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Structural features of pectic polysaccharides from the skin of *Opuntia ficus-indica* prickly pear fruits

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Abstract—After removal of the mucilage with water at room temperature, pectic polysaccharides were solubilized from *Opuntia ficus-indica* fruit skin, by sequential extraction with water at 60 °C (WSP) and EDTA solution at 60 °C (CSP). Polysaccharides with neutral sugar content of 0.48 and 0.36 mol/mol galacturonic acid residue were obtained, respectively, in the WSP and CSP extracts. These pectic polysaccharides were de-esterified and fractionated by anion-exchange chromatography, yielding for each extract five fractions, which were thereafter purified by size-exclusion chromatography. Two of these purified fractions were characterized by sugar analysis combined with methylation and reduction–methylation analysis. The study was then supported by ^{1}H and ^{13}C NMR spectroscopy. The results showed that the water-soluble fraction WSP3 and the EDTA soluble fraction CSP3, consisted of a disaccharide repeating unit →2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→ backbone, with side chains attached to O-4 of the rhamnosyl residues. The side chains contained highly branched α-(1→5)-linked arabinan and short linear β-(1→4)-linked galactan.

Keywords: *Opuntia ficus-indica*; Prickly pear; Pectin; Galactan; Arabinan; Rhamnogalacturonan; ^{1}H and ^{13}C NMR

1. Introduction

Pectins are the most abundant polysaccharides in the cell wall of many higher plants. They are localized in the middle lamellae where they regulate intercellular adhesion. They also influence growth, development and senescence. They are a family of complex polysaccharides that contain (1→4) linked D-galacturonic acid residues. Three pectic polysaccharides, homogalacturonan, rhamonogalacturonan-I and substituted galacturonans have been isolated from primary cell walls and structurally characterized.[1] The dominant feature of pectins is a linear chain of α-(1→4)-linked D-galactopyranosyl acid in which varying proportions of the acid groups are present as methoxyl esters. These homogalacturonan sequences may be interspersed at intervals with β-L-rhamnopyranosyl residues carrying the major part of neutral sugar side chains, mainly arabinans, galactans or arabinogalactans.[1–3]

Pectins have a number of applications in the pharmaceutical, cosmetic and food industries and new sources of these polysaccharides are constantly seeked.[4–6]

Opuntia ficus-indica (OFI) is a tropical or subtropical plant, which belongs to the *Cactaceae* family and is mainly used for fruit production.[7] Because of its high adaptation to the harsh desert environment and its different applications, the OFI fruit, commonly known as prickly pear, is an important and abundant potential raw material for the Moroccan industry. Within the last decade, prickly pear fruits have become an important crop in the semi-arid lands of Morocco, where they play a strategic role in subsistence agriculture. Efforts are currently made to develop the fruit production and to find new applications in the food industries.

A preliminary sugar composition of the pectic polysaccharides extracted from the peel of OFI cactus fruits

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has already been published by Forni et al.[8] They found that these polysaccharides are characterized by high neutral sugar content (rhamnose, arabinose and galactose) and large amounts of galacturonic acid. They suggested their applications as thickening additives in food components as well as in cosmetic and pharmaceutical preparations. However there is no structural study on the pectic polysaccharides of the prickly pear fruits. The aim of this work was to study in detail the chemical composition and structural features of pectic polysaccharides isolated from the skin of prickly pear fruit.

2. Results and discussion

2.1. Isolation of cell-wall material (CWM)

In a whole *O. ficus-indica* prickly pear fruit, the amount of skin is important, and can vary from 15% to 20% on a dry weight basis. The constituents and chemical composition of the skin are given in Table 1. An important amount of minerals (11.5 wt.%), as well as fats and waxes (11 wt.%) are observed. The lignin content is low (2.4 wt.%), and the main constituents are polysaccharides (66.1 wt.%), including cellulose (27 wt.%). The morphological study carried out by scanning electron microscopy after critical point drying showed that the cells of the prickly pear fruit skins were mainly made up of collenchyma and parenchyma cells (Fig. 1). These typical tissues are usually rich in pectic polysaccharides.

In order to prepare the cell-wall material (CWM), the dry skin was first refluxed in a toluene–EtOH mixture to remove fats and waxes, and then extracted with de-ionized water at 20 °C to eliminate the mucilage exudates (unpublished results). The CWM represented 84.5% of skin dry matter. Its sugar composition is reported in Table 2. The high content of galacturonic acid (35.6%) suggested the presence of an important amount of pectic polymers. Glucose and xylose were also detected, confirming the presence of cellulose and xylan in the cell wall. A 4-*O*-methylglucuronoxylan has already been isolated and characterized from the skin.[9]

Table 1. Chemical composition of skin of prickly pear fruits

Constituents	Dry wt.%
Ash	11.5
Fat and wax	11.0
Lignin	2.4
Protein ($N \times 6.25$)	8.6
Mucilage	4.1
Other polysaccharides	35.0
Cellulose	27.0

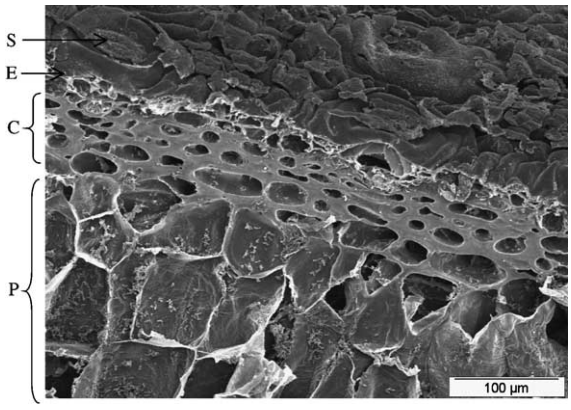


Figure 1. SEM of a cross-section of the skin of *O. ficus-indica* fruit. S: Stomata, E: Epidermi cells, C: Collenchyma cells, P: Parenchyma cells.

2.2. Extraction of pectic polysaccharides

The pectic polysaccharides were extracted from the CWM by sequential extraction with water at 60 °C and aqueous solution of calcium chelator agent at 60 °C (disodium EDTA). The resulting polysaccharides in the extracts are named, water-soluble pectin (WSP) and chelating-soluble pectin (CSP) as shown in the fractionation scheme (Fig. 2). The relative amounts of polymers solubilized and their sugar composition are reported in Table 2. The results indicated that the largest extract (CSP) was solubilized by EDTA (12.4 wt.%), the fractions solubilized by distilled water (WSP) corresponded to 6.1 wt.%. These two extracts accounted for 18.5% of the CWM. The different fractions had a relatively high content of galacturonic acid (52.0% and 64.5% in WSP and CSP, respectively), and the neutral sugars were present in varying amounts. WSP contained quite high amount of arabinose (14.2%) and galactose (10.8%), whereas CSP contained mainly arabinose (17.2%). Furthermore, one can notice the poor yields of total neutral sugars, due to incomplete hydrolysis of the

Table 2. Sugar composition of fractions obtained by sequential extraction of skin of prickly pear fruits

Fraction	Yield[a]	Uronic acid[b]	Neutral sugars[b]					
			Rha	Glc	Gal	Ara	Xyl	Man
CWM	84.5	35.6	3.3	29.3	8.4	12.3	4.5	1.6
WSP	6.1	52.0	1.4	3.3	10.8	14.2	0.9	0.5
CSP	12.4	64.5	3.7	—	4.0	17.2	0.9	—

[a] As percentage of skin dry matter.
[b] Expressed in relative weight percentages.

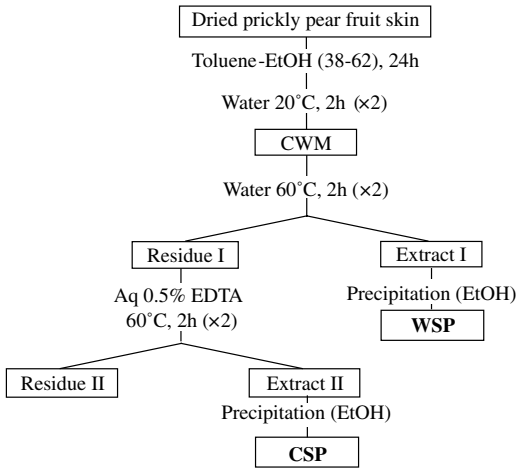


Figure 2. Scheme of extraction of pectic polysaccharides from the skin of prickly pear fruits.

Gal*p*A → Rha*p* linkage, which induced an under-estimation of the rhamnose and galactose content, as already reported.[10]

2.3. Fractionation and characterization of various pectin fractions

The different pectin fractions were partially esterified. The methyl and acetyl ester groups of WSP and CSP polysaccharides were saponified before fractionation. The resulting de-esterified WSP^+ and CSP^+ into their H^+ form, were fractionated by anion-exchange chromatography. An example of this fractionation is summarized in Figure 3. After removal of the salts, 70% of WSP^+ and 58% of CSP^+ were recovered. The yields and sugar compositions of all fractions are reported in Table 3. The major fractions were WSP3* (35%) and CSP3* (28%).

2.4. Purification and characterization of major pectin fractions

The major fractions WSP3* and CSP3* were purified by size-exclusion chromatography, and gave purified WSP3 and CSP3 fractions, respectively. We reported in Figure 4 a typical chromatogram corresponding to the purification of CSP3*.

The sugar composition of fractions WSP3 and CSP3 are reported in Table 4. The original acidic fractions and their carboxyl-reduced forms were methylated. In order to differentiate the galactose arising from the reduction of the galacturonic acid residues and the galactose residues already in the side chain, the carboxyl groups of each acidic fraction were reduced with $NaBD_4$ into the corresponding 6,6′-dideutero-D-galactosyl residues before methylation. The methylation data of unreduced and $NaBD_4$ carboxyl reduced samples are reported in Table 5.

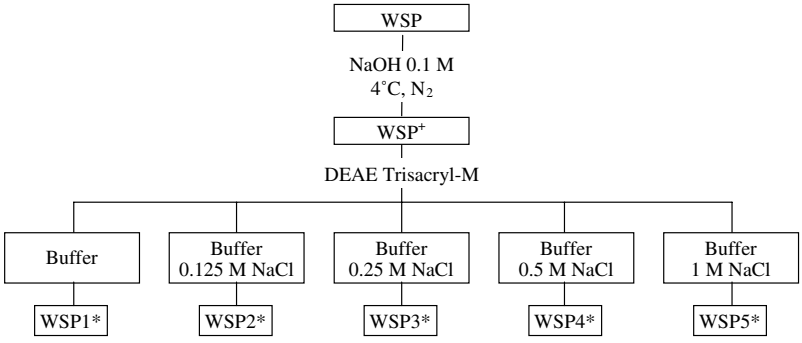


Figure 3. Ion-exchange chromatography fractionation of water-soluble pectic fraction.

Table 3. Yields and sugar composition of WSP and CSP fractions

Fraction	Yield (%)	Sugar composition[a]						
		GalA	Rha	Glc	Gal	Ara	Xyl	Man
WSP1*	8	0	4.2	4.5	38.5	40.4	9.5	—
WSP2*	13	12	3	1.5	19.1	22.4	2.5	—
WSP3*	35	25	4.3	0	8.2	15	0	—
WSP4*	7	39	5	0	4.6	9.7	0	—
WSP5*	7	45	3.4	0	3.1	7.3	0	—
CSP1*	4	0	0	4.1	50.1	43	2.3	—
CSP2*	11	18	5.2	1.4	11.4	50.2	1.1	—
CSP3*	28	29.4	7	2	12	37	2.5	1
CSP4*	6	48.5	6	0	5.2	10.3	0	—
CSP5*	9	60.5	5	0	2.5	7.5	0	—

[a]Expressed in relative weight percentages.

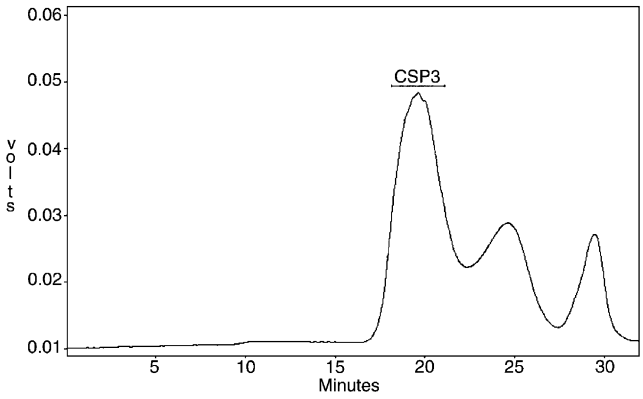


Figure 4. Size-exclusion chromatogram of CSP3*.

These results are also confirmed by ^{1}H and ^{13}C NMR spectroscopy. Powerful 2D-NMR techniques are now available and greatly assist the structural analysis of pectic polysaccharides. Among these methods, homonuclear Correlated Spectroscopy (COSY), and shift correlation using either Heteronuclear Multiple Quantum Coherence (HMQC) or Heteronuclear Multiple Bond Correlation (HMBC) were used in order to assign unambiguously most of ^{1}H and ^{13}C resonances. The NMR spectra of the two fractions studied are given in Figures 5 and 6, the corresponding NMR data are reported in Tables 6 and 7.

2.4.1. Characterization of WSP3. The sugar composition of native WSP3 fraction reported in Table 4 after acid hydrolysis, showed a poor yield of total neutral sugar (20%). The data were much better after two carbodiimide treatments and reduction with $NaBD_4$ (96%). Galacturonic acid, rhamnose, galactose and arabinose in a 25:24:6:41 molar ratio were the main sugars detected.

The results of methylation of native and reduced WSP3 sample were given in Table 5. The reduction of galacturonic acid residues resulted in increased values for $(1\rightarrow2)$- and $(1\rightarrow2,\ 1\rightarrow4)$-linked rhamnose residues. The presence of 2,3,6-tri-*O*-methyl galactitol 6,6′-d_2 in approximately equal amount to the sum of the 3-*O*-methyl rhamnitol and 3,4-di-*O*-methyl rhamnitol, indicated that there is one galacturonic acid per rhamnose residue. Regarding the rhamnosyl residues, the 2,4-linked rhamnose accounted for 29% of the total rhamnose.

The detection of only 2,3,6-tri-*O*-methyl galactitol and 2,3,4,6-tetra-*O*-methyl galactitol in proportion 5.0:4.8, respectively, indicated that there is a two

Table 5. Partially methylated alditol acetates of native and $NaBD_4$ carboxyl reduced WSP3 and CSP3 fractions

Alditol	WSP3		CSP3	
	Native[a]	Reduced[a]	Native[a]	Reduced[a]
3,4-Me_2-Rha[b]	4.1	17.4	3.8	12.4
3-Me-Rha	3.6	7.2	3.9	5.2
Total	7.7	24.6	7.7	17.6
2,3,5-Me_3-Ara	5.3	10.0	15.4	18.3
2,3-Me_2-Ara	4.7	11.9	19.0	18.1
2-Me-Ara	2.3	2.5	5.2	4.5
3-Me-Ara	2.1	3.1	4.8	2.7
Ara	5.0	5.5	6.3	6.8
Total	19.4	33.0	50.7	50.4
2,3,4,6-Me_4-Gal	3.2	4.8	2.8	4.1
2,3,6-Me_3-Gal	1.7	5.0	7.6	10.2
Total	4.9	9.8	10.4	14.3
2,3,6-Me_3-Gal 6,6′-d_2	0.0	24.0	0.0	17.6

[a] Relative mole ratio.
[b] 3,4-Me_2-Rha = 1,2-di-*O*-acetyl-3,4-di-*O*-methyl-rhamnitol, etc.

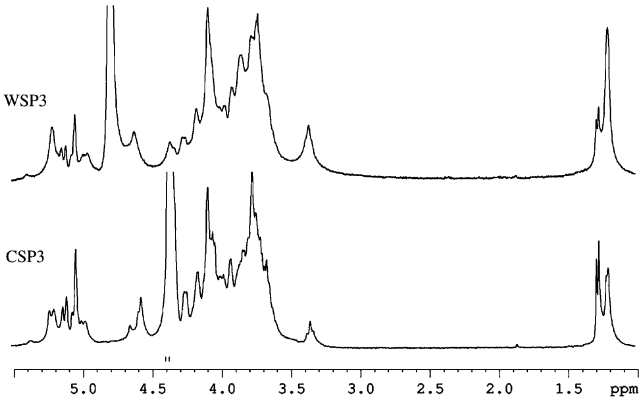


Figure 5. ^{1}H NMR spectra of WSP3 and CSP3 (333 K, 400 MHz).

Table 4. Sugar composition[a] of native and $NaBD_4$ carboxyl reduced WSP3 and CSP3 fractions

Fraction	GalA	Neutral sugars						
		Gal 6,6′-d_2	Rha	Glc	Gal	Ara	Xyl	Man
WSP3 native	27	—	2	0	5	13	0	—
WSP3 reduced	—	25	24	0	6	41	0	—
CSP3 native	26.4	—	3	2	10	47	2	1
CSP3 reduced	—	23	19	3	9	41	1	1

[a] Expressed in relative weight percentages.

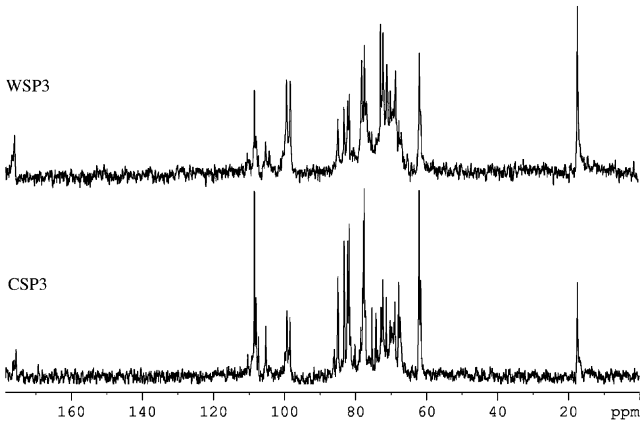


Figure 6. ^{13}C NMR spectra of WSP3 and CSP3 (333 K, 400 MHz).

galactose units side chain. In addition, the nondetection of other *O*-methyl galactitol residues indicated the absence of arabinogalactan side chain, already detected in other rhamnogalacturonan. Indeed, several authors,[11–20] reported the presence of two types of arabinogalactans in apple pectic hairy regions: pectins rich in $(1\rightarrow3,6)$-linked galactan side chains preferentially extracted by cold buffer, and pectins with $(1\rightarrow4)$-linked galactan side chains subsequently extracted under more drastic conditions.

The side chains are attached to the backbone at the O-4 position of rhamnose residues, as shown by the presence of the 3-*O*-methyl rhamnitol. The remaining 3-*O*-methyl rhamnitol are substituted by other side chains constituted by arabinose oligosaccharide. The proportions of 2,3,5-tri-*O*-methyl arabinitol, 2,3-di-*O*-methyl arabinitol, 2-mono-*O*-methyl arabinitol, 3-mono-*O*-methyl arabinitol and penta-*O*-acetyl arabinitol in the carboxyl reduced WSP3, were, respectively, in the ratio 30:36:7.5:9.3:16.5. These results suggested that the arabinan side chains contained a central core of α-$(1\rightarrow5)$-linked arabinofuranosyl residues, as 70% of the units were 1,5-linked, from which 36% were exclusively 1,5-linked. The degree of branching (around 33.3%) was relatively high, either on O-2 (9.3% of the units being 1,2,5-linked) or on O-3 (7.5% of the units being 1,3,5-linked), or both on O-2 and O-3 (16.5% of the units being 1,2,3,5-linked). The proportion of terminal nonreducing arabinose was 30%, and the average length of the arabinan side chains, inferred from the relative amounts of terminal to mid-chain residues, was of 9–10 arabinose units.

The NMR data for WSP3 are reported in Table 6, and the ^{1}H and ^{13}C spectra in Figures 5 and 6. The ^{13}C NMR spectrum contained: (a) in the anomeric region, (i) strong signals at 99.40, 98.34 and 108.46 ppm characteristics of alternating rhamnose and galacturonic acid units linked, respectively, $(1\rightarrow2, 1\rightarrow4)$, with α-$(1\rightarrow5)$-linked arabinofuranose residues in the arabinan side chains, (ii) small signals at 110.30, 107.71 and 107.21 ppm corresponding to branched or terminal arabinofuranosyl units, and at 105.26 ppm corresponding to β-$(1\rightarrow4)$-linked galactopyranose residues; (b) in the region 80.20–85 ppm signals characteristics to C-2, C-3, C-4 of arabinofuranosyl units; (c) in the region 60–64 ppm signals characteristics to C-5 of terminal arabinofuranosyl units (62.26 ppm) and C-6 of galactopyranose residues (61.85 ppm); (d) in the high-field region, C-6 signal at 17.45 ppm for the methyl group of rhamnose units; (e) in the low field region, typical signals were observed for the C-6 carboxyl group of galacturonic acid units at 176.2 and 175.4 ppm. The occurrence of two carboxyl signals can be explained by the presence of branched and unbranched rhamnosyl residues in the main chain.

Table 6. Chemical shift data[a] (333 K) for related glycosyl residues of WSP3 fraction

Glycosyl residues		Assignment					
		1	2	3	4	5	6
α-Galacturonosyl residues							
→4)-α-Gal*p*A(l→2)-α-L-Rhap-(l→	$^{1}H/^{13}C$	4.96/98.34	3.97/69.10	3.95/69.72	4.37/77.90	4.60/72.01	175.40
α-Rhamnosyl residues							
→4)-α-Gal*p*A(l→2)-α-L-Rhap-(l→	$^{1}H/^{13}C$	5.21/99.40	4.09/77.70	3.87/70.80	3.34/71.30	3.78/69.35	1.20/17.45
→4)-α-Gal*p*A(l→2,4)-α-L-Rhap-(l→	$^{1}H/^{13}C$	5.21/98.84	4.15/78.75	3.87/70.80	4.32/80.18	3.72/68.47	1.26/17.45
α-Arabinosyl residues							
→5)-α-Araf-(l→	$^{1}H/^{13}C$	5.04/108.46	4.08/81.85	4.03/77.48	4.18/83.06	3.87/68.22	—
→3,5)-α-Araf-(l→	$^{1}H/^{13}C$	5.09/107.82	4.23/80.38	4.04/84.90	4.24/82.82	3.72/68.47	—
→2,5)-α-Araf-(l→	$^{1}H/^{13}C$	4.97/108.52	4.32/84.96	4.32/83.20	4.18/83.06	3.86/67.92	—
→2,3,5)-α-Araf-(l→	$^{1}H/^{13}C$	5.13/107.71	4.27/84.96	3.99/77.56	4.03/82.79	3.67/68.13	—
T-α-Araf-(l→2	$^{1}H/^{13}C$	5.19/107.21	4.09/82.62	3.91/77.47	3.97/84.93	3.80/62.26	—
T-α-Araf-(l→3	$^{1}H/^{13}C$	5.19/107.21	4.09/82.62	3.91/77.47	3.97/84.93	3.80/62.26	—
β-Galactosyl residues							
→4)-β-Gal*p*-(l→	$^{1}H/^{13}C$	4.58/105.26	3.48/72.89	3.70/74.36	4.11/78.53	na	3.80/61.85

[a] In ppm relative to the signal of internal acetone in deuterium oxide, at 2.1 ppm (^{1}H) or at 31.5 ppm (^{13}C).

Table 7. Chemical shift data[a] (333 K) for related glycosyl residues of CSP3 fraction

Glycosyl residues		Assignment					
		1	2	3	4	5	6
α-Galacturonosyl residues							
→4)-α-Gal*p*A(1→2)-α-L-Rha*p*-(1→	$^1H/^{13}C$	4.96/98.45	3.97/68.80	4.02/69.80	4.37/77.90	4.56/72.30	175.50
α-Rhamnosyl residues							
→4)-α-Gal*p*A(1→2)-α-L-Rha*p*-(1→	$^1H/^{13}C$	5.22/99.24	4.03/77.50	3.87/70.20	3.35/71.30	3.80/69.80	1.20/17.45
→4)-α-Gal*p*A(1→2,4)-α-L-Rha*p*-(1→	$^1H/^{13}C$	5.22/99.04	4.15/78.75	3.87/70.20	4.23/80.15	3.68/67.80	1.25/17.45
α-Arabinosyl residues							
→5)-α-Araf-(1→	$^1H/^{13}C$	5.03/108.40	4.08/82.4	4.11/78.53	4.16/83.20	3.81/68.22	—
→3,5)-α-Araf-(1→	$^1H/^{13}C$	5.10/107.98	4.23/80.15	3.98/84.98	4.22/82.80	3.72/68.17	—
→2,5)-α-Araf-(1→	$^1H/^{13}C$	5.03/108.40	4.23/85.00	4.37/78.04	4.16/83.20	3.86/67.92	—
→2,3,5)-α-Araf-(1→	$^1H/^{13}C$	5.13/107.87	4.25/85.00	4.06/84.98	4.20/83.20	3.67/68.13	—
T-α-Araf-(1→5	$^1H/^{13}C$	5.19/107.21	4.08/82.40	3.93/77.61	4.00/84.98	3.65/62.25	—
T-α-Araf-(1→3	$^1H/^{13}C$	5.23/110.34	4.08/82.40	3.93/77.61	4.00/84.98	3.76/62.25	
β-*Galactosyl residues*							
→4)-β-Gal*p*-(1→	$^1H/^{13}C$	4.57/105.21	3.48/72.70	3.70/74.36	4.11/78.53	3.65/75.30	3.80/61.94

[a] In ppm relative to the signal of internal acetone in deuterium oxide, at 2.1 ppm (^1H) or at 31.5 ppm (^{13}C).

In the proton spectrum the signals in the range 1.20–1.26 ppm were easily assigned to the CH_3 of the rhamnose units. These methyl rhamnose signals appeared generally as two well-resolved doublets, due to the presence of two different rhamnose residues. The two pairs of doublets, centred at $\delta = 1.20$ and 1.26 ppm, were, respectively, assigned to the rhamnosyl residues linked only at O-2 and to the rhamnosyl residues linked both at O-2 and O-4, as already observed.[10] Their relative proportions indicated that 71% of the rhamnosyl residues were linked only at O-2, the remaining rhamnosyl residues (29%) being linked both at O-2 and O-4. These results corroborated the methylation data.

According to the above characteristic signals, the ^1H and ^{13}C spectra of WSP3 were completely assigned by two-dimensional COSY, HMQC and HMBC NMR experiments, and the corresponding chemical shifts are reported in Table 6. These data are in good agreement with literature data already published.[10,21–34]

The NMR results corroborated the methylation data and demonstrated that WSP3 consisted of repeating units →2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→, with 71% of rhamnose units linked in O-2 and 29% both in O-2 and O-4, and with side chains of either arabinan (average of 9–10 arabinose units) or galacto-oligosaccharides (average of 2 galactose units) attached to O-4 of the backbone rhamnose units.

2.4.2. Characterization of CSP3. The sugar composition of native and reduced CSP3 is reported in Table 4. Galacturonic acid, rhamnose, arabinose and galactose were the main sugars detected, suggesting the presence of a rhamnogalacturonan substituted by a typical side chain (arabinan, galactan or arabinogalactan). The methylation data of native and $NaBD_4$ carboxyl-reduced samples (Table 5) showed that CSP3 presented a composition similar to WSP3, but with a larger amount of arabinose. Indeed, ratios of 5.2:12.4 of (1→2,4)- to (1→2)-linked rhamnose in $NaBD_4$ reduced CSP3, indicated that the 2,4-linked rhamnose accounted for 29.5% of the total rhamnose. The presence of 2,3,6-tri-*O*-methyl galactitol 6,6′-d_2 in approximately equal amount to the sum of 3-*O*-methyl rhamnitol and 3,4-di-*O*-methyl rhamnitol suggested that CSP3 is constituted of a disaccharide repeating unit →2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→. The side chains, attached to the backbone at the O-4 position of rhamnose residues, consisted of oligoarabinan and short galactans. The proportion of 2,3,6-tri-*O*-methyl galactitol and 2,3,4,6-tetra-*O*-methyl galactitol indicated that the side-chain galactan have an average length of 3–4 galactose units. The proportions of 2,3,5-tri-*O*-methyl arabinitol, 2,3-di-*O*-methyl arabinitol, 2-mono-*O*-methyl arabinitol, 3-mono-*O*-methyl arabinitol and penta-*O*-acetyl arabinitol in the carboxyl-reduced CSP3 were, respectively, in the ratio 36.3:35.9:8.9:5.4:13.5. These results suggested that the arabinan side chains contained a central core of α-(1→5)-linked arabinofuranosyl residues, as 63.7% of the units were 1.5 linked, from which 35.9% were exclusively 1,5-linked. The degree of branching (around 27.8%) was relatively high but less than in WSP3, either on O-2 (5.4% of the units being 1,2,5-linked) or on O-3 (8.9% of the units being 1,3,5-linked), or both on O-2 and O-3 (13.5% of the units being 1,2,3,5-linked). The proportion of terminal nonreducing arabinose was 36.3% and the average length of the arabinan side chains, inferred from the relative amounts of terminal to mid-chain residues, was of 25–30 arabinose units for CSP3, which was the main difference with WSP3.

The ^1H and ^{13}C spectra of CSP3 reported in Figures 5 and 6, showed the same general features as already observed in the case of WSP3, but one can notice that the ^{13}C spectrum is dominated by signals of α-L-arabinofuranosyl moieties. According to methylation data and

previous assignments for WSP3, the intense peaks at 108.40, 83.20, 82.40, 78.53 and 68.22 ppm are to be ascribed, respectively, to C-1, C-4, C-2, C-3 and C-5 of 5-linked arabinofuranosyl residues, confirming the preeminence of an arabinan-like structure as side chain. The NMR data are collected in Table 7, and confirmed that CSP3 is composed of a rhamnogalacturonan backbone. The backbone is indicated by the presence in the anomeric regions of the characteristic C-1 signals of galactopyranosyl acid α-(1→4) linked at 99.24 ppm and rhamnopyranosyl α-(1→2) linked at 98.45 ppm. The occurrence of two carboxyl signals at 174.9 ppm (small) and more intense at 175.50 ppm, can be explained by the presence of unbranched and branched rhamnosyl residues in the rhamnogalacturonan backbone. Characteristic signals at 17.45 and 1.2 ppm were easily identified to C-6 and H-6 from rhamnopyranose methyl group. ^{13}C NMR data indicated the presence of β-(1→4)-linked galactopyranosyl residues, and it was easy to attribute the signals at 105.21, 78.53, 75.30, 74.36, 72.70 and 61.94 ppm to C-1, C-4, C-5, C-3, C-2 and C-6, respectively. A resonance in the anomeric region at 105.21/4.57 ppm in the $^{1}H/^{13}C$-HMQC spectrum (Fig. 7) indicated the presence of a terminal β-galactopyranose residues β-(1→4)-linked to another galactopyranose residue. The characteristic signals of H-2 at 3.48 ppm and C-6 at 61.85 ppm of this terminal galactopyranose residue confirmed this interpretation. The one-bond correlation in the $^{1}H/^{13}C$-HMQC spectrum (Fig. 7) allowed the following attribution of the protons of the β-(1→4)-Gal*p* residues: 4.57 (H-1), 3.48 (H-2), 3.70 (H-3), 4.11 (H-4), 3.65 (H-5) and 3.80 ppm (H-6) (Table 6). According to the literature data published for pectic arabinan side chains,[33,35] it was possible to confirm the structure of arabinan side chains in CSP3. Thus, it was possible to identify the anomeric ^{1}H and ^{13}C for all residues: T-α-L-Ara*f*-(1→5 (5.19/107.21), T-α-L-Ara*f*-(1→3 (5.23/110.34), (1→5)-α-L-Ara*f* (5.03/108.40), (1→3,5)-α-L-Ara*f* (5.10/107.98), (1→2,5)- α-L-Ara*f* (5.03/108.40), (1→2,3,5)- α-L-Ara*f* (5.13/107.87).

The NMR and methylation results are in good agreement and demonstrated that CSP3 consisted of repeating units →2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→, with 71% of rhamnose units linked in O-2 and 29% both in O-2 and O-4, and with side chains of either arabinan

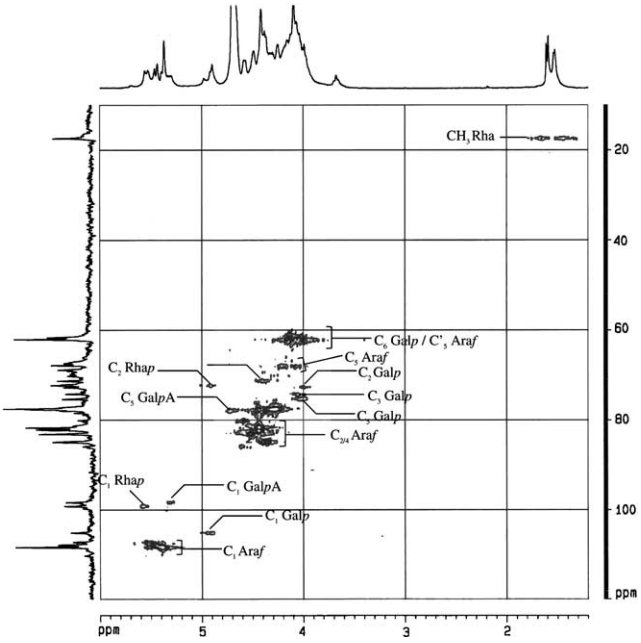
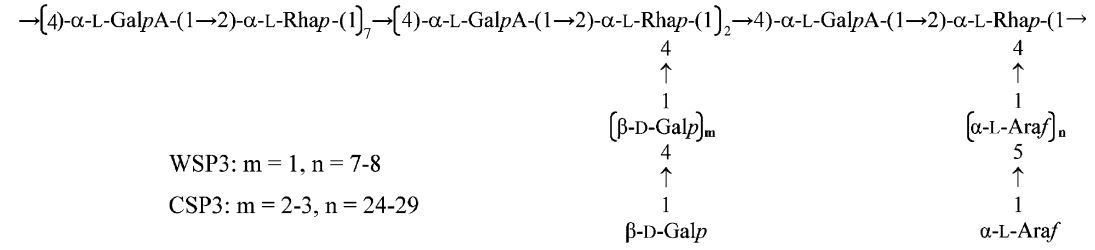


Figure 7. ^{13}C and ^{1}H heteronuclear correlation NMR spectrum (HMQC) of CSP3 (in D_2O at 333 K).

(average of 25–30 arabinose units) or galacto-oligosaccharides (average of 3–4 galactose units) attached to O-4 of the backbone rhamnose units.

The pectic polysaccharides reported so far consisted of 'smooth' regions with blocks of homogalacturonan and 'hairy' regions with blocks of rhamnogalacturonan carrying side chain oligo- and polysaccharides.[11] From the foregoing results it can be concluded that WSP3 and CSP3 had similar structural features, and consisted of repeating units of *O*-(α-D-galacturonic acid)-(1→2)-L-rhamnose, with 29% of the rhamnosyl units carrying side chains attached via position 4. The arabinan side chains were branched with a main core of (1→5)-linked-Ara*f* carrying 2-O- and/or 3-O-substituents. The arabinose side chain were longer in CSP3 (25–30 arabinose units) than in WSP3 (9–10 arabinose units). The galactose side chains were (1→4)-linked, with average of 2 galactose units in WSP3 and 3–4 units in CPS3. We can propose for the structure of WSP3 and CSP3 the following repeating unit, but other variations are possible:



3. Experimental

3.1. Materials

Fresh mature prickly pear fruits of *O. ficus-indica* (OFI) were collected in November 2000 from the experimental station plantation located in the vicinity of Marrakech (Morocco). The harvested fruits were washed, carefully hand-peeled and the peels (with a thickness of about 3–4 mm) were cut up into small pieces and dried in a ventilated oven adjusted at 50 °C. After drying, they were ground for a few minutes in a domestic coffee grinder and sieved to produce a homogeneous powder.

3.2. Analytical methods

Uronic acid content was determined according to Blumenkrantz and Asboe-Hansen method.[36] Neutral sugars were analyzed, after H_2SO_4 hydrolysis, by GLC as their corresponding alditol acetates,[37] using a Packard and Becker 417 instrument coupled to a Hewlett–Packard 3380 A integrator. Glass columns (3 mm × 2 m) packed with 3% SP 2340 on Chromosorb W-AW DMCS (100–120 mesh), or 3% OV 17 on the same support were used. The carboxyl groups of the D-galactosyluronic acid were reduced according to the method of Taylor and Conrad.[38] The carboxyl reduced and the neutrals samples were methylated twice by the Hakomori procedure, as described by Jansson et al.[39] The partially methylated carbohydrates were then converted into their alditol acetates by successive treatments with $NaBH_4$ and pyridine–Ac_2O and analyzed on a fused-silica widebore column (30 m/0.53 m) half bonded with SP-2380. Peak identification was based on retention times using partially methylated alditol acetates standard and confirmed by GLC by using a SP 2380 capillary column (0.32 mm) coupled to a Nermag R1010C mass spectrometer. Peak areas were corrected by using the molar response factors according to Sweet et al.[40]

3.3. Preparation of cell-wall material (CWM)

Fats, waxes and oils (11 wt.% of dry material) were removed from skin powder by refluxing in a Soxhlet apparatus during 24 h with 38:62 toluene–EtOH. The mucilage exudate was removed from defatted residue of skin by treatment with water (2 × 2h) at room temperature in order to prepare CWM.

3.4. Isolation of pectic polysaccharides

Pectic polysaccharides were sequentially extracted from CWM by water (2 × 2 h at 60 °C) and aqueous solution of calcium chelator agent 0.5% EDTA (2 × 2 h at 60 °C). All extracts were precipitated with EtOH (4 volumes) resulting, respectively, in a water-soluble pectin (WSP) and a chelating-soluble pectin (CSP). The extraction scheme is given in Figure 2.

3.5. Ion-exchange chromatography

The different pectic fractions (WSP and CSP) were partially esterified, and were saponified with 0.1 M NaOH (overnight, N_2, 4 °C) in order to hydrolyze the acetyl and methyl esters. The solution was then acidified to pH 4–5 by the addition of 0.5 M HCl solution and extensively dialyzed against distilled water and freeze-dried to yield (WSP^+ and CSP^+) in H^+ form. Thereafter, a sample (500 mg) of each fraction (WSP^+, CSP^+) was suspended in 100 mL of 0.05 M phosphate buffer (pH = 6.3) and the solution was loaded onto a DEAE-Trisacryl M column (20 × 200 mm, phosphate form) eluted at 40 mL/h flow rate and previously equilibrated with the same buffer. The column was eluted with 300 mL of buffer and then successively with buffer containing, respectively, 0.125, 0.25, 0.5 and 1 M NaCl, 300 mL each. The fractions were then desalted by ultrafiltration with a membrane having a molecular weight cut-off of 500 and freeze-dried. For each extract five fractions were collected and the amounts of sample recovered in each fraction were for WSP: buffer, 40 mg (WSP1*, 8%); 0.125 M, 65 mg (WSP2*, 13%); 0.25 M, 175 mg (WSP3*, 35%); 0.5 M, 35 mg (WSP4*, 7%) and 1 M, 35 mg (WSP5*, 7%). For CSP: buffer, 20 mg (CSP1*, 4%); 0.125 M, 55 mg (CSP2*, 11%); 0.25 M, 140 mg (CSP3*, 28%); 0.5 M, 30 mg (CSP4*, 6%) and 1 M, 45 mg (CSP5*, 9%). An example of this fractionation procedure for WSP is summarized in Figure 3.

3.6. Size-exclusion chromatography

The major fractions (WSP3* and CSP3*) were purified by size-exclusion chromatography on a Shodex-OHpak B-804 (7.5 × 500 mm) and Shodex-OHpak B-803 (7.5 × 500 mm) columns carrying up in series, eluted at 1 mL/min flow rate with 0.05 M $NaNO_3$ solution, and at room temperature. The column effluent was monitored using a refractive index detector. The salts were removed by dialysis and the solution freeze-dried, to give the purified fractions WSP3 and CSP3.

3.7. Scanning electron microscopy

Small cubes were cut out from fresh skin of OFI, fixed with glutaraldehyde and dried under critical point conditions in a Polaron Critical Point Dryer operated with liquid CO_2. Before observation, the samples were sputtered with gold palladium alloy in a JEOL JFC sputterer. The observations were made with a JEOL JMS-6100 SEM operating at an accelerating voltage ranging from 5 to 8 kV and in secondary electron mode.

3.8. NMR spectroscopy

^{1}H experiments were recorded on a Bruker Avance 400 spectrometer (operating frequency of 400.13 MHz). Samples were examined as solution in D_2O at 333 K in 5 mm o.d. tube (internal acetone ^{1}H (CH_3) at 2.1 ppm relative to Me_4Si). ^{13}C NMR experiments were obtained on the same spectrometer (operating frequency: 100.57 MHz). Samples were recorded as solution in D_2O at 333 K in 5 mm o.d. tube (internal acetone ^{13}C (CH_3) at 31.5 ppm relative to Me_4Si). Two-dimensional spectra COSY, HMBC and HMQC were recorded using the standard Bruker procedures. COSY experiments were performed in the phase-sensitive mode. A 2048 (t_2)×512 (t_1)×2 data matrix was used with spectral widths of 2.5×2.5 kHz. A double quantum filter was used so that all signals could be phased to the pure absorption mode. ^{13}C–^{1}H Shift-correlation experiments were performed using the conventional Bruker sequence (with ^{13}C detection), with a J-filter value of 135 Hz for HMBC experiments. A 2048 (t_2 ^{1}H)×256 (t_1 ^{13}C) data matrix was used, with spectral widths of 2.5 kHz (^{1}H)×2.5 kHz (^{13}C). Delay times were 0.7 s between scans. A conventional ^{13}C–^{1}H dual probe was used and the 90° pulse lengths were 8 (^{13}C) and 16 μs (^{1}H).

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References

1. O'Neill, M. A. The pectic polysaccharides of primary cell walls. In *Methods in Plant Biochemistry*; Harborne, D., Ed.; Academic: London, 1990; pp 415–444.
2. Ridley, B. L.; O'Neill, M. A.; Mohnen, D. *Phytochemistry* **2001**, *57*(6), 929–967.
3. Stephen, A. M. Other plant polysaccharides. In *The Polysaccharides*; Aspinall, G. O., Ed.; Academic: Orlando, 1983; pp 98–193.
4. May, C. D. *Carbohydr. Polym.* **1990**, *12*, 79–99.
5. May, C. D. *Hydrocolloids* **2000**, 169–188.
6. Novosel'skaya, I. L.; Voropaeva, N. L.; Semenova, L. N.; Rashidova, S. S. *Chem. Nat. Compd.* **2000**, *36*(1), 1–10.
7. De Cortazar, V. G.; Nobel, P. S. *J. Am. Soc. Hor. Sci.* **1992**, *117*(4), 558–562.
8. Forni, E.; Penci, M.; Polesello, A. *Carbohydr. Polym.* **1994**, *23*(4), 231–234.
9. Habibi, Y.; Mahrouz, M.; Vignon, M. R. *J. Carbohydr. Chem.* **2003**, *22*(5), 331–337.
10. Vignon, M. R.; Garcia-Jaldon, C. *Carbohydr. Res.* **1996**, *296*, 249–260.
11. De Vries, J. A.; Rombouts, F. M.; Voragen, A. G. J.; Pilnik, W. *Carbohydr. Polym.* **1982**, *2*(1), 25–33.
12. De Vries, J. A.; Den Uijl, C. H.; Voragen, A. G. J.; Rombouts, F. M.; Pilnik, W. *Carbohydr. Polym.* **1983**, *3*(3), 193–205.
13. Westerlund, E.; Aaman, P.; Andersson, R.; Andersson, R. E.; Rahman, S. M. M. *Carbohydr. Polym.* **1991**, *15*(1), 67–78.
14. Huisman, M. M. H.; Schols, H. A.; Voragen, A. G. J. *Carbohydr. Polym.* **1999**, *38*(4), 299–307.
15. Schols, H. A.; Vierhuis, E.; Bakx, E. J.; Voragen, A. G. J. *Carbohydr. Res.* **1995**, *275*(2), 343–360.
16. Stevens, B. J. H.; Selvendran, R. R. *Carbohydr. Res.* **1984**, *135*(1), 155–166.
17. Stevens, B. J. H.; Selvendran, R. R. *Carbohydr. Res.* **1984**, *128*(2), 321–333.
18. Saulnier, L.; Thibault, J. F. *Carbohydr. Polym.* **1987**, *7*(5), 329–343.
19. Guillon, F.; Thibault, J. F.; Rombouts, F. M.; Voragen, A. G. J.; Pilnik, W. *Carbohyd. Res.* **1989**, *190*(1), 97–108.
20. Guillon, F.; Thibault, J. F. *Carbohyd. Res.* **1989**, *190*(1), 85–96.
21. Colquhoun, I. J.; de Ruiter, G. A.; Schols, H. A.; Voragen, A. G. *Carbohydr. Res.* **1990**, *206*(1), 131–144.
22. Davis, E. A.; Derouet, C.; Herve du Penhoat, C.; Morvan, C. *Carbohydr. Res.* **1990**, *197*, 205–215.
23. Huisman, M. M. H.; Fransen, C. T. M.; Kamerling, J. P.; Vliegenthart, J. F. G.; Schols, H. A.; Voragen, A. G. J. *Biopolymers* **2001**, *58*(3), 279–294.
24. Keenan, M. H. J.; Belton, P. S.; Matthew, J. A.; Howson, S. J. *Carbohydr. Res.* **1985**, *138*(1), 168–170.
25. Saulnier, L.; Brillouet, J. M.; Joseleau, J. P. *Carbohydr. Res.* **1988**, *182*(1), 63–78.
26. Polle, A. Y.; Ovodova, R. G.; Shashkov, A. S.; Ovodov, Y. S. *Carbohydr. Polym.* **2002**, *49*(3), 337–344.
27. Polle, A. Y.; Ovodova, R. G.; Chizhov, A. O.; Shashkov, A. S.; Ovodov, Y. S. *Biochemistry* **2002**, *67*(12), 1371–1376.
28. Redgwell, R. J.; Selvendran, R. R. *Carbohydr. Res.* **1986**, *157*, 183–199.
29. Catoire, L.; Goldberg, R.; Pierron, M.; Morvan, C.; Herve du Penhoat, C. *Eur. Biophys. J.* **1998**, *27*(2), 127–136.
30. Golovchenko, V. V.; Ovodova, R. G.; Shashkov, A. S.; Ovodov, Y. S. *Phytochemistry* **2002**, *60*(1), 89–97.
31. Odonmazig, P.; Badga, D.; Ebringerova, A.; Alfoldi, J. *Carbohydr. Res.* **1992**, *226*(2), 353–358.
32. Ryden, P.; Colquhoun, I. J.; Selvendran, R. R. *Carbohydr. Res.* **1989**, *185*(2), 233–237.
33. Cardoso Susana, M.; Silva Artur, M. S.; Coimbra Manuel, A. *Carbohydr. Res.* **2002**, *337*(10), 917–924.
34. Bushneva, O. A.; Ovodova, R. G.; Shashkov, A. S.; Ovodov, Y. S. *Carbohydr. Polym.* **2002**, *49*(4), 471–478.
35. Navarro, D. A.; Cerezo, A. S.; Stortz, C. A. *Carbohydr. Res.* **2002**, *337*(3), 255–263.
36. Blumenkrantz, N.; Asboe-Hansen, G. *Anal. Biochem.* **1973**, *54*(2), 484–489.
37. Selvendran, R. R.; March, J. F.; Ring, S. G. *Anal. Biochem.* **1979**, *96*(2), 282–292.
38. Taylor, R. L.; Conrad, H. E. *Biochemistry* **1972**, *11*(8), 1383–1388.
39. Jansson, P. E.; Kenne, L.; Liedgren, H.; Lindberg, B.; Lonngren, J. *Chem. Commun. (Stockholm Univ.)* **1976**, *8*, 1–20.
40. Sweet, D. P.; Shapiro, R. H.; Albersheim, P. *Carbohydr. Res.* **1975**, *40*(2), 217–225.