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Cell wall polysaccharides in black currants and bilberries—characterisation in berries, juice, and press cake

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Abstract

Cell wall polysaccharides from black currants and bilberries were characterised in three approaches. First, compositions of skin, pulp, and seeds show the distribution of polysaccharides over these tissues. A sequential extraction of cell wall material with different aqueous extractants informs about the extractability of the different polysaccharides, viz. pectins, hemicellulose, and cellulose. Finally, by isolation of cell wall polysaccharides from juice and press cakes obtained by the conventional juice manufacturing. The polysaccharide distribution was followed during juice processing. The main difference between bilberries and black currants is the dominant sugar residue in seeds: mannose for black currants and xylose for bilberries. Most of the hemicellulolytic sugars and cellulose can be found back in the press cake. The sugar composition of the press cake is similar to the composition of the residue after sequential extraction. Black currants contain more pectic sugars than bilberries. Consequently, a commercial enzyme used during processing releases more pectic material into the juice.

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

1. Introduction

In the food industry most of black currants (*Ribes nigrum* L.) and bilberries (*Vaccinium myrtillus* L.) are processed to juice. This juice can be used directly for human consumption or as a food ingredient. With only pressing, juice yields from these berries are very low because a highly viscous pectin gel is formed after mashing. Therefore, cell wall degrading enzymes, mainly pectinolytic enzymes, are used during conventional berry processing (Grassin & Fauquembergue, 1996). An additional effect of enzyme treatment is improved colour extraction (Grassin & Fauquembergue, 1996), currently one of the most valuable characteristics of berry juice next to flavour.

In the future not only yield, flavour, and colour, but also the presence of bio-functional constituents might be of importance for berry juice. Anthocyanins and other polyphenolic compounds present in berries probably promote human health (Frankel, 1999). Next to polyphenols, cell wall polysaccharides may be valuable healthy compounds since they are belonging to the class of dietary fibre (Anderson, 1990; Sembries et al., 2003; Yamada, 1994; Yamada, 1996).

Plant cell walls consist of a firm network of hemicelluloses and cellulose, which is embedded in a matrix of pectins (McCann & Roberts, 1991). The main

Abbreviations: 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; DMSO, dimethyl sulfoxide; dRGII-B, dimeric rhamnogalacturonan II cross-linked by boron; EDTA, ethylenediaminetetraacetic acid; HBSS, hot buffer soluble solids; HGA, homogalacturonan; HPAEC, high performance anion exchange chromatography; HPSEC, high performance size exclusion chromatography; MHR, modified hairy regions; NBPC, non-berry press cake; RG, rhamnogalacturonan; TFA, trifluoro acetic acid.

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hemicellulolytic polysaccharides in dicotyledonous plants are xyloglucans, which mainly consist of a β-1,4-linked glucose backbone that is substituted at O-6 with xylose residues (McNeill, Darvill, Fry, & Albersheim, 1984). Pectic polysaccharides consist of two main structural elements: rhamnogalacturonan I (RG I, 'hairy regions') and homogalacturonan (HGA, 'smooth regions'; Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Rhamnogalacturonan I contains a backbone of alternating rhamnose and galacturonic acid residues to which neutral side chains such as arabinans and type I and II arabinogalactans are attached (Schols & Voragen, 2002). Homogalacturonan is a long linear chain of 1,4-linked galacturonic acids, which can be methyl esterified or in position 2 or 3 acetylated (Quemener, Pino, Ralet, Bonnin, & Thibault, 2003). Embedded in homogalacturonan is rhamnogalacturonan II (RG II; Ishii & Matsunaga, 2001), which has a backbone of 8–10 galacturonic acids with four complex side chains consisting of 12 different sugars and 20 different linkages. Rhamnogalacturonan II can form a dimer through an apiose-boron ester (Kobayashi, Matoh, & Azuma, 1996; Rodriguez Carvajal, du Penhoat, Mazeau, Doco, & Perez, 2003), which influences the pore size of the cell wall (Fleischer, O'Neill, & Ehwald, 1999).

The first studies on cell wall carbohydrates in black currants and bilberries were limited to pectin contents, which vary between 0.20 and 1.79 g/100 g for black currants and between 0.10 and 0.78 g/100 g for bilberries (Fuchs & Wretling, 1991; Lampitt & Hughes, 1928; Letzig, 1950; Money & Christian, 1950). Salo and Suomi (1972) investigated hemicellulolytic sugars in bilberries and black currants and found high contents of mannose in black currant seeds and of xylose in bilberry seeds. However, they do not give a distribution of hemicellulolytic sugars over the different tissues of the berries.

During the juice manufacturing process high amounts of press cake are obtained. The press cake, which contains not only 70% of polyphenols originally present in berries (Meyer, 2002) but also large amounts of cell wall polysaccharides, might be a valuable source for health promoting and colour giving compounds. To date, press cake is burned, since its value is not accessible yet. However, some valuable compounds can be released using cell wall degrading enzymes (Meyer, 2002). On the one hand, this can lead to high quality juices with improved colour and potential health promoting effects and enables higher yields. On the other hand, the lower amount of press cake can be further upgraded by enzyme treatment to yield new products such as dietary fibre.

In this paper we present the first characterisation of cell wall polysaccharides in bilberries and black currants. We separated berries in skin, pulp, and seeds, extracted berry cell wall material with different aqueous extractants, and also included material from current juice processing in our study.

2. Materials and methods

2.1. Plant material

Commercial berries, juice, and press cake from bilberries (*Vaccinium myrtillus* L.) and black currants (*Ribes nigrum* L.) were obtained from Kiantama Ltd, Finland. Damaged fruits were discarded. Frozen berries were separated into skin and pulp containing seeds. Pulp and seeds were separated after preparation of alcohol insoluble solids (AIS; vide infra). The press cake from black currants had to be separated into berry material and stams and wooden parts. The last part is further referred to as non-berry press cake (NBPC).

2.2. Preparation of alcohol insoluble solids and sequential buffer extraction

Cell wall material was precipitated with 70% aqueous ethanol (alcohol insoluble solids, AIS) and sequentially extracted with 0.05 M sodium acetate buffer at pH 5.2 and 70 °C (hot buffer soluble solids, HBSS), 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), 0.05 M sodium hydroxide at 0 °C (diluted alkali soluble solids, DASS), and 6 M sodium hydroxide at 0 °C (concentrated alkali soluble solids, CASS) as described by De Vries, Voragen, Rombouts, and Pilnik (1981) adapted by Vierhuis, Schols, Beldman, and Voragen (2000). The juice was ultrafiltrated through a 10 kDa membrane (Milipore Pellicon 2 MINI 10k B-10AQ 0.1 m^2) in a Masterflex Consoledrive L/S 77250-62 system to obtain the polysaccharides from juice (juice MHR; vide infra).

2.3. Sugar composition

Sugar composition was determined after Seaman hydrolysis. After a prehydrolysis step using 72% w/w sulphuric acid at 30 °C for 1 h the samples were hydrolysed with 1 M sulphuric acid at 100 °C for 3 h. Afterwards the sugars were derivatised as alditol acetates and determined by gas chromatography (Englyst & Cummings, 1984) using inositol as internal standard.

2.4. Uronic acid content

The total uronic acid content was determined with the automated m-hydroxydiphenyl assay (Thibault, 1979). Differential determination of galacturonic acid and glucuronic acid was carried out by HPAEC after methanolysis according to De Ruiter, Schols, Voragen, and Rombouts (1992).

2.5. Degree of acetylation and methyl esterification

Degree of acetylation and methyl esterification were determined by HPLC after hydrolysis with 0.4 N sodium

hydroxide in isopropanol/water (50/50 v/v; Voragen, Schols, & Pilnik, 1986). The degree of acetylation and methyl esterification are calculated as mole methyl/acetyl groups per 100 mole galacturonic acid. One mole galacturonic acid can carry only one mol methyl esters and two moles acetyl esters.

2.6. Protein content

Protein content was determined using the combustion method (Dumas, 1831) on a Thermo Quest NA 2100 Nitrogen and Protein Analyser (Interscience, The Netherlands) according to the instructions of the manufacturer. 5–6 mg sample were weighed into a sample cup and directly analysed. D-Methionine was used as external standard. The protein content was calculated using 6.25 as nitrogen to protein conversion factor.

2.7. Soluble polyphenols

To determine the content of soluble polyphenols 1 ml water was added to 50 mg of the sample. This suspension was placed into an ultrasonic bath for 20 min and then centrifuged. The photometric absorption was measured after reaction with Folin-Ciocalteu-Reagent (Swain & Hillis, 1959).

2.8. Sugar linkage analysis

Linkage analysis was carried out as described by Carpita and Shea (1989) adapted by Oosterveld, Beldman, Schols, and Voragen (1996). The samples were methylated and hydrolysed using 2 M TFA (2 h, 121 °C). After evaporation in a stream of air, the partially methylated sample was converted to alditol acetates and analysed by GC-FID. Identification of the compounds was performed using GC–MS.

2.9. High performance size exclusion chromatography (HPSEC)

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).

3. Results and discussion

3.1. Berries

The contents of dry matter and berry AIS, the latter representing cell wall material, show differences between black currants and bilberries. Both contents are higher for black currants when compared to bilberries (Table 1).

Table 1
Relative amounts of the various tissues and processing products in fresh berries [g/100 g]

	Fresh	Dry weight	AIS
Black currant skin	23.1	5.2	2.1
Black currant pulp		8.7	2.9
Black currant seeds		4.8	3.6
Black currant pulp and seeds	71.7	13.6	6.2
Black currant berries	100.0	18.7	9.3
Black currant juice MHR	2.9		3.4[a]
Black currant press cake	4.7		30.1
Black currant non-berry press cake	6[b]		7.4[c]
Bilberry skin	17.6	2.7	0.7
Bilberry pulp			1.4
Bilberry seeds			2.0
Bilberry pulp and seeds	60.0	7.9	3.1
Bilberry berries	100.0	12.4	3.8
Bilberry juice MHR	1.7		1.9[a]
Bilberry press cake	3.2		37.0

[a] Freeze dried after ultrafiltration.
[b] % of fresh press cake.
[c] AIS of non-berry press cake.

The determined values of dry weight material are in the range typically found for black currants (18.7 g/100 g) and bilberries (15.4 g/100 g; Scherz, Sensor, & Souci, 2000).

Cell wall polysaccharides present in AIS can be characterised by their sugar composition (Tables 2 and 3). The major sugar moiety in black currant AIS is galacturonic acid in contrast to bilberries, which contain glucose as major sugar. From the typical pectic sugars only galactose is present in both berries to the same relative amount. The relative amounts of rhamnose, arabinose, and galacturonic acid are larger in black currants. These findings indicate that less pectic material is present in bilberries (Table 3). The galacturonic acid content found in bilberries is comparable to what has been found before for bilberries (Fuchs & Wretling, 1991; Letzig, 1950), low bush blueberries (*Vaccinium angustifolium* Ait.; Chen & Camire, 1997), or blackthornton berries (*Hippophae rhamnoides* L.; Dongowski, 1996). The galacturonic acid content found in black currants is comparable to the findings of Letzig (1950), but three to four times higher than what has been found by Fuchs and Wretling (1991).

3.2. Different berry tissues

Three different types of tissue comprise a berry: strong flexible skin, soft pulp, and firm and inflexible seeds. Since cell wall polysaccharides vary in different tissues within one fruit (Lecas & Brillouet, 1994; Vidal, Williams, O'Neill, & Pellerin, 2001), these different tissues were separated (Table 1).

Table 2
Sugar composition of black currants

	Yield [g/100 g AIS]	Total sugars[a] [% w/w]	Rha[a] [mol%]	Fuc[a] [mol%]	Ara[a] [mol%]	Xyl[a] [mol%]	Man[a] [mol%]	Gal[a] [mol%]	Glc[a] [mol%]	GalA[a] [mol%]	GlcA[a] [mol%]	DM [%]	DA [%]	Protein[a] [% w/w]	Soluble polyphenols[b] [% w/w]
Berry AIS		38 (34,875)	2 (558)	0 (93)	11 (3255)	6 (1767)	13 (4464)	6 (2232)	20 (6975)	40 (15,277)	1 (440)	55	25	11 (10,230)	0.3
Skin AIS		47 (9954)	2 (147)	1 (42)	12 (966)	6 (483)	4 (420)	5 (462)	26 (2541)	46[c] (4935)	–[d]	69	–[d]	10 (2163)	–[d]
Pulp AIS		51 (14,863)	1 (290)	0 (29)	8 (957)	10 (1160)	2 (290)	6 (928)	21 (3045)	51[c] (8207)	–[d]	70	–[d]	9 (2697)	–[d]
Seeds		32 (11,340)	1 (108)	0 (0)	6 (576)	4 (360)	37 (4248)	6 (684)	20 (2304)	26[c] (3132)	–[d]	45	–[d]	16 (5688)	–[d]
HBSS	12.1	62 (7011)	1 (79)	0 (11)	6 (349)	2 (79)	0 (23)	3 (169)	1 (56)	84 (6086)	3 (182)	91	8	2 (214)	–[d]
ChSS	4.1	65 (2463)	1 (19)	0 (4)	6 (114)	0 (8)	0 (0)	2 (50)	0 (11)	88 (2215)	2 (50)	58	4	14[e] (545)	–[d]
DASS	10.4	41 (3985)	3 (97)	0 (10)	19 (609)	1 (39)	0 (0)	17 (648)	1 (39)	58 (2496)	2 (77)	2	2	15[e] (1480)	0.8
CASS	4.0	63 (2344)	1 (15)	2 (48)	6 (112)	37 (818)	16 (446)	8 (205)	23 (632)	6 (203)	2 (48)	3	1	13[e] (495)	0.3
Residue	46.9	35 (15,179)	1 (174)	0 (0)	8 (1003)	9 (1134)	33 (5147)	4 (654)	34 (5278)	11 (1791)	0 (84)	2	5	13 (5670)	1.2
Juice MHR		40 (2090)	10 (182)	0 (5)	24 (405)	2 (31)	2 (36)	7 (140)	9 (187)	47 (1084)	1 (23)	57	20	3 (130)	28.5
Press cake AIS		34 (15,980)	1 (141)	0 (47)	6 (799)	9 (1081)	24 (3854)	5 (846)	34 (5499)	20 (3553)	1 (160)	24	52	17 (7755)	0.1
NBPC AIS		39	2	0	6	17	10	5	41	20	1	–[d]	–[d]	12	0.1

[a] Values in brackets gives mg/kg fresh berries.
[b] Calculated as tannic acid.
[c] Total uronic acids.
[d] Not determined.
[e] EDTA adulterates the result.

Table 3
Sugar composition of bilberries

	Yield [g/100 g AIS]	Total sugars[a] [%w/w]	Rha[a] [mol%]	Fuc[a] [mol%]	Ara[a] [mol%]	Xyl[a] [mol%]	Man[a] [mol%]	Gal[a] [mol%]	Glc[a] [mol%]	GalA[a] [mol%]	GlcA[a] [mol%]	DM [%]	DA [%]	Protein[a] [%w/w]	Soluble polyphenols[b] [% w/w]
Berry AIS		40 (15,238)	1 (190)	0 (38)	5 (684)	29 (3800)	2 (380)	5 (760)	35 (5624)	21 (3626)	1 (250)	60	102	14 (5320)	0.1
Skin AIS		52 (3654)	1 (35)	0 (14)	6 (189)	9 (273)	4 (133)	7 (252)	31 (1127)	42[c] (1645)	_[d]	65	_[d]	17 (1169)	_[d]
Pulp AIS		49 (6874)	1 (70)	0 (14)	5 (266)	22 (1162)	2 (168)	5 (448)	31 (2198)	32[c] (2436)	_[d]	77	_[d]	16 (2170)	_[d]
Seeds		70 (13,920)	1 (160)	0 (0)	5 (400)	44 (5460)	2 (300)	3 (480)	37 (5700)	7[c] (1160)	_[d]	40	_[d]	21 (4220)	_[d]
HBSS	6.0	64 (1464)	1 (16)	0 (2)	4 (48)	1 (9)	0 (0)	6 (82)	2 (27)	83 (1242)	3 (44)	87	2	4 (96)	0.5
ChSS	4.4	61 (1020)	1 (6)	0 (2)	5 (33)	2 (17)	1 (17)	4 (40)	3 (33)	83 (861)	2 (25)	77	3	22 (361)	0.6
DASS	4.7	36 (650)	3 (16)	0 (2)	10 (54)	1 (7)	0 (0)	10 (61)	3 (20)	70 (479)	2 (16)	3	1	32[e] (570)	0.3
CASS	3.9	66 (978)	1 (5)	1 (12)	3 (31)	28 (237)	11 (117)	12 (123)	38 (397)	5 (65)	1 (15)	4	1	9[e] (138)	1.9
Residue	68.2	41 (10,608)	1 (104)	0 (0)	6 (572)	37 (3432)	1 (130)	3 (390)	41 (4654)	10 (1279)	1 (73)	1	5	11 (2730)	0.4
Juice MHR		40 (902)	6 (54)	0 (2)	34 (267)	3 (23)	1 (11)	20 (192)	13 (129)	21 (213)	1 (13)	58	38	4 (86)	30.9
Press cake AIS		36 (11,584)	1 (128)	0 (32)	4 (448)	33 (3264)	3 (320)	4 (480)	41 (5088)	14 (1816)	1 (104)	27	157	17 (5440)	0.6

[a] Values in brackets gives mg/kg fresh berries.
[b] Calculated as tannic acid.
[c] Total uronic acids.
[d] Not determined.
[e] EDTA may adulterates the result.

Black currants were easily separated in pulp and seeds after drying of the total fruit. Due to smaller seeds this was not possible in bilberries. During separation of fresh tissue some berry material, mainly juice, was lost. The softer the pulp tissue the higher the loss: 22 g/100 g in bilberries compared to 5 g/100 g in black currant. The smallest part of berry AIS in bilberries and black currants originates from the skin, the largest from the seeds. Black currants contain more skin than bilberries and black currant skin contains more cell wall material (2.1 g/100 g skin) than bilberry skin (0.7 g/100 g skin) or grape skin (0.6–0.5 g/100 g skin; Lecas & Brillouet, 1994). The weight content of seeds is higher and their size is larger in black currants than in bilberries.

3.2.1. Sugar composition

The sugar compositions of skin and pulp tissue differ only slightly in black currants, while in bilberries the pulp contains a larger amount of xylose residues when compared to the skin. The compositions of skin and pulp of both kinds of berries are similar to the findings for grapes (Lecas & Brillouet, 1994; Vidal et al., 2001).

In black currants a high mannose content distinguishes seeds from the other tissues, where mannose is almost absent. In contrast, seeds of bilberries contain mannose only in traces while xylose is the major sugar moiety present. Bilberry seeds contain double the relative amount of xylose when compared to pulp AIS. Salo and Suomi (1972) showed the same difference between black currants and bilberries. Bilberry seeds differ also form black currant seeds in the total amount of sugars, which is 70% in bilberry seeds and 32% in black currant seeds.

3.2.2. Degree of methyl esterification and acetylation

The degree of methyl esterification (DM) in pulp and skin (65–77%) is slightly higher than in berry AIS (55–60%) of both kinds of berries. The seeds of bilberries and black currants show a DM of approximately 40%. These data correspond with a DM of 82.5% found for pectins present in fresh black currant pulp and a DM of 57% in mature black currants, respectively (Green, 1971).

In contrast, the degree of acetylation (DA) is much higher in bilberries than in black currants. With around 100% the DA is extremely high in bilberry AIS. Although this is possible—per molecule uronic acid there are two binding sites for an acetyl group (O-2 and O-3; Quemener et al., 2003)—in case of bilberries it is more likely that the acetyl groups are not only bound to galacturonic acid, but to hemicellulolytic polysaccharides, as well (vide infra).

3.3. Extracted fractions

Alcohol insoluble solids (AIS) of berries were sequentially extracted with different aqueous extractants. Seeds were left intact, as during juice processing. Four aqueous extractants were chosen: hot buffer releases not tightly

bound pectins (hot buffer soluble solids, HBSS), while chelating agent solubilises pectins bound in cell walls via calcium (chelating agent soluble solids, ChSS). 50 mM sodium hydroxide releases pectins that are bound tightly to hemicelluloses or cellulose (diluted alkali soluble solids, DASS) and 6 M sodium hydroxide extracts hemicelluloses (concentrated alkali soluble solids, CASS). Cellulose and intact seeds remain in the residue. Tables 2 and 3 show how much material and total sugars were recovered in the five fractions. In both berries the residues are the largest fractions, which is partly due to the intact seeds. The amount of residue is larger in bilberries than in black currants, caused partly by a larger amount of seeds in AIS of bilberries (53%) when compared to black currant AIS (39%; Table 1). The smallest fractions are CASS. Although bilberries gave higher AIS yields when compared to high bush blueberries (*Vaccinium corymbosum* L.), the yields of HBSS and ChSS from AIS are similar (Kader, Rovel, Girardin, & Metche, 1994). However, the yield of DASS in high bush blueberries was less than half of the DASS yield of bilberries. This can be due to difference between the two species or to β-eliminative degradation of homogalacturonan at higher temperatures (Kravtchenko, Arnould, Voragen, & Pilnik, 1992), as used by Kader et al. (1994).

3.3.1. Sugar composition

The sugar compositions of the extracted fractions inform about the extraction behaviour of different kinds of polysaccharides, viz. pectins and (hemi-)cellulose. HBSS and ChSS contain more than 80 mol% galacturonic acid (Tables 2 and 3), which is the building block of homogalacturonan. Around 50% of galacturonic acid present in black currant and bilberry AIS is extracted in these two fractions. More or longer homogalacturonan segments are present compared to neutral segments ((Ara + Gal)/GalA > 0.12). Black currants contain more arabinose than galactose in HBSS and ChSS (Ara/Gal ≈ 3), while the relative amounts are equal in bilberries (Ara/Gal ≈ 1). Apples (Schols, Vierhuis, Bakx, & Voragen, 1995) and olives (Huisman, Schols, & Voragen, 1996) contain more neutral segments compared to homogalacturonan segments, although the latter still predominate ((Ara + Gal)/GalA > 0.18 in apple and > 0.6 in olives). In the neutral segments arabinose dominates galactose (Ara/Gal ≈ 3 in apple and ≈ 7 in olives).

In DASS the relative amount of rhamnose is the largest of all fractions. The relative amounts of arabinose and galactose are large, either. This indicates the presence of large amounts of rhamnogalacturonan I, the pectic hairy regions. The relative amounts of galacturonic acid are large, although smaller than in HBSS and ChSS. DASS contain around 15% of all galacturonic acids present in berries. Pectins in black currant DASS contain more or longer neutral segments and less or shorter homogalacturonan segments than pectins in bilberry DASS ((Ara + Gal)/GalA = 0.62 and 0.29, respectively), but the Ara/Gal ratio is around 1 and about the same in bilberry and black currant DASS. This was not the case in HBSS or ChSS.

With 6 M sodium hydroxide stronger hydrogen bounds are broken leading to the solubilisation of hemicelluloses (Roland, Reis, Vian, & Roy, 1989). In black currant CASS xylose is the major sugar, followed by glucose and mannose. In contrast, bilberry CASS contain more glucose than xylose. The galacturonic acid content is the lowest of all fractions, so that the ratio of glucuronic acid to galacturonic acid is the highest in CASS. Glucuronic acid is commonly present in acidic hemicelluloses such as xylans (Rosell & Svensson, 1975). While in black currant CASS almost 50% of the xylose is extracted, bilberry CASS contain only 6% of the xylose present in berry AIS. Most of the xylose is present in the seeds, which remain in the residue. The same counts for the content of mannose in black currant CASS, which represents only 10% of the mannose present in berry AIS.

In the residues the main sugar is glucose, but hemicellulolytic sugars play a major role due to their presence in seeds, as well. In black currant residue the content of mannose is even as high as the content of glucose and in bilberry residue the content of xylose reaches almost the content of glucose. Approximately 80% of glucose and approximately 90% of the major hemicellulolytic sugars—mannose in black currants and xylose in bilberries—present in berry AIS remain in the residue, which is an indication for limited extraction of cell wall polysaccharides from the seeds. Galacturonic acid is still present in the residues (11 mol%). In bilberries this amount represent still 35% of galacturonic acid present in berry AIS, while this value is only 12% for black currants.

3.3.2. Degree of methyl esterification and acetylation

For bilberries and black currants the DM is higher in HBSS than in ChSS, although ChSS of bilberries still have a DM of 77%. This has been shown in olives and apple (Huisman et al., 1996; Schols et al., 1995), as well, although chelating agents are supposed to extract calcium sensitive pectins, viz. pectin with a low DM (Ralet, Crepeau, Buchholt, & Thibault, 2003). Recent studies showed evidence that calcium is necessary to stabilise boron cross-linked rhamnogalacturonan II (dRGII-B; Ishii et al., 1999; Kobayashi, Nakagawa, Asaka, & Matoh, 1999; Wimmer & Goldbach, 1999). Rhamno-galacturonan II is embedded in a homogalacturonan chain (Ishii & Matsunaga, 2001; Reuhs et al., 2004). The removal of calcium could destabilise dRGII-B, which could fall apart and set two pectic molecules free, independently of their DM. If this happens in muro, which still has to be proven, it can explain why ChSS contain high DM pectins.

Alkali treatment splits all methyl and acetyl ester linkages present. In HBSS and ChSS 82% of the methyl groups and 28% of the acetyl groups from black currant AIS

are recovered. Thus, all methyl groups are linked to pectic galacturonic acids, the only sugar present that can form methyl esters. However, the missing acetyl groups can be either linked to galacturonic acids or to neutral polymers present in the (hemi-)cellulolytic fractions. In bilberries 84% of methyl groups, but only 3% of the acetyl groups are recovered in HBSS and ChSS. Too many acetyl groups remain in the other three fractions to be attached only to galacturonic acid. The main part of the acetyl groups has to be esterified with hemicellulolytic polysaccharides. This explains the high DA values for berry AIS and press cake AIS, because we calculated the DA as acetyl groups per galacturonic acid residues.

3.3.3. Sugar linkage analysis

To gain information about branching and structure of polysaccharides, the linkage composition was analysed (Tables 4 and 5). Because some fractions are hardly soluble and almost all fractions contain large amounts of uronic acids, quantitative methylation was even after repeated methylation (three times) not possible. The results allow general conclusions about the polysaccharides present in the different fractions.

In all fractions less rhamnose was found than determined by alditol acetates (Tables 2 and 3). This is due to a strong linkage between rhamnose and galacturonic acid in the rhamnogalacturonan I backbone, which cannot be

Table 4
Sugar linkage composition of black currants

	AIS	HBSS	ChSS	DASS	CASS	Residue	Juice MHR	PC AIS	NBPC AIS
t-araf	1	6	5	9	1	1	12	1	1
1,5-araf	5	23	21	21	2	2	31	2	2
1,3-araf	1	1	2	3	1	0	4	1	1
1,3,5-araf	2	8	13	10	0	1	8	1	1
1,2,5-araf	1	2	3	3		0	2		0
1,2,3,5-araf	1	2	4	7		0		2	1
Total ara	9	43	48	54	5	5	58	6	5
t-xylp	1	0		0	3	0	0	1	1
1,4-xylp	6	9	2	3	26	11	1	7	20
1,3,4-/1,2,4-xylp	1	1[a]		0[a]	4	1		1	2
1,2,3,4-xylp	4							5	2
Total xyl	12	11	3	3	32	12	2	14	25
t-rhap				0			5		
1,2-rhap				1					
Total rha	0	0	0	1	0	0	5	0	0
t-fucp	0				1			0	0
1,2,4-Fucp				1					
Total fuc	0	0	0	1	1	0	0	0	0
t-glcp	1	0		0		1	4	1	1
1,6-glcp					0	0	11		
1,4-glcp	39	9	10	3	19	39	5	35	46
1,3-glc							2		
1,2,4-glcp	1				1	1		2	1
1,4,6-glcp	7				16	2		6	6
1,3,4-glcp						1		1	
1,2,3,4,6-glc	3	8	12	4	0	2	4	3	1
Total glc	50	18	22	8	35	46	26	47	56
1,6-Manp					1				
1,4-manp	17	1	1		14	32	1	17	7
1,4,6-manp	2				4	2		2	1
1,3,4-manp						0			
1,2,3,4,6-man	2	2		4	0	1	1	7	0
Total man	20	3	1	4	19	34	2	26	8
t-galp	1	2	1	1	4	1	1	1	1
1,6-galp		0					2		
1,4-galp	4	10	9	17	2	1		2	2
1,3-galp	1	0		2	2	0	1	2	2
1,4,6-galp		3	2	1			1		
1,3,4-galp	1				0	1		2	2
1,3,6-galp	2	11	15	5	1		4	2	1
1,2,3,4,6-galp	0	1		4	0	1		1	
Total gal	9	27	27	31	9	4	9	9	8
Ration t/b	0.1	0.2	0.1	0.3	0.2	0.2	0.8	0.1	0.2

[a] Only 1,2,4-xylp.

Table 5
Sugar linkage composition of bilberries

	AIS	HBSS	ChSS	DASS	CASS	Residue	Juice MHR	PC AIS
t-araf	1	2	3	5	1	1	6	
1,5-araf	2	5	20	15	0	2	22	2
1,3-araf	0	1	3	4	2	0	9	0
1,3,5-araf	2	1	9	11	0	2	12	1
1,2,5-araf			2	1			1	
1,2,3,5-araf	2	3		3	4	0		1
Total ara	8	13	38	40	7	5	49	4
t-xylp	1	0		1	4	1	2	1
1,4-xylp	23	2	1	4	14	34	2	19
1,3,4-/1,2,4-xylp	5	0[a]		0[a]	1	3		8
1,2,3,4-xylp	5				0	0		12
Total xyl	35	3	1	5	19	39	3	39
t-rhap	0						0	
1,2-rhap				0				
Total rha	0	0	0	0	0	0	0	0
t-fucp	0				1			0
1,2,4-Fucp				1				
Total fuc	0	0	0	1	1	0	0	0
t-glcp	1	0	0	1	1	1	4	1
1,6-glcp			7			0		
1,4-glcp	34	19	19	13	23	43	11	35
1,3-glc							2	
1,2,4-glcp	1				1	2		1
1,4,6-glcp	7				23	5		6
1,3,4-glcp								
1,2,3,4,6-glcp	3	3	3	4	1	0	2	3
Total glc	46	22	29	19	49	51	20	46
1,6-Manp								
1,4-manp	1	0			8	1		2
1,4,6-manp	1				6			1
1,3,4-manp								
1,2,3,4,6-manp	1	0	1	2	0			0
Total man	2	1	1	2	13	1	0	3
t-galp	1	2	2	3	7	1	4	1
1,6-galp		3			0		11	
1,4-galp	2	18	16	17	1	1		1
1,3-galp	1	0		1	3	1	3	1
1,4,6-galp		4	4	5				
1,3,4-galp	3		10		1	2		2
1,3,6-galp	1	36		7	1		9	2
1,2,3,4,6-galp	1			1	0			0
Total gal	9	63	32	34	13	5	27	7
Ration t/b	0.1	0.1	0.2	0.2	0.3	0.2	0.6	0.1

[a] Only 1,2,4-xylp.

hydrolysed with TFA. Most of the arabinose residues found in the different fractions are part of 1,5-linked arabinan side chains of rhamnogalacturonan I or type I arabinogalactan (Schols & Voragen, 2002). In the latter they can be attached to O-6 of the 1,4-linked galactan backbone. The contents of 1,4- and 1,4,6-linked galactose indicate that less type I arabinogalactans are present in HBSS and ChSS of black currants than of bilberries. The large amount of arabinose in black currants (Tables 2 and 3) is probably due to arabinan side chains of pectins.

Bilberry HBSS and black currant HBSS and ChSS contain type II arabinogalactan, as concluded from a high content of 1,3,6-linked galactose. Hot buffer and chelating agent extract most of type II arabinogalactan next to type I arabinogalactan. With diluted alkali the remaining type I arabinogalactan is extracted. Type II arabinogalactans may not only be parts of pectic polysaccharides, but of arabinogalactan proteins, as well (Majewska-Sawka & Nothnagel, 2000). From black currant AIS more type II arabinogalactans are extracted with chelating agent than with hot buffer, while for bilberries it is the reverse.

The hemicellulolytic polysaccharides, viz. xylans, mannans, and glucans, are present as long linear 1,4-linked chains. Xylose, galactose, and fucose are present as terminal sugars, which is in combination with 1,4,6-linked glucose typical for xyloglucans (Vincken, Beldman, & Voragen,

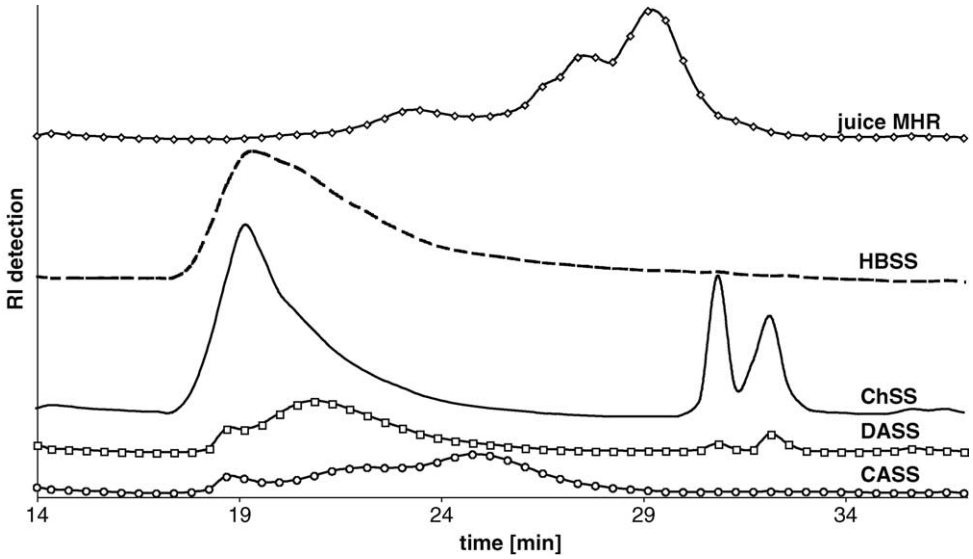

Fig. 1. High performance size exclusion chromatography pattern of black currant polysaccharide fractions.

1994). Mannans, cellulose, xylans, and xyloglucans are present in black currants and bilberries.

3.3.4. Molecular weight distribution

High performance size exclusion chromatography (HPSEC) is an established tool to view the molecular weight distribution of soluble polysaccharides. In case of HBSS and ChSS in black currants and bilberries, which contain mainly acidic homogalacturonan, the first molecules elute in the excluding volume (Figs. 1 and 2). The chromatograms show a broad molecular weight distribution. With these non-destructive extraction conditions very large molecules, mainly homogalacturonans, are extracted. The molecular weight is comparable for HBSS and ChSS. The harsher the extraction conditions, the less polymeric are the polysaccharides in the corresponding fractions. Diluted alkali treatment opens bonds in pectic polysaccharides releasing them form hemicelluloses and leads to a shift in molecular weight. This shift can be due to extraction of natively smaller polymers or to slight degradation in diluted alkali. Concentrated sodium hydroxide extracts only some polysaccharides with very high molecular weight, while most of the extracted hemicellulolytic polysaccharides are of medium size. These trends are reported for olives (Vierhuis et al., 2000), as well. With the peaks at 31 and 32 min in ChSS of black currants and DASS of both kinds of berries remaining EDTA is shown, which was not sufficiently removed during dialysis. The peaks do not represent cell wall polysaccharides.

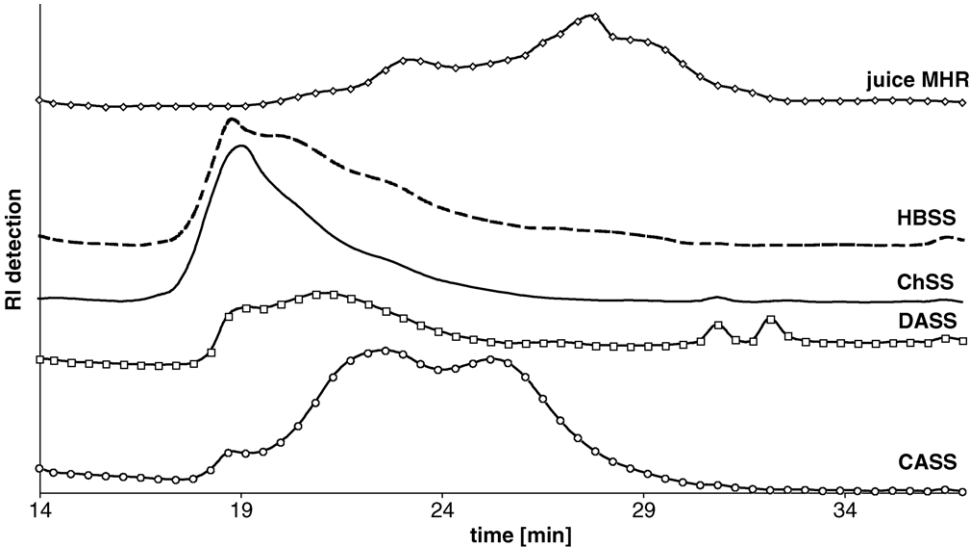

Fig. 2. High performance size exclusion chromatography pattern of bilberry polysaccharide fractions.

3.4. Material from juice processing

The influence of current juice processing on cell wall carbohydrate composition and distribution is shown by a comparison of AIS from press cake with polysaccharides isolated from juice concentrate by ultra filtration (juice modified hairy regions–juice MHR; vide infra). Many different juices contain dissolved polysaccharides not degradable by enzymes used during processing (Schols & Voragen, 1994), although producers worked on the improvement of their enzymes to lower the amount of undegradable polysaccharides. These enzyme resistant pectic polysaccharides are called modified hairy regions. They contain rhamnogalacturonan I with arabinan and arabinogalactan side chains and stubs of homogalacturonan. Commercial enzyme mixtures used during juice production may have cut off some sugar residues.

In bilberries polysaccharide in press cake and juice account for 82% of the total polysaccharides present in the berries. The losses are due to enzymatic degradation of homogalacturonans to small fragments which were lost during the ultra filtration step. The recovery is much lower in black currants (52%) where more pectic polysaccharides are present. In black currants wooden parts and stems are present in the mechanical harvested bunches of black currants. They contribute up to 6 g/100 g of the press cake's fresh weight and are further referred to as non-berry press cake (NBPC).

3.4.1. Sugar composition

The polysaccharides isolated from black currant and bilberry juice concentrate show the typical pattern of modified hairy regions (MHR) with large relative amounts of arabinose, galactose, and rhamnose (Tables 2 and 3). Bilberry juice MHR contain less galacturonic acids than black currant juice MHR. The total sugar content is quite low in bilberry and black currant juice MHR (40%), representing 6% of total cell wall sugars present in black currant or bilberry AIS. Juice MHR contain around 30% polyphenols measured by the colour assay, which can be more or less firmly attached to the polysaccharides or polymerised, giving juice MHR a red colour.

The compositions and contents of cell wall carbohydrates in the press cake are comparable to the composition of the residue after sequential extraction. From total cell wall sugars present in black currants 44% can be found in the residue and 46% in the press cake (70 and 76% for bilberries, respectively). Press cake AIS from black currants contain less mannose and more galacturonic acid when compared to the extraction residue.

With the enzyme treatment used it is possible to solubilise as many polysaccharides from AIS as could be extracted with the sequential extraction process. Up to 69% of the pectic sugars (galacturonic acid, arabinose, and galactose) are lost during juice processing due to enzymatic degradation. The power of the enzyme treatment is not only dependent on the kind of enzyme preparation used, but also on the kind of polysaccharide present in the fruits. In bilberries the composition of residue and press cake AIS are very similar, in black currants there are slight differences. The DM and DA of press cake AIS may indicate what can be expected for the esterification of polysaccharides remaining in the residue. For bilberry press cake AIS a DA of 157 seems very improbable confirming the assumption that acetyl groups are mainly bound to hemicellulolytic sugars.

However, it should be realised that non-berry material is present during processing of black currants in an industrial process. This non-berry press cake AIS (NBPC AIS) show a completely different pattern in sugar composition than all berry fractions. The tissue is much firmer and, as expected, NBPC contains a larger amount of glucose and xylose, viz. hemicellulose and cellulose, but less mannose when compared to berry AIS.

3.4.2. Molecular weight distribution

The juice MHR pattern is different for bilberries and black currants (Figs. 1 and 2). Both are similar till approx. 27 min, where an external standard of rhamnogalacturonan II elutes. The highest peak of black currant juice MHR elutes after approx. 29 min, while in bilberry juice MHR only a shoulder is detected. The HPSEC pattern of bilberry MHR is similar to the patterns of apple MHR (Schols, Posthumus, & Voragen, 1990).

3.5. Conclusions

In this paper we are the first to present a detailed characterisation of cell wall polysaccharides in bilberries and black currants. Although the general tendencies are similar, some important differences were found.

The seeds of black currants contain mannose as major sugar, while bilberries contain xylose. After sequential buffer extraction mannose remains in the residue, where the whole seeds are recovered. The same counts for xylose in bilberries. Bilberries contain a much larger amount of residue than black currants, which can partly be due to the larger amount of seeds in bilberry AIS. Black currants contain more arabinose side chains in HBSS and ChSS.

The press cakes are in amount and composition very similar to the residues. With the enzyme preparation used it is possible to solubilise as many polysaccharides as could be extracted from berry AIS with the sequential extraction method. The polysaccharide fraction left in the juice show the typical composition of modified hairy regions as found in other fruits before.

Acetyl groups are present in a very large amount in bilberry polysaccharides that are extracted by alkali. These acetyl groups are mainly attached to hemicellulolytic sugars.

The findings described in this article are essential for the recognition of changes in cell wall polysaccharides during

berry processing. Analysis of press cake AIS and juice MHR from conventional juice processing shows that the major part of cell wall polysaccharides remain in the press cake after juice processing (76% in bilberries and 46% in black currants, respectively).

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References

Anderson, J. W. (1990). Dietary fiber and human health. *Hortscience*, *25*(12), 1488–1495.

Carpita, N. C., & Shea, E. M. (1989). Linkage structure of carbohydrates by gas chromatography–mass spectrometry (GC–MS) of partially methylated alditol acetates. In C. J. Biermann, & G. D. McGinnis (Eds.), *Analysis of carbohydrates by GLC and MS* (pp. 157–216). Boca Raton, FL: CRC Press, Inc., 157–216.

Chen, H. C., & Camire, M. E. (1997). Recovery of anthocyanins, pectin, and dietary fiber from cull lowbush blueberries. *Journal of Food Quality*, *20*(3), 199–209.

Chen, Z., Schols, H. A., & Voragen, A. J. G. (2004). Differently sized granules from acetylated potato and sweet potato starches differ in the actyl substitution pattern of their amylose population. *Carbohydrate Polymers*, *52*(2), 219–226.

De Ruiter, G. A., Schols, H. A., Voragen, A. G. J., & Rombouts, F. M. (1992). Carbohydrate analysis of water-soluble uronic acid-containing polysaccharides with high-performance anion-exchange chromatography using methanolysis combined with TFA hydrolysis is superior to four other methods. *Analytical Biochemistry*, *207*, 176–185.

De Vries, J. A., Voragen, A. G. J., Rombouts, F. M., & Pilnik, W. (1981). Extraction and purification of pectins from alcohol insoluble solids from ripe and unripe apples. *Carbohydrate Polymers*, *1*, 117–127.

Dongowski, G. (1996). Investigation on polysaccharide, pectin and dietary fiber fractions of buckthornton berries. *Zeitschrift fur Lebensmittel Untersuchung und Forschung*, *203*(3), 302–308.

Dumas, J. B. A. (1831). Procedes de L'analyse Organique. *Annales de Chimie et de Physique*, *47*, 198–205.

Englyst, H. N., & Cummings, J. H. (1984). Simplified method for the measurement of total non-starch polysaccharides by gas–liquid chromatography of constituent sugars as alditol acetates. *Analyst*, *109*, 937–942.

Fleischer, A., O'Neill, M. A., & Ehwald, R. (1999). The pore size of non-graminaceous plant cell walls is rapidly decreased by borate ester cross-linking of the pectic polysaccharide rhamnogalacturonan II. *Plant Physiology*, *121*(3), 829–838.

Frankel, E. N. (1999). Food antioxidants and phytochemicals: Present and future perspectives. *Fett - Lipid*, *101*(12), 450–455.

Fuchs, G., & Wretling, S. (1991). Chemical composition of Swedish blueberries, raspberries, strawberries, lingonberries and black currants. *Vaar Foeda*, *43*(8), 425–438.

Grassin, C., & Fauquembergue, P. (1996). Fruit juices. In T. Godfrey, & S. West (Eds.), *Industrial enzymology* (pp. 225–264). London: MacMillan Press Ltd, 225–264.

Green, A. (1971). Soft fruits. In A. C. Hulme, *The biochemistry of fruits and their products* (Vol. 2) (pp. 375–410). Norwich, England: Academic Press, 375–410.

Huisman, M. M. H., Schols, H. A., & Voragen, A. G. J. (1996). Changes in cell wall polysaccharides from ripening olive fruits. *Carbohydrate Polymers*, *31*, 123–133.

Ishii, T., & Matsunaga, T. (2001). Pectic polysaccharide rhamnogalacturonan II is covalently linked to homogalacturonan. *Phytochemistry*, *57*(6), 674–696.

Ishii, T., Matsunaga, T., Pellerin, P., O'Neill, M. A., Darvill, A., & Albersheim, P. (1999). The plant cell wall polysaccharide rhamnogalacturonan II self-assembles into a covalently cross-linked dimer. *Journal of Biological Chemistry*, *274*(19), 13098–13104.

Kader, F., Rovel, B., Girardin, M., & Metche, M. (1994). Composition of the fruit of highbush blueberry (*Vaccinium corymbosum* L) grown in the East of France (Vosges). *Sciences Des Aliments*, *14*(3), 281–290.

Kobayashi, M., Matoh, T., & Azuma, J. I. (1996). Two chains of rhamnoglacturonan II are cross linked by borate-diol ester bonds in higher plant cell walls. *Plant Physiology* 110, 1017–1020.

Kobayashi, M., Nakagawa, H., Asaka, T., & Matoh, T. (1999). Borate-rhamnogalacturonan II bonding reinforced by Ca2+ retains pectic polysaccharides in higher-plant cell walls. *Plant Physiology*, *119*(1), 199–203.

Kravtchenko, T. P., Arnould, I., Voragen, A. G. J., & Pilnik, W. (1992). Improvement of the selective depolymerization of pectic substances by chemical beta-elimination in aqueous solution. *Carbohydrate Polymers*, *19*(4), 237–242.

Lampitt, L. H., & Hughes, E. B. (1928). The composition of fruit. *Analyst*, *53*, 32–34.

Lecas, M., & Brillouet, J. M. (1994). Cell wall composition of grape berry skins. *Phytochemistry*, *35*(5), 1241–1243.

Letzig, E. (1950). Die Uronsaeurebestimmung, eine vernachlaessigte Methode in der Lebensmittelchemie. *Zeitschrift fuer Lebensmittel Untersuchung und Forschung*, *91*(5), 325–338.

Majewska-Sawka, A., & Nothnagel, E. A. (2000). The multiple roles of arabinogalactan proteins in plant development. *Plant Physiology*, *122*(1), 3–10.

McCann, M. C., & Roberts, K. (1991). Architecture of the primary cell wall. In C. W. Lloyd (Ed.), *The cytoskeletal basis of plant growth and form* (pp. 109–129). London: Academic Press, 109–129.

McNeill, M., Darvill, A. G., Fry, S. C., & Albersheim, P. (1984). Structure and function of the primary cell walls of plants. *Annual Review of Biochemistry*, *53*, 625–663.

Meyer, A. S. (2002). Enhanced extraction of antioxidant phenols from wine and juice press residues via enzymatic polysaccharide hydrolysis. *Fruit Processing*, *12*(1), 29–33.

Money, R. W., & Christian, W. A. (1950). Analytical data of some common fruits. *Journal of the Science of Food and Agriculture*, *1*(1), 8–12.

Oosterveld, A., Beldman, G., Schols, H. A., & Voragen, A. G. J. (1996). Arabinose and ferulic acid rich pectic polysaccharides extracted from sugar beet pulp. *Carbohydrate Research*, *288*, 143–153.

Quemener, B., Pino, J. C. C., Ralet, M. C., Bonnin, E., & Thibault, J. F. (2003). Assignment of acetyl groups to O-2 and/or O-3 of pectic oligogalacturonides using negative electrospray ionization ion trap mass spectrometry. *Journal of Mass Spectrometry*, *38*(6), 641–648.

Ralet, M. C., Crepeau, M. J., Buchholt, H. C., & Thibault, J. F. (2003). Polyelectrolyte behaviour and calcium binding properties of sugar beet pectins differing in their degrees of methylation and acetylation. *Biochemical Engineering Journal*, *16*(2), 191–201.

Reuhs, B. L., Glenn, J., Stephens, S. B., Kim, J. S., Christie, D. B., Glushka, J. G., et al. (2004). L-Galactose replaces L-fucose in the pectic polysaccharide rhamnogalacturonan II synthesized by the L-fucose-deficientmur1 Arabidopsis mutant. *Planta*, *219*(1), 147–157.

Rodriguez Carvajal, M. A., du Penhoat, C. H., Mazeau, K., Doco, T., & Perez, S. (2003). The three-dimensional structure of the mega-oligosaccharide rhamnogalacturonan II monomer: A combined molecular modeling and NMR investigation. *Carbohydrate Research*, *338*(7), 651–671.

Roland, J. C., Reis, D., Vian, B., & Roy, S. (1989). The helicoidal plant cell wall as a performing cellulose-based composite. *Biology of the Cell*, *67*(2), 209–220.

Rosell, K. G., & Svensson, S. (1975). Studies of the distribution of the 4-*O*-methyl-glucuronic acid residues in birch xylan. *Carbohydrate Research*, *42*(2), 297–304.

Salo, M. L., & Suomi, K. (1972). Carbohydrate and acid composition of Finnish berries. *Journal of the Scientific Agricultural Society of Finland*, *44*(2), 68–75.

Scherz, H., Senser, F., & Souci, S. W. (2000). *Deutsche Forschungsanstalt für Lebensmittelchemie*. *Food composition and nutrition tables; Die Zusammensetzung der Lebensmittel, Nährwert-Tabellen; La composition des aliments, tableaux des valeurs nutritives*. Stuttgart: Medpharm Scientific Publications.

Schols, H. A., Posthumus, M. A., & Voragen, A. G. J. (1990). Structural features of hairy regions of pectins isolated from apple juice produced by the liquefaction process. *Carbohydrate Research*, *206*, 117–129.

Schols, H. A., Vierhuis, E., Bakx, E. J., & Voragen, A. G. J. (1995). Different populations of pectic hairy regions occur in apple cell walls. *Carbohydrate Research*, *275*, 343–360.

Schols, H. A., & Voragen, A. G. J. (1994). Occurence of pectic hairy regions in various plant cell wall materials and their degradability by rhamnogalacturonase. *Carbohydrate Research*, *256*, 83–95.

Schols, H. A., & Voragen, A. G. J. (2002). The chemical structure of pectins. In G. B. Seymour, & J. P. Knox (Eds.), *Pectins and their manipulation Sheffield biological sciences* (pp. 1–29). Oxford: Blackwell Publishing, 1–29.

Sembries, S., Dongowski, G., Jacobasch, G., Mehrlander, K., Will, F., & Dietrich, H. (2003). Effects of dietary fibre-rich juice colloids from apple pomace extraction juices on intestinal fermentation products and microbiota in rats. *The British Journal of Nutrition*, *90*(3), 607–616.

Swain, T., & Hillis, W. E. (1959). The phenolic constituents of prunus domestica. I. - The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture* 10, 63–68.

Thibault, J. F. (1979). Automatisation du dosage des substances pectiques par la méthode au méta-hydroxydiphenyl. *Lebensmittel Wissenschaft und Technologie - Food Science and Technology*, *12*, 247–251.

Vidal, S., Williams, P., O'Neill, M. A., & Pellerin, P. (2001). Polysaccharides from grape berry cell walls. Part I: Tissue distribution and structural characterization of the pectic polysaccharides. *Carbohydrate Polymers*, *45*, 315–323.

Vierhuis, E., Schols, H. A., Beldman, G., & Voragen, A. G. J. (2000). Isolation and characterisation of cell wall material from olive fruit (Olea europaea cv koroneiki) at different ripening stages. *Carbohydrate Polymers*, *43*, 11–21.

Vincken, J. P., Beldman, G., & Voragen, A. G. J. (1994). The effect of xyloglucans on the degradation of cell-wall-embedded cellulose by the combined action of cellobiohydrolase and endoglucanases from *Trichoderma viride*. *Plant Physiology*, *104*, 99–107.

Voragen, A. G. J., Pilnik, W., Thibault, J. F., Axelos, M. A. V., & Renard, C. M. G. C. (1995). Pectins. In A. M. Stephen (Ed.), *Food polysaccharides and their applications* (pp. 287–339). New York, Basel, Hong Kong: Marcel Dekker Inc., 287–339.

Voragen, A. G. J., Schols, H. A., & Pilnik, W. (1986). Determination of the degree of methylation and acetylation of pectins by h.p.l.c.. *Food Hydrocolloids*, *1*(1), 65–70.

Wimmer, M. A., & Goldbach, H. E. (1999). Influence of Ca2+ and pH on the stability of different boron fractions in intact roots of Vicia faba L. *Plant Biology*, *1*(6), 632–637.

Yamada, H. (1994). Pectic polysaccharides from Chinese herbs: Structure and biological activity. *Carbohydrate Polymers*, *25*, 269–276.

Yamada, H. (1996). Contribution of pectins on health care. *Pectins and pectinases, proceedings of an international symposium*. Wageningen, The Netherlands: Elsevier Science.