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Alkaline extractability of pectic arabinan and galactan and their mobility in sugar beet and potato cell walls

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Abstract

The organisation of sugar beet and potato cell walls was studied using alkaline extractions following a response surface methodology, simultaneously with solid-state ^{13}C NMR spectroscopy. The influence of two extraction parameters: NaOH concentration (0.05, 0.275, 0.5 M) and temperature (40, 65, 90 °C) on the composition (neutral and acidic sugars) of the residues recovered was established. Treatments of increasing harshness progressively washed off non-cellulosic polysaccharides from the cell walls. Alkaline treatments applied to sugar beet cell wall material (SB-CWM) revealed the presence of diverse pectin populations. The existence of distinct pectin populations in potato cell wall material (P-CWM) was less outstanding. Solid-state ^{13}C NMR applied to SB-CWM and P-CWM and residues after treatment by 0.275 M NaOH at 65 °C revealed two fractions of pectic arabinan and galactan side chains. One fraction was highly mobile, whereas the other one displayed restricted mobility.

Keywords: Pectin; Arabinan; Galactan; Xyloglucan; Experimental design; Solid-state ^{13}C NMR spectroscopy

1. Introduction

Plant cell wall is a highly complex and dynamic structure that undergoes changes during cell division, expansion and differentiation (Carpita & Gibeaut, 1993). In dicotyledons cell walls, two different layers can be distinguished. The most external one, containing mostly pectic polysaccharides, constitutes the middle lamella. The inner one, characterised by a higher degree of organisation, is known as the primary cell wall. Primary walls of higher plant tissues are predominantly composed of polysaccharides (O'Neill & York, 2003).

Pectins are complex carbohydrate polymers consisting of a backbone of (1 → 4)-linked α-D-GalA*p* units. This linear homogalacturonan (HG) region is interrupted by a type I rhamnogalacturonan (RG I) consisting of the repeating disaccharide unit: (1 → 2)-α-L-Rha*p*-(1 → 4)-α-D-GalA*p* (Renard, Crépeau, & Thibault, 1995). A type II rhamnogalacturonan (RG II), a complex polysaccharide composed of GalA, Rha, Gal and some unusual sugars, constitutes also a part of the pectic molecule (Ishii & Matsunaga, 2001). RG II, although present as a quantitatively minor pectic subunit, is thought to play a key role in cell wall architecture (O'Neill, Ishii, Albersheim, & Darvill, 2004). Primary cell wall pectins are characterised by a high quantity of neutral sugar side chains mostly composed of Ara and Gal that are mainly branched at *O*-4 of Rha residues (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Besides pectins, the other major polysaccharide of primary cell wall is cellulose. The cellulosic linear chains of parallel

Abbreviations: CP/MAS, cross polarisation magic angle spinning; C-WM, cell wall material; HG, homogalacturonan; P, potato; P-CWM, potato cell wall material; RG I, rhamnogalacturonan I; RG II, rhamnogalacturonan II; SB, sugar beet; SB-CWM, sugar beet cell wall material; SPE/MAS, single pulse excitation magic angle spinning.

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alignment are composed of (1 → 4)-linked β-D-Glc*p* residues, which are tightly linked by hydrogen bonds to form microfibrils. Xyloglucan, the most abundant hemicellulosic polysaccharide in the primary cell walls of dicotyledons, is composed of a cellulose-like backbone, branched at *O*-6 by α-D-Xyl*p* residues, which can be further substituted at *O*-2 by β-D-Gal*p* residues (Fry, 1989). Some of the Gal residues may be substituted at *O*-6 by α-D-Fuc*p* residues.

Although the fine structure of primary cell wall polysaccharides is rather well known, the way in which they are assembled within the cell wall is still controversial. The most common "sticky network" model (Cosgrove, 2000) assumes that the xyloglucan chains not only bind to the cellulose surface but also cross-link the cellulose microfibrils. However, some theoretical difficulties with the "sticky network" model and discrepancies between predicted behaviour and experimental results were reported (Cosgrove, 2000; Thompson, 2005). Moreover, in some dicotyledons cell walls (sugar beet and celery for example), the very low xyloglucan content was claimed to be insufficient to provide neither complete coating of cellulose microfibrils surface nor tethering of cellulose microfibrils (Renard & Jarvis, 1999; Thimm et al., 2002). It is noteworthy that pectins are particularly abundant polymers in these cell walls so that they could replace xyloglucans in binding the gaps between microfibrils and maintaining their separation. Pectic arabinan and galactan side chains were indeed recently shown to form in vitro associations with cellulose (Zykwinska, Ralet, Garnier, & Thibault, 2005).

In the present work, we used an extraction procedure following an experimental central composite design simultaneously with solid-state ^{13}C NMR spectroscopy. The experimental design applied allowed us to study the influence of alkaline extraction parameters (concentration and temperature) on the composition of the arabinan- and galactan-rich pectins remaining in the cellulose-enriched residues. Alkaline conditions, contrary to acidic ones, allow the preservation of pectic neutral sugar side chains and are particularly advantageous to extract HG regions by β-elimination and oxidative peeling reactions (Bonnin et al., 2001). To better understand how the primary cell wall polysaccharides interact together, solid-state NMR was applied (Rondeau-Mouro, Crepeau, & Lahaye, 2003a). Solid-state NMR can provide data on the dynamic of cell wall polysaccharides by the analysis of their mobility in situ (Bootten, Harris, Melton, & Newman, 2004; Ha, Apperley, & Jarvis, 1997). This can be done by comparing single pulse excitation magic angle spinning (SPE/MAS) experiment to CP/MAS one (Lahaye, Rondeau-Mouro, Deniaud, & Buléon, 2003; Rondeau-Mouro et al., 2003a). SPE/MAS detects the highly mobile polymers while CP/MAS reveal the cell wall components of limited mobility. These experiments were conducted to examine the structure of untreated cell walls and their residues recovered after alkaline treatments.

2. Experimental

2.1. Material

Sugar beet CWM (SB-CWM) from fresh sugar beet pulp (sugar factory in Cagny, France) and potato CWM (P-CWM) from potato pulp (Roquette, France) were both prepared as described elsewhere (Zykwinska et al., 2005).

2.2. Experimental design and data analysis

A Response Surface Methodology was used following an experimental central composite design to study the effects of two variables (temperature and concentration) on the composition of residues recovered after alkaline extractions. The experimental design was constructed from a full two-level factorial design for two factors and the variables were coded as levels (−1, 0, +1) (Table 1). Central points (CP) were performed in triplicate and the quadratic effects were determined by four "star" points (SP). The model coefficients obtained revealed the linear, quadratic and interactive effects.

The data were analysed using StatGraphics 3.0 for Windows program in order to determine the significance of the chosen variables effects. An empirical model was built

$$Y = b_0 + b_1X_1 + \cdots + b_iX_i + b_{12}X_1X_2 + \cdots + b_{ij}X_iX_j + \cdots,$$

where Y is the estimated response, b_0 the model constant, b_i and b_{ij} the model coefficients reflecting the simple and interactive effects, respectively, and the coded independent reaction variables, $X_1 \ldots X_i$. The b coefficients were calculated by multiple linear regression and their significance checked by variance analysis.

Table 1
Extraction conditions and coding of the residues

Variable No. of variable	NaOH concentration (M)	Temperature (°C)
−1	0.05	40
0	0.275	65
+1	0.5	90
1	0.05	40
2	0.5	40
3	0.05	90
4	0.5	90
SP1	0.275	40
SP2	0.05	65
SP3	0.5	65
SP4	0.275	90
CP1	0.275	65
CP2	0.275	65
CP3	0.275	65

SP = star points, CP = central points.

2.3. Alkaline treatment of CWM

The SB-CWM (5 g) and P-CWM (4 g) were stirred with 150 mL of 0.05 M, 0.275 M or 0.5 M NaOH at 40, 65 or 90 °C for 1 h. The extraction was performed three times successively. The final sugar beet (SB) residues and potato (P) residues were recovered after filtration through G3 sintered glass, abundantly washed with distilled water, dried by solvent exchange (ethanol, acetone) and left overnight at 40 °C.

2.4. Analytical

The individual neutral sugars were analysed as their alditol acetate derivatives by gas–liquid chromatography (Blakeney, Harris, Henry, & Stone, 1983). SB-CWM, P-CWM, SB-residue, and P-residue were hydrolysed by 2 M H_2SO_4 at 100 °C for 2 h. Cellulosic Glc was measured as the difference in Glc content with and without prehydrolysis by 72% (w/v) H_2SO_4. The conditions for cellulosic Glc quantification were optimised (data not shown) and a time of 1 h 30 min, and a temperature 25 °C were used for the prehydrolysis step. Inositol was added as an internal standard.

Uronic acid (as GalA) was determined colorimetrically by the automated *m*-hydroxybiphenyl method (Thibault, 1979).

2.5. NMR spectroscopy

NMR experiments were performed on a Bruker DMX-400 spectrometer operating at a ^{13}C frequency of 100.62 MHz and equipped with a double resonance H/X CP/MAS 4 mm probe. The spinning rate was fixed at 7000 Hz and each experiment was recorded at ambient temperature (294 ± 1 K). The cross polarisation (CP) transfer was achieved using 4.25 μs 90° proton pulse, a 1 ms contact time at 62.5 kHz and a 5 s recycle time. A total number of 5120 and 12,288 scans was acquired for water saturated samples: SB-CWM/SB-residue and P-CWM/P-residue, respectively. The SPE/MAS experiments were conducted with a 4 μs ^{13}C pulse, a 1 s recovery delay and a WALTZ16 proton decoupling during data acquisition. A total number of 25,600 and 10,240 scans was acquired for SB-CWM/SB-residue and P-CWM/P-residue, respectively.

To estimate the quantity of arabinan and galactan fractions in SPE/MAS and CP/MAS spectra (fractions with high and restricted mobilities, respectively), the C-1 and C-6 resonances were fitted with a software based on the SIMPLEX method (dimfit2000 – Massiot, Thiele, & Germanus, 1994). Lorentzian type lines were chosen for highly crystalline components, whereas the Gaussian type lines were used for less-ordered components. 50/50 mixture of the Lorentzian and Gaussian shape lines were used when separate type lines did not fit the components well (Rondeau-Mouro et al., 2003b). To compare the signal intensity in CP/MAS to the one acquired in SPE/MAS, the signal to noise ratio S_0/σ_0 was calculated for one experiment (one scan), on glycine and ethanol, in CP/MAS and SPE/MAS, respectively. S_0 represents the methylene signal amplitude of the two components and σ_0 characterises the noise standard deviation. The ratio S_0/σ_0 for CP/MAS experiment was determined 2.32 less than the one for SPE/MAS. Then, from the fitted spectra, the percentage (P) of arabinan and galactan in CP/MAS and SPE/MAS, was calculated as follows:

$$P_{\mathrm{CP/MAS}} = 2.32 \cdot \sqrt{n} \cdot \left(\frac{S_0}{\sigma_0}\right)_{\mathrm{CP/MAS}} = 2.32 \cdot k \cdot \left(\frac{S}{N}\right)_{\mathrm{CP/MAS}},$$

$$P_{\mathrm{SPE/MAS}} = \sqrt{n'} \cdot \left(\frac{S_0}{\sigma_0}\right)_{\mathrm{SPE/MAS}} = k' \cdot \left(\frac{S}{N}\right)_{\mathrm{SPE/MAS}},$$

$$\%\ \text{restricted mobility} = \frac{P_{\mathrm{CP/MAS}}}{P_{\mathrm{CP/MAS}} + P_{\mathrm{SPE/MAS}}},$$

$$\%\ \text{mobile} = \frac{P_{\mathrm{SPE/MAS}}}{P_{\mathrm{CP/MAS}} + P_{\mathrm{SPE/MAS}}},$$

where n is the scan number; S_0/σ_0 is the theoretical signal-to-noise ratio for one scan; k and k' are the calculated ratios that fulfil the condition $n = n'$; $(S/N)_{\mathrm{CP/MAS}}$ and $(S/N)_{\mathrm{SPE/MAS}}$ are the signal-to-noise ratios measured on the C-1 of Ara (3,5-) at 107.7 ppm in SB-CWM and SB-residue, and on the C-6 of Gal at 61.3 ppm in P-CWM and P-residue.

3. Results

3.1. Cell wall material composition

Chemical composition of SB-CWM revealed that three main sugars are present, Ara (225 mg/g), GalA (225 mg/g) and cellulosic Glc (237 mg/g). Some Rha (16 mg/g) and Gal (54 mg/g) were also detected. The low amount of non-cellulosic Glc (13 mg/g), Xyl (19 mg/g) and Fuc (2 mg/g) indicates low xyloglucan and/or xylan contents, as previously reported by Renard and Jarvis (1999). This composition shows that the major polysaccharides present in this cell wall are arabinan-rich pectins and cellulose.

P-CWM is rich in galactan-rich pectin and cellulose, with 234 mg/g of Gal, 162 mg/g of GalA and 281 mg/g of cellulosic Glc. Some Rha (13 mg/g) and Ara (52 mg/g) were also present. P-CWM contains low amount of xyloglucan and/or xylan as only 34 mg/g of non-cellulosic Glc, 26 mg/g of Xyl and 2 mg/g of Fuc were detected.

3.2. Experimental design approach

Alkaline extraction conditions of increasing severity were applied to SB-CWM and P-CWM to study the composition of the residues recovered after extraction of non-cellulosic polysaccharides (Tables 2 and 3). Residues weight recoveries varied widely with respect to the

Table 2
Weight and sugar[a] recoveries (%) of alkali treated sugar beet CWM

	Weight	GalA	Rha	Ara	Gal	Xyl	Glc	
							Cellulosic	Non-cellulosic
1	80	74	74	78	95	88	85	80
2	75	77	73	70	79	67	75	100
3	28	7	9	8	12	49	62	88
4	11	1	0	2	0	13	32	55
SP1	79	80	88	79	93	83	84	100
SP2	64	49	68	60	70	70	76	100
SP3	42	39	36	38	45	51	62	77
SP4	14	1	0	2	0	26	40	70
CP1	48	43	64	49	58	66	63	88
CP2	57	52	66	55	69	75	68	100
CP3	52	46	58	52	63	76	65	80

[a] Sugar recovery (%) = calculated by taking into account the weight recovery, the amount (mg/g) of each sugar present in the sugar beet residues recovered and the amount (mg/g) of each sugar present in the untreated sugar beet CWM (Zykwinska et al., 2005).

Table 3
Weight and sugar[a] recoveries (%) of alkali treated potato CWM

	Weight	GalA	Rha	Ara	Gal	Xyl	Glc	
							Cellulosic	Non-cellulosic
1	89	76	82	83	77	100	99	84
2	70	45	44	48	40	84	93	74
3	34	6	0	16	8	59	77	70
4	26	3	0	7	2	47	76	56
SP1	74	66	56	72	65	85	95	78
SP2	72	62	57	67	56	91	94	82
SP3	48	38	37	31	24	74	92	66
SP4	26	3	0	7	2	45	69	55
CP1	57	43	43	44	37	84	93	84
CP2	58	47	47	49	40	98	94	81
CP3	57	45	41	50	40	93	100	74

[a] Sugar recovery (%) = calculated by taking into account the weight recovery, the amount (mg/g) of each sugar present in the potato residues recovered and the amount (mg/g) of each sugar present in the untreated potato CWM (Zykwinska et al., 2005).

conditions used. A progressive extraction of cell wall polysaccharides was observed with increasing severity of treatments and the lowest weight recoveries were observed for extractions carried out in the harshest conditions. The central point (CP) of the experimental design was performed in triplicate and was reproducible, both with respect to weight and sugars recoveries in the residues.

3.2.1. Cellulosic glucose recovery

The cellulosic Glc recovery from SB-CWM and P-CWM varied only slightly with the conditions used (Tables 2 and 3). About 32–85% and 69–100% of the cellulosic Glc initially present in SB-CWM and P-CWM, respectively, were recovered in the residues. It appeared that temperature has a significant effect on cellulose recovery. At 40 and 65 °C, residues recoveries were quite high, whereas at 90 °C some cellulose was removed during treatment. In order to better visualise the effects of extraction conditions onto cellulose recovery, response surfaces were constructed, as shown for P-residues in Fig. 1. The *R*-squared value (97% and 91% for SB-CWM and P-CWM, respectively)

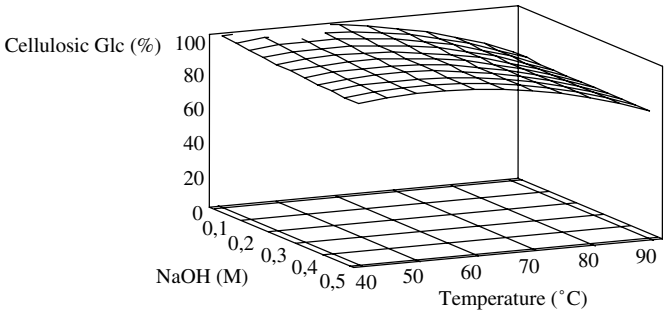

Fig. 1. Influence of NaOH concentration and temperature on cellulosic Glc recovery in potato residues.

indicated that the models correlate well with the experimental results. The simplified regression equations for the cellulose recovery are:

$$\text{SB-CWM}: \ \text{Glc}(\%) = 82.1 - 9.7\text{NaOH} - 0.4T - 49.9\text{NaOH}^2 - 0.9\text{NaOH} \cdot T - 0.01T^2,$$

$$\text{P-CWM}: \ \text{Glc}(\%) = 59.7 - 28.3\text{NaOH} - 1.6T + 13\text{NaOH}^2 + 0.2\text{NaOH} \cdot T - 0.02T^2.$$

3.2.2. Hemicellulosic sugars recovery

Two main hemicellulosic sugars (Xyl and non-cellulosic Glc) recoveries also showed low variations in the different residues (Tables 2 and 3). NaOH concentration as well as temperature had only minor effects on the Xyl and non-cellulosic Glc recoveries. Even the extraction performed at 90 °C was not strong enough to solubilise all hemicelluloses present. It is noteworthy that 55–88% of the non-cellulosic Glc and 13–49% of the Xyl initially present in SB-CWM, as well as 56–70% of the non-cellulosic Glc and 47–59% of the Xyl initially present in P-CWM were still present in those residues. The loss of hemicelluloses was simultaneous with the loss of cellulose as described by the response surface shown in Fig. 2. The simplified regression equations for the Xyl recovery are:

$$\text{SB-CWM}: \ \text{Xyl}(\%) = 78.8 - 2.5\text{NaOH} - 0.8T - 68.6\text{NaOH}^2 - 0.1\text{NaOH} \cdot T - 0.01T^2 (R^2 = 95\%$$

$$\text{P-CWM}: \ \text{Xyl}(\%) = 54.6 - 128.7\text{NaOH} - 2.3T + 39\text{NaOH}^2 + 0.9\text{NaOH} \cdot T - 0.02T^2 (R^2 = 96\%).$$

3.2.3. Pectic sugars recovery

In contrast to cellulosic and hemicellulosic sugars, the amount of pectins recovered in the residues varied largely with the conditions used (Tables 2 and 3). A moderate influence of NaOH concentration on the amount of GalA and Rha remaining in the residues was observed at 40 °C. The NaOH concentration had less significant effect than the temperature, since a more important solubilisation was observed with increasing temperature. Residues recovered at 90 °C contain only minor fragments of pectic backbone sugars: 1–7% of the GalA and 0–9% of the Rha initially present in SB-CWM, and 3% to 6% of the GalA and 0% of Rha initially present in P-CWM. The simplified regression equations for the GalA recovery are:

$$\text{SB-CWM}: \ \text{GalA}(\%) = 97.1 - 41.2\text{NaOH} - 0.2T - 38.5\text{NaOH}^2 - 0.4\text{NaOH} \cdot T - 0.01T^2 (R^2 = 96\%),$$

$$\text{P-CWM}: \ \text{GalA}(\%) = 71.1 - 90.5\text{NaOH} - 1.1T + 97.7\text{NaOH}^2 + 0.2\text{NaOH} \cdot T - 0.02T^2 (R^2 = 98\%).$$

Ara and Gal are the main sugars representative of pectic side chains in SB-CWM and P-CWM, respectively. Their recoveries were similar and showed comparable variations with the conditions used (Tables 2 and 3). After NaOH treatments performed at increasing temperatures (40, 65 and 90 °C), Ara and Gal were progressively washed off from the CWMs. The residues recovered at 90 °C contained only 2–8% of the Ara and Gal initially present in SB-CWM and P-CWM, respectively. To better visualise the effects of extraction conditions on Ara and Gal recoveries, their respective response surfaces are shown in Figs. 3 and 4. The simplified regression equations for the Ara recovery in SB-CWM and for the Gal in P-CWM are:

$$\text{SB-CWM}: \ \text{Ara}(\%) = 75.6 - 2.8\text{NaOH} - 0.9T - 47.3\text{NaOH}^2 - 0.5\text{NaOH} \cdot T - 0.02T^2 (R^2 = 98\%),$$

$$\text{P-CWM}: \ \text{Gal}(\%) = 117.7 - 83.1\text{NaOH} - 0.6T + 58.7\text{NaOH}^2 + 0.2\text{NaOH} \cdot T - 0.01T^2 (R^2 = 97\%).$$

These results suggest that different pectin populations may be present in SB-CWM and P-CWM. The composition of Ara and GalA (mg/g) in SB-residues was expressed in mol and the Ara to GalA molar ratios are presented in Fig. 5. When alkaline extractions were performed at 40 and 65 °C, the Ara recovery in the res-

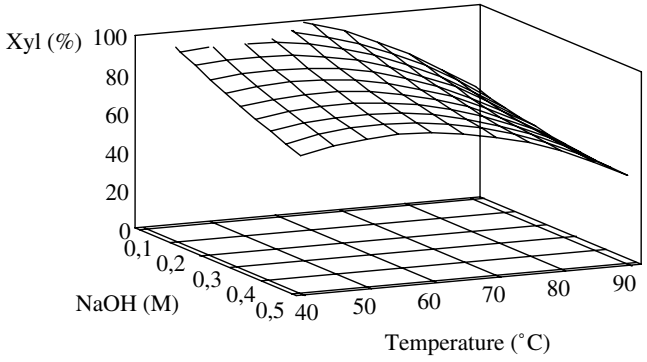

Fig. 2. Influence of NaOH concentration and temperature on Xyl recovery in potato residues.

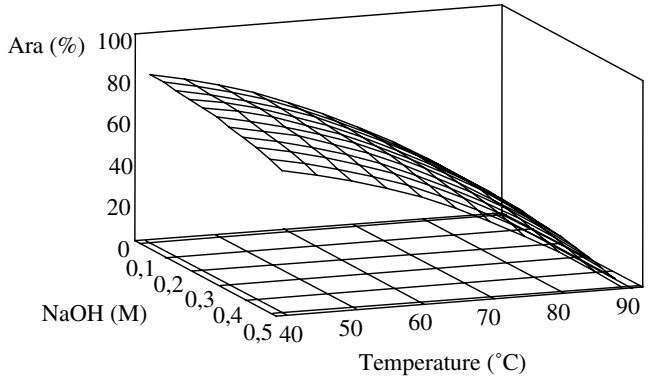

Fig. 3. Influence of NaOH concentration and temperature on Ara recovery in sugar beet residues.

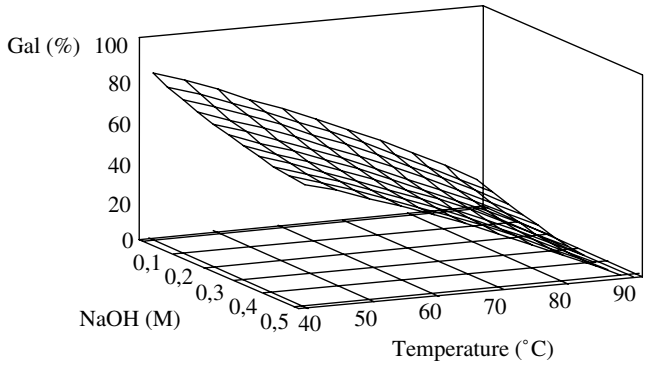

Fig. 4. Influence of NaOH concentration and temperature on Gal recovery in potato residues.

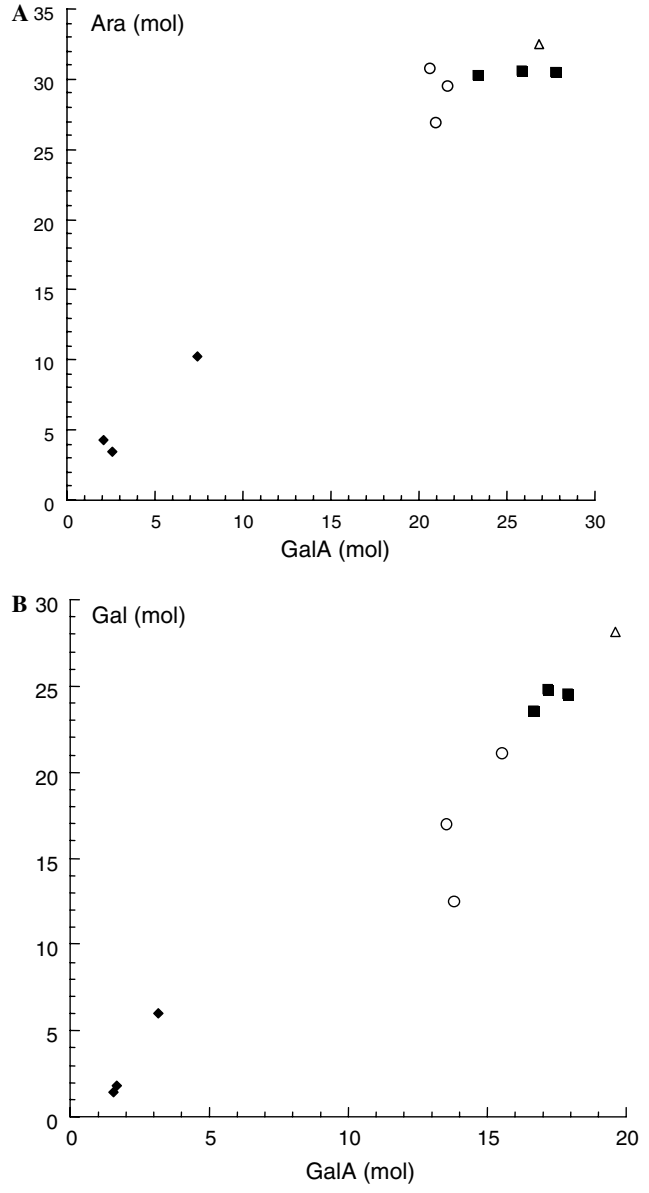

Fig. 5. (A) Ara amount (mol) versus GalA amount (mol) in sugar beet CWM (△) and sugar beet residues recovered after alkaline treatments at 40 °C (■), 65 °C (○) and 90 °C (◆). (B) Gal amount (mol) versus GalA amount (mol) in potato CWM (△) and potato residues recovered after alkaline treatments at 40 °C (■), 65 °C (○) and 90 °C (◆).

idues appeared almost constant (~30 mol of Ara), while a loss of GalA was observed at the same time (from ~29 to ~21 mol of GalA). Considerable loss of Rha was also measured. This observation suggests that the pectin population extracted in these conditions was enriched in HG and RG I regions that are only poorly substituted by arabinan side chains. Increasing the extraction harshness (0.05, 0.275, and 0.5 M NaOH at 90 °C) eliminated pectin fragments that are rich in arabinan side chains and the remaining residues appeared almost free of pectins, since they contained only ~4 and ~3 mol of Ara and GalA, respectively (Fig. 5A). The presence of distinct pectin populations in P-CWM was not as obvious as in SB-CWM. At each temperature (40, 65, 90 °C) the NaOH treatment extracted pectin fractions rich in galactan side chains (Fig. 5B).

3.3. Solid-state NMR characterisation

The CP/MAS NMR spectrum of water saturated SB-CWM (Fig. 6A) shows mainly carbons arising from cellulose: C-1 (104.7 ppm), C-4 (82.9 and 84.2 ppm), C-2,3,5 (72–75 ppm) and C-6 (62.5 and 65 ppm) (Table 4). Some minor signals from pectins: galacturonan C-6 carboxy (173 and 175 ppm) and methoxy carbon (53.2 ppm), as well as rhamnogalacturonan C-6 (21 ppm) were also present. The characteristic peak at 107.7 ppm (pointed out with an arrow) was assigned to sugar beet arabinan C-1 (Renard & Jarvis, 1999). The resonances detected in CP/MAS experiment are characteristic for polymers of restricted mobility, such as cellulose or cellulose-associated polymers (Ha, Evans, Jarvis, Apperley, & Kenwright, 1996). The presence of a measurable signal at 107.7 ppm suggests that part of the arabinan is characterised by a sufficiently restricted mobility to appear in CP/MAS spectrum (Vignon, Heux, Malainine, & Mahrouz, 2004). Fig. 6B presents the CP/MAS NMR spectra of water saturated SB-residue extracted by 0.275 M NaOH at 65 °C. This residue was particularly interesting since it was still enriched in Ara (224 mg/g) and cellulose (295 mg/g). The resonances at 79.3 ppm assigned to C-4 of galacturonan and methoxy carbon at 53.2 ppm, as well as the resonance at 21 ppm assigned to C-6 of rhamnogalacturonan, disappeared (Fig. 6B). However, the signal at 107.7 ppm assigned to arabinan was still present (pointed out with an arrow). This result confirms the existence of arabinan fractions with restricted motions.

Polysaccharide components containing more mobile fractions can be studied by SPE/MAS experiments (Lahaye et al., 2003; Rondeau-Mouro et al., 2003a). The peaks observed (Fig. 7) can be assigned to pectic galacturonan and rhamnogalacturonan, but mostly to pectic side chains (Table 5). The assignment of arabinan signals is in good agreement with that reported by Renard and Jarvis (1999). The galactan peaks were assigned according to Navarro, Cerezo, and Stortz (2002). The SPE/MAS spectra of SB-CWM and SB-res-

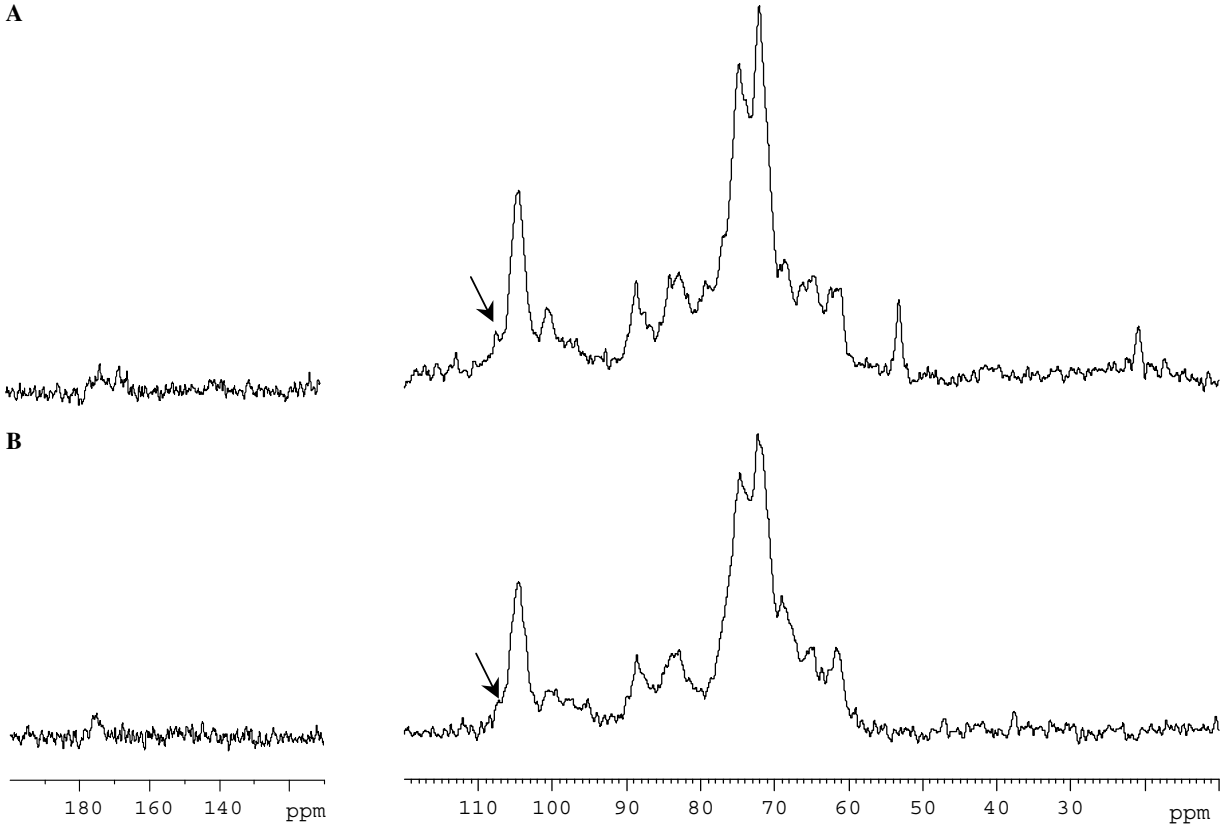

Fig. 6. CP/MAS spectra of sugar beet CWM (A) and sugar beet residues recovered after 0.275 M NaOH treatment at 65 °C (B).

Table 4
Peak assignments in CP/MAS spectra of sugar beet and potato CWM, sugar beet and potato residues

Sugar	Carbon	δ (ppm)
Galacturonan	C-6	173.0–175.0
Arabinan	C-1 (3,5-)	107.7
Cellulose/galactan	C-1	104.7
Galacturonan/xyloglucan	C-1	100.5
Cellulose (crystalline)	C-4	84.2
Cellulose (amorphous)	C-4	82.9
Galacturonan	C-4	79.3
Cellulose	C-2,3,5	72.0–75.0
Galacturonan/xyloglucan	C-5	68.6
Cellulose (crystalline)	C-6	65.0
Cellulose (amorphous)	C-6	62.5
Xyloglucan	C-6	62.5
Galactan	C-6	61.3
Galacturonan	C-6	53.2
Rhamnogalacturonan	C-6	21.0

idue (Figs. 7A and B) clearly show that most of the pectic arabinan side chains are highly mobile. It was roughly estimated that in untreated SB-CWM, about 69% (±3%) of Ara was found highly mobile, whereas about 31% (±3%) showed restricted dynamics. Alkaline treatment of SB-CWM by 0.275 M NaOH at 65 °C yielded a residue containing about 52% of the Ara initially present in CWM (CP3, Table 2). From this, about 80% (±2%) (41.6/52) of Ara was highly mobile when about 20% (±2%) (10.4/52) was found with limited mobility.

The CP/MAS spectra of water saturated P-CWM and P-residue extracted by 0.275 M NaOH at 65 °C are presented in Fig. 8. Both CP/MAS and SPE/MAS (data not shown) spectra were very similar to that recorded for SB-CWM and SB-residue (Figs. 6, 7A and B). Some differences were however observed, in particularly the absence of clearly measurable Ara signal at 107.7 ppm and the presence of the characteristic peak at 61.3 ppm, assigned to C-6 of Gal in galactan (pointed out with an arrow) (Figs. 8A and B). Indeed, the severe extraction conditions used solubilised some pectic and hemicellulosic sugars but the residue was still enriched in Gal (165 mg/g) and cellulose (500 mg/g) (Table 3). The SPE/MAS spectra clearly show that a major fraction of galactan side chains is highly mobile. The percentage of galactan in the highly mobile fractions and in the fractions of restricted mobility was estimated, as described above for sugar beet. In P-CWM, about 70% (±2%) of Gal was found highly mobile, while about 30% (±2%) of Gal was detected in the fractions of restricted mobility. The residue obtained by alkaline extraction contained still about 40% of Gal initially present in CWM (CP3, Table 3). It was estimated that about 67% (±3%) (26.8/40) of Gal in this residue was present in mobile fraction whereas about 33% (±3%) (13.2/40) was found with limited mobility.

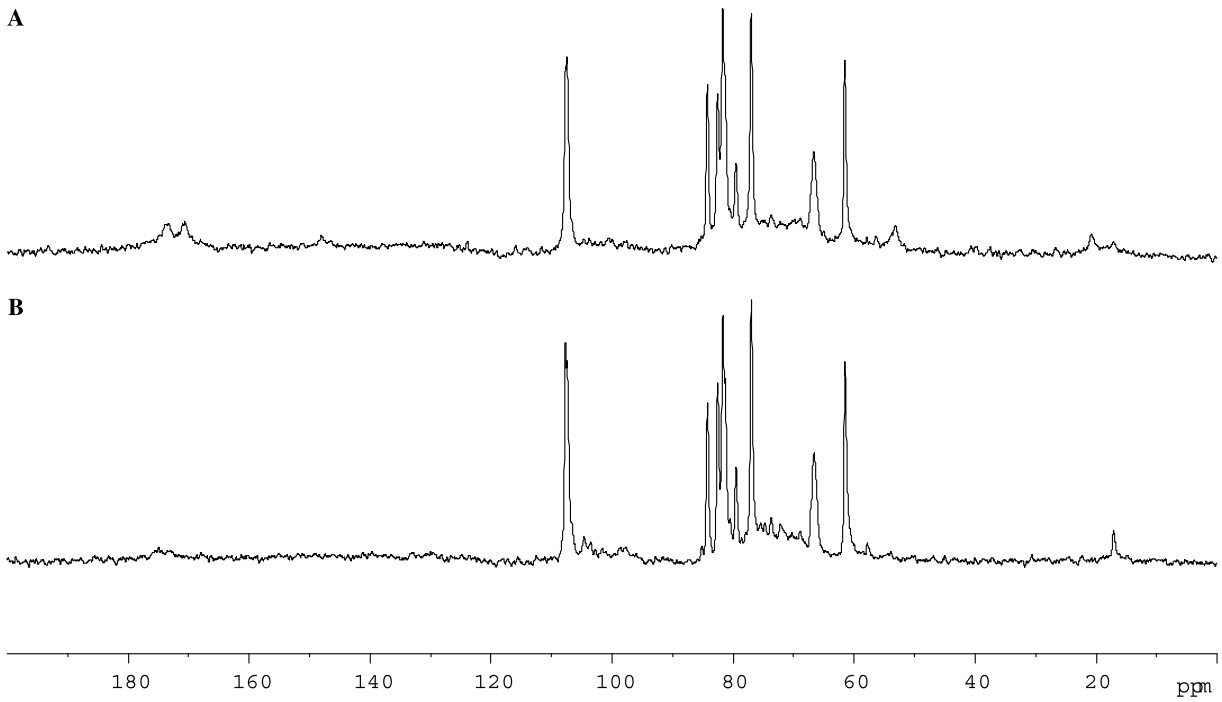

Fig. 7. SPE/MAS spectra of sugar beet CWM (A) and sugar beet residues recovered after 0.275 M NaOH treatment at 65 °C (B).

Table 5
Peak assignments in SPE/MAS spectra of sugar beet and potato CWM, sugar beet and potato residues

Sugar	Carbon	δ (ppm)
Galacturonan	C-6 (COO^-/COOH)	174.4
Galacturonan	C-6 ($COOCH_3$)	170.6
Arabinan	C-1 (3,5-)	107.7
Galactan	C-1	104.8
Arabinan	C-4 (t-)	84.2
Arabinan	C-4 (5-/3,5-)	82.5
Arabinan	C-2 (5-/3,5-)	81.7
Arabinan	C-3 (3,5-)	79.5
Galactan	C-4	78.1
Arabinan	C-3 (t-/5-)	77.0–77.2
Galactan	C-5	74.8
Galactan	C-3	73.8
Galactan	C-2	72.3
Arabinan	C-5 (5-)	67.3
Arabinan	C-5 (t-)	62.0
Galactan	C-6	61.3
Galacturonan	C-6	53.0-53.3
Rhamnogalacturonan	C-6	17.7–20.0

The NMR quantification of arabinan and galactan in CP/MAS and SPE/MAS spectra suggests the existence of pectin populations with more and less restricted mobilities. It can be supposed that the alkaline treatment applied to SB-CWM extracted not only the highly mobile fractions of the arabinan side chains but also the fractions with limited mobility. In contrast, the extraction performed on P-CWM indicated more efficient solubilisation of the fractions showing higher motions than the fractions of restricted mobility. However, the possibility of changes in pectic side chains accessibility and thus mobility induced by alkaline treatments must be taken into consideration.

4. Discussion

The aim of this work was to study the potential interactions between pectic neutral sugar side chains, and cellulose. For this purpose, cell walls that are particularly abundant in arabinan- or galactan-rich pectins and cellulose were chosen. To preserve RG I regions including neutral sugar side chains and induce an important degradation of HG regions, alkaline treatment appeared the most appropriate. Indeed, hot alkali conditions were successfully applied for undegraded RG I recovery (Bonnin et al., 2001). In harsh alkali conditions, HG regions are seriously degraded by β-elimination and oxidative peeling. In the present work, similar conditions were intentionally reproduced. The other major treatments inducing pectin solubilisation are chelating agents and mild acid. It was demonstrated that chelating agents induce only a limited solubilisation of sugar beet pectins (Renard & Thibault, 1993). Acid extractions that are well known to lead to high pectin solubilisation, provoke however an important side chains degradation (Levigne, Ralet, & Thibault, 2002).

A response surface methodology following an experimental central composite design allowed to quantify the effects of the extraction variables used (concentration and temperature) on the composition of the residues. Residues enriched in cellulose and hemicelluloses were recovered whatever the extraction conditions applied. Indeed, alkaline treatments performed were not strong enough to completely extract the hemicellulosic polysaccharides present in

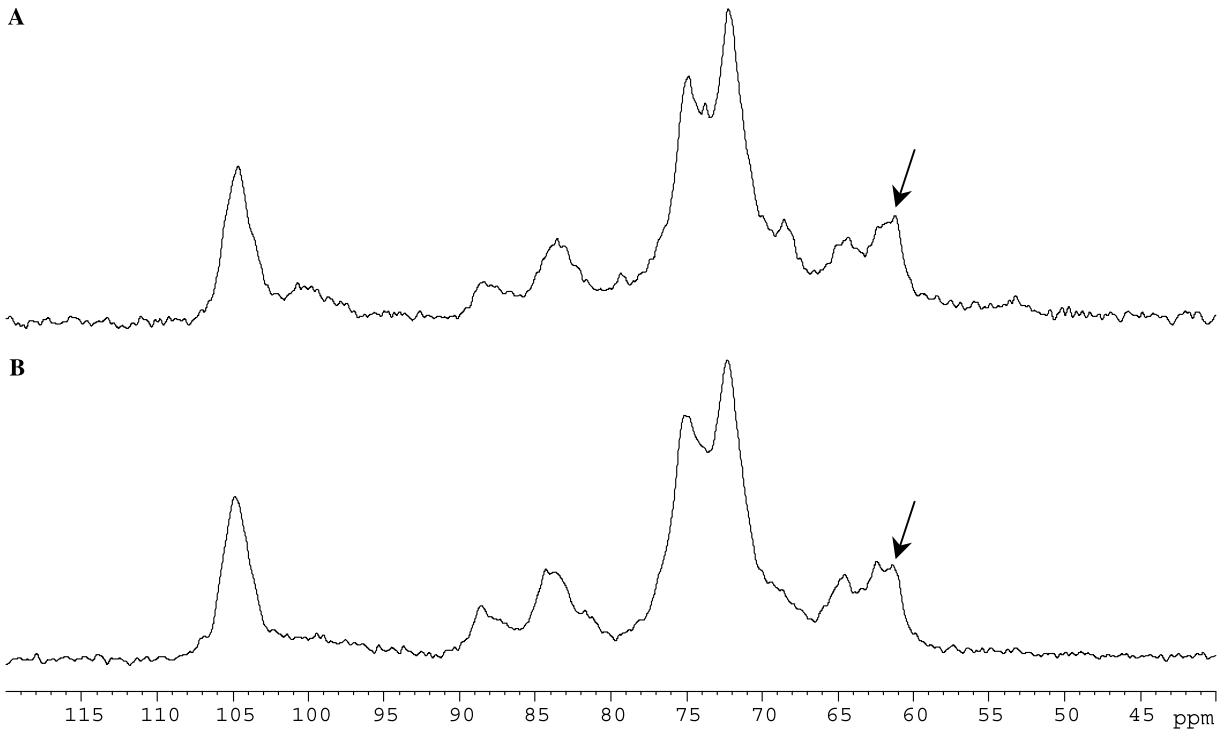

Fig. 8. CP/MAS spectra of potato CWM (A) and potato residues recovered after 0.275 M NaOH treatment at 65 °C (B).

CWM. It was previously shown that only very severe alkaline conditions effectively solubilise xyloglucan from pea cell walls, probably because xyloglucan binds not only to the surface of cellulose microfibrils but is also entrapped within the microfibrils (Hayashi & Maclachlan, 1984). In the present work, alkaline conditions of increasing severity were shown to progressively extract pectins from the cell walls.

Results of alkaline extractions suggest that distinct pectin populations co-exist in SB-CWM, which appeared less evident in P-CWM. Pectins easily extracted with alkali at 40 and 65 °C were enriched in homo- and rhamnogalacturonans with arabinan side chains in limited amounts. This fraction may correspond to the one that is loosely associated to cellulose microfibrils, as it was recently shown (Zykwinska et al., 2005). Another fraction was enriched in arabinan side chains and seemed to be less accessible, as it withstands extraction in harsher conditions. Acidic extraction conditions of increasing severity also revealed the presence of different pectin fractions in SB-CWM (Levigne et al., 2002). The pectin fraction extracted for very smooth acid conditions was poor in arabinose, whereas mild acid treatment led to the extraction of another pectic fraction particularly rich in arabinan. Stronger acid conditions completely hydrolysed the arabinan side chains.

Solid-state ^{13}C NMR seems to confirm the presence of pectin domains differing in molecular mobilities. The extraction procedure applied to SB-CWM and P-CWM does however not preclude artefacts formation. The possibility that arabinan and galactan side chains rearrange after HG degradation has to be taken into account. This modification may create less or more mobile arabinan- and galactan-rich populations. To minimize undesirable phenomena, the harshness of extraction conditions was increased progressively. Additionally, solid-state NMR was applied both to untreated cell walls and to residues obtained with intermediate extraction conditions. It came out, that some minor populations of arabinan and galactan quantified in CP/MAS spectra were characteristic for polymers of limited mobility. These fractions could constitute domains tightly associated with cellulose or domains with a high degree of entanglement. It is likely that only limited domains of arabinan and galactan chains are bound to cellulose microfibrils while others are highly mobile. Indeed, the SPE/MAS clearly showed that most of the pectic side chains are highly mobile. The percentage of arabinan and galactan fractions calculated in SB-CWM and P-CWM before and after alkaline extractions showed that this treatment solubilised not only the highly mobile arabinan and galactan fractions but also fractions with restricted movements. This finding suggests that arabinan or galactan side chains closely associated with cellulose surface (i.e., with restricted mobility) belong to the same pectin molecule than side chains of high mobility, and are extracted at the same time.

Vignon et al. (2004) also reported the presence of at least three fractions of arabinan in the arabinan/cellulose composite (1:1, w/w) recovered after alkaline treatments (0.5 M NaOH for 2 h at 80 °C) and chlorite bleaching of prickly pear spines from *Opuntia ficus-indica*. Solid-state NMR relaxation experiments revealed a fraction of around 15% of the arabinan, which was claimed to strongly

interact with cellulose and exhibited a solid-like behaviour, whereas 25% undergoes hindered motions and 60% is in liquid-like state (Vignon et al., 2004). Oechslin, Lutz, and Amado (2003) isolated a cellulosic residue enriched in Gal from apple cell walls treated by 1 and 4 M NaOH for 16 h at 20–22 °C and proposed non-covalent interactions between galactan and cellulose. Redgwell, Fischer, Kendal, and MacRae (1997) found considerable amounts of Ara and Gal that were not extracted by 4 M NaOH and supposed to be non-covalently associated with the cellulosic residues of nine different fruit species.

It is likely that interactions between pectic side chains and cellulose are mediated by hydrogen bonds as suggested for xyloglucan. Mishima, Hisamatsu, York, Teranishi, and Yamada (1998) demonstrated that polymers presenting a high affinity for cellulose were β-D-(1 → 4)-linked glucans and suggested interaction mechanisms based on surface complementarity. Because of their important length, xyloglucan molecules may bind to the cellulose surface and tether adjacent microfibrils. It is likely that they are also entrapped within the microfibrils during their biosynthesis (Hayashi, 1989). Debranched arabinans were recently shown to adopt a 2-fold helical conformation with a pitch of 0.868 nm (Janaswamy & Chandrasekaran, 2005). This conformation, although compatible with potential binding to cellulose (1.036 nm pitch; Sugiyama, Vuong, & Chanzy, 1991), does probably not allow surface complementarities as good as those presumed between xyloglucan and cellulose. Moreover, arabinan/cellulose associations are most likely to be limited only to the cellulose surface, since arabinan chains are rather "short" (~100 Ara residues in sugar beet pulp; Oosterveld, Beldman, Schols, & Voragen, 2000). These facts may explain why pectins are more easily removed from the cell walls than xyloglucans.

It can be hypothesised that arabinan and galactan chains display a *continuum* between cellulose and pectic network together with or instead of xyloglucan. This can be of importance for cell walls that are poor in xyloglucans. In those cases, fractions of arabinan and galactan side chains could be associated with cellulose, as recently proposed using in vitro approach (Zykwinska et al., 2005). From a biological point of view, the setting up of associations between pectic side chains and cellulose could be a dynamic process, which depends on the structure and location of pectic chains attached to RG I. McCartney, Ormerod, Gidley, and Knox (2000) suggested that pea cotyledon RG I is a *continuum* of heterogeneous molecules, which could have various functions within the primary cell wall matrix. Indeed, the immunochemistry study of developing pea cotyledons revealed that galactan side chains are deposited close to the plasma membrane during cell expansion. Their appearance after cell expansion and intercellular space formation, but before cell dehydration and maturation, was correlated with an increase in cell firmness (McCartney et al., 2000). In apple, galactan is lost primarily during the cell enlargement phase and the lowest levels were detected upon maturation (Peña & Carpita, 2004). On the opposite, two arabinan populations attached to RG I regions were distinguished at different locations in the cell walls during ripening. A first population of highly branched arabinan decreases rapidly in overripe apples, whereas the amount of (1 → 5)-α-linked arabinan remains almost unchanged. It was then proposed that the linear arabinan is tightly associated with cellulose and is therefore less accessible for cell wall enzymes (Peña & Carpita, 2004).

In conclusion, the experimental design, simultaneously with solid-state ^{13}C NMR, emphasize the possibility of associations between pectins, particularly their neutral sugar side chains, and cellulose. Although pectins/cellulose interactions are most likely weaker than the xyloglucan/cellulose ones, their presence may be of great significance in the building up of primary cell walls of plants. It can be envisioned that arabinan and galactan chains are loosely attached to cellulose surfaces through non-covalent linkages. Microfibrils are then hold together only by the cohesive forces between successive layers of laterally associated pectins and xyloglucans, as already proposed in the "multi-coat" model (Cosgrove, 2000). Further elucidation of arabinan/galactan domains associated with cellulose is in progress.

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