European Food Research and Technology (2026) 252:150
https://doi.org/10.1007/s00217-026-05081-0

ORIGINAL PAPER

Impact of pulsed electric field pretreatment on quality parameters of aronia and haskap berry juices

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Received: 30 October 2025 / Revised: 6 January 2026 / Accepted: 31 January 2026
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Abstract
This study examined the effect of pulsed electric field pre-treatment at three intensities (1.78, 5.3, and 10.7 kJ/kg) on the yield and physicochemical properties of aronia and haskap juices. PEF significantly increased juice yield by 18.0% points in aronia (from 39.3% to 57.3%) and 30.6% points in haskap (from 29.2% to 59.8%) at 10.7 kJ/kg. Physicochemical properties were also affected: pH, total soluble solids, and viscosity increased, while both juices retained pseudoplastic flow behaviour. PEF induced significant colour changes, with total colour difference increasing progressively with treatment intensity. PEF enhanced sugar extractability, increasing total sugars from 169.4 to 210.0 g/L in aronia (mainly glucose and sorbitol) and from 115.2 to 142.1 g/L in haskap (mainly glucose and fructose). Organic acids increased by 26-27%, with higher citric, malic, and quinic acids. Phenolic compounds, especially anthocyanins, increased from 8.60 to 15.12 g/L in aronia and from 5.65 to 10.18 g/L in haskap, along with increases in flavonols and total phenolics. Correlation analysis revealed strong positive relationships between juice yield, sugar content, total acids, and phenolic compounds in aronia, while moderate positive correlations were observed between these parameters in haskap. Overall, PEF at 10.7 kJ/kg effectively improved yield and compositional quality of both aronia and haskap juice.

Keywords Non-thermal processing · Fruit juice · Bioactive compounds · Anthocyanins · Extraction · Berries

Introduction

Berry crops, particularly underutilized varieties such as elderberry, honeyberry, and black chokeberry, are widely recognized for their bioactive compounds, which serve as natural food colourants and contribute to various health-promoting activities [1]. The high content of phenolic compounds in berries, especially anthocyanins, enhance their strong antioxidant capacity, which is associated with significant health benefits, including reducing the risk of cardiovascular diseases, inflammation, and cancer [2, 3]. While extensively studied berries such as blueberries and cranberries are well established as rich sources of bioactive compounds, less attention has been given to emerging northern berry species such as haskap (*Lonicera caerulea* L.) and aronia (*Aronia melanocarpa* L.), despite their equal or superior levels of phenolics and antioxidant capacity [4, 5]. Haskap contains unique anthocyanins such as cyanidin-3,5-diglucoside and cyanidin-3-glucoside, which are rarely found in other berries and exhibit strong antioxidant and anti-inflammatory activities [4]. This berry has traditionally been valued for its therapeutic benefits, such as lowering hypertension, heart attack risk, and gastrointestinal diseases, due to its rich composition of bioactive compounds [6]. Similarly, aronia is recognized for having one of the highest antioxidant levels among berries and diverse phenolic composition, including procyanidins, phenolic acids, and anthocyanins, making it a promising candidate for functional food applications [7]. Juice production from aronia is well established at an industrial level, as the fruit is widely processed into juices, concentrates, nectars, and functional beverages due to its high polyphenol content and intense colour [8]. Large-scale aronia juice production is well established in Poland, Germany, the Baltic countries, and North

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Published online: 24 February 2026

America, where mechanized harvesting and conventional pressing technologies are routinely applied [9]. In contrast, industrial processing of haskap remains relatively limited, despite its comparable or even higher concentrations of anthocyanins such as cyanidin-3-glucoside and cyanidin-3,5-diglucoside [4, 10]. Previous studies have demonstrated the technical feasibility of juice extraction from haskap berries using pressing, enzymatic treatment, and non-thermal technologies, although optimization of extraction efficiency and quality preservation remains an important challenge [4]. Beyond their nutritional potential, these berries remain underutilized crops with increasing agricultural and economic interest, especially in cold climates, where they offer resilience to abiotic stress and opportunities for diversification of berry production systems [5]. Their intense pigment content also makes them a valuable material for use as natural colourants and functional ingredients in the food and nutraceutical industries [5, 11]. Recent study by Ayuso-Yuste et al. (9) has further emphasized the importance of these berries, demonstrating that aronia exhibits significantly higher total phenolic content than haskap and goji, and that anthocyanins dominate the phenolic profile of both aronia and haskap, whereas goji lacks these pigments. This finding confirms that aronia and haskap are rich sources of anthocyanins and polyphenols, making them particularly valuable for juice production due to their naturally high acidity and astringency, which limit their fresh consumption [12]. Recent market data support the industrial relevance of juice production from these fruits. The global aronia berry market was valued at USD 779 million in 2021 and is projected to reach USD 1.29 billion by 2028, including the juice, concentrate, and functional beverage production [13]. Similarly, commercial cultivation of haskap has expanded rapidly, European estimates indicate approximately 5500 ha of cultivated area in 2021 (excluding Asia), while China alone reached around 5000 ha by 2022, with berries increasingly processed into juices and related products [14]. As health-conscious consumers increasingly seek high-quality foods that retain their nutritional and sensory properties while minimizing harsh processing methods, the demand for innovative food technologies has grown [15]. In this context, Pulsed Electric Field (PEF) technology presents an effective solution. PEF involves the application of electrical pulses that preserve the nutritional, functional, and sensory attributes of food products while ensuring microbial safety [16]. Unlike thermal processing, PEF minimizes the degradation of bioactive compounds, thereby maintaining the nutritional and functional value of foods [17]. In addition, PEF is more energy-efficient and environmentally friendly compared to traditional thermal methods, contributing to sustainability in the food industry [18]. Its ability to modify cellular structures enhances mass transfer and the extraction efficiency of bioactive compounds, outperforming traditional extraction methods [19]. The process of PEF-induced electroporation disrupts cell membranes, creating pores that facilitate the release of intracellular compounds, such as phenolics and antioxidants [20]. This mechanism relies on the dielectric breakdown of the cell membrane, increasing permeability to small molecules and enhancing the recovery of pigments, antioxidants, and other functional compounds in food and byproducts [19, 21]. Some studies have reported the use of Pulsed Electric Field (PEF) as a pretreatment before extraction because it has desirable traits such as minimal operating time and efficient power consumption [22]. However, the use of PEF to increase the extraction rate in the food and nutraceutical industry is still in its infancy [22].

Current research on PEF-assisted extraction is mainly focused on foods and bioactive substances that have not yet been detected or on optimizing the parameters of the PEF system to improve yields [23, 24]. Most studies on the effects of PEF treatment on the quality parameters of fruit juices have focused on common fruits, such as apples, grapes, or oranges [24–30], but only few studies have been performed on juices from berry fruit species. There is limited data in the literature on the use of PEF as a pretreatment in the production of aronia and haskap juices, indicating that further research is needed to investigate the effect of PEF technology on the quality parameters of these juices. Therefore, the aim of this study is to evaluate the effect PEF pretreatment of fruit mash on improving the yield and physicochemical properties of aronia and haskap fruit juices.

Materials and methods

Juice production and PEF treatments

Aronia (*Aronia melanocarpa* L. cv. 'Nero') and haskap (*Lonicera caerulea* L. cv. 'Aurora') berries were harvested in July 2024 from farms located in Novo Mesto and Maribor, Slovenia, respectively. Frozen berries (−18 °C) were thawed overnight at 4 °C and cold-crushed using a stainless-steel mill to obtain the mash. Prior to PEF treatment, the fruit mash was preheated in a water bath to 50 °C to improve its electrical conductivity. This step was necessary because, in the production of 100% fruit juice, the mash composition cannot be modified by adding electrolytes such as salts or sugars; therefore, temperature adjustment is the only practical way to achieve sufficient electrical conductivity for effective energy transfer during PEF treatment. A moderate increase in temperature enhances conductivity and uniform electric field distribution within the mash, thereby

improving electroporation efficiency [31–33]. The preheating step was applied uniformly to all samples, including the control treatment, ensuring that the differences observed between treatments were attributable to PEF and not heat exposure. PEF treatments were performed using a laboratory-scale EPULSUS®-LBM1B-15 system (Energy Pulse Systems, Lisbon, Portugal), which delivers monopolar or bipolar square wave pulses up to 15 kV and 400 A with a 250 ns rise time and short-circuit protection. The treatment chamber consisted of parallel stainless-steel plate electrodes with a 10 cm gap and a total volume of 1 L. Approximately 500 ± 10 g of aronia or haskap mash was placed in the chamber for each run. The electric field strength (E), pulse duration (τ), and pulse frequency (f) were fixed at 1 kV/cm, ± 10 µs, and 1 Hz, respectively. Treatments included: Control (no PEF), PEF 1 (W = 1.78 kJ/kg), PEF 2 (W = 5.3 kJ/kg), and PEF 3 (W = 10.7 kJ/kg). The specific energy input and electrical parameters (electric field strength, energy per pulse, and total applied energy) were calculated according to the equations and methodology described by [34] using real-time monitoring of voltage and current signals with a PicoScope® 2000 Series mixed-signal oscilloscope connected to the PEF system (Pico Technology, UK). Signals were recorded and analyzed using PicoScope 7 T&M software. The temperature and electrical conductivity of the mash were measured using a portable conductivity meter (mod. 913, Knick Elektronische, Berlin, Germany). Only minor changes were observed, with a temperature increase of 2–3 °C after PEF treatment. To avoid the risk of thermal effects, all PEF treatments were conducted under mild processing conditions. The temperature of the mash was continuously monitored throughout treatment and did not exceed 52–53 °C. These conditions were selected based on previous studies demonstrating that elevated temperatures adversely affect anthocyanins, phenolic compounds, and ascorbic acid in berry products [35, 36]. Therefore, the experimental design focused on isolating the impact of PEF pretreatment on juice quality parameters rather than evaluating thermal degradation effects. The influence of thermal pretreatments on berry bioactive compounds has been comprehensively reported elsewhere and was not the focus of the present study. Finally, juices were extracted using a Hydro PARA-Press (Paul Arauner GmbH, Germany) at 3.5 bar for 15 min. The resulting juices were then pasteurized at 85 °C for 1 min in plastic bottles immersed in a thermostatic water bath (MPM Instruments s.r.l., Bernareggio, Italy) and subsequently cooled in an ice bath prior to further analysis.

Juice yield

The effectiveness of juice pressing was determined according to the following equation:

$$Wj\ (\%) = \frac{Mj}{Mi} \times 100$$

where *Wj* refers to the efficiency of pressing expressed in %, *Mj* denotes the mass of the juice after pressing expressed in kg, and *Mi* refers to the mass of the input material, i.e., fruit mass in kg.

Measurement of pH and soluble solids

The content of total soluble solids (TSS) in juice samples was determined using a portable sugar/Brix refractometer, model no. 300,001 by Sper Scientific Ltd. (Scottsdale, AZ, USA), and expressed as °Brix. pH values were determined using a Crison pH meter (Basic 20, Crison Instruments, Barcelona, Spain).

Viscosity measurements

Juice viscosity was measured using an Anton Paar MCR 102e rheometer (Graz, Austria) with a concentric cylinder probe and thermostatic bath to maintain 20 °C. Shear rates ranged from 10 to 300 s^{-1}, and data were recorded via RheoCompass™ 1.31 software. Shear stress vs. shear rate data were fitted to the Ostwald–de Waele power-law model:

$$\mu\left(\dot{\gamma}\right) = K\dot{\gamma}^{n-1}$$

where *K* denotes the viscosity of a sample at a shear rate of 1 s^{-1}, and *n* is the power-law index indicating the rate of shear thinning when $n<1$.

Colourimetric analysis

Juice colour was analyzed with a Konica Minolta CM-36dG colourimeter (Osaka, Japan) in transmittance mode using D65 illumination. Measurements were performed in a clear rectangular plastic cuvette provided by the manufacturer, with a fixed optical path length of 1 cm, filled with approximately 14 mL of juice. Results were expressed as L^* (lightness), a^* (redness), and b^* (yellowness) using Spectramagic™ 3.6 software. Total colour difference (ΔE^*) was calculated as:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^*, Δa^*, and Δb^* denote the differences between juice samples processed with different treatments. Colour differences were classified as described in Cserhalmi et al. [37]: ΔE^*=0–0.5: not noticeable, ΔE^*=0.5–1.5: slightly

noticeable, ΔE^*=1.5–3.0: noticeable, ΔE^*=3.0–6.0: well visible, ΔE^*=6.0–12.0: great.

Extraction and quantification of sugars and organic acids

Sugars and organic acids were extracted and quantified following method by Mikulic-Petkovsek et al. (31). Juice samples were centrifuged (Eppendorf 5810 R, Germany) at 6000 rpm for 5 min at 4 °C, then filtered using 0.20 µm cellulose filters (Macherey-Nagel, Germany). Sugars were analyzed using HPLC (Vanquish™ Flex UHPLC, Thermo Fisher, USA) with a Rezex RCM-monosaccharide Ca+ (2%) column (150 × 7.8 mm, Phenomenex, USA), using bidistilled water as the mobile phase (0.8 mL/min, 80 °C, 20 min run time). Sugars were quantified using calibration curves prepared from reference standards (fructose, glucose, sucrose, sorbitol; all purchased from Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

Organic acids were analyzed using a Rezex ROA–Organic Acid H+ (8%) column (150 × 7.8 mm) at 65 °C, with 4 mM sulfuric acid as the mobile phase (0.6 mL/min, 15 min run time). Concentrations were determined using calibration curves prepared with corresponding standards purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Standards included citric, malic, oxalic, quinic, shikimic, and tartaric acids for aronia, and citric, fumaric, malic, quinic, shikimic, and tartaric acids for haskap. A refractive index detector was used for sugars, and a UV detector at 210 nm for organic acids. Concentrations were expressed in g/L.

Extraction and quantification of phenolic compounds

Phenolic compounds were extracted as described in Chap. 2.6, according to the method by [39]. Analysis was performed using HPLC (Finnigan Surveyor, Thermo Fisher, USA) with a photodiode array detector (280, 350, 530 nm) and mass spectrometer. Mobile phases were A: bidistilled water/acetonitrile/formic acid (96.9/3.0/0.1, v/v/v) and B: acetonitrile/bidistilled water/formic acid (96.9/3/0.1, v/v/v). A Gemini C18 column (Phenomenex, USA) at 25 °C was used. Elution involved a gradient from 5% to 90% B over 30 min, followed by a return to initial conditions. Anthocyanins were detected in positive ionization mode, the rest of the phenolics in negative mode (scan range: m/z 115-1900). MS parameters included 250 °C capillary temperature, sheath/auxiliary gas at 60/15, source voltage 3 kV, and collision energy 20-35%. Data were processed using Xcalibur™ 4.7 (Thermo Scientific). Phenolics were identified by retention time, PDA spectra, MSn fragmentation, and standards corresponding to the compounds detected in each juice:

- aronia: anthocyanins (six major cyanidin- and pelargonidin-derived glycosides), flavones (apigenin-dirhamnoside), flavanones (eriodictyol- and aaringenin-hexosides), flavanols (catechin, epicatechin, and procyanidin oligomers), flavonols (kaempferol, quercetin, and isorhamnetin glycosides), hydroxycinnamic acids (caffeic, *p*-coumaric, feruloyl, and caffeoylquinic acid derivatives), and stilbenes (resveratrol hexoside);
- haskap: anthocyanins (cyanidin-, delphinidin-, pelargonidin-, and peonidin-derived glycosides), flavones (genistein- and luteolin-glycosides), flavanones (naringenin-hexoside), flavonols (quercetin and isorhamnetin glycosides), flavanols (catechin and procyanidin oligomers), hydroxybenzoic acids (ellagic acid hexoside), and hydroxycinnamic acids (caffeic, dicaffeoylquinic, *p*-coumaric, and feruloylquinic acid derivatives).

Standards were purchased from Sigma–Aldrich, Fluka, Extrasynthese, or Merck, prepared at appropriate concentrations (0.1-5 mg/mL), and used for quantification. Total and individual phenolics were expressed in g/L.

Statistical analysis

Statistical analysis was performed using the R-commander statistical software version 4.3.0 (R Formation for Statistical Computing, Auckland, New Zealand). Differences between the treatments were examined using one-way ANOVA, along with Tukey's post-hoc test for multiple comparisons at a 95% confidence level. All measurements were performed in four repetitions, and the results are presented as mean values with standard error (mean ± SE). Pearson correlation analysis was performed to examine potential correlations among sugars, organic acids, and phenolic compounds in both aronia and haskap juices in relation to PEF treatment. Correlation matrices were visualized using the corrplot package in R (version 4.3.0).

Results and discussion

Yield

As shown in Fig. 1, PEF treatment significantly enhanced juice yield in both aronia and haskap fruits ($p < 0.05$). In aronia, yield increased from 39.3% ± 0.24 in the control to 57.3% at the highest energy level (10.7 kJ/kg), representing

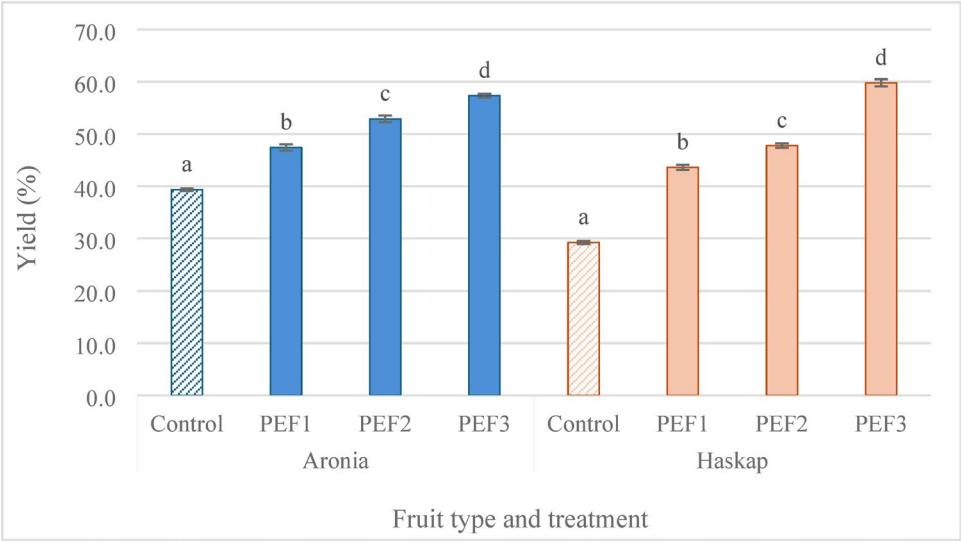


Fig. 1 Juice yield of aronia and haskap juices compared to untreated (control) juice. The values are the means of four repetitions. Mean values followed by a different letter are significantly different according to Tukey's test ($p \leq 0.05$)

an increase of 18.0% points. Similarly, haskap juice yield increased from 29.2% ± 0.32 in control to from 29.2% in the control to 59.8% at PEF3, corresponding to an increase of 30.6% points, which represents a significant improvement in extraction efficiency. Overall, PEF3 produced the highest yields in both fruits, confirming a positive correlation between applied energy and juice extraction efficiency, consistent with previous findings in sweet cherry [40].

These results can be attributed to electroporation of cellular membranes, which enhances mass transfer and facilitates the release of intracellular liquid during pressing [41]. Similar mechanisms have been widely reported, with PEF treatments shown to increase both yield and juice purity in apples, carrots, and grapes [42–45]. In grapes, yield gains of 5–27% have been documented depending on cultivar [28, 46], while in raspberries, increases of up to 25% were achieved at 3 kV/cm and 12 kJ/kg [27]. Additionally, optimized PEF conditions were used in production of Sohiong juice, where juice yield increased from 53.9% to 74.4%, indicating influence of process parameters and fruit matrix on extraction efficiency [47].

Colour

PEF treatment significantly influenced the colour attributes of both aronia and haskap juices ($p < 0.01$ for all parameters except chroma in haskap, $p = 0.558$). In aronia juice, lightness (L^*) decreased consistently with increasing treatment intensity, from 14.92 in the control to 12.87 in PEF3 ($p < 0.001$), reflecting a darker appearance that can be attributed to enhanced extraction and concentration of pigments, particularly anthocyanins (Table 3). Similar PEF-induced darkening has been reported in 'Concord' grape juice and Sohiong juice, where pigment release from disrupted cell structures intensified colour expression [47, 48]. Redness (a^*) also increased significantly in aronia, from 3.61 in the control to 4.47 at PEF3, while blueness (b^*) shifted from −15.03 to −16.90 ($p < 0.001$), indicating greater colour saturation. These changes were accompanied by significant increases in chroma (C^*) at PEF1 and PEF3 ($p = 0.003$) and an increase in hue angle (h^*), particularly at PEF2 (29.80) and PEF3 (24.85), compared with the control (15.47). These results are consistent with findings in grape and pomegranate juices, where PEF has been shown to intensify purplish-red tones through enhanced anthocyanin liberation [46, 49].

In haskap juice, L^* values also decreased progressively with PEF intensity, from 22.29 in the control to 20.15 in PEF3 ($p < 0.001$), indicating darkening of the juice, likely caused by increased pigment extraction or transformation (Table 3). Redness (a^*) was slightly increased, from 1.08 to 1.41, while b^* values had a slight decrease at PEF1 (–0.05) compared to the control (0.03), and these differences were significant ($p = 0.009$). Chroma (C^*) remained statistically unchanged ($p = 0.558$), suggesting overall colour intensity was not affected. However, hue angle (h^*) increased significantly with treatment, from 16.26 (control) to 28.28 (PEF3) ($p < 0.001$), indicating a shift toward more bluish-red tones. Comparable increases in hue have been documented in tomato and Sohiong juices, where PEF promoted pigment release but with variable effects depending on cultivar and matrix composition [47, 50].

Regarding total colour difference (ΔE^*; Fig. 2), PEF treatment resulted in perceptible and statistically significant changes in both aronia and haskap juices ($p < 0.001$). In aronia, ΔE^* values increased progressively with treatment intensity, from 1.85 at PEF1 to 2.49 at PEF2 and 2.91 at PEF3, indicating a clear intensification of colour at higher energy inputs. Similarly, haskap juice exhibited

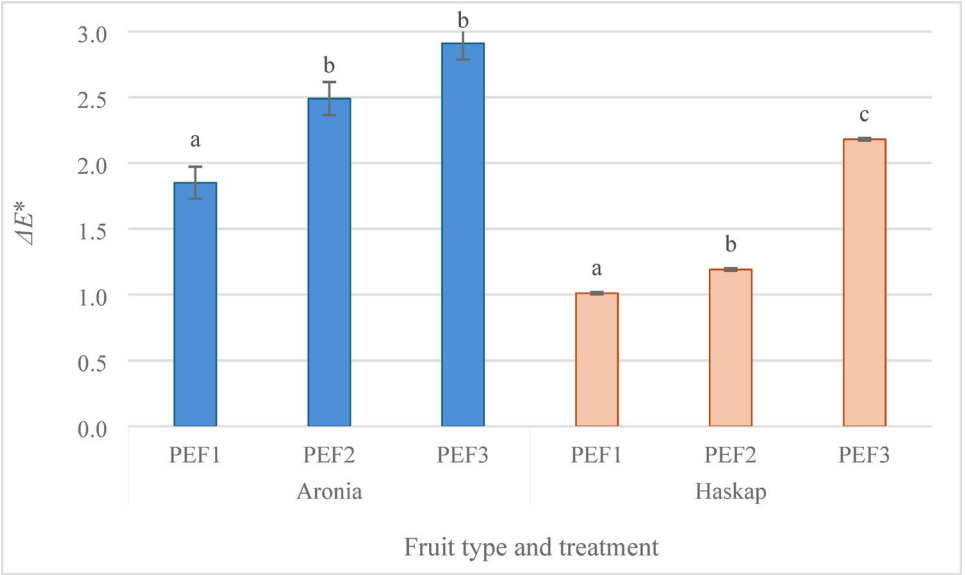


Fig. 2 Total colour difference (ΔE^*) of aronia and haskap juices compared to untreated control juice. The values are the means of four repetitions. Mean values followed by a different letter are significantly different according to Tukey's test ($p \leq 0.05$)

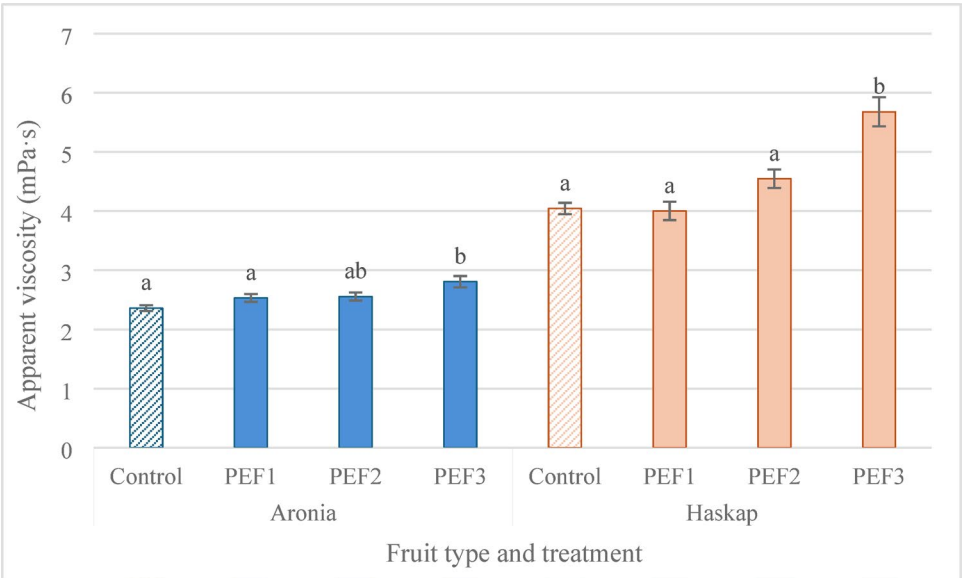


Fig. 3 Apparent viscosity of aronia and haskap juices compared to untreated control at a shear rate of 100 s^{-1}. The values are the means of four repetitions. Mean values followed by a different letter are significantly different according to Tukey's test ($p \leq 0.05$)

significant increases in ΔE^*, from 1.01 at PEF1 to 1.19 at PEF2 and 2.18 at PEF3. These values fall within the range considered perceptible to the human eye, confirming that PEF pre-treatment induced noticeable colour enhancement. The greater colour difference observed at higher field intensities is consistent with the enhanced release of anthocyanins through electroporation-induced disruption of cellular structures, as reported in grape and pomegranate juices [46, 49].

Although colour changes were evaluated instrumentally in the present study, it should be noted that the activity of oxidative enzymes, such as polyphenol oxidase and peroxidase, was not quantified. These enzymes are known to catalyse oxidation of phenolic substrates and contribute to pigment degradation and browning reactions in berry juices, and their measurement could provide mechanistic insight into the colour changes observed following PEF pretreatment [51]. Therefore, future studies should integrate enzymatic activity assays in combination with PEF processing to better understand the relationship between pigment stability, enzyme activity, and electric field-induced cellular disruption.

Viscosity

PEF treatment significantly influenced the rheological properties of both aronia and haskap juices (Fig. 3). As shown in the apparent viscosity results, aronia juice exhibited a slight

Table 1 Consistency index (k), flow index (n) and determination index (R^2) of Aronia and Haskap juice samples obtained under different treatments.

Treatment	Aronia	Haskap
	k	
Control	0.004 ± 0.000 [a]	0.103 ± 0.097 [a]
PEF 1	0.004 ± 0.001 [a]	0.526 ± 0.294 [ab]
PEF 2	0.006 ± 0.002 [a]	0.829 ± 0.203 [ab]
PEF 3	0.005 ± 0.001 [a]	1.308 ± 0.279 [b]
p-value	0.411	0.020
	n	
Control	0.919 ± 0.030 [a]	0.767 ± 0.142 [a]
PEF 1	0.938 ± 0.061 [a]	0.469 ± 0.137 [ab]
PEF 2	0.839 ± 0.071 [a]	0.271 ± 0.049 [b]
PEF 3	0.903 ± 0.048 [a]	0.258 ± 0.017 [b]
p-value	0.620	0.014
	R^2	
Control	0.949 ± 0.011 [a]	0.953 ± 0.017 [a]
PEF 1	0.950 ± 0.019 [a]	0.913 ± 0.020 [a]
PEF 2	0.926 ± 0.030 [a]	0.901 ± 0.025 [a]
PEF 3	0.964 ± 0.013 [a]	0.932 ± 0.010 [a]
p-value	0.596	0.281

The values are means of four repetitions. Mean values followed by a different letter in the column are significantly different according to Tukey's test ($p \leq 0.05$).

but statistically significant increase in viscosity at the highest energy input (PEF3), increasing from approximately 2.3 mPa·s in the control to 2.8 mPa·s ($p < 0.05$). Lower-intensity treatments (PEF1 and PEF2) did not differ significantly from the control, suggesting that only high-energy input effectively enhances the viscosity of aronia juice, a trend also observed in other fruit juices subjected to PEF and HIPEF treatments [52, 53].

In haskap juice, a more pronounced response to PEF was observed, particularly under PEF3, where viscosity increased significantly from 4.0 mPa·s (control) to nearly 5.8 mPa·s ($p < 0.05$). This more substantial viscosity change in haskap may reflect a greater susceptibility of its cell structure to electroporation and subsequent release of soluble solids and structural components, as previous studies have reported at electroporation-induced cell wall disruption promotes solubilization of pectic and colloidal material in plant tissues [52]. These viscosity changes are likely the result of multiple mechanisms. Primarily, solubilization of pectin, cell wall polysaccharides, and other colloidal materials contributes to thickening, as previously reported for watermelon and tomato juices after HIPEF [52, 53]. In addition, the significant increase in phenolic compounds observed under PEF3, particularly anthocyanins and flavonols (Fig. 6), may further enhance viscosity. Phenolics can interact with pectins and soluble sugars via hydrogen bonding and other non-covalent interactions, promoting aggregation and network formation, which contributes to the observed thickening of the juices [54].

Table 2 pH and TSS values of aronia and haskap juices compared to untreated juice (control).

Treatment	Aronia	Haskap
	pH	
Control	3.71 ± 0.00[a]	3.33 ± 0.04[ab]
PEF 1	3.72 ± 0.00[a]	3.41 ± 0.02[b]
PEF 2	3.73 ± 0.00[a]	3.39 ± 0.04[b]
PEF 3	3.62 ± 0.02[b]	3.22 ± 0.01[a]
p-value	0.000	0.006
	TSS	
Control	17.68 ± 0.31[a]	13.15 ± 0.12[a]
PEF 1	18.58 ± 0.23[ab]	14.15 ± 0.12[b]
PEF 2	18.53 ± 0.28[ab]	14.23 ± 0.28[b]
PEF 3	18.93 ± 0.22[b]	14.40 ± 0.13[b]
p-value	0.032	0.001

The values are the means of four repetitions. Mean values followed by a different letter in the column are significantly different according to tukey's test ($p \leq 0.05$)

These observations were further supported by the rheological parameters obtained from the power-law model (Table 1). Both juices demonstrated shear-thinning behaviour across all treatment groups, as indicated by flow behaviour index (n) values below 1, confirming pseudoplastic flow typical of fruit juices containing suspended particulates and macromolecules [55, 56]. In aronia juice, the consistency coefficient (k) slightly increased with higher PEF intensity, reaching the highest value under PEF3, while n values remained relatively stable, suggesting thickening without a major change in flow character. In contrast, haskap juice exhibited a more pronounced effect: k increased progressively with PEF intensity, particularly for PEF3, whereas n decreased significantly, indicating a stronger shear-thinning tendency. These results suggest that PEF3 treatment enhanced apparent viscosity and modified flow behaviour, likely through solubilization of pectin, cell wall polysaccharides, and other colloidal materials [55].

pH and TSS

PEF treatment had a statistically significant impact on juice pH in both aronia and haskap ($p = 0.000$ and 0.006, respectively), as presented in Table 2. In aronia juice, the pH results did not show any significant differences from the control (pH = 3.71) across PEF1 and PEF2 treatments. However, PEF3 led to statistically significant decrease in pH to 3.62 ($p < 0.05$), which may reflect increased acid release due to greater cellular disruption at higher energy input. In contrast, haskap juice showed a variable response, with moderate increases at lower intensities (PEF1–PEF2) and a significant decrease

at the highest intensity (PEF3). The observed pH reduction at PEF3 corresponds with the measured increase in total organic acids (particularly citric, malic, and quinic acids) and °Brix, suggesting that high-intensity PEF promoted the simultaneous release of both sugars and acids into the juice phase. This is consistent with prior reports indicating that PEF has variable influence on juice acidity, with some studies noting negligible effects [57, 58], while others have reported statistically significant changes depending on fruit matrix and processing intensity [59].

Total soluble solids (TSS) were also significantly influenced by PEF treatment in both juices ($p = 0.032$ for aronia; $p = 0.001$ for haskap). In aronia, a significant TSS increase was observed only at the highest energy level (PEF3 = 18.93), while in haskap all PEF treatments resulted in higher TSS compared to the control. These increases are consistent with the enhanced extractability of sugars and acids observed in both matrices (Figs. 4 and 5) suggesting that PEF effectively improves the release of soluble constituents, such as sugars and acids, thereby contributing to higher °Brix values in the final juice. Similar increases in °Brix following PEF have been reported for orange, tomato and grape juices [60], and raspberry juice [59], with the increase depending on field strength, energy input, and tissue characteristics.

Sugars

PEF treatment significantly influenced both individual sugars and total sugar content in aronia and haskap juices (Fig. 4). In aronia juice, fructose, glucose, and sorbitol concentrations increased significantly with PEF application ($p < 0.01$), while sucrose remained statistically unchanged ($p = 0.931$). Fructose content increased from 39.91 g/L in control to 52.68 g/L in PEF2 and 52.06 g/L in PEF3, showing clear enhancement with higher treatment intensities. A similar pattern was observed for glucose, which increased significantly from 56.53 g/L (control) to around 69.0 g/L in PEF2 and PEF3 ($p = 0.002$). Sorbitol, the dominant carbohydrate (sugar alcohol) in aronia, showed a notable rise from 65.71 g/L in control to 81.18 g/L in PEF2 ($p = 0.009$), though no further increase was observed at PEF3 (Table 4). These changes led to a significant increase in total sugar content, from 169.36 g/L in control to over 210 g/L in PEF2 and 207.75 g/L in PEF3 ($p < 0.05$), likely due to improved cell wall permeability and sugar diffusion [61]. This is consistent with the findings from Oziembłowski et al. (54), who reported an increase in the total sugar content after PEF processing of aronia juice. Additionally, comparable sugar profiles have been reported in fresh

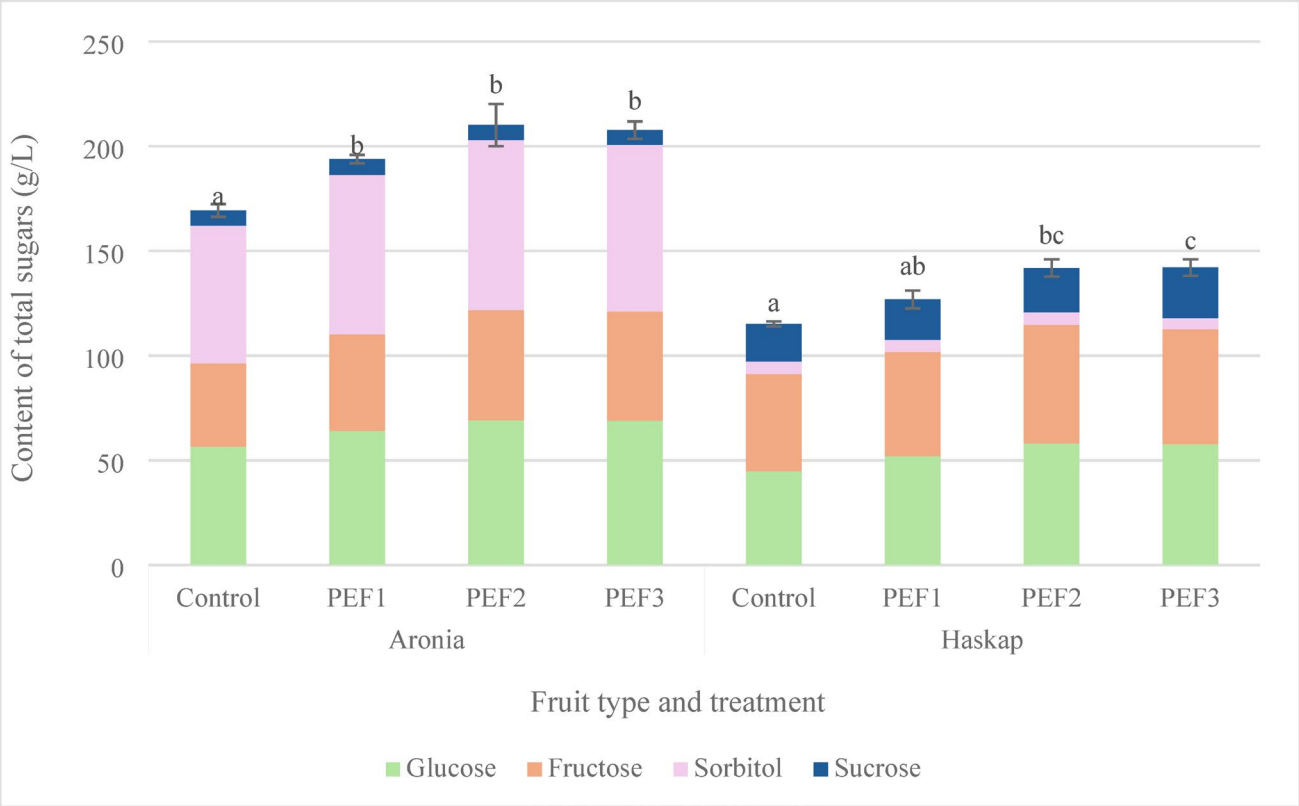


Fig. 4 Content of sugars in aronia and haskap juices compared to the untreated control (g/L). The values are the means of four repetitions. Mean values followed by a different letter are significantly different according to Tukey's test ($p \leq 0.05$)

chokeberries, where fructose and glucose are the predominant sugars, sorbitol (a sugar alcohol) is also abundant, and sucrose is present only in minor amounts [63–65]. Denev et al. (57) identified sucrose alongside the major sugars, similar to our findings.

In contrast, haskap juice showed a different pattern (Table 4). Significant increases were observed in fructose, glucose, and sucrose with PEF ($p < 0.01$ for all), while sorbitol remained statistically unchanged ($p = 0.733$) and low in absolute amounts. Fructose contents increased from 46.60 g/L in control to approximately 56.70 g/L in PEF2 and 54.95 g/L in PEF3. Glucose increased from 44.74 to 58.18 g/L in PEF2, while sucrose increased steadily from 17.93 to 24.08 g/L in PEF3, indicating that sucrose may be more responsive to higher PEF intensities in haskap. These increases resulted in a significant enhancement of total sugar content, rising from 115.16 g/L in control to 142.10 g/L in PEF3 ($p < 0.05$). Our results are consistent with previous reports on blue honeysuckle berries, in which fructose and glucose were identified as the predominant sugars (together > 80% of total), while sucrose was only present in trace amounts [66, 67]. Reported total sugar contents in haskap range from 1557 to 2585 mg/100 g fresh weight, which are substantially lower than in many other berry species such as bilberry, raspberry, or strawberry [38], suggesting that haskap is among the less sweet berries. Overall, PEF treatment was effective in improving sugar extractability in both juices, particularly fructose and glucose. In addition to enhanced mass transfer, PEF treatment may also contribute to higher concentrations of simple sugars through structural modifications of complex carbohydrates, increasing porosity and accessibility, which may facilitate carbohydrate mobilization or partial hydrolytic processes [68, 69]. Although enzymatic activity and polysaccharide degradation were not investigated in the present study, such mechanisms have been reported in other plant-based matrices and could plausibly contribute to the observed increase in sugar content.

Organic acids

As presented in Fig. 5, PEF treatment significantly influenced the organic acid composition of both aronia and haskap juices (Table 5). In aronia juice, six organic acids were identified, including citric, malic, quinic, tartaric, oxalic, and shikimic acids. Among these, citric, quinic,

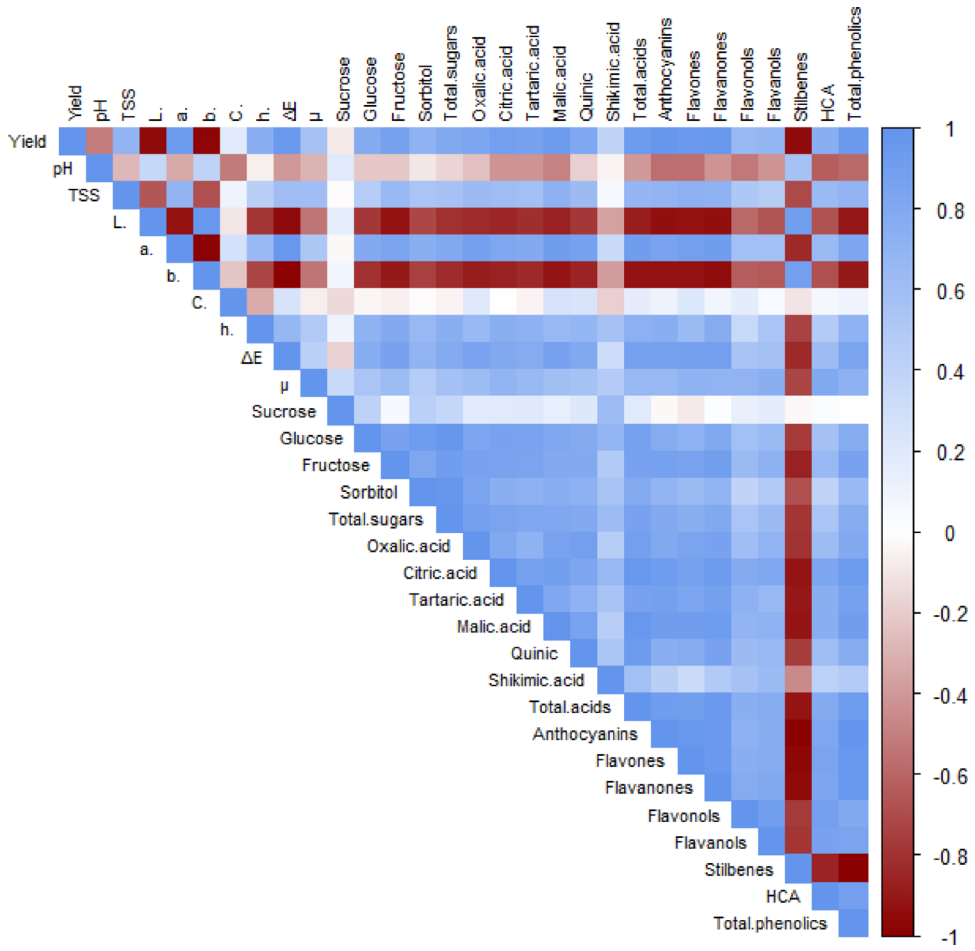


Fig. 7 Heatmap of aronia juice quality attributes

and malic were the dominant contributors, which is consisted with previous studies on aronia berries, which have reported quinic and malic acids as the major representatives, together accounting for the largest share of total organic acids, although some variability in their relative dominance has been noted across cultivars and growing conditions [65]. Application of PEF resulted in a progressive and statistically significant increase in citric, malic, quinic, tartaric, and oxalic acid concentrations ($p < 0.001$), while shikimic acid remained statistically unchanged. Specifically, citric acid increased from 20.93 g/L in the control to 25.94 g/L in PEF3-treated samples, and malic acid content increased from 16.85 to 22.49 g/L. Similarly, the content of quinic acid increased from 18.29 to 22.61 g/L, and smaller but consistent increases were also observed for tartaric acid and oxalic acids. These increases led to a significant increase in total acid content, from 61.99 g/L in the control to 78.44 g/L in PEF3 ($p < 0.001$).

A total of five organic acids were detected in haskap juice, including citric, malic, quinic, tartaric, shikimic, and minor levels of fumaric acid. There were no significant differences in citric acid content following the PEF treatments compared to the control ($p = 0.24$). However, significant increases were observed in quinic acid (from 15.44 to 21.66 g/L; $p < 0.001$) and malic acid (from 5.00 to 6.66 g/L; $p = 0.004$). Other acids, including tartaric, shikimic, and fumaric, remained unaffected by PEF treatment. As a result, total acid content in haskap juice increased from 43.96 g/L in the control to 55.79 g/L in PEF3 ($p < 0.05$), caused primarily by the release of quinic and malic acids. These results observed in aronia and haskap suggest that PEF effectively promotes the release of vacuole and wall-bound organic acids through electroporation-mediated cell disruption, also demonstrated in jabuticaba fruits pretreated with PEF [70].

Phenolic compounds and anthocyanins

Aronia juice contained seven phenolic groups comprising a total of 54 individual compounds, including 6 anthocyanins, 15 flavonols, 15 flavanols, 3 flavanones, 1 flavone, 12 hydroxycinnamic acids (HCA) and 1 stilbene (Fig. 6). PEF treatment had a significant effect on most phenolic compounds and among them, anthocyanins were the most abundant phenolic group and showed a significant increase with higher PEF intensity ($p < 0.05$), ranging

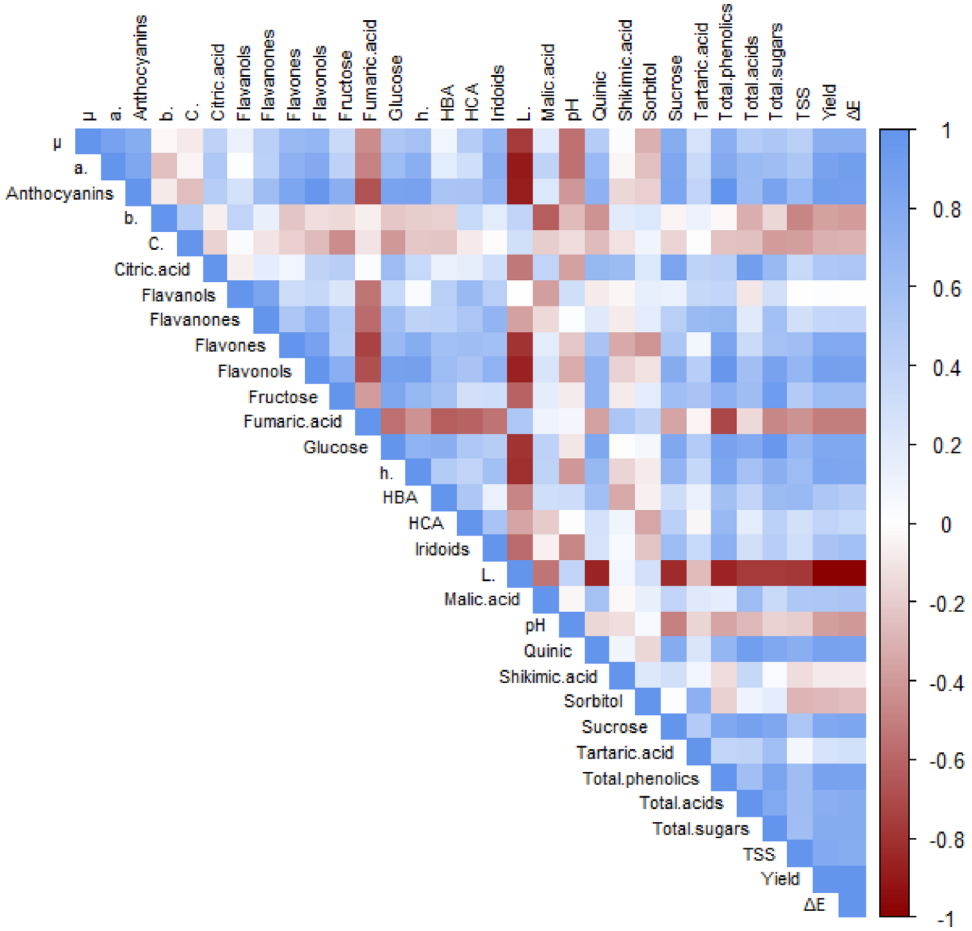


Fig. 8 Heatmap of haskap juice quality attributes

from 6.14 g/L in the control to 10.80 g/L in the PEF3 treatment. These findings are in line with previous studies on blueberry juice, where anthocyanin content increased by 21–55% with PEF pre-treatments of 1–10 kJ/kg, although significant improvements were most pronounced at higher energy inputs [40]. Similarly, Bobinaitė et al. [62] reported that PEF pre-treatment at 10 kJ/kg and field strengths of 1–5 kV/cm significantly enhanced total anthocyanin content in by 44–78%, without additional increases beyond 1–3 kV/cm, confirming that moderate field strengths (around 1 kV/cm) combined with energy inputs of 10 kJ/kg are particularly favorable for promoting anthocyanin release, supporting results obtained in our study in aronia juice.

Similarly, total phenolics increased significantly from 9.23 g/L in the control to 16.07 g/L in PEF3 ($p < 0.05$), representing a substantial enhancement in phenolic content, in line with previous findings on Sohiong, barberry and blueberry juices produced with PEF pre-treatment [47, 71, 72]. Other phenolic subgroups such as flavanols, flavonols, flavones and HCA also showed statistically significant increases with PEF3 treatment. These results are consistent with studies from Pataro et al. and Bobinaitė et al. who reported that PEF at moderate intensities (1 kV/cm) can significantly increase the release of phenolic compounds in cherry and blueberry juices [40, 71]. In contrast, stilbenes in aronia juice showed a slight decrease among treatments, whereas López-Alfaro et al. reported significant increases, up to 200% in Tempranillo grapes, following PEF treatment during must production [73]. To further clarify these compositional changes, analysis of individual phenolic compounds detected in aronia and haskap juices, including major anthocyanins, flavonols, flavanols/proanthocyanidins, and hydroxycinnamic acids is presented in tables S1–S2. Several compounds increased significantly with PEF intensity. For example, cyanidin-3-glucoside increased from 3.40 ± 0.08 g/L (control) to 5.13 ± 0.05 g/L (PEF3, $p < 0.001$), cyanidin-3-arabinoside increased from 0.53 ± 0.01 to 1.04 ± 0.02 g/L, and cyanidin-3-galactoside increased from 1.79 ± 0.03 to 3.79 ± 0.07 g/L. Among hydroxycinnamic acids, 5-caffeoylquinic acid (chlorogenic acid), the predominant HCA in aronia, increased from 0.656 ± 0.003 g/L to 1.012 ± 0.060 g/L, while 4-caffeoylquinic acid increased from 0.082 ± 0.0003 to 0.111 ± 0.004 g/L. Concentrations of proanthocyanidins, i.e., condensed tannins, were similarly enhanced, including increases in procyanidin dimers, trimers, and hexamers; for example, procyanidin dimer 2 increased from 0.196 ± 0.002 g/L to 0.272 ± 0.013 g/L at PEF3, procyanidin dimer 1 from 0.117 ± 0.001 to 0.161 ± 0.010 g/L, and procyanidin hexamer 6 from 0.0249 ± 0.0002 to 0.0385 ± 0.0020 g/L. Although some monomeric flavanols, such as epicatechin (0.182 ± 0.014 to 0.237 ± 0.011 g/L), showed minor or non-significant changes, the majority of compounds within the anthocyanin, HCA, and proanthocyanidin groups increased with higher PEF treatment. Overall, the applied PEF treatments in our study proved effective at enhancing the phenolic content of aronia juice.

Haskap juice contained eight phenolic groups comprising a total of 46 compounds, including 10 anthocyanins, 12 flavonols, 10 flavanols, 1 flavanone, 3 flavones, 1 hydroxybenzoic acid (HBA), 9 hydroxycinnamic acids and 1 iridoid. As with aronia, anthocyanins were the predominant phenolic class and increased significantly with treatment intensity, from 5.65 g/L in control to 10.19 g/L in PEF3 ($p < 0.05$). Comparable results were obtained in grape juice, where PEF pre-treatment of Pinot Noir grapes promoted a greater release of the predominant anthocyanin, malvidin-3-*O*-glucoside, compared with untreated samples [46].

The content of total phenolics also increased from 8.04 g/L (control) to 12.84 g/L (PEF3), with statistically significant differences between the control, PEF2, and PEF3 ($p < 0.05$), in agreement with previous results showing improved total polyphenol recovery in grape, barberry and Sohiong, juices after PEF pre-treatment [46, 47, 71]. Other phenolic groups such as flavonols and HCA also showed significant increases in PEF2 and PEF3, whereas flavanols, flavanones, and iridoids remained statistically unchanged across treatments, reflecting the compound-specific nature of PEF extractability [74]. The increase in HBA content in PEF1 and PEF2 suggests enhanced extractability of bound phenolics, although values remained relatively low in absolute terms, as previously reported for phenolic acids in plant matrices [75]. As for the analysis of individual compounds, concentration of pelargonidin-3-glucoside increased from 2.98 ± 0.02 g/L (control) to 5.82 ± 0.08 g/L under PEF3 ($p < 0.001$). Similarly, cyanidin-3-glucoside increased from 1.30 ± 0.01 to 2.44 ± 0.05 g/L, and cyanidin-3,5-diglucoside from 0.52 ± 0.01 to 0.69 ± 0.01 g/L across the same treatments ($p < 0.001$). Cyanidin-rhamnosyl-hexoside also increased from 0.067 ± 0.009 to 0.169 ± 0.010 g/L, while delphinidin-3-rutinoside increased from 0.107 ± 0.002 to 0.163 ± 0.003 g/L ($p < 0.001$). Flavonols showed significant increases at higher PEF intensities, particularly isorhamnetin-3-rutinoside (0.033 ± 0.001 to 0.069 ± 0.001 g/L) and quercetin-3-rutinoside (0.051 ± 0.003 to 0.068 ± 0.002 g/L). Smaller but significant increases were observed for several procyanidin oligomers, including procyanidin dimer 5 (from 0.091 in control to 0.133 g/L in PEF3, $p = 0.010$). Among hydroxycinnamic acids, 5-caffeoylquinic acid increased from 0.92 ± 0.08 to 1.14 ± 0.06 g/L at PEF2 ($p = 0.037$). Overall, PEF treatment effectively enhanced the phenolic composition of haskap juice, particularly anthocyanins and flavonols, thereby improving juice bioactivity.

Correlation analysis of juice quality parameters

To better understand the relationships among juice quality attributes affected by PEF treatment, Pearson correlation analyses were performed for aronia and haskap juices (Figs. 7 and 8, Supplementary tables S3 and S4). Correlations with $r \geq 0.7$ were considered strong, $0.4 \leq r < 0.7$ moderate, and $r < 0.4$ weak. In aronia juice, strong positive correlations were observed between juice yield and major compositional variables, including total sugars, glucose, fructose, sorbitol, total organic acids, total phenolics, and anthocyanins. These relationships confirm that the increase in juice yield induced by PEF (Sect. 3.1) was associated with enhanced extraction of intracellular compounds. Similarly, strong positive correlations among sugars, organic acids (particularly citric and quinic acids), and phenolic compounds imply that these compounds were released as cell membranes and vacuolar structures were disrupted. Colour parameters in aronia juice exhibited pronounced negative correlations with compositional variables. Lightness (L^*), chroma-related parameters, and total colour difference (ΔE^*) were inversely correlated with sugars, yield, phenolics, and, most notably, anthocyanins, indicating that the darker and more intensely coloured juices resulted from enhanced pigment extraction. These correlations are consistent with the observed increases in anthocyanin concentration (Sect. 3.7) and the colour changes described in Sect. 3.2, suggesting that anthocyanins largely account for the colour changes observed following PEF treatment. pH also showed negative correlations with organic acids and phenolics, aligning with the modest pH reduction observed at the highest PEF intensity (Sect. 3.4).

In haskap juice, correlation was generally weaker and more dispersed, implying a less coordinated response to PEF treatment compared with aronia. Moderate positive correlations were observed between juice yield, sugars (glucose and fructose), total acids, and total phenolics, indicating that PEF enhanced extractability of these compounds, although to a lesser extent and with greater variability. Anthocyanins and total phenolics were positively correlated with sugars and yield, supporting the observed increases in these parameters under higher PEF intensities (Sect. 3.5 and 3.7). In contrast to aronia, colour attributes (L^*, a^*, b^*, ΔE^*, hue) displayed weaker and less consistent correlations with compositional variables. This observation is consistent with the minor changes in chroma and the more moderate ΔE^* values reported in Sect. 3.2.

Viscosity showed positive correlations with sugars, phenolics, and yield in both juices, particularly in haskap, supporting the discussion in Sect. 3.3 that increased solubilization of sugars, pectic substances, and phenolic-polysaccharide interactions promoted viscosity enhancement. Overall, correlation analysis confirms that PEF treatment induced coordinated changes in juice yield, chemical composition, and selected physical properties. Additionally, the observed relationships were more pronounced and consistent in aronia compared with haskap, implying that matrix composition also modulated the efficiency of PEF treatment, which should be of interest in future studies.

Conclusions

The aim of this study was to evaluate the effect of pulsed electric field pre-treatment (PEF 1 = 1.78 kJ/kg, PEF 2 = 5.3 kJ/kg, and PEF 3 = 10.7 kJ/kg) on the yield and physicochemical properties of aronia and haskap juices, with emphasis on extraction efficiency and bioactive compound enhancement. PEF pre-treatment significantly increased juice yield in both aronia and haskap, with aronia increasing from 39.3% to 57.3% and haskap from 29.2% to 59.8% at PEF3, confirming a strong positive correlation between applied energy and extraction efficiency. PEF also modified physicochemical attributes, including viscosity, pH, total soluble solids, and colour, with higher treatment intensities leading to darker, more saturated juices with decreased L^*, and increased a^* and hue values. PEF enhanced the release of sugars and organic acids, with significant increases in fructose, glucose, sorbitol (aronia), sucrose (haskap), and dominant acids such as citric, malic, and quinic acids, implying improved cell wall permeability and vacuolar disruption. Similarly, concentration of anthocyanins in aronia increased significantly from 6.14 to 10.80 g/L and in haskap juice from 5.65 to 10.19 g/L in control to PEF3 treatment, respectively. Correlation analysis revealed strong positive relationships in aronia between juice yield and several analyzed variables, including sugar content, total acids, and phenolic compounds. In haskap, moderate positive correlations were observed between juice yield, glucose and fructose, total acids, and total phenolics, suggesting that PEF enhanced the extractability of these compounds, though to a lesser extent and with greater variability. Overall, PEF pre-treatment proved highly effective at improving both yield and compositional quality of aronia and haskap juices, particularly by increasing sugars, organic acids, phenolics, anthocyanins, and colour intensity. However, future work should focus on sensory quality, stability, enzymatic activity, and industrial scalability to fully assess the applicability of PEF for juice production.

Appendix

Table 3 CIELAB colour parameters of aronia and haskap juice.

	Aronia	*Haskap*
Treatment	*L**	
Control	14.92±0.24[a]	22.29±0.01[a]
PEF 1	13.91±0.10[b]	21.28±0.01[b]
PEF 2	13.08±0.02[c]	21.13±0.01[c]
PEF 3	12.87±0.02[c]	20.15±0.01[d]
p-value	0.000	0.000
	*a**	
Control	3.61±0.04[a]	1.08±0.02[a]
PEF 1	4.27±0.01[b]	1.15±0.01[ab]
PEF 2	4.33±0.01[b]	1.16±0.02[b]
PEF 3	4.47±0.01[c]	1.41±0.02[c]
p-value	0.000	0.000
	*b**	
Control	-15.03±0.01[a]	0.03±0.01[a]
PEF 1	-16.16±0.01[b]	-0.05±0.01[b]
PEF 2	-16.57±0.01[c]	0.01±0.01[ab]
PEF 3	-16.90±0.01[d]	-0.01±0.01[ab]
p-value	0.000	0.009
	*C**	
Control	0.03±0.01[ab]	0.12±0.01[a]
PEF 1	0.06±0.00[b]	0.05±0.01[a]
PEF 2	0.01±0.01[a]	0.03±0.00[a]
PEF 3	0.06±0.01[b]	0.05±0.01[a]
p-value	0.003	0.558
	*h**	
Control	15.47±0.21[a]	16.26±0.18[a]
PEF 1	17.86±2.67[a]	19.13±1.70[ab]
PEF 2	29.80±0.83[b]	24.46±2.04[bc]
PEF 3	24.85±0.81[b]	28.28±1.33[c]
p-value	0.000	0.000

The values are the means of four repetitions. Mean values followed by a different letter in the column are significantly different according to Tukey's test ($p \leq 0.05$).

Table 4 Concentration of individual sugars in aronia and haskap juice (g/L).

Fruit type	*Treatment*	*Fructose*	*Glucose*	*Sucrose*	*Sorbitol*
Aronia	*Control*	39.91±1.63[a]	56.53±1.97[a]	7.21±0.43[a]	65.71±1.35[a]
	PEF1	46.12±0.52[b]	64.16±0.92[ab]	7.47±0.40[a]	76.17±0.60[ab]
	PEF2	52.68±1.48[c]	69.21±3.15[b]	7.08±0.66[a]	81.18±4.85[b]
	PEF3	52.06±0.84[c]	69.01±1.20[b]	7.03±0.53[a]	79.66±2.38[b]
	p-value	0.000	0.002	0.931	0.009
		Fructose	*Glucose*	*Sucrose*	*Sorbitol*
Haskap	*Control*	46.60±2.29[a]	44.74±0.96[a]	17.93±0.72[a]	5.90±0.20[a]
	PEF1	49.93±1.68[ab]	51.93±1.75[b]	19.23±0.68[a]	5.76±0.20[a]
	PEF2	56.70±1.71[b]	58.18±1.18[c]	21.18±0.96a[b]	5.83±0.74[a]
	PEF3	54.95±1.80[b]	57.82±1.53[c]	24.08±0.81[b]	5.26±0.40[a]
	p-value	0.010	0.000	0.001	0.733

The values are the means of four repetitions. Mean values followed by a different letter in the column are significantly different according to Tukey's test ($p \leq 0.05$).

Table 5 Concentration of individual acids in aronia and haskap juice (g/L).

Fruit type	*Treatment*	*Citric*	*Malic*	*Oxalic*	*Quinic*	*Shikimic*	*Tartaric*
Aronia	*Control*	20.93±0.40^{a}	16.85±0.38^{a}	0.67±0.01^{a}	18.29±0.31^{a}	0.33±0.03^{a}	4.93±0.14^{a}
	PEF1	22.72±0.17^{ab}	19.98±0.22^{b}	0.78±0.01^{b}	21.87±0.52^{b}	0.33±0.01^{a}	5.36±0.22^{ab}
	PEF2	24.77±0.68^{bc}	20.59±0.48^{b}	0.79±0.02^{b}	22.33±0.67^{b}	0.37±0.01^{a}	5.90±0.14^{bc}
	PEF3	25.94±0.66^{c}	22.49±0.55^{c}	0.81±0.02^{b}	22.61±0.61^{b}	0.36±0.02^{a}	6.24±0.11^{c}
	p-value	0.000	0.000	0.000	0.000	0.000	0.000
		Citric	*Fumaric*	*Malic*	*Quinic*	*Shikimic*	*Tartaric*
Haskap	*Control*	20.50±1.05^{a}	0.02±0.00^{a}	5.00±0.24^{a}	15.44±0.51^{a}	0.06±0.00^{a}	2.94±0.08^{a}
	PEF1	22.44±1.38^{a}	0.02±0.00^{a}	6.66±0.32^{b}	20.14±0.52^{b}	0.06±0.00^{a}	2.91±0.09^{a}
	PEF2	22.26±1.65^{a}	0.02±0.00^{a}	5.62±0.30^{ab}	20.31±0.80^{b}	0.05±0.01^{a}	3.10±0.26^{a}
	PEF3	24.62±1.17^{a}	0.02±0.00^{a}	6.26±0.16^{b}	21.66±0.52^{b}	0.06±0.00^{a}	3.18±0.22^{a}
	p-value	0.24	0.059	0.004	0.000	0.833	0.674

The values are the means of four repetitions. Mean values followed by a different letter in the column are significantly different according to Tukey's test ($p \leq 0.05$).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00217-026-05081-0.

Acknowledgements We would like to thank Sveva Cesari for her invaluable assistance in performing the laboratory experiments, which significantly contributed to the success of this research.

Author contributions Alema Puzovic: Conceptualization, Validation, Formal analysis, Data Curation, Visualization, Writing - Original Draft, Writing - Review & Editing; Massimiliano Rinaldi: Writing - Review & Editing, Supervision, Resources; Neamtallah Assaf: Investigation, Data Curation, Writing - Review & Editing; Maja Mikulic-Petkovsek: Conceptualization, Supervision, Methodology, Writing - Review & Editing, Project administration, Funding acquisition.

Funding This work received funding from the European Union Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement (no. 956257). The authors also acknowledge the financial support of Slovenian Research and Innovation Agency (ARIS) within the research program Horticulture (P4-0013).

Data availability The datasets generated and/or analyzed during the study are available from the corresponding author upon request.

Declarations

Conflict of interest The authors declare no conflicts of interest.

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