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Microwave-assisted extraction of turkish *Aronia melanocarpa* fruit: modeling, optimization and characterization of commercial products

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Abstract: *Aronia melanocarpa* (black chokeberry) is a fruit rich in phenolic compounds and well-known for its potent antioxidant capacity; however, comparative investigations comparing fresh berries and their processed counterparts remain scarce. In this work, microwave-assisted extraction (MAE) was optimized *via* response surface methodology (RSM) to enhance the recovery of antioxidant constituents from *Aronia* fruits cultivated in Türkiye. Extraction time (5–30 min), temperature (40–100 °C), solvent-to-solid ratio (mL/0.1 g dry sample, DS), and solvent composition (20–100%) were systematically modeled, while antioxidant performance was assessed using the CUPRAC assay. The optimal conditions (100 °C, 24 min, 34% ethanol, 14 mL per 0.1 g DS) yielded a maximum TAC value of 0.998 mmol TE/g DS. Both raw fruits and commercial preparations (juice, concentrate, dried fruit, jam, vinegar, gummies, herbal capsules) were then characterized by spectrophotometric assays (CUPRAC, Folin–Ciocalteu, pH differential) and by chromatographic analysis (HPLC-PDA). Major anthocyanins—including cyanidin-3-*O*-xyloside, cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-glucoside, and cyanidin-3-*O*-galactoside—and phenolics such as chlorogenic acid, neochlorogenic acid, epicatechin, and gallic acid were identified and quantified. Substantial compositional differences were observed between fresh fruit and processed products, with pronounced losses of anthocyanins attributed to industrial processing. This study provides the first integrated assessment of Turkish *Aronia* fruits and related commercial products, by uniting MAE optimization with comprehensive antioxidant and phytochemical profiling, and offering new insights into their nutritional and functional potential.

Keywords: *Aronia melanocarpa*; response surface methodology; microwave-assisted extraction; antioxidant capacity; anthocyanins; HPLC-PDA.

1. Introduction

The shrub-like species *Aronia melanocarpa*, belonging to the Rosaceae family, is cultivated for fruit production. Commonly known as "chokeberry," it has two well-recognized species: *Aronia arbutifolia* (red chokeberry) and *Aronia melanocarpa* (black chokeberry). Their fruits are widely used in different regions of Europe for the production of fruit syrup, juice, soft marmalades, jams, extracts, and teas [1,2]. In Türkiye, *Aronia* cultivation began in 2012, and due to its rich chemical composition and high antioxidant activity, the fruit has been regarded as a functional food, with increasing global popularity in both usage and cultivation. Because of its significant antioxidant activity, it plays a role in the prevention and treatment of many chronic and degenerative diseases [3-6]. Interest in the health effects of anthocyanins has increased over time, and *Aronia* fruits, with their high anthocyanin content, have been shown to exhibit substantial protective and beneficial effects on human health, based on their antioxidant and other biological activities [7].

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Anthocyanins, which give *Aronia* fruits their characteristic color, along with other phenolic compounds, are the major bioactive constituents responsible for the plant's biological activities. Examination of their chemical composition reveals that proanthocyanidins, predominantly (−)-epicatechin, constitute approximately 66% of the total polyphenol content of *Aronia* berries [8]. Fruits of *Aronia melanocarpa* represent a notable reservoir of polyphenolic constituents, particularly anthocyanins such as cyanidin-3,5-*O*-diglucoside, cyanidin-3-*O*-xyloside, cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-glucoside, and cyanidin-3-*O*-galactoside, in addition to flavanols, procyanidins (catechin, epicatechin), and phenolic acids like chlorogenic and neochlorogenic acids [3,4,9]. In addition to being consumed as a nutritionally rich fruit, it has also been used in traditional herbal medicine. Numerous studies have reported on the therapeutic potential of *Aronia* berries, highlighting not only their antioxidant activity but also their anti-inflammatory, antidiabetic, anticancer, antimutagenic, and antibacterial properties, as well as their potential benefits against obesity, cardiovascular diseases, and autoimmune disorders [1,10].

Free radicals may be generated as a result of normal energy metabolism in the body or due to various environmental factors (e.g., radiation, smoking, certain drugs, environmental pollution). While reactive oxygen and nitrogen species are necessary for several physiological functions, excessive production or impaired elimination of these radicals disrupts the natural balance and leads to oxidative stress. Oxidative stress is regarded as an important contributor to the progression of degenerative and chronic disorders, including cardiovascular diseases, cataracts, autoimmune conditions, cancer, aging, and neurodegenerative diseases such as Alzheimer's [11]. Compounds with antioxidant properties play a crucial role in protection against such conditions and in repairing the associated damage. To assess antioxidant activity, various *in vitro* methods based on radical scavenging capacity and metal-reducing power have been developed. *Aronia* berries, being rich in phenolics such as anthocyanins and flavonoids, have been extensively evaluated in this context and consistently shown to possess remarkable antioxidant activity (Table 1).

Table 1. Antioxidant potential of *Aronia* berries determined by different assays.

Method	Result	Reference
Free Radical Scavenging Activity (DPPH)	1.8 (EC_{50})	[12]
	36.3 g/kg (TE), (DW)	[13]
CUPRAC	257.2 g/kg (TE), (DW)	[13]
ABTS	7.4 g/kg (TE)	[13]
ORAC	160.2 μmol/g (TE)	[14]
FRAP	36.64 mM/100 g (TE), (DW)	[15]
Lipophilic ORAC Method	2.42 μmol/g (TE)	[16]
Hydrophilic ORAC Method	158.2 μmol/g (TE)	[16]

EC_{50}: The amount of fruit (mg) required to scavenge 50% of the initial DPPH•; DPPH: 1,1-Diphenyl-2-picrylhydrazyl; CUPRAC: Cupric ion reducing antioxidant capacity; ORAC: Oxygen radical absorbance capacity, FRAP: Ferric Reducing Antioxidant Power; TE: Trolox equivalent; DW: dry weight.

Aronia (*Aronia melanocarpa*) has gained significant scientific interest among various fruit species because of its diverse protective properties and promising health-promoting potential. Moreover, the consumption of *Aronia*-based products (juice, tea, fruits, etc.) has increasingly become part of a healthy lifestyle. Owing to its rich chemical composition and strong antioxidant activity, it is classified as a functional food, and its cultivation and use are expanding worldwide. Although numerous scientific studies have been conducted to determine the phytochemical composition of *Aronia* fruit, studies focusing on the comparison of commercial herbal products remain rather limited. Therefore, assessing the authenticity of such products and verifying whether they truly contain the bioactive components of the claimed plant is of great importance for public health. In this context, *Aronia* fruits cultivated in Türkiye and their commercial derivatives (dried *Aronia*, juice, concentrate, jam, herbal capsules, etc.) were evaluated using spectrophotometric methods (CUPRAC, Folin–Ciocalteu, and pH differential) and HPLC-PDA.

2. Experimental

2.1. Materials and Instruments

The chemicals used in this work were obtained from standard suppliers: Copper(II) chloride dihydrate (98%, $CuCl_2$), neocuproin (Nc), ammonium acetate (NH_4Ac), sodium carbonate (Na_2CO_3), sodium potassium tartarate ($NaKC_4H_4O_6$), copper sulfate ($CuSO_4$), methanol (MeOH), ethanol (EtOH), acetonitrile (ACN), phosphoric acid (o-H_3PO_4), NaOH, HCl, sodium acetate trihydrate ($NaCH_3COOH{\cdot}3H_2O$), and were purchased from Merck (Darmstadt, Germany). Cyanidin-3-*O*-glucoside chloride (cy-3-glc) was provided by Extrasynthese (Genay, France). Potassium chloride (KCl) was obtained from Riedel-de-Haën (Germany) while formic acid was supplied by Sigma-Aldrich (Steinheim, Germany). Milli-Q deionized water (Millipore, Bedford, USA) was used for the preparation of all reagents and calibration solutions.

Spectrophotometric analyses were performed with a UV-1900i UV–Vis spectrophotometer (Shimadzu, Japan, spectral resolution ≈1 nm). Chromatographic analyses were conducted on a Waters Breeze 2 HPLC system (Milford, MA, USA). The instrument was configured with a 2998 photodiode array (PDA) detector (Chelmsford, MA, USA), a 1525 binary pump, and a column oven with temperature control. Separations were performed on a Zorbax C8 column (150 × 4.6 mm, 5 µm, USA), and sample injections (25 µL) were introduced using a Hamilton syringe (Reno, NV, USA). Data collection and processing were managed through Empower PRO software (Waters Associates, Milford, MA).

Aronia and commercial products (juice, dried *Aronia*, concentrate, jam, herbal capsules, etc.) grown in Türkiye were procured from local companies. The *Aronia* berries used in the experimental studies were freeze-dried and ground until homogeneous. Samples were sieved (<300 µm) to obtain the desired ground product size. Dry-ground samples were extracted and used under the specified optimum conditions. Liquid samples were diluted with 30% ethanol. All extracts were filtered through 0.45 µm membranes (Chromafil GF/PET 45/25) and stored at +4 °C until analysis.

2.2. Microwave-Assisted Extraction (MAE)

MAE can be carried out in either open-vessel or closed-vessel setups. In this study, the closed-vessel approach was preferred. Within this system, temperature regulation was achieved by an infrared sensor, and the equipment automatically adjusted its power output (0–1500 W) to maintain constant operating conditions. Compared to open-vessel techniques, the closed-vessel MAE configuration offers significant advantages, such as the ability to operate above the solvent's normal boiling point, improved extraction efficiency, reduced processing time, and the capacity to treat several samples in parallel [17]. The extractions were performed using a Milestone Ethos Easy closed-vessel apparatus. To isolate antioxidant compounds from *Aronia* fruits, various experimental parameters were systematically investigated. These parameters included solvent composition (20–100%), type of solvent (ethanol, methanol, or water), extraction time (5–30 min), extraction temperature (40–100 °C), and the solvent-to-solid ratio (10–30 mL per 0.1 g sample). The influence of these variables on the efficiency of extraction was evaluated, and the optimized conditions were subsequently modeled to describe the process in detail.

2.3. Spectrophotometric Analyses

2.3.1. TAC of Aronia Samples with CUPRAC Assay

The total antioxidant capacity (TAC) of the samples was determined by the CUPRAC assay (cupric ion reducing antioxidant capacity) as originally introduced by Apak *et al*. (2004) [18]. For the test, 1 mL each of 1.0×10^{-2} Cu(II) solution, 7.5×10^{-3} M neocuproine (Nc) solution, and 1 M ammonium acetate (NH_4Ac) buffer were successively pipetted into a test tube. Then, x mL of the extract and (1.1 – x) mL of solvent were added to bring the total reaction volume to 4.1 mL, and the mixture was vortexed. The tubes were left capped at room temperature for 30 min, after which the absorbance was read at 450 nm against a blank without sample. TAC values were calculated and expressed as mg TE per g sample (mg TE/g), using the molar extinction coefficient (ε_{TR}) of the Trolox reference standard.

$$TAC\ (mg\ TE/g) = \left(\frac{A}{\varepsilon_{TR}}\right) x\ \left(\frac{V_{total}}{V_{sample}}\right) x\ D.F. x\ \left(\frac{V_{extract}}{m_{sample}}\right) x\ MW_{TR} \quad (1)$$

2.3.2. TPC of Aronia Samples with Folin-Ciocalteu Method

The total phenolic content (TPC) of the extracts was determined by employing the Folin–Ciocalteu assay originally described by Singleton et al. (1999) [19]. For each measurement, *x* mL of the sample solution and 2.5 mL of Lowry C reagent (prepared from 50 mL of Lowry A containing 2% Na_2CO_3 in 0.1 M NaOH and 1 mL of Lowry B containing 0.5% $CuSO_4$ in 1% $NaKC_4H_4O_6$) were transferred into the test tube, followed by the addition of $(2 - x)$ mL of deionized water. After allowing the mixture to stand for 10 min, 0.25 mL of Folin reagent (prediluted with water in a 1:3 ratio) was added. The tubes were then kept closed at room temperature for 30 min, followed by absorbance measurement at 750 nm against a blank solution. The TPC results were expressed as mg gallic acid equivalents per g sample (mg GE/g), using the molar extinction coefficient (ε_{GA}) determined for gallic acid as the calibration standard.

$$TPC\ (mg\ GE/g) = \left(\frac{A}{\varepsilon_{GA}}\right) x\ \left(\frac{V_{total}}{V_{sample}}\right) x\ D.F. x\ \left(\frac{V_{extract}}{m_{sample}}\right) x\ MW_{GA} \quad (2)$$

2.3.3. TAnC of Aronia Samples with pH Differential Method

The total anthocyanin content (TAnC) of the extracts was assessed using the pH differential method. Anthocyanins are known to undergo reversible structural conversions, which produce distinct absorption characteristics at different pH levels. In highly acidic media (pH 1.0), the oxonium ion predominates as the colored form, whereas at pH 4.5 the pigment largely exists in a nearly colorless hemiketal structure [20]. Following the procedure described by Wrolstad et al. (2005), each sample was diluted with buffer solutions at the two pH values, and the absorbance at the λ_{max} of the pH 1.0 solution was recorded. The difference in absorbance between the two buffered states was then used to calculate the monomeric anthocyanin content. The calculation employed the molecular weight (MW = 449.2 g/mol) and molar extinction coefficient ($\varepsilon = 2.69 \times 10^4$ L/mol cm) of cyanidin-3-glucoside (cy-3-glc), the most representative anthocyanin pigment, and results were expressed as cyanidin-3-glucoside equivalents (CGE) [21]. For the assay, extracts were diluted tenfold with buffers at pH 1.0 and at pH 4.5. After incubation for 15 min, absorbance values were measured at 520 and 700 nm against distilled water, and the TAnC values were calculated accordingly.

$$Absorbance = (A_{520} - A_{700})_{pH\ 1} - (A_{520} - A_{700})_{pH\ 4.5} \quad (3)$$

$$TAnC\ (mg\ CGE\ /g) = \left(\frac{A}{\varepsilon_{Cy-3-glc}}\right) x\ \left(\frac{V_{total}}{V_{sample}}\right) x\ D.F. x\ \left(\frac{V_{extract}}{m_{sample}}\right) x\ MW_{Cy-3-glc} \quad (4)$$

$$TAnC\ (mg\ CGE/L) = \left(\frac{A}{\varepsilon_{Cy-3-glc}}\right) x\ \left(\frac{V_{total}}{V_{sample}}\right) x\ D.F. x\ MW_{Cy-3-glc}\ x\ 1000 \quad (5)$$

2.4. Chromatographic Analysis

To quantify the target phenolic constituents and anthocyanins, reversed-phase HPLC-PDA analysis was conducted under a gradient elution program (Table 2). Before injection, extracts were diluted with 0.02% HCl. The analyses were carried out at a flow rate of 0.6 mL/min, with spectral monitoring across 210–600 nm, using a binary solvent system composed of acetonitrile (A) and 0.2% o-H_3PO_4 (B). The injection volume was set to 20 µL, and the column was thermostated at 35 °C.

Table 2. Gradient elution program used for HPLC-PDA analysis.

Time (min)	Flow rate (mL/min)	A% (Acetonitrile)	B% (0.2% o-H_3PO_4)
0	0.6	5	95
5	0.6	10	90
20	0.6	15	85
30	0.6	40	60
35	0.6	60	40
40	0.6	5	95

2.5. Statistical Evaluation

A face-centered composite design (FCCD) was applied with the aid of Design-Expert® Software v11 (Stat-Ease Inc., Minneapolis, USA) in order to optimize the extraction conditions and assess the influence of the studied factors, namely temperature (A), extraction time (B, min), solvent composition (C, %), and solid-to-solvent ratio (D, mL/0.1 g dry sample, DS), with the total antioxidant capacity (TAC, mmol TE/g DS) of powdered *Aronia* set as the response variable; the tested factors and their coded levels are presented in Table 3. Statistical calculations of means and standard deviations were carried out using Microsoft Excel (Office 2002), and results are reported as mean ± standard deviation (SD). Moreover, significance testing of the data was performed through two-way ANOVA implemented in SPSS for Windows (version 13). In addition, both Pearson and Spearman correlation analyses were performed to evaluate the agreement and concordance among spectrophotometric methods.

Table 3. Operational factors for RSM, their corresponding values, and the coded symbols representing these factors.

Operational factor	Units	Symbol	Levels		
			-1	0	1
Temperature	°C	A	40	70	100
Time	min	B	5	17.5	30
Solvent composition	%	C	0	50	100
Solvent-to-solid ratio	mL/0.1 g	D	10	15	20

3. Results and Discussion

3.1. RSM Results for the Optimization of MAE of Aronia

To enhance the recovery of bioactive compounds from *Aronia melanocarpa*, MAE was applied, and the experimental conditions were optimized through RSM. In these optimization trials, the total antioxidant capacity (TAC) determined by the CUPRAC assay was chosen as the response parameter. Before establishing the RSM model, preliminary tests compared EtOH and MeOH as solvents. Since EtOH produced the highest extraction efficiency, the EtOH-to-H_2O ratio was selected as a critical variable in the modeling process.

The influence of independent parameters on the TAC values of powdered *Aronia* samples is presented in Table 4. The regression model for TAC yielded a *p*-value below 0.0001, confirming that the response was strongly associated with the tested variables. Among these, solvent composition was identified as the dominant factor shaping TAC outcomes. According to Table 5, the relevance of each coefficient was evaluated through the F-test and its corresponding *p*-values, where greater absolute F-values combined with lower *p*-values indicated higher significance of the variables. Collectively, these findings demonstrate that the model was statistically valid at the 95% confidence level, and the ANOVA analysis supported the use of a second-order model to describe the response.

The model produced predicted and adjusted R^2 values of 0.7541 and 0.9096, respectively. Since the gap between these two was below 0.2, the predictive performance of the model was considered

consistent with the adjusted outcome. Adequate precision, which evaluates the signal-to-noise ratio, was calculated as 14.299—well above the minimum acceptable value of 4—indicating that the model possessed a strong signal and could be used with confidence. In addition, the F-value was 21.84, confirming the overall statistical significance of the regression, with only a 0.01% probability that such a high value could be attributed to random error. Figure 1 compares the experimental TAC data with the values predicted by the model, showing close agreement between the two sets. Figure 2 displays three-dimensional surface plots illustrating how operational factors interact to influence TAC. The optimal extraction conditions predicted by the model corresponded to a TAC yield of 0.998 mmol TE/g DS, obtained at 100 °C (A), 24 min (B), 34% solvent composition (C), and a solvent-to-solid (SS) ratio of 14 mL per 0.1 g DS (D).

Table 4. Experimental TAC values obtained under the FCCD design of independent parameters for MAE.

No	Factors				TAC*
	A	B	C	D	mmol TE/g DS
1	40	5	0	10	0.438
2	100	5	0	10	0.641
3	40	30	0	10	0.606
4	100	30	0	10	0.902
5	40	5	100	10	0.326
6	100	5	100	10	0.336
7	40	30	100	10	0.431
8	100	30	100	10	0.475
9	40	5	0	20	0.617
10	100	5	0	20	0.700
11	40	30	0	20	0.516
12	100	30	0	20	0.855
13	40	5	100	20	0.310
14	100	5	100	20	0.402
15	40	30	100	20	0.353
16	100	30	100	20	0.443
17	40	17.5	50	15	0.830
18	100	17.5	50	15	0.993
19	70	5	50	15	0.801
20	70	30	50	15	0.86
21	70	17.5	0	15	0.666
22	70	17.5	100	15	0.629
23	70	17.5	50	10	0.727
24	70	17.5	50	20	0.920
25	70	17.5	50	15	0.851
26	70	17.5	50	15	0.870
27	70	17.5	50	15	0.858
28	70	17.5	50	15	0.851
29	70	17.5	50	15	0.862
30	70	17.5	50	15	0.851

*DS: dried sample

Table 5. ANOVA for the quadratic FCCD model describing TAC.

	Sum of Squares	df	Mean Square	F-value	*p*-value
Model	1.25	14	0.0894	21.84	< 0.0001
A (Temperature)	0.0968	1	0.0968	23.66	0.0002
B (Extraction time)	0.0420	1	0.0420	10.28	0.0059
C (Solvent composition)	0.2778	1	0.2778	67.88	< 0.0001
D (SS ratio)	0.0030	1	0.0030	0.7434	0.4021
AB	0.0091	1	0.0091	2.22	0.1572
AC	0.0293	1	0.0293	7.17	0.0172
AD	0.0002	1	0.0002	0.0397	0.8447
BC	0.0015	1	0.0015	0.3670	0.5537
BD	0.0179	1	0.0179	4.37	0.0540
CD	0.0016	1	0.0016	0.3959	0.5387
A^2	0.0011	1	0.0011	0.2732	0.6088
B^2	0.0094	1	0.0094	2.30	0.1504
C^2	0.1533	1	0.1533	37.46	< 0.0001
D^2	0.0117	1	0.0117	2.86	0.1114
Residual	0.0614	15	0.0041		
Lack of Fit	0.0611	10	0.0061	100.84	< 0.0001
Pure Error	0.0003	5	0.0001		
Total Correlation	1.31	29			

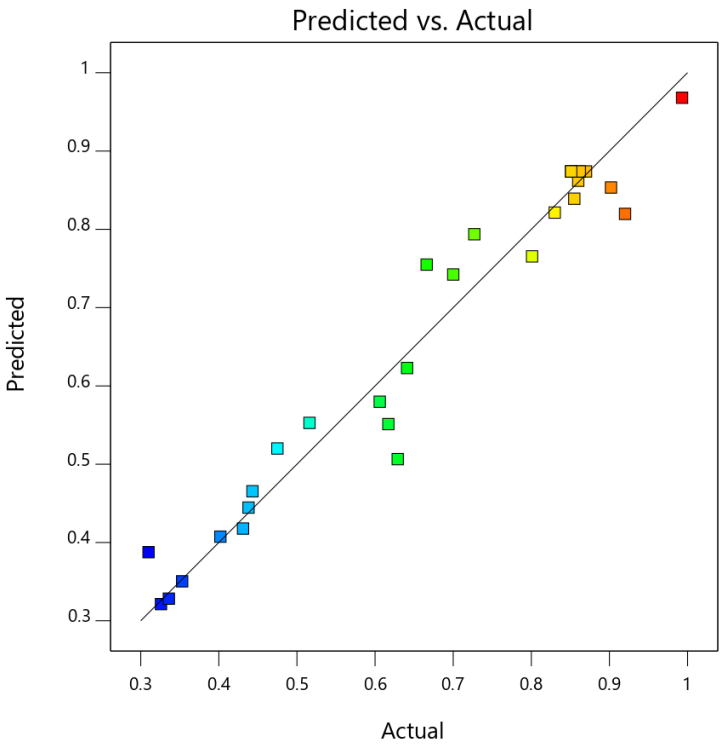

Figure 1. Correlation between predicted and actual TAC values.

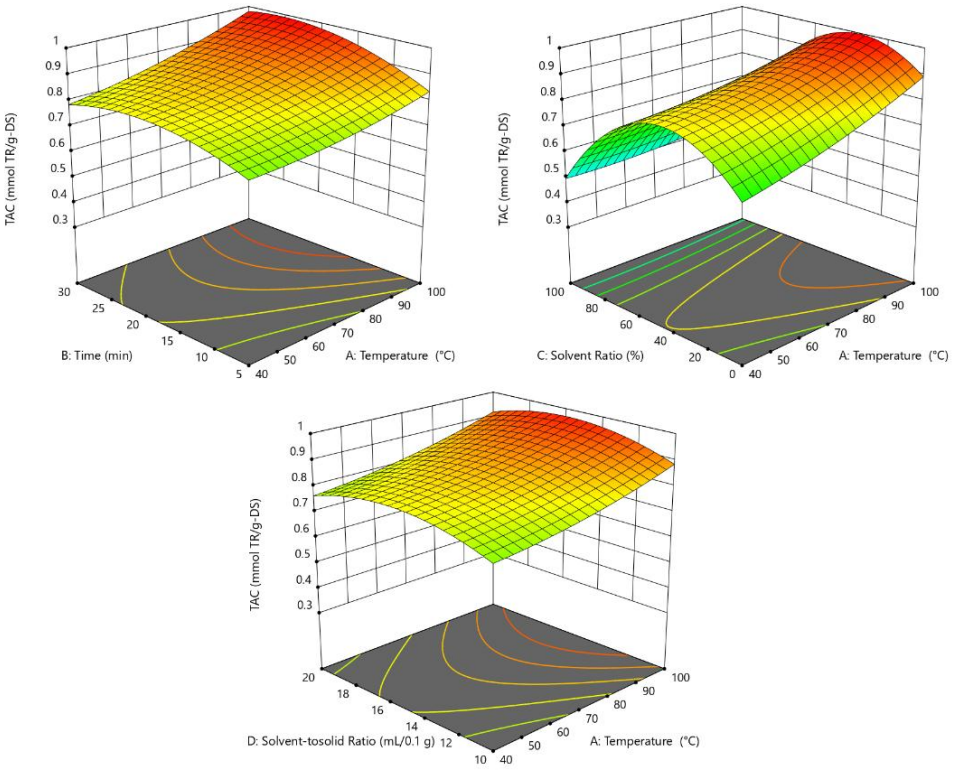

Figure 2. Three-dimensional plots of TAC of the extract as a function of operational factors.

3.2. Comparative Spectrophotometric Analysis of Antioxidant Capacity, Phenolics, and Anthocyanins in Aronia Samples

The calibration characteristics of the investigated methods, comprising linear range, regression parameters, correlation coefficients, and sensitivity values (LOD and LOQ), are given in Table 6 to demonstrate the performance criteria of the applied methods. The spectrophotometric findings for the raw *Aronia* sample and its commercial derivatives are summarized in Table 7. The parameters assessed included TAC (CUPRAC method), TPC (Folin–Ciocalteu method), and TAnC (pH differential method).

Table 6. Calibration and performance criteria of the applied methods.

Method	Calibrant	Range (M)	Slope (S) (L/mol cm	Intercept	r^2	LOD (µM)	LOQ (µM)
pH differential (TAnC)	Cy-3-glc (CGE)	$4.0\times10^{-6} – 6.1\times10^{-4}$	2.69×10^{4}	0.01	0.9997	0.18	0.60
CUPRAC (TAC)	Trolox (TE)	$3.2\times10^{-6} – 8.4\times10^{-5}$	1.67×10^{4}	0.03	0.9999	0.89	2.94
Folin-Ciocalteu (TPC)	Gallic acid (GE)	$5.8\times10^{-6} – 2.9\times10^{-4}$	5.31×10^{3}	0.04	0.9997	3.39	11.19
HPLC (total CGE)	Cy-3-glc	$1.0\times10^{-5} – 1.2\times10^{-4}$	2.38×10^{10}	2.08×10^{4}	0.9990	1.81	5.99

The raw *Aronia* sample exhibited markedly higher values (TAC: 217.76 mg TE/g, TPC: 168.3 mg GE/g, TAnC: 21.29 mg CGE/g) compared to the commercial formulations. Among processed products, dried *Aronia* retained relatively high levels of bioactive compounds (TAC: 197.73 mg TE/g, TPC: 147.9 mg GE/g, TAnC: 15.57 mg CGE/g), whereas effervescent tablets, dietary capsules, gummies, and fruit spreads (jam and marmalade) demonstrated drastically reduced values, indicating substantial

losses during processing. Beverages such as juice and concentrate preserved higher levels of anthocyanins, with the concentrate in particular yielding notably elevated TAnC (284.02 mg CGE/L), consistent with the concentration effect. In contrast, vinegars contained only modest levels of phenolics and anthocyanins.

When compared with literature data, the TAC value of the dried *Aronia* sample in the present study (197.73 mg TE/g) appears somewhat lower than the CUPRAC results reported by [13], who measured 257.2 and 233.2 g TE/kg for dried *Aronia*. Similarly, the TPC values obtained (147.9 mg GE/g) were within the broad range (127–197 mg GE/g) reported by Taheri *et al.* [22]. However, the total anthocyanin content observed in our raw sample (21.29 mg CGE/g) was lower than the 39.2 mg CGE/g reported by Samoticha *et al.* [23], yet higher than values presented by Lin *et al.* [24] (2.9 mg/g DW). In addition, Bushmeleva *et al.* [25] documented substantially higher TAnC values (93.6 mg/g DW), further highlighting the large variability in anthocyanin quantification across different studies.

Such discrepancies can be attributed to multiple factors, including the extraction methodology employed, genetic diversity of plant material, geographical origin, cultivation practices, and post-harvest handling [24]. Furthermore, environmental influences such as temperature, precipitation, relative humidity, and solar radiation have been shown to affect the biosynthesis and accumulation of phenolic compounds in *Aronia* [4]. Considering these parameters, the values obtained in our raw sample are largely consistent with literature trends, supporting the robustness of the present analytical approach while underscoring the influence of external factors on phytochemical composition.

Table 7. Spectrophotometric results of raw *Aronia* sample and commercial products.

Samples	**TAC (mg TE/g)**	**RSD (%)**	**U (%)**	**TPC (mg GE/g)**	**RSD (%)**	**U (%)**	**TAnC (mg CGE/g)**	**RSD (%)**	**U (%)**
Raw *Aronia*	217.76±8.71	4.01	8.01	168.3±10.09	6.02	12.01	21.29±0.31	1.46	2.90
Dried *Aronia*	197.73±5.93	3.11	6.02	147.9±2.21	1.49	3.05	15.57±0.38	2.44	4.91
Effervescent tablet	30.03±0.90	3.21	6.11	6.29±0.22	3.50	7.11	0.40±0.01	2.50	5.00
Dietary capsule	20.55±0.41	2.15	3.50	17.10±0.59	3.45	6.90	0.26±0.01	3.85	7.70
Gummy	0.63±0.02	3.17	6.35	3.40±0.05	1.47	2.94	0.03±0.002	6.67	13.31
Jam	7.76±0.31	4.05	8.12	11.73±0.47	4.01	8.11	0.02±0.001	5.00	10.01
Marmalade	10.54±0.32	3.04	6.08	6.80±0.11	1.62	3.24	0.03±0.001	3.33	6.66
Samples	**TAC (g TE/L)**	**RSD (%)**	**U (%)**	**TPC (g GE/L)**	**RSD (%)**	**U (%)**	**TAnC (mg CGE/L)**	**RSD (%)**	**U (%)**
Aronia juice	19.27±0.38	1.97	3.95	17.51±0.70	4.01	8.01	179.57±2.69	1.50	3.01
Aronia concentrate	85.10±3.40	4.00	8.01	65.28±1.95	2.99	6.11	284.02±7.10	2.50	5.02
Vinegar 1	9.51±0.28	2.94	5.88	7.31±0.18	2.46	4.93	20.78±0.72	3.46	6.92
Vinegar 2	7.01±0.27	3.85	7.70	6.80±0.26	3.82	7.64	7.76±0.18	2.32	4.64

* Results are given as mean ± SD (n=3). Relative standard deviation (RSD, %) and expanded uncertainty (U%, k=2) are reported.

The agreement between spectrophotometric methods was evaluated using both Spearman's rank correlation (ρ) and Pearson's correlation (r). For solid samples (n = 7), a strong correlation was observed between TAC (CUPRAC) and TPC (Folin–Ciocalteu) values (ρ = 0.964, p = 0.002; r = 0.997, p < 0.001). Likewise, TAC and TAnC (pH differential) also showed high concordance (ρ = 0.929, p = 0.006; r = 0.963, p < 0.01), with the consensus ranking of samples being raw > dried > capsule ≳ effervescent >

marmalade ≳ jam > gummy. For liquid samples (n = 4), both TAC *vs* TPC and TAC *vs* TAnC exhibited perfect correlations (ρ = 1.000, p < 0.05; r = 0.996 and 1.000, respectively), reflecting an identical hierarchical ordering (concentrate > juice > vinegar-1 > vinegar-2). These results clearly demonstrate that the ranking of samples is highly consistent across spectrophotometric assays, supporting the robustness and reliability of the comparative evaluation.

3.3. Chromatographic Characterization of Anthocyanins and Phenolic Acids in Aronia Products

Through HPLC-PDA analysis, multiple anthocyanins (λ = 520 nm; Figure 3) and phenolic acids (λ = 300 nm; Figure 4) were detected in both raw *Aronia* fruits and their commercial products. The composition of the commercial samples was then assessed in a comparative manner.

Anthocyanin quantification in *Aronia* samples was performed using a calibration curve constructed for cyanidin-3-*O*-glucoside (y = 2.378 × 10^{10} c + 20775; r = 0.9990). Analysis of fresh fruits revealed four principal anthocyanins—cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, and cyanidin-3-O-xyloside (Figure 3)—with concentrations expressed as cyanidin-3-glucoside equivalents (CGE) (Table 8). Literature sources consistently report cyanidin-3-O-arabinoside and cyanidin-3-*O*-galactoside as the dominant pigments, whereas cyanidin-3-*O*-glucoside and cyanidin-3-*O*-xyloside usually occur in smaller amounts [26]. Consistent with these findings, our data identified cyanidin-3-*O*-galactoside (67.5%) and cyanidin-3-*O*-arabinoside (24.8%) as the most abundant compounds, while cyanidin-3-*O*-xyloside (4.0%) and cyanidin-3-*O*-glucoside (3.8%) were detected at relatively low proportions [8,27].

In both raw *Aronia* fruits and commercial preparations, the main phenolic constituents detected were gallic acid, neochlorogenic acid, chlorogenic acid, and epicatechin (Figure 4). Their concentrations were expressed as gallic acid equivalents (GE), based on the calibration curve for gallic acid (y = 8.6 × 10^{9} c − 7870, r = 0.9989). Earlier studies similarly identified *Aronia* as a valuable source of chlorogenic and neochlorogenic acids [3,4,8,9], with reported levels of 3.0 g/kg DW for chlorogenic acid and 2.9 g/kg DW for neochlorogenic acid, corresponding to about 7.5% of the total polyphenols in the berries. Alongside these dominant acids, smaller amounts of (−)-epicatechin were also observed [28]. Additional phenolics reported in the literature include cinnamic acid (0.34 g/kg DW), gallic acid (0.016 g/kg DW), p-coumaric acid (0.069 g/kg FW), and caffeic acid (0.75 g/kg FW) [29]. The current findings are consistent with these previously published results.

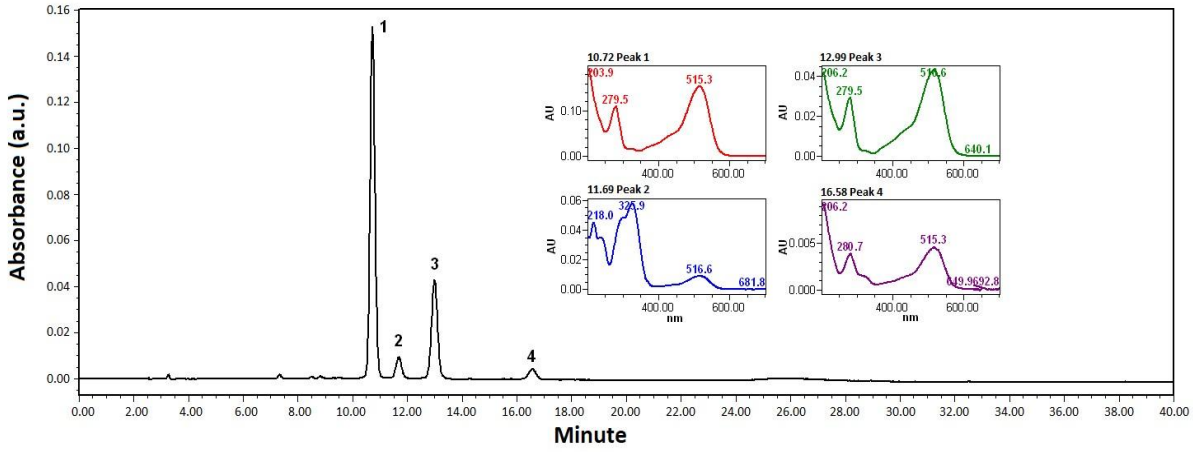

Figure 3. Anthocyanins in powdered *Aronia* at 520 nm (1: cyanidin-3-*O*-galactoside, 2: cyanidin-3-*O*-glucoside, 3: cyanidin-3-*O*-arabinoside, 4: cyanidin-3-*O*-xyloside).

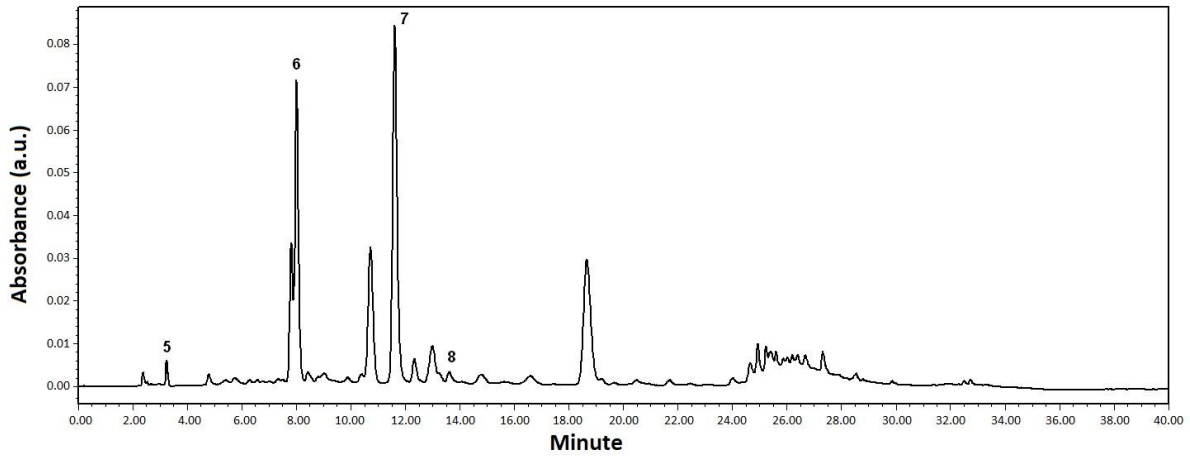

Figure 4. Phenolic compounds in powdered *Aronia* at 300 nm (5: gallic acid, 6: neochlorogenic acid, 7: chlorogenic acid, 8: epicatechin).

Table 8. Anthocyanin (CGE) and phenolic (GE) contents of commercial products of *Aronia* determined by HPLC.

Product (solid)	**mg CGE/g sample**				**mg GE/g sample**			
	Cy-3-galactoside	Cy-3-glucoside	Cy-3-arabinoside	Cy-3-xyloside	Gallic acid	Neochlorogenic acid	Chlorogenic acid	Epicatechin
Raw *Aronia*	10.45	0.67	3.68	0.46	0.20	2.57	5.54	0.75
Dried *Aronia*	8.95	0.50	2.86	0.39	0.16	1.98	5.43	0.67
Effervescent tablet	0.13	0.02	0.13	0.06	1.33	0.90	1.25	0.29
Dietary capsule	0.14	0.01	0.12	0.07	0.35	0.82	1.15	0.12
Gummy	0.009	0.002	0.004	0.006	0.03	0.10	0.13	0.004
Jam	<0.001	<0.001	<0.001	n.d.	0.124	0.15	0.31	0.17
Marmalade	<0.001	<0.001	<0.001	n.d.	0.164	0.39	0.72	0.17
Product (liquid)	**mg CGE/L sample**				**mg GE/L sample**			
Aronia juice	28.85	11.25	15.19	1.22	106.72	742.84	731.51	28.84
Aronia concentrate	43.77	21.54	31.73	5.28	504.41	1260.31	1671.93	58.66
Vinegar 1	5.19	1.58	1.18	0.22	17.96	130.44	31.43	16.12
Vinegar 2	2.21	0.10	1.22	0.14	2.65	125.91	102.51	37.09

To evaluate the comparability of methods, the total anthocyanin content (TAnC) determined by the pH differential method was compared with the sum of anthocyanins quantified by HPLC (Figure 5). Correlation analysis revealed excellent agreement between the two approaches (Pearson $r = 0.997$, Spearman $\rho = 0.963$, $p < 0.001$).

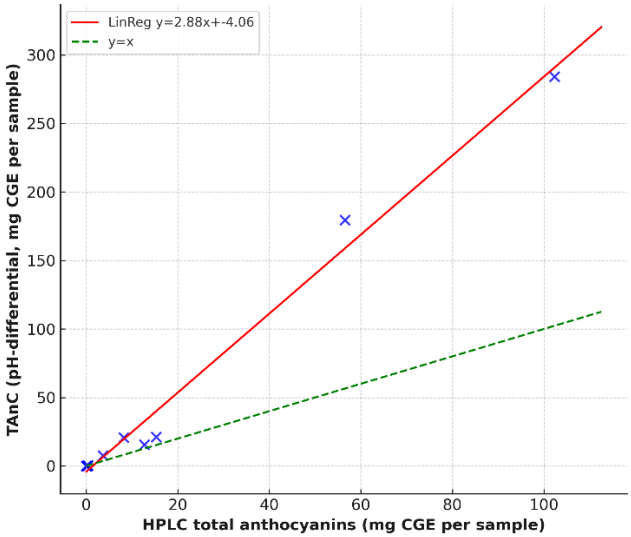

Figure 5. Correlation between total anthocyanins determined by the pH differential method (TAnC) and the sum of anthocyanins quantified by HPLC.

4. Conclusions

This study provided a comprehensive assessment of *Aronia* fruits and their commercial derivatives, focusing on phenolic composition, antioxidant capacity, and anthocyanin content. By employing optimized microwave-assisted extraction in combination with spectrophotometric assays (CUPRAC, Folin–Ciocalteu, and pH differential) and chromatographic analysis (HPLC-PDA), the work delivered an integrated view of the bioactive profile of both fresh fruit and processed products. Cyanidin-3-*O*-galactoside was consistently identified as the predominant anthocyanin across all samples, and chlorogenic acid derivatives emerged as the major phenolic constituents. The comparative evaluation further showed that fresh *Aronia* fruits retain the highest bioactive potential, while industrially processed products display marked reductions, particularly in anthocyanins. These losses were largely attributable to thermal treatments such as boiling, drying, and pasteurization, which can reduce phenolic stability but also inactivate degradative enzymes. Despite these reductions, the overall phenolic fingerprint of *Aronia* remained recognizable across product forms, confirming that processing alters concentrations but not the qualitative composition. The study also highlighted other influential variables—including extraction efficiency, pH, acidity, sugar levels, and formulation additives—that may affect both flavonoid determinations and antioxidant measurements. By systematically linking optimized extraction with comparative profiling, this work provides valuable insights into the compositional integrity of *Aronia* -based products. The results not only contribute new knowledge on the nutritional quality of commercially available *Aronia* derivatives but also offer a methodological framework for authenticity verification and quality control in functional food development.

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References

[1] S. E. Kulling and H. M. Rawel (2008). Chokeberry (*Aronia melanocarpa*) – A review on the characteristic components and potential health effects, *Planta Med*. **74**, 1625–1634.

[2] C. Chrubasik G. Li and S. Chrubasik (2010). The clinical effectiveness of chokeberry: A systematic review, *Phytother. Res*. **24**, 1107–1114.

[3] J. Oszmiański and S. Lachowicz (2016). Effect of the production of dried fruits and juice from chokeberry (Aronia melanocarpa L.) on the content and antioxidative activity of bioactive compounds, *Molecules* **21**, 1098.

[4] M. T. Tolić, I. P. Krbavčić, P. Vujević, B. Milinović, I. L. Jurčević and N. Vahčić (2017). Effects of weather conditions on phenolic content and antioxidant capacity in juice of chokeberries (*Aronia melanocarpa* L.), *Pol. J. Food Nutr. Sci.* **67**, 67–74.

[5] A. Sidor, A. Drożdżyńska and A. Gramza-Michałowska (2019). Black chokeberry (*Aronia melanocarpa*) and its products as potential health-promoting factors – An overview, *Trends Food Sci. Technol.* **89**, 45–60.

[6] T. Tanaka and A. Tanaka (2001). Chemical components and characteristics of black chokeberry, *J. Jpn. Soc. Food Sci. Technol.* **48**, 606–610.

[7] A. Konić-Ristić, K. Šavikin, G. Zdunić, T. Janković, Z. Juranic, N. Menković and I. Stanković (2011). Biological activity and chemical composition of different berry juices, *Food Chem.* **125**, 1412–1417.

[8] J. Oszmiański and A. Wojdylo (2005). Aronia melanocarpa phenolics and their antioxidant activity, *Eur. Food Res. Technol.* **221**, 809–813.

[9] J. Beattie, A. Crozier and G. Duthie (2005). Potential health benefits of berries, *Curr. Nutr. Food Sci.* **1**, 71–86.

[10] T. Jurikova, J. Mlcek, S. Skrovankova, D. Sumczynski, J. Sochor, I. Hlavacova, L. Snopek and J. Orsavova (2017). Fruits of black chokeberry *Aronia melanocarpa* in the prevention of chronic diseases, *Molecules* **22**, 1–23.

[11] L. A. Pham-Huy, H. He and C. Pham-Huy (2008). Free radicals, antioxidants in disease and health, *Int. J. Biomed. Sci.* **4**, 89–96.

[12] S. Benvenuti, F. Pellati, M. Melegari and D. Bertelli (2004). Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of Rubus, Ribes, and Aronia, *J. Food Sci.* **69**, 164–169.

[13] B. Kapci, E. Neradová, H. Čížková, M. Voldřich, A. Rajchl and E. Capanoglu (2013). Investigating the antioxidant potential of chokeberry (*Aronia melanocarpa*) products, *J. Food Nutr. Res.* **52**, 219–229.

[14] W. Zheng and S. Y. Wang (2003). Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries, *J. Agric. Food Chem.* **51**, 502–509.

[15] M. Teleszko and A. Wojdyło (2015). Comparison of phenolic compounds and antioxidant potential between selected edible fruits and their leaves, *J. Funct. Foods* **14**, 736–746.

[16] X. Wu, L. Gu, R. L. Prior and S. McKay (2004). Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity, *J. Agric. Food Chem.* **52**, 7846–7856.

[17] C. S. Eskilsson and E. Björklund (2000). Analytical-scale microwave-assisted extraction, *J. Chromatogr. A* **902**, 227–250.

[18] R. Apak, K. Güçlü, M. Özyürek and S. E. Karademir (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method, *J. Agric. Food Chem.* **52**, 7970–7981.

[19] V. L. Singleton, R. Orthofer and R. M. Lamuela-Raventós (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, *Methods Enzymol.* **299**, 152–178.

[20] R. E. Wrolstad, R. W. Durst and J. Lee (2005). Tracking color and pigment changes in anthocyanin products, *Food Sci. Technol.* **16**, 423–428.

[21] F. J. Francis and P. C. Markakis (1989). Food colorants: anthocyanins, *Crit. Rev. Food Sci. Nutr.* **28**, 273–314.

[22] R. Taheri, B. A. Connolly, M. H. Brand and B. W. Bolling (2013). Underutilized chokeberry (*Aronia melanocarpa, Aronia arbutifolia*, *Aronia prunifolia*) accessions are rich sources of anthocyanins, flavonoids, hydroxycinnamic acids, and proanthocyanidins, *J. Agric. Food Chem.* **61**, 8581–8588.

[23] J. Samoticha, A. Wojdyło and K. Lech (2016). The influence of different drying methods on chemical composition and antioxidant activity in chokeberries, *LWT–Food Sci. Technol.* **66**, 484–489.

[24] S. Lin, X. Meng, C. Tan, Y. Tong, M. Wan, M. Wang, Y. Zhao, H. Deng, Y. Kong and Y. Ma (2022). Composition and antioxidant activity of anthocyanins from *Aronia melanocarpa* extracted using an ultrasonic-microwave-assisted natural deep eutectic solvent extraction method, *Ultrason. Sonochem.* **89**, 106102.

[25] K. Bushmeleva, A. Vyshtakalyuk, D. Terenzhev, T. Belov, E. Nikitin and V. Zobov (2022). Antioxidative and immunomodulating properties of *Aronia melanocarpa* extract rich in anthocyanins, *Plants* **11**, 3333.

[26] L. Jakobek, M. Seruga, M. Medvidovic-Kosanovic and I. Novak (2007). Anthocyanin content and antioxidant activity of various red fruit juices, *Dtsch. Lebensm.-Rundsch.* **103**, 58.

[27] M. Bräunlich, R. Slimestad, H. Wangensteen, C. Brede, K. E. Malterud and H. Barsett (2013). Extracts, anthocyanins and procyanidins from Aronia melanocarpa as radical scavengers and enzyme inhibitors, *Nutrients* **5**, 663–678.

[28] K. R. Määttä-Riihinen, A. Kamal-Eldin, P. H. Mattila, A. M. González-Paramás and A. R. A. Törrönen (2004). Distribution and contents of phenolic compounds in eighteen Scandinavian berry species, *J. Agric. Food Chem.* **52**, 4477–4486.

[29] J. Hudec, R. Kochanova, M. Burdova, L. Kobida, G. Kogan, I. Turianica and P. Chlep (2009). Regulation of the phenolic profile of berries can increase their antioxidant activity, *J. Agric. Food Chem.* **57**, 2022–2029.