MORPHOLOGICAL AND STRUCTURAL CHARACTERIZATION OF BAMBOO FIBER INTO CULM – *Guadua angustifolia* KUNTH

CARACTERIZAÇÃO MORFOLÓGICA E ESTRUTURAL DE FIBRAS NA COLMO DO BAMBU DENTRO DE COLMO – *Guadua angustifolia* KUNTH

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ABSTRACT

Studies about lignocellulosic materials have gained importance in the last decades due to the outstanding characteristics that their fibers offer, which becomes in a good alternative to replace synthetic fibers. Lignocellulosic materials such as bamboo have high availability in most of regions around the world due to the adaptation capacity to grow in different areas and diverse climatic conditions. Despite of this fiber has being widely used; their microstructural organization into the culm has not been fully studied. The focus of this research is to study the morphology, distribution and crystalline compounds of fiber in native Colombian Bamboo called *Guadua angustifolia* Kunth for two varieties *Rayada Amarilla* and *Macana*. Scanning electron microscopy was used to observe *Guadua* fiber morphology and their variation throughout the culm, X-ray diffraction was used to identify crystalline compounds present in *Guadua angustifolia* Kunth and AOAC-2000 methodology (Association Official Agricultural Chemist) was used to get quantitative information about the insoluble and soluble fiber content into *Guadua* culm. Findings indicated that the insoluble fiber distribution is not homogenous between the internal and external layer of bamboo culm; the fiber content inside of *Guadua angustifolia* culms increases from inner to outer layer. X-ray patterns showed that the insoluble fiber has a preferential crystalline orientation in relation to the growth direction of bamboo. The combination of scanning electronic microscope and X-ray diffraction offers important information about the localization and morphologic distribution of components inside bamboo culms.
Keywords: *Guadua angustifolia* Kunth; fiber distribution; structural characterization.

RESUMO

O estudo sobre os materiais para obter fibras naturais tem tido grande importância nas últimas décadas devido às características excepcionais que essas fibras oferecem, as quais as convertem em uma alternativa interessante para substituir as fibras sintéticas em diferentes aplicações. O Bambu tem alta disponibilidade em várias regiões do mundo, devido à capacidade de adaptação e de crescimento em diferentes áreas. Apesar de as fibras de bambu estarem sendo altamente aplicadas, sua organização microestrutural dentro das hastes não tem sido estudada amplamente. O objetivo desta pesquisa está baseado no desenvolvimento e estudo das características e distribuição das fibras dentro do colmo no bambu nativo colombiano *Guadua angustifolia* Kunth. Neste estudo foram consideradas duas variedades de *Guadua: Guadua angustifolia* Kunth *Rayada Amarilla* e *Guadua angustifolia* Kunth *Macana*. As *Guaduas* foram analisadas por microscopia eletrônica de varredura, esta técnica é usada para observar a morfologia da fibra de *Guadua* e a sua variação através do colmo. Difração de raios X é usada para observar variação dos compostos através do colmo. Finalmente, a metodologia da "Association Official Agricultural Chemist – 2000" (AOAC-2000) é usada para obter

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informação quantitativa do conteúdo de fibra não solúvel. Os resultados indicaram que a distribuição de fibras não solúveis não é homogênea entre as camadas internas e externas da haste do bambu, isso aumenta na direção radial da parede interna até a parede externa; os padrões de difração de raios X mostram que a celulose presente no colmo tem uma preferência de ligação cristalina relacionada à direção do crescimento da planta. A combinação da análise de microscopia eletrônica de varredura e a difração de raios X oferecem informações importantes sobre a distribuição e a morfologia dentro do colmo dos componentes do Bambu.
Palavras-chave: *Guadua angustifolia* Kunth; distribuição de fibras; caracterização estrutural.

INTRODUCTION

In the last decades, research studies have developed materials where different fibers are used to reinforce organic or inorganic matrices. These materials, called composites, are stronger, lighter, or less expensive when compared to traditional materials. For these kinds of materials, vegetable fibers appear as a good alternative of reinforcement due to their outstanding mechanical properties (SAVASTANO et al., 1999; TONOLI et al., 2010; CRAVO et al., 2013; YU et al., 2013; COSTA et al., 2014; 2015a; BERARDI; IANNACE, 2015; BINOJ et al., 2016; GUEDES; FLORENTINO; MULINARI, 2016).

In the search of vegetable fibers to use as reinforcement, different lignocellulosic materials arise, which behave as natural composites. Bamboo is a natural composite that consists mainly in long cellulose fibers joins by lignin and hemicellulose matrix (JAIN; KUMAR; JINDAL, 1992; GHAVAMI; MARINHO, 2005). This structure is much stronger and stiffer than wood fiber, and comparable in extensibility due to its unique cell wall structure (YU et al., 2013). In the last three decades, bamboo has increased in importance around the world because of its mechanical, esthetic, and ecological properties, making it versatile for many applications. Moreover, the wide variety in appearance and chemical composition are some of the reasons why bamboo is more attractive, even than wood, for several purposes (SANCHEZ-ECHEVERRI et al., 2014). Not only the uses of bamboo as structural material are studied; some studies have shown the importance of bamboo leaves as pozzolanic materials to remove Cadmium I (Cd(I)) ions from aqueous solution (VILLAR-COCIÑA et al., 2011; PANDEY et al., 2015). Other research works use bamboo cellulose fibers to reinforce different matrices to develop composite materials (OKUBO; FUJII; YAMAMOTO, 2004; FRÍAS et al., 2012; MORAIS et al., 2015). Those studies show the importance of this plant and their components.

Regarding with bamboo fibers, these fibers have two principal compounds: insoluble and soluble fiber. The insoluble fiber constituted mainly by cellulose, hemicelluloses, and lignin while gums, mucilage and pectins constitute the soluble part and they are known as extractives (MARINHO, 2012). Other authors have reported starch into bamboo culms as another invited component related with soluble fiber (TOLEDO; AZZINI; REYES, 1987). There are different studies which provide detailed information related with bamboo fibers and their chemical compounds. Ghavami and Marihno (2005) and Marinho, Nisgoski and Muñiz (2014) proved that fiber distribution into bamboo culms is different across the internal to external wall. On the other hand, different studies showed that fiber distribution and quality depend of culm age, diameter, plant location, and the bamboo species (LO; CUI; LEUNG, 2004; AHMAD; KAMKE, 2005; RAY et al., 2005; OBATAYA; KITIN; YAMAUCHI, 2007; SANCHEZ-ECHEVERRI; CONTRERAS-PADILLA; RODRIGUEZ-GARCÍA, 2011). Not only environmental aspects affect fiber quality, the quality is highly affected by the fiber extraction method. Different authors have studied extraction methods and demonstrated that fiber properties are affected by these procedures. Finally, vegetable fiber properties affect directly mechanical properties of the composite materials where those fibers are used as reinforcement (SGRICCIA; HAWLEY; MISRA, 2008; COSTA et al., 2015b).

Despite of the aforementioned reports which clearly indicate that Bamboo fibers have an important role on different applications; their microstructural characterization is still unknown. The objective of this paper is to show fiber content distribution, morphological changes and crystalline orientation of fiber within the culm of two varieties of Colombian bamboo called *Guadua angustifolia* Kunth.

MATERIALS AND METHODS

Sample description

Two *Guadua angustifolia* varieties *Rayada Amarilla* and *Macana* were studied. Samples come from an experimental field of Colombia, harvested on February 15th, 2009. They were dried in a furnace during 8h at 40°C to reduce the moisture content and decrease the microorganism proliferation. The culms in these kinds of bamboos are hollow in the center as a cylinder and the wall thickness is in average 1.5cm. For some analysis, the whole culm was used and two different directions Z and Y were defined as shown in Figure 1A. Z- direction corresponds to growth direction of the *Guadua angustifolia* while Y- direction goes from the internal wall to external wall. In addition, in order to determine fiber distribution and morphological changes inside bamboo's culms, four equal culm sections along Y-direction were cut (Figure 1B).
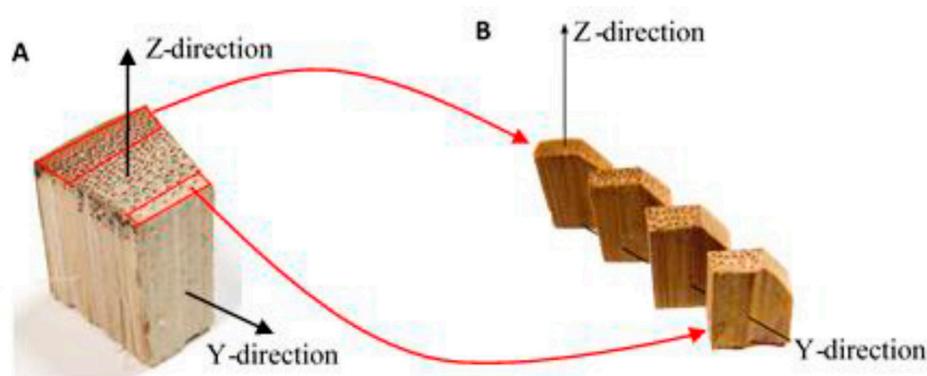

FIGURE 1: (A) Small piece taken from the whole culm of *Guadua angustifolia* Kunth, letters X-Y and Z show the three directions in culm. Z-direction is the direction of growth and Y-direction goes from the internal wall to the external wall (B) Four equal sections taken from whole culm (A) where R1 is the internal layer and R4 is the external layer.

FIGURA 1: (A) Pequena amostra tomada do colmo inteiro do Bambu (*Guadua angustifolia* Kunth), As letras Y-Z representam as duas direções do colmo. A direção Z indica a direção de crescimento da planta, e a direção Y indica a espessura do colmo o qual vai desde a parede interna até a externa. (B) Quatro seções tomadas do colmo inteiro em que R1 é a seção interna e R4 a seção externa.

Fiber determination

Insoluble and soluble fiber determination was done using the official Method 992.16, AOAC 2000 (Sigma Aldrich TDF-100A Kit, Saint Louis Missouri, USA) (AOAC-2005, 1997). The fiber content determination was carried out in whole sample (Figure 1A) as well as in each one of the section shown in Figure 1B. All measurements were done by triplicate.

Scanning electron microscopy (SEM)

The morphology analysis of the samples was performed with a low vacuum scanning electron microscope (LV-SEM, JSM 5600LV) with resolution of 5 nm in LV mode, fitted with an energy dispersive X-ray spectrometer (Noran model Voyager 4.2.3). Prior to the analysis, samples were polished with different grain sizes of sandpaper, 400, 600 and 1200 (Fandeli SIC A-99-600, 1200). This procedure guarantees that the damage produced by the machine cut is removed, and the surface structure is not affected. After this process, samples were cleaned to remove sandpaper particles and the excess of organic materials. As bamboo culms are organic materials, it is necessary to cover them with a gold layer in order to make them conductive and obtain good resolution SEM images. For their observation in SEM, samples were fixed on the specimen holder with conductive tape of Carbon. The analyses conditions used were 15kV electron

acceleration voltages and 12–20Pa of pressure in the specimen chamber with the backscattering electron signal. The SEM images were taken from whole sample and four equal cuts showed in the Figure 1A and 1B, respectively.

X-ray diffraction (XRD)

X-ray diffraction patterns of both *Guadua angustifolia* varieties *Macana* and *Rayada Amarilla* were done as follow: A first study was carried out in the whole sample two for Z and Y directions shown in Figure 1A; and then, patterns were taken for each one of the internal faces of the samples shown in Figure 1B. The analysis of the crystalline structures of *Guadua angustifolia* were recorded on a diffractometer (Siemens D5000) operating at 35K and 15mA, with Cu Kα radiation wavelength of $\lambda = 1.5406$Å. Data was collected from 4º to 70º on a 2θ scale with a step size of 0.05s. Room temperature was used in this study.

RESULTS AND DISCUSSIONS

Fiber determination

The anatomical properties and the unique structure of Bamboo make it superior than other known natural ligno-cellulose fibers; that fibers consist in a complex carbohydrates, comprising various amounts of cellulose, hemicellulose, pectin and lignin (SELVENDRAN, 1984). Compounds into bamboo culms are classified in two great groups of components: soluble and insoluble fiber. Cellulose, some lignin, and hemicelluloses are main components of insoluble fiber (PEREIRA; BERALDO, 2007). While gums, pectins, and mucilages compose soluble fiber. The proportion of those compounds varies between bamboo species as well as the bamboo culm sections. Table 1 shows soluble and insoluble fiber content inside bamboo culms (Figure 1A) for both *Guadua angustifolia* varieties *Rayada Amarilla* and *Macana*.

TABLE 1: Insoluble and soluble fiber content of *Guadua angustifolia* Kunth culm for *Macana* and *Rayada Amarilla* varieties.

TABELA 1: Conteúdo de fibra solúvel e insolúvel no colmo da *Guadua angustifolia* Kunth para as variedades *Macana* e *Rayada Amarilla*.

Sample	Insoluble Fiber (%)	Soluble Fiber (%)
Macana	74.41 ± 0.23	10.6 ± 0.35
Rayada Amarilla	83.22 ± 0.04	2.16 ± 0.06

The results showed in Table 1 agree with those results founds by Liese (1998), who argued that the most compound into the *Guadua angustifolia* culm is insoluble fiber. He showed that parenchymal cells represent nearly 60% of a bamboo volume, and other 20% are constituted by Bamboo fibers, and both, parenchyma and bamboo fiber are components of insoluble fiber. Those results were previously present at the International Conference on Sustainable Construction Materials and Technologies Conference (SANCHEZ-ECHEVERRI; MEDINA-PERILLA; RODRIGUEZ-GARCÍA, 2016). Insoluble fiber content was also determined for each segment throughout radial direction of *Guadua angustifolia's* culms (Figure 1B). Figure 2 shows the insoluble fiber content distribution across the culm for both varieties studied. The results displayed correspond to an average of three measurements per point.
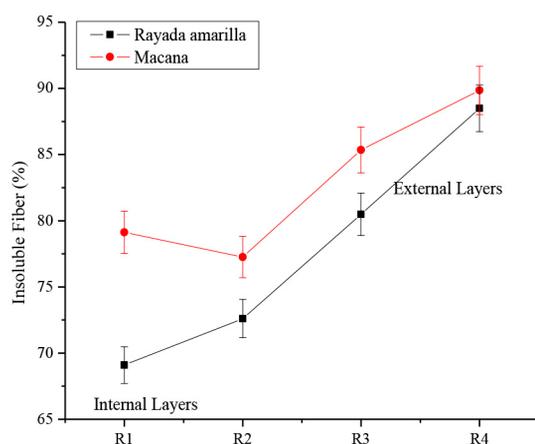
FIGURE 2:   Insoluble Fiber distribution across *Guadua angustifolia* Kunth culm for *Rayada Amarilla* (black line) and *Macana* (Red line) varieties. Distribution is shown from internal section R1 to external section R4.

FIGURA 2:   Distribuição de fibra insolúvel através do colmo do bambu (*Guadua angustifolia* Kunth), para as variedades *Rayada Amarilla* (linha preta) e *Macana* (linha vermelha). A distribuição se mostra desde a seção interna R1 até a seção externa R4.

According to those results, the most external layers of the culm possess the higher insoluble fiber content and this concentration increases from the internal to the external layers. Results about how the insoluble fiber is varying throughout the culm are in accordance with Ghavami and Marinho (2005); they studied the fiber distribution across the culm thickness of *Guadua angustifolia* by means of optic and electronic microscopy techniques and found that the fiber increases from internal to external wall. Wang et al. (2011), studied moso bamboo (*Phyllostachys pubescens*) and define that the higher amount of vascular bundles in the outer culm wall is a perfect structural adaptation towards a high bending stiffness and strength of the bamboo culm.

It is also possible define from the Figure 2 that *Macana* variety has a higher insoluble fiber content in each bamboo culm section than *Rayada Amarilla*; however, as a whole, insoluble fiber content is higher in *Guadua angustifolia Rayada Amarilla* as was shown in Table 1. As was found by Wang et al. (2011) and Sanchez-Echeverri et al. (2014) the mechanical response in bamboo culms is a correlation between both fibers; therefore, according with that results *Macana* culms are more appropriated for structural applications while *Rayada Amarilla* could be thinking for other applications such cellulose and pulp production.

Scanning electron microscopy

In order to have an idea about morphology and the variation of internal components inside bamboo culms, several micrographs were taken. Figure 3A shows 3D SEM image of *Guadua angustifolia Rayada Amarilla* took at 13x. Vascular bundles are many in numbers and scattered all over any piece of bamboo. In this figure, it is possible to see how the tissues around them are becoming more compact across the radial direction of bamboo culm, it means, from internal to external walls (red arrow). Fiber bundles and pitted vessels compose vascular bundles, and they are surrounding with parenchyma tissue (Figure 3B) (RAY et al., 2004; MARINHO; NISGOSKI; MUÑIZ, 2014). The distribution gradient of vascular bundles across culm is the responsible that an insoluble fiber distribution inside bamboo culms increases from internal to external walls, as was defined in Figure 2. Lo, Cui and Leung (2004) demonstrated that fiber density affects strength capacity bamboo; therefore, based on these results, the external part of bamboo has more strength than the internal part. Another important feature on this SEM image is that it shows that fibers are oriented in the growth direction of the plant (blue arrow Figure 3A); this behavior allows *Guadua angustifolia* to have better mechanical response in longitudinal direction than in transversal direction (JAIN; KUMAR; JINDAL, 1992).
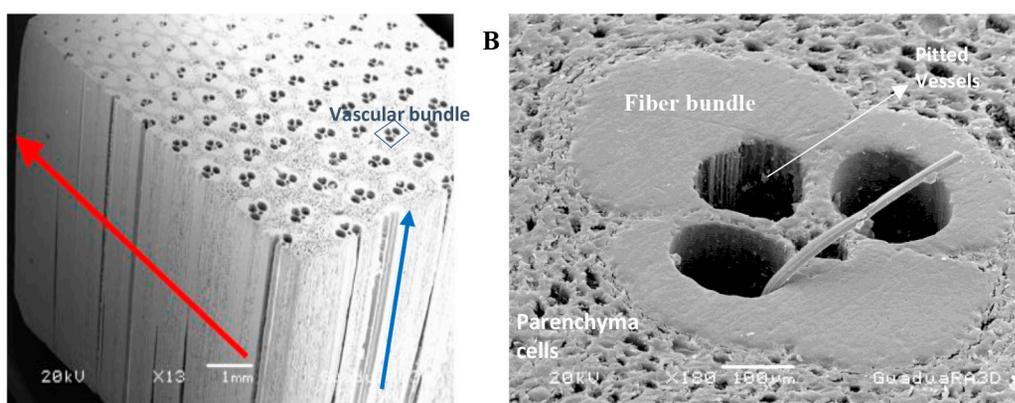
FIGURE 3:   3D SEM image (13X) bamboo culm *Guadua angustifolia* Kunth *Rayada Amarilla* showing vascular bundles. Red arrow indicates the direction where tissues around of the vascular bundle are becoming more compact. Blue arrow shows fiber orientation into *Guadua* culm, which is growth direction. (B) Vascular bundle components.

FIGURA 3:   (A) Imagem de MEV (13X) em 3D do colmo de bambu (*Guadua angustifolia* Kunth *Rayada Amarilla*) mostrando os feixes vasculares. A seta vermelha indica a direção na qual os tecidos ao redor dos feixes vasculares se tornam mais compactos. A seta azul indica a direção da orientação das fibras dentro do colmo, a qual é a direção de crescimento da planta. (B) Componentes dos feixes vasculares.

It is very important to establish the reason why the insoluble fiber content changes as function of the radius. Those changes may be related with variation throughout the culm of the main fiber compound contents like starch, cellulose, hemicellulose, and gums. In order to know how the morphology across the culm is changing, a SEM analysis of the four sections of culm shown in the Figure 1B was performed. Figures 4A and 4B show the morphology found in the internal face of the four sections called R1, R2, R3, and R4 (Figure 1B) of *Guadua angustifolia Rayada Amarilla*; the same structures were found in *Guadua angustifolia Macana*. For all sections, in both bamboo culms, it is possible to see a rectangular network with semi-spherical structures within them. These networks are identified as parenchyma cells (LIESE, 1998), and the semispherical structures are identified as starch (TOLEDO; AZZINI; REYES, 1987). Parenchyma cells do not have the same dimension in each of the four sections. The changes of the cell dimension explain the variation of fiber content across the culm due to those cells are composed by cellulose which is the main compound of insoluble fiber. Regarding to the starch, starch granules into the bamboo vary from 3 to 10μm with semispherical and polygonal shapes; this result agrees with the starches found by (TOLEDO; AZZINI; REYES, 1987); also, it is possible to see that section R3 showed the major quantity of these structures (Figure 4C).
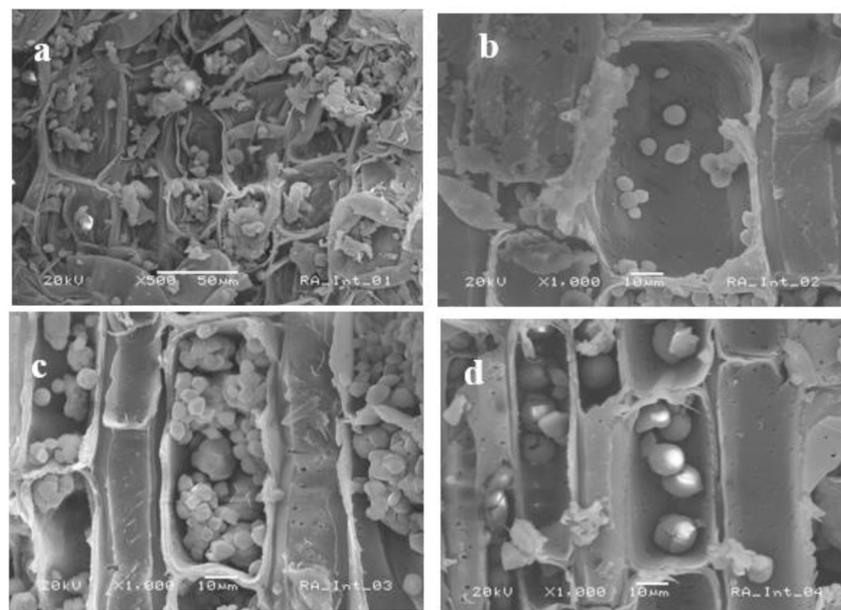
FIGURE 4:   SEM Images of starch morphological variation and cell walls across of four sections of *Guadua angustifolia* Kunth *Rayada Amarilla*. (A) Section R1; (B) Section R2; (C) Section R3 and (D) Section R4.

FIGURA 4:   Imagens de MEV da variação morfológica de amido e paredes celulares através das quatro seções de colmo de *Guadua angustifolia* Kunth *Rayada Amarilla* (A) Seção R1, (B) Seção R2, (C) Seção R3 e (D) seção R4.

Figures 5A and 5B show the starch granules into the parenchyma cells for *Guadua angustifolia Macana* and Figures 5C and 5D show the starch granules into the parenchyma cells for *Guadua angustifolia Rayada Amarilla*. A noticeable point is that in the case of *Macana* variety the starch granules are spherical with dimension between 3 and 10μm located in separated way while in the case the starch granules present in the *Rayada Amarilla* variety those are in agglomerates formed by polygonal starch granules with dimension around 5μm.
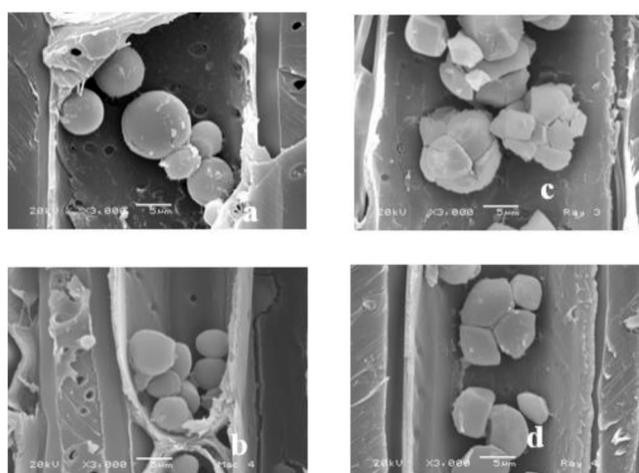

FIGURE 5:   SEM images (3000X) showing starch granules in both *Guadua angustifolia* varieties (A and B) *Macana* (C and D) *Rayada Amarilla*. The granules are identified as starch.

FIGURA 5:   Imagens de MEV (3000X) mostrando grânulos de amido em ambas as variedades de *Guadua angustifolia* (A e B) *Macana* e (C e D) *Rayada Amarilla*.

According to the results from SEM analysis, it is possible identify that *Guadua angustifolia* has the same structure than other Bamboo species studied (LIESE, 1998; WANG et al., 2011). Finally, SEM micrographs show unique microstructures and alignment of bamboo fiber into culm, which accounts for the mechanical behavior of it.

X-ray Diffraction

Bamboo culm has two well-established directions (Z: growth direction and Y: transversal going from internal to external layer), in both directions it is possible to find the same chemicals compounds, however due to the anisotropy of this material; these compounds could be organized differently. X-ray diffraction technique was used to understand how chemical compounds inside bamboo culms are oriented. Due to the crystalline structure of cellulose, the main compound of insoluble fiber, it is possible identify it using X-ray diffraction technique; however, other important compounds like lignin and hemicelluloses are amorphous and it is not possible to establish a crystalline direction (GHAVAMI; MARINHO, 2005). Andress (1928) classified native cellulose as monoclinic lattice. He also published the International Diffraction Data, classified it with the pattern number 00-003-0289. On the other hand, Imberty et al. (1988) published the possible crystalline structure of α-amylose classified with the patter number 00-043-1858 by International Diffraction Data; this pattern is widely use to have an identification of starch. To determine crystalline compounds inside Bamboo culms, those works were referenced. Figure 6 shows the X-Ray Diffraction patterns of *Guadua angustifolia* (A) *Rayada Amarilla* and (B) *Macana* for z and y directions mentioned in Figure 1A. Continuous lines in this figure represent the identification of different crystalline orientations for native cellulose and amylose. According to these results, it is clear that *Guadua angustifolia Rayada Amarilla* and *Macana* have the same components in both directions but these components have different preferential crystalline orientation.
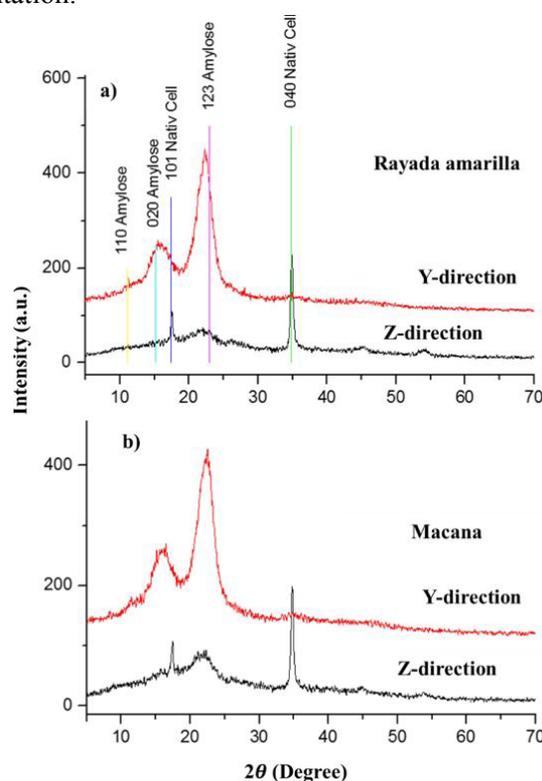

FIGURE 6:   X- Ray diffraction patterns in two directions for both *Guadua angustifolia* Kunth varieties: (A) *Rayada Amarilla* and (B) *Macana*. Z-direction represents plant growth direction while Y-direction goes from the internal wall to external wall.

FIGURA 6:   Padrões de difração de raios X em duas direções para as duas variedades do *Guadua angustifolia* (A) *Rayada Amarilla* e (B) *Macana*. A direção Z representa a direção do crescimento da planta, enquanto a direção Y indica a espessura do colmo, que vai desde a parede interna até a externa.

These results demonstrate that the main crystalline contribution is coming from cellulose, one of the components of insoluble fiber. It is possible to see how the cellulose shows a defined orientation in Z-direction, which is the growth Bamboo direction. This fact could explain the better mechanical performance in this direction than transverse direction. In Figure 6, it is also possible to see an amylose contribution; this compound is one of the compounds of starch, which was observed in SEM images taken from Y-direction (Figure 4). Starches are formed by amylose and amylopectin; despite amylopectin has not been identified by International Diffraction Data, Rojas-Molina et al. (2007) proposed that amylopectin could be identified with the detection of the following peaks: 15.214º and 21.154º. The inspection of the X- ray patterns shown in Figure 6 let establish us that in both studied samples amylose is main compound of starch granules even though its morphology is different as was shown in Figure 5.

Figure 7 shows X-ray diffraction patterns on Y-direction of *Guadua angustifolia* (A) *Rayada Amarilla* and (B) *Macana* for four sections identified before as R1, R2, R3, and R4 (Figure 1B). In Figure 7, it is possible to see that chemicals compounds: Cellulose (C) and Amylose (A) do not have the same distribution and preferential orientation throughout culm. Another important point in this figure is the pattern of internal layers R1 in both *Guadua angustifolia* varieties; these layers have the most amorphous behavior, which agrees with the SEM images showing in the Figure 4A, where R1 images showed not organized structures. Figure 7 also shows that sections R3 and R2 have the most intense peaks in both varieties, according to Figure 4, those sections have considerable quantity of starch, and this could influence the intensity of the X-ray pattern.
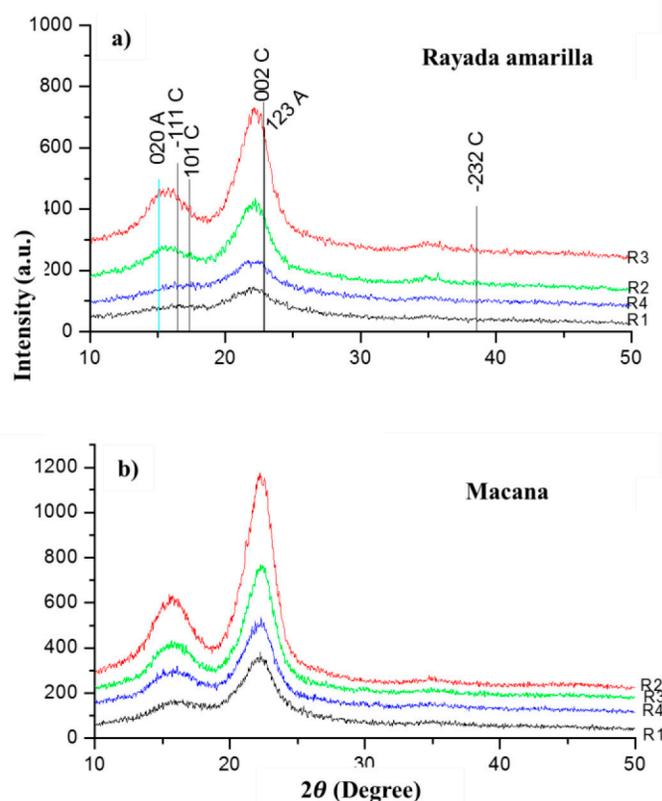

FIGURE 7:   X- Ray diffraction patterns on four sections of *Guadua angustifolia* Kunth through the culm from internal layer R1 to external layer R4 (A) *Rayada Amarilla* and (B) *Macana*. The patterns were taken in Y-direction, which goes from the internal wall to the external one.

FIGURA 7:   Padrões de difração de raios X nas quatro seções do *Guadua angustifolia* Kunth através do colmo, desde a parede interna R1 até a parede externa R4. (A) *Rayada Amarilla* (B) *Macana*.

The most important point illustrated in Figure 6 and Figure 7 is that cellulose has preferential growth direction but not uniform distribution throughout the culm. Variation of the main compounds of Bamboo throughout the culm turns on that the degree of lignification also varies, and it is an important feature for pulp production.

CONCLUSIONS

The soluble and insoluble fiber content was quantified for the macro whole specimens of *Guadua angustifolia* Kunth and in microscale along his radius. In both scales, insoluble fiber content is greater than soluble fiber.

The soluble and insoluble fiber content across the culm in both varieties is not uniform. Insoluble fiber content increases while soluble fiber content decreases from internal to external wall.

Guadua angustifolia variety *Rayada Amarilla* has more insoluble-fiber content than *Guadua angustifolia* variety *Macana* in the whole culm; however, across the culm making a comparison in each section, *Macana* variety has higher insoluble fiber content than Rayada Amarilla.

According to X-ray patterns, the composition of starches in both varieties of *Guadua angustifolia* is similar even though their morphology is different as was determined in the Scanning Electronic Microscopy images.

The cellulose within *Guadua angustifolia* culms has a preferential orientation in the crystalline directions 040 and 101. This preferential orientation is related with the *Guadua angustifolia* growth direction as was shown in X-Ray patterns

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