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Abstract

Cell wall (CW) degradation causes texture loss of plant-based products after processing. However, these losses differ in intensity, which could be due to cell wall structure or plant tissue internal pH conditions. To distinguish these two factors, CWs isolated from apple, beet and kiwifruit were subjected to boiling at pH 2.0, 3.5 and 6.0. Pectin depolymerization was the least pronounced at pH 3.5, while galacturonic acid contents of all CWs decreased at pH 6.0 due to the β-elimination. Pectins were solubilized, and their size decreased with increased pH during CW treatment. At pH 6.0, degrees of methylation decreased mostly in apple and beet CWs while galactose decreased more in kiwifruit CW. At pH 2.0, arabinan was lost in apple and beet CW due to acid hydrolysis. Apple CW was the most susceptible to degradation either at pH 2.0 or 6.0, while beet CW was more degraded at pH 2.0. In contrast, kiwifruit CW was the least susceptible to degradation whichever the pH. Acid hydrolysis and β-elimination appeared to be common mechanisms that cause loss of neutral sugars, often from pectin side chains, and galacturonic acid, respectively, but their effects were of different intensities. This work has a guiding significance for improving texture in the thermally canning process.

Keywords: *Malus x domestica* Borkh; *Beta vulgaris* L.; *Actinidia deliciosa*; processing; pectin; molar mass

Abbreviations:

AIS, alcohol insoluble solids; ATR-FTIR, Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy; CW, Cell Wall; DW, dry weight; HPSEC-MALLS, High Performance Size-Exclusion Chromatography combined with Multi Angle Laser Light Scattering; Solubilized Polysaccharides, SP; PCA, Principal Component Analysis; SEM, Scanning Electron Microscopy.

1 Introduction

Cell walls and their constituent polysaccharides play an important role in plant-based foods, where they control the texture and its evolution during maturation and processing (Ranganathan, Subramanian, & Shanmugam, 2016). Plant cell walls are a complex, porous, polysaccharidic material composed of cellulose, hemicelluloses and pectins. The relative amounts of these constituents are dependent upon the species and maturity of plant tissue. Plant-based foods are commonly processed, e.g. cooking and canning, before being consumed to improve palatability and for a microbiological stability (M. Li, Ho, Hayes, & Ferruzzi, 2019). The first steps of cell wall modification during processing, which involve the endogenous pectinases and their synergies, have been extensively studied as a function of temperature and pressure (Sila et al., 2009). However, it is difficult to control the action of endogenous enzymes during the first phase of heating. Many articles also describe texture loss of plant tissues after enzyme inactivation or thermal processing, but each concerns a single plant material at its natural pH (from 3.0 – 6.5), such as apple (Le Bourvellec, Watrelot, Ginies, Imberty, & Renard, 2012), apricot (Ella Missang, Maingonnat, Renard, & Audergon, 2012), pear (Renard, 2005a), radish (Ando, Hagiwara, & Nabetani, 2017; X. Li, Liu, Tu, Li, & Yan, 2019), potato (Zhao, Shehzad, Yan, Li, & Wang, 2017) or asparagus (Peng, Song, Zhang, Pan, & Tu, 2019). Different plant sources with their natural pH values may have specific responses to processing, and common responses may occur at distinct extents. However, it is difficult from the existing literature to ascertain whether these different responses are

48 due to the structure of the cell walls or to the conditions during fruit and vegetable
49 (F&V) processing. Notably, the natural internal pH is known to be a major driver for
50 the degradation of polysaccharides, and primarily of pectins. It is therefore important
51 to obtain a clear picture differentiating between the effects of the cell wall structure
52 and the pH conditions during F&V processing.

53 Non-enzymatic cell wall modifications during processing involve an increased
54 solubilization accompanied by a depolymerization of pectins (Fraeye et al., 2007).
55 Cellulose and hemicelluloses show minimal structural changes compared to pectins,
56 which therefore appear to be a key to texture loss (Houben, Jolie, Fraeye, Van Loey, &
57 Hendrickx, 2011). Many studies were done using purified pectins heated in model
58 solutions, at different pHs (Chen et al., 2015; Diaz, Anthon, & Barrett, 2007; Fraeye
59 et al., 2007), but we did not find any comparable model studies on complex cell wall
60 matrices. Moreover, work on the impact of thermal processing on the cell wall is
61 mainly focused on the entire plant tissue, but confounding parameters such as the cell
62 wall structure and degradation in pH conditions were not controlled (at natural pH)
63 (Renard, 2005a; Ella Missang et al., 2012; Zhao et al., 2017; X. Li et al., 2019). The
64 chemical mechanisms vary with pH conditions. β-elimination, which is specific for
65 methoxylated pectins, cleaves the methoxylated homogalacturonan chains creating an
66 unsaturated bond (between C4 and C5 of a methoxylated uronic acid) with absorption
67 at 235 nm (Albersheim, Neukom, & Deuel, 1960). Pectins with a higher degree of
68 methylation (DM) are more sensitive to β-elimination (Fraeye et al., 2007).
69 β-elimination is in competition with saponification of methoxylated galacturonic acids,

which is favoured by high pH. As temperature increases, the rate of β-elimination increases faster than that of demethylation, so that heating at a neutral to slightly acidic pH (> 4.5) may lead to an extensive pectin depolymerization. During thermal treatment in acidic conditions (pH < 3.0), acid hydrolysis occurs, leading to a loss of pectin side-chains, starting with arabinans, and a cleavage in the rhamnogalacturonan I regions of pectins, in the order of sensitivity of the glycosidic bonds in a following order: Ara - Ara > Gal - Gal > Rha - GalA > GalA - Rha > GalA - GalA (Renard, Crépeau, & Thibault, 1995). In this condition, the rate of hydrolysis of pectins with low DM is faster (Fraeye et al., 2007). The β-elimination reaction and acid hydrolysis may occur simultaneously when the heating is performed at a weakly acidic pH, with the maximal stability of pectins being observed at circa pH 3.5. As the pH increases, the reaction rates of β-elimination increase whereas they decrease for the acid hydrolysis (Fraeye et al., 2007; Smidsrød et al., 1966). Therefore, in order to avoid confounding factors like action of endogenous enzymes at the beginning of heating, tissue structure and natural pH without control, three pH values (2.0, 3.5 and 6.0) were chosen to explore how the balance between β-elimination and hydrolysis affects pectins within the multi-scale structure of purified cell walls.

Two fruits (apple and kiwifruit) and one vegetable (beet) were chosen because the structure and composition of their cell walls are different (Latorre, de Escalada Plá, Rojas, & Gerschenson, 2013; Le Bourvellec et al., 2011; Redgwell, Melton, & Brasch, 1988), as well as their responses to processing and their internal pH values. Since there is little information about the modification of the kiwifruit cell wall, we

92 separately compared the phenomenon at two maturity stages. To schematize, apple
93 cell walls are rich in highly methylated pectins, beet cell walls are rich in arabinans
94 and pectins of intermediate DM, while kiwifruit cell walls have few pectins with very
95 few side-chains. Apple rapidly loses its texture upon heating (less than 30 min, (Kebe,
96 Renard, El Maâtaoui, Amani, & Maingonnat, 2015)), while beet fails to soften
97 completely after several hours, which may be due to the ferulic acid cross-linking of
98 pectins (Waldron, Ng, Parker, & Parr, 1997). The texture loss of kiwifruit is also
99 limited but with different responses to processing according to ripe and overripe
100 stages, and show extensive swelling after ripening (Redgwell et al., 1997).

101 Therefore, our aim was to identify whether common chemical mechanisms
102 (β-elimination and acid hydrolysis) or specific cell wall composition and structure had
103 a higher impact on the cell wall susceptibility to degradation. The originality of this
104 study is that purified cell walls from apple, beet and kiwifruit, were first isolated then
105 subjected to boiling (20 min) at pH 2.0, 3.5 and 6.0, allowing an independent
106 variation of cell wall structure and pH. The surface topography of the cell walls was
107 visualized using scanning electron microscopy (SEM). Native and modified cell walls
108 as well as extracted solubilized polysaccharide compositions were analyzed. The
109 extent of degradation of solubilized polysaccharides was studied using
110 HPSEC-MALLS. This work provides some implications for the fruit and vegetable
111 processing, such as the fine-tuning of pH conditions that may improve the quality of
112 thermally processed plant-based products.

113 **2 Materials and methods**

2.1 Standards and Chemicals

Ethanol and acetone were from Fisher Scientific (Strasbourg, France). Acetonitrile of HPLC grade was obtained from VWR International (Radnor, USA). Hexane, methanol, hydrochloric acid and acetic acid were from Merck (Darmstadt, Germany). Sugar standards (arabinose, fucose, galactose, xylose, mannose and rhamnose) and polygalacturonic acid were from Fluka (Buchs, Switzerland). Formic acid, benzyl mercaptan, sodium carbonate, sodium hydroxide, $NaBH_4$, N-methylimidazole, acetic anhydride, lignin alkali, toluene-α-thiol, (+)-catechin, (-)-epicatechin, inositol and galacturonic acid were provided from Sigma-Aldrich (Saint Quentin Fallavier, France). Methanol-d_3 was from Acros Organics (Geel, Belgium).

2.2 Plant Material

Apple fruits (*Malus* × *domestica* Borkh.) from the 'Golden Delicious' cultivar, beets (*Beta vulgaris* L.) from the round red beetroot type, and kiwifruits (*Actinidia deliciosa)* from the 'Hayward Green' cultivar were purchased at commercial maturity in September 2017 at the supermarket (Auchan, Avignon, France). For kiwifruits, two maturity stages were studied: "Ripe" corresponding to kiwifruits at the date of purchase and "Overripe" (described as "soft under the fingers") obtained after three weeks at 10 °C followed by one week at room temperature (26 °C). To quantify this evolution, the kiwifruit flesh firmness was measured using a multi-purpose TAplus Texture Analyser (Ametek, Lloyd Instruments Ltd., Fareham, UK). Kiwifruits were

peeled in a 10 × 10 mm area on the upside and downside, placed on a stationary steel plate and penetrated to depth of 50 mm with a 12.57 mm^2 probe at a speed of 5 mm/s. A batch of five fruits was constituted for each maturity stage. Two determinations were conducted on the two opposite sides of each fruit. Clear texture differences were obtained with respectively 1.4 ± 0.41 N (eating-ripe, soft) and 0.6 ± 0.31 N (overripe, very soft). All plant materials were peeled, apples were cored, kiwifruits were cored and deseeded, and cortex tissues were used for the preparation of cell wall materials.

2.3 Preparation of cell wall

Alcohol-insoluble solids (AIS) were prepared from apple (pH 3.8), beet (pH 5.0) and kiwifruit (pH 3.4 for ripe and 3.3 for overripe) according to the method of Renard (2005). More than 10 batches of 0.5 to 1.0 kg were processed for each plant material, then mixed to obtain an uniform sample. The samples were named as apple native cell wall (ACN), beet native cell wall (BCN), ripe kiwifruit native cell wall (KCRN) and overripe kiwifruit native cell wall (KCON), respectively.

2.4 Modification of cell walls by boiling at different pH values

The cell walls (25 g/L) were separately incubated in a citrate/phosphate buffer (pH 2.0, 3.5, and 6.0; 0.1 M) in sealed glass bottles in a boiling water bath for 20 min. After treatments, the cell walls and the buffer solution were separated by hot filtration under vacuum in a G0 sintered glass filter. The cell walls were dried by solvent

exchange using 96% ethanol (three times) and acetone (three times), then overnight in an oven at 40 °C.

The cell wall samples after boiling at pH 2.0, 3.5, and 6.0 are designated (AC, BC, KCR or KCO) - 2, (AC, BC, KCR or KCO) -3, and (AC, BC, KCR or KCO) - 6, respectively.

The buffer solutions containing solubilized polysaccharides (SP) were dialyzed (a dialysis tube of theoretical porosity of 12 kDa, Sigma Chemical Co., St. Louis, MO, USA) against 0.1 M NaCl during 48 h, and then against water before freeze-dring. SP at pH 2.0, 3.5, and 6.0 are designated (AP, BP, KPR or KPO) - 2, (AP, BP, KPR or KPO) -3, and (AP, BP, KPR or KPO) - 6, respectively (Fig. 1).

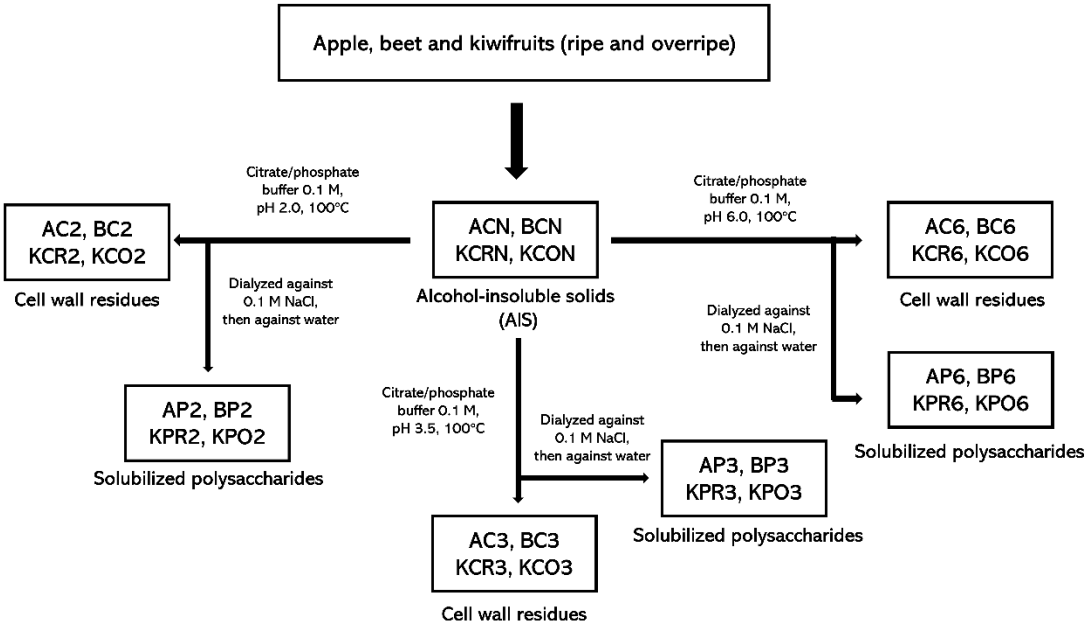

Figure 1. Scheme of AISs preparation, cell walls modification and solubilized polysaccharides extraction at three pHs from apple, beet and kiwifruit. Abbreviations: A, Apple; B, Beet; K, Kiwifruit; C, cell walls; P, solubilized polysaccharides; 2, pH 2.0; 3, pH 3.5; 6, pH 6.0; R, Ripe; O, Overripe.

2.5 Surface morphology analysis by scanning electron microscopy (SEM)

Samples were mounted on SEM specimen stubs with a carbon double-sided, carbon-conductive, adhesive tape prior to coating. Samples were then coated with 20 nm gold layers by ion sputtering using Balzers SCD 004 sputter coater (Balzers, Bal Tec.AG, Furstentum, Lichtenstein) and subsequently photographed using a PhilipsXL30 (FEI/Philips, Eindhoven, The Netherlands) scanning electron microscope operated at an accelerating voltage of 10 kV.

2.6 ATR-FTIR spectra

The spectral data of cell walls and solubilized polysaccharides were both acquired with a Tensor 27 FTIR spectrometer (Bruker Optics®, Wissembourg, France). Native cell walls and modified cell walls were stored in P_2O_5 atmosphere before analysis to remove residual water. ATR-FTIR spectra were acquired at room temperature on sample powder using a single-reflectance horizontal ATR cell (Golden Gate equipped with a diamond crystal, Bruker Optics) as described by Bureau et al. (2012), by scanning from 4000 to 600 cm^{-1} and correcting against the background spectrum of air. Each sample was analyzed three times, and each spectrum was an average of 16 scans. Spectral pre-processing and data treatment using multivariate analyses were performed with Matlab 7.5 (Mathworks Inc. Natick, MA) software using SAISIR package (Cordella & Bertrand, 2014). The spectral data were transformed with baseline correction and standard normal variate (SNV) to correct multiplicative interferences and variations in the baseline shift before any multivariate analysis. A Principal Component Analysis (PCA) was applied using FT-IR spectra in the range between 2000 and 600 cm^{-1} to evaluate the distribution of the different samples

192 (species and pH treatments).

193 **2.7 High-Performance Size-Exclusion Chromatography Coupled with**
194 **Multi-Angle Laser Light Scattering (HPSEC-MALLS)**

195 The molar mass distribution of SP was determined using a HPSEC system
196 involving a Ultra Fast Liquid Chromatography Prominence system (Shimadzu, Kyoto,
197 Japan) including a LC-2OAD pump, a DGU-20A5 degasser, a SIL-20ACHT
198 autosampler, a CTO-20 AC column oven, a SPD-M20A diode array detector and a
199 RID-10A refractive index detector. In addition, a multi-angle laser light scattering
200 (MALLS) detector DAWN HELEOS 8+ (fitted with a K5 flow cell and a GaAs laser
201 at λ = 660 nm) from Wyatt Technology Corporation (Santa Barbara, CA) was coupled
202 with the HPSEC system. Separations were achieved at 40 °C using three HPSEC
203 columns in series (PolySep-GFC-P3000, P5000 and P6000 300 ×7.8 mm) and a guard
204 column from Phenomenex (Torrence, CA, USA). 100 µL of SP (2.5 mg/mL) solutions
205 were injected and eluted at 0.6 mL/min using a 0.1 M citrate/phosphate buffer at pH
206 3.8, previously filtered through a 0.1 µm, Omnipore^{TM} membrane (Millipore, Milford,
207 USA) and degassed. Before injection, SP were solubilized under magnetic stirring in
208 the filtered eluant at 4°C overnight. The solutions were centrifuged 10 min at 8000 g
209 and the supernatant was filtered through a 0.45 µm hydrophilic PTFE syringe filter
210 (Macherey-Nagel, Düren, Germany) before injection. M_i, the molar mass at each slice
211 of the chromatogram, was determined using the concentration and the light scattering
212 signal from 5 angles (from 20.4° to 90°) and data extrapolation to zero angle using
213 the Zimm formalism with a one order polynomial fit (Rolland-Sabaté, Colonna,

Potocki-Véronèse, Monsan, & Planchot, 2004). Then, the weight-average ($\overline{M}_w$) was calculated using the summations taken over the whole peaks using ASTRA® software from Wyatt Technology Corporation (version 7.1.4 for PC) as previously described (Rolland-Sabaté et al., 2004). A value of 0.146 mL/g was used as the refractive index increment (dn/dc) for glucans and the normalization of photodiodes was achieved using a low molar mass pullulan standard (P20) from Showa Denko K.K. (Tokyo, Japan).

2.8 Chemical composition analysis

2.8.1 Hydrolysis

For cell walls, approximately 10 mg of AIS were prehydrolyzed with 72% sulfuric acid (250 µL) for 1 h at 26 °C (Saeman, Moore, Mitchell, & Millett, 1954), before dilution to 1 M sulfuric acid by the addition of water and an internal standard (inositol); simple hydrolysis was also carried out to estimate cellulose content by difference. For SP, the sample was directly dissolved in 1 M sulfuric acid with internal standard without prehydrolysis. All samples were hydolysed at 100 ° C for 3 hours.

2.8.2 Neutral sugar composition

Neutral sugars were analyzed as alditol acetates (Canteri, Renard, Le Bourvellec, & Bureau, 2019; Englyst, Wiggins, & Cummings, 1982). The samples were injected in a GC-FID HP 5890 Series II (Agilent, Inc., Palo Alto, USA) equipped with a capillary column (30 m × 0.25 mm i.d. coated with DB225 MS, 0.25 µm film thickness) using: split mode (1:25 ratio); injector temperature 250 °C; hydrogen as

carrier gas at 45 cm/s (215 °C); column flow 1.3 mL/min and oven temperature 215 °C (isothermal).

2.8.3 Galacturonic acid content

Uronic acids were measured by the meta-hydroxyl-diphenyl assay as described by (Blumenkrantz & Asboe-Hansen, 1973; Canteri et al., 2019) after Saeman hydrolysis (§ 2.8.1). The absorbance was read at 520 nm using a spectrophotometer (V-530 Jasco, Tokyo, Japan), and concentrations were calculated against a calibration curve with galacturonic acid as external standard.

2.8.4 Methanol and degree of methylation

The methanol content was measured by stable isotope dilution assay using headspace-GC-MS (QP2010 Shimadzu, Kyoto, Japan) as described by Renard & Ginies (2009) after saponification. The degree of methylation (DM) was calculated as molar ratio of methanol to galacturonic acid.

2.8.5 Lignin content

Lignin was measured spectrophotometrically (V-530Jasco, Tokyo, Japan) as described by Metaxas, Syros, Yupsanis, & Economou (2004) with alkali lignin as external standard. Cell wall samples (15 mg) were digested in 1 mL 25 % acetyl bromide in acetic acid containing 2.7% (v/v) perchloric acid and incubated for 30 minutes at 70 °C. The samples were cooled and 10 µL were transferred into a test tube, then 570 µl of 17 % 2N NaOH and 83 % acetic acid were added, followed by 20 µL of 7.5 M hydroxylamine hydrochloride to stop the reaction. The volume was corrected

to 2 mL with acetic acid and the absorbance was read at 280 nm using a spectrophotometer V-530 (Jasco, Tokyo, Japan). The amount of lignin was calculated from a linear calibration curve with commercial alkali lignin as standard.

2.8.6 Procyanidin content

Procyanidins were measured by HPLC-DAD after thioacidolysis as described by Guyot, Marnet, & Drilleau (2001). Their characterization and quantification were performed using an Ultra Fast Liquid Chromatography Prominence system (Shimadzu, Kyoto, Japan) controlled by the LabSolutions software (Version 5.57, Shimadzu, Kyoto, Japan). Separation conditions, identification and quantification were performed as described (Guyot et al., 2001; Le Bourvellec et al., 2011).

2.8.7 Acetic acid content

Acetic acid was released by saponification and quantified according to the acetic acid assay kit (K-ACET, ACS Manual Format, Megazyme International, Ireland). Samples (10 mg) were incubated in 5 ml NaOH 0.2 M for 3 h. 2 ml deionized water, 0.1 ml sample, 0.5 ml buffer (30mL, pH 8.4) plus L-malic acid and sodium azide (0.02 % w/v), and 0.2 ml NAD+/ATP/PVP/CoA solution were added into cuvettes and mixed. The absorption was read at 340 nm (A0) after approx. 3 min using a spectrophotometer V-530 (Jasco, Tokyo, Japan). Then the reaction was started by addition of 20 µl L-malate dehydrogenase plus citrate synthase suspension. The absorption was read at 340 nm (A1) after approx. 4 min and the reaction was started again by addition of 20 µl acetyl-coenzyme a synthetase suspension. The reaction

misture was mixed and the absorbance of the solutions (A2) at the end of the reaction (approx. 12 min) was read at 340 nm. The acetic acid content was calculated based on an acetic acid standard curve using *Mega-Calc*™ software tool (Megazyme International, Ireland).

2.8.8 Ferulic acid content

Ferulic acid was released by saponification according to the method of Micard, Renard, & Thibault (1994). Samples (25 mg) were incubated in 25 mL of 2 M NaOH at room temperature for 1 h under a stream of argon followed by two hours at 35 °C. The extracts were filtered on G1 sintered glass filters, adjusted to pH 2.0 by fuming hydrochloric acid and extracted by ethyl acetate (3 times). The upper organic phases were recovered, dried over anhydrous Na_2SO_4, filtered and evaporated. The dry extracts were taken up in 1 mL of methanol and 20 µL were injected into the HPLC-DAD system (§2.8.5) with detection at 320 nm.

2.9 Statistical Analysis

Results are presented as mean values of analytical triplicates and the reproducibility of the results is expressed as pooled standard deviations (Pooled SD). Pooled SD were calculated for each series of replicates using the sum of individual variances weighted by the individual degrees of freedom (Box, Hunter, & Hunter, 1978). Analysis of variance (ANOVA) was performed using Excelstat package of Microsoft Excel. Spectral pre-processing and Principal Component Analysis (PCA) were performed using MATLAB 7.5 (Mathworks Inc. Natick, MA) software using the

298 SAISIR package (Cordella & Bertrand, 2014).

299 **3 Results and discussion**

300 **3.1 Surface morphology of cell walls**

301 Apple, beet and kiwifruit cell wall preparations had thin cell walls and showed a
302 network of empty cells of irregular shape and size (Fig. 2). The kiwifruit cell wall
303 preparations exhibited the smallest cavities, non-porous and compact, with a wrinkled
304 appearance comparable to tree cuticles or the surface of mountain heavily eroded by
305 wind and sand. These preparations contained sieve tubes in xylem and phloem tissues
306 and thin rods, probably raphide crystals of calcium oxalate, as described previously in
307 kiwifruit (Redgwell et al., 1988). The surface of the apple cell walls was the flattest
308 and had large empty spaces, followed by beet cell walls. The tissular structure of beets
309 was similar to a honeycomb, with thin interconnected walls, matching earlier studies
310 (Dongowski, 2001).

311 All treatments, whichever the pH and cell wall, seemed to have similar effects on
312 the surface morphology of cell walls, i.e., all cell walls became more wrinkled and
313 crumpled. The appearance of cell walls treated at pH 3.5 was the most similar to those
314 of the initial cell walls. In apple and beet cell walls treated at pH 6.0, the cell walls
315 became less smooth and some regular patterns were faintly visible, so that a
316 substructure seemed to emerge, probably linked to the organization of the cellulose
317 microfibrils (McCann, Wells, & Roberts, 1990), but the treatment was not sufficient to
318 clearly reveal it. Rougher surfaces were obtained for kiwifruit cell walls, notably after

overripening. Another remarkable point was the persistence of calcium oxalate crystals in the kiwifruit cell wall preparations after treatment at pH 3.5. Heating and drying can modify the physical properties of the cell wall material by inducing collapse of β-glucans and pectins (Le Bourvellec & Renard, 2005). However, in the present case, all cell walls were subjected to hydrothermal treatment and gentle drying. Therefore, less modifications were observed, compared with apple cell walls dried in harsh conditions (Le Bourvellec & Renard, 2005).

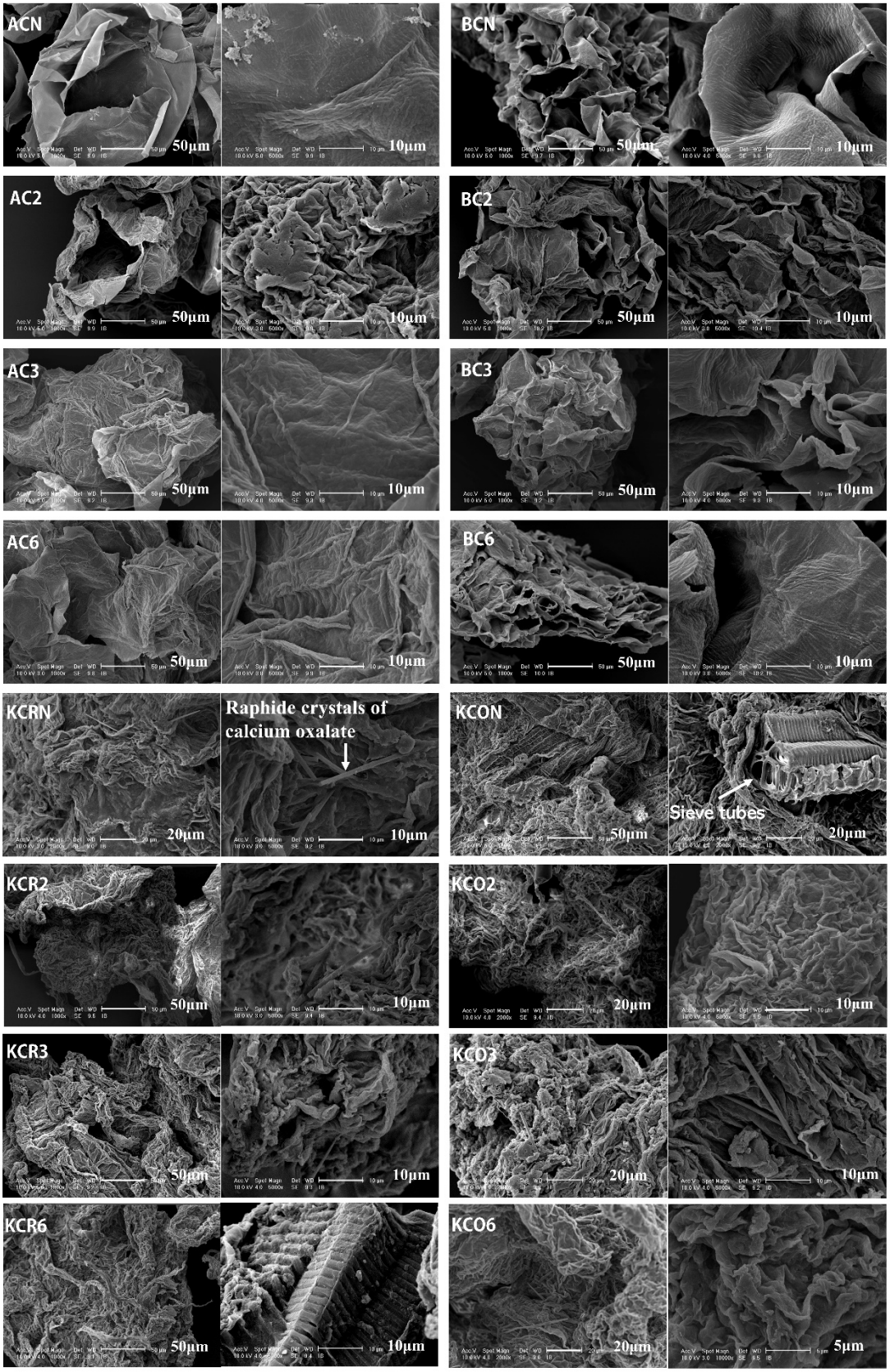

Figure 2. Scanning Electron Microscopy showing the differences among native and three pH treatments (2.0, 3.5 and 6.0) after boiling for 20 min in apple, beet and kiwifruit cell walls. Abbreviations: A, Apple; B, Beet; K, Kiwifruit; C, cell walls; P, solubilized polysaccharides; 2, pH

2.0; 3, pH 3.5; 6, pH 6.0; R, Ripe; O, Overripe.

Table 1. Yields (mg/g fresh weight), neutral sugars, galacturonic acid, lignin and ferulic acid compositions (mg/g cell walls) and ANOVA results of the different fruit flesh cell walls before and after modifications by boiling at different pH values.

Sample	pH	Maturity	Yields[b]	Rha	Fuc	Ara	Xyl	Man	Gal	CGlc	NCGlc	Gal A	Ac. A	MeOH	DM (%)	Lig	FA
ACN	Native[a]	Ripe	14.3	14	16	66	65	16	65	286	13	206	21	31	82	26	-
AC2	2.0	Ripe	-	12	14	27	75	19	65	347	5	191	19	30	86	26	-
AC3	3.5	Ripe	-	13	15	57	67	16	63	302	15	140	19	24	89	20	-
AC6	6.0	Ripe	-	12	16	66	74	18	67	332	16	120	18	13	60	18	-
BCN	Native	Ripe	31.4	15	7	178	10	17	52	220	2	225	45	27	65	46	7.0
BC2	2.0	Ripe	-	14	5	111	13	20	57	292	9	205	41	21	58	44	7.7
BC3	3.5	Ripe	-	15	4	174	11	17	54	255	6	153	39	20	72	47	9.6
BC6	6.0	Ripe	-	13	4	189	11	18	54	262	6	141	32	11	42	45	8.8
KCRN	Native	Ripe	13.3	6	5	10	47	23	51	241	21	210	11	26	67	49	-
KCR2	2.0	Ripe	-	4	5	7	63	30	41	334	18	133	12	15	62	55	-
KCR3	3.5	Ripe	-	4	5	9	60	25	43	279	26	98	11	12	70	72	-
KCR6	6.0	Ripe	-	3	4	7	62	29	38	329	24	58	11	7	69	56	-
KCON	Native	Overripe	13.6	4	7	8	52	22	25	223	16	173	11	22	72	26	-
KCO2	2.0	Overripe	-	2	4	5	66	31	24	335	11	73	13	9	58	46	-
KCO3	3.5	Overripe	-	2	4	6	62	30	22	316	17	74	11	9	65	49	-
KCO6	6.0	Overripe	-	2	4	5	60	30	21	332	14	53	11	5	55	37	-
		Pooled SD		0.7	0.8	2.1	2.0	0.9	1.0	13.2	1.0	8.7	0.8	0.4	4.1	3.8	0.2
ANOVA																	
CW	Species			170***	159***	4401***	898***	674***	401***	79***	241***	38***	1319***	628***	21***	91***	99***
	pH			5**	2	173***	13***	65***	6**	73***	9***	79***	26***	1072***	10***	19***	2
	Species*pH			0.1	1	52***	3**	8***	14***	3*	8***	3*	14***	43***	4*	4**	3*

CW-K	pH	40***	6**	14***	28***	16***	38***	14***	11***	148***	3	603***	2	56***	10**
	Maturity	158***	1	34***	2	3	1284***	0.2	91***	37***	0.3	136***	2	17***	37***
	pH*Maturity	6**	4*	1	1	1	16***	1	2	4*	0.1	7**	2	2	1

Fisher's Value F value, P≤0.05: *, P≤0.01: **, P≤0.001: ***. Pooled SD: pooled standard deviation (degree of freedom: 32 except for ferulic acid, DF = 8). a: Natural pH values, Apple (pH 3.8); beet (pH 5.0); kiwifruit (pH 3.4 for ripe and 3.3 for overripe). b: Yields (mg/g fresh weight). Rha: rhamnose, Fuc: fucose, Ara: arabinose, Xyl: xylose, Man: mannose, Gal: galactose, Glc: glucose, Gal A: galacturonic acid, CGlc: glucose from cellulose, NCGlc: glucose from non-cellulose, Ac.A: acetic acid, MeOH: methanol, DM: degree of methylation, FA: ferulic Acid, Lig: lignin. CW: Apple, beet, kiwifruit cell walls at ripe stage. CW-K: Kiwifruit cell walls at ripe and overripe stages. Abbreviations: A, Apple; B, Beet; K, Kiwifruit; C, cell walls; 2, pH 2.0; 3, pH 3.5; 6, pH 6.0; R, Ripe; O, Overripe.

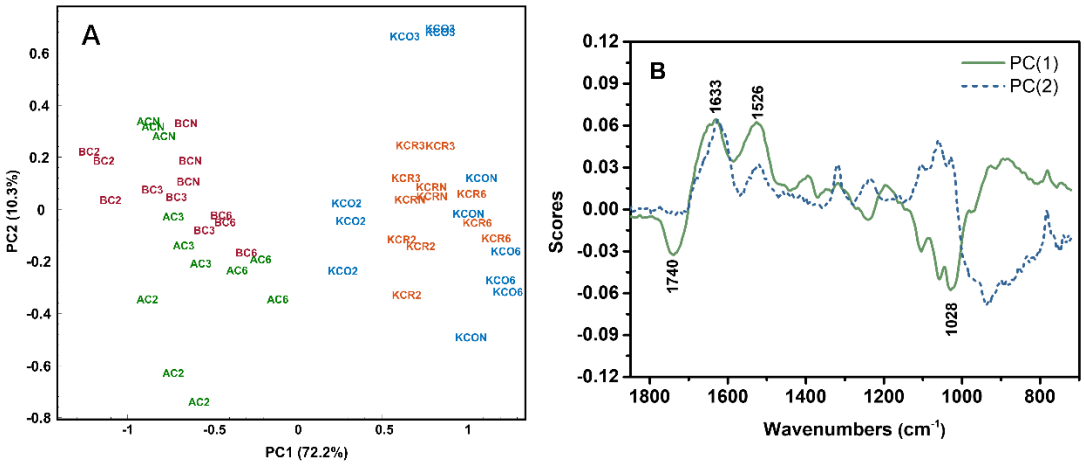

Figure 3. Principal component analysis (PCA) of (A) Apple, beet and kiwifruit cell walls using mid-infrared spectral data between 2000 to 600 cm^{-1} and (B) score loadings of PC1 and PC2. Abbreviations: A, Apple; B, Beet; K, Kiwifruit; C, cell walls; 2, pH 2.0; 3, pH 3.5; 6, pH 6.0; R, Ripe; O, Overripe.

3.2 Characterization of native and modified cell walls after boiling at three different pHs

3.2.1 Global characterization by FTIR spectra

A principal component analysis (PCA) was carried out using the spectral data (Fig. 3) to highlight the distribution of all AIS samples. The first two axes (PC1 and PC2) explained more than 82% of the total variance and allowed the discrimination of the three species, apple, beet and kiwifruit (Fig. 3A). Since the composition of apple and beet cell walls are very different (Latorre et al., 2013; Le Bourvellec et al., 2011), one would have expected the samples according to their spectra to be separated on the PC1 x PC2 plane. However, this was not the case, indicating that the complex internal cross-linking type in cell walls may also influence the spectral data and not only chemical composition *per se*. Concerning the treatments (at pH 2.0, 3.5 and 6.0), the samples were also separated in four groups (one for each treatment plus the non treated cell walls) for each species (Fig. 3A). PC1 discriminated cell walls at pH 2.0

358 on the left and pH 6.0 on the right in each species group, highlighting a common
359 impact. Samples treated at pH 3.5 were close to the native cell walls, at least along
360 PC1. The study of eigenvectors (Fig 3B) and especially of PC1 showed two negative
361 peaks at 1740 and 1028 cm^{-1} which could be due to galacturonic acid and its ester
362 carbonyl group absorptions whereas the positive peak at 1633 cm^{-1} could be due to its
363 free carbonyl group (Gnanasambandam, Proctor, 2000; Kohn, 1975;
364 Szymanska-Chargot & Zdunek, 2013). The positive peak at 1526 cm^{-1}, characteristic
365 of kiwifruit cell walls, might be due to lignin (Huck, 2015). PC1 thus differentiated
366 apple and beet cell walls (pectin-rich) from kiwifruit cell walls (pectin-poor), and
367 within each species group common mechanisms, probably also linked to the pectic
368 backbone.

369 PC2 only expressed 10% of the variability and presented bands linked to
370 galacturonic acid and neutral sugars, but sample discrimination was more variable per
371 species. Two kinds of samples stood out along PC2, namely apple cell walls treated at
372 pH 2.0 (lower) and kiwifruit cell walls treated at pH 3.5 (higher than the initial cell
373 walls). For apple and beet cell walls, all the treatments resulted in a shift in the same
374 direction along PC2, but not for the kiwifruit cell walls. This PC could thus be more
375 related to specific effects.

376 **3.2.2 Native cell wall yields**

377 The cell walls of the apples, beets and kiwifruits were pure white, reddish gray
378 and light grayish white, respectively. The beet cell wall yields (Table 1) were twice

379 those of apple and kiwifruit. The cell wall yields were comparable to, though
380 generally slightly lower than, those reported earlier: for apple 17.2 to 27 mg/g (FW,
381 fresh weight) (Le Bourvellec et al. (2011), 12 cultivars); for beet 30.4 mg/g
382 (Dongowski (2001) and 36 mg/g (FW) (Latorre et al. (2013), after 10 min boiling);
383 for kiwifruit 32.1 mg/g FW with whole, unpeeled kiwifruits (Sauvageau, Hinkley,
384 Carnachan, & Sims, 2010), and 16 mg/g FW with outer parenchyma (Fischer,
385 Wegryzn, Hallett, & Redgwell, 1996).

386 **3.2.3 Compositions of native and modified cell walls**

387 Native cell wall compositions were in agreement with literature data (Le
388 Bourvellec et al., 2011; Redgwell et al., 1988; Renard & Thibault, 1993). Concerning
389 polysaccharides, the cell walls of all three species were rich in glucose and
390 galacturonic acid (Table 1). Glucose was mainly derived from cellulose. Contents of
391 mannose and galactose were very similar in all cell walls, though differences were
392 still statistically significant. Rhamnose and fucose were minor components (5-16
393 mg/g CW).

394 One marked difference between the cell walls was the arabinose content,
395 particularly high in the cell walls from beet, while it was very low in the cell walls of
396 kiwifruit (10 mg/g CW). The pectic substances of apple were more highly methylated
397 (DM 82) than those of beet or kiwifruit, while acetyl groups were higher in beet,
398 giving apparent degrees of acetylation (for pectins) > 100, and higher than reported in
399 beet varieties used for sugar productions (DAc from 66 to 69 %) (Renard & Jarvis,

1999; Renard & Thibault, 1993). It should be mentioned that most cell wall polymers can be acetylated, especially hemicelluloses and pectins (Gille & Pauly, 2012), and that reporting acetylation relative only to galacturonic acid might lead to overestimated degree of acetylation.

Cell walls can contain polyphenols or be contaminated by intracellular polyphenols, and these patterns were different per species. All three native cell walls contained some low amounts of lignin, with higher concentrations in the kiwifruit cell wall. These were the only phenolic compounds detected in the kiwifruit cell wall. Beet cell walls contained ester-bound phenolic acids, primarily ferulic acid (7.0 mg/g CW) lower than reported earlier (13-20 mg/g CW) (Renard & Jarvis, 1999; Renard & Thibault, 1993). Ferulic acid is an intrinsic component of beet cell walls, where it is bound to arabinans and galactan side chains of pectins (Fry, 1986). The native apple cell wall was slightly contaminated by vacuolar procyanidins (0.26 mg/g CW) of high $\overline{DP}_{n}$ (15). These procyanidins became non detectable in the modified apple cell walls perhaps due to their removal with soluble polysaccharides as procyanidins have a high affinity, among polysaccharides, for pectins (Le Bourvellec, Bouchet, & Renard, 2005). The AIS preparation used here includes a specific step for elimination of this artefact, the efficiency of which was validated.

What about their common and specific responses after modification? For all cell walls, all the treatments led to a marked loss of arabinose and galacturonic acid, and by balance a relative increase in xylose, mannose and cellulose (Table. 1). Whichever

421 the treatment and the species, xylose and mannose from hemicelluloses and glucose
422 from cellulose were not affected by acid hydrolysis nor β-elimination reactions, so
423 that their contents increased. The rhamnose and galactose contents were stable, so that
424 their losses appeared to be close to the average cell wall loss. Intensity of loss of the
425 different sugars appeared to depend primarily on pH, then on species. The least
426 pronounced pectin depolymerization was observed at pH 3.5, that is, the neutral sugar,
427 galacturonic acid, lignin contents were intermediate between values obtained in pH
428 2.0 and 6.0. This is a common response for all cell walls. It suggested that the
429 β-elimination reaction and acid hydrolysis occur simultaneously at pH 3.5, but both
430 had a low intensity. This is consistent with Ella Missang et al. (2012) who reported the
431 composition of the cell wall material from cooked apricot at natural pH (c.a. 4.0) for
432 10 min did not change significantly, compared with the cell wall isolated from fresh
433 apricot fruit. The same result was also observed in the pear cell walls (cooked at 85 °
434 C for 20 min) (Renard, 2005). Therefore, thermal processing at this pH can protect
435 fruits and vegetables by reducing the pectin degradation reaction rates by both
436 β-elimination and hydrolysis.

437 At pH 2.0, the most marked difference concerned arabinose, while the
438 galacturonic acid was mostly retained, in accordance to the order of sensitivity to
439 hydrolysis of glycosidic linkages. Arabinose is in the furanose form in the cell walls,
440 and bonds involving furanose sugars are particularly susceptible to acid hydrolysis
441 (Fry, 1988). Moreover, significant specific responses were observed in the degree of
442 arabinose loss in different cell walls at this pH. The percentages of arabinose loss for

AC2, BC2 and KCR2 were 59 %, 38 % and 30 %, respectively. Therefore, the arabinose in the apple cell walls was the most sensitive to acid hydrolysis. At this pH, the content of ferulic acid in the beet cell walls was also relatively low, which is attributed to the degradation of arabinan side chains. Ferulic acid can participate in intra-/inter- molecular crosslinking with arabinan chains (Morris & Ralet, 2012; Wefers, Gmeiner, Tyl, & Bunzel, 2015) and limit beet texture loss (Waldron et al., 1997). Therefore a more acidic pH, by enhancing arabinan degradation and loss of ferulic cross-links, might favour softening of beets. Li, Liu, Tu, Li, & Yan (2019) also confirmed that the addition of ferulic acid led to better preservation of the cell wall structure of cooked radish. This may due to ferulic acid reaction with neutral sugar side chains, preventing the leaching of pectins from the cell wall matrix. Compared with the other two pH treatments and untreated cell walls, pH 2.0 processing increased the pectin linearity (GalA/Rha molar ratio) and decreased the RG-I branching ((Ara+Gal)/Rha molar ratio) in all cell walls.

With regard to treatment at pH 6.0, the galacturonic acid content decreased significantly after treatment in all cell walls, especially the kiwifruit cell walls, due to β-elimination. The percentage of galacturonic acid loss for KCR6, AC6 and BC6 was 72 %, 42 % and 37 % in order. The galactose in AC6 and BC6 was not significantly different from the untreated cell walls, while KCR6 lost 25 %. The common response under this condition is that the modified cell walls had the lowest pectin linearity and the highest RG-I content. The cell walls also had significantly lower methanol and slightly lower lignin contents. The acetyl groups of the cell walls were more stable in

apple and kiwifruit cell walls than beet cell walls, and also more stable than methyl-esters during the processing (Broxterman, Picouet, & Schols, 2017), probably because some O-acetyl-substituents were carried by other cell wall constituents (Gille & Pauly, 2012). Lower acetic acid content of beet cell walls after treatment at pH 6.0 may be due to the initial presence of acetylated pectins and loss during treatment. Acid hydrolysis and β-elimination appeared to be common mechanisms that cause loss of neutral sugars, often from pectin side chains, and galacturonic acid, respectively, but their effects were of different intensities.

This was confirmed by the ANOVA (Table 1): while the species was the most significant factor, pH values and ripeness (for kiwifruit) had also significant effects. Only fucose was not significantly modified by treatment at different pHs, while the effect on galactose and rhamnose was less marked than for all other parameters. The interaction 'species × pH' influenced significantly acetic acid, methanol and neutral sugars such as arabinose, mannose, galactose and non-cellulosic glucose. This confirmed the existence of common effects on galacturonic acid (and absence of effect on cellulose) and more specific effects on pectic substituents and some neutral sugars.

3.2.4 Comparison of kiwifruit cell walls at two maturity stage

After harvest, the kiwifruit underwent a marked softening. As is shown in Table 1, the neutral sugars, galacturonic acid, acetic acid, methanol and lignin contents were reduced from KCRs to KCOs, and the most relevant differences with maturity

concerned rhamnose, arabinose, galactose, glucose and galacturonic acid in accordance with literature data on fruit softening: ripening involves hydrolysis of neutral sugars from pectin side chains, depolymerization and increased solubilization of pectins and hemicelluloses (Brummell, 2006). The degrees of methylation and acetylation of pectins increased, signaling potential preferential degradation of the less methylated and acetylated pectins during overripening. The cell walls of ripe kiwifruit were more sensitive to the treatment at different pHs than that of overripe as the main modifications had occurred during ripening. The interaction 'pH × maturity' influenced significantly galactose.

Table 2. Yields (mg/g cell wall), neutral sugars, galacturonic acid and ferulic acid compositions (mg/g solubilized polysaccharide), macromolecular characteristics and ANOVA results of solubilized polysaccharides from the different cell walls before and after modifications by boiling at different pHs.

Sample	pH	Maturity	Yields	Rha	Fuc	Ara	Xyl	Man	Gal	NCGlc	Gal A	Ac. A	MeOH	DM (%)	DAc (%)	FA	$\bar{M}_w$ ($\times10^3$ g·mol^{-1})	M_p ($\times10^3$ g·mol^{-1})
AP2	2.0	Ripe	9.6	13	4	89	26	3	55	18	456	17	71	86	17	-	431	541
AP3	3.5	Ripe	16.1	10	6	63	27	3	40	13	419	14	59	77	15	-	149	82
AP6	6.0	Ripe	16.5	14	4	56	16	2	35	10	452	15	78	95	16	-	217	32
BP2	2.0	Ripe	17.3	6	1	187	2	3	27	3	325	47	49	83	67	8.1	147	120
BP3	3.5	Ripe	9.9	6	2	96	1	4	19	4	402	64	66	90	73	4.3	117	112
BP6	6.0	Ripe	15.4	9	3	54	1	4	19	3	491	71	73	81	67	2.2	65	38
KPR2	2.0	Ripe	13.9	7	2	13	10	3	73	51	473	5	65	73	5	-	287	88
KPR3	3.5	Ripe	11.2	8	3	16	6	3	67	19	616	4	89	75	3	-	285	74
KPR6	6.0	Ripe	22.1	11	2	16	6	3	75	20	449	6	67	79	6	-	161	37
KPO2	2.0	Overripe	14.4	4	3	10	12	4	23	34	465	2	72	82	2	-	80	28
KPO3	3.5	Overripe	16.4	7	4	13	12	4	28	28	567	8	89	82	7	-	137	31
KPO6	6.0	Overripe	18.3	9	3	11	9	4	26	15	465	4	72	81	4	-	79	15
		Pooled SD	-	0.6	0.3	2.7	1.5	0.2	1.8	2.1	16.1	1.1	2.2	3.3	1.3	0.1	11.3	6.4
ANOVA																		
PC	Species			55***	45***	724***	127***	9**	441***	1229***	43***	3046***	22***	9**	1940***	4128***	394***	1076***
	pH			21***	7**	235***	7**	5*	20***	345***	15***	53***	25***	2	0.4	507***	279***	1994***
	Species*pH			2	6**	132***	4*	5**	7***	215***	30***	62***	36***	4*	4*	507***	123***	1183***
PC-K	pH			56***	56***	47***	87***	15***	4*	39***	28***	0.04	67***	0.312	0.4	-	602***	305***
	Maturity			26***	134***	239***	183***	97***	3447***	3	1	3	7*	5	2	-	90***	65***
	pH*Maturity			3	5*	3	23***	6*	21***	10**	1	6	1	1	8*	-	35***	18***

Fisher's Value F value, P≤0.05: *, P≤0.01: **, P≤0.001: ***. Pooled SD: pooled standard deviation (degree of freedom: 24). PC: Apple, beet, kiwifruit solubilized polysaccharides at ripe stage. PC-K: Kiwifruit solubilized polysaccharides at ripe and overripe stages. Species: Apple, beet and kiwifruit; pH treatments: 2.0, 3.5 and 6.0; Maturity: Ripe and overripe stages. Rha: rhamnose, Fuc: fucose, Ara: arabinose, Xyl: xylose, Man: mannose, Gal: galactose, Glc: glucose, Gal A: galacturonic acid, NCGlc: glucose from non-cellulose, Ac. A: acetic acid, DAc: degree of acetylation, MeOH: methanol, DM: degree of methylation, FA: ferulic Acid. $\bar{M}_w$: weight average molar mass; M_p: molar mass at the apex of the major peak. Abbreviations: A, Apple; B, Beet; K, Kiwifruit; P, solubilized polysaccharides; 2, pH 2.0; 3, pH 3.5; 6, pH 6.0; R, Ripe; O, Overripe.

3.3 Characterization of solubilized polysaccharides

Solubilized polysaccharides represented a minor part of the original cell walls, with recoveries between 9.6 and 22% of the original cell walls, however, they were of interest as indicators of involved mechanisms.

3.3.1 Composition of the solubilized polysaccharides

Similar to cell walls, solubilized polysaccharides (SP) from different cell walls or after treamtents at different pHs also showed both common and specific features. Galacturonic acid was the main component in all SP, which were thus clearly mainly of pectic nature, but significant differences were observed between the various pH treatments and species (Table 2). Beet solubilized polysaccharides (BPs) had the highest arabinose content with less xylose, galactose and galacturonic acid than apple and kiwifruit. They were also the richest in glucose and acetic acid. Kiwifruit solubilized polysaccharides (KPs) had the highest galactose and glucose contents, and apple solubilized polysaccharides (APs) the highest rhamnose and xylose contents. For all species, rhamnose content rose with increasing pH of the treatment. For apple and beet cell walls treated at pH 6.0 SP were relatively enriched in galacturonic acid. This could be linked to a larger amount of galacturonic acid solubilized from the cell wall at pH 6.0. In addition, for APs and BPs, more acidic pH led to more extraction of arabinose (Ara) and, for BPs, ferulic acid (FA), with a constant ratio of Ara/FA indicating that there was no specific extraction nor degradation of feruloylated moieties during extraction. In the case of kiwifruit, the highest content of galacturonic

523 acid was at pH 3.5, and there were less differences as a function of pH. The Gal
524 A/(Rha+Ara+Gal) ratio of KPs (from 3.9 to 10.9) was larger than APs (from 2.4 to 3.5)
525 and BPs (from 1.1 to 4.7) indicating KPs were less ramified. The DM showed
526 differences between the various species and pH in Table 2. For the ANOVA result of
527 apple, beet, kiwifruit solubilized polysaccharides, arabinose, galactose, glucose, acetic
528 acid and ferulic acids were the most relevant compounds for differentiating pH values
529 (Table 2). The interaction 'species × pH' influenced significantly arabinose, acetic
530 acid and methanol.

531 Less arabinose, galactose and galacturonic acid were solubilized in SP from
532 overripe kiwifruit cell walls, probably due to preliminary degradation of pectins by
533 endogenous enzymes during overripening (Table 2). The most statistically significant
534 differences that distinguished maturity were rhamnose, fucose, arabinose, xylose,
535 mannose and galactose contents. The interaction 'pH × maturity' influenced
536 significantly xylose and galactose (Table 2).

537 **3.3.2 Molar mass distribution of the solubilized polysaccharides**

538 HPSEC-MALLS chromatograms showed generally one main peak at 20-26 mL
539 with shoulders, representing high molar mass polymers, and a peak around 29-30 mL
540 which could be attributed to oligomers (Fig. 4a, b, e and f). With increasing pH value,
541 the main peaks of APs, BPs, KPRs and KPOs shifted to higher elution volumes
542 indicating a decrease in hydrodynamic volume, which was generally accompanied by
543 a decrease of the molar mass of the main fraction, M_p (Table 2).

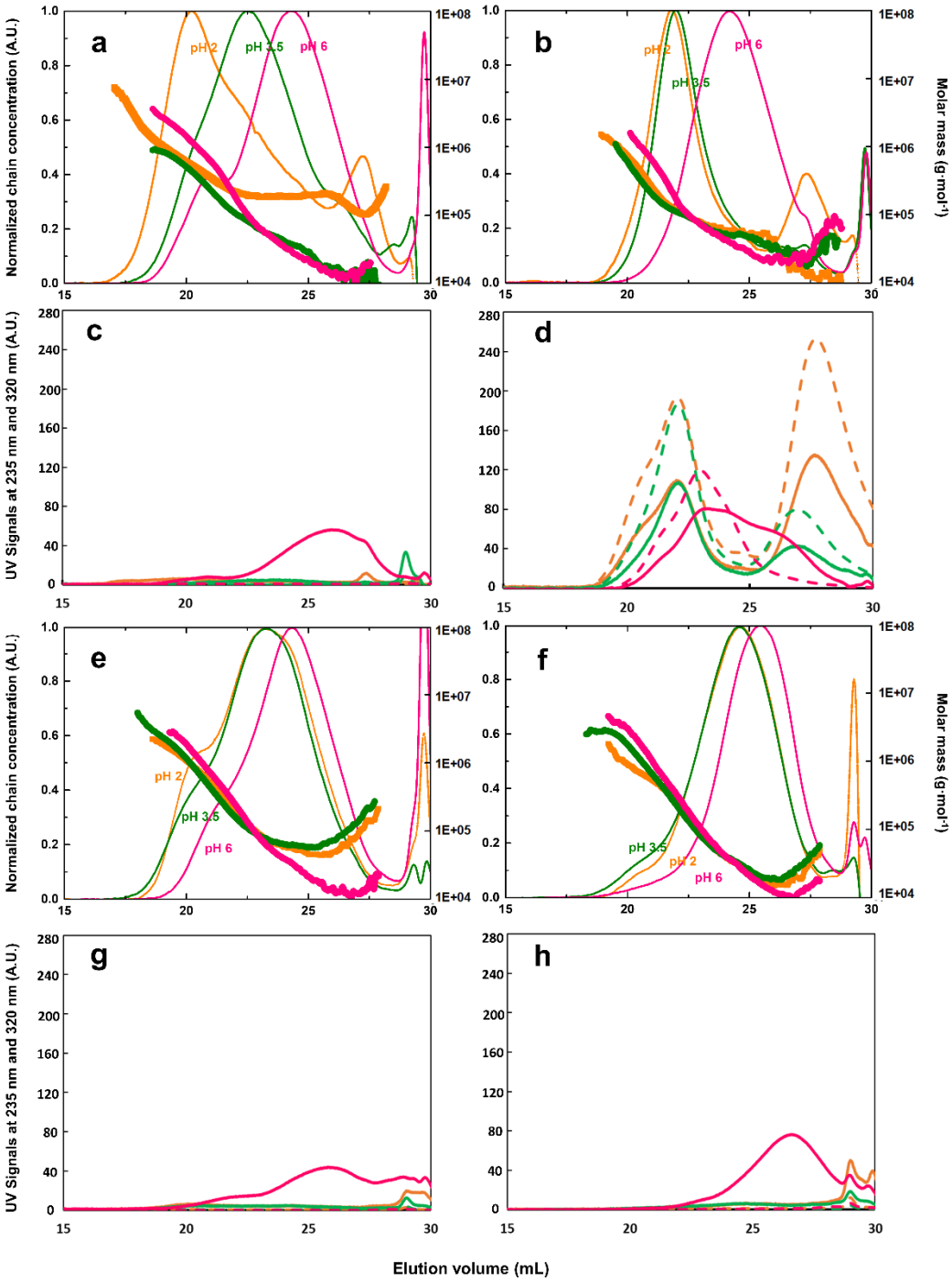

Figure 4. HPSEC-MALLS chromatograms (RID and DAD signals) and molar mass *vs* elution volume of the solubilized polysaccharides samples. a, c: Apple; b, d: Beet; e, g: Kiwifruit at ripe stage; f, h: Kiwifruit at overripe stage. a, b, e and f: pH 2.0; pH 3.5; pH 6.0 represent normalized chain concentration and pH 2.0; pH 3.5; pH 6.0 represent molar mass. c, d, g and h: pH 2.0; pH 3.5; pH 6.0 represent UV signal at 235 nm and pH 2.0; pH 3.5; pH 6.0 represent the UV signal at 320 nm.

APs had higher $\bar{M}_w$ than BPs, KPRs and KPOs especially at pH 2.0 (AP2) (Table 2). The difference of molar mass and size between AP2 and AP3 (Table 2, Fig. 4a, b, e

553 and f) was much more marked than that of the three series of three pHs for beet and
554 kiwifruit, while all pectins extracted at pH 6.0 were clearly differentiated. Apple cell
555 walls thus appeared to react differently from beet and kiwifruit upon acidic treatment,
556 which was also coherent with the analysis of their infrared spectra (Fig. 3).

557 For AP2 and BP2, there were two distinct populations of SP, one major peaking at
558 20-22 mL with high molar masses, and a minor population peaking at 27-28 mL
559 corresponding to lower molar masses probably arising acid hydrolysis (Fig. 4a and b).
560 In BP2, these two fractions were also marked by relatively intense UV signals at 235
561 and 320 nm, which can be attributed to the presence of ferulic acid covalently
562 bounded to SP molecules (Fig. 4d). Moreover, the highest UV signals at 235 and 320
563 nm were found for low molar mass fractions of BP extracted at pH 2.0 (Fig. 4d),
564 mainly produced by acid hydrolysis. As ferulic acid is known to link preferentially to
565 arabinose side chains of pectins, this may indicate that this low molar mass fraction is
566 richer in arabinans. The higher extraction of Ara observed at pH 2.0 for BP and AP
567 might then be due to higher hydrolysis of arabinan during acid extraction in line with
568 the higher sensitivity of Ara-Ara linkages to acid hydrolysis (Renard et al., 1995). KPs
569 chromatograms exhibited one main peak with a shoulder at a lower elution volume
570 (24 mL) than APs and BPs, i.e. lower sized SP, but no peak at 27-28 mL, even for
571 KPR2. Moreover, even if the shoulder in the main peak diminished when pH
572 increased, the molar mass observed for KPR2 and KPR3 were not significantly
573 different (Table 2), this indicates that very low acid hydrolysis occurred during
574 extraction at pH 2.0, probably because KPs contain fewer neutral sugars (Table 2).

AP2, BP2 and KPR2 exhibited a higher $\bar{M}_w$ than their homologs extracted at higher pHs (Table 2), which suggested that larger SP were extracted at pH 2.0 even if a small amount of hydrolysis was observed as well. Interestingly, the specific extraction of high molar mass pectins from apple cell walls might explain the preferential industrial use of apples for pectin extraction, and imply the existence of specific, acid-labile bonds retaining pectins in apple cell walls.

After extraction at pH 6.0, all SP absorbed at 235 nm indicating β-elimination. APs size and molar mass distributions were broader and tighter for the others (Fig. 4a, b, e and f). This suggests that apple SP were the most sensitive to pH treatment. This may be attributed to the origin of the cell walls, i.e., to the intrinsic 3D cell wall network and especially to the intermolecular interactions of the wall polysaccharides. In particular, the pectin populations in the cell walls can interact with cellulose or hemicelluloses, with variation between different species (Broxterman & Schols, 2018). These more or less strong interactions between the polysaccharides of the cell wall drive the response to the different pH extraction conditions. For KPs in particular, when treatment pH increased the shoulder observed at 20 mL generally decreased. These results are in agreement with previous observations on SP extraction by heating at pH 6.0 which showed that the extraction led to one pectic fraction for beet and two pectic fractions for apple: one of high hydrodynamic volume, rich in neutral sugars, and one of low hydrodynamic volume rich in galacturonic acid (Renard & Thibault, 1993). The main fraction of AP6 peaking at 3.2 x 10^4 g/mol might then be enriched in galacturonic acid. In addition to the molar mass decrease, a signal at 235 nm was

obvious in the middle molecular size part of the chromatograms (elution volume around 24-27 mL) for all the SP extracted at pH 6.0 (Fig. 4c, d, g and h), indicating the presence of unsaturated double bonds such as produced by β-elimination. As for APs, KPRs and KPOs extracted at pH 2.0 and pH 3.5 absorbances at 235 nm were quite minors, this confirmed that the degradation of pectic fractions mainly occurred through β-elimination at pH 6.0 for APs, KPRs and KPOs. Nevertheless, for BPs UV signals at 235 nm were present for all pHs: at pH 2.0 and 3.5 they closely followed those at 320 nm, suggesting minor absorption from ferulic acid. At pH 6.0 however, the two bands were not superimposable, with a shoulder at higher elution volumes for the signal at 235 nm, closely following the RI signal and again suggesting extraction of homogalacturonan-rich pectins after β-elimination.

All SP from overripe kiwifruit were markedly smaller than their analogs from ripe kiwifruit ($\overline{M}_w$ of 6.4 x 10^4 g/mol and 1.53 x 10^5 g/mol for KPO6 and KPR6 respectively, Table 2) confirming SP degradation during maturation as a result of endogeneous enzymic process (Matsumoto, Obara, & Luh, 1983). Nevertheless, the variation of molar mass observed between the extraction at different pHs occurred in the same proportions for KPs (Table 2) whatever their maturity.

4 Conclusions

We explored the interactions between cell wall structures, proxied by plant origin, and pH in a model system miming F&V processing. Cell wall modifications and polysaccharide solubilization occurring in commonly used fruits (apple and kiwifruit)

and vegetable (beet) revealed some common features but also some striking differences for the same pH. In all cases, pectins were clearly the polysaccharides the most impacted, highlighted either from sugar loss in the modified cell walls or the composition of the solubilized polysaccharides. The acid treatment at pH 2.0 removed the arabinan side chains but the solubilized polysaccharides had a higher hydrodynamic volume, especially in apple. Galactose in kiwifruit cell walls was the most sensitive to boiling treatment, especially at pH 6.0. The main skeleton of pectins was significantly degraded after treatments at pH 6.0 by β-elimination reaction, leading to a higher extraction of smaller molecules, especially from kiwifruit cell walls. Under this condition, the molar mass of solubilized polysaccharides with more unsaturated double bonds was the smallest. The effectiveness of the pH treatments on different fruits and vegetables was different, and this was particularly marked for apple.

This study improved the understanding of structure/processing relationships and pointed out important differences concerning the behavior of individual species. This aspect means that each fruit or vegetable species should be regarded to have putatively some specificities in relation to their modified cell walls. The knowledge of such specificities in different natural sources can improve the quality of canned fruit and vegetable processing and open up prospects for their fine-tuning of functional characteristics.

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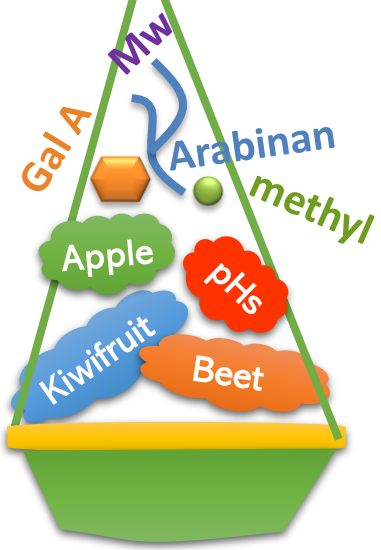

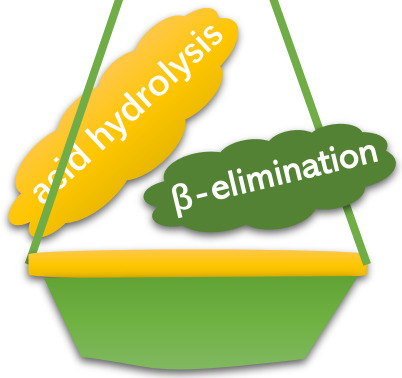

Same mechanisms

Specific responses?

Common response?

F&V processing