JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Structural and Water-Holding Characteristics of Untreated and Ensiled Chicory Root Pulp

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ABSTRACT: Cell wall polysaccharides (CWPs) from chicory root pulp (CRP) and the effect of ensiling on CWP structure to reduce the water-holding capacity (WHC) were studied. Sequential extractions of CRP showed that hot water extraction solubilized arabinan-rich pectin and inulin, each representing 6% of all CRP sugars. A significant amount of pectic sugars (46%) rich in uronic acid from CRP was solubilized by chelating agents. Both dilute alkali extraction, which solubilized branched pectin (14% from CRP), and concentrated alkali extraction, which solubilized hemicellulose dominant in xyloglucans (2.5%) mostly of the XXXG type and mannan (0.9%), from CRP CWPs seemed to influence the WHC of CRP. Alkali-insoluble residue (39% of CRP sugars) mainly comprised cellulose and some branched pectin (17% from CRP). Ensiling reduced the methyl esterification of pectin, caused degradation of homogalacturonan and rhamnogalacturonan, and possibly modified the xyloglucan, mannan, and glucan network, reducing the WHC from 6 mL/g to 3.4 mL/g.

KEYWORDS: *chicory root, extraction, cell wall polysaccharides, pectin, water-holding capacity, ensiling*

1. INTRODUCTION

Chicory roots (*Cichorium intybus*) are industrially used for the extraction of inulin, a prebiotic fiber ingredient used in many food applications.[1] Chicory root pulp (CRP) obtained after inulin extraction is rich in cell wall polysaccharides (CWPs), predominantly pectin.[2,3] The use of CRP as a fiber supplement in food applications could be promising since plant fibers have been claimed to contribute to health benefits.

Supplementing foods with fiber from such byproducts requires the understanding of its functional properties, such as water-holding capacity (WHC) and swelling behavior. These physical properties are determined by the molecular structure of the cell wall polysaccharides and the cell wall architecture.[4] The constituent monosaccharide composition of chicory root pulp has been studied before.[2,5] Differences were found in the degree of esterification, sugar contents, and average molar mass of pectins extracted with acid and those with enzymes, such as cellulases and proteases.[5] Despite the differences between the pectins obtained by the two treatments, they have in common that they were highly methyl esterified (49−53) and acetylated (12−17). Besides the characterization of extracted pectins from CRP, not much information is present on the interactions between different subpopulations of pectin and other CWPs present in the chicory root cell wall.

Processing of raw pulp materials may cause different water-binding capacities, as has been illustrated for autoclaving of sugar beet pulp,[6] grinding of sugar beet pulp and citrus pulp,[7] and ensiling of potato pulp with inoculants involving degradation of starch and pectin.[8] This clearly points toward interaction between CWPs, which modulates the water-binding capacity of the material. Different polysaccharides within the cell wall can be studied by sequentially solubilizing polysaccharides with extractants of increasing severity.[9]

The aim of this present research was to characterize the composition of CWPs of CRP and their role toward the WHC of the pulp. Furthermore, the effect of ensiling on the CWP structure and WHC was investigated.

2. MATERIALS AND METHODS

2.1. Plant Material. CRP was obtained industrially after extraction of inulin from chicory root with hot water at 80 °C. The pulp was dried at 120 °C and was kindly provided by Sensus B.V. (Roosendaal, The Netherlands). The pulp was milled using a 0.5 mm sieve in a Retsch mill (ZM 200, Retsch, Haan, Germany). Ensiling of wet, unheated CRP (ECRP) was performed at Cosun Food Technology Center (Roosendaal, The Netherlands). A container of 20 L was filled completely with the pulp, and air was evacuated by applying pressure on the pulp before sealing the container. The fermentation by endogenous bacteria was performed at 20−25 °C for 21 days. The ensiled pulp was subsequently heat dried at 120 °C and milled to a particle size of 0.5 mm.

2.2. Sequential Extraction of Cell Wall Polysaccharides from Plant Materials. The first treatment involved extracting solids from CRP and ECRP with hot water at 80 °C for 1 h. The substrate:extractant ratio was 1:30 (w/v). The suspension was centrifuged (20 min, 38000*g*, 20 °C) to obtain the extract. The extraction was repeated until no sugars could be detected in the extract using the phenol sulfuric acid color assay.[10] This check was also performed for other extractions. The extracts were combined and dialyzed against demineralized water using cellulose dialysis membranes (cutoff 12−14 kDa for proteins, Visking, Medicell International, London, UK), freeze-dried, and denoted as hot water soluble solids (HWSS). The final residue obtained was also freeze-dried and denoted as water unextractable solids (WUS).

The WUS was treated with chelating agents (0.05 M EDTA/0.05 M ammonium oxalate) in 0.05 M sodium acetate buffer, pH 5.2 at 70 °C for 1 h. The substrate:extractant ratio was 1:50 (w/v). Next, the suspension was centrifuged (20 min, 38000*g*, 20 °C) to obtain the extracts. The combined extracts and the residue were dialyzed

Received: April 12, 2013
Revised: May 31, 2013
Accepted: May 31, 2013
Published: May 31, 2013

Table 1. Sugar Composition of Chicory Root Pulp (CRP) and Fractions Derived from the Pulp[a]

	carbohydrate yield [g in fraction per 100 g in pulp]	mol %								total sugars, % w/w	DM	DA	protein, % w/w
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA				
CRP	100	1	0	15	4	4	7	31	38	64	70	43	7.6
HWSS	11	1	0	31	1	10	7	13	37	50	90	27	3.5
WUS	87	1	0	12	4	2	7	33	40	68	70	46	7.5
CHSS	29	1	0	11	0	1	4	1	82	52	49	14	
CHUS	52	1	0	10	6	3	7	53	19	59	52	91	
DASS	9	2	0	31	1	1	17	2	46	52			
DAUS	45	1	0	9	8	3	7	61	11	62			
CASS	4	0	2	5	29	17	7	35	5	56			
CAUS	39	1	0	10	4	1	7	67	10	67			

[a]DM/DA: degree of methyl/acetyl esterification expressed as moles of methanol esters/acetyl groups per 100 mol of uronic acid. Rhamnose (Rha), fucose (Fuc), arabinose (Ara), xylose (Xyl), mannose (Man), galactose (Gal), glucose (Glc), uronic acid (UA).

subsequently against 0.1 M ammonium acetate buffer, pH 5.2, and against demineraliszed water before freeze-drying. The dialyzed extract, denoted as chelating agent soluble solids (CHSS), and the residue, denoted as chelating agent unextractable solids (CHUS), were freeze-dried.

The CHUS was further treated with dilute alkali (0.05 M NaOH containing 0.02 M $NaBH_4$) at 0 °C for 1 h. The extracts obtained after centrifugation (20 min, 38000*g*, 20 °C) were combined and, independently from the residue, neutralized and dialyzed subsequently against 0.05 M sodium acetate buffer, pH 5.2, and demineralized water before freeze-drying. The dialyzed extract, denoted as dilute alkali soluble solids (DASS), and the residue, denoted as dilute alkali unextractable solids (DAUS), were freeze-dried. The DAUS was finally treated with concentrated alkali (4 M NaOH with 0.02 M $NaBH_4$) at 0 °C for 1 h. The extracts obtained after centrifugation (20 min, 38000*g*, 20 °C) were combined and, independently from the residue, treated the same as for the dilute alkali extraction. This dialyzed extract was denoted as concentrated alkali soluble solids (CASS), and the residue was denoted as concentrated alkali unextractable solids (CAUS).

2.3. Enzyme Treatments of CASS. Hemicellulose digests of CASS were obtained by incubating 2.5 mg of CASS in 1 mL of 10 mM sodium acetate buffer (pH 5.0) with enzymes. Separate enzyme incubations were performed at 40 °C for 24 h using xyloglucan-specific endoglucanase (XEG, EC 3.2.1.151 from *Aspergillus aculeatus,* 2.26U, [11]), endoxylanase (X, EC 3.2.1.8 from *Aspergillus awamori* 0.00047U, [12]), and endomannanase (M, EC 3.2.1.78 from *Aspergillus niger* 0.046U[13]). Following incubation, the enzymes were inactivated at 100 °C for 5 min.

2.4. Characterization. *2.4.1. Sugar Composition.* The total constituent monosaccharide content and composition were determined after prehydrolysis with 72% w/w sulfuric acid at 30 °C for 1 h followed by hydrolysis with 1 M sulfuric acid at 100 °C for 3 h. The monosaccharides formed upon hydrolysis were derivatized to alditol acetates and analyzed by gas chromatography using inositol as an internal standard.[14] The automated colorimetric *m*-hydroxydiphenyl assay was used to determine the total uronic acid (UA) content.[15] Measurements were performed in duplicate. Overall, the coefficient of variation for the measurement of the sugar composition was below 6%. Rhamnose (Rha) levels were used to calculate the *rhamnogalacturonan (RG) backbone* content of a fraction assuming the *RG backbone* consists of Rha:UA of 1:1. The *rhamnogalacturonan (RG)* content was calculated as *RG backbone* plus arabinose plus galactose. The *homogalacturonan (HG)* content was calculated as total UA content minus UA content present in *RG backbone*.[16]

2.4.2. Molecular Weight Distribution. High-performance size exclusion chromatography (HPSEC) was performed on an Ultimate 3000 HPLC (Dionex, Sunnyvale, CA, USA) using three TSK-Gel columns connected in series (4000−3000−2500 SuperAW; 150 × 6 mm). The columns were preceded by a TSK Super AW-L guard column (35 × 4.6 mm). All columns were from Tosoh Bioscience (Tokyo, Japan). Sodium nitrate (0.2 M) was used as an eluent at a flow rate of 0.6 mL/min. A volume of 20 μL of the sample (2.5 mg/mL in 0.01 M sodium acetate buffer, pH 5.0) was injected and eluted at 55 °C. Solubles were detected using a refractive index detector, Shodex type RI 101 (Showa Denko, Japan). The software used for acquiring the data was Chromeleon version 7. The molecular mass distribution of polysaccharides was determined using pullulan standards (Polymer Laboratories, Varian Inc., Palo Alto, CA, USA) in the molecular mass range 0.18−790 kDa.

2.4.3. Fructan Content. Samples of 1 mg/mL in 0.05 M sodium acetate buffer, pH 4.7, were treated with 10 μL of inulinase (Fructozyme L, Novozymes, Bagsvaerd, Denmark). The hydrolysis was performed at 50 °C for 18 h. The enzymes were inactivated by boiling for 10 min. After 20 times dilution, 25 μL of the digest was injected into a Dionex ICS 3000 system (Dionex) for high-performance anion exchange chromatography (HPAEC) using a Dionex ICS 3000 autosampler. The system was equipped with a Dionex CarboPac PA-1 column (2 × 250 mm) in combination with a Carbopac PA-1 guard column (2 × 50 mm). The system was equipped with pulsed amperometric detection. Fructose, glucose, and saccharose were eluted at 0.3 mL/min using a gradient with 0.1 M NaOH (A) and 1 M NaOAc in 0.1 M NaOH (B): 0−15 min from 100% A to 85% A and 15% B, followed by a washing step for 9 min with 100% B and an equilibration step for 14 min with 100% A.

Total fructan concentration (Cf) was calculated using the equation $Cf = k(Ff + Gf)$, in which Ff is total fructose released from the fructans, Gf is total glucose released from the fructans, and k is a correction factor for water uptake of monosugars after hydrolysis. $k = [180DP - 18(DP - 1)]/(180DP)$[17] in which DP is the degree of polymerization.

2.4.4. MALDI-TOF MS. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed for oligomer analysis using an Ultraflextreme workstation (Bruker Daltonics, Bremen, Germany). Mass spectra were obtained in positive mode using a nitrogen laser of 337 nm. After a delayed extraction in a time of 200 ns, the ions were accelerated to a kinetic energy of 12 kV and detected using the reflector mode. The laser intensity was adjusted to obtain clear mass spectra. A minimum of 100 mass spectra were used. Prior to obtaining mass spectra of unknown samples, a series of maltodextrins (mass range of 350−2350 Da) were used for calibration. Samples were desalted using AG 50W-X4 resin, and 1 μL of the desalted sample was added to a dried spot of matrix of 1 μL of 10 mg/mL 2,5-dihydroxybenzoic acid (Bruker Daltonics) in 50% (v/v) acetonitrile on the MALDI plate. The sample was dried, and 1 μL of matrix was spread over the dried spot and dried.

2.4.5. Degree of Acetylation and Methyl Esterification. The degree of methyl esterification (DM) and degree of acetylation (DA) of polysaccharides were determined by adding 0.8 mL of 0.4 N sodium hydroxide in 2-propanol/water (50/50 v/v) to 10 mg for 4 h and analyzing the acetic acid and methanol released by HPLC.[18] The DA and DM were calculated as moles of acetic acid or methanol per 100 mol of UA.

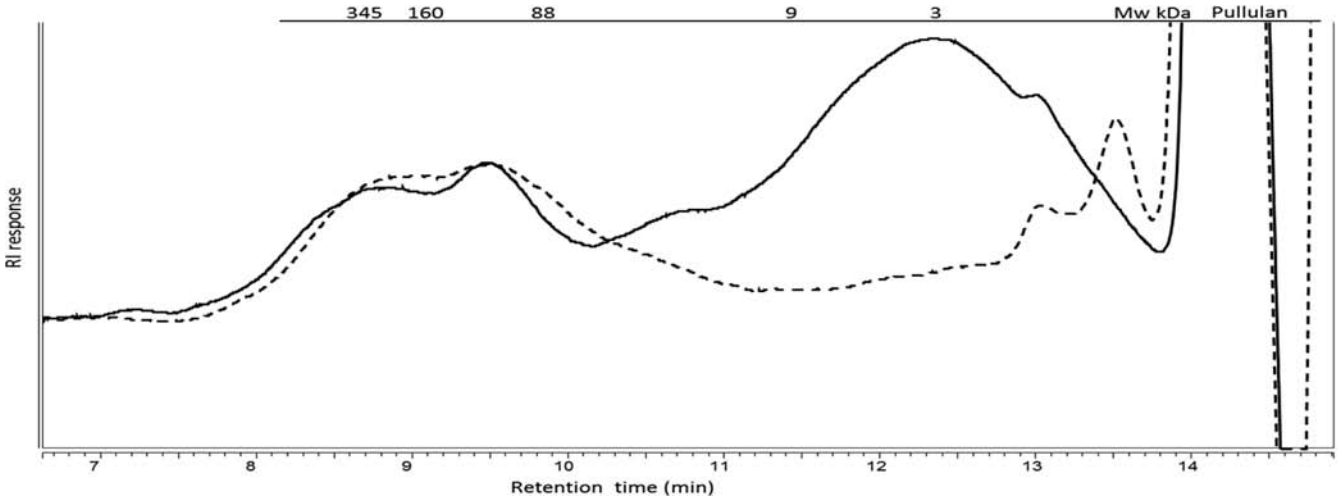

Figure 1. HPSEC elution patterns of HWSS before (solid line) and after treatment with inulinase (dotted line).

2.4.6. Protein Content. The protein content ($N \times 6.25$) was determined on a Thermo Quest NA 2100 nitrogen and protein analyzer (Interscience, Breda, The Netherlands) by combustion of the sample. D-Methionine (Acros Organics, NJ, USA) was used for calibration, and cellulose (Fluka, Buchs, Switzerland) was used as a blank.

2.4.7. Water-Holding Capacity. The WHC of the material was determined using Baumann's apparatus.[19] The apparatus was equipped with a glass filter of porosity level G2 (Duran, Wertheim, Germany). The apparatus was set at 25 °C prior to analysis. A blank reading without the substrate was set as the starting point for measurement. Approximately 10−80 mg of sample was placed on the glass filter, and the amount of water absorbed until saturation was determined. All samples were analyzed in triplicate. Evaporation of water over time was measured for a blank filter in triplicate. This loss in water was used to correct the amounts of water held by samples.

3. RESULTS AND DISCUSSION

3.1. Carbohydrates in Chicory Root Pulp. The carbohydrate contents and molar sugar compositions of CRP and its extracts obtained by sequential extraction are shown in Table 1. The carbohydrate content of CRP is 64% w/w. Dominant sugars are uronic acid (UA, 38 mol %), glucose (Glc, 31 mol %), and arabinose (Ara, 15 mol %). The values are in agreement with earlier findings.[2] The high pectin content makes CRP a good alternative to all cereal-based fibers, especially due to the absence of off flavors compared to other fiber-rich agricultural byproducts such as sugar beet pulp.

CRP contains pectins that are highly methyl esterified (DM of 70). CRP is highly acetylated (DA of 43), as has been seen before for sugar beet pectin (DA of 35).[20] Similar high levels of DM and DA have been reported for chicory root Alcohol Insoluble Residue (AIR), 68 and 36, respectively.[5]

The protein content (Table 1) in CRP is 7.6% w/w and is similar to the protein content of chicory root AIR (7.4% w/w).[5] About 81% of proteins are recovered in the WUS fraction. The presence of proteins in acid-extracted high-M_w pectins (500 kDa) from chicory root AIR has been reported before.[5]

Yields of carbohydrates extracted sequentially from CRP are shown in Table1. HWSS represents only 11% of all carbohydrates present in CRP and indicates a poor extractability of CWP in hot water. CHSS contained 29% of the carbohydrates, while DASS and CASS represented 9% and 4% of the CRP carbohydrate yield, respectively. The final residue represented 39% of the carbohydrates present in CRP.

3.1.1. HWSS. The constituent monosaccharide composition of HWSS revealed (Table 1) water-soluble pectins rich in both galacturonic acid (37 mol %) and arabinose (31 mol %). Pectin is dominantly present in homogalacturonan (HG) segments, as concluded from the ratio UA:Rha 35:1. Among the pectic sugars present in CRP (GalA, Ara, Gal), 24% of all Ara is soluble in hot water. These water-soluble pectins are highly methyl esterified (DM 90) and highly acetylated (DA 27).

The hot water treatment resulted in the extraction of mannose- and glucose-containing material (Table 1). Since inulin might be present,[2] and fructose from inulin is reduced with $NaBH_4$ to produce a mixture of mannitol and glucitol during the analysis of alditol acetates,[21] the extract was treated with inulinase and analyzed by HPSEC (Figure 1). It clearly showed degradation of a population with an average molecular mass of 3 kDa. This indicated that inulin is present in HWSS and has approximately an average DP of 18. HPAEC analysis confirmed the presence of inulin and DP range (data not shown). The inulin content in this dialyzed extract was determined to be 24.2% w/w, representing 5.2% of all carbohydrates present in CRP carbohydrates. Dialysis did not remove much inulin since inulin in the undialyzed extract represented 5.9% of all sugars from CRP. This indicated that industrial extraction removed most of the inulin oligomers with DP $\leq$ 18 from CRP.

3.1.2. Sugar Composition and Distribution of CWP in Extracts from WUS. CRP mostly comprises water-insoluble carbohydrates (Table 1), as 87% of all carbohydrates from CRP were retained in WUS. The WUS contained 68% w/w carbohydrates and was dominant in UA (40 mol %), Glc (33 mol %), and Ara (12 mol %). Pectins in WUS are rich in methyl esters (DM 70) and acetyl groups (DA 46). The extracts obtained from WUS are described below.

CHSS. As the major extract in terms of yield from the insoluble cell wall network, the pectic sugars (GalA+Rha+Ara +Gal) in CHSS constitute 46% of all pectic sugars from CRP. The extract is abundant in HG (82 mol % UA). While representing only 26% of all rhamnogalacturonan (RG) backbones from CRP, 60% of all HG from CRP is represented in CHSS. Ara + Gal side chains constitute only 18% of all Ara +Gal from CRP.

Although CHSS pectins are less methyl esterified than HWSS pectins, they still contain a significant amount of methyl

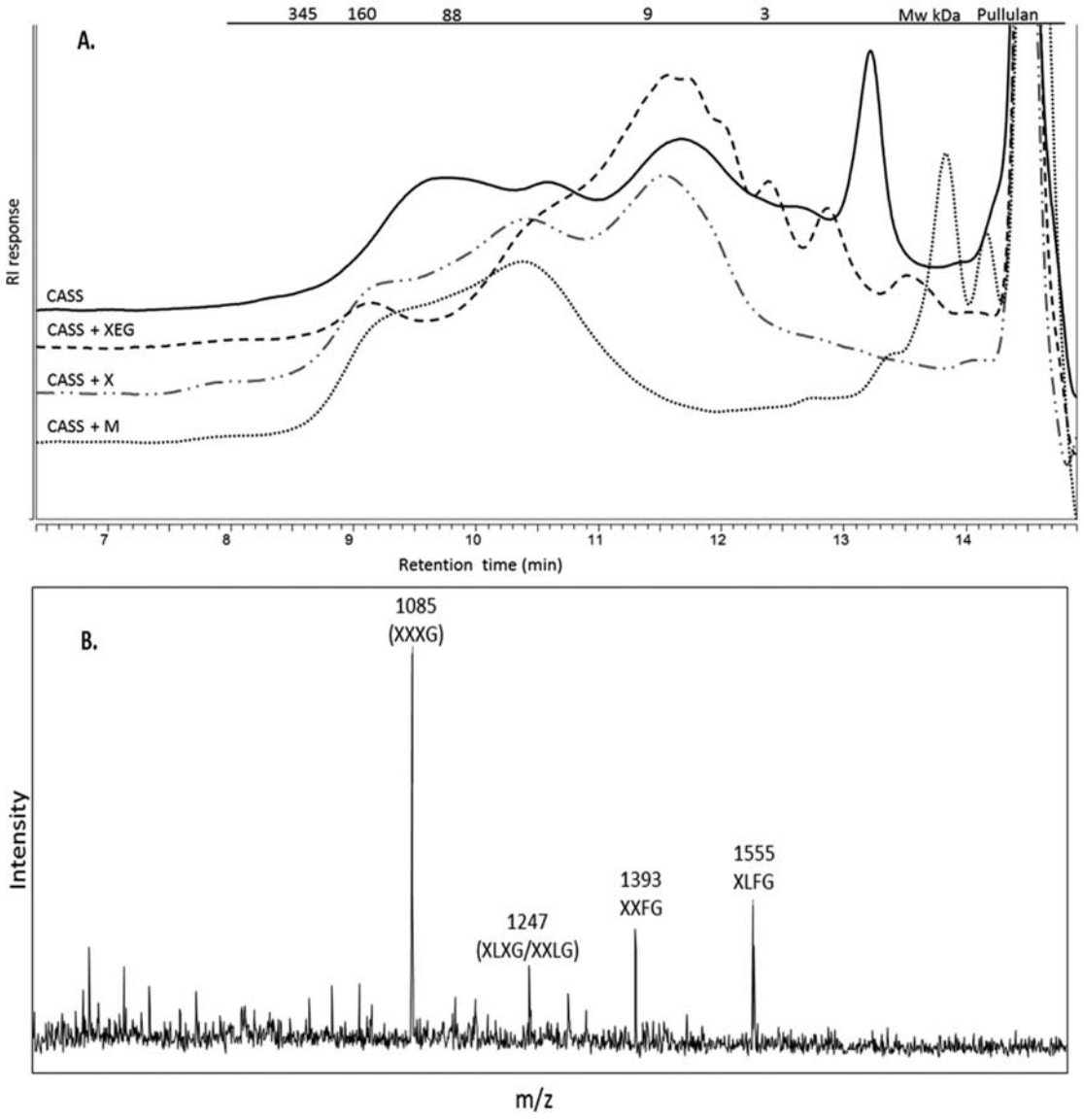

Figure 2. (A) HPSEC elution pattern of CASS from chicory root pulp (CRP) treated with xyloglucan-specific endoglucanase (XEG), xylanase (X), and mannanase (M). (B) MALDI-TOF mass spectrum of xyloglucan oligomers obtained after XEG treatment of CASS.

esters (DM 49). Besides methyl esterification, CHSS pectins are also acetylated (DA 14). Following the extraction of calcium-bound pectin by the chelating agent, pectins still containing esters may remain unextracted by the chelating agent in the residue (CHUS). Since sequential alkali extraction will remove methyl esters and acetyl groups, CHUS was analyzed for DA and DM (Table 1). It was found that pectin in CHUS (with only 27% of all UA from CRP) was highly methyl esterified (DM 52). Acetyl groups constituted 57% of all acetyl groups from CRP. If all the acetyl groups were assumed to be present on pectin, the DA could be estimated as 91. This is quite high, but possible.[22] Nevertheless, it should be taken into account that some nonpectic CWPs also bear acetyl groups.[23]

DASS. DASS is a minor fraction, rich in branched pectin compared to the other extracts. It represents 14% of the CRP pectic sugars. An increased proportion of RG backbone over HG (HG:RG backbone 9) compared to CHSS (HG:RG backbone 30) and higher branching of the RG backbone with side chains of Ara and Gal (Ara+Gal/Rha 21) compared to CHSS (Ara+Gal/Rha 11) are seen. Among the side chains, the Gal:Ara ratio is higher in DASS (0.6) than in CHSS (0.4) and HWSS (0.2). This increase in Gal:Ara ratio with extraction severity indicates an increase in pectin complexity containing galactose in the entangled network of pectins.

CASS. Upon extraction with 4 M alkali, CASS was found to mainly contain hemicellulose, as seen from glucose (35 mol %), xylose (29 mol %), and mannose (17 mol %). Hemicelluloses from CRP have not been studied before. The xylose:glucose ratio in CASS is 0.8. The ratio of xylose:glucose of 0.75 is indicative of xyloglucan of the XXXG type in which three out of four glucose units (G) are substituted with xylose units (X).[24] Although indicative for xyloglucans, the ratio of xylose:glucose of 0.8 cannot rule out the presence of xylan. The presence of XXXG-type xyloglucan in CRP CASS was confirmed by digestion with xyloglucan-specific endoglucanase. HPSEC analysis showed degradation of the high molecular mass material after digestion with XEG (Figure 2A). Oligosaccharides formed upon degradation of xyloglucan were identified by

the mass to charge ratios of their sodium adducts with MALDI-TOF MS (Figure 2B). The spectrum showed four peaks corresponding to sodium adducts of XXXG (m/z = 1085) as the major peak, XLXG or XXLG (m/z = 1247) in which L corresponds to XXXG substituted with Gal, and XXFG (m/z = 1393) and XLFG (m/z = 1555) in which F corresponds to XLXG or XXLG further substituted with fucose (Fuc). Gal and Fuc decorations on xyloglucan were also indicated from the sugar composition of the extract: Gal (7 mol %) and Fuc (2 mol %).

Apart from xyloglucans, the presence of mannans in CASS was indicated from the sugar composition. Digestion of CASS with mannanase or xylanase showed degradation of the material with apparent molecular masses of 9 and 3−160 kDa, respectively (Figure 2A). These digestions further indicate the presence of mannans and xylans in CASS. This was confirmed from HPAEC and MALDI-TOF MS, which indicated the presence of hexose oligomers for mannans and pentose oligomers substituted with 4-*O*-methylglucuronic acid for xylans (data not shown).

CAUS. The residue retained as the major fraction after 4 M alkali treatment is rich in Glc (67 mol %); 87% of all Glc from CRP is retained in the residue. Although only 4 mol % xylose (Xyl) is present, 44% of all Xyl from CRP is present in CAUS. This residual Xyl could arise from xylans or xyloglucans bound to cellulose microfibrils that remain unextracted with concentrated alkali.[25−27]

Next to cellulose, this fraction still contains some RG branched with Ara and Gal side chains (Ara+Gal/Rha 18), as also seen for DASS pectins. CAUS contains 30% of all Ara+Gal present in CRP, thereby constituting a significant proportion of Ara- and Gal-rich pectin. Most of the Gal is believed to originate from pectin side chains, since xyloglucans bearing Gal decorations would be present in minor levels due to the low content of Xyl (4 mol %). Side chains of Ara and Gal may play a role in anchoring pectins to cellulose microfibrils through covalent bonds.[28]

3.3. WHC of Residues Obtained from Sequential Extractions. In order to understand how CWPs and their network contribute to the WHC of CRP, residues obtained after sequential extractions of CWPs were analyzed for their WHCs (Figure 3). The WHC for CRP was 6 mL/g. This value is in the same range as found for other pulp materials, such as sugar beet pulp (5.5 mL/g).[29] The WHCs for WUS, CHUS, DAUS, and CAUS were 13, 14, 22, and 13 mL/g, respectively.

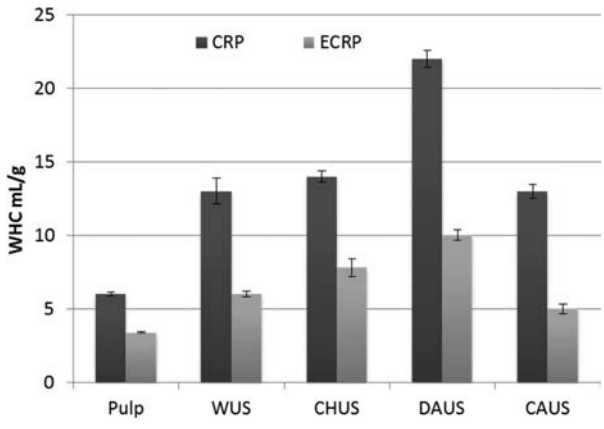

Figure 3. Water-holding capacity (WHC) of chicory root pulp (CRP) and ensiled CRP (ECRP) and fractions derived from the pulps where WUS is water unextractable solids, CHUS is chelating agent unextractable solids, DAUS is dilute alkali unextractable solids, and CAUS is concentrated alkali unextractable solids.

Despite recovering most of CRP polysaccharides in WUS (87% of CRP PS), the increase in the WHC of WUS can be due to an increase in the porosity of the cell wall caused by hot water treatment.[30,31] Removal of 35% of WUS CWPs by the chelating agent did not alter the WHC of the network, as seen from similar values of WHC for WUS and CHUS. Thus the removal of HG-rich pectin has no influence on the WHC of CRP. The high WHC for DAUS, despite representing still 87% of CWPs from CHUS, could arise from a different arrangement of CWPs in the network due to removal of branched pectins (DASS). Although such a network is abundant in cellulose, it contains hemicellulose and still some branched pectin, which may be important for hydration. Since the residue has been subjected to dilute alkali, esters lost from polysaccharides may have altered the WHC of the network. Removal of even minor amounts of CASS CWPs (representing only 9% of DAUS CWPs) resulted in a much lower WHC for CAUS compared to DAUS. CASS CWPs dominant in xyloglucan followed by mannan-rich hemicellulose (representing 2.5% and 0.9% of all CRP CWPs, respectively) possibly suggest their positive role in the WHC of DAUS. In addition, entrapped xyloglucans have been found to show motional properties intermediate between cellulose and pectin.[32] Thus, they may contribute to WHC when bound to cellulose microfibrils. The observed WHC for CAUS may be an overestimation because of alkali-swollen cellulose.[33,34]

3.4. Effect of Ensiling on the Cell Wall Network of CRP. Ensiling of CRP was performed to determine if such a treatment could reduce the WHC of the pulp. A significant reduction in the WHC of 44% from 6 mL/g to 3.4 mL/g was indeed seen (Figure 3). In order to determine the role of CWPs and their network toward the WHC of the pulp, ensiled chicory root pulp was further characterized. Analysis of the sugar composition of ECRP (Table 2) showed that it was quite similar to that of CRP (Table 1). However, ensiling caused demethyl esterification, as observed by the lower DM (22) of ECRP compared to CRP (70). The acetylation of CWPs remained similar to that of CRP. Despite the similarity in sugar composition and acetylation, possible modifications on the cell wall network caused by ensiling might have caused a reduction in the WHC. Hence, sequential extractions were performed on ECRP.

Effect of Ensiling on Solubility of the CWP Network and WHC. Ensiling of CRP increased the hot water extractability of CWPs. This was indicated (Table 2) by the higher carbohydrate yield (39%) for ECRP HWSS compared to CRP HWSS (11%) (Table 1).

Dominant among the sugars solubilized in ECRP HWSS are pectic sugars, as seen from the sugar composition (Table 2). ECRP HWSS had 4 times more pectic sugars than in CRP HWSS (Figure 4). This proves that ensiling increased the extractability of pectic sugars from the insoluble network of CRP, which are extractable only under more severe conditions for CRP (Figure 4A). The pectins in ECRP HWSS were shown to be degraded during ensilage, as can be seen from the elution profile by HPSEC (Figure 5A). The dominant population of low molecular mass (M_w) less than 3 kDa indicated that dialysis was not sufficient to remove soluble material of low molecular mass. Removal of higher proportions of pectin from the cell wall network and reduced M_w and DM of pectin could explain a

Table 2. Sugar Composition of Ensiled Chicory Root Pulp (ECRP) and Fractions Derived from the Pulp[a]

	carbohydrate yield [g in fraction per 100 g in pulp]	mol %								total sugars, % w/w	DM	DA	protein, %
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA				
ECRP	100	2	0	15	4	3	7	31	38	56	22	49	9.2
HWSS	39	2	0	35	0	3	10	3	47	72	24	36	3.1
WUS	55	1	0	5	7	3	5	56	21	55	6	79	10.4
CHSS	9	1	0	5	0	1	4	1	88	43	6	8	
DASS	2	3	0	19	4	3	26	14	31	33			
CASS	8	0	2	7	28	15	10	31	7	54			
CAUS	35	1	0	3	4	1	4	83	5	59			

[a]DM/DA: degree of methyl/acetyl esterification expressed as moles of methanol esters/acetyl groups per 100 mol of uronic acid. Rhamnose (Rha), fucose (Fuc), arabinose (Ara), xylose (Xyl), mannose (Man), galactose (Gal), glucose (Glc), uronic acid (UA).

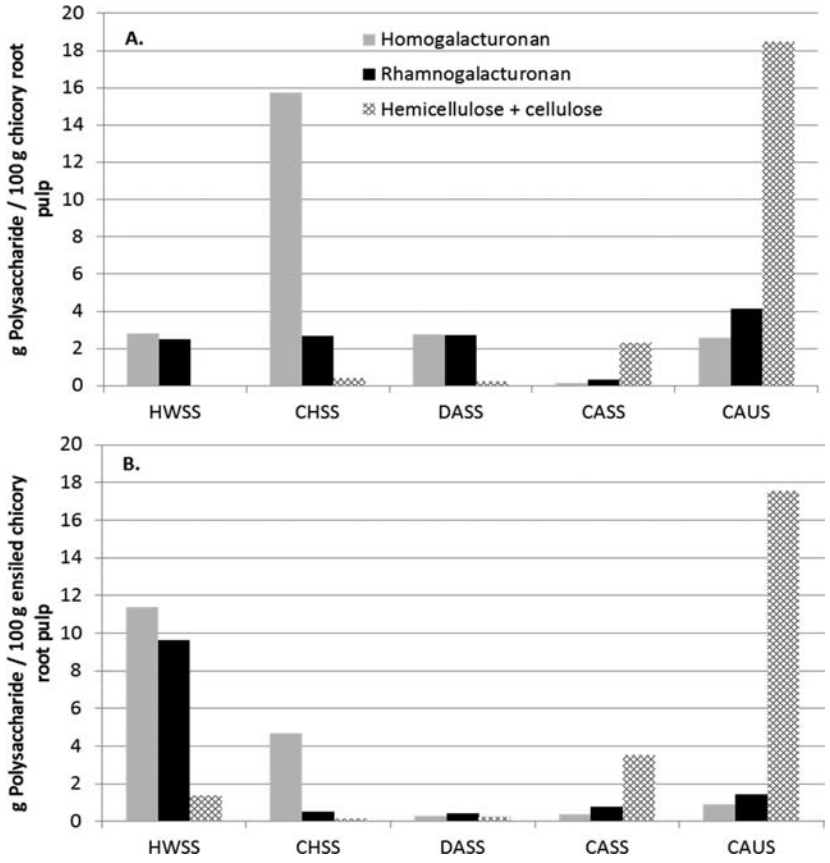

Figure 4. Distribution of polysaccharides in fractions derived from (A) 100 g of chicory root pulp (CRP) and (B) 100 g of ensiled chicory root pulp (ECRP).

lower WHC for ECRP WUS (6 mL/g) compared to CRP WUS (13 mL/g). Furthermore, the characteristics of the residual CWPs in the insoluble network may also contribute to a lower WHC for ECRP WUS.

Effect of Ensiling on the Extractability of CWPs and WHC. Fractions obtained from the insoluble cell wall network (WUS) of ECRP had lower WHCs compared to those from CRP (Figure 3).

Ensiling increased the extractability of HG by 4 times in HWSS than CRP HWSS, having the consequence of a 4 times lower HG recovery in ECRP CHSS compared to CRP CHSS (Figure 4). HPSEC analysis of the extracted HG-rich pectin (88 mol % UA) in ECRP CHSS pointed to a lower M_w compared to CRP CHSS (Figure 5B). Thus, increased removal of HG from WUS and degradation of residual HG-rich pectin in WUS during ensiling lowered the WHC of ECRP WUS (6 mL/g) compared to CRP WUS (13 mL/g).

ECRP CHUS (sum of DASS, CASS, and CAUS) contains 3 times less RG and 4 times less HG than the fractions in CRP CHUS (Figure 4). Ara-rich pectin was degraded in CHUS during ensiling, as indicated from low Ara:Rha in ECRP DASS (7:1) and CAUS (5:1) (Table 1) compared to CRP DASS (13:1) and CAUS (11:1), respectively (Table 2). The lower proportion of HG and RG and degraded Ara-rich pectin reduced the WHC of ECRP CHUS (7.8 mL/g) compared to CRP CHUS (14 mL/g) (Figure 3).

Furthermore, the WHCs for ECRP DAUS (10 mL/g) and CAUS (5 mL/g) were lower compared to CRP DAUS (22 mL/g) and CAUS (13 mL/g) (Figure 3), respectively. ECRP CAUS derived from ECRP DAUS contains 3 times less pectic

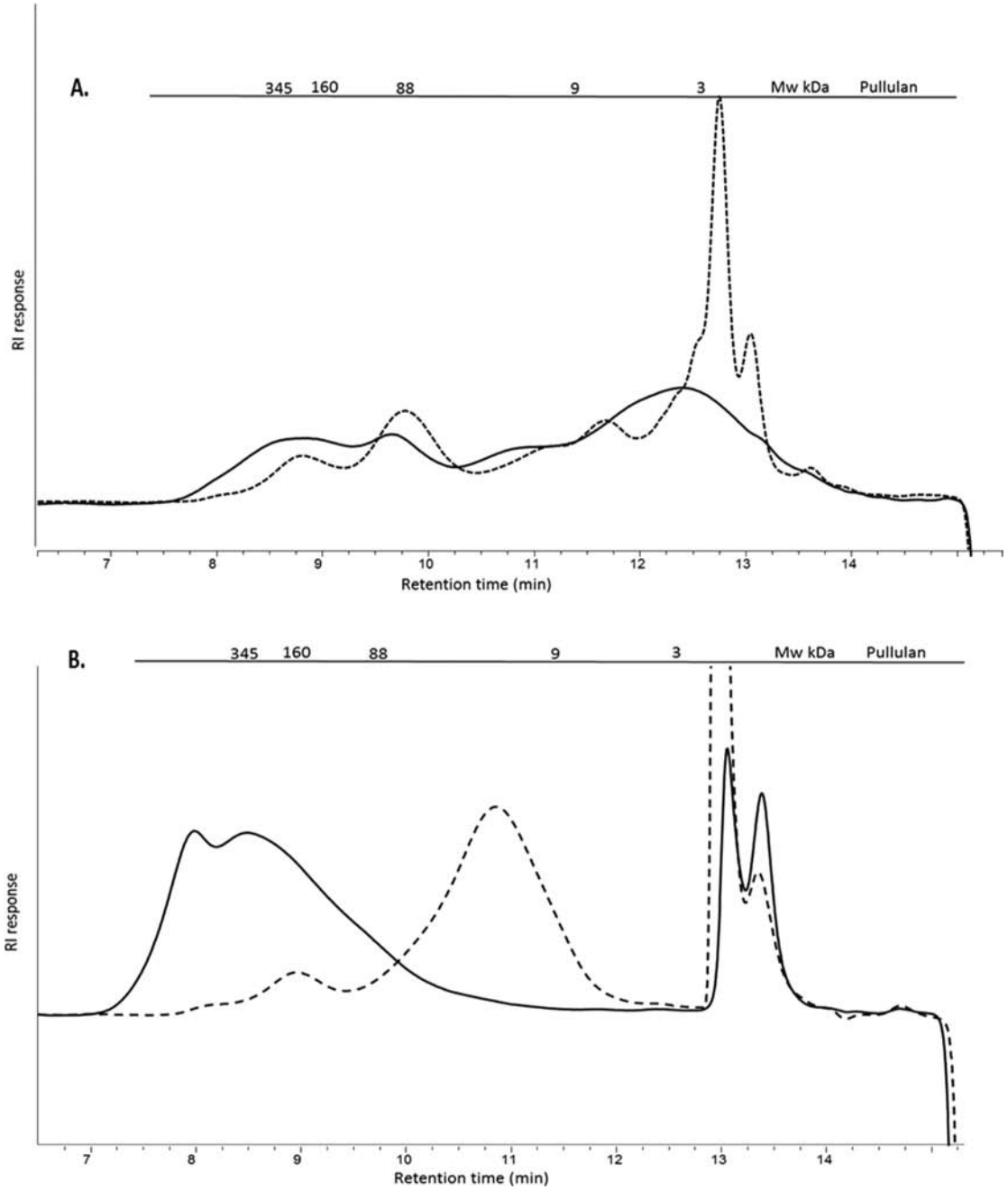

Figure 5. HPSEC elution patterns of (A) HWSS from chicory root pulp (CRP) (solid line) and HWSS from ensiled CRP (ECRP) (dotted line). (B) CHSS from chicory root pulp (CRP) (solid line) and CHSS from ensiled CRP (ECRP) (dotted line).

sugars than CRP CAUS (Figure 4). Other polysaccharides, such as xyloglucan, glucan, and mannan were also modified in ECRP CAUS, increasing their extractability in ECRP CASS, as indicated from a higher carbohydrate yield (Table 2) compared to CRP CASS and similar sugar composition to CRP CASS (Table 1).

Studies on WHC showed that the WHC of chicory root pulp may change depending on the arrangement of cell wall polysaccharides within the plant cell wall network. The removal of branched pectin seemed to influence the WHC strongly. Besides, alkali-soluble xyloglucan and mannan-rich hemicellulose were indicated to contribute positively to the WHC. Ensiling degraded arabinose-rich pectin, homogalacturonan, and rhamnogalacturonan and modified the network consisting of xyloglucan, mannan, and glucan, all of which caused a significant reduction in the WHC.

Following the characterization of chicory root pulp before and after ensiling, the use of CRP as a fiber supplement may have become closer to application. We now know that small changes in architecture as a result of mild processing or even by a targeted enzyme treatment will enable modulation of the WHC of CRP, resulting in improved properties of the CRP in the final fiber-rich product.

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Funding

This project is jointly financed by the European Union, European Regional Development Fund and The Ministry of Economic Affairs, Agriculture and Innovation, Peaks in the Delta, the Municipality of Groningen, the Province of Groningen, and the Dutch Carbohydrate Competence Center (CCC WP2a) and Royal Cosun.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

CRP, chicory root pulp; HG, homogalacturonan; RG, rhamnogalacturonan; ECRP, ensiled chicory root pulp; Ara, arabinose; Gal, galactose; Glc, glucose; Xyl, xylose; Man, mannose; Fuc, fucose; UA, uronic acid; Rha, rhamnose; WHC, water-holding capacity; CWPs, cell wall polysaccharides; PS, polysaccharides; HWSS, hot water soluble solids; WUS, water unextractable solids; CHSS, chelating agent soluble solids; CHUS, chelating agent unextractable solids; DASS, dilute alkali soluble solids; DAUS, dilute alkali unextractable solids; CASS, concentrated alkali soluble solids; CAUS, concentrated alkali unextractable solids

■ REFERENCES

(1) Roberfroid, M. B. Inulin-type fructans: functional food ingredients. *J. Nutr.* **2007**, *137*, 2493S−2502S.

(2) Femenia, A.; Robertson, J. A.; Waldron, K. W.; Selvendran, R. R. Cauliflower (*Brassica oleracea L*), globe artichoke (*Cynara scolymus*) and chicory witloof (*Cichorium intybus*) processing by-products as sources of dietary fibre. *J. Sci. Food Agric.* **1998**, *77*, 511−518.

(3) Robert, C.; Devillers, T.; Wathelet, B.; Van Herck, J. C.; Paquot, M. Use of a Plackett-Burman experimental design to examine the impact of extraction parameters on yields and compositions of pectins extracted from chicory roots (*Cichorium intybus L.*). *J. Agric. Food Chem.* **2006**, *54*, 7167−7174.

(4) Renard, C. M. G. C.; Crépeau, M. J.; Thibault, J. F. Influence of ionic strength, pH and dielectric constant on hydration properties of native and modified fibres from sugar-beet and wheat bran. *Ind. Crops Prod.* **1994**, *3*, 75−84.

(5) Panouille, M.; Thibault, J. F.; Bonnin, E. Cellulase and protease preparations can extract pectins from various plant byproducts. *J. Agric. Food Chem.* **2006**, *54*, 8926−8935.

(6) Guillon, F.; Barry, J.-L.; Thibault, J.-F. Effect of autoclaving sugar-beet fibre on its physico-chemical properties and its in-vitro degradation by human faecal bacteria. *J. Sci. Food Agric.* **1992**, *60*, 69−79.

(7) Auffret, A.; Ralet, M. C.; Guillon, F.; Barry, J. L.; Thibault, J. F. Effect of grinding and experimental conditions on the measurement of hydration properties of dietary-fibers. *Food Sci. Technol.-Lebensm.-Wiss. Technol.* **1994**, *27*, 166−172.

(8) Okine, A.; Hanada, M.; Aibibula, Y.; Okamoto, M. Ensiling of potato pulp with or without bacterial inoculants and its effect on fermentation quality, nutrient composition and nutritive value. *Animal Feed Sci. Technol.* **2005**, *121*, 329−343.

(9) Voragen, A. G. J.; Coenen, G. J.; Verhoef, R. P.; Schols, H. A. Pectin, a versatile polysaccharide present in plant cell walls. *Struct. Chem.* **2009**, *20*, 263−275.

(10) DuBois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350−356.

(11) Pauly, M.; Andersen, L. N.; Kauppinen, S.; Kofod, L. V.; York, W. S.; Albersheim, P.; Darvill, A. A xyloglucan-specific endo-beta-1,4-glucanase from *Aspergillus aculeatus*: expression cloning in yeast, purification and characterization of the recombinant enzyme. *Glycobiology* **1999**, *9*, 93−100.

(12) Kormelink, F. J. M.; Searle-Van Leeuwen, M. J. F.; Wood, T. M.; Voragen, A. G. J. Purification and characterization of three endo-(1,4)-β-xylanases and one β-xylosidase from *Aspergillus awamori*. *J. Biotechnol.* **1993**, *27*, 249−265.

(13) Düsterhöft, E. M.; Bonte, A. W.; Voragen, A. G. J. Solubilisation of non-starch polysaccharides from oil-seed meals by polysaccharide-degrading enzymes. *J. Sci. Food Agric.* **1993**, *63*, 211−220.

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

(15) Thibault, J. F. Automated-method for the determination of pectic substances. *Lebens.-Wiss. Technol.* **1979**, *12*, 247−251.

(16) Ramaswamy, U. R.; Kabel, M. A.; Schols, H. A.; Gruppen, H. Structural features and water holding capacities of pressed potato fibre polysaccharides. *Carbohydr. Polym.* **2013**, *93*, 589−596.

(17) Stöber, P.; Bénet, S.; Hischenhuber, C. Simplified enzymatic high-performance anion exchange chromatographic determination of total fructans in food and pet food limitations and measurement uncertainty. *J. Agric. Food Chem.* **2004**, *52*, 2137−2146.

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

(19) Baumann, H. Apparatur nach Baumann zur bestimmung der flüssigkeitsaufnahme von pulvrigen substanzen. *Fette, Seifen, Anstrichm.* **1966**, *68*, 741−743.

(20) Rombouts, F. M.; Thibault, J. F. Sugar beet pectins: chemical structure and gelation through oxidative coupling. In *Chemistry and Function of Pectins*; American Chemical Society: 1986; Vol. *310*, pp 49−60.

(21) Davison, P. K.; Young, R. Gas chromatography of carbohydrates the quantitative determination of the free sugars of plants as their trimethylsilyl ethers. *J. Chromatogr. A* **1964**, *41*, 12−21.

(22) Schols, H. A.; Posthumus, M. A.; Voragen, A. G. J. Hairy (ramified) regions of pectins 0.1. Structural features of hairy regions of pectins isolated from apple juice produced by the liquefaction process. *Carbohydr. Res.* **1990**, *206*, 117−129.

(23) Carpita, N. C.; Gibeaut, D. M. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant J.* **1993**, *3*, 1−30.

(24) Hilz, H.; de Jong, L. E.; Kabel, M. A.; Schols, H. A.; Voragen, A. G. J. A comparison of liquid chromatography, capillary electrophoresis, and mass spectrometry methods to determine xyloglucan structures in black currants. *J. Chromatogr. A* **2006**, *1133*, 275−286.

(25) Pauly, M.; Albersheim, P.; Darvill, A.; York, W. S. Molecular domains of the cellulose/xyloglucan network in the cell walls of higher plants. *Plant J.* **1999**, *20*, 629−639.

(26) Hayashi, T.; Maclachlan, G. Pea xyloglucans and cellulose. 1. Macromolecular organisation. *Plant Physiol.* **1984**, *75*, 596−604.

(27) Bootten, T. J.; Harris, P. J.; Melton, L. D.; Newman, R. H. WAXS and ^{13}C NMR study of *Gluconoacetobacter xylinus* cellulose in composites with tamarind xyloglucan. *Carbohydr. Res.* **2008**, *343*, 221−229.

(28) Zykwinska, A.; Thibault, J. F.; Ralet, M. C. Competitive binding of pectin and xyloglucan with primary cell wall cellulose. *Carbohydr. Polym.* **2008**, *74*, 957−961.

(29) Rosell, C. M.; Santos, E.; Collar, C. Mixing properties of fibre-enriched wheat bread doughs: a response surface methodology study. *Eur. Food Res. Technol.* **2006**, *223*, 333−340.

(30) Leboeuf, E.; Thoiron, S.; Lahaye, M. Physico-chemical characteristics of cell walls from Arabidopsis thaliana microcalli showing different adhesion strengths. *J. Exp. Bot.* **2004**, *55*, 2087−2097.

(31) Cosgrove, D. J. Relaxation in a high-stress environment: the molecular bases of extensible cell walls and cell enlargement. *Plant Cell Online* **1997**, *9*, 1031−41.

(32) Dick-Pérez, M.; Zhang, Y.; Hayes, J.; Salazar, A.; Zabotina, O. A.; Hong, M. Structure and interactions of plant cell-wall

polysaccharides by two- and three-dimensional magic-angle-spinning solid-state NMR. *Biochemistry* **2011**, *50*, 989−1000.
(33) Dinand, E.; Vignon, M.; Chanzy, H.; Heux, L. Mercerization of primary wall cellulose and its implication for the conversion of cellulose I cellulose II. *Cellulose* **2002**, *9*, 7−18.
(34) Sarko, A.; Nishimura, H.; Okano, T. Crystalline alkali-cellulose complexes as intermediates during mercerization. In *The Structures of Cellulose*; American Chemical Society: Washington, DC, **1987**; Vol. *340*, pp 169−177.