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Investigation of using natural deep eutectic solvents (NADES) for the extraction of bioactive compounds from *Aronia melanocarpa* fruit

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Abstract: In this study, for the extraction of polyphenolic compounds found in high amounts in aronia fruit, three different extraction methods (classical, ultrasound, and ultrasonic), three different traditional solvents (water, ethanol, acidified 80% methanol) as well as five different natural deep eutectic solvents (NADES) were used. Color indices as L*, a*, b*, C*, and H° values, total phenolic content (TPC), total flavonoid content (TFC), total monomeric anthocyanin (TMA), total antioxidant capacity (1,1-diphenyl-2-picrylhydrazyl, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid, and ferric ion reducing antioxidant potential assays), and phenolic compounds of the aronia fruit extracts were analyzed. There were significant differences in the measured parameters depending on the extraction method and solvent type. Extracts in ultrasound and classical extraction had similar and higher TPC than those in ultrasonic extraction. The highest TFC (66.87 mg of QE/g) was determined in the extracts obtained by ultrasound-assisted extraction, and the highest TMA content (1551.08 mg of Cyn-3-glu/kg) was determined in the extracts by ultrasonic extraction. In the statistical evaluations, the highest average TPC (66.71 and 60.97 mg of GAE/g, respectively) was determined in the aronia extracts prepared with choline chloride:acetic acid and choline chloride:citric acid. Using NADES increased the TPC, and TFC while providing results close to methanol in the TMA content. Based on these results, better results will be obtained using these solvents in obtaining extracts used in the production of functional foods in the food industry with high TPC, TFC, and monomeric anthocyanin content. The present study shows the potential for creating ecofriendly solvents with enhanced extraction capabilities compared to classical solvents for the extraction of bioactive compounds from aronia fruits.

Key words: Aronia, extraction method, solvent type, antioxidant activity, phenolic profile, natural deep eutectic solvents

1. Introduction

Nowadays, the preference for foods rich in bioactive compounds with human health promoting effects in nutrition is increasing (Renard, 2018; Abanoz and Okcu, 2022; Dawadi et al., 2022). In the plant kingdom, horticultural plants are found on all continents of the world with big diversity in terms of their morphological traits, and biochemical and bioactive contents (Sahin et al., 2002; Ercisli et al., 2003; Altindag et al., 2006; Celik et al., 2007; Sarkar and Rakshit, 2021; Urün et al., 2021; Sadeghinejad et al., 2022). Horticulture plants are rich in bioactive compounds (phenolics, alkaloids, phenylpropanoids, terpenoids, polysaccharides, lipids, peptides, and others) and these compounds are obtained by extraction procedures mostly using solvents such as hexane, benzene, methanol, chloroform, petroleum ether, and acetone (Benvenutti et al., 2019). Conventional solvents usually display toxicity and flammability. Hence, they may harm the environment and human health (Bubalo et al., 2018).

Increasing awareness of environmental impacts (increase in energy, water, solvent consumption, and carbon emissions) has encouraged research to develop green extraction as an alternative and preferable process (Koraqi et al., 2024). The innovation and planning of extraction processes based on the optimal use of solvents, energy, and resources constitute the basis of green extraction. Therefore, research has focused on designing new, environmentally friendly, naturally derived, and tunable solvents that serve various biosustainable purposes (Usmani et al., 2023). The terminology of natural deep eutectic solvents (NADES), among these solvents, represents eutectic mixtures of two or more natural compounds among choline chloride (ChCl), citric acid, malic acid, maleic acid, acetic acid, glucose, fructose, sucrose, trehalose, and water. Nevertheless, some researchers continue to use the term deep eutectic solvents (DES) for mixtures of natural compounds (Benvenutti et al., 2019). NADES is usually prepared from two

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components, one called hydrogen bond donors (HBDs) and the other called hydrogen bond acceptors (HBAs). In general, HBAs are nontoxic quaternary ammonium salts or amino acids (e.g., alanine, proline, glycine, and betaine), whereas HBDs are organic acids (e.g., oxalic acid, lactic acid, malic acid, etc.) or carbohydrates (e.g., glucose, fructose, maltose, etc.). Alcohol, amine, aldehyde, ketone, and carboxylic groups act dually as HBDs and HBAs (de los Ángeles Fernández et al., 2018).

NADES are usually characterized by very low vapor pressure, higher biodegradability (>68% in 28 days), nontoxicity, compositional tunability, compatibility with foods/drugs/cosmetics, and recyclability in comparison with conventional solvents (Benvenutti et al., 2019; Mišan et al., 2020; Athanasiadis et al., 2023). ChCl, which is the main HBA utilized, has features such as biodegradability, nontoxicity, and low cost. The abovementioned amine salt is naturally found in the cell lipid membrane. For commercial offerings, ChCl is also easily synthesized with trimethylamine, ethylene oxide, and hydrochloric acid (HCl). Since this chemical reaction produces no residues, the environmental (E) factor (mass ratio of waste to desired product) is zero. Hence, it is considered a clean and sustainable process (Benvenutti et al., 2019). Moreover, NADES have been revealed to have exceptional solubilization properties against a range of structurally diversified polyphenolic compounds, and polyphenol extraction with NADES has been reported to outperform common conventional solvents in several cases (Lu and Liu, 2020).

Horticultural plants including fruits and vegetables are important food sources as well as essential sources of various bioactive molecules, particularly polyphenolic compounds, and are known for their pharmacological properties, including antioxidant, antibacterial, antiinflammatory, and anticancer impacts (Benjak et al., 2005; Erturk et al., 2012; Sarkar et al., 2022; Nekkaa et al., 2023). *Aronia melanocarpa* is a good antioxidant food source due to its high polyphenol content. (Boyaci et al., 2023; Esatbeyoglu et al., 2023). Furthermore, black chokeberry, or aronia, contains higher amounts of anthocyanins than dark colored berries such as blackberries, raspberries, black currants, elderberries, and blueberries (Jang and Koh, 2023). Aronia, is a shrub belonging to the family *Rosaceae*, a plant native to North America and transferred to Europe approximately a century ago. The edible parts of the black chokeberry are primarily its small, dark, cherry-like fruits (Sidor et al., 2019). Fresh, unprocessed black chokeberry fruits are rarely consumed because of their astringent taste. However, they are used in the food industry to produce juices, syrups, wines, jams, fruit teas, dietary supplements, and food colorants (Sidor et al., 2019; Esatbeyoglu et al., 2023; Wang et al., 2023a). Concerning considerable health and economic interest in chokeberry fruits, significant research efforts have been made to develop efficient methods that will obtain an extract with the desired features.

As one of the outcomes of the increasing interest in natural product research worldwide, the extraction of bioactive compounds represents a crucial step in natural product research. It has become a bottleneck in accelerating the screening of an increasing number of products. At present, extraction includes the separation of medicinally active molecular components of plant tissues from inert components by employing conventional solvent extraction techniques or standard modern and green extraction procedures. The choice of an extraction technique is essential since it determines the reliability and quality of subsequent analytical activities. Therefore, the current study aimed to determine the chemical differences of the extracts prepared by 3 methods (classic, ultrasonic, ultrasound) from the lyophilized dried form of the aronia fruit, which displays high antioxidant properties, using three conventional solvents (water, ethanol, and acidified 80% methanol) and five NADES solutions prepared with ChCl. In this way, it would be possible to determine whether green solvents could be used instead of conventionally used solvents. First, the NADES solutions were prepared and then, it was attempted to determine the changes in some physical and antioxidant properties of the aronia fruit extracts using the solution type and extraction method.

2. Materials and methods

2.1. Materials

Fresh aronia (*Aronia melanocarpa*) fruits harvested from Kırklareli, Vize, Türkiye were used. Prior to the extraction, the fruits were lyophilized with a lyophilizer (FDU-8612, Operon Co., Ltd., Gimpo, Korea) and ground with a Waring device (HGB2WTS3; Waring Commercial, Stamford, CT, USA). The ground fruits were placed in sealed metallic bags and used for extraction. The chemicals used in the extraction were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) as food-grade acetic acid, glycerol, oxalic acid, propylene glycol, and citric acid.

2.2. Methods

2.2.1. Preparation of the solutions and NADES

Distilled water (W) and ethanol (E) were used directly for extraction in the study. Methanol (M) was used by preparing an 80% M-distilled water (V:V) mixture acidified with 0.01% HCl (M). When preparing the NADES, ChCl, which was dried at 40 °C for 1 h and kept in a desiccator, was used as the HBD, and acetic acid, glycerol, oxalic acid, citric acid, and 1,2-propanediol were used as the HBAs. The prepared NADES were kept in a shaking water bath at

90 rpm at 80 °C until a homogeneous colorless liquid was formed (12 h on average). The mixtures were diluted using 20%–30% (v/v) distilled water for the purpose of acquiring a uniform and transparent, colorless solution that did not display crystallization. The solutions prepared in the study were stored in amber-colored, airtight glass bottles at room temperature. Table 1 lists the abbreviated names of the NADES and corresponding mole ratios.

2.2.2. Preparation of the fruit extracts

2.2.2.1. Classic extraction

For the classic extraction, 0.5 g of the lyophilized aronia fruit powder was weighed, and 20 mL of the different solvents (W, E, M, NA, NG, NO, NP, and NC) were added. This mixture was mixed using a vortex mixer (Heidolph Reax Top, D-91126; Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at 1000 rpm for 3 h to prepare the extracts. Afterward, it was centrifuged in a cooled centrifuge (Hettich Mikro 22 R; Hettich GmbH & Co.KG, Tuttlingen, Germany) at 6000 rpm and 4 °C for 15 min. After centrifugation, it was filtered through Whatman No. 42 filter paper.

2.2.2.2. Ultrasonic extraction

For the ultrasonic extraction, 0.5 g of lyophilized aronia fruit powder was weighed and 20 mL of the different solvents (W, E, M, NA, NG, NO, NP, and NC) were added. To prepare the ultrasonic extracts, the mixture was kept in an ultrasonic water bath (Bandelin Sonorex Super RK 103H; Bandelin Electronic GmbH & Co. KG, Heinrichstraße, Berlin, Germany) for 30 min. Afterward, it was centrifuged in a cooled centrifuge (Hettich Mikro 22 R; Heidolph Instruments GmbH & Co. KG) at 6000 rpm and 4 °C for 15 min. After centrifugation, it was filtered through Whatman No. 42 filter paper (Karakütük et al., 2023).

2.2.2.3. Ultrasound-assisted extraction

For the ultrasound-assisted extraction, 0.5 g of the lyophilized aronia fruit powder was weighed, and 20 mL of the different solvents (W, E, M, NA, NG, NO, NP, and NC) were added. To prepare ultrasonic extracts, the mixture was exposed to ultrasound at an interval of 0.6 s at 80% amplitude power for 5 min in an ultrasound-assisted extraction device (UP400S; Hielscher USA, Inc., Ringwood NJ, USA) (González-Silva et al., 2022). Afterward, it was centrifuged in a cooled centrifuge (Hettich Mikro 22 R; Heidolph Instruments GmbH & Co. KG) at 6000 rpm and 4 °C for 15 min. After centrifugation, it was filtered through Whatman No. 42 filter paper (Karakütük et al., 2023). The extracted samples were coded as specified in Table S1.

2.2.3. Color determination

A Konica Minolta CR-400 colorimeter (Konica Minolta, Inc., Chiyoda-ku, Tokyo, Japan) was utilized to determine the color of the extracts. The L* value was evaluated with scales between 0 (black) and 100 (white), the a* value was evaluated with scales between –60 (green) and +60 (red), and the b* value was evaluated with scales between –60 (blue) and +60 (yellow) (Zor et al., 2022). The chroma (C*) value expresses the tone of the product color, and it is low in pale colors and high in vivid colors. It was reported that if the hue angle (H°) value is 0°, 90°, 180°, and 270°, the product is red, yellow, green, and blue, respectively, and intermediate colors are formed in the parts that coincide with these angle values (Zor and Sengul, 2022).

2.2.4. Total monomeric anthocyanin (TMA)

The TMA content was calculated as cyanidin-3-glycoside using the pH differential method. The extracts were filtered by being diluted separately with potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5), and at the end of the 30-min incubation period, absorbance values were read at 515 nm and 700 nm on an ultraviolet-visible (UV-Vis) spectrophotometer (TV60; PG Instruments Ltd., Alma Park, Leicestershire, UK). The TMA was calculated as mg Cyn-3-glu/kg using the equation given below (Zor and Sengul, 2022).

TMA content (mg Cyn-3-glu / kg) = $A \times 10^3 \times M_A \times SF / (\varepsilon \times L)$

A (absorbance difference) = $(A_{515} - A_{700})_{pH1} - (A_{515} - A_{700})_{pH4.5}$

Here, L is the layer thickness of the reading cuvette (cm), SF is the dilution factor, M_A is the molecular weight (Cyn-3-glu), and Ɛ is the molar absorbance

2.2.5. Total phenolic content (TPC)

The TPC was measured colorimetrically with Folin–Ciocalteu reagent. After adding 0.2 N 2.5 mL of Folin–Ciocalteu reagent to 100 µL of the extract placed in a volumetric flask, it was shaken thoroughly and incubated

Table 1. Contents of the prepared NADES.

The abbreviated names of the NADES	Hydrogen bond donor (HBD)	Hydrogen bond acceptor (HBA)	Molar ratio	Water (%)
NA	Choline chloride	Acetic acid	1:2	20
NG	Choline chloride	Glycerol	1:2	20
NO	Choline chloride	Oxalic acid	1:1	25
NP	Choline chloride	1,2-Propanediol	1:4	30
NC	Choline chloride	Citric acid	1:1	30

for 3 min. Then, 2 mL of 7.5% Na_2CO_3 solution was added and incubated in a dark environment for 2 h. Absorbances were read at 760 nm in a UV-Vis (TV60; PG Instruments Ltd.) spectrophotometer. Using a standard gallic acid curve, the phenolic content of the extracts was expressed as the gallic acid equivalent (mg GAE/kg of sample) (Karakütük et al., 2023).

2.2.6. Total flavonoid content (TFC)

The determination of the TFC was performed spectrophotometrically following the method described by Koçak et al. (2018). Initially, 250 µL of the sample extracts were taken, and 1250 µL of distilled water was added to them. Afterward, 0.075 mL of 0.05 g/mL of $NaNO_2$ was added and incubated for 6 min following vortexing. At the end of the vortexing, 0.15 mL of 0.1 g/mL of $AlCl_3 \cdot 6H_2O$ was added and vortexed, following which it was left for 5 min. Finally, 0.5 mL of 1 mol/L of NaOH was added, mixed, and incubated for 15 min. At the end of the incubation, the absorbance values of the samples were read using a spectrophotometer (TV60; PG Instruments Ltd.) at 510 nm. The equation acquired from the calibration curve drawn as a result of the measurements, carried out by preparing 10–250 mg/L of quercetin, was employed when performing the calculations. The TFC was expressed as mg quercetin equivalent (QE)/g (Şengül et al., 2023).

2.2.7. 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) assay

To determine the $DPPH^{\cdot}$ free radical scavenging activity of the extracts, 10–30 µg/mL was taken from the stock extracts and then after bringing it up to 2 mL with M and adding 500 µL $DPPH^{\cdot}$ (0.1 mM), a homogeneous mixture was obtained using a vortex. The mixture was incubated in a dark environment at room temperature for 30 min, and the mixture's absorbance value was read at 517 nm. The % inhibition was calculated using the formula given below. Additionally, the half-maximal inhibitory concentration (IC_{50}) values were determined using the % inhibition values (Zor and Sengul, 2022).

$$\% \text{ Inhibition} = (\text{Control}_{ABS} - \text{Extract}_{ABS} / \text{Control}_{ABS}) \times 100$$

2.2.8. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+\cdot}$) assay

The $ABTS^{+\cdot}$ radical scavenging activity was determined as described by Zor et al. (2022). First, a 7-mM ABTS solution was prepared. The solution was then mixed with 2.45 mM of potassium persulfate and incubated in the dark for 12–16 h. The $ABTS^{+\cdot}$ radical solution was added to the extracts taken at a concentration of 10–30 µg/mL, making the final volume 2500 µL, and incubated at room temperature in the dark for 6 min. The resulting absorbances were recorded at 734 nm. The % inhibition values were calculated using the absorbance values obtained, and the IC_{50} values were determined using these values.

2.2.9. Ferric ion reducing antioxidant potential (FRAP) assay

It is based on the principle that the Fe^{3+} ions in the Fe $(TPTZ)^{3+}$ mixture found in the radical to be utilized in determining the antioxidant activity of the aronia extracts using the FRAP method are reduced to the blue-colored Fe $(TPTZ)^{2+}$ complex in an acidic environment (Koçak et al., 2018). Antioxidant activity was determined using the FRAP method by making some changes to the method reported by Koçak et al. (2018). Three solutions utilized in the research were prepared daily, including:

Solution 1: Acetate buffer (pH 3.6), including 3.1 g sodium acetate + 16 mL of acetic acid in 1 L of solution

Solution 2: 0.156 g TPTZ (2,4,6-tripyridyl-s-triazine) dissolved in 50 mL of E

Solution 3: 0.5404 g $FeCl_3 \cdot 6H_2O$+2 mL HCl (37% m/m) in 100 mL of solution

After preparing the solutions, 80 mL of solution 1, 8 mL of solution 2, and 8 mL of solution 3 were mixed, and thus, the FRAP reagent was prepared. Then, 2.4 mL of the FRAP reagent was added to 0.1 mL of the sample extract, and the mixture was vortexed. After 4 min, absorbance was measured at 593 nm. A calibration curve was obtained using 5 to 25-µmol solutions of Trolox prepared with M. The results from the calibration curve were expressed as mM TE/100 g (Şengül et al., 2023).

2.2.10. Determination of the phenolic compounds by liquid chromatography tandem mass spectrometry (LC-MS/MS)

The phenolic components of the extracts were analyzed on an Agilent 6460 Triple Quadrupole LC-MS/MS device (Agilent Technologies, Santa Clara, CA, USA). A ZORBAXTB C18 4.6 × 100-mm column (Agilent Technologies) with a 3.5-µm particle size was utilized in the analysis. Deionized water (A) containing 0.1% formic acid and acetonitrile (B) were used as the mobile phases. The mobile phase program was as follows: the linear gradient was set from 95:5 A:B to 5:95 A:B in the range of 0–4 min, from 80:20 A:B to 20:80 A:B in the range of 4–7 min, from 10:90 A:B to 90:10 A:B in the range of 7–14 min, from 10:90 A:B to 90:10 A:B in the range of 14–15 min, and from 5:95 A:B to 95:5 A:B in the range of 15–15.10 min, and the mobile phase flow rate was set at 0.4 mL/min. The column kept at 30 °C throughout the study. The injection volume was set at 5 µL, the nebulizer gas flow at 12 L/min, the dryer gas flow at 5 L/min, the detector at 350 °C, and the air block at 250 °C. Electrospray ionization and Agilent Jet Stream ionizers (Agilent Technologies) were utilized for ionizing the molecules. Multiple reaction monitoring (MRM) mode was employed to identify the molecules. After MRM optimization was carried out for each phenolic substance (quinic acid, fumaric acid, gallic acid, pyrogallol, keracyanin chloride, cyanidin-3-

o-glucoside (C_3G), chlorogenic acid (CGA), catechin, peonidin-3-o-glucoside, 4-OH-benzoic acid, epicatechin, epigallocatechin gallate, caffeic acid, vanillic acid, syringic acid, vitexin, naringin, ellagic acid, hesperidin, p-coumaric acid, sinapic acid, taxifolin, ferulic acid, rosmarinic acid, vanillin, myricetin, resveratrol, luteolin, quercetin, apigenin, naringenin, isorhamnetin, chrysin, galangin, and curcumin) without a column, the mixture that contained concentrations of 1, 5, 10, 25, 50, 100, 200, and 500 ppb of the standards was analyzed, and calibration curves were drawn (Karakütük et al., 2023). Then, the limit of detection (LOD) and limit of quantification (LOQ) values were calculated (Table S2).

2.2.11. Statistical analysis

The data acquired in 3 replicates were analyzed with IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA). The results were expressed as the mean values with the standard deviation (±SD). Two-way analysis of variance (ANOVA) was performed to determine significant group differences ($p \leq 0.05$, $p \leq 0.01$) between the means. Duncan's multiple range test was carried out to compare the mean values. Principal component analysis (PCA) was implemented on some data to facilitate the identification of similarities and differences between the samples (SIMCA-P + 14.1, UMETRICS).

3. Results and discussion

The L*, a* and b*, values of the extracts were between 28.12 and 38.47, 10.97 and 29.32, and (–4.68) and (+18.22). The highest L* value was in the NPP, the lowest L* value was detected in the NOC. The highest a*, b*, and C* values were determined in NCS and the lowest in WS. For the H° values, WS had the lowest (Figure 1). The average L*, a*, b*, C*, and H° values of the extracts of the aronia fruit prepared with 3 extraction methods and 8 different solvents are given in Table 2. The different extraction methods and solvent types caused significant changes in the L*, a*, b*, C*, and H° values. The average L* value of the extracts prepared with ultrasound-assisted extraction was higher than those of the extracts prepared with the classical and ultrasonic methods, and therefore, they were darker in color (Table 2).

When the a*, b*, and C* values were examined, higher values were determined in the extracts prepared with the ultrasonic method. The H° value was quite higher with the ultrasound-assisted extraction compared to the other methods (Table 2).

According to the solvent type, the average L* and b* values of the extracts prepared with NG were the highest. When looking at the a* and C* values, the highest values were from the extracts prepared with NC. The extracts prepared with W had the lowest values. The H° value, which indicates the opacity and brightness of the color, was the highest in the extracts made with water and lowest in the extracts made with NC (Table 2).

The composition of NADES determines their physicochemical properties and thus affects the extraction efficiency. Therefore, the performance of NADES commonly reported in the literature in the extraction of bioactive compounds from aronia fruit was compared in this study. The TPC, TFC, TMA, DPPH, ABTS, and FRAP values of the prepared extracts were determined as 17.86–

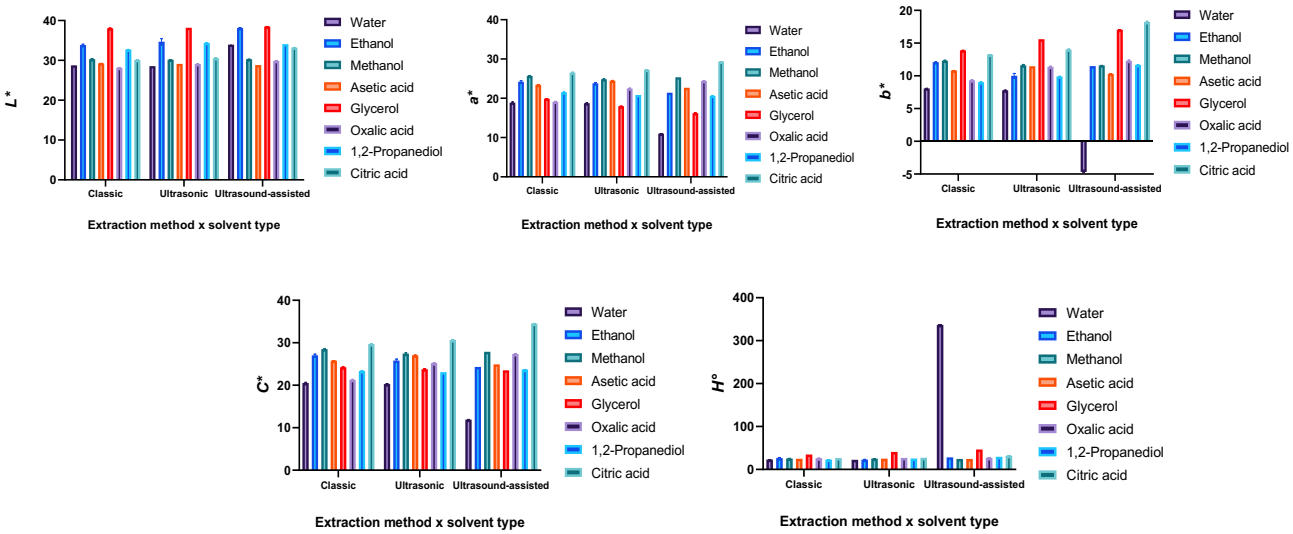

Figure 1. L*, a*, b*, C*, and H° values of the extracts according to the extraction method × solvent type. W: water, E: ethanol, M: 80% methanol-distilled water (V:V) mixture acidified with 0.01% HCl, NA: ChCl:acetic acid (1:2 + 20% water), NG: ChCl:glycerol (1:2 + 20% water), NO: ChCl:oxalic acid (1:1 + 25% water), NP: ChCl:1,2-propanediol (1:4 + 30% water), and NC: ChCl:citric acid (1:1 + 30% water).

Table 2. Effect of extraction method and solvent type on L^*, a^*, b^*, C^*, and H° values of aronia fruit extracts

Extraction method (EM)	**L***	**a***	**b***	**C***	**H°**
Classic	31.39 ± 3.18^{c}	22.41 ± 2.86^{b}	11.09 ± 2.03^{b}	25.05 ± 3.16^{b}	26.28 ± 3.61^{c}
Ultrasonic	31.84 ± 3.34^{b}	22.55 ± 3.04^{a}	11.48 ± 2.34^{a}	25.41 ± 3.00^{a}	27.00 ± 5.61^{b}
Ultrasound-assisted	33.35 ± 3.47^{a}	21.37 ± 5.42^{c}	10.99 ± 6.64^{c}	24.76 ± 6.04^{c}	68.57 ± 103.82^{a}
Significance	**	**	**	**	**
Solvent type (ST)					
W	30.41 ± 2.66^{e}	16.20 ± 3.93^{h}	3.72 ± 6.30^{h}	17.58 ± 4.25^{h}	127.53 ± 157.03^{a}
E	35.55 ± 2.00^{b}	23.14 ± 1.32^{d}	11.20 ± 0.94^{d}	25.73 ± 1.22^{d}	25.85 ± 2.40^{e}
M	30.25 ± 0.07^{f}	25.30 ± 0.38^{b}	11.84 ± 0.36^{c}	27.94 ± 0.48^{b}	25.07 ± 0.45^{f}
NA	29.06 ± 0.19^{g}	23.53 ± 0.78^{c}	10.86 ± 0.51^{f}	25.92 ± 0.92^{c}	24.77 ± 0.30^{g}
NG	38.24 ± 0.19^{a}	18.02 ± 1.60^{g}	15.50 ± 1.37^{a}	23.85 ± 0.32^{f}	40.76 ± 4.99^{b}
NO	29.01 ± 0.75^{g}	22.01 ± 2.33^{e}	11.00 ± 1.32^{e}	24.61 ± 2.67^{e}	26.53 ± 0.43^{d}
NP	33.73 ± 0.76^{c}	21.00 ± 0.41^{f}	10.20 ± 1.16^{g}	23.37 ± 0.27^{g}	25.87 ± 2.92^{e}
NC	31.29 ± 1.46^{d}	27.71 ± 1.24^{a}	15.16 ± 2.32^{b}	31.61 ± 2.21^{a}	28.54 ± 2.51^{c}
Significance	**	**	**	**	**
EM X ST	**	**	**	**	**

- Data were shown as the mean ± SD (n= 3).
- ** $p < 0.01$
- a-h; Mean values with different letters are significantly different from each other.

70.90 mg GAE/g, 29.00–135.50 mg QE/g, 347.80–1944.42 mg Cyn-3 glu/kg, 74.45–256.01 µg/mL, 34.68–111.40 µg/mL, and 0.92–29.77 mM TE/100 g, respectively. When the TFC values were examined, it was seen that TFC was not be detected in the NAC, NOC, NAU, NOU, NAS, and NOS extracts (data not shown). The highest values for the TPC, TFC, TMA, DPPH, ABTS, and FRAP values were detected in the NAS, NPU, MU, ES, ES, and NAS samples, respectively (Figure 2).

In parallel with the current study, Bosiljkov et al. (2017) used NADES and ultrasound-assisted extraction to obtain anthocyanin from wine residues and reported that most NADES were more efficient than ethanol at a certain water content and molar ratio. Successful extraction of anthocyanins is highly related to the acidity of the extraction solvent, as anthocyanins exist in different chemical forms under different pHs (Castañeda-Ovando et al., 2009). For this reason, it is thought that there are differences in these values depending on the extraction solutions.

The average TPC, TFC, and TMA values and antioxidant properties of the extracts of the aronia fruit prepared with 3 extraction methods and 8 different solvents are given in Table 3. Different extraction methods and solvent types caused significant changes in the TPC, TFC, and TMA values and antioxidant properties. It was observed that the extracts with the ultrasound-assisted and classical extraction had similar and higher TPC averages than with the ultrasonic extraction. The highest average TFC was (66.87 mg QE/g) in the extracts obtained with ultrasound-assisted extraction, and the highest average TMA was (1551.08 mg Cyn-3-glu/kg) in extracts obtained with ultrasonic extraction (Table 3). Bubalo et al. (2016) used microwave and ultrasound-assisted extraction methods to extract phenolic compounds from grape skins using NADES (ChCl:glycerol (1:2), ChCl:oxalic acid, ChCl:malic acid, ChCl:sorbose, and ChCl: proline:malic acid). It was reported that most NADES lead to an increase in the recovery of extracted phenolic compounds compared to water and methyl alcohol.

Additionally, as a result of their study, it was emphasized that the ultrasound-assisted extraction technique is the best method, similar to the results of the present study. Tong et al. (2021) used different NADES combined with ultrasound-assisted extraction to extract bioactive compounds from safflower and test their bioavailability, and tested the oral bioavailability of hydroxysafflor yellow A (HSYA) and anhydrosafflor yellow B (ASYB) on rats. Pharmacokinetic studies revealed that blood levels of HSYA and ASYB after oral administration of L-proline-acetamide extract were significantly higher than those of the aqueous extract. In antioxidant activity analyses, according to the DPPH and ABTS methods, the highest IC_{50} values were detected in extracts prepared with the classical extraction method, while, according to the FRAP analysis, the highest antioxidant activity was observed in the extracts prepared with classical extraction (Table 3). When looking at the solvent type, the highest average TPC value was in the aronia extracts prepared with NA and NC, while that for the TFC was in the extracts prepared with NP, and for the TMA was in the extracts

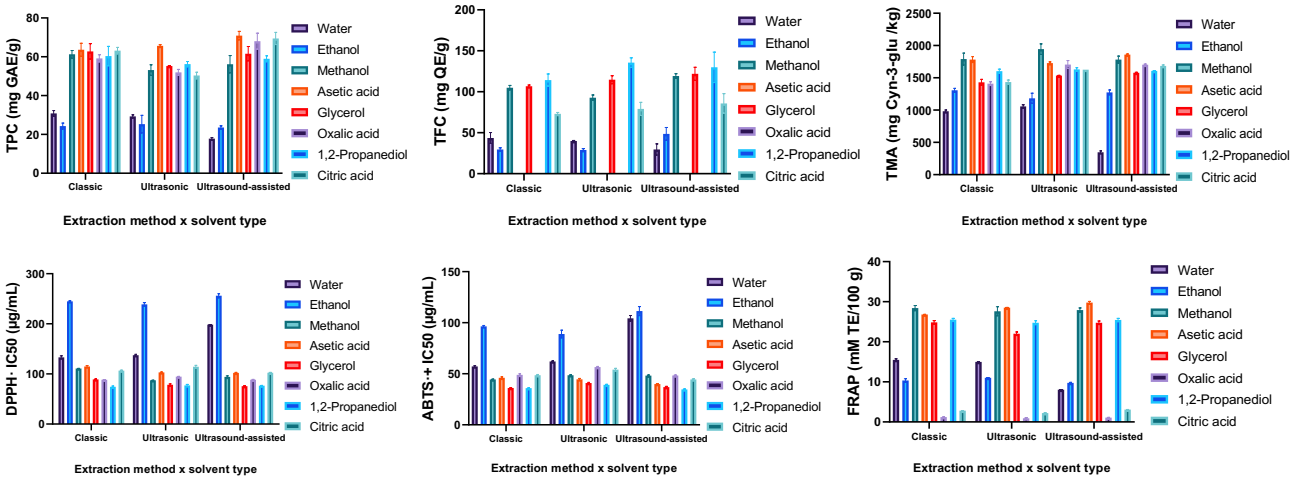

Figure 2. TPC, TFC, TMA, DPPH, ABTS, and FRAP values of the extracts according to the extraction method × solvent type.

Table 3. Effect of extraction method and solvent type on TPC, TFC, TMA, and antioxidant properties (DPPH, ABTS, FRAP) of aronia fruit extracts.

Extraction method (EM)	TPC (mg GAE/g)	TFC (mg QE/g)	TMA (mg Cyn-3-glu/kg)	DPPH· IC_{50} (µg/mL)	$ABTS^{\cdot+}$ IC_{50} (µg/mL)	FRAP (mM TE/100 g)
Classic	53.18 ± 15.46^{a}	59.04 ± 45.40^{b}	1467.56 ± 255.28^{b}	120.28 ± 51.16^{b}	51.72 ± 18.50^{c}	16.93 ± 10.58^{a}
Ultrasonic	48.39 ± 13.35^{b}	61.37 ± 49.47^{b}	1551.08 ± 282.99^{a}	116.39 ± 51.01^{c}	54.37 ± 15.38^{b}	16.50 ± 10.52^{b}
Ultrasound-assisted	53.31 ± 20.04^{a}	66.87 ± 52.61^{a}	1479.54 ± 468.28^{b}	124.23 ± 63.26^{a}	58.53 ± 29.56^{a}	16.23 ± 11.36^{c}
Significance	**	**	**	**	**	**
Solvent type (ST)						
W	26.00 ± 6.20^{d}	37.71 ± 7.92^{e}	796.02 ± 338.19^{f}	156.33 ± 31.72^{b}	74.50 ± 22.50^{b}	12.85 ± 3.60^{d}
E	24.40 ± 2.53^{d}	35.70 ± 10.48^{e}	1254.29 ± 71.86^{e}	246.53 ± 8.07^{a}	98.94 ± 10.31^{a}	10.37 ± 0.59^{e}
M	56.87 ± 4.53^{c}	105.66 ± 11.76^{c}	1839.44 ± 103.55^{a}	97.46 ± 10.58^{d}	47.05 ± 2.01^{e}	27.98 ± 0.79^{b}
NA	66.71 ± 3.85^{a}	ND	1790.14 ± 62.50^{b}	106.65 ± 6.25^{c}	43.50 ± 2.85^{f}	28.37 ± 1.29^{a}
NG	59.86 ± 4.43^{b}	114.49 ± 8.10^{b}	1513.54 ± 69.81^{d}	81.09 ± 6.28^{f}	38.02 ± 2.31^{g}	23.87 ± 1.45^{d}
NO	59.72 ± 7.36^{b}	ND	1606.66 ± 151.05^{c}	90.43 ± 3.23^{e}	51.29 ± 4.01^{c}	1.07 ± 0.15^{g}
NP	58.50 ± 3.26^{bc}	126.55 ± 14.09^{a}	1613.05 ± 25.32^{c}	76.03 ± 1.78^{g}	36.64 ± 2.08^{h}	25.24 ± 0.50^{c}
NC	60.97 ± 8.66^{a}	79.30 ± 9.12^{d}	1582.01 ± 114.29^{c}	107.86 ± 5.45^{c}	49.05 ± 4.26^{d}	2.69 ± 0.39^{f}
Significance	**	**	**	**	**	**
EM X ST	**	**	**	**	**	**

- Data were shown as the mean ± SD (n= 3).
- **, $p < 0.01$
- a-h: Mean values with different letters are significantly different from each other.
- ND: Not detected.
- W; Water, E; Ethanol, M; 80% methanol-distilled water (V:V) mixture acidified with 0.01% HCl, NA; Choline chloride:Acetic acid + %, NG; Choline chloride:Glycerol, NO; Choline chloride:Oxalic acid, NP; Choline chloride:1,2-Propanediol, and NC; Choline chloride:Citric acid.

prepared with M. According to the antioxidant activity analyses, the highest antioxidant activities were in the aronia extracts prepared with NP, according to the DPPH and ABTS analysis results. According to the average results of the FRAP analysis, it was in aronia extracts prepared with NA (Table 3).

The use of NADES increased the extraction of the TPC and TFC, while providing results close to M in the TMA contents (Figure 2). According to the results of the DPPH and ABTS analysis, the NGC extract showed the second highest antioxidant activity. Here, it can be thought that

the amount of water in the formulation of the NG solvent, which had a viscous structure, affected this result. In parallel with this, Vieira et al. (2018) reported that the main factor affecting the extraction efficiency of currant anthocyanins is the water content in NADES, followed by the extraction temperature and the less noticeable extraction time. It has been reported that increasing the water content in NADES results in improved extraction, reduced viscosity, intensified mass transfer, and easier solvent diffusion in plant matrices (Percevault et al., 2021). In addition, the NADES composition and the compound to be extracted are of great importance. Chen and Lahaye (2021) reported that they pretreated apple pulp with NADES (ChCl:lactic acid, ChCl:oxalic acid and ChCl:urea, 1:2 molar ratio) and then extracted pectin with hot water. At the end of their study, they determined that the extracts made using ChCl:lactic acid and ChCl:urea provided yields close to the control group made using water. They observed that while the ChCl:oxalic acid pretreatment led to the degradation and loss of polysaccharides, ChCl:lactic acid pretreatment provided high methoxylated pectin, similar to that obtained by the classical method. In the same study, they noted that ChCl:urea pretreatment affected the composition of the pectin by adding choline, causing saponification.Aronia fruit is rich in polyphenols and mainly contains proanthocyanins, anthocyanins, phenolic acids, and flavonoids (Meng et al., 2019; Wang et al., 2023b). The anthocyanin profile of the aronia fruit consists of cyanidin-3-o-galactoside, C_3G, cyanidin-3-o-arabinoside, and cyanidin-3-o-xyloside (Andrade et al., 2021).

In the extracts, 35 phenolic compounds (quinic acid, fumaric acid, gallic acid, pyrogallol, keracyanin chloride, C_3G, CGA, catechin, peonidin-3-o-), peonidin-3-o-glucoside, 4-OH-benzoic acid, epicatechin, epigallocatechin gallate, caffeic acid, vanillic acid, syringic acid, vitexin, naringin, ellagic acid, hesperidin, p-coumaric acid, sinapic acid, taxifolin, ferulic acid, rosmarinic acid, vanillin, myricetin, resveratrol, luteolin, quercetin, apigenin, naringenin, isorhamnetin, chrysin, galangin, and curcumin) were screened.

As a result of the screening, 8 phenolic compounds (quinic acid, fumaric acid, C_3G, CGA, epicatechin, ellagic acid, hesperidin, and quercetin) were detected in the extracts. In general, when looking at the amounts of phenolic compounds detected, the order was quinic acid > C_3G > CGA > hesperidin > epicatechin > quercetin > ellagic acid > fumaric acid.

Looking at the results of the quinic acid, it was seen that the highest amount was detected in the EC extract, and for C_3G, the highest contents were obtained in the MC extract (Table 4). However, when the C_3G results are examined according to the extraction method, although the C_3G contents of the aronia extracts prepared with M in the classical and ultrasonic extraction were high, the C_3G contents of the extracts prepared with NA in the ultrasound-assisted method were lower. It was determined to be higher than the extracts prepared with M (Table 4). Lin et al. (2022) developed a timesaving, efficient, and environmentally friendly ultrasonic-microwave-assisted natural deep eutectic solvent extraction method for the extraction of anthocyanins from aronia, and reported that the extraction rate of anthocyanins was faster than those obtained by the traditional ethanol method, the natural deep eutectic solvent extraction method and the ultrasonic microwave-assisted ethanol method. Looking at Table 4, it can be seen that solvent type has different effects on the detected phenolic compounds.

It has been reported that some natural compounds obtained from plants such as piperine, curcumin, or baicalin extracted by traditional extraction methods show low solubility, poor stability, low adsorption in the gastrointestinal tract and poor bioavailability, and it has been emphasized that this may affect their pharmacological activities. NADES as an extraction solvent can increase the solubility and bioactivity of these compounds compared to conventional solvents. The use of NADES can also increase the stability and shelf life of compounds in extracts (Hikmawanti et al., 2021). In addition to improving solubility, the presence of NADES has been found to improve the stability of phytochemicals dissolved in NADES and preserve their bioactivity (Cao et al., 2020). Dai et al. (2016) extracted anthocyanins from *Catharanthus roseus* with a series of NADES and found that the lactic acid:glucose and 1,2-propanediol:ChCl for anthocyanins had extraction power similar to that of conventional organic solvents, and even NADES provided higher stability compared to conventional organic solvents.

PCA was performed to determine the differences among the antioxidant properties, TPC, phenolic substance profile, TFC, and TMA contents of the aronia extracts obtained by 3 different extraction methods and 8 different dissolution methods.

Figures 3A–3C show the score distribution plot, loading distribution plot, and biplot plot of the lyophilized aronia fruit extracts. The first 2 principal components (PC1 = 52.50% and PC2 = 20.00%) explained 72.50% of the total variance.

As a result of the analyses, the lyophilized aronia fruit extracts were divided into 3 main groups (Figures 3A–3C). Samples were prepared with 3 different extraction methods using E and W as the solvents (WC, WU, WS, EC, EU, and ES) and extracts prepared using the classical and ultrasonic methods using citric acid ChCl and oxalic acid ChCl as the solvents (NCC, NCU, NOC, and NOU) was

Table 4. LC-MS analysis results of aronia extracts.

Phenolic component (mg/kg)	Quinic acid	Fumaric acid	Cyanidin-3-o-glucoside	Chlorogenic acid	Epicatechin	Ellagic Acid	Hesperidin	Quercetin
WC	8080.79	ND	4887.26	1131.92	12.74	ND	44.02	ND
EC	12438.06	ND	7092.08	548.49	21.89	ND	62.45	ND
MC	3755.56	ND	11041.70	883.32	98.56	ND	112.10	ND
NAC	699.74	ND	9349.30	1411.58	32.42	ND	90.80	ND
NGC	1224.63	ND	8842.08	1251.84	29.40	ND	80.08	1.32
NOC	ND	ND	6120.37	1328.10	ND	ND	58.98	26.82
NPC	1268.49	ND	7885.88	1480.63	30.78	ND	88.04	ND
NCC	285.18	ND	6126.88	1165.96	1.56	ND	80.82	1.11
WU	10173.66	ND	4526.25	1390.74	15.83	ND	82.97	ND
EU	7298.02	6.55	6911.20	624.37	28.33	ND	86.28	ND
MU	4831.50	ND	8070.11	687.71	68.40	33.96	100.55	ND
NAU	ND	ND	7744.44	1291.14	34.16	ND	88.12	ND
NGU	1461.11	ND	6974.78	1101.72	24.32	ND	68.00	ND
NOU	ND	7.51	6400.55	1313.45	ND	ND	63.88	39.69
NPU	1413.57	ND	6977.99	1366.98	24.99	ND	91.20	ND
NCU	287.80	ND	5823.34	1066.90	2.82	ND	88.32	0.76
WS	8701.33	ND	2437.43	1166.96	17.89	ND	75.29	ND
ES	6962.07	ND	6221.12	598.07	26.06	ND	86.96	ND
MS	5058.32	ND	7835.16	729.60	64.17	30.53	112.78	ND
NAS	899.32	ND	7936.60	1356.26	24.01	ND	105.49	ND
NGS	1565.34	ND	7845.44	1408.05	34.18	ND	95.33	ND
NOS	25.54	ND	7485.35	1464.81	ND	ND	72.84	305.72
NPS	1369.60	5.76	7598.14	1454.06	36.98	ND	106.59	0.77
NCS	314.36	13.71	6822.22	1273.90	ND	ND	112.39	15.88

- ND: Not detected
- In table: WC: Aronia extract prepared with water using the classic method, EC: Aronia extract prepared with ethanol using the classic method, MC: Aronia extract prepared with methanol using the classic method, NAC: Aronia extract prepared with NA using the classic method, NGC: Aronia extract prepared with NG using the classic method, NOC: Aronia extract prepared with NO using the classic method, NPC: Aronia extract prepared with NP: using the classic method, NCC: Aronia extract prepared with NC using the classic method, WU: Aronia extract prepared with water using the ultrasonic method, EU: Aronia extract prepared with ethanol using the ultrasonic method, MU: Aronia extract prepared with methanol using the ultrasonic method, NAU: Aronia extract prepared with NA using the ultrasonic method, NGU: Aronia extract prepared with NG using the ultrasonic method, NOU: Aronia extract prepared with NO using the ultrasonic method, NPU: Aronia extract prepared with NP using the ultrasonic method, NCU: Aronia extract prepared with NC using the ultrasonic method, WS: Aronia extract prepared with water using the ultrasound-assisted method, ES: Aronia extract prepared with ethanol using the ultrasound-assisted method, MS: Aronia extract prepared with methanol using the ultrasound-assisted method, NAS: Aronia extract prepared with NA using the ultrasound-assisted method, NGS: Aronia extract prepared with NG using the ultrasound-assisted method, NOS: Aronia extract prepared with NO using the ultrasound-assisted method, NPS: Aronia extract prepared with NP using the ultrasound-assisted method, and NCS: Aronia extract prepared with NC using the ultrasound-assisted method.

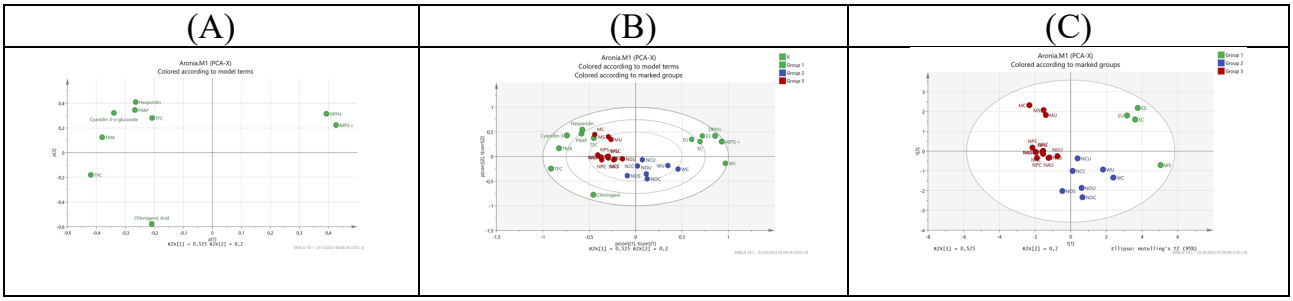

Figure 3. Loading scatter plot (A), score scatter plot (B), and biplot (C) of the PCA (PC1 vs. PC2) for the attributes in aronia extracts. WC: aronia extract prepared with W using the classic method, EC: aronia extract prepared with E using the classic method, MC: aronia extract prepared with M using the classic method, NAC: aronia extract prepared with NA using the classic method, NGC: aronia extract prepared with NG using the classic method, NOC: aronia extract prepared with NO using the classic method, NPC: aronia extract prepared with NP using the classic method, NCC: aronia extract prepared with NC using the classic method, WU: aronia extract prepared with W using the ultrasonic method, EU: aronia extract prepared with E using the ultrasonic method, MU: aronia extract prepared with M using the ultrasonic method, NAU: aronia extract prepared with NA using the ultrasonic method, NGU: aronia extract prepared with NG using the ultrasonic method, NOU: aronia extract prepared with NO using the ultrasonic method, NPU: aronia extract prepared with NP using the ultrasonic method, NCU: aronia extract prepared with NC using the ultrasonic method, WS: aronia extract prepared with W using the ultrasound-assisted method, ES: aronia extract prepared with E using the ultrasound-assisted method, MS: aronia extract prepared with M using the ultrasound-assisted method, NAS: aronia extract prepared with NA using the ultrasound-assisted method, NGS: aronia extract prepared with NG using the ultrasound-assisted method, NOS: aronia extract prepared with NO using the ultrasound-assisted method, NPS: aronia extract prepared with NP using the ultrasound-assisted method, and NCS: aronia extract prepared with NC using the ultrasound-assisted method.

located on the right side of PC1, while all the remaining extracts were located on the left side of PC1 (Figure 3A).

Since the TPC analysis was located close to the sample (MC) extracted with the acidified M as the solvent and the classical extraction method, it can be said that the highest amount of TPC was in these samples. On the other hand, samples extracted with 3 different methods, acetic acid:ChCl, glycerol:ChCl, and propylene glycol:ChCl, were located on the left side of PC1 and close to the samples extracted with M. These results showed that green solvents could be an alternative to M.

4. Conclusion

It has been known for years that solvents used to obtain phytochemicals from various plants have negative effects on the environment. For this reason, discovering the most suitable extraction techniques and solvent types for the environment, and therefore, human health, will be an important step in terms of sustainability. For this reason, in this study, extracts from *A. melanocarpa* were prepared with different methods (classic, ultrasonic, and ultrasound-assisted) using 5 different NADES (NAC, NGC, NOC, NPC, and NCC) as well as generally used W, E, and M solvents. Color properties, TPC, TFC, TMA, and antioxidant capacities (DPPH, ABTS, and FRAP) were determined. It was observed that there were significant changes in the measured values according to the extraction method and solvent type. Extracts obtained with ultrasound and classical extraction had similar and higher TPC than those obtained with ultrasonic extraction. The highest TFC was determined in the extracts obtained with ultrasound-assisted extraction, and the highest TMA content was determined in the extracts obtained with ultrasonic extraction. In the statistical evaluations, the highest average TPC was determined in the aronia extracts prepared with ChCl:acetic acid (NA) and ChCl:citric acid (NC).

With the PCA analysis, it was concluded that NADES can be used as an alternative to M. These results are an important step for protecting the environment and human health. It is thought that the study can be expanded with NADES extracts prepared in formulations other than the NADES extracts prepared in this study. Additionally, extracts need to be evaluated in terms of stability and bioavailability in future studies.

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Supplementary Information

Table S1. Extract codes.

No	Extract code	W: Water M: Methanol E: Ethanol C: Classic U: Ultrasonic S: Ultrasound
1	WC	Aronia extract prepared with water using the classic method
2	EC	Aronia extract prepared with ethanol using the classic method
3	MC	Aronia extract prepared with methanol using the classic method
4	NAC	Aronia extract prepared with NA using the classic method
5	NGC	Aronia extract prepared with NG using the classic method
6	NOC	Aronia extract prepared with NO using the classic method
7	NPC	Aronia extract prepared with NP using the classic method
8	NCC	Aronia extract prepared with NC using the classic method
9	WU	Aronia extract prepared with water using the ultrasonic method
10	EU	Aronia extract prepared with ethanol using the ultrasonic method
11	MU	Aronia extract prepared with methanol using the ultrasonic method
12	NAU	Aronia extract prepared with NA using the ultrasonic method
13	NGU	Aronia extract prepared with NG using the ultrasonic method
14	NOU	Aronia extract prepared with NO using the ultrasonic method
15	NPU	Aronia extract prepared with NP using the ultrasonic method
16	NCU	Aronia extract prepared with NC using the ultrasonic method
17	WS	Aronia extract prepared with water using the ultrasound-assisted method
18	ES	Aronia extract prepared with ethanol using the ultrasound-assisted method
19	MS	Aronia extract prepared with methanol using the ultrasound-assisted method
20	NAS	Aronia extract prepared with NA using the ultrasound-assisted method
21	NGS	Aronia extract prepared with NG using the ultrasound-assisted method
22	NOS	Aronia extract prepared with NO using the ultrasound-assisted method
23	NPS	Aronia extract prepared with NP using the ultrasound-assisted method
24	NCS	Aronia extract prepared with NC using the ultrasound-assisted method

Table S2. LOD and LOQ recovery for the minerals (ng/mL).

	Component	LOD	LOQ
1	Quinic acid	0.84	2.80
2	Fumaric acid	0.41	1.38
3	Gallic acid	1.29	4.29
4	Pyrogallol	1.33	4.42
5	Keracyanin chloride	1.68	5.59
6	Cyanidin-3-*O*-glucoside	0.77	2.56
7	Chlorogenic acid	0.41	1.37
8	Catechin	1.16	3.88
9	Peonidin-3-*O*-glucoside	1.09	3.63
10	4-OH-Benzoic acid	1.24	4.13
11	Epicatechin	1.09	3.65
12	Epigallocatechin gallate	2.98	9.94
13	Caffeic acid	0.87	2.90
14	Vanillic acid	2.30	7.67
15	Syringic acid	3.20	10.79
16	Vitexin	0.40	1.34
17	Naringin	0.33	1.08
18	Ellagic acid	1.55	5.16
19	Hesperidin	1.31	4.36
20	p-Coumaric acid	0.90	3.00
21	Sinapic acid	1.27	4.23
22	Taxifolin	0.94	3.13
23	Ferulic acid	1.30	4.35
24	Rosmarinic acid	1.70	5.67
25	Vanillin	0.63	2.09
26	Myricetin	0.57	1.91
27	Resveratrol	0.69	2.29
28	Luteolin	0.54	1.81
29	Quercetin	0.98	3.27
30	Apigenin	1.09	3.65
31	Naringenin	0.81	2.70
32	Isorhamnetin	2.15	7.17
33	Chrysin	1.70	5.68
34	Galangin	1.17	3.92
35	Curcumin	0.57	1.89