ± 0.18 | 3.16 ± 0.24 | 3.17 ± 0.29 | 2.03 ± 0.16 | 2.42 ± 0.21 | 2.48 ± 0.17 |
| Quercetin-3-O-vicianoside | 3.89 ± 0.28 | 4.28 ± 0.36 | 4.56 ± 0.39 | 3.38 ± 0.25 | 3.12 ± 0.29 | 3.09 ± 0.21 | 4.04 ± 0.27 | 4.32 ± 0.36 | 4.59 ± 0.42 | 3.42 ± 0.28 | 3.15 ± 0.15 | 3.12 ± 0.25 |
| Quercetin 3-robinobioside | 2.23 ± 0.14 | 2.33 ± 0.18 | 2.15 ± 0.12 | 1.44 ± 0.09 | 1.47 ± 0.09 | 1.59 ± 0.08 | 2.27 ± 0.21 | 2.36 ± 0.19 | 2.18 ± 0.09 | 1.47 ± 0.08 | 1.51 ± 0.07 | 1.62 ± 0.05 |
| Quercetin 3-O-rutinoside | 9.87 ± 0.06 | 10.45 ± 0.94 | 9.56 ± 0.64 | 8.26 ± 0.36 | 9.09 ± 0.74 | 8.76 ± 0.62 | 9.92 ± 0.36 | 10.51 ± 0.83 | 9.59 ± 0.64 | 8.28 ± 0.28 | 9.12 ± 0.85 | 8.82 ± 0.34 |
| Quercetin-3-O-galctoside | 31.99 ± 0.20 | 37.99 ± 2.11 | 35.97 ± 3.12 | 30.83 ± 2.96 | 30.56 ± 2.1 = 83 | 31.96 ± 2.67 | 32.05 ± 2.99 | 38.12 ± 3.18 | 36.03 ± 2.86 | 30.88 ± 2.56 | 30.62 ± 2.44 | 31.99 ± 2.78 |
| Quercetin-3-O-glucoside | 26.79 ± 0.13 | 26.21 ± 1.43 | 26.14 ± 2.04 | 22.94 ± 1.98 | 22.37 ± 1.66 | 23.02 ± 1.94 | 26.92 ± 1.37 | 26.33 ± 1.43 | 26.38 ± 2.13 | 23.12 ± 1.75 | 22.58 ± 1.55 | 23.16 ± 1.83 |
| Isorhamnetin pentosylhexoside | 5.81 ± 0.44 | 5.67 ± 0.48 | 5.91 ± 0.44 | 5.36 ± 0.46 | 5.39 ± 0.51 | 5.42 ± 0.42 | 5.89 ± 0.34 | 5.62 ± 0.47 | 6.05 ± 0.54 | 5.42 ± 0.49 | 5.48 ± 0.46 | 5.54 ± 0.44 |
| Isorhamnetin rhamnosylhexosideisomer | 2.95 ± 0.22 | 2.91 ± 0.19 | 2.83 ± 0.21 | 2.86 ± 0.20 | 2.61 ± 0.18 | 2.75 ± 0.17 | 3.02 ± 0.29 | 2.99 ± 0.22 | 2.92 ± 0.21 | 2.9 ± 0.16 | 2.69 ± 0.19 | 2.82 ± 0.15 |

**Table 4.** *Cont.*

| Compounds | Juice | | | | | | PEF | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **J 1** | **J 2** | **J 3** | **J 4** | **J 5** | **J 6** | **PEF 1** | **PEF 2** | **PEF 3** | **PEF 4** | **PEF 5** | **PEF 6** |
| | | | | | | **mg/100 mL** | | | | | | |
| | | | | | | Flavan-3-ole | | | | | | |
| (+) Catechin | 17.67 ± 1.33 | 17.78 ± 1.18 | 17.99 ± 0.92 | 15.78 ± 1.12 | 15.75 ± 0.99 | 16.21 ± 0.86 | 17.98 ± 0.02 | 17.76 ± 0.39 | 17.82 ± 0.39 | 15.94 ± 0.39 | 15.98 ± 0.39 | 16.46 ± 0.39 |
| Procyanidin B2 | 3.78 ± 0.22 | 3.27 ± 0.18 | 3.29 ± 0.32 | 3.03 ± 0.26 | 3.09 ± 0.24 | 3.06 ± 0.31 | 3.94 ± 0.31 | 3.53 ± 0.29 | 3.43 ± 0.30 | 3.09 ± 0.26 | 3.11 ± 0.29 | 3.08 ± 0.24 |
| (−) Epicatechin | 154.94 ± 13.33 | 158.52 ± 12.96 | 163.12 ± 14.39 | 153.89 ± 15.01 | 152.86 ± 13.33 | 153.86 ± 14.88 | 156.44 ± 15.12 | 159.99 ± 15.36 | 165.84 ± 15.88 | 154.32 ± 14.43 | 153.12 ± 14.88 | 153.24 ± 14.32 |
| Procyanidin polymers | 908.22 ± 69.09 | 889.95 ± 71.11 | 909.85 ± 81.75 | 828.38 ± 79.44 | 827.46 ± 78.98 | 825.39 ± 76.19 | 921.83 ± 91.04 | 892.13 ± 85.11 | 914.36 ± 89.17 | 832.32 ± 71.99 | 833.87 ± 72.67 | 839.31 ± 79.82 |
| | | | | | | Flavonones | | | | | | |
| Eriodictyol-glucuronide | 43.93 ± 2.98 | 47.12 ± 4.07 | 44.99 ± 3.32 | 38.22 ± 2.43 | 39.08 ± 3.16 | 40.56 ± 3.95 | 44.01 ± 3.96 | 47.19 ± 3.33 | 45.04 ± 3.85 | 38.34 ± 2.66 | 39.16 ± 2.43 | 40.82 ± 3.18 |
| Total | 2435.02 [a] | 2393.89 [b] | 2350.62 [c] | 2209.22 [d] | 2146.45 [e] | 2139.55 [e] | 2483.46 [a] | 2409.88 [b] | 2365.22 [c] | 2225.87 [d] | 2159.93 [e] | 2164.78 [e] |

Mean values with different letters (a–e) within same row were statistically different ($p = 0.05$); values expressed as mean ± standard deviation.

### 3.2. Analysis of Sugars

The content of sugar and its types depends on the cultivar, weather conditions during harvest time, and the degree of ripeness of the fruit. Therefore, fruits of the same species and cultivar may present differing total sugar values. Depending on their ripeness, fruits may also contain different amounts of sugars.

The samples were analyzed in terms of the content of three sugars—fructose, sorbitol, and glucose. Additionally, in three juices obtained that were subjected to PEF treatment, we also determined trace amounts of sucrose (Table 3). Other studies indicate the presence of fructose, glucose, and sorbitol in fresh chokeberries and chokeberry juice [29,30,33]. Denev et al. [33], apart from fructose, glucose, and sorbitol, also determined sucrose. In our present study, the higher content of sugar in chokeberry juice was glucose (1.87–3.76 g/100 mL in fresh juices and 2.62–3.51 g/100 mL in juices subjected to PEF treatment) with the exception of two samples, PEF 2 and PEF 3, whereby determination indicated more sorbitol. As you can see, the total sugar content increased after PEF processing, but the value varied significantly between results, and at this stage of the study, no reason for this change was found. It seems, however, that the increased dry matter content after PEF treatment resulted in higher sugar content values. The course of the impact of the PEF process on the content of sugars and their passing in juices has not yet been thoroughly investigated.

Lachowicz et al. [34] showed that the sugar content of chokeberry juices obtained using different enzyme products was slightly higher than in the present study. This may be due to the place of cultivation, a sunnier ripening period, later harvest, and the method by which the juices were made.

### 3.3. Comparison of Phenolic Compounds Detected in Juice of Black Chokeberry

The results in Table 4 showed no significant difference between fresh juice and PEF juice, and again this small difference could be related to the difference in dry matter content. Growing conditions had an impact on the content of particular fractions of polyphenols. Juices obtained from samples 1–3 contained more total polyphenols. Chokeberries from cultivation 1 had the highest number of polyphenols, 2435.02 mg/100 mL, while juice from fruits from conventional cultivation had the lowest amount: 6–2139.55 mg/100 mL. As in the work of Ternka et al. [4], the content of individual fractions was as follows: anthocyanins > procyanidin polymers >> phenolic acids $\geq$ flavonols > flavan-3-ols > flavanone. The content of polyphenols in fruit and later in juices is strongly influenced by atmospheric conditions. Although the plantations from which chokeberries were sourced were located in one region, there were small local differences in the number of rainy and sunny days, which was reflected in differences in particular polyphenol fractions content.

The most important group of identified polyphenolic compounds in our juice samples were anthocyanins (seven cyanidin derivatives), which accounted for about 40% of total polyphenols (Table 4). The predominant compound was cyanidin-3-O-galactoside, whose content ranged from 543.48 to 606.36 mg/100 mL, and in PEF-treated juices from 549.98 to 616.32 mg/100 mL. It follows that PEF treatment slightly increased the content of cyanidin-3-O-galactoside analogously to the other anthocyanins.

The second-largest group of compounds present in our chokeberry juice samples was procyanidin polymers, which accounted for ~35% of all polyphenolic compounds. They cause the tart taste of chokeberry juices and, at the same time, constitute an important group of prohealth compounds. These compounds are also interesting for their strong antioxidant activity and health benefits, as they may have, for example, anticancer and antiproliferative effects [9].

Another important group of compounds was phenolic acids (Table 4). Based on ultra-performant liquid chromatography techniques, five phenolic acids were identified in the samples. The dominant compound was chlorogenic acid, and its determined content in the juice samples ranged from 88.29 to 159.38 mg/100 mL in fresh juices and slightly more in PEF-treated juices: from 89.86 to 166.24 mg/100 mL. These results correspond to

those obtained by Trenka et al. [4] in fresh chokeberry fruit from organic and conventional cultivation. In addition, analogous correlations were reported in the study on various chokeberry fruit products by Oszmiański and Lachowicz [32]. Flavonols are another group of polyphenolic compounds determined in fresh and PEF-treated chokeberry juices. Eight flavonols were identified in the samples, including six quercetin derivatives and two isorhamnetin derivatives.

Flavan-3-ols were obtained for ~7.0% of the total polyphenolic compound content in our juices. Three compounds were identified in the samples, two monomers ((+)—catechins and (-)—epicatechins) and dimer—procyanidin B2 (Table 4). The biggest content was determined for (-)epicatechin, which was fairly stable irrespective of the place of chokeberry cultivation, which the effect of PEF treatment on juice found to be insignificant.

## 4. Conclusions

Our research confirmed previous research results indicating that black chokeberry juices are rich in bioactive compounds and preserves with high health properties. The treatment of juices with a pulsed electromagnetic field (PEF) as a method of preservation did not have a negative effect on the content of bioactive compounds and slightly increased it. In fresh and preserved juices, there was a clear difference in the content of bioactive compounds depending on the place of the crop. The PEF method offers new possibilities for application in the food industry of the nonthermal preservation of juices with high-quality preservation of health compounds.

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