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No/moderate pectin removal
Extensive pectin removal

High Pressure Homogenization

High Pressure Homogenization

H₂O

**Physico-chemical and viscoelastic properties of high pressure homogenized lemon peel fiber
fraction suspensions obtained after sequential pectin extraction**

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Abstract

The viscoelastic properties of high pressure homogenized lemon peel cell wall fiber suspensions, obtained after sequential selective pectin extraction, were investigated in the current study. For comparison, a general pectin extraction was additionally performed on lemon peel under acid thermal conditions. The obtained insoluble residues and solubilized pectin fractions were characterized based on their neutral sugar and galacturonic acid content, degree of methoxylation and molar mass distribution. Subsequently, microstructure and viscoelastic characteristics of the diverse insoluble residue suspensions were investigated by means of microscopic visualization, determination of the particle size distribution and rheological properties after high pressure homogenization.

Due to the use of different extraction conditions, the insoluble cell wall residues were found to have diverse pectin characteristics and contents. While homogenization of non- or moderate-depleted cell wall suspensions resulted in small viscoelastic moduli due to the disruption of the stiff microfibrillar cell wall network, extensive pectin removal was found to correlate to large viscoelastic moduli of the cell wall suspension upon high pressure homogenization. It is suggested that an extensive pectin extraction resulted in the creation of interfibrillar voids within the cellulose-hemicellulose network, increasing the cell wall flexibility and hydration of the fibers upon suspension. Due to the increased flexibility of this fiber network, the interfibrillar voids in the pectin depleted cell wall material were proposed to expand with increasing pressure level during high pressure homogenization of the generated cell wall suspensions, thereby increasing its viscoelastic properties.

Keywords: pectin fractions, cell wall, lemon peel, physico-chemical properties, high pressure homogenization, microstructural and viscoelastic properties

1. Introduction

The primary cell wall of fruits and vegetables is constituted of a complex polysaccharide network of cellulose, hemicellulose and pectin, within which an additional independent structuring network of proteins such as extensins can be present in minor amounts (Debon, Wallecan, & Mazoyer, 2012; Lamport, Kieliszewski, Chen, & Cannon, 2011). The main structural element of the cell wall is cellulose, a linear homopolymer of D-glucose linked by β -1,4 glucosidic bonds, that can tightly associate with other cellulose chains through hydrogen bonds to form microfibrils (Carpita & McCann, 2000). Cellulose microfibrils can additionally interact with hemicellulose, a heteropolymer containing a backbone of 1,4-linked β -D-pyranosyl residues including mainly xyloglucans, xylans and mannans. Current cell wall models propose that the loadbearing cellulose-hemicellulose network is embedded in a pectin matrix (Cosgrove, 2005). Pectin is a complex polysaccharide consisting of three covalently linked structures including homogalacturonan (HG), rhamnogalacturonan I (RGI) and rhamnogalacturonan II (RGII). Homogalacturonan is composed of a linear backbone of 1,4-linked α -D-galacturonic acid (GalA) units that can be methyl esterified at C-6. Non-methyl esterified pectin polymers can interact through ionic cross-links with calcium ions, creating a calcium pectate gel that considerably affects the structural properties of the plant cell wall. In contrast with HG, both RGI and RGII are characteristic for its branched pectin structures. RGI consists of a backbone of repeating GalA and rhamnose (Rha) disaccharides containing linear or branched sidechains of α -L-arabinose and β -D-galactose units that form heteropolysaccharides such as arabinans, galactans and arabinogalactans (Mohnen, 2008). Lastly, RGII is composed of a backbone of seven to nine α -(1-4) linked GalA units that can be methyl esterified. Furthermore, four types of hetero-oligomeric side chains can be present including up to twelve different sugars (O'Neill, Ishii, Albersheim, & Darvill, 2004).

During maturation, structural properties of the primary cell wall are influenced by changes in the cell wall composition, polymer interactions and organization (Agoda-Tandjawa, Durand, Gaillard, Garnier, & Doublier, 2012; Vanstreels et al., 2005; Waldron, Parker, & Smith, 2003). Pectin is believed to play an important role in these structural changes as the degradation and solubilization of pectin is linked to cell wall hydration and swelling during fruit ripening (Redgwell et al. 1997; Redgwell et al. 1991; Ben-Arie et al. 1979). In the literature, both model and real cell wall systems are exploited to gain insight in the effect of cell wall composition on its structural and mechanical properties upon

hydration. In model systems, the dispersion of cellulose fibers in water was found to be facilitated by the application of high shear forces, resulting in the formation of a gel-like structure (Agoda-Tandjawa et al., 2010; Lowys, Desbrières, & Rinaudo, 2001; Pääkko et al., 2007) that in presence of pectin is dependent on the pectic macromolecular characteristics (Agoda-Tandjawa et al., 2012; Lopez-Sanchez et al., 2017). Furthermore, enzymatic pectin removal was described to be crucial for viscoelastic property improvement of real plant tissue suspensions (Sankaran et al., 2015).

In this study, it is investigated if differences in both the macromolecular composition as well as the amount of pectin in real plant cell wall tissue influences its microstructural and viscoelastic properties upon dispersion. To obtain cell wall material with diverse residual pectin composition and content, pectin was removed from the cell wall material based on its solubility under different extraction conditions. Although this chemical pectin removal is often used in literature to investigate the properties of the solubilized pectin fractions (Christiaens et al., 2015; De Roeck, Sila, Duvetter, Vanloey, & Hendrickx, 2008; Houben, Jolie, Fraeye, Van Loey, & Hendrickx, 2011; Jamsazzadeh Kermani et al., 2014; Van Buggenhout et al., 2015), little research focusses on the characterization of the residual pectin polymers in the non-solubilized cell wall material, and its effect on the microstructural and mechanical properties upon dispersion. Lemon peel was selected as fiber-rich plant source because of its large industrial production as by-product after juice extraction. While different pectin families were selectively removed from the lemon peels during a sequential fractionation under specific extraction conditions, pectin was removed non-selectively from lemon peel by the application of acid and thermal conditions. These (non)-specific pectin extractions resulted in the creation of cell wall residues with different pectin content and characteristics. First, the pectin properties of all cell wall residues as well as the extracted pectin families were determined to physico-chemically characterize the cell wall material. Subsequently, the microstructural and viscoelastic role of pectin in the cell wall was investigated by suspending the different cell wall residues. In addition, the effect of shear forces on the microstructural and rheological properties was investigated by high pressure homogenization of the different cell wall suspensions.

2. Materials and methods

2.1 Experimental design

To understand the role of pectin in the functionalization of lemon peel, the alcohol insoluble residue (AIR) was first extracted from the dry lemon peels in order to isolate its polysaccharide polymeric fraction (Figure 1). Subsequently, pectin was extracted from the AIR sequentially under different extraction conditions and under acid conditions, thereby obtaining various pectin depleted cell wall fractions and pectin enriched filtrates. Insight in the physico-chemical composition of both the extracted pectin and the insoluble residual material was obtained. In addition, the functionalization potential of the pectin-depleted residues was investigated by means of high pressure homogenization.

2.2 Sample preparation

2.2.1 Alcohol insoluble residue

Lemon peel (LP) was received in dried and milled state from a local supplier. First, cell wall material was isolated as the alcohol insoluble residue (AIR) using the method described by McFeeters & Armstrong (1984). Approximately 30 g lemon peel was wetted with 30 g demineralized water, suspended in 192 ml 95% ethanol while mixing (Buchi mixer B-400, Flawil, Switzerland) and filtered over a paper filter (Machery-Nagel MN 615 Ø 90 mm). Subsequently the residue was mixed again in 96 ml 95% ethanol and filtered. Finally the residue was suspended in 96 ml acetone under magnetic stirring and filtered to obtain the corresponding AIR (residue). The AIR was dried overnight at 40 °C, grounded with a mortar and pestle and stored in a desiccator until further use.

2.3 Lemon peel fractionation

Lemon peel fractions with different pectin composition and content were obtained by sequential fractionation of AIR based on the extractability of pectin in different solutions (i.e. water, 0.05 M CDTA in 0.1 M potassium acetate solution, 0.05 M Na₂CO₃ containing 0.02 M NaBH₄).

2.3.1 Water insoluble cell wall fraction

Water extractable pectin was separated from the AIR according to a procedure described by Braga et al. (1998). Approximately 4000 ml of hot distilled water (95 °C) was added to 60 g AIR. After stirring (10 min), the suspension was cooled in iced water and filtered over a paper filter (Machery-Nagel MN 615 Ø 90 mm), resulting in the water insoluble residue (WR) and a filtrate containing hot water extracted pectin (WF).

2.3.2 Chelator insoluble cell wall fraction

The second fractionation step was performed following the method of Chin et al. (1999). In particular, the WR was resuspended in 4000 ml of 0.05 M CDTA in 0.1 M potassium acetate solution with pH 6.5. After incubation at 28 °C for 6 h under stirring, the suspension was filtered over filter paper (Machery-Nagel MN 615 Ø 90 mm), resulting in the chelator insoluble residue (CR) and a pectin rich chelator extracted filtrate (CF).

2.3.3 Alkaline insoluble cell wall fraction

During a final fractionation, previously obtained CR was resuspended in 4000 ml 0.05 M Na₂CO₃ containing 0.02 M NaBH₄, incubated for 16 h at 4 °C and subsequently for 6 h at 28 °C while stirring. The suspension was filtered (Paper filter, Machery-Nagel MN 615 Ø 90 mm), resulting in the sodium carbonate insoluble residue (NR) and a filtrate containing sodium carbonate extractable pectin (NF).

2.3.4 Acid insoluble cell wall fraction

Besides the sequential solubilization of pectin from lemon peel using different extraction solutions, pectin was also removed from lemon peel under acid conditions during an acid extraction procedure based on Kaya et al. (2014). In particular, 60 g of AIR was suspended in 4000 ml distilled water and incubated for 30 min at 80 °C under stirring. Subsequently, the pH of the suspension was adjusted to 1.6 with 7 N HNO₃, and the incubation was continued for 1 h. Thereafter, the suspension was cooled in an ice bath and centrifuged at room temperature for 10 min at 4000 g. The pectin rich acid supernatant (AF) was separated and the pellet was washed with 1 L distilled water before filtration over a paper filter (Machery-Nagel MN 615 Ø 90 mm).

2.3.5 High pressure homogenization

First, fractionation residues, namely WR, CR, NR and AR, were suspended at a 2% (dry matter w/w) concentration in standardized tap water (0.2% NaCl and 0.015% CaCl₂·H₂O in ultrapure water (organic free, 18 MΩ cm resistance)). Thereafter, these suspensions were mixed with an Ultra-Turrax (IKA, Staufen, Germany) for 10 min at 8000 rpm to obtain homogeneous suspensions. Finally, samples were homogenized using a high pressure homogenizer (Panda 2k, GEA Niro Soavi) at 20 or 80 MPa while non-homogenized samples (0 MPa) were considered as control.

2.3.6 Lyophilization and rehydration

Despite the possible effect on the microstructural and therefore viscoelastic properties of the residue suspensions (Borchani, Besbes, Masmoudi, Blecker, & Paquot, 2011; Oikonomopoulou, Krokida, &

Karathanos, 2011; Ratti, 2001), the non-homogenized and homogenized samples were lyophilized for logistical reasons. Samples were first frozen in liquid nitrogen, and dried (Christ alpha 2-4, Osterode, Germany) during 24 hours (shelf temp. 20 °C, cond. temp. -80 °C, pressure 0.100 mbar). Lyophilized samples were stored in a desiccator at room temperature until further use.

2.4 Physico-chemical characterization

Non-homogenized LP, AIR, as well as fractionation residues and filtrates were analyzed for their physico-chemical characteristics. In particular, the pectin content and structural characteristics were determined by measuring the galacturonic acid (GalA) content, the degree of methoxylation (DM), the neutral sugar (NS) content and ratio and molar mass (MM) distribution.

2.4.1 Determination of galacturonic acid content

The galacturonic acid (GalA) content of LP, AIR, as well as fractionation residues and filtrates was determined according to a procedure described by Ahmed (1977). In particular, 10 mg (dry weight) of a non-lyophilized sample was hydrolyzed in 8 ml H₂SO₄ (98%) with a drop-wise addition of 2 ml demineralized water while stirring. After 4 h of hydrolysis and diluting to 50 ml, the GalA content was measured according to a procedure described by Blumenkrantz & Asboe-Hansen (1973) by means of spectrophotometric analysis. Hereto, 3.6 ml of sulfuric acid-sodium borate solution (0.0125 M sodium tetraborate in 98% H₂SO₄) was added to 0.6 ml of the hydrolyzed sample, and heated for 5 min at 100 °C. After cooling to room temperature in an ice bath, the sample was mixed with 60 µL of m-hydroxydiphenyl-solution (0.15% metahydroxydiphenyl in 0.5% NaOH) forming a chromogenic product absorbing at 520 nm. Based on a calibration curve from known amounts of GalA solutions and correlated absorbances, the GalA content of the samples was determined. A blank was included for each sample using 60 µl of 0.5% NaOH instead of the m-hydroxydifenyl solution.

2.4.2 Determination of degree of methoxylation

To determine the amount of GalA methyl esterification, the molar ratio of methyl over GalA was calculated and expressed as degree of methoxylation (DM). Pectin saponification to pectate and MeOH was performed, according to the procedure of Ng & Waldron (1997). Here, 1 ml of liquid pectin extracted filtrate or 50 mg lyophilized insoluble residue was suspended in 1 ml ultrapure water (organic free, 18 MΩ cm resistance) and was added in a 10 ml vial. Furthermore, each sample was spiked with 10 µmol of an internal methanol d₃-standard and subsequently saponified forming MeOH.

Saponification was performed in duplicate with 0.4 ml 2 M NaOH for 1 h at 20 °C, followed by neutralization with 0.4 ml 2 M HCl. Lastly, a total volume of 5 ml was obtained by adding ultrapure water. To account for the amount of free methanol formed during previous processing, a blank was included for each sample. In this blank, NaOH and HCl were replaced by similar amounts of ultrapure water during the sample preparation.

The intrinsic and formed amounts of methanol were measured by means of head-space gas-chromatography (GC) (7890B, Agilent Technologies, Diegem, Belgium) equipped with a mass spectrometer (MS) (Keysight Technologies, Santa Rosa, California) and a CTC Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland). First, the samples were equilibrated at 40 °C for 5 min before exposure to a solid-phase microextraction (SPME) fiber (Supelco, Bellefonte, Pennsylvania) with an 85 µm carboxen/polydimethylsiloxane (CAR/PDMS) sorptive coating. Subsequently, the absorbed compounds were thermally desorbed for 2 min at 300 °C in the GC injection port, followed by a thermal cleaning for 5 min at 300 °C in the autosampler conditioning station. Next, the desorbed volatiles were injected in split mode and separated on a HP-PLOT Q column (30 m × 320 µm, 20 µm film thickness, Keysight Technologies, Santa Rosa, California) using helium as carrier gas at a constant 1.5 ml/min flow rate. During the elution, a stable column oven temperature of 40 °C was maintained for 1.6 min, then elevated to 250 °C at a rate of 50 °C/min for 5 min and subsequently cooled to the initial temperature. Finally, mass spectra were obtained by electron ionization (EI) at 70 eV, in a combined SCAN and SIM mode. Here, the MS ion source and quadrupole temperatures were 230 and 150 °C, respectively. For calibration, stock solutions with known concentrations of 0 to 70 µmol methanol/ml were spiked with 10 µmol methanol d₃-standard and subsequently analyzed. The calibration curve was obtained by plotting the ratio of MeOH concentrations in its normal and its deuterated form to the peak area ratios of ion pairs (m/z 31/35_d).

2.4.3 Determination of pectin methyl esterase activity

Pectin methyl esterase (PME) activity was measured in LP by means of titration using an automatic pH stat titrator (Metrohm, Herisau, Switzerland). First, PME from LP was extracted by suspending LP in a Tris-NaCl extraction buffer (2:1 w/v ratio, 0.2 M Tris in 1 M NaCl solution, pH 8.0). Subsequent centrifugation (10000 g, 60 min, 4 °C) resulted in a PME rich crude enzyme extract. Apple pectin (degree of esterification, 70-75%; Fluka, Buches, Switzerland) solution (0.35% w/v containing 0.117

M NaCl) was prepared as a substrate for PME activity measurement. Hereto 100 μ L of crude enzyme extract was added to 30 ml of apple pectin solution. The enzymatically-formed demethoxylated carboxyl group was titrated with 0.01 N NaOH at pH 7.5 and 30°C for 16 min.

2.4.4 Determination of neutral sugar content

High performance anion exchange chromatography (HPAEC) was used to determine the amount of neutral monosaccharides fucose, rhamnose, arabinose, galactose, glucose, xylose and mannose in the LP, AIR, and fractionation residues and filtrates. First, approximately 5 mg (dry weight) of non-lyophilized sample was hydrolyzed using 8 M trifluoro-acetic acid during 1.5 h at 110 °C. After cooling to room temperature, trifluoro-acetic acid was evaporated from the samples at 45 °C using N₂-gas. Further elimination and neutralization of trifluoro-acetic acid was obtained by diluting the dried samples with demineralized water to a 0.1% w/v concentration. The analysis was performed using a Dionex DX600 coupled to a ED50 electrochemical detector with a reference pH-electrode and gold-electrode. Hydrolyzed samples (100 μ l) were eluted with 4 mM NaOH at a flow rate of 0.5 ml/min at 3 °C on a CarboPac PA20 column equipped with a CarboPac PA20 guard column (Dionex, Sunnyvale, California). Commercially available neutral sugar standards (Sigma-Aldrich, St.-Louis MO, USA) were used to identify and quantify the eluted sugars. In addition, neutral sugar standards were treated with similar pre-treatment conditions as the samples, to determine the correction factors relevant for the hydrolysis of monosaccharides during acid hydrolysis.

2.4.5 Determination of pectin molar mass distribution

Molar mass (MM) distributions of pectin in the fractionation residues, filtrates and AIR were determined by means of high performance size exclusion chromatography (HPSEC) coupled to multi-angle laser light scattering (MALLS) (PN3621, Postnova analytics, Germany), a refractive index (RI) detector (Shodex RI-101, Showa Denko K.K., Kawazaki, Japan), and diode array detector (DAD) (G1316A, Agilent technologies, Diegem, Belgium), according to the method of Shpigelman et al. (2014). An Agilent 1200 Series was used with 3 Waters columns (Waters, Milford, MA): Ultrahydrogel 250, 1000 and 2000, with exclusion limits 8×10^4 , 4×10^6 and 1×10^7 g/mol respectively. First, lyophilized samples were solubilized (0.25% (w/v) in a 1 M acetic acid elution buffer (pH 4.4) with 0.1 M NaCl) while stirring for 4 h. Subsequently, the samples were filtered using a 0.45 μ m filter (Milex-HV), injected (100 μ l) and eluted over the columns at 35 °C (flow rate 0.5 ml/min) (Corredig,

Kerr, & Wicker, 2000). The molar masses were calculated by a second order Debye fitting method using the instrument software (Nova Mals, version 1.0.0.18, Postnova analytics, Germany) (Shpigelman et al., 2014).

2.5 Microstructural and viscoelastic characterization

Prior to microstructural and viscoelastic characterization, lyophilized LP, AIR and residues suspensions (both homogenized and non-homogenized) were resuspended in ultrapure water (2% w/w) and magnetically stirred overnight. The microstructural and viscoelastic characteristics of these suspensions were investigated by laser diffraction, microscopy and rheological analysis.

2.5.1 Laser diffraction analysis

Laser diffraction (Beckman Coulter, LS 13 320, Miami, Florida) was used to determine the particle size distribution of LP, AIR, WR, CR, NR and AR suspensions before and after homogenization. Samples were added in a stirring tank, filled with demineralized water and circulated 2 times into the measuring cell (pumping rate 30%) before measurement. Laser light with a wavelength of 750 nm was used as the main laser light source, whereas laser light with wavelength 450, 600, and 900 nm was used for polarization intensity differential scattering (PIDS) giving a detection range of 0.04-2000 μm . The volumetric particle size distributions and D_{50} -value of the samples were calculated from the intensity distributions of the scattered light according to the Fraunhofer optical model using the instrument software (plant cell wall RI = 1.6, water RI = 1.33 and dispersion absorption coefficient = 1) (Verrijssen et al., 2014).

2.5.2 Microscopy analysis

The microstructure of non-homogenized and homogenized LP, AIR, and fractionation residues suspensions was visualized by means of microscopy, using cell wall specific dyes and epifluorescent light, as well as normal light. Light microscopy was performed using an Olympus BX-41 microscope (Olympus, Optical Co. Ltd, Tokyo, Japan), equipped with an Olympus XC-50 digital camera and photo-analyzing software in differential interference contrast mode (DIC). Epifluorescent samples were stained with acridine orange (dilution of 1:100 (w/v) from 2% concentrated dye) and analyzed using an Olympus BX-41 microscope equipped with epifluorescence illumination (X-Cite® Fluorescence Illumination, Series 120Q, EXFO Europe, Hants, United Kingdom). Acridine orange was used as a cationic dye which associates with polyanionic compounds while emitting a green

fluorescence when illuminated. At least ten micrographs were taken with objective 10 x to obtain a representative overview of the cell wall suspension.

2.5.3 Rheological analysis

Rheological measurements were performed with a stress controlled rheometer (MCR 501, Anton Paar, Graz, Austria) at 25 °C. Samples were analyzed using a parallel plate geometry of 40 mm diameter with a gap of 2 mm, within 24 h after preparation. In order to limit wall slip effects, sand-paper (roughness 46.2 μm) was glued on both upper and lower plate, and was regularly changed. Confinement effects were considered to be negligible for all measurable suspensions since the gap was ten times larger than the particles and/or viscoelastic measurements with a vane geometry were comparable (data not shown). To avoid potential effects of sample manipulation and loading history on the measurements, the samples were pre-sheared at 10 s^{-1} for 30 s, and allowed to rest for 30 s before the measurement. Given the time required for the tests (20-30 min), solvent evaporation was considered to be negligible. Oscillatory measurements were performed to characterize the viscoelastic behavior of the samples. At first, a strain sweep from 0.01 to 1000% strain at an angular frequency of 6.28 rad/s was carried out to identify the linear viscoelastic region (LVR) and the critical strain. After identification of the LVR, frequency sweep tests were performed at constant strain amplitude of 1% (within the LVR) from 628 to 0.628 rad/s to determine the dependence of the storage and loss moduli (G' and G'') on frequency.

2.5.4 Statistical analysis

All samples were measured minimally two times and results are presented as averages including standard deviation. Analyses of variance (ANOVA) was performed with the significance level set to $P < 0.05$ (Statistica for Windows, ver. 5.1, Statsoft Inc. Tulsa, USA, 1997) whereas Tukey's procedure was used to test for differences between means.

3. Results and Discussion

3.1 Physico-chemical characterization

3.1.1 Galacturonic acid content as guidance for the pectin content

Considering the high linearity of citrus pectin, the pectin content of each LP fraction was estimated based on its GalA-content since homogalacturonan is considered the most abundant pectic polysaccharide present (Voragen, Verhoef, Coenen, & Schols, 2009). Table 1 presents the amount of

GalA in LP, AIR and its fractionation residues and filtrates, as well as the relative amount of pectin in the fractions to the entire pectin population in the AIR. With a GalA-content of 1.29 mmol/g dry LP, citrus peel is considered to be particularly rich in GalA as also described by Ros et al. (1996), Koubala et al. (2008) and Kaya et al. (2014), reporting a GalA content of 1.25 mmol/g in lemon peel. Although an ethanol-acetone extraction procedure was performed on lemon peel to remove small sugars and other ethanol-soluble compounds such as lipids and pigments (Selvendran & Du Pont, 1980), no significant losses of pectin were measured in the AIR fraction in comparison with the initial pectin content in lemon peel. Because of the similarity in GalA-content of LP and AIR, the AIR was used as starting material for the fractionation and pectin extraction procedure since possible interfering components were removed during the AIR extraction procedure. During the subsequent fractionation of AIR with water, chelator and alkaline solutions, pectin polysaccharides were solubilized and removed depending on the fractionation medium used. In particular, a decrease in pectin content was measured in the insoluble residues after each fractionation step. The highest pectin content of the fractionation residues was measured in the water residue (WR) whereas the alkaline residue (NR) was almost fully depleted of pectin. In contrast to the insoluble fractionation residues, the corresponding water filtrate (WF) included the lowest pectin content whereas the highest pectin content was solubilized in the alkaline filtrate (NF), indicating the presence of high amounts of alkaline soluble pectin in lemon peel. Deviations from 100% of the combined extraction yields of the residues and filtrates, for example the combined pectin yield of NR, WF, CF and NF, can be ascribed to unavoidable losses during the actual performance of the fractionation procedure. Due to the extensive amount of handlings for each fractionation step, an accumulation of losses was obtained further down the fractionation procedure.

In comparison, the use of acidic extraction conditions resulted in the extraction of an extensive amount of pectin from the AIR to the acid filtrate (AF). Furthermore, the pectin content of the insoluble acid residue (AR) decreased by 71.9% in comparison with the initial pectin content of AIR. In previous studies, a pectin depletion up to 90% was reported by the application of harsher extraction conditions including longer extraction time and/or higher extraction temperature than those commonly applied in industry (Kaya et al., 2014). Further comparison of the pectin content of all insoluble residues showed that AR contained a considerably lower pectin content than CR but a higher pectin content than NR.

Despite the fact that pectin extraction under the current acid conditions is commonly used in industrial processes to remove pectin from citrus peel, in this study the utmost reduction in pectin could be obtained only after the total sequential fractionation up to the removal of alkaline soluble pectin (NR).

3.1.2 Degree of methoxylation of pectin

The functionality of pectin polysaccharides can vary significantly depending on their degree of methoxylation (DM) (Voragen et al., 2009). The latter is influenced not only by the fractionation conditions, such as pH and temperature, but also by the presence of active pectin methyl esterase (PME) (Fraeye et al., 2007). Nevertheless, no PME activity was detected in lemon peel during this study and was therefore considered as either absent or negligible. Differences in the DM of pectin in LP, AIR, residues and filtrates can thus be attributed to the sole effect of the fractionation conditions (Table 1). LP and AIR were found to be comparable not only based on their GalA content, but also based on their DM. With a DM of 68.36% and 69.48% for LP and AIR respectively, citrus pectin is considered as high methoxylated pectin ($DM > 50\%$). Performing a first fractionation step on AIR with water resulted in the solubilization of pectin polysaccharides in WF with a slightly higher degree of methoxylation (DM 89.86%), which is in close agreement with the results of Koubala et al. (2008) for lime (DM 82%). Considering the small amount of pectin solubilized in the WF (Table 1), no significant changes in DM were observed regarding the pectin family in the residual WR material (DM 69.47%). Further fractionation involving a chelator solution resulted in the solubilization of pectin polymers in CF with a DM of 71.56%. Depending on the pH ($pH > pK_a$), free carboxyl groups of pectin can be electrostatically charged and therefore associate with free Ca^{2+} ions to form pectate-calcium gels. The presence of a chelator prevents the electrostatic pectin- Ca^{2+} interactions, consequently solubilizing calcium bound pectin (Jamsazzadeh Kermani et al., 2014; Shpigelman et al., 2014). Consistent with WR, also CR pectin showed to be not significantly different from AIR based on its DM. Subsequently, the alkaline conditions used during the final fractionation step are reported to demethoxylate and solubilize non-calcium sensitive pectin polymers that interact with other cell wall polymers (Redgwell et al., 1997). Therefore, pectin polymers with extremely low degree of methoxylation were found in NF (DM 3.58%) whereas no DM was detected for NR. Lastly, the use of acid extraction conditions resulted in a slight decrease in DM for AR pectin (DM 59.86%) polysaccharides but no significant demethoxylation of AF pectin (DM 71.84%). Although Van Buren

(1979) reported that the combination of harsh thermal and acid conditions could result in a chemical pectin demethoxylation, the acid conditions used in this study were not that severe. As a consequence, a large family of pectin polymers could be extracted under the present acid conditions without extensive demethoxylation of the extracted pectin.

3.1.3 Neutral sugar content

In general, the structural composition of pectin can be estimated based on its monosaccharide content determined after hydrolysis (Caffall & Mohnen, 2009). Although pectin polymers are composed of a limited group of neutral sugars, their total neutral sugar content can vary greatly as their side chains can differ in composition and extent of branching (Voragen et al., 2009). Table 2 presents the neutral sugar content of LP, AIR, and the different residues and filtrates. Comparison of the native monosaccharide content of LP and AIR shows that both have a similar neutral sugar composition except for glucose. The latter decreased after performing the alcohol treatment, indicating a removal of free sugars from lemon peel. Once more, AIR was found to be a suitable control and starting material for the pectin fractionation procedure. Furthermore it can be seen that lemon peel is particularly rich in GalA, arabinose (Ara) and galactose (Gal) in good agreement with Kaya et al. (2014). Conversely, only small amounts of fucose (Fuc), rhamnose (Rha), xylose (Xyl) and mannose (Man) were detected. Different “sugar ratios” can be introduced to provide an indication of some pectin molecular features. In particular, sugar ratio 1 represents the contribution of homogalacturonan in the different pectin fractions by expressing the ratio of pectic backbone sugar GalA to the sum of the major pectin-associated neutral sugars Rha, Ara, Fuc, Gal and Xyl (Figure 2A). While sugar ratio 1 gives an indication of the pectin linearity, sugar ratio 2 is defined to estimate the presence of branched RG structures by the ratio of Rha over GalA (Figure 2B). Figure 2C shows sugar ratio 3, comprising the ratio of both Ara and Gal over Rha, which correlates with the length of the RGI branches (Caffall & Mohnen, 2009; De Roeck et al., 2008; Houben et al., 2011). As seen in Figures 2A-C, the linearity of the pectin polysaccharides in the residues decreased during the fractionation procedure, while the RGI contribution and branching increased for the residual pectin families. The use of mild extraction conditions during the fractionation of water soluble pectin resulted in the solubilization of a limited amount of highly branched pectin (lowest sugar ratio 1 and highest sugar ratio 2) in WF. In agreement with Houben et al. (2011), pectin polymers with both a high amount of branches and high DM (Table

1) undergo only limited pectin interactions, hereby facilitating the removal of this pectin to the WF despite the mild extraction conditions. On the other hand, pectin with a significantly high linearity and corresponding low RGI contribution was solubilized in the CF. Since pectin-Ca²⁺ interactions are stimulated by linear HG blocks in combination with a low DM, chelator interaction resulted in the solubilization of pectin polysaccharides with according characteristics. Subsequent fractionation of the residual cell wall material under alkaline conditions resulted in the solubilization of ester-bound pectin with intermediate linearity and RGI contribution while being extensively branched. Pectin is described to interact with cellulose through its neutral sugar side chains (Cosgrove, 2014; Lin, Lopez-Sanchez, & Gidley, 2015, 2016; Redgwell & Percy, 1992; Zykowska et al., 2007), whereas also hemicellulose-pectin interactions are suggested (Seymour & Knox, 2002). Extraction of pectin from lemon peel under intermediate acid conditions resulted in the removal of pectin polymers with a similar structural composition as the control. Contrarily, pectin remaining in AR showed a decreased RGI contribution and extent of branching. In previous work, Garnier et al. (1993) and Jamsazzadeh Kermani et al. (2014) suggested that pectin extracted under acidic conditions undergoes debranching. In particular, it is suggested that the neutral sugar linkages are more susceptible to acid hydrolysis than linkages between galacturonic acid residues, resulting in a decreased extent of RGI branches in the AR. This is consistent with the increased amount of Ara and Gal found in the AF (Table 2), which also affected the first and third sugar ratios (Figure 2A and C).

3.1.4 Molar mass distribution

In Figure 3 A&B, the concentration distribution over the elution time and the molar masses (weight average molar mass, M_w) of pectin polymers in LP, AIR, filtrates and residues are shown. Since HPSEC-RI-MALLS-DAD analysis requires the samples to be filtered, large polymers and insolubilized material are sometimes withheld from analysis during pre-treatment (Jamsazzadeh Kermani et al., 2014) resulting in a lack of signal response what is the case for CR and NR in the present study. Due to the low degree of methoxylation, pectin chains in the NF are likely to crosslink, resulting in larger polymers which were withheld during the mandatory filtration step prior to analysis. As shown in the molar mass profile in Figure 3A, both LP and AIR show great similarity in retention time. With a weight average molar mass of 655 kDa and 548 kDa for LP and AIR respectively, the AIR was once more found to be a suitable control and starting material for the fractionation and acid

pectin extraction procedure. Despite the similar retention time of the filterable pectin of WR and AIR, a lower M_w of 270 kDa was measured for WR. As the retention time depends on the hydrodynamic volume, WR is suggested to contain pectin polymers with lower molecular weight and less compact structure, occupying a hydrodynamic volume similar to the polymers in LP and AIR. In contrast with the WR, the molar mass distribution of solubilized pectin from AR showed an increased retention time compared to AIR. Therefore, pectin polymers in AR are suggested to have a substantially compacter conformation and lower M_w (214 kDa) than the native pectin polymers. Taking into account the results of the neutral sugar analysis, also the molar mass distribution analysis of AR is consistent with a debranching of pectin under the acid extraction conditions. In fact, debranched pectin polymers can encounter less sterical hindrance, resulting in a more compact conformation of the pectin polysaccharide. While detection of the polymers in the insoluble residues was impaired by low concentrations of solubilized pectin, the detection of polymers in the fractionation filtrates was facilitated due to the high concentrations of solubilized pectin. Figure 3B shows that fractionation with hot water (WF) resulted in the solubilization of pectin polymers with M_w of 361 kDa and a slightly longer retention time than the AIR, suggesting the solubilization of pectin with a slightly smaller hydrodynamic volume and lower molar weight than the native AIR polymers. According to the neutral sugar analysis (Figure 2), WF pectin is characterized by a large extent of branching. Further fractionation using a CDTA-solution resulted in the solubilization of CF pectin polymers with lower M_w (250 kDa) but higher hydrodynamic volume than WF. In accordance with the neutral sugar composition of CF pectin, suggesting the presence of large HG sections, the decrease in M_w can be attributed to the lack of branches. Opposed to the previously discussed elution profiles, a polydisperse concentration peak with molar mass ranging between 128 and 361 kDa was obtained for AF pectin. The occurrence of a polydisperse mixture of pectin polymers can be ascribed to the partial depolymerization and debranching of the pectin side chains under acid conditions. This finding supports previous suggestions concerning the presence of free oligosaccharides in AF, influencing its neutral sugar profile (Figure 2).

3.2 Microstructural and viscoelastic characterization

To gain insight in the role of pectin on the viscoelastic properties of the cell wall suspensions, LP and the insoluble residues were first suspended (2% w/w) in water and mechanically disrupted by high

pressure homogenization. Subsequently, the influence of pectin content and characteristics on the microstructural and viscoelastic properties of lemon peel suspensions were investigated by determination of the particle size, microscopic appearance and storage modulus of the LP, WR, CR, NR and AR fraction suspensions. Additionally, the effect of pressure level was investigated by homogenizing the suspensions at 20 or 80 MPa. Non-homogenized suspensions were considered as controls.

3.2.1 Effect of pectin extraction conditions and pressure level on the microstructural characteristics of high pressure homogenized lemon peel residues.

Particle size distributions of LP, WR, CR, NR and AR suspensions with and without homogenization (at 20 MPa and 80 MPa) are shown in Figure 4. To quantitatively compare the differences in particle size among the different fractions and homogenization pressures, the average particle diameter (D_{50}) was additionally considered (Table A (supplementary data)). First, it can be observed that the average particle diameter of the different non-homogenized residue suspensions decreased during the fractionation procedure, whereas a D_{50} value between that of the WR and CR suspension was found for the non-homogenized AR suspension. It can be observed that homogenization of the LP suspension resulted in a shift of the particle size distribution towards smaller particle sizes as well as in a narrowing of the size distribution profile. Similar findings are described by Van Buggenhout et al. (2015), who linked the degree of orange pulp disintegration to the pressure level applied during high pressure homogenization. A large standard deviation can be noticed on the particle size distribution of non-homogenized LP, suggesting the presence of a polydisperse mixture of particles with different sizes (D_{50} of $521 \pm 19 \mu\text{m}$). The polydispersity of the particle size distribution is deduced from the width of the volumetric distribution peak and is reflected in the large standard deviation of the D_{50} value. Homogenization of LP at 20 and 80 MPa resulted in a more uniform particle distribution as the samples were subjected to large mechanical stresses, thereby destroying large particle clusters. Furthermore a decrease in average particle diameter from 212 ± 4 to $87 \pm 2 \mu\text{m}$ was noticed with increasing homogenization pressure from 20 to 80 MPa. Additionally, the polydispersity of LP decreased with increase in homogenization pressure. Similarly, the average particle size of the WR suspension decreased with increasing homogenization pressure from 372 ± 17 to 179 ± 6 and $76 \pm 1 \mu\text{m}$. In addition, the non-homogenized WR fraction suspension presented the widest particle size

distribution as compared to the other fractions, where the particle size of the non-homogenized fractionation residues decreased during the subsequent pectin extraction. While an extensive decrease in particle size and polydispersity with increasing homogenization pressure was observed for WR, no effect of homogenization pressure could be noticed for CR. Here, the average particle size of the non-homogenized CR suspension was $99 \pm 1 \mu\text{m}$, while a decrease with homogenization to 74 ± 0.1 and $75 \pm 1 \mu\text{m}$ could be observed at 20 and 80 MPa, respectively. In contrast to LP, WR and CR, an increase in particle size and polydispersity with increasing homogenization pressure was noticed for NR and AR. More specifically, the average D_{50} of NR increased from 81 ± 1 to 115 ± 1 and $129 \pm 6 \mu\text{m}$, while the average D_{50} value of AR increased even more substantially from 106 ± 1 to 155 ± 6 and $240 \pm 6 \mu\text{m}$. Furthermore it can be noticed that the non-homogenized NR fraction presented the narrowest particle size distribution. These results suggest that high pressure homogenization has different effects on the microstructure of the suspensions depending on the cell wall residue composition. During high pressure homogenization, the cell wall residue suspension is pushed through a very small nozzle, inducing high velocity and pressure as well as high shear forces, thereby decreasing the fiber size (Abdul Khalil et al., 2014). In particular, in the case of the LP, WR and CR fractions, high pressure homogenization induced a disruption of the matrix because of the high velocity and shear forces created. By contrast, the increasing apparent particle sizes of the NR and AR fraction suspensions suggest the swelling or aggregation of the cell fragments upon high pressure homogenization.

Additional information on the effect of the cell wall residue composition on microstructural changes upon high pressure homogenization was obtained by microscopy analyses (Figures 5 and 6). Figure 5 includes epifluorescence microscopy images using acridine orange to stain cell wall polymers. This cationic dye specifically associates with cell wall material, coloring it green under fluorescent light. Differences in color intensity of the epifluorescence micrographs can be ascribed to the fact that each residue was obtained after fractionation or extraction under different pH conditions, which slightly influenced the dye. In agreement with the particle size distribution, also epifluorescence microscopy images of the non-homogenized LP and WR suspensions showed presence of large cell clusters (Figure 5, intact cell indicated with arrow). Although no large cell clusters could be observed in the CR suspension, intact cells can be distinguished in the micrographs of the non-homogenized CR suspension. While high pressure homogenization of the CR suspension resulted in a complete

disruption of the cell tissue, some intact cells could still be distinguished for LP and WR even after high pressure homogenization at 80 MPa.

Similar observations could be made under light microscopy (Figure 6). Hence, the removal of water soluble and chelator soluble pectin from LP seemed to weaken the cell wall structure to the point that intact cells could not withstand the high shear forces generated during high pressure. In contrast with the images of the LP, CR and WR suspensions, light and fluorescence images of the NR and AR fraction suspensions showed the presence of disrupted cells in the non-homogenized samples. In fact, in both the non-homogenized NR and AR micrographs (Figures 5 and 6), more uniformly distributed cell fragments were observed, resulting in an overall green blurriness under fluorescent light (Figure 5). High pressure homogenization of the NR and AR suspensions increased this effect, where cell fragments seem to hydrate and aggregate. Similar observations were made by Van Buggenhout et al. (2015) after high pressure homogenization of orange pulp suspensions at 80 MPa. While upon increasing homogenization pressure a disintegration was observed for LP, WR and CR fraction suspensions, aggregation was observed for NR and AR fraction suspensions. Despite the differences in pectin composition due to the extraction conditions, both NR and AR suspensions showed very similar microstructural behavior. The latter can be attributed to the rather extensive depletion of pectin in the NR and AR samples. In correlation with these findings, Redgwell et al. (1997) reported hydration and swelling of cell walls after the solubilization of pectin polymers under alkaline conditions. Due to the strong alkaline conditions (such as those of the NR fractionation), interpolymeric crosslinks between pectin and cellulose and/or pectin and hemicellulose are suggested to be interrupted. In good agreement with Redgwell et al. (1997), it is suggested that by this pectin removal, voids in between the cellulose-hemicellulose network can be formed, later on referred to as interfibrillar spaces, giving water the possibility to penetrate the cell wall. On the other hand, pectin debranching is suggested to occur due to breakage of arabinose and galactose linkages under acid conditions, such as those applied in the present work (AR) (Garnier et al., 1993; Jamsazzadeh Kermani et al., 2014). In line with previous studies attributing pectin-cellulose interactions to pectic arabinan and/or galactan sidechains (Lin et al., 2015; Zykwiniska et al., 2007), debranching under acid conditions is suggested to facilitate the removal of cellulose-linked pectin and the formation of interfibrillar spaces. In contrast, the physico-chemical characterization of WR and CR revealed the presence of extensive amounts of

pectin in both fractions, decreasing the possibility of interfibrillar spaces. A similar effect of homogenization pressure on the microstructure of cell wall fragments was discussed by Sankaran et al. (2015) where pectin was removed enzymatically from the cell wall of carrots, creating structurally weak zones throughout the cell wall. In good agreement with our findings, cell wall material without (such as LP) or with minimal occurrence of weak zones (such as WR and CR), were suggested to fragment into smaller pieces after high pressure homogenization whereas cell wall material with partially removed polysaccharide cross-links (such as NR and AR) will rupture along these defects and swell.

3.2.2 Effect of pectin extraction conditions and pressure level on the viscoelastic properties of high pressure homogenized lemon peel suspensions

The viscoelastic properties of the different (non)-homogenized samples were investigated by means of dynamic shear rheological analysis. To achieve reliable results, the linear viscoelastic region of all measurable cell wall fraction suspensions was identified by strain sweep experiments (Figure A (supplementary data)). In this region, both the storage modulus (G') and loss modulus (G'') are strain independent at constant angular frequency (ω). Furthermore, G' of the measurable suspensions was found to be higher than G'' , indicating that these suspensions presented a more elastic than viscous behavior. Frequency sweeps of the measurable samples (Figure B (supplementary data)) were performed within the linear viscoelastic region. Figure 7 shows the frequency sweep of AR-80MPa-L-R, which is representative for all measurable samples. No dependence of the G' values on frequency can be noticed, while a slight increase in the G'' values was observed with increasing frequency. Since the G' values reflect the stiffness of the network and the slope of G' and G'' the network type, the AR and NR suspensions can be considered as strong gels with a moderate stiffness. To compare the effect of homogenization pressure and pectin characteristics on lemon peel suspensions, average G' values were considered for the different lemon peel fractions after high pressure homogenization. Because G' values were found to be independent of the frequency, G' corresponding to an angular frequency of 6.28 rad/s was considered for sample comparison, shown in Figure 8. Since no logical trend between G' and D_{50} values could be identified, G' values were assumed not to be dominated by particle dimensions. Besides the particle size, the rheological properties of plant-based suspensions can be

influenced by other factors such as the particle hardness, morphology, concentration of insoluble solids and particle interactions (Lopez-Sanchez et al., 2011). No reliable rheological results could be obtained for both homogenized and non-homogenized lemon peel suspensions as well as non-homogenized WR suspension because of fast sedimentation. First, it can be observed that the G' values of the (non-) homogenized WR and CR suspensions are very low (Figure 8). Despite these low G' values, the CR suspension was found to have a better suspension stability than the WR suspension since a measurement of the non-homogenized CR was feasible before sedimentation. The viscoelastic behavior of the WR and CR suspensions is consistent with the disruption rather than the swelling of the cell wall after high pressure homogenization due to the presence of pectin, observed by microscopy analysis and previously described (Figures 5 and 6). Conversely, higher G' values were detected for both the pectin depleted NR and AR suspensions after high pressure homogenization. Since both samples are characterized by low pectin content (Table 1), the physico-chemical differences of the residual pectin polymers do not have a dominant effect on the viscoelastic properties of the suspensions. While non-homogenized AR suspensions showed G' values of 38 Pa, homogenization at 20 MPa and 80 MPa increased G' values to 115 and 135 Pa, respectively. Viscoelastic characterization of non-homogenized NR showed that G' values of NR reached nearly two times the G' value of non-homogenized AR, whereas homogenization at 20 and 80 MPa stabilized at G' values of 216 Pa and 229 Pa, respectively. While differences in homogenization pressure resulted in distinct differences in microstructure of both NR and AR, differences in viscoelastic behavior upon homogenization at different pressure levels was only observed for NR. These results suggest that homogenization at 20 MPa is sufficient to overcome the interfibrillar interactions if the cell wall is previously weakened, inducing the swelling of the cell wall fibers. While NR was characterized as the most pectin depleted residue and the highest G' value is reported for NR, it is suggested that pectin is of crucial importance for the cell wall integrity. Similar findings were reported by Sankaran et al. (2015), stating that pectin removal is a key factor for the cell wall fibers of carrots to be loosened by high pressure homogenization.

4. Conclusions

Although pectin extraction from lemon peel, by using different extraction conditions, resulted in insoluble residues containing pectin polysaccharides with various chemical characteristics, it is concluded that the amount of pectin in the residues was of prominent importance with regard to its viscoelastic behavior upon dispersion. Since pectin surrounds the loadbearing cellulose-hemicellulose network, no or only partial pectin removal did not weaken the cell wall fiber interactions intensively. Due to high shear forces and the high amount of these polymer interactions in the cell wall material, high pressure homogenization resulted in the disruption of the stiff cell wall material. This disruption was visually observed under microscopy and characterized by a decrease of particle size. In contrast, an extensive removal of pectin caused the creation of interfibrillar spaces within the cellulose-hemicellulose network, allowing for fiber hydration and improvement of the viscoelastic properties upon suspension. In agreement with Redgwell et al. (1997) it is suggested that the presence of interfibrillar spaces causes the cell wall to be less densely packed, and thus more flexible. Microstructurally such decrease in cell wall density resulted in an increased particle diameter while microscopically cell wall swelling and aggregation of the cell wall fibers can be ascribed to the extensive removal of pectin. In addition, it is suggested that the increased viscoelasticity of the cell wall suspensions is influenced by the creation of an increased free fibrillar surface since both the acid and alkaline conditions used in this study facilitated the removal of ester cellulose bound pectin polymers. Nevertheless, shearing is required to increase the viscoelastic properties of pectin depleted cell wall suspensions. Although the application of shear forces is reported to interfere with hydrogen bonds and potentially affect fiber-fiber interactions, additional removal of pectin seems to be crucial to expand the interfibrillar spaces, increasing the fiber-water interactions.

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Table 1: Total galacturonic acid content (GalA), relative pectin content and degree of methoxylation (DM) of lemon peel (LP), alcohol insoluble residue (AIR), water residue and filtrate (WR and WF), chelator residue and filtrate (CR and CF), alkaline residue and filtrate (NR and NF), and acid residue and filtrate (AR and AF), $\pm$ standard deviation. Significant differences (Tukey test, $P < 0.05$) among residues, LP and AIR are indicated with different capital letters (A,B,C,D) while differences among filtrates, LP and AIR are indicated with different small letters (a,b,c). Not detectable (n.d)

		mmol GalA/g dry LP	pectin content ¹ (%)	DM (%)
	LP	1.29 ± 0.07^{Aa}		68.36 ± 1.95^{ABa}
	AIR	1.37 ± 0.11^{Aa}		69.48 ± 0.06^{ABa}
Residue	WR	1.22 ± 0.05^A	89.1^A	69.47 ± 0.68^A
	CR	0.63 ± 0.10^B	46.2^B	67.48 ± 0.90^{AB}
	NR	0.12 ± 0.01^C	8.7^C	n.d.
	AR	0.39 ± 0.01^{BC}	28.1^D	59.86 ± 1.20^B
Filtrate	WF	0.17 ± 0.01^b	12.3^a	89.54 ± 2.10^b
	CF	0.25 ± 0.08^b	18.4^{ab}	71.56 ± 1.30^a
	NF	0.34 ± 0.01^b	24.8^b	3.58 ± 4.79^c
	AF	0.77 ± 0.05^c	56.3^c	71.84 ± 4.75^a

¹ GalA content fraction/ GalA content AIR

Table 2: Monosaccharide composition of lemon peel (LP), alcohol insoluble residue (AIR), water residue and filtrate (WR and WF), chelator residue and filtrate (CR and CF), alkaline residue and filtrate (AR and AF) and acid residue and filtrate (AR and AF) expressed per gram dry lemon peel $\pm$ standard deviation. Not detectable (n.d.)

	Fuc	Rha	Ara	Gal	Glu	Xyl	Man
LP	0.019 ± 0.001	0.070 ± 0.004	0.524 ± 0.001	0.292 ± 0.022	0.452 ± 0.009	0.102 ± 0.037	0.025 ± 0.012
AIR	0.020 ± 0.000	0.060 ± 0.001	0.540 ± 0.001	0.307 ± 0.006	0.222 ± 0.004	0.123 ± 0.005	0.033 ± 0.003
WR	0.016 ± 0.004	0.076 ± 0.019	0.468 ± 0.118	0.267 ± 0.087	0.037 ± 0.002	0.091 ± 0.009	0.016 ± 0.004
CR	0.019 ± 0.002	0.088 ± 0.024	0.510 ± 0.099	0.338 ± 0.088	0.040 ± 0.018	0.103 ± 0.055	0.016 ± 0.023
NR	0.007 ± 0.001	0.021 ± 0.001	0.131 ± 0.014	0.104 ± 0.008	0.012 ± 0.002	0.045 ± 0.035	0.005 ± 0.015
AR	0.015 ± 0.002	0.036 ± 0.005	0.089 ± 0.017	0.196 ± 0.027	0.038 ± 0.012	0.111 ± 0.023	0.025 ± 0.004
WF	0.002 ± 0.000	0.024 ± 0.000	0.123 ± 0.005	0.054 ± 0.000	0.229 ± 0.009	0.023 ± 0.015	0.021 ± 0.017
CF	0.001 ± 0.000	0.008 ± 0.001	0.038 ± 0.002	0.012 ± 0.001	0.018 ± 0.001	0.001 ± 0.000	0.001 ± 0.000
NF	0.002 ± 0.000	0.026 ± 0.001	0.275 ± 0.001	0.108 ± 0.001	0.010 ± 0.000	0.005 ± 0.000	n.d.
AF	0.003 ± 0.001	0.028 ± 0.003	0.305 ± 0.032	0.112 ± 0.015	0.132 ± 0.017	0.007 ± 0.001	0.008 ± 0.001

Figure captions:

Figure 1: Experimental design implemented to study the effect of pectin fractionation and extraction and the effect of different homogenization pressure levels on the activation of lemon peel fractions.

Figure 2: Sugar ratios calculated based on the neutral sugar composition of the alcohol insoluble residue (AIR), water residue and filtrate (WR and WF), chelator residue and filtrate (CR and CF), alkaline residue and filtrate (AR and AF) and acid residue and filtrate (AR and AF). (A) *Sugar ratio 1* = $\frac{GalA}{Fuc+Rha+Ara+Gal+Xyl}$, (B) *Sugar ratio 2* = $\frac{Rha}{GalA}$, (C) *Sugar ratio 3* = $\frac{Ara+Gal}{Rha}$. Significant differences (Tukey test, $P < 0.05$) among residues, LP and AIR are indicated with different capital letters (A,B,C,D,E) while differences among filtrates, LP and AIR are indicated with different small letters (a,b,c,d).

Figure 3: Concentration profiles and corresponding weight average molar mass (M_w) of pectin polymers in lemon peel (LP), alcohol insoluble residue (AIR), water residue and filtrate (WR and WF), chelator residue and filtrate (CR and CF), alkaline residue and filtrate (NR and NF), and acid residue and filtrate (AR and AF).

Figure 4: Average particle size distribution of lemon peel (LP), water residue (WR), chelator residue (CR), alkaline residue (NR), acid residue (AR) of lemon peel, homogenized (HPH) at 20 MPa (▲), 80 MPa (■) and non-homogenized control (●), lyophilized and rehydrated (L-R). Error bars represent the standard deviation.

Figure 5: Epifluorescence microscopy with acridine orange images of lemon peel (LP), water insoluble (WR), chelator insoluble (CR), alkaline insoluble (NR), acid insoluble (AR) lemon peel residues non-homogenized (0 MPa) and homogenized at 20 and 80 MPa, lyophilized (L) and rehydrated (R). White arrows indicate the presence of intact cells. Scale bars correspond to 500 μ m. For color images, the reader is referred to the online version of this article.

Figure 6: Light microscopy images of lemon peel (LP), water insoluble (WR), chelator insoluble (CR), alkaline insoluble (NR), acid insoluble (AR) lemon peel residues non-homogenized (0 MPa) and homogenized at 20 and 80 MPa, lyophilized (L) and rehydrated (R). Black arrows indicate the presence of intact cells. Scale bars correspond to 500 μ m. For color images, the reader is referred to the online version of this article.

Figure 7: Frequency sweep at a constant strain amplitude of 1% of acid residue suspension homogenized at 80 MPa lyophilized and rehydrated. Average storage modulus (●) and loss modulus (▲) are shown while error bars represent the standard deviation.

Figure 8: Storage modulus at 6.28 rad/s angular frequency of water residue (WR), chelator residue (CR), alkaline residue (NR) and acid residue (AR) suspensions non-homogenized (0 MPa) (full) and homogenized (HPH) at 20 (stripped) and 80 MPa (dotted), lyophilized (L) and rehydrated (R). Error bars represent the standard deviation. Significant differences (Tukey test, $P < 0.05$) are indicated with different letters (a,b,c,d,e,f). Not detectable (n.d.)

Figure 1

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 2

Highlights

- Selective pectin extraction results in residues with diverse pectic characteristics.
- Extensive pectin removal is key to improve viscoelasticity of residue suspensions.
- Interfibrillar voids in cell wall network are created by extensive pectin removal.
- HPH increases the interfibrillar spaces within the cell wall network.