AN ANATOMICAL COMPARISON BETWEEN BUNCH AND FRUIT OF OIL PALM WITH PINEAPPLE LEAF AND THREE WOODS FROM PLANTATIONS IN COSTA RICA

RÓGER MOYA*; FREDDY MUÑOZ*; JULIO MATA, S** and ROY SOTO, F‡

INTRODUCTION

Costa Rica is a small country in Central America. Its enviable tropical climates make it possible to grow a great variety of crops there. There are 47 000 ha of oil palm, 40 000 ha of pineapple in the country and 40 000 ha of plantations for timber, among other crops. Agricultural crops in the country, however, suffer various problems. Three of the most important are:

- the crops in general belong to many producers and are distributed throughout the country (Bertsch, 2006). For this reason, the harvesting process or material transportation (products or residues) to the collection centre is complex, which combined with the poor road infrastructure increases harvest costs (GFA Consulting Group, 2010);
- post-harvest residues, with the exception of sugar-cane, are not being used currently, thus their disposal becomes a problem (Acuña, 2009); and

ABSTRACT

Elaeis guineensis, Ananas comusus and forest plantations are planted widely in Costa Rica; however their residues (8000 t) are not utilised. The main objective of this work was to undertake an anatomical description of the structure of the oil palm’s empty fruit and the leaves from the crown and plant of the pineapple. Fibre dimensions of these agricultural crops were also compared with three main timber species planted. The anatomy of the fruit and the bunch of the oil palm are different from wood anatomy or pineapple leaves. The fibre dimensions and the anatomical distribution of the vascular strands are different for the pineapple leaf, the oil palm fruit and bunch. Large quantities of vascular strands are found in the oil palm bunch, and their frequency is similar in the oil palm fruit. A large proportion of parenchyma is observed in the pineapple leaf compared with the oil palm bunch and fruit. Oil palm fibre is different from the fibre dimension of the pineapple leaves and of wood. Wood and oil palm fruit and bunch present similar fibre dimensions. The pineapple leaf fibres are shorter fibres with wider diameter and thicker wall than oil palm and wood species.

Keywords: fibre dimensions, tropical species, biofibre, cell structure.

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some crops have been blamed for environmental problems. In the case of pineapple, it is a crop that leaves behind great ecological damage mainly during the plant’s growth stage and the post-harvest period (Kissinger and Rees, 2010).

The solution to the three problems described, given the characteristics of the country, should be oriented to joining the residues resulting from the harvesting processes (pineapple plant) and the processes at the collection centre (oil palm), in one type of industry or one attractive product (Acuña, 2009). Although these crops are lignocellulose materials, their anatomic and chemical structure are different and thus reduces compatibility. Wood is over 90% fibre (Bowyer et al., 2007), oil palm (its trunk and fruit) is composed of a great amount of vascular axes (Weiner and Liese, 1990) and the pineapple plant consists of bundles immersed in parenchymal tissue (Bismarck et al., 2005).

On the other hand, particleboards were traditionally produced from wood residues or a variety of raw lignocellulose materials (James, 2010), as agricultural residues or the combination of wood residues (Hashim et al., 2010; Onuorah, 2005).

The anatomy of wood is one of the most studied among lignocellulose materials with production purposes (Bowyer et al., 2007). Oil palm has been studied in general (Juliano, 1926; Roberson, 1977) but most research has focused on the anatomy of the trunk, leaving aside the anatomy of the fruit and the stalk. The material coming from the fruit has the advantage of being centralised in a collection centre where it is easier to use it. Although the anatomy of the pineapple plant is well-known (Py et al., 1987), the anatomical description focuses on the development of the tissue during the growth stage, without aiming at industrial exploitation, as is the case of wood.

The objective of the present study is to undertake the anatomical description of the bunch and fruit of *Elaeis guineensis* and of the leaves and the crown of *Ananas*. Also, to make a comparison between the parts studied and then a comparison of the length of the fibres of these materials with the wood of the three main timber species used for commercial reforestation in Costa Rica (*Gmelina arborea*, *Tectona grandis* and *Cupressus lusitanica*). The understanding of the anatomy of the palm and its relation to the anatomy of the pineapple leaves and wood will allow establishing best combinations of these wastes in a commercial product and increase the potential of these crops wastes. So it will be given a viable environmental solution to waste of these crops.

**MATERIAL AND METHODS**

**Materials**

Oil palm fruits were collected from two different palms in two different production sites in Costa Rica: one plantation in the South Pacific coast and the other in the Central Pacific of Costa Rica. Pineapple plants without fruit were obtained from two pineapple plantations in two different production sites in Costa Rica: one plantation was located in a tropical humid region of the Caribbean coast of Costa Rica; the second plantation was located in a tropical dry zone in the Central Pacific coast of Costa Rica. It was selected two different palm and two pineapple plants for to have variation. Wood samples of *Gmelina arborea*, *Tectona grandis* and *Cupressus lusitanica* were collected from mature plantations in Costa Rica for characterisation of wood coming from a plantation (Moya and Muñoz, 2010).

**Samples**

Two different oil palm fruits from two palms were sampled and were separated in samples from the bunch and the fruit bunch set (Figure 1a), then four samples were prepared. Then two 12-15 µm thick cross-sections were cut from these parts. Smaller pieces 10 cm long were extracted also from each position of the oil palm fruit and fruit bunch. The two pieces 10 cm long were extracted too from basal and top parts. These small pieces were macerated materials for fibre dimensions measurement. The two pineapple plants were divided in two parts: leaves from the plant and leaves from the crown, two leaves were sampled in each part (Figure 1b), then four samples were prepared and observed. Again four samples were selected for to have variation. Cross-sections 12-15 µm thick were obtained from the basal and the top parts of each leaf (two samples from each part). Finally, small samples from average breast height diameter were extracted from trees of three plantations of different species for wood maceration (Moya and Muñoz, 2010).

**Permanent Slides and Macerated Material Preparation**

Customary methods of microtomy were followed. Using Franklin’s method, macerations were prepared using glacial acetic acid and hydrogen peroxide (1:1 volume/volume) for determining the fibre dimension (Ruzin, 1999). Permanent slides of bunch and fruit of oil palm were...
prepared following the methodology proposed by Johansen and Sass. Cross-sections were stained with safranin and glued using Canadian-balsam gelatin (Ruzin, 1999). Pineapple leaf cross-sections were prepared following the methodology proposed by Jerson. To summarise: leaf washing in a FAA solution (formaldehyde, acetic acid, alcohol and water), sequential dehydration with alcohol (30, stain with safranin, 50%, 70%, 90% and 100%) and afterwards immersion in paraffin as follows: ethanol: xilol for 12 hr, 2 part xilol: 1 part of paraffin for 12 hr, 1 part xilol: 1 part of paraffin for 12 hr and 0 part xilol: 1 part of paraffin for 24 hr.

Fibre Dimension Measurement

Five samples were macerated and stained with safranin to measure fibre dimensions. A digital camera on an optical microscope was used to photograph anatomical features. Fifty fibres (10 fibres per replica) were measured for each anatomical feature: length, fibre diameter, lumen diameter and cell wall thickness. A 250X magnification for length and 1000X for fibre diameter, lumen diameter and cell wall thickness were used.

Statistical Analysis

The normality and presence of extreme values or outliers were verified for each fibre dimension. A general statistical description (average and variation coefficient) was performed for the different variables. An analysis of variance (ANOVA) was used to test differences among fibre dimensions of lignocelluloses types. Mean differences were evaluated using Tukey test (P < 0.01). The statistical SAS program was used to evaluate the meaning of the ANOVA model.

RESULTS

Anatomical Description for Oil Palm Fruit

*Oil palm bunch*. The anatomical description was carried out in the pericarp zone. The seed was not analysed because it was difficult to obtain cross-sections. The pericarp is divided in two parts: the outer part of fruit called the epicarp or hypocarp and the mesocarp zone. The pericarp is the largest part, with more parenchymal tissue and it is the most commercial (Figure 2a). The epicarp has two parts: (1) the outer part, called external hypodermis, which is formed by one to four papillate cells; the cells present rectangular and square shapes and thin cell wall (Figure 2a) and tanniferous canals were observed in some parts of the fruit (Figure 2b); (2) the inner part, called subjacent hypodermis, which is well defined and formed by one to five papillate cells, rectangular and square shaped and oriented in a tangential pattern throughout the mesocarp. The cell wall is lightly defined and filled with tanniferous substances (Figure 2a).

The mesocarp zone is composed by the following anatomical elements: (1) parenchymal tissue, which is a major proportion of the transversal sections, its diameter is irregular and its wall is thin (Figure 2b); (2) bund fibres, (Figure 2c) which are rounded and endorsed with poorly lignified cell wall. They are distributed in the inner mesocarp and
alternating with fibrovascular strands with raphide sacs; (3) fibrovascular strands, (Figure 2c) which are rounded and endorsed in transversal sections and are formed by vascular tissue (conduction function) associated with a stegmatiferous massive sheath of lignified fibres, with poorly lignified cell wall and concentric distribution. The fibrovascular sheath in the outer mesocarp is tangentially extended and marginally fused to form a sclerotic cylinder. In addition to the inner mesocarp, the fibrovascular bundles are also seen distributed along with fibre and concentric vascular bundles and raphide sacs in the massive outer mesocarp; (4) raphide sacs (Figure 2d) are thin-walled and rarely distributed along with bundle fibres and fibrovascular strands. They were observed in the outer mesocarp, but they were not observed in the inner mesocarp. They are large, numerous and form conspicuous features of the mesocarp; and Tanniferous cells (Figure 2b) are distributed in the outer mesocarp and they are abundant next to the epidermis. They are round and of variable diameter and they can be confused with bundle fibres.

**Palm empty fruit.** The anatomical structure of the central part of the oil palm empty fruit, as expected, is typical of monocotyledon tissue. The epidermis is formed by one to two layers of rectangular and square cells. The cortex zone is located in the inner part of the epidermis and is formed by parenchymal cells and fibre bundles with less diameter, extended and aligned tangentially, the diameter increasing inwards (Figure 3a). Following, diffuse vascular strands can be seen inside the
cortex, containing poorly-defined protoxylem and protophloem (Figure 3b). The vascular strands increase their abundance inside the core, with one to two layers of medullar parenchyma present between two vascular strands (Figure 3d). They are round shaped when the frequency is low, but an increment of the abundance produces strands with oval shapes (Figure 3c). A vascular strand is formed by metaxylem, metaphloem and a round fibre sheath (Figure 3d). Diffuse sieve tubes are located in the metaphloem tissue and two vessels are located in the metaxylem. However, one or three vessels can be observed in some vascular strands.

**Pineapple Plant**

*Crown leaf*. There is variation in the wood anatomy along the leaf. The basal zone of the leaf is formed by epidermis zone covered by one to two layers of cells with poorly-defined and poorly lignified wall at the top of the leaf, while three to five layers of cells were observed in the bottom part (Figure 4a). At the bottom of the leaf, the upper epidermis is formed by two to three layers of thin-walled cells, with elliptical or polygonal shape (Figure 4b). On the contrary, the cells of the bottom epidermis are elongated columnar- or tapered radially oriented. The cells of the epidermis are covered with a thick cuticle, which contains silica bodies (Figure 5c). Chemical analysis showed that they were aluminium oxalate. Ground parenchyma cells with thin walls and round shape were observed within the upper epidermis (Figure 4b). Palisade parenchyma with small diameter was observed inside bottom epidermis, as well as mesophyll spongy lightly circular, slightly thick-walled cells, round with irregular diameter (Figure 4b). Small aerating canals were observed in the mesophyll in both parts. Vascular strands and fibre bundles were found in the pineapple leaf (Figures 4c and 4d, respectively). Fibre bundles present varied diameter, with smaller diameter in the upper part and an increasing diameter when approaching the bottom part of leaf. Vascular strands are scarce, rounded and well-defined in transversal sections. They are formed by vascular tissue (conductive function) covered with a massive sheath of lignified fibres, with poorly lignified cell wall and concentric

Figure 3. (a) Cross-section of empty fruit of *Elaeis guineensis* in the inner of epidermis, (b) fibre bundles and fibrovascular strands in the epidermis zone, (c) cross-section of fibrovascular strands and (d) anatomical element of fibrovascular strands.
distribution. The vascular tissue contains poorly-defined protoxylem and protophloem (Figure 4c). In the tip area of the leaf, the anatomical structure is slightly similar to the basal part. Two to three layers of thin-walled cells with elliptical or polygonal shape are present in the upper part, but unlike the basal part of the leaf, the lower epidermis is as thick as the upper epidermis. The lower epidermis of the basal area is thicker than in the tip area. Fibre bundles are more frequent in the basal area, and the diameter of fibre bundles in the basal area is greater than in the tip part (Figure 4). Fibre bundles are concentrated in the central part (Figure 4a). Vascular strands contain poorly-defined protoxylem and protophloem.

**Plant leaf.** There are no big differences along the leaf. Both upper and lower epidermis is formed by thin-walled cells with elliptical or polygonal shape, although there are differences in the quantity of layers. A two to three cell layers are observed in the lower epidermis, while only one cell layer was observed in the upper epidermis. There are irregularities on the surface of the epidermis, produced by fibre bundles (Figure 5a). Within the epidermal layer there is a palisade parenchyma layer formed by two to three layers of cells, which can be considered the beginning of cortex. They are poorly lignified, thin-walled and rectangular and round shaped, with a small diameter (Figure 5b). The middle part of the leaf is formed by fundamental parenchyma, vascular strands and aerating canals and scarce fibre bundles. Fundamental parenchyma presents thin-walled cells, large diameter and round shape. Fibre bundles have a small diameter, they are scarce, rounded in transversal sections and they are located next to the epidermis. The spongy mesophyll is formed by round cells, irregular diameter and poorly lignified wall. Aerating canals were observed (Figure 5a). Vascular strands contain defined protoxylem and protophloem covered with a massive sheath of lignified fibres, and poorly lignified cell wall. They have a concentric distribution (Figure 5a). The cells of the epidermis contain silica bodies (Figure 5c), which are aluminium oxalate (Figure 5c).
TABLE 1. FIBRE DIMENSION OF PINEAPPLE LEAF AND OIL PALM FRUIT AND ITS RELATION WITH OTHER WOODY FIBRES

<table>
<thead>
<tr>
<th>Fibre parameters</th>
<th>Oil palm</th>
<th>Pineapple</th>
<th>Cupressus lusitanica*</th>
<th>Gmelina arborea*</th>
<th>Tectona grandis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fruit</td>
<td>Bunch</td>
<td>Crown</td>
<td>plant</td>
<td>Fruit</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>0.86a</td>
<td>0.62bc</td>
<td>3.99c</td>
<td>4.68d</td>
<td>2.41e</td>
</tr>
<tr>
<td></td>
<td>(0.21)</td>
<td>(0.20)</td>
<td>(0.65)</td>
<td>(0.82)</td>
<td>(0.64)</td>
</tr>
<tr>
<td>Diameter (µm)</td>
<td>23.88a</td>
<td>16.91b</td>
<td>5.97c</td>
<td>6.31d</td>
<td>29.50f</td>
</tr>
<tr>
<td></td>
<td>(5.22)</td>
<td>(3.04)</td>
<td>(1.59)</td>
<td>(1.71)</td>
<td>(9.81)</td>
</tr>
<tr>
<td>Lumen (µm)</td>
<td>16.11a</td>
<td>9.83b</td>
<td>2.41c</td>
<td>2.00d</td>
<td>21.20e</td>
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<tr>
<td></td>
<td>(4.94)</td>
<td>(2.37)</td>
<td>(1.00)</td>
<td>(1.05)</td>
<td>(7.47)</td>
</tr>
<tr>
<td>Cell wall (µm)</td>
<td>3.89a</td>
<td>3.54bc</td>
<td>1.88bc</td>
<td>2.15d</td>
<td>4.15e</td>
</tr>
<tr>
<td></td>
<td>(0.66)</td>
<td>(0.71)</td>
<td>(0.42)</td>
<td>(0.47)</td>
<td>(1.51)</td>
</tr>
</tbody>
</table>

Note: *Data from Moya et al. (2010). The lower case letters next to this value indicates that the values are statistically different at a confidence level of 95%.

Figure 5. (a) Cross-section of the leaf of a pineapple plant, (b) epidermis zone showing cells with elliptical or polygonal shape and thin-walled cells, (c) cells of epidermis covered with a thick cuticle and (d) presence of silica bodies and spectrum of composition of silica bodies showing high content of oxygen and aluminium elements.
Fibre Dimension Comparison of Pineapple Leaf and Oil Palm Fruit with Woody Fibres

Fibre dimension of different agriculture or forestry residues are detailed in Table 1. The fibre length varied from 0.67 to 4.68 mm. Oil palm fibres, from fruit or bunch, are significantly shorter than the fibres of pineapple or of the three plantation species used in Costa Rica (Table 1). Oil plant fibre bunches are the shortest and pineapple plant fibres are the longest. Fibres of hardwood species, such as *Gmelina arborea* or *Tectona grandis*, are typically shorter than fibres of softwood, like *Cupressus lusitanica* (Moya et al., 2010). The fibre diameter ranged from 5.97 to 29.50 µm and lumen diameter varied from 2.00 to 21.70 µm. Pineapple leaf fibres, both from the crown and plant, are the smallest in diameter and the largest are woody fibres, except for lumen diameter of *Tectona grandis*, which presents lumen diameter smaller than *Gmelina arborea* and *Cupressus lusitanica*. While the fibre and lumen diameter are significantly different between bunch and fruit part of the oil palm, the fruit part presents greater diameter than the bunch part. Both parts are statistically different for pineapple and wood fibres (Table 1). Finally, the variation of the cell wall was from 1.88 to 4.88 µm, where pineapple presented thin-walled fibres and *Tectona grandis* presented thick walled fibres (Table 1). There are no differences between cell walls of the different parts of oil palm and the different parts of pineapple leaf. Thick cell walls of fruit from oil palm are different to those of woody species. However, thick cell walls from bunch of oil palm fruit are similar to *Gmelina arborea*, but they are statistically different from *Cupressus lusitanica* and *Tectona grandis* (Table 1).

**DISCUSSION**

**Oil Palm Fruit Anatomy**

The anatomy description of oil palm bunch agreed with Reddy’s and Kulkarni’s (1987) description and with other anatomy descriptions of palm bunch set (Juliano, 1926; Roberson, 1977). The bulky and fundamental tissue of the pericarp zone represents the major volume of the oil palm bunch set. The mesocarp is the most commercially important part. It contains storing cells, mechanical, conducting and protective tissue (Reddy and Kulkarni, 1987). The fibrovascular strands (Figure 2c) serve as conductive and mechanical tissue and they are typical tissue of the mesocarp. Raphide sacs are considered a protective tissue and fibre bundles are developed in the mesocarp for support function. The most abundant tissue is made up of parenchyma cells for oil storing (Juliano, 1926; Roberson, 1977; Reddy and Kulkarni, 1985; Khalil et al., 2006). Tanniferous cells have certainly a mixed function, involving a defence mechanism against plant enemies and the delay of decomposition when the plant tissue becomes litter (Zucker, 1983).

On the other hand, the central part of the oil palm empty fruit corresponds to the general structure of the monocotyledons, represented by collateral vascular bundles embedded in ground parenchyma (Weiner and Liese, 1990). The stem is covered by epidermis cells composed of one to two cell layers (Bhat et al., 1993). The cortex is located in the core direction, and it is formed by parenchyma cells, fibres bundles and incomplete fibrovascular strands (Weiner and Liese, 1990). The central part of oil palm empty fruit contains cells for storing (fundamental parenchyma), for mechanical support (fibres bundles), for conducting (vessels in metaxylem and sieve tubes in metaphloem), and protective tissue (epidermis). Although both the bunch and the central part of the oil palm empty fruit contain storing, mechanical, conductive and protective tissue, there are large differences in distribution and frequency of each tissue: (1) parenchyma cells are abundant in the fruit, but scarce in the bunch (Figure 2b); (2) fibrovascular strands (vascular tissue associated with a steigmatiferous massive sheath of lignified fibres) are present in fruits, but vascular strands in the bunch are formed by metaxylem, metaphloem and fibres sheath; (3) tanniferous cells are not present in the bunch.

**Pineapple Leaf Anatomy**

The anatomical description of the pineapple leaf agreed with the descriptions of Py et al. (1987) and Khalil et al. (2006). However, there are some differences. Py et al. (1987) indicated large proportions of water storage tissue and we did not find this characteristic. Khalil et al. (2006) made no difference between fibre bundles and vascular strands, nor did they mention the spongy mesophyll tissue. They observed vascular strands instead of fibre bundles. The anatomical structure found in the pineapple leaf is the typical anatomy of the Bromeliaceae species, with vascular strands, spongy mesophyll, epidermis tissue and parenchyma cells (Martin, 1994; Proença and Sajo, 2007). The leaf is the primary photosynthetic organ for the pineapple plant and its anatomy reflects the different functions developed during growth (Mauzeth, 2009). The cross-sections showed several anatomy features: storage cells, mechanical, conductive, interchange and protective tissue. The fibrovascular strands (Figure 2c) serve as conductive and mechanical tissue while fibre strands have a mechanical support function. These structures are adaptations developed by...
the plant, to provide strength and rigidity to the leaf (Mauseth, 2009). Parenchyma cells form the most abundant tissue and its function is storing, spongy mesophyll allows for gaseous interchange, palisade cells are responsible for photosynthesis because they contain chloroplasts. The epidermis is a protective (Krauss, 1949; Bartholomew et al., 2003) and waterproof tissue (Py et al., 1987). The cuticle protects not only the outer walls, but also the lateral and interior walls. The aerating canals located into the mesophyll can play a role in gaseous interchange (Krauss, 1949).

There are some anatomical differences between the leaves from the crown and from the plant. For example, the vascular strands are poorly-defined in the crown leaf (Figure 4a), but they are better defined in the plant leaf (Figure 5a). The palisade parenchyma in the plant leaf is thicker than in the crown leaf. The frequency of fibre bundles is higher in the plant leaf and the area of the spongy mesophyll tissue is larger for the plant leaf than for the crown leaf. However, an important feature for industrial utilisation of pineapple leaf, or other natural fibres, is the dimension of the fibres (Bismarck et al., 2005). For example, a longer fibre has greater strength (Reddy and Yang, 2005). Lumen, fibre diameter and cell wall are similar in the plant leaf and the crown leaf, but leaf fibre length is statistically different between the crown and the plant (Table 1).

The leaves are the largest proportion of the pineapple plant and they are slightly different from the crown leaves, mainly regarding the length of fibres. The leaf from the plant has higher proportions of fibres than the crown, so we can produce higher quantity of raw material from the plant than from the crown. Due to the availability of raw material, products manufactured with plant leaves can be more easily produced with plant leaves than with crown leaves. Nevertheless, in view of the similarity between fibre dimension regarding physical and mechanical properties, both materials can be mixed together.

An inconvenience of the epidermis tissue in the pineapple leaf is that this is an impermeable tissue (Py et al., 1987) and as such is not easy to dry. Water is not removed easily from the leaf because the epidermis cells do not allow water flowing through the cuticle (Py et al., 1987). Thus, to remove water from the leaf, it is necessary firstly to remove or tear the epidermis (Figure 4d), to allow water flowing through it.

Potential Mixing Wastes

A large proportion of parenchyma is found in the pineapple leaf in relation to the bunch of fruit of oil palm and wood. Agriculture residues with higher parenchyma content produce higher swelling ratio because they attract more water due to attractive OH groups (Akgül et al., 2010), thus we can expect the same result in fibreboard manufactured with pineapple leaf residues.

Physical and mechanical properties of the fibreboards are affected by the anatomical characteristics of the raw material they are made of (Lee et al., 2006). Consequently, fibreboards manufactured with pineapple leaf can be different from fibreboards made of oil palm fruit. On the other hand, woody fibres present similar fibre dimensions to fruit and bunch of oil palm fruit. The similarity between fibre dimensions of these agricultural materials could permit the mixing of wood and oil palm fruit, however, although there is anatomical compatibility between these materials, further studies on chemical compatibility are necessary.

CONCLUSION

The anatomy of the pineapple leaf is different from the anatomy of the fruit and the bunch of the oil palm fruit. Both of them are different from wood anatomy. The main differences are the fibre bundles and the vascular strands. The fibre dimensions and the anatomical distribution of the vascular strands are different between the pineapple and the oil plant. Large quantities of vascular strands are found in the oil plant bunch, but the frequency of vascular strands is similar in the oil palm fruit. Other differences are the parenchyma abundance and silica abundance.

The fibre dimensions of the pineapple leaf are very different from oil palm (fruit and bunch) and wood fibres. The oil palm and the wood present shorter fibre, wider diameter and thicker wall than the pineapple leaf.

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