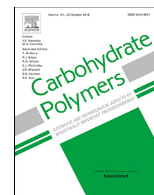
Carbohydrate Polymers 192 (2018) 263–272

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol



Interactions between pectin and cellulose in primary plant cell walls

Suzanne E. Broxterman, Henk A. Schols*

Laboratory of Food Chemistry, Wageningen University and Research, Bornse Weilanden 9, 6708 WG, Wageningen, The Netherlands

ARTICLE INFO

Keywords:
Cross-link
Sequential alkali extraction
Enzymatic digestion by glucanases
Carrot
Tomato
Strawberry

ABSTRACT

To understand the architecture of the plant cell wall, it is of importance to understand both structural characteristics of cell wall polysaccharides and interactions between these polysaccharides. Interactions between polysaccharides were studied in the residue after water and chelating agent extraction by sequential extractions with H_2O and alkali.

The 6 M alkali residue still represented 31%, 11% and 5% of all GalA present in carrot, tomato and strawberry, respectively, and these pectin populations were assumed to strongly interact with cellulose. Digestion of the carrot 6 M alkali residue by glucanases released ~27% of the 6 M residue, mainly representing pectin. In tomato and strawberry alkali residues, glucanases were not able to release pectin populations. The ability of glucanases to release pectin populations suggests that the carrot cell wall contains unique, covalent interactions between pectin and cellulose.

1. Introduction

The primary plant cell wall is essential for strength, growth and development of the plant (Caffall & Mohnen, 2009). In edible tissue it is also of major importance for texture. The plant cell wall predominantly consists of pectin, hemicellulose and cellulose. Pectin consists of galacturonic acid as the most prevailing building block, mostly present in the homogalacturonan (HG) and in the rhamnogalacturonan I (RG-I) structural elements. Whereas the HG backbone is only composed of galacturonic acid residues, the RG-I backbone is composed of alternating rhamnose and galacturonic acid residues. The rhamnose residues in RG-I can be substituted with neutral sugar side chains, composed of arabinose and galactose (Voragen, Coenen, Verhoef, & Schols, 2009). Hemicelluloses are composed of xylans, xyloglucans and mannans (Scheller & Ulvskov, 2010). Xyloglucan is the major hemicellulosic polysaccharide in primary plant cell walls of fruits and vegetables, and is composed of a cellulose-like backbone branched at O-6 by xylosyl residues. The xylose units can be substituted by several other monosaccharides such as galactose, fucose and arabinose (Fry, 1989b). Cellulose consists of a linear chain composed of β-(1 → 4)-linked glucose residues (Scheller & Ulvskov, 2010).

The plant cell wall is long believed to be composed of two separate networks: a pectin network and a hemicellulose/cellulose network (Cosgrove, 2005). Although this model of the plant cell wall is still generally accepted, increasing evidence shows interactions between these two networks and a more dominant role for pectin as part of the load-bearing cell wall structures (Höfte, Peaucelle, & Braybrook, 2012). The cell wall components involved and the exact nature of the interactions are still unknown, although evidence is found for both covalent and for non-covalent interactions between both networks (Cosgrove, 2001; Mort, 2002). The most well-known and fully accepted interaction between cell wall polysaccharides is the adsorption of xyloglucan onto cellulose by H-bonds, hereby coating cellulose (Hayashi, 1989). Similarly, many other interactions are also suggested such as interactions between xyloglucan and RG-I side chains or between xylan and RG-I side chains (Popper & Fry, 2005; Ralet et al., 2016). Interactions between RG-I and cellulose were shown in vitro, by adsorption of RG-I side chains to cellulose (Zykwinska, Ralet, Garnier, & Thibault, 2005). Linkages between cellodextrins and HG have been described, but the precise annotation and allocation has not been presented (Nunes et al., 2012). Next to polysaccharide interactions, interactions involving cell wall proteins such as extensin and AGP have been found (Mort, 2002; Tan et al., 2013). The nature of the potential interactions between cell wall polysaccharides and proteins remains unclear, although it is speculated that many of these covalent and non-covalent interactions are based on ester linkages and H-bonds (Jarvis, Briggs, & Knox, 2003).

Most of the dicot primary plant cell models indicate a dominant role for hemicellulose within the network. Therefore it was chosen to study the cell wall architecture of carrot, tomato and strawberry, 3 sources with a different hemicellulose content and composition (Houben, Jolie, Fraeye, Van Loey, & Hendrickx, 2011; Voragen, Timmers, Linssen, Schols, & Pilnik, 1983). Since both ester linkages and H-bonds are not stable under strong alkali conditions, sequential alkali extraction was used as a method to degrade possible ester cross-links and characterise

* Corresponding author.
E-mail address: Henk.Schols@wur.nl (H.A. Schols).

https://doi.org/10.1016/j.carbpol.2018.03.070
Received 14 February 2018; Received in revised form 19 March 2018; Accepted 19 March 2018
Available online 20 March 2018
0144-8617/ © 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

solubilised polysaccharide populations from the cell wall of carrot, tomato and strawberry. Pectinase and glucanase digestions were performed to release strongly interacting pectin populations from the alkali residues.

2. Materials and methods

2.1. Plant material

Carrots (Daucus carota cv. Romance) and strawberries (Fragaria ananassa cv. Elsanta) were purchased from a local vegetable store. Tomatoes (Solanum lycopersicum cv. H2401) were kindly donated by Heinz (Heinz, Nijmegen, The Netherlands).

2.2. Extraction of cell wall polysaccharides

Cell wall polysaccharides were extracted using the procedure as described before (Broxterman, Picouet, & Schols, 2017; Houben et al., 2011).

Shortly, Alcohol Insoluble Solids (AIS) were extracted by blending carrots, tomatoes and strawberries in a 1:3 w/v ratio in 96% ethanol. Prior to blending, only for peeled tomatoes, microwave pretreatment was performed to inactivate pectinases (10 min, 900W). The suspension was filtered and the residue was washed with 70% ethanol until the filtrate gave a negative reaction in the phenol-sulfuric acid test (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956). The water soluble solids (WSS) and chelating agent soluble solids (ChSS) were subsequently extracted from AIS according to the references mentioned above. The residue after WSS and ChSS was extensively dialysed, first against potassium acetate, followed by distilled water. After freeze-drying the Chelating agent Unextractable Solids (ChUS) were obtained. This fraction was used to identify potential interactions between pectin, hemicellulose and cellulose. All fractions were milled for 30 s in a Retsch Cryomill MM440 at a frequency of 20 Hz to obtain homogeneous material (Retsch GmbH, Haan, Germany). Dry matter content of starting materials was determined in triplicate by drying ∼500 mg of sample at 105 °C for 3 h.

2.3. Sequential water-alkali extraction to yield 6 M NaOH and 0.1 M NaOH residues

In order to selectively degrade alkali-labile interactions in the primary plant cell wall, sequential water-alkali extraction was performed according to the extraction diagram shown in Supporting information Fig. S-1. 30 ml water was added to 300 mg ChUS from carrot, tomato or strawberry. Extraction was done overnight at 40 °C, the suspension centrifuged (20 min, 20 °C, 30.000 × *g*) and the supernatant was freeze-dried. 30 ml 0.1 M NaOH containing 25 mM $NaBH_4$ was added to the residue and extraction was done for 6 h at 4 °C. After centrifugation (20 min, 4 °C, 30.000 × *g*), the residue was washed with 30 ml 0.1 M NaOH containing 25 mM $NaBH_4$ for 30 min at 4 °C and centrifuged again (20 min, 4 °C, 30.000 × *g*). Supernatants were pooled.

Both supernatant and residue were neutralized to pH 6. The supernatant was ultrafiltered by using a 10 kDa filter (Millipore centrifugal filter units, Merck, Billerica, Massachusetts, United States) and subsequently freeze-dried. 30 ml H_2O was added to the residue and water extraction was performed at 40 °C overnight. The suspension was centrifuged (20 min, 20 °C, 30.000 × *g*) and the supernatant was freeze-dried after ultrafiltration with a 10 kDa filter.

The same procedure was repeated with 1 M NaOH containing 0.25 M $NaBH_4$ followed by water, and 6 M NaOH with 0.25 M $NaBH_4$ followed by water. All alkali extractions were done at 4 °C for 6 h, water extractions overnight at 40 °C, and all with head-over-tail rotation.

To obtain the 0.1 M alkali residue, the same procedure was followed as described above. However, after water extraction following the 0.1 M NaOH extraction, the residue was neutralised, ultrafiltrated and freeze-dried to obtain the 0.1 M alkali Residue.

2.4. Sugar composition of the extracts

To determine the pectin content of the extracted fractions, the uronic acid content was determined by the automated colorimetric m-hydroxydiphenyl method (Blumenkrantz & Asboe-hansen, 1973). Neutral carbohydrate composition was analysed after pretreatment with 72% (w/w) H_2SO_4 (1 h, 30 °C) followed by hydrolysis with 1 M H_2SO_4 (3 h, 100 °C). Sugars released were derivatised and analysed as their alditol acetates using gas chromatography (Englyst & Cummings, 1984), inositol was used as internal standard.

2.5. Starch digestion

The presence of starch in AIS, WSS, ChSS and ChUS was analysed by using the Megazyme total starch assay procedure for resistant starch (Megazyme, Wicklow, Ireland). After digestion of the sample with amylase and amyloglucosidase, samples were filtered using a 10 kDa filter to remove glucose originating from starch and freeze-dried. Starch was not removed prior to the fractionation of AIS into WSS, ChSS and ChUS. Starch levels were determined in isolated fractions, and all monosaccharide compositions given represent destarched fractions.

2.6. Enzymatic digestion of pectin populations in the 0.1 M and 6 M alkali residue

In order to test the accessibility of pectin in the 0.1 M and 6 M alkali residues, incubations with pectinases and glucanases were performed. The pectinases used were rhamnogalacturonan hydrolase (RG-H) from *Aspergillus aculeatus*, *endo*-polygalacturonase (PG) from *Aspergillus aculeates* (Limberg et al., 2000), *endo*-β-(1,4)-galactanase from *Aspergillus niger* (Schols, Posthumus, & Voragen, 1990), β-galactosidase from *Aspergillus niger*, and *endo*-arabinanase from *Aspergillus aculeates* and *exo*-arabinanase from *Chrysosporium lucknowense* (Kühnel et al., 2010). The glucanases used were *endo*-glucanase from *Trichoderma viride* and *exo*-glucanase/CBH from *Trichoderma viride* (Vincken, Beldman, & Voragen, 1997). Digestion was done at 5 mg/ml in 50 mM sodium citrate buffer pH 5 at 40 °C (pectinases) or at 50 °C (glucanases) by head-over-tail rotation for 24 h. Enzymes were dosed to fully degrade the specific substrate in 6 h. Isolation of solubilised polysaccharides > 10 kDa was done using centrifugal filter units with a cut-off of 10 kDa.

All enzymes used were well characterised and extensively tested for their purity including the different pectin structure elements (HG, RG-I backbone and side chains), and did not show side activity.

2.7. Structural characterisation of the extracts

2.7.1. High performance size exclusion chromatography (HPSEC)

Extracted pectin fractions before and after enzymatic digestion were analysed for their molecular weight distribution using an Ultimate 3000 system (Dionex, Sunnyvale, CA, USA) coupled to a Shodex RI-101 detector (Showa Denko K.K., Tokyo, Japan). A set of TSK-Gel super AW columns 4000, 3000, 2000 (6 mm × 150 mm) preceded by a TSK-Gel super AW guard column (6 mm ID × 40 mm) (Tosoh Bioscience, Tokyo, Japan) was used in series. The column temperature was set to 55 °C. Samples (5 mg/ml) were injected (10 μl) and eluted with 0.2 M $NaNO_3$ at a flow rate of 0.6 ml/min. Pectin standards from 10 to 100 kDa were used to estimate the molecular weight distribution (Voragen et al., 1982).

2.7.2. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

The oligosaccharides in the glucanase digests of the 6 M and 0.1 M alkali residues were analysed by MALDI-TOF MS. MALDI-TOF mass spectra were recorded using an Ultraflextreme workstation controlled

Download English Version:

https://daneshyari.com/en/article/7782767

Download Persian Version:

https://daneshyari.com/article/7782767

Daneshyari.com