## Influence of a Biological Soaking in the Presence of Microbes on the Tensile Strength of Açaí Fibers

Antonio de Lima Mesquita,<sup>a</sup> Felipe Nascimento Arroyo,<sup>b,\*</sup> André Luis Christoforo,<sup>b</sup> Francisco Antônio Rocco Lahr,<sup>c</sup> Nubia Garzon Barrero,<sup>d</sup> Elza Brandão Santana,<sup>e</sup> and Lênio José Guerreiro de Faria <sup>e</sup>

In order to contribute to the dissemination of lignocellulosic residues in the composite materials manufacturing, this study aimed to investigate the influence of a soaking in the presence of natural bacteria and fungi on the resistance to axial traction of acaí fibers (Euterpe oleracea Mart.). The stipulated factors and levels were the source of water, *i.e.*, Rio Guamá, Pará Sanitation Company COSANPA and Cassava, as a catalyst and the mass concentration of water on the açaí cores, i.e., 2%, 4%, 6%. In this way, 9 different experimental conditions were performed, together with the reference condition, *i.e.*, acaí fibers without the soaking. In all, 200 specimens were cast, with 10 of each composition. The analysis of variance results revealed that the individual factors were not significant in obtaining the axial tensile strength, providing equivalent results regardless of the concentration and type of water. However, the interaction between the factors was considered significant, showing that the best treatment for the fibers came from the use of 2% cassava scraps. The axial tensile strength was 47% greater than the axial tensile strength obtained from the reference sample. Furthermore, 59% surpassed the reference sample, showing the efficiency of the soaking in the presence of natural bacteria and fungi treatment.

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Contact information: a: Coordenação de Engenharia de Materiais, State University of Amazonas (UEA), avenue Djalma Batista, 3578, Manaus, Amazonas Brazil; b: Department of Civil Engineering, Federal University of São Carlos (UFSCar), Washington Luís Highway, km 235, São Carlos, São Paulo Brazil; c: Department of Structural Engineering, University of São Paulo (USP), School of Engineering of São Carlos, avenue Trabalhador Sãocarlense, 400, São Carlos, São Paulo Brazil; d: School of Engineering and Architecture, Universidad Pontificia Bolivariana (UPB), Cra 6 no 97A-99, Montería Colombia; e: Chemical Engineering College, Federal University of Pará (UFPA), Street Augusto Corrêa, 1, Guamá, Belém Brazil; \*Corresponding author: lipe.arroyo@gmail.com

#### INTRODUCTION

There is growing consensus among industrialized nations about the need for research to convert unused agricultural byproducts and crop surpluses into profitable new products. The need to develop and commercialize composite-type materials based on constituents of natural origin, *i.e.*, bio-based composites and bio composites, is now considered urgent. In this way it will have an impact in terms of reducing dependence on materials from non-renewable sources, *i.e.*, fossil fuels, as well as from a social, environmental, and economic point of view (Joshi *et al.* 2004; Mohanty *et al.* 2006). Normally all countries that produce lignocellulosic fibers use them in conventional ways,

*e.g.*, inputs in the industrial and domestic sectors and handicraft articles, carpets, wallets, *etc.* Sometimes such inputs are used in composite materials when combined with clay in the construction industry (Satyanarayana *et al.* 2007).

The particleboard and fiberboard industries in Brazil preferably use reforested wood chips from the *Pinus* sp. and some species of *Eucalyptus*. This also yields a better quality product, in terms of better homogeneity control of the raw material. However, lignocellulosic materials from agro-industrial residues have become a new economic, social, and environmental alternative for the manufacture of medium density particleboard (MDP) and medium density fiberboard (MDF) panels (Iwakiri 2003).

The Brazilian agribusiness presents countless lignocellulosic residues with the potential for use in the manufacturing of new materials, e.g., green coconut shells (Iwakiri 2003; Khedari et al. 2003; Brito et al. 2004), peanut shells (Caraschi et al. 2009; Gatani et al. 2014), and sugarcane bagasse (Widyorini et al. 2005; Silva 2006; Battistelle et al. 2009; Caraschi et al. 2009; Fiorelli et al. 2012; Gatani et al. 2014; Garzón-Barrero et al. 2016). The results of national and international research with sugarcane bagasse indicate its viability in different polymer matrices for making composites (Teixeira et al. 1997; Paiva and Frollini 1999; Lee et al. 2004; Widyorini et al. 2005). The açaí agro-industry is an important economic source in the northern region of Brazil. Pará is currently the largest producer in Brazil, accounting for 87.1% of all pulp consumed in the country, with approximately 80% to 90% coming from the northeast of that state (Souza et al. 2006; Fonseca et al. 2013). Approximately 24% of this production is obtained from cultivated areas and 76% from extractive sources, revealing an increase in the proportion of cultivated areas and managed native açai sources, which is interesting in terms of environmental and productive efficiency (Souza et al. 2006; Fonseca et al. 2013). Approximately 16000 tons of fibrous açaí tissue (Euterpe oleracea Mart.) is discarded in the metropolitan region of Belem (PA) after the production of juice. This agro-industrial residue is infrequently used in boilers as an energy source.

The physical-mechanical properties of some lignocellulosic fibers have been evaluated in the world and in Brazil, as part of the development of new composite materials. Some studies present the axial tensile strength results of various natural fibers of agro-industrial origin, *e.g.*, Jacitara (24.2 to 113.2 MPa), banana (142.9 MPa), sugarcane bagasse (222 MPa), Curauá (500 to 1150 MPa), and green coconut (65 MPa) (Satyanarayana *et al.* 2007; Pereira 2012; Fonseca *et al.* 2013). Treatments conventionally performed on the surface of plant fibers aim to decrease their hygroscopicity and/or increase the ability of the fibers to interact with the resin, which may influence the final particulate composite characteristics. Several methods can be used to improve fiber/resin adhesion. Currently, this step is considered extremely important in the development of these materials.

The surface modification methods can be physical, chemical, or biological, according to the way in which the fiber surface is modified. An example of a widely used method is the biological soaking process (sometimes called maceration), in which the fibers are submitted to the reaction and decomposition process of the pectic substances through the action of enzymes, resulting in the liberation of microfibrils. Research on the biological mercerization process for obtaining Amazonian fibers is scarce in the literature (Fonseca *et al.* 2013). Since it deals with the production of acai fibers *by* soaking in the presence of natural bacteria and fungi treatment, the present research was a contribution to the development of non-conventional materials in the application of treatment these processes, aiming at improving the physical-mechanical properties of acai fibers. The objective of this

paper is to investigate the influence of soaking treatment on açaí fibers using water from two different sources, *i.e.*, Guamá River water and water from the Sanitation Company of Pará, and in three concentrations, *i.e.*, 2%, 4%, and 6%, on the axial tensile strength. Furthermore, cassava scraps (CS) were used as the catalyst.

#### EXPERIMENTAL

The fibrous tissue samples came from the species *Euterpe oleracea* Mart. These were collected from an agroindustry that prepares açaí juice and disposes of the fruit residues by packaging them in polypropylene bags in front of the establishments to be collected by the urban waste collection company, in the city of Belem, Pará.

The raw material obtained *in natura* was washed in running water, dried in an oven at a temperature of 60 °C, packed in plastic containers, and placed in a refrigerator at a temperature of 15 °C until the beginning of the soaking in the presence of natural bacteria and fungi step (Sivasubramanian *et al.* 2021). The process of obtaining açaí fibers by soaking was carried out both spontaneously and induced. A manufactured prototype was used containing bench scale tanks with a 5 L capacity and a Lutron Ph-201 portable pH meter, according to the normative prescription (Zenebon *et al.* 2008). In addition, during the experiments, various properties were analyzed, *e.g.*, the pH, solution temperature, ambient temperature, and relative humidity. Three soaking treatments were performed, classified as follows: spontaneous soaking using either Guamá River water (GR) or water from the Sanitation Company of Pará COSANPA (CO) as the catalyst for fiber disintegration, and induced soaking, in which cassava scraps (CS) were used as the catalyst. The reason for using two different waters is because of their properties. In this way, it will be possible to analyze the differences in the results obtained.

The work involved the spontaneous soaking in the presence of natural bacteria and fungi conditions using either water from the Guamá River (GR) or water from COSANPA (CO) as catalyzing agents. Three proportions were employed, based on published findings (Gatani *et al.* 2014; Garzón-Barrero *et al.* 2016), between the solution mass and kernel mass were used, *i.e.*, 2 to 1, 4 to 1, and 6 to 1. These were distributed as follows: in tank 1, the soaking was performed at a ratio of 2 to 1, which corresponded to 800 g of solution for 400 g of stones; in tank 2, the soaking was performed at a ratio of 4 to 1, which corresponded to 1600 g of solution and 400 g of stones, and in tank 3 the ratio used was 6 to 1, which corresponded to 2400 g of solution and 400 g of stones (as shown in Fig. 1).



Fig. 1. Fabricated prototype for the biological soaking process

The process of soaking in the presence of natural bacteria and fungi is considered handmade, in which cassava scraps were used catalyst agent at a proportion of 10% (Souza *et al.* 2016) with respect to the mass of the stones, which corresponded to 40 g of cassava. These were added to the three tanks in catalyst agent to stones ratios of 2 to 1, 4 to 1, and 6 to 1.

During the three processes (*i.e.*, Guamá River, COSANPA Water and cassava scraps), the fiber samples were extracted by hand and dried under laboratory conditions at room temperature (28 °C) and 80% relative humidity for subsequent tensile testing.

#### **Research Design**

The factors and experimental levels investigated in the evaluation of the tensile strength of açaí fibers were the type of soaking in the presence of natural bacteria and fungi, *i.e.*, Guamá River (GR), COSANPA Water (CO), and cassava scraps (CS). The mass concentration of water for the açaí kernels was 2%, 4%, and 6%, leading to a type 32 full factorial design that resulted in nine distinct experimental conditions (EC) (as shown in Table 1).

EC	Medium for Soaking (M)	Mass Concentration of Water (%)		
1	GR	2%		
2	GR	4%		
3	GR	6%		
4	СО	2%		
5	СО	4%		
6	СО	6%		
7	CS	2%		
8	CS	4%		
9	CS	6%		

**Table 1.** Experimental Conditions for the Soaking in the Presence of Natural

 Bacteria and Fungi of Açaí Fibers

Analysis of variance (ANOVA) was used to investigate the influence of the single factors, *i.e.*, mercerization and water concentration, as well as the interaction between them in terms of the açaí fiber tensile strength. The ANOVA was evaluated at a significance level ( $\alpha$ ) of 5%, with an equivalence of means between treatments as the null hypothesis (H<sub>0</sub>) and the non-equivalence between the means as the alternative hypothesis (H<sub>1</sub>). A *p*-value lower than the significance level implied the H<sub>0</sub> was rejected, while it was accepted otherwise.

To validate the ANOVA, the Anderson-Darling test was used to check the normality of the distribution of the residuals and the F tests, *i.e.*, Bartlett's and Levene, were used to assess the homogeneity of the variances between the experimental conditions investigated. The tests were formulated at the 5% significance level. For the Anderson-Darling test, the null hypothesis consisted of the normality of the distribution, and the non-normality as the alternative hypothesis. A *p*-value equal to or greater than the significance level of the test implied the H<sub>0</sub> was accepted, while it was refuted otherwise. For the F tests, the null hypothesis consisted of the homogeneity of the ANOVA residuals, and the inhomogeneity as an alternative hypothesis. A *p*-value equal to or greater than the significance level implied the null hypothesis was accepted, while it was rejected otherwise. For the F tests, the null hypothesis consisted of the null hypothesis was accepted, while it was rejected otherwise. Once the best experimental conditions in terms of the optimal axial tensile

strength of the açaí fibers were found, the test results were compared to the axial tensile strength of the reference condition, *i.e.*, the untreated fibers, again using the analysis of variance.

#### Fiber Bundle Axial Tensile Tests

The axial tensile strength tests were performed for a bundle of fibers with the aid of an EMIC-DL-3000 universal testing machine with a maximum capacity of 300 kN, with a data acquisition system, a 1 kN load cell, at a speed of 0.3 mm/min, and pneumatic grippers of 200 kgf. Twenty specimens were used for each of the nine experimental conditions plus the reference (natural açaí fibers), for a total of 200 specimens.

The samples were prepared with kraft paper supports. The supports were used to evenly distribute the load applied to the fibers and also to protect the fibers from damage when positioning the grips in the testing machine. The kraft paper supports (a basis weight of 200 g/m<sup>2</sup>) had dimensions of 25 mm  $\times$  55 mm and were glued at the fiber ends along the effective length, adapted from the recommendations of the ASTM standard D3822 (2010).

#### **RESULTS AND DISCUSSION**

Table 2 presents the results of the maximum axial tensile strength as a function of the experimental conditions arising from the soaking in the presence of natural bacteria and fungi treatment along with the reference condition. This table shows the sample mean  $(\bar{x})$ , coefficient of variation (CV) and the minimum (Min) and maximum (Max) values obtained in the tests.

	EC1	EC2	EC3	EC4	EC5	EC6	EC7	EC8	EC9	REF
$\overline{x}$	18.20	16.82	17.18	17.00	18.73	16.85	26.26	18.46	16.33	16.48
CV	34%	36%	33%	35%	31%	28%	25%	34%	27%	25%
Min	11.79	10.74	9.48	9.28	10.43	11.70	17.78	10.72	9.75	9.96
Max	29.94	29.03	25.26	25.50	32.29	26.19	36.13	26.83	23.54	24.13

 Table 2. Maximum Axial Tensile Strength Results (MPa)

The average fiber bundle tensile strength values of the experimental conditions, with the exception of condition EC7, were close to that of the reference condition (untreated fiber). Experimental condition EC7 showed an axial tensile strength 47% higher (looking at the average value) than the axial tensile strength obtained from the other eight experimental conditions, as well as an axial tensile strength 59% higher than the reference condition.

From the normality and homogeneity of the variance tests for the maximum axial tensile strength of the açaí fibers, the *p*-value found in the Anderson-Darling test was less than 0.05 (0.007). Thus, it is implied that the distribution of the tensile strength values was not normal. The same was found for the F test (Bartlett and Levene), where the *p*-value was greater than 0.05 (0.982 and 0.919, respectively) in both cases, *i.e.*, the null hypothesis was accepted (normality of distribution).

In order to meet the normality requirement demanded by ANOVA, the Johnson transformation was performed. With the transformed data, the distribution normality and the equivalence of variances were evaluated. The results revealed that the transformed data for the maximum axial tensile strength showed a normal distribution (R<sup>2</sup> of 0.986919) and

the equivalence of variances between the experimental conditions was investigated. This is due to the fact that it presented, in both cases, a p-value greater than the significance level adopted (0.05), validating the ANOVA model.

The individual factors were not non-significant (a p-value less than 0.05), leading to equivalent tensile strength results regardless of the water type or concentration. However, the interaction between the factors was significant (a p-value higher than 0.05), and the interaction results are depicted in Fig. 2. In this figure, equal lowercase letters indicate that the results do not differ (at a 5% significance level) according to the Tukey test.



Fig. 2. Unfolding effects of soaking in the presence of natural bacteria and fungi treatments for each water concentration on maximum axial tensile strength

There were no significant differences in the tensile strength values at concentrations of 4% and 6%. However, there was a significant difference at 2%, in which the use of cassava scraps (experimental condition 7) as a catalyzing agent (induced soaking) led to the best results.

Regarding the results of the normality and homogeneity of variance tests for the maximum axial tensile strength of the reference conditions (natural fiber) and the EC7 (2% water CS) conditions, the *p*-values were greater than 0.05 (0.425, 0.193, and 0.084 for the Anderson-Darling, Bartlett and Levene tests, respectively). Thus, the normality of the distribution for the maximum axial tensile strength and the equivalence of variances between the conditions were verified, validating the ANOVA model. The ANOVA *p*-value was within 5% (0.001), and this implied that the mean tensile strength values of the two compared conditions (reference and EC7) were significantly different.



Fig. 3. Main effects plot for maximum axial tensile strength (reference × EC7)

The primary effects plot for the maximum axial tensile strength is presented in Fig. 3. The experimental condition EC7 (2% water CS) provided a higher tensile strength than the reference condition. Thus, the effectiveness of the treatment of açaí fibers *via* biological induced mercerization with cassava chips as a catalyst at a 2% concentration was evident.

Table 3 presents recent research with results on the maximum axial tensile strength of agro-industrial vegetable fibers under natural conditions. The results obtained for the axial tensile strength of acaí fibers (26.9 MPa) were close to the results obtained by Fonseca et al. (2013), whose average minimum tensile strength for Jacitara fiber was 24.2 MPa. In this study, the average value found for the açaí fibers in the process of spontaneous soaking in the presence of natural bacteria and fungi for the extraction of the fibers in the three experimental conditions, showed that the best condition was EC7 (CS) with 2% water, which presented an average maximum tensile strength value of 26.26 MPa and a 25% coefficient of variation (CV%). Based on these results, it was verified that this process was unique and unprecedented in this research. As such, it contributed above all to the understanding of the behavior of the mechanical performance of açaí fibers submitted to the process of induced soaking in the presence of natural bacteria and fungi, *i.e.*, the disintegration and obtaining of fibrous tissue, using as cassava chips as the catalyst agent. The results of this research showed similar values to Sivasubramanian et al. (2021), i.e., 25.84 and 26.95 MPa, when developed with alkaline mercerization, using 2% NaOH and NaOH + autoclave, respectively.

Fiber	Tensile Strength (MPa)			
Jacitara (Fonseca <i>et al.</i> 2013)	24.2 to 113.2			
Banana (Satyanarayana <i>et al.</i> 2007)	142.9			
Green coconut (Pereira 2012)	65			
Sugarcane bagasse (Satyanarayana et al. 2007)	222			
Curauá (Satyanarayana et al. 2007)	500 - 1150			
Açaí - experimental data	26.9			

 Table 3. Maximum Tensile Strength of Agro-Industrial Vegetable Fibers

As shown in Fig. 4, after performing the axial tensile strength tests, the açaí fibers were analyzed *via* scanning electron microscopy (SEM), for experimental conditions EC1 (water from Guamá river and concentration of 2%), EC4 (water treated by the Sanitation Company of Pará - COSANPA and concentration of 2%), and EC7 (cassava chips and 2% concentration). The fiber obtained from the CS process presented numerous surface silica (SiO<sub>2</sub>), while on the surface of the fibers from the Guamá River condition (GR) (Fig. 4b) the removal of silica was observed, evidenced by the empty spaces of the globular cavities, as well as the degradation of the fibrous tissue (as shown in Fig. 4b). This is probably because of the presence of microorganisms in the water. On the fiber surface of the experimental conditions using COSANPA water (CO); in addition, the presence of few silica (SiO<sub>2</sub>) was verified, as well as voids in the cavities. This fact is certainly related to the chemical composition of the COSANPA water and as a consequence of the chemical compounds in the water, the probable degradation of the cuticle cell layer was also noted (as shown in Fig. 4c).

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Fig. 4. SEM images: a) Cassava scraps (CS); b) Guamá River (GR); and c) COSANPA Water (CO)

## CONCLUSIONS

- 1. The individual factors stipulated (*i.e.*, soaking in the presence of natural bacteria and fungi Guamá River, COSANPA Water and cassava scraps) in the treatment of the açaí fibers were found to be non-significant for the axial tensile strength.
- 2. The interaction between the factors (*i.e.*, soaking and water concentration, as well as the interaction between them in terms of the açaí fiber tensile strength) was considered significant by ANOVA. The best treatment condition was obtained with the use of 2% cassava scraps, since it yielded a higher axial tensile strength values (47%) compared to the other experimental conditions.
- 3. The analysis of variance showed that the treatment of açaí fibers was significant in terms of the axial tensile strength when compared to the reference condition.
- 4. The axial tensile strength of the açaí fibers from condition EC7 was significantly higher (59%) than the reference condition fibers, showing the efficiency of the treatment with 2% cassava scrap in the açaí fibers.

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