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Antioxidant capacity, phenolic composition and microbial stability of aronia juice subjected to high hydrostatic pressure processing

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ABSTRACT

The aim of this study was to characterize the effect of high hydrostatic pressure (200–600 MPa/15 min) and storage (4 °C/80 days) on aronia juice quality. The total antioxidant capacity, phenolic content and composition were assessed using an updated analytical strategy. Microbial growth was also analyzed following juice storage. Among all the analyzed juices, the untreated aronia juice had the greatest reduction (36%) in total polyphenols over the entire storage period. At the end of the storage period, the pressurized juices demonstrated ABTS and FRAP values higher by 14% and 5% as compared to the untreated juices. The main antioxidants identified in the aronia juice were: chlorogenic acid; neochlorogenic acid; cyanidin 3-galactoside; cyanidin 3-xyloside; cyanidin 3-arabinoside; cyanidin 3-glucoside. Cyanidin 3-glucoside was the most stable compound during juice storage. Microorganism growth in juices pressurized at 400–600 MPa was below the detection limit (<1 CFU mL^{-1}) upon storage.

Industrial relevance: Aronia berries are rarely consumed fresh since they give off several negative sensory attributes. Multiple health-promoting properties of aronia berries make them a valuable raw material for juice production. However, processing involves pasteurization or hot-filling strongly diminishes the product quality due to the changes in quantity and quality of thermolabile phytochemicals. The objective of this work was to characterize the effect of high hydrostatic pressure on the antioxidant capacity, polyphenol content and composition and microbial stability of aronia juice. Results of this study may be useful for the juice industry commercialize this technology for the development healthy, nonclarified aronia juices with desired level of quality.

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1. Introduction

Aronia (chokeberry) (*Aronia melanocarpa*) constitutes an attractive research material due to its multiple health-promoting properties (Kedzierska et al., 2013). It has a unique composition of bioactive compounds including high levels of anthocyanins and procyanidins; with their concentrations being as high as 2% and 5% in the berries' dry matter, respectively (Kokotkiewicz, Jaremicz, & Luczkiewicz, 2010). Polymeric flavan-3-ols of the chokeberry are mainly composed of (−)epicatechins which are constitutive units of procyanidins. In turn, anthocyanins are a mixture of four different cyanidin glycosides: 3-galactoside, 3-glucoside, 3-arabinose and 3-xylose (Kokotkiewicz et al., 2010). In addition to these two main groups of phenolic compounds, aronia berries are also rich in phenolic acids (chlorogenic and neochlorogenic acids) (Slimestad & Solheim, 2002). In contrast, flavonols were found to constitute only 1.3% of the total phenolic compounds in aronia (7849.21 mg/100 g of dried matter) (Oszmiański & Wojdyło, 2005). The content and unique composition of aronia phenolics is strongly correlated with their high functional properties and biological activities (Zheng & Wang, 2003).

The production of fresh aronia is limited to a relatively short time period. Its black berries are borne in clusters and ripen in early September in Northern and Eastern Europe. Aronia berries are rarely consumed fresh because of their several negative sensory attributes like bitterness and astringency (Troszyńska, Lamparski, & Kmita-Głażewska, 2003). They are rather used for the production of jams, juices, wines and anthocyanin colorants (Oszmiański & Wojdyło, 2005). In the case of jam and juice production, a heat treatment is required to prolong the shelf life of these aronia products. However, the processing involves temperature treatment, such as blanching of berries and pasteurization and/or hot-filling of wine/juices, which strongly diminishes the product's quality due to changes in the quantity and quality of thermolabile phytochemicals (Cao et al., 2012). As reported in literature, approximately 80% loss of anthocyanins from blackberries may be attributed to thermal degradation. The pasteurization of non-clarified and clarified juices obtained from black raspberries was reported to lead to the loss of anthocyanins by 19% and 23%, respectively (Howard, Prior, Liyanage, & Lay, 2012).

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Likewise, aronia juice subjected to pasteurization (100 °C) yielded a similar drop in anthocyanin content, which in turn was accompanied by a reduction in its antioxidant capacity (Arancibia-Avila et al., 2012).

High hydrostatic pressure (HP) is an innovative processing technology, wherein food is exposed to pressure (up to 600 MPa) for a short duration with or without exposure to different temperatures (Nguyen et al., 2010). A number of attempts have been made to use HP instead of high temperatures to inactivate food-spoiling microorganisms (Liu, Li, Wang, Bi, & Liao, 2014) and undesired food enzymes (Mujica-Paz, Valdez-Fragoso, Samson, Welti-Chanes, & Torres, 2011) while maintaining all the quality and safety parameters of the products (Zhang et al., 2012; Ferrari, Maresca, & Ciccarone, 2010). Because chemical or enzymatic reactions can be enhanced or retarded by HP, the content of some bioactive compounds may be indirectly altered upon pressurization (Oey, Lille, Van Loey, & Hendrickx, 2008; Corrales, Butz, & Tauscher, 2008). It has been reported that the levels of phenolics, anthocyanins, flavonols and tannins in red wine are distinctly affected by HP (250–650 MPa for 15–120 min at ambient temperature) (Tao et al., 2012). In contrast, no significant changes in anthocyanin and ascorbic acid contents were found after pressurization (400–600 MPa for 15 min at 10–30 °C) of strawberry and blackberry purees (Patras, Brunton, da Pieve, & Butler, 2009). Blueberry juices pressurized at 600 MPa and at 42 °C for 5 min had reduced levels of ascorbic acid, which accounted for <5% of the initial content (Barba et al., 2012).

The effect of high pressure on the quality of aronia juice has not been extensively studied in literature. The aim of the present study was, therefore, to characterize the effect of high hydrostatic pressure (200–600 MPa/15 min) on the antioxidant capacity, polyphenol content and composition, as well as microbiological stability of aronia juice. Taking into consideration that the storage of berries (temperature, time, amount of light and oxygen) may also be a key factor affecting phytochemicals stability (Patras, Brunton, O'Donnell, & Tiwari, 2010; Howard et al., 2012), all the aforementioned evaluations were also performed on pressurized juices that were stored at 4 °C for 0, 20, 40, 60 and 80 days.

2. Materials and methods

2.1. Aronia juice

Aronia juice was procured from the Farm Specialist Plantation of Aronia (*Aronia melanocarpa*) in Bielawki, Pelplin, Poland. It produces cold-pressed juices without any addition of sugar, water or preservatives. According to the producer, the juice had a pH of 3.5, and a total solids content of 15.4 °Brix.

Naturally cloudy, cold-pressed juice was immediately packed in dark glass bottles to a volume of 0.75 L, and subsequently transported in cold state (6 ± 2 °C) to the Institute of Animal Reproduction and Food Research, PAS, Olsztyn, Poland. The juice was stored (for no longer than 3 days) in the dark at 4 °C before high pressure treatment.

2.2. Chemicals

All solvents used were of HPLC or analytical grade unless otherwise specified. 2.2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tris(pyridyl-s-triazine) (TPTZ), $FeSO_4$, acetic acid, sodium acetate trihydrate, Folin and Ciocalteu's phenol reagent and catechin were obtained from Sigma Chemical Co. (Poznań, Poland). Chlorogenic acid, neochlorogenic acid and cyanidin 3-glucoside were purchased from Extrasynthese (Genay Cedex, France). The remaining reagents (all of reagent-grade quality) were supplied by POCh (Gliwice, Poland). Water was purified using the Milli-Q system (Millipore, Bedford, USA).

2.3. Experiments

The juice samples were enclosed in Teflon tubes (50 mL), deaerated, tightly sealed and subjected to HP treatment using a high pressure device (Unipress U-303,Warsaw, Poland). The Teflon tubes were put into a high pressure chamber (with a capacity of approximately 100 mL) filled with a pressure-transmitting medium (water–propylene glycol (propane-1.2-diol), 1:1, v/v), which also minimized adiabatic heating. Compression and decompression rates were 8 MPa/s and 10 MPa/s, respectively. The samples were pressure-treated at 200, 400 and 600 MPa for 15 min (Tao et al., 2013). Respectively of the pressure volume applied, the temperature inside the pressure chamber averaged from 26 ± 2 °C to 38 ± 2 °C. The pressure treatment was performed in two replicates of each combination. The HP-treated juices were stored in airtight vials in the dark at 4 °C for 0, 20, 40, 60 and 80 days until chemical analysis.

The same conditions of processing were applied for the microbial determination of indigenous microbiota of the aronia juice. After pressurization, the juices were poured into previously sterilized (120 °C, 0.1 MPa) vials that were sealed and stored under the same condition as mentioned above.

2.4. Total phenolic content

The content of phenolic compounds in the juice was measured using Folin and Ciocalteu's phenol reagent (Singleton & Rossi, 1965). Quantification was done at 725 nm (Beckman DU 7500 spectrophotometer, California, USA) with catechin as a standard in the range of 0.015–1.00 mg mL^{-1} ($R^2 = 0.998$).

2.5. Total antioxidant capacity measured with the ABTS assay

The ABTS assay described by Re et al. (1999) was used to assess the ABTS cation radical scavenging activity. Juice was dissolved in methanol, and the results were expressed in mmol of Trolox equivalents per mL of juice. The linearity range of the calibration curve was from 0.0 to 2.0 mmol ($R^2 = 0.999$).

2.6. Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was performed as described by Benzie and Strain (1999). The sample solution analyzed was first properly diluted with deionized water to fit within the linearity range of Fe^{2+}. FRAP values were expressed as mmol of Fe^{2+} equivalents per mL of juice using the calibration curve of Fe^{2+}. The linearity range of the calibration curve was from 0.1 to 1.0 mmol ($R^2 = 0.9979$).

2.7. Reverse-phase high performance liquid chromatography (RP-HPLC)

For the RP-HPLC fingerprint analysis of individual phenolic compounds present in the aronia juice, a Shimadzu system (Shimadzu Corp., Kyoto, Japan) consisting of two LC-10AD pumps, an SCTL 10A system controller, an SPD-M 10 A photo-diode array detector and a prepacked LUNA C 18 column (4 × 259 mm, 5 μm, Phenomenex) was used. A flow rate of 1 mL min^{-1}, injection volume of 20 μL, a gradient elution of acetonitrile-water-acetic acid (5:93:2, v/v/v) [solvent A] and acetonitrile-water-acetic acid (40:58:2, v/v/v) [solvent B], and a 0–50 min solvent B from 0% to 100% were applied (Crozier, Jensen, Lean, & McDonald, 1997). Aronia juice was dissolved in methanol (1:19, v/v) and filtrated through a 0.45-μm filter (CHROMAFIL Extra PET-45/25, Macherey-Nagel). The separation of compounds was monitored at 320 and 520 nm. For the quantitative analysis, the external standard method was used with concentrations between 0.01 and 0.05 mg mL^{-1} for chlorogenic and neochlorogenic acid and between 0.01 and 0.10 mg mL^{-1} for cyanidin 3-glucoside. The identification was based on retention times and UV spectra of the standards and the samples.

2.8. Microbiological analysis

Total aerobic mesophilic count, yeast and mold counts were analyzed in fresh, untreated juice and in juices subjected to HP treatment upon their storage at 4 °C for a period of 0, 20, 40, 60, and 80 days. A sample of juice (10 mL) was diluted with 90 mL of saline peptone (SP, 1 g L^{-1} peptone, 8.5 g L^{-1} NaCl), and homogenized in a Stomacher (Model 400, Seward, London, UK) at a regular speed for 2 min.

To determine the total aerobic mesophile count of juice samples, homogenates were serially diluted and plated on Plate Count Agar (Merck, No. 105463) followed by incubation at 30 ± 1 °C for 3 days. Molds and yeasts were determined by plating the homogenates in Dichloran Rose-Bengal Chloramphenicol Agar (Merck, No. 100466) followed by incubation at 25 °C ± 1 °C for 5 days.

After incubation, the plates were counted and the results were expressed as colony forming units per 1 mL (CFU mL^{-1}). Determinations were carried out in triplicate (3 from each treatment). Detection limit was <1 CFU mL^{-1}.

2.9. Statistical analysis

Data on the total phenolic content, ABTS, FRAP, and HPLC analysis was analyzed using one-way ANOVA and post-hoc Tukey's test. Simple linear regression was used to find a correlation between the total phenol content (TPC) and antioxidant properties (ABTS, FRAP). The data obtained from these studies was also assessed using a *t*-test for significance ($p < 0.01$) (IBM SPSS Statistics v.22).

3. Results and discussion

3.1. Total phenolic content

The fresh and untreated juice yielded a significantly higher total phenolic content compared to juices treated with the pressures of 200, 400 and 600 MPa for 15 min (Table 1). However, the reduction in total phenols noted for the HP-treated juices was not correlated with the pressure applied. Juice pressurized at 200 MPa yielded a 12% drop in total phenols, whereas juice pressure-treated at 600 MPa yielded an 8% loss in antioxidants compared to the untreated and fresh samples. The opposite results were obtained by Barba et al. (2012), wherein high pressure did not influence the total phenolic concentration in blueberry juice. Those results could be, however, related to the fact that these authors significantly reduced the processing time to 5 min when compared to our studies. As expected, all the pressurized juices had significantly higher levels of phenolic compounds than their untreated samples upon storage. After the first 20 days of storage, the concentration of total polyphenols in the HP-treated juices was by 5% higher compared to the untreated juice, and after the next 40 and 60 days of storage, the pressurized juices yielded on an approximately 11% increase in antioxidants concentration.

Among all the analyzed juices, the untreated aronia juice was characterized by the greatest reduction (36%) in total polyphenols over the entire storage period (Table 1). At the end of storage period, the reduction in antioxidants concentration noted for the juice pressurized at 200 MPa was approximately 18%, whereas juices pressurized at 400 MPa and 600 MPa manifested 22% and 23% decrease in phenolic concentration, respectively, as compared to the fresh juices treated at a proper level of pressure.

The higher level of antioxidants noted in the HP-treated juices upon storage was likely to result from the inactivation of the enzymes responsible for degradation of the phenolic compounds, i.e. polyphenoloxidase (PPO) or peroxidase (POD) (Barba et al., 2012). Inactivation of PPO and POD in strawberries was already observed at pressures of 600 and 800 MPa for 15 min and 10 min, respectively (Garcia-Palazon, Suthanthangjai, Kajda, & Zabetakis, 2004).

3.2. Total antioxidant capacity as measured by ABTS assay

A variety of analytical methods are used to analyze the antioxidant properties of plants including the Trolox equivalent antioxidant capacity assay (TEAC, also referred to as the ABTS assay). In this assay, radicals are deactivated by single electron transfer, and it is the most common and easiest assay to quickly evaluate the antioxidant capacity in plant material (Bonarska-Kujawa, Sarapuk, Bielecki, Oszmiański, & Kleszczyńska, 2012).

The results of the ABTS assay used to analyze the aronia juices are shown in Table 2. Significant differences were observed in the total antioxidant capacity (TAC) between the fresh untreated juice and the HP-treated juice. The TAC values obtained for the fresh and HP-treated (200–600 MPa for 15 min) juices were on average by 8% lower compared to the values determined for the untreated juices. The results shown in Table 2 indicate also that the effect of pressure treatment for a fixed period of time on the antioxidant capacity of aronia juices was insignificant.

However, the TAC values of the HP-treated juices were significantly higher than the antioxidant capacity of untreated juices following 80 days of storage at 4 °C (Table 2). In the first 20 days, the antioxidant capacity of juice pressurized at 200 MPa was approximately 10% higher than that of the untreated juice. However, the radical scavenging activity of juices pressure-treated at 400 and 600 MPa was distinctly reduced after 20 days of storage. Nevertheless, the antioxidant capacity of juices processed using the higher pressure was on average by 6% higher than that of the untreated samples. The TAC values obtained for the HP-treated juices after 40 and 60 days of storage were on average by 17% and 14% higher, respectively, than the values noted for the untreated juices. Similarly, the HP-treated juices had an approximately 14% higher ABTS radical scavenging capacity compared to the untreated juices at the end of the storage period.

The effect of the storage period on the antioxidant capacity of individual aronia juice samples was also analyzed in the present study (Table 2). A significant decrease in the TAC value (40%) was noted for the untreated fresh juice over the entire storage period. The antioxidant capacity of the HP-treated (200–600 MPa) juices was also significantly reduced over the 80 days of storage. However, the reduction in TAC values observed during the storage of pressurized juices was distinctly lower and had a more gradual character as compared to the changes noted for the untreated juices.

Table 1
Effect of HP treatment on the total phenolic concentration (mg catechin mL^{-1}) during aronia juice storage at 4 °C.

	Storage time (days)				
Sample	0	20	40	60	80
Untreated/reference	9.35 dD* ± 0.09	7.76 aC ± 0.13	6.24 aB ± 0.06	6.00 aA ± 0.09	5.95 aA ± 0.19
200 MPa	8.24 aC ± 0.04	8.14 bC ± 0.13	7.26 cB ± 0.04	6.82 bA ± 0.13	6.76 cA ± 0.08
400 MPa	8.44 bD ± 0.18	8.18 bC ± 0.13	6.93 bB ± 0.13	6.72 bA ± 0.07	6.59 bA ± 0.09
600 MPa	8.60 cD ± 0.11	8.09 bC ± 0.13	6.88 bB ± 0.15	6.72 bA ± 0.10	6.61 bA ± 0.10

* Small letters indicate difference (uniform Tukey's groups) between treatment type (in column) in particular days of storage, whereas capital letters indicate difference between storage time for individual sample (in lines).

Table 2
Effect of HP treatment on the antioxidant capacity in the aronia juice ($ABTS^{\bullet+}$, mmol Trolox mL^{-1}) during storage at 4 °C.

	Storage time (days)				
Sample	0	20	40	60	80
Untreated/reference	54.33 bD* ± 0.78	44.37 aC ± 0.50	37.01 aB ± 1.46	33.60 aA ± 0.98	32.53 aA ± 1.04
200 MPa	50.08 aE ± 0.39	48.66 cD ± 0.55	43.35 bC ± 1.12	37.79 bB ± 1.08	36.52 bA ± 1.05
400 MPa	50.18 aD ± 0.93	47.27 bC ± 0.44	43.41 bB ± 0.74	37.74 bA ± 1.06	37.42 bA ± 0.98
600 MPa	50.30 aE ± 1.03	47.11 bD ± 0.52	43.74 bC ± 1.00	38.89 bB ± 1.06	37.48 bA ± 0.80

* Small letters indicate difference (uniform Tucey's groups) between treatment type (in column) in particular days of storage, whereas capital letters indicate difference between storage time for individual sample (in lines).

The results obtained are consistent with those reported in literature. It was found that pressurization (400 MPa for 15 min) of strawberry purees resulted in a significant reduction (22%) in the antioxidant capacity (Patras et al., 2009). Similarly, plum puree pressurized at 500 MPa for 150 s had a lower antioxidant capacity (13%) compared to the untreated sample (Gonzales-Cebrino, Duran, Delgado-Adamez, Contador, & Ramirez, 2013). Barba, Esteve, and Frigola (2013) also observed a reduction in the antioxidant capacity (determined as TEAC value) of blueberry juice HP-treated at 400 MPa (15 min) and 600 MPa (5–15 min), which was estimated in the range of 8–16%.

3.3. Ferric reducing antioxidant power (FRAP)

Almost all the results discussed above were also reflected in the antioxidant capacity of the aronia juices as measured by the FRAP assay (Table 3). The ability of the fresh and untreated juices to reduce Fe^{3+} to Fe^{2+} was approximately by 3% higher compared to the reducing power of the pressurized and fresh juices. In contrast, the HP-treated juices (200–600 MPa for 15 min) had a significantly higher reducing power over the entire storage period compared to the untreated juices. The antioxidant capacity of the HP-treated juices was on average by 8% and 5% higher than the capacity of reference juice upon 40–60 and 80 days of storage, respectively. On the contrary, Keenan et al. (2010) demonstrated that pressurized (450 MPa/5 min) fruit smoothies, stored for 20 and 30 days at 4 °C, manifested by 3% and 7% lower FRAP values, respectively, compared to the values determined for the untreated sample. The drop in reducing power of pressurized fruit smoothies was ascribed by these authors directly to antioxidants degradation upon storage as a result of indirect oxidation by phenolic quinones generated by PPO and peroxidase.

The effect of storage on the reducing antioxidant power of individual juices was analyzed as well (Table 3). In the case of the untreated juice, the ability to reduce Fe^{3+} to Fe^{2+} decreased by 21% over the entire storage period. Over the 80 days of storage, the juices pressurized at 200–400 MPa and 600 MPa had by 15% and 19%, respectively a lower reducing capacity as compared to the fresh and treated samples. These results are consistent with a previous study by Liu, Wang, Li, Bi and Liao (2014), which indicated that storage significantly affected the total antioxidant capacity of mango nectars as measured by FRAP or DPPH assays. The authors demonstrated that mango nectar that had been HP-treated (600 MPa for 1 min) and stored (112 days at 4 °C) yielded a significant reduction (21%) in the antioxidant capacity as measured by the FRAP assay.

The statistical analysis showed that the total phenolic concentration in all the aronia juices studied was significantly ($p < 0.01$) correlated with the antioxidant capacity, measured by both $ABTS^{\bullet+}$ and FRAP assays, after storage. In fact, the regression analysis proved explicitly that - irrespective of treatment - antioxidants had a large impact on the antiradical potential. A very strong correlation was observed between $ABTS^{\bullet+}$ values and the total phenolic concentration for fresh and untreated samples ($R^2 = 0.988$) as well as for juices pressurized at 200–400 MPa ($R^2 = 0.974$–0.902). A strong correlation between the analyzed quality attributes was found for the juice pressurized at 600 MPa ($R^2 = 0.888$). The correlation between FRAP values and antioxidant concentrations measured in the untreated juice and juice treated at 600 MPa was $R^2 = 0.965$ and $R^2 = 0.933$, respectively. However, a distinctly lower correlation was determined for the juices treated at 200 MPa ($R^2 = 0.727$) and 400 MPa ($R^2 = 0.816$).

Table 3
Effect of HP treatment on the ferric reducing antioxidant power in the juice (FRAP, μmol Fe^{2+} mL^{-1}) during storage at 4 °C.

	Storage time (days)				
Sample	0	20	40	60	80
Untreated/reference	134.61 bD* ± 1.60	115.98 aC ± 1.68	107.89 aB ± 1.69	106.63 aAB ± 0.76	106.02 aA ± 1.27
200 MPa	130.58 aD ± 0.79	119.66 bC ± 1.12	118.39 bC ± 1.50	115.13 bB ± 1.50	111.01 bA ± 1.04
400 MPa	130.07 aE ± 0.09	120.14 bD ± 0.91	116.82 bC ± 1.44	114.66 bB ± 1.11	111.11 bA ± 1.38
600 MPa	131.36 aD ± 1.90	122.55 cC ± 1.22	116.75 bB ± 1.57	115.38 bB ± 1.44	112.34 bA ± 1.42

* Small letters indicate difference (uniform Tucey's groups) between treatment type (in column) in particular days of storage, whereas capital letters indicate difference between storage time for individual sample (in lines).

3.4. RP-HPLC analysis

The quantification of individual phenolic compounds in untreated and HP-treated (200–600 MPa for 15 min) aronia juices after storage was carried out using RP-HPLC, and the results were presented in Fig. 1A, B and Table 4.

The first two compounds identified in the aronia juices (Table 4) were chlorogenic and neochlorogenic acid. The more abundant compound, however, was chlorogenic acid (5′-caffeoyl quinic acid). These results are in agreement with studies by Oszmiański and Wojdyło (2005) who found that the concentrations of chlorogenic and neochlorogenic acid in aronia juice were 416 and 393 mg/100 g dried weight, respectively. Given that the dry weight in aronia juice can vary from 19 to 24 g/100 g, the concentrations of chlorogenic and neochlorogenic acid measured in the untreated and fresh juices are in accordance with results presented by Oszmiański and Wojdyło (2005).

We also detected four other phenolic compounds coded as numbers 3 to 6 (Table 4), which were obtained at retention times of 22.5, 23.6, 25.2 and 27.5 min, respectively. The absorption spectra of these compounds peaked at the visible region with a maximum at 516–518 nm. Considering the HPLC profiles obtained for these compounds as well as literature data (Oszmiański & Wojdyło, 2005; Cujic et al., 2016), we can classify these compounds as anthocyanins. According to Oszmiański and Wojdyło (2005), anthocyanins are the main phenolic compounds in aronia fruits (25% of total polyphenols), being a mixture of four different cyanidin glycosides including 3-galactoside, 3-

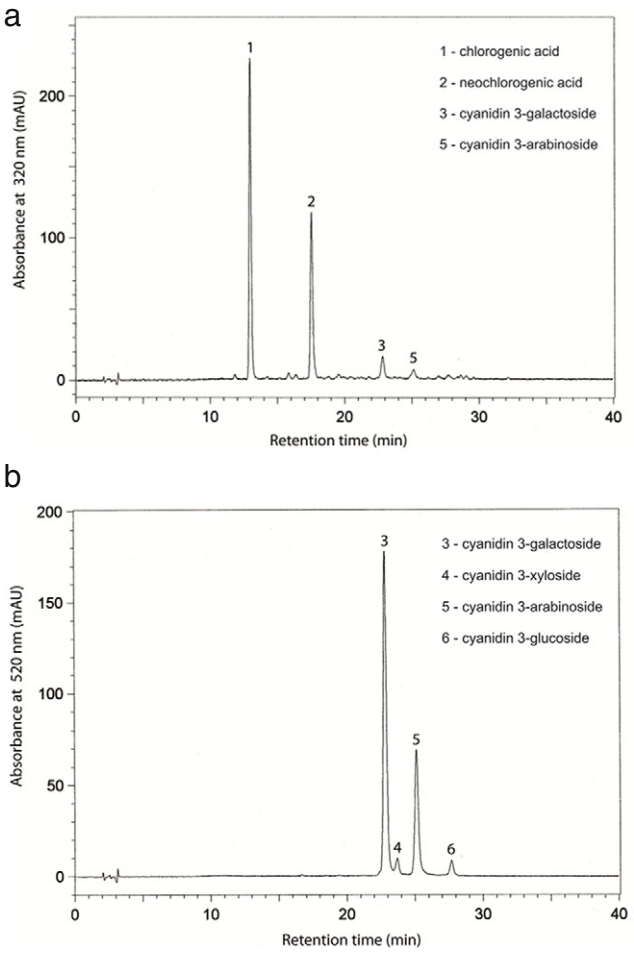

Fig. 1. RP–HPLC chromatogram of aronia juice phenolic compounds.

glucoside, 3-arabinoside and 3-xyloside, of which cyanidin 3-galactoside is the main compound. Following the authors' findings and our results, we identified compounds 3 to 6 (Table 4) as cyanidin 3-galactoside, cyanidin 3-xyloside, cyanidin 3-arabinoside and cyanidin 3-glucoside, respectively.

These results suggest that the HP treatment makes the anthocyanins more stable than chlorogenic and neochlorogenic acid (Table 4). Irrespective of the applied volume of pressure, no significant changes in anthocyanins were found in the fresh and treated juices. In contrast, the fresh pressurized juices demonstrated significant decreases in chlorogenic and neochlorogenic acids with average reductions of 10% and 11%, respectively, compared to the untreated sample.

The pressure-treated (200–600 MPa for 15 min) juices contained chlorogenic acid at the level comparable to the untreated samples after 20, 40 and 80 days of storage. Whereas, concentrations of neochlorogenic acid in the pressure-treated juices were higher than in the untreated samples at days 40 and 80 of storage (on average by 16% and 4% higher, respectively).

Some differences were also found in anthocyanins concentration (except cyanidin 3-glucoside) between the untreated and pressure-treated juices after storage. The concentration of cyanidin 3-galactoside was found to be significantly higher in the pressurized juices than in the untreated ones over the entire storage period. Almost two-fold higher concentration of cyanidin 3-xyloside after 40 and 60 days of storage was found for pressurized juices compared to the untreated sample. At the end of the storage period, concentrations of cyanidin 3-xyloside in the HP-treated juices were still higher (by 33%, on average) than in the untreated juice. The pressurized juices stored for 40 and 60 days also had higher concentrations of cyanidin 3-arabinoside (58% and 10%, respectively) compared to the untreated juice.

Based on the results listed in Table 4, we can conclude that among all the anthocyanins detected in the untreated and treated juices, cyanidin 3-glucoside was the most stable compound over the 80 days of storage. On the contrary, the most labile one with regard to storage was cyanidin 3-xyloside. The untreated juice was characterized by on average 70% decrease in cyanidin 3-xyloside in the 40th day of storage compared to the fresh sample. The pressurized juices also showed a significant decrease in the concentrations of cyanidin 3-xyloside over the storage period; for the treated juices (200–600 MPa for 15 min) these decreases ranged from 30 to 40%. This trend was maintained over the next 40 days of storage.

The results described above are consistent with data found in the literature and indicating that the anthocyanins are stable after HP

Table 4
HPLC analysis and individual concentration (mg/mL $^{-1}$) of the main phenolic compounds (1 - chlorogenic acid; 2 - neochlorogenic acid; 3 - cyanidin 3-galactoside; 4 - cyanidin 3-xyloside; 5 - cyanidin 3-arabinoside; 6 - cyanidin 3-glucoside) as measured in untreated and HP-treated (200–600 MPa for 15 min) aronia juice upon storage at 4 °C.

Storage (days)	Pressure (MPa)	Compounds					
		1	2	3	4	5	6
Fresh	Untreated	0.82 ± 0.05 Aa*	0.44 ± 0.03 Aa	3.93 ± 0.34 Aa	0.23 ± 0.01 Aa	1.79 ± 0.014 Aa	0.13 ± 0.01 Aa
	200	0.72 ± 0.03 Ab	0.38 ± 0.02 BC a	3.46 ± 0.10 Aa	0.21 ± 0.06 Aa	1.55 ± 0.019 Ba	0.12 ± 0.01 ABa
	400	0.76 ± 0.01 Aab	0.40 ± 0.01 Bab	3.55 ± 0.20 Aa	0.23 ± 0.01 Aa	1.65 ± 0.02 Aa	0.13 ± 0.01 Aa
	600	0.75 ± 0.01 Aab	0.40 ± 0.02 Aab	3.64 ± 0.27 Aa	0.21 ± 0.04 Aa	1.65 ± 0.02 Aa	0.13 ± 0.01 Aa
20	Untreated	0.75 ± 0.01 ABa	0.39 ± 0.01 ABa	2.93 ± 0.04 Bb	0.19 ± 0.05 Aa	1.38 ± 0.07 Ba	0.11 ± 0.01Aa
	200	0.80 ± 0.04 Aa	0.43 ± 0.03 ABa	3.34 ± 0.29 Aab	0.21 ± 0.04 Aa	1.54 ± 0.18 BCa	0.11 ± 0.01ABa
	400	0.78 ± 0.04 Aa	0.41 ± 0.02 Ba	3.41 ± 0.15 ABa	0.22 ± 0.02 Aa	1.63 ± 0.11 Aa	0.13 ± 0.01 Aa
	600	0.79 ± 0.03 Aa	0.41 ± 0.01 Aa	3.47 ± 0.08 Aa	0.17 ± 0.05 ABa	1.63 ± 0.08 Aa	0.11 ± 0.01 Aa
40	Untreated	0.63 ± 0.07 BCa	0.31 ± 0.03 Cb	2.27 ± 0.27 Cb	0.07 ± 0.01 Bb	1.10 ± 0.15 BCc	0.12 ± 0.01 Aa
	200	0.77 ± 0.09 Aa	0.48 ± 0.03 Aa	3.08 ± 0.13 Aa	0.14 ± 0.01 ABa	1.95 ± 0.07 Aa	0.15 ± 0.01 Aa
	400	0.76 ± 0.06 Aa	0.49 ± 0.03 Aa	3.22 ± 0.07 Ba	0.13 ± 0.01 BCa	1.57 ± 0.10 Ab	0.13 ± 0.01 Aa
	600	0.75 ± 0.07 Aa	0.45 ± 0.07 Aa	3.54 ± 0.24 Aa	0.13 ± 0.02 Ba	1.68 ± 0.15 Aab	0.13 ± 0.01 Aa
60	Untreated	0.61 ± 0.07 Cb	0.38 ± 0.03 ABa	2.36 ± 0.06 Cc	0.08 ± 0.02 Bb	1.14 ± 0.05 BCb	0.11 ± 0.01 Aa
	200	0.77 ± 0.05 Aa	0.40 ± 0.02 BCa	2.63 ± 0.14 Bb	0.12 ± 0.01 Ba	1.21 ± 0.07 CDab	0.10 ± 0.01 Ba
	400	0.77 ± 0.03 Aa	0.40 ± 0.03 Ba	2.72 ± 0.08 Cab	0.14 ± 0.01 Ba	1.26 ± 0.03 Bab	0.10 ± 0.01 Aa
	600	0.79 ± 0.01 Aa	0.41 ± 0.01 Aa	2.90 ± 0.03 Ba	0.12 ± 0.01 Ba	1.31 ± 0.01 ABa	0.10 ± 0.01 Aa
80	Untreated	0.71 ± 0.03 ABCa	0.33 ± 0.01 BCb	2.14 ± 0.08Cb	0.07 ± 0.00 Bb	1.03 ± 0.07 Ca	0.11 ± 0.01 Aa
	200	0.69 ± 0.03 Aa	0.34 ± 0.02 Cab	2.43 ± 0.04 Bab	0.09 ± 0.00 Bab	1.12 ± 0.02 Da	0.10 ± 0.01 Ba
	400	0.78 ± 0.07 Aa	0.39 ± 0.04 Ba	2.63 ± 0.19 Ca	0.10 ± 0.01Ca	1.20 ± 0.12 Ba	0.12 ± 0.01 Aa
	600	0.73 ± 0.06 Aa	0.39 ± 0.01 Aab	2.62 ± 0.10 Ba	0.09 ± 0.01 Ba	0.79 ± 0.08 Ba	0.12 ± 0.01 Aa

* Small letters indicate difference (uniform Tucey's groups) between treatment type (in column) in particular days of storage, whereas capital letters indicate difference between storage time for individual sample (in column).

treatment at ambient temperature, however their stability is rather transient (Patras et al., 2009; Ferrari et al., 2010).

3.5. Microbiological quality

The evolution of microbial load in the untreated and HP treated (200–600 MPa for 15 min) aronia juice upon 80 days of storage at 4 °C was presented in Fig. 2. The microbial counts determined for the untreated juice ranged from 2.1×10^3 CFU mL^{-1} (fresh sample) to 3.95×10^4 CFU mL^{-1} (sample stored for 80 days) (Fig. 2A). The juice treatment at 200 MPa resulted in one order of magnitude reduction of microbial growth as compared to the untreated sample. Given the microbial counts determined in the untreated and HP-treated juices, it is clear that pressurization already at 200 MPa significantly delayed the microbial growth over the entire storage period of the analyzed samples.

A significant inhibition of yeast (almost three-fold) and mold (one order of magnitude) counts was observed after the fresh juice was pressurized at 200 MPa. In opposite to the untreated sample, three and two order of magnitude lower total yeast count was detected in pressurized juices on the 40th and 80th day of storage, respectively (Fig. 2B). The mold population was distinctly reduced in the juice pressurized at 200 MPa at the early stage of its storage (Fig. 2C). However, the analysis performed on samples stored for 60 and 80 days indicated a distinct decrease in the mold population both in untreated and treated juices. This decrease could be attributed to i) the competition effect between yeast and mold because both have special nutritional requirements; ii) competition for space due to the higher growth rate of yeast; or iii) toxicity of yeast towards molds due to specific excreted metabolites (Ubeda, Maldonado, Briones, & Gonzalez, 2014).

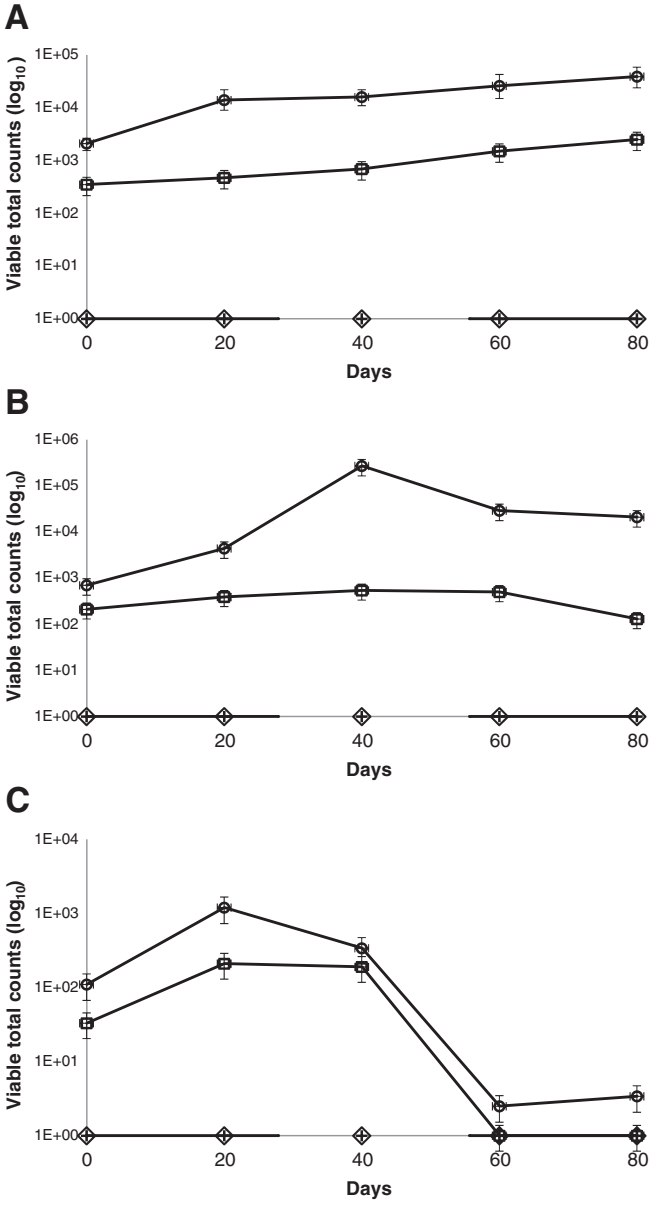

Fig. 2. Average total bacterial count (A), total yeast count (B), total mold count (C) as analyzed in untreated and HP-treated aronia juice upon storage at 4 °C: untreated; 200 MPa; 400 MPa*; 600 MPa* (*below detection limit <1 CFU mL^{-1}).

In contrast to the results above, other levels of pressure (400 and 600 MPa) were equally effective at inhibiting the growth of microorganisms to a level below the limit of detection (<1 CFU/mL) over the entire storage period.

The results obtained in this research are consistent with literature data indicating that treatment of pomegranate juice at 350 MPa for 2.5 min was effective enough to maintain the microbial population (aerobic mesophilic bacteria, molds and yeasts count) below the detection limit (<1 $\log_{10}$ CFU mL^{-1}) (Varela-Santos et al., 2012). Additionally, these same authors noted that the low pH range of the pomegranate juice (2.98–3.20) might enhance the lethality of HP treatment. It was also demonstrated that orange juice treated at 600 MPa for 3 min showed a reduction in the growth of aerobic microorganisms, yeasts and molds to a level below the limit of detection (<10 CFU mL^{-1}) (Bisconsin-Junior, Rosenthal, & Monteiro, 2014).

4. Conclusions

The results presented here characterized and compared the antioxidant concentration, composition and antiradical potential of aronia juice subjected to HP treatment (200–600 MPa for 15 min at ambient temperature) and storage (4 °C for 80 days). Irrespective of the pressure applied, pressurization significantly affected the phenolic concentration in aronia juice. The regression analysis proved explicitly that the antioxidants concentration greatly affected the antiradical potential. A total of 6 types of polyphenolic compounds were identified in the aronia juice. Anthocyanins were more stable following pressure treatment as compared to chlorogenic and neochlorogenic acids. Cyanidin 3-glucoside was the most stable compound in the stored juice, whereas cyanidin 3-xyloside was the most labile.

Taking into consideration the results obtained as well as cost efficiency of the process that should be an advantage for the producer, we may conclude that the pressure of 400 MPa was effective enough to distinctly increase the microbial stability of the product and to make the phenolic compounds more stable upon storage.

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