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Structural features and water holding capacities of pressed potato fibre polysaccharides

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ABSTRACT

Pressed potato fibre (PPF) has a high water holding capacity (WHC) affecting its processing as an animal feed. The aim of this study was to characterize cell wall polysaccharides (CWPs) in PPF and investigate their WHC. This was done via sequential extractions. Half of all CWPs were recovered in the hot buffer soluble solids extract as pectins (uronic acid and rhamnose) and galactans wherein most pectins (76%) from PPF were water soluble. Most likely, the network of CWPs is loosened during processing of potatoes. PPF showed a WHC of 7.4 expressed as the amount of water held per g of dry matter (mL/g). Reconstituting hot buffer soluble solids with buffer insoluble solids in water gave a WHC comparable to that of PPF. Removal of alkali soluble solids, which mainly comprised xyloglucans, lowered the WHC of the final residue. The results indicated that interactions between CWPs could affect the WHC of PPF.

1. Introduction

Pressed potato fibre (PPF) is a major by-product of industrial potato starch production. PPF exhibits a high water holding capacity (WHC) that makes it colloid-like, bulky and difficult to process (e.g. pumping). This hinders its use as cattle feed. An approach to enable the handling of PPF would be to reduce its WHC. WHC is affected by factors related to the potato cell wall, such as polysaccharide composition and architecture (Serena & Knudsen, 2007). Other factors include particle size, pore volume, thermo-mechanical treatments and drying conditions (Pejic, Kostic, Skundric, & Praskalo, 2008; Serena & Knudsen, 2007; Thibault, Renard, & Guillon, 2001).

PPF is composed of potato flesh and potato skin fragments (Lisinska & Leszczynski, 1989). Potato flesh is largely composed of parenchyma that contains thin walled, non-lignified, primary cell walls. Skin fragments typically consist of periderm, which includes epidermal and suberized cells containing some secondary cell walls (McDougall, Morrison, Stewart, & Hillman, 1996). PPF contains 20–40% (w/w) starch (Lisinska & Leszczynski, 1989) and 48% (w/w) cell wall polysaccharides (CWPs; Mayer & Hillebrandt, 1997). Detailed structural characteristics of PPF polysaccharides are not yet known.

In general, the plant cell wall architecture in dicotyledonous plants, such as potato, consists of a firm network of cellulose and xyloglucans that entrap pectic polysaccharides (McCann & Roberts, 1991). Pectins include linear 1,4-linked galacturonans, also named homogalacturonans (HG) or smooth regions, and branched galacturonans, such as xylogalacturonans, rhamnogalacturonan type I (RG-I) and rhamnogalacturonan type II (RG-II). RG-I and RG-II are often referred to as hairy regions.

In potato cell walls, RG-I constitutes about 75% of total pectins (Oomen et al., 2003). The backbone of RG-I has repeats of [→α-D-GalA-1,2-α-L-Rha-1,4→] (Voragen, Coenen, Verhoef, & Schols, 2009). Most of the structural analyses on potato cell walls has been related to RG-I and its side chains predominantly composed of (1,4)-β-galactans next to arabinogalactans (Harris, Jaspreet, & Lovedeep, 2009). The degree of acetylation (DA) and the degree of methyl esterification (DM) of potato pectins were reported to be 14 and 31, respectively (Voragen, Schols, & Pilnik, 1986). Analysis of other cell wall polysaccharides reveal the presence of crystalline microfibrills of cellulose in an interwoven network (Kirby, Ng, Waldron, & Morris, 2006), and the occurrence of XXGG type of xyloglucans where the repeating unit consists of two glucosyl

Abbreviations: PPF, pressed potato fibres; WHC, water holding capacity; CWPs, cell wall polysaccharides; HBSS, hot buffer soluble solids; BIS, buffer insoluble solids; EDTA, ethylenediaminetetraacetic acid; CHSS, chelating agent soluble solids; CHUS, chelating agent un-extractable solids; DASS, dilute alkali soluble solids; DAUS, dilute alkali un-extractable solids; CASS, concentrated alkali soluble solids; Res, residue; HPLC, high performance liquid chromatography; HPSEC, high performance size exclusion chromatography; MALDI-TOF MS, matrix assisted laser desorption/ionization time of flight mass spectrometry; DA, degree of acetylation; DM, degree of methyl esterification; RG, rhamnogalacturonan.

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residues substituted with xylose (X) adjacent to two un-substituted glucosyl (Glc) residues (G). Substitutions on X gives several forms of XXGG as XSGG (S is arabinosyl substitution of X), XLGG (L is galactosyl substitution of X) and LSGG (Vincken, Wijsman, Beldman, Niessen, & Voragen, 1996). Also, heteromannans and heteroxylans have been indicated to be present in potato cell walls, but have not yet been characterized (Jarvis, Hall, Threlfall, & Friend, 1981).

Hydration of CWPs has been expressed in terms of swelling, water retention power and WHC. The arabinan side chains of potato RG-I have been suggested to be hydrated more readily than galactan side chains, although long galactan side chains have been implicated to be extremely mobile. Also longer side chains were shown to impart a hydrophilic nature to RG-I (Larsen et al., 2011). In hemp fibres, a removal of 59% of the hemicelluloses decreased the water retention power by 7% (Pejic et al., 2008). For PPF, hydration capacities previously reported were water binding capacity of 7 g/g and swelling of 10 mL/g freeze dried material (Serena & Knudsen, 2007).

However, no information is available yet about the contribution of individual CWPs to the WHC of PPF. Such information is important if the WHC of PPF is to be lowered. In addition, the composition of CWPs, their organization and structural aspects in PPF is unknown. In this study, CWPs from PPF have been extracted and characterized for sugar composition, levels of esterification and types of CWPs present. In addition, the WHCs of CWPs in different residues from PPF has been investigated.

2. Materials and methods

Pressed potato fibre (PPF) was provided by Avebe, Foxhol, The Netherlands on January, 2010. The potatoes were harvested in autumn 2009.

2.1. Extraction of non-starch polysaccharides from potato pulp fibre

2.1.1. De-starching potato pulp fibre

Freeze dried PPF (particle size < 1 mm; 90 g; provided by Avebe, Foxhol, The Netherlands) was suspended in 0.2 M sodium acetate buffer, pH 5.2 (1520 mL) heated to 85 °C and 0.006 g of α-amylase (Sigma A4551-1G, USA; 0.548 U/g, *Bacillus licheniformis*) was added. The suspension was stirred at 85 °C for 30 min. After adjusting the pH to 5.5 (using 1 M NaOH), residual starch was hydrolyzed with 0.006 g of α-amylase and 0.3 g of amyloglucosidase (Sigma; 11.6 U/g, *Rhizopus mold*) and stirred at 30 °C for 20 h. The suspension was centrifuged (20 min, 29,400 × *g*, 20 °C) and the procedure was repeated for the residue. The residue was washed 3 times with demineralized water; freeze dried and denoted as buffer insoluble solids (BIS). All washings and supernatants were combined into one extract and dialyzed. Dialysis was performed against distilled water (cutoff size 12–14 kDa; Visking, Medicell International Ltd., UK) and freeze dried. After dialysis, the extract was denoted hot buffer soluble solids (HBSS).

2.1.2. Extraction of buffer insoluble solids (BIS)

BIS was extracted with 0.05 M EDTA and 0.05 M sodium acetate in 0.05 M sodium oxalate at pH 5.2 at 70 °C for 1 h. The suspension was centrifuged (20 min, 38,000 × *g*, 20 °C). The residue was re-extracted once and all supernatants were combined. Supernatants and residue were dialyzed twice against 0.1 M ammonium acetate buffer, pH 5.2 and subsequently against demineralized water and after freeze drying denoted as chelating agent soluble solids (CHSS) and chelating agent un-extractable solids (CHUS). CHUS was treated with 0.05 M NaOH containing 20 mM $NaBH_4$ at 0 °C for 1 h. The suspension was centrifuged (20 min, 38,000 × *g*, 20 °C), the residue was re-extracted twice and all supernatants were combined. Supernatants and residue were neutralized with acetic acid, dialyzed twice against 0.05 M sodium acetate buffer, pH 5.2 and against demineralized water. After freeze drying, they were denoted dilute alkali soluble solids (DASS) and dilute alkali un-extractable solids (DAUS). Finally, DAUS was treated with 4 M NaOH + 20 mM $NaBH_4$ (concentrated alkali) at 0 °C for 1 h. The suspension was centrifuged (20 min, 48,000 × *g*, 20 °C) and the residue was re-extracted twice. Supernatants and residue were treated as described above for DASS and DAUS and after freeze drying denoted concentrated alkali soluble solids (CASS) and Res.

2.2. Analytical methods

All analyses were performed in duplicates.

2.2.1. Carbohydrate composition

Samples were pre-hydrolysed with 72% (w/w) sulphuric acid for 1 h at 30 °C followed by hydrolysis with 1 M sulphuric acid for 3 h at 100 °C. The monosaccharides released were derivatized into their corresponding alditol acetates and determined by gas chromatography (Englyst & Cummings, 1984) using inositol as an internal standard. The total uronic acid content was determined using an automated m-hydroxydiphenyl assay (Thibault, 1979). Starch content (including resistant starch) was determined enzymatically using the Megazyme total starch kit (Megazyme, Ireland). The starch glucose (Glc) was subtracted from the total Glc to obtain cell wall Glc.

2.2.2. Protein content

Protein content (N × 6.25 (Van Gelder, 1981)) was determined on a Thermo Quest NA 2100 Nitrogen analyzer (Interscience, The Netherlands). D-Methionine (Acros Organics, USA) was used as an external standard.

2.2.3. Acetic acid and esterified methanol content

Contents of acetic acid and methanol were determined using HPLC after saponification of 0.01 g sample by 0.4 N NaOH (1 mL) in isopropanol/water (ratio 1:1) for 4 h. For HPLC, an Ultimate 3000 system (Thermo Scientific, USA) equipped with an Aminex HPX-87H ion exclusion column (300 mm × 7.8 mm; Bio-rad Laboratories, USA) was used in combination with a self-packaged guard column (50 mm × 7.8 mm, AG 50W-X4 Resin; Bio-Rad). Elution was performed with 0.005 M sulphuric acid at a flow rate of 0.6 mL/min. 20 μL of each sample was injected and eluted at 40 °C and a refractive index detector, Shodex type RI 101 was used for detection. The degree of acetylation (DA) and methyl esterification (DM) were calculated as moles of acetic acid or methanol per 100 mol of uronic acid, respectively, and were corrected for free acetic acid and methanol.

2.2.4. Ferulic acid content

PPF, BIS and HBSS (0.01 g each) were suspended in 200 μL methanol and 1 mL 0.5 M KOH (flushed with N_2). Samples were submerged in N_2 and stored in the dark at 20 °C for 16 h. After 16 h, 0.75 mL of 6 M HCl was added to adjust the pH to <2. The ferulic acid released was extracted twice using 4 mL of ethyl acetate. The ethyl acetate fractions were combined and dried under N_2. The dried residue was dissolved in 1 mL of methanol prior to analysis. Analysis was performed on an Acella UHPLC system (Thermo Scientific, USA) as described by Appeldoorn, Kabel, Van Eylen, Gruppen, and Schols (2010). Ferulic acid (FA) was identified and quantified using standards and corrected for free FA. The recoveries ($n = 3$) were 89.5% (±0.003 standard deviation) for FA.

2.3. Molecular weight characterization of cell wall polysaccharides

2.3.1. High performance size exclusion chromatography

Analyses of molecular weight distribution of oligo- and polysaccharides were performed using high performance size exclusion chromatography (HPSEC). For this, an Ultimate 3000 HPLC (Thermo Scientific) was used with three TosoHaas TSK-gel columns in series (4000, 3000 and 2500 SuperAW; each 150 mm × 6 mm; TosoHaas, Japan) preceded by a TSK Super AW-L guard column (3.5 cm × 4.6 mm; TosoHaas). 20 μL of sample (0.002 g/mL) was injected and eluted with 0.2 M sodium nitrate at a flow rate of 0.6 mL/min at 55 °C. Detection was performed with a refractive index detector Shodex R101 (Showa Denko, Japan). The system was controlled using Chromeleon (version 7) software (Dionex, USA; now ThermoScientific, USA). For calibration, pullulan standards (Sigma; mass range of 180 Da to 790 kDa) and pectin standards (mass range of 2.8–100 kDa) were used.

2.3.2. Matrix assisted laser desorption/ionization time of flight mass spectrometry

Oligosaccharides were profiled using Matrix Assisted Laser Detection Time of Flight Mass Spectrometry (MALDI-TOF MS). An Ultraflextreme workstation (Bruker Daltonics, Germany) equipped with a nitrogen laser of 337 nm was used in positive mode. After a delayed extraction time of 200 ns, the ions were accelerated to a kinetic energy of 12 kV and were detected using reflector mode. Data were collected by averaging at least 100 laser shots. The lowest laser power needed to obtain sufficient spectra intensity was used. The apparatus was calibrated using a mixture of maltodextrins (Avebe, The Netherlands). Samples (0.001 g/mL) were desalted using ion exchange material (AG 50W-X8 resin; Bio-Rad Laboratories, CA, USA). 1 μL of desalted sample was mixed with 1 μL of matrix solution on an MS target plate. The matrix solution used was 2,5-dihydroxybenzoic acid (Bruker Daltonics) in a concentration of 0.01 g/mL of acetonitrile/water ratio of 3:7. The sample mix was dried under a stream of warm air before analysis.

2.4. Enzymatic profiling of cell wall polysaccharides

Pure and well characterized enzymes (Table 1) were used to specify the type of polysaccharides present in the HBSS, CHSS and CASS extracts. 0.002 g/mL (total sugars) of HBSS, saponified HBSS (sHBSS), CHSS and CASS were prepared in 10 mM sodium acetate buffer, pH 5. Saponification of HBSS was carried out by incubating HBSS in 0.05 N NaOH for 24 h at 4 °C. Afterwards, the solution was acidified with 0.1 M acetic acid to pH 5 and the volume was adjusted with 10 mM sodium acetate buffer, pH 5 to obtain a final concentration of 0.002 g/mL (total sugars).

All enzyme digestions were carried until endpoint incubation. Polygalacturonase (PGII) and a combination of endo-galactanase (endo-Gal) and β-galactosidase (exo-Gal) were applied to HBSS and sHBSS in a dose of 0.01% (w/w) of enzyme protein to total sugar. PGII was also applied to CHSS in the same dosage and all incubations were carried for 24 h. CASS was incubated with 0.0128 mL/g (total sugars) of a xyloglucan specific endo-glucanase (EGII, Table 1) for 48 h. After this, CASS was incubated separately with 0.01% (w/w of enzyme protein to total sugar) endo-xylanase (xylanase, Table 1) and separately with 0.01% (w/w of enzyme protein to total sugar) endo-mannanase (Mannanase, Table 1). The incubations were carried at 40 °C in a head over tail mixer and enzyme digests were inactivated at 100 °C for 10 min. Samples were centrifuged (20 min, 19,500 × *g*, 20 °C) before analysis on HPSEC and MALDI-TOF MS.

2.5. Water holding capacity

WHC measurements were performed using the Baumann's apparatus (Baumann, Germany; Baumann, 1966). A small quantity (minimum 0.01 g) of dried sample was placed on the filter (G2; Duran, Germany) and the volume of water absorbed to hydrate the sample until saturation was recorded.

Reference substrates tested were potato starch (Sigma Aldrich, USA), polygalacturonic acid (ICN Biochemicals, USA), cellulose (filter paper; Whatman, Grade 3, UK), tamarind seed xyloglucan (Dainippon Sumitomo Pharma, Japan), linear arabinan and branched arabinan (British Sugar, UK) and potato galactan (Megazyme).

For HBSS, a filter paper (grade 595 Whatman, Germany) was used above the filter and water absorbed by the filter was corrected accordingly. In addition, the WHC was also measured for a combination of HBSS and BIS (in a ratio of 1:1) and a combination of HBSS and BIS mixed with water (in a ratio of 1:1; 0.07 g/mL) at 200 rpm in a shaking incubator (Innova 40 R, New Brunswick, USA) for 2.5 h at 40 °C before freeze drying.

The WHC was expressed as the volume of water held in mL per g dry matter of sample analyzed. Corrections were made for evaporation losses by measuring water uptake without sample. All measurements were performed in triplicates.

3. Results and discussion

3.1. Cell wall polysaccharides in PPF extracts

PPF contains 74% (w/w) carbohydrates of which 30% is starch (Table 2). The most abundant CWPs in PPF are built from glucosyl (Glc), galactosyl (Gal) and uronyl (UA) residues.

3.1.1. Distribution and composition of CWPs in extracts

After de-starching, 46% of all CWPs from PPF are recovered in HBSS (dialyzed), while 41% remains in BIS. This is consistent with earlier data on PPF where Thomassen, Vigsnæs, Licht, Mikkelsen, and Meyer (2011) reported a high solubilization of dry matter in phosphate buffer. From undialyzed HBSS (51% of all CWPs from PPF), 5% of CWPs are removed upon dialysis as Rha, Ara, Gal and UA. Oligomers of these CWPs were present prior to de-starching PPF (data not shown). Since HBSS is composed of 85% (w/w) of CWPs, of

Table 1
Enzymes employed in screening of polysaccharide structures in extracts.

Enzyme	Organism	Specific activity	Literature
Polygalacturonase II (PG II)	*Aspergillus niger*	8700 U/mg	Kester and Visser (1990)
Endo-galactanase (Endo-Gal)	*Aspergillus aculeatus*	988 U/mg	Van de Vis, Searle-van Leeuwen, Siliha, Kormelink, and Voragen (1991)
Betagalactosidase (Exo-Gal)	*Aspergillus niger*	0.8 U/mg	Laboratory of Food Chemistry, unpublished data
Endo-Xylanase I	*Aspergillus awamori*	7.8 U/mg	Kormelink et al. (1993)
Endo-mannanase	*Aspergillus niger*	86.4 U/mg	Düsterhöft, Bonte, and Voragen (1993)
Xyloglucan specific endo-glucanase (EGII)	*Aspergillus aculeatus*	2259 U/mL	Pauly et al. (1999)

Table 2
Composition of cell wall polysaccharides from PPF.

Fraction	Yield% CWPs (dry PPF)	NSPs: Sugar composition (mol%); yield% sugar residues[a,b]								mol%		w/w% dry matter		
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc[d]	UA	DA[c]	DM[c]	Starch	CWP	Protein
PPF	100	4 (100)	0 (100)	8 (100)	3 (100)	2 (100)	27 (100)	32 (100)	24 (100)	25	17	30	44	4
HBSS	46	5 (53)	0 (19)	8 (48)	1 (9)	1 (26)	41 (68)	1 (2)	43 (79)	20	1	0.4	85	4.4
BIS	41	2 (23)	0 (23)	5 (25)	7 (92)	3 (66)	4 (6)	73 (96)	6 (10)	156	2	0.7	59	5.7
CHSS	1.2	4 (1)	0 (0.9)	6 (0.9)	2 (0.7)	3 (2)	13 (0.6)	9 (0.3)	64 (3)	6	3	n.d.	32	n.d.
DASS	0.5	5 (0.6)	1 (2)	15 (1)	9 (1)	2 (0.8)	31 (0.7)	19 (0.3)	18 (0.4)	n.d.	n.d.	4.6	21	n.d.
CASS	9.7	2 (5)	0 (9)	7 (9)	19 (60)	8 (51)	7 (3)	54 (18)	2 (0.9)	n.d.	n.d.	0.1	42	n.d.
Res	30.6	2 (12)	0 (17)	3 (10)	1 (13)	0 (6)	1 (1)	90 (87)	3 (4)	n.d.	n.d.	1	68	n.d.

[a] Values in brackets indicate yields of sugar residues; "n.d." is not determined.
[b] Rha, rhamnose; Fuc, fucose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acid.
[c] DA is degree of acetylation and DM is degree of methyl esterification calculated as moles of acetyl/methyl esters per 100 mol of uronic acid.
[d] Cell wall Glc.

which 41 mol% is Gal, 8 mol% is arabinose (Ara) and 43 mol% is UA, it is expected to be rich in pectins (Table 2). About 76% of pectins (UA and Rha) from PPF were extracted into HBSS.

Earlier studies on cooking or de-starching of intact potato cell walls solubilized much less pectins. Ball milling of uncooked cells released much more cell wall material as small and large fragments than decompression of cooked potato cells (Van Marle, Stolle-Smits, et al., 1997). De-starching of non-processed potato cell walls show much less solubilization of CWPs (less than 6%; Ring & Selvendran, 1978) than that of processed cell walls (our data). The composition of pectins in HBSS is similar to the Na_2CO_3 extract of intact potato cell walls as shown by Jarvis et al. (1981) after extraction of calcium bound pectins. Pectin solubilization into HBSS without prior chelation is possible if the middle lamella (rich in calcium bound pectins (Caffall & Mohnen, 2009)) is ruptured. This indicates that disruption of the cell wall interactions in potatoes may have occurred during processing in industrial starch extraction. In PPF, a lower ratio of Rha:Gal (about 1:7) is observed compared to whole potato cell walls (about 1:35), indicating a relatively lower occurrence of Gal in PPF cell walls (Van Marle, Recourt, Van Dijk, Schols, & Voragen, 1997). This could imply differences in the packaging of CWPs between PPF and potato cell walls.

Less than 2% (w/w) CWPs are solubilized into CHSS and DASS, 9.7% in CASS and 30.6% remains in Res. In prior studies on potato cell walls, 50% CWPs were left in Res (Ryden & Selvendran, 1990; Van Marle, Recourt, et al., 1997). This difference supports the observation that a less rigid CWP architecture exists in PPF compared to non-processed potato cell walls.

Only 0.7% of starch remains in BIS, which contains 59% (w/w) as CWPs, mainly comprising cell wall Glc and Xyl. UA is the major sugar in CHSS (64 mol%, Table 2), which contains 2.7% (w/w) of HG (based on UA) from PPF. A Rha:UA ratio of 1:17 points that CHSS is rich in HG.

Dilute alkali (0.05 M NaOH) treatment of CHUS extracts mainly Gal, UA, Ara, and Rha (Table 2) in DASS and most of the starch from BIS (4.6% on dry matter; Table 2). Compared to HBSS, the ratio of (Ara + Gal)/Rha (8.5) is lower, indicating lesser branching of RG and an Ara:Gal ratio of 1:2 indicates lesser dominance of Gal residues. The ratio of Rha:UA (2:7) is higher than that of HBSS suggesting that DASS is richer in RG. DASS is thus distinct from HBSS with respect to abundance and composition of RG.

CASS mainly comprises Xyl (19 mol%) and Glc (54 mol%) (Table 2) pointing to the presence of xyloglucans (XGs) reported earlier as XXGG type, in which the Xyl:Glc ratio is 1:2 for potato cell walls (Vincken et al., 1996). Since CASS shows a ratio of 1:3, it indicates possible presence of β-glucans or glucomannans. In previous studies, a Xyl:Glc ratio of 1:9 for the 6 M NaOH extract by Jarvis et al. (1981) suggests differences in alkali soluble glucans between PPF in our study and potato cell walls.

Res is abundant in cell wall Glc (90 mol%) (Table 2) and contains 87% of all cell wall Glc from PPF which is possibly cellulose.

In general, our results suggest that most pectins are easily extractable from PPF due to a less rigid cell wall network. Few pectins are extracted in CHSS and DASS extracts, while hemicelluloses are mainly extracted in the CASS extract, leaving glucans, most likely cellulose, in Res.

3.2. Esterification of cell wall polysaccharides

The degree of acetyl esterification (DA) and methylation (DM) of PPF is 25 and 17, respectively. This is consistent with earlier data (Turquois, Rinaudo, Taravel, & Heyraud, 1999). Since PPF has a lower DM than reported for potato cell walls (49–53, Van Marle, Recourt, et al., 1997), most likely methyl esters are removed during the industrial starch extraction process. Also, although the experimental set up should have inactivated the native pectin methyl esterase, extracts from PPF even show a lower DM due to losses in methyl esters. The DA of BIS (156, Table 2) suggests a high level of substitution of acetic acid ester-groups over pectin as reported previously for potato pectic hairy regions (DA of 90; Schols & Voragen, 1994). However, it cannot be excluded that non-pectic CWPs could also be acetylated, such as xyloglucans in tomato (Jia, Cash, Darvill, & York, 2005). Next to acetylation and methyl esterification of CWPs, ferulic acid esterification was determined for potato CWPs. Not much information on feruloyl substitution is known for potato CWPs. The ferulic acid (FA) content is 0.01% ((w/w) dry matter) in PPF and 0.03% in BIS, which is slightly higher than those for potato peels (0.007%; Nara, Miyoshi, Honma, & Koga, 2006). 8-*O*-4 linked di-FA (*m*/*z* 385 (M−H)), which was one-fifth the response of FA, was observed in PPF and BIS, and has not been reported previously for potato cell walls. In BIS, 1 mol of FA corresponds to 233 mol of Ara + Gal. For sugar beet pulp, pectin populations are reported containing 1 mol of FA per 67 mol of Ara + Gal (Oosterveld, Beldman, & Schols, 1996). For wheat arabinoxylans, in which 1 mol of FA corresponds to 308 mol of arabinose + xylose (Gruppen, Marseille, Voragen, Hamer, & Pilnik, 1989), feruloyl substituents were indicated to maintain the quarternary structure of CWPs (Smith & Hartley, 1983). In our PPF and BIS, LC–MS detection of (-di)FA esters could indicate presence of quarternary structures in PPF. Further

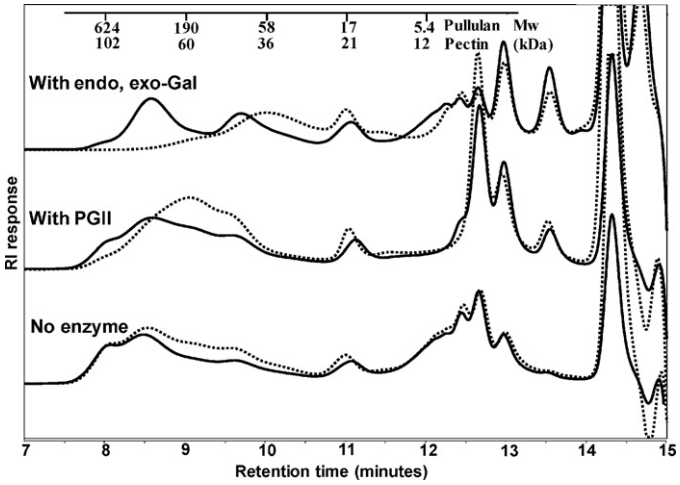

Fig. 1. HPSEC elution patterns of enzymatic profiling after 24 h of HBSS (—) and saponified HBSS (s.HBSS; ···) with polygalacturonase (PGII) and a combination of endo-galactanase and β-galactosidase (endo, exo-Gal). Molecular weight (Mw) annotations on top x-axis are based on pullulan and pectin standards.

research on these (-di)FA's may prove their contribution to quarternary structures and effect on WHC.

3.3. Size distribution of polysaccharides in soluble extracts

To compare the molecular weight (Mw) distributions of extracts, HPSEC was performed (HBSS in Fig. 1). For HBSS, two major populations of CWPs are observed where the larger population is distributed over a broad molecular weight (Mw) range of 50–570 kDa next to a smaller population of 12–170 kDa. Based on sugar composition, these populations could represent pectin of different Mw. For CHSS and DASS (not shown), a large population from 50 to 570 kDa is observed. CASS (in Fig. 2A) shows a broad population distributed from a low to high Mw range (5–572 kDa) representing hemicelluloses. Thus, HPSEC profiling of extracts show main differences in Mw distributions of HBSS, CHSS and DASS on one hand and CASS on the other hand. The HPSEC data indicates that next to sugar composition data, pectins and hemicelluloses have distinct Mw populations.

3.4. Enzymatic profiling of cell wall polysaccharides in extracts

To obtain more information on the structure of CWPs present in extracts, pure and well characterized enzymes (Table 1) were used. It was confirmed that the digestion was an endpoint incubation based on elution patterns of defined substrates treated with these enzymes. Therefore, it was assumed that CWPs were targeted by the enzymes as far as possible, although the extent of degradation of the extract is far from complete. Nevertheless, the enzymatic profiling described is well usable to study CWP structural characteristics in various PPF extracts.

3.4.1. Profiling CWPs in HBSS

Sugar composition data of HBSS shows dominance of UA and Gal. Therefore, HBSS was profiled using polygalacturonase (PGII) and a combination of endo-galactanase (endo-Gal) and beta-galactosidase (exo-Gal). CWPs in HBSS are acetylated (DA of 20). Therefore, after saponification, PGII shows more degradation (Fig. 1) in the high Mw region eluting between 7.5 and 8.5 min. Irrespective of saponification, comparable profiles are observed in the low Mw region eluting between 11.5 and 12.5 min. This low Mw region in undigested HBSS may be abundant in low Mw HG. Surprisingly, only saponified HBSS (s.HBSS) shows a complete shift in HMw material when digested with a combination of endo-Gal and exo-Gal. This indicates high esterification of RG-I (Table 2) in HBSS and is in line with earlier findings where for potato RG-I (Schols & Voragen, 1994). Removal of acetyl groups increases accessibility of these enzymes to galactan side chains. Due to this, it is possible that a significant distortion in the secondary structure of pectic hairy regions occurs resulting in a complete shift in the high Mw region of HBSS. These results suggest that HBSS contains acetylated CWPs mainly rich in pectic galactans and HG.

3.4.2. Profiling CWPs in CHSS using polygalacturonase

As the degree of esterification of HG in CHSS is very low (Table 2), PG II seems to degrade CHSS effectively to produce low Mw material less than 10 kDa (added as supplementary figure). This data supplements sugar composition of CHSS indicating that it is mainly dominant in HG.

3.4.3. Profiling CWPs in CASS using hemicellulases

Digestion of CASS with xyloglucan specific endoglucanase (EGII) degrades 59% of soluble material eluting between 8 and 12 min (Fig. 2A). This indicates that CWPs in soluble CASS largely consist of xyloglucans (XGs). The abundance of xyloglucans in CASS is supported by previous studies on potato xyloglucans by Vincken et al. (1996), wherein linkage analyses of a purified alkali extract showed to consist exclusively of terminal and 1,2-linked Xyl, together with 1,4 and 1,4,6-linked Glc. Oligomer profiling of EGII treated CASS using MALDI-TOF MS shows that the main fragments in the low Mw region are H_3P_2, H_3P_3, H_4P_2, H_4P_3, H_5P_2, H_5P_3, H_6P_2 and H_6P_3 (H is hexose and P is pentose (Fig. 2B)). These are expected to correspond to XXG, XSG, XXGG or LXG, XSGG, XLGG, LSGG and LLGG (where G is glucose, X is xylose linked to glucose, L is galactose and S is arabinose linked to xylose–glucose) building blocks reported previously by Vincken et al. (1996). After treatment with EGII, treatment of CASS with mannanase and xylanase further degrades CASS, indicating the presence of mannans and xylans. Earlier studies on potato CWPs also have reported the presence of 4-linked mannans and 4-linked xylans (Jarvis et al., 1981), which are yet to be confirmed. Further investigations are necessary to characterize their structures.

In general, enzymatic profiling revealed that HBSS mainly contains acetylated pectins comprising HG and pectic galactans which were easily extractable while CHSS exclusively contains HG. On the other hand, CASS is abundant in hemicellulose, mainly xyloglucans with lesser abundance of mannans and xylans.

3.5. Water holding capacity

The water holding capacity (WHC) is defined as the amount of water held (in mL) per g dry matter of sample. The WHC is determined using the Baumann's method based on the principle that a certain amount of water hydrates a known amount of dried sample material (usually in the form of a powder) until equilibrium or saturation. The amount of water held by defined substrates and PPF substrates (extracts and residues sequentially derived from PPF and representing different populations of CWPs) was determined (Figs. 3 and 4). Also, the relative contribution of each of the PPF substrates to the total water held by PPF was determined (Fig. 4) and expressed as "the WHC contributed by a substrate to the WHC of PPF". This relative contribution of PPF substrates to the total WHC of PPF (Fig. 4) is calculated based on the CWP yield per substrate (extract or residue) and the mL of water held per g of CWPs in the substrate (denoted in Fig. 4 as "mL/g CWPs in sample for 1 g total CWPs in PPF").

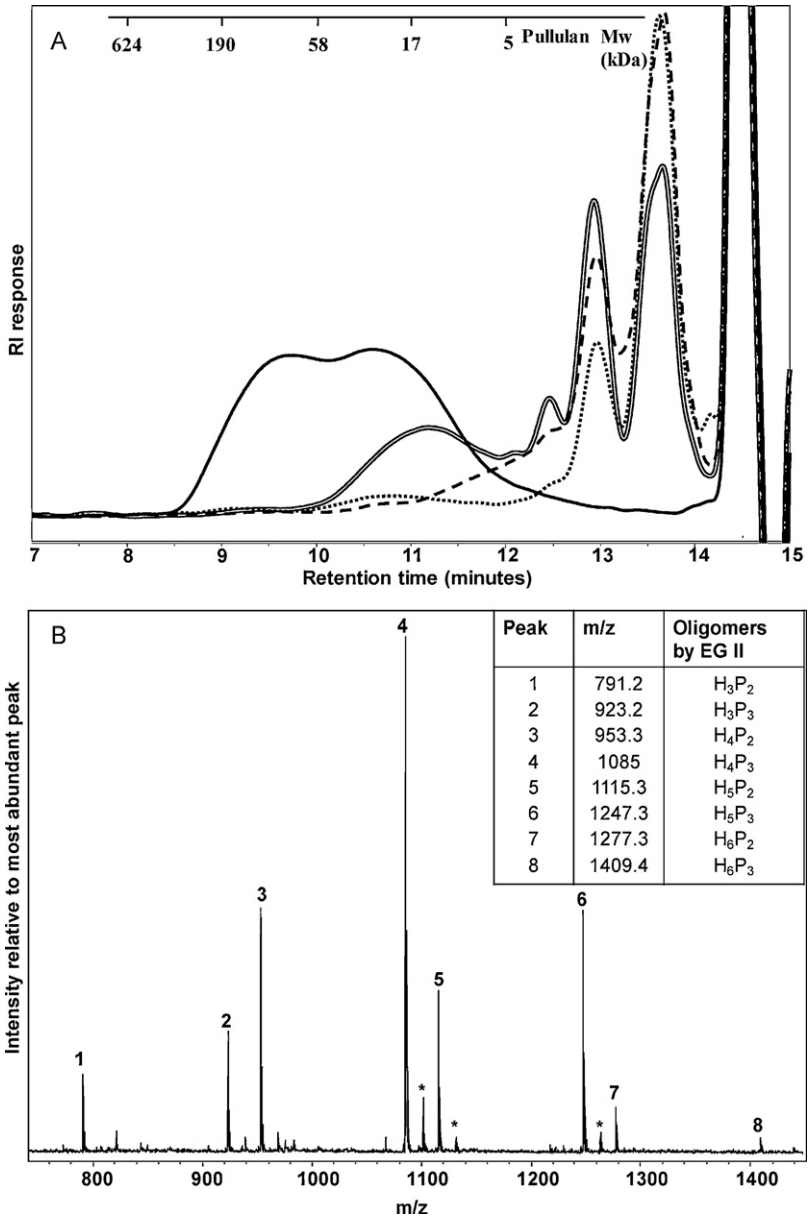

Fig. 2. (A) HPSEC elution patterns of CASS before (—) and after digestion with EGII (=), EGII and xylanase (– –) and EGII and mannanase (· · ·). (B) MALDI-TOF-mass spectrum of CASS after digestion with EG II. P is pentose and H is hexose (sodium adducts),* represent potassium adducts.

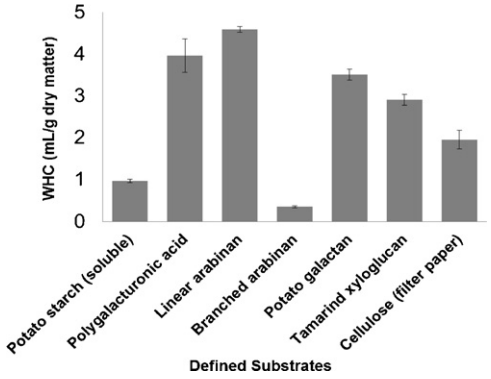

Fig. 3. Water holding capacity (WHC) values (mL/g) of reference substrates with the Baumann's apparatus. See Section 2 for more details on substrates.

3.5.1. *Water holding capacity of reference substrates*

Of all reference substrates tested, linear arabinan shows the highest WHC (4.6 mL/g); α-(1,5)-linked backbone of L-arabinosyl residues (Beldman, Schols, Pitson, Searle-van Leeuwen, & Voragen, 1997). The WHC of branched arabinan (0.4 mL/g); arabinan substituted with α-(1,2) and/or α-(1,3) linked L-arabinosyl residues (Beldman et al., 1997) is much lower than that of linear arabinan. This could be related to the observation that longer chains of linear arabinan are more flexible than highly branched arabinan, and, therefore, more mobile to interact with water giving them a higher radius of gyration and hydrodynamic volume (Chaplin, 2003). Potato galactan has a lower WHC (3.5 mL/g) than linear arabinan, but higher than branched arabinan. This may be due to increased mobility of galactan side chains in solution. The results are in line with prior studies in which arabinan side chains hydrate more readily than galactan and shorter side chains impart RG-I with a lesser affinity to water (Larsen et al., 2011). Compared to galactan, filter paper cellulose shows a lower WHC. Our observation matches

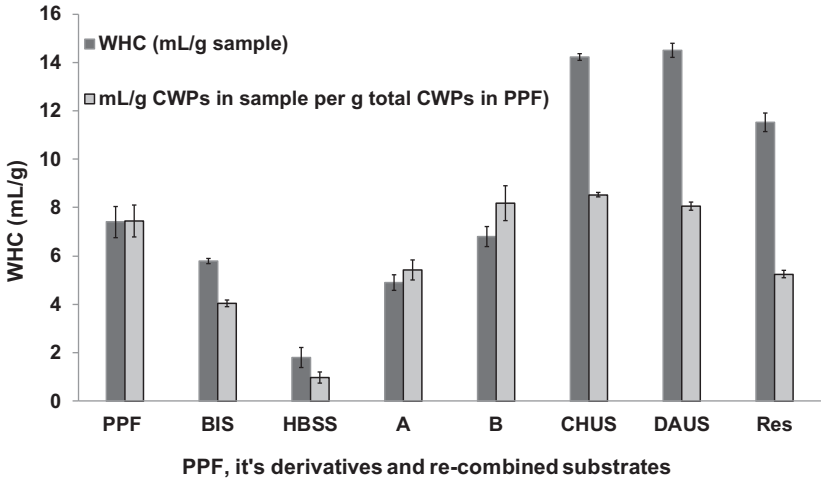

Fig. 4. Water holding capacity (WHC) values (mL/g) of PPF and substrates derived from PPF. "A" is reconstitution of HBSS and BIS by mixing them without water and "B" is re-constitution of HBSS and BIS in water. See Section 2 for more details on A and B.

with the finding that for onion cell wall material solid state NMR revealed a lower hydration for cellulose than for pectic galactan. This was not only due to increased mobility of the latter but also due to easier penetration of water into galactan (Hediger, Emsley, & Fischer, 1999).

3.5.2. Water holding capacity of substrates derived from PPF

In general, substrates derived from PPF have higher WHCs than reference substrates (Fig. 4 versus Fig. 3). This was expected since PPF substrates represent different populations of CWPs, rather than pure reference substrates. Either entrapment of water within the matrix of CWM or water binding due to interactions between CWPs, most likely contributes to the high WHC (Fig. 4).

PPF has a WHC of 7.4 mL/g. After extracting 50% CWPs from PPF in HBSS, the cell wall network is loosened. The resulting BIS has a WHC, which is 22% lower than the WHC of PPF. HBSS, in which both UA and Gal (Table 2) are abundant and present in equal proportions, shows a lower WHC than that of reference substrates potato galactan and polygalacturonic acid. When a mix of HBSS and insoluble BIS is reconstituted by hydrating for two and a half hours followed by freeze-drying, the WHC of the recombined sample is higher than the mix reconstituted in the absence of water. In presence of water, it is likely that galactan side chains in HBSS become mobile due to which RG-I in HBSS is hydrated as observed previously (Larsen et al., 2011). After hydration, these polysaccharides may interact with CWPs in BIS forming a network similar to that in PPF. This indicates that the interactions are at least partly restored in this mix and play a role in the WHC. The exact role of HG remains unclear, although HG and galactan are present in equal amounts in HBSS. Since soluble potato starch had a very low WHC (1 mL/g; Fig. 3), the presence of residual amounts of starch is not expected to have much effect on the WHC of PPF. Removal of calcium bound pectins from BIS modifies the cell wall network resulting in CHUS with a very high WHC (14.2 mL/g). Modification of the network could be because of spatial rearrangement of the packaging of CWPs instead of only removing HG. Therefore, the role of HG is not discussed further. Removal of hairy pectins from CHUS did not have any major effect on the WHC seen in DAUS. Removal of CASS from DAUS, lowers the WHC further by 21% seen for Res. CASS is found to consist of hemicelluloses mainly comprising xyloglucans (XGs), which could contribute positively to a higher WHC. Alkali treatment is expected to increase the swelling and therefore, most likely, the apparent WHC of the Res which is abundant in cellulose (Pott, 2003). In view with these findings and as discussed earlier for reference substrates, cellulose alone is not expected to contribute much to the WHC of PPF.

In general, the results suggest that among the most influential interactions of CWPs in regulating the WHC of PPF, are those between HBSS and BIS CWPs. In more detail, the results implicate that galactans, and xyloglucans in the CHUS and DAUS residues could be important in regulating the WHC of PPF.

4. Conclusions

The cell wall architecture in PPF is less rigid than in non-processed potato cell walls as observed from the high solubilization of pectins and pectic galactans in HBSS. In addition, the composition of CWPs in PPF is different than in non-processed potato cell walls with respect to alkali soluble glucans and a lower proportion of galactans. Profiling of the main CWP extracts, HBSS and CASS, using enzymes showed that the most easily extractable CWPs from PPF are acetylated homogalacturonan and rhamnogalacturonan rich in galactans while hemicelluloses, mainly xyloglucans, are extracted under harsher conditions. The WHC of PPF is restored at least partly after hydrated mixing of HBSS with BIS. The WHC of PPF, may be governed by interactions between CWPs in which the water soluble galactans and alkali soluble xyloglucans are implicated to be important.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.12.057.

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