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Revisiting the contribution of ATR-FTIR spectroscopy to characterize plant cell wall polysaccharides

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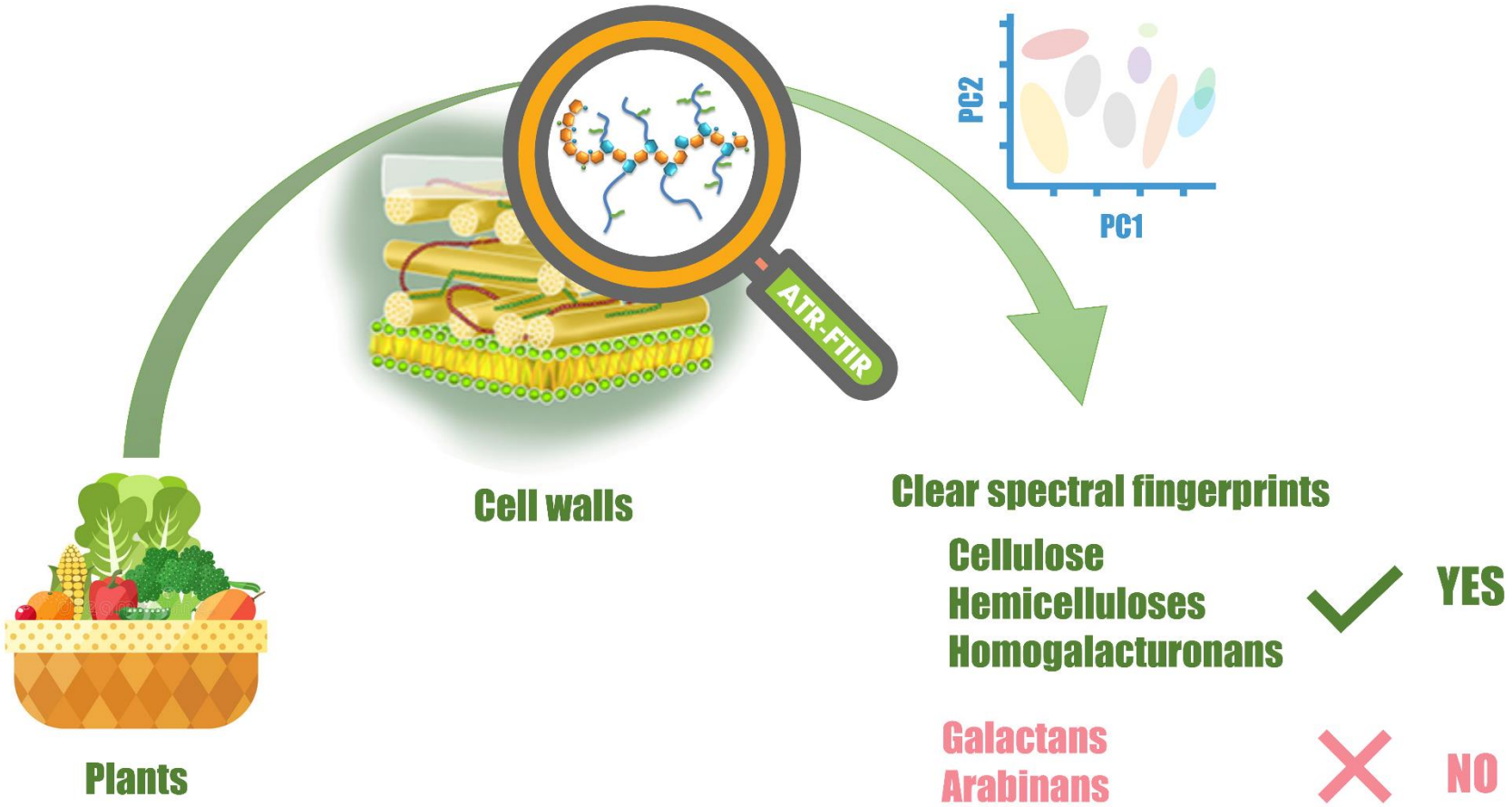
Graphical Abstract



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Abstract

The contribution of ATR-FTIR spectroscopy to study cell wall polysaccharides (CWPs) was carefully investigated. The region 1800-800 cm^{-1} was exploited using principal component analysis and hierarchical clustering on a large range of different powders of CWPs based on their precise chemical characterization. Relevant wavenumbers were highlighted for each CWP: 1035 cm^{-1} was attributed to xylose-containing hemicelluloses, 1065 and 807 cm^{-1} to mannose-containing hemicelluloses, 988 cm^{-1} to cellulose, 1740 and 1600 cm^{-1} to homogalacturonans according to the degree of methylation. Some band positions were affected by macromolecular arrangements (especially hemicellulose-cellulose interactions). However, as arabinan and galactan did not reveal distinctive absorption bands, ATR-FTIR spectroscopy did not allow the discrimination of cell walls differing by the abundance of these polysaccharides, e.g., those extracted from apple and beet. Therefore, the application of ATR-FTIR could remain sometimes limited due to the complexity of overlapping spectra bands and vibrational coupling from the large diversity of CWP chemical bonds.

Keywords: ATR-FTIR; Polysaccharides; Cell walls; Cellulose; Pectins; Hemicelluloses

Abbreviations:

AIS, alcohol insoluble solids; ATR-FTIR, Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy; DW, dry weight; PCA, Principal Component

Analysis; HCA: Hierarchical Cluster Analysis.

1. Introduction

Plant cell walls of the primary walls of dicots and non-grass monocots are dynamic and ordered networks of natural carbohydrate polymers, constituted by an amorphous matrix mainly composed of pectins embedded in a network of cellulose and hemicelluloses, as well as minor amounts of structural glycoproteins, phenolic compounds and enzymes (Carpita, Sabularse, Montezinos, & Delmer, 1979). Cell walls are highly variable according to species, developmental and maturity stages, plant organs and environmental conditions (Anderson & Kieber, 2020; Burton, Gidley, & Fincher, 2010). Therefore, this makes it difficult to easily identify and quantify cell wall components.

In general, the structure and composition of plant cell walls are characterized after sample extraction, pretreatment (e.g., acid hydrolysis) and diverse specific biochemical analyses (e.g., chromatography, mass spectrometry, spectrophotometry), which are expensive and time-consuming. An advanced tool based on mid-infrared spectroscopy would provide the advantages of rapid and easy analysis of the prepared samples. Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) has been increasingly used for the rapid characterization of cell walls of fruits and vegetables (Chylinska, Szymanska-Chargot, & Zdunek, 2016; Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1998; Coimbra, Barros, Rutledge, & Delgadillo, 1999; Ferreira, Barros, Coimbra, & Delgadillo, 2001; Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000; Szymanska-Chargot, Chylinska, Kruk, & Zdunek, 2015). Especially in recent years, it has become a powerful research

technology to clarify the composition of dry carbohydrate samples (Canteri, Renard, Le Bourvellec, & Bureau, 2019). This method appears really convenient insofar as it avoids undesirable structural changes that may occur during sample analysis, e.g., extraction and preparation. Moreover, ATR-FTIR can detect changes in the fruit and vegetables during processing at the cell wall level (Lan, Renard, Jaillais, Leca, & Bureau, 2020).

The identification of cell wall polysaccharides by infrared spectroscopy is generally carried out on the different polysaccharide fractions obtained by sequential extractions (the extraction of pure polysaccharides is imperfect), followed by ethanol precipitation and anion exchange chromatography. These extracted polysaccharides are then characterized using chemical and biochemical methods (Brahem, Renard, Gouble, Bureau, & Le Bourvellec, 2017; Coimbra et al., 1999; Renard, 2005; Szymanska-Chargot et al., 2015; Szymanska-Chargot & Zdunek, 2013). However, these studies do not use purified polysaccharides to confirm the absorption bands identified by comparison with the literature. Moreover, some studies have also performed polysaccharide analysis using spectral data but obtained from KBr pellets or aqueous solutions, the classical way before the development of the ATR method (Kacurakova et al., 2000). Fruits and vegetables are highly hydrated and susceptible to environmental conditions. Drying, not only prevents samples from oxidation and hydrolysis under the action of endogenous enzymes, but also concentrates samples by water elimination, so it significantly improves the reflectance spectra of some specific components present in lower content than water (Lan et al., 2020). Therefore, the

systematic analysis of purified solid materials from cell wall polysaccharides by ATR-FTIR may improve their identification.

Moreover, some challenges exist due to the ATR-FTIR response of the different cell wall polysaccharides. For example, according to our previous research, in spite of very different structures and compositions, apple and beet cell walls were poorly discriminated by Principal Component Analysis (PCA) based on ATR-FTIR spectra (Liu, Renard, Rolland-Sabaté, Bureau, & Le Bourvellec, 2021). Therefore, we need to reconsider these results, knowing that the interactions between the internal components of the cell walls (Le Bourvellec & Renard, 2012; Liu, Le Bourvellec, & Renard, 2020) may affect the absorption of these very complex mixtures. This study combined ATR-FTIR and stoichiometry to characterize the abundance and composition of cell wall polysaccharides, taking into account the heterogeneity and interactions between different cell wall components. To track the characteristic peaks of each cell wall component, spectral data and conventional chemical methods are used to study the composition of cell walls and internal structures. In order to evaluate the available information based on extracted samples, powders of cellulose, hemicelluloses, and pectins were also scanned in ATR-FTIR. To identify the typology of the cell walls, both PCA and Hierarchical Cluster Analysis (HCA) were performed. This study provided new explanations and experimental ideas for studying complex natural polymer systems, and guidance for using ATR-FTIR data to clarify carbohydrate structures, physical properties and interactions.

2. Materials and methods

2.1. Monosaccharide and polysaccharide samples

Monosaccharides (D-(+)-Arabinose, D-(−)-Fucose, D-(+)-Xylose, D-(+)-Mannose, L-Rhamnose, D-(+)-Glucose, and D-(+)-galactose) and D-(+)-Galacturonic acid monohydrate were obtained from Fluka (Buchs, Switzerland). Arabinan (sugar beet), linear 1,5-α-L-arabinan (sugar beet), debranched arabinan, galactan (potato), rhamnogalacturonan I (from potato pectic fibre), and rhamnogalacturonan (from soybean pectic fibre), xylan, arabinoxylan, glucomannan, and xyloglucan were provided from Megazyme (Bray, Ireland). Commercial apple and citrus peel pectins (degrees of methylation ~75%), microcrystalline cellulose and poly-galacturonic acid were provided by Sigma-Aldrich (Deisenhofen, Germany). Homogalacturonan DM 70 was supplied by Watrelot et al. (2013). The common names of cell wall components and their abbreviations used in this study are presented in Table 1.

Native and modified cell walls and pectins from apple, beet and kiwifruit were supplied and characterized by Liu et al. (2021). The native cell wall samples were named as apple cell wall (ACN), beet cell wall (BCN), ripe kiwifruit cell wall (KCRN) and overripe kiwifruit cell wall (KCON), and 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. Extracted pectins at pH 2.0, 3.5 and 6.0 from apple, beet and two kiwifruit cell walls 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.

Table 1. The common names of cell wall components, their abbreviations and their ATR-FTIR frequencies (cm^{-1}) determined with our spectrometer of the studied plant cell wall polysaccharides.

	Sample names	Abbreviations	Linkable peaks or regions (cm^{-1})
Monosaccharides	D-(-)-Arabinose	Ara	1312, 1128*s*, 1088*s*, 1050***vs***, 991***vs***, 940, 890*s*, 841*s*
	D-(+)-Xylose	Xyl	1146, 1123*s*, 1034***vs***, 1016*s*, 930*s*, 902*s*
	D-(+)-Mannose	Man	1110*s*, 1064*s*,1034***vs***,1016***vs***, 966*s*, 949*s*, 912, 879
	D-(+)-Galactose	Gal	1154*s*, 1142, 1100*s*, 1056***vs***, 1039***vs***, 990, 971, 953*s*, 827*s*
	L-Rhamnose	Rha	1375, 1290, 1226, 1145, 1116*s*, 1074*s*, 1026***vs***, 976*s*, 907, 874, 827*s*
	D-(+)-Glucose	Glc	1228, 1206, 1150*s*,1100*s*, 1052*s*, 1016***vs***, 991***vs***, 912*s*, 840*s*
	D-(-)-Fucose	Fuc	1334s, 1140s, 1095*s*, 1083***vs***, 1050***vs***, 976***vs***, 921, 868, 814
	D(+)-Galacturonic acid monohydrate	Gal A	1756*s*, 1708*s*, 1275, 1218, 1155, 1095***vs***, 1062***vs***, 1025***vs***, 823*s*
β-glucans	Microcrystalline cellulose	MCCE	1640, 1428, 1367, 1320, 1308, 1200, 1160, 1052*s*, 1030***vs***, 988*s*, 893
	Yeast β-glucan	YGLU	1640, 1428, 1367, 1308, 1200, 1160, 1068*s*, 1030***vs***, 988*s*, 886
	Curdlan (1,3-β-o-glucan)	CGLU	1640, 1428, 1367, 1308, 1200, 1160, 1068*s*, 1030***vs***, 988*s*, 886
Hemicelluloses	Rye Arabinoxylan (59% xylose)	ARHV	1164, 1035***vs***, 983*s*, 890
	Wheet Arabinoxylan (64% xylose)	AXMB	1164, 1035***vs***, 983*s*, 890
	Wheet Arabinoxylan (77% xylose)	AXLB	1164, 1035***vs***, 983*s*, 890
	Xylan (Beechwood)	XYBW	1164, 1035***vs***, 983*s*, 890
	1,4-β-D-Mannan	MANB	1367, 1065***vs***, 1035***s***, 1013***vs***, 938*s*, 890, 870*s*, 807*s*
	Galactomannan (Carob)	GAMA	1065***vs***, 1027***vs***, 870*s*, 807*s*
	Xyloglucan (from tamarind seed)	XYGT	(1040-1010)***vs***, 939, 890
	Xyloglucan Oligosaccharides	XYGO	(1040-1010)***vs***, 939, 890
	Xyloglucan (Hepta-, +Octa, +Nona-saccharides)	XYGH	(1040-1010)***vs***, 939, 890
Pectins	Citrus peel pectin	CPPC	1740*s*, 1600, 1440, 1230, 1141, 1097*s*, 1014***vs***, 954, 914, 831

(Continued)

Table 1. (Continues)

Sample names	Abbreviations	Linkable peaks or regions (cm^{-1})
Commercial apple pectin	APPC	1740*s*, 1600, 1440, 1230, 1141, 1097*s*, 1014***vs***, 954, 914, 831
Arabinan (sugar beet)	ARSB	1600, the region of (1100 - 950)
Linear 1,5-α-L-arabinan (sugar beet)	LNAR	1208, 1115, 1086*s*, 1071*s*, 1043***vs***, 1022***vs***, 1004***vs***, 982***vs***, 948*s*
Debranched arabinan (sugar beet)	DBAR	1600, 1208, 1115, 1086*s*, 1071*s*, 1043***vs***, 1022***vs***, 1004***vs***, 982***vs***, 948*s*
Galactan (Potato)	GTAN	1600, 1405, 1039***vs***, 884*s*
Rhamnogalacturonan I (from potato pectic fibre)	RGPP	1740, 1600***s***, 1410, 1238, 1141*s*, 1097*s*, 1074s, 1014***vs***, 954
Rhamnogalacturonan (from soybean pectic fibre)	RGSP	1600***s***, 1410, 1141*s*, 1097*s*, 1074s, 1014***vs***, 954
Homogalacturonan DM 70	HGTN	1740***vs***, 1440, 1230, 1140, 1097*s*, 1014***vs***, 970*s*, 914
Poly-galacturonic acid	GALN	1590***s***, 1410*s*, 1330, 1141*s*, 1097*s*, 1014***vs***, 954*s*

114 * IR band intensity: *vs*, very strong; *s*, strong.

2.2. Characterization of carbohydrate composition

Sugar analysis was performed as previously described by Liu et al. (2021). For neutral sugars analysis, 10 mg of cell walls or cellulose were submitted to a Saeman acid hydrolysis (Saeman, Moore, Mitchell, & Millett, 1954) and then to simple hydrolysis (dissolved in 1 mol/L sulfuric acid) whereas soluble polysaccharides (10 mg) were only submitted to simple hydrolysis. The derivatization to alditol acetates (Englyst, Wiggins, & Cummings, 1982) allows the detection of sugars by gas chromatography with a flame ionization detector (Agilent, Inc., Palo Alto, USA). Galacturonic acid was measured by a meta-hydroxyl-diphenyl assay (Blumenkrantz & Asboe-Hansen, 1973). The methanol was measured by a stable isotope dilution assay using headspace-GC-MS (QP2010 Shimadzu Kyoto, Japan) as described by Renard & Ginies (2009). The degree of methylation (DM) was then calculated as the molar ratio of methanol to galacturonic acid.

2.3. ATR-FTIR spectra

All cell wall polysaccharide samples, in the form of dry powder, were stored in P_2O_5 atmosphere before analysis to remove residual water. ATR-FTIR spectra data (4000 to 600 cm^{-1}) were acquired at room temperature in a Tensor 27 FTIR spectrometer (Bruker Optics®, Wissembourg, France), using a single-reflectance horizontal ATR cell (Golden Gate with a diamond crystal, Bruker Optics®) equipped with a system to press the dried homogenized samples on the crystal surface (Bureau et al. 2012). Each sample was analyzed three times (using after homogenization three

different aliquots of the powders) to consider its heterogeneity, and each spectrum was the 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 the SAISIR package (Cordella & Bertrand, 2014). The spectral data were pretreated with baseline correction and standard normal variate (SNV) to correct multiplicative interferences and variations in baseline shift before any multivariate analysis.

2.4. Statistical analysis

All biochemical analyses were presented as mean values of analytical triplicates and the reproducibility of the results was expressed as pooled standard deviations (Pooled SD). Pooled SD was calculated per series of replicates using the sum of individual variances weighted by the individual degrees of freedom (Box, Hunter, & Hunter, 1978). A PCA was applied on the ATR-FTIR spectra in the range between 1800 and 800 cm^{-1} in order to study the repartition of the cellulose, hemicelluloses and pectins in a space according to their composition and absorption bands. Spectral data pre-processing and PCA were performed using MATLAB 7.5 (Mathworks Inc. Natick, MA) software using the SAISIR package (Cordella & Bertrand, 2014). HCA was performed using R software using FactoMineR (for computing) and factoextra (for visualizing the results) (R Core Team., 2014).

3. Results and discussion

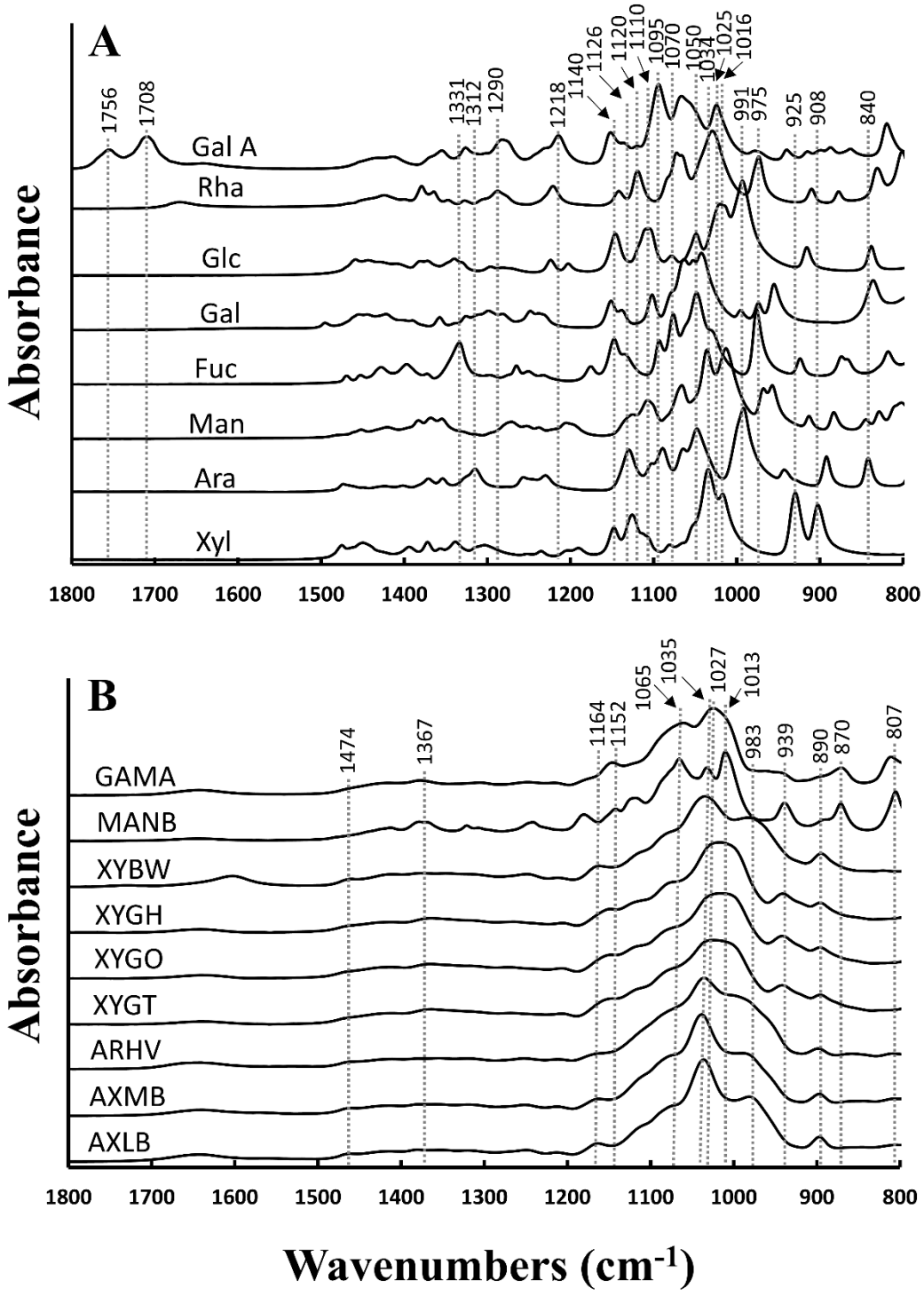
3.1. Characteristic bands of cell wall polysaccharides in the ATR-FTIR spectra

157 The compositions of the 58 cell wall polysaccharides from extracted and
158 commercial origin were determined in this study by both, the classical methods (Table
159 2, see Liu et al., 2021 for cell walls and extracted pectins) and ATR-FTIR
160 spectroscopy (Table 1, Figure 1). Detailed peak positions and assignments of each
161 pure cell wall polysaccharide were limited to the specific bands in the range of
162 1800-800 cm^{-1} (detected in solid or liquid form) in agreement with the previous
163 works (Canteri et al., 2019; Coimbra et al., 1998, 1999; Ferreira et al., 2001; Filippov
164 & Kohn, 1975; Gnanasambandam, R., Proctor, 2000; Kacurakova et al., 2000;
165 Kyomugasho, Christiaens, Shpigelman, Van Loey, & Hendrickx, 2015; McCann,
166 Hammouri, Wilson, Belton, & Roberts, 1992; Monsoor, Kalapathy, & Proctor, 2001;
167 Szymanska-Chargot et al., 2015; Szymanska-Chargot & Zdunek, 2013) and
168 summarized in Table 3. Strong absorption bands in this region corresponding to the
169 specific wavenumbers assigned to pectins (e.g., rhamnogalacturonan and
170 homogalacturonan), hemicelluloses (e.g., xyloglucan, mannan, galactomannan,
171 arabinoxylan and xylan) and cellulose (Figure 1), are detailed below.

Table 2. Composition of extracted cell walls and pectins from fruits and vegetables and commercial purified cellulose, hemicelluloses and pectin components (mg/g dry weight, except for degree of methylation expressed in %).

Codes	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Gal A	MeOH	DM
β-glucans										
MCCE	3	0	0	17	13	0	861	-	-	-
CGLU	0	0	0	0	0	0	705	-	-	-
YGLU	0	0	0	10	6	0	650	-	-	-
Hemicelluloses										
GAMA	0	0	5	0	776	190	7	-	-	-
ARHV	0	0	321	486	0	17	0	-	-	-
AXMB	1	0	288	693	0	7	5	-	-	-
AXLB	1	0	209	733	0	6	5	-	-	-
XYBW	12	0	6	716	0	10	8	-	-	-
MANB	0	0	0	0	960	18	8	-	-	-
XYGT	1	0	12	282	0	140	399	-	-	-
XYGO	3	0	37	287	0	123	515	-	-	-
XYGH	1	0	3	281	0	106	506	-	-	-
Commercial pectins										
CPPC	20	1	21	4	0	138	33	535	74	73
APPC	12	0	20	8	1	88	63	564	77	79
ARSB	52	0	584	0	0	114	0	83	-	-
LNAR	25	0	841	0	0	78	0	51	-	-
DBAR	68	0	504	0	0	193	48	98	-	-
GTAN	51	0	23	4	0	628	9	79		
RGPP	54	0	10	3	0	105	0	473	-	-
RGSP	81	64	21	120	0	96	0	403	-	-
HGTN	-	-	-	-	-	-	-	814	100	68
GALN	-	-	-	-	-	-	-	850	0	0
Pooled SD	*1.0*	*0.3*	*8.3*	*7.0*	*5.8*	*2.3*	*8.6*	*4.0*	*2.5*	*3.2*

Pooled SD: pooled standard deviation. Rha: rhamnose, Fuc: fucose, Ara: arabinose, Xyl: xylose, Man: mannose, Gal: galactose, Glc: glucose, Gal A: galacturonic acid, MeOH: methanol, DM: degree of methylation. Hemicelluloses (ARHV: Rye Arabinoxylan (59% Xylose), AXMB: Wheet Arabinoxylan (64% Xylose), AXLB: Wheet Arabinoxylan (77% Xylose), XYBW: Xylan (Beechwood), MANB: 1,4-β-D-Mannan, XYGT: Xyloglucan (from tamarind seed), XYGO: Xyloglucan Oligosaccharides, XYGH: Xyloglucan Oligosaccharides (Hepta-, +Octa, +Nona-saccharides), GAMA: Galactomannan (Carob)); Pectins (APPC: Apple pectin, CPPC: Citrus peel pectin, HGTN: Homogalacturonan DM 70, GALN: Polygalacturonic acid, DBAR: Debranched arabinan, ARSB: Arabinan, LNAR: Linear arabinan, RGPP: Rhamnogalacturonan I, RGSP: Rhamnogalacturonan, GTAN: Galactan (Potato)); β-glucans (MCCE: Microcrystalline cellulose, CGLU: 1,3-beta-o-glucan and YGLU: Yeast beta-glucan).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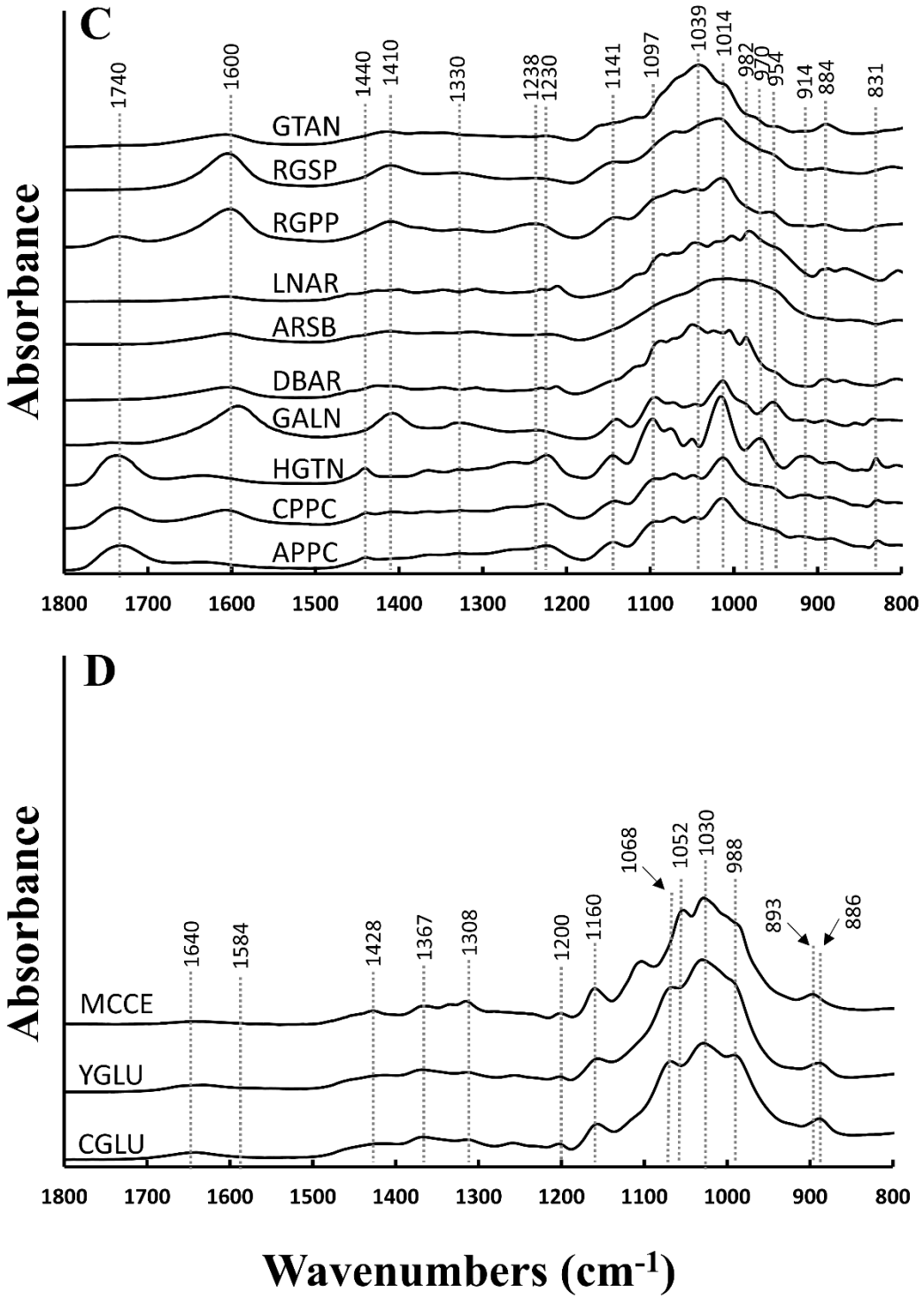


Figure 1 ATR-FTIR spectra (pre-processed with Standard Normal Variate) of commercial purified and extracted cell wall polysaccharides in solid form: A. Monosaccharides (Rha: rhamnose, Fuc: fucose, Ara: arabinose, Xyl: xylose, Man: mannose, Gal: galactose, Glc: glucose, Gal A: galacturonic acid); B. Hemicelluloses (ARHV: Rye Arabinoxylan (59% Xylose), AXMB: Wheet Arabinoxylan (64% Xylose), AXLB: Wheet Arabinoxylan (77% Xylose), XYBW: Xylan (Beechwood), MANB: 1,4-β-D-Mannan, XYGT: Xyloglucan (from tamarind seed), XYGO: Xyloglucan Oligosaccharides, XYGH: Xyloglucan Oligosaccharides (Hepta-, +Octa, +Nona-saccharides), GAMA: Galactomannan (Carob)); C. Pectins (APPC: Apple pectin, CPPC: Citrus peel pectin, HGTN: Homogalacturonan DM 70, GALN: Polygalacturonic acid, DBAR: Debranched arabinan, ARSB: Arabinan, LNAR:

Linear arabinan, RGPP: Rhamnogalacturonan I, RGSP: Rhamnogalacturonan, GTAN: Galactan (Potato)); D. β-glucans (MCCE: Microcrystalline cellulose; CGLU: curdlan 1,3-beta-o-glucan; YGLU: Yeast beta-glucan).

196 **Table 3.** The main ATR-FTIR absorption bands, the polysaccharides in which they were detected and their tentative assignment. For polysaccharide identification (detailed in Table 1).

Wavenumber range (cm^{-1}) (detected)	**Mono- or polysaccharide in which it was detected**	**Band assignments**	**Corresponding Wavenumber range (cm^{-1})***	**References**
1756 & 1708	Gal A	Galacturonic acid (absence of glycosidic bond)	-	-
1740	APPC, CPPC and HGTN	C=O stretching vibration of alkyl ester (pectin)	1745-1730	(Filippov & Kohn, 1975; Gnanasambandam, R., Proctor, 2000; McCann et al., 1992; Monsoor et al., 2001; Szymanska-Chargot & Zdunek, 2013)
1640	MCCE, CGLU and YGLU	H–O–H bending vibration absorbed water	1640	(Szymanska-Chargot et al., 2015)
1605 - 1595	GALN, CPPC, ARSB, DBAR, RGPP and RGSP	COO^- antisymmetric stretching polygalacturonic acid, free carboxyl group	1630-1600	(Filippov & Kohn, 1975; Gnanasambandam, R., Proctor, 2000; McCann et al., 1992; Monsoor et al., 2001; Szymanska-Chargot & Zdunek, 2013)
1525	KCR/Os	Amid II N–H deformation (proteins); lignin and phenolic back bone	1550	(McCann et al., 1992)
1474	ARHV, XYBW and XYGT	Xylose-containing hemicellulose	-	-
1440	APPC, CPPC and HGTN	Asymmetric stretching modes vibration of methyl esters (pectin)	1440	(Canteri et al., 2019; Szymanska-Chargot et al., 2015)
1428	MCCE, CGLU and YGLU	CH_2 symmetric bending (cellulose)	1428	(Szymanska-Chargot & Zdunek, 2013)
1410	GALN, RGPP and RGSP	COO^- symmetric stretching, free carboxyl group (rhamnogalacturonan and homogalacturonan)	1410	(Szymanska-Chargot & Zdunek, 2013)
1367	MCCE, CGLU and YGLU, XYGT, GAMA and MANB	C–H vibrations and CH_2 bending (cellulose, hemicelluloses)	1370, 1362	(Szymanska-Chargot et al., 2015; Szymanska-Chargot & Zdunek, 2013)

(Continued)

Table 3. (Continues)

Wavenumber range (cm^{-1}) (detected)	Mono- or polysaccharide in which it was detected	Band assignments	Corresponding Wavenumber range (cm^{-1})*	References
1331	Fuc	Fucose (absence of glycosidic bond)	-	
1330	HGTN, GALN, RGPP and RGSP	Bending of O–H groups in pyranose ring of pectins	1331-1320	(Szymanska-Chargot et al., 2015; Szymanska-Chargot & Zdunek, 2013)
1312	Ara	Arabinose (absence of glycosidic bond)	-	
1308	MCCE, CGLU and YGLU	CH_2 symmetric bending or CH_2 rocking vibration (cellulose)	1317-1313	(Szymanska-Chargot et al., 2015; Szymanska-Chargot & Zdunek, 2013)
1290	Rha	Rhamnose (absence of glycosidic bond)	-	
1238	RGPP	Rhamnogalacturonan	-	
1230	APPC, CPPC and HGTN	C–O stretching (pectins)	1240, 1230	(Szymanska-Chargot et al., 2015; Szymanska-Chargot & Zdunek, 2013)
1218	Gal A	Galacturonic acid (absence of glycosidic bond)	-	-
1164	ARHV, XYBW and XYGT	Glycosidic bond vibrations (O–C–O) (xylose-containing hemicellulose)	1173, 1153, 1147	(Coimbra et al., 1999; Kacurakova et al., 2000)
1160	KCR/Os	Glycosidic bond vibrations (O–C–O) (cellulose in cell walls)	1160	(Canteri et al., 2019; Szymanska-Chargot & Zdunek, 2013)
1152	MANB and GAMA	Glycosidic bond vibrations (O–C–O) (mannose-containing hemicellulose)	1150	(Szymanska-Chargot et al., 2015)
1141	APPC, CPPC, GALN, HGTN, RGPP and RGSP	Glycosidic bond vibrations (O–C–O) (pectin)	1150-1143	(Coimbra et al., 1998, 1999)
1126	Ara	Arabinose (absence of glycosidic bond)	-	

(Continued)

Table 3. (Continues)

Wavenumber range (cm^{-1}) (detected)	Mono- or polysaccharide in which it was detected	Band assignments	Corresponding Wavenumber range (cm^{-1})*	References
1097	APPC, CPPC, HGTN, GALN, RGPP and RGSP	C–O stretching, C–C stretching ring pectin	1100-1090	(Coimbra et al., 1998; Szymanska-Chargot et al., 2015; Szymanska-Chargot & Zdunek, 2013)
1074	GTAN, RGPP and RGSP	C–C stretching ring (galactan and rhamnogalacturonan)	1072, 1070	(Kacurakova et al., 2000)
1068	MCCE, CGLU and YGLU	C–O stretching, C–C stretching, C6–H2–O6 (cellulose)	1059, 1047	(Szymanska-Chargot et al., 2015)
1065	MANB and GAMA	C–O stretching, C–C stretching (mannose-containing hemicellulose)	1064	(Kacurakova et al., 2000)
1039	GTAN	C–C stretching ring (galactan)	1038	(Kacurakova et al., 2000)
1035	ARHV, XYBW and XYGT	C–O stretching, C–C stretching (xylose-containing hemicellulose)	1042, 1041, 1038	(Canteri et al., 2019; Coimbra et al., 1999; Kacurakova et al., 2000)
1030	MCCE, CGLU and YGLU	C–O stretching, C–C stretching, C6–H2–O6 (cellulose)	1034, 1030	(Kacurakova et al., 2000; Szymanska-Chargot & Zdunek, 2013)
1027	GAMA	C–O stretching, C–C stretching, C6–H2–O6 (galactomannan)	1034	(Kacurakova et al., 2000)
1014	APPC, CPPC, GALN, HGTN, DBAR, ARSB, GTAN, RGPP and RGSP	C–O stretching, C–C stretching pectin (C2–C3, C2–O2, C1–O1) backbone vibrations (pectin)	1020, 1015, 1014	(Coimbra et al., 1998, 1999; Szymanska-Chargot et al., 2015; Szymanska-Chargot & Zdunek, 2013)
1013	MANB	C–O stretching, C–C stretching (mannan)	-	
991	Glc and Ara	Glucose and arabinose (absence of glycosidic bond)	-	
988	MCCE, CGLU and YGLU	C–O stretching, C–C stretching cellulose (C6–H2–O6)	1000, 985	(Canteri et al., 2019; Szymanska-Chargot & Zdunek, 2013)

(Continued)

Table 3. (Continues)

Wavenumber range (cm^{-1}) (detected)	Mono or polysaccharide in which it was detected	Band assignments	Corresponding Wavenumber range (cm^{-1})*	References
983	ARHV and XYBW	Xylan and arabinoxylan	-	
982	DBAR, ARSB and LNAR	Arabinan	-	
970	APPC, CPPC and HGTN	Pectins	972	(Kacurakova et al., 2000)
954	RGSP	CO bending (pectins)	952	(Coimbra et al., 1999; Szymanska-Chargot & Zdunek, 2013)
939	XYGT, GAMA and MANB	Ring vibration (hemicellulose and arabinan)	941	(Szymanska-Chargot et al., 2015; Szymanska-Chargot & Zdunek, 2013)
925 and 908	Xyl	Xylose (absence of glycosidic bond)	-	
914	APPC, CPPC, HGTN and GALN	Ring vibration (pectin)	-	
890	XYGT, ARHV and XYBW	C1–H bending (xylose-containing hemicellulose)	893	(Szymanska-Chargot & Zdunek, 2013)
886	KCR/Os	C1–H bending (cellulose)	899, 895	(Canteri et al., 2019; Szymanska-Chargot & Zdunek, 2013)
884	GTAN	C1–H bending (galactan)	883	
870	GAMA and MANB	C1–H bending (mannose-containing polysaccharide)	-	
840	Ara	Arabinose (absence of glycosidic bond)	-	
831	APPC, CPPC, GALN and HGTN	Ring vibration (pectin)	833-830	(Szymanska-Chargot et al., 2015; Szymanska-Chargot & Zdunek, 2013)
807	GAMA and MANB	Ring vibration (mannose-containing hemicellulose)	-	

197 * Reference from the literatures. Monosaccharides (Rha: rhamnose, Fuc: fucose, Ara: arabinose, Xyl: xylose, Man: mannose, Gal: galactose, Glc: glucose, Gal A: galacturonic acid);

Hemicelluloses (ARHV: Rye Arabinoxylan (59% Xylose), AXMB: Wheet Arabinoxylan (64% Xylose), AXLB: Wheet Arabinoxylan (77% Xylose), XYBW: Xylan (Beechwood), MANB: 1,4-β-D-Mannan, XYGT: Xyloglucan (from tamarind seed), XYGO: Xyloglucan Oligosaccharides, XYGH: Xyloglucan Oligosaccharides (Hepta-, +Octa, +Nona-saccharides), GAMA: Galactomannan (Carob)); Pectins (APPC: Apple pectin, CPPC: Citrus peel pectin, HGTN: Homogalacturonan DM 70, GALN: Polygalacturonic acid, DBAR: Debranched arabinan, ARSB: Arabinan, LNAR: Linear arabinan, RGPP: Rhamnogalacturonan I, RGSP: Rhamnogalacturonan, GTAN: Galactan (Potato)); β-glucans (MCCE: Microcrystalline cellulose; CGLU: curdlan, 1,3-beta-o-glucan; YGLU: Yeast beta-glucan). Bands of monosaccharides such as mannose and galactose were not shown due to their overlapping with bands of polysaccharide polymers.

3.1.1. Monosaccharides

Monosaccharides, without any glycosidic linkage to other units, are the simplest component units of the cell wall polysaccharides, and have been shown to be important in polymer analysis and structure elucidation (Wiercigroch et al., 2017). It was necessary to consider here hexopyranoses (glucose, mannose and galactose), dehydro-hexopyranoses (rhamnose and fucose), pentopyranose (xylose), and pentofuranose (arabinose), standards with different positions of hydroxyl groups on C-2, C-3, and C-4 (Figure 1A). The spectra of pentoses (arabinose and xylose) were dominated by a band at 991 and 1034 cm^{-1}, respectively, mainly due to the ν(C-C), ν(C-O) and β(C-CH) vibrations (Edwards, 1976). For hexoses, D-(+)-glucose was observed by the main band at 991 cm^{-1}. D-(+)-galactose is almost identical in structure to D-(+)-glucose, with a different orientation in the C-4 OH group, but with a distinct spectral difference due to their free crystalline structure (Figure 1A). Similarly, the spectrum of other hexopyranoses with different hydroxyl group orientations on C-2, C-3, and C-4 was also significantly different. Therefore, the relative positions of (C-OH) essentially affected the spectrum through variations in their spatial arrangement and interactions. The detailed ATR-FTIR bands of monosaccharides with modes assignments are listed in Table 1.

3.1.2. Pectic components

Pectins are acidic hetero-polysaccharides mainly composed of homogalacturonan, rhamnogalacturonan I and II, and different neutral sugar side chains (e.g., arabinan

and galactan). The spectra of apple pectins (APPC) and citrus peel pectins (CPPC) had very similar characteristic bands (Figure 1C) centering at 1740, 1600, 1440, 1230, 1141, 1097, 1014, 954, 914 and 831 cm^{-1}. They have also similar levels of neutral sugars, galacturonic acid and degree of methylation (Table 2). The band at 1740 cm^{-1} is assigned to the esterified galacturonic acids. This band is characteristic of pectins with a high degree of methylation (DM) such as high methylated homogalacturonan (DM=70) (HGTN). Inversely, the band of poly-galacturonic acid (GALN), which is non-esterified, was at 1600 cm^{-1} due to the COO^- carboxylate ion stretching. These two main characteristic bands of pectins are fixed and similar to those previously reported (Coimbra et al., 1999; Filippov & Kohn, 1975; Gnanasambandam, R., Proctor, 2000; Kacurakova et al., 2000; Reintjes, Musco, & Joseph, 1962; Szymanska-Chargot et al., 2015; Wojdyło, Figiel, Lech, Nowicka, & Oszmiański, 2014).

For a further interpretation of these spectra, pure pectic components were characterized in the same conditions. Notably, although these parts of the polysaccharide components were not a complete pectin structure found in the plant, they are representative of different subunits of the pectins. For rhamnogalacturonan (RGPP and RGSP), the band at 1014 cm^{-1} was the strongest (Figure 1C). However, this peak overlapped with the main peak of commercial pectins (APPC and CPPC). Some other specific bands, assigned to rhamnogalacturonan (RGPP and RGSP) can be used such as 1410, 1238 and 1074 cm^{-1}. In fact, RGSP contained more xylose than RGPP (120 mg/g *vs* 3 mg/g respectively) (Table 2). Based on the maximum peak of

246 xylose at 1035 cm^{-1} for RGSP, but not for RGPP (Figure 1C), 1035 cm^{-1} may be used
247 to differentiate the abundance of xylose in cell wall polysaccharides.

248 In the case of the main neutral sugar side chains of pectins (Figure 1C): (i)
249 galactan (GTAN) was characterized by bands at 1039 cm^{-1}, 1014 and 884 cm^{-1}; and
250 (ii) arabinan, such as present in sugar beet arabinan (ARSB), linear arabinan (LNAR)
251 and debranched arabinan (DBAR), were more visible in the region between 1100 and
252 950 cm^{-1}. The arabinans with different linearity and branching degrees had some
253 minor peak intensity differences in this region. For example, the main band of LNAR
254 (with the highest arabinose content, Table 2) was close to 980 cm^{-1}, which may be
255 used to assess arabinan. However, the characteristic peaks of arabinan (sugar beet)
256 were in the region and also within the range of the main peaks of other pectins (e.g.,
257 commercial pectins, rhamnogalacturonan and galactan) not allowing a clear
258 assignment of bands between 1100 and 950 cm^{-1}. In aqueous solutions, Kacurakova et
259 al. (2000) observed bands at 1070 and 1043 cm^{-1} for rhamnogalacturonan, 1072 cm^{-1}
260 for galactan and 1039 cm^{-1} arabinan, which were too close for reliable discrimination.
261 These differences may be due to the different states (solid or solution) of the cell wall
262 compounds and probably also to the specificity of the used spectrometers.

263 For pectic homogalacturonans with more or less methylation, the main
264 characteristic peaks (especially 1740, 1600, 1097 and 1014 cm^{-1}) were stable
265 regardless of whether they are in a solid crystalline state or in an aqueous solution.
266 However, the identification of spectral characteristic peaks of arabinan, galactan, and

rhamnogalacturonan required caution as their peaks were dependent on the sample state, which could lead to their overlapping with those of hemicelluloses and pectins.

3.1.3. Hemicelluloses and cellulose

Bands at 1474, 1367, 1164, 1152, 1065, 1035, 1027, 1013, 983, 939, 890, 870 and 807 cm^{-1} were identified for hemicelluloses (Figure 1B). Xylan (XYBW) had characteristic absorption bands at 1035 cm^{-1} and 983 cm^{-1}. The arabinoxylans with different xylose contents (Table 2), e.g., ARHV (59%), AXMB (64%) and AXLB (77%), showed a main band at 1035 cm^{-1} for which the intensity varied with the xylose content. Xyloglucan (XYGT, XYGO and XYGH) absorbed in the region of 1040-1010 cm^{-1}, like hemicelluloses and cellulose making it difficult to be identified. Galactomannan (GAMA) and mannan (MANB) had bands at 1027 and 1013 cm^{-1}, respectively. In addition, they had a common secondary band at 1065 cm^{-1}, and two specific peaks at 870 and 807 cm^{-1} (also found in softwood sample, Simonović, Stevanic, Djikanović, Salmén, & Radotić, 2011), which allowed them to be distinguished from others (Figure 1B). Therefore, the bands which could be assigned to xylose- and mannose- containing hemicelluloses were 1474, 1164, 1035, 983 and 890 cm^{-1} for xylose-, and 1152, 1065, 1027, 1013, 939, 870 and 807 cm^{-1} for mannose-, respectively (Table 3). This was confirmed for xylose- containing hemicelluloses including XYBW, ARHV, AXMB, AXLB, XYGT, XYGO and XYGT, and mannose- containing hemicelluloses including MANB and GAMA.

The bands characteristic of cellulose, β-(1→4)-linked glucan, for MCCE, were at

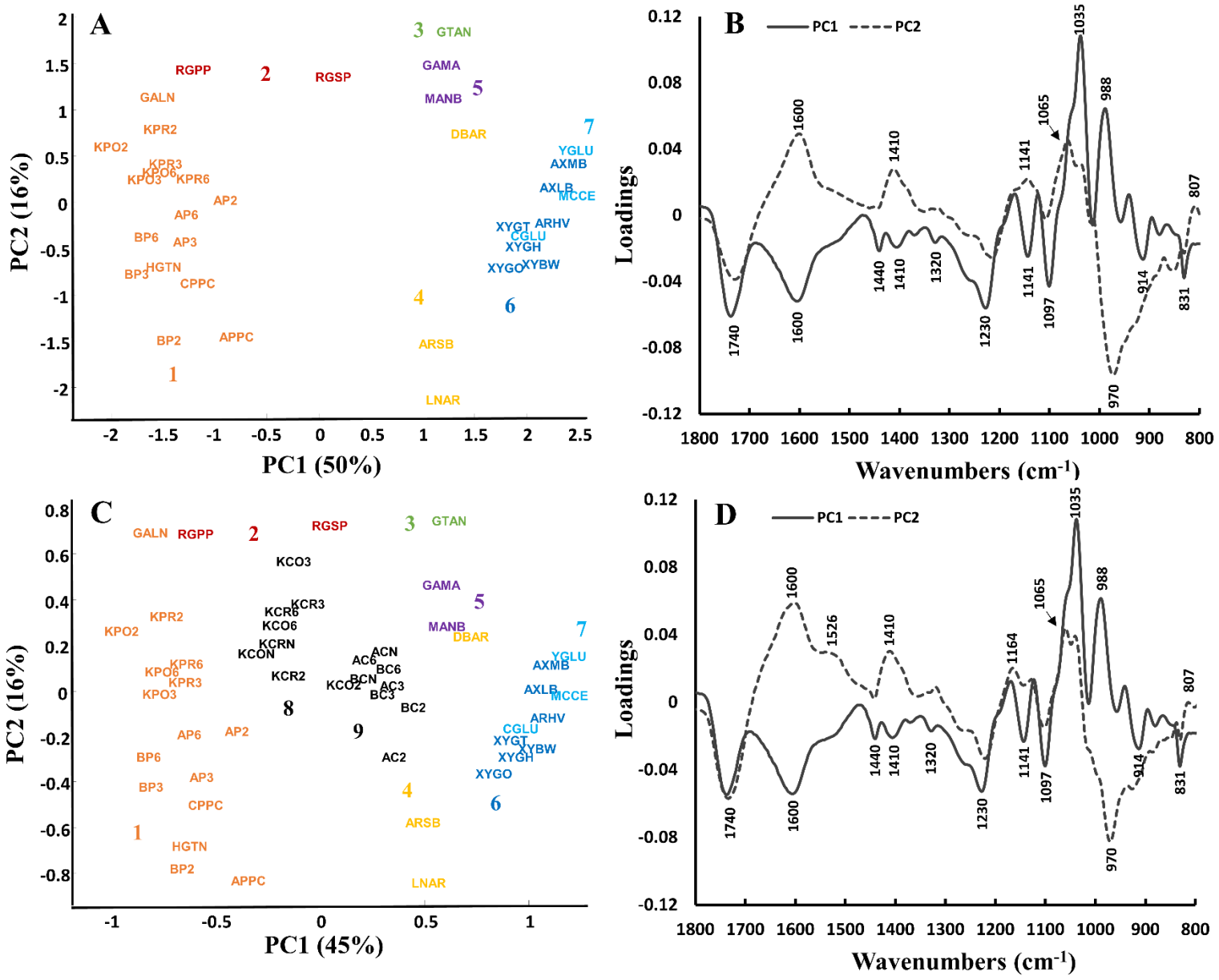
1640, 1428, 1367, 1320, 1308, 1200, 1160, 1052, 1030, 988 and 893 cm^{-1} (Figure 1D), whereas bands at 1068 and 886 cm^{-1} (instead of 1052 and 893 cm^{-1}) were identified for β-(1→3)-glucans (YGLU and CGLU). These slight differences for two peaks may contribute to distinguish 1,3-β- and 1,4-β- bonds.

3.1.4. Glycosidic linkage

The glycosidic linkages are an important characteristic structure in polysaccharides and influence the spectral changes in aqueous solutions (Jockusch et al., 2004; Kačuráková & Mathlouthi, 1996; Kanou, Nakanishi, Hashimoto, & Kameokaj, 2005; Nikonenko, Buslov, Sushko, & Zhbankov, 2000; Wiercigroch et al., 2017). For example, the spectra of the glycosidic bonds with different positions and configurations of oligo- and poly- saccharides in aqueous solution differ markedly from monosaccharides, with stretching vibrations [ν(CO)] of the C-O-C glycosidic linkage being the marker of the polysaccharide configuration. These vibrations appear in the two spectral ranges 1160-1130 and 1000-960 cm^{-1} (Kacurakova et al., 2000; Kačuráková & Mathlouthi, 1996; Wiercigroch et al., 2017). Similarly, these bands appear in cell wall polysaccharides in the solid states when compared with monosaccharides (Figure 1). For xylose units with β-(1→4) glycosidic linkage (XYBW) bands were found at about 1164 cm^{-1}, for glucose units with β-(1→4) glycosidic linkage (YGLU, CGLU and MCCE) bands were found at 1160 cm^{-1} and for mannose units with β-(1→4) glycosidic linkage (MANB) bands were found at 1152 cm^{-1}. Bands at 1141 cm^{-1} for pectins originate from the stretching motion of the

309 CO bond within the glycosidic linkage (Coimbra et al., 1999). Therefore, the
310 involvement of glycosidic bonds influenced the spectrum through changes in their
311 spatial arrangement, type and position.

312 However, some monosaccharides also showed spectral bands in the region
313 between 1100 to 1000 cm $^{-1}$ presenting overlapping with those of polysaccharides
314 (Figure1, Table 1).


A
PC2 (16%)
PC1 (50%)
RGPP
RGSP
GTAN
GAMA
MANB
DBAR
GALN
KPR2
KPO2
KPR3
KPO6
KPO3
KPR6
AP2
AP6
BP6
AP3
HGTN
BP3
CPPC
BP2
APPC
ARSB
LNAR
YGLU
AXMB
AXLB
MCCE
XYGT
ARHV
CGLU
XYGH
XYGO
XYBW
1
2
3
4
5
6
7
B
PC1
PC2
Loadings
Wavenumbers (cm-1)
1740
1600
1440
1410
1320
1230
1141
1097
1065
1035
988
970
914
831
807
C
PC2 (16%)
PC1 (45%)
KCO3
KCR3
KCR6
KCO6
KCRN
KCON
KCR2
KCO2
ACN
AC6
BC6
BCN
AC3
BC3
BC2
AC2
8
9
D
PC1
PC2
Loadings
Wavenumbers (cm-1)
1600
1526
1410
1164
1065
1035
988
1440
1410
1320
1141
1097
1230
914
831
807
970

315

Figure 2 PCA scores scatter plots of 1) commercial and extracted pectins, 2) rhamnogalacturonan, 3) galactan, 4) arabinan, 5) mannose- containing hemicelluloses, 6) xylose- containing hemicelluloses and 7) cellulose (A), and all cell wall materials (excluding monosaccharides): 8) kiwifruit cell walls, 9) apple and beet cell walls (C). ATR-FTIR spectra in the range 1800 to 800 cm^{-1} with their PCA loading profile of components PC1 and PC2 (B) and (D). Monosaccharides (Rha: rhamnose, Fuc: fucose, Ara: arabinose, Xyl: xylose, Man: mannose, Gal: galactose, Glc: glucose, Gal A: galacturonic acid); Hemicelluloses (ARHV: Rye Arabinoxylan (59% Xylose), AXMB: Wheet Arabinoxylan (64% Xylose), AXLB: Wheet Arabinoxylan (77% Xylose), XYBW: Xylan (Beechwood), MANB: 1,4-β-D-Mannan, XYGT: Xyloglucan (from tamarind seed), XYGO: Xyloglucan Oligosaccharides, XYGH: Xyloglucan Oligosaccharides (Hepta-, +Octa, +Nona-saccharides), GAMA: Galactomannan (Carob)); Pectins (APPC: Apple pectin, CPPC: Citrus peel pectin, HGTN: Homogalacturonan DM 70, GALN: Polygalacturonic acid, DBAR: Debranched arabinan, ARSB: Arabinan, LNAR: Linear arabinan, RGPP: Rhamnogalacturonan I, RGSP: Rhamnogalacturonan, GTAN: Galactan (Potato)); β-glucans: (MCCE: Microcrystalline cellulose; CGLU: curdlan, 1,3-beta-o-glucan; YGLU: Yeast beta-glucan).

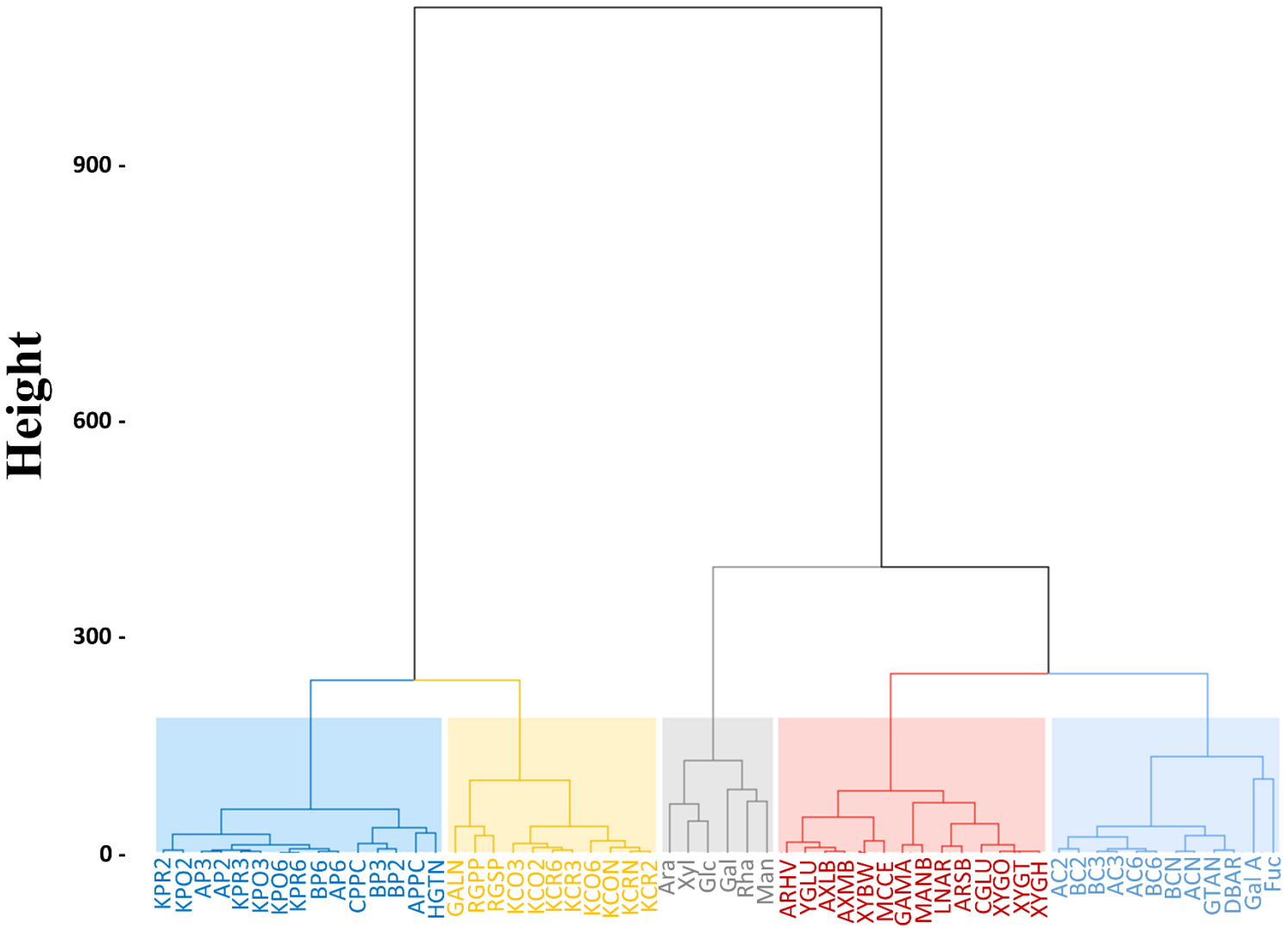
3.2. Discrimination of cell walls

Two multivariate analyses were used to study the discrimination of complex fruit cell walls in comparison with that obtained on standard compounds. A Principal component analysis (PCA) was carried out using the spectral data (Figure 2A) to identify the mapping of cell wall polysaccharides (excluding extracted cell walls and monosaccharides). PC1 and PC2 explained respectively 50% and 16% of the total variance. Along the PC1, the samples were well discriminated due to the different types of cell wall polysaccharides. Positive loadings of PC1 covered wavenumbers characteristic of xylose-containing (1035 cm^{-1}) hemicellulose and cellulose (988 cm^{-1}) (Figure 2B). The negative high values of PC1 were obtained for wavenumbers at 1740 and 1600 cm^{-1} characteristic of esterified and non-esterified carboxyl groups in pectins (Figure 2B), respectively and of 1440, 1320, 1230, 1141, 1097, 914 and 831 cm^{-1} bands characteristic of pectins (Figure 2B). In addition, negative PC1 loading appeared for wavenumbers at 1410 cm^{-1} characteristic for rhamnogalacturonan. The PC2 separated the esterified pectins and arabinan on the negative side (at the bottom) from the less esterified pectins, galactan and mannose-containing hemicelluloses (at the top). The bands at (1600 and 1141 cm^{-1}), 1410 cm^{-1}, and (1065 and 807 cm^{-1}) were attributed to respectively free carbonyl group of pectins, rhamnogalacturonan and mannose-containing hemicelluloses with a positive correlation to PC2. The region closes to 1740 and 970 cm^{-1} corresponded to the esterified pectins with a negative correlation to PC2.

345 The same approach was used on the extracted cell walls from apple, beet and
346 kiwifruit (Figure 2C). The cell wall polysaccharides were divided into nine groups: 1)
347 commercial and extracted pectins; 2) rhamnogalacturonans; 3) galactans; 4) arabinans;
348 5) mannose- containing hemicelluloses; 6) xylose- containing hemicelluloses; 7)
349 cellulose; 8) kiwifruit cell walls; 9) apple and beet cell walls. The first seven groups
350 are similar to Figure 2A, with the later addition of the extracted cell walls in the
351 center of the Figure 2C. This is consistent with the expected results as they contain
352 intact cell wall polysaccharide components. However, the extracted cell walls issued
353 from different species and extraction conditions were not well separated, especially
354 for apple and beet cell walls. How can we explain the lack of discrimination of cell
355 walls between apple and beet, whereas their composition differ significantly (Liu et
356 al., 2021)? As shown on the PCA, the kiwifruit cell walls were at the upper left in the
357 middle of the Figure 2C, while the apple and beet cell walls were at the bottom right
358 in the middle of the Figure 2C. This was probably linked to the high cellulose and
359 relatively low galacturonic acid for kiwifruit, and the high galacturonic acid for both
360 apple and beet (140 to 206 mg/g for apple, 141 to 225 mg/g for beet) (Liu et al., 2021).
361 However, the cell walls of apple and beet were not distinguished, in spite of marked
362 chemical and structural differences (Liu et al., 2021). Even a PCA performed on the
363 ATR-FTIR spectra of the cell walls of apple and beet alone did not separate them well
364 (data not shown). Beet cell walls are rich in arabinan (111 to 189 mg/g), contain
365 ferulic acid, and have only minor amounts of rhamnose, fucose, xylose and mannose.
366 Apple cell walls contain as much or more xylose (65 to 75 mg/g) and galactose (63

-67 mg/g) than arabinose (27 to 66 mg/g). However, the expected separation of apple and beet cell walls spectra by the characteristic peaks of arabinan (982 cm^{-1}) or galactan (1039 cm^{-1}) was not observed. Probably the characteristic peaks were not detected or were overlapped with those of other cell wall components, e.g., hemicelluloses or cellulose (Figure 1). This may be a limitation of ATR-FTIR in cell wall characterization, because arabinan and galactan are well known to change during ripening or processing.

PC loadings (Figure 2D) were very close to those obtained on the standard compounds (Figure 2B), probably reflecting the fact that the cell walls are by themselves combinations of pectins, hemicelluloses and cellulose. PC2 loadings had an extra shoulder peak at 1526 cm^{-1}, which may be attributed to lignin and ferulic acid, but occurring in combination with hemicelluloses (e.g., xylans and xyloglucans). The non-polysaccharide compounds in the cell wall, such as proteins (e.g., C = O of amides at 1655 cm^{-1} and N-H of amides at 1540, and 1234 cm^{-1}), lignins (e.g., 1520, 1410 and 921 cm^{-1}) and other phenolic compounds (e.g., 1520, 1440, 1284, 1196 and 1075 cm^{-1}) have absorption bands which mare interfere with those of polysaccharides. This needs to be taken into account when characterizing cell walls using ATR-FTIR. Moreover, the structure of xylans varies with the source of the plant, such as wheat or beechwood. As an example, some phenolic compounds can couple beechwood xylan chains via ferulate dimerization (dehydrodiferulate cross-links) and/or incorporation into lignin, thus affecting their spectral bands (Kačuráková et al., 1999).


Height
900 -
600 -
300 -
0 -
KPR2
KPO2
AP3
AP2
KPR3
KPO3
KPO6
KPR6
BP6
AP6
CPPC
BP3
BP2
APPC
HGTN
GALN
RGPP
RGSP
KCO3
KCO2
KCR6
KCR3
KCO6
KCON
KCRN
KCR2
Ara
Xyl
Glc
Gal
Rha
Man
ARHV
YGLU
AXLB
AXMB
XYBW
MCCE
GAMA
MANB
LNAR
ARSB
CGLU
XYGO
XYGT
XYGH
AC2
BC2
BC3
AC3
AC6
BC6
BCN
ACN
GTAN
DBAR
Gal A
Fuc

388

Figure 3 Hierarchical cluster analysis dendrogram of 58 cell wall and cell wall polysaccharide and monosaccharide samples based on average ATR-FTIR spectra in the range 4000 to 600 cm^{-1} using Ward's clustering algorithm with Euclidian distance. From left to right, the groups are (i) commercial and extracted pectins; (ii) kiwifruit cell walls and RG; (iii) monosaccharides; (iv) cellulose and hemicelluloses and (v) apple and beet cell walls. Monosaccharides (Rha: rhamnose, Fuc: fucose, Ara: arabinose, Xyl: xylose, Man: mannose, Gal: galactose, Glc: glucose, Gal A: galacturonic acid); Hemicelluloses (ARHV: Rye Arabinoxylan (59% Xylose), AXMB: Wheet Arabinoxylan (64% Xylose), AXLB: Wheet Arabinoxylan (77% Xylose), XYBW: Xylan (Beechwood), MANB: 1,4-β-D-Mannan, XYGT: Xyloglucan (from tamarind seed), XYGO: Xyloglucan Oligosaccharides, XYGH: Xyloglucan Oligosaccharides (Hepta-, +Octa, +Nona-saccharides), GAMA: Galactomannan (Carob)); Pectins (APPC: Apple pectin, CPPC: Citrus peel pectin, HGTN: Homogalacturonan DM 70, GALN: Polygalacturonic acid, DBAR: Debranched arabinan, ARSB: Arabinan, LNAR: Linear arabinan, RGPP: Rhamnogalacturonan I, RGSP: Rhamnogalacturonan, GTAN: Galactan (Potato)); β-glucans: (MCCE: Microcrystalline cellulose; CGLU: curdlan, 1,3-beta-o-glucan; YGLU: Yeast beta-glucan).

Hierarchical cluster analysis (HCA) is widely applied as an unsupervised classification method to calculate distances between samples and cluster them according to this distance, based here on their spectra (Granato, Santos, Escher, Ferreira, & Maggio, 2018). HCA highlighted five groups (Figure 3). Group 1 clustered samples with high galacturonic acid contents from apple, beet, citrus and kiwifruit pectins. Group 2 contained samples with linear pectins and less side chains from kiwifruit cell walls, polygalacturonic acid and rhamnogalacturonan. Group 3 associated monosaccharide samples with absence of glycosidic bonds. Group 4 clustered samples including hemicelluloses and cellulose. Group 5 clustered samples with high methylated pectins and rich in arabinose and galactose, extracted from apple, beet cell walls, galactan and arabinan. Therefore, ATR-FTIR spectroscopy coupled with chemometrics allowed a good discrimination of cell walls related to their compositions and structures, giving some classes according to the different kinds of pectins, hemicelluloses and cellulose.

The cell walls represented a polymer system in a complex mixture with a diversity of both compositions and structures. PCA and HCA performed on the spectral data allowed to discriminate samples according to their cell wall polysaccharides. ATR-FTIR could be considered as a fast and easy way to distinguish different types of cell walls. Due to their complexity and the numerous spectral bands for each component, with the addition of overlapping, it was therefore difficult to assign each band to a compound chemical structure. However, the observed changes in intensity or presence/absence of some bands reflected the differences in

419 composition between the cell walls. And, slight changes in the strength and position
420 of individual bands could also be due to the different conformations of cell wall
421 polymers and the interactions between individual components.

422 **4. Conclusions**

423 ATR-FTIR spectra in the region between 1800 and 800 cm^{-1} combined with PCA
424 has been widely applied to study the main polysaccharides present in the complex cell
425 walls. However, it is not always possible to analyze the structural changes in cell wall
426 polysaccharides at the molecular level and not all absorption bands allow
427 differentiation. Their complex structures, compositions, glycosidic linkage patterns
428 and the interactions between polysaccharides (or even with polyphenols and proteins)
429 make the application of ATR-FTIR to plant cell walls still challenging.

430 What can be determined is that: 1) xylan, arabinoxylan and xyloglucan all had the
431 same characteristic band at 1035 cm^{-1}; 2) cellulose showed a characteristic band at
432 988 cm^{-1}; 3) the mannan and galactomannan were identified by the bands at 1065 and
433 807 cm^{-1}; 4) the degree of methylation of pectin homogalacturonans was easily
434 determined by the relative height of the two bands at 1740 and 1600 cm^{-1}. According
435 to these results, the analysis of purified cell wall polysaccharides could be easily and
436 successfully performed directly with these bands giving information on the main cell
437 wall compounds: pectins, hemicelluloses and cellulose.

438 However, some difficulties remain to identify intact cell wall components and in
439 particular to discriminate cell walls of apple and beet in relation to the bands of

arabinan and galactan. The main chain made of arabinan did not give available characteristic peaks. The specific band of galactan at 1039 cm^{-1} was overlapped with the bands of hemicelluloses. Therefore, the application of ATR-FTIR spectroscopy for the characterization of cell wall polysaccharides requires more in-depth research and should be used in combination with other analytical techniques.

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