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Arabinan-rich polysaccharides isolated and characterized from the endosperm of the seed of *Opuntia ficus-indica* prickly pear fruits

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Abstract

After removal of starch, the reserve storage polysaccharide of the endosperm seed of *Opuntia ficus-indica* fruit was studied. Cell Wall Material (CWM) was extracted successively by boiling water (WSF), hot calcium chelating agent solution (CSF) and cold mild alkaline solution (CASF). All polysaccharides extracted were fractionated by ion-exchange chromatography into five fractions. The resulting major fractions were purified by size-exclusion chromatography and analyzed by sugar composition and glycosyl linkage analyses. The investigations were also supported by ^{1}H and ^{13}C NMR spectroscopy analysis. The results showed that the major fraction of WSF consisted of an arabinan. The backbone contained α-(1→5)-linked arabinofuranosyl residues with high percentage of arabinose units substituted at O-2. The predominant fractions from CSF and CASF were related to rhamnogalacturonan type I which consisted of a disaccharide repeating unit →2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→backbone with α-(1→5)-linked arabinan side-chains attached to O-4 of the rhamnosyl residues.

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

1. Introduction

Cactaceaes are a plant family which exhibit *Crassulacean* Acid Metabolism (CAM) and are drought-tolerant. This explains why they are successively cultivated in arid and semi-arid regions. *Opuntia ficus-indica* (OFI) is the most widespread specie of the *Cactaceaes* family. This prickly pear cactus was exploited essentially for its fruits, which can constitute an important and abundant raw material for the food industry in arid regions (Ingelse, Basile, & Schirra, 1997).

The amount of seeds is important as it varies from 20 to 40% dry-weight of the whole fruit, depending on the cultivars (Barbera, Inglese, & La Mantia, 1994). Indeed, seeds of *O. ficus-indica* can play a key role in the valorization of the whole fruit. For that reason, different studies have been carried out on the chemical composition of seeds in the last decade. Their nutritive value was determined by Sawaya, Khalil, and Al-Mohammad (1983), and their reserve proteins have been studied by Uchoa, Souza, Zarate, Gomes-Filho, and Campos (1998). Their main conclusion is that such proteins have an amino acid composition similar to the 2S albumin storage protein family. The oil extracted from the seeds of prickly pear fruit was studied by numerous groups (Barbagallo & Spagna, 1999; Krifa, Villet, Krifa, & Alary, 1993; Sawaya & Khan, 1982). The results of physical and chemical analysis showed that their fatty acid composition is similar to other common edible vegetable oils. They suggested that *O. ficus-indica* seeds presented a good potential source of edible oil for human consumption.

The polysaccharides of the seeds of prickly pear fruit have attracted our attention and in a previous study we isolated several glucuronoxylan from their pericarp, with molar ratios of 4-*O*-methyl-glucuronic acid to xylose varying from 1:12 to 1:65 (Habibi, Mahrouz, & Vignon, 2002).

Arabinans were found in primary cell walls of different parts of plants of many families notably in seeds, fruits

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and roots. They appear either associated with pectic polysaccharides especially rhamnogalacturonan or as 'free' polysaccharides (Capek, Toman, Kardosova, & Rosik, 1983; Cardoso, Silva, & Coimbra, 2002; Churms et al., 1983; Eriksson, Andersson, Westerlund, & Aaman, 1996; Herve du Penhoat, Michon, & Goldberg, 1987; Jiang & Timell, 1972; Joseleau, Chambat, Vignon, & Barnoud, 1977; Larm, Theander, & Aman, 1975; Navarro, Cerezo, & Stortz, 2002; Petkowicz, Sierakowski, Ganter, & Reicher, 1998; Siddiqui & Wood, 1974; Stevens & Selvendran, 1980; Tharanathan, Bhat, Krishna, & Paramahans, 1985; Zawadzki-Baggio, Sierakowski, Correa, & Reicher, 1992). They usually carry a backbone of α-(1→5)-linked L-arabinofuranosyl units, more or less branched by single or multiple stubs of the same kind on positions 2 and/or 3. Various degrees of branching were found; a linear (1→5)-arabinan was found in apple juice (Churms et al., 1983) and in the coat of several legume seeds (Petkowicz, Reicher, Chanzy, Taravel, & Vuong, 2000; Petkowicz et al., 1998; Zawadzki-Baggio et al., 1992), while an almost linear one was found in the hypocotyl cells of *Vigna radiate* (Herve du Penhoat et al., 1987). In early papers, most of the branching was encountered on C-3 (Aspinall & Cottrell, 1971; Cardoso et al., 2002; Siddiqui & Wood, 1974) and more recently from the olive pomace (Cardoso et al., 2002). An arabinan with a low degree of branching mainly on C-2, was found in the roots of the horsebean (Joseleau, Chambat, & Lanvers, 1983).

In this work we describe the chemical composition and structural features of arabinan-rich polysaccharides isolated from the endosperm of the seeds of prickly pear fruit.

2. Experimental

2.1. Materials

Fresh mature prickly pear fruits of *O. ficus-indica* (OFI) were collected from the experimental station plantation located in the vicinity of Marrakech (Morocco). The harvested fruits were washed, carefully hand-peeled and the pulp was mixed for a few minutes in a mixer grinder. The seeds were recovered from the resulting pulp juice by straining through metallic strainer and cleaned by several washings in distilled water. After drying, they were cracked in an analytical grinder for a few minutes and the endosperm was recovered after sieving on 60 mesh sieve.

2.2. Analytical methods

Uronic acid content was determined according to Blumenkrantz and Asboe-Hansen method (Blumenkrantz & Asboe-Hansen, 1973). Neutral sugars were analyzed, after H_2SO_4 hydrolysis, by GLC as their corresponding alditol acetates, 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 (1972). The carboxyl-reduced and the neutral samples were methylated twice by the Hakomori procedure, as described by Jansson, Kenne, Liedgren, Lindberg, and Lonngren (1976). 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, Shapiro, and Albersheim (1975).

2.3. Preparation of cell wall material (CWM)

Fats, waxes and oils were removed from endosperm powder by refluxing in a Soxhlet apparatus during 24 h with 38:62 toluene–EtOH. The endosperm powder was suspended in water (water to pulp ratio of 20:1) and ground in a warring blender apparatus for 10 min. The slurry was then poured on 0.25 mm sieve and washed with water to remove the starch granules. The residual starch content was removed by treatment with pancreatic α-amylase in order to prepare CWM. The sample was suspended in 0.2 M NaOAc buffer solution (pH 6.1) containing a 1% α-amylase in order to make 1 mg amylase for 10 mg of sample. The mixture was incubated at 37 °C for 36 h. The suspension was centrifuged and the supernatant was removed. The resulting residue was washed exhaustively with water and freeze-dried to give an amylose-free sample, CWM (52% of dry matter).

2.4. Isolation of polysaccharides

Polysaccharides were sequentially extracted from CWM by water (2×2 h at 100 °C) and aqueous solution of calcium chelator agent 0.5% EDTA (2×2 h at 100 °C) and finally by 0.5 M NaOH aqueous solution (2×1 h at 20 °C). The alkaline extract was neutralized by acetic acid solution 20% at pH≈5–6. The different extracts were precipitated with EtOH (4 vol) resulting, in a water-soluble fraction (WSF), a chelating soluble fraction (CSF) and a cold alkaline soluble fraction (CASF). The extraction scheme is given in Fig. 2.

2.5. Ion-exchange chromatography

The different fractions (WSF and CSF) 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 addition

of 0.5 M HCl solution and extensively dialyzed against distilled water and freeze-dried to yield (WSF^+ and CSF^+) in H^+ form. The CASF fraction was acidified, dialyzed and freeze-dried to yield $CASF^+$. Thereafter, a sample (300 mg) of each fraction (WSF^+, CSF^+ and $CASF^+$) 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 300 ml of buffer containing, respectively, 0.125, 0.25, 0.5 and 1 M NaCl, 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 WSF: buffer, 195 mg (WSF1*, 65%); 0.125 M, 18 mg (WSF2*, 6%); 0.25 M, 9 mg (WSF3*, 3%); 0.5 M, 0 mg (WSF4*, 0%) and 1 M, 6 mg (WSF5*, 2%). For CSF: buffer, 0 mg (CSF1*, 0%); 0.125 M, 45 mg (CSF2*, 15%); 0.25 M, 174 mg (CSF3*, 58%); 0.5 M, 12 mg (CSF4*, 4%) and 1 M, 0 mg (CSF5*, 0%). For CASF: buffer, 45 mg (CASF1*, 15%); 0.125 M, 18 mg (CASF2*, 6%); 0.25 M, 126 mg (CASF3*, 42%); 0.5 M, 13.5 mg (CASF4*, 4.5%) and 1 M, 21 mg (CASF5*, 7%).

2.6. Size-exclusion chromatography

The major fractions (WSF1*, CSF3* and CASF3*) were purified by size-exclusion chromatography on a polyacrylamide Biogel P6 column (4–100 cm) column, eluted at 80 ml/h 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 WSF1, CSF3 and CASF3.

2.7. NMR spectroscopy

^1H 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 OD tube (internal acetone ^1H (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 OD 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–^1H shift-correlation experiments were performed using both the conventional Bruker sequence (with ^{13}C detection). A 2048 (t_2 ^1H)×256 (t_1 ^{13}C) data matrix was used, with spectral widths of 2.5 kHz (^1H)×2.5 kHz (^{13}C). Delay times were 0.7 s between scans. A conventional ^{13}C–^1H dual probe was used and the 90° pulse lengths were 8 μs (^{13}C) and 16 μs (^1H).

3. Results and discussion

3.1. Preliminary studies of endosperm seed

The optical micrographs of transversal cross-section of seeds of *O. ficus-indica* showed that the seed consisted of two different tissues, the endosperm (E) and the pericarp (P) as shown in Fig. 1A. The endosperm was observed by scanning electron microscopy and showed that it was mainly made up of starch granules enclosed in thin cellulose cell walls (Fig. 1B).

In Table 1 we report the results of sugar analysis of the whole seed and of the endosperm. Sugar composition of the whole seed showed a predominance of xylose and glucose residues corresponding to xylan and cellulose already found in the pericarp of the seed. The pericarp corresponded to 90–95% of the whole seed and indeed constitutes a natural xylan–cellulose composite (unpublished results). The endosperm corresponded to 5–10% of the whole seed and its sugar composition showed that it contained mainly arabinose and glucose. The glucose was found at high levels and originated essentially from starch and cellulose.

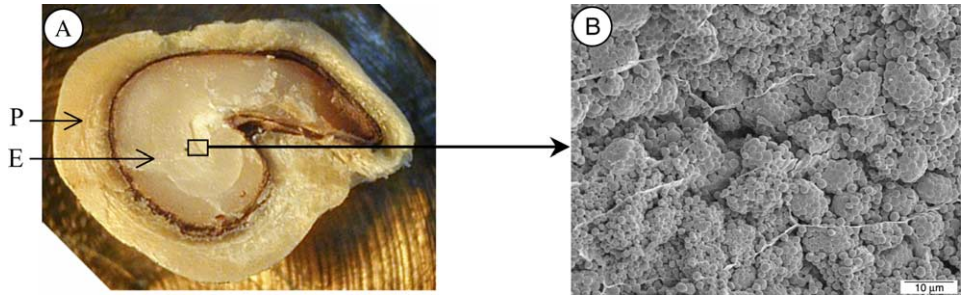

Fig. 1. (A) Optical micrograph of a seed cross-section P, pericarp; E, endosperm. (B) Scanning electron micrograph of seed endosperm.

Table 1
Sugar composition of the seed and endosperm of seed

Component	Uronic acid	Neutral sugars[a]					
		Rha	Glc	Gal	Ara	Xyl	Man
Whole seed	9	0.6	40.6	1.0	3.1	44.8	1.0
Endosperm	5.5	7	35	8	39	5.5	Traces

[a] Expressed in relative weight percentages.

3.2. Extraction and characterization of polysaccharides from endosperm

The largest part of starch granules was removed by sieving and the residual amount was eliminated by enzymatic digestion in order to prepare Cell Wall Material (CWM) of endosperm. The sugar composition of CWM reported in Table 2 showed a decrease in the amount of glucose corresponding to removal of starch. The CWM was extracted sequentially by boiling water, hot calcium chelating agent solution and cold mild alkaline solution. The extracted polymers are named Water Soluble Fraction (WSF), Chelating Soluble Fraction (CSF) and Cold Alkaline Soluble Fraction (CASF) as shown in the extraction procedure (Fig. 2).

The yields and sugar composition of all the extracts are given in Table 2. The results of sugar analysis revealed that the arabinose was a predominant neutral sugar in CWM, WSF, CSF and CASF. The different fractions contained also uronic acid in varying amounts, 10.4, 25.3 and 26% in WSF, CSF and CASF, respectively.

3.3. Fractionation of isolated polysaccharides

The methyl and acetyl ester groups of WSF and CSF were saponified and the resulting de-esterified WSF^+ and CSF^+ as well as $CASF^+$ into their H^+ form were fractionated by ion-exchange chromatography. For each extract, five fractions were collected. We can notice that each fractionation is characterized by the predominance of only one fraction, WSF1* (65%), CSF3* (58%) and CASF3* (42%). These major fractions were purified by size-exclusion chromatography. The resulting purified fraction WSF1, CSF3 and CASF3 were characterized by sugar, methylation and NMR analysis.

3.4. Characterization of major polysaccharide fractions

The results of sugar and methylation analysis of native fractions WSF1, CSF3 and CASF3 are reported in Tables 3 and 4. In another experiment, the carboxyl groups of each acidic fraction were reduced with $NaBD_4$ into the corresponding 6,6′-dideutero-D-galactosyl residues before hydrolysis or methylation, in order to differentiate the galactose arising from the reduction of the galacturonic acid residues and the native galactose residues already in the side-chain.

The investigations are supported by 1D ^1H and ^{13}C NMR spectroscopy and also by several 2D-NMR techniques such as Correlated Spectroscopy (COSY), shift-correlation using either Heteronuclear Multiple Quantum Coherence (HMQC) or Heteronuclear Multiple Bond Correlation (HMBC). The ^1H and ^{13}C NMR spectra of WSF1, CSF3 and CASF3 fractions are given in Figs. 3 and 4.

3.4.1. Characterization of WSF1

The sugar analysis of WSF1 reported in Table 3 showed that this fraction contained exclusively arabinose and thus corresponded to an arabinan. The methylation data are reported in Table 4. The results suggested that the arabinan contained a (1→5)-arabinofuranose backbone, with 44.7% of the units being (1→5)-linked, from which only 6.7% were exclusively (1→5)-linked. The degree of branching was around 85% and exclusively in O-2 (37.7% of 2-Me-Ara). The presence of 2,5-di-*O*-methyl arabinitol (15.3%) suggested that the L-arabinose residues in the side-chains are 1,3-linked. The relatively high proportion of terminal sugars (38.3% of 2,3,5-tri-*O*-methyl arabinitol) combined with 15.3% of arabinose units (1→3)-linked in the side-chains, indicated that the side-chain contains either a (1→3)-linked arabinose disaccharide or only one arabinose unit.

The NMR data for WSF1 are reported in Table 5, and the ^1H and ^{13}C spectra in Figs. 3 and 4. The ^1H spectrum showed great similarity with the spectrum given by Eriksson et al. (1996). The region for anomeric signals in Fig. 3 contained at least four signals at 5.14, 5.04, 5.21 and 4.97 ppm and were assigned, to terminal-α-Ara*f*, α-(1→5), α-(1→2,5), and α-(1→3) linked arabinose, respectively. The assignments of the proton signals reported in Table 5 were made according to 2D COSY experiments and

Table 2
Yield and sugar composition of CWM, WSF, CSF and CASF from endosperm of seed

Fraction	Yield[a]	Uronic acid[b]	Neutral sugars[b]					
			Rha	Glc	Gal	Ara	Xyl	Man
CWM	52.7	10.5	3.5	12.5	7.3	55.5	9.6	1.2
WSF	6.0	10.4	3.4	–	2.5	76.3	1.2	–
CSF	8.5	25.3	4.5	1.3	3.7	40.1	2.0	–
CASF	7.0	26.0	3.8	2.1	4.1	37.2	3.7	–

[a] As % of endosperm dry matter.
[b] Expressed in relative weight percentages.

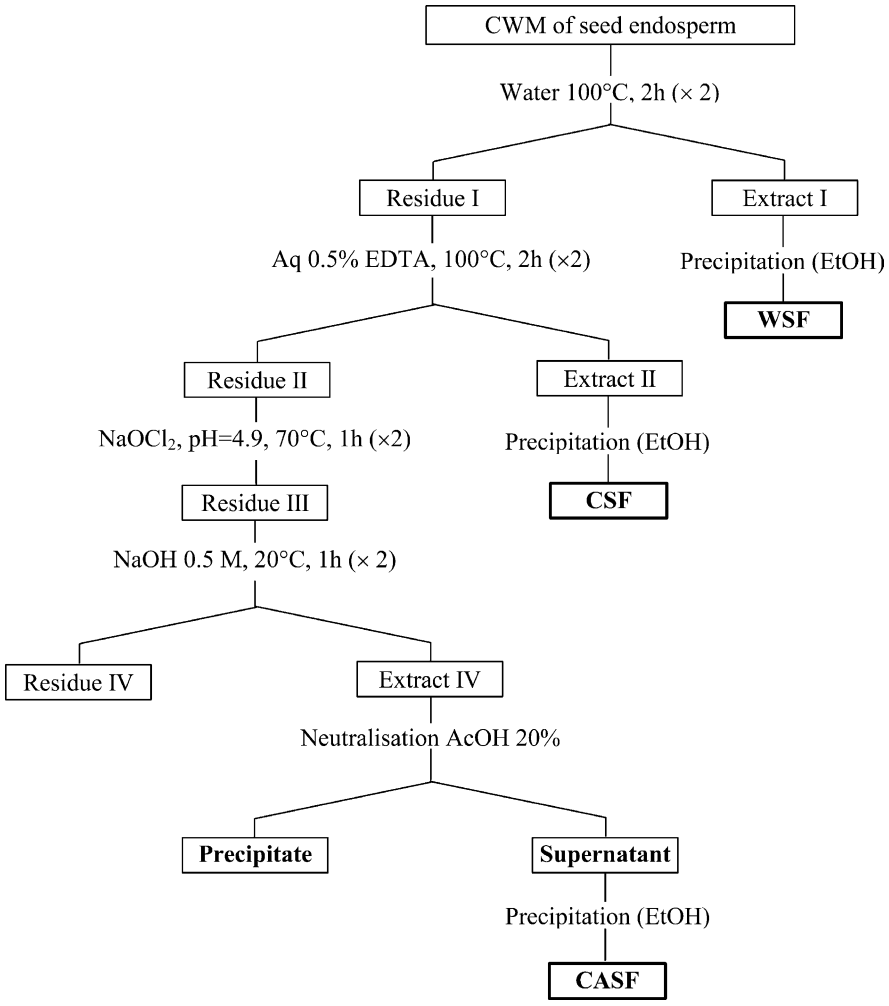

Fig. 2. Scheme of extraction of polysaccharides from the endosperm.

literature data (Cardoso et al., 2002; Herve du Penhoat et al., 1987; Navarro et al., 2002; Saulnier, Brillouet, Moutounet, Herve du Penhoat, & Michon, 1992). The region for signals of anomeric carbons in the ^{13}C NMR spectrum (Fig. 4) contained at least four signals at 107.82, 107.21, 108.46 and 108.52 ppm, assigned, respectively, to terminal-α-Ara*f*, α-(1→2,5), α-(1→5) and α-(1→3) linked arabinose, with approximate relative intensities of 1:2:3:1, respectively. The assignments of the carbon-13 signals (Table 5) were done according to 2D hetero-correlated experiments, and confirmed previous work (Bakinovskii, Nepogod'ev, & Kochetkov, 1985; Capek et al., 1983; Eriksson et al., 1996; Joseleau et al., 1977; Vignon, Heux, Malainine, & Mahrouz, 2004). The NMR results corroborated the methylation data and demonstrated that WSF1 consisted of a central core of α-(1→5)-linked arabinofuranosyl residues, with side-chains exclusively linked in O-2. We can propose for WSF1 the following repeating unit (Fig. 5), but other variations are possible.

3.4.2. Characterization of CSF3

The sugar composition of native and reduced CSF3 is reported in Table 3 and showed that this fraction contained high amount of arabinose (55%) suggesting the presence of

Table 3
Sugar composition[a] of native WSF1 and native and $NaBD_4$ carboxyl reduced CSF3 and CASF3

Fraction	Uronic acid	Neutral sugars					
		Gal 6,6′-d_2	Rha	Glc	Gal	Ara	Xyl
WSF1 native	–	–	–	–	–	98.5	–
CSF3 native	15	–	2.5	–	4.4	48.2	2.56
CSF3 reduced	–	18	17.6	3	4	55	1
CASF3 native	30	–	4.5	–	1.2	49.3	1.9
CASF3 reduced	–	35	11	–	2	50	1.4

[a] Expressed in relative weight percentages.

Table 4
Partially methylated alditol acetates of native WSF1 and $NaBD_4$ carboxyl reduced CSF3 and CASF3

Alditol	Native WSF1[a]	Reduced CSF3[a]	Reduced CASF3[a]
2,3,5-Me_3-Ara[b]	38.3	20.3	18.8
2,3-Me_2-Ara	6.7	17.2	17.7
2,5-Me_2-Ara	15.3	–	–
2-Me-Ara	0.1	5.8	7.2
3-Me-Ara	37.7	5.1	3.6
Ara	0.2	4.3	5.0
Total	98.3	52.7	52.3
2,3,4,6-Me_4-Gal	–	4.9	4.2
2,3,6-Me_3-Gal	–	5.1	–
Total	–	10.0	4.2
3,4-Me_2-Rha	–	10.6	7.7
3-Me-Rha	–	6.7	5.9
Total	–	17.3	13.6
2,3,6-Me_3-Gal 6,6′-d_2	–	20	30.2

[a] Relative mole ratio.
[b] 2,3,5-Me_3-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-arabinitol, etc.

an arabinan rich polysaccharide. Other many sugars are also detected such as galacturonic acid, rhamnose and galactose in the ratios 15:2.5:4.4. The poor yield of rhamnose can be explained by incomplete hydrolysis of Gal*p*A→Rha*p* linkage previously observed by Vignon and Garcia-Jaldon (1996). The data were much better after two carbodiimide treatments and reduction with $NaBD_4$. Galacturonic acid, rhamnose, galactose and arabinose in 18:17.6:4:55 molar ratio, were the main sugars detected. These results suggested the presence of a rhamnogalacturonan substituted by arabinan and galactane side-chains which is confirmed by the methylation data of $NaBD_4$ carboxyl reduced CSF3

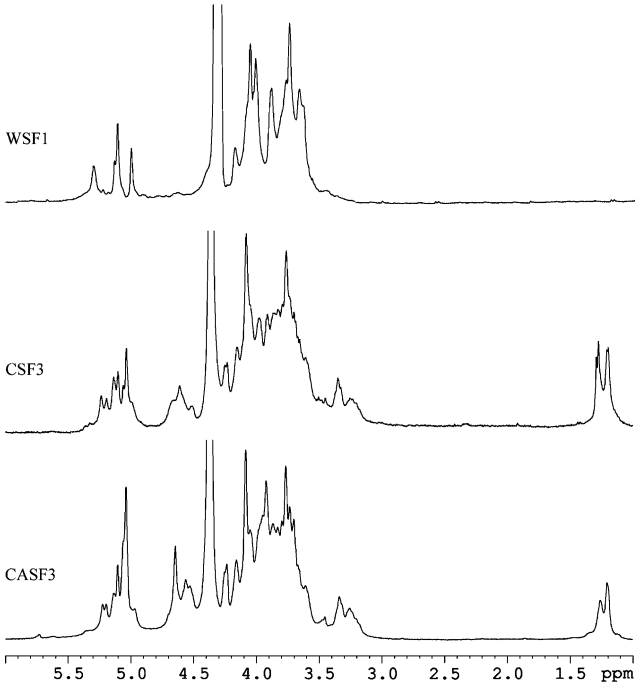

Fig. 3. ^{1}H NMR spectra of WSF1, CSF3 and CASF3 (333 K, 400.13 MHz).

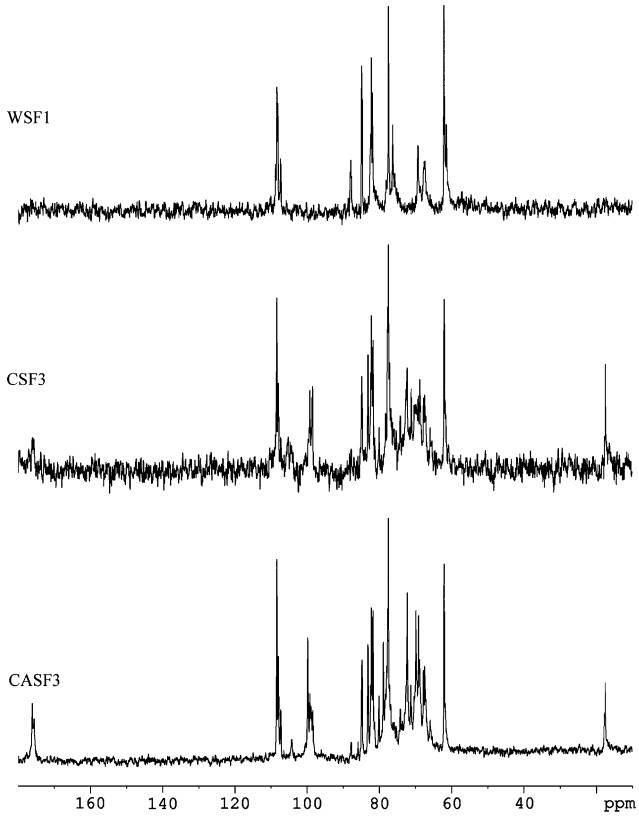

Fig. 4. ^{13}C NMR spectra of WSF1, CSF3 and CASF3 (333 K, 100.57 MHz).

(Table 4). In fact, 3-*O*-methyl rhamnitol and 3,4-di-*O*-methyl rhamnitol were detected in ratios of 6.7:10.7 in $NaBD_4$ reduced CSF3, indicating that the (1→2,4)-linked rhamnose accounted for 38.5% 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 indicated that there is one galacturonic acid per rhamnose residue, suggesting that CSF3 backbone 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 (4.9%) and 2,3,4,6-tetra-*O*-methyl galactitol (5.1%) indicated that the galactan side-chains have an average length of two galactose units.

The methylated isomers of arabinose found in CSF3 indicated some structural differences with the arabinan of the WSF1 fraction. Indeed, 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 CSF3 were, respectively, in the ratio 20.3:17.2:5.8:5.1:4.3. These data which are very similar to the results already obtained in the case of CSP3 (Habibi, Heyraud, Mahrouz, & Vignon, 2004) suggested that the backbone of the arabinan side-chains of CSF3 consisted of a central core of α-(1→5)-linked

Table 5
Chemical shift data[a] (333 K) for related α-arabinosyl residues of WSF1

Glycosyl residues	Assignment					
	1	2	3	4	5	
→5)-α-L-Ara*f*-(1→	$^{1}H/^{13}C$	5.04/108.46	4.08/81.85	4.03/77.48	4.18/83.06	3.87/68.22
→2,5)-α-L-Ara*f*-(1→	$^{1}H/^{13}C$	5.21/107.21	4.32/80.38	4.04/84.90	4.24/82.82	3.72/68.47
→3)-α-L-Ara*f*-(1→	$^{1}H/^{13}C$	4.97/108.52	4.09/84.96	4.16/83.20	4.18/83.06	3.86/67.92
T-α-L-Ara*f*-(1→	$^{1}H/^{13}C$	5.14/107.82	4.16/82.62	3.91/77.47	3.97/84.93	3.80/62.26

[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).

arabinofuranosyl residues from which 53% were exclusively (1→5)-linked, 18% were (1→3,5)-linked, 15.7% (1→2,5)-linked and 13.2% (1→2,3,5)-linked. The proportion of terminal non-reducing arabinose was relatively high (20.3%). The average length of the branches in the arabinan side-chains, inferred from the relative amounts of terminal to branched arabinose residues was of one, indicating that the branches in the arabinan side-chains consisted in fact of single arabinose unit.

The NMR data for CSF3 are reported in Table 6. The ^{1}H and ^{13}C spectra of CSF3 in Figs. 3 and 4, showed the same general features as already observed in the case of CSP3 (Habibi et al., 2004). The ^{13}C spectrum is indeed dominated by signals of α-L-arabinofuranosyl moieties with major peaks at 108.45 (C-1), 83.15 (C-4), 82.22 (C-2), 78.53 (C-3) and 68.22 ppm (C-5) of α-(1→5)-linked arabinofuranosyl residues, confirming the presence of an arabinan-like structure as side-chain. In addition to the presence in the anomeric region of the characteristic C-1 signals of galactopyranosyl acid α-(1→4)-linked at 98.52 ppm and rhamnopyranosyl α-(1→2)-linked at 99.21 ppm, indicated that CSF3 is composed of rhamnogalacturonan backbone. In the ^{1}H spectra, two signals at 1.20 and 1.25 ppm were assigned to the CH_3 of the rhamnose units, respectively, to the rhamnosyl residues linked only at O-2 and to the rhamnosyl residues linked both at O-2 and O-4. The side-chains are constituted by galactan and arabinan oligosaccharides and the structure of CSF3 was given in Fig. 6A.

From the different results we can also propose for arabinan side-chain in CSF3 the structure reported in the Fig. 6B, but other possibilities are conceivable.

3.4.3. Characterization of CASF3

The results of sugar analysis of native or $NaBD_4$ carboxyl reduced CASF3 (Table 3) showed that the amount of galacturonic acid or 6,6′-d_2-galactosyl was larger than the amount of rhamnose, indicating that CASF3 consisted of homogalacturonan blocks and rhamnogalacturonan blocks. Methylation analysis confirmed these results, and we noticed that the proportion of 2,3,6-tri-*O*-methyl galactitol 6,6′-d_2 (30.2%) arising from the reduced 4-linked D-galacturonic acid was larger than the sum of 3-*O*-methyl rhamnitol (5.9%) and 3,4-di-*O*-methyl rhamnitol (7.7%). These data indicated that 55% of galacturonic acid residues were involved in galacturonan blocks and 45% in rhamnogalacturonan blocks. Among the rhamnose residues, 43% were substituted by arabinan or galactan side-chains. The detection of only 2,3,4,6-tetra-*O*-methyl-galactitol

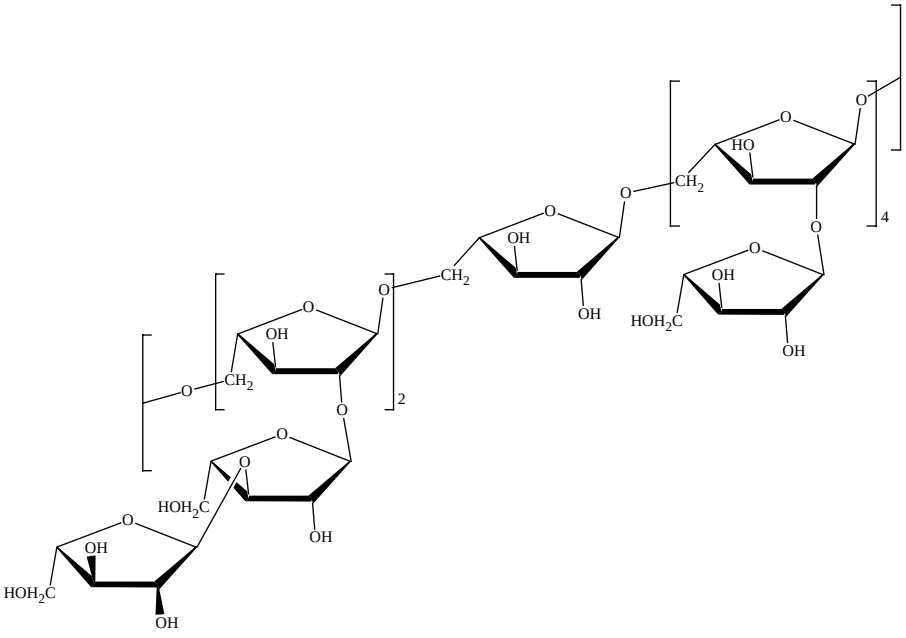

Fig. 5. Schematic structure of arabinan WSF1.

Table 6
Chemical shift data[a] (333 K) for related glycosyl residues of CSF3

Glycosyl residues	Assignment						
	1	2	3	4	5	6	
α-Arabinosyl residues							
→5)-α-L-Ara*f*-(1→	$^{1}H/^{13}C$	5.05/108.45	4.08/82.22	4.11/78.53	4.16/83.15	3.81/68.22	–
→3,5)-α-L-Ara*f*-(1→	$^{1}H/^{13}C$	5.09/108.38	4.23/80.01	3.92/84.95	4.25/82.13	3.76/68.17	–
→2,5)-α-L-Ara*f*-(1→	$^{1}H/^{13}C$	5.21/107.33	4.13/85.00	4.37/78.04	4.16/83.20	3.86/67.65	–
→2,3,5)-α-L-Ara*f*-(1→	$^{1}H/^{13}C$	5.09/107.95	4.25/85.00	4.06/84.98	4.20/83.20	3.67/68.13	–
T-α-L-Ara*f*-(1→	$^{1}H/^{13}C$	5.14/108.02	4.08/82.40	3.98/77.66	4.00/84.98	3.79/62.09	–
β-Galactosyl residues							
→4)-β-D-Gal*p*-(1→	$^{1}H/^{13}C$	4.56/105.26	3.48/72.70	3.70/74.18	4.11/78.34	3.65/75.3	3.76/61.78
α-Galacturonosyl residues							
→4)-α-D-Gal*p*-A-(1→2)-α-L-Rha*p*-(1→	$^{1}H/^{13}C$	4.96/98.52	3.97/68.82	4.02/69.8	4.37/77.9	4.56/72.3	176.50
α-Rhamnosyl residues							
→4)-α-D-Gal*p*-A-(1→2)-α-L-Rha*p*-(1→	$^{1}H/^{13}C$	5.23/99.21	4.08/77.69	3.87/70.32	3.35/71.20	3.80/69.81	1.20/17.43
→4)-α-D-Gal*p*-A-(1→2,4)-α-L-Rha*p*-(1→	$^{1}H/^{13}C$	5.23/99.02	4.15/78.75	3.87/70.32	4.23/80.05	3.68/67.76	1.25/17.64

[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).

showed that galactan side-chain contained only one galactose unit.

The same methyl arabinitol acetates already detected in CSF3 were found in the case of CASF3, but in different proportions, suggesting a similar arabinan structure. The results showed that 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 CASF3 were, respectively, in the ratio 18.8:17.7:7.2:3.6:5.0. The backbone of the arabinan side-chain consisted of a (1→5)-linked arabinose residues, in which the repeating unit contained, in average, five not substituted units, two units substituted at O-3, one unit substituted at O-2 and one unit substituted both at O-2 and O-3.

The NMR spectra presented the same general features as already observed in the case of CSF3, but we can notice the presence in the ^{13}C spectrum, in addition the presence of the characteristic signals of α-(1→4)-linked galacturonic acid of homogalacturonan blocks. These signals were identified at 99.78, 69.15, 69.90, 78.86, 72.25 and 176.24 ppm and can be assigned, respectively, to C-1, C-2, C-3, C-4, C-5 and

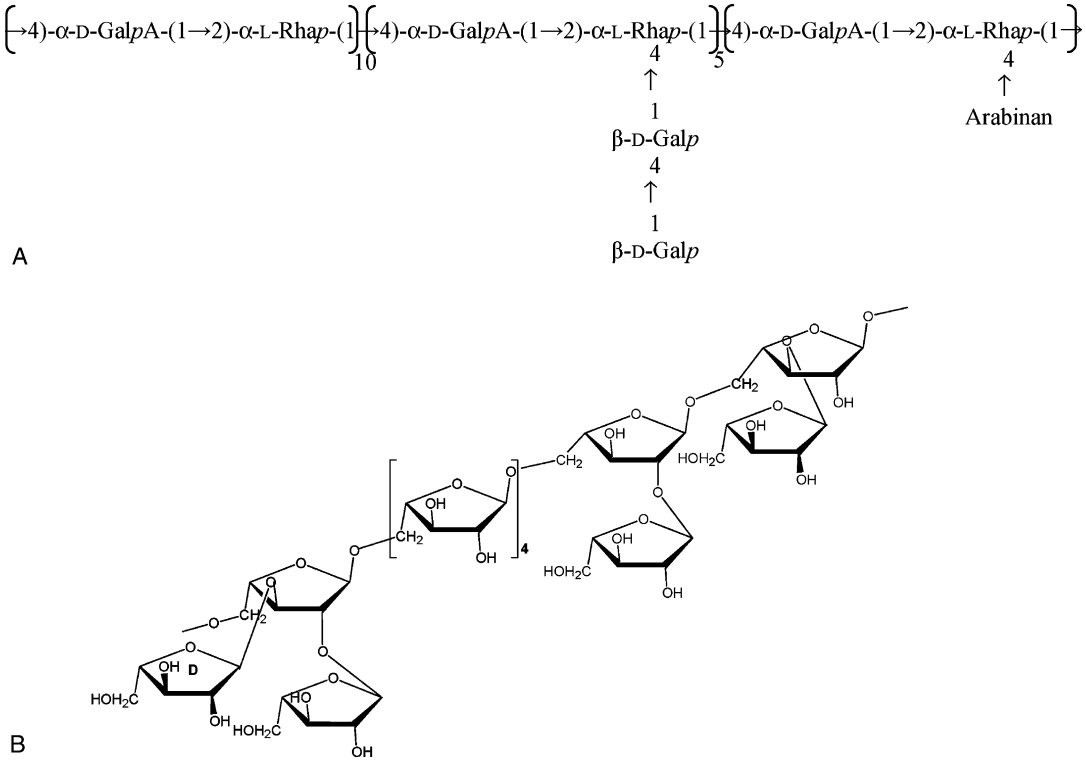

Fig. 6. (A) Schematic structure of CSF3. (B) Schematic structure of arabinan side-chains in CSF3.

Table 7
NMR data[a] (333 K) for related glycosyl residues of CASF3

Glycosyl residues	Assignment						
	1	2	3	4	5	6	
α-Arabinosyl residues							
→5)-α-L-Ara*f*-(1→	$^{1}H/^{13}C$	5.03/108.40	4.08/82.40	4.11/78.53	4.16/83.20	3.81/68.22	–
→3,5)-α-L-Ara*f*-(1→	$^{1}H/^{13}C$	5.08/108.37	4.23/80.15	3.98/84.98	4.22/82.80	3.72/68.17	–
→2,5)-α-L-Ara*f*-(1→	$^{1}H/^{13}C$	5.20/107.28	4.23/85.00	4.37/78.04	4.16/83.20	3.86/67.92	–
→2,3,5)-α-L-Ara*f*-(1→	$^{1}H/^{13}C$	5.10/107.87	4.25/85.00	4.06/84.98	4.20/83.20	3.67/68.13	–
T-α-L-Ara*f*-(1→	$^{1}H/^{13}C$	5.13/107.98	4.08/82.40	3.93/77.61	4.00/84.98	3.76/62.25	–
B-Galactosyl residues							
→4)-β-D-Gal*p*-(1→	$^{1}H/^{13}C$	4.57/105.21	3.48/72.70	3.70/74.36	4.11/78.53	3.65/75.30	3.80/61.94
α-Galacturonosyl residues							
→4)-α-D-Gal*p*-A(1→	$^{1}H/^{13}C$	5.02/99.78	NA/69.15	NA/69.90	NA/78.86	4.64/72.25	176.24
→4)-α-D-Gal*p*-A(1→2)-α-L-Rha*p*-(1→	$^{1}H/^{13}C$	4.95/98.44	3.97/68.80	4.02/69.80	4.37/77.90	4.55/72.35	175.5
α-Rhamnosyl residues							
→4)-α-D-Gal*p*-A(1→2)-α-L-Rha*p*-(1→	$^{1}H/^{13}C$	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-D-Gal*p*-A(1→2,4)-α-L-Rha*p*-(1→	$^{1}H/^{13}C$	5.22/99.04	4.15/78.75	3.87/70.20	4.23/80.15	3.68/67.83	1.25/17.45

[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).

carboxyl group of α-(1→4)-galacturonan. We noticed the presence in the anomeric regions of the characteristic signals of rhamnose and galacturonic acid residues involved in rhamnogalacturonan blocks at 99.24 and 98.44 ppm assigned, respectively, to (1→2)-linked rhamnose and (1→4)-linked galacturonic acid residues.

The galactan side-chains were characterized by the minor signals at 105.21, 72.70, 74.36, 78.53, 75.30 and 61.94 ppm assigned to C-1–C-6, respectively.

The NMR data collected are given in Table 7 and this confirmed that CASF3 consisted of alterning homogalacturonan and rhamnogalacturonan blocks which can be substituted by short galactan and arabinan side-chains (Fig. 7A). According to HSQC and HMBC correlation, it was possible to confirm the structure of the arabinan side-chains in CASF3. Thus, it was possible to identify the anomeric ^{1}H and ^{13}C for all residues: T-α-L-Ara*f* (5.13/107.98), (1→5)-α-L-Ara*f* (5.03/108.40) (1→3,5)-α-L-Ara*f* (5.08/108.37) (1→2,5)-α-L-Ara*f* (5.20/107.28) (1→2,3,5)-α-L-Ara*f* (5.10/107.87).

From these results, we can propose for the arabinan side-chain, in CASF3 the structure reported in Fig. 7B, but other isomer structures are possible.

It is worth noting that our previous studies on the pectic polysaccharides from the peel of prickly pear fruit of *O. ficus-indica* showed that the arabinan side-chains have

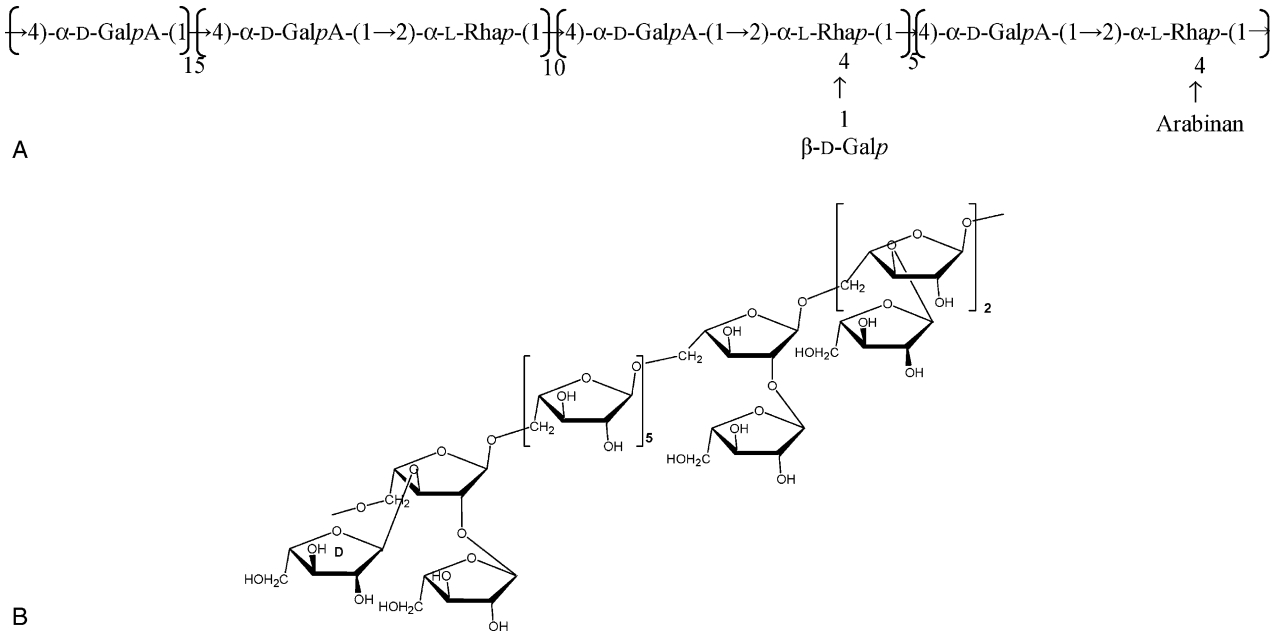

Fig. 7. (A) Schematic structure of CASF3. (B) Schematic structure of arabinan side-chains in CASF3.

a structure with branching on C-3 and/or C-2 (Habibi et al., 2004). Also Vignon et al. (2004) have demonstrated that the free arabinan isolated from cladode spines of *O. ficus-indica* presented a similar structure with branching point both on C-2 and C-3.

To conclude, we have demonstrated that the polysaccharides isolated and purified from endosperm seed of *O. ficus-indica* show the presence of either a free arabinan (WSF1) or arabinan-rich polysaccharides attached to rhamnogalacturonan type I blocks (CSF3 and CASF3).

From a structural point of view, the WSF1 arabinan showed a difference, notably in the branching point that are only in O-2 and in the stubs (linked 1→3), with other arabinans isolated from higher plants (Churms et al., 1983; Eriksson et al., 1996; Jiang & Timell, 1972; Joseleau et al., 1983; Joseleau et al., 1977; Larm et al., 1975; Stevens & Selvendran, 1980; Swamy & Salimath, 1991; Tharanathan et al., 1985).

We can notice similarity in the structure of arabinan side-chains linked to rhamnogalacturonan blocks in the case of CSF3 and CASF3 fractions with arabinan side-chains in rhamnogalacturonan from sugar beet (Guillon & Thibault, 1989; Guillon, Thibault, Rombouts, Voragen, & Pilnik, 1989). We can explain this similitude as the *Cactaceaes* family is very close to the *Amaranthaceae* family (sugar beet family) within the *Caryophylalles* order.

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