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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.

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.