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Combined Enzymatic and High-Pressure Processing Affect Cell Wall Polysaccharides in Berries

HAUKE HILZ,† MARTINA LILLE,§ KAISA POUTANEN,§ HENK A. SCHOLS,† AND ALPHONS G. J. VORAGEN*,†

Department of Agrotechnology and Food Sciences, Laboratory of Food Chemistry, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands, and VTT Biotechnology, P.O. Box 1500, 02044 VTT (Espoo), Finland

The effect of high-pressure processing (HPP) on cell wall polysaccharides in berries was investigated. HPP decreased the degree of methyl esterification (DM), probably by activation of pectin methyl esterase (PME), and improved the extractability of pectins. When commercial enzyme mixtures were added to mashed berries, a synergistic effect was observed between treatment with commercial enzymes and HPP. Compared to treatment at atmospheric pressure, pectic polysaccharides were degraded to a larger extent when HPP was used. In contrast, hemicelluloses were hardly affected by the added enzymes when HPP was included, although they were degraded during similar treatment at atmospheric pressure. Additionally, the activity of rhamnose-releasing enzymes present in minor quantities might be enhanced after HPP, resulting in a decrease of rhamnose in the polymeric cell wall material. These results exploring the effect of HPP at representative conditions clearly point out the potential of HPP for polysaccharide modification.

KEYWORDS: ***Vaccinium myrtillus*; bilberry; *Ribes nigrum*; black currant; cell wall polysaccharides; fractionation; berries; high pressure; enzyme mixture; enzymes; cell wall**

INTRODUCTION

The first experiments with high-pressure processing (HPP) on food were carried out with the purpose of enhancing the microbial stability of food products such as milk, meat, and fruit juices without changing the sensorial properties (*1*). New interest in this field rose at the end of the 1980s with the development of high-pressure devices suitable for broad industrial food applications (*2*). Today, HPP is investigated with the aim of preserving foods, changing food texture, preserving flavor, or influencing enzymatic reactions (*2*, *3*). Some HPP products are already on the market (*4*−*8*). Fruit juices, fruits, jams, or other fruit products can be treated with high pressure for pasteurization or high-pressure freezing (*4*, *8*). However, HPP remains an expensive treatment.

High pressure encourages those chemical reactions and structural rearrangements that lead to a decrease of the total volume, for example, by changing the tertiary structure of proteins. Covalent bonds in proteins are hardly affected by high pressure, but ionic and hydrogen bonds and with them the tertiary structure can change drastically (*3*, *9*). HPP-induced gelling was reported for proteins and amidated, low methyl esterified pectins (*2*, *10*).

To date, the effect of HPP on cell wall polysaccharides is unclear. The plant cell wall is composed of three main groups of polysaccharides: cellulose, hemicellulose, and pectins (*11*). Cellulose forms together with hemicelluloses the firm backbone of the wall, which is embedded in a pectic network. This pectic network mainly influences the firmness of tissue and is responsible for textural changes during food processing, although the mechanism is not always clear.

How HPP affects endogenous pectinolytic enzymes was studied for polygalacturonase (PG) and pectin methyl esterase (PME) isolated from different fruits and vegetables (*12*−*18*). All investigations show a fast decrease of PG activity with increasing pressure, but PME activity appears to be pressure stable or is even activated. How other cell wall degrading enzymes, for example, of fungal origin or hemicellulases, behave under high pressure is not known.

Bilberries (*Vaccinium myrtillus* L.) and black currants (*Ribes nigrum* L.) are important crops in northern Europe. In the food industry most bilberries and black currants are processed to juice. This juice can be consumed as such or used as a food ingredient. During juice production the cell walls are ruptured and pectic polysaccharides form a strong pectin gel, from which juice can be obtained only with difficulty (*19*). For better juice yields (*19*) and improved extraction of phenolic compounds (*20*−*23*) it has become common practice to use commercial enzyme mixtures, usually of fungal origin, which contain a variety of cell wall degrading enzymes, to degrade the pectin gel. In a

* Corresponding author (telephone +31 317 482888; fax +31 317 484893; e-mail fons.voragen@wur.nl).
† Wageningen University.
§ VTT Biotechnology.

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Table 1. Composition of Cell Walls and Cell Wall Fractions Isolated from Bilberry Mash Treated without (C, Control) and with High Pressure (HPP)

	yield per wet mash (g/100 g)		mol %																total sugar residues (% w/w)		DM (%)	
			Rha		Fuc		Ara		Gal		UA		Xyl		Man		Glc					
	C	HPP	C	HPP	C	HPP	C	HPP	C	HPP	C	HPP	C	HPP	C	HPP	C	HPP	C	HPP	C	HPP
AIS	5.1	5.1	1	1	0	0	6	7	7	6	22	27	27	26	3	3	34	29	36	34	67	58
HBSS	0.4	0.6	2	1	0	0	5	5	9	10	71	66	4	6	3	3	8	10	53	44	96	106
ChSS	0.1	0.3	2	2	0	0	12	11	10	7	68	71	2	2	2	3	5	5	38	37	86	73
DASS	0.2	0.2	3	3	1	0	19	20	17	18	51	43	3	5	2	2	7	9	28	27		
CASS	0.4	0.4	1	2	1	1	7	7	14	14	8	7	25	27	12	11	32	31	57	54		
residue	3.1	3.2	1	1	0	0	6	6	4	3	8	8	37	35	1	2	43	46	44	50		

recent publication we described the detailed composition of cell wall polysaccharides from bilberries and black currants (*24*).

The research described in this study is part of a bigger research project aiming at maximizing the yield and functionality of berry products by using novel processing technologies such as high pressure or high-power ultrasound. Preliminary observations using HPP and commercial enzyme mixtures showed a clear effect on viscosity and extractability of bioactive compounds. The aim of this study was to investigate how enzymes affect cell wall polysaccharides in food when treated under high-pressure conditions. This paper describes these effects observed for representative HPP conditions to better understand the potential of HPP to modify cell wall polysaccharides. We investigated changes in different classes of cell wall polysaccharides caused by HPP alone and in combination with commercial enzyme mixtures.

MATERIALS AND METHODS

Materials. Commercial bilberries (*V. myrtillus* L.) were obtained from Kiantama Ltd., Suomussalmi, Finland. Black currants (*R. nigrum* L. cv. Öjeby) were obtained from Peltohermanni Ltd., Ilomantsi, Finland. Both berries were of the 2002 harvest and were stored frozen at −23 °C. Three commercial enzyme mixtures were used: (1) Biopectinase CCM, pectinolytic mixture (Kerry Biosciences, Tralee, Ireland); (2) Pectinex Ultra SP-L, pectinolytic enzyme (Novozymes, Bagsvaerd, Denmark); and (3) Econase CE, hemicellulolytic enzyme (AB Enzymes, Darmstadt, Germany). Specific activities of glycanases present in the enzyme mixtures have been described before (*22*).

High-Pressure Processing. Frozen bilberries or black currants (200 g) were spread onto a dish and allowed to thaw at 21 °C for 20 or 35 min, respectively. The partly thawed berries were mashed for 8 s (bilberries) or 40 + 5 s (black currants) in a kitchen mixer. For HPP, 20 g of the mash was packed into six small plastic pouches. The rest of the puree was left at room temperature during the pressure treatment and used as control sample in the analysis described below.

HPP was performed on a laboratory-scale, multivessel high-pressure device (HPIU-10000-AT, Resato International, Roden, The Netherlands). One sample pouch was placed into each of the six pressure vessels. The vessels were filled with pressure-transmitting medium and closed. The samples were pressurized to 400 MPa at a rate of 50 MPa/min. The pressure was kept constant for 15 min. Afterward, the pressure was released within a few seconds and the vessels were opened. The temperature of the pressure vessels was controlled by a circulating thermostatic liquid (35 °C) from an external bath through the jackets surrounding the vessels. The temperature was not constant during the pressure treatment, due to adiabatic heating (maximum temperature of 43 °C) and cooling (minimum temperature of 15 °C) during pressure buildup and release, respectively. Samples were frozen after HPP.

HPP in Combination with Enzyme Treatment. When HPP was combined with enzyme treatment, the enzyme mixtures were added to the puree before the plastic pouches were sealed. Enzyme dosages were standardized on 100 nkat of PG activity for Biopectinase CCM (4.4 μL/g berries) and Pectinex Ultra SP-L (3.4 μL/g berries) and on 100 nkat of endoglucanase activity for Econase CE (6.0 μL/g berries), respectively. The commercial enzyme mixtures contain other enzymes as well (*22*). After HPP, the samples were kept for 2 h at room temperature and atmospheric pressure to allow the enzymes to work further on their substrates and frozen at −23 °C afterwards. One control was kept in the vessel but was not pressurized (mash TP), one control was kept at room temperature and atmospheric pressure (mash RT), and one control was pressurized without the addition of enzyme (mash + HPP).

Preparation of Alcohol Insoluble Solids (AIS) and Sequential Buffer Extraction. Cell wall material was precipitated with 70% aqueous ethanol at 50 °C (AIS) and sequentially extracted with 0.05 M sodium acetate buffer at pH 5.2 and 70 °C (hot buffer soluble solids, HBSS), with 0.05 M EDTA and 0.05 M sodium acetate in 0.05 M sodium oxalate at pH 5.2 and 70 °C (chelating agent soluble solids, ChSS), with 0.05 M sodium hydroxide at 0 °C (diluted alkali soluble solids, DASS), and with 6 M sodium hydroxide at 0 °C (concentrated alkali soluble solids, CASS). The remaining residue was included in the analysis as well (*25*, *26*).

Sugar Composition. Sugar composition was determined after Saeman hydrolysis: after prehydrolysis using 72% w/w sulfuric acid at 30 °C for 1 h, the samples were hydrolyzed with 1 M sulfuric acid at 100 °C for 3 h (*27*). Afterwards, the sugars were derivatized to alditol acetates and determined by gas chromatography (*28*) using inositol as internal standard.

Uronic Acid Content. The total uronic acid content was determined photometrically with the automated *m*-hydroxydiphenyl assay (*29*).

Degree of Methyl Esterification (DM) and Degree of Acetylation (DA). DM and DA were determined by HPLC after hydrolysis with 0.4 N sodium hydroxide in 2-propanol/water 50:50 v/v (*30*). In addition, the DM was also determined by headspace gas chromatography (*31*). DM and DA were calculated as moles of methyl/acetyl groups per 100 mol of galacturonic acid. One mole galacturonic acid can carry only 1 mol of methyl esters and 2 mol of acetyl ester.

RESULTS

Effect of HPP on Cell Wall Polysaccharides. To investigate if HPP changes the structure, composition, or extractability of cell wall polysaccharides, bilberries and black currants were mashed and treated at 400 MPa for 15 min, whereas the control remained at atmospheric pressure and room temperature. Cell walls present in these samples were isolated and their polysaccharide structure, composition, and extractability analyzed.

The sugar compositions of bilberry cell walls with and without HPP were very similar (**Table 1**). No considerable differences in molar contents between the sugar residues were observed. The DM of pectins, however, was affected by HPP. It decreased after HPP of bilberry mash from 67 to 58% in AIS. Such a decrease was similar in ChSS (from 86 to 73%). In HBSS the DM remained remarkably high (96 and 106%). Obviously, a DM above 100% is theoretically impossible, but was shown with two different methods to measure methanol. We have no explanation for this aberrant value and consider the DM unchanged in HBSS.

The sugar composition of black currant AIS (**Table 2**) did not change considerably after HPP. An exception is an

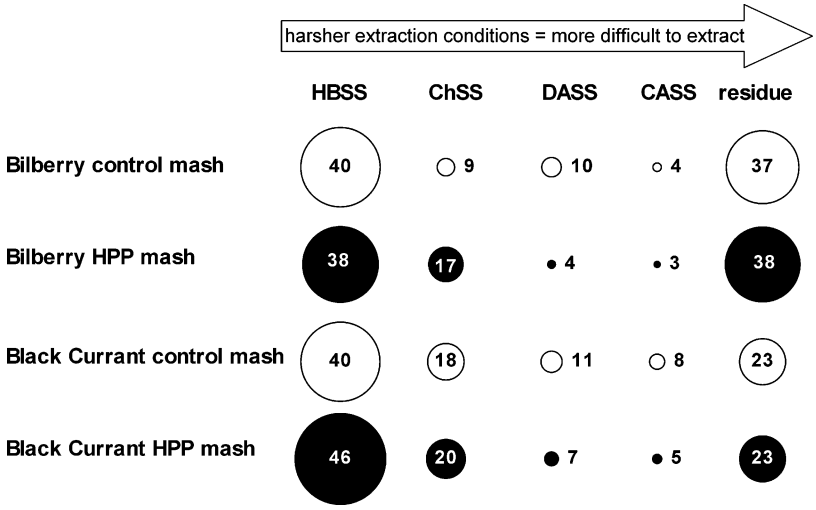

Figure 1. Content of uronic acids in the different cell wall fractions obtained from mashes treated and nontreated with HPP (percent of extracted uronic acid).

Table 2. Composition of Cell Walls and Cell Wall Fractions Isolated from Black Currant Mash Treated without (C) and with High Pressure (HPP)

	yield per wet mash (g/100 g)		mol %																total sugar residues (% w/w)		DM (%)	
			Rha		Fuc		Ara		Gal		UA		Xyl		Man		Glc					
	C	HPP	C	HPP	C	HPP	C	HPP	C	HPP	C	HPP	C	HPP	C	HPP	C	HPP	C	HPP	C	HPP
AIS	8.3	8.1	2	2	0	0	9	8	7	6	36	36	6	7	23	19	19	23	34	39	47	39
HBSS	0.8	1.1	2	2	0	0	12	12	5	7	76	69	3	2	2	5	2	5	48	50	70	67
ChSS	0.4	0.5	2	2	0	0	14	12	5	5	75	77	1	1	2	3	1	2	48	42	66	57
DASS	0.6	0.4	3	4	0	0	27	25	20	20	44	44	3	3	2	2	2	2	31	30		
CASS	1.2	0.7	1	2	2	2	8	8	13	13	9	10	25	23	13	13	28	29	47	49		
residue	4.6	4.4	1	1	0	0	8	7	5	5	8	8	8	9	38	26	33	35	46	47		

unexplainable decrease of the mannose content in AIS and the residue. In HBSS of the HPP mash, mannose and glucose were present in higher molar contents compared to control HBSS. However, this was not due to an increase in these sugar contents, but to a decrease in uronic acid content (vide infra). Like in bilberries, the most remarkable change was the decrease of DM after HPP. This was shown for pectins present in AIS and ChSS, but not for pectins in HBSS.

With a decrease in DM, a change in extractability of uronic acid after HPP was observed. **Figure 1** shows how much uronic acid is extracted from AIS into the different fractions. The highest amount of uronic acid was found in HBSS for all four mashes. In bilberry control mash, uronic acid was extracted into the ChSS and DASS fractions to the same extent, whereas the lowest content of uronic acid was extracted into CASS. After HPP, the content of uronic acid decreased in DASS, whereas it increased in ChSS. This means a better extractability of uronic acid after HPP, because ChSS is extracted under milder conditions than DASS. The high amount of uronic acid in the residue is due to pectic polysaccharides, which are known to be present in the seeds (*24*) that remained intact during extraction.

An enhanced extractability of uronic acid was observed for HPP mash of black currants compared to black currant control mash, as well. The uronic acid content decreased in DASS and CASS from HPP mash of black currants, whereas the uronic acid content increased in HBSS and hardly changed in ChSS, which is in contrast to bilberry mash.

Combination of HPP and Treatment with Commercial Enzymes. To test if HPP and commercial enzymes have a synergistic effect on degradation of cell wall polysaccharides, black currants were homogenized and three different commercial enzymes mixtures (Econase CE, Biopectinase CCM, and Pectinex Ultra SP-L) were added directly before HPP.

Comparing the sugar compositions of AIS indicates how enzymes affect cell wall polysaccharides (**Table 3**). The sugar compositions of AIS did not differ significantly among the three controls. Treatment with Biopectinase CCM and Pectinex Ultra SP-L caused a decrease in the content of hemicellulolytic sugars (xylose, mannose, glucose) at atmospheric pressure compared to all three control samples, but not at high pressure.

Pectins behaved differently from hemicelluloses. The content of uronic acid in AIS decreased after treatment with Biopectinase CCM and Pectinex Ultra SP-L. The uronic acid content after treatment at high pressure was even lower than after treatment at atmospheric pressure. The content of rhamnose decreased in AIS after treatment with Biopectinase CCM and Pectinex Ultra SP-L at atmospheric pressure. After treatment at high pressure, the decrease was much stronger: almost 90% of rhamnose was removed. The content of galactose and arabinose decreased in AIS after treatment with Biopectinase CCM and Pectinex Ultra SP-L at atmospheric pressure and at high pressure. The decrease of galactose content was stronger after treatment at atmospheric pressure (>50%), whereas the arabinose content decreased equally after treatment at atmospheric and high pressures.

The sample treated with Econase CE showed a decrease of hemicellulolytic sugars (xylose, mannose, glucose) after treatment at atmospheric pressure. If Econase was used in combination with high pressure, there was hardly any change in the content of hemicellulolytic sugars. The different pectic sugar residues showed similar behavior as after treatment with the pectinolytic enzyme mixtures, but to a lesser extent.

Table 3. Composition of Cell Walls (AIS) Isolated from Black Currant Mash Treated with Different Commercial Enzyme Mixtures with and without HPP

	g/kg of mash								
	Rha	Fuc	Ara	Gal	UA	Xyl	Man	Glc	total sugar residues
mash RT	0.8	0.1	2.9	3.3	13.7	2.5	12.0	11.2	46.5
mash TP	0.9	0.1	3.0	3.0	15.3	2.6	12.8	12.3	50.0
mash + HPP	0.8	0.1	3.4	3.4	16.4	2.5	13.4	13.4	53.4
mash + Econase CE	0.4	0.1	2.6	2.4	11.7	1.8	7.1	8.7	34.8
mash + Econase CE + HPP	0.8	0.1	2.4	2.8	11.3	2.0	11.3	10.5	41.2
mash + Biopectinase CCM	0.3	0.1	1.2	1.5	6.4	1.9	7.3	9.6	28.3
mash + Biopectinase CCM + HPP	0.1	0.1	1.5	2.2	3.7	2.2	12.6	11.8	34.2
mash + Pectinex Ultra SP	0.3	0.1	1.4	1.7	6.9	2.1	8.5	10.7	31.7
mash + Pectinex Ultra SP + HPP	0.1	0.1	1.4	2.1	3.9	2.1	11.0	10.7	31.3

Table 4. Sugar Composition of Black Currant AIS and Extractability to HBSS and ChSS of Cell Wall Polysaccharides in Control Sample and Samples Treated with Biopectinase CCM at Atmospheric and High Pressure

	Rha	Fuc	Ara	Gal	uronic acid	Xyl	Man	Glc	total
A. Mash TP									
AIS (g/kg of mash)	0.9	0.1	3.0	3.0	15.3	2.6	12.8	12.3	49.9
HBSS (% sugar residue relative to AIS)	11	11	10	10	18	4	3	1	8
ChSS (% sugar residue relative to AIS)	4	5	4	3	12	2	1	1	5
B. Biopectinase CCM									
AIS (g/kg of mash)	0.3	0.1	1.2	1.5	6.4	1.9	7.3	9.6	28.4
HBSS (% sugar residue relative to AIS)	27	0	31	24	37	5	7	2	14
ChSS (% sugar residue relative to AIS)	5	0	7	3	14	1	1	0	4
C. Biopectinase CCM + HPP									
AIS (g/kg of mash)	0.1	0.1	1.5	2.2	3.7	2.2	12.6	11.8	34.4
HBSS (% sugar residue relative to AIS)	39	4	7	10	6	3	3	1	3
ChSS (% sugar residue relative to AIS)	7	0	2	2	28	1	1	1	4

A more detailed picture of the nondegradable polysaccharides gives their extractability to HBSS and ChSS (**Table 4**). In the control (mash TP) only a minor part of the hemicellulolytic sugars (xylose, mannose, glucose) was extracted to HBSS and ChSS (2−5%), and ∼15% of the neutral pectic sugar residues (rhamnose, galactose, arabinose) were extracted with these two agents.

Table 4 shows data for the degradation with Biopectinase CCM. The data for Pectinex Ultra SP-L were similar (not shown). Data for Econase CE are not shown, because this enzyme mixture is mainly active on hemicelluloses, which are not extracted with hot buffer or chelating agent. Although only a minor part of the hemicelluloses was extracted in the pectic fractions (HBSS and ChSS), a decreased extractability of hemicelluloses was observed after treatment with Biopectinase CCM at high pressure.

The uronic acid that remained after treatment with Biopectinase CCM at atmospheric pressure was more easily extractable from AIS with hot buffer and chelating agent compared to the control mash, but after treatment at high pressure, uronic acid was mainly extracted with chelating agent. Apparently, enzymes solubilized tightly bound uronic acids and degraded easily extractable pectins (HBSS). The pectin remaining after HPP was mainly calcium bound (ChSS). Rhamnose was removed after treatment with Biopectinase CCM, and the remaining rhamnose was extracted into HBSS. Compared to the control, relatively more of the remaining galactose residues were extracted to HBSS after treatment at atmospheric pressure, whereas no differences in relative extractability were observed after HPP. From the remaining arabinose a higher percentage was extracted with hot buffer after treatment at atmospheric pressure. More tightly bound pectins, probably rhamnogalacturonan I with arabinan and arabinogalactan side chains, were degraded at atmospheric pressure.

Table 5. Degree of Methyl Esterification (DM) and Acetylation (DA) in Black Currant Mash Treated with Different Commercial Enzyme Mixtures with and without HPP

	AIS		HBSS		ChSS	
	DM	DA	DM	DA	DM	DA
mash RT	56	31	84	14	47	6
mash TP	54	16	79	13	45	5
mash + HPP	47	15	71	14	43	5
mash + Econase	35	23	102	11	55	5
mash + Econase + HPP	60	25	73	11	27	5
mash + Biopectinase CCM	37	53	36	19	37	7
mash + Biopectinase CCM + HPP	20	69	6	43	5	4
mash + Pectinex Ultra SP-L	43	49	60	18	32	7
mash + Pectinex Ultra SP-L + HPP	23	51	22	41	12	8

The DM decreased in AIS and ChSS and remained the same in HBSS after HPP of black currant control mash (**Table 5**). This is similar to the results from the previous trial (**Table 2**). No change in DM was observed when the control mash followed the same temperature profile as the HPP control. The DA differed in the three controls (**Table 5**). It decreased in AIS at higher temperature of the treatment, but not after HPP. The DA did not change with temperature or pressure in the pectic fractions (HBSS, ChSS). Thus, the change in acetylation probably occurred in hemicelluloses (vide infra).

The DM decreased in AIS after treatment with Biopectinase CCM at atmospheric pressure and at high pressure even more. The DM was also drastically reduced in HBSS and ChSS after treatment with Biopectinase CCM at high pressure. The DA increased in AIS and HBSS after treatment with Biopectinase CCM. This increase was higher after treatment at high pressure.

Because the DA is calculated per mole of uronic acid, this increase may correlate with the decrease of the uronic acid content. Whether the remaining uronic acid was acetylated is not clear. Acetyl groups could also be attached to hemicelluloses.

DISCUSSION

Effect of HPP on Pectin Structure in Berries. Our current study gives an overview of the polysaccharide composition of cell walls and cell wall fractions in bilberries and black currants before and after HPP. From this overview it can be deduced if HPP has an impact on berry processing. Cell walls are important for the texture of berries. High pressure was not applied on whole berries but on berry mash to have a more homogeneous sample.

The main components that influence the structure and viscosity of berry mash are pectic polysaccharides. After mashing, a highly viscous pectin gel is formed, which hinders pressing and reduces the juice yield (*19*). After HPP, pectic polysaccharides lose methyl esters and, therefore, get more free carboxyl groups. The DM decreases. This results in a higher calcium sensitivity of the pectins (*32*) and, therefore, formation of a stronger pectin gel after HPP, which hampers the release of juice. HPP of tomato products results also in a firmer texture and higher viscosities (*33*−*35*). This is mainly related to the increased activity of pectin methyl esterase (PME), which is supposed to result in a decreased DM (*33*, *36*). Unfortunately, in these studies the DM of tomato pectins was not determined. A decreased DM accompanied by a firmer texture of vegetables was reported for carrots (*37*) and Japanese radish (*38*) after HPP. PME activity of Japanese radish, however, remains the same before and after HPP (*38*). HPP of synthetically amidated, low methyl esterified pectin solutions gives a stronger gel (*10*). Amide groups are known to enhance the hydrogen bonds (*39*), which are particularly affected by HPP (*2*).

It has been suggested that pectic polysaccharides become easier to extract after HPP (*33*). Our results show this clearly. In the first instance it was expected that a decrease in DM would occur in easily extractable and, therefore, easily accessible pectins. This would cause a shift of pectins from HBSS to ChSS (calcium sensitive fraction). However, our results show a different effect. In bilberries the amount of pectic polysaccharides in HBSS remained the same, whereas it increased in HBSS of black currants. A decrease was observed only in DASS. The DM remained the same in HBSS and decreased only in ChSS. This is in contrast to the findings for guava juice, for which the DM remains unchanged in all pectic populations after HPP (*40*). Surprisingly, these authors found the highest DM in DASS, which is very unlikely and raises doubts about the analytical methodology, because alkali saponifies the methyl esters. In mashes of bilberries and black currants, HPP seems to cause modifications in specific populations within the pectic polysaccharides. This is probably an effect of high pressure on pectinolytic enzymes. A possible explanation would be that HPP favors the formation of complexes between enzymes such as PME and pectin substrate, which is deposited in the cell wall as calcium complexes or as complexes with the hemicellulose−cellulose network through hydrogen bonds.

Effect of HPP on Commercial Enzymes. Processing berry mashes with high pressure improves extractability and increases calcium sensibility, leading to a stronger pectin gel. During juice production commercial enzyme mixtures containing mainly pectinolytic enzymes of fungal origin are used to degrade such a pectin gel (*19*). Combining HPP and treatment with commercial enzyme mixtures might have a synergistic effect on the degradation of cell wall polysaccharides. This would lead to a higher juice yield and a better extractability of phenolic compounds into the juice (*20*−*23*). At the same time minor enzyme activities present in commercial enzyme mixtures might be activated and affect cell wall polysaccharides differently than at atmospheric pressure.

Our results show that hemicellulases were active only if the enzymatic treatment is carried out at atmospheric pressure. If high pressure was applied to the sample immediately after the addition of the enzymes, no degradation of hemicelluloses was observed. Apparently, hemicellulases are inactivated by the high pressure applied. This has been shown for polygalacturonases (PG) from different plants as well (*12*, *13*, *17*, *18*).

High pressure did not seem to inactivate exogenous pectinolytic enzymes in the berry mash matrix. This is in contrast to results of isolated plant PG, which are very quickly inactivated with increasing pressure (*12*, *17*, *18*). Commercial pectinolytic enzyme mixtures are mostly of fungal origin. It was expected that they would be inactivated by HPP just like plant PGs. Perhaps fungal PGs differ structurally from plant PGs and are pressure resistant up to 400 MPa. Although similarities in tertiary structure can be observed between fungal and plant PGs, there are major differences in the loop regions (*41*). Another explanation might be that soluble solids in the berry mash protect PG against inactivation, as seen for isolated PME under pressure (*16*). The carrot matrix is able to protect PME under pressure as well (*15*).

However, added pectinolytic enzymes appeared not only pressure stable; in most cases even a further degradation of pectins was observed after HPP. Degradation of uronic acid residues present in homogalacturonan is dependent not only on PG activity but on PME or pectin lyase (PL) activity as well. PG can degrade homogalacturonan only if it is deesterified, for example, by PME. PL degrades only methyl-esterified homogalacturonan. Homogalacturonan degradation was observed after treatment with Biopectinase CCM and Pectinex Ultra SP-L at atmospheric pressure. HPP decreased the content of uronic acid even further. This activation can have several causes. First of all, we showed a decrease of the DM in AIS after HPP of berry mash. A lower DM facilitates the action of PG. Next to endogenous PME, PME was added to the mash by adding commercial enzyme mixtures. Although this PME is from fungal origin, it might also be activated under high pressure in a similar way as plant PME (*13*−*16*). The observed further decrease of the DM after HPP supports this possibility. From our results it appears that PG retains its activity up to 400 MPa. It might even be activated under high pressure and would then be able to degrade the substrate further during the incubation time after HPP. Another option is the formation of an energetically beneficial complex between enzyme and substrate under high pressure, resulting in more degradation when both substrate and enzyme are present during HPP. In this case PG activity may not necessarily change after the pressure is released.

The content of rhamnose, which is mainly present in the pectic polysaccharide rhamnogalacturonan I, decreased after treatment with Biopectinase CCM or Pectinex Ultra SP-L at atmospheric pressure and decreased even further after treatment at high pressure. This is an indication for an activation of rhamnogalacturonan I degrading enzymes such as rhamnogalacturonases present in these two enzyme mixtures (*42*). Rhamnogalacturonan I was degraded into small alcohol soluble oligomers that are removed during alcohol extraction. The question of whether improved degradation of rhamnogalacturonan I is really due to activation of the enzyme or improved

accessibility cannot be answered, although such a difference between treatments at atmospheric pressure and at high pressure is a strong indication for enzyme activation.

The DA increased after treatment with pectinolytic enzymes and even more when in combination with HPP. This increase in DA correlates with the decrease of uronic acid. Because the DA was calculated per mole of uronic acids, a decrease in uronic acid content while retaining the acetyl groups results in an increased DA. The amount of acetyl esters present did not change. We assumed before that a significant part of the acetyl substitution is on the hemicelluloses (*24*). Why the DA decreases in the control when the same temperature profile is followed as under HPP is, however, not clear.

If high pressure is applied to bilberry or black currant mash, to which a commercial enzyme mixture is added, hemicelluloses are not affected, probably due to an inactivation of hemicellulases. Pectic polysaccharides (mainly homogalacturonan and rhamnogalacturonan I) are, however, degraded to a larger extent compared to treatment at atmospheric pressure. Thus, treatment with commercial enzymes in combination with controlled HPP opens possibilities to tailor enzymatic modification of polysaccharides and, therefore, the texture or other properties such as extractability of phenolic compounds into juice. Detailed analysis of juice yields and polyphenol composition of juices produced by HPP will be reported later. Our study included only one condition for the HPP treatment that was shown to be effective in preliminary experiments. Nevertheless, evidence is presented that HPP has the potential to affect cell wall polysaccharides, particularly in combination pectinolytic enzymes, possibly improving processing of bilberries and black currants.

ABBREVIATIONS USED

AIS, alcohol insoluble solids; CASS, concentrated alkali soluble solids; ChSS, chelating agent soluble solids; DA, degree of acetylation; DASS, diluted alkali soluble solids; DM, degree of methyl esterification; HBSS, hot buffer soluble solids; HPAEC, high-performance anion exchange chromatography; HPP, high-pressure processing; PG, polygalacturonase; PL, pectin lyase; PME, pectin methyl esterase.

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