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Caracterización química y morfología de la madera de *Moringa oleifera* como materia prima potencial para biorrefinerías

Chemical characterization and morphology of *moringa oleifera*'s wood as potential raw material for biorefineries

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Resumen

La creciente demanda de biomasa forestal ha motivado el cultivo de plantaciones forestales de corta rotación en países desarrollados y en desarrollo. La *Moringa oleifera* es una especie de rápido crecimiento que se adapta a un amplio rango de suelos. La incorporación de esta especie como cultivo forestal en los sistemas silvo-pastoriles permitiría el uso de las semillas y hojas como forraje y productos medicinales/alimentos de bajo costo, obteniendo una mejor rentabilidad de los productores y mayor sustentabilidad de la actividad. El objetivo de este trabajo es conocer la composición química y la estructura morfológica de la madera de *Moringa oleifera* de dos edades diferentes (3 años y 8 años) para analizar su potencial uso como materia prima fibrosa de biorrefinerías.

Palabras clave: Composición química; Análisis microscópico; Relaciones biométricas; Biorrefinería.

Abstract

The growing demand for forest biomass has motivated the culture of forest plantations of short rotation in developed and in developing countries. *Moringa oleifera* is a fast-growing species that is to a wide range of soils. The incorporation of this species as forest cultures in silvopastoral systems would allow the use of seeds and leaves as forage and as low-cost medicinal products and foods, improving the profitability of producers and the greater sustainability of the activity. The aim of this study is to know the chemical composition and morphological structure of the wood of *Moringa oleifera* of two different ages (3 years-old and 8 years-old) to analyze its potential as raw material for biorefineries.

Keywords: Chemical composition; Microscopic analysis; Biometric Relationships; Biorefineries..

Introduction

The growing demand of forest biomass for conventional (forest industry and as energy resource) and non-conventional uses (new materials and foods), has motivated the cultivation of short-rotation forest plantations in developed and developing countries (1-3). The application of sustainable forestry practices of fast-growing plantations for the production of woody biomass in agricultural or forest lands, fertile but degraded, enables the environmental and economic use of natural resources.

Moringa oleifera is native from the southern Himalayas, northeast India, Pakistan, Bangladesh, and Afghanistan (4), and currently it is distributed in South America (Peru, Paraguay and Brazil) as an ornamental tree and as a "green" curtain (5, 6). It is a fast growing species, reaching 10-12 m in height and 20-40 cm in diameter at maturity (7), which adapts to a wide range of soils without requirements of natural fertilizers or agrochemicals. Its

cultivation brings a high amount of nutrients to the soil, in addition to protect it from external factors such as erosion, dehydration and high temperatures (8, 9).

This culture can be used in silvopastoral systems (forestation together with livestock) as an alternative to improve the profitability of the land through the diversification of the production. The incorporation of this species as a forest culture would offer the possibility of using the seeds and leaves as forage and low cost products for human consumption, improving the profitability of the producers and the sustainability of the activity. Additionally, the use of the log would generate a 100% utilization of this forest resource.

There are few precedents of the use of *Moringa oleifera* wood (10, 11). *Moringa oleifera*'s wood could be an alternative lignocellulosic material since it is characterized by its rapid growth and adaptability (10) in South America, where the leaves and seeds are already sold. In recent years, the cultivation of this species has aroused great

interest in Argentina due to its nutritional and medicinal properties. The optimal conditions for its cultivation are average annual temperatures greater than 12°C and average annual rainfalls of 500 mm, which could be found in Misiones, Corrientes, Entre Ríos, Santa Fe, half the surface of the provinces of Formosa and Chaco, and part of Buenos Aires and Salta (11).

The *Moringa oleifera* is cultivated in many developing countries as a low cost food resource to prevent malnutrition and pathologies associated with shortages of essential dietary components (vitamins and minerals) (5). Its leaves and seeds can be used for the production of supplements for human food and animal forage (livestock, poultry, and pigs) due to its high nutritional value (12, 13). Its seed contains 35-40% of oil (70% of oleic acid) (14), which also makes it an important resource for biodiesel production (15, 16). The powder of seeds contains a cationic coagulant protein that can be used to decrease turbidity in water treatment (17). Also, the seed cake is composed of a natural and not toxic polypeptide that can be used to absorb and retain volatile substances, therefore it is a valuable additive in the fragrance industry to stabilize aromas (18), in the cleaning of vegetable oil, and in the sedimentation of fibers in the fruit juice and brewing industries (19). The extraction of natural antioxidant from leaves and stems has also been studied for potential applications in the food and pharmaceutical industries (20). There are few reports about its chemical composition, wood structure, fiber morphology, and aptitude for pulp and paper production (11, 21-24).

Cellulose (40-45%), hemicelluloses (25-40%), lignin (15-30%) and extractives are the main chemical components of wood (25, 26). Cellulose is a linear polymer (homopolysaccharide) composed of D-glucose units linked by β -1,4-glycosidic bonds, with a degree of polymerization (DP) higher than 10,000. Its structure is semi-crystalline because it is formed by amorphous and crystalline regions (25, 26). Hemicelluloses are amorphous polymers and they are composed by low molecular weight branched hetero-polysaccharide polymers (DP 50 - 500) from hexoses (glucose, mannose and galactose) and pentoses (xylose and arabinose), and different types of uronic acids (25, 26). Their proportion and composition depends on the type of species, age, variability of species, and others. Lignin is a complex three-dimensional phenolic and amorphous polymer constituted of phenyl propane units (27). The extractives are composed mainly of fatty acids, alcohols, phenols, terpenes, steroids, resinic acids, among others, and can be removed from wood by extraction with different solvents (26).

The *Moringa oleifera* is a hardwood species. The main morphological characteristic of hardwood is that they present vessels which have the function of conduction. These vessels can be seen in the cross section of the stem as "holes" called porous. Its structure is more complex than

that softwood due to the greater number of types of cellular elements which are highly variable in type, size, shape and arrangement (vessels, tracheids, fibers and parenchyma) (28). Unlike, softwood is formed mainly by longitudinal tracheids (approximately 90%) (29). The morphological characteristics of wood can be studied through the observation of the microscopy structure of the cross, radial and tangential sections in which it is possible to see the size and distribution of porous (vessels), the fibers and parenchymal cells.

The aim of this work was to determine the chemical composition and morphological structure of *Moringa oleifera*'s wood of two different ages (3 and 8 years-old) to assess its potential as a fibrous raw material for biorefineries, including paper production.

Materials and methods

Raw Materials

The three-years-old and eight-years-old stems of *Moringa oleifera* were extracted from experimental regional plantations. The samples without bark were milled in a laboratory knife mill, collecting the sawdust passing through an ASTM sieve N° 20 (0.840mm) and retained on ASTM sieve N° 80 (0.177mm). The fraction of the material retained on ASTM sieve N° 80 was used for the chemical determinations.

Physical and chemical characterization

Bark content was determined as percentage considering the weight of bark of a disk and the weight of the initial disk with bark. The basic density and dry density of wood were gravimetrically determined according to TAPPI T258 om-94.

Laboratory Analytical Procedures of the National Renewable Energy Laboratory (NREL - LAP) used for the chemical characterization were: "Preparation of samples for Compositional Analysis" NREL/TP-510-42620, "Determination of Extractives in Biomass" NREL/TP-510-42619, "Determination of Ash in Biomass" NREL/TP-510-42622, and "Determination of Structural carbohydrates and lignin in Biomass" NREL/TP-51042618. The different chemical components determined are detailed in Figure 1.

The determination of the structural carbohydrates was carried out by liquid chromatography (HPLC) using SHOD-DEX SP810 and AMINEX-HPX87H (BIO-RAD) columns with detectors of refractive index and the diode array.

Starch content was determined by a colorimetric technique (30). The oligomers and inorganic anionic content were quantified by HPLC in the hot water extract using an AMINEX-HPX87H (BIO-RAD) column with a refractive index detector. Soluble inorganic were characterized by HPLC (Hamilton PRP-X-100 column) using a conductivity detector.

Holocellulose was determined by the modified acid chlorite method (acetate buffer) (31) and alpha, beta, and gamma cellulose were quantified by applying the TAPPI T203 cm-99.

Morphological characterization of wood

Histological sections (tangential, radial, and transverse cuts) were observed by an optical microscope (Zeiss) with image analyzer. The samples were disintegrated with chlorine dioxide and sodium carbonate (32) for the study of the morphological parameters of fibers. The average values of length, width, and lumen of about 200 fibers were measured by optical microscopy and the coefficients of variation were calculated. The wall thickness was determined as the difference between the width and the lumen of the fibers.

The aptitude of *Moringa oleifera* for pulp and paper can be predicted from the biometric relationships calculated from the dimensions of the fibers (33). The biometric relationships are listed below.

Flexibility coefficient: Relationship between lumen width and fiber width (Eq. 1).

$$FC = \frac{l}{W} * 100 \text{ (Eq. 1)}$$

Felting index: Relationship between fiber length and fiber width (Eq. 2).

$$Fel = \frac{L}{W} * 100 \text{ (Eq. 2)}$$

Runkel ratio: Relationship between the fiber wall thickness and lumen diameter (Eq. 3):

$$R = \frac{2w}{l} \text{ (Eq. 3)}$$

Where:

W = fiber width

L = fiber length

l = lumen width

w = fiber wall thickness

Results and discussion

Physical and chemical characterization

Physical and chemical characteristics of *Moringa oleifera* samples are shown in Table 1. The percentage of bark was lower in the 8 years-old tree as compared with the youngest one because of its growth in diameter. The average density was lower than other species used for the pulp and paper production, which usually ranges between 0.30 and 0.60 g.cm⁻³ (34). The *Moringa oleifera* density was almost half of eucalyptus one, which is about 0.48 g.cm⁻³ in trees from the Northeast Region of Argentina (35).

The extractives content was variable depending on the age, the sample, and the solvent used. The substances extracted by dichloromethane are usually waxes, fats, resins, phytosterols and non-volatile hydrocarbons, whereas the extractives in hot water are usually phenolic substances and some carbohydrates. The extractives content in

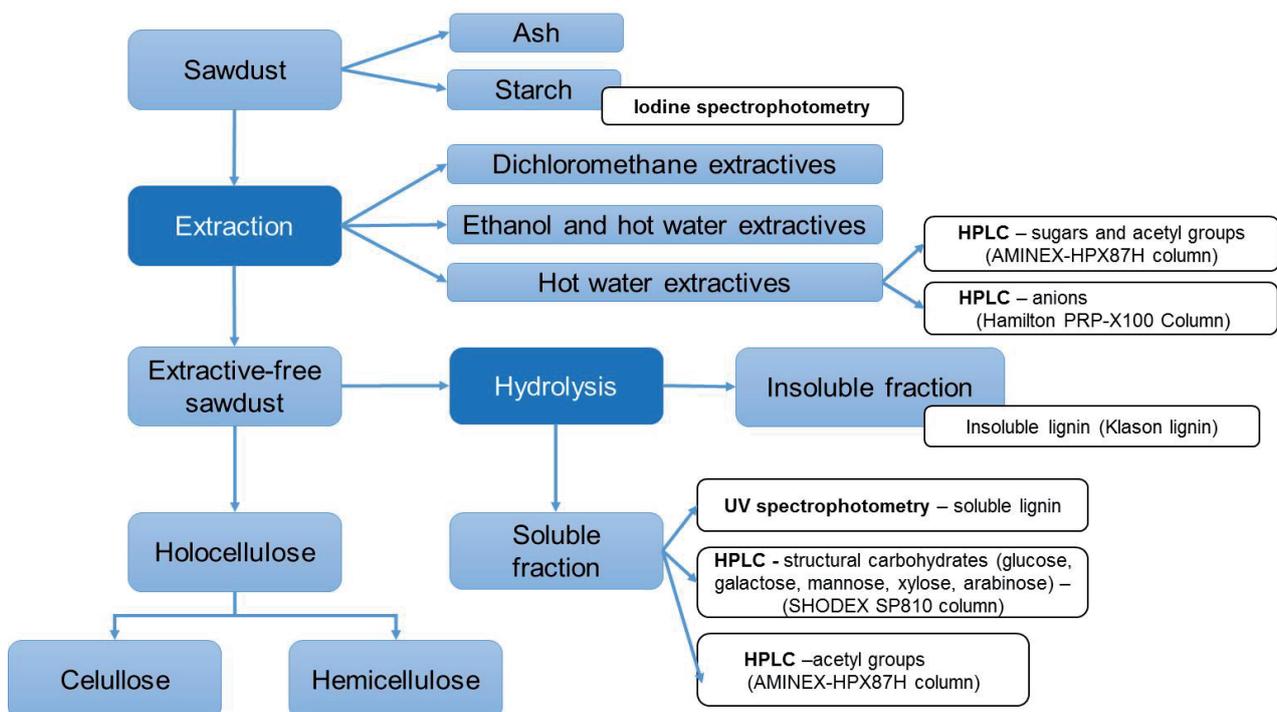


Figure 1: Methodology for the chemical characterization of *Moringa oleifera*.

ethanol was similar for both ages, although the extractive content in dichloromethane and in hot water showed a great difference between them. The sequential extraction (ethanol followed by hot water) in the 8 years-old sample has extracted a similar amount of substances than those extracted in hot water (single extraction) indicating that the components extracted with ethanol are also soluble in hot water (Table 1). The hot water extractives of the 8 years-old wood (23.89%) are almost two-folds of those of the 3 years-old, consisting of 18.86% oligomers (13.07% glucans and 5.79% xylans), and 1.89% of inorganic compounds (0.61% carbonates, 0.32% chloride, 0.75% phosphate, and 0.21% sulfate). Acetyl groups and simple sugars were not detected by HPLC. The total extractives content is very high as compared, for example, with that of eucalyptus (4.80% over dry wood) (36).

Table 1: Physical and chemical characterization of 3 years-old and 8 years-old *Moringa oleifera*'s wood.

	3 years	8 years
Bark (%)	21.68	16.16
Basic density (gcm ⁻³)	0.21 ± 0.01	0.24 ± 0.02
Chemical composition		
Hot water extractives (% otw)*	16.46 ± 1.45	23.89 ± 0.36
Total ethanol and hot water extractives ^(a)	16.90 ± 0.34	23.88 ± 0.48
Ethanol Extractive state 1 (% otw)*	8.50 ± 0.34	8.86 ± 0.32
Hot water Extractive state 2 (% otw)*	8.40 ± 0.00	15.02 ± 0.16
Dichloromethane extractives (% otw)*	0.71 ± 0.00	1.12 ± 0.01
Extractable carbohydrates (Starch) (% otw)*	0.41 ± 0.03	12.44 ± 0.31
Total Lignin	19.60 ± 0.05 (23.6)**	20.37 ± 0.3 (26.8)**
Soluble Lignin (% otw)*	1.30 ± 0.03 (1.56)**	1.23 ± 0.03 (1.62)**
Insoluble Lignin (% otw)*	18.30 ± 0.02 (22.0)**	19.14 ± 0.27 (25.1)**
Total structural carbohydrates	50.00 ± 0.05 (60.2)**	45.17 ± 0.05 (59.3)**
Glucan (% otw)*	38.40 ± 0.08 (46.2)**	34.95 ± 0.08 (45.9)**
Xylans (% otw)*	8.10 ± 0.08 (9.75)**	7.99 ± 0.08 (10.5)**
Arabinans (% otw)*	0.80 ± 0.02 (0.96)**	0.48 ± 0.02 (0.63)**
Galactans (% otw)*	1.70 ± 0.10(2.04)**	1.05 ± 0.11 (1.38)**
Mannan (% otw)*	1.00 ± 0.00 (1.20)**	0.70 ± 0.00 (0.92)**
Acetyl groups (% otw)*	1.70 ± 0.00 (2.05)**	2.17 ± 0.00 (2.85)**
Hemicelluloses (% otw)****	13.30 ± 0.28 (16.0)**	12.39 ± 0.29 (16.3)**
Ash at 525°C (% otw)*	10.40 ± 0.03**	3.92 ± 0.04

(a) Sequential extraction. Percentages are expressed on dry base of: * total wood (% otw), ** extracted wood (% oew). **** Hemicelluloses were calculated based on the sum of xylans, galactans, arabinans, mannans, and acetyl groups.

Significant differences were also found in ashes content, being much higher in the 3 years-old tree as compared to the 8 years-old one. The starch content extracted in hot water shows the opposite behavior.

The contents of alfa, beta, and gamma cellulose (holocellulose) of total and extracted wood are shown in Table 2.

Table 2: Analysis of the holocellulose fraction.

	Holocellulose (% oth*)	Holocellulose (% oew**)
Alpha cellulose	59.93 ± 0.06	43.93
Gamma cellulose	31.29 ± 0.26	22.94
Beta cellulose	8.78 ± 0.32	6.43
Total	100.00	73.31

Percentages are expressed on dry base of: * total holocellulose (% oth), ** extracted wood (% oew).

Holocellulose content was 73.31% oew (± 0.25), higher than that reported by Khider *et al.* (37) for *Moringa oleifera* in Sudan (68.5%). The alpha cellulose amount (43.93% oew) was similar to the glucans quantified by HPLC (45.9% oew), which would indicate that glucans content corresponds mainly to cellulose. This result could be interesting to produce nanofibrilated or microfibrilated cellulose.

Morphological characterization

The images of the histological sections of the samples of 8 years-old obtained by optical microscopy are shown in Figures 2 to 4. Fibers of very thin walls and solitary vessels are observed in the cross sections (Figure 2).

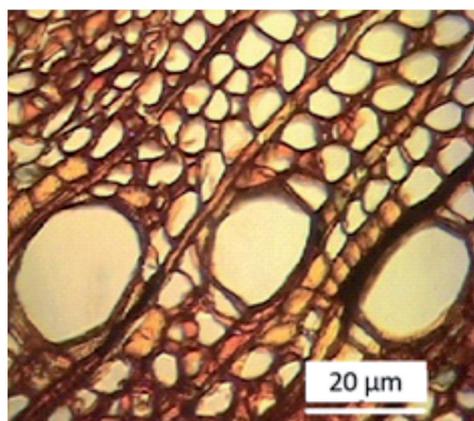
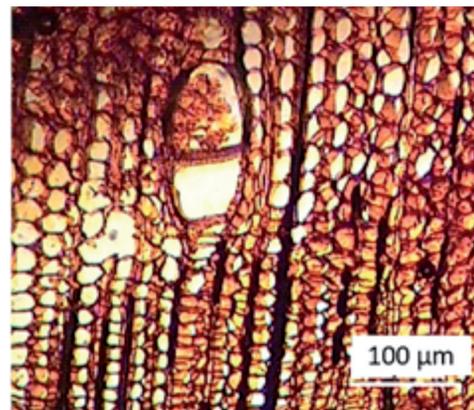


Figure 2: Cross sections of *Moringa oleifera*'s wood (objectives 4X and 10X).

Uniseriate and biseriate woody rays of 1 to 9 cells in height, solitary vessels, and multiples vessel can be observed in the tangential sections (Figure 3).

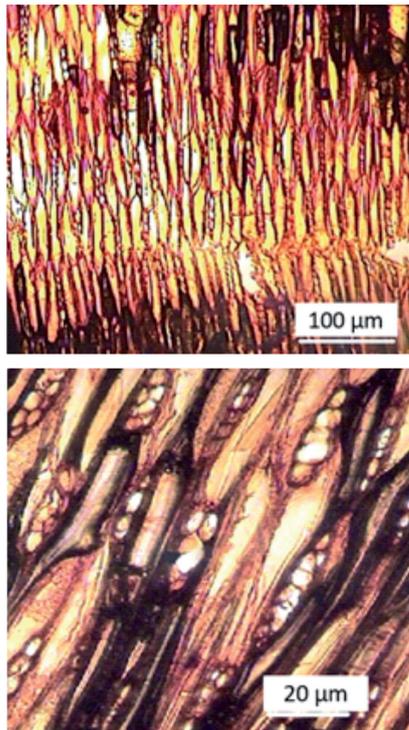


Figure 3: Tangential sections of *Moringa oleifera*'s wood (objectives 4X and 10X).

The radial sections show inhomogeneous woody rays and fibers widened in their central part can be observed in Figure 4. This and other peculiar forms of fibers are shown in Figure 5. A large quantity of parenchymal cells can be observed, which justify the high extractives content of the wood. The fibers are very wide at the center (a and b). Some fibers have bifurcations at the ends (f, h, i, and k). Vessels have also peculiar shapes (c, d, e, g, and j). Fibers are broad and thin walled so they are supposed to easily collapse (28).

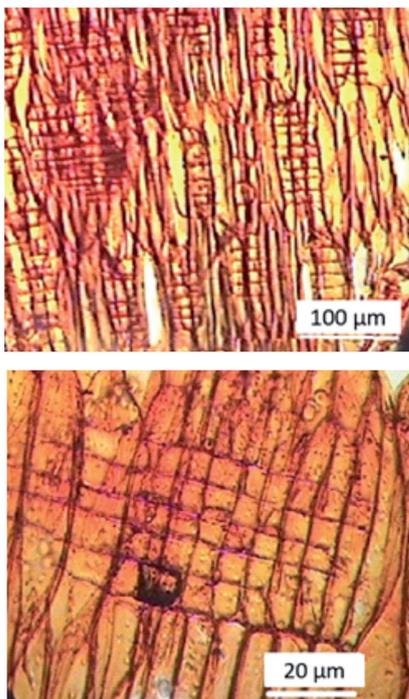


Figure 4: Radial sections of *Moringa oleifera*'s wood (objectives 4X and 10X).

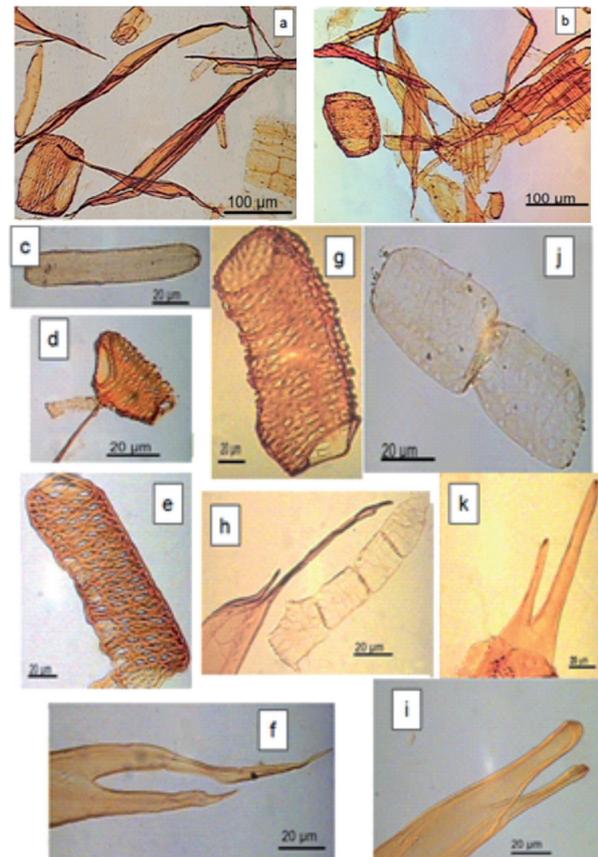


Figure 5: Optical microscopy images of fibers (a, b) (objective 4X). Fiber ends (f, h, i, and k) and vessel elements (c, d, e, g, and j), (objective 20X).

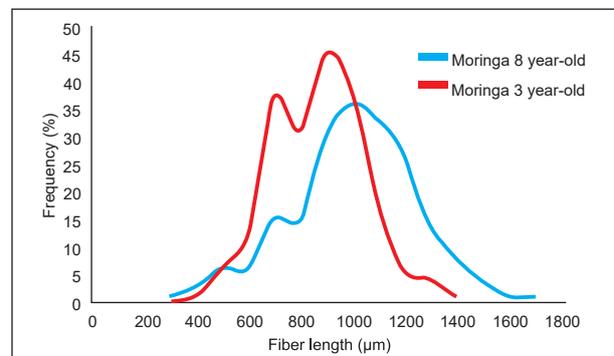


Figure 6: Length distribution of pulp fibers (200 fibers for each type of pulp).

The fiber length distribution for each of the pulps is presented in Figure 6. The MO 3 year-old shows a bimodal fiber length distribution. The range of fiber length the MO 3 year-old comprises 76% of the fibers between 700 - 1100 µm, whereas, in the MO 8 year-old 63% of the measured fibers are longer, between 900 - 1300 µm, which is understandable considering the difference in age of trees.

Fiber dimensions of *Moringa oleifera* samples are detailed in Table 3. Other species are included for comparative purposes. The dimensions of the fibers vary between species and in the same species depend on climate, soil, and position in the stem. Thus, it is very difficult to make comparisons between fibers that, for example, do not come from the same region. However, broadly speaking, it can

be said that *Moringa oleifera* fibers have a typical length of hardwoods, although the fibers appear to be shorter than those of Ekhuemelo and Udo (38) originating from Nigeria. On the contrary, the fibers are much wider than those of other species, according to this work and those of Olson and Carlquist (39) and Cobas and Molina Tirado (21). The values reported by Ekhuemelo and Udo (38) are atypical, considering the shape of the fibers as shown in Figure 5.a and 5.b.

Table 3: Fiber dimensions of the *Moringa oleifera*'s samples and other wood species.

Source of fibers	Average dimensions			Origin	Reference
	L (mm)	W (µm)	w (µm)		
<i>Moringa oleifera</i> (8 year-old)	1.06	73.5 (± 15.5)*	4.19 (± 0.98)*	NE Argentina	This work
<i>Moringa oleifera</i> (3 year-old)	0.92	55.8 (± 12.5)*	4.08 (± 0.80)*	NE Argentina	This work
<i>Moringa oleifera</i>	0.59	50.0	2.14	Arabia - India	(39)
<i>Moringa oleifera</i> (1 year-old)	0.78	76.0	2.63	Misiones	(21)
<i>Moringa oleifera</i> (1 year-old)	1.21	15.0	3.11	Nigeria	(38)
<i>Moringa oleifera</i> (3 years-old)	1.23	15.0	3.80	Nigeria	(38)
<i>Moringa oleifera</i> (5 years-old)	1.27	15.1	4.60	Nigeria	(38)
<i>Eucaliptus grandis</i>	0.96	13.6	3.20	India	(28)
Sugarcane bagasse	1.59	21.0	5.63	India	(28)

*The values in parentheses correspond to the standard deviations.

The biometric relationships of the samples and of the studied *Moringa oleifera* samples and those of *Sugarcane bagasse* and eucalyptus are shown in Table 4.

Table 4: Main biometric relationships of fibers from *Moringa oleifera*'s wood, *Sugarcane bagasse* and eucalyptus.

	3 year-old moringa	8 years-old moringa	Sugarcane bagasse (40)	Sugarcane bagasse (41)	Eucalyptus (42)
FC (%)	16.5	11.4	52.2	56.5	34.0 – 72.0
Fel. (%)	16.5	14.4	83.0	60.0	25.3 – 55.2
R	0.17	0.13	0.80	0.67	0.40 – 1.76

The Flexibility coefficient (Eq.1), indicates the capacity of fibers to collapse and form a paper web. Table 4 shows that *FC* values for *Moringa oleifera* are really low considering the general rule (it has to be more than 50) and also as compared with usual papermaking species. Felting index (Eq. 2). Table 4 shows that *Fel* which valorizes the length of the fiber, presents the same behavior. Runkel Index (Eq. 3) indicates the rigidity of the fiber and the strength of fiber walls. It *R* was significantly lower than those of the other species in Table 4.

Considering the fiber quality of both studied ages, typical of juvenile wood, this raw material would be better used as a source of nano or microfibrillated cellulose or as material for the manufacture of high-value chemical products.

Conclusions

Moringa oleifera wood presents higher starch content than usual commercial hardwoods.

Due to the high content of extractives, an extraction in hot water is recommended as the first step of fractionation.

Since fibrous and biometric parameters indicate that *Moringa oleifera* is not suitable for paper production, it can be exploited as source of high-valuable chemicals.

The high content of alpha cellulose makes *Moringa oleifera* an interesting raw material for the production of nanofibrillated cellulose or microfibrillated cellulose.

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