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Elucidation of the fibrous structure of *Musaceae* maturate rachis

Piedad Gañán · Robin Zuluaga · Javier Cruz · Juan M. Vélez · Aloña Retegi · Iñaki Mondragon

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Abstract In the last years several composites and high performance materials with woody and non-woody natural fibers have been developed. In this study, a morphological study of agricultural residues as rachis from *Musaceae* plants cultivated in Colombia has been carried out. Fibrous structures as fiber bundles, elementary or ultimate fibers and even cellulose microfibrils grouped together into microfibril bundles have been observed. Both biological retting and chemical procedures like alkali treatments combined with alkaline peroxide and acid addition have been used. Different microscopic techniques as optical (OM), confocal (CM), scanning electron (SEM), and atomic force (AFM) ones have been used for analysis of isolated samples. A hierarchical arrangement from conducting tissues and fiber bundles to cellulose microfibrils in *Musaceae* rachis has been noted. All of these structures can be isolated by biological and chemical processes at the corresponding arrangement level. This means that *Musaceae* rachises constitute a source of new interesting biodegradable raw materials with multiple possibilities in dimensions and morphologies for several industries. A strong presence of crystal structures exists on fiber surfaces, being their occurrence related to the maturate state of rachis samples. Additionally, a top-down scheme is proposed for understanding the structuration of rachis at each length scale.

Keywords Agricultural residues · Cellulose microfibrils · Fiber bundles · Fibrous structure · Hierarchical arrangement · Morphology · *Musaceae* plants

Abbreviations

AFM	Atomic force microscopy
CM	Confocal microscopy
FTIR	Fourier transform infrared spectroscopy
OM	Optical microscopy
SEM	Scanning electron microscopy

P. Gañán (✉) · R. Zuluaga · J. Cruz
New Materials Group, Universidad Pontificia Bolivariana, Circular 1 # 70-01, Medellin, Colombia
e-mail: piedad.ganan@upb.edu.co

R. Zuluaga
Agro-Industrial Engineering Programme, Pontificia Bolivariana University, Circular 1 # 70-01, Medellin, Colombia
e-mail: robin.zuluaga@upb.edu.co

J. M. Vélez
Grupo de Ciencia e Ingeniería de Materiales, Universidad Nacional de Colombia, Carrera 80, # 65-223, Medellin, Colombia
e-mail: jmvelez@unalmed.edu.co

A. Retegi · I. Mondragon
"Materials + Technologies" Group, Dpto. Ingeniería Química y M. Ambiente, Escuela Politécnica, Universidad del País Vasco/Euskal Herriko Unibertsitatea, Pza. Europa, 1, 20018 Donostia-San Sebastian, Spain

I. Mondragon
e-mail: inaki.mondragon@ehu.es

fb Fiber bundles
pq Parenchyma
ct Conducting tissues
mf Macrofibrils

Introduction

Nowadays, vegetable sources can be used to develop materials with high performance (Qian et al. 2004; Greil et al. 2004; Hoppe and Petrova 2004), even it is recognized by institutions like the National Academy of Science of USA, which declares that "hierarchical structures in biology as a guide for new materials technology" should be used (National Academy of Science 1994). This tendency is associated with particular advantages that combine low density with good mechanical properties, low cost and biodegradability (Qian et al. 2004; Greil et al. 2004; Hoppe and Petrova 2004). Other important aspect is associated with the mimics of their hierarchically built anatomies developed and optimized in a long-term evolution process (Hoppe and Petrova 2004). Wood and a wide variety of non-woody plants offer multiple possibilities in dimensions, composition and morphology of fibrous structures (Schurz 1999) than can be useful for pulp and paper making industries (Cordeiro et al. 2004), novel microcellular ceramics with unidirectional porous structures (Qian et al. 2004; Hoppe and Petrova 2004; Qian et al. 2005) or nanocellulosic fiber composites (Malainine et al. 2005; Kvien et al. 2005). The tubular cell disposition of wood for example offers an opportunity to use infiltration with gas, liquid (Greil et al. 1998) or sol-gel techniques (Drosched et al. 2000) to transform the bioorganic source into cellular ceramic reinforcement for composites materials useful for many applications that include high-temperature resistance gas filters, catalyst support, heat insulation components or structural applications (Qian et al. 2004; Greil et al. 1998). Additionally, natural fiber bundles are broadly evaluated as reinforcement for recyclable composites in transport applications (Joshi et al. 2004; Rouison et al. 2006) or for production of environmental friendly polymers (Sun and Tomkinson 2003).

Plants are characterized by the presence of several hierarchically ordered structures ranging from millimeter, fiber bundle, up to nanometer scale, cellulose microfibrils, and all of these structures are constituted by cellulose joined to non-cellulosic components as hemicellulose, lignin, pectins and proteins.

In this environmental tendency, agricultural wastes present a vast potential being a cheap feedstock due to the huge amount generated all around the world as it happens with wheat straw (Sun and Tomkinson 2003), sugarcane (Baudel et al. 2005) or *Musaceae* residues (Gañán et al. 2004). In the case of Colombian *Musaceae* crops more than four millions tons of agricultural residues per year are generated. In spite of this, few morphological studies about this kind of materials have been developed (Gañán et al. 2004; Pothan et al. 2006; Jimenez et al. 2005). Many applications could be envisaged by taking advantage of their hierarchical constitution that include cellulosic microfibrils and other components. This study is focused to investigate the fibrous structure of agro-industrial residues like rachis of two different *Musaceae* plants cultivated in Colombia. Using an up-bottom approach, rachises, fiber bundles and conducting tissues, elementary or ultimate fibers, microfibrils bundles and cellulose microfibrils have been isolated. Techniques as optical (OM), confocal (CM), scanning electron (SEM) and atomic force (AFM) microscopies have been used. Biological retting and chemical process using alkali treatment combined with alkaline peroxide and acid addition have been utilized to isolate the above outlined products.

Experimental

Samples

Maturate rachises from two types of *Musaceae* plants cultivated in Colombia were the raw material of this work. They were collected two or three days after fruit harvesting. Two commercial *Musaceae* plants as banana (*Musa AAA, cv "Valery"*)) and plantain (*Musa AAB, cv "Dominico Harton"*) were used. Fiber bundles were extracted from these rachises by biological retting using an innocule composed by *fusarium spp, trychoderma spp* and acidogenic and metanogenic bacterias. The inocule was obtained by previous fermentation of several *Musaceae* rachises. Rachis sample was put in a flask and a 5 L fresh

water solution with 10 wt% of innocule was added. Fiber bundle samples were obtained at different exposure times from 10 days to 60 days. These fiber bundles isolated were cut into small portions between 100 mm and 300 mm using a milling machine, and then cleaned with toluene-ethanol using Soxhlet extraction for 6 h, and then exposed to sequential alkali solution treatments. Scheme 1 shows details of each step and indicates the residue obtained in each steps. Three residues were obtained and they are named as residue 1, 2 or 3, according with sequential step. Some of them were combined with the addition of few amounts of H_2O_2, see procedure A, and HCl, see procedure B. These processes were followed in according with Sun et al. (2004, p. 331). Before morphological analysis, each sample was dried at 105 ± 5 °C during 24 h.

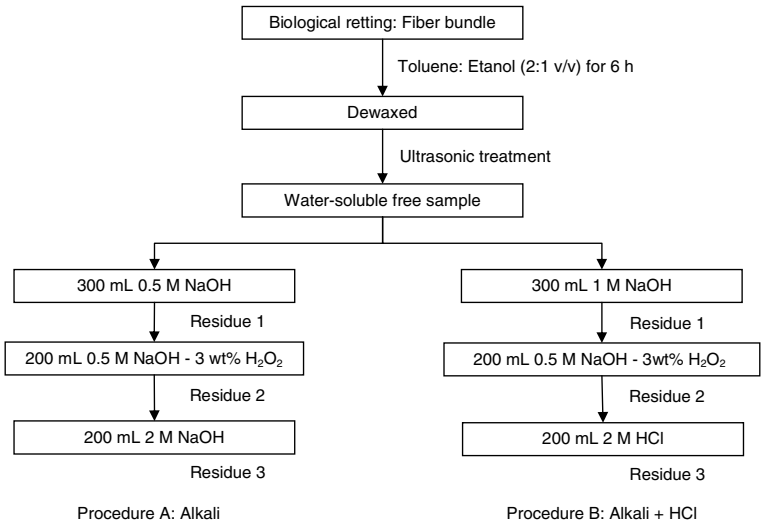
Sample test methods

Rachises and fiber bundles were analyzed using an optical microscope, Leica MLDL, in transmission mode l, a confocal laser scanning microcope, Leica DM RXE-TCS SP2 and scanning electron microscopy, Jeol JSM 5910 LV. In this case, the samples were coated with gold using the sputtering technique. Cellulose microfibrils were observed using atomic force microscopy, NanoScope IIIa, Multimode™ from Digital Instruments, in tapping mode. A drop of each suspension (A or B) was put down onto freshly cleaved mica and left to dry in a silica gel ambient for 12 h. Both height and phase images were captured. A resonance frequency of 200 kHz, and a spring constant of 12–103 N/m were used.

Fiber bundles and both residues 3 were analyzed using Fourier transform infrared spectroscopy (FTIR), Perkin Elmer PC1600. This test was useful to identify the nature of non-organic components on the surface of different samples. Spectra were taken at a resolution of 4 cm^{-1} with twenty scans for each specimen. For each material, five samples were tested.

Results and discussion

Musaceae crops grow in tropical and humidity areas. As it can be seen in Figs. 1–3, both banana and plantain plants have an important amount of vascular bundles that are formed by conducting tissues (ct) and fiber bundles (fb). Parenchyma cells are observed nearly to vascular tissues. Fiber bundles are formed by elementary or ultimate fibers with cell wall thickness higher than that of other parenchyma cells (see Fig. 2a, b). These micro-structural aspects are related with their function in the plant: the parenchyma cell acts in storage activities, conducting tissues in conduction functions and elementary fibers inside fiber bundles acts as support (Dinwoodie



Scheme 1 Isolation steps for *Musaceae* rachis fibrous configuration

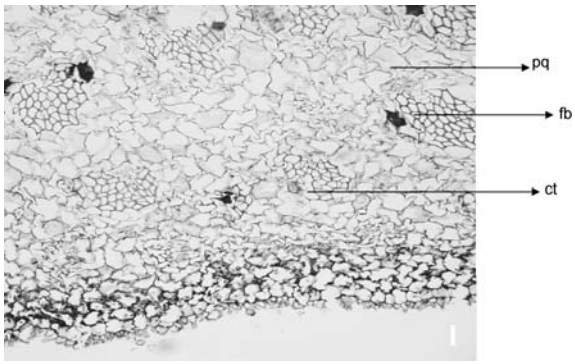


Fig. 1 OM micrograph with details of a half cross-section of banana maturate rachis. ct, conducting tissue; pq, parenchyma; fb, fiber bundle. Scale bar: 100 μm

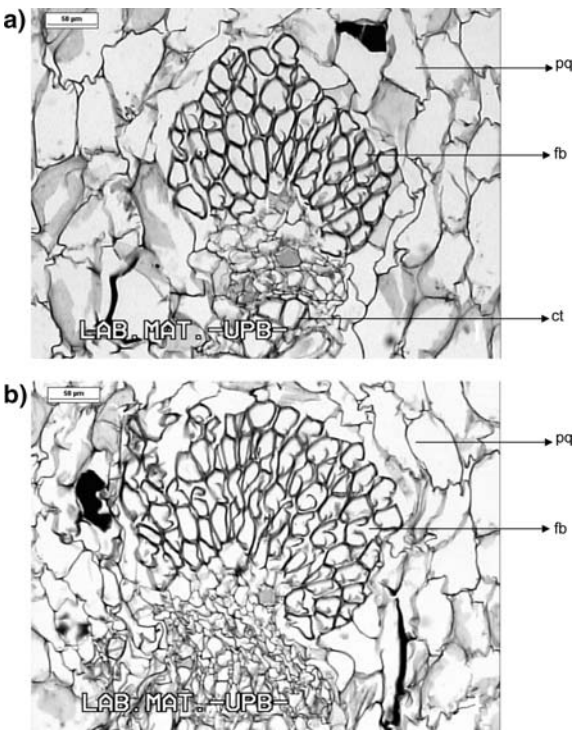


Fig. 2 Detail of cross section of *Musaceae* maturate rachis. 200×. (**a**) banana maturate rachis, (**b**) plantain maturate rachis. ct, conducting tissue; pq, parenchyma; fb, fiber bundle

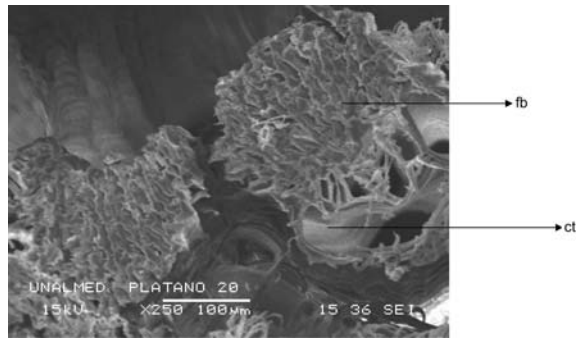


Fig. 3 SEM micrograph of a half cross-section of plantain maturate rachis

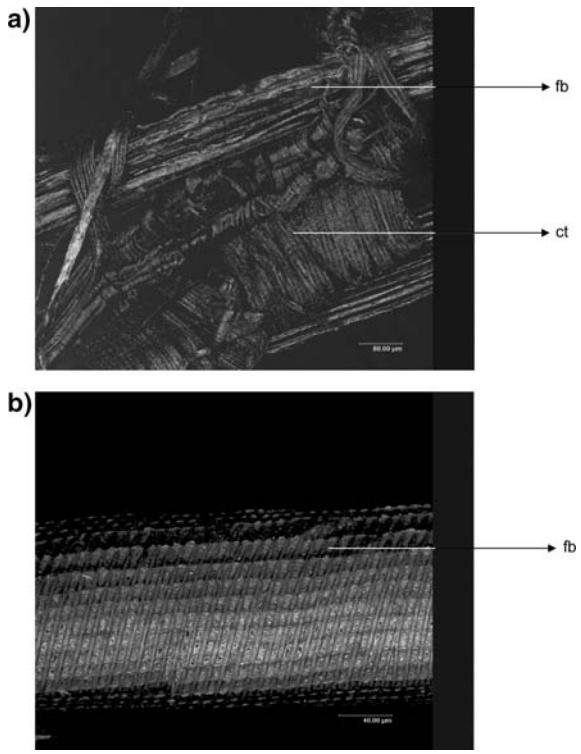


Fig. 4 Confocal micrograph of fiber bundle isolated from banana maturate rachis

2002). Other kind of plants such as rattan genus *Calamus* (Tomlinson et al. 2001) or sugarcane (Dong et al. 1997) also present similar structures. Fiber bundles and conducting tissues can be isolated from rachis using processes as hand scrapping, mechanical decortication and biological retting. Figure 4a, b shows a CM image of a fiber bundle isolated by biological retting. As shown in Fig. 4a, conducting tissues appear still together with fiber bundle, which suggests that this process is non-homogeneous to obtain a complete separation of fiber bundles. This fact is related with their position on the rachis transversal section, as shown in Figs. 1–3. Conducting tissues with spiral structural backbone have a

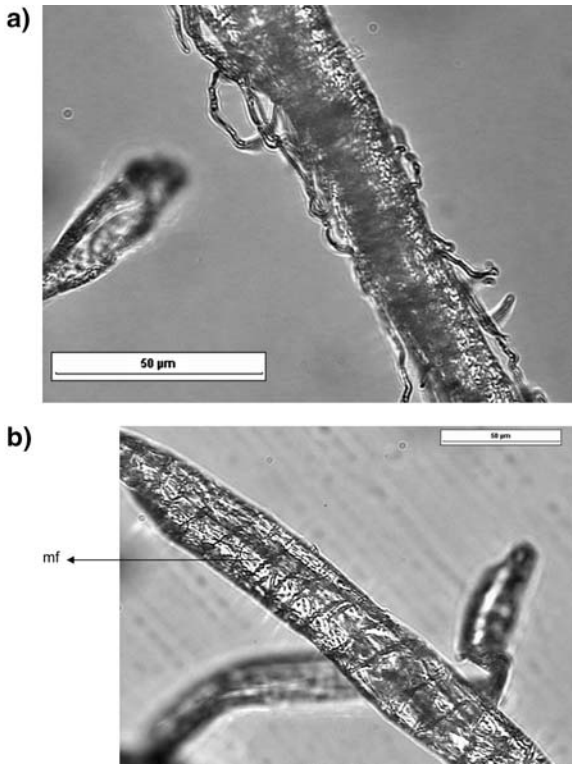


Fig. 5 OM micrographs of fiber bundles residues 1 obtained from plantain maturate rachis exposed both treatments. 500×. (**a**) Procedure A, (**b**) Procedure B

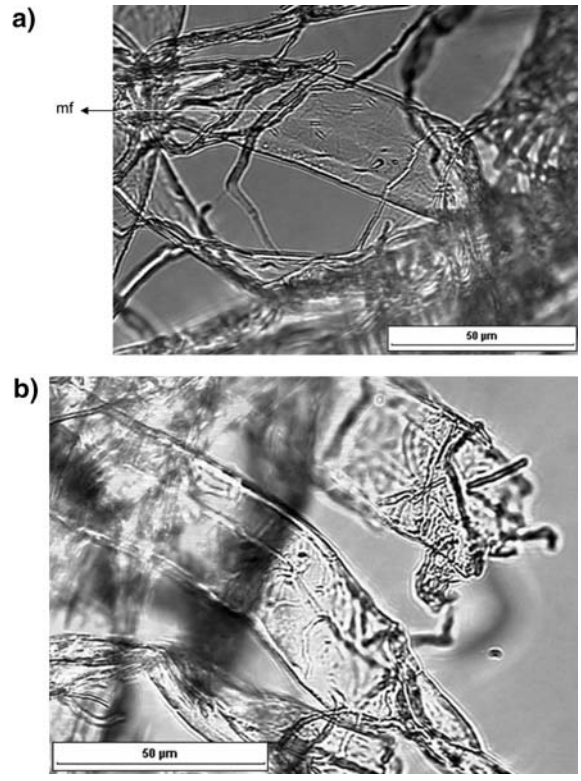


Fig. 6 OM micrographs of fiber bundles residues 2 obtained from plantain maturate rachis exposed both treatments. 500×. (**a**) Procedure A, (**b**) Procedure B

roughly diameter larger than that of elementary fibers (see Fig. 3). Comparable tissues have been observed by Yu et al. (2005, p. 5689) and Liu et al. (2005, p. 25) during cellulose isolation of wheat straw. As shown in Fig. 5b, elementary or ultimate fibers inside the fiber bundles are disposed parallel to the axis of the rachis. This aspect is one of the large anisotropy characteristics observed in the rachis fibrous structure. This arrangement has been observed for both *Musaceae* rachises.

For elementary fiber isolation, different chemical treatments can be utilized. One of them is an alkali process using several steps. In this work, alkali treatment has been combined with alkaline peroxide and hydrochloric acid addition. Both treatments are described in Scheme 1 and correspond to procedure A and procedure B, respectively. Three different residues for each procedure have been analyzed using optical microscopy (Figs. 5–6), scanning electron microscopy (Figs. 7–8) and atomic force microscopy (Fig. 8).

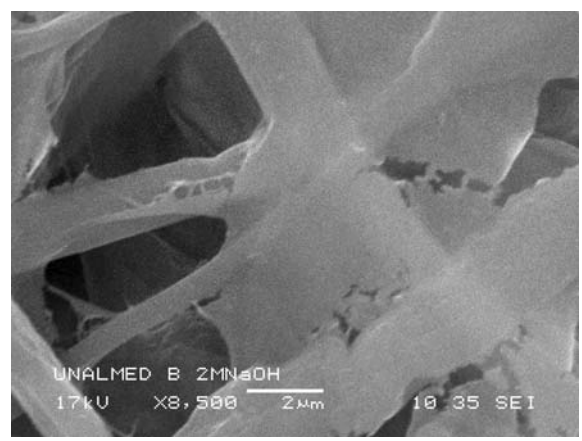


Fig. 7 SEM micrograph of fiber bundles residue 3 obtained from plantain maturate rachis exposed of procedure A

After first alkaline step, both residues 1 show fibrillation (see Fig. 5). The fibrillation increases with the following alkaline peroxide step, as shown in Fig. 6. Additionally, the residues have more fibril

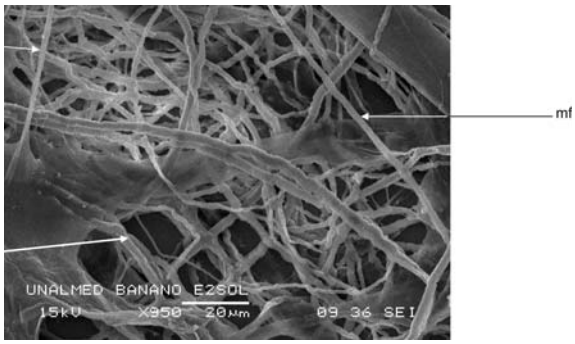


Fig. 8 SEM micrographs of fiber bundles residue 3 obtained from banana maturate rachis exposed at of procedure B

aggregation than fiber bundles (Fig. 4b). These phenomena have been related with the progressive dissolution of non-cellulosic components as pectins and hemicellulose, and a small portion of lignin (Kondo and Sarkanen 1984; Hult et al. 2001). This dissolution increases during the alkaline peroxide step and a progressive separation of elementary fibers takes place (Fig. 6). These elementary fibers have rough diameter between 10 µm to 20 µm. Both chemical procedures attack primary cell wall and even secondary cell wall (Fig. 6). Thus, macrofibrils (mf) showing a helicoidal arrangement inside the secondary cell wall is observed (Fig. 5b). This disposition has been observed for both *Musaceae* samples.

Both residues 3 for all *Musaceae* samples analyzed in this study are basically formed by macrofibrils isolated with rough diameter less than 1 µm (Figs. 7, 8). According with Fig. 8, macrofibril bundles are conformed by a hierarchical arrangement of macrofibrils. Microfibril bundles with rough diameter of 40 nm to 60 nm (Fig. 9a) and even cellulose microfibrils with rough diameter around 5 to 10 nm (Fig. 9b) are observed. Both procedures are effective to isolate cellulose microfibrils, specially from

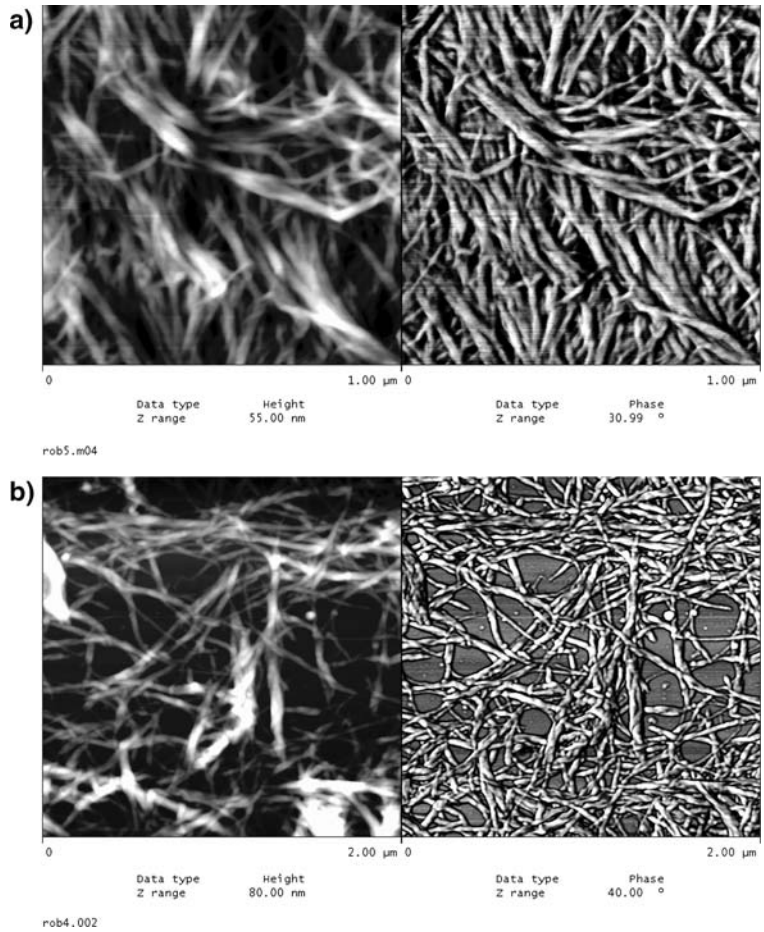


Fig. 9 AFM micrographs of both fiber bundles residues 3 obtained from banana maturate rachis. (**a**) Procedure A, (**b**) Procedure B

primary cell wall of banana and plantain samples. Additional steps, using mechanical equipment as homogenizer machine could be risen the amount of cellulose microfibrils.

During each extraction step, matrix residues joined to cellulose fibrous structures are observed (Figs. 6–8). This indicates that non-cellulosic components are strongly joined to cellulose along of fibrous structures that form the rachis.

Additionally, Fig. 10 reveals a strong presence of crystal structures on fiber surface that are associated with mineral salts present in the cell walls. Moreover, as shown in Fig. 11, FTIR spectra of fiber bundles and both residues 3 present vibrations around 670, 617 and 560 cm^{-1}, all of them being related to calcium oxalate salts. This kind of biomineral is common in plants (Monje and Baran 2005) as *Musaceae* (Osuji and Ndukwu 2005). Small vibration in 1700 cm^{-1} region is observed, but it could be affected by non-cellulosic component still present. Their occurrence on residue surface is related to the maturate state of vegetable (Dickison 1990), and treatment conditions used as alkali dosage or hydrogen peroxide concentration, that contribute to their precipitation (Sjöde et al. 2005; Ulmgren and Rådeström 2000). This observation agrees with Dufresne et al. (1997, p. 1185), who found that chemical treatments are not enough for fully removal of these crystals.

All observations indicate that the hierarchical arrangement of the rachis offers several fibrous structures that each could be useful for many applications. Scheme 2 proposes a top-down approach for the structuration at several scales of the rachis and its fibrous structures. They can be grouped in two levels: microscopic level formed by vascular bundles and elementary fibers, and nanoscopic or ultrastructural level formed by macrofibrils, microfibril bundles and cellulose microfibrils. This scheme suggests that several reinforcements with different aspect ratio and their composites with a broad range of mechanical properties could be obtained starting from Musaceae rachis wastes. For completion of ultrastructural level shown in Scheme 2 we have also made use of models presented by other authors (Emons and Mulder 1998; Fengel and Wegener 1983; Bruck et al. 2002).

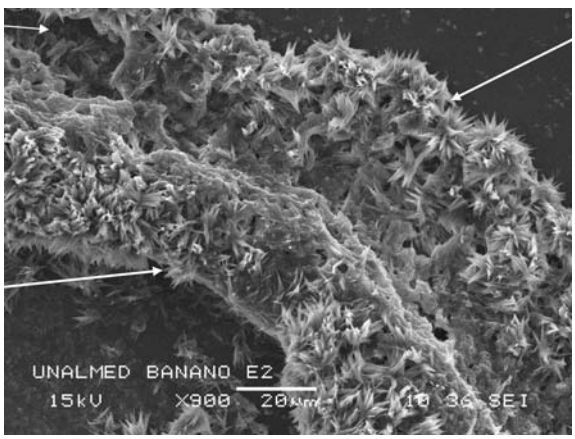


Fig. 10 SEM micrograph of crystals on fiber bundles residue 3 obtained from banana maturate rachis exposed at procedure B

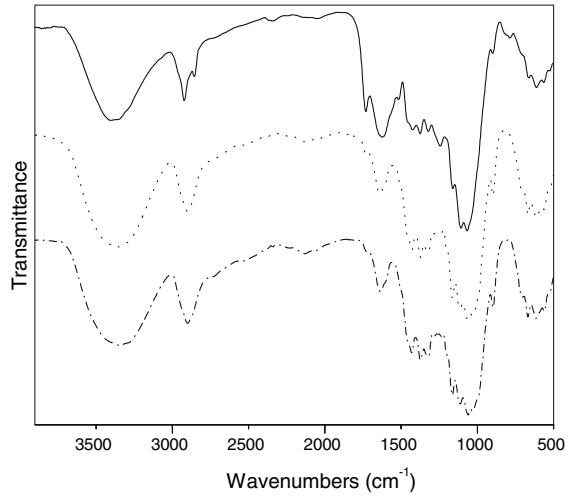
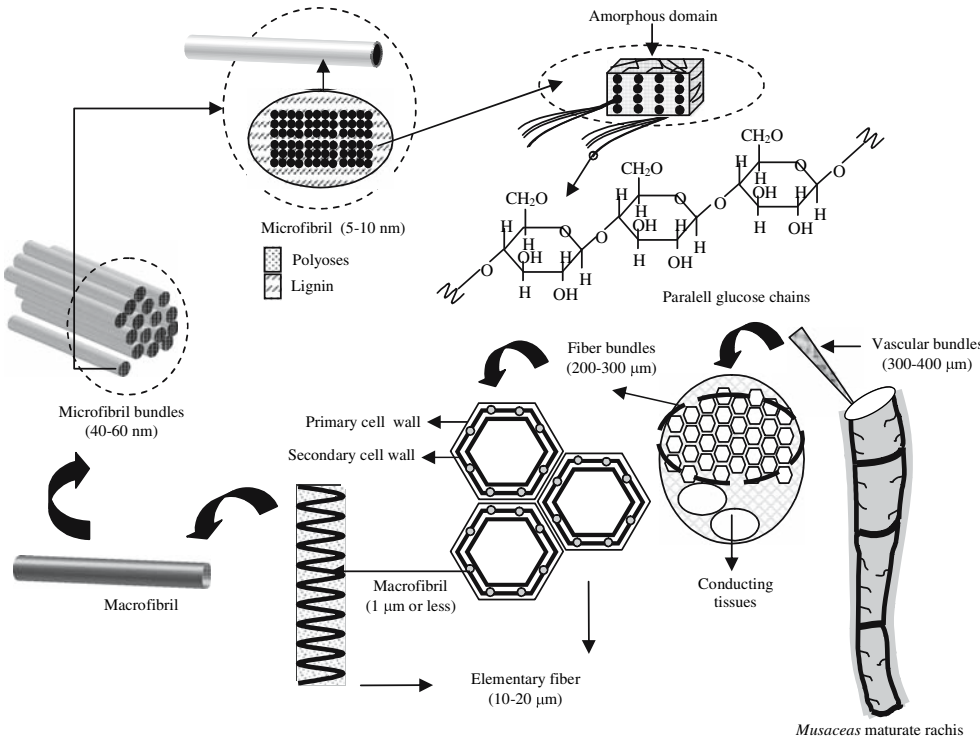


Fig. 11 FTIR spectra of banana fiber bundles and their both residues 3. (—) fiber bundle, (– –) Procedure A, (– — –) Procedure B

Conclusions

In this study, the fibrous structure of rachis residues has been analyzed. For this, two types of *Musaceae* maturate waste rachises have been used. A hierarchical structure has been demonstrated by microstructural analysis using different microscopic techniques. All results suggest that these fibrous structures can be grouped at two levels: microscopic level formed by conducting tissues, fiber bundles and their elementary fibers, and nanoscopic or ultrastructural level where cellulose microfibrils are grouped in microfibril bundles. Both plant structural levels have been isolated. Thus, using of controlled treatments can



Scheme 2 Diagram of *Musaceae* rachis fibrous configuration

allow to isolate fibers at different scales that can be useful to develop traditional natural fiber composites but also new nanocomposites based on cellulose microfibrils useful for new applications in several industrial sectors.

A strong presence of crystal structures on fiber surface has been observed. FTIR analysis suggested that it can be related with calcium oxalates. Their occurrence on residue surfaces is related to the maturate state of samples, also suggesting that both treatments are not enough for their fully elimination.

Additionally, a top-down scheme has been proposed for understanding the structuration of rachis at each length scale.

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