Institut für Ernährungs- und Lebensmittelwissenschaften
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# Ultrasound-assisted enzymatic maceration and valorization of by-products

## A contribution to sustainable production of berry juices

**Dissertation**

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“Gib das, was dir wichtig ist, nicht auf, nur weil es nicht einfach ist.” -Albert Einstein

**Abstract**

Red berry juice, rich in secondary plant metabolites such as anthocyanins, represents a good alternative to the consumption of red berries. According to the German Association of Nutrition, a consumption of five portions of fruits and/or vegetables per day is recommended. However, the average intake of the German citizens only amounts to 50 – 60% of daily fruit and/or vegetable intake. Thus, fruit juice producers are interested in reaching for the potential in berry juice consumption as a part of the daily intake and to increase the content of bioactive compounds in the juices. The extraction of phenolic compounds (PCs), facilitated by the enzymatic maceration, is decisive in juice production resulting in a by-product with low PC content. In juice production, more than 20% of the fruit accrue as pomace. However, the great potential of pomace has not been fully exploited, yet. Currently, pomace is used as animal feed, discarded, or only parts of it are utilized for PCs extraction. Optimization of the juice production by ultrasound (US) or valorization of by-products is of relevance in juice industry to produce sustainably and to preserve heat-sensitive PCs.

The present thesis aimed to investigate the effects of US treatment during enzymatic maceration (UAEM) conducted at gentle conditions on chokeberry juice production on pilot-plant scale. Further, a strategy for the valorization of pomace was investigated by producing coloring foodstuffs originating from dried berry pomace.

UAEM resulted in lower yields of pomace making juice production more resource-efficient and further improved cell wall polysaccharide degradation. Thermal and storage stability of anthocyanins were improved by UAEM.

Milling of the seedless fraction of dried berry pomace presents a promising valorization technique. The intact cell structure and a high content of PCs ensured an improved storage stability of anthocyanins in pomace powders. Compared to other coloring foodstuffs, red pomace powders showed acceptable color stability and enables a wide application in food industry.

Hence, the present dissertation provides new insights into the promising potential of an advanced conventional chokeberry juice production by applying US at gentle conditions resulting in juices rich in PCs and enabling the valorization of berry pomace on pilot-plant scale with a transfer towards a resource-efficient production.

**Kurzfassung**

Roter Beerensaft, der reich an sekundären Pflanzenstoffen wie Anthocyanen ist, stellt eine gute Alternative zum Verzehr von Beeren dar. Laut der Deutschen Gesellschaft für Ernährung wird ein Verzehr von fünf Portionen Obst und/oder Gemüse pro Tag empfohlen. Jedoch beträgt der durchschnittliche Verzehr der deutschen Bevölkerung dieser Empfehlung lediglich 50 – 60 % der täglichen Obst- und/oder Gemüsezufuhr. Die Fruchtsaftindustrie sieht daher ein hohes Potenzial darin, den Saftkonsum als Teil der täglichen Zufuhr zu nutzen und den Gehalt an bioaktiven Substanzen in den Säften zu erhöhen. Die Extraktion phenolischer Verbindungen (PCs) stellt einen entscheidenden Punkt bei der Saftherstellung dar, welcher durch die enzymatische Mazeration erleichtert wird und den Gehalt an PCs im Trester verringert. Während der Saftherstellung fallen mehr als 20 % der Beeren als Trester an. Das große Potenzial des Tresters ist jedoch noch nicht ausgeschöpft, denn nur Teile werden in der Industrie weiterverwertet und der Rest zu Futtermittel verarbeitet oder entsorgt. Die Optimierung der Saftherstellung mit Hilfe des Einsatzes von Ultraschall (US) oder der Aufwertung des Tresters kann die Saftproduktion umweltfreundlicher und nachhaltiger gestalten sowie hitzelabile PCs erhalten. Das Ziel der vorliegenden Arbeit war, die Auswirkungen der US-Behandlung während der enzymatischen Mazeration (UAEM) unter schonenden Bedingungen auf die Aroniasaftproduktion im Technikumsmaßstab zu untersuchen. Weiterhin wurde eine mögliche Strategie zur Aufwertung von Beerentrester in Form eines färbenden Lebensmittels untersucht. Die UAEM führte zu einer verminderten Tresterausbeute sowie zu einem begünstigten Zellwandpolysaccharidabbau. Durch die UAEM wurden die Hitze- und Lagerstabilität von Anthocyanen in Säften verbessert. Als vielversprechende Aufwertungsmethode wird das Vermahlen der kernlosen Fraktionen getrockneter Trester zu feinen Pulvern angesehen. Hohe Gehalte an PCs und die intakte Zellstruktur sorgen für eine hohe Lagerstabilität der Anthocyane. Im Vergleich zu anderen färbenden Lebensmitteln zeigte die Verwendung von rotem Tresterpulver eine akzeptable Farbstabilität, was ein breites Anwendungsspektrum in der Lebensmittelindustrie bietet.

Die vorliegende Dissertation liefert neue Erkenntnisse über das vielversprechende Potenzial, die konventionelle Aroniasaftproduktion durch die Anwendung von US unter schonenden Bedingungen voranzutreiben, was zu Säften führt, die reich an PCs sind, sowie der Verwertung von Beerentrester, um den Transfer in Richtung ressourceneffizienter Produktion zu ermöglichen.

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# Preliminary remarks

## List of Figures

## List of Abbreviations

| | |
|---|---|
| AIR | Alcohol insoluble residue |
| Ara | L-Arabinose |
| Frc | Fructose |
| Gal | D-Galactose |
| GalA | Galacturonic acid |
| Glc | D-Glucose |
| HHP | High hydrostatic pressure |
| HG | Homogalacturonan |
| HMW | High molecular weight |
| HP-SEC | High-performance size exclusion chromatography |
| IQF | Individual quick frozen |
| LMW | Low molecular weight |
| Man | Mannose |
| MW | Molecular weight |
| MMW | Medium molecular weight |
| PCs | Phenolic compounds |
| PEF | Pulsed electric fields |
| PG | Polygalacturonase |
| PL | Pectin lyase |
| PME | Pectin methylesterase |
| PP | Pomace powder |
| RG | Rhamnogalacturonan |
| Rha | Rhamnose |
| UAEM | Ultrasound-assisted enzymatic maceration |
| UHPLC | Ultra-high performance liquid chromatography |
| US | Ultrasound |
| XG | Xylogalacturonan |
| Xyl | Xylose |

## List of Publications

Nemetz, N.J., Schieber, A., & Weber, F. (2021). Application of crude pomace powder of chokeberry, bilberry, and elderberry as a coloring foodstuff. *Molecules*, 26, 2689. 10.3390/molecules26092689

Nemetz, N.J., Winter, A.R., Hensen, J.-P., Schieber, A., & Weber, F. (2023). Toward gentle chokeberry juice production by ultrasound-assisted enzymatic maceration. *Current Research in Food Science*, 6, 100518. 10.1016/j.crfs.2023.100518

## Conferences

Nemetz, N.J., Weber, F., & Schieber, A. (2021). Strategies for the valorization of berry pomace in the food industry. 14$^{th}$ Polyphenols Applications 2021 World Congress, Online, September 22-24, 2021, *Abstracts*, 43. [Short Oral Presentation]

Nemetz, N.J., Weber, F., & Schieber, A. (2022). Innovative processes for berry juice production and side stream valorization. 15$^{th}$ Polyphenols Applications 2022 World Congress, Valencia, Spain, September 28-30, 2022, *Abstracts*, 24. [Oral Presentation]

Nemetz, N.J., Haas, P.P., Schieber, A., & Weber, F. (2022). UHPLC-MS-basiertes on-line Monitoring der enzymatischen Oxidation phenolischer Säuren und Anthocyane. 50. Deutscher Lebensmittelchemikertag in Hamburg, Germany, September 19-21, 2022, *Lebensmittelchemie*, *76*, S2 118. [Poster]

## Declaration of contribution as co-authors

The papers presented in the **Chapter 2** and **Chapter 3** were done with the contribution of the following co-authors as described below:

**Prof. Dr. Andreas Schieber** contributed to the publications as the supervisor of this PhD projects and proofread all manuscripts. Further, he assisted in the conceptualization of the funding proposal.

**Prof. Dr. Fabian Weber** assisted in conceptualization, data interpretation, and publication of the results. He advised on experimental work and proofread all manuscripts, gave critical feedback, and was the corresponding author of the publications.

**Anne Winter** conducted the chokeberry juice production and contributed to the subsequent analysis.

**Jan-Peter Hensen** performed the anthocyanin extraction of chokeberries.

# Chapter 1

## General introduction

Red berry juice is an important food in the human diet. Juice consumption is predominantly because of its nutritional value and not because of its organoleptic properties (Havas et al., 1995). The German Association of Nutrition recommends a consumption of five portions of fruits and/or vegetables per day, comparably to the campaign of the National Cancer Institute of the USA (Havas et al., 1995). The consumption of those portions might have a positive effect on human health and further are a relevant feature in human diet (Taylor, 2016). Thus, fruit juice producers are interested in seizing the high potential in berry juice consumption as a part of the daily intake. Red berry juice shows a comparable composition to the raw material and is rich in secondary plant metabolites such as anthocyanins, which are responsible for the intensive color of these berry juices. Red berry juices are gaining in popularity since, additionally to the bright color, anthocyanins bear various health beneficial effects (Quast, 2008; He and Giusti, 2010). To obtain a final product which is rich in those value adding bioactive compounds, the juice production for each fruit needs to be adjusted, individually. The selection of suitable pectinolytic enzymes during berry juice production is crucial for an increase in juice yield and the release of cell wall bound compounds. A disintegration of cell wall matrices is mandatory for the release of these compounds, resulting in an increased extraction yield of juice and plant metabolites (Landbo and Meyer, 2001; Buchert et al., 2005; Tchabo et al., 2015; Weber and Larsen, 2017).

The optimization of the juice production by modifying processing steps leads to an environmentally friendly and sustainable production coming along with energy savings. By applying alternative technologies such as high hydrostatic pressure (HHP), pulsed electric fields (PEF), or ultrasound (US), the structure and the effects on the matrix of the raw material has to be studied and the relationship between the structures must be controlled, resulting in a tailor-made production (Knorr et al., 2011). Besides using HHP and PEF, US facilitates the activation or inactivation of enzymes in berries and the disintegration of cell wall matrices (Weber and Larsen, 2017; Knorr et al., 2011). An optimization of the berry juice production can be achieved by performing ultrasound-assisted enzymatic maceration (UAEM) (**Chapter 3**). The application of US bears several potential benefits. First, US may affect the enzyme conformation, resulting in an increase in enzyme activity. Second, US may facilitate

the generation of polysaccharide fragments with medium molecular weight (MMW), which subsequently can be degraded by enzymes. As previously reported, the mentioned effects of US may be synergistic on polysaccharide degradation (Larsen et al., 2021). Moreover, the use of UAEM during juice production improved the cell wall degradation, enhanced juice yield, and showed a stabilizing effect on juice anthocyanins. Further, by applying UAEM, pomace yield can be reduced (Larsen et al., 2019; Larsen et al., 2021; Tomas et al., 2020; Shirsath et al., 2012; Le Lieu and van Le, 2010).

In addition to the minimization of by-products, their valorization is also of importance (**Chapter 2**). To omit adverse effects of artificial dyes on human health, which have long been common practice, the application of natural food colorants has been of interest (McCann et al., 2007; Müller-Maatsch and Gras, 2016; He and Giusti, 2010; Stich, 2016; Kendrick, 2016; Bridle and Timberlake, 1997).

The main purpose for adding colorants to food is either to restore or enhance the natural color, standardize batches, or color originally non-colored foods like yoghurt (Lakshmi, 2014; Demirkol and Tarakci, 2018; Jovanović et al., 2020). By using natural sources like red berry fruits and vegetables instead of food dyes, consumer demands on healthier and more natural foods with vivid colors can be satisfied (Reißner et al., 2019; Struck et al., 2016; Khanal et al., 2010; May and Guenther, 2020). Berry pomace, which is rich in anthocyanins, is an excellent source of coloring foodstuffs. Berry pomace shows an acceptable color stability compared to other coloring foodstuffs like black carrot concentrate and purple sweet potato anthocyanins. Further, berry pomace has a wide application potential in several food applications (Struck et al., 2016; Reißner et al., 2019).

# 1. Chokeberry, bilberry, and elderberry – composition

## 1.1 General information of red berries

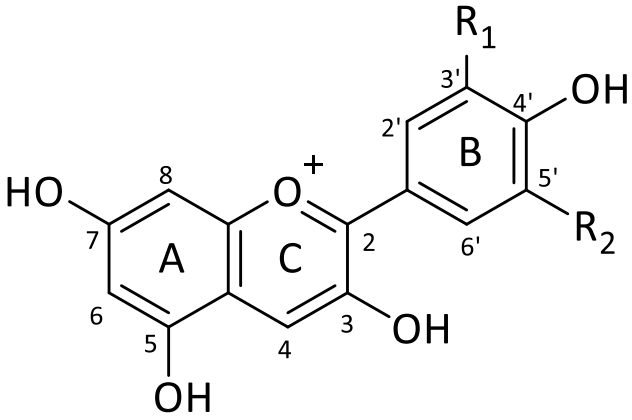
Chokeberries (*Aronia melanocarpa* Michx.) are originated in the eastern parts of North America and Canada. They belong to the Rosaceae family and contain a high amount of anthocyanins (Kulling and Rawel, 2008). Total anthocyanin content of chokeberries can be up to 1500 mg/100 g per fresh fruit (Sójka et al., 2013). The main anthocyanins found in these berries are cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, and cyanidin-3-*O*-xyloside. Further, chokeberries have a high content in fiber, which remains in the pomace after pressing during juice production (Trost et al., 2008; Georgiev and Ludneva, 2009; Sójka et al., 2013; Oszmianski and Wojdylo, 2005; Oszmianski and Sapis, 1988). Total anthocyanin content in the pomace accounts for 1800 mg/100 g per dry weight (Oszmianski and Wojdylo, 2005).

Bilberries (*Vaccinium myrtillus* L.) belong to the Ericaceae family and are often mixed up with the cultivated blueberries. Bilberries are wild berries, growing in low bushed forest areas, especially in Eastern Europe. These berries are known for their red flesh and high anthocyanin content (Kressmann, 2001). The bright red color originates from 15 different anthocyanins, namely delphinidin, cyanidin, petunidin, peonidin, malvidin in combination with three sugars (D-Galactose (Gal), D-Glucose (Glc), L-Arabinose (Ara)) (Kader et al., 1998; Lätti et al., 2008). Total anthocyanin content of bilberries amounts to 600 mg/100 g per fresh weight (Buchert et al., 2005).

European black elder (*Sambucus nigra* L.) is a native herb and belongs to the Adoxaceae family in the order of Dipsacales and grows in subtropical temperate regions (Dulf et al., 2013; Domínguez et al., 2020; Mikulic-Petkovsek et al., 2014). Elderberries show high contents of anthocyanins and other phenolic compounds (PCs) bearing antibacterial, anti-inflammatory, and antioxidant effects (Dulf et al., 2013; Salvador et al., 2015; Domínguez et al., 2020; Mikulic-Petkovsek et al., 2014). Elderberry anthocyanins are mostly cyanidin glucosides namely 3-sambubioside, 3-glucoside, 3-sambubioside-5-glucoside, and 3,5-diglucoside derivatives which are further acetylated (Bridle and Timberlake, 1997; Galic et al., 2009). The total anthocyanin content in elderberries amounts to 200 – 1000 mg/100 g per fresh weight (Bridle and Timberlake, 1997).

## 1.2 Anthocyanins of red berries

Anthocyanins, a subclass of flavonoids, are the most abundant PCs in red berry fruits. The six main anthocyanidins, the aglycones of anthocyanins, are cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin (Figure 1). The skeletal structure consists of a $C_6$-$C_3$-$C_6$ backbone with an aromatic ring (A-ring) connected with a heterocyclic ring (C-ring) which contains an oxygen atom. This heterocyclic ring is linked to another aromatic ring (B-ring) *via* a carbon bridge (Castañeda-Ovando et al., 2009).




| Anthocyanidin | $R_1$ | $R_2$ |
|---|---|---|
| Pelargonidin | H | H |
| Cyanidin | OH | H |
| Delphinidin | OH | OH |
| Peonidin | $OCH_3$ | H |
| Petunidin | $OCH_3$ | OH |
| Malvidin | $OCH_3$ | $OCH_3$ |

**Figure 1. General anthocyanidin structure depicted as flavylium cation and the six main anthocyanidins characterized by the substituents. Modified after Castañeda-Ovando et al. (2009).**

The substituents $R_1$ and $R_2$ at the B-ring allow a differentiation between the six anthocyanidins. A hydroxylation or methoxylation at the B-ring identifies the flavylium cation derivate. Glycosylation with a sugar moiety on $C_3$, $C_5$ or $C_7$ allows the formation of an ester bond with sugars or organic acids (He and Giusti, 2010).

### 1.2.1 Health aspects of anthocyanins

Anthocyanins are located in the vacuole of plant cells with the function to protect the organelle material from ultraviolet radiation. Anthocyanins accumulate during stress situations like drought, cold stress, and/or high salt concentrations (Koes et al., 1994; Takahashi et al., 1991; Harborne and Grayer, 1988). The protective effects of anthocyanins also bear some health benefits such as antioxidant properties, antimicrobial effects, skin protection, and anti-angiogenesis (Rechner and Kroner, 2005; Pojer et al., 2013). The quinoidal base and anthocyanin chalcone are free radical quenchers due to their conjugated double bonds (Bors et al., 1990). Further, glycosylation on the C-ring and methoxylation on the B-ring enhances the antioxidant properties (Wang et al., 1997).

### 1.2.2 Stability of anthocyanins

Low pH values (pH 3–4), which are typical for red berries, favor an enhanced stability of anthocyanins. Besides covalent bonds, the stability can be improved by inter- or intramolecular co-pigmentation, involving other anthocyanins or phenolic acids as well as in the presence of pectin fragments. Interactions of anthocyanins and pectin fragments are based on hydrogen bonds between the dissociated galacturonic acid (GalA) chains of homogalacturonan (HG) and the hydroxy groups of the anthocyanin B-ring (Rein and Heinonen, 2004; Gras et al., 2017; Fernandes et al., 2014; Buchweitz et al., 2013a; Buchweitz et al., 2013b; Belitz et al., 2008). Especially at pH 3–4 the interaction between the negatively charged GalA chains and the positively charged flavylium cation *via* ionic bonds is promoted (Castañeda-Ovando et al., 2009; Fernandes et al., 2014).

## 1.3 Cell wall composition of red berries

The cell wall of fruits and vegetables is mainly composed of 90% polysaccharides and 10% proteins forming a heterogenous mixture including bound PCs (Cosgrove, 2005; Ochoa-Villarreal et al., 2012; McNeil et al., 1984). The cell wall of the three berries contain water soluble polysaccharides composed of GalA and neutral monosaccharides such as Gal, Glc, Ara, Fructose (Frc), Xylose (Xyl), Rhamnose (Rha), and Mannose (Man) and a high content of pectin (Stroev and Martynov, 1980; Kobus et al., 2019; Veloso et al., 2022). Pectin of bilberry is highly acetylated and contains a dimer of Rhamnogalacturonan II (RG II) (Hilz et al., 2005; Hilz et al., 2006b; Hilz et al., 2007).

### 1.3.1 Pectin

Pectin is a multifunctional, soluble, complex polysaccharide, and essential for the fruit structure. It mainly consists of α-1,4-D-GalA chains and is divided in several structural classes *i.a.*: HG, RG I and II, and xylogalacturonan (XG) (Figure 2) (Ochoa-Villarreal et al., 2012; Cosgrove, 2005; Taylor, 2016).

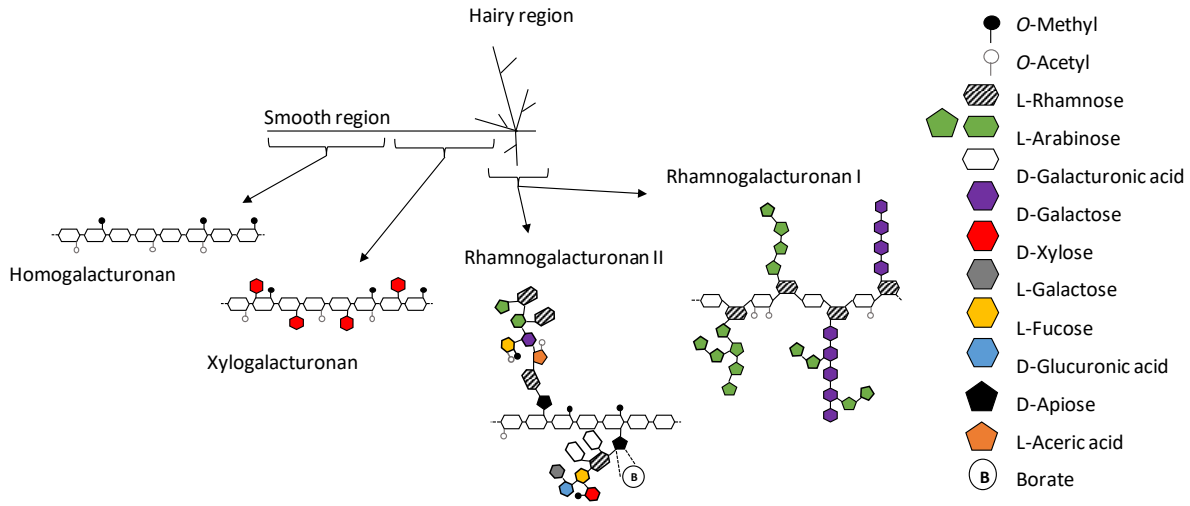



**Figure 2. Schematic structures of pectin and its classes, namely HG (smooth region), RG I and II (hairy region), and XG. Adapted from Ropartz and Ralet (2020) and Harholt et al. (2010).**

HG as the most abundant polymer accounts for 60% of pectin. It consists of a linear α-1,4-D-GalA chain, which can further be methyl-esterified or *O*-acetylated. HG is also called "smooth region". If HG is methylated at a low degree it forms stable gels with calcium ions (egg-box model) (Grant et al., 1973; McNeil et al., 1984; Voragen et al., 2009; Ochoa-Villarreal et al., 2012). RG I contains a disaccharide backbone of repetitive [→2)-α-L-Rha-(1→4)-α-D-GalA-(1→] units. RG II is a complex and branched polysaccharide, that controls the cell wall porosity and thickness (Ochoa-Villarreal et al., 2012; Cosgrove, 2005; Voragen et al., 2009; Lara-Espinoza et al., 2018; Belitz et al., 2008). The ratio of branched Rha units depends on the polysaccharide origin (Gawkowska et al., 2018). RG I is called "hairy region" because of its branched chains (Lara-Espinoza et al., 2018). The structurally complex RG II contains a linear backbone consisting of GalA units substituted with Rha, Gal, Ara, and other sugars (Figure 2). RG II improves gel formation by the crosslink of two pectin molecules. This mechanism might hinder berry processing due to a higher viscosity of the resulting mash. Furthermore, RG II accumulates in the juice after enzymatic degradation (Hilz et al., 2006b; O'Neill et al., 2004). XG consists of a GalA chain substituted with Xyl (Ochoa-Villarreal et al., 2012). The pectin network effects tissue firmness and is responsible for textural changes during food processing and maturation (Hilz et al., 2006a). During maturation, insoluble polysaccharides gradually break down to more soluble fragments. In that way, maturation fully optimizes flavor. To enhance juice yield, enzyme preparations with a pectin degradation activity need to be used. Pectinolytic enzymes are the enzyme preparations of choice (**Section 2.2**) and commonly used during maceration (Taylor, 2016).

Besides pectin, hemicellulose and cellulose are major polysaccharides of the primary cell wall in which cellulose and hemicellulose bring rigidity, whereas pectin provides fluidity (Cosgrove, 2005; Ochoa-Villarreal et al., 2012; McNeil et al., 1984).

### 1.3.2 Hemicellulose

Hemicellulose is tightly bound to the cellulose surface and combined forming a resilient and strong network which is embedded in the pectin network (Hilz et al., 2006a; Cosgrove, 2005). Individual cells are bound by combining pectin and hemicellulose to form the fruit tissue (Taylor, 2016). The low molecular weight (LMW) and heterogeneous polysaccharide consists of a β-1,4-linked backbone (Ochoa-Villarreal et al., 2012).

### 1.3.3 Cellulose

Cellulose consists of an unbranched and insoluble β-1,4-D-Glc chain, which forms a crystalline and mechanically strong microfibril (Ochoa-Villarreal et al., 2012; Cosgrove, 2005). Cellulose chains are connected *via* hydrogen bonds and Van der Waals forces as the main cell wall polymer (Ochoa-Villarreal et al., 2012).

## 1.4 Pomace of red berries

Pomace should be considered as an inhomogeneous material with differently sized particle fractions. Processing of pomace for further use must include the separation of different fractions (seeds, seedless, agglomerates). Pomace is characterized by a substantial content of cellulose (35%), hemicellulose (34%), and pectin (7%) and may be applied as dietary fiber in functional food (Sójka et al., 2013). Most of the cell wall polysaccharides remain in the pomace after juice production (about 76%) (Hilz et al., 2005). Further, the seeds of especially elderberry have a unique phytochemical composition and antioxidant properties (Dulf et al., 2013). The seeds are accumulated as a by-product of the juice production and disposed without further use. Elderberry seed residue can be used for non-conventional oil production because of its fatty acid composition and the position of the fatty acid in the triacylglycerides. In this way, the nutritional potential of these seed oils can be exhausted and an optimized and sustainable production achieved (Dulf et al., 2013). The polyunsaturated fatty acid content (omega 3 and 6) comprises around 40% (Domínguez et al., 2020).

# 2. Berry juice production

## 2.1 Production steps

Juice production includes several steps such as crushing, short thermal treatment, enzymatic maceration, pressing, clarification, filtration, and juice pasteurization (Holtung et al., 2011; Szalóki-Dorkó et al., 2016) (Figure 3).

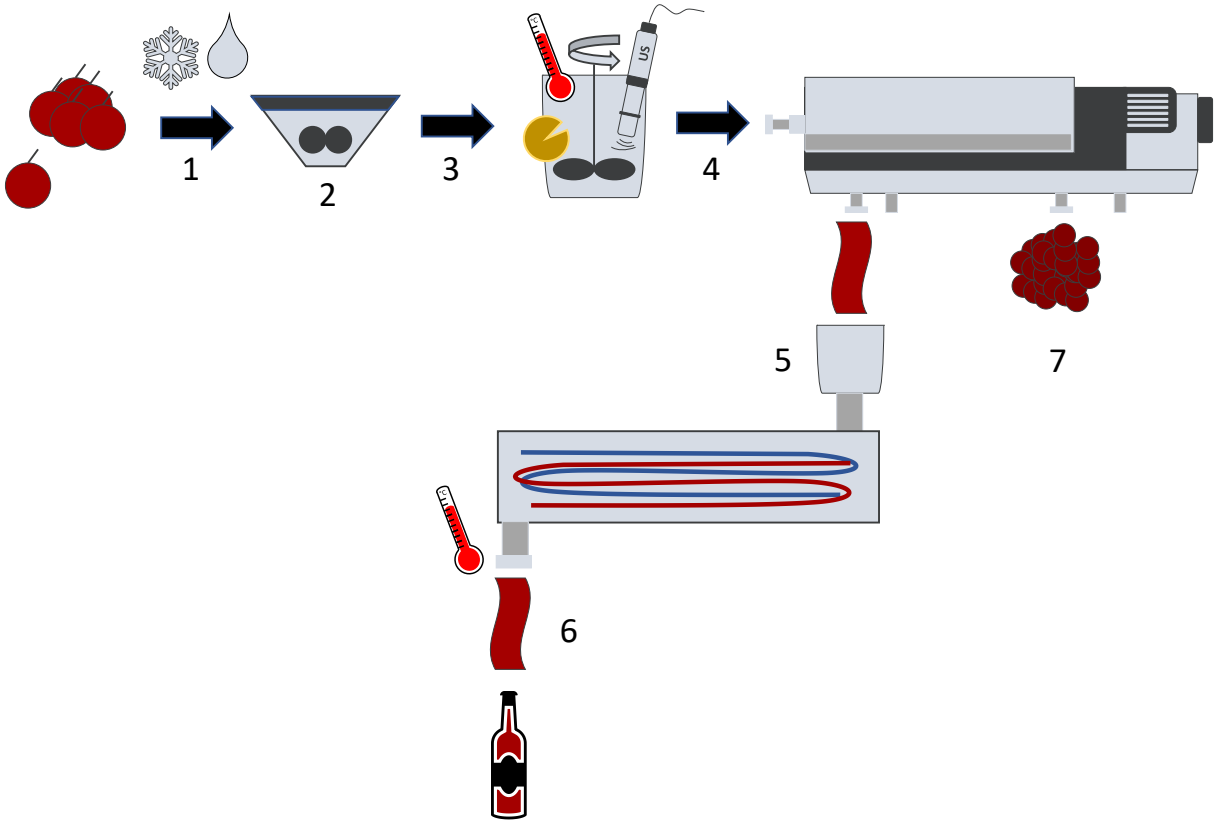



**Figure 3. Berry juice production steps: Thawing of the individual quick frozen (IQF) berries to ambient temperature (1), crushing (2), followed by a heating step at the corresponding maceration temperature. Maceration of berry mash and enzymatic treatment with optional use of ultrasound (US) application (3). Pressing of mash (4) resulting in raw juice (5) and pomace (7). Raw juice is pasteurized and bottled (6). Scheme adapted from Grassin and Coutel (2010), Flottweg SE (2023) and Hielscher Ultrasonics (2013).**

In detail, individually quick frozen (IQF) berries are thawed (Figure 3, Step 1) followed by a crushing (pre-disintegration, Figure 3, Step 2) and heating step at the corresponding maceration temperature. Freezing effects allow a better pre-disintegration of the cell wall material and the release of cell wall bound compounds (Weber and Larsen, 2017). Maceration is conducted at about 40 – 50 °C for 1 – 2 h with an additional enzymatic treatment under stirring (Figure 3, Step 3). This production step is required for the disintegration of cell wall

material followed by the release of the juice. Subsequently, the mash is pressed (Figure 3, Step 4) resulting in pomace (Figure 3, Step 7) and berry juice (Figure 3, Step 5), followed by a pasteurization step (Figure 3, Step 6) of the juice (Kobus et al., 2019; Oszmianski and Lee, 1990; Skrede et al., 2000).

### 2.1.1 Influence of production steps on composition and structure of compounds

Freezing, thawing, and storage of berries and/or juice can influence the composition and structure of compounds (van Buggenhout et al., 2009). The disintegration of cell wall material can additionally be facilitated by using pectinolytic enzyme preparations which improve the release of cell wall bound anthocyanins and the yield, and reduce mash viscosity (**Section 2.2**). The disruption of cell wall polysaccharides increases the permeability and enhances the extractability of PCs from the cell wall (Buchert et al., 2005; Tchabo et al., 2015). The pectinolytic enzymes may affect the anthocyanin stability and increase their total content, which positively influences the quality of juice due to the antioxidative properties of anthocyanins (Buchert et al., 2005; Koponen et al., 2008; Acosta-Estrada et al., 2014; He and Giusti, 2010). Besides the positive effects of these enzymes, anthocyanins might be degraded due to side activities of enzymes, such as β-glucosidase. This causes a deglycosylation of anthocyanins, resulting in labile aglycones associated with a loss in total anthocyanin content (Landbo and Meyer, 2004; Acosta-Estrada et al., 2014). Anthocyanin loss is also caused during pressing of red berry mash and the removal of the anthocyanin rich skin. Further, free anthocyanins might form complexes with cell wall polysaccharides. Changes in total anthocyanin content in the juice results in a retention of anthocyanins in the pomace and may cause changes in the nutritional value (Acosta-Estrada et al., 2014; Howard et al., 2012; Weber and Larsen, 2017).

Pasteurization may influence the anthocyanin concentration and stability in the juice. However, the results obtained so far are ambiguous. The complexity of the interactions between the food matrix, process parameters, and the anthocyanin structure, but also clarification processes to produce clear juices, have a high impact on the total anthocyanin content and their stability. During clarification processes, copigments crucial for anthocyanin stability are removed (Weber and Larsen, 2017). Storage of red berry juices at ambient temperature shows higher losses in total anthocyanin content compared to other compounds like flavonols, proanthocyanidins, and hydroxycinnamic acids (Wilkes et al., 2014). Chilled storage temperature (4 °C) causes less total anthocyanin degradation compared to storage at

ambient temperature (Hellström et al., 2013). The mechanisms of anthocyanin degradation during storage are not fully elucidated. One hypothesis is a nucleophilic attack of water at position $C_2$ of the flavylium cation, resulting in the formation of colorless adducts (Castañeda-Ovando et al., 2009). Similarly, the degradation might be caused by the formation of anthocyanin-tannin polymers (Howard et al., 2012). Moreover, a complexation of anthocyanins with other compounds or a derivatization might hamper the analytical detection (Weber and Larsen, 2017).

Anthocyanin losses need to be minimized either by the optimization of juice production processes, increasing extraction yield and quality of juice (**Chapter 3**), or by their recovery from the pomace, which may also lead to the valorization of by-products in the actual context of circular economy (**Chapter 2**).

### 2.1.2 Effect of cell wall degradation and pectin fragments

The content of soluble solids in the juice depends on the pressing step and pre-treatment of the berries. A decrease in soluble solids can be achieved by using frozen berries and crushing prior to enzymatic maceration (Kobus et al., 2019). Juices with particles larger than 1 mm show a strong tendency to sedimentation and turbidity after a storage time of five months at 5 °C. Further, a positive correlation between turbidity and total PCs has been observed (Lachowicz et al., 2018). An undesired increase in gel formation during juice production can be overcome by split off methanol from HG *via* pectinolytic enzymes because of their sensitivity to temperature and enzymatic treatment (Kobus et al., 2019; Taylor, 2016). The interaction of anthocyanins with pectin fragments generates anthocyanin-pectin complexes. Interactions are mainly hydrogen bonds, hydrophobic interactions, and Van der Waals forces (Pinelo et al., 2006). Galacturonans of pectin interact with the hydroxy groups of anthocyanins. These groups show a higher interaction rate compared to the methoxy groups of the B-ring of anthocyanins (Weber, 2022). Depending on the number of hydroxy groups on anthocyanins, the stabilization effect is more pronounced. These interactions further stabilize the flavylium cation, resulting in more stable anthocyanins (Buchweitz et al., 2013b; Fernandes et al., 2014). Changes in pH and the ionic composition may additionally influence the anthocyanin-pectin binding process. In acidic conditions, like in red berry juices, the flavylium cation is formed, which boosts the binding affinity and enhances interactions between anthocyanins and pectin fragments. The highest binding affinity of anthocyanin-pectin was observed at pH 3 (Koh et al., 2020; Fernandes et al., 2014; Castañeda-Ovando et al., 2009). Another stabilization

mechanism is the stacking effect. Anthocyanins bind to pectin followed by further interactions between several anthocyanins. The higher the binding constant observed, the stronger are the interactions between anthocyanins and the lower methyl-esterified fractions. Anthocyanins bearing more hydroxy groups show greater anthocyanin-pectin binding. The different binding affinities might correlate with the color stability of anthocyanins (Fernandes et al., 2020; Padayachee et al., 2012). Pectin has a higher affinity to build complexes with PCs compared to cellulose and other high molecular weight (HMW) molecules (Liu et al., 2020; Renard et al., 2017). Further, the stability of anthocyanins is enhanced, followed by an improved color stability of anthocyanins containing foods and beverages (Koh et al., 2020; Tomas et al., 2020). Moreover, high amounts of pectin result in a greater depletion of free anthocyanins (Padayachee et al., 2012). It bears protective effects which lead to a greater total anthocyanin content due to the creation of soluble anthocyanin-pectin complexes (Larsen et al., 2019). Colloids which transfer into the juice can be used as "natural" stabilizers and maintain the content of PCs in the juice (Lösche, 2000). The content of anthocyanins in pomace is not negligible due to interactions of PCs with cell wall polymers and the retention of those in the pomace after juice production (Renard et al., 2017; Hilz et al., 2005). Therefore, an optimization of berry juice production might be carried out during the maceration step. One possibility is adjusting the pectinolytic enzyme preparations or temperature. Another approach is improving the enzyme-assisted extraction by the simultaneous application of US (**Chapter 3**) (Larsen et al., 2021; Larsen et al., 2019). UAEM might reduce maceration time, while increasing extraction yield (Tchabo et al., 2015; Le Lieu and van Le, 2010). Moreover, total anthocyanin content can be improved by applying a combination of US and enzymes (Dalagnol et al., 2017a), which results in an improved juice yield and quality (Nguyen et al., 2013; Le Lieu and van Le, 2010) (**Section 3.3**).

## 2.2 Enzymes used during berry juice production

The main function of pectinolytic enzymes is degrading cell wall and plant tissue, improving juice yield and enabling the producer to increase the value of food material and to reduce waste (Tchabo et al., 2015; Aehle, 2008). Disrupting the cell wall matrix increases permeability and further enhances the extraction of phytochemicals such as phenolics, flavonoids, and anthocyanins (Tchabo et al., 2015). Based on the fruit type, different enzyme preparations are required to break down the cell wall matrix. Therefore, biochemical processes are supplemented to the mechanical treatment involving enzyme preparations. Depending on the

enzymes and temperature applied, enzyme activity can be influenced and juice yield, soluble solids content, and chemical composition of the juice adjusted (Ashurst, 2016; Dietrich, 2004). The optimal activity and stability of enzymes depends on the temperature and pH value. An inactivation of enzymes occurs in an acidic environment (low pH values) and depends on the maceration time and temperature (Lösche, 2000). Moreover, enzymatic treatment influences pomace composition (Vagiri and Jensen, 2017). The wide range of processing conditions allows changes in the processes toward new conditions. One trend is applying gentle process conditions to optimize production (Aehle, 2008). Enzyme-assisted extraction enhances the content of PCs in the juice but is more time-consuming than traditional thermal treatments. Enzyme activity can be influenced by ultrasonic waves. The sonication might show positive effects on enzymatic maceration. Therefore, a combination of enzyme-assisted extraction and the application of US is preferred and is discussed in **Section 3** (Tchabo et al., 2015).

The pectinases used in the juice industry are mainly derived from fungal strains of *Aspergillus* sp.. Production, purification, and concentration of enzymes is conducted during the fungal growth. Pectinases can be subdivided by their activity and catalytic reactions in: pectin methylesterase (PME), polygalacturonase (PG), and pectin lyase (PL) (Aehle, 2008; Lösche, 2000) (Figure 4).

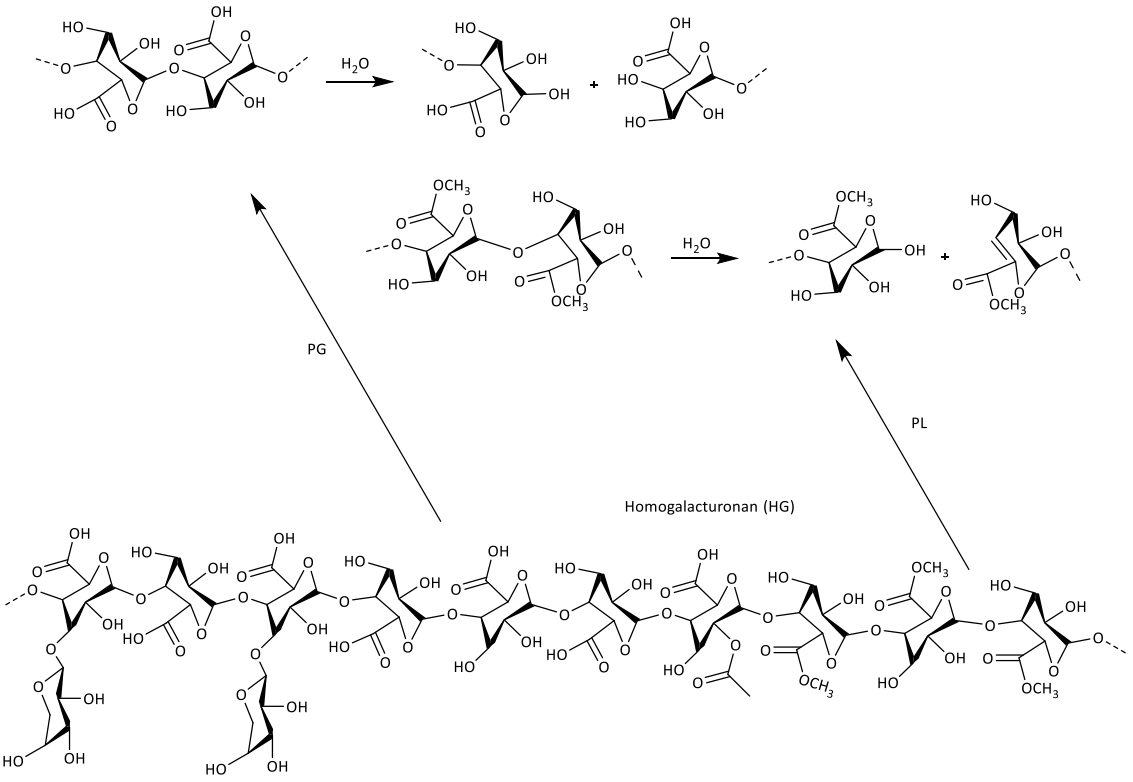



**Figure 4. Cleavage sites on homogalacturonan (HG) of enzymes (polygalacturonase (PG) and pectin lyase (PL)) used during juice production. Adapted from Kanungo and Bag (2019).**

### 2.2.1 Pectin lyase

PL (EC 4.2.2.10) degrades long chains of high esterified pectin and splits methylated α-1,4-HG in oligosaccharides at their non-reducing end, resulting in a β-elimination of HG. It thereby decreases viscosity. PL also regulates fruit softening (Aehle, 2008; Grassin and Coutel, 2010; Bonnin and Pelloux, 2020).

### 2.2.2 Pectin methylesterase

PME (EC 3.1.1.11) cleaves methoxy groups of pectin, resulting in a release of methanol and a reduction in the degree of esterification. The PME action on pectin allows the attack of PG showing the synergistic effect between those two enzymes (Aehle, 2008; Grassin and Coutel, 2010; Bonnin and Pelloux, 2020).

### 2.2.3 Polygalacturonase

PG catalyzes the hydrolysis of α-(1,4) glycosidic bonds of GalA units in non-methylated sections of HG. Two types of PG exist: Endo-PG (EC 3.2.1.15), which randomly cuts within the α-1,4-polygalacturonan backbone of de-esterified pectin and causes a decrease in viscosity. In contrast, exo-PG (EC 3.2.1.67) splits GalA monomers from the non-reducing end. PG only acts on pectin chains with a low degree of methylation. PG is frequently used for juice production due to its high enzyme activity and ability to function at pH values characteristic of red berries (Aehle, 2008; Ma et al., 2015; Cho et al., 2001; Bonnin and Pelloux, 2020).

### 2.2.4 Hemicellulase and cellulase

Hemicellulase is responsible for the hydrolysis of arabinogalactan, galactan, xyloglucan, and xylan. The use of cellulases (EC 3.2.1.4) in juice production is prohibited, but some enzyme preparations showing cellulolytic side activities allow the hydrolysis of β-1,4-glycosidic bonds (Aehle, 2008; Whitaker, 2018; Dalagnol et al., 2017b).

## 2.3 Berry juice by-product and its valorization

Pomace is used for the extraction of polyphenols and anthocyanins which can further be used in technological applications. Apart from that, pomace represents a good raw material to produce pomace powder and to be applied as coloring foodstuff (**Chapter 2**) (Oszmiański and Lachowicz, 2016; Reißner et al., 2019).

### 2.3.1 Valorization of pomace

#### 2.3.1.1 General information

Consumer demands on products and colorants regarding naturalness, encourage the food industry to provide a large variety of coloring foodstuffs and ingredients from natural sources. Besides the most abundant coloring foodstuffs black carrot concentrate (Gras et al., 2016) and purple sweet potato anthocyanins (Gras et al., 2017), the use of berry pomace is emerging (Reißner et al., 2019; Struck et al., 2016; Khanal et al., 2010; May and Guenther, 2020). Berry pomace has less limitations regarding the applicability of anthocyanins compared to isolated or concentrated anthocyanins, which show a lower stability of these towards temperature, light, changes in pH, heat, oxygen, and storage conditions (Kendrick, 2016; Weber et al., 2017; Stintzing and Carle, 2004; Robert and Fredes, 2015). As the by-product of the red berry juice production, berry pomace is rich in anthocyanins, and introduces a possible way to improve color properties of coloring foodstuff (Bridle and Timberlake, 1997; Weber et al., 2017; Robert and Fredes, 2015; Silva et al., 2013; Reißner et al., 2019; Struck et al., 2016; Khanal et al., 2010; May and Guenther, 2020). Between 20 – 30% of fresh material used in berry juice production remain as pomace. It consists of flesh, skin, and seeds and is a rich source of bioactive compounds and dietary fiber (Struck et al., 2016; Rohm et al., 2015). Due to its high water activity, berry pomace is a perishable food. The moisture content of the pomace depends on the composition of the raw material, the enzymatic treatment, and the conditions used during juice production. Therefore, immediate processing of the by-product is crucial to ensure a stable and safe product. One possible way to valorize the pomace is drying, followed by separating the seeds and seedless fraction or milling the pomace into a fine powder with a water activity less than 5% (Skrede et al., 2000; Struck et al., 2016; May and Guenther, 2020; Sójka et al., 2013; Rohm et al., 2015; Schieber, 2017; Seabra et al., 2010). Further, pomace can either be used as a powder to enrich food with dietary fiber and bioactive compounds, or for the release of PCs *via* e.g. liquid or ultrasound-assisted extraction (Struck et al., 2016). Commonly, dried or freeze-dried samples are used and extracted using an aqueous mixture of

methanol, ethanol or acetone (Kapasakalidis et al., 2006; Landbo and Meyer, 2001). Due to the increase in juice production, a high amount of pomace production is expected in the future. Therefore, processing options need to be considered for the use of pomace as a valuable source of anthocyanins or fiber (Khanal et al., 2010).

#### 2.3.1.2 Process conditions

During the drying step of the pomace, time and temperature must be adjusted to ensure the stability of anthocyanins (Skrede et al., 2000; Struck et al., 2016; Rohm et al., 2015). Processing conditions of pomace influence the composition and degradation of PCs. Therefore, processing methods have to be adjusted to achieve the best outcome (Struck et al., 2016; Horszwald et al., 2013). For the application of pomace powder in food, techno-functional properties, microbial stability, anthocyanin content, color, porosity, and particle size need to be determined (Reißner et al., 2019; Struck et al., 2016).

#### 2.3.1.3 Application

Berry pomace is frequently composted, recycled as animal feed, or used for biogas production. After analyzing the value of berry pomace, the recovery of PCs can be optimized and the incorporation as powder into baked or cereal-based products, yoghurt, or beef can be facilitated. Berry pomace is used in pastries to replace flour, sugar, or fat. Moreover, pomace is applied to increase fiber content, add color, or to enhance the nutritional and sensory properties of food (Schieber, 2017; Reißner et al., 2019; Struck et al., 2016; May and Guenther, 2020; Quiles et al., 2018a; Rohm et al., 2015; Seabra et al., 2010; Sójka et al., 2013; Perez-Gregorio and Simal-Gandara, 2017; Reißner et al., 2020; Demirkol and Tarakci, 2018; Wang et al., 2019; Quiles et al., 2018b; Garzón et al., 2021; Irigoytia et al., 2022). Besides the great consumer acceptance towards products containing berry pomace, the whole production can be converted to a more sustainable food chain (Rohm et al., 2015; Demirkol and Tarakci, 2018; Wang et al., 2019; Irigoytia et al., 2022; Garzón et al., 2021; Quiles et al., 2018b). The valorization of berry pomace as a powder, the effects of drying conditions, milling, techno-functional properties, and its application in yoghurt are evaluated and discussed in **Chapter 2**.

# 3. Ultrasound

## 3.1 General information

Ultrasound is based on the principle of cavitation. Pressure of a sonic wave which goes through a medium is not constant, resulting in the formation of cavitation bubbles. Sonication waves are transmitted through the medium, causing stretching and compression of the molecular structures by the generation of zones with compression and rarefaction. Depending on the frequency and amplitude, many different physical, chemical, and biochemical reactions take place (Mason and Lorimer, 1989; Bermudez-Aguirre, 2017).

In stretching cycles, bubbles first grow (Figure 5, Step 1) and turn apart to form microbubbles which is followed by the collapse of these bubbles and the generation of local temperature hotspots. The collapse results in an implosion of bubbles due to an intense acoustic field. The force of the implosion is strong, causing local pressure and temperature hotspots reach about 5000 K and 2000 bar, respectively. Cavitation bubbles further split into smaller bubbles, resulting in a transient cavitation (Figure 5, Step 2). If a transient cavitation is caused at the surface, microjets can be formed (Figure 5, Step 3). Bubble size depends on the applied frequency and bubble count is subjected to the amplitude and power input. Due to increased temperature and mass transfer caused by the implosions, enormous shear forces are created and chemical reactions can be initiated, which may catalyze the formation of reactive species. Therefore, US can be used to cut food material, activate or inactivate enzymes, or mechanically degrade cell wall polysaccharides (Mason and Lorimer, 1989; Bermudez-Aguirre, 2017). The energy amount of the generated field sound is characterized by the intensity ($W/m^2$), sound power (W), or sound energy ($Ws/m^3$) (Knorr et al., 2004). The collapse of cavitation bubbles leads to cell wall disruption (Figure 5, Step 4), which enhances the penetration of solvents into the cell through US jets.

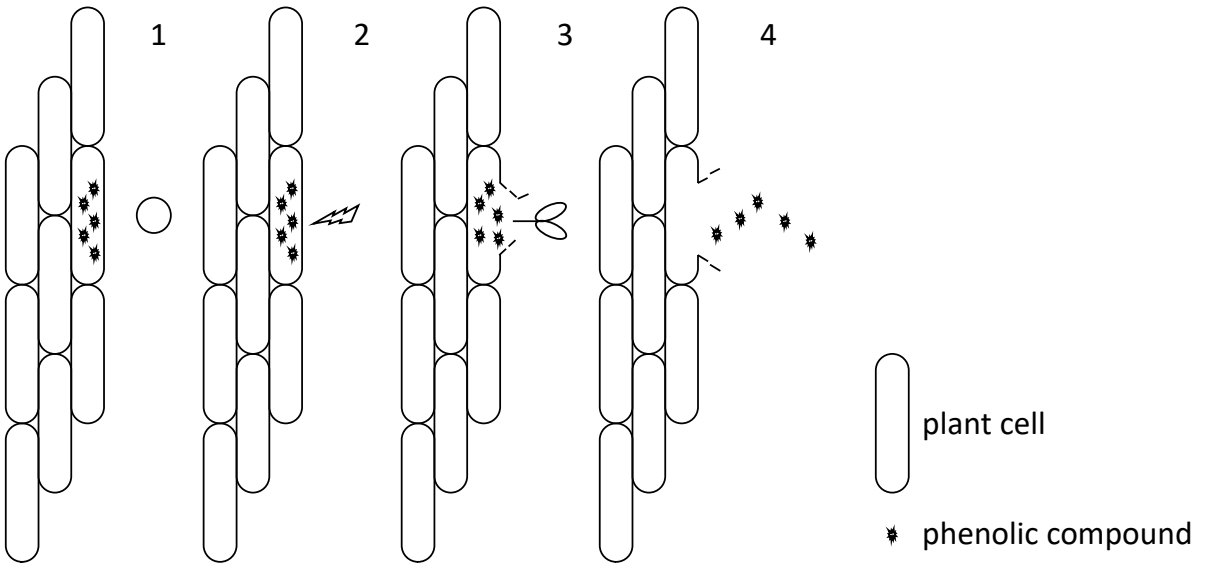



**Figure 5. Cell wall disruption caused by US. Bubble formation (1), grow (2), and implosion (3) resulting in a release of cell wall compounds (4). Adapted from Chemat et al. (2011).**

This tissue effect is caused by shear disruption and local hot spots. US directly affects vegetal material, showing an increase in swelling index after US treatment. After cell wall degradation, the increase in the surface area is followed by an enhanced mass transfer rate of targeted compounds into the medium. Further, a depolymerization effect is determined resulting in lower viscosity (Toma et al., 2001; Jambrak et al., 2007; Grönroos et al., 2004; Chemat et al., 2011).

## 3.2 Application of ultrasound in food industry

US can be used for the disintegration of plant material and release of valuable compounds (Etzbach et al., 2019). US is applied at low frequencies (20 – 100 kHz) and has been used in the food industry for several years (Soria et al., 2017; Chemat et al., 2011; Lapornik et al., 2005). It shows positive effects on the extraction, filtration, and preservation of food (Lapornik et al., 2005). US focuses on three aspects: The stimulation of enzymatic reactions, preservation, and alteration of the food matrix, e.g., the extraction by improving heat and mass transfer (Knorr et al., 2011). An enhanced extraction may be achieved by optimizing the process applying US. Cavitation with local hot spots occur during very short periods and may cause plant cell wall disruption. These effects enhance the release of intracellular substances into the extraction medium, and increase mass transfer (Mason et al., 1996; Knorr et al., 2004). US causes the loss of cellular adhesion and the production of large cell interspaces (Fernandes et al., 2009). Moreover, combining enzymes with US may increase enzyme activities *via* synergistic effects

and extraction yields. Especially by using the pulsed version, low intensity US can improve the temperature stability of PG without affecting its optimum temperature. The cavitation bubbles generated by US provoke shear forces, resulting in a change in enzyme configuration. The configuration change might expose more active sites of the enzyme enhancing substrate availability (Ma et al., 2015; Ma et al., 2016; Mason et al., 1996; Islam et al., 2014; Larsen et al., 2021). Applying US is conducted at lower process temperatures compared to conventional processes in food industry and is an environmentally friendly option for juice production. Moreover, it enhances the quality of juices and offers advantages in terms of yield and stability. Sonication is generally performed by using simple immersion probes at 20 kHz (Chemat et al., 2011; Abid et al., 2014; Mason et al., 2005). The described green technology can be used under gentle physical conditions (Soria et al., 2017).

## 3.3 US in juice production

The application of US is an innovative processing technology, improving the extraction and stabilization of PCs during juice production (Zou and Jiang, 2016). US application depends on various parameters such as the temperature, US power, amplitude, frequency, and the raw material (Bermudez-Aguirre, 2017). Using US during juice production enhances plant cell wall degradation and improves the release of cell wall bound compounds. Because of cavitation and the occurrence of local hot spots, process temperature can be reduced (Mason et al., 1996; Bermudez-Aguirre, 2017; Le Lieu and van Le, 2010). These changes have an influence on the content and profile of juice compounds. Moreover, US may positively affect the nutritional value of a product but is ineffective for complete peroxidase inactivation (Etzbach et al., 2019).

### 3.3.1 Ultrasound-assisted enzymatic maceration (UAEM)

UAEM can improve polysaccharide degradation, resulting in LMW polysaccharides compared to the conventional process without using US (Larsen et al., 2021). The molecular weight (MW) distribution alters, which is influenced by US treatment time and, especially, by applying low frequencies. The degradation of carbohydrates is predominantly caused by mechanical cavitation effects rather than by radical formation (Soria et al., 2017). During UAEM, pulsed low intensity US (about 22 kHz, amplitude of 2 – 20%) and a low maceration temperature are applied (35 °C), which positively affects the activity, optimum temperature, stability, and shelf-life of enzymes (Soria et al., 2017; Ma et al., 2015; Dalagnol et al., 2017b; Larsen et al.,

2021). Using US at low pH values improves the enzyme activity compared to mechanical stirring alone. Further, US affects enzyme conformation, resulting in a higher activity and catalytic efficiency of the enzyme at lower temperatures (Dalagnol et al., 2017b). By applying US, an increased enzyme activity, which is called sonoenzymolysis, was determined by UAEM in a pectin model solution. The effect of UAEM at 30 °C was comparable to the control sample at 50 °C showing an improvement in pectin degradation (Larsen et al., 2021). UAEM might change the polysaccharide composition by generating smaller fragments which interact with anthocyanins and cause stabilizing effects. The reduction of MW does not affect the generation of monomers or the monosaccharide composition of the juice (Larsen et al., 2021). Pectin fragments, which are released especially after UAEM treatment, tend to interact with anthocyanins. After US treatment, linear polymers form insoluble complexes with red pigments, whereas after enzymatic maceration, oligosaccharides are formed, resulting in soluble complexes with protecting properties to anthocyanins (Larsen et al., 2019). Pectin degradation is enhanced by UAEM (Zhang et al., 2013; Ma et al., 2016). An enhanced pectin degradation improves the extractability of anthocyanins and forms more soluble anthocyanin-pectin complexes, increasing the total anthocyanin content of the juice. Further, a decrease in the degree of methylation and acetylation of pectin has a positive effect on anthocyanin complexation (Kohn and Kovác, 1978; Larsen et al., 2019; Larsen et al., 2021). Furthermore, quality parameters and extraction yield were enhanced conducting UAEM. The juice showed an increase in total anthocyanin content and no differences in pH value and titratable acids compared to the control (Dalagnol et al., 2017a; Carrera et al., 2012; González-Centeno et al., 2015). Overall, applying US may improve juice production due to the reduced temperature, energy-saving potential, and protection of heat-sensitive compounds (Larsen et al., 2021). UAEM is a promising technology to improve the extractability and stability of anthocyanins and may facilitate cell wall polysaccharide degradation under gentle process conditions. This effect is evaluated and discussed in **Chapter 3**.

# 4. Aims of the thesis

The quality of red berry juice is connected with the composition and content of dietary fiber and secondary plant metabolites such as PCs and more specifically anthocyanins because of the nutritional value. Berry juice is an important part of the human diet. Consumers prefer drinking juice rather than eating the whole fruits.

Production processes may lead to a degradation of PCs which are present in fresh fruits. Therefore, an optimization of the production is crucial to fruit juice industry, aiming to produce sustainably with less energy consumption, protection of heat-sensitive compounds, and a resource-saving production including the valorization of by-products. Optimization of industry processes may increase juice yield and total anthocyanin content, and further preserve bioactive compounds. Total anthocyanin content depends especially on the degree of cell wall degradation during maceration. Besides the preservation of anthocyanins, the valorization of berry pomace by alternative uses has to be developed to reduce food waste and to establish new protocols in industry.

The present thesis addresses some key aspects of the actual berry juice production like the potential application of US on cell wall degradation and the effects of different process conditions on total anthocyanin content (**Chapter 3**) and the valorization of by-products accumulating in this industry (**Chapter 2**). In detail, the project aims to investigate the effects of gentle maceration treatment using US on the pectin degradation during enzymatic maceration for chokeberry juice production, anthocyanin extraction and storage stability (**Chapter 3**). The valorization of the by-products of red berry juice production was investigated by applying crude pomace powder as a coloring foodstuff (**Chapter 2**).

Among alternative technologies, US was applied on juices not only because of its preservative effect. US influences enzyme activity (activation or inactivation), as well as the cell wall degradation. These effects enable an increased extraction of PCs during juice production. The extraction of PCs facilitated by pectinolytic enzymes is important. Providing the food industry with optimized production protocols is crucial to enhance the nutritional quality of the juice and to guide juice industry towards an energy-saving production. Particularly, consumer awareness has grown in recent years towards natural ingredients, non-artificial colors, and a resource-efficient production of food. The present study evaluates the influence of UAEM on the juice production under gentle process conditions, more precisely on the cell wall degradation and total anthocyanin content (**Chapter 3**).

However, a high content of PCs remains in the pomace, which has so far been used only for animal feed, biogas production, discarded, or partially extracted. For this purpose, valorizing berry pomace can be implemented towards a more sustainable production. The use of less artificial colorants, which are rejected by consumers, is driving the food industry to use coloring foodstuffs. Coloring foodstuffs mainly originate from vegetable sources such as black carrot and purple sweet potato. A new trend is the use of berry pomace in a dried form as powder to color yoghurt or pastry. The composition of the pomace, drying processes, and milling as well as powder characteristic are the main aspects to focus on. The present study analyzed the effects of drying and storage on the total anthocyanin content and focused on the application of crude pomace powder in yoghurt (**Chapter 2**).

The specific aims of this thesis are:

- To valorize berry pomace from chokeberry, bilberry, and elderberry by producing pomace powder (**Chapter 2**)
- To use pomace powder as coloring foodstuff and apply pomace powder as an ingredient in yoghurt (**Chapter 2**)
- To evaluate the techno-functional properties of pomace powders for potential food applications (**Chapter 2**)
- To study the stability of anthocyanins in pomace powder at elevated storage conditions (**Chapter 2**)
- To optimize the enzymatic maceration during chokeberry juice production by ultrasound assistance under gentle process conditions (**Chapter 3**)
- To study the effect of ultrasound on cell wall polysaccharide degradation (**Chapter 3**)
- To evaluate the thermal and storage stabilities of anthocyanins in juices treated with polygalacturonase and pectin lyase after ultrasound-assisted enzymatic maceration (**Chapter 3**)

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# Chapter 2

## Application of Crude Pomace Powder of Chokeberry, Bilberry, and Elderberry as a Coloring Foodstuff

**Abstract**

Berry pomace, rich in polyphenols, especially anthocyanins, accumulates during the production of red juices. Pomace from chokeberry (*Aronia melanocarpa* Michx.), bilberry (*Vaccinium myrtillus* L.), and elderberry (*Sambucus nigra* L.) represent good sources of coloring foodstuffs. Pomace powders (PP) were prepared by milling the seedless fractions of the three dried berry pomaces (50 °C, 8 h). Techno-functional properties of the powders such as particle size distribution, bulk density, sedimentation velocity, and swelling capacity were determined to evaluate the powders for possible food applications. Total anthocyanin content was quantified by UHPLC-DAD before and during a storage experiment to monitor the degradation of anthocyanins in the PP and in a yoghurt model application. The high content of phenolic compounds and the still intact cell structure ensured a high stability of anthocyanins over 28 days of storage. In the model application, color saturation was stable over the whole storage time of 14 days. Regarding the techno-functional properties, only few differences between the three PP were observed. Particle size of elderberry PP was larger, resulting in lowest bulk density (0.45 g/mL), high cold-water solubility (16.42%), and a swelling capacity of 10.16 mL/g dw. Sedimentation velocity of the three PP was fast (0.02 mL/min), due to cluster formation of the particles caused by electrostatic and hydrophobic properties. Compared to other high intensity coloring foodstuffs, the use of PP, showing acceptable color stability with potential health-promoting effects, represents a wide applicability in different food applications especially in products with a longer shelf-life.

**Keywords:** berry pomace; powder; anthocyanins; recovery; sustainability; coloring foodstuff; yoghurt

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## 1 Introduction

In order to preserve the natural color of food, application of artificial dyes has long been common practice. In recent years, the use of these dyes has faced growing concerns due to several adverse effects on human health [1–4]. Therefore, application of natural food colorants like anthocyanins or carotenoids has been favored. These natural food dyes are obtained from sources like fruits and vegetables by physical processing, generally including separation, pasteurization, and concentration [2; 4–6]. The intention of adding colorants to food is either to enhance or and restore natural color, standardizing differing batches, or to color originally non-colored food [7]. As a consequence of the increasing consumer awareness regarding healthier and more natural food and life style, the food industry seeks to avoid application of food additives mandating the use of E-numbers [4–6; 8; 9]. The labeling of coloring foodstuffs as additives is not compulsory if no selective enrichment of the pigment had taken place [5], rendering coloring foodstuffs very attractive ingredients. Concentrates from black carrot [10] and purple sweet potato [11] are some of the most abundant coloring foodstuffs. The applicability of anthocyanins, not only in isolated form but also in such concentrates, is limited due to their low stability toward light, temperature, oxygen, pH changes, heat, and other storage conditions [5; 8; 12; 13]. Because of the high instability, the application as microencapsulated powders turned out to be a possible way to improve color properties and also to maintain the potential health benefits of anthocyanins [6; 8; 13; 14].

To fulfill consumer demands regarding naturalness and vivid, diverse colors of food, the industry requires a high variety of coloring foodstuffs from many different sources. One of the upcoming trend is the use of berry pomaces because they are rich in anthocyanins and further bioactive compounds showing potential health-promoting effects [15–18].

Red berry fruits represent a good source not only for obtaining anthocyanin-rich spray-dried powders. During fruit juice processing, the pomace accumulates as a by-product. It contains high amounts of valuable components [15–17; 19; 20] such as phenolic compounds which are located in the skin and seeds [21; 22]. Several berries contain very high amounts of anthocyanins but a great share of these are not extracted during juice production [23]. The remaining pomace consequently can be used as a good source for these pigments [21; 24]. The transformation of by-products into valuable products and food ingredients should be conducted rapidly to ensure a safe and stable product [9; 18; 25]. The first step of the valorization process is drying of the pomace. This is followed by separation into the seeds and

a seedless fraction for further processing into valuable seed oils and powders rich in phenolic compounds [18; 26]. Subsequent processing steps of the seedless fraction commonly include solvent extraction, resulting in extracts that may be used as bread additives, in beef, or cereal-based products [16; 18; 24–27]. The residual fiber of pomaces is applied in bakery products to reduce the amount of flour and to increase the dietary fiber content [19]. The incorporation of berry pomace or parts of it contributes to the enhancement of nutritional and sensory properties of food [9; 15; 28].

The present study demonstrates the production of pomace powder from chokeberry (*Aronia melanocarpa* Michx.), elderberry (*Sambucus nigra* L.) and bilberry (*Vaccinium myrtillus* L.) for their application as a coloring foodstuff. Anthocyanin degradation and changes in color were determined during storage and in a model application. Moreover, techno-functional properties of the powders were investigated. The determination of these characteristics of pomace powder will facilitate its food application.

## 2 Results and Discussion

### *2.1 Techno-functional properties of pomace powder (PP)*

Drying of the three different berry pomaces at 50 °C (0% rH, 8 h) followed by milling of the seedless fraction resulted in fine pomace powders (PP) of chokeberry, bilberry and elderberry. Techno-functional characterization of these PP are presented and discussed in the following. Dry matter of the berry pomace and the mass balance during the valorization process of the pomace to PP is shown in Table S1 (supplement material).

#### 2.1.1. Moisture content and microbiological status of the pomace powders

The residual moisture content of the three PP was less than 5.30% (Table 1). Significant differences were determined between elderberry PP and the other two PP. Such low residual moisture content was not determined for apple, carrot, and beetroot pomace powders dried at 50 °C to 65 °C for 6 – 7 h [29] or red grape pomace powder dried at 50 °C. Those powders resulted in a residual moisture content between 5 and 10% [30].

**Table 1.** Moisture content (%) and microbiological status (PC total bacterial count, YGC yeast and mold count in colony forming units (CFU)) of pomace powders. Different letters indicate significant differences ($p \leq 0.05$) within each row. Values are mean ± standard deviation (n = 3).

| | | **chokeberry** | **bilberry** | **elderberry** |
|---|---|---|---|---|
| dry matter content (%) | | 94.70 ± 0.14$^{B}$ | 94.76 ± 0.15$^{B}$ | 96.23 ± 0.36$^{A}$ |
| microbiological status (CFU/g) | PC | < 1 · 103 | < 1 · 103 | 4.6 · 104 |
| | YGC | < 1 · 103 | < 1 · 103 | < 1 · 103 |

Microbiological status was determined by total bacterial count on PC and YGC culture medium. Results showed overall low colony forming units (< $1 \cdot 10^3$ CFU/g) except for elderberry PP. Elderberry PP showed higher colony forming unit for total bacterial count (Table 1) but did not exceed the standard value of $1 \cdot 10^7$ CFU/g considering microbiological safety [31]. The values were even lower compared to results from Reißner et al. [15] were the lowest values were $1.6 \cdot 10^3$ CFU/g for berry pomace powders produced from the whole pomace and dried at 60 °C for 24 h.

2.1.2. Particle size distribution and morphology

Figure 1 shows the particle size distribution and light microscope pictures of PP obtained from drying and milling of chokeberry, bilberry and elderberry pomace, respectively. Milling of the seedless fraction of dried pomace resulted in fine powders with a mean particle size $d_{50}$ of 10.36 µm (chokeberry), 11.53 µm (bilberry) and 13.28 µm (elderberry) (Table 2). Similar results for $d_{50}$ of 14.55 µm were obtained for red grape pomace powder dried at 50 °C [30]. Larger particles (86 – 112 µm) were obtained by Reißner et al. who prepared powders by milling the whole pomace after drying at 60 °C for 24 h in an ultra-centrifugal mill [15]. Powders obtained from milling in a domestic grinder followed by sieving through a sieve of 250 µm particle size of dried apple, carrot, and beetroot pomace resulted in powders with particle sizes below 150 µm [29]. The milling conditions have an impact on the size of the particles resulting in the differences between the compared studies.

**Table 2.** Particle size distribution of pomace powder with the characteristic particle size $d_{10}$, $d_{50}$ and $d_{90}$. Different letters indicate significant differences ($p \leq 0.05$) within each column. Values are mean ± standard deviation (n = 6).

| | **$d_{10}$ (µm)** | **$d_{50}$ (µm)** | **$d_{90}$ (µm)** |
|---|---|---|---|
| Chokeberry | $5.21 \pm 0.04^C$ | $10.36 \pm 0.06^C$ | $21.93 \pm 0.74^B$ |
| Bilberry | $6.50 \pm 0.46^B$ | $11.53 \pm 0.83^B$ | $20.93 \pm 2.67^B$ |
| Elderberry | $7.57 \pm 0.41^A$ | $13.28 \pm 0.66^A$ | $24.69 \pm 1.54^A$ |

Despite similar milling conditions, the $d_{50}$ differed significantly which may be explained by the different fruit structure of the seedless fraction of the dried pomace. The constitution of this fraction of berry pomace caused the heterogeneous particle size distribution of the three PP. With regard to the 90% percentile $d_{90}$, differences between elderberry and the other two were more prominent.

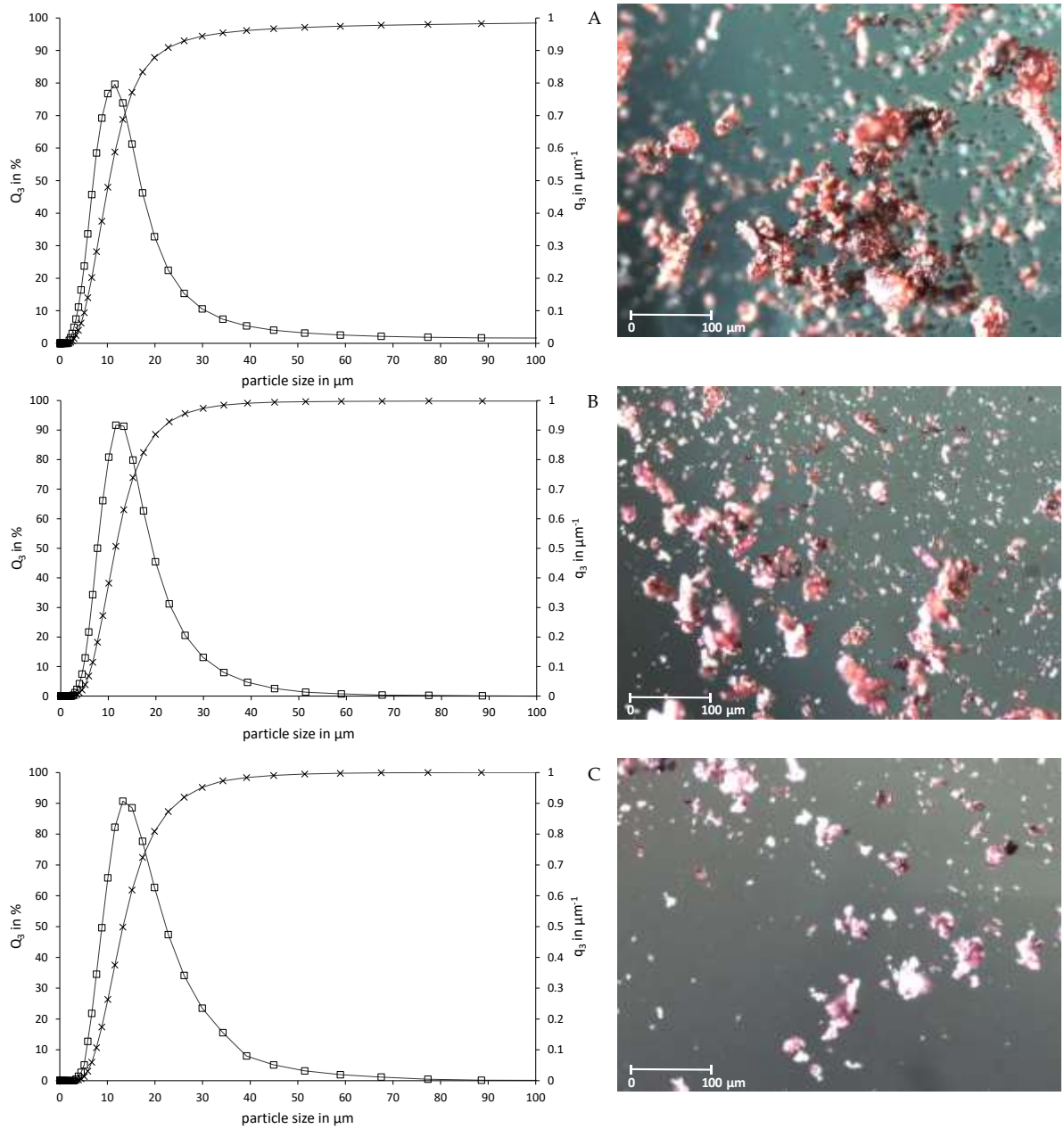


**Figure 1**. Particle size distribution plotted as the cumulative $Q_3$ in % (×) and density distribution $q_3$ in µm$^{-1}$ (□) over particle size in µm and light microscope pictures of chokeberry (**A**), bilberry (**B**) and elderberry (**C**).

Compared to spray-dried powders, the particle size of all PP is in the lowest quantile of the range of values [32].

Light microscope pictures of the three PP (Figure 1) show spherically shaped particles with a tendency to cohesiveness and the formation of clusters. Smaller particles appear to adhere to the surface of larger ones. Moreover, PP particles showed coarse surfaces of high heterogeneity in shape and size.

The particle morphology and tendency to form clusters affect several technological properties like bulk density, water-binding and oil absorption capacity, cold-water solubility and sedimentation velocity, which have an impact on the applicability of the pomace powders.

### 2.1.3. Hygroscopicity

Moisture absorption (%) after 24 h was comparable between the three PP. Although it was lowest in dry matter, chokeberry PP showed the highest moisture absorption after 24 h among all powders (Table 3). Significant differences in the moisture absorption were determined between chokeberry and bilberry. Chokeberry PP tended to form agglomerates resulting in higher moisture absorption. This behavior was already determined for spray-dried powders [32]. Hygroscopicity also depends on the bulk density of the powder. Densely packed powders, like elderberry PP (section 2.1.4.), enhance the barrier properties toward water and reduce moisture absorption [14]. Differences between the moisture absorption values of elderberry PP and bilberry PP were not significant, but elderberry showed a denser powder.

**Table 3**. Techno-functional properties of the pomace powders. Different letters indicate significant differences ($p \leq 0.05$) within each row. Values are mean ± standard deviation (n = 3).

| | **chokeberry** | **bilberry** | **elderberry** |
|---|---|---|---|
| moisture absorption after 24 h (%) | $4.09 \pm 0.13^{A}$ | $3.31 \pm 0.12^{B}$ | $3.63 \pm 0.29^{A,B}$ |
| bulk density (g/mL) | $0.73 \pm 0.03^{A}$ | $0.63 \pm 0.00^{C}$ | $0.45 \pm 0.01^{B}$ |
| water-binding capacity (g water/g dw) | $2.43 \pm 0.10^{A}$ | $3.10 \pm 0.07^{A}$ | $2.38 \pm 0.76^{A}$ |
| oil absorption capacity (g canola oil/g dw) | $1.74 \pm 0.18^{B}$ | $1.79 \pm 0.09^{B}$ | $2.24 \pm 0.10^{A}$ |
| oil absorption capacity (g sunflower oil/g dw) | $1.76 \pm 0.08^{B}$ | $1.85 \pm 0.05^{B}$ | $2.13 \pm 0.03^{A}$ |
| cold-water solubility (%) | $10.98 \pm 0.03^{B}$ | $10.98 \pm 0.17^{B}$ | $16.42 \pm 0.26^{A}$ |
| sedimentation velocity (mL/min) | $0.02 \pm 0.00^{A}$ | $0.02 \pm 0.00^{A}$ | $0.02 \pm 0.00^{B}$ |
| swelling capacity (mL/g dw) | $5.08 \pm 0.10^{B}$ | $5.16 \pm 0.10^{B}$ | $10.16 \pm 0.18^{A}$ |

### 2.1.4. Bulk density

All three pomace powders significantly differed in their bulk density, whereby the values of chokeberry PP and bilberry PP only differed by 0.10 g/mL. Elderberry PP resulted in the densest and largest particles with a bulk density of 0.45 g/mL, although the milling process was similar (Table 3). Comparable results for bulk density were determined for goldenberry waste powder (0.63 g/mL) [33] or for apple, carrot, and beetroot pomace powders which had densities of 0.56 g/mL, 0.52 g/mL, and 0.63 g/mL, respectively [29].

Densely packed powders possess several advantages, like the necessity of smaller containers as well as less gas binding due to limited space between particles, which might delay oxidative reactions.

2.1.5. Water-binding and oil absorption capacity

PP showed little and not significant differences in water-binding capacities (Table 3). Water-binding and oil absorption capacities of chokeberry PP were comparable to those described by Reißner et al. [15]. Overall, Reißner et al. found higher values for these techno-functional properties of chokeberry, but still in a comparable range. It has to be considered that these authors obtained chokeberry powder from the whole pomace with broader particle size distribution [15]. Water-binding capacity determined for bilberry PP showed the highest value, which is related to the narrow particle size distribution. The presented results are generally lower compared to previous studies on pomace powders of several fruits and vegetables, whereby some differences in the study design have to be considered. The mentioned studies used the whole pomace or the particle sizes were larger [29; 33–35]. Particle size affects the hydration properties of the powders which is related to the preservation of the structure in powders showing large particle size resulting in higher values for water-binding capacity [29; 36]. Oil absorption capacity determined using canola and sunflower oil was not significantly different, and significant differences were observed only between elderberry PP and the other two powders. For elderberry PP, an oil absorption capacity of 2.24 and 2.13 g oil/g dw for canola oil and sunflower oil was determined, respectively (Table 3). The oil absorption capacity depends on the porosity of the generated powder, which can be deduced from different properties shown for elderberry PP, especially the swelling capacity (see 2.1.8.). These findings are similar to those reported by Reißner et al. [15] and are comparable to previous studies on goldenberry waste powder, apple pomace powder, and jaboticaba pomace [33; 35].

2.1.6. Cold-water solubility

PP proved to have only poor cold-water solubility (Table 3). Compared to spray-dried powders which showed high cold-water solubility of over 80% [32], PP shows low applicability in aqueous solutions. This behaviour may be explained by the high amount of insoluble cell wall polysaccharides like cellulose and pectin. The powders have a high hydrophobicity and electrostatic property which further lowers the solubility due to the formation of aggregates.

Moreover, the porosity of the particles (Figure 1, light microscope pictures) affects the solubility characteristics.

2.1.7. Sedimentation velocity

The stability of the PP suspensions was determined by the sedimentation velocity (Table 3). Significant differences were observed between elderberry PP and the other two PP. The high values for sedimentation velocity are related to the low cold-water solubility of the PP. Both properties render the application of PP in drinks with low viscosity challenging. Etzbach et al. described similar observations for spray-dried powders with low cold-water solubility and fast emulsion separation [32]. Although PP is a fine powder, cluster formation is a critical issue and causes the low stability in solutions with low viscosity. Cluster formation caused by electrostatic and hydrophobic properties of the powders favor the fast sedimentation. The surface properties of particles have a strong influence on their behavior in solutions. This was determined in light microscopic pictures of the three PP in dry and wet conditions (Figure 1, light microscope pictures).

2.1.8. Swelling capacity

Swelling capacity of elderberry PP was significantly higher (10.16 mL/g dw) compared to bilberry PP and chokeberry PP (5.16 and 5.08 mL/g dw, respectively) (Table 3). Swelling capacity of the latter two PP were comparable to results obtained for goldenberry and Mexican apple powder with values of 5.24 mL/g and 3.2 mL/g, respectively [33; 34]. The swelling capacity depends on the surface characteristics of particles, showing pronounced porous particles in elderberry PP. These particles showed less electrostatic properties compared to particles from chokeberry PP and bilberry PP, resulting in remarkable swelling capacity between the three powders. Moreover, the densely packed elderberry PP enhanced the barrier properties toward water, leading to a distinct swelling capacity. These results were also observed in a previous study on fruit pomace subjected to bakery products [36].

*2.2 Anthocyanin and total phenolic contents in pomace powder (PP)*

The degradation process of total anthocyanins and total phenolics was compared with the stability of reference substances under the same storage conditions. This comparison was performed to evaluate the resistance of individual berry anthocyanins toward degradation compared to anthocyanins in the PP matrix.

The UHPLC-DAD chromatograms of the untreated pomace and the three pomace powders as well as the yoghurt applications are shown in Figures S1 and S2, respectively (supplement material).

2.2.1. Storage stability of anthocyanins and phenolic compounds in PP

In Figures 2A-C, the total anthocyanin (dark grey bars) and total phenolic content (white bars) of each PP is shown.

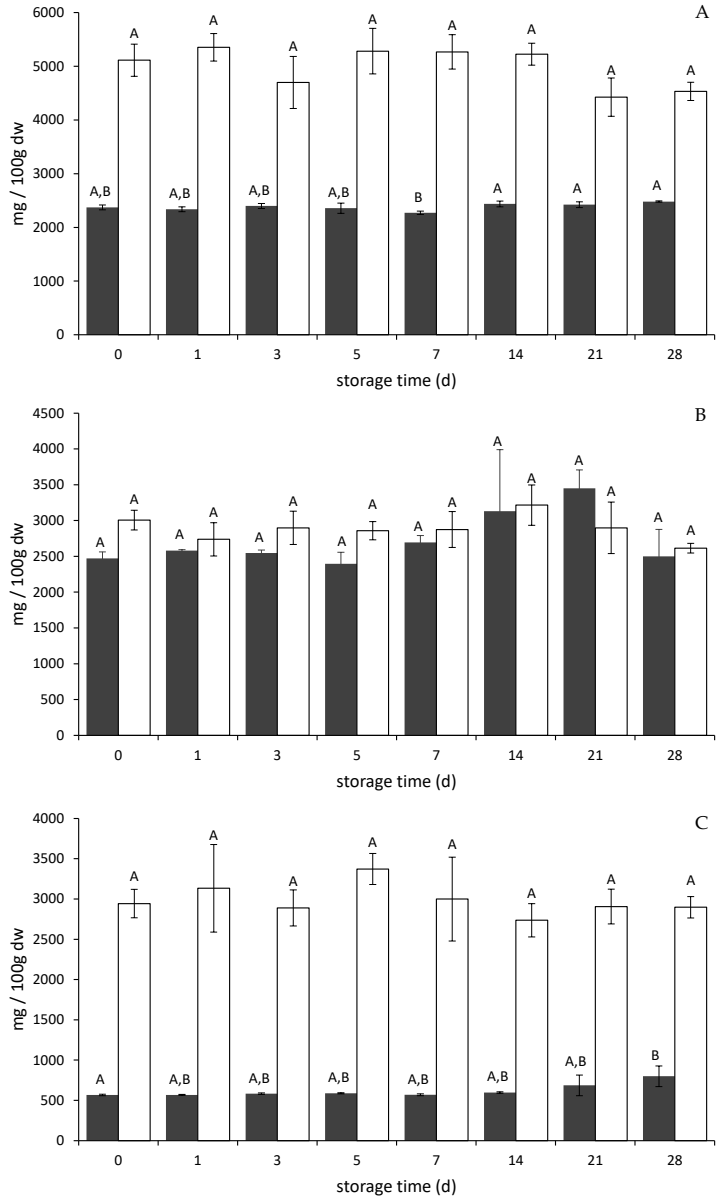


**Figure 2.** Total anthocyanin content (dark grey) in mg Cya-Glc eq. per 100 g dw of powder and total phenolic content (white) in mg gallic acid eq. per 100 g dw of powder over storage time (in days) of pomace powder of chokeberry (**A**), bilberry (**B**) and elderberry (**C**). Different letters indicate significant differences ($p \leq 0.05$) within one powder. Values are mean ± standard deviation (n = 3).

Total phenolic content showed no significant decrease during storage for two PP (Figures 2A and B, white bars) and a constant content for elderberry PP (Figure 2C, white bars). For chokeberry PP and bilberry PP, the storage experiment revealed a constant total anthocyanin content over 28 days of storage (Figures 2A and B, dark grey bars). The temporal changes during storage were not considerable. Total anthocyanin content in elderberry PP was constant during the first half of the storage period and increased subsequently with a significant difference compared to the beginning (Figure 2C, dark grey bars). The constant or slightly higher content of total anthocyanins can be related to the strong binding to cell wall components which might attenuate over storage time rendering anthocyanins more accessible for quantification. It has already been shown that the non-extractable polyphenols, which are associated to cell wall material, may be released at the end of the storage period [37]. The stability of anthocyanins in the three PP may be explained by the high and constant content of other phenolic compounds and the partly intact cell structures. The gentle drying temperature ensured a PP with high anthocyanin content after processing. Furthermore, elderberries contain anthocyanin diglycosides, which are more resistant towards degradation [38]. Both XAD7 extracts (chokeberry and bilberry) showed comparable results to the PP with constant values of total anthocyanins (supplement material, Table S2). This may be explained by the high amount of other phenolic compounds, which act as copigments preventing hydrolysis and oxidation. These protective effects of copigments have already been shown for blackberry XAD7 extract in spray dried samples [8]. The purified chokeberry and bilberry anthocyanins showed a slight decrease in total anthocyanin content over the storage time, especially in the first half. This may be explained by the removal of stabilizing components during membrane chromatography used for the isolation of anthocyanins.

### 2.2.2. Color parameters of the three PP

Color parameters were subjected to considerable changes, which are expressed as color difference $\Delta E$, hue $h°$, and Chroma $C^*$ (Table 4). The highest measured color difference was determined for chokeberry PP with a change of $\Delta E$ = 3.55 ± 0.03. The three PP differed significantly in their color changes, with the lowest measurable and observed change for bilberry PP (Table 4).

**Table 4.** Color difference ΔE, hue angle h°, and Chroma C* of pomace powder of chokeberry, bilberry and elderberry over storage time and day 0 and day 28. Values are mean ± standard deviation (n = 3).

| | | **chokeberry** | **bilberry** | **elderberry** |
|---|---|---|---|---|
| *C** | day 0 | 17.44 ± 0.02 | 14.40 ± 0.02 | 9.99 ± 0.02 |
| | day 28 | 14.72 ± 0.01[a] | 14.21 ± 0.02[a] | 8.93 ± 0.04[a] |
| *h°* | day 0 | 15.03 ± 0.09 | 13.71 ± 0.06 | 14.26 ± 0.01 |
| | day 28 | 13.97 ± 0.05[a] | 12.82 ± 0.06 | 11.55 ± 0.26[a] |
| | *ΔE*[b] | 3.55 ± 0.03[A] | 0.38 ± 0.05[C] | 2.32 ± 0.19[B] |

[a]: Value at day 28 is significantly different compared to the value at day 0. [b]: Different letters indicate significant differences ($p \leq 0.05$) within the row.

Comparable results were found for the hue angle. The greatest difference was calculated for elderberry PP with a decrease of 2.72 ± 0.19 (Table 4). However, these slight changes in color in storage experiments conducted in the dark were not considerable with respect to the generally intense color of PP. Color changes are not necessarily correlated with the degradation of anthocyanins and vice versa [8], which may be explained by the formation of colored polymers or complexation of several phenolic compounds with other pomace constituents in PP. A slow but constant release of bound anthocyanins from PP into the surrounding environment can be explained by the techno-functional properties of the three powders and the high hydrophobicity and electrostatic property which hindered hydrolysis and oxidation. This constant release apparently outweighs the degradation of anthocyanins in elderberry PP, resulting in an increase in anthocyanin content at the end of storage.

### *2.3 Anthocyanin and total phenolic content in a yoghurt model application over storage and the effect on color parameters*

The degradation process of total anthocyanins and total phenolics was assessed by comparison with the stability of reference substances under the same storage conditions. Since purple sweet potato anthocyanins and black carrot concentrate were applied in amounts necessary to equate the color saturation of the yoghurt with PP, the samples contained only approximately 0.3% of the colorant and coloring foodstuff, respectively.

#### 2.3.1. Storage stability of anthocyanins during a yoghurt model application

For samples containing chokeberry PP and bilberry PP (Figures 3A and B, dark grey bars), total anthocyanins decreased over the storage time, with no significant differences between initial total anthocyanin content on day 0 and day 14 (chokeberry: $\Delta_{0\text{-}14}$total anthocyanin

content = - 14.89% or 7.07 mg/100g fw yoghurt; bilberry: $\Delta_{0\text{-}14}$total anthocyanin content = - 35.21% or 19.98 mg/100g fw yoghurt).

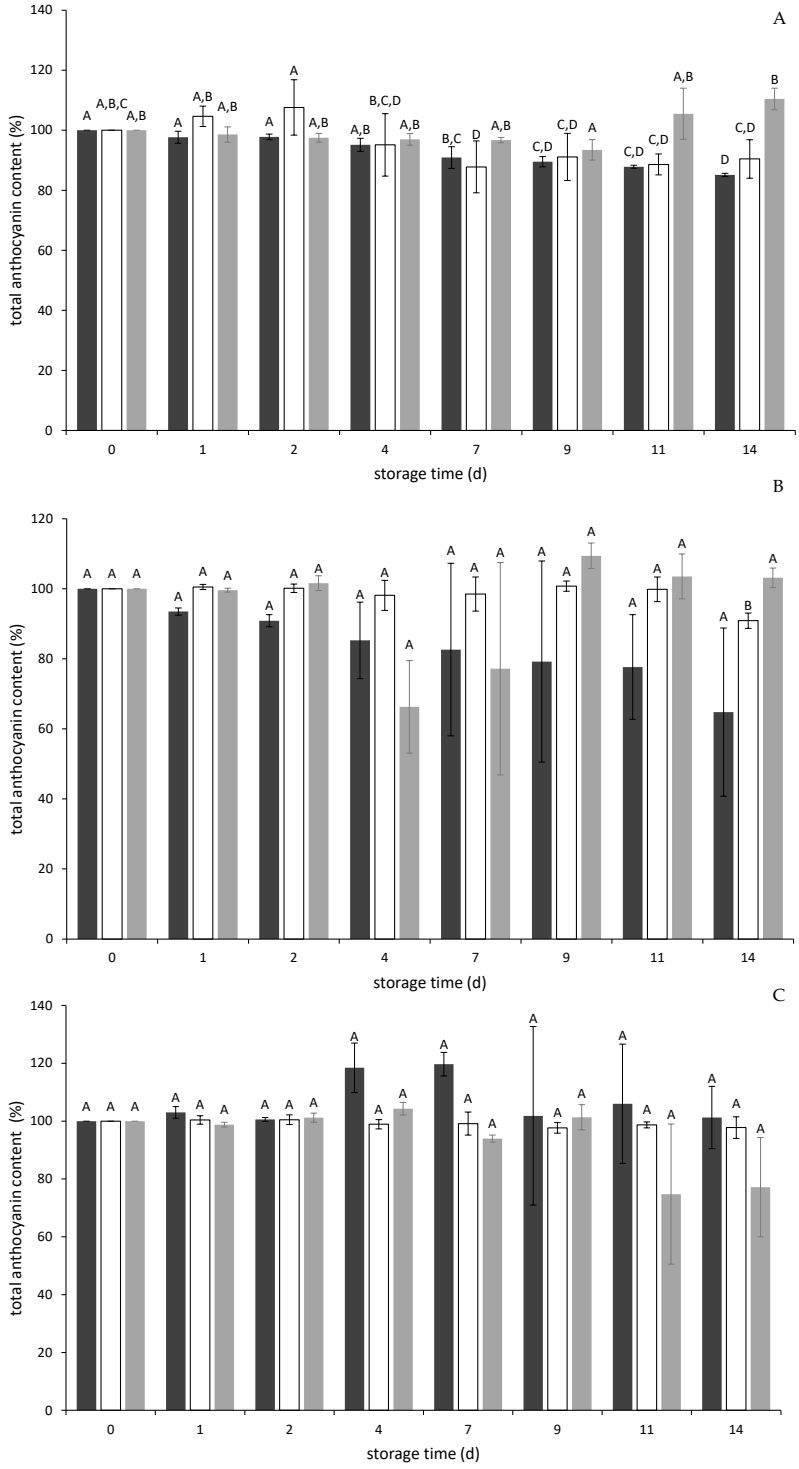


**Figure 3**. Anthocyanin degradation (%) referred to the initial total anthocyanin content on day 0 in yoghurt applications stored for 14 days of pomace powders (dark grey) of chokeberry (**A**), bilberry (**B**) and elderberry (**C**) as well as reference substances, namely purple sweet potato anthocyanins (white) and black carrot concentrate (bright grey). Different letters indicate significant differences ($p \leq 0.05$) within one yoghurt application. Values are mean ± standard deviation (n = 3).

In contrast to these changes, the total anthocyanin content of samples containing elderberry PP remained relatively stable, with no significant differences over the storage time ($\Delta_{0\text{-}14}$total anthocyanin content = + 1.26% or 0.17 mg/100g fw yoghurt) (Figure 3C, dark grey bars). These findings reflected the higher stability of the total anthocyanin content in PP storage experiments (section 2.2.1.). Nevertheless, the high standard deviation on some storage days has to be emphasized. This might be explained by inhomogeneous distribution of the PP and reference substances in the plain yoghurt. The homogenization was conducted with gentle agitation to keep the yoghurt matrix intact to prevent syneresis. Compared with the PP yoghurt samples, yoghurt blended with purple sweet potato anthocyanins showed a constant total anthocyanin content with marginal changes over storage time. Similar results were obtained for black carrot concentrate samples (Figures 3A-C, white and light grey bars). Purple sweet potato and black carrot contain acylated anthocyanins, which have been shown to be more stable than non-acylated derivatives. These acylated anthocyanins are more stable towards temperature, changes in pH, and can be stabilized inter alia by intramolecular copigmentation [10; 11]. It should be noted that the absolute anthocyanin content of the reference substances in the yoghurt applications was much lower compared to those of PP, because a great part of the anthocyanins in PP are bound to the still intact cell structures and are released only slowly during storage. It has to be considered that there is an inhomogeneous dispersion of anthocyanins between the yoghurt matrix and the PP particles. Bound anthocyanins might contribute less to the overall color than those that are already released into the yoghurt. This might explain the discrepancy between anthocyanin content and color yield.

2.3.2. Storage stability of phenolic compounds during a yoghurt model application

The total phenolic content remained constant with no significant differences in bilberry and chokeberry PP and increased slightly in elderberry PP samples (Table 5). Comparable results were published for yoghurt fortified with freeze-dried grape pomace powder and a storage period of 21 days [39].

**Table 5.** Total phenolic content of yogurt applications containing chokeberry, bilberry, and elderberry PP and purple sweet potato (PSP), and black carrot concentrate (BC) stored for 14 days with expression of values of day 0 and day 14. Values are mean ± standard deviation (n = 3).

| | **total phenolic content (mg GAE / 100 g fw yoghurt)** | |
|---|---|---|
| | **day 0** | **day 14** |
| Chokeberry PP | 71.27 ± 2.85 | 73.45 ± 2.49 |
| PSP reference chokeberry | 23.62 ± 4.66 | 29.68 ± 7.13 |
| BC reference chokeberry | 17.24 ± 4.66 | 29.75 ± 7.13 |
| Bilberry PP | 71.97 ± 11.43 | 65.14 ± 9.09 |
| PSP reference bilberry | 25.09 ± 3.33 | 17.32 ± 0.71 |
| BC reference bilberry | 21.54 ± 2.50 | 11.21 ± 0.80 |
| Elderberry PP | 65.38 ± 8.47 | 85.75 ± 4.15[a] |
| PSP reference elderberry | 18.91 ± 3.09 | 37.17 ± 12.41 |
| BC reference elderberry | 10.92 ± 4.54 | 17.27 ± 5.71 |

[a]: Value at day 14 is significantly different compared to the value at day 0.

Compared with the PP, yoghurt applications blended with purple sweet potato anthocyanins and black carrot concentrate showed for two trials a non-significant increase in total phenolic content. The difference in total phenolic content between day 0 and day 14 were more pronounced in the reference substances than in the PP. The stability of anthocyanins in the three yoghurt blends with PP can be explained by the high content of phenolic compounds and the partly intact cell structures. An increase in total phenolic compounds was previously described [40]. The increase can be explained by the limitations of the Folin-Ciocalteu assay used for the analysis as well as by the partial hydrolysis of lactose forming reducing sugars during the fermentation. Additionally, a continuous release of pomace polyphenols over the storage period was shown [40]. These non-extractable polyphenols, which interact with cell wall material, may be gradually released during storage. This may contribute to the increase in total phenolic compounds during the 14 days of storage. Although the method is generally not well suited for comparing phenolic content of different samples, it can be assumed from Table 5 that the PP colored yoghurts contained considerably higher amounts of phenolic compounds, compared to the reference yoghurts with purple sweet potato anthocyanins or black carrot concentrate.

2.3.3. Color parameters of the yoghurt model applications

Color parameters determined for each yoghurt application resulted in measurable changes over the storage time. Color saturation in yoghurt containing PP did not change considerably and decreased only for chokeberry PP and bilberry PP. Samples containing elderberry PP even

showed an increase in Chroma (Table 6). Similar changes were observed for the color difference and hue angle. Color stability with slight changes were previously observed for yoghurt fortified with freeze-dried grape pomace powder [39].

**Table 6.** Color parameters namely color difference ΔE, Chroma C* and hue angle h° of yoghurt applications containing chokeberry, bilberry and elderberry PP and purple sweet potato (PSP), black carrot (BC) over storage time and day 0 and day 14.

| | ***ΔE*** | ***h°*** | | ***C**** | |
|---|---|---|---|---|---|
| | | **day 0** | **day 14** | **day 0** | **day 14** |
| Chokeberry PP | 1.87 | 1.79 | 0.46 | 19.74 | 19.68 |
| PSP reference chokeberry | 3.72 | 12.92 | 10.37 | 19.61 | 17.39 |
| BC reference chokeberry | 1.96 | 1.75 | 0.70 | 20.29 | 19.11 |
| Bilberry PP | 2.17 | 10.42 | 9.98 | 16.78 | 16.30 |
| PSP reference bilberry | 2.94 | 10.05 | 7.62 | 16.27 | 14.83 |
| BC reference bilberry | 2.61 | 0.75 | 2.97 | 16.04 | 14.90 |
| Elderberry PP | 4.89 | 3.59 | 4.20 | 15.16 | 15.61 |
| PSP reference elderberry | 5.72 | 9.20 | 4.20 | 15.50 | 13.17 |
| BC reference elderberry | 2.94 | 2.79 | 5.35 | 14.33 | 13.30 |

In contrast, both reference substances showed considerable changes in color parameters compared to the PP. Especially the color of the yoghurt colored with purple sweet potato anthocyanins remained less stable and showed a strong decrease in saturation over the storage time (Table 6). This argues for a lower applicability compared to PP due to the strong decrease in color saturation over storage time. Similar results were determined for croissants fortified with 4% elderberry juice. The product provided the same benefits compared to those with the addition of black carrot commercial dye [41]. The powder properties and the surface composition have an influence on the release of anthocyanins. High hydrophobicity and incorporation capability of PP influenced the release of anthocyanins into the yoghurt followed by lasting color saturation. Visual appearance was additionally influenced by light scattering effects caused by the particles.

## 3 Materials and Methods

### *3.1 Materials*

#### 3.1.1. Chemicals and standards

Ultrapure water was obtained from a PURELAB flex 2 water purification system (ELGA LabWater, Paris, France). Anthocyanin extraction and analysis was conducted using methanol (HPLC grade) from Fisher Scientific GmbH (Schwerte, Germany), acetic acid glacial (ACS reagent 99.9%) and acetonitrile (LC-MS grade, 99.9%) all from VWR International GmbH

(Darmstadt, Germany), formic acid 99.9% (Merck KGaA, Darmstadt, Germany), ethanol 99% (denatured with benzene, Julius Hoesch GmbH, Düren, Germany) and cyanidin-3-*O*-glucoside > 97% (Phytoplan, Heidelberg, Germany). *n*-Hexane (VWR International GmbH, Darmstadt, Germany) and Span® 65 (Merck KGaA, Darmstadt, Germany) were used for particle size distribution analysis. Total phenolic content was determined using Folin-Ciocalteu´s phenol reagent (Merck KGaA, Darmstadt, Germany), sodium carbonate > 99% (Carl Roth, Karlsruhe, Germany) and gallic acid 98% (Alfa Aesar, Kandel, Germany). Sodium chloride ≥ 99.5% (Carl Roth, Karlsruhe, Germany) was used for the determination of the hygroscopicity. Purple sweet potato anthocyanins were obtained from Vitiva d.d. (Markovci, Slovenia), black carrot concentrate was kindly provided by Döhler GmbH (Darmstadt, Germany). Plain yoghurt (3.8% fat), canola and sunflower oil were purchased from a local supermarket.

Anthocyanin powder reference substances - namely chokeberry and bilberry XAD7 extracts and their further purified anthocyanin extracts - were isolated as described by Larsen et al. [42]. Anthocyanins were extracted at ambient temperature from chokeberry and bilberry juice provided by Haus Rabenhorst O. Lauffs GmbH & Co. KG (Unkel, Germany). The XAD7 extracts were obtained using column chromatography with Amberlite XAD7 HP (Sigma-Aldrich, Munich, Germany). The polyphenols were eluted with ethanol/acetic acid (19:1, *v/v*) and subsequently lyophilized. The resulting XAD7 extract was further purified by membrane chromatography with a membrane adsorber Sartobind S IEX 150 mL (Sartorius Stedim Biotech, Göttingen, Germany) obtaining the chokeberry and bilberry anthocyanins.

3.1.2. Berry pomace as a raw material for the production of pomace powder (PP)

The berry pomaces (*Aronia melanocarpa* Michx., chokeberry; *Vaccinium myrtillus* L., bilberry; *Sambucus nigra* L., elderberry) were kindly provided by Haus Rabenhorst O. Lauffs GmbH & Co. KG (Unkel, Germany) and were stored in 5 kg batches at -20 °C until further use. Samples of approximately 100 g were thawed overnight (at 20 °C) in an aluminum bowl covered with a cling film. After thawing, the pomace was dried in a constant climate chamber (KBF P, Fa. BINDER GmbH, Tuttlingen, Germany) for about 8 h (50 °C, 0%rH) under manually stirring every 30 min. After drying to a residual moisture content of approx. 5%, the individual batches of each berry were pooled. The drying procedure was repeated three times resulting in 2.4 kg, 4.4 kg and 3 kg dried berry pomace from bilberry, elderberry and chokeberry, respectively. Dried material was subjected twice to a malt grinder (grinder size: 0.635 – 2.54 mm, Brewferm, Brouwland, Beverlo, Belgium) followed by sieving of the pomace into five fractions

for elderberry and chokeberry and six fractions for bilberry. The sieve tower (100% intensity, 10 min, Retsch Technology GmbH, Haan, Germany) was built up with sieves of size (mm) 2.5, 2, 1, 0.5, 0.355, and base for bilberry, sieves of size (mm) 2.5, 2, 1, 0.71, and base for elderberry and sieves of size (mm) 2, 1, 0.71, 0.5, and base for chokeberry. The seedless fraction was obtained from the base sieve in case of chokeberry and bilberry and from the 0.71 mm sieve and base sieve for elderberry. Other fractions were the seed fraction on sieves (mm) 0.5 and 0.355 for bilberry, 0.71 and 0.5 for chokeberry, and 1 for elderberry. The agglomerate fraction was located on sieves (mm) 2.5 and 2 for bilberry, 2 and 1 for chokeberry, and 2.5 and 2 for elderberry. The seedless fraction was pulverized in a vibratory disc mill, type RS200 (Retsch Technology GmbH, Haan, Germany) at 14 000 rpm for 40 seconds (ambient temperature). The resulted powders were stored at -20 °C until further use.

*3.2 Methods*

3.2.1. Storage of pomace powder (PP)

To evaluate the stability of the produced PP of chokeberry, bilberry, and elderberry, approximately 10 g of each PP was packed in eight vacuum bags, sealed (vacuum sealer, Vac-Star 2000GSL, Bern, Swiss) and were stored at elevated temperature (35 °C, 0% rH, dark) in a constant climate chamber (KBF P, Fa. BINDER GmbH, Tuttlingen, Germany) for a period of 28 days to simulate prolonged storage. Samples were taken after 1, 3, 5, 7, 14, 21 and 28 days. As references during storage of PP, chokeberry and bilberry XAD7 extracts (approximately 1 mg) and the corresponding purified anthocyanin extracts (approximately 0.5 mg) was packed in eight vacuum bags each (see above) and was subjected to the same storage conditions. After sampling, PP and reference bags were stored at -80 °C until analysis.

3.2.2. Storage of colored yoghurt

To evaluate the applicability of the produced PP of chokeberry, bilberry, and elderberry, the three powders (6 g) were mixed manually into approximately 315 g plain yoghurt (2% PP, *w/w*). Followed by transferring approximately 35 g yoghurt sample into eight 50 mL sealable test tubes, the head space was flushed with $N_2$ and sealed with Parafilm® M. Yoghurt samples were stored at 4 °C in a constant climate chamber in the dark at 0% rH (KBF P, Fa. BINDER GmbH, Tuttlingen, Germany) for 14 days, with samples taken on days 1, 2, 4, 7, 9, 11 and 14. After sampling, yoghurt samples were stored at -80 °C after spectrophotometrical determination of CIE L*a*b* parameters. As a comparison, yoghurt was colored with two

reference substances, which were the frequently used colorant from purple sweet potato and the coloring foodstuff from black carrot concentrate. The concentration was set to adjust the color saturation (Chroma, C*) similar to each PP (Table 7). The mixing and test tube preparation was conducted according to the yoghurt samples blend with PP.

**Table 7.** Amount of reference substance (%), purple sweet potato anthocyanins and black carrot concentrate used, for the adjustment of color saturation of each yogurt application from the three PP.

| | **chokeberry** | **bilberry** | **elderberry** |
|---|---|---|---|
| Purple sweet potato anthocyanins (%) | 0.43 | 0.33 | 0.27 |
| Black carrot concentrate (%) | 0.32 | 0.18 | 0.14 |

3.2.3. Extraction and quantification of anthocyanins in stored PP and yoghurt

Anthocyanin extraction and quantification from PP and yoghurt samples were performed as reported previously by Heffels et al. [43]. Extraction solvent B with a ratio of methanol/water/acetic acid (80:15:5, *v/v/v*) was used. Lyophilization prior to extraction was omitted. Anthocyanins were extracted in triplicate by weighing approximately 1.2 g PP or 10 g yoghurt samples in test tubes. Powder samples were homogenized with an Ultra-Turrax, yoghurt samples were vortexed. Centrifugation was carried out at 4 °C (11,000 g, 10 min). The extraction procedure was repeated twice, first with 20 mL extraction solvent and after transferring the supernatant into a 50 mL volumetric flask, the pallet was extracted again with 10 mL extraction solvent. After the second centrifugation (4 °C (11,000 g, 10 min), both supernatants were pooled and made up to the mark with water. Ultrahigh-performance liquid chromatography diode array detector (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) as well as on a Kinetex C-18 column (1.7 µm, 150 × 2.1 mm, Phenomenex, Inc., Aschaffenburg, Germany) equipped with a security guard cartridge of the same material (2.1 × 5 mm, 1.7 µm). The following gradient was used where eluent A was water/formic acid (97:3, *v/v*) and eluent B was acetonitrile/formic acid (97:3, *v/v*) at a flow rate of 0.4 mL·min$^{-1}$/(min/% B): 0/4, 2/4, 7/8, 13/10, 19/17, 23/30, 23.3/100, 25.3/100, 25.8/4

and 0/4, 2/4, 5.5/6, 13/8, 18/9, 23/14, 25/30, 25.3/100, 27.3/100, 27.8/4 for HSS T3 and Kinetex, respectively. Anthocyanins were detected at 520 nm and quantified as cyanidin-3-*O*-glucoside equivalents (Cya-Glc eq.) by external calibration. Total anthocyanin content was calculated as the sum of individual anthocyanins. Analytes were identified by comparing elution order and UV/Vis spectra with those in previous studies [42–45]. The HSS T3 column was used for all samples containing anthocyanins from chokeberry namely all PP of chokeberry, the XAD7 extracts of chokeberry and the corresponding purified anthocyanin extracts. All other samples containing anthocyanins from elderberry or bilberry were analyzed on the Kinetex C-18 column. Purple sweet potato anthocyanin and black carrot concentrate samples were analyzed on both columns to compare the total anthocyanin content with each berry due to the use of two columns. Values measured on HSS T3 were compared with the values for chokeberry samples, values from Kinetex C-18 were compared with samples from bilberry and elderberry.

3.2.4. Determination of total phenolic content by the Folin-Ciocalteu assay

For the determination of total phenolic content, sample solutions of PP and the yoghurt samples were prepared. Therefore, a 0.1% PP in ethanol/water solution (50/50, *v/v*) was centrifuged and the obtained supernatant was used for further analysis of PP samples. 1 mL of extracts obtained from anthocyanin extraction (section 3.2.3) of yoghurt samples were used for the Folin-Ciocalteu assay. Results were expressed as mg gallic acid equivalents/100 mg dw (mg GAE/100 mg dw) by external calibration. Total phenolic content in PP and yoghurt samples were determined as reported previously [46–48] with some modifications. 840 µL ultra-pure water and 10 µL sample solution was mixed with 50 µL Folin-Ciocalteu reagent in a semi-micro cuvette (1 cm). After 3 minutes (ambient temperature), 100 µL saturated sodium carbonate solution was added, mixed and left for 60 minutes (ambient temperature). After incubation, the absorbance at 720 nm was measured in triplicate runs using a Genesys 6 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

3.2.5. Determination of techno-functional properties of PP

The techno-functional properties of the processed pomace powders were determined to evaluate the powders for possible food applications.

Particle size.

Particle size distribution of the PP was analyzed using a laser scattering particle size distribution analyzer (Horiba Scientific Partica LA-960, Retsch Technology GmbH, Haan, Germany). Powder was dispersed in 0.1% Span® 65 *n*-hexane solution and subjected to the measuring chamber. Cumulative and density distribution as well as particle parameters such as $d_{10}$, $d_{50}$ and $d_{90}$ were determined sixfold. Particle morphology (surface, cluster formation and structure) of the powders were assessed with a light microscope (AXIO Lab.A1, Carl Zeiss AG, Oberkochen, Germany) and top light (KL 750, Schott AG, Mainz, Germany) and observed at 10x magnification (0.25 Ph1, N-Achroplan).

Hygroscopicity.

The hygroscopicity of the PP was determined in triplicate as described by Silva et al. [14] with modifications. Samples were stored in flat aluminum trays in a desiccator and were weighed after 1, 3, 7, and 24 h. Moisture absorption was calculated according to Etzbach et al. [32].

Color parameters (CIELab color metrics).

The determination of color parameters (according to CIE Lab color metrics) of PP and yoghurt samples was conducted using a Chromameter CR-400/410 with illuminant $D_{65}$ (Konica Minolta, Langenhagen, Germany). 5 g PP and 35 g yoghurt were subjected to a glass cuvette with a diameter of 60 mm. Color loss during storage was calculated as overall color difference $\Delta E$ according to the equations mentioned previously [8; 14] and the readings obtained for color parameters Chroma C* and hue h°.

Microbiological status.

1 g of each PP was suspended in 9 mL physiological saline solution and homogenized in blender bags (400 mL, 190 mm x 300 mm, Corning Life Science B.V., Amsterdam, Netherlands) for 2 minutes. The solution was decimally diluted ($10^{-1}$ – $10^{-5}$) and pour-plated onto a plate count agar as well as on a yeast plate count agar to determine total bacteria counts after 48 h (30 °C) and 3 – 5 days (25 °C) incubation, respectively.

Water-binding and oil absorption capacity.

Water-binding and oil absorption capacity were determined according to Reißner et al. [15] with modifications. Besides canola oil, also sunflower oil was used to determine the oil absorption capacity.

Solubility.

Cold water solubility of the three PP was determined in triplicate according to Etzbach et al. [32] with slight modifications. A 50 mL (2%) powder suspension was vortexed for 3 min in a test tube. After centrifugation (11,000 g, 4 °C, 10 min) 25 g supernatant was transferred to an aluminum bowl and dried at 110 °C for 24 h. The cold water solubility was calculated as described previously [32].

Bulk density.

Bulk density was determined in triplicate according to Carneiro et al. [49] with some modifications. 5 g of PP was subjected to a 50:1 mL graduated cylinder and tapped by hand on the lab bench 50 times from a height of 10 cm.

Dry matter.

Dry matter (%dw) was determined thermogravimetrically in triplicate using a Sartorius moisture analyzer (MA100Q000230V1, Göttingen, Germany) by drying about 5 g of PP each.

Sedimentation velocity.

Sedimentation velocity was determined in triplicate by modifying two published suspension stability experiments [32; 49]. PP (2%, *w/w*) was dissolved in water, and 25 mL of the solution was transferred to test tubes, and stored at ambient temperature for one day. The separation (%) was calculated according to Carneiro et al. [49].

Swelling capacity.

Swelling capacity was carried out in triplicate following the experimental setup from Reißner et al. [15] with some modifications. 0.2 g PP was mixed with 10 mL water for 30 sec in a test tube and placed in a rack for 18 h (ambient temperature). The volume of the swollen powder was determined according to Reißner et al. [15].

3.2.6. Statistical analysis

Statistical analysis was conducted using XLSTAT software version 2019 (Addinsoft, Paris, France). An ANOVA with Tukey test was performed to determine significant differences ($p \leq 0.05$). In case the data did not follow a normal distribution, the Kruskal-Wallis test with Dunn test for multiple comparisons was performed.

## 4 Conclusion

Berry pomace, obtained as a side product of juice processing, represents a better raw material for producing an anthocyanin and phenolic rich powder compared to the whole fruit. The gentle drying temperature and short time followed by subsequent milling of the seedless fraction of red berry pomace ensured fine, red colored powders with considerable amounts of anthocyanins and total phenolic compounds without using any solvent or long processing lines. The chokeberry, bilberry and elderberry pomace powders (PP) showed promising techno-functional properties with the exception of a rather poor applicability in solutions with low viscosity due to a high sedimentation velocity. Despite the considerably higher dosages, compared to food colorants and coloring foodstuff, that are necessary to obtain comparable colors, berry PP still has a great potential as a coloring foodstuff since it represents an inexpensive side stream product of the berry juice production. The results obtained in the storage experiments suggest the applicability of the three PP, showing acceptable color stability with additional benefits with regard to phenolic compounds. It can be assumed that PP further increases the dietary fiber content in food applications. The three PP provided the same coloring effect like the purple sweet potato anthocyanins or the black carrot concentrate, but presumably added fiber and antioxidative compounds at the same time, raising the potential of PP to be used at industrial level. Compared to other high intensity coloring foodstuffs, the use of PP shows a wide applicability in dry or liquid, savory or sweet, bakery or dairy food applications, where the higher amounts are not an issue. Moreover, the release of anthocyanins in PP into the surrounding environment is slow and, thus, PP can be used in a wide range of applications especially in products with a longer shelf-life. Based on the conducted yogurt model application, it is likely that an increased dosage level of PP could influence the techno-functional and sensory properties. Therefore, a validation of the recipe may be conducted to determine the threshold of acceptability. An enhanced standardization of the particle size, obtained by an optimized milling process, might further amend the techno-functional properties. It might be concluded that the presented strategy can be applied as a valorization process to obtain berry PP suitable to improve the color and the nutritional value of food.

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# Chapter 3

## Toward gentle chokeberry juice production by ultrasound-assisted enzymatic maceration

**Abstract**

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.

**Keywords:** Ultrasound-assisted maceration; Chokeberry juice; Pectin degradation; Polygalacturonase; Pectin lyase; Anthocyanin extraction

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## 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 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).

**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 |

2.2.2. Extraction and quantification of anthocyanins

Anthocyanin extraction and quantification of samples collected 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, 2,217g), 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 (11,000g, 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, 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.

**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 ± 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 ± 1.15$^{A,B}$ | 16.43 ± 0.06$^{B}$ | 2.64 ± 0.06$^{A}$ | 11.08 ± 0.04$^{B}$ | 3.43 ± 0.00$^{A,B}$ |
| 35_PG | 43.33 | n.a. | 14.40 ± 2.89$^{A}$ | 67.54 ± 0.52$^{B,C}$ | 15.93 ± 0.06$^{C}$ | 2.54 ± 0.04$^{B}$ | 11.43 ± 0.04$^{A,B}$ | 3.42 ± 0.01$^{B}$ |
| 50_PL | 36.67 | 18.20 | 12.97 ± 1.16$^{A}$ | 68.01 ± 0.89$^{B,C}$ | 16.30 ± 0.00$^{D}$ | 2.58 ± 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 ± 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 ± 0.02$^{A,B}$ | 10.28 ± 0.15$^{C}$ | 3.51 ± 0.00$^{E}$ |

n.a.: not available.

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.

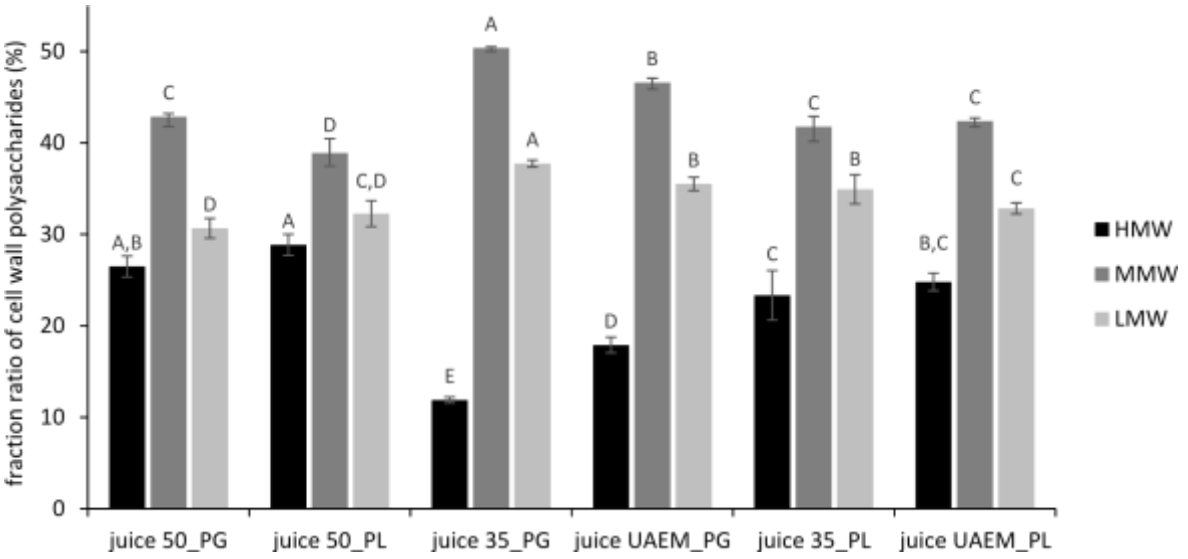


**Fig. 1.** Fraction ratio (%, HP-SEC) of cell wall polysaccharides of the six chokeberry juices produced at commercial standard procedure at 50 °C, ultrasound-assisted enzymatic maceration (UAEM, 35 °C), and temperature control 35 °C using two different enzyme preparations showing either polygalacturonase (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 ($n = 5$, $p \leq 0.05$).

### 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., 2006).

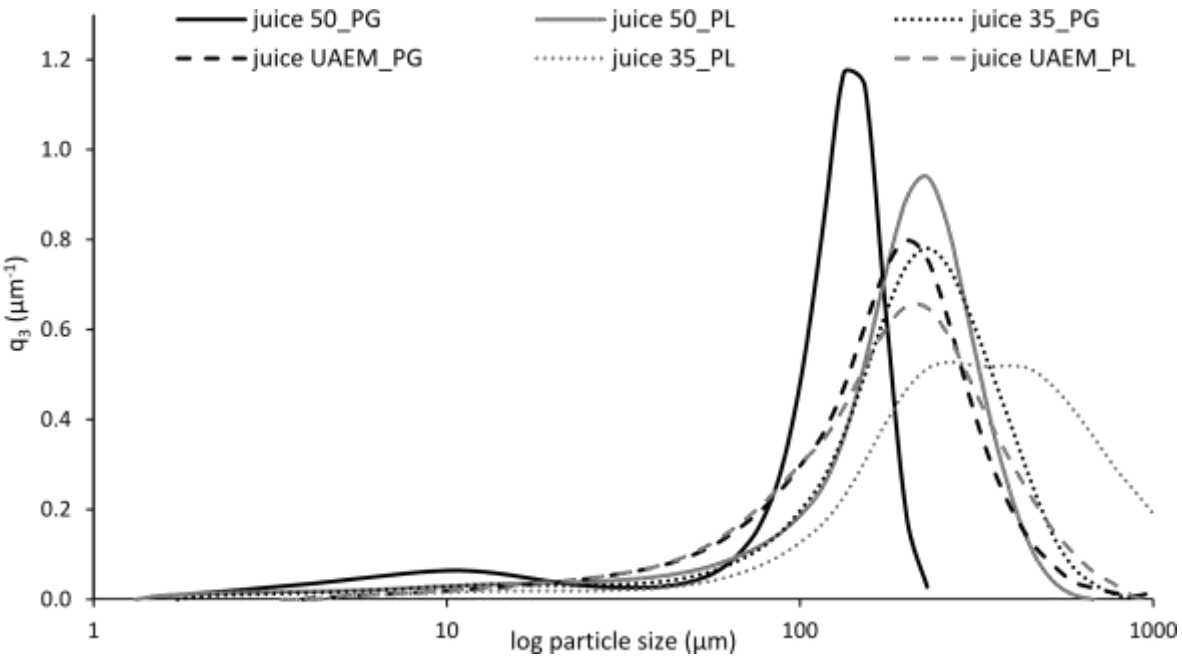


**Fig. 2.** Particle size distribution (PSD) plotted as density distribution $q_3$ ($\mu m^{-1}$) over logarithmic particle size (µm) of six pasteurized chokeberry juices. Maceration at commercial standard procedure at 50 °C (solid lines), ultrasound-assisted enzymatic maceration (UAEM, dashed lines), 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).

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).

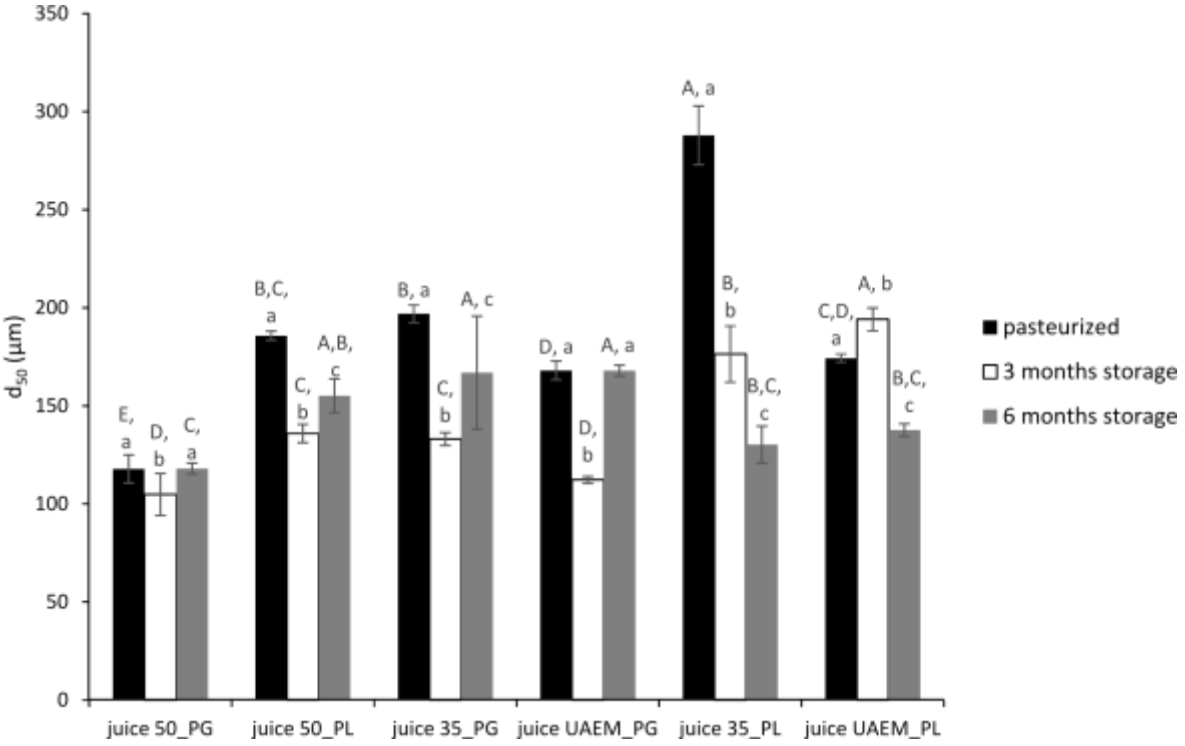


**Fig. 3.** Particle diameter $d_{50}$ (µm) of the six produced chokeberry juices after pasteurization (black), after three months of storage (white), and after six months of storage (grey). Maceration conditions at commercial standard procedure at 50 °C, ultrasound-assisted enzymatic maceration (UAEM), 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 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.

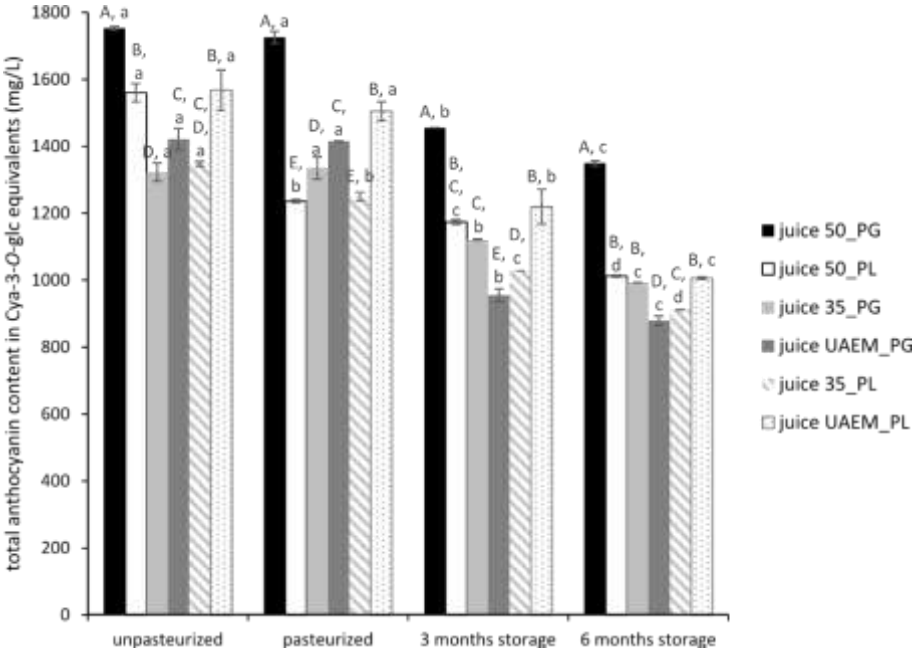


**Fig. 4.** Total anthocyanin content (Cya-3-*O*-glc equivalents mg/L) of the six chokeberry juices (unpasteurized, pasteurized, three and six months of storage) of the commercial standard procedure at 50 °C, ultrasound-assisted enzymatic maceration (UAEM) and temperature control 35 °C using two different 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, $p \leq 0.05$).

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.

## 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100518.

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# Chapter 4

## Concluding remarks

The high quality requirements of red berry juices, which are mostly determined by the qualitative and quantitative composition of the secondary plant metabolites and dietary fiber and the nutritional value must be ensured in respect to the human diet. Extraction of PCs, particularly anthocyanins, is currently facilitated by treating berry mash with pectinolytic enzymes. Cell wall polysaccharide degradation may be enhanced by applying US, resulting in a high extraction yield and quality of the final juice. This might increase the release of cell wall bound anthocyanins. Since juice production causes a by-product, which is rich in secondary plant metabolites, the pomace can be used as a source of PCs. Besides the extraction of PCs, the valorization of pomace with minor processing might be one alternative.

In this work, conventional red berry juice production was optimized by applying US during enzymatic maceration. The optimization was conducted with chokeberries under gentle process conditions (**Chapter 3**). Besides the optimization of red berry juice production, the valorization of berry pomace by its application as a coloring foodstuff in yoghurt was investigated (**Chapter 2**). Specifically, total anthocyanin content, thermal and storage stability of anthocyanins were determined.

## 1. Potential of US application during enzymatic maceration

In addition to enzymatic maceration, ultrasonication of mash proved to be a promising technology for the juice industry because of its beneficial effects on PCs extraction and cell wall degradation.

### 1.1. Effect of US on cell wall degradation and anthocyanin stability

The simultaneous use of US with the enzymatic treatment during maceration demonstrated to be more effective in terms of cell wall degradation. The combined application of UAEM during chokeberry juice production, as investigated in **Chapter 3**, has not previously been described for pilot-plant scale in literature. There are only a few studies considering US application in small laboratory scale and/or model solutions (Larsen et al., 2021; Tchabo et al., 2015; Le Lieu and van Le, 2010; Dalagnol et al., 2017; Nguyen et al., 2013).

The results showed improved cell wall degradation under gentle process conditions as well as by applying US with a significant improvement compared to conventional standard procedure. UAEM treatment, conducted at 35 °C, resulted in improved cell wall degradation with a higher ratio of the MMW fraction. Fragments of the MMW fraction exert protective effects on anthocyanins. Juices produced by applying US and using PG enzyme preparation as well as by conducting gentle process conditions showed larger particle diameters. These juices contained fragments low in MW which tend to form stable complexes with anthocyanins. These stable anthocyanin-pectin complexes stabilize PCs, ensure a product containing a high amount of valuable compounds, and might affect the thermal and storage stability of anthocyanins. In fact, total anthocyanin content was not significantly affected by pasteurization in PG-treated juices showing thermal stability. Additionally, by applying US, storage stability was improved especially in PG-treated juice. Differences in storage stability between PG and PL were less pronounced compared to the effects on thermal stability of anthocyanins.

From an industrial point of view, less sedimentation provides advantages, such as fewer need of adding hydrocolloids to the juice, reducing the number of ingredients, simplifying the process, and meeting consumer's demand to reduce the use of additives (Rojas et al., 2016). Further, UAEM might contribute to reducing maceration time resulting in an equal outcome and less energy consumption. The successful implementation of US in berry juice production in juice industry is challenging especially during continuous production. Currently, US is used in food industry to cut food material. Additionally, US can be applied for the valorization of pomace for further cell wall degradation or taking advantage of the positive effects of UAEM by applying US during enzymatic treatment of pomace. In respect to the altered composition of pomace after UAEM, resulting in a higher ratio of HMW fraction, changed polysaccharide profile, and dietary fiber content, the application of enzyme preparations for pomace treatment has to be selected from new perspectives.

# 2. Valorization of pomace and the application of pomace powder as a coloring foodstuff

At present, pomace is used for animal feed, discarded, or partially extracted. From a sustainable perspective, pomace can be valorized as a food alternative such as coloring foodstuff. The demand on coloring foodstuff increased in the past decade compared to artificial dyes, as the latter may lead to low consumer acceptance because of their negative image. Pomace powder introduces an alternative to currently used coloring foodstuffs such as black carrot concentrate and purple sweet potato anthocyanins (Gras et al., 2016; Gras et al., 2017).

## 2.1. Production of pomace powder and its application

The production of coloring foodstuff is mainly conducted by concentrating anthocyanins from vegetables or fruits. One novel approach is using berry pomace, which is rich in bioactive compounds (e.g. anthocyanins) to generate a coloring powder. The recovery of by-products from the vegetable and fruit juice industry is more resource-efficient. Nevertheless, process conditions, especially the drying process of the by-product, needs to be individually adjusted for each vegetable/fruit to ensure a product with high value adding compounds. Fast processing of the raw material, including gentle drying, is crucial to ensure a safe product (Reißner et al., 2019; Struck et al., 2016; Khanal et al., 2010; May and Guenther, 2020; Sójka et al., 2013). The implementation of the valorization process of pomace in juice industry is challenging because new equipment and process conditions are needed.

The still intact cell wall material and high total anthocyanin content of the generated crude pomace powders ensured high stability of anthocyanins over a storage period of 28 days. Color stability with additional benefits in regard to PCs was maintained. Pomace powders provided an equal coloring effect as purple sweet potato anthocyanins or black carrot concentrate. The techno-functional properties of the three berry pomace powders were similar to these of other powders generated from different sources. Pomace powder is an inexpensive side stream product with the potential to be used as coloring foodstuff at industrial level with a wide applicability especially in food with higher viscosity. Coloring foodstuffs have so far been applied in several foods. Most applications were bread ingredients, cereal-based products, beef, or baked goods to reduce flour or fat amount (Struck et al., 2016; May and Guenther, 2020; Quiles et al., 2018; Rohm et al., 2015; Seabra et al., 2010; Sójka et al., 2013; Perez-

Gregorio and Simal-Gandara, 2017). A novel approach is coloring yoghurt by adding pomace powder. Besides coloring the originally non-colored food, the nutritional value of the product (antioxidant properties and dietary fiber) is increased. The results demonstrated no significant changes in total anthocyanin content in the fortified yoghurt over a storage period of 14 days. Nevertheless, dosage level and threshold of acceptability need to be determined and optimized and the applicability in other food matrices needs to be studied. Pomace powder might be additionally treated with enzymes, generating an ingredient for a dough used for the production of a tortilla-like wrap or an edible muffin tin. Untreated pomace may be applied as an ingredient in fruit bars, increasing dietary fiber content and the nutritional value of the final product without adversely affecting the sensory perception.

## 3. Conclusion

In order to assess the effects of US on cell wall degradation and anthocyanin stability, applying US during enzymatic maceration on a pilot-plant scale is of utmost importance. Based on studies conducted in model solutions and the application in small scale, upscaling is the next step for the implementation and transfer to industry. To ensure the best possible use of PCs, red berry juice production needs to be optimized. This optimization should be simple and effective, as well as feasible for the fruit juice industry. Further, high yield has to be achieved in an ecological and resource-saving production. A sustainable red berry juice production involves not only a consistently high total anthocyanin recovery, but also the reutilization of by-products. Red berry juice by-products need to be valorized. The pomace may be used as a whole or as a coloring foodstuff in the food industry, adding value to the food by coloring originally non-colored food and/or enriching the food with dietary fiber and bioactive compounds like PCs.

As the present studies meet the requirements for the application and optimization of red berry juice production, comprehensive insights into anthocyanin stability and valorization of the pomace were obtained. The present thesis provides new information for the fruit juice industry regarding the application of US during enzymatic maceration, processing under gentle process conditions and valorization of pomace, and its application in yoghurt. These results offer additional benefits such as an energy saving potential, reduced pomace yield and transfer towards a resource-efficient production. Upscaling and implementation of the valorization process in the fruit juice industry is challenging, especially implementing US in a

continuous process. Further studies in respect to demethylation, deacetylation, and the sugar monomer profile of degraded cell wall material as well as the copigmentation of anthocyanins with PCs and cell wall fragments should be performed. Moreover, additional experiments regarding time reduction during UAEM should be conducted. The application of US during valorization, the liquefaction process and the implementation of pomace in food (fruit bars, wraps, or burger patties) should be investigated. In fruit bars, pomace can be implemented as a whole and replaces about 20% of other fruits, making the fruit bar more sustainable. After liquefaction of pomace powder, the dough-like ingredient can be used to produce gluten-free wraps.

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## Summary

Red berry juices are a possible strategy to increase the consumers' intake of secondary plant metabolites such as anthocyanins. To increase the juice yield and to release cell wall bound phenolic compounds (PCs), pectin degradation *via* enzymatic maceration is indispensable. Besides the enhanced cell wall degradation, the activity of pectinolytic enzyme preparations can positively be affected by applying ultrasound (US). Gentle maceration conditions and the application of US during enzymatic maceration (UAEM) improved juice production in terms of cell wall polysaccharide degradation and extraction yield compared to the conventional standard procedure. UAEM bears economic benefits because of lower production temperature and enhanced juice yield, increasing the profit of the juice industry. Applying UAEM generated smaller pectin fragments which, due to the formation of anthocyanin-pectin complexes, showed a protective effect on anthocyanins. The formation of these complexes resulted in an improved storage stability and thermostability of anthocyanins.

The present thesis reveals the potential of valorizing berry pomace into a coloring foodstuff. At present, pomace is used for animal feed, discarded, or only partly extracted. Pomace, which is rich in PCs especially anthocyanins, obtained as an inexpensive by-product from the juice production, represents an alternative for the production of coloring foodstuff in powdered form compared to using the whole berry fruit. Process conditions for generating pomace powder have to be adjusted to gentle and short drying steps, ensuring a stable and safe product. Subsequent milling of the seedless fraction generates a fine red-colored powder with considerable amounts of anthocyanins. In the whole production process, solvents or long processing lines are avoided. Pomace powders of chokeberry, bilberry, and elderberry show a great potential as a coloring foodstuff compared to currently used black carrot concentrate and purple sweet potato anthocyanins. Applying pomace powder in yoghurt showed acceptable color stability over storage and slow release of anthocyanins into the surrounding environment. Pomace powder bears a wide applicability in dry or liquid, sweet or savory, dairy or baked food. Further, pomace not only adds color to food but also increases the content of PCs and dietary fiber. In regards to the valorization of berry pomace, the dosage of pomace powder in food may be examined to determine its influence on sensory and techno-functional properties and the threshold of acceptability in applications. Valorization strategies raise the potential of pomace powder to be used at industrial level.

The present dissertation introduces new possibilities to optimize berry juice production and valorize its by-products. Besides the investigation of the effects of US on cell wall degradation and total anthocyanin content, this thesis provides a deeper understanding of the berry juice production and valorization of berry pomace on pilot-plant scale and a transition toward a resource-efficient production.

## Zusammenfassung

Rote Beerensäfte stellen eine mögliche Strategie zur Erhöhung der Aufnahme von sekundären Pflanzenstoffen wie Anthocyanen dar. Für die Erhöhung der Saftausbeute und die Freisetzung zellwandgebundener phenolischer Verbindungen (PCs) ist der Pektinabbau durch die enzymatische Mazeration unerlässlich. Die ultraschallgestützte enzymatische Mazeration (UAEM) hat eine verbesserte Pressbarkeit der Maische zur Folge, schützt hitzeempfindliche PCs und gewährleistet die Hitze- und Lagerstabilität der Anthocyane. Neben dem verbesserten Zellwandabbau kann die Aktivität pektinolytischer Enzympräparate durch den Einsatz von US positiv beeinflusst werden. Schonende Mazerationsbedingungen und die Anwendung von US wirken sich positiv auf die Saftproduktion hinsichtlich des Zellwandpolysaccharidabbaus und der resultierenden Extraktionsausbeute im Vergleich zu herkömmlichen Standardverfahren aus. Die Einführung der UAEM bietet aufgrund der milden Prozesstemperaturen, der erhöhten Saftausbeute und somit des gesteigerten Profits wirtschaftliche Vorteile. Zusätzlich wirken die durch die Anwendung von schonenden Bedingungen und US gebildeten kleineren Pektinfragmente durch die Bildung von Anthocyan-Pektin-Komplexen schützend auf die Saftanthocyane. Durch die gebildeten Komplexe zeigen Anthocyane eine verbesserte Lagerstabilität. Des Weiteren verbessert die UAEM die Hitzestabilität der Anthocyane in Säften, die mit PL hergestellt wurden.

Die vorliegende Arbeit bringt das Potenzial der Beerentresteraufwertung als färbendes Lebensmittel zum Vorschein. Aktuell werden Trester verworfen, finden Anwendung als Viehfutter oder werden zu Teilen extrahiert. Beerentrester, der reich an phenolischen Verbindungen, insbesonders aber an Anthocyanen ist und als kostengünstiges Nebenprodukt bei der Saftproduktion anfällt, stellt im Vergleich zur ganzen Beerenfrucht einen besseren Rohstoff für die Herstellung von färbenden Lebensmitteln in Pulverform dar. Um ein stabiles und sicheres Produkt gewährleisten zu können, müssen die Prozessbedingungen für die Herstellung von Tresterpulvern auf schonende Trocknungstemperaturen und kurze Trocknungszeiten abgestimmt sein. Durch die anschließende Vermahlung der kernlosen Fraktion entsteht ein feines, rot gefärbtes Tresterpulver mit erheblichen Mengen an Anthocyanen. Die gesamte Produktion erfolgt ohne den Einsatz von Lösungsmitteln oder zeitintensiven Prozessschritten. Deshalb zeigt rotes Beerentresterpulver ein vergleichbar großes Potenzial als färbendes Lebensmittel zu den derzeit verwendeten Konzentraten aus

schwarzer Karotte und Anthocyanen der violetten Süßkartoffel. Zusätzlich zeigte die Verwendung von Beerentresterpulver in Joghurt eine akzeptable Farbstabilität während der Lagerung. Dies macht eine breite Anwendbarkeit von Tresterpulver in trockenen oder flüssigen, süßen oder herzhaften, Milch- oder Backwarenprodukten denkbar. Darüber hinaus verleiht Tresterpulver den Lebensmitteln nicht nur Farbe, sondern erhöht auch den Gehalt an phenolischen Verbindungen und Ballaststoffen. Hinsichtlich der Beerentresteraufwertung sollten die Dosage des Tresterpulvers im Lebensmittel, die sensorischen und techno-funktionalen Eigenschaften sowie die Akzeptanzschwelle untersucht werden. Diese Strategien erhöhen das Anwendungspotential von Trester auf industrieller Ebene.

Die vorliegende Dissertation bietet daher der Beerensaftindustrie neue Möglichkeiten zur Optimierung der Saftproduktion und zur Verwertung des Nebenprodukts Trester. Neben der Untersuchung von US-Effekten auf den Zellwandabbau und den Gesamtanthocyangehalt liefert diese Arbeit ein tieferes Verständnis der Produktion von roten Beerensäften und der Verwertung von Beerentrester im Technikumsmaßstab und somit für den Transfer in Richtung einer ressourceneffizienten Produktion.

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