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Article

Stability of Chokeberry Bioactive Polyphenols during Juice Processing and Stabilization of a Polyphenol-Rich Material from the By-Product

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Abstract: Chokeberries (*Aronia melanocarpa*) are nowadays believed to exhibit potential cardioprotective and antidiabetic effects principally due to their high content in bioactive phenolic compounds. The stability of the phenolic compounds was studied during different stages of a juice production line and a method for the valorization of pomace was evaluated. Samples were taken from a commercial juice production plant, extracted and analyzed for phenolic constituents and antioxidant potential. Prototypes of functional food ingredients were produced from the pomace by wet milling and micro-milling. Alongside juice processing, the contents of phenolic berry constituents did not vary to a great extent and the overall antioxidant activity increased by about 34%. A high quality juice and a by-product still rich in polyphenols resulted from the process. The phenolic compounds content and the overall antioxidant activity remained stable when milling and micro-milling the pomace. During coarse milling, extractability of total phenolic compounds increased significantly (40% to 50%). Nanosized materials with averaged particle sizes ($x_{50,0}$) of about 90 nm were obtained by micro-milling. These materials showed significantly enhanced extractability of total phenolic compounds (25%) and total phenolic acid (30%), as well as antioxidant activity (35%), with unchanged contents of total procyanidins and anthocyanins contents.

Keywords: polyphenols; procyanidins; anthocyanins; juice processing; wet micro-milling

1. Introduction

Aronia, also known as chokeberry, is a member of the Rosaceae family, a native of Eastern North America. Black Chokeberry (*Aronia melanocarpa*), one of the Aronia species, is cultivated commercially principally in Eastern Europe and used for the production of juice, jam, syrup, wine and natural food colorants. Nowadays the berries of this species are considered an important source of natural antioxidants. The study of the postulated cardio-protective, anti-diabetic and immune-regulatory effects of chokeberries, which are generally related to their high content in phenolic compounds, principally procyanidins and anthocyanins, are current research topics [1–3].

In berries, such as grapes, procyanidins are found associated with the cell walls mainly in the skin and seeds [4]; in apples procyanidins are also present in the flesh [5,6]. For juice production, berries are squeezed or coarsely ground, heated and finally pressed. An important part of the phenolic compounds contained in the berries is found in the juice, but the pomace, the solid by-product of juice production, is still very rich in these bioactive compounds [7–11].

The stability of anthocyanins and other polyphenolic compounds during processing of berries into juice seems to be highly correlated with the kind of processed berry. During the processing of cranberries into juice, the flavonols and procyanidins presented greater thermal stability than anthocyanins (40%–50% retention). However, significant losses of procyanidins and anthocyanins were observed as a result of the separation of skin and seeds during pressing [12]. During processing of highbush blueberries into juice substantial decrease of both anthocyanins (32% retention) and procyanidins levels were observed, while only a small amount of the procyanidins remained in the press-cake residue [13]. Regarding by-product valorization, some food ingredients, obtained mainly by drying chokeberry pomace and used for example in fruit teas, jams or dietary supplements are currently commercially available. However, the pomace is generally used as animal feed or is simply discarded. In general, the strategies for pomace valorization are based on the development procedures for the extraction of bioactive compounds, and nowadays efforts are made towards improving the extraction process, e.g., using water instead of organic solvents as extraction medium [9,14].

Mechanical and thermal processing of plant material, leading to the disruption of the natural matrix, may enhance the bioaccessibility of bioactive food components [15]. Disruption of plant cell walls, by mechanical size reduction or by adding pectinolytic enzymes, significantly enhanced the amounts of extracted polyphenols [7] and increased bioaccessability of carotenoids [16]. However, the enhanced bioaccessibility of β-carotene from carrot tissue was only observed when the particle size obtained by mechanical disruption was smaller than the size of the carrot cells [16].

Wet micro-milling processes providing nanometer-sized materials have been reported to improve the bioavailability of poorly water soluble drugs [17]. However, literature data concerning micro-milling of plant materials rich in secondary plant metabolites are certainly quite scarce.

The first part of this paper deals with the investigation of the stability of polyphenols, mainly anthocyanins and procyanidins, along the different steps of the production line of a commercial

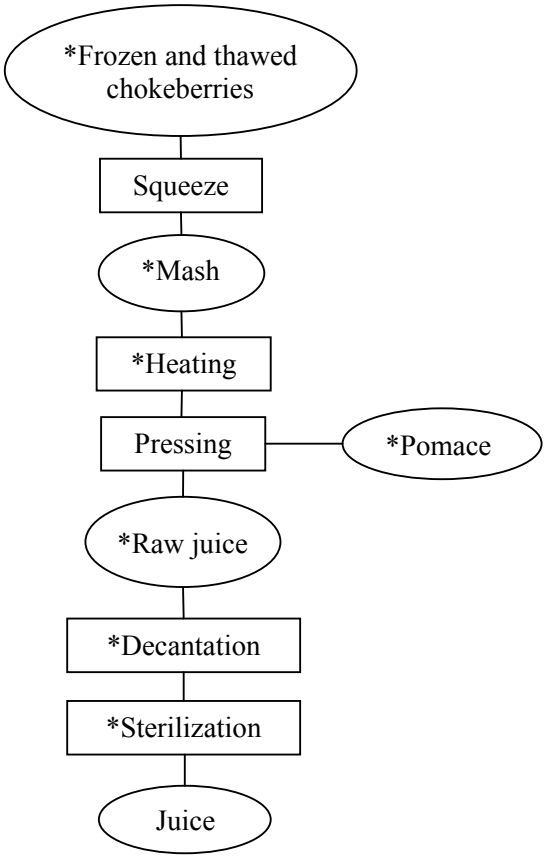
chokeberry juice. As the resulting solid by-product of juice production was still very rich in polyphenols, particle size reduction of the pomace by wet milling and micro-milling was studied as possible processes to obtain procyanidin-rich materials intended as functional food ingredients.

2. Results and Discussion

2.1. Commercial Juice Production

Several authors studied the stability of anthocyanins and procyanidins alongside the processing of berries into juice at laboratory scale [10,12,13]. However, little information has been published concerning the stability of bioactive compounds along the different processing steps of the production line of commercial berry juices. Therefore, our interest relied in the evaluation of the stability of the phenolic constituents of chokeberries during the processing into juice (Figure 1).

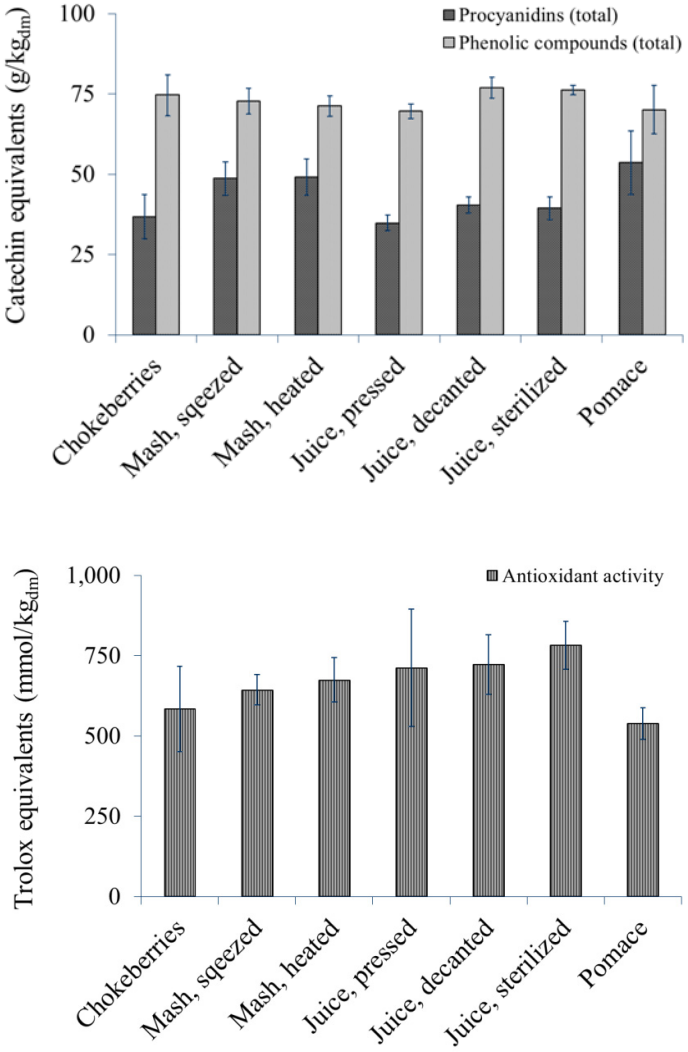
Figure 1. Chokeberry juice processing (sampling points marked by asterisks).



2.1.1. Processing Effects on Polyphenols

The total phenolic compounds and total procyanidins content as well as the overall antioxidant activity (expressed on a dry matter base) suffer no significant changes throughout all stages of the commercial juice processing plant investigated (Figure 2).

Figure 2. (**a**) Total phenolic compounds and total procyanidins contents (dry matter base: g/kg_{dm}); (**b**) antioxidant activity during the different stages of commercial juice processing (mean values; standard deviation; $n = 3–6$).



Squeezing significantly increased total procyanidins content of the mash by about 30% (dm base; $P \leq 0.05$) as compared to the berries. Further heating did not affect the total procyanidins contents of the mash ($P \geq 0.05$). After solid matter separation by pressing, similar contents of total procyanidins were determined for the raw juice, the decanted juice and the sterilized juice ($P \geq 0.05$). The procyanidins content in the sterilized juice and in the berries was similar but significantly lower than in the heated mash (about 20%, $P \leq 0.05$). The procyanidins content in the pomace was significantly higher than in the berries (about 45%, $P \leq 0.05$) and also higher than in the heated mash (about 10%, $P \geq 0.05$).

The observed higher total procyanidin content within the mash after mechanical disruption of the berries is most likely due to an enhanced extractability and subsequent release of monomeric units (predominantly epicatechin) and of oligo- and polymeric procyanidins. The procyanidins contained in

the chokeberries used in this study were distributed as follows: 70% in the flesh, 25% in the skin and 5% in the kernels. This may indicate a comparatively low overall content of polymeric procyanidins in our material. In studies with apple pomace procyanidins were detected within the flesh, being a large part of the polymeric procyanidins tightly bounded to the cell wall polysaccharides [5,6]. After pressing, most of the released procyanidins remain in the pomace mainly composed of insoluble cell wall material, berry skins and kernels. Our results are in accordance with Oszmiański *et al.* [10], who reported 60% higher procyanidin content in the pomace than in the chokeberries. No procyanidin degradation was detected during thermal treatment of either mash or raw juice. Actually, total procyanidins remained stable after heating chokeberry purees up to 100 °C within 20 minutes and holding at 100 °C for further 15 minutes (own unpublished data). The procyanidins of heated grape seed flour were stable at 120 °C for up to 60 minutes [18]. Even at the very extreme processing conditions applied during extrusion, which combined a high temperature with a high mechanical energy input, about 88% of the initial polymeric procyanidins content were retained in an extrusion product containing blueberry pomace (160 °C, screw speed 200 min^{-1}) [19].

The total phenolic compounds content of all process intermediates was similar, no significant increase of total phenolic compounds within juice or by-product was detected ($P \geq 0.05$). Oszmiański *et al.* [10], using a pectinolytic enzyme treatment in a laboratory scale study, observed that total phenolic content increased by about 35% within chokeberry pomace as compared to the chokeberries. Enzymatic depectinization of fruit mashes is often performed to improve the pressing process efficacy, leading in general to higher juice yields [20]. Polyphenols may be extracted from the fruit matrices to a higher extend after enzyme treatment, but are finally not extracted into the juice during pressing.

The overall antioxidant activity of the juice was 34% greater than in berries (Figure 2b) and was significantly lower within the pomace as compared to the heated mash ($P \leq 0.05$). The extraction of anthocyanins into the juice may account for this effect. This is also reflected in the results concerning anthocyanins contents of several lots of frozen chokeberries, juice and pomace (Table 1). In contrast, as concluded by other authors, the antioxidant activity in chokeberry juice was 30% lower as compared to the berries, with about 40% lower anthocyanins content [10]. As pectinolytic enzymes were used to produce this juice the lower antioxidant activity may be an effect of the enzyme treatment. On the other hand, an enzyme treatment produced no reduction in the anthocyanins content of blackcurrant juice as compared to the berries [21].

The sterilized chokeberry juice exhibited total procyanidins content of 4.6 ± 0.4 g/L, total phenolic compounds content of 9.0 ± 0.5 g/L, and an antioxidant activity of 85 ± 11 mmol/L.

2.1.2. Lot Specific Diversity of Polyphenols Contents of Berries, Juice and Pomace

In addition to the samples obtained from the juice processing plant, different lots of frozen chokeberries, juice and pomace (all from Kelterei Walther) were analyzed for the content of total phenolic compounds, total procyanidins, anthocyanins, phenolic acids, sugar, sugar alcohols and dry masses as well as for antioxidant activity and polymerization degree of procyanidins (Table 1). Although generally data concerning constituents of plant material are affected by cultivar, harvest time,

habitat, maturation and in particular by analytical problems, our results correspond well with the data currently found in the literature, as reviewed elsewhere [1,10,22].

The mean procyanidin polymerization degree (mDP) determined for berries was 19 ± 4 and for the juice 23.9 ± 0.4, corresponding quite well to literature data, 23 for both materials. For the pomace, however, the value obtained for the mDP, 18 ± 5, was lower as the ones found elsewhere, 34 [10] and 25–30 [23,24].

Table 1. Phenolic constituents, sugar, sugar alcohols content (g/kg_{wb} for berries and pomace, g/L for juice), dry mass (%, w/w), and antioxidant activity ($mmol/kg_{wb}$) in chokeberries, commercial juice and pomace (one to three lots; mean values).

	Berries [1]	**Juice**	**Pomace**
Phenolic compounds (total)	15–17.9	4.7–9.0	31–63
Procyanidins (total)	8–178	4.6–15	24–129
- Polymerization degree (mDP)	19	24	18
Anthocyanins (total)	6.2–6.7	58–473	11.9–19.5
- Cyanidin-3-galactoside	4.1–4.4	43–341	7.6–12.5
- Cyanidin-3-arabinoside	1.9–2.1	14–108	3.7–5.7
- Cyanidin-3-xyloside	0.13–0.14	1–13	0.3–0.6
- Cyanidin-3-glucoside	0.08–0.09	0.5–9.9	0.24–0.44
Phenolic acids (total)	1.4–1.5	0.45–0.59	0.72–0.82
- Chlorogenic acid	0.69–0.74	0.2–0.3	0.42–0.50
- Neochlorogenic acid	0.71–0.72	0.21–0.29	0.31–0.32
Sugar /sugar alcohols (total)	68–158	110–143	84.0
- Glucose	10.9–40.1	31.7–40.4	22.8
- Fructose	13.8–41.6	30.2–39.1	23.6
- Sorbitol	43.6–76.1	47.8–63.8	37.6
Antioxidant activity	119–165	48–85	240–600
Dry mass	17.9–26	11.1–17.4	44.6–50

[1] after coarse milling.

2.2. Juice Processing By-Product Valorization Strategy

According to our results, the residual pomace from processing chokeberry into juice is still rich in phenolic compounds (Table 1), and these are correlated with several health enhancement effects. Therefore, we investigated the used of different wet milling processes for the reduction of the particle size of chokeberry pomace down to the sub-micrometer range. The aim was the production of a high nutritional quality material to be used as food ingredient and containing not only the polyphenol fraction, but also all other berry constituents that remain in the pomace.

2.2.1. Wet Milling and Micro-milling Processing

Chokeberry pomace was coarsely milled, obtaining a material with an averaged particle size ($x_{50,0}$) of 6.7 ± 0.4 µm. Further wet micro-milling of the coarsely milled material was performed according to two different protocols (A, B), which differ mainly in the rotation speed of the agitator shaft. The dry

matter content of the milling suspension was kept constant at 7.7 ± 0.1% (w/w). The mean mass specific energies applied were significantly different, about 7600 kJ/kg_{dm} for protocol A and 10830 kJ/kg_{dm} for protocol B (Table 2).

Table 2. Mass specific energy input, particle sizes (µm), spans, total phenolic compounds contents (g/kg_{dm}) and antioxidant activity (mmol/kg_{dm}) of untreated, milled and micro-milled (protocols A, B) chokeberry pomace (mean values; standard deviation; $n \geq 2$; means with different letters (a, b, c, d) within the same row are significantly different; $P \leq 0.05$).

	control	Milling	Micro-milling A [1]	Micro-milling B
Mass specific energy input	ND [2]	ND [1]	7599 ± 1794	10826 ± 1336
Particle size ($x_{50,0}$)	ND [2]	6.7 ± 0.4 a	0.0858 ± 0.0003 b	0.0861 ± 0.0004 b
Span	ND [2]	1.44 ± 0.09 a	1.18 ± 0.04 b	1.16 ± 0.01 b
Phenolic compounds (total)	56 ± 9 a	86 ± 11 b	106 ± 7 c	108 ± 4 c
Anthocyanins (total)	19 ± 5 a	22 ± 2 a	24	22.8 ± 0.5 a
- Cyanidin-3-galactoside	12 ± 3 a	13.6 ± 0.2 a	16	14.7 ± 0.3 b
- Cyanidin-3-arabinoside	6 ± 2 a	6.6 ± 0.1 a	8	7.1 ± 0.1 b
- Cyanidin-3-xyloside	0.4 ± 0.2 a	0.44 ± 0.01 a	0.6	0.52 ± 0.01 b
- Cyanidin-3-glucoside	0.3 ± 0.1 a	0.38 ± 0.03 a	0.4	0.47 ± 0.01 b
Procyanidins (total)	132 ± 36 a	186 ± 22 b	190 ± 18 b	190 ± 15 b
Phenolic acids (total)	1.2 ± 0.2 a	1.62 ± 0.03 a	ND 2	2.1 ± 0.1 b
- Chlorogenic acid	0.7 ± 0.1 a	0.94 ± 0.01 a	ND 2	1.2 ± 0.1 b
- Neochlorogenic acid	0.5 ± 0.1 a	0.69 ± 0.01 a	ND 2	0.88 ± 0.02 b
Antioxidant activity	631 ± 122 a	958 ± 111 b	1296 ± 36 c	1144 ± 28 d

[1] $n = 1$ for anthocyanins; [2] ND not determined.

An increase in the rotation energy from 2000 to 3000 min^{-1} resulted in an increase of about 40% in the mechanical energy input. For both milling protocols the resulting mean particle sizes were in the same range (about 0.086 µm) with spans (width of the distribution) of about 1.2, indicating quite uniform particle size distributions. The overall size reduction of the coarsely milled material to the sub-micrometer range was of the order of 80-fold. It is to mention, that a higher energy input does not necessarily result in a better size reduction. Instead, a specific energy input based on material properties seems to be sufficient. Regarding the particle sizes generated and the reduction rates described, our results are in accordance with previously published data. Liao *et al.* [25,26] used a wet bead based micro-milling process in order to study the effect of particle reduction on the *in vivo* bioavailability of bioactive lignin glucosides from sesame meal, a by-product from sesame oil production. After 39 minutes of milling with a rotation speed of 3600 min^{-1}, a sub-micrometer-sized preparation with a mean particle size of 0.2 µm was obtained from the base material with a particle size of 2 µm (10-fold size reduction). Rice fermented with *Monascus* sp. (red mold rice), used as a food colouring agent in Asia, was processed using a high energy wet micro-milling method to improve the bioavailability of the bioactive *Monascus* sp. metabolites, e.g., monacolin K [27]. Milling was performed by using grinding media with different diameters (0.65 mm for 1 h and 0.2 mm for 3 h) at a

rotation speed of 3000 min^{-1}. A 50-fold size reduction (0.15–0.41 μm) to the sub-micrometer range was determined.

2.2.2. Stability and Extractability of Polyphenols as Affected by Wet Milling and Micro-milling

The contents of all studied bioactive constituents contained in the chokeberry pomace as well as the overall antioxidant activity remained stable during the milling and micro-milling processes (Table 2). The extraction of total phenolic compounds and total procyanidins from the coarsely milled pomace was improved as compared to the pomace; the values obtained were slightly but significantly ($P \leq 0.05$) higher, about 40% and 50%, respectively. A similar trend was determined for the anthocyanins and the phenolic acids content. The higher extractability of bioactive components was also reflected in a significantly 50% higher antioxidant activity of the coarsely milled material as compared to the untreated pomace. Further micro-milling of the coarsely milled material resulted in a further significant extractability increase of total phenolic compounds, total phenolic acids and antioxidant activity by about 25%, 30% and 35%, respectively, but the total procyanidins and anthocyanins contents remained unchanged. However, the contents of the main cyanidin glycosides detected were significantly higher, about 20%, after micro-milling. The increased extractability of bioactive chokeberry components from pomace may be explained by their location within the fruit skin and kernels. The size reduction performed by the milling and micro-milling processes involves a simultaneous surface enlargement of the pomace particles and supports the solubility of particle components within the extraction media. Literature data concerning solubility and bioavailability enhancements due to micro-milling are predominantly limited to drug delivery applications [17] and will not be discussed here. A food application aiming at a bioavailability enhancement by wet micro-milling is described in which, contrary to our results, after milling, the metabolites monacolin K (a bioactive component) and citrinin (a mycotoxin) of *Monascus* sp. were degraded by about 20% and 52% in the nanometer-sized red mold fermented rice [27].

Our results showed, furthermore, that it is non-essential whether micro-milling is performed according to protocol A or B. Both milling processes differ in the mechanical and thermal energies applied as well as in milling times. Thermal energy inputs were not estimated; however, product temperature during milling was kept constant at 20 °C (A) or 40 °C (B), milling times were 2 h (A) or 1 h (B), always under a reduced oxygen atmosphere. Therefore, it can be stated that the bioactive constituents of chokeberry pomace were not degraded by thermal effect or the presence of oxygen during the milling process.

According to our results, milling and micro-milling processes are suitable for the size reduction of the pomace resulting from chokeberry juice production. Furthermore, using these processes, the bioactive compounds with potential health enhancement properties contained in the pomace are not degraded. The observed enhanced extraction of bioactive compounds suggests a possible better bioaccessibility from the milled pomace matrix. All these facts make the milling processes very interesting for the valorization of chokeberry pomace aiming at the development of food ingredients.

3. Experimental Section

3.1. Materials and Reagents

Chokeberries (*Aronia melanocarpa*), chokeberry juice, pomace and process intermediates from a commercial juice production line were kindly supplied by Kelterei Walther GmbH & Co. KG (Arnsdorf, Germany). For the commercial juice production (Figure 1) freshly harvested or deep frozen and thawed chokeberries were mashed and heated to around 60 °C. Juice was separated from the mash by subsequent pressing and decanting, and finally sterilized and bottled. Representative samples (berries, juice, pomace and process intermediates) were taken directly from the production line throughout ongoing production, packaged within gas tight bags, vacuum sealed, immediately chilled and transported to our laboratories on dry ice and stored at −50 °C in the dark until further use. Bottled juice samples were stored at room temperature in the dark. All chemicals used for extraction and HPLC analysis were purchased from Merck Millipore/VWR, Darmstadt, Germany, except where otherwise stated.

3.2. Wet Milling, Micro-milling

The deep frozen pomace was coarsely milled in a cutting mill (Grindomix GM 200, Retsch GmbH, Haan, Germany) at 4000 min^{-1} for 2 minutes. For further micro-milling, aliquots of about 0.5 kg on a wet base (wb) were mixed with 2.5 kg of ice cold desalted and degassed water. Micro-milling experiments were conducted using a continuously operating laboratory scale agitator bead-mill Labstar LS1 (Netzsch Feinmahltechnik GmbH, Selb, Germany) consisting of a 0.55 L grinding chamber with a Zeta® agitator shaft for high speed circulation, equipped with a preparation tank with stirrer and gear pump. The pomace suspension was filled into the preparation tank and circulated through the grinding chamber back to the tank throughout the whole grinding duration (closed loop operation). Experiments were performed using two different standard protocols (A, B). The rotation speed of the agitator shaft and grinding duration were 2000 min^{-1} for 2 h and 3000 min^{-1} for 1 h for protocols A and B, respectively. The filling degree of the grinding chamber and the agitator speed of the gear pump were 65% and 500 min^{-1}, respectively, for both protocols. Zirconium oxide micro milling beads (1.4–2 mm; Jyoti Ceramic Inds. Pvt. Ltd., Nashik, India) were used as grinding media. All experiments were performed at 20 °C and 40 °C for A and B, respectively, under an oxygen reduced atmosphere implemented by continuous supply of nitrogen gas. Each experiment was performed three times.

3.3. Particle Size Analysis

Particle size distributions were analyzed by static light scattering (LS13320 PIDS Laser Diffraction Particle Size Analyzer, Beckman Coulter GmbH, Krefeld, Germany). Prior to the measure, the milled material was passed through a sieve (mesh aperture 1.000 μm), therefore, and milled and micro-milled samples were dispersed in ultrapure water (Milli-Q Advantage A10, Millipore, Schwalbach, Germany). A refractive index of 1.46 was used for the particles [28]. Particle diameters (median; $x_{50,0}$) were calculated from particle size distribution densities on the basis of numbers (q_0). The width of the particle size distribution (span) was calculated from $x_{10,0}$ (10% of the particles of a particle collective

are below this particle size) and $x_{90,0}$ (90% of the particles of a particle collective are below this particle size).

3.4. Polyphenols Extraction and Analysis

3.4.1. Extraction Methods

To extract procyanidins and other polyphenols (except anthocyanins, hydroxycinnamic acids) from chokeberries, juice and pomace a published extraction method [29] was modified. Berries and pomace were first homogenized in a cutting mill (B 400, Büchi GmbH, Essen, Germany), while juice was extracted directly. Aliquots of 2 g of each sample were mixed with 30 mL of an ice-cold mixture of acetone and water (60/40 v/v), stirred for 30 minutes and centrifuged at 14.225× *g* (Sorvall RC 26 plus, Thermo Fisher Scientific, Dreieich, Germany) for 10 minutes at 4 °C. The supernatants were filtered (Filtrac 3 w, Sartorius, Göttingen, Germany) and kept in the dark at 8 °C. The centrifugates were re-extracted twice (berry, juices samples) or fourfold (pomace samples), either, with 30 mL of acetone/water (60/40, v/v). The pooled filtrates were brought to a volume of either 100 mL (berry, juices samples) or 250 mL (pomace samples) and analyzed. Anthocyanin and hydroxycinnamic acids were extracted according to the procedure described above except that an extraction medium consisting of methanol, water and formic acid (85/12/3 v/v/v) was used [30]. All experiments were carried out under subdued light to prevent photo-degradation.

3.4.2. Analysis of Total Phenolic Compounds Content

Total phenolic contents were determined according to Singleton *et al.* [31] In brief, 125 µL of an appropriate sample dilution in acetone/water (60/40, v/v) was mixed with 625 µL of Folin-Ciocalteus reagent and, after 3 minutes at room temperature, with 500 µL of a sodium carbonate solution (6.5% in water). After incubation for 15 minutes at 45 °C, samples were rapidly cooled in an ice bath to 20 °C and absorption was measured at 750 nm (Lambda 40, Perkin Elmer, Rodgau, Germany). Sample and reagent blanks were prepared using acetone/water (60/40, v/v) instead of sample and Folin-Ciocalteus reagent, respectively. Triplicate runs were made for samples and blanks. Catechin hydrate (≥98%; Sigma-Aldrich Chemie GmbH, München, Germany) dissolved in ultrapure water was used as a standard to generate calibration curves for total phenolics quantification as catechine equivalents.

3.4.3. Analysis of Total Procyanidins Content and Polymerization Degree

Total procyanidins contents were measured on the basis of a method described by Swain *et al.* [32].Sample aliquots of 200 µL were mixed with 500 µL of sulphuric acid (30% in methanol) and 500 µL of vanillin reagent (1% in methanol), successively, and incubated at 20 °C in the dark for 10 minutes. Absorption was measured at 500 nm using a spectrometer (Lambda 40, Perkin Elmer, Rodgau, Germany). Reagent blanks were prepared using aceton/water (60/40, v/v) instead of sample and sample blanks were prepared using methanol instead of sulphuric acid and vanillin reagent, respectively. Triplicate runs were made for samples and blanks. The results were calculated as catechine equivalents using Catechin hydrate (≥98%; Sigma-Aldrich Chemie GmbH, München, Germany) dissolved in aceton/water (60/40, v/v) as a standard.

The mean polymerization degree (mDP) of procyanidins was estimated according to Kennedy *et al.* [33].

3.4.4. Anthocyanins Contents

For HPLC analysis of monomeric anthocyanins, aliquots of extracts were dried using nitrogen gas, re-dissolved in 1% hydrous formic acid with or without concentration by 2- to 10-fold, filtered (membrec 0.45 µm, membraPure, Bodenheim, Germany), and measured twice according to a modified method described by Kammerer *et al.* [34] using a HPLC equipment (1100 series, Agilent Technologies, Waldbronn, Germany) consisting of a quarternary pump, a solvent degasser, a column oven and a photodiode array detector combined with a software for controlling, data acquisition and analysis (HP ChemStation, Agilent, Waldbronn, Germany). All analytes were separated on a Synergi Hydro C18 reversed phase column (150 mm × 4.6 mm; 4 µm) (Phenomenex, Aschaffenburg, Germany) at 20 °C with a linear gradient of acetonitrile in formic acid for 15 minutes (starting phase composition: 87% water, 10% formic acid, 3% acetonitrile; end phase composition: 65.5% water, 10% formic acid, 24.4% acetonitrile) followed by a 3 minutes isocratic flow at a flow rate of 0.5 mL/min. Anthocyanins were recorded at 520 nm, respectively, and were identified based on retention time and HPLC-MSMS identification using the same separation system described using an HPLC/MSMS system (1200 series, Agilent Technologies, Waldbronn, Germany) equipped with a linear ion trap quadrupole with an electrospray ionization chamber using the positive mode (QTrap 3200, Applied Biosystems, Darmstadt, Germany) and QTrap software (Analyst, Applied Biosystems, Darmstadt, Germany). Duplicate runs were made for all samples. Cyanidin-3O-glucoside chloride (≥96%; HPLC), (Roth, Karlsruhe, Germany) was used as calibration standard to quantify cyanidin-3O-glycosides.

3.4.5. Hydroxycinnamic Acids Contents

For HPLC analysis of hydroxycinnamic acids, dried and re-dissolved (1% FA) extract aliquots were filtered (membrec 0.45 µm, membraPure, Bodenheim, Germany), and measured twice according to a modified method described by Kennedy *et al.* [34]. An HPLC equipment (LaChrom Elite, VWR/Hitachi, Darmstadt, Germany) consisting of a quarternary pump, a solvent degasser, a column oven and a photodiode array detector combined with a software for controlling, data acquisition and analysis (EZChrom Elite, VWR/Hitachi, Darmstadt, Germany) was used to separate the analytes on an Aqua C18 reversed phase column (150 mm × 4.6 mm; 4 µm) (Phenomenex, Aschaffenburg, Germany) at 25 °C with a combination of isocratic and linear gradient separation steps of methanol in 1% acetic acid (t = 0–6 min: isocratic, 5% methanol; t = 6–18 min: gradient, 20% methanol; t = 18–33 min: gradient, 40% methanol; t = 33–34.5 min: gradient, 90% methanol; t = 34.5–40.5 min: isocratic, 90% methanol) at a flow rate of 1 mL/min. Hydroxycinnamic acids were recorded at 325 nm and were identified based on retention time and HPLC-MSMS identification using the same separation system described using an HPLC/MSMS system (1200 series, Agilent Technologies, Waldbronn, Germany) equipped with a linear ion trap quadrupole with an electrospray ionization chamber using the negative mode (QTrap 3200, Applied Biosystems, Darmstadt, Germany) and QTrap software (Analyst, Applied Biosystems, Darmstadt, Germany). Duplicate runs were made for all samples. Chlorogenic acid (≥96%; HPLC, Roth, Karlsruhe, Germany) was used to quantify chlorogenic and neochlorogenic acid, respectively.

3.4.6. Antioxidant Activity

Antioxidant activity was determined according to Re *et al.* [35]. To prepare the ABTS-radical reagent, 10mL 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (≥98%; Sigma-Aldrich Chemie GmbH, München, Germany; 7 mM in ultrapure water) were mixed with 180 µL potassium persulphate (140 mM in ultrapure water) and incubated over night at room temperature in the dark. Extract aliquots of 500 µL with dry mass contents in the range of 0.5–2.5 µg were mixed with 1 mL ABTS-radical reagent, incubated for 2.5 minutes at 30 °C, and absorption was measured at 734 nm using a spectrometer (Lambda 40, Perkin Elmer, Rodgau, Germany). To guarantee linearity of the test, every extract sample was measured threefold, using three different dry mass contents thereby. Sample blanks for each dry mass content and reagent blanks were prepared using ethanol instead of ABTS reagent and sample, respectively. Trolox (6-hydroxy-2,5,7, 8-tetramethylchroman-2-carboxylic acid; ≥97%; Sigma-Aldrich Chemie GmbH, München, Germany) was used as calibration standard.

3.5. Analysis of Sugar, Sugar Alcohol

Sucrose, glucose, fructose and sorbitol contents were determined by spectrophotometry (Enzyme-based test kits: 10 139 041 035, 10 670 057 035; R-Biopharm AG, Darmstadt, Germany).

3.6. Analysis of Dry Matter

Dry matter (dm) content was determined using a gravimetric method. Aliquots of about 5 g of berry or pomace were weight and dried at 105 °C until constant weight. About 5–10 g of juice samples were mixed with about 25 g of sea sand and dried as described above. Triplicate runs were made for all samples.

3.7. Statistical Analysis

Results are expressed as mean values ± standard deviations. Statistical analysis was performed using the one-way ANOVA procedure (SigmaPlot 11.0, Systat Software GmbH, Erkrath, Germany).

4. Conclusions

A high quality juice rich in health related polyphenols (e.g., procyanidins, anthocyanins, phenolic acids) is produced during commercial juice processing of *Aronia melanocarpa*. The remaining processing by-product (pomace), still rich in phenolic constituents with contents comparable to berries or even higher, is shown to be a potential source for the production of healthy food ingredients with a high content of bioactive compounds.

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