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The pectic polysaccharide rhamnogalacturonan II is present as a dimer in pectic populations of bilberries and black currants in muro and in juice

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Abstract

Rhamnogalacturonan II (RG II) can play an important role during processing of berries due to its enzyme resistance and its possible role as a pectic cross-linker. This article describes the presence of RG II in cell walls, in juice and in press cake of bilberries and black currants. RG II was identified and quantified via its diagnostic sugar residues. RG II, which was released from homogalacturonan, was probably present in its dimeric form in muro. Juice contained the free RG II dimer, while from press cake dimeric RG II was released by enzymatic degradation of homogalacturonan. A higher amount of RG II was present in juice than in press cake. During juice processing a cross-linker RG II might improve gel formation, which hinders the processability of berries. In addition, enzymes used during juice processing release dimeric RG II from pectin molecules and accumulate RG II in the juice.

Keywords: *Vaccinium myrtillus*; Bilberry; *Ribes nigrum*; Black currant; Cell wall polysaccharides; Rhamnogalacturonan II; Juice; Berries

1. Introduction

Bilberries and black currants are important crops in the Scandinavian countries. They have a strong and typical flavour and are rich in polyphenols (Buchert et al., 2005). Most berries are processed to juice. Due to the formation of a strong pectin gel after mashing, pectinolytic enzyme mixtures are used for juice (Grassin & Fauquembergue, 1996) and polyphenol extraction (Bagger-Jorgensen & Meyer, 2004; Landbo & Meyer, 2001; Meyer, 2002). After degradation with commercial pectinolytic enzymes, some polysaccharides resistant to enzymatic degradation remain in the juice (Hilz, Bakx, Schols, & Voragen, 2005).

Pectic polysaccharides are a major part of the plant cell wall. They form a network independent from the hemicellulose/cellulose network (McCann & Roberts, 1991), and influence cell wall properties such as porosity or tensile strength (Fleischer, O'Neill, & Ehwald, 1999; Ryden et al., 2003; Titel & Ehwald, 1999). Pectins consist of three major structural elements: homogalacturonan, which is a linear chain of α-1,4-linked galacturonic acids, rhamnogalacturonan I, which consists of a back bone of altering

Abbreviations: AIS, alcohol insoluble solids; CASS, concentrated alkali soluble solids; ChSS, chelating agent soluble solids; DASS, diluted alkali soluble solids; DHA, 3-deoxy-D-lyxo-heptulosaric acid; DM, degree of methyl esterification; EI, electro ionisation; FID, flame ionisation detector; GC, gas chromatography; HBSS, hot buffer soluble solids; HPSEC, high-performance size-exclusion chromatography; KDO, 3-deoxy-D-manno-octulosonic acid; MHR, modified hairy regions; MS, mass spectrometry; PC, press cake; RG II, rhamnogalacturonan II; TMS, trimethyl silyl.

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α-1,4-linked galacturonic acid and β-1,2-linked rhamnose units (De Vries, 1988), and the complex rhamnogalacturonan II (RG II).

RG II is a well-defined structural element of pectin. It is of low molecular weight and was first described in sycamore cells (Darvill, McNeill, & Albersheim, 1978). Its 12 different sugars that are connected via 20 different linkages make the polysaccharide the most complex known in nature to date. Attached to a backbone of 8–10 galacturonic acid residues (Melton, McNeill, Darvill, & Albersheim, 1986; Vidal et al., 2000) rhamnogalacturonan II contains four side chains with rare and therefore diagnostic sugars: 2-*O*-methyl fucose (Barrett & Northcot, 1965; Darvill et al., 1978), 2-*O*-methyl xylose (Barrett & Northcot, 1965; Darvill et al., 1978), apiose (Darvill et al., 1978), 3-*C*-carboxy-5-deoxy-L-xylose (aceric acid) (Spellman, McNeill, Darvill, Albersheim, & Hendrick, 1983), 3-deoxy-D-lyxo-2-heptulosaric acid (KDO) (Stevenson, Darvill, & Albersheim, 1988), and 3-deoxy-D-manno-2-octulosonic acid (DHA) (York, Darvill, McNeill, & Albersheim, 1985). In cell walls, the galacturonic acid backbone of RG II is unesterified when RG II is located near the plasma membrane and methyl esterified when RG II is located in the primary cell wall, as shown by immunolabelling (Williams, Freshour, Darvill, Albersheim, & Hahn, 1996). In the middle lamella no RG II could be immunolabelled (Matoh, Takasaki, Takabe, & Kobayashi, 1998). The apiose units in side chain A (2-*O*-methyl xylose containing side chain) of two RG II monomers can be esterified with boric acid (Ishii et al., 1999; Matoh, Ishigaki, Ohno, & Azuma, 1993; Mazeau & Perez, 1998; O'Neill et al., 1996). This may be a covalent cross-link between two pectin molecules in muro (O'Neill et al., 1996).

RG II can be released from homogalacturonan by enzymatic degradation with *endo*-polygalacturonase (Darvill et al., 1978; Ishii & Matsunaga, 2001). Polygalacturonase (PG) is involved in different processes in food manufacture such as vinification (yeast PG) or berry juice production (fungal PG as processing aid). During vinification RG II is released and not degraded by naturally occurring enzymes. This results in accumulation of RG II in wine, where it is stable for even more than 10 years (Doco & Brillouet, 1993; Doco, Quellec, Moutounet, & Pellerin, 1999). After liquefaction of fruits and vegetables, RG II is the dominant polysaccharide in apple, carrot and tomato juice (Doco, Williams, Vidal, & Pellerin, 1997). Consequently RG II, if present in berry cell walls, is expected to be present in berry juice produced with the help of enzymes, as well.

The research described in this study is part of a project aiming at maximising yield and functionality of berry products by using novel enzyme-aided extraction technologies. Due to its presence in different juices, RG II has been a target of degradation studies with fungal enzyme extracts (Vidal, Salmon, Williams, & Pellerin, 1999). This study describes the presence of RG II in cell wall material of berries, juice and press cake of bilberries and black currants. Pectins were sequentially extracted from cell wall material. In three obtained fractions the content of RG II was determined and a mass balance of RG II over the various pectic populations is presented. Finally, we investigated in which polysaccharide fractions RG II was bound to homogalacturonan and where it was present as monomer or as a dimer.

2. Materials and methods

2.1. Material

Bilberries (*Vaccinium myrtillus* L.) and black currants (*Ribes nigrum* L.) and their commercial juice concentrates and press cakes were obtained from Kiantama Ltd., Suomussalmi, Finland. Alcohol insoluble solids (AIS), hot buffer soluble solids (HBSS), chelating agent soluble solids (ChSS), diluted alkali soluble solids (DASS), concentrated alkali soluble solids (CASS), the remaining residue and modified hairy regions (MHR) were prepared as described before (Hilz et al., 2005).

2.2. Neutral sugar composition as alditol acetates

Neutral sugars were determined as alditol acetates after TFA hydrolysis by GLC (Harris, Henry, Blakeney, & Stone, 1984). Separation was carried out on a DB225 column (15 m × 0.53 mm ID; 1.0 μm film; J&W Scientific) with helium as carrier gas (0.6 bar inlet pressure). Inositol was used as internal standard.

2.3. Sugar composition as trimethyl silyl derivatives

The determination of the sugar composition including common and rare acidic sugars, as present in rhamnogalacturonan II, was done after methanolysis and derivatisation to trimethyl silyl (TMS) derivatives (Doco, O'Neill, & Pellerin, 2001). The TMS derivatives were prepared using hexamethyldisilazane–trimethyl chlorosilane–pyridine, 3:1:9 (Syl-PREP Kit, Alltech), and analysed by GC-EI-MS using a Hewlett Packard mass selective detector 5970-B coupled to a Hewlett Packard 5890 GLC equipped with a DB-1 capillary column (30 m × 0.25 mm, 0.25 μm film; J&W Scientific). Quantification was done on a Carlo Erba HRGC 5160 system using the same column and flame ionisation detection.

2.4. Formation of monomers and dimers of RG II

Rhamnogalacturonan II dimers were converted to the monomer in 0.1 M hydrochloric acid and back to the dimer by incubation in the presence of boric acid at pH 3.5 (Ishii et al., 1999). High-performance size-exclusion chromatography (HPSEC) was performed on three TosoHaas TSK-Gel G columns in series (4000PWXL–3000PWXL–2500PWXL) using 0.2 M sodium nitrate as eluent (Chen, Schols, & Voragen, 2004).

2.5. Pectin degradation with polygalacturonase in combination with pectin methyl esterase and with polygalacturonase in combination with pectin lyase

Five milligrams of the different fractions was dissolved in 1 mL sodium acetate buffer (50 mM, pH 5) and incubated with addition of 5 μL of *endo*-polygalacturonase (EC 3.2.1.15 from *Kluyveromyces fragilis*, 16 U/mL) and 1 μL of pectin methyl esterase (EC 3.1.1.11 from *Aspergillus niger*, 180 U/mL) or 5 μL of pectin lyase (EC 4.2.2.10 from *A. niger*, 8.8 U/mL) overnight, respectively. The incubated samples were analysed on HPSEC and compared with non-treated material.

3. Results

3.1. Rhamnogalacturonan is present in all pectic fractions

Rhamnogalacturonan II (RG II) contains six diagnostic sugars, of which 2-*O*-methyl fucose and 2-*O*-methyl xylose were determined after hydrolysis with trifluoro acetic acid as alditol acetates using GC. Alcohol insoluble solids (AIS) and all three pectic fractions from bilberries and black currants contained 2-*O*-methyl fucose and 2-*O*-methyl xylose as minor sugar residues (Tables 1 and 2).

Black currant AIS contained more 2-*O*-methyl fucose and 2-*O*-methyl xylose than bilberry AIS, probably because less pectin is present in bilberry AIS (Hilz et al., 2005). In bilberries and black currants, hot buffer soluble solids (HBSS) contained the highest amount of 2-*O*-methyl fucose and 2-*O*-methyl xylose of the three pectic fractions, whereas chelating agent soluble solids (ChSS) contained the smallest amount. 2-*O*-Methyl fucose and 2-*O*-methyl xylose in the residue probably originated from pectic polysaccharides present in the seeds. The latter remained intact during extraction and were recovered in the residue. Although black currants contain higher amounts of seeds (Hilz et al., 2005), more 2-*O*-methyl fucose was found in bilberry residue than in black currant residue.

In the pectic fractions of bilberries 2-*O*-methyl xylose was present in similar concentrations as 2-*O*-methyl fucose. In most fractions of black currants, however, more 2-*O*-methyl fucose than 2-*O*-methyl xylose was present. This is in contrast to the commonly reported molar ratio of these sugars in an RG II molecule of 1:1. In the residue of bilberries and black currants even no 2-*O*-methyl xylose could be detected. The recovery of 2-*O*-methyl xylose in the different fractions is too high (147%). Probably, RG II was incompletely hydrolysed with TFA in AIS. With methanolysis followed by TMS derivatisation the obtained ratio was approximately 1:1, as expected (Table 4). Therefore, the content of RG II was estimated on the basis of 2-*O*-methyl fucose. Although being a minor component, RG II was present up to 9% of polysaccharides in AIS and the different pectic fractions.

By analysing juice modified hairy regions (MHR) and press cake (PC), it was possible to trace RG II during juice processing. In juice MHR of bilberries and black currants approximately the same amounts of the 2-*O*-methyl fucose and 2-*O*-methyl xylose were found. RG II is the major polysaccharide present, representing about 34% of the total polysaccharides. In PC of bilberries 2-*O*-methyl fucose and 2-*O*-methyl xylose were present in the same amounts, while in black currant PC the amount of 2-*O*-methyl fucose exceeded the amount of 2-*O*-methyl xylose (similar to AIS). The content of these diagnostic sugars indicates that more RG II was extracted into the juice than remained in the press cake.

In AIS, HBSS, ChSS, DASS, the residue, juice MHR and PC AIS, the diagnostic sugars (apiose, aceric acid, KDO and DHA) were unambiguously identified using GC-FID-MS after methanolysis and derivatisation to TMS sugars. The cell wall fractions of bilberries (Table 3) and black currants (Table 4) contained the diagnostic sugars in similar molar ratios, although in low amounts. With the exception of PC AIS of bilberries, 2-*O*-methyl fucose and 2-*O*-methyl xylose are present in the same amounts. The RG II molecule contains two apiose residues, one 2-*O*-methyl fucose residue and one 2-*O*-methyl xylose residue, respectively. However, per mole 2-*O*-methyl fucose (2-*O*-methyl xylose, respectively) only 1 mol apiose was present. This is due to difficulties in peak separation of apiose and arabinose, which was present in 100-fold higher amounts. Difficulties in peak separation caused deviations in the molar percentages of the acidic sugars, as well.

Table 1
Content of 2-*O*-methyl fucose, 2-*O*-methyl xylose and rhamnogalacturonan II in pectic fractions of bilberries

Bilberry	2-*O*-Methyl fucose[a] (mg/kg berry)	2-*O*-Methyl xylose[a] (mg/kg berry)	Total CWPS (mg/kg berry)	app. RG II (% of PS)
AIS	5	5	15,238	2
HBSS	2 (29%)	1 (20%)	1,464	8
ChSS	1 (20%)	1 (13%)	1,020	6
DASS	1 (25%)	1 (19%)	650	9
CASS	0 (3%)	1 (17%)	978	1
Residue	3 (49%)	0 (0%)	10,608	2
Juice MHR	5 (87%)	4 (67%)	902	33
Press cake AIS	3 (60%)	3 (50%)	11,584	2

[a] In brackets sugars present in the fraction as % of sugar present in AIS.

Table 2
Content of 2-*O*-methyl fucose, 2-*O*-methyl xylose and rhamnogalacturonan II in pectic fractions of black currants

Black currants	2-*O*-Methyl fucose[a] (mg/kg berry)	2-*O*-methyl xylose[a] (mg/kg berry)	Total CWPS (mg/kg berry)	App. RG II (% of PS)
AIS	19	9	34,875	3
HBSS	9 (47%)	5 (48%)	7,011	8
ChSS	3 (15%)	2 (18%)	2,463	7
DASS	5 (29%)	5 (57%)	3,985	8
CASS	1 (4%)	0 (0%)	2,344	3
Residue	2 (12%)	0 (0%)	15,179	1
Juice MHR	12 (64%)	9 (95%)	2,090	34
Press cake AIS	9 (51%)	5 (51%)	15,980	3

[a] In brackets sugars present in the fraction as % of sugar present in AIS.

Table 3
Sugar composition of bilberries and fraction derived from them analysed as TMS derivatives using GC-FID (mol%)

	2-*O*-Meth fuc	Rha	Fuc	2-*O*-Meth xyl	Ara	Api	Xyl	Man	Gal	Glc	GalA	GlcA	AceA	DHA	KDO	Total sugars (w/w%)
AIS	0.2	17.9	0.5	0.3	22.8	0.3	2.5	1.1	14.0	11.4	27.7	0.3	0.2	0.5	0.2	11.9
HBSS	0.1	2.0	0.4	0.1	7.1	0.2	0.8	1.0	12.9	2.1	72.5	0.7	0.2	0.0	0.3	39.2
ChSS	0.0	1.0	0.2	0.0	3.1	0.1	0.7	0.3	7.8	2.6	83.2	0.6	0.1	0.1	0.4	30.1
DASS	0.3	3.8	0.6	0.2	10.4	0.2	1.8	2.4	18.7	3.2	55.9	0.8	0.2	0.2	0.2	20.0
CASS	n.d.	2.3	0.3	n.d.	2.6	0.0	0.6	1.7	27.6	35.9	24.8	0.8	n.d.	n.d.	n.d.	4.0
Residue	0.4	26.0	0.4	0.5	33.3	0.1	1.8	1.0	8.8	12.1	14.8	0.3	0.4	0.2	0.4	18.4
Juice MHR	0.4	8.0	0.7	0.3	30.2	0.4	1.6	1.2	21.2	14.4	19.3	0.6	0.4	0.5	0.7	45.7
Press cake AIS	0.1	22.5	0.6	0.4	28.1	0.1	2.1	1.2	13.1	10.6	20.3	0.3	0.4	0.1	0.5	15.9

n.d., not detected.

Table 4
Sugar composition of black currants and fraction derived from them analysed as TMS derivatives using GC-FID (mol%)

	2-*O*-Meth fuc	Rha	Fuc	2-*O*-Meth xyl	Ara	Api	Xyl	Man	Gal	Glc	GalA	GlcA	AceA	DHA	KDO	Total sugars (w/w%)
AIS	0.6	5.1	0.8	0.7	17.0	0.7	1.4	14.1	7.8	29.0	0.9	0.9	0.7	0.6	0.0	23.8
HBSS	0.4	2.4	1.3	0.3	9.1	0.4	1.0	1.1	2.8	0.7	78.9	0.4	1.0	0.7	0.1	49.0
ChSS	0.3	1.9	0.5	0.2	10.2	0.2	2.5	0.4	4.4	0.5	77.0	0.7	0.2	1.2	0.3	34.9
DASS	0.3	3.4	0.4	0.1	17.6	0.1	2.3	1.0	30.0	0.6	42.6	0.6	0.5	0.9	0.1	28.7
CASS	n.d.	3.2	0.5	n.d.	3.9	0.0	0.1	12.6	29.1	34.2	12.9	2.4	n.d.	n.d.	n.d.	4.7
Residue	0.1	3.9	0.2	0.1	8.3	0.2	0.7	50.3	12.3	14.9	8.6	0.3	0.4	0.3	0.0	22.0
Juice MHR	0.5	9.0	0.6	0.4	20.9	0.5	0.5	0.8	6.2	7.2	50.4	0.4	0.7	0.4	0.5	50.0
Press cake AIS	0.1	4.4	0.7	0.2	9.8	0.2	0.7	38.8	13.4	13.0	16.8	0.4	1.2	0.4	0.0	23.1

n.d., not detected.

3.2. Rhamnogalacturonan II is present as a dimer in juice

To investigate whether RG II is present as monomer or dimer in juice, RG II was monomerised and redimerised in bilberry and black currant juice MHR and analysed on by HPSEC. The dimer of RG II elutes in our HPSEC system after 27.0 min and the monomer after 27.8 min (Figs. 1 and 2).

When bilberry juice MHR was dissolved at pH 3.5, peaks at retention times of both monomer and dimer were present. After treatment of bilberry juice MHR with 0.1 N hydrochloric acid, the peak at 27.0 min disappeared, although the RI response did not reach the baseline. The borate-diol ester of the RG II dimer was hydrolysed by hydrochloric acid treatment. Apparently, other material with the same hydrodynamic volume was present, since pure RG II was converted quantitatively by the same treatment. The shoulder at 27.8 min seemed to be enlarged after acid treatment, due to the formation of monomeric RG II. After incubation with boric acid, the peak at 27.0 min reappeared and the shoulder at 27.8 min decreased slightly. The RG II monomers were esterified by borate to form the dimer again. The remaining peak at 27.8 min was probably another population of polysaccharides. However, for bilberry MHR it cannot be excluded that RG II was partly present in monomeric form, as well.

The pattern of black currant juice MHR showed a distinct peak at 27.8 min (Fig. 2). The conversion from dimer to monomer after acid treatment was, however, only visible by the disappearance of a shoulder at 27.0 min. This shoulder reappeared after incubation with boric acid. The difference between the shoulder at 27.0 min and the peak at

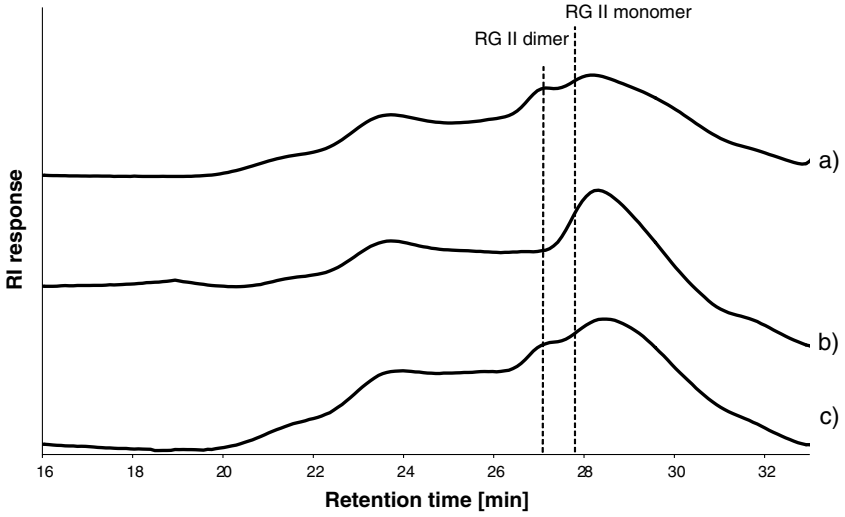

Fig. 1. HPSEC elution patterns of bilberry juice MHR (a) in sodium acetate buffer, pH 3.5, (b) after treatment with 0.1 M HCl dialysed with sodium acetate buffer, pH 3.5, and (c) after treatment with 0.1 M HCl dialysed with sodium acetate buffer, pH 3.5, containing 50 mM boric acid. Vertical lines indicate the elution time of RG II monomer and dimer.

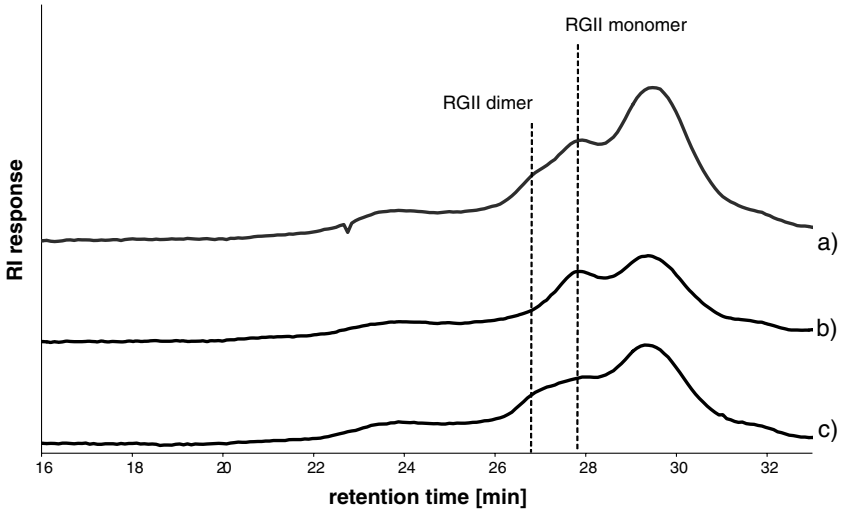

Fig. 2. HPSEC elution patterns of black currant juice MHR in (a) sodium acetate buffer, pH 3.5, (b) after treatment with 0.1 M HCl followed by dialysis against sodium acetate buffer pH 3.5, and (c) after treatment with 0.1 M HCl followed by dialysis against sodium acetate buffer, pH 3.5, containing 50 mM boric acid. Vertical lines indicate the elution time of RG II monomer and dimer.

27.8 min is much smaller after acid treatment and incubation with boric acid compared to black currant MHR dissolved at pH 3.5. Thus, monomeric RG II might be present in black currant MHR, as well.

3.3. Rhamnogalacturonan II is released by enzymatic degradation of pectin

RG II is linked to pectin and can be released by enzymatic degradation of homogalacturonan (Darvill et al., 1978; Ishii & Matsunaga, 2001). Thus, AIS and the pectic fractions of the berries were incubated with homogalacturonan degrading enzymes to release RG II. Polygalacturonase (PG) combined with pectin methyl esterase (PME) or PG combined with pectin lyase (PL) were chosen to degrade methyl esterified and non-methyl esterified homogalacturonan.

As an example, Fig. 3 shows the degradation of bilberry HBSS with PG/PME and PG/PL. After enzyme incubation a peak at 27.0 min appeared, representing dimeric RG II. Dimeric RG II was found after degradation of black currant HBSS and of bilberry and black currant AIS, as well. After pectin degradation in ChSS and DASS of bilberries and black currants, not only a peak at 27.0 min, but also a peak at 27.8 min appeared (data not shown), indicating the presence of monomeric and dimeric RG II. With HPSEC it was possible to estimate the contents of RG II by dividing the area of RG II (monomer and dimer) by

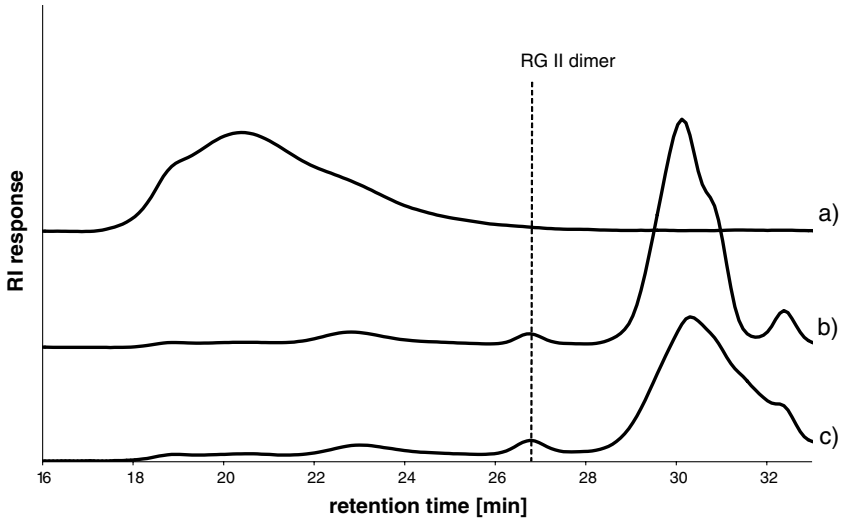

Fig. 3. HPSEC elution pattern of bilberry HBSS (a) before and after incubation with (b) PG/PME and (c) PG/PL at pH 5.

the total area of degradation products (data not shown). Similar RG II contents were obtained as calculated via the content of 2-*O*-methyl fucose (Table 1).

4. Discussion

Rhamnogalacturonan II (RG II) is known to be present in the cell walls of various angiosperms, as recently reviewed (O'Neill, Ishii, Albersheim, & Darvill, 2004). We have shown that black currants and bilberries make no exception. All pectic fractions contain RG II, as shown by the presence of all its diagnostic sugar residues. RG II as a possible cross-linker might influence the extractability of cell walls, which can be an important factor in juice processing. If RG II is present as a monomer, extraction of pectic polysaccharides is easier (Kakegawa, Ishii, & Matsunaga, 2005). In ChSS and DASS of radish roots, RG II was identified and structurally analysed (Matoh et al., 1998). However, RG II was not quantified in cell wall material of bilberries and black currants before.

For quantification of RG II in different polysaccharide samples, TFA hydrolysis and derivatisation as alditol acetates is a suitable method. We were able to quantify RG II in pectin-rich fractions, in juice and in press cake of bilberries and black currants, although the analysis was carried out close to the detection limit. The diagnostic acidic sugars present in RG II were identified after methanolysis of the different fractions followed by TMS derivatisation. This method is suitable for quantification of soluble material such as juice MHR. Compared to previous results, where Saeman-hydrolysis and derivatisation as alditol acetates was performed (Hilz et al., 2005), hardly any cellulose and hemicellulose could be determined.

In juice production, RG II is an important structural element because of its possible role as a cross-linker of pectins and because of its enzyme resistance. By analysing juice MHR and cell wall polysaccharides in the press cake, we traced RG II during juice processing. In juice MHR of bilberries and black currants, RG II was the main polysaccharide and was present in higher relative amounts than in the cell wall fractions. During juice production pectinolytic enzymes are used to degrade pectins. Because the applied enzyme preparation was not able to degrade RG II, this pectic element accumulated and was extracted into the juice (about 60% of total RG II). The press cakes contained still about 40% of the RG II present in the berries. This RG II was, however, still linked to homogalacturonan and probably originated from the seeds, which ended up intact in the press cake. RG II accumulates in wine after enzymatic degradation of pectins during vinification, as well (Ayestaran, Guadalupe, & Leon, 2004). We showed that at least the major part of RG II is present as a dimer in juice, although berry juice is quite acidic (pH 2.8). Neither the applied enzyme preparation nor the acidity saponified the boron ester that cross-links the two RG II molecules.

By degradation with pectinolytic enzymes RG II was released from homogalacturonan in cell wall material and in all pectic fractions. This confirms that RG II is covalently linked to homogalacturonan (Ishii & Matsunaga, 2001; Reuhs et al., 2004). While RG II was released as only dimer from HBSS and AIS, RG II was released as both monomer and dimer from pectins present in ChSS and DASS. These fractions were treated with the chelating agent EDTA. EDTA forms a complex with calcium and removes calcium from the calcium–pectin complex. Insoluble, calcium-sensitive pectins become soluble. Calcium was found to stabilise the RG II diester (Ishii et al., 1999; Kobayashi, Nakagawa, Asaka, & Matoh, 1999; Wimmer & Goldbach, 1999). With the help of chelating agents, monomeric RG II is formed from the dimer. EDTA is not able to complete this conversion (Ishii et al., 1999), whereas CDTA is (Matoh et al., 1998). Monomerisation of RG II is probably due to complexation of stabilising calcium and not due to interaction of chelating agent with the boron ester itself. The boron ester might help to retain chelating agent soluble pectins in the cell wall (Kobayashi et al., 1999). We suggested that

the release of pectins with a high degree of methyl esterification (DM), thus calcium-insensitive pectin, with chelating agent may partly be caused by hydrolysing the boron diester of RG II that is linked to homogalacturonan with a high DM (Hilz et al., 2005). Thus, the RG II monomers in ChSS and DASS of bilberries and black currants were probably formed during extraction. However, the possibility that the monomeric form is present in muro cannot be ruled out.

The results of this study may indicate that RG II is an important polysaccharide for the juice industry. It is present in all pectic population of bilberries and black currants, in muro probably always in the dimeric from. It might cross-link two pectin molecules and, therefore, improve gel formation, which hinders the processability of berries. Furthermore, RG II is released from pectin molecules and is accumulated in the juice during processing. Enzymatic degradation of the covalent, pectic RG II might enable the production of special fruit juices.

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