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Microfibrillated cellulose from the peel of prickly pear fruits

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ABSTRACT

Cellulose microfibrils were isolated from the skin of *Opuntia ficus indica* (prickly pear fruits). Defatted skin powder was processed through consecutive extraction steps in order to remove mucilage, pectin and hemicelluloses. The cellulosic residue was made up of disencrusted cell-ghosts, having ovoid or elongated shapes, as revealed by optical microscopy. Transmission electron microscopy showed that, at the ultrastructural level, the cell walls of these cell-ghosts consisted of a loose network of cellulose microfibrils. This residue was subjected to mechanical homogenisation, leading to a stable and non-flocculating suspension by cellulose microfibril individualisation. This cellulosic material was characterised in terms of chemical composition, morphology and crystallinity, using sugar analyses, transmission electron microscopy and X-ray diffraction, as well as solid state NMR ^{13}C spectroscopy.

1. Introduction

Cellulose in the form of suprastructures, such as micro-fibres and amorphous constructs, constitutes the most abundant renewable polymer available in the biosphere. In fact, cellulose represents about 1.5×10^{12} tons of the total annual biomass produced in nature, and is therefore considered as an inexhaustible source for the manufacture of environmentally-friendly products. There is now a new type of cellulose material termed microfibrillated cellulose (MFC; Turbak, Snyder, & Sandberg, 1982, 1983). Through a homogenisation process, wood pulp is disintegrated, giving a material whose fibres are moderately degraded and opened into their substructural fibrils and microfibrils. More recently, a novel and simple way to produce MFCs, by combining enzymatic hydrolysis and mechanical shearing of wood pulp was proposed. The process resulted in long and entangled nanoscale cellulose elements which led to stronger networks and gels (Henriksson, Henriksson, Berglund, & Lindstroem, 2007). MFCs were also prepared from parenchyma cell cellulose (PCC) from sugar beet pulp (SBP; Dinand, Maureaux, Chanzy, Vincent, & Vignon, 1996), and were reported to show excellent gel behaviour (Dinand, Chanzy, & Vignon, 1999).

MFCs possess several interesting properties, such as extremely large specific surface area and very high aspect ratios of the fibrillated material; thus, they have found application as rheology modifiers in foods, paints, cosmetics and pharmaceutical products. Moreover, MFCs can be used as fillers in composite materials and paper products (Berglund, 2005; Hamad, 2006; Kumar, 2002; Matsuda, Hirose, & Ueno, 2001; Wegner & Jones, 2006). However, despite a large number of publications within the field of MFCs, few commercial applications exist today. To our knowledge, only MFCs from wood pulp and bacterial cellulose are presently commercialised under the trade names of Celish® and Cellulon®, respectively.

Cactaceaes are a plant family which exhibit *Crassulacean acid metabolism* (CAM) and are drought-tolerant. This explains why they are successfully cultivated in arid and semi-arid regions. *Opuntia ficus indica* (OFI) is the most widespread of species of the Cactaceaes family. In north Africa, the cultivation of the cactus OFI is used to prevent soil erosion in arid areas and as a forage substitute during periods of dryness. This prickly pear cactus was exploited essentially for its fruits, which are generally consumed fresh. Young shoots are also eaten as a vegetable (Nopalitos) in Mexico and southern USA. The most important product extracted from the OFI plant, which is used in the food industry, is carminic acid, also known as red carmin or grana colorant (E-120). There is a growing interest in the non-food usage of OFI, mainly for medical applications. Indeed, numerous scientific studies have reported that different parts of the OFI species have several medical effects, such as diuretic (Galati, Tripodo, Trovato, Miceli, & Monforte, 2002), antigotous, antinflammatory (Park, Kahng, Lee, & Shin, 2001), analgesic, and antiulcerous (Galati, Monforte, Tripodo, d'Aquino & Mondello, 2001). Moreover, antihyperglycemia and

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hypocholesterolaemia (Frati, Jimènez, & Ariza, 1990; Perfumi & Tacconi, 1996; Roman-Ramos, Flores-Saenz, & Alarcon-Aguilar, 1995), attributed to the 'nopalitos' of OFI, are the most important effects studied. In fact, ingestion of raw and cooked OFI extracts of cladodes presents beneficial effects on total cholesterol, without any secondary effect on glucose or lipoprotein levels in blood. The most important progress in the chemistry, technology and use of cactus is reported by Stintzing and Carle in a recent review (Stintzing & Carle, 2005).

Within the past decade, cactus plants have become an important crop in the semi-arid lands of Morocco, where they play a strategic role in subsistence agriculture. Because of its good adaptation to the harsh desert environment and its different applications, economic interest in prickly pear fruits has increased considerably. Several bilateral projects and local programmes have promoted *Opuntia ficus indica* for its use in multiple applications. Efforts are currently underway to develop the production of fruit and to foster its introduction into various food and non-food products. For better understanding of their chemical composition, we have been engaged in numerous studies on the characterisation of their components, particularly the cell-wall polysaccharides of different parts of the cactus fruit. In seeds of the fruit, we have analysed several xylans which are characterised by a large glucuronic acid content (Habibi, Mahrouz, & Vignon, 2002). The mucilage from the skin was structurally characterised to be an arabinogalactan, type I (Habibi, Mahrouz, Marais, & Vignon, 2004b) and different fractions of pectin type polysaccharides were extracted and their structures were identified (Habibi, Heyraud, Mahrouz, & Vignon, 2004a; Habibi, Mahrouz, & Vignon, 2005).

We note that the consumption of fresh fruit has resulted in the production of a huge amount of peel. This is an abundant, renewable resource which has been used essentially for animal alimentation. It is expected that high-value products can be developed and therefore, in this work, we aimed at investigating the isolation, preparation and morphological characterisation of the microfibrillated cellulose from the skin of OFI fruits. To our knowledge, no studies have been conducted in this area.

2. Materials and methods

2.1. Materials

Fresh and mature prickly pear fruits of OFI were collected from an experimental plantation in the vicinity of Marrakech (Morocco). The fruits were carefully hand-peeled, and the peels (with a thickness of about 3–4 mm) were cut into small pieces and dried in a ventilated oven adjusted to 50 °C. After drying, the peel fragments were ground for a few minutes in a domestic coffee grinder and sieved.

2.2. Analytical methods

Neutral sugars, after H_2SO_4 hydrolysis, and the corresponding alditol acetates were analysed by gas liquid chromatography (GLC) 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. Uronic acid content was determined (Blumenkrantz & Asboe-Hansen, 1973). The lignin analysis was achieved according to the TAPPI standard T222-03-75.

2.3. Purification

Fats, waxes and oils were removed from the skin powder sample by refluxing in a Soxhlet apparatus for 24 h with 38:62 (v:v) toluene–ethanol. The defatted residue was extracted twice with de-ionised water at room temperature for 2 h in order to extract mucilage polysaccharides. The pectic polysaccharides were extracted sequentially with hot water (2 × 2 h at 60 °C), aq 0.5% ammonium oxalate (2 × 2 h at 60 °C), and 0.05 N HCl solution (2 × 1 h at 80 °C). The residue was then extracted with a 2% NaOH solution at 80 °C (2 × 2 h) and the final cellulosic residue was bleached (Wise, Murphy & D'Addieco, 1946).

2.4. Manton–Gaulin treatment

Purified and bleached cellulosic residue was dispersed in water at a concentration between 1% and 1.5% and disrupted in a Waring blender operated at full speed for 5 min. The slurry, which had reached a temperature of 60 °C, was immediately treated with a laboratory scale Manton–Gaulin homogenizer 15MR-8TBA (APV Gaulin Inc., Wilmington, Mass). Fifteen passes were applied at a pressure of 500 bars, and the temperature was kept below 95 °C to avoid cavitations. The resulting creamy suspensions were freeze–dried for further use.

2.5. Scanning electron microscopy (SEM)

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

2.6. Optical microscopy

Cellulose cells residues were observed under a computer-controlled Zeiss Axiophot 2 optical microscope equipped with a camera and operated in Nomarski's contrast.

2.7. Transmission electron microscopy (TEM)

For TEM analysis, the samples of either the individualised cell-ghosts or the microfibril suspensions, were deposited on carbon-coated electron microscope grids and allowed to dry. 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, using a low dose electron beam for observation and recording.

2.8. 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 films obtained by water evaporation of the suspensions of cellulose microfibril residues.

2.9. Determination of molecular weights

The cellulose samples were dissolved in 1 M cupriethylenediamine diluted from the 3 M stock solution purchased from Prolabo (France). The viscosimetric average degree of polymerisation (DP_v) of cellulose samples was calculated from the intrinsic viscosities of the corresponding solutions at 25 °C, using the relationship (Rinaudo, 1968): $[\eta] = 0.891 DP_v^{0.936}$.

2.10. 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-polarisation (CP). CP transfer was achieved using a ramped amplitude sequence (RAMPCP) for an optimised total contact time of 2 ms. The spinning speed was set at 6 kHz, sweep width 50,000 Hz, recycle delay 4 s and 10,000 scans. The ^{13}C chemical shifts were measured relative to the carbon chemical shift of the glycine carboxyl group (176.0 ppm).

3. Results and discussion

Fruits of OFI were peeled, and the skin tissues were observed by scanning electron microscopy (SEM), as shown in Fig. 1. These images, obtained after critical point drying, revealed the organisation and morphology of the different types of cells. The tissues constituting the skin of prickly pear fruits consist essentially of collenchyma and parenchyma cells. From the outside to the inside of the skin in Fig. 1B, we can observe thin-walled epidermal cells, known as chlorenchyma cells. Two or three layers of chollenchyma cells were observed whilst the rest were identified as parenchyma cells. The collenchyma cells, which are rich in non-cellulosic components (considered as young parenchyma cells) are characterised by their thick cell walls. On the other hand, the parenchyma cells (Fig. 1C) typically showed thin walls. Within the tissues, in the collenchyma and parenchyma, there are mucilaginous cells that store mucilage polysaccharide, as shown in Fig. 1D. This material, commonly known as 'cactus dribble', is very common in succulent plants and exhibits large (osmotic) water retention. Mineral inclusions are also observed, mainly made up of calcium oxalate crystals

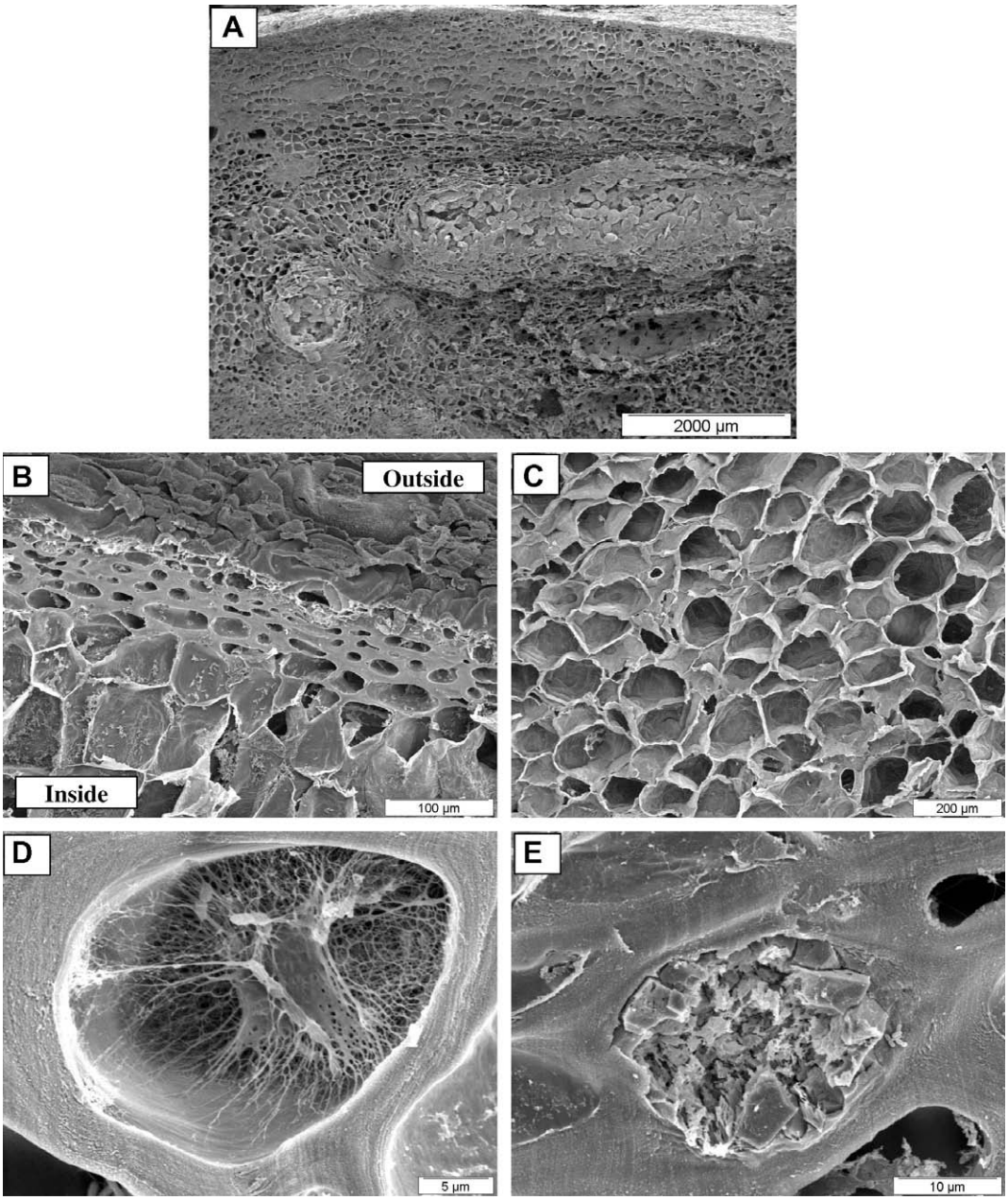

Fig. 1. SEM micrographs of a cross-section of the skin of OFI fruit: (A) global view, (B) surface layers view, (C) parenchyma cells, (D) mucilaginous cell, and (E) calcium oxalate crystal inclusions.

Table 1
Chemical composition of skin of prickly pear fruits.

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

(Fig. 1E). These crystallites, with various degrees of hydration, are known to occur in plant tissues, especially in succulent plants (though their role is still poorly understood). In *Opuntia* species, the calcium oxalate crystallites are habitually present in whewellite form. The first evidence of the weddellite crystalline form in the parenchyma tissues of OFI stems was reported by Malainine, Dufresne, Dupeyre, Vignon, and Mahrouz (2003).

In the whole prickly pear fruit, the amount of skin is 40 wt% (wet weight basis) or 25 wt% (dry weight basis). Important amounts of fats and waxes (11 wt%), as well as of minerals (11.5 wt%) were measured in the skin, whilst the lignin content was low (2.4 wt%), due to the predominance of primary cell walls. The main constituents of the skin were polysaccharides (66.1 wt%), including 27 wt% of cellulose. Detailed constituents and chemical composition of this skin are given in Table 1. The sugar composition, as shown in Table 2, revealed the presence of important amounts of uronic acid and glucose, confirming the presence of cellulose and pectic polymers in the cell wall.

A method previously developed by Dinand et al. (1999) for the extraction of cellulose microfibrils from sugar beet roots was applied to the skin of OFI. In order to remove the mucilage, pectin and hemicelluloses polysaccharides, as well as calcium oxalate crystals, a slight modification of this method was implemented. The peels were disencrusted by two extractions with cold and hot water and two extractions with ammonium oxalate (to extract the mucilage and pectin polysaccharides). One extraction with hot, dilute HCl was carried out to remove protopectin (which remained intimately linked to cellulose fibrils) and also to solubilise calcium oxalate crystals. Finally, hemicelluloses were removed by two alkaline treatments with 2% NaOH aqueous solution. Under these conditions, the various cells lost most of their non-cellulosic constituents. Sugar analysis of the final residue (see Table 2) demonstrated that glucose was the main sugar present in this residue, confirming its cellulosic nature. The molecular weight was around 130×10^3 g/mol, which corresponds to a degree of polymerisation of 850. These values corroborated the weight–average molecular weight reported earlier for cellulose extracted from primary cell walls of higher plants (Stone, 2007).

When observed by optical microscopy, in Nomarski's contrast, the disencrusted samples of skin of OFI fruit consisted essentially of flattened cells commonly called "cell-ghosts" and having various shapes, ranging from oval to spherical (Fig. 2A), with diameters between 50 and 200 μm. TEM images of their cell walls (after shadowing) showed a microfibrillar structure (Fig. 2B). It is observed that the cellulose microfibrils in the cell-ghost walls have an interwoven network texture, in which the cellulose microfibrils interweave tightly and randomly without any preferred orientation. This arrangement of cellulose microfibrils helps to keep the parenchyma cell in shape and strengthens the cell walls. The cellulose microfibrils, which consist of individual elements associated in bundles, are typically 20–30 nm in diameter and several micrometres in length. This texture, known as "foliate texture", is classical in the primary wall of isodiametric cells of higher plants (Roelofsen, 1959). Such cell walls are isotropic in surface view but anisotropic in their section.

The homogenisation of purified cells from OFI skins, after several passages through the Manton Gaulin apparatus, induced a complete disruption of parenchyma and collenchyma cell-ghosts and the release of the cellulose microfibrils in the medium. This is illustrated in the low dose TEM image shown in Fig. 2C and D, obtained after negative staining with uranyl acetate, revealed cellulose microfibrils, individual or associated into bundles. These microfibrils are no more than 2 to 5 nm in diameter and a few microns in length. The bundles consisted of a loose parallel arrangement of microfibrils ranging in size from only a few elements. No changes were observed, either in the sugar composition or in the molecular weight, as shown in Table 2. Glucose was the main sugar detected with a degree of polymerisation between 850 and 830.

The CP/MAS ^{13}C-NMR spectrum of microfibrillated cellulose from skin of OFI is shown in Fig. 3A. The spectrum presented the characteristic signals of native cellulose (Atalla, Gast, Sindorf, Bartuska, & Maciel, 1980). The anomeric carbon, C-1, appears furthest downfield, at around 105 ppm. Characteristic signals of C-2, C-3 and C-5 carbons are shown between 70 and 78 ppm. The signal between 87 and 90 ppm corresponds to C-4 of the highly ordered cellulose crystallite, whereas the broader upfield signals, 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 (Larsson, 2004). Also, the signal centred at 64.5 ppm is attributed to C-6 of ordered cellulose chains and the slight shoulder, between 60 and 63 ppm, is attributed to C-6 of cellulose in the amorphous and disordered component of cellulose microfibres.

Solid state NMR was used to investigate the ratio of cellulose I_α and I_β in cellulose from different origins. In particular, the C-4 region was used for this analysis, the signals from ordered and less ordered regions being well separated. Larsson (2004) used spectral fitting for the C-4 region of cotton cellulose and assigned three Lorentzian's lines for the signals from celluloses I_α, $I_{(\alpha+\beta)}$, and I_β and four Gaussian's lines for the signals from para-crystalline 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 microfibrillated cellulose from the skin of OFI, were determined using the areas of the crystalline and amorphous C-4 signals according to Larsson (2004) fitting. The results indicated that cellulose from the skin of OFI contained 60% of cellulose I_α and 40% of cellulose I_β, with a degree of crystallinity near to 38%.

Table 2
Sugar composition and molecular weights of different residues from skin of prickly pear fruit.

Fraction	Sugar composition[a] (% wt/wt)						$\bar{M}_w$ ($\times 10^3$) (g mol^{-1})
	Uronic acid	Rhamnose	Arabinose	Xylose	Galactose	Glucose	
Defatted skin	35.6	3.30	9.30	4.50	8.40	29.3	–
CR-I[b]	0.91	–	Traces	4.90	2.25	92.8	130
CR-II[b]	0.90	–	Traces	4.85	2.30	92.9	128

[a] Expressed in relative weight percentages.
[b] CR-I and CR-II are cellulosic residues, before and after homogenisation, respectively.

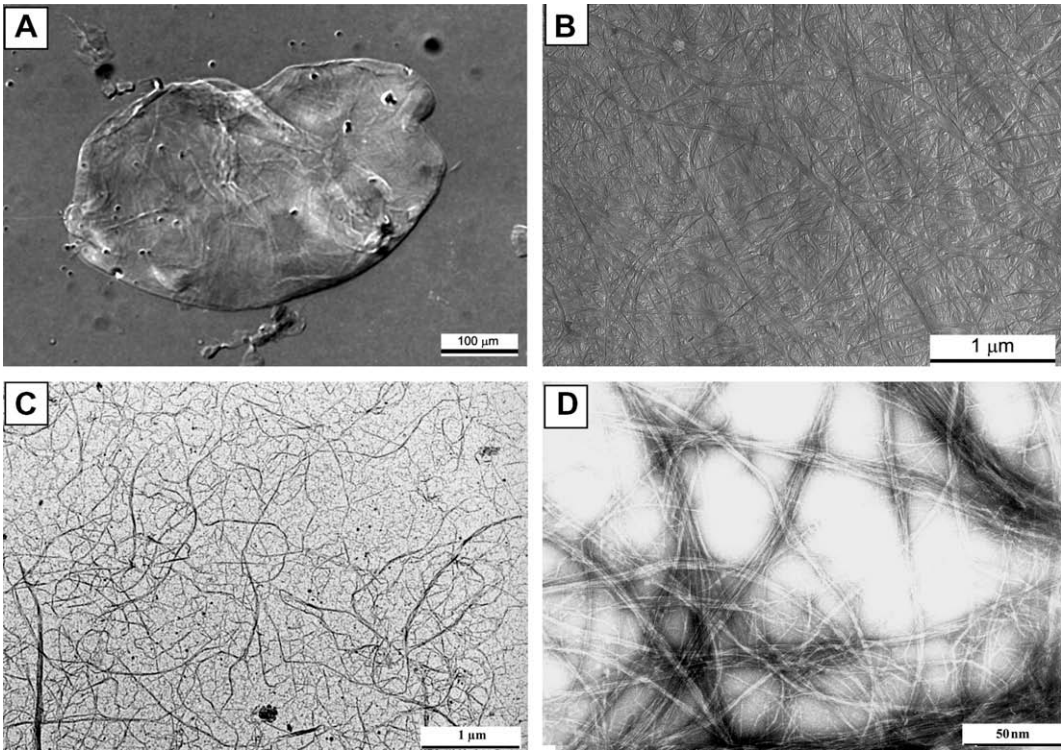

Fig. 2. Optical micrographs, in Nomarski's contrast (A), and TEM micrograph after shadow casting with W/Pa (B) of purified cell-ghosts from prickly pear skin. Low dose (C) and high magnification (D) TEM micrographs of microfibrillated cellulose from prickly pear skin after negative staining.

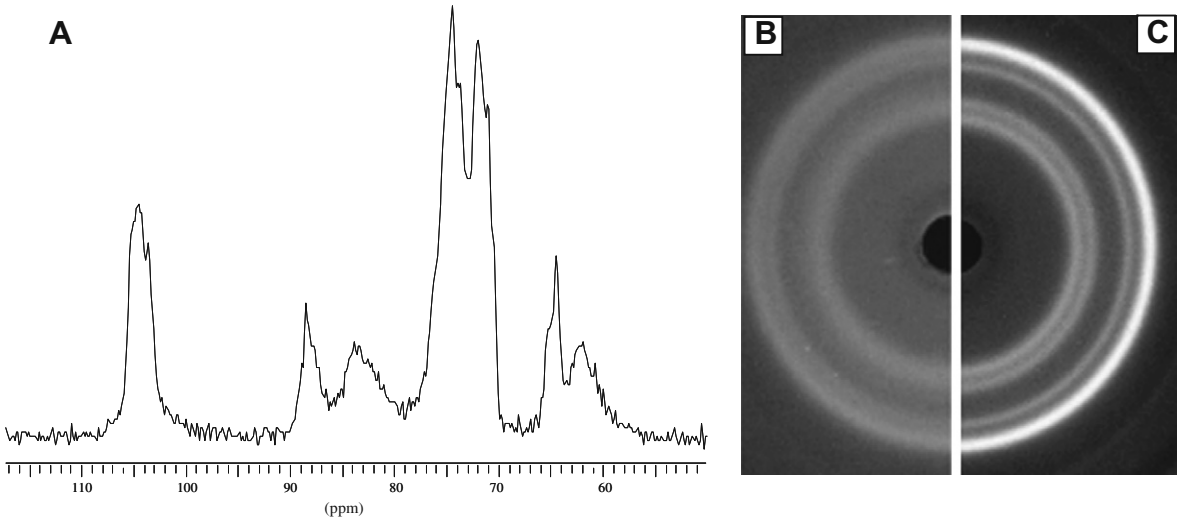

Fig. 3. ^{13}C-NMR spectrum (A) and X-ray diffraction patterns of microfibrillated cellulose from prickly pear fruit skin. X-ray diffraction patterns for cotton lintners reference (C).

The microfibrillated cellulose obtained from skins of OFI fruits was also analysed by X-ray diffraction. The diffraction pattern, depicted in Fig. 3B, is classical for this type of cellulosic material. It is characterised by one very broad ring centred at a *d*-spacing of 0.57 nm and two sharper rings positioned at *d*-spacings of 0.44 and 0.40 nm. The diffraction pattern of cellulose I, from high crystalline cellulose from cotton fibres, is shown as a reference (Fig. 3C) and is characterised by the four sharp rings with *d*-spacings at 0.60, 0.54, 0.44, and 0.40 nm. One can observe that the sharp rings at 0.60 and 0.54 nm, typical of cellulose I, are merged and only one sharp ring appears centred at 0.57 nm for microfibrillated cellulose. This observation is believed to be a consequence of the small diameter of the cellulose microfibres, evident in the TEM images shown Fig. 2D. Having diameters only around 2–5 nm, the primary wall microfibrils contain only between 16 and 30 cellulose chains. With such a small number of cellulose chains, most of them are located at the surface of the microfibrils and, therefore, only crystals with disorganised lateral cohesion, and no periodic lateral hydrogen bonds, occur. Most authors consider that this cellulosic microfibre corresponds to cellulose IV_I and not to the classical native cellulose I.

Fig. 4. Aqueous suspension 0.25 % (w/v) of cellulose residue from skin of OFI prickly pear fruit, before (A) and after the Manton Gaulin homogenisation (B).

Within experimental error, a measured degree of crystallinity or crystallinity index (CrI) of 40% (calculated from $CrI = 100\times (I_{002} - I_{am})/I_{002}$; (Segal, Creely, Martin, & Conrad, 1959)) corroborates solid state NMR results and shows the low crystallinity of this cellulosic substrate.

One of the most interesting properties of the mechanically homogenised cellulose suspensions was that they did not flocculate or settle down (see Fig. 4). The dynamical rheology measurements show that the suspension in water of these MFCs presents a typical shear-thinning behaviour and they can be considered as weak gels. As described in Section 1, the suspension, prepared from MFCs extracted from sugar beet pulp, presented similar gel behaviour (Dinand et al., 1996; Dinand et al., 1999). The stability of these microfibrils from sugar beet pulp was attributed to the presence of residual hemicelluloses (4-*O*-Me-glucuroxylan) and pectin in the microfibril surface. Other authors have established that a residual amount of 1% to 3% of acid polysaccharides is necessary to obtain a stable (non-flocculating) suspension of cellulose microfibrils. Below this amount, the suspensions flocculate and accordingly lose some of their rheological properties. A similar 4-*O*-Me-glucuroxylan, with the same glucuronic acid molar ratio, was previously detected in the skin of *Opuntia ficus indica* prickly fruits (Habibi, Mahrouz, & Vignon, 2003). The residual amount of these xylans in the surface of cellulose microfibrils, as well pectin polysaccharides, may explain their colloidal stability. It is interesting to note that suspensions of Celish® (Fukui, 1985) and Cellulon® (Johnson & Neogi, 1989) MFCs, which have ultrastructures similar to those of MFCs from prickly pear skin, display rheological properties similar to those presented in this work.

4. Conclusion

The morphologies of the cells from the skin of *Opuntia ficus indica,* before and after disencrustation and homogenisation steps, were followed by optical and transmission electron microscopy. The microfibrillated cellulose, obtained after mechanical disintegration, contained collenchyma and parenchyma cells which were shown to be very stable gels in water. These cellulose microfibrils presented great similarities, in terms of composition and morphology, to those extracted from primary walls (particularly microfibrils made of parenchyma cells such as sugar beet pulp). These observations help to explain similarities in the rheological properties, as well. Thus, as stated in the different patents, MFCs from skins of OFI should find the same range of applications as those from other sources, such as sugar beet pulp, wood pulp and bacterial cellulose, all of which are used for their thickening, suspending, binding and coating properties. A noteworthy fact is that prickly pear fruits have better nutritional properties than have sugar beet roots. Finally, we note that OFI plants have minimal soil and water requirements and therefore are an interesting alternative for the agricultural economy of arid and semi-arid regions.

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