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

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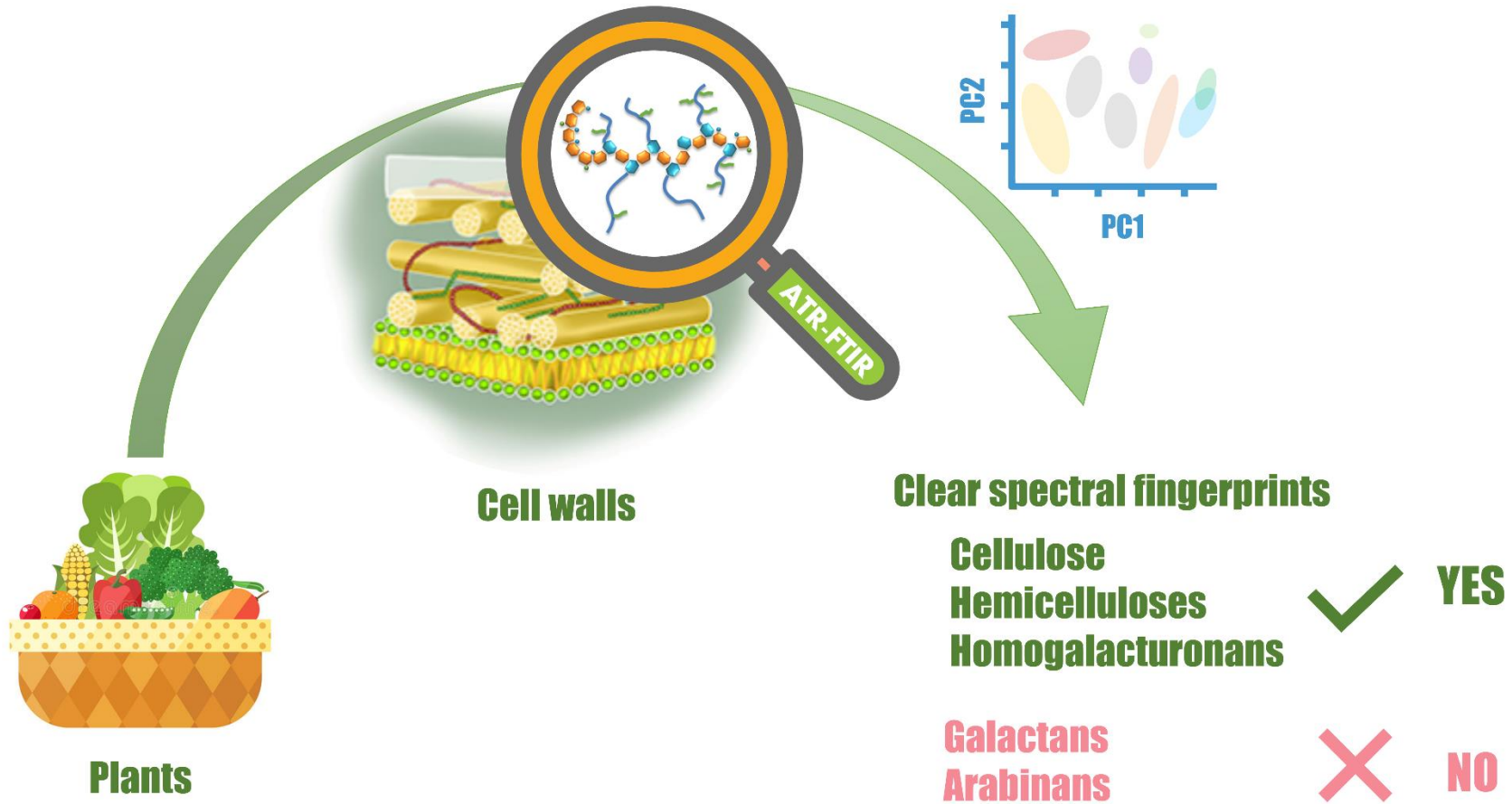
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1

Graphical Abstract



2

3 **Abstract**

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

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

21 **Abbreviations:**

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

24 Analysis; HCA: Hierarchical Cluster Analysis.

25 **1. Introduction**

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

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

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

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

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

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

90 **2. Materials and methods**

91 2.1. Monosaccharide and polysaccharide samples

92 Monosaccharides (D-(+)-Arabinose, D-(-)-Fucose, D-(+)-Xylose, D-(+)-Mannose,
93 L-Rhamnose, D-(+)-Glucose, and D-(+)-galactose) and D-(+)-Galacturonic acid
94 monohydrate were obtained from Fluka (Buchs, Switzerland). Arabinan (sugar beet),
95 linear 1,5- α -L-arabinan (sugar beet), debranched arabinan, galactan (potato),
96 rhamnogalacturonan I (from potato pectic fibre), and rhamnogalacturonan (from
97 soybean pectic fibre), xylan, arabinoxylan, glucomannan, and xyloglucan were
98 provided from Megazyme (Bray, Ireland). Commercial apple and citrus peel pectins
99 (degrees of methylation ~75%), microcrystalline cellulose and poly-galacturonic acid
100 were provided by Sigma-Aldrich (Deisenhofen, Germany). Homogalacturonan DM
101 70 was supplied by Watrelot et al. (2013). The common names of cell wall
102 components and their abbreviations used in this study are presented in Table 1.

103 Native and modified cell walls and pectins from apple, beet and kiwifruit were
104 supplied and characterized by Liu et al. (2021). The native cell wall samples were
105 named as apple cell wall (ACN), beet cell wall (BCN), ripe kiwifruit cell wall (KCRN)
106 and overripe kiwifruit cell wall (KCON), and samples after boiling at pH 2.0, 3.5, and
107 6.0 are designated (AC, BC, KCR or KCO) - 2, (AC, BC, KCR or KCO) - 3, and (AC,
108 BC, KCR or KCO) - 6, respectively. Extracted pectins at pH 2.0, 3.5 and 6.0 from
109 apple, beet and two kiwifruit cell walls at pH 2.0, 3.5, and 6.0 are designated (AP, BP,
110 KPR or KPO) - 2, (AP, BP, KPR or KPO) - 3, and (AP, BP, KPR or KPO) - 6,
111 respectively.

112 **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
 113 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	1334 _s , 1140 _s , 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
	Wheat Arabinoxylan (64% xylose)	AXMB	1164, 1035 _{vs} , 983 _s , 890
	Wheat 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 , 1074 _s , 1014 _{vs} , 954
Rhamnogalacturonan (from soybean pectic fibre)	RGSP	1600 _s , 1410, 1141 _s , 1097 _s , 1074 _s , 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.

115 **2.2. Characterization of carbohydrate composition**

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

128 **2.3. ATR-FTIR spectra**

129 All cell wall polysaccharide samples, in the form of dry powder, were stored in
130 P₂O₅ atmosphere before analysis to remove residual water. ATR-FTIR spectra data
131 (4000 to 600 cm⁻¹) were acquired at room temperature in a Tensor 27 FTIR
132 spectrometer (Bruker Optics®, Wissembourg, France), using a single-reflectance
133 horizontal ATR cell (Golden Gate with a diamond crystal, Bruker Optics®) equipped
134 with a system to press the dried homogenized samples on the crystal surface (Bureau
135 et al. 2012). Each sample was analyzed three times (using after homogenization three

136 different aliquots of the powders) to consider its heterogeneity, and each spectrum was
137 the average of 16 scans. Spectral pre-processing and data treatment using multivariate
138 analyses were performed with MATLAB 7.5 (Mathworks Inc. Natick, MA) software
139 using the SAISIR package (Cordella & Bertrand, 2014). The spectral data were
140 pretreated with baseline correction and standard normal variate (SNV) to correct
141 multiplicative interferences and variations in baseline shift before any multivariate
142 analysis.

143 **2.4. Statistical analysis**

144 All biochemical analyses were presented as mean values of analytical triplicates
145 and the reproducibility of the results was expressed as pooled standard deviations
146 (Pooled SD). Pooled SD was calculated per series of replicates using the sum of
147 individual variances weighted by the individual degrees of freedom (Box, Hunter, &
148 Hunter, 1978). A PCA was applied on the ATR-FTIR spectra in the range between
149 1800 and 800 cm^{-1} in order to study the repartition of the cellulose, hemicelluloses
150 and pectins in a space according to their composition and absorption bands. Spectral
151 data pre-processing and PCA were performed using MATLAB 7.5 (Mathworks Inc.
152 Natick, MA) software using the SAISIR package (Cordella & Bertrand, 2014). HCA
153 was performed using R software using FactoMineR (for computing) and factoextra
154 (for visualizing the results) (R Core Team., 2014).

155 **3. Results and discussion**

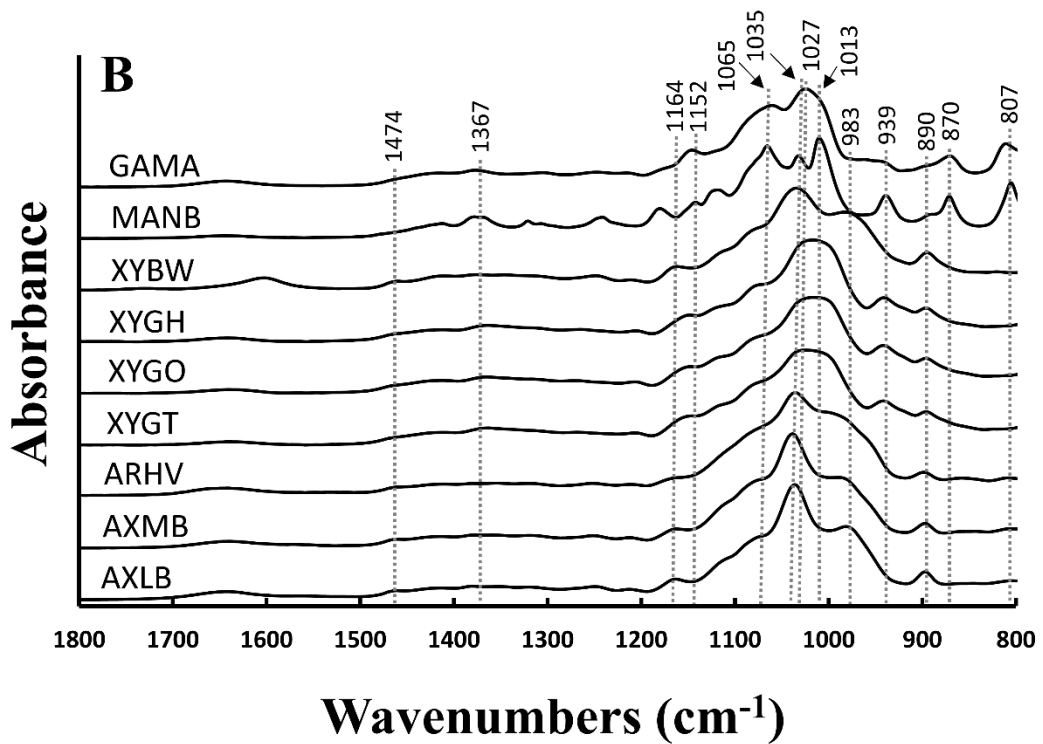
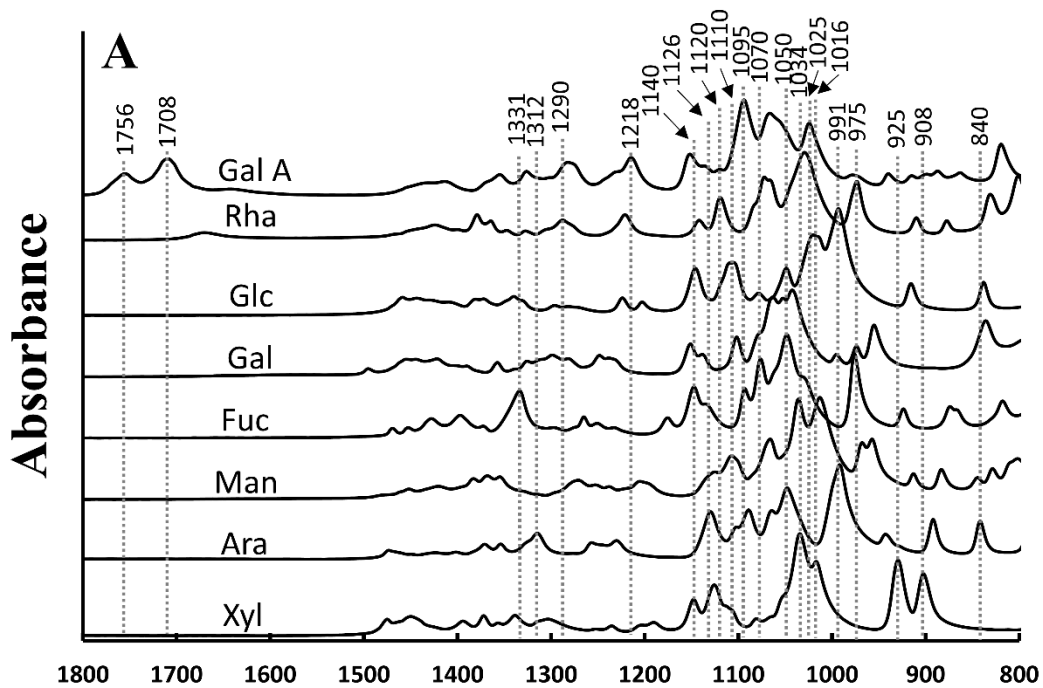
156 **3.1. Characteristic bands of cell wall polysaccharides in the ATR-FTIR spectra**

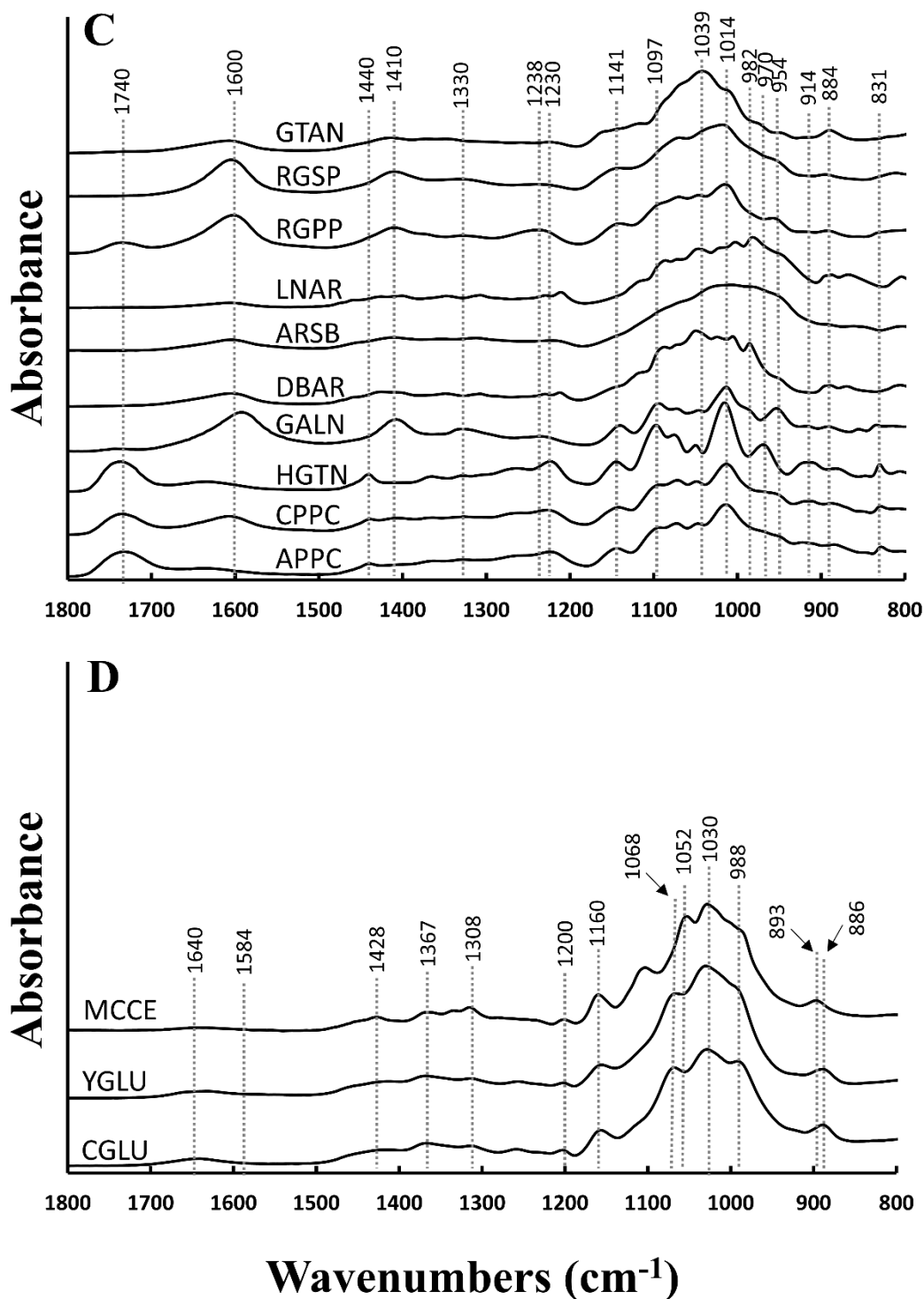
157 The compositions of the 58 cell wall polysaccharides from extracted and
158 commercial origin were determined in this study by both, the classical methods (Table
159 2, see Liu et al., 2021 for cell walls and extracted pectins) and ATR-FTIR
160 spectroscopy (Table 1, Figure 1). Detailed peak positions 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.

172 **Table 2.** Composition of extracted cell walls and pectins from fruits and vegetables and commercial purified
 173 cellulose, hemicelluloses and pectin components (mg/g dry weight, except for degree of methylation expressed
 174 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
<i>Pooled SD</i>	<i>1.0</i>	<i>0.3</i>	<i>8.3</i>	<i>7.0</i>	<i>5.8</i>	<i>2.3</i>	<i>8.6</i>	<i>4.0</i>	<i>2.5</i>	<i>3.2</i>

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

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

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)	-	

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

198 Hemicelluloses (ARHV: Rye Arabinoxylan (59% Xylose), AXMB: Wheat Arabinoxylan (64% Xylose), AXLB: Wheat Arabinoxylan (77% Xylose), XYBW: Xylan (Beechwood), MANB:
199 1,4-β-D-Mannan, XYGT: Xyloglucan (from tamarind seed), XYGO: Xyloglucan Oligosaccharides, XYGH: Xyloglucan Oligosaccharides (Hepta-, +Octa, +Nona-saccharides), GAMA:
200 Galactomannan (Carob)); Pectins (APPC: Apple pectin, CPPC: Citrus peel pectin, HGTN: Homogalacturonan DM 70, GALN: Polygalacturonic acid, DBAR: Debranched arabinan, ARSB:
201 Arabinan, LNAR: Linear arabinan, RGPP: Rhamnogalacturonan I, RGSP: Rhamnogalacturonan, GTAN: Galactan (Potato)); β-glucans (MCCE: Microcrystalline cellulose; CGLU: curdlan,
202 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.

203 **3.1.1. Monosaccharides**

204 Monosaccharides, without any glycosidic linkage to other units, are the simplest
205 component units of the cell wall polysaccharides, and have been shown to be
206 important in polymer analysis and structure elucidation (Wiercigroch et al., 2017). It
207 was necessary to consider here hexopyranoses (glucose, mannose and galactose),
208 dehydro-hexopyranoses (rhamnose and fucose), pentopyranose (xylose), and
209 pentofuranose (arabinose), standards with different positions of hydroxyl groups on
210 C-2, C-3, and C-4 (Figure 1A). The spectra of pentoses (arabinose and xylose) were
211 dominated by a band at 991 and 1034 cm^{-1} , respectively, mainly due to the $\nu(\text{C-C})$,
212 $\nu(\text{C-O})$ and $\beta(\text{C-CH})$ vibrations (Edwards, 1976). For hexoses, D-(+)-glucose was
213 observed by the main band at 991 cm^{-1} . D-(+)-galactose is almost identical in structure
214 to D-(+)-glucose, with a different orientation in the C-4 OH group, but with a distinct
215 spectral difference due to their free crystalline structure (Figure 1A). Similarly, the
216 spectrum of other hexopyranoses with different hydroxyl group orientations on C-2,
217 C-3, and C-4 was also significantly different. Therefore, the relative positions of
218 (C-OH) essentially affected the spectrum through variations in their spatial
219 arrangement and interactions. The detailed ATR-FTIR bands of monosaccharides with
220 modes assignments are listed in Table 1.

221 **3.1.2. Pectic components**

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

224 and galactan). The spectra of apple pectins (APPC) and citrus peel pectins (CPPC)
225 had very similar characteristic bands (Figure 1C) centering at 1740, 1600, 1440, 1230,
226 1141, 1097, 1014, 954, 914 and 831 cm^{-1} . They have also similar levels of neutral
227 sugars, galacturonic acid and degree of methylation (Table 2). The band at 1740 cm^{-1}
228 is assigned to the esterified galacturonic acids. This band is characteristic of pectins
229 with a high degree of methylation (DM) such as high methylated homogalacturonan
230 (DM=70) (HGTM). Inversely, the band of poly-galacturonic acid (GALN), which is
231 non-esterified, was at 1600 cm^{-1} due to the COO^- carboxylate ion stretching. These
232 two main characteristic bands of pectins are fixed and similar to those previously
233 reported (Coimbra et al., 1999; Filippov & Kohn, 1975; Gnanasambandam, R.,
234 Proctor, 2000; Kacurakova et al., 2000; Reintjes, Musco, & Joseph, 1962;
235 Szymanska-Chargot et al., 2015; Wojdyło, Figiel, Lech, Nowicka, & Oszmiański,
236 2014).

237 For a further interpretation of these spectra, pure pectic components were
238 characterized in the same conditions. Notably, although these parts of the
239 polysaccharide components were not a complete pectin structure found in the plant,
240 they are representative of different subunits of the pectins. For rhamnogalacturonan
241 (RGPP and RGSP), the band at 1014 cm^{-1} was the strongest (Figure 1C). However,
242 this peak overlapped with the main peak of commercial pectins (APPC and CPPC).
243 Some other specific bands, assigned to rhamnogalacturonan (RGPP and RGSP) can
244 be used such as 1410, 1238 and 1074 cm^{-1} . In fact, RGSP contained more xylose than
245 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

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

269 **3.1.3. Hemicelluloses and cellulose**

270 Bands at 1474, 1367, 1164, 1152, 1065, 1035, 1027, 1013, 983, 939, 890, 870 and
271 807 cm^{-1} were identified for hemicelluloses (Figure 1B). Xylan (XYBW) had
272 characteristic absorption bands at 1035 cm^{-1} and 983 cm^{-1} . The arabinoxylans with
273 different xylose contents (Table 2), e.g., ARHV (59%), AXMB (64%) and AXLB
274 (77%), showed a main band at 1035 cm^{-1} for which the intensity varied with the
275 xylose content. Xyloglucan (XYGT, XYGO and XYGH) absorbed in the region of
276 1040-1010 cm^{-1} , like hemicelluloses and cellulose making it difficult to be identified.
277 Galactomannan (GAMA) and mannan (MANB) had bands at 1027 and 1013 cm^{-1} ,
278 respectively. In addition, they had a common secondary band at 1065 cm^{-1} , and two
279 specific peaks at 870 and 807 cm^{-1} (also found in softwood sample, Simonović,
280 Stevanic, Djikanović, Salmén, & Radotić, 2011), which allowed them to be
281 distinguished from others (Figure 1B). Therefore, the bands which could be assigned
282 to xylose- and mannose- containing hemicelluloses were 1474, 1164, 1035, 983 and
283 890 cm^{-1} for xylose-, and 1152, 1065, 1027, 1013, 939, 870 and 807 cm^{-1} for
284 mannose-, respectively (Table 3). This was confirmed for xylose- containing
285 hemicelluloses including XYBW, ARHV, AXMB, AXLB, XYGT, XYGO and XYGT,
286 and mannose- containing hemicelluloses including MANB and GAMA.

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

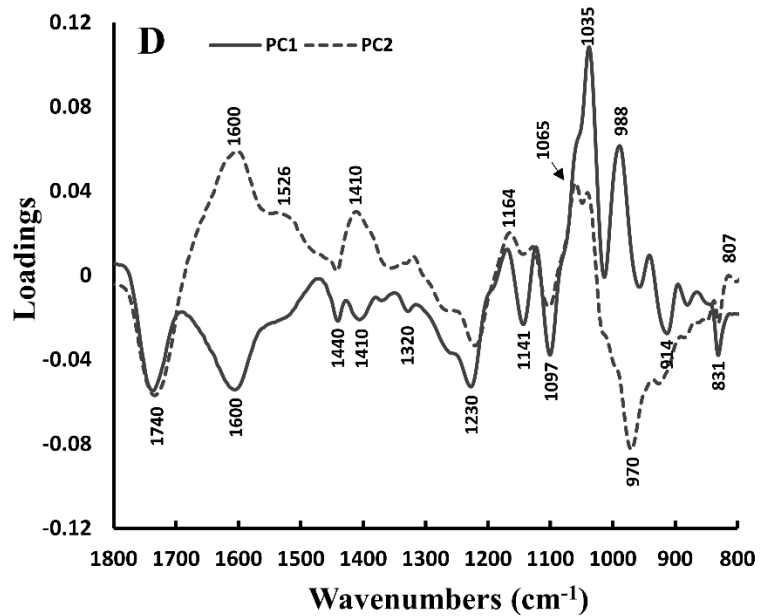
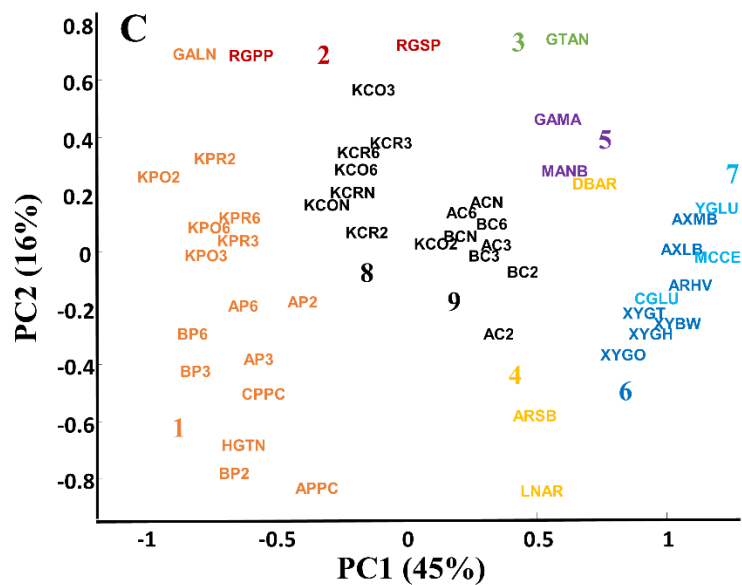
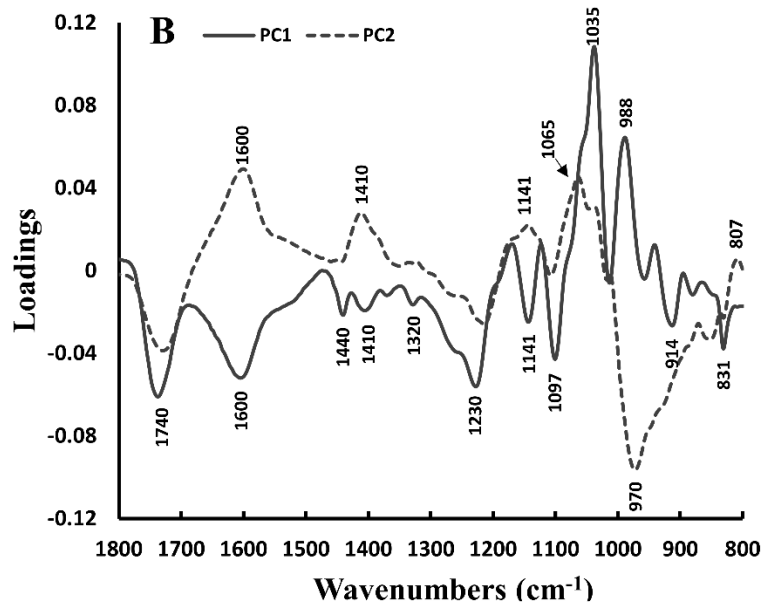
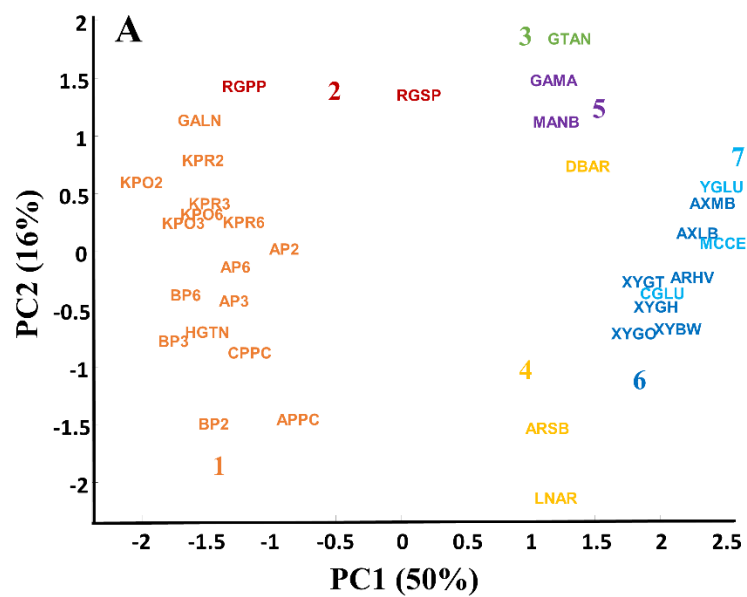
288 1640, 1428, 1367, 1320, 1308, 1200, 1160, 1052, 1030, 988 and 893 cm^{-1} (Figure 1D),
289 whereas bands at 1068 and 886 cm^{-1} (instead of 1052 and 893 cm^{-1}) were identified
290 for β -(1 \rightarrow 3)-glucans (YGLU and CGLU). These slight differences for two peaks may
291 contribute to distinguish 1,3- β - and 1,4- β - bonds.

292 **3.1.4. Glycosidic linkage**

293 The glycosidic linkages are an important characteristic structure in
294 polysaccharides and influence the spectral changes in aqueous solutions (Jockusch et
295 al., 2004; Kačuráková & Mathlouthi, 1996; Kanou, Nakanishi, Hashimoto, &
296 Kameokaj, 2005; Nikonenko, Buslov, Sushko, & Zhbakov, 2000; Wiercigroch et al.,
297 2017). For example, the spectra of the glycosidic bonds with different positions and
298 configurations of oligo- and poly- saccharides in aqueous solution differ markedly
299 from monosaccharides, with stretching vibrations [$\nu(\text{CO})$] of the C-O-C glycosidic
300 linkage being the marker of the polysaccharide configuration. These vibrations appear
301 in the two spectral ranges 1160-1130 and 1000-960 cm^{-1} (Kacurakova et al., 2000;
302 Kačuráková & Mathlouthi, 1996; Wiercigroch et al., 2017). Similarly, these bands
303 appear in cell wall polysaccharides in the solid states when compared with
304 monosaccharides (Figure 1). For xylose units with β -(1 \rightarrow 4) glycosidic linkage
305 (XYBW) bands were found at about 1164 cm^{-1} , for glucose units with β -(1 \rightarrow 4)
306 glycosidic linkage (YGLU, CGLU and MCCE) bands were found at 1160 cm^{-1} and
307 for mannose units with β -(1 \rightarrow 4) glycosidic linkage (MANB) bands were found at
308 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).



316 **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
317 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
318 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:
319 glucose, Gal A: galacturonic acid); Hemicelluloses (ARHV: Rye Arabinoxylan (59% Xylose), AXMB: Wheat Arabinoxylan (64% Xylose), AXLB: Wheat Arabinoxylan (77% Xylose), XYBW:
320 Xylan (Beechwood), MANB: 1,4- β -D-Mannan, XYGT: Xyloglucan (from tamarind seed), XYGO: Xyloglucan Oligosaccharides, XYGH: Xyloglucan Oligosaccharides (Hepta-, +Octa,
321 +Nona-saccharides), GAMA: Galactomannan (Carob)); Pectins (APPC: Apple pectin, CPPC: Citrus peel pectin, HGTN: Homogalacturonan DM 70, GALN: Polygalacturonic acid, DBAR:
322 Debranched arabinan, ARSB: Arabinan, LNAR: Linear arabinan, RGPP: Rhamnogalacturonan I, RGSP: Rhamnogalacturonan, GTAN: Galactan (Potato)); β -glucans: (MCCE: Microcrystalline
323 cellulose; CGLU: curdlan, 1,3-beta-o-glucan; YGLU: Yeast beta-glucan).

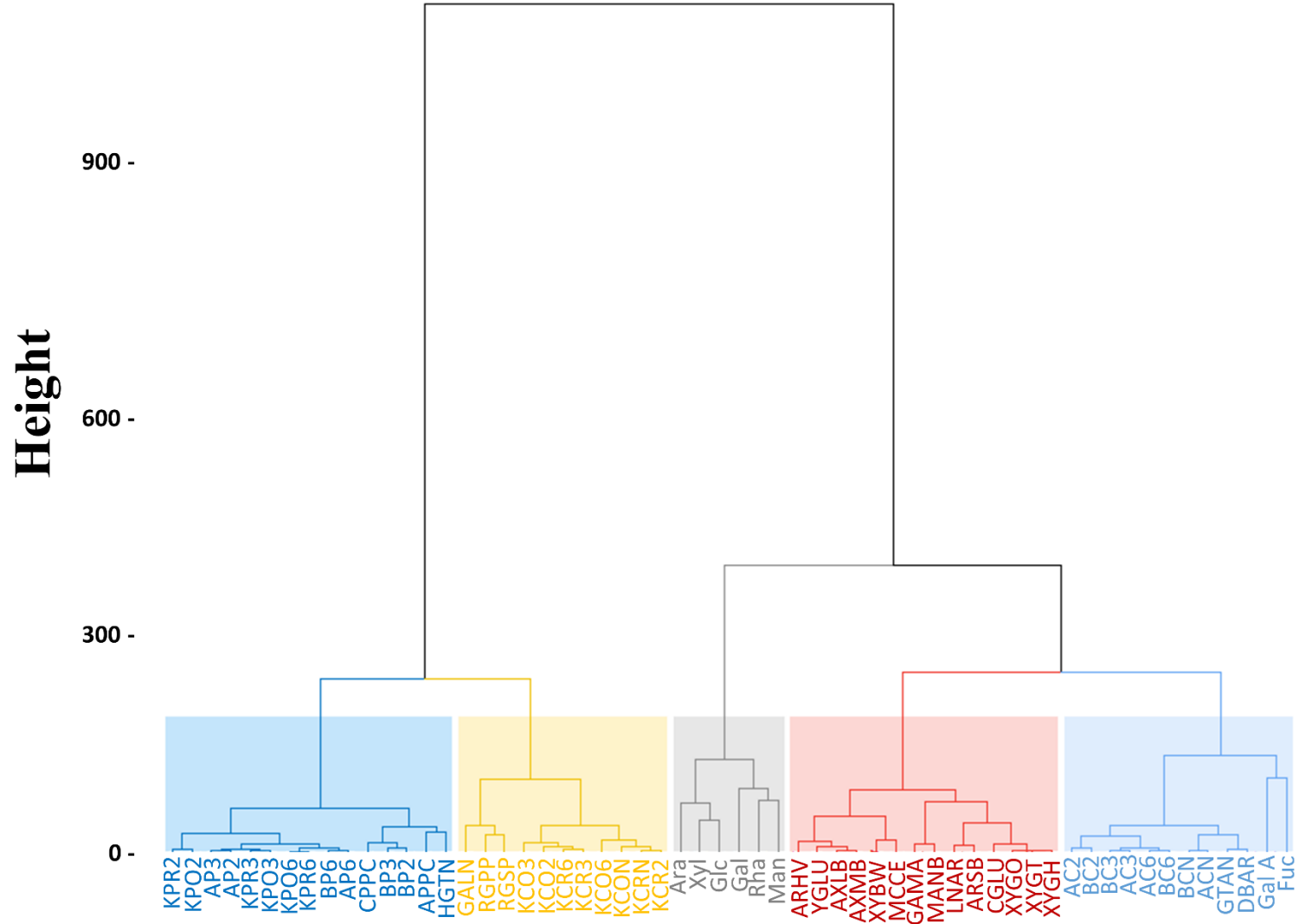
324 3.2. Discrimination of cell walls

325 Two multivariate analyses were used to study the discrimination of complex fruit
326 cell walls in comparison with that obtained on standard compounds. A Principal
327 component analysis (PCA) was carried out using the spectral data (Figure 2A) to
328 identify the mapping of cell wall polysaccharides (excluding extracted cell walls and
329 monosaccharides). PC1 and PC2 explained respectively 50% and 16% of the total
330 variance. Along the PC1, the samples were well discriminated due to the different
331 types of cell wall polysaccharides. Positive loadings of PC1 covered wavenumbers
332 characteristic of xylose-containing (1035 cm^{-1}) hemicellulose and cellulose (988 cm^{-1})
333 (Figure 2B). The negative high values of PC1 were obtained for wavenumbers at 1740
334 and 1600 cm^{-1} characteristic of esterified and non-esterified carboxyl groups in
335 pectins (Figure 2B), respectively and of 1440, 1320, 1230, 1141, 1097, 914 and 831
336 cm^{-1} bands characteristic of pectins (Figure 2B). In addition, negative PC1 loading
337 appeared for wavenumbers at 1410 cm^{-1} characteristic for rhamnogalacturonan. The
338 PC2 separated the esterified pectins and arabinan on the negative side (at the bottom)
339 from the less esterified pectins, galactan and mannose-containing hemicelluloses (at
340 the top). The bands at (1600 and 1141 cm^{-1}), 1410 cm^{-1} , and (1065 and 807 cm^{-1})
341 were attributed to respectively free carbonyl group of pectins, rhamnogalacturonan
342 and mannose-containing hemicelluloses with a positive correlation to PC2. The region
343 closes to 1740 and 970 cm^{-1} corresponded to the esterified pectins with a negative
344 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

367 -67 mg/g) than arabinose (27 to 66 mg/g). However, the expected separation of apple
368 and beet cell walls spectra by the characteristic peaks of arabinan (982 cm^{-1}) or
369 galactan (1039 cm^{-1}) was not observed. Probably the characteristic peaks were not
370 detected or were overlapped with those of other cell wall components, e.g.,
371 hemicelluloses or cellulose (Figure 1). This may be a limitation of ATR-FTIR in cell
372 wall characterization, because arabinan and galactan are well known to change during
373 ripening or processing.

374 PC loadings (Figure 2D) were very close to those obtained on the standard
375 compounds (Figure 2B), probably reflecting the fact that the cell walls are by
376 themselves combinations of pectins, hemicelluloses and cellulose. PC2 loadings had
377 an extra shoulder peak at 1526 cm^{-1} , which may be attributed to lignin and ferulic acid,
378 but occurring in combination with hemicelluloses (e.g., xylans and xyloglucans). The
379 non-polysaccharide compounds in the cell wall, such as proteins (e.g., C = O of
380 amides at 1655 cm^{-1} and N-H of amides at 1540, and 1234 cm^{-1}), lignins (e.g., 1520,
381 1410 and 921 cm^{-1}) and other phenolic compounds (e.g., 1520, 1440, 1284, 1196 and
382 1075 cm^{-1}) have absorption bands which may interfere with those of polysaccharides.
383 This needs to be taken into account when characterizing cell walls using ATR-FTIR.
384 Moreover, the structure of xylans varies with the source of the plant, such as wheat or
385 beechwood. As an example, some phenolic compounds can couple beechwood xylan
386 chains via ferulate dimerization (dehydrodiferulate cross-links) and/or incorporation
387 into lignin, thus affecting their spectral bands (Kačuráková et al., 1999).



389 **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
390 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)
391 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:
392 galacturonic acid); Hemicelluloses (ARHV: Rye Arabinoxylan (59% Xylose), AXMB: Wheat Arabinoxylan (64% Xylose), AXLB: Wheat Arabinoxylan (77% Xylose), XYBW: Xylan
393 (Beechwood), MANB: 1,4-β-D-Mannan, XYGT: Xyloglucan (from tamarind seed), XYGO: Xyloglucan Oligosaccharides, XYGH: Xyloglucan Oligosaccharides (Hepta-, +Octa,
394 +Nona-saccharides), GAMA: Galactomannan (Carob)); Pectins (APPC: Apple pectin, CPPC: Citrus peel pectin, HGTN: Homogalacturonan DM 70, GALN: Polygalacturonic acid, DBAR:
395 Debranched arabinan, ARSB: Arabinan, LNAR: Linear arabinan, RGPP: Rhamnogalacturonan I, RGSP: Rhamnogalacturonan, GTAN: Galactan (Potato)); β-glucans: (MCCE: Microcrystalline
396 cellulose; CGLU: curdlan, 1,3-beta-o-glucan; YGLU: Yeast beta-glucan).

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

411 The cell walls represented a polymer system in a complex mixture with a
412 diversity of both compositions and structures. PCA and HCA performed on the
413 spectral data allowed to discriminate samples according to their cell wall
414 polysaccharides. ATR-FTIR could be considered as a fast and easy way to distinguish
415 different types of cell walls. Due to their complexity and the numerous spectral bands
416 for each component, with the addition of overlapping, it was therefore difficult to
417 assign each band to a compound chemical structure. However, the observed changes
418 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

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

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