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Toward gentle chokeberry juice production by ultrasound-assisted enzymatic maceration

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A R T I C L E I N F O

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A B S T R A C T

Sustainable processes accompanied by high extraction yields and minimized amounts of by-products are a major goal of current fruit juice production. Controlled degradation of cell wall polysaccharides, in particular pectin, may contribute to reduced emergence of side streams. Possible strategies for the optimization are the selection of enzyme preparations based on comprehensive studies of their activities, the adjustment of maceration temperature toward more gentle conditions, and the application of alternative technologies such as ultrasound (US) during maceration. The present study provides insights into the effects of ultrasound-assisted enzymatic maceration (UAEM) on pectin degradation, total anthocyanin content, thermal and storage stability, and juice yield during chokeberry juice production on pilot-plant scale. The two enzyme preparations applied predominantly possessed polygalacturonase or pectin lyase activity. Cell wall polysaccharide degradation was improved by US and resulted in a 3% increase in juice yield by UAEM using an enzyme preparation that shows mostly polygalacturonase activity. Thermostability of anthocyanins was improved in juices produced using pectin lyase and applying US and matched the stability of anthocyanins in juices produced using polygalacturonase. Storage stability of anthocyanins was improved in juice produced using polygalacturonase during UAEM. UAEM also resulted in lower yields of pomace making the production more resource-efficient. Overall, the use of polygalacturonase has promising potential to advance conventional chokeberry juice production by applying US at gentle conditions.

1. Introduction

The degradation of cell wall polysaccharides and the extraction of phenolic compounds are crucial aspects of fruit juice production. Both degradation and extraction are greatly influenced by processing parameters like temperature, time, and processing aids such as enzyme preparations. The goal is to achieve high amounts of the value-adding phenolic compounds in the final product, good storage stability, and high sensory quality (Padayachee et al., 2012; Renard et al., 2017; Weber and Larsen, 2017; Kobus et al., 2019; Larsen et al., 2019; Liu et al., 2020).

Pectinolytic enzyme preparations increase juice yields and especially the extraction of phenolic compounds bound to the cell wall by the hydrolysis of polysaccharides (Landbo and Meyer, 2001; Buchert et al., 2005; Tchabo et al., 2015). These enzyme preparations mostly show polygalacturonase (PG), pectin methylesterase (PME), and pectin lyase (PL) activities (Aehle, 2008). The extent of cell wall degradation depends on both these major enzyme activities, and the prevailing process conditions (Tchabo et al., 2015).

While polysaccharide degradation enhances the release of phenolic compounds, it needs to be considered that the native pectin as well as the pectin fragments may interact with the phenols, in particular with anthocyanins. These interactions depend on the pH value of the medium and are favored in acidic conditions, that is, at approximately pH 3 (Dalagnol et al., 2017b; Larsen et al., 2019). Then, formation of pectin-anthocyanin complexes occurs mainly due to ionic forces. In addition, weak hydrogen bonds and hydrophobic interactions may stabilize these complexes (Holzwarth et al., 2012; Fernandes et al., 2020; Koh et al., 2020). Complexation stabilizes and protects anthocyanins against oxidation and degradation. Degradation of pectin results in pectin-derived polysaccharides of different molecular weights (MW), which affect the solubility of the complexes formed, resulting in juices

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that differ in nutritional value and color quality (Fernandes et al., 2014, 2020; Renard et al., 2017; Larsen et al., 2019; Koh et al., 2020; Liu et al., 2020; Tomas et al., 2020). However, these polysaccharides and the complexes formed may also cause negative effects like increased turbidity and sedimentation (Lachowicz et al., 2018), which may also affect color stability. During juice processing, the major part of cell wall polysaccharides, in particular high MW polysaccharides, remain in the press cake (Hilz et al., 2005), whereas smaller polymers and oligomers will transfer into the juice. Besides the above stabilizing effects, these soluble polysaccharides and oligosaccharides increase the fiber content of the juice (Koh et al., 2020; Liu et al., 2020).

Ultrasound (US) technology is a possible approach for the optimization of berry juice production by primarily minimizing heat-intensive processing steps, preserving heat-sensitive compounds, and enhancing the extraction of valuable compounds in the juice (Le Lieu and van Le, 2010; Carrera et al., 2012; Shirsath et al., 2012; Tchabo et al., 2015; Dalagnol et al., 2017a; Larsen et al., 2021). The advantages US provides include its energy-saving potential at lower, more gentle conditions, increased enzyme activities (sonoenzymolysis), and the enhanced degradation of cell wall polysaccharides such as pectin. The resulting poly- and oligosaccharides possess stabilizing effects on phenolic compounds, especially anthocyanins (Larsen et al., 2019, 2021; Tomas et al., 2020). MW distribution of these fragments needs to be controlled during maceration, e.g., by lower maceration temperatures and the use of ultrasound-assisted enzymatic maceration (UAEM). The MW of the oligomers formed should not exceed 30 kDa to ensure the formation of soluble, stabilizing complexes with anthocyanins (Larsen et al., 2021). Incorporation of US during enzymatic maceration has already been applied for several fruits on small laboratory scale (50–500 g) such as mulberry and acerola (Tchabo et al., 2015), grapes (Le Lieu and van Le, 2010; Tchabo et al., 2015; Dalagnol et al., 2017a), or guava (Nguyen et al., 2013). However, the use of UAEM during berry juice production has not been shown on pilot-plant scale so far. Processing of chokeberries containing a high amount of pectin is particularly challenging because the resulting high pulp viscosity hampers pressing. Thus, pre-treatment such as blanching and enzymatic treatment of the mash is required (Kobus et al., 2019).

In the present study, chokeberry (*Aronia melanocarpa* Michx.) juice was produced on pilot-plant scale (15 kg) using either conventional standard procedures or UAEM at 35 °C. The degradation of cell wall polysaccharides as well as total anthocyanin contents and anthocyanin stability in juices resulting from those productions were compared.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals and standards

Ultra-pure water was obtained from a PURELAB flex 2 water purification system (ELGA LabWater, Paris, France). Methanol (>99.9%), hydrochloric acid, acetic acid (99.9%), and acetonitrile (LC-MS grade, 99.9%) were purchased from VWR (Mannheim, Germany). Methanol (UHPLC grade) and sulphuric acid (95%) were from Th. Geyer (Renningen, Germany). Acetone (99%) was sourced from Juli.O GmbH (Jülich, Germany); ethanol (99.7%, denatured with petroleum ether) was from Julius Hoesch GmbH & Co. KG (Düren-Hoven, Germany); formic acid (99.9%) and sodium azide (>99%) were obtained from Merck GmbH (Darmstadt, Germany); sodium carbonate monohydrate was supplied by Sigma-Aldrich GmbH (Seelze, Germany); sodium nitrate (99%) was from Acros Organics (Geel, Belgium), and ReadyCal Kit Pullulan SEC-Standards (Mp 9600–708000 Da Lot No. Pulkit 1–02) from PSS Polymer Standards Service GmbH (Mainz, Germany). Cyanidin-3-*O*-glucoside (>97%) was purchased from Phytoplan (Heidelberg, Germany).

2.1.2. Raw materials for the chokeberry juice production

The chokeberries (*Aronia melanocarpa* Michx.; Lot No. 10092021) were kindly provided by Haus Rabenhorst O. Lauffs GmbH & Co. KG (Unkel, Germany) and were stored in 15 kg batches at −20 °C (pH 3–4, total anthocyanin content 1330.8 ± 20.3–1905.8 ± 111.1 mg/100 g dm). The food grade enzymes used for maceration were Natuzym BE +200 (Lot No. 20-W0029), which contains acid-stable pectinases (polygalacturonase (2700.60 ± 90.60 nkat/mL), pectinmethylesterase (2290.25 ± 157.56 nkat/mL) with side activity of pectin lyase (2.11 ± 0.28 nkat/mL)) and proteases from *Aspergillus niger,* and Rohapect PTE 100 (Lot No. R161293ST), which contains a pectin lyase from *Trichoderma reseei*, GMO (4543.40 ± 278.10 nkat/mL and side activity of polygalacturonase 815.30 ± 98.00 nkat/mL). Enzyme activities were determined according to Larsen et al. (2021). The enzymes were kindly provided by WeissBioTech GmbH (Ascheberg, Germany) and AB Enzymes GmbH (Darmstadt, Germany), respectively.

2.2. Methods

2.2.1. Chokeberry juice production

Juice production was conducted in different batches on pilot-plant scale, with conventionally applied maceration conditions (50 °C) being used as benchmark. UAEM was conducted at 35 °C, and a third batch was produced at 35 °C without US to reveal effects caused solely by the reduced temperature.

For each batch, 15 kg berries were thawed overnight followed by crushing in a berry crusher (roller gap: 8 mm, Grifo Macchine enologiche s.n.c., Piadena, Cremona, Italy). The mash was heated at the corresponding maceration temperature and the enzyme preparation (0.3 mL/kg) was added. Maceration was conducted in a jacked tank (Schwarte-Werk GmbH, Ahlen, Germany) for 2 h, with a mixing cycle of 20/10 min (stirring/standing). Subsequently, the mash was pressed using a membrane press (europress, Scharfenberger GmbH & Co. KG, Bad Dürkheim, Germany) with the following pressing program: 1.5 h, pressure 0.2–2.0 bar, eight pressure stages. The fresh juice was pasteurized at 85 °C for 30 s (UHT plant FT74X, Armfield Ltd., Ringwood, Hampshire, England) and hot filled in brown glass bottles closed with crown caps. These samples were stored at 4 °C for six months.

Enzyme dosage, maceration time, and temperature were applied according to the manufacturers' recommendation. Other production steps were adjusted based on preliminary experiments and reports on black chokeberry processing (Kobus et al., 2019).

UAEM treatment was conducted similarly, except for the use of an US probe of 9 cm^2 which was dipped into the mash (depth: 2 cm). The US processor (UIP 1000hdT, 1000 W, 20 kHz, Hielscher, Teltow, Germany) was equipped with a US booster horn (100% amplitude: 35 μm) and a sonotrode (BS4d34). The amplitude of the US generator was set at 90% (445.5–486 W) and pulsed operation (10 s on/off).

All three treatments were performed with both enzyme preparations, resulting in six different juices (Table 1).

2.2.2. Extraction and quantification of anthocyanins

Anthocyanin extraction and quantification of samples collected

Table 1
Maceration conditions during chokeberry juice production applying different temperatures and ultrasound-assisted enzymatic maceration (UAEM) and two different enzyme preparations (polygalacturonase, PG, and pectin lyase, PL).

Temperature	Maceration condition	Enzyme preparation	Chokeberry juice
50 °C	Conventional standard procedure	PG	50_PG
		PL	50_PL
35 °C	UAEM	PG	UAEM_PG
		PL	UAEM_PL
	Temperature control	PG	35_PG
		PL	35_PL

during juice production were performed as reported previously (Heffels et al., 2015) with some modifications. Whereas concentrations of anthocyanin in juices were sufficiently high for direct injection, berries and pomace needed to be extracted. Extractions were performed in triplicate with two solvents containing methanol/water/acetic acid in different ratios. The first extraction was carried out with 10 mL solvent A (20:75:5, *v/v/v*). For this purpose, approximately 0.6 g sample was homogenized in test tubes with an Ultra-Turrax. Subsequently, the sample was centrifuged at 4 °C (10,947*g*, 10 min). The pellet was extracted again using 10 mL extraction solvent B (80:15:5, *v/v/v*). Both supernatants were combined and the volume was made up to 25 mL. Ultra-high performance liquid chromatography diode array detection (UHPLC--DAD) analysis was performed on a Prominence UFLC system (Shimadzu, Kyoto, Japan) equipped with two Nexera X2 LC-30AD high-pressure gradient pumps, a Prominence DGU-20A5R degasser, a Nexera SIL-30AC Prominence autosampler (15 °C, injection volume 5 μL), a CTO-20AC Prominence column oven (40 °C), and a SPDM20A Prominence diode array detector. Data acquisition and processing were performed using LabSolutions software version 5.85 (Shimadzu, Kyoto, Japan). Anthocyanin separation was carried out on an ACQUITY UPLC HSS T3 column (2.1 μm, 150 × 1.8 μm; Waters, Milford, MA, USA) equipped with a security guard cartridge of the same material. For analysis two eluents were used. Eluent A was water/formic acid (97:3, *v/v*) and eluent B was acetonitrile/formic acid (97:3, *v/v*). The flow rate was set at 0.4 mL min^{-1} using the following gradient: 0 min, 4% B; 2 min, 4% B; 7 min, 8% B; 13 min, 10% B; 19 min, 17% B; 23 min, 30% B; 23.3 min, 100% B; 25.3 min, 100% B; 25.8 min, 4% B; 30 min, 4% B. Anthocyanins were detected at 520 nm and semi-quantified as cyanidin-3-*O*-glucoside equivalents (Cya-Glc eq.) by external calibration. The total anthocyanin content was calculated as the sum of the four main anthocyanins found in chokeberry samples.

2.2.3. Characterization of physicochemical parameters

Dry matter (% dm) was determined thermogravimetrically using a Sartorius moisture analyzer (MA100Q000230V1, Göttingen, Germany) by drying 1 g of sample each. Soluble solids (°Brix) were examined *via* a digital refractometer (PAL-α ATAGO Co. LTD., Tokyo, Japan). All measurements were conducted in triplicate. Viscosity measurements of the juices were performed according to Larsen et al. (2019) using a rotary viscometer (V-Pad, Fungilab, New York City, NY) equipped with an LCP-spindle. The analysis was carried out for 1 min and 200 U/min (20 °C) in six-fold. The titratable acidity of the processed juices was determined according to Cliff et al. (2007). For this purpose, 10 mL of juice was diluted with 90 mL ultra-pure water followed by endpoint titration with 0.1 M NaOH to pH 8.1.

2.2.4. Characterization of polysaccharides in berries, juices, and pomaces

2.2.4.1. Preparation of alcohol insoluble residue (AIR). For the preparation of the AIR of berries and pomaces, samples were lyophilized and mortared to obtain a fine powder. Quantities of 10 g of the prepared powder were homogenized in 100 mL ethanol (80% *v/v*) using an Ultra-Turrax (5000 rpm, 10 min). Subsequently, the suspension was heated at 40 ± 2 °C and stirred for 1 h followed by vacuum filtration. The filter cake was then suspended again with 100 mL ethanol (80% *v/v*) and stirred for 1 h at 40 ± 2 °C. This procedure was repeated 10 times in triplicate. The final extraction was performed with acetone (25 mL acetone/g filter cake). The final filter cake was suspended in acetone for 22 h at room temperature followed by vacuum filtration and drying in a petri dish for 24 h at 40 °C in a drying cabinet. The yield was measured gravimetrically. The dry matter of the AIR was determined *via* a moisture analyzer (92.95 ± 0.47–98.34 ± 0.61%).

The AIR of the pasteurized juices was obtained by lyophilizing 20 mL of the juice. The residue was weighed and dissolved in 50 mL ultra-pure water. The sample was mixed with 250 mL of an ethanol-hydrochloric acid mix (96% *v/v* ethanol, 4% *v/v* 0.3% HCl) and incubated in an incubator at 4 °C for 22 h while shaking in an orbital shaker (150 rpm). Subsequently, the sample was centrifuged (20 °C, 20 min, 2,217*g*), the pellet was dispersed in 300 mL ethanol (80% *v/v*) for 30 min at room temperature in an orbital shaker (250 rpm) and centrifuged again. This procedure was repeated three times. The AIR (remaining pellet) was dissolved in 50 mL ultrapure water and freeze dried. The AIR yield was determined gravimetrically and the preparation of the AIR was also conducted in triplicates.

2.2.4.2. Molecular weight distribution. The MW distribution of the polysaccharides was analyzed *via* high-performance size exclusion chromatography (HP-SEC) with refractive index (RI) detection (Houben et al., 2011). AIR (100 mg) was suspended in 5 mL water and heated at 50 °C for 5.5 h under stirring. The solution was dialyzed (MWCO: 12–14 kDa) for 24 h against demineralized water. Subsequently, 2 mL of the retentate was centrifuged (11,000*g*, 10 min) and the pH and sample concentration were adjusted using 1 M NaOH, 1 M HCl, and 500 mM $NaNO_3$ + 0.025% NaN_3. The dialysis and the HP-SEC analysis were repeated five times. Three different polymer mixtures (Pullulan ReadyCial-Kit) with known molecular weight (9.6–708 kDa) were used for calibration. The analyses were conducted on a Smartline HPLC system with a RI detector 2300 (Knauer, Berlin, Germany) equipped with two different, connected SEC-Diol columns (300 and 120 Å, 3 μm; YMC, Kyoto, Japan). Samples (20 μL) were injected and eluted with 50 mM sodium nitrate and 0.025% sodium azide (*w/w*) at pH 7 for 30 min at a flow rate of 0.3 mL min^{-1} and isocratic conditions. The HP-SEC chromatograms were divided into three segments: high (>208 kDa), medium (37–208 kDa), and low (19–37 kDa) molecular weight (HMW, MMW, LMW) fractions (supplemental data, Figure A.1).

2.2.5. Determination of particle size in the juices

Size distribution of particles in the juices was analyzed using a laser scattering particle size distribution analyzer (Horiba Scientific Partica LA-960, Retsch Technology GmbH, Haan, Germany). For this purpose, 0.5 mL juice was diluted in 14 mL ultrapure water and introduced into the measuring chamber. Density distribution and particle parameter d_{50} were determined fivefold.

2.2.6. Statistical analysis

Statistical analysis was conducted using XLSTAT software version 2019.1.1 (Addinsoft, Paris, France). An ANOVA with Tukey post-hoc test was performed ($p \leq 0.05$).

3. Results and discussion

3.1. General juice composition and yields

Juice yield, pomace yield and dry matter as well as physicochemical parameters of the six juices are shown in Table 2. Although statistically significant, the differences observed in the general juice composition were not pronounced, which demonstrates the substantial equivalence of the juices. However, the three juices produced using PL showed notable differences in total acidity, with lower values compared to the juices produced using PG. This correlates with the higher pH values of 3.47–3.52 in juices produced using PL. Nevertheless, all six juices showed similar values of physicochemical parameters that had been reported for chokeberry juices (Tolic et al., 2015). The application of other novel techniques such as pulsed electric fields (PEF) showed slightly increased anthocyanin contents and had no negative effect on chokeberry juice (Oziembłowski et al., 2022).

High juice yields and total anthocyanin contents (supporting information in Figure A.2 and Table A.1) pose a major goal during juice production. Juice production using PG enzyme preparation resulted in highest juice yield applying US and under gentle conditions (Table 2,

Table 2
General juice parameters of the six chokeberry juice productions. The treatments were conducted using either polygalacturonase (PG) or pectin lyase (PL) during conventional standard procedure at 50 °C, during ultrasound-assisted enzymatic maceration (UAEM, 35 °C) or temperature control at 35 °C. Different letters indicate significant differences within a column (n = 3, $p \leq 0.05$).

Treatment	Yield (% *w/w*)		Dry matter (% *w/w*)		Soluble solids (°Brix)	Viscosity (mPas)	Total acidity (g/L)	pH value (−)
	Juice	Pomace	Juice	Pomace				
50_PG	40.00	17.27	12.33 ± 3.96^{A}	74.23 ± 1.93^{A}	16.87 ± 0.06^{A}	$2.62 \pm 0.03^{A,B}$	11.71 ± 0.00^{A}	3.43 ± 0.00^{A}
UAEM_PG	43.33	15.80	10.66 ± 1.08^{A}	$71.23 \pm 1.15^{A,B}$	16.43 ± 0.06^{B}	2.64 ± 0.06^{A}	11.08 ± 0.04^{B}	$3.43 \pm 0.00^{A,B}$
35_PG	43.33	n.a.	14.40 ± 2.89^{A}	$67.54 \pm 0.52^{B,C}$	15.93 ± 0.06^{C}	2.54 ± 0.04^{B}	$11.43 \pm 0.04^{A,B}$	3.42 ± 0.01^{B}
50_PL	36.67	18.20	12.97 ± 1.16^{A}	$68.01 \pm 0.89^{B,C}$	16.30 ± 0.00^{D}	$2.58 \pm 0.03^{A,B}$	10.46 ± 0.11^{C}	3.52 ± 0.00^{C}
UAEM_PL	41.33	17.80	8.13 ± 0.34^{A}	$68.81 \pm 2.08^{B,C}$	16.50 ± 0.00^{B}	2.65 ± 0.02^{A}	10.51 ± 0.30^{C}	3.47 ± 0.00^{D}
35_PL	43.33	19.00	8.85 ± 1.48^{A}	66.84 ± 1.32^{C}	16.27 ± 0.06^{D}	$2.59 \pm 0.02^{A,B}$	10.28 ± 0.15^{C}	3.51 ± 0.00^{E}

n.a.: not available.

UAEM_PG, 35_PG) compared to the conventional conditions (50 °C). A similar yield was achieved for juice production using PL at gentle process conditions (35_PL), which decreased only slightly by US treatment (UAEM_PL). In addition, low pomace yield, with a high dry matter content and low total anthocyanin content, is desired as a trait of sustainable juice production. The pressability of pomace was enhanced after UAEM using PG enzyme preparation, resulting in lowest pomace yield and pomace with high dry matter content (Table 2, UAEM_PG). The efficacy of the processes was assessed by the mass balance, high anthocyanin recovery, and the quality of pomace such as high dry matter content, low yield and low anthocyanin content. Table 3 shows the anthocyanin recovery of all juices, the corresponding pomaces, and the loss, the latter accumulating during all production steps. Anthocyanin recovery was highest in unpasteurized juice after conventional standard procedure using PG (Table 3, 50_PG). At gentle conditions, anthocyanin recovery was lower but considerably enhanced by application of US (UAEM_PG). Noteworthy, processing at lower temperatures led to higher anthocyanin recoveries in PL-treated juices compared to the conventional procedure (50_PL) and were even enhanced by US (UAEM_PL). The latter resulted in yields comparable to those using PG (50_PG). Furthermore, anthocyanin loss was reduced applying gentle temperatures (35_PL) and US (UAEM_PL) during juice production using PL compared to the conventional standard procedure (50_PL). Thermal and storage stability of anthocyanins are discussed in section 3.4.

3.2. Cell wall polysaccharide degradation analyzed via HP-SEC

The HMW fraction (>208 kDa) defined by the analysis of the HP-SEC chromatograms presumably consists of non-degraded native polysaccharides such as pectin, which tend to form insoluble complexes with phenolic compounds such as anthocyanins (Larsen et al., 2019). The MMW fraction (37–208 kDa) is made up mainly of released, highly branched polymers (Larsen et al., 2019, 2021). The LMW fraction (19–37 kDa) likely consists of oligosaccharides, which tend to form soluble complexes due to hydrogen bonds and hydrophobic interactions (Hilz et al., 2005; Holzwarth et al., 2012). Fig. 1 shows the ratio of the fractions in pasteurized juices.

Table 3
Anthocyanin recovery in unpasteurized juice, pomace, and inevitable loss of the six chokeberry juice productions referred to the corresponding berry content. The treatments were conducted using either polygalacturonase (PG) or pectin lyase (PL) during enzymatic maceration at 50 °C, during ultrasound-assisted enzymatic maceration (UAEM, 35 °C) or temperature control at 35 °C.

Treatment	Unpasteurized juice (%)	Pomace (%)	Loss (%)
50_PG	42.83	17.88	39.29
UAEM_PG	35.66	11.32	53.02
35_PG	29.63	n.a.	n.a.
50_PL	23.44	12.70	63.86
UAEM_PL	41.82	18.15	40.02
35_PL	35.00	15.21	49.80

n.a.: not available.

Cell wall degradation for all juices was characterized by lower ratios of the HMW fraction and higher ratios of the MMW and LMW fractions. According to Larsen et al. (2021), polysaccharide degradation was positively affected by US *via* two mechanisms. First, US may facilitate the generation of fragments with MMW, which may subsequently be degraded by the enzymes. Second, US affects the enzyme conformation, resulting in higher activity. Noteworthy, both effects can be synergistic. Juice 35_PG showed the lowest ratio of the HMW fraction and highest ratios of the MMW and LMW fractions. Juices produced using PL at lower temperatures showed improved cell wall degradation compared to the conventional standard procedure with or without applying US (35_PL and UAEM_PL). Cell wall polysaccharide degradation was generally improved at gentle conditions for both enzyme preparations compared to the conventional standard procedure at 50 °C. Applying US in PL-treated juices facilitated the degradation compared to the temperature control and showed improvement in PL- and PG-treated juices compared to the conventional standard procedure. Ma et al. (2015) demonstrated that US enhances the activity and thermostability of enzymes, in particular the activity of PG, introducing US as a potential option for enhancing PG activity at gentle process conditions.

3.3. Particle size distribution of juices

Particle size distribution (PSD) of pasteurized juices of the six treatment conditions was analyzed *via* laser scattering PSD analyzer (Fig. 2).

Smallest particles were found in juices after conventional standard procedure using PG (50_PG, Fig. 2, black line). Furthermore, this juice showed a narrow PSD. Juice UAEM_PG (Fig. 2, black dashed line) contained larger particles compared to the conventional standard procedure but smaller particles compared to the temperature control (35_PG, Fig. 2, black dotted line). Applying US during production using PL (UAEM_PL, Fig. 2, grey dashed line) resulted in juices with smaller particles but not significantly different compared to the conventional standard procedure (50_PL, Fig. 2, grey line). The small polysaccharide fragments, which make up the MMW and LMW fractions determined by HP-SEC, tend to form particle aggregates, which were detected in PSD analysis. US reduces particle size and changes the surface of the generated fragments, which alters the potential interactions of the particles. The balance between surface interactions such as attractive Van der Waals and repulsive electrostatic forces between the particles determine the formation, growth, or breakdown of aggregates (Genovese et al., 2007; Rojas et al., 2016, 2017). Extensive aggregation, depending on the size of the fragments, was observed in juices due to a large particle size. These aggregates may alter the appearance of the juices, resulting in turbidity, the formation of sedimentation layers, and a changed mouthfeel.

Reduction in particle diameter (Fig. 3) might be explained by the breakdown of particle aggregates during storage. The aggregates were formed from particles of a polydisperse distribution, resulting in aggregates with lower stability and the tendency to break down (Liu et al.,

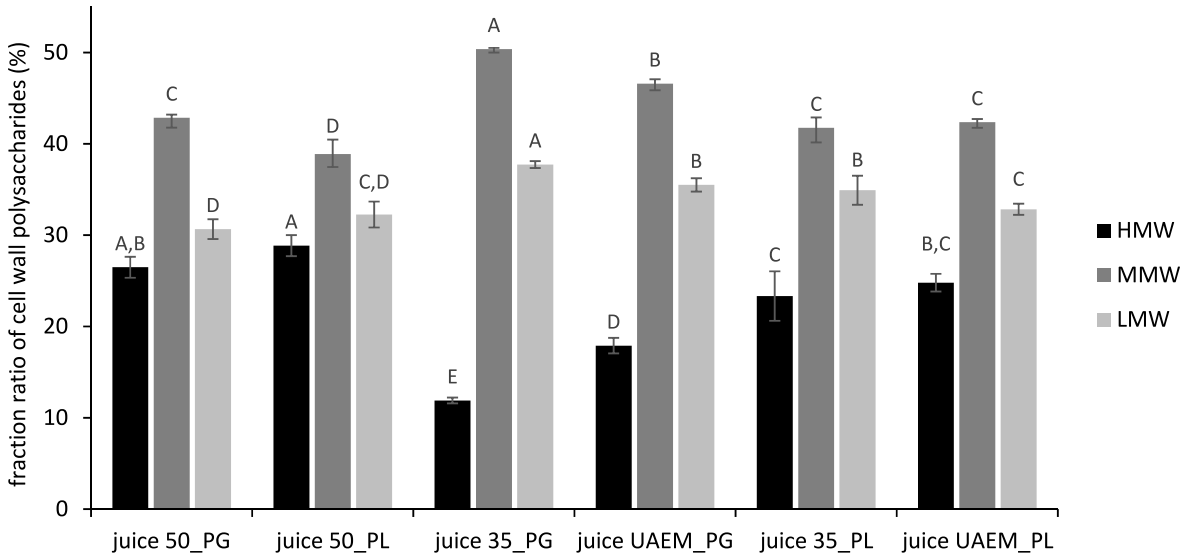

Fig. 1. Fraction ratio (%, HP-SEC) of cell wall polysaccharides of the six chokeberry juices produced at commercial standard procedure at 50 °C, ultrasound-assisted enzymatic maceration (UAEM, 35 °C), and temperature control 35 °C using two different enzyme preparations showing either polygalacturonase (PG) or pectin lyase (PL) activity. Molecular weight (MW) was divided into three fractions: High (>208 kDa, HMW, black), medium (37–208 kDa, MMW, grey), and low (19–37 kDa, LMW, light grey). Different letters indicate significant differences of the same fraction in the different juices (n = 5, p ≤ 0.05).

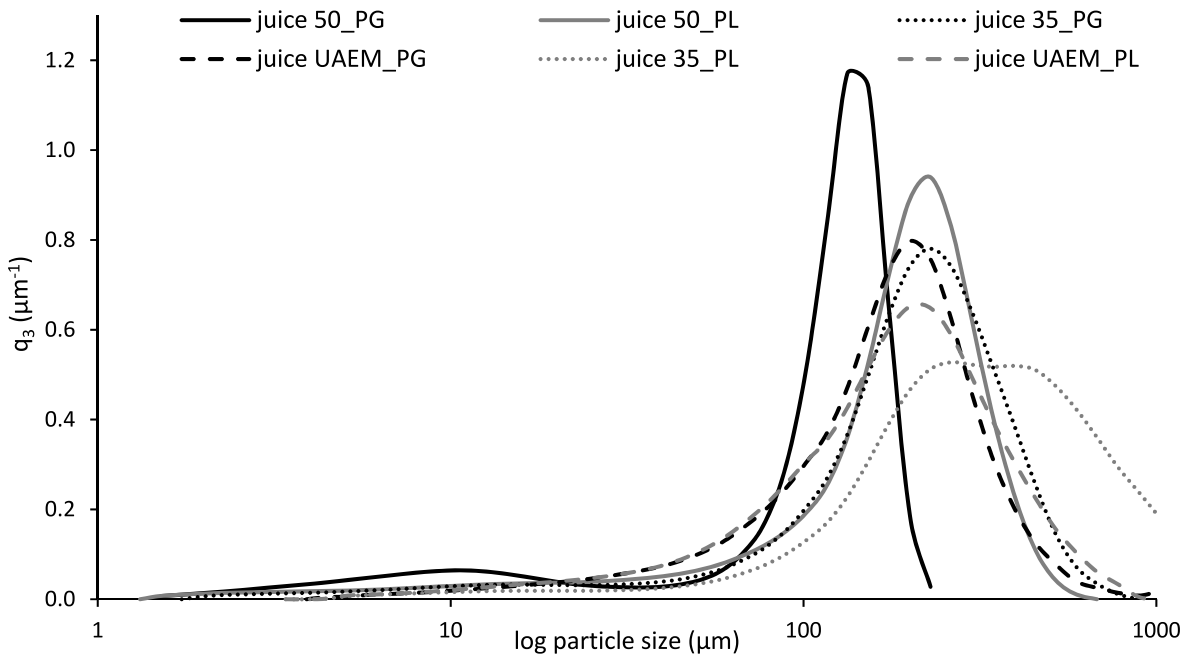

Fig. 2. Particle size distribution (PSD) plotted as density distribution q_3 (μm^{-1}) over logarithmic particle size (µm) of six pasteurized chokeberry juices. Maceration at commercial standard procedure at 50 °C (solid lines), ultrasound-assisted enzymatic maceration (UAEM, dashed lines), and temperature control at 35 °C (dotted lines) using two different enzyme preparations showing either polygalacturonase (PG, black) or pectin lyase (PL, grey) activity (n = 5).

2006). The smallest particle diameter was found in juices after six months of storage using PL during juice production and applying US (Fig. 3, UAEM_PL), the temperature control (Fig. 3, 35_PL) as well as in juice of the conventional standard procedure using PG (Fig. 3, 50_PG). The two PL-treated juices (UAEM_PL and 35_PL) showed higher ratios in HMW fraction. Larger fragments of these HMW fractions might form loose aggregates, which tend to break down more easily over the storage time. In contrast, larger particle diameters were determined in juices after six months of storage produced using PG and applying US (Fig. 3, UAEM_PG), the temperature control (Fig. 3, 35_PG) as well as in juice of the conventional standard procedure produced using PL (Fig. 3, 50_PL). Especially juices of the production using PG contained fragments low in MW, which tend to form stable complexes and might also affect the storage stability of anthocyanins over the storage period. While small oligomers possess positive properties, it should be considered that they might cause increased turbidity and sedimentation (Lachowicz et al., 2018). The formation of aggregates of small fragments led to visible sedimentation layers in stored juice bottles, which had previously been observed by Campoli et al. (2018) especially after US treatment. In contrast, high pressure processing (HPP) applied on chokeberry juice instead of thermal treatment after pressing resulted in juices stable to sedimentation (Yi et al., 2022).

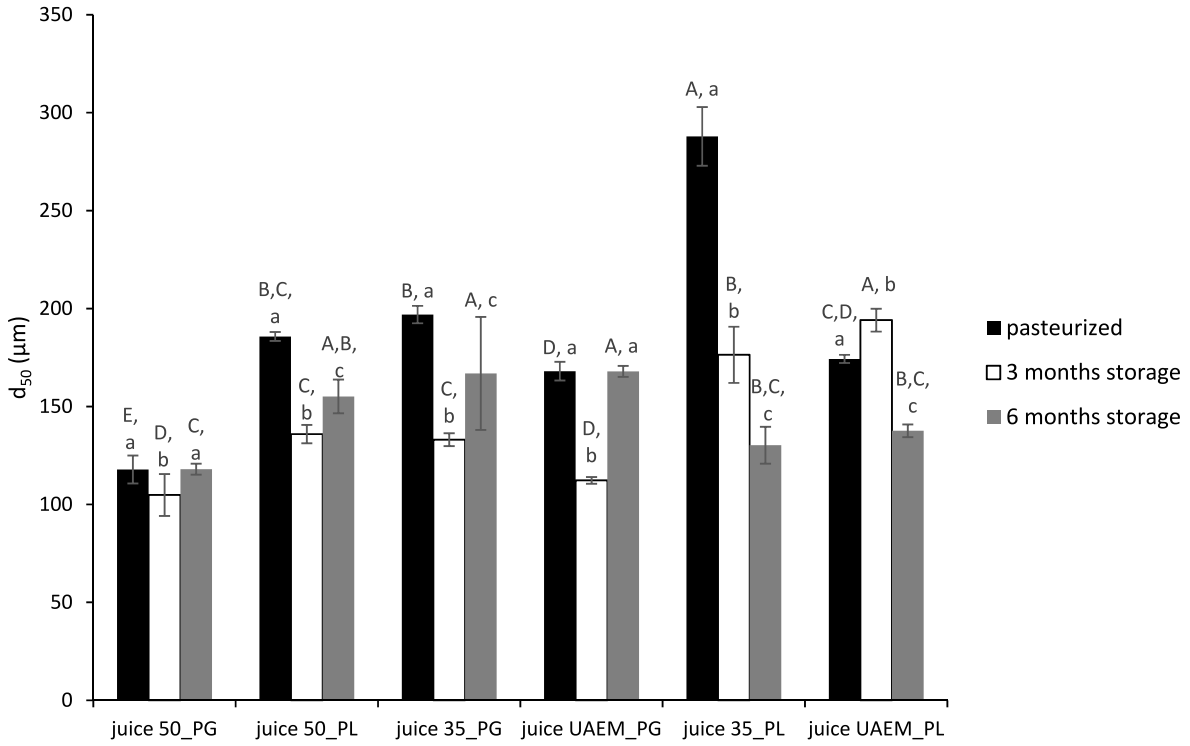

Fig. 3. Particle diameter d_{50} (μm) of the six produced chokeberry juices after pasteurization (black), after three months of storage (white), and after six months of storage (grey). Maceration conditions at commercial standard procedure at 50 °C, ultrasound-assisted enzymatic maceration (UAEM), and temperature control at 35 °C using two different enzyme preparations showing either polygalacturonase (PG) or pectin lyase (PL) activity. Different capital letters indicate significant differences between the different juices at the same stage; different small letters indicate significant differences between the pasteurized juice and storage time ($n = 5$, $p \leq 0.05$).

3.4. Anthocyanin stability

Anthocyanin stability during thermal treatment (pasteurization at 85 °C, 30 s) and storage at 4 °C up to six months (Fig. 4) revealed differences based on the enzyme preparation applied.

Total anthocyanin content was not significantly affected by pasteurization in PG-treated juices, suggesting higher thermostability of the anthocyanins due to complexation by the PG-generated polysaccharide fragments. Anthocyanins in juice produced at 50 °C using PL (conventional standard procedure) had low stability. At lower temperatures, their stability was increased especially by US treatment. The improvement in thermostability of anthocyanins in these juices can be explained by the enhanced cell wall degradation, as evidenced by HP-SEC analysis. According to previous studies, a favorable distribution of

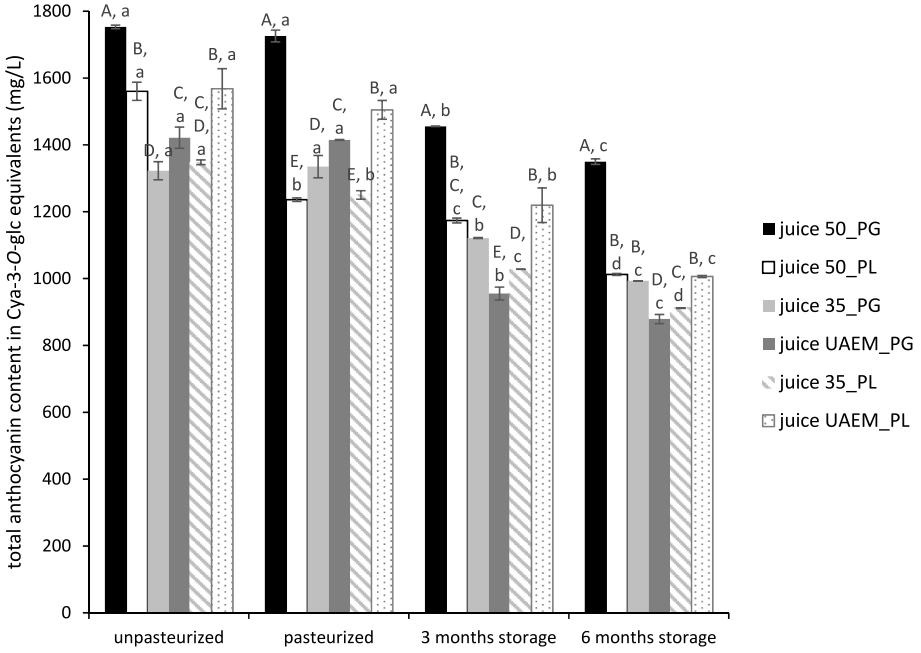

Fig. 4. Total anthocyanin content (Cya-3-O-glc equivalents mg/L) of the six chokeberry juices (unpasteurized, pasteurized, three and six months of storage) of the commercial standard procedure at 50 °C, ultrasound-assisted enzymatic maceration (UAEM) and temperature control 35 °C using two different enzyme preparations showing either polygalacturonase (PG) or pectin lyase (PL) activity. Different capital letters indicate significant differences between the six juices at the same stage; different small letters indicate significant differences between the different steps of one juice ($n = 3$, $p \leq 0.05$).

the resulting oligosaccharides and polysaccharides is characterized by low HMW and high MMW and LMW fractions. This distribution improves the thermostability of anthocyanins. Smaller fragments bear protective effects by enhanced non-covalent interactions, which are attributed to the more linear and more negatively charged fragments (Koh et al., 2020; Liu et al., 2020). Pasteurization only affected anthocyanins in the juices of the conventional standard procedure and temperature control produced using PL (50_PL and 35_PL). Anthocyanins are more sensitive toward heat when no protecting polysaccharides are present. The breakdown of stable anthocyanin-pectin complexes accordingly decreases heat stability (Koh et al., 2020; Liu et al., 2020). These juices showed higher ratios of the HMW fraction, which are less protective during thermal treatment.

Anthocyanins were stable in the later stage of storage in juices produced using PG as well as after UAEM treatment (UAEM_PG). The small fragments in these juices exhibited a protective effect on anthocyanins and thus contributed to their enhanced retention. Previous studies on strawberry juice and puree treated with PG showed highest anthocyanin retention during storage at 4 °C and a stabilizing effect of pectin fragments on anthocyanins (Hartmann et al., 2008; Holzwarth et al., 2012). Wilkes et al. (2014) produced chokeberry juice under conventional conditions (45 °C) and found that pasteurization had a greater effect on anthocyanin content than storage.

Accordingly, the poor stability of anthocyanins observed in some juices may result from the weaker complexes formed by anthocyanins and larger polysaccharide fragments, which were generally more abundant in juices treated with PL compared to juices treated with PG. However, the differences in storage stability between the two enzyme treatments were less pronounced compared to the effects on thermostability of anthocyanins.

4. Conclusion

The results demonstrate that US and gentle conditions showed a positive influence on juice production regarding general juice composition and chemical composition compared to the conventional standard procedure. UAEM enhanced the degradation of cell wall polysaccharides and thus the generation of smaller pectin fragments. Based on findings of other studies, these fragments might form aggregates which possess protective and stabilizing effects by non-covalent interactions with anthocyanins (Larsen et al., 2019). The determination of such interactions in real food matrix is complex and beyond the scope of this study. Thermostability of anthocyanins was improved in PL-treated juices by US treatment (UAEM_PL), which resulted in a stability comparable to that of PG-treated juices. Pasteurization affected anthocyanins only in the juices of the conventional standard procedure and temperature control produced using PL (50_PL and 35_PL). At later stages of storage, anthocyanin stability was improved in juices treated with PG especially after UAEM (UAEM_PG), whereas juices using PL showed higher losses of anthocyanins during storage.

The use of an enzyme preparation which possesses PG activity seems preferable for chokeberry juice production due to the specific pectin composition, which varies greatly between different fruits. Accordingly, the slightly better effects of US on PG cell wall degradation might be different during processing of other fruits. However, implementation of gentle production conditions by UAEM offers additional benefits such as energy saving potential and reduced pomace yield. Finally, optimized production results in juices containing valuable compounds with improved thermal and storage stability.

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CRediT authorship contribution statement

Nicole Jasmin Nemetz: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Anne Ruth Winter:** Investigation, Formal analysis. **Jan-Peter Hensen:** Investigation. **Andreas Schieber:** Resources, Supervision, Writing – review & editing, Funding acquisition. **Fabian Weber:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Nemetz, Nicole and Hensen, Jan-Peter reports financial support was provided by Federal Ministry of Food and Agriculture.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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