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Morphological and structural study of seed pericarp of *Opuntia ficus-indica* prickly pear fruits

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Abstract

The morphological study of pericarp of *Opuntia ficus-indica* (OFI) seeds showed that the cells were mainly made up of spindle-shaped sclerenchyma fibers. The chemical composition of the pericarp revealed a significant amount of polysaccharides, with cellulose (35%) and xylan (27%). The structure of xylan and cellulose, both in isolated form and as a component of seed pericarp of OFI were studied by X-ray and CP/MAS ^{13}C NMR spectroscopy. The supramolecular structure of xylan is very sensitive to the surrounding environment, in particular to the presence of water and of cellulose fibers. The cellulose fibers presented X-ray diagrams typical of secondary wall cellulose but they were sensitive toward NaOH since they started to be converted into cellulose II at a NaOH concentration as low as 8%. In seed pericarp, cellulose fibers interact with xylan polymers, causing these to adopt a conformation different to the one observed for xylan both in dry or hydrated form, suggesting that xylans were probably present as composites with cellulose fibers.

Keywords: Cellulose; CP/MAS; Morphology; *Opuntia ficus-indica*; Seed; X-ray; Xylan

1. Introduction

Cellulose is the most abundant biopolymer on earth and the major component in the cell walls of wood and terrestrial plants. In contrast to cellulose, which is a homopolysaccharide, hemicelluloses which also occur in the cell walls are heteropolysaccharides. Xylans are the most abundant of the hemicelluloses found in the cell walls of plants, of which they can constitute more than 30% of the dry weight. Xylans are characterized by a β-(1 → 4)-D-Xyl*p* backbone to which arabinosyl, glucuronic acid, and acetyl substituents can be attached. The main xylan in softwoods is arabino-4-*O*-methylglucuronoxylan, while 4-*O*-methyl glucuronoxylan free from arabinose substituents dominates in hardwoods (Stephen, 1983; Whistler & Chen, 1991; Wilkie, 1979).

Strong interactions are present between xylan and cellulose as evidenced by the fact that the synthesis and deposition of xylan are intimately linked with cellulose during the assembly of the cell wall. The xylan retention phenomenon has been explained by co-crystallization of xylan segments with cellulose and by the formation of strong xylan–cellulose hydrogen bonds. Several researchers have observed the strong tendency of xylans to self-associate (Brett, 2000; Joseleau, Comtat, & Ruel, 1992; Mora, Ruel, Comtat, & Joseleau, 1986).

The structures of cellulose in wood and pulp as well as interaction between cellulose and hemicelluloses have been extensively studied by CP/MAS ^{13}C NMR spectroscopy (Hult, Larsson, & Iversen, 2000; Larsson, Hult, Wickholm, Pettersson, & Iversen, 1999; Larsson, Wickholm, & Iversen, 1997; Lindgren, Edlund, & Iversen, 1995; Maunu,

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Liitiä, Kauliomäki, Hortling, & Sundquist, 2000; Newman, 1998; Newman & Hemmingson, 1990; Newman, Hemmingson, & Suckling, 1993; Van der Hart & Atalla, 1984; Wickholm, Larsson, & Iversen, 1998). The most important progress in this context is reported by Larsson in a recent review (Larsson, 2004).

Opuntia ficus-indica is a tropical or subtropical plant, which belongs to the *Cactaceae* family and is mainly used for fruit production (De Cortazar & Nobel, 1992). Because of its high adaptation to the harsh desert environment and its different applications, the *Opuntia ficus-indica* 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. Several bilateral projects and local programs have promoted the establishment of *Opuntia ficus-indica* to orient the utilization of the plant for multiple purposes. A better understanding of their chemical composition could allow opening new opportunities for both food and non-food applications of this abundant resource. In the present study, we explored the morphological organization of cells in seed pericarp of *Opuntia ficus-indica* prickly pear fruit. We used X-ray and CP/MAS ^{13}C NMR spectroscopy to characterize the structure of xylan and cellulose fibers in both isolated form and as component of pericarp seed of OFI.

2. Experimental

2.1. Materials

Fresh mature prickly pear fruits of *Opuntia ficus-indica* (Fig. 1a) were collected from an 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 a metallic strainer and cleaned by several washings with distilled water. After drying, they were cracked in an analytical grinder for a few minutes and the pericarp was recovered after sieving on 60 mesh sieve.

2.2. Analytical methods

Neutral sugars were analyzed, after H_2SO_4 hydrolysis, by Gas Liquid Chromatography (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 lignin analysis was achieved according to the TAPPI standard T222-03-75.

2.3. Isolation and purification

The scheme of extraction and purification of xylan and cellulose from pericarp seed of *Opuntia ficus-indica* is given in Fig. 2. Defatted seed pericarp was treated by water (2 × 1 h at 100°C) and a sodium chlorite treatment according to Wise et al. (Wise, Murphy, & D'Addieco, 1946) was performed to remove residual protein and lignin. The bleached residues were treated, respectively, by 2%, 4% and 6% NaOH aqueous solutions. All extracts were neutralized (pH 5–6) and the precipitates were recovered by centrifugation. The crude extracted fractions were purified by solubilization in 1% NaOH solution and precipitation with Fehling solution according to Jones and Stoodley (Jones & Stoodley, 1965).

For cellulose residue purification, the resulting residue IV is treated according to two methods: (i) Alkaline extraction: residue IV was extracted sequentially by 8% and 12% NaOH solution at 80 °C for 2 h to give Residue V-A and Residue VI-A, respectively. (ii) Mechanical treatment combined with an alkaline extraction: residue IV at 1% wt concentration in water was disintegrated for 15 min in a Waring Blender operated at full speed where a final temperature of 60 °C was reached. The suspension was then homogenized by 15 passes through a Manton Gaulin laboratory homogenizer operated at 500 bars at a temperature that was controlled at 80 °C to give the homogenized residue noted Residue IV-G. This residue was then treated by 6% NaOH solution to give a final residue (Residue V-G).

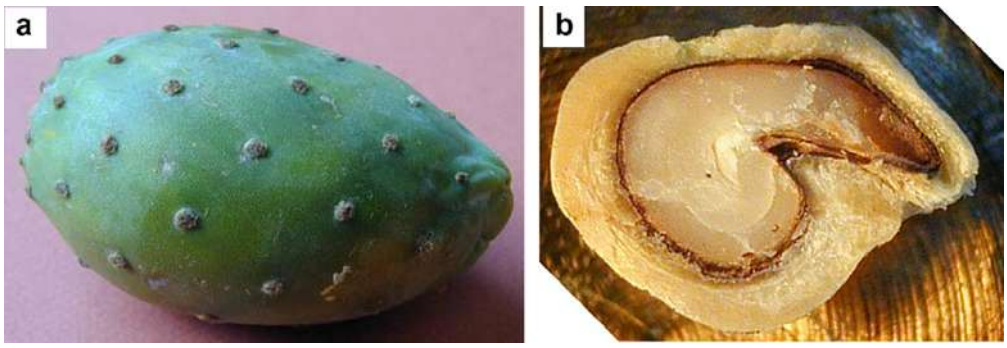

Fig. 1. (a) *Opuntia ficus-indica* fruit, (b) cross-section of seed from *Opuntia ficus-indica* fruit.

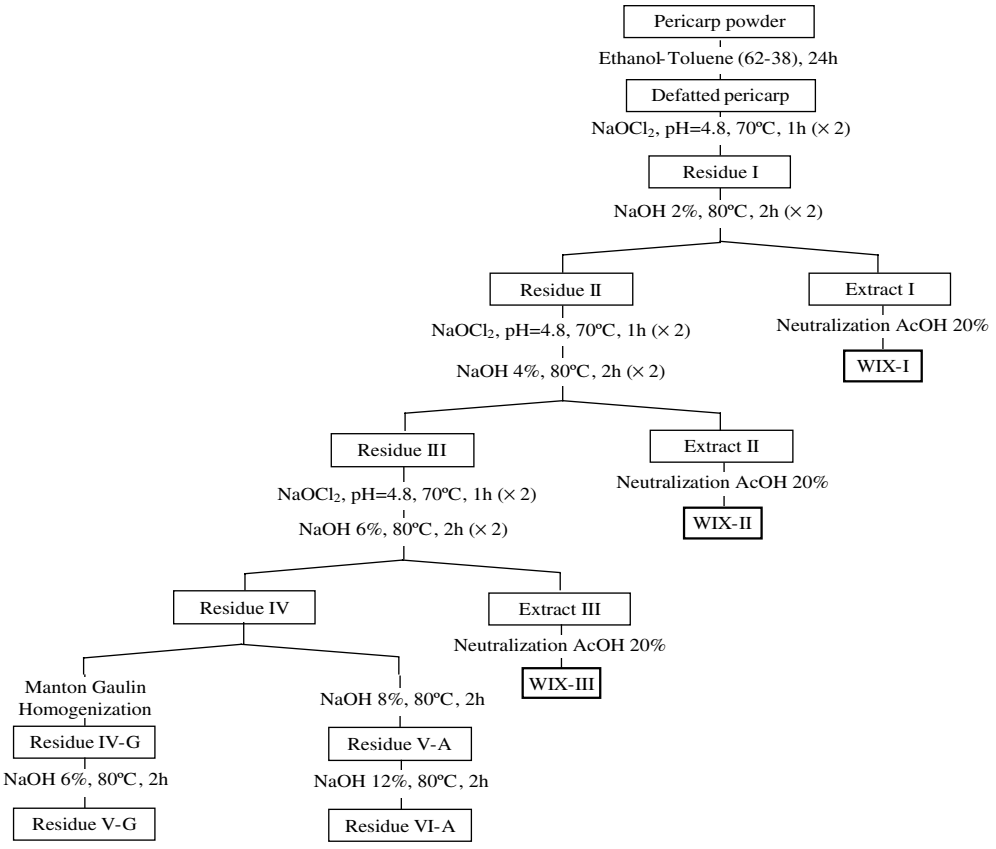

Fig. 2. Scheme of fractionation and purification of xylan and cellulose from pericarp seed of OFI.

2.4. CP/MAS ^{13}C solid state NMR spectroscopy

The NMR experiments were performed on a Bruker Avance spectrometer (^{13}C frequency of 100 MHz), using proton dipolar decoupling (DD), magic angle spinning (MAS) and cross-polarization (CP). CP transfer was achieved using a ramped amplitude sequence (RAMPCP) for an optimized total contact time of 2 ms. The spinning speed was set at 6 kHz, sweep width 50,000 Hz, recycle delay 4 s. An average number of 10,000 scans was acquired for each spectrum. The various xylan samples were examined under different conditions either dried or analyzed wet in the presence of liquid water. Each cellulose residue sample was analyzed in the presence of liquid water. The ^{13}C chemical shifts were measured relative to carbon chemical shift of glycine carboxyl group (176.03 ppm).

2.5. X-ray diffraction

The X-ray diagrams were recorded on a Warhus flat film vacuum X-ray camera mounted on a Philips PW 1720 X-ray generator operated at 20 mA and 30 kV. X-ray measurements were made on dry xylan powder or hydrated xylan inserted and sealed in thin wall glass capillaries and on films obtained by water evaporation of the suspensions for cellulose residues.

2.6. Scanning electron microscopy

Seeds were embedded in LR White resin (hard mixture; London Resin Co., Woking, UK) polymerized for 24 h at 50 °C. Ultra-thin (250 nm) longitudinal and transversal cross-sections were cut with a microtome (MTX RMC Ultrotome) and fixed in a freshly prepared mixture of 0.3% glutaraldehyde, 2% paraformaldehyde in 0.05 M phosphate buffer pH 7–7.2. Samples were dehydrated through a graded series of ethanol. Before viewing, 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.

2.7. Optical microscopy

Observations of the cellulose fiber residues were achieved with a Zeiss Axiophot 2 optical microscope operated in Nomarski contrast, equipped with a camera and controlled by computer.

2.8. Determination of molecular weight

The purified and freeze-dried xylans were dissolved in DMSO. The solutions were filtered directly into light scattering cells through 0.2 μm nylon membranes (Pall Gelman

Laboratory). The concentration of the solutions for each sample was chosen so that the experiment could be carried out under dilute regime conditions. Static light scattering (SLS) experiments were performed with a spectrometer equipped with an argon ion laser (Spectra Physics, model 2020, $\lambda = 488$ nm) and fitted with a variable-angle detection system operated by a stepping motor (ALV, Langen-Germany Instruments). The sample temperature was set at 25 ± 0.1 °C, and the scattered intensity was measured through a band-pass filter (488 nm) and a 200-μm pinhole with a photomultiplier tube (ALV).

3. Results and discussion

3.1. Morphological and chemical analysis

The micrographs of transversal cross-section of seeds of *Opuntia ficus-indica* showed that the seed consisted of two different tissues, the endosperm (E) and the pericarp (P), as shown in Figs. 1b and 3a. In a whole *Opuntia ficus-indica* prickly pear fruit, the amount of seed is important, and can vary from 30% to 40% on a dry weight basis. The pericarp corresponded to 90–95% of the whole seed. The constituents and chemical composition of the pericarp are given in Table 1.

An important amount of lignin (20 wt%), as well as fats and waxes (8 wt%) are observed. The minerals and protein content are low (2.5 wt%), and the main constituents are polysaccharides (62 wt%), including cellulose (35 wt%).

The morphological study carried out by scanning electron microscopy showed that the cells of pericarp seed of *Opuntia ficus-indica* were mainly made up of spindle-shaped sclerenchyma fibers organized into two distinct orientations: P1 and P2 (Fig. 3b, c, and d). These tissues are typical constituent of the secondary walls. We can also observe some spiral conducting vessels in simple helix (Fig. 3e and f).

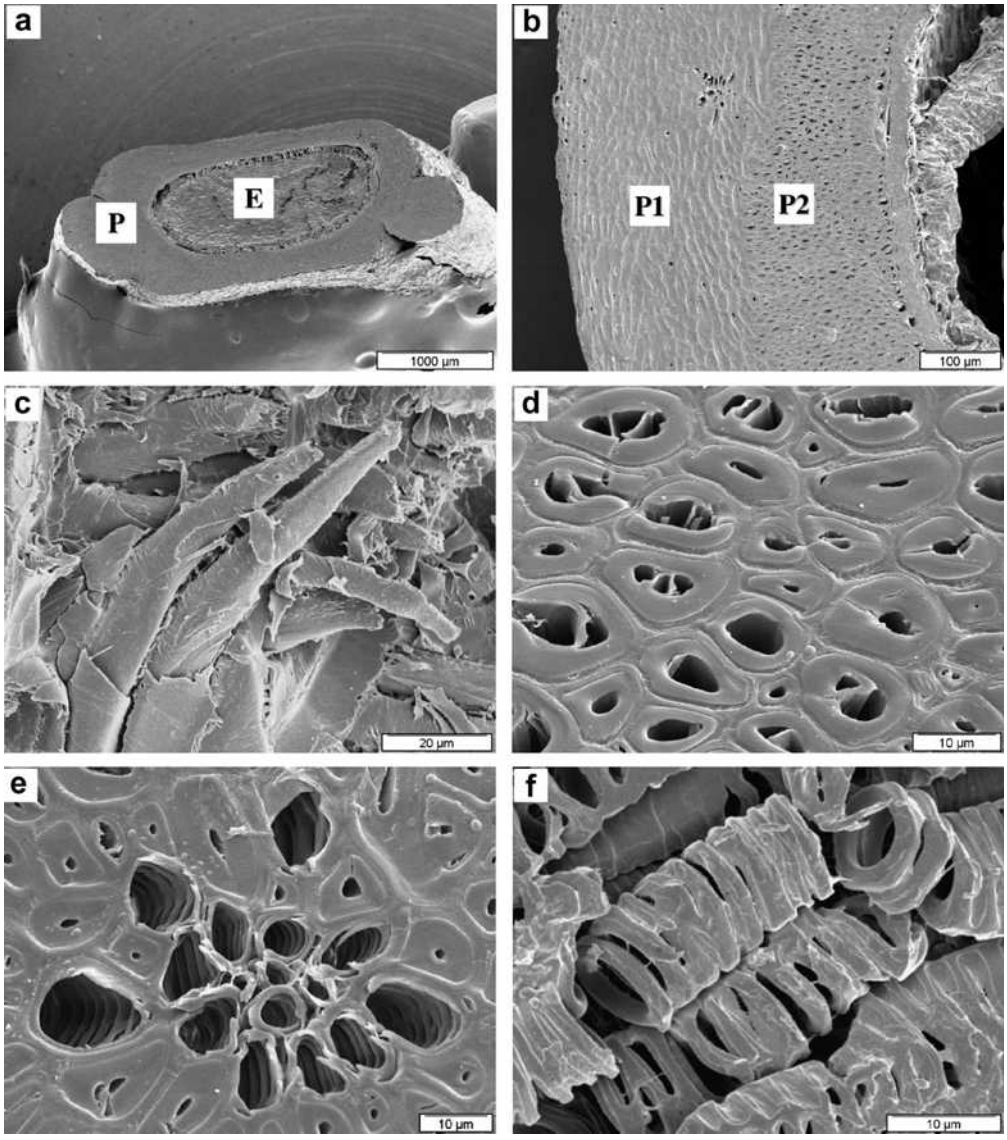

Fig. 3. SEM micrographs of transverse sections of: (a) an *Opuntia ficus-indica* seed; (b) the pericarp of the seed with the two orientations P1 and P2; (c) and (d) enlargement of P1 and P2, respectively; (e) and (f) longitudinal and transversal cross-sections, respectively, of spiral conducting vessels of P2.

Table 1
Chemical composition of pericarp seed of OFI

Constituents	Dry wt%
Ash	2.5
Fat and wax	8
Lignin	20
Protein	1.5
Other polysaccharides	27
Cellulose	35

In Table 2 we reported the results of sugar analysis of the whole seed and of the pericarp. Sugar composition showed a predominance of xylose (42.7%) and glucose (47.1%) residues which indicated that the pericarp seed consisted of a natural xylan–cellulose composite.

3.2. Extraction of xylan content and purification of cellulose fibers

Xylan can be extracted directly from wood with aqueous alkali, although their alkali-extractability cannot be complete because xylans were differently associated physically and chemically with lignin and cellulose. A higher efficiency of extraction is reached with higher alkali concentrations, but high concentrations of sodium hydroxide can cause mercerization of cellulose. Mercerization process involves swelling of native cellulose I fibers in concentrated sodium hydroxide with formation of cellulose–NaOH complexes. These complexes can recrystallize in an anti-parallel manner to form the energetically favorable cellulose II polymorph after removal of the swelling agent with water (O'Sullivan, 1997). In addition, it has been demonstrated that the mercerization of cellulose fibers depends on their origin and their morphology. As an example, mercerization of secondary wall cellulose such as cotton linters does not occur if the concentration of NaOH is below 10% (w/w) (Lindgren et al., 1995) and starts at 9% NaOH (w/w) in the case of primary wall cellulose extracted from sugar beet pulp (Dinand, Vignon, Chanzy, & Heux, 2002). Other alternative methods, such as enzymatic degradation, chemical acidic hydrolysis, or thermo-mechanical treatments, are used to remove xylan and purify cellulose.

The seed pericarp of *Opuntia ficus-indica* is composed essentially of xylan and cellulose. The extraction of xylan content is carried out by alkaline treatments as show in Fig. 2. The efficiency of the extraction is controlled by sugars dosage and CP/MAS ^{13}C NMR. Sugars analysis,

Table 2
Sugar composition of the seed and of seed pericarp

Component	Uronic acid[a]	Neutral sugars[a]					
		Rha	Glc	Gal	Ara	Xyl	Man
Whole seed	–	0.6	40.6	1.0	3.1	44.8	1.0
Pericarp	9	–	47.1	–	1.3	42.7	0

[a] Expressed in relative weight percentages.

Table 3
Sugars content of different treated cellulose fibers from pericarp seed of *Opuntia ficus-indica*

Residue	Sugars[a]	
	Xylose	Glucose
Defatted pericarp	42.7	47.1
Residue IV	19.5	80.5
Residue V-A	10.6	89.4
Residue VI-A	1.0	99.0
Residue IV-G	18.8	82.2
Residue V-G	1.2	98.8

[a] Expressed in relative weight percentages.

reported in Table 3, indicated that 80% of the xylan could be extracted by 2, 4, and 6% NaOH extractions.

Cellulosic residues were analyzed by CP/MAS ^{13}C NMR and we reported in Fig. 4 the spectra of defatted residue and of different alkali treated residues. We noticed the presence, in addition to characteristic signals of cellulose, of several signals in the spectrum of the defatted pericarp at 20.94, 56.06, and 173.21 ppm. The two signals at 20.94 and 173.21 ppm disappeared after the first alkali treatment suggesting that they were removed during the treatment. This observation suggested that they corresponded to acetate groups carried by the xylan. It indicates that the native xylans of seed pericarp are partially acetylated. After bleaching treatment, the signals at 56.06, and between 112 and 152 ppm disappeared indicating that they corresponded more likely to lignin residues. The spectrum of residue IV corresponds roughly to cellulose-rich polysaccharide. Nevertheless, the sugars analysis showed that it contained an important amount of xylan (19.5%), which required purification. Thereafter, we were interested in the purification of this cellulosic fraction.

In order to purify cellulose fibers from pericarp seed of *Opuntia ficus-indica*, we studied two extraction methods as shown in Fig. 1: (i) entirely alkaline treatments and (ii) a combination of Manton Gaulin mechanical treatment and alkaline extractions. A study was achieved to check the effect of alkaline concentrations and optimize the xylan extraction and cellulose purification.

3.3. Alkaline treatment of seed pericarp of OFI

Before purification of cellulose fibers from pericarp seed of OFI, we were interested by their mercerization. We determined by X-rays and CP/MAS ^{13}C NMR spectroscopy the alkali concentration threshold where the cellulose I $\rightarrow$ cellulose II transformation starts. After washing and drying, the X-ray investigation of the various alkali treated samples gave typical variations in the diffraction patterns. This is illustrated in Fig. 5, where the four patterns corresponded to samples treated with different alkali concentration. The patterns in Fig. 5a–d are those of residues treated, respectively, with 2%, 6%, 8%, and 12% NaOH. The pattern in Fig. 5b has the same features as the one in Fig. 5a, but with a slightly better resolution. Both

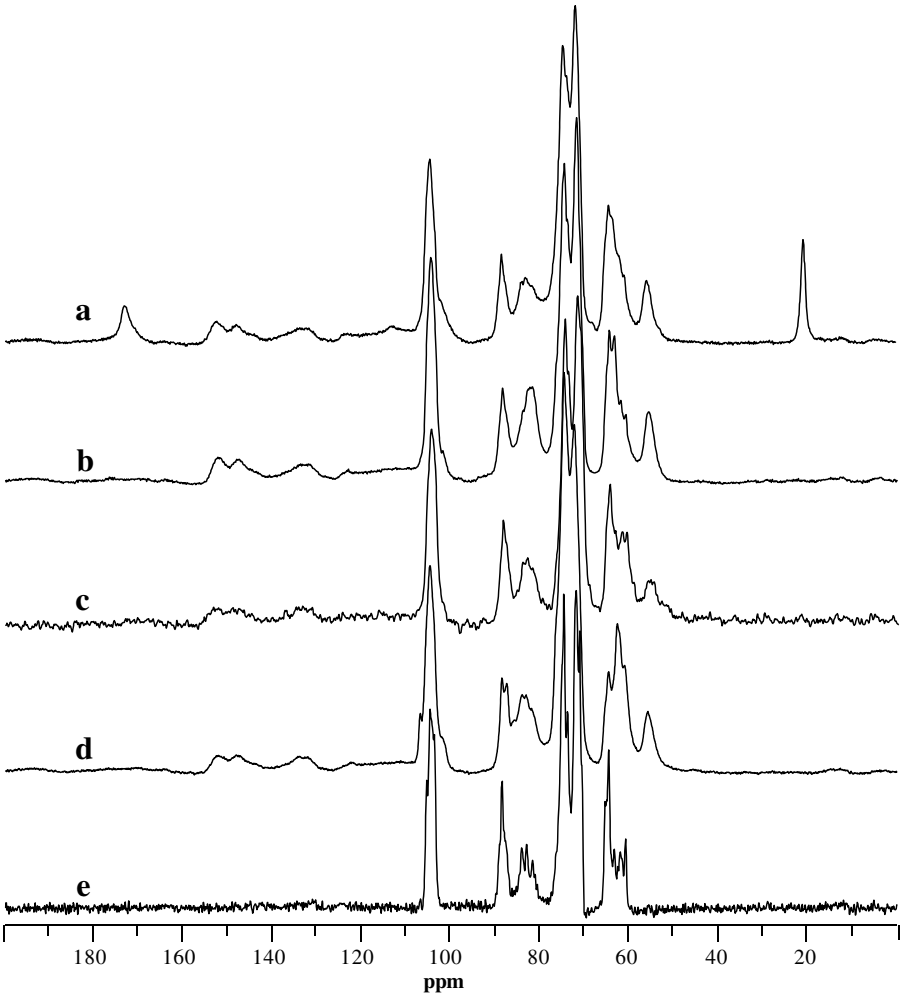

Fig. 4. CP/MAS ^{13}C NMR spectra of: (a) defatted pericarp; (b) aq. 2% NaOH – Residue II; (c) aq. 6% NaOH – Residue IV; (d) aq. 8% NaOH – Residue V-A; (e) aq. 6% NaOH – Gaulin Residue V-G.

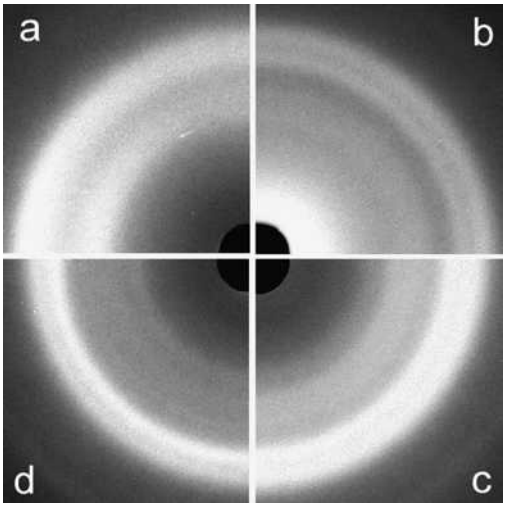

Fig. 5. X-ray diffraction patterns of alkali-treated pericarp seed of *Opuntia ficus-indica*: (a) aq. 2% NaOH, (b) aq. 6% NaOH, (c) aq. 8% NaOH, and (d) aq. 12% NaOH.

Fig. 5a and b are typical cellulose I patterns. These patterns consist of four diffraction rings: (i) a sharp weak ring at a *d*-spacing of 0.258 nm, (ii) two stronger and slightly broader rings at $d = 0.43$ and 0.40 nm, the ring at 0.40 nm being more intense than the one at 0.43 nm, and (iii) a broad ring of medium intensity centered at 0.57 nm.

The sample treated with 8% NaOH solution gave a diagram (pattern Fig. 5c) that differed somewhat from the patterns in Fig. 5a and b. The pattern 5c presented four diffraction rings, located at nearly the same position as those in 5a and 5b, but with different relative intensities. Indeed, in 5c the rings at 0.43 and 0.40 nm have practically the same strong intensity, the one at 0.43 nm being somewhat more intense than the one at 0.40 nm. On the other hand, the sharp ring at 0.258 nm and the broad one at 0.57 nm became extremely weak. This pattern demonstrated clearly the cellulose I → cellulose II conversion. The pattern 5d was different from the other three patterns. It showed only two rings at *d*-spacing of 0.40 and 0.43 nm, the ring at 0.43 nm being slightly stronger than the one at 0.40 nm. This pattern corresponded to mercerized cellulose II.

According to the X-ray diagrams, the cellulose I → cellulose II transformation of cellulose fibers from seed

pericarp of OFI starts at 8% NaOH concentration, which is unusual in the case of secondary wall cellulose fibers.

The various alkali-treated residues were also analyzed by CP–MAS ^{13}C NMR spectroscopy and the spectra of various residues are shown in Fig. 4. The spectrum 4-C presented the characteristic signals of native cellulose (Atalla, Gast, Sindorf, Bartuska, & Maciel, 1980; Earl & VanderHart, 1980). In the spectrum treated with 8% NaOH aqueous solution (Fig. 3d), the apparition of some signals, particularly the doublets near 104.80 and 107.02 ppm together with the doublet near 62.86 and 62.40 ppm, demonstrated clearly the onset of cellulose conversion. The concomitant decrease of the cellulose I doublet centered near 64.95 ppm was also observed. The percentage of cellulose II increased steadily with the concentration in NaOH to reach 100% conversion with the sample treated with 12% NaOH (spectra not showed). These results confirmed that mercerization of pericarp seed cellulose started with 8% NaOH.

3.4. Mechanical treatment of seed pericarp of OFI

We chose to combine alkaline extraction with mechanical treatment in order to purify cellulose from seed pericarp of OFI. This mechanical treatment makes it possible to individualize fibers in microfibrils and to increase their accessibility and their alkaline extraction without cellulose mercerization.

The residue IV, according to the preceding extraction scheme (Fig. 2), was treated in a Manton Gaulin homogenizer. The homogenized residue, noted Residue IV-G, was then treated another time by 6% alkaline aqueous solution to give Residue V-G. The mechanical treatment and purification were controlled by microscopy, sugar analysis and also by CP/MAS ^{13}C NMR.

Fig. 6 showed the optical micrograph of residue IV before and after mechanical treatment in a Manton Gaulin homogenizer. These images indicated that the fibers have been completely disrupted during the homogenization treatment and the homogenized residue consisted of disencrusted cellulose fibers.

As shown in Table 3, the xylose content of 6% NaOH treated residue (residue IV) is important (19.5%), demonstrating the presence of an important amount of residual xylan. No variation in the sugar composition was observed after the mechanical treatment (Residue IV-G). However, the sugar composition of the cellulosic residue was extensively modified after the last alkaline treatment (residue V-G). The glucose was the dominant constituent (98.1%) of residue V-G showing that the removal of residual xylan was facilitated by the mechanical treatment which allowed a better accessibility. The mechanical treatment induced a better access to fibers' surface and allowed the extraction of the residual xylan (20%), without mercerization of cellulose as demonstrated by X-ray experiments (not shown) as well as CP/MAS ^{13}C NMR (Fig. 4e). Indeed, the CP/MAS ^{13}C NMR spectrum presented the characteristic signals of native cellulose.

These results showed the efficiency of the mechanical–chemical combined treatments used for purification of cellulose from pericarp seed of OFI.

3.5. Ratio of I_α and I_β cellulose content and degree of cellulose crystallinity

Solid state NMR was generally used to investigate the ratio of cellulose I_α and I_β in cellulose species. In particular the C-4 region was used for this analysis, the signals from ordered and less ordered regions being well separated. The signals between 86 and 92 ppm corresponded to C-4 of the highly ordered cellulose crystallite, whereas the broader upfield signal between 79 and 86 ppm were assigned to the C-4 of disordered cellulose as well as to the less ordered cellulose chains of the crystallite surfaces (Van der Hart & Atalla, 1984; Wickholm et al., 1998). Larsson used spectral fitting for the C-4 region of cotton cellulose and assigned three Lorentzian lines for the signals from cellulose I_α, $I_{(\alpha+\beta)}$, and I_β and four Gaussian lines for the signals from para-cristalline cellulose, inaccessible fibril surfaces, and two accessible fibril surfaces.

The ratio of I_α and I_β cellulose polymorphs and the degree of cellulose crystallinity in the case of seed pericarp

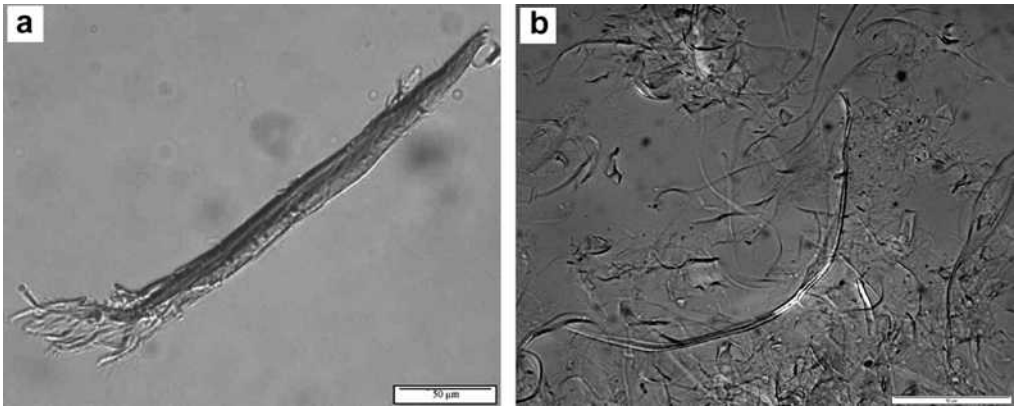

Fig. 6. Optical micrographs in Nomarski contrast of disencrusted cells (Residue IV): (a) before and (b) after homogenization.

of OFI were determined using the areas of the crystalline and amorphous C-4 signals according to Larsson fitting. The results indicated that cellulose of seed pericarp was constituted of 73% of cellulose I_α and 27% of cellulose I_β with a degree of crystallinity near to 60%.

3.6. Characterization and organization of extracted xylan

The xylan content of pericarp seed of OFI was characterized essentially by high resolution ^1H and ^{13}C NMR spectroscopy in our previous work (Habibi, Mahrouz, & Vignon, 2002). We distinguished two types of xylans from pericarp seed of OFI. The water soluble xylans are characterized by a molar ratio of xylose to uronic acid varying from 8/1 to 12/1. The water insoluble xylans which are in larger amount, have a low uronic acid content (varying from 26/1 to 65/1 Xyl/UA).

In the present work, we are interested by the water insoluble xylans (WIX) which were therefore isolated by sequential alkaline extractions with aqueous 2%, 4%, and 6% (w/w) NaOH solutions, according to Habibi et al. (Habibi et al., 2002) with minor modifications as reported in Fig. 2.

The sugar composition and average molecular weight of each insoluble xylan were reported in Table 4. We noticed that the molar ratio of xylose to uronic acid as well as molecular weight of xylan fractions increased with the NaOH concentration. The average molecular weight ranged from 11,250 g/mol for WIX-I, and 13,500 g/mol and 24,000 g/mol for WIX-III which correspond to degree of polymerization values of approximately 75, 90, and 160 for WIX-I, WIX-II, and WIX-III, respectively. These values corroborated the weight-average molecular weight reported earlier for xylan extracted from plants holocellulose with aqueous alkaline solutions (Ebringerova & Heinze, 2000).

We were interested in the organization of xylan and cellulose, both in isolated form and as a component of seed pericarp of OFI. Xylan and cellulose residues were studied essentially by X-ray and CP/MAS spectroscopy.

Xylan type polysaccharides are not thought to be crystalline in situ in the wood cell wall, but these polymers can crystallize under certain conditions (Chanzy, Dube, & Marchessault, 1979; Nieduszynski & Marchessault, 1972; Roelofsen, 1954). The backbone of xylan in crystalline form in aqueous solution has a three-fold, left-handed conformation. In Fig. 7 we reported, as an example, the

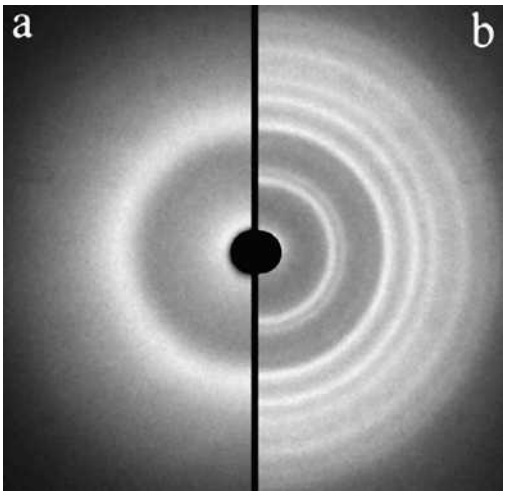

Fig. 7. X-ray diffraction patterns of WIX-II from pericarp seed of OFI (a) dried form; (b) hydrated form.

X-ray diffraction patterns of xylan extracted with aqueous 4% NaOH (fraction WIX-II) in dry and hydrated form. We can observe that hydration caused fundamental change in X-ray diffraction pattern showing a re-organization of xylan under hydration. The X-ray pattern of dry xylan shows one broad diffraction ring centered at 0.416 nm indicating that xylan does not present any organization. After hydration we noticed the apparition of several diffraction rings at 0.790, 0.698, 0.531, 0.460, 0.391, 0.355, 0.309, and 0.280 nm which corroborated with values published by Horio and Imamura (1964) and Nieduszynski and Marchessault (1972). No difference is observed between the various xylan fractions, what is due probably to their low uronic acid content. The behavior observed suggests that water induces a helical conformation and re-organization of the xylan polysaccharide. This phenomenon has already been reported for several helix-forming polysaccharides, such as β-(1 → 3)-linked D-glucans (Pelosi et al., 2003; Saito, Tabeta, Yokoi, & Erata, 1987; Saito, Yokoi, & Yoshioka, 1989), β-(1 → 3)-linked D-xylans (Lahaye, Rondeau-Mouro, Deniaud, & Buleon, 2003; Saito, Yamada, Yoshioka, Shibata, & Erata, 1991), and amylases (Cheetham & Tao, 1998).

This phenomenon was also observed by CP/MAS ^{13}C NMR spectroscopy. Hydration of plant cell wall materials (Rondeau-Mouro, Crepeau, & Lahaye, 2003), xylan (Lahaye et al., 2003; Saito et al., 1991), and galactans (Saito, Yokoi, & Yamada, 1990) or other polysaccharides, such

Table 4
Yields, sugar composition and average molecular weight ($\bar{M}_w$) of water insoluble xylans from seed pericarp

Xylan fraction	Yields	Sugars[a]				$\bar{M}_w$ ($\times 10^3$) (g mol^{-1})
		Uronic acid	Glucose	Arabinose	Xylose	
WIX-I	5.1	11.5	Tr	–	88.3	11.25
WIX-II	2.2	5.9	1.1	–	93.0	13.50
WIX-III	7.0	2.5	Tr	–	98.2	24

[a] Expressed in relative weight percentages.

as starch (Cheetham & Tao, 1998; Tanner, Ring, Whittam, & Belton, 1987) and β-glucans (Fyfe et al., 1984; Saito et al., 1987, 1989, 1991; Saito, Yoshioka, Yokoi, & Yamada, 1990; Stipanovic, Giammatteo, & Robie, 1985) has also been reported to increase the CP/MAS ^{13}C NMR signal resolution. The CP/MAS ^{13}C NMR spectra of WIX-I xylan fraction in the dry and hydrated states are shown in Fig. 8a and b, respectively, and that of WIX-III xylan fraction at hydrated form is reported in Fig. 8c. We can notice that hydration of xylan induced a marked increase in the signal resolution in the CP/MAS NMR spectrum, demonstrating the re-organization of the xylan chains under hydration. The spectrum of the dry sample showed the three broad unresolved peaks. These peaks are centered toward 101.9, 74.4, and 63.5 ppm and are assigned, respectively, to C-1 (C-2, C-3, C-4), and C-5 of β-(1 → 4)-linked D-xylose residues. Hydratation of xylan from pericarp of OFI markedly improved the resolution of the signals and affected also the chemical shifts of broad resonances, particularly of C-1 (101.4 ppm) and C-5 (62.83 ppm). Also no difference is observed between the spectra of various xylan fractions both in dried or hydrated states.

3.7. Organization and interaction of xylan–cellulose in seed pericarp

The organization of xylan–cellulose composite in seed percicarp of OFI was also studied. Different residues have been examined by CP/MAS ^{13}C NMR in hydrated state. We reported in Fig. 9 the spectra of defatted residue, of different alkali treated residues and of a sample of extracted xylan. As first interesting observation, the signal at 101.4 ppm, corresponding to C-1 of hydrated xylan, was not observed in the spectra of the different residues specially defatted residue and 2% NaOH treated residue (Residue II), whereas these residues are rich in xylan. Apparently, the structure of xylan in pericarp residues differed from that of hydrated xylan.

Furthermore, we noticed a continuous decrease of the signal at 81.9 ppm with the progression of xylan extraction. However this signal is not present in the spectrum of hydrated xylan and dry xylan. This signal was assigned to xylan interacting with cellulose; probably xylan presents as co-aggregates with cellulose fibril aggregates.

These observations were already reported by Wickholm et al. (1998), Larsson et al. (1999), Teleman, Larsson, and Iversen (2001), as well as Liitiae et al. (2003). They concluded that the xylan adopted, in the presence of the cellulose, an organization in co-aggregations. This organization is different to the one adopted by isolated xylan either in dry, hydrated form or in the presence of other substances (such as methanol, isopropanol). This change in conformation induced in the CP/MAS NMR spectrum principally a shift toward the weak fields of the signals assigned to C-4 and C-1 of the xylose residues. The shift of C-4 signal from 74.38 ppm for hydrated xylan to 81.87 ppm for xylan-composite in pericarp indicated a large difference in the molecular environment of the xylan. The xylan backbone itself imposed certain minimal structural constraints, but in fact the interactions between xylan and cellulose chains determined the final conformation.

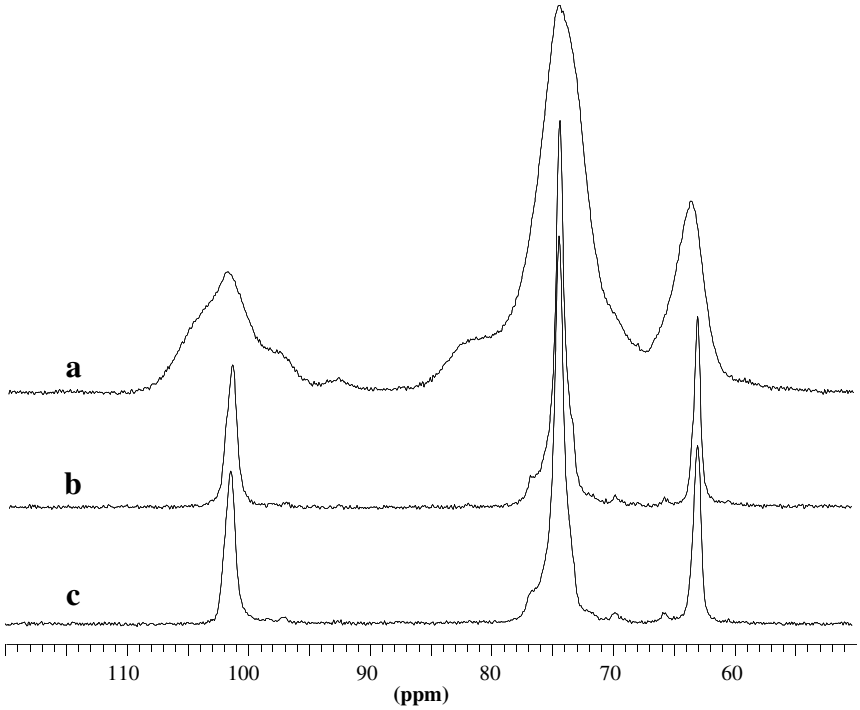

Fig. 8. ^{13}C CP/MAS NMR spectra of xylan extracted from pericarp seed of OFI: (a) WIX-I dried form; (b) WIX-I hydrated form and (c) WIX-III hydrated form.

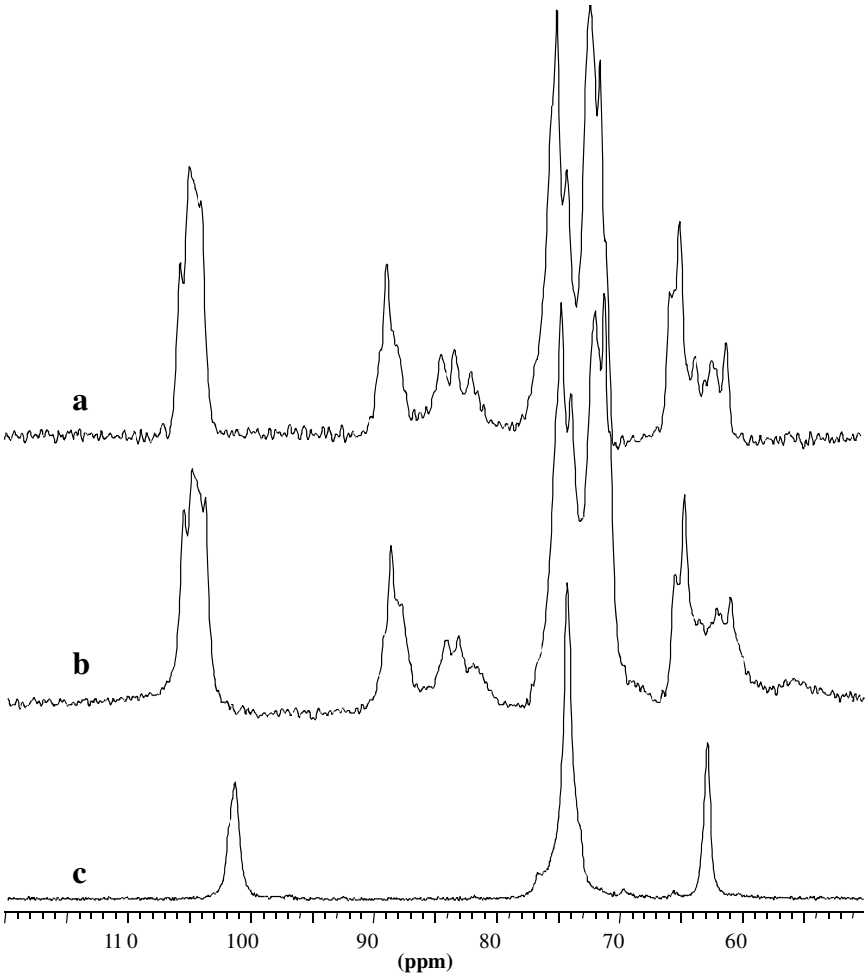

Fig. 9. CP/MAS ^{13}C NMR spectra of xylan or treated pericarp seed of OFI. (a) Aq. 6% NaOH – Residue IV; (b) aq. 6% NaOH – Gaulin Residue V-G; (c) 6% NaOH extracted xylan.

4. Conclusion

The study of pericarp of *Opuntia ficus-indica* (OFI) seeds showed that it is mainly made up of spindle-shaped sclerenchyma fibers and consisted of a natural xylan–cellulose–lignin composite. After lignin elimination, the xylan and cellulose were studied by X-ray and CP/MAS ^{13}C NMR spectroscopy. The water induced a better organization of supramolecular structure of isolated xylan. The sclerenchyma cellulose fibers are typical of secondary wall cellulose, with polymorphs ratios of 73% I_α and 27% I_β and of crystallinity near to 60%, but they were sensitive toward NaOH because they started to be converted into cellulose II at NaOH concentration as low as 8%. As component of seed pericarp, cellulose fibers interact with xylan polymers, causing these to adopt a conformation different to the one observed for xylan both in dry or hydrated form.

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