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Carbohydrate Polymers 51 (2003) 77–83

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Structure and morphology of cladodes and spines of *Opuntia ficus-indica*. Cellulose extraction and characterisation

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Received 21 January 2002; revised 18 April 2002; accepted 24 April 2002

Abstract

The morphology and structure of cladodes and spines from cactus *Opuntia ficus-indica* (OFI) were investigated by transmission and scanning electron microscopy together with electron and X-ray diffraction analysis. This paper focused also on the characterisation of cellulose from cladodes and spines of OFI. This cellulose can be found either as parenchyma cell cellulose (PCC) in cladodes, or as fibre in spines. Its localisation within the initial cladode was revealed by optical microscopy together with scanning electron microscopy (SEM). The surface of the cell wall is made of thin microfibrils easily observed by transmission electron microscopy (TEM) after shadowing. The purified preparations of cladodes PCC were mechanically homogenised leading to a stable and non flocculating suspension by microfibrils individualisation. The spines decorating the cladodes of OFI cactus consisted of a compact parallel arrangement of slender cellulosic fibres (0.4 mm long, 6–10 μm diameter) with very small lumens. The mechanical properties of these natural spines were investigated by tensile tests at various conditioning relative humidities. The morphology and structure account well for the high mechanical properties of these spines. It was observed that the tensile modulus decreases as the moisture content increases.

Keywords: Cactus; *Opuntia ficus-indica*; Morphology; Structure; Cellulose; Microscopy

1. Introduction

OFI cactus originates from the American continent and is mainly used for fruit production (Nobel, Garcia-Moya, & Quero, 1992). Young shoots are also eaten as a vegetable (Nopalitos) in Mexico and south of USA. In North Africa, the cultivation of OFI cactus is also used on the one hand against the soil erosion in arid areas, and on the other hand as a forage substitute during drought. There is a growing interest in non-food uses of OFI, mainly in medical applications. Ethanol extract of OFI shows potential analgesic and anti-inflammatory effects (Park, Kahng, & Paek, 1998). Ingestion of raw and cooked OFI extracts presents beneficial effects on growth and total cholesterol, without any secondary effect on glucose and lipoproteins amounts in blood (Medellin, Salvidar, & De la Garza, 1998).

Cellulose is the ubiquitous structural polymer that controls the mechanical properties of all higher plant cells. In these, natural cellulose is usually found in the form of microfibrils that are themselves organised in fibres, cell walls, etc. In the cellulose microfibrils the cellulose chains are aligned parallel to the microfibril axis. This perfect organisation confers to the microfibrils mechanical properties that are close to the theoretical limit for cellulose. In the cellulosic fibres, it is the ultrastructural organisation and orientation of the microfibrils that are responsible of their mechanical strength.

The chemical compositions of *Opuntia* mucilages have been described by several research groups, with important contradictions in their results. For some authors, the mucilage was neutral with mainly D-galactose and L-arabinose residues (Harlay, 1902). Others suggest the mucilage was acidic and contained L-arabinose, D-galactose, L-rhamnose and D-galacturonic acid (Anderson, Sands, & Sturgis, 1925; Sands & Klaas, 1929). More recently Parikh and Jones (Parikh & Jones, 1965, 1966a,b) have reported that the mucilage of *Opuntia fulgida* consisted of a backbone of $\beta(1 \rightarrow 3)$-linked galactose units with branches on carbon C-6 containing D-galacturonic acid, D-galactose, D-xylose, L-rhamnose and L-arabinose units. The mucilage

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0144-8617/03/$ - see front matter
PII: S0144-8617(02)00157-1

of OFI Mill. was analysed by Amin et al. (Amin, Awad, & El-Sayed, 1970) and they found that it was neutral and contained arabinose, galactose, rhamnose and xylose residues. However, the mucilage of OFI cv "Burbank's spineless" contained both neutral and acidic fractions (Paulsen & Lund, 1979). Trachtenberg and Mayer (Trachtenberg & Mayer, 1987) have isolated carbohydrate polymers from OFI nopals. They contained galacturonic and rhamnose residues, which were probably pectin polysaccharides. This study focused on the cellulosic residue, after removal by water and alkaline extractions of the mucilageneous and hemicellulosic polysaccharides.

Whatever the application may be, spines corresponding to about 8.4 wt% on a dry basis of the whole cladode have to be removed and are consequently a by-product. On a dry weight basis the spines of OFI consist of 96% polysaccharides composed mainly of cellulose (49.7%) and arabinan (50.3%). This cellulose is organised as fibres with small lumens well aligned along the spine main axis, which should lead to high strength.

A method previously developed by Dinand et al. (Dinand, Chanzy, & Vignon, 1996) for the extraction of cellulose microfibrils from sugar beet roots has been used for OFI. It has been adapted in order to remove most of the calcium oxalate crystals. Stable and non-flocculated cellulose microfibrils suspensions in water were obtained from OFI cladodes.

The aim of this research was to study the structure, the morphology and the chemical composition of cladodes and spines from cactus *Opuntia ficus-indica,* in order to find non-food applications.

2. Materials and methods

2.1. Materials and chemical analysis

Fresh cladodes were gathered in a pilot plantation in Amezmiz, 30 km from Marrakech (Morocco). Spines were removed manually from cladodes. The neutral sugars were released by Seaman hydrolysis and analysed by GLC as their corresponding alditol acetates (Selvendran, March, & Ring, 1979), using a Packard and Becker 417 instrument coupled to a Hewlett–Packard 3380A integrator. Glass column (3 mm × 2 m) packed with 3% SP 2340 on Chromosorb W-AW DMCS (100–120 mesh) was used. The uronic acid content was determined by the *m*-hydroxydiphenyl method, according to Blumenkrantz and Asboe-Hansen (Blumenkrantz & Asboe-Hansen, 1973). The ash content was measured by heating the samples overnight at 600 °C. This content corresponded in fact to only 66% of the minerals in the case of $CaCO_3$, to account for the loss of CO_2. The true amount of mineral is then determined by multiplying the experimental value by 1.5 (100/66). The amount of fats and waxes was obtained from the loss of weight after 24 h extraction with a soxhlet apparatus operated with boiling toluene/ethanol (38/62, v/v). The lignin analysis was achieved according to the TAPPI standard T222-03-75.

2.2. Purification

The protocol of Dinand et al. (Dinand et al., 1996) was followed with slight modifications. The cladodes (100 g) were dispersed for 10 min with a Warring Blender into 500 ml of water. This suspension was shaken for 2 h at 50 °C, and then filtered and washed with water. This treatment was performed twice. Then the residue was dispersed into 500 ml of a 2% NaOH solution, and the suspension was shaken for 2 h at 80 °C, then filtered and washed with water. A second extraction was performed under the same alkaline conditions, and the alkali-insoluble product was recovered on a 60 μm sieve and was extensively washed with water while being stirred. This washing removed the soluble polysaccharides and one part of the inorganic material consisting essentially of calcium oxalate crystals. The washed product was then bleached with sodium chlorite following the method of Wise et al. (Wise, Murphy, & D'Addieco, 1946). This treatment removed most of the residual lignin and proteins, and the resulting bleached product consisted essentially of individualised cells, with calcium oxalate crystals. The bleached pulp was treated with a nitric acid solution 0.05N for 1 h at 70 °C, filtered on a 60 μm sieve and washed extensively with water in order to remove most of the residual oxalate crystals.

The spines (10 g) were suspended in 2% NaOH solution, dispersed for 15 min with a Warring Blender, shaken for 2 h at 80 °C and then filtered and washed. A second extraction was performed under the same alkaline conditions and the alkali-insoluble fibres were bleached with sodium chlorite (Wise et al., 1946). The resulting fibres were treated with a trifluoroacetic acid (TFA) 2N for 1 h at 100 °C, then filtered and extensively washed with water to remove degraded arabinan.

For tensile tests, the spines were treated two times for 2 h at 80 °C in 2% NaOH solution under slow mechanical agitation. After filtration and extensive washing with water, the spines were bleached with sodium chlorite. The aspect of the spines remains similar, except the colour that dries white. After drying at 105 °C for 4 h, the samples were stored in a dry atmosphere (dessicator with P_2O_5) and weighed (M_o). The moisture content of both untreated and treated spines was achieved by conditioning the samples at room temperature in dessicators at controlled humidities containing saturated salt solutions. Three relative humidity (RH) conditions were used, namely, 0, 43 and 75%. Conditioning was performed for a time ensuring the equilibration of the water content in the spines with that of the atmosphere (stabilisation of the sample weight). For this, the samples were removed at specific intervals (t) and weighed (M_t) up to an equilibrium value (M_∞). The water

Table 1
Chemical composition of OFI cladodes and spines before and after purification.

Constituents	Cladodes (dry wt%)			Spines (dry wt%)		
	Initial raw sample	After 2% alkali	After HNO_3	Initial raw sample	After 2% alkali	After TFA[a]
Ash	19.6	19.5	9.7	1.3	0.3	–
Fat and wax	7.2	–	–	1.2	–	–
Lignin	3.6	–	–	1.2	–	–
Cellulose	21.6	63.7	75.2	47.9	51.2	96.6
Other polysaccharides	48.0	10.05	15.1	48.4	47.5	3.4

[a] TFA: trifluoroacetic acid.

content or water uptake (wu) of the samples was calculated by dividing the gain in weight ($M_t - M_0$) by the initial weight (M_0).

2.3. Preparation of microfibril suspensions

A 2 wt% water suspension was prepared from the purified cell wall of cladodes. The cells were dispersed in a Warring Blender until a final temperature of 60 °C was reached (15 min). The suspension was then homogenised by 15 passes through a Manton Gaulin laboratory homogeniser operated at 500 bars at a temperature that was controlled at 90–95 °C.

2.4. Microscopies

Observations of the disencrusted pulp or fibres were achieved with a Zeiss Axiophot 2 optical microscope operated in Nomarski contrast. For SEM analysis, small cubes were cut out from fresh cactus cladodes, fixed with glutaraldehyde and dried under critical point conditions in a Polaron Critical Point Dryer operated with liquid CO_2.

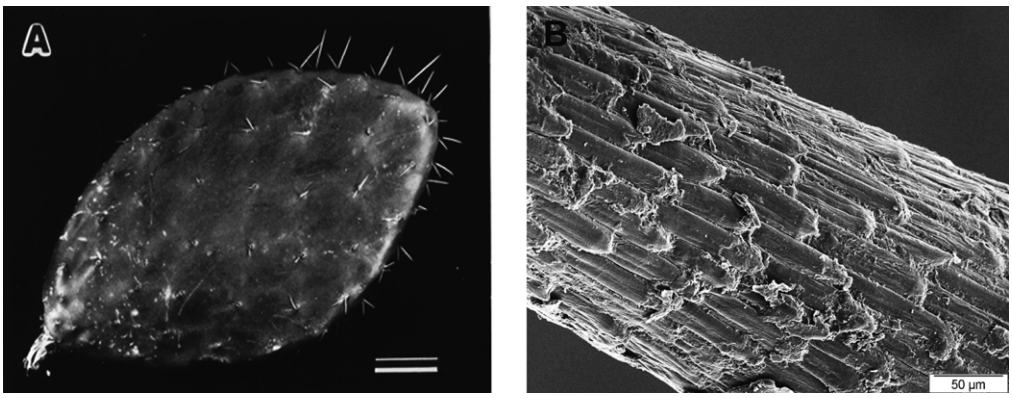

Fig. 1. (A) Cladode of *Opuntia ficus-indica* prickly pear cactus with its spines. Scale bar: 5 cm. (B) SEM micrograph of one cactus spine showing the surface cuticle cells.

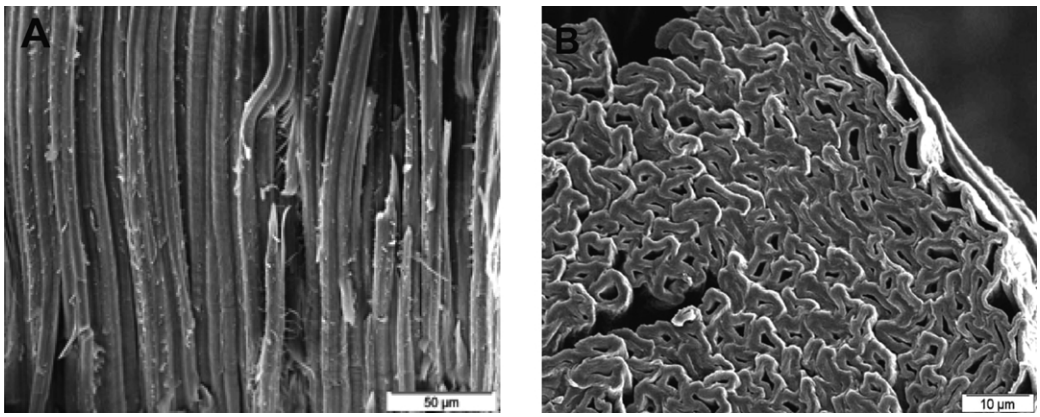

Fig. 2. SEM micrographs of a cactus spine. (A) Cross-sectioned parallel to the spine axis. (B) Cross-sectioned perpendicular to the spine axis showing the arrangement of the fibres.

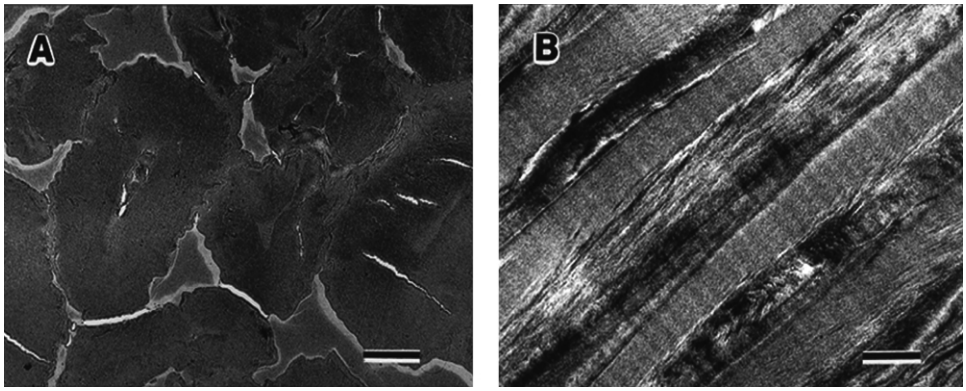

Fig. 3. TEM micrographs of ultra thin sections of a cactus spine. (A) cross-sectioned perpendicular to the spine axis showing the arrangement of the cellulosic fibres. (B) cross-sectioned parallel to the spine axis. Scale bars: 2 μm.

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.

For TEM analysis the samples, either the individualised cell-ghosts or the microfibrils suspensions, were deposited on carbon coated electron microscope grids. Some of these specimens were shadowed with tungsten/tantalum alloys before observations and others were observed as such. The electron microscopy was performed with a Philips CM200 CRYO transmission electron microscope operated at an acceleration voltage of 80 kV for imaging and 200 kV for diffraction purposes. Non-shadowed samples were observed under low electron dose conditions in order to minimise the beam damage. The electron diffraction diagrams were calibrated with gold rings as standards.

2.5. X-ray diffraction

X-ray measurements were made on films obtained by water evaporation of the suspensions of cellulose microfibrils. 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.

2.6. Tensile tests

The mechanical behaviour of the cactus spines was analysed using an Instron 4301 testing machine in tensile mode, with a load cell of 100N capacity. The stress–strain curves of conditioned samples were obtained at room temperature at a strain rate $d\epsilon/dt = 8.3 \times 10^{-3}\ s^{-1}$ (cross-head speed = 5 mm min^{-1}). The true strain ϵ was determined by $\epsilon = \ln(L/L_0)$, where L and L_0 are the length during the test, and the length at zero time, respectively. The true stress σ was calculated by $\sigma = F/S$, where F is the applied load and S is the cross-sectional area. S was determined assuming that the total volume of the sample remained constant, so that $S = S_0 \times L_0/L$, where S_0 is the initial cross-sectional area. The Young's modulus (E) was measured from the slope of the low strain region in the vicinity of $\sigma = \epsilon = 0$. Ultimate mechanical properties, stress at break ($\sigma_B = F_b/S$, where F_b is the applied load at break), and strain at break ($\epsilon_b = \ln[1 + (\Delta L_b/L_0)]$, where ΔL_b is the elongation at break), were also characterised. Mechanical tensile data were averaged over at least 8 specimens.

3. Results and discussion

The constituents and chemical composition of OFI

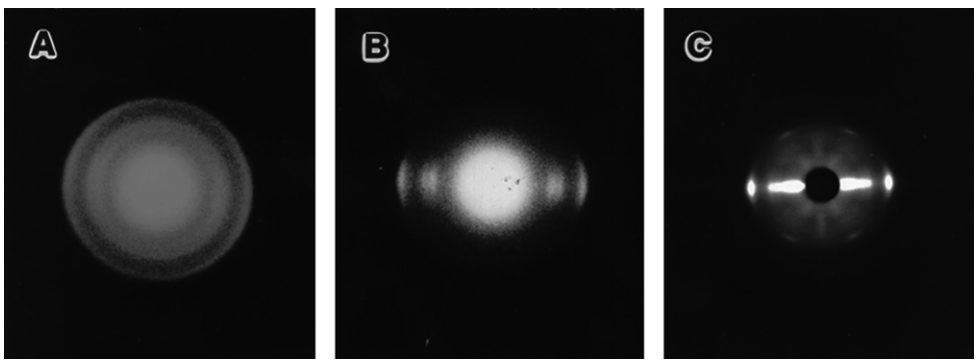

Fig. 4. (A and B) Electron diffraction diagrams recorded on one square micron of a fibre from cactus spine sectioned respectively as in Fig. 3A and B. (C) X-ray fibre diagram of one cactus spine oriented along its axis vertical.

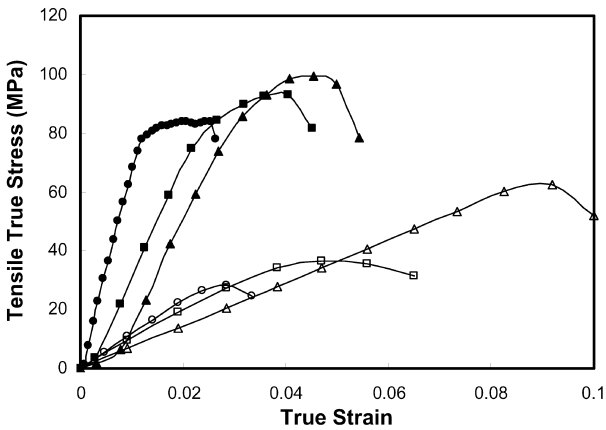

Fig. 5. Typical true stress versus true strain curves ($T = 25\,°C$, $d\epsilon/dt = 8.3 \times 10^{-3}\ s^{-1}$) of untreated (filled symbols) and treated (empty symbols) OFI spines, conditioned at 0% (•, ○), 43% (■, □) and 75%RH (▲, △).

cladodes are collected in Table 1. A large amount of minerals (19.6 wt%) as well as waxes and fats (7.2 wt%) are observed. The lignin content is low (3.6 wt%) and the main constituents are polysaccharides (69.6 wt%), including cellulose (21.6 wt%). Upon disencrustation (2% alkali and sodium chlorite bleaching), the composition became 63.7% cellulose, 17.3% hemicelluloses and 19% minerals. Carbohydrates constitute the main part of cactus cladodes, before and after purification. At this stage the amount of minerals was still high (19%), and a specific treatment with a nitric acid solution for 1 h at 70 °C, followed by filtration on a 60 μm sieve and extensive washing with water were required in order to remove about half of the residual oxalate crystals (9.7%).

The composition of OFI spines, given in Table 1, indicates that cellulose is the main constituent (47.9) with hemicelluloses (48.4%), lignin, ashes, fat and waxes being in very small amounts. After alkali purification with 2% NaOH solution, the ratio cellulose/hemicelluloses is approximately the same (51.2/47.5). A trifluoroacetic acid treatment was required to hydrolyse selectively the hemicellulosic constituents. The degraded material appeared to be exclusively arabinose, which corresponds to an arabinan that will be isolated and characterised later.

Fig. 1A is a typical prickly pear cactus cladodes showing its system of spines that can reach several centimetres in length. The SEM micrograph in Fig. 1B shows how the surface of each spine is covered with cuticle cells that protect the spines from drying. These cuticle cells are stacked and shifted circularly as a result of the growth of the spine from the cladode, leading to a thin section at the extremity and a larger one near the cladode. Fig. 2 corresponds to SEM micrographs of purified OFI spines. Fig. 2A and B show a cross-section cut perpendicularly and longitudinally to the main axis of the spine. These micrographs reveal the organisation of the fibres constituting the body of the spine, which consists of a compact parallel arrangement of slender cellulosic fibres. Each fibre has a lateral dimension of the order of 6–10 μm, a very small lumen and a convoluted surface. This near perfect organisation is also confirmed from TEM observation (Fig. 3) of ultra-thin perpendicular and longitudinal sections of embedded spine.

Fig. 4 presents a series of diffraction diagrams recorded on the spines. Fig. 4A and B are electron diffraction diagrams that reveal the near perfect organisation of the cellulose microfibrils parallel to the spine axis. This high orientation is confirmed by the X-ray diffraction diagram in Fig. 4C, which shows that in the spines, the cellulose system is of high crystallinity and that the crystalline cellulose microfibrils are almost perfectly aligned parallel to the spine axis. This organisation should be responsible for high mechanical properties of the spines.

The mechanical behaviour of the spines was characterised by tensile tests performed at room temperature. Fig. 5 shows typical stress versus strain curves obtained for both initial raw and purified spines conditioned at different humidity levels. The experimental data are collected in Table 2. The water content and required time to ensure the equilibration of the water content in the spines are also reported in Table 2. The moisture content or water uptake is higher for treated spines compared to untreated ones. This is most probably ascribed to both the presence of a more

Table 2
Mechanical properties obtained from tensile tests and water uptake of raw and purified spines.

RH (%)	Untreated				Treated[a]				t (h)[b]
	E (MPa)[c]	σ_B (MPa)[d]	ϵ_B (%)[e]	WU (%)[f]	E (MPa)[c]	σ_B (MPa)[d]	ϵ_B (%)[e]	WU (%)[f]	
0	6091	84.1	2.5	0.11	1168	27.3	2.9	0.10	48
43	4705	93.3	4.2	2.5	936	35.1	5.3	3.2	72
75	4054	99.4	5.0	3.6	687	62.3	8.7	4.6	168

[a] Purified by 2% alkali and chlorite bleaching.
[b] t: required time to ensure the equilibration of the water content.
[c] E: tensile modulus.
[d] ϵ_B: stress at break.
[e] ϵ_B: elongation at break.
[f] WU: water uptake or water content.

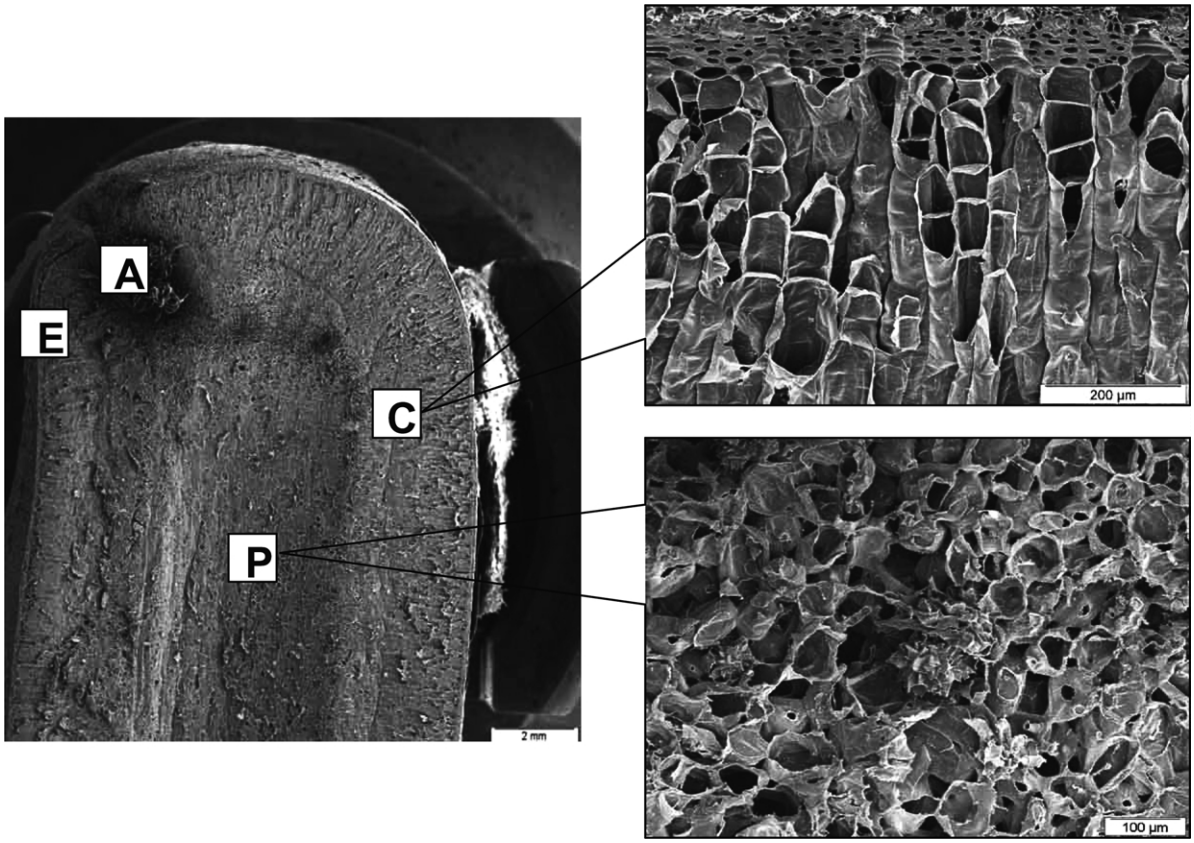

Fig. 6. SEM of cross-section of a OFI cladode. E: epidermic cell; A: areola domains; P: parenchyma and C: chlorenchyma cells.

hydrophobic cuticle around the untreated spines and to the close packing of the fibres inside the untreated spines. It is worth noting that treatment most likely alters the water partition between the hemicelluloses and the cellulose and that an increase of the water available to the latter should result in a decrease of the mechanical properties.

A decrease of the tensile modulus with moisture content is observed for both untreated and treated spines. It is ascribed to the well-known water-induced softening of polysaccharides. The cohesion of polysaccharides is mainly due to hydrogen-bonding forces, which are very sensitive to the presence of water. Increasing the water content results in higher stress and elongation at break. These parameters are more strongly sensitive to the moisture conditions for treated spines. The treatment of the spines and then the elimination of hemicelluloses results in a strong decrease of both the tensile modulus and tensile strength. It is clear that cellulose fibres are stiffer in the presence of hemicelluloses. We previously observed this phenomenon with pectins, and reported that pectins act as a binder between cellulose fibres and improve the mechanism of load transfer towards fibres when the sample is subjected to a mechanical stress (Dufresne, Cavaillé, & Vignon, 1997). This binding mechanism is governed by hydrogen bonding and/or covalent connections between hemicelluloses and cellulose fibres. In fact, the values reported for treated spines should be corrected to account for the porosity induced by the purification treatment. This porosity was observed by SEM and was found to represent about 25% of the cross-sectional area. This correction results in a slight increase (25%) of both the tensile modulus and strength.

The morphology of both the cladode and isolated cell walls were studied by SEM. Fig. 6 shows a cross-section cut perpendicular to the main axis of a OFI cladode. The epidermic cell (E), areola domains (A), parenchyma (P) and chlorenchyma (C) cells are clearly identified. Inserts show magnifications of P and C cells, which display very thin wall having thickness as low as 0.1 µm, in which oxalate crystals can be observed. After disencrustation, the various P and C cells lost most of the non cellulosic polysaccharides (the cellulose content is close to 74 wt%, without taking into account the amount of mineral), and occur as cell ghosts

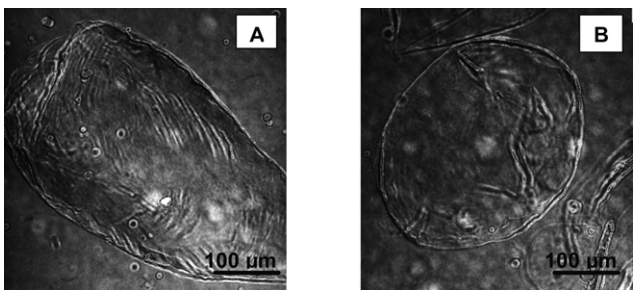

Fig. 7. Optical micrograph in Nomarsky contrast of (A) chlorenchyma and (B) parenchyma purified cell-ghosts.

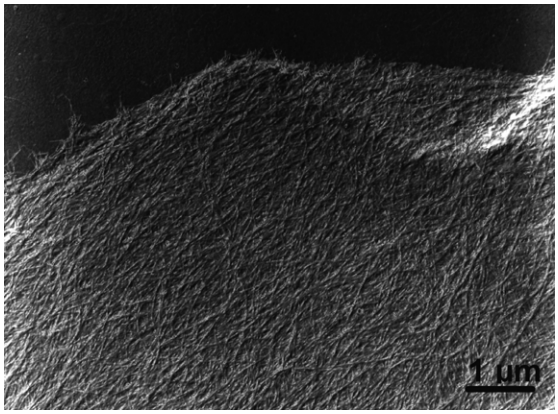

Fig. 8. TEM observation after shadow casting with W/Pa of the border purified cell-ghosts.

having either circular or ovoid forms as recorded on Fig. 7 by optical microscopy in Nomarski contrast. At this stage, we still have a large amount of calcium oxalate crystals (20 wt%), which can be partially removed by a nitric acid treatment followed by extensive washing with water, leading to a purified material containing 9.5% of calcium oxalate crystals. TEM observation after shadowing of P or C cells clearly shows the microfibrillar cellulose network which consists of microfibrils either isolated or associated in bundles (Fig. 8).

The homogenisation of OFI cladode cells after several passages through the Manton Gaulin apparatus induces the total disruption of the P or C cell-ghosts and the release of the cellulose microfibrils in the medium. This is illustrated in the low dose TEM observation shown in Fig. 9, and we can observe that some microfibrils remain still associated into bundles.

One of the most interesting properties of the homogenised cladode cell suspensions is that they did not sediment nor flocculate. As described earlier in the case of sugar beet pulps (Dinand et al., 1996, 1999), these suspensions present shear thinning properties together with pseudo plasticity. Nanocomposite materials will be prepared from these high aspect ratio cellulose microfibrils suspensions and poly(styrene-co-butylacrylate) as the matrix. High mechanical performances are expected from these systems.

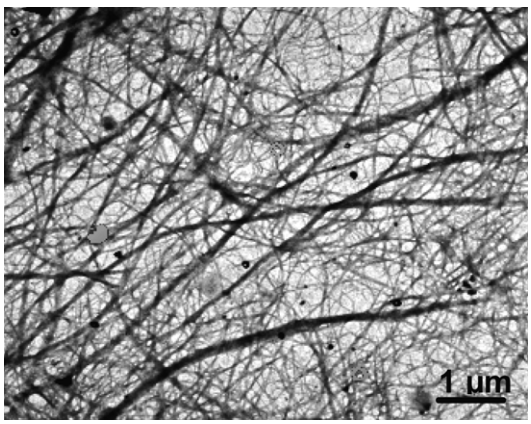

Fig. 9. TEM observation of homogenized cellulose microfibrils suspension.

Acknowledgments

The authors acknowledge the financial help of the Rhône–Alpes region (programme TEMPRA Sud-Méditerranée) and the Comité Mixte Franco-Marocain (action intégrée 236/SVS/00).

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