Tech Science Press

Morphological Characterization of Brazil Nut Tree (*Bertholletia excelsa*) Fruit Pericarp

Gustavo P. Petrechen[1,4,*], Marcos Arduin[3] and José D. Ambrósio[1,2]

[1]Materials Science and Engineering Postgraduate Program, Federal University of São Carlos, São Carlos, Brazil.

[2]Center of Characterization and Materials Development, Federal University of São Carlos, São Carlos, Brazil.

[3]Department of Botany, Federal University of São Carlos, São Carlos, Brazil.

[4]Citlab-Science, Innovation and Technology Laboratory, Sorocaba, Brazil.

[*]Corresponding Author: Gustavo P. Petrechen. Email: gustavo.petrechen@citlab.com.br.

Abstract: This article presents the overall morphological structure of the Brazil nut tree (*Bertholletia excelsa*) fruit pericarp, from macro to nano scale. The acquired knowledge would be used for the development of new applications, like using the materials as fillers for biocomposites, or as a hierarchical architecture model for biomimetics. This research was performed using stereo and light microscopy and conventional and force field emission scanning electron microscopy. The pericarp presents three layers: the exocarp, a dark gray, brittle and fragile outer layer; the mesocarp, a beige, dry, rigid, impermeable and fibrous intermediate layer; and the endocarp, an inner layer with similar characteristic as the exocarp, but formed next to the seeds. Morphologically, the exocarp and the endocarp presented minor regions of sclereids, fibers and vascular cell bundles, inside major regions of parenchyma cells. The mesocarp presents a structure of fiber cells regions alternating with sclereids and vascular cells regions, arranged in a composite like arrangement, with the fibers cells bundles acting as randomly oriented disperse phases in a sclereid cells matrix. This arrangement was associated with the mesocarp relative superior proprieties, indicating a great material for using as fillers for biocomposites or in biomimetics applications.

Keywords: *Bertholletia excelsa*; brazil nut; fruit pericarp; morphological structure; microscopy

1 Introduction

Brazil nut tree (*Bertholletia excelsa*), also known as *Pará* nut tree or *castanheira*, is an Amazon region native plant from which the edible seed endosperm, commercially known as Brazil nuts, shown in the Fig. 1(c), is extracted. Considered one of the tallest species in the Amazon rainforest, this tree can reach sixty meters height and one hundred and eighty centimeters of trunk diameter [1]. The Brazil nut trees lives mainly in dry land forests and hot and humid weather, with precipitation between 1500 and 2800 mm/year [2]. They form clusters around twenty individuals per hectare, where each tree produces about two hundred and fifty fruits per year, reaching more than two thousand in trees with more than two hundred years old [3]. The Brazil nut tree is considered productive from three years old, when grafted (agronomy technique), to eight, if native [4], and the fruit maturation takes about fifteen months, when the mature fruits falls up to 50 meters height, to be collected in the ground [5-7].

The Brazil nut tree fruits consists of a dry, woody, dehiscent (opens when mature), imperfect (it does not spontaneously free the seeds) and globular kind fruit, which weighs around 500 g and carries around 18 seeds inside [8]. The Brazil nut tree fruit pericarp, popularly called "*ouriço*", shown in the Fig. 1(a), is a dark gray, dry, woody and rigid globular shaped fruit, that weights around 250 g (without seeds) and

DOI: 10.32604/jrm.2019.04588

measure around 11 cm in diameter [8]. The seeds, whose seed shells (teguments) are shown in the Fig. 1 (b), have a brown, dry, woody, rigid and triangular shell, that involves the seed endosperm, the so-called Brazil nut, a white-yellowish, oily and very nutritive edible food, that is shown in the Fig. 1(c) [8].

Figure 1: Brazil nut lignocellulosic residues and the edible so-called Brazil nuts: (a) the greater quantity residue, the fruit pericarps; (b) the minor quantity residues, the seed shell (tegument); (c) the highly commercialized edible Brazil nuts (the endosperms) [8]

According to a study [8] based on data acquired from the United Nations (UN) Food and Agriculture Organization (FAO) [9], the world production of Brazil nuts endosperm (only the edible part) in 2014 was of 69900 tons, with Brazil and Bolivia accounting for about 80% of this production. In order to estimate the amount of the lignocellulosic residues generated in the Brazil nut harvest and processing, the study [8] evaluated the weights of the main fruit parts. The study founds a total fruit weight, with all pericarp layers and seeds, of about 480 g, and the corresponding fruit parts percentages of this value was of 25% for the exocarp, 50% for the mesocarp, 1% for the endocarp and 12% each for the seed shell and the edible endosperm. This results that about 88% of the total fruit weight corresponds to lignocellulosic residues and 86% of that (76% of the fruit) relates just to the pericarp. In absolute values, that corresponds about 512.600 tons/year of total lignocellulosic residues and 442.700 tons/year derived from the fruit pericarps, a considerable amount. Actually, the seed shells accumulate in large quantities in the producers, are burnt for space allocation or for heat, are dumped in the forest or, in some cases, are used as natural fertilizer. The fruit pericarps, by the way, mostly are left behind in the forest during the harvest or, in a very small amount, are burnt for heat [8].

The Brazil nut tree fruit lignocellulosic materials can be classified as a natural lignocellulosic filler (NLF), for using in polymer matrix biocomposites. Some examples of lignocellulosic fillers are particles and fibers of hemp, bamboo, wood, bagasse and coconut shells [10]. The use of NLF is promising for several factors, such as cost-effectiveness, renewable nature, low density, and good mechanical properties [11]. As disadvantages, lignocellulosic fillers are hygroscopic (absorbs moisture), hydrophilic (bonds on a molecular level with water), release volatile substances (steam and others) during processing and relatively degrades easily, resulting in a low processing temperature (less than 220°C) [10].

Considering some characteristics of the Brazil nut tree fruit lignocellulosic residues materials (fruits pericarps and seed shells), like the relative high stiffness and good mechanical behavior [8], besides the large amount generated, it is important to understand its morphological structures in the macro, micro and even nano scale, to find ways to add value to them. This effort collaborates with sustainable development, especially in the Amazon region. Several applications such as the use as fillers for bio composites or even biomimetics, for example, needs a deeper understand of the structural characteristics of these materials. Due to this, the aim of this study was characterized the Brazil nut tree (*Bertholletia excelsa*) fruit pericarp morphology. For this purpose, some microscopic analysis were used, allowing describe its structure in the macro, micro and nano scale. This research can open new perspectives to several researches related of using this natural, renewable and eco-friendly material.

2 Materials and Methods

For this study, some fruit samples were obtained from a local producer from *Juina* region, located in the Brazil's state of *Mato Grosso*. For the morphological analysis, were used images captured with a stereo microscope, with a light (optical) microscope, with a conventional scanning electron microscope (SEM) and with a field emission scanning electron microscope (FESEM).

For the stereo microscope images, some samples of the pericarp layers were fractured to be observed just as they are, and others were cut and embedded in a commercial polyester resin, cured at room temperature, and were grinded an polished respectively with sandpapers up to 1200 mesh and with 1 μm alumina solution. All the samples were photographed on a stereo microscope, model TXB2-D10, Srate brand, set configured for increases from 7X to 180X, with a coupled 5 MP CMOS camera.

For the light microscope images, samples of the mesocarp were grinded and polished in the same way as prepared for obtain the stereo microscope images. These samples were photographed on an Olympus, BX41M-LED light microscope, with light set configured for increases from 40X to 1000X, and a coupled 5 MP CMOS camera.

For the conventional scanning electron microscope (SEM) and for the field emission scanning electron microscope (FESEM) images, samples of the pericarp layers were fractured to expose its interior, and the surface of this samples were coated with gold, in order to become electrically conductive for the image formation in the equipment. Then, images of the samples surfaces were obtained in the SEM, a FEI brand, model Inspect S-50, with magnifications up to 100,000X, and in the FESEM, a FEI brand, model Magellan 400L, with magnifications up to 1,000,000X.

3 Results and Discussion

The Brazil nut tree (*Bertholletia excelsa*) fruit pericarp is mainly formed by three layers: the exocarp, a dark gray color, brittle and fragile outer layer; the mesocarp, a beige, dry, rigid, impermeable and fibrous intermediate layer; and the endocarp, an inner layer with similar characteristic as the exocarp, but formed next to the seeds. The Fig. 2 shows a sliced sample of an entire fruit, fully filled with polyester resin, to allow the visualization of its internal structure. The pictures show the three layers of the pericarp together with the seeds.

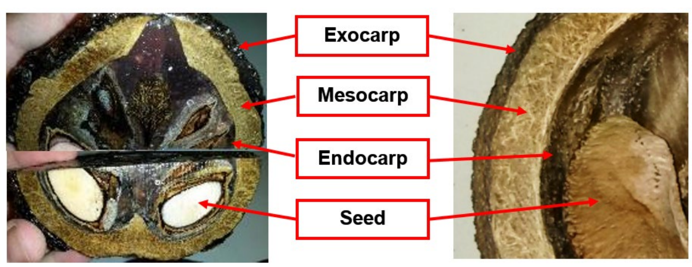


Figure 2: Sliced Brazil nut tree fruit, embedded in polyester resin, showing the pericarp three layers: the exocarp, the mesocarp and the endocarp, along with the seeds [8]

The outermost layer of the pericarp is the exocarp. The Fig. 3 shows images taken with the stereo microscope of exocarp samples embedded in resin, grinded, and polished. The analysis of the microstructure showed that the exocarp is composed of two regions: one formed of parenchyma cells, shown in the darker region in the Fig. 3 images, mostly found in a decomposing state, present even with fungal hyphae; and another, shown in the lighter region, formed by some sclereids and mostly by fiber and vascular cells bundles. This layer is fragile and brittle and this occurred mainly due the decomposition state in which the parenchyma cells regions are found when mature [8].

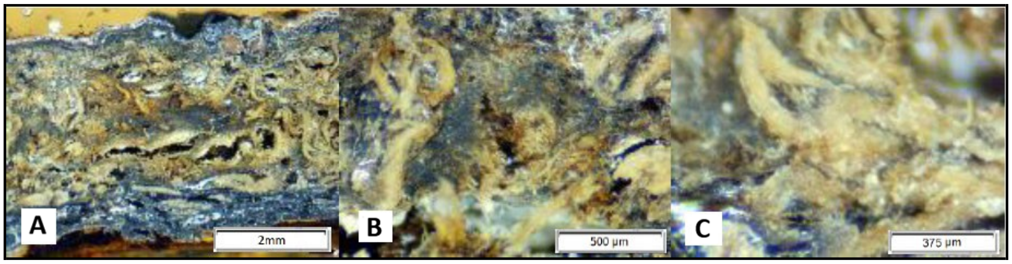


Figure 3: Stereo microscopy images of the exocarp: (a) the parenchyma cells region (darker color) and the fiber and vascular bundles regions (lighter color); (b) the parenchyma cells region in the center; (c) the fiber and vascular bundles regions [8]

With the obtained SEM images of this layer, shown in Fig. 4, it is possible to view the different cell regions that builds the exocarp. The Fig. 4(a) shows the parenchyma cells region, composed with isodiametric rounded shape cells, that measures about 20 µm in diameter, present only primary cell walls, have a large lumen and are found together with a relative vast intercellular space. The Fig. 4(b) shows a parenchyma cell in detail. The Fig. 4(c) shows a fiber and vascular cell bundle, also containing some sclereid cells. The Fig. 4(d) shows in detail a tracheal element, of the vascular bundle, with an inner lignified spiral structure.

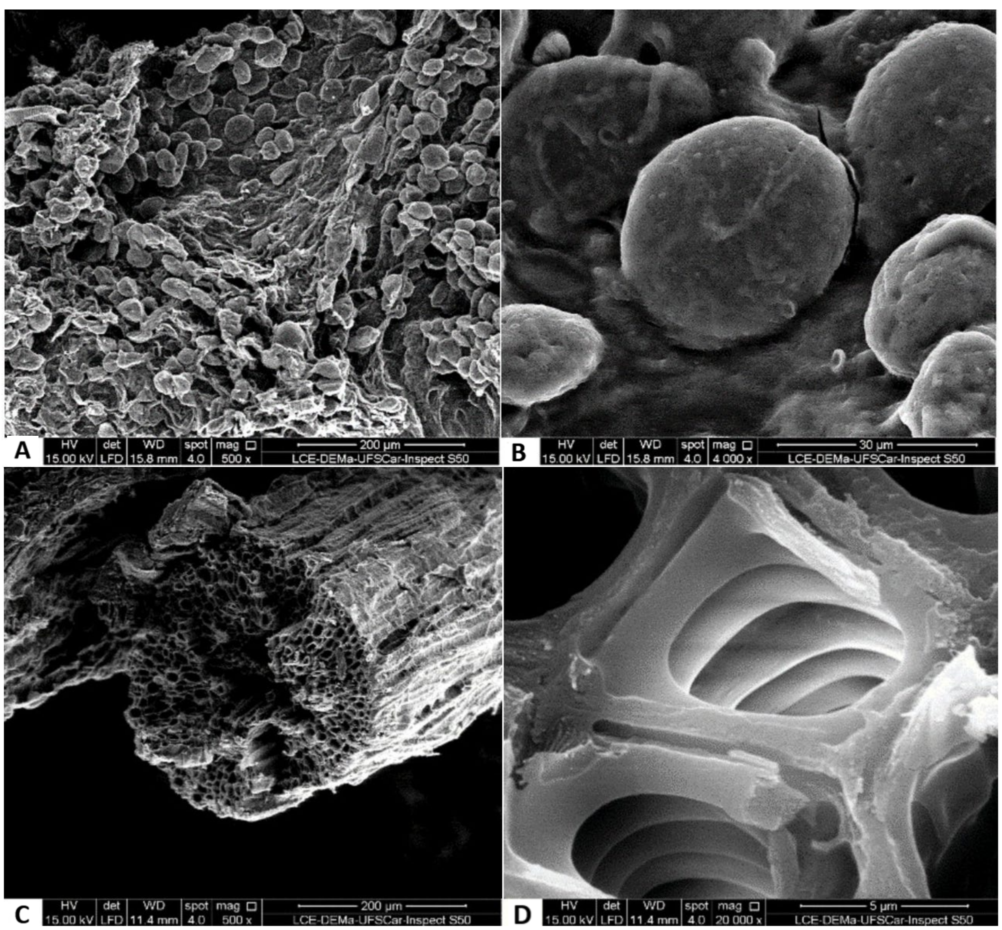


Figure 4: SEM microscopy images of the exocarp: (a) the parenchyma cells region; (b) a parenchyma cell; (c) a fiber and vascular cells bundle; (d) the detail of a tracheal element (vascular cell) in the fiber and vascular cells bundle, showing its inner lignified spiral structure [8]

Representing the inner layer of the pericarp, the endocarp stays in close contact with the seeds. It has a dark brown color and a dry, porous and brittle structure. Its morphological composition is similar as the found in the exocarp, with parenchyma cells regions among some vascular and fiber cells bundles regions. The stereo microscopy of the endocarp, presented on Fig. 5(a), shows a dark-colored parenchyma cells, surrounded by a yellowish-white color fibers and vascular bundles, in a fractured region. At the surface of the endocarp, parenchyma cells among fiber and vascular cells bundles can be seen in the Fig. 5(b). This layer represents about 1% of the total fruit weight and, as the exocarp, is fragile and brittle by the same reasons. Due this, as well as the exocarp, results a low interest for using this layer with new applies.

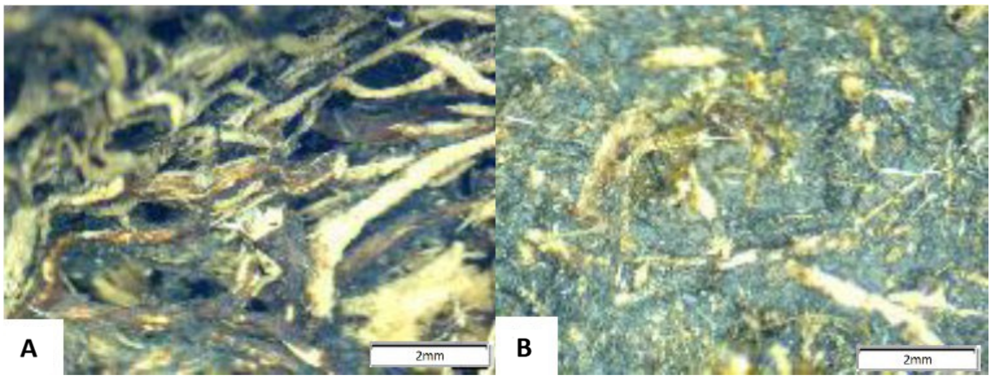


Figure 5: Stereoscopy microscopy images of the endocarp: (a) internal fractured region, showing the parenchyma cells regions, in a darker color, and the fiber and vascular cells bundles, in a yellowish-white color; (b) external surface showing the same composition [8]

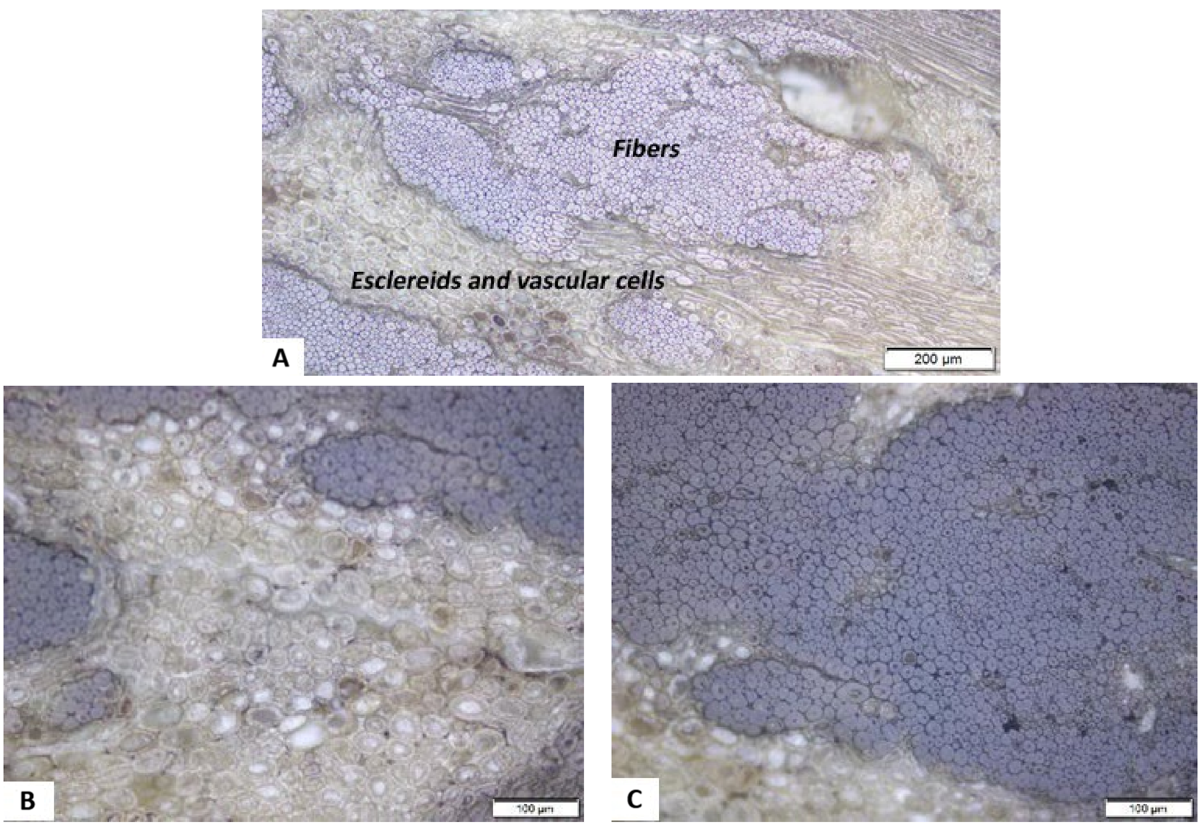
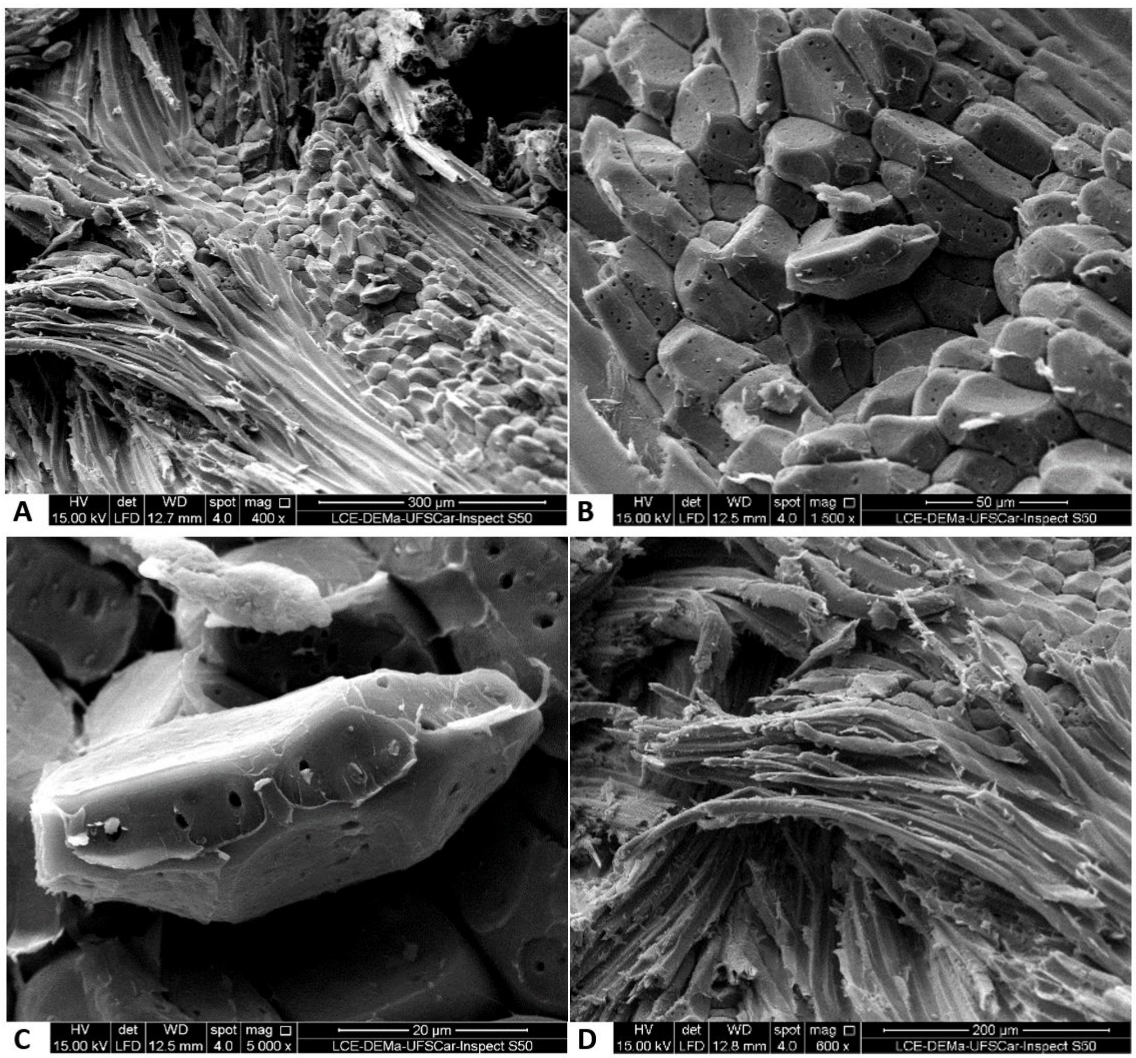


Figure 6: Light microscopy images of the mesocarp: (a) the fiber cells region and the sclereids and vascular cells region; (b) the sclereids and vascular cells region; (c) the fiber cells region [8]

The mesocarp is the central layer of the fruit pericarp. It presents a beige color and a dry, rigid, hard, impermeable and fibrous structure. The Fig. 6(a) shows an image, obtained by light microscopy, of the grinded and polished resin-embedded mesocarp sample. Through this image, it is possible to view in detail two kind of regions: one constituted of fiber cells, and another, constituted of sclereid cells and some scattered vascular cells. The Fig. 6(b) shows the sclereids cells region and the Fig. 6(c) shows the fiber cells region.

With the SEM microscope acquired images, shown on Fig. 7, it is possible to view, with more detail, the arrangement of the fiber cells and the sclereids cells regions. The Fig. 7(a) shows how this arrangement is ordained. Compared with a typical composite material structure, it is possible to see randomly oriented fiber cells bundles, acting as a disperse phase, involved with sclereid cells regions, acting as a continuous phase, all separated by a well-defined interface. In the Fig. 7(b) it is possible to view the sclereids cells region, with its typical polyhedral and barely elongated shape sclereids cells. The Fig. 7(c) shows in detail a sclereid cell. In the Fig. 7(d) it is possible to view the fiber cell region, with the fiber cells bundles randomly oriented, and the typical thin and elongated shape of the fiber cells. The Fig. 7(e) shows some fractured fiber cells with their typical thick cell wall and reduced lumen.



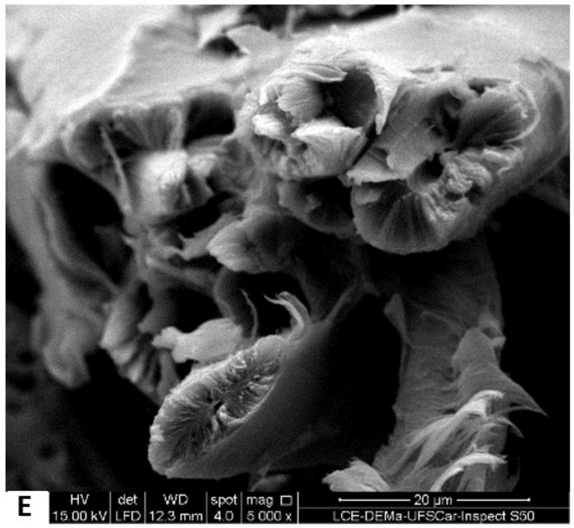


Figure 7: SEM microscopy images of the mesocarp fractured surface: (a) fiber cells bundles and sclereids cells forming a composite like structure; (b) the sclereid cells region; (c) a sclereid cell in detail, with its typical polyhedral shape; (d) the fiber cell bundles region; (e) some fractured fiber cells showing its typical thick cell wall and reduced lumen [8]

Through FESEM microscope obtained images, shown in the Fig. 8, it is possible to view in detail the sclereid cell wall and its microfibrils organization. Sclerenchyma cells, as the sclereids and fibers cells found in the mesocarp, typically have a thick, hard and lignified cell wall. This occurred due the formation of the secondary cell wall, a well-oriented cellulose fibril layer, and due the lignification process, coinciding with the death of the cells. The Fig. 8(a) shows a cell wall of a fractured sclereid. It is possible to view clearly the presence of a well formed secondary cell wall. In the Fig. 8(b), it is possible to view the different layers and the structure of the cell wall, formed by structural polymers (cellulose, hemicelulose and lignin) arranged as microfibrils bundles.

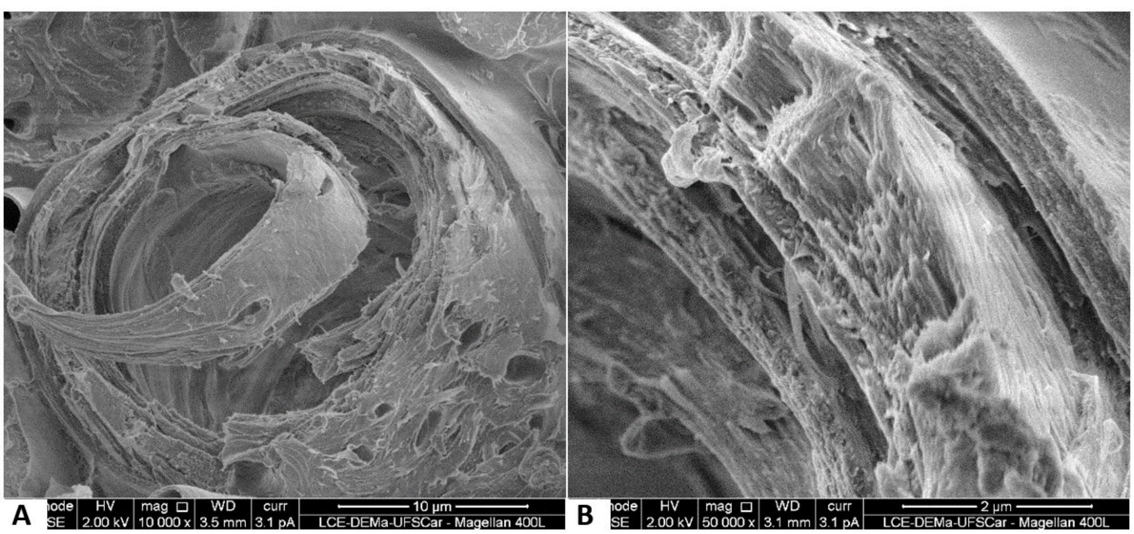


Figure 8: FESEM microscopy images of a sclereid cell wall detail: (a) A fractured sclereid cell showing its cell wall with different inner layers; (b) the structure of the sclereid cell wall formed by microfibrils bundles [8]

With a greater increase in resolution, it was possible to view the organization of the microfibrils in the sclereid cell wall at nanoscale. The Fig. 9(a) shows a microfibril bundle, with a diameter around 500 nm and the Fig. 9(b) shows some broken microfibrils, with around 20 nm in diameter, in the microfibril bundle.

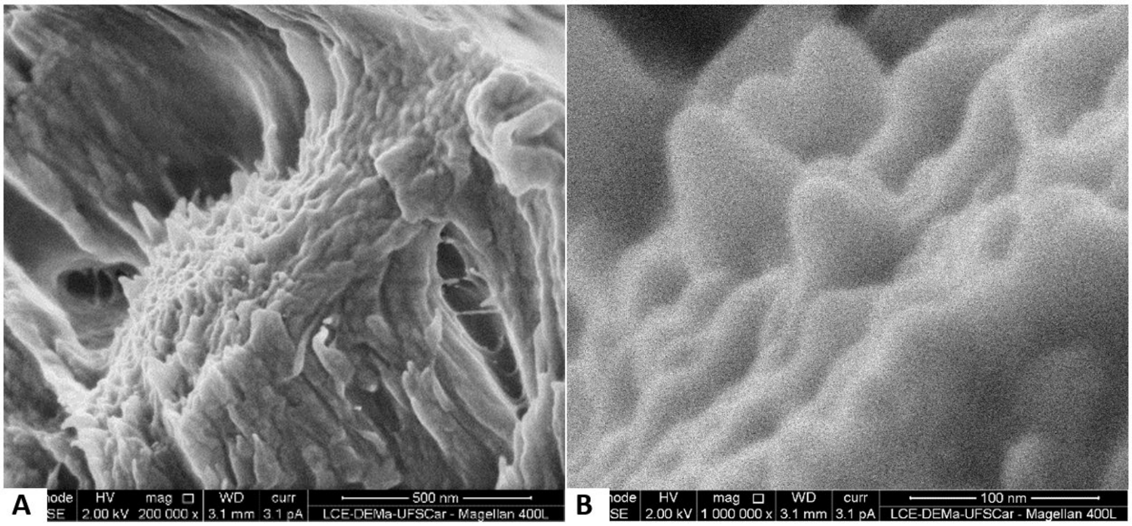


Figure 9: FESEM microscopy images, in greater resolution, of the sclereid cell wall: (a) a cellulose microfibril bundle in the sclereid cell wall; (b) some broken microfibrils in a microfibril bundle [8]

At the cell walls ultrastructural level, the structural polymers (cellulose, hemicelulose and lignin) arrangement could be compared as a composite material, with the cellulose fibrils acting as a fiber disperse phase, involved by and lignin matrix, with the hemicelluloses acting as a compatibilizer [12,13,14]. In this way, cellulose determines the tensile strength [15-17], while the hemicelluloses and lignin play a more dominant role in the compressive strength [16,18]. At the cellular level, the fibers cells, due to its thin and elongated morphology, tend to determine the tensile strength in the material, while the sclereids cells, due to their thick and lignified (rigid) cell walls, ensure a good behavior related to the compressive strength and fracture toughness [19]. Regarding Fig. 8(a) and Fig. 7(e), the sclereids and fibers cell walls found are thick, occupying a vast volume of the cell. Considering the rigid and lignified [8] characteristic of the secondary walls found, typical of the sclerenchyma tissues [20,21], combined with the cell arrangement found in the Fig. 7(a), where the fiber cell bundles are acting together with the sclereid cells as a composite structure, it was proposed to be to that two characteristics the reason why the mesocarp shows a relative high stiffness and good mechanical behavior.

Schüler et al. [22] compared the crack resistance of different nuts shells/coats and found a much higher strength for the Macadamia (*Macadamia integrifolia*) seed coat. Unlike others nuts shells/coats, formed by compact arrangements of sclereid cells [23], the cell arrangement found in the Macadamia seed coat was similar as the found in the Brazil nut fruit mesocarp, where fiber cell bundles and sclereids are acting together as a composite structure, as shown in the Fig. 7(a). Considering the natural selection of the species, one possible reason for the Brazil nut fruit mesocarp to present a high mechanical resistance, including the composite like cell arrangement found, would be the fact that the mature fruit had to withstand a drop of around 50 meters, weighing about 500 g [8], without breaking, a quite peculiar requirement compared to other species.

Based on this differentiated characteristics of the Brazil nut fruit mesocarp, it is expected that the proper incorporation with an adequate polymer matrix can generate great NLF composites, with differentiated proprieties. Petrechen [8], incorporated Brazil nut mesocarp particles (NLF) with different polypropylene (PP) matrices and the biocomposites obtained showed significant improvements in several mechanical properties, compared with the PP matrix and with other similar particulate NLF biocomposites [24]. In that way, as already stated for the case of Macadamia seed shell [22,25,26], the Brazil nut fruit mesocarp, due its outstanding mechanical characteristics, could well serve as NFL for biocomposites, or as a hierarchical architecture model for biomimetics materials.

4 Conclusion

Brazil nut tree (*Bertholletia excelsa*) fruit pericarp is an abundant source of inexpensive, renewable and high quality lignocellulosic material. It presents three layers: the exocarp, a dark gray, brittle and fragile outer layer; the mesocarp, a beige, dry, rigid, impermeable and fibrous intermediate layer; and the endocarp, an inner layer with similar characteristic as the exocarp, but formed next to the seeds. Morphologically, the exocarp and the endocarp presented minor regions of sclereids, fibers and vascular cell bundles, inside major regions of parenchyma cells. The mesocarp presents a structure of fiber cells regions alternating with sclereids and vascular cells regions, arranged in a composite like arrangement, with the fibers cells bundles acting as randomly oriented disperse phases in a sclereid cells matrix. This arrangement was associated with the mesocarp relative superior mechanical proprieties, indicating the mesocarp as being a very promissory lignocellulosic material for using as filler in biocomposites or as a hierarchical architecture model for biomimetic materials, among others newer applications.

Acknowledgement: The authors would like to thank the CNPq for the scholarship of the student Gustavo P. Petrechen and the CCDM/UFSCar for the material and laboratory resources.

References

1. Donadio, L. C., Môro, F. V., Servidone, A. A. (2002). *Frutas Brasileiras.* Novos Talentos.
2. Martins, L., Silva, G. Z. P., Silveira, B. C. (2008). Produção e comercialização da castanha do Brasil (*Bertholletia excelsa, hbk*) no estado do Acre-Brasil, 1998-2006. *XLVI Congress of the Brazilian Rural Economy, Administration and Sociology Society.*
3. Shanley, P., Medina, G. (2005). *Frutíferas e plantas úteis na vida amazônica.* Center for International Forestry Research-CIFOR.
4. de Souza, M. (1984). *Estudos de processos tecnológicos para obtenção de produtos da Castanha-do-Brasil (Bertholletia excelsa, HB K.) (Ph.D. Thesis).* Food Technology Department, Federal University of Ceará, Fortaleza-CE, Brazil.
5. Soares-Filho, B. S., Nepstad, D. C., Curran, L. M., Cerqueira, G. C., Garcia, R. A. et al. (2006). Modelling conservation in the Amazon basin. *Nature, 440(7083),* 520.
6. Rivero, S., Almeida, O., Ávila, S., Oliveira, W. (2009). Pecuária e desmatamento: uma análise das principais causas diretas do desmatamento na Amazônia. *Nova Economia, 19(1),* 41-66.
7. Angelo, H., Pompermayer, R. S., de Almeida, A. N., Moreira, J. M. M. Á. P. (2013). O custo social do desmatamento da Amazônia brasileira: o caso da castanha-do-brasil (*Bertholletia excelsa*). *Ciência Florestal, 23(1),* 183-191.
8. Petrechen, G. P. (2017). *Caracterização dos materiais lignocelulósicos da Castanha do brasil (Bertholletia excelsa), preparação e caracterização de seus compósitos com polipropileno (Msc. Dissertation). Materials engineering department.* Federal University of São Carlos, São Carlos-SP, Brazil.
9. United Nations (2015). *2014 database*. Agriculture Statistics Division-FAOSTAT.
10. Lucas, A. A., Ambrósio, J. D., Bonse, B. C., Bettini, H. S. P. (2011). Natural fiber polymer composites technology applied to the recovery and protection of tropical forests allied to the recycling of industrial and urban residues. *Advances in Composite Materials-Analysis of Natural and Man-Made Materials*, pp. 163-194.
11. Ramamoorthy, S. K. (2015). A review of natural fibers used in biocomposites: plant, animal and regenerated cellulose fibers. *Polymer Reviews, 55,* 107.
12. Gibson, E. J. (1992). Wood: a natural fibre reinforced composite. *Metals and Materials, 8(6),* 333-336.
13. Rowell, R. M. (2012). *Handbook of wood chemistry and wood composites*. CRC Press.
14. Pothan, L. A. (2009). Natural fibre reinforced polymer composites: from macro to nanoscale. *Archives*

Contemporaines.

15. Salmén, L. (2004). Micromechanical understanding of the cell-wall structure. *Comptes Rendus Biologies, 327, 9(10),* 873-880.
16. Bergander, A., Salmén, L. (2002). Cell wall properties and their effects on the mechanical properties of fibers. *Journal of Materials Science, 37(1),* 151-156.
17. Fratzl, P. (2003). Cellulose and collagen: from fibres to tissues. *Current Opinion in Colloid & Interface Science, 8(1),* 32-39.
18. Hult, L. E., Larsson, P. T., Iverson, T. (2001). Cellulose fibril aggregation-an inherent property of kraft pulps. *Polymer, 42(8),* 3309-3314.
19. Mishnaevsky, J., Leon, R., Qing, H. (2008). Micromechanical modelling of mechanical behavior and strength of wood: state-of-the-art review. *Computational Materials Science*, *44(2),* 363-370.
20. Raven, P. H., Evert, R. F., Eichhorn, S. E. (2005). Biology of plants. *Macmillan*.
21. Appezzato-da-Glória, B., Carmello-Guerreiro, S. M. (2006). *Anatomia Vegetal.* UFV.
22. Schüler, P., Speck, T., Bührig-Polaczek, A., Fleck C. (2014). Structure-function relationships in Macadamia integrifolia seed coats-fundamentals of the hierarchical microstructure. *PLoS One, 9(8),* e102913.
23. Esau, K. (1977). *Anatomy of seed plants*. Wiley-VCH.
24. Sobczak, L., Lang, L., Reinhold, W., Haider, A. (2012). Polypropylene composites with natural fibers and wood-General mechanical property profiles. *Composites Science and Technology, 72(5),* 550-557.
25. Fleck, C., Schüler, P., Meinel D., Zaslansky, P., Currey J. D. (2012). Microstructural features influencing failure in Macadamia nuts. *Bioinspired, Biomimetic and Nanobiomaterials, 1,* 67.
26. Wang, C., Mai, Y. (1995). Deformation and fracture of Macadamia nuts. *International Journal of Fracture, 69,* 67.