Chusquea quila, a Natural Resource from Chile: Its Chemical, Physical, and Nanomechanical Properties

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Chusquea quila or “quila”, is one of the most abundant lesser-known species from Chile, and for many years it has created problems for farmers in the southern part of this country. In this study, it was examined as a promising resource for high-tech materials. The chemical and physical properties were determined by ASTM standards. The extractives, ash content, lignin, and alpha-cellulose were 4.55%, 2.17%, 13.78%, and 54.65%, respectively. The higher heating value and basic density obtained were 5,106 kcal/kg and 290 kg/m^3, respectively. The moisture content was studied during four seasons and found to be the highest in winter (73%). Regarding the nanomechanical profiles, hardness varied from 0.16 GPa in the cortex to 0.21 GPa in the nodule. The average elastic modulus in the nodule and internode was 12.5 GPa, while in the cortex it was 7.45 GPa. Considering the high cellulose content and structural features of the lignocellulosic matrix, it could be possible to extract cellulose fibers for commercial use and crude lignin for testing new applications. Thus, the entire quila structure is a potential biomass resource.

Keywords: Chusquea quila; Chilean bamboo; Invasion problem; Characterization; Nanoindentation

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INTRODUCTION

Bamboo is a perennial woody grass that grows abundantly in Asia, with an annual output of 6 to 7 million tons. It grows quickly (up to several centimeters per day), and its fibers have excellent mechanical properties (Chung and Yu 2002). Due to the several advantages associated with bamboo, such as rapid growth, short renewal, and easy spreading, it has gained increasing attention in applications related to the development of energy from biomass, including methane (Kobayashi *et al*. 2004) and ethanol (Shimokawa *et al*. 2009) production. Moreover, bamboo is also an excellent candidate for the development of sustainable natural fiber composites (Chen *et al*. 2011). D’Oliveira *et al*. (2013) studied the largest formation of neotropical bamboo forests in a lightly logged bamboo-dominated forest in Brazilian western Amazon, with the objective to evaluate the sustainability of the applied forest management regime in terms of tree density, above-ground dried biomass, tree bole volume stocks recovery rates, and species groups.

Chusquea is a genre of bamboo that includes about 150 species. In Chile, it is represented by approximately 11 species, of which two have high economic potential: colihue (*Chusquea culeou*) and quila (*Chusquea quila*). Normally, these native species, especially quila, are treated like common weeds or underbrush. The main habitats of quila are the forests in the provinces of Araucanía, Valdivia, Llanquihue, and Chiloe at an altitude of up to 800 to 900 meters above the sea level (Fig. 1).

Related species such as *Chusquea ramosissima* can also be found in the rainforests of Argentina, especially in the provinces of Neuquen and Rio Negro. This is a native monocarpic bamboo species growing in the subtropical forests of northeastern Argentina, which can dominate gaps and open forests in the region (Montti *et al.* 2011).

Fig. 1. Digital photograph of *Chusquea quila,* taken at Catholic University of Temuco, Araucanía, Chile

Quila is characterized by its high abundance and capacity to easily spread. Quila can grow attached to trees and reach over 20 meters high. In Chile, the Chusquea species is estimated to cover around 4 million hectares, of which approximately 900,000 hectares are of economic potential (INBAR 2005) (Table 1).

Table 1. Chusquea Species in Chile; Regions from V to XII (INBAR 2005)

Species	Surface (ha)
Chusquea culeou	305,382
Chusquea quila	396,507
Chusquea cumingii	179,207
Chusquea uliginosa	188,39
Total	**899,935**

To date, research and use of quila are scarce, only including handicraft work (Guioteca 2010), mapuche medicinal treatment (Serindigena 2007), and edible shoots (Gunckel 1948). Additionally, studies led by Intec (2003) indicated the structural potential of *Chusquea culeou* (colihue) and *Chusquea quila* (quila).

For years, *Chusquea quila* has created environmental and economic problems for farmers in southern Chile. In the flowering period, the presence of quila increases the mouse population (Schlegel 1993). In the summer season, it causes fires because of the dry biomass accumulated (CONAF 2012). Finally, as underbrush, it occupies hectares that could be otherwise used for agriculture activities (Pinto and Barrientos 1993).

Ly *et al.* (2012) evaluated bamboo as an alternative cropping strategy in the northern central upland of Vietnam. They analyzed the above-ground carbon fixing capacity of bamboo, accumulation of soil organic carbon, and socio-economic aspects as compared to other land use systems. Over the long term, a bamboo-based cropping system compared favorably to several other land use alternatives in the area.

The present study provides a fundamental characterization of quila in order to explore the potential of its lignocellulosic structure and composition. The development of a technological application for quila and its commercial use would contribute to the reduction of quila overcrowding and, simultaneously, promote a sustainable balance of other native species. Zhang *et al.* (2014) studied a site-specific nutrient management (SSNM) for a field-specific approach with dynamically applying nutrients. This study could show the further increases in bamboo yield and improve ecological environment, with good economic returns, and most important, feasibility in most favourable growth environments of China through relatively straightforward adjustments in crop and nutrient management. In Chile, the study of Chusquea species could help the economy of the farmers and provide new sources of income.

EXPERIMENTAL

Raw Material

The maturity state of quila was chosen according to Campos Roasio *et al.* (2003). The samples, before be collected, when still are shrub, changed color, from duller dark to bright green, and were usually covered with algae, lichens, and mosses. This stage lasts between one and a half to 2 years and is the most appropriate for the harvest of mature canes having a relatively strong structure. The bamboo was collected from local farmers in Temuco and Freire cities (located in Araucania, Chile) (Fig. 2).

Fig. 2. *Chusquea quila* sources in Chile

The samples measuring about 2 meters high were removed and were cut at 20 cm from the ground to the top. Surface impurities were removed by washing and retaining its outer peel, and then the samples were transformed into chips for experimental analysis (Fig. 3). The material was then placed in a ZM200 Ultra-Centrifugal Mill (Retsch GmbH, Haan, Germany) passed through a No. 40-mesh sieve (425 μm), and retained on a No. 60-mesh sieve (250 μm).

The preparation and characterization of the samples was carried out at the Bioprocess Laboratories of Catholic University of Temuco (UCT) and also at the Biomaterials and Nanotechnology Center of Bío-Bío University (UBB), both in Chile. For this study, 3 replicates were performed for each parameter analyzed.

Fig. 3. *Chusquea quila* splinters used for analysis

Determination of the Physical Profiles

Physical profiles were intended to demonstrate the structural potential of quila compared with other members of Poaceae. The moisture content was determined according to ASTM D4442-07 (2007) because it is one of the most important variables affecting the properties of wood and wood-based materials. The test method is structured in a way to permit the full range of use, from fundamental research to industrial processing. The moisture content was evaluated not only for physical analysis, but also to assess species' behavior in the four seasons of the year. The specimen density was determined by ASTM D2395-14 (2002), which is calculated from the weight of a given substance volume divided by the weight of an equal volume of water. Both the weight and volume of the sample vary with the amount of contained moisture. Basic density, as applied to wood, is an indefinite quantity, unless the conditions under which it is determined are clearly specified. Higher heating value was determined by ASTM E711-87(2004), which covers the determination of gross calorific value of a prepared sample of solid forms of refuse-derived fuel (RDF) by the bomb calorimeter method.

Determination of the Chemical Profiles

The chemical profile of quila was assessed mainly regarding its lignocellulosic characteristics. The extractives, holocellulose, lignin, ash, and alpha-cellulose contents were determined according to ASTM D1108-96 (2001), ASTM D1104-56 (1978), ASTM D1106-96 (2007), ASTM D1102-84 (2013), and ASTM D1103-60 (1977), respectively. The extractives-free quila was the starting material for lignin and holocellulose content testing. Holocellulose testing is also a necessary preparatory stage to determine the alpha-cellulose content (Maya and Narasimhamurthy 2015).

Morphological and Nanomechanical Studies of Quila

Scanning electron microscopy (SEM; Tex TSL Laboratories, Inc., Saskatchewan, Canada; with 15 kV and 20 to 25 mm working distance) was used to determine the morphology of quila specimens with dimensions of 2 × 5 mm (width by length), and original thickness of quila stalk 2 to 4 cm, which were cut using sharpened blades. Each sample was sputtered (in the cross-section) with gold coating using a sputter coater (Wang *et al.* 2013).

The nanomechanical properties of quila were studied by means of a nanoindentation test, which was carried out on sclerenchyma tissue from nodules. First, a target region for nanomechanical characterization was selected (with a light microscope attached to the instrument), which was then scanned with an indenter tip to obtain the high magnification image. Subsequently, from the digital image, points for nanoindentation were selected and precisely located on the cell wall.

For the nanoindentation studies, another set of samples with similar size was cut from quila cross-sections using an ultramicrotome equipped with a diamond knife. Nanoindentation testing on cell walls of small specimens was performed using a Hysitron Triboscope nanoindenter (Minneapolis, MN, USA) equipped with a force transducer. The indentations were made with a Berkovich type of indenter (the Berkovich indenter is designed to have the same area as the Vickers indenter at any given indentation depth) and using a loading cycle with maximum rated power of 150 μN. To determine the elastic modulus and yield stress, 10 fibers were indented per cube with less than 1% moisture content. In a normal nanoindentation test, the maximum load indenter penetration and initial stiffness (slope of the discharge curve) were obtained. The known geometry of the indenter enables the determination of the area of contact between the indenter and the material. The reduced modulus (E_r) was determined according to Eq. 1 (Wang *et al.* 2013),

$$E_r = \left[\frac{1 - v_s^2}{E_s} + \frac{1 - v_i^2}{E_i} \right]^{-1} \qquad (1)$$

where the subscripts *s* and *i* represent the sample (S2 cell wall layer) and indenter, respectively, v is the Poisson's ratio, and the indenter modulus E_i is constant and equal to 1240 GPa, with a Poisson's ratio of 0.07. Finally, the modulus of elasticity or Young’s modulus of the sample (E_s) was determined (unit of Young’s modulus of elasticity).

The load-displacement data of each nanoindentation test was used to calculate the hardness *H* from the equation (Xinzhou *et al*. 2013),

$$H = \frac{P_{\max}}{A} \qquad (2)$$

where P_{max} is the load measured at a maximum depth of penetration for each cycle of indentation (N/mm), and A is the projected area of contact between nanoindenter and the sample at the value P_{max} (N/mm).

RESULTS AND DISCUSSIONS

Physical Profiles

Moisture content (*MC*) is an important factor that affects the mechanical properties of bamboo species when used as raw material for structural composite materials (Ahmad and Kamke 2005). In quila, *MC* was determined under four-humidity conditions simulating the four Chilean weather seasons (Fig. 4).

The highest *MC* values of 73% and 67% were found in winter (July 2014 to September 2014) and autumn (April 2015 to June 2015), respectively. However, in spring, *MC* remained above 50%. This data is consistent with the climatic information of the southern part of Chile, particularly in the last two years, when there has been an above-normal rainfall period (ONEMI 2014). In the summer season, *MC* only reached 22%. This low moisture value can explain the persistent fire occurrences, which cause substantial economic and ecological losses, compromising many hectares of wild forest.

To examine the use of *Chusquea quila* as an energy source, its higher heating value (HHV) was determined. Exothermic reactions of carbon and hydrogen release large amounts of energy per unit mass, independent of biomass origin, and this is very important when looking for biomass sources (Millar 2009).

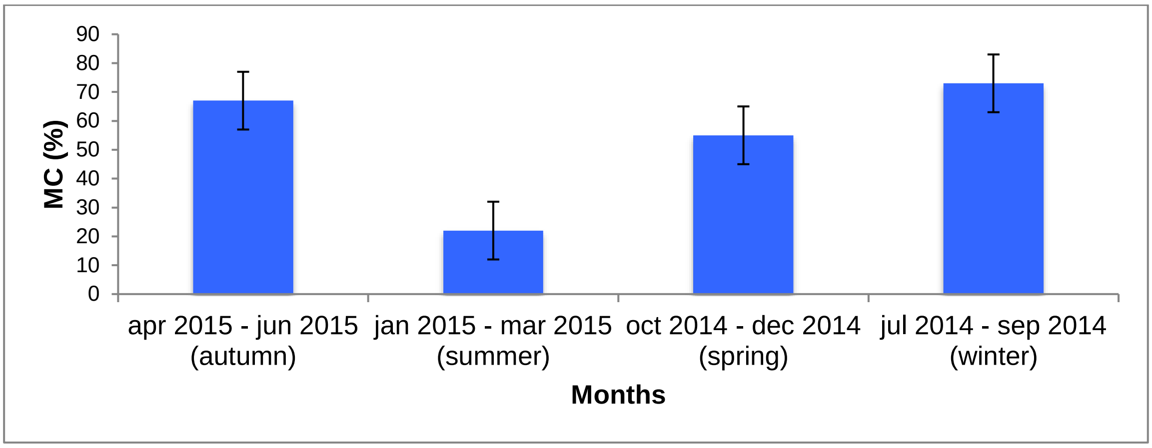

Fig. 4. Moisture content of quila specimens during the four weather seasons in Chile

The utilization of bamboo species as energy feedstock is not new (especially in rural households), yet it has been neglected by the bioenergy industry as a sustainable energy alternative (Engler *et al.* 2012). A possible application for quila can be its reasonable use as an energy source. The average higher heating value of *Chusquea quila* and other bamboo species is shown in Table 2. Compared with other types of bamboo, quila has good potential as fuel source energy.

Density is an important parameter in what concerns biomass because usually it is traded by volume in the market. Ideally, the cubic meter of biomass should have the highest amount of material possible (Li *et al.* 2007). The density of different bamboo species clearly varies depending on their origin, as shown in Table 3.

Table 2. Higher Heating Values of Different Bamboo Species

	Higher Heating Value (Kcal/kg)	Reference
Bambusa vulgaris	6,620	ENC 2012
Phyllostachys bambusoides	4,664	Scurlock *et al.* 2000
Phyllostachys bissetii	4,657	Scurlock *et al.* 2000
Phyllostachys nigra	4,652	Scurlock *et al.* 2000
Chusquea quila	5,106±0.50	------

Table 3. Basic Density of Different Bamboo Species

	Basic Density (kg/m^3)	Country	Reference
Bambusa pervariabilis	346	China	Chung and Yu 2002
Phyllostachys pubescens	213	Japan	Chung and Yu 2002
Phyllostachys edulis	630	China	Li 2004
Dendrocalamus strictus	651	India, Cuba	Wu *et al.* 2002
Bambusa vulgaris	207	Colombia	ENC 2012
Chusquea quila	290±0.23	Chile	-----

Quila has a fairly high density of about 300 Kg/m^3. Future studies should be conducted to determine the optimum density range for quila according to its management objectives based on the size-density relationship (Liu *et al*. 2016).

Chemical Profiles

The chemical composition of quila is shown in Table 4. The amount of extractives, lignin, and alpha-cellulose in quila is comparable to that in other types of lignocellulosic raw materials, currently studied as promising sources for the development of new products.

Table 4. Chemical Characteristics of Quila, Bamboo Species, Biomass, and Wood

Parameters	*Chusquea quila* (%)	*Phyllostachys pubescens* (%)	*Phyllostachys nigra* (%)	Grape Pomace (%)	Conifer Woods (%)	Broadleaf Woods (%)
Extractives	4.5±0.55	5.0	2.3	26.0	0.2–8.5	0.1–7.7
Ash	2.0±0.43	1.5	0.9	---	0.02–1.1	0.1–5.4
Lignin	13.7±0.51	22.8	28.3	42.0	21.7–37.0	14.0–34.6
Holocellulose	67.2±0.38	71.4	---	45.7	59.8–80.9	71.0–89.1
Alpha-cellulose	54.6±0.22	47.0	42.3	25.6	30.1–60.7	31.1–64.4
Reference	---	Li *et al*. 2007	Scurlock *et al*. 1999	Rodríguez *et al*. 2011	Ross 2010	Ross 2010

The bamboo extractives consist of soluble components not generally considered part of the bamboo, which are primarily waxes, fats, resins, some gums, and some water-soluble substances. The epidermis of bamboo has an attractive green color due to its chlorophyll (Li 2004). After the solvent extraction of quila, the color of the extracted solution was dark green due to the presence of extracted chlorophyll. Previous studies have revealed that the chlorophyll in the epidermis is easily degraded, and, thus, treatment with inorganic salts, such as chromates, nickel salts, and copper salts, have been used to preserve the green color of bamboo surfaces (Scurlock *et al.* 2000).

The ash content in bamboo samples was between 1 to 2%. This ash content is comparable to that of woody biomass materials (Table 4). Many herbaceous biomass materials, grasses, and straws have higher ash content (Li *et al*. 2007). The low ash content of the bamboo samples makes them attractive for biomass combustion applications in electricity production.

Its 13.7% lignin content placed quila at the low end of the normal range of 11 to 27% reported for non-woody biomass (Bagby *et al.* 1971), and very far from ranges reported for softwoods (24 to 37%) and hardwoods (17 to 30%) (Li *et al.,* 2007). This would suggest that quila, as an underbrush, should have similar chemistry properties and useful to conventional biomass, more than woods species. Indeed, its low lignin content probably contributed to high heating value, but its structural rigidity makes quila not valuable as building material.

The holocellulose content comprises both alpha-cellulose and hemicelluloses. Alpha-cellulose is the main constituent of quila, accounting for approximately 55% of its dry matter. Cellulose is a homopolysaccharide composed of β-D-glucopyranose units, which are linked together by (1→4)-glycosidic bonds. The cellulose structure is completely linear, and it has a strong tendency to form intra- and intermolecular hydrogen bonds due to the high abundance of hydroxyl groups. As a consequence, bundles of cellulose molecules are aggregated together in the form of microfibrils, in which crystalline regions alternate with amorphous regions (Sjöström 1981).

Hemicelluloses, however, are heterogeneous polysaccharides, and, like cellulose, most hemicelluloses function as supporting materials in the cell walls (Janssen 1995). Alpha-cellulose is the main responsible for the mechanical properties of bamboo species and woods species (Cortés Rodríguez 2012). The high cellulose content in quila, which exceeds that in most wood species, gives good prospects for quila as source of cellulose nanofibrils and/or nanocrystals.

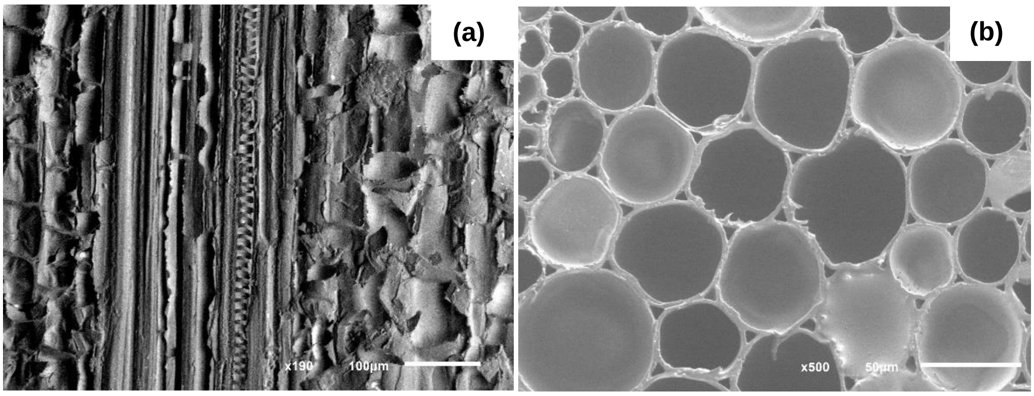

Fig. 5. SEM micrographs from (a) longitudinal and (b) transversal quila fiber

Morphological and Nanomechanical Studies

The surface of longitudinal quila fiber and cross-sectional area of quila block samples were observed by SEM (Fig. 5). The structure of a bamboo transverse section is characterized by numerous vascular bundles embedded in the sclerenchyma tissue. The sclerenchyma is cellular tissue present in many plants and is a supporting tissue of plants. It is a relatively elastic tissue which can be deformed by tension or pressure, but it can return to its original shape when the plant is alive (Londoño *et al*. 2002). The parenchyma cells are mostly thin-walled and connected to each other by numerous simple pits. These cells are vital for storage and mobilization of energy culm because they contain significant amounts of starch and also the pits of the tangential walls of parenchyma cells, which facilitate the radial diffusion of liquids. Pits are located predominantly on the longitudinal walls, but the sclerenchyma cells are thick-walled. Sclerenchyma generally forms packages of fibers below and around the vascular bundles (Maya and Narasimhamurthy 2015).

The values of elastic modulus (*E*) and hardness (*H*) of quila were analyzed in the cortex zone, nodule, and internode. The elastic modulus varied from 7.45 GPa in the cortex to 12.80 GPa in the nodule and 12.20 GPa in internode (Figs. 6a and 6b). The lower hardness was found in the cortex (0.16 GPa), followed by the internode (0.18 GPa), and finally in the nodule (0.21 GPa).

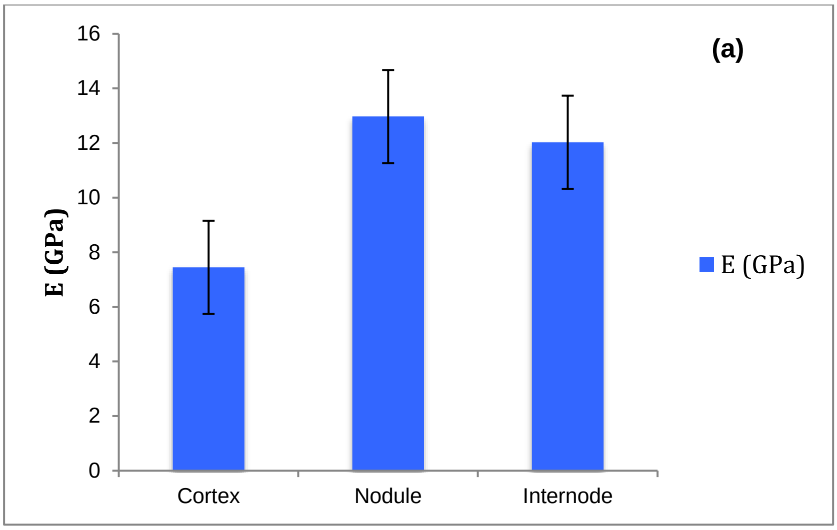

Fig. 6. (a) *E* from quila in cortex, nodule, and internode

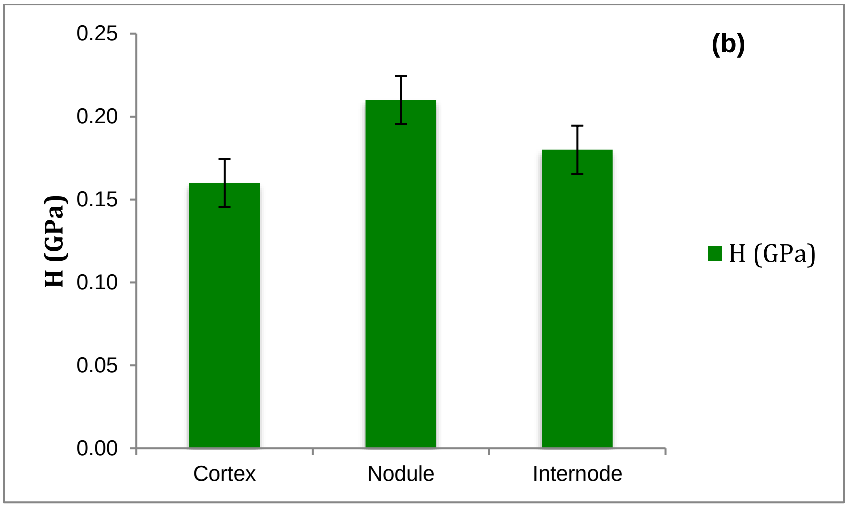

Fig. 6. (b) *H* from quila in cortex, nodule, and internode

When compared with other bamboo species, quila shows a lower *E* value than the average; for instance, compared to 16.01 GPa in nodule for *Phyllostachys edulis* (Li 2004) or 17.87 GPa in the internode for *Dendrocalamus latiflorus* (Wu *et al.* 2002). Quila has lower values for *E* and *H*, which may restrict its commercial value as raw material in its natural condition; however, its chemical potential remains attractive for the processing industry.

CONCLUSIONS

1. A Chilean bamboo was thoroughly studied for the very first time. Whereas its physical and nanomechanical profiles do not seem particularly attractive, its chemical structure shows great potential as a source for cellulose, biomass, or even high-tech materials, especially those comprising cellulose nanofibrils or nanocrystals.
2. For most species of bamboos studied here, nanomechanical and physical properties are highly required in terms of construction and textile manufacturing. For quila, its

low values of basic density (290 kg /m^3) and low values of elasticity and hardness results in few commercial possibilities. However, its impressive high cellulose content (54.6%) and low lignin (13.7%) and ash (2.0%) contents exemplify how important it is to pay attention to species without current commercial use.

3. To date, quila is considered a species that competes for agricultural land, and compared with the chemical potential of other lignocellulosic sources currently available in Chile, these bamboos can be advantageous.

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