

Evaluation of Microstructure of Açai Seeds Biomass Untreated and Treated with H₂SO₄ and NaOH by SEM, RDX and FTIR

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The açai seed is an abundant byproduct of açai productive chain, Brazil's northern region important fruit, especially in Pará and Amazonas states. However, it has been characterized as a hygienic-sanitary inconvenience to the region big cities, respectively Belém and Manaus. The use of this material to generate high added value products depends on better knowledge of its microstructure. This work aimed to analyse açai seeds biomass using Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) in order to better understand its structural and chemical changes during treatment stages. For this purpose untreated, treated with H₂SO₄ and treated with H₂SO₄ and subsequently delignified with NaOH açai seeds were used. SEM photomicrographs comparison showed that treatment with H₂SO₄ resulted in partial disruption of açai seed structure, which became more evident after NaOH treatment. The crystallinity index for untreated açai seeds was 47.05%, reducing after the treatments. FTIR provided spectra in the region of 4000 to 400 cm⁻¹. The used techniques allowed to observe significant structure changes caused by açai seeds biomass treatment and provided information about microstructure of the untreated material.

1. Introduction

Non-renewable fuels utilization is responsible for major environmental problems over time, such as global warming, climate change and health related problems. Along with global awareness about clean technology needs, a series of research efforts has emerged intending to propose viable alternatives economically and environmentally clean (Aransiola et al., 2014).

Around the world, lignocellulosic material is the most diverse and has shown great potential to ethanol fuel production (Rabelo et al., 2011). Besides its large availability as agroindustry residue, these materials are advantageously renewable (Singh and Trivedi, 2013).

In Brazil sugarcane is intensely used in ethanol production. Therefore its residues (bagasse and straw) utilization would be the first alternative to lignocellulosic ethanol production (Moraes et al, 2015). However Brazil's north region does not produce sugarcane, impeding these material use for ethanol production. Still it has a strong açai production agroindustry, regional fruit widely traded. Much of the post processed açai (juice production) is disposed as seed, which is rich in lignocellulosic material (Oliveira, 2014).

Açai seeds biomass utilization to produce fermentable sugars by chemical treatment (Oliveira et al., 2014) and enzymatic hydrolysis (Oliveira et al., 2015) are one of ways to obtain ethanol fuel. Besides being an excellent alternative on utilizing the large volume of this residue generated daily in northern Brazil big cities, such as Belém and Manaus (Oliveira, 2014).

Lignocellulosic biomass ethanol production demands good knowledge of the material structure used in the biotechnological transformation. During ethanol production steps a serie of modifications occur in the utilized biomass, changing its microstructures due to the transformation agents (Alnuami et al., 2014).

Good understanding of these transformations enable the creation of enhanced methods and processes in the material utilization.

A few techniques already widely known in scientific community such as Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) can be used to deeply observe chemical and structural modifications in the transformed material, in the treatment and enzymatic hydrolysis steps (Balaji et al., 2015)

Thereby it is proposed to observe the chemical and microstructural behavior of açai seed biomass before and after treatments, in order to obtain new information on this little known biomass, as well as the chemical treatment effects on açai seed microstructure. Thus seeking more information about the potential of açai seed for the production of ethanol.

2. Material and methods

2.1 Raw material

The açai seeds were obtained in Belém - Pará - Brazil (01° 27' 21" S, 48° 30' 16" W) and were dried for 48h at 50°C in an air circulating oven and left for 48h at room temperature. The material was prepared according to Oliveira et al. (2015).

2.2. Treated and analyzed açai seeds

Açai seeds used on this work were: (1) - Raw açai seed obtained according to item 2.1. (2) – Açai seed treated with H₂SO₄ (10% of solid, 1% of acid for 60 minutes at 121°C), (3) - Açai seed treated with H₂SO₄ (10% of solid, 1% of acid for 60 minutes at 121°C) and subsequently delignified (25% of solids, 1% of peroxide at 70°C during 60 minutes). Both H₂SO₄ treatment and sequential delignification were performed in retort at 120°C.

2.3 Imaging and spectroscopic techniques applied in açai seeds characterization.

Untreated açai seeds, treated with diluted H₂SO₄ and delignified, were analyzed using Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD) and *Fourier Transform Infrared Spectroscopy* (FTIR).

2.3.1. Scanning Electron Microscopy (SEM)

The micrographs were obtained using a Scanning Electron Microscope (SEM) with Energy Dispersive X-Ray Detector (EDX), model Leo 440i and EDS 6070, brand MEV/EDS: LEO Electron Microscopy/Oxford (Cambridge, England). Initial acceleration tension of 20kV and beam current of 100 pA were used.

2.3.2. X-Ray Diffraction (XRD)

Cellulose crystallinity index were determined through X-Ray Diffraction (XRD) to açai seeds treated with diluted H₂SO₄ and delignified. Biomass crystallinity index (CrI) is defined as biomass crystalline material percentage and was calculated according to Segal et al., (1959).

2.3.3. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was used in chemical structures and functional groups identification in the evaluated samples based on Silva (2009) methodology.

3. Results and Discussions

3.1. Scanning Electron Microscopy (SEM) results

As observed in Figure 1(a) the material has some cut points and ruptures as a grinding process result. Comparing Figures 1(a) and 1(b) a partial disorganization in the material structure is verified, due to the acid treatment, occasioning ruptures and fissures, which are related to lignin and carbohydrates chemical bonds disruption (Hendriks and Zeeman, 2009).

Great disorder of biomass structure was observed in Figure 1(c) after delignification of H₂SO₄ treated açai seeds. It becomes visibly more porous and amorphous in comparison to untreated material (a) or H₂SO₄ treated (b). As also verified by Zúñiga (2010) this material heterogeneity is a complicating factor to more efficient MEV biomass structure evaluation. In Figure 1 (a, b and c) are presented photomicrographies of treated and untreated açai seeds.

3.2. X-Ray Diffraction (XRD) crystallinity index results

In Figure 2 is shown X-Ray Diffraction calibration curves used to calculate the crystallinity index observed in Table 1. Studied samples analysis showed açai seeds crystallinity index of 47.05%, smaller then described by Rodriguez-zúñiga (2010) to sugarcane bagasse (58.18%) and by Ayala (2012) to sugarcane straw (49.01%). Crystallinity index after acid treatment (in analyzed conditions) reduced only 11% in comparison to untreated material. treated and subsequently delignified material displayed 64% reduction in comparison to raw material. As showed in Table 1, crystallinity index fixed to yield basis was 41.64% to H₂SO₄ treated material and

17.01% after delignification. Similar results were founded by Samuel et al. (2012) who noted that diluted H_2SO_4 treatment promoted 12% reduction in switchgrass biomass crystallinity. To the treated and subsequently delignified material showed 64% reduction in comparison to untreated material. As shown in Table 1, crystallinity index corrected to yield basis is 41.64% to acid treated material and 17.01% after delignification.

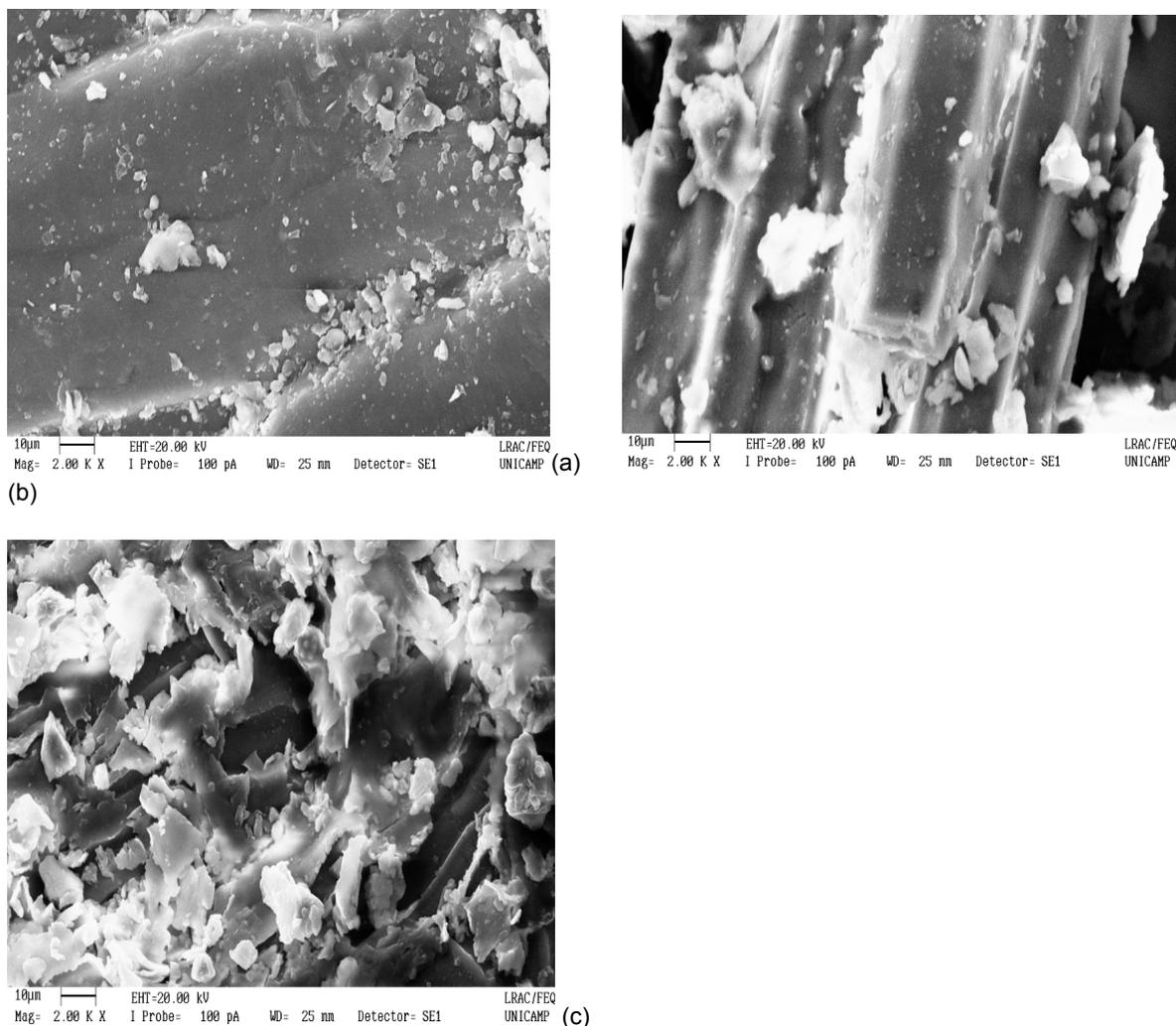


Figure 1. Untreated açai seed (a), post diluted H_2SO_4 treatment (b) and post diluted H_2SO_4 and subsequently delignified (c) photomicrographs.

Thereby, it is possible to claim the delignification was highly effective in reducing açai seed crystallinity, which happens most likely due to lignin and hemicellulose removal than for cellulose I to cellulose II conversion. The evidence of this standpoint is in the diffractogram peaks in untreated material and treated and subsequently delignified material, it shows 2θ peaks in 16° (002 plan) and 18° (101) which corresponds to cellulose I (Terinte et al., 2011). Therefore it is not possible to claim the reduction in crystallinity is result of cellulose I to cellulose II conversion.

Ben Sghaier et al. (2012) treated American Agave fibers and observed NaOH concentrations above 5% cause crystallinity reduction of cellulose fibers around 15%, which was caused by fiber swelling after alkaline agent penetration.

The same authors claim that, during treatment, cellulose fibers are rearranged from cellulose I (parallel aligned chains) to cellulose II (anti-parallel). What was not observed to the açai seed mercerizing probably as result of the alkaline agent low concentration. Previous research report cellulose I to cellulose II transformation occurs in NaOH concentrations above 6% (Moigne and Navard, 2010).

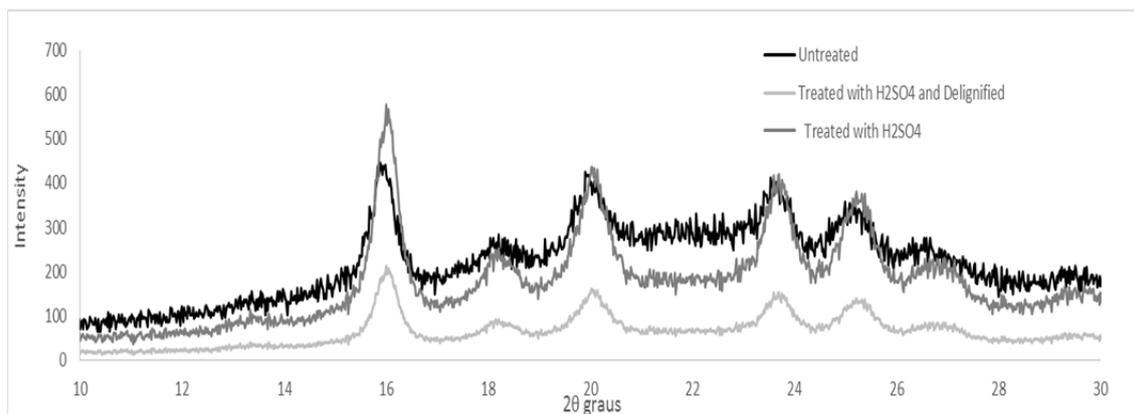


Figure 2. X-Ray Diffraction calibration curves used to calculate the crystallinity index

Table 1. Crystallinity index (Crl) obtained to untreated açai seeds, treated with diluted H_2SO_4 and treated with acid and subsequently delignified.

Açai seeds	Crystallinity Index	Treatment Yield (%)	Treatment Correction (%)
Untreated	47,05	-	-
Treated with H_2SO_4 dilute	64,07	65	41,64
Treated with H_2SO_4 and delignified	71,35	23,85	17,01

3.3. Fourier Transform Infrared Spectroscopy (FTIR) results

FTIR was used to investigate açai seeds physical structure and functional groups. Thereby, in Figure 3, it is possible to verify FTIR spectra in the 4000 to 400 cm^{-1} range in the present work.

The spectra main characteristics were assigned to presence of lignin, hemicellulose and cellulose, natural fibers typical components. In this kind of material, modified or not, is common to observe a 3100-3500 cm^{-1} band appearing, a broadband, associated to the O-H group axial deformation. In this way untreated açai seed fibers spectra was characterized by a 3411 cm^{-1} band, which refers to the OH groups vibrational stretch (Merlini, 2011). To the treated or delignified analysed material a drastic decrease in absorptivity is observed, due to cellulose removal during treatment.

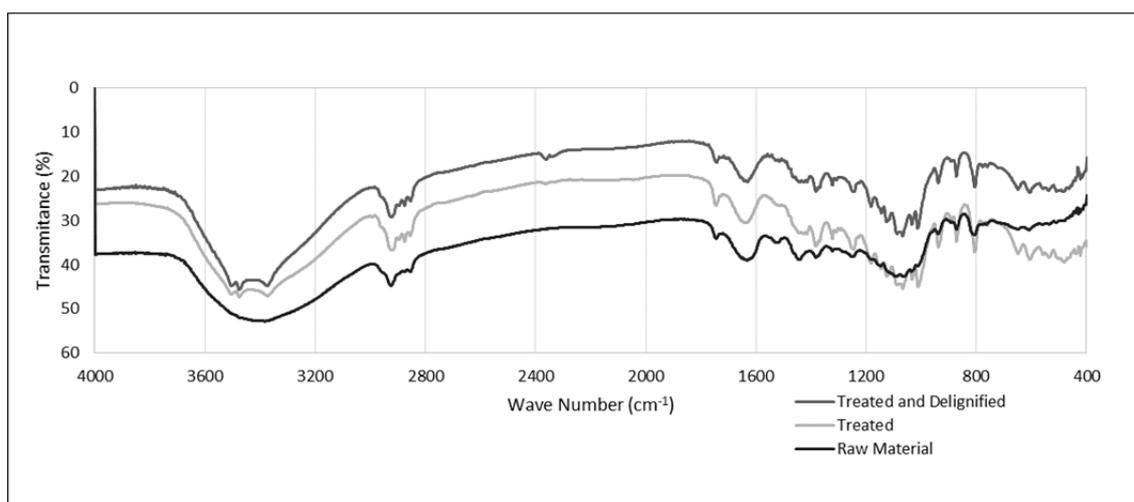


Figure 3. FTIR spectra to untreated açai seed (raw material), treated and treated and subsequently delignified. Each of the following numbers points to identified and discussed peaks: (1) 3411; (2) 2921; (3) 2875; (4) 2852; (5) 1743 (6) 1629 (7)1517 (8)1445 (9)1378 (10) 1321; (11)1245; (12) 1160; (13) 128; (14)1099; (15) 1010; (16)1060; (17) 937; (18)871; (19) 804.

The 2894-2950 cm^{-1} can be assigned to the C-H stretching, such as observed by Khalil et al. (2001). On this band the 2921, 2875 and 2852 cm^{-1} peaks were observed, which are related to the C-H aliphatic stretching of the methyl (CH₃) and methylene (CH₂) groups (Sinha and Rout, 2008). As pointed by Kim et al. (2003) the bands intensity related to characteristic lignin peaks (1502-1600 cm^{-1} range) was bigger to the acid treated material and much bigger to the treated and subsequently delignified material, which proves the lignin removal treatments effect.

The 1742 cm^{-1} peak in untreated material is characteristic of the carbonyl (C=O) stretching, assigned to the hemicellulose (Owen and Thomas, 1989) in açai seeds and which presented lower intensity to the treated and delignified material, showing hemicellulose removal in the treatment process. The 1629 cm^{-1} peak represent residual water in all presented spectra (Nacos et al., 2006; Troedec et al., 2008).

Intensity difference between 1510 and 1600 cm^{-1} bands can be used to distinguish conifer lignin (high content of guaiacyl groups) from broadleaf lignin (higher content of syringyl) (Owen and Thomas, 1989; Colom et al., 2003; Fengel and Wegener, 2003). Comparing the açai seed existing bands, it is possible to observe the 1600 cm^{-1} band presents the highest intensity, which indicates the analysed material lignin has higher content of syringyl units.

According to the literature, the 1517, 1445, 1378 and 1321 cm^{-1} peaks are related to the C-C bond in the aromatic, methyl group asymmetric deformation, C-H stretching in polysaccharides and hydroxyl angular deformation (-OH) in the lignin aromatic ring (Nacos et al., 2006; Troedec et al., 2008).

The 1245 cm^{-1} peak band is related to acetyl group stretching of hemicellulose, however lignin also contributes to this band formation. The 1160, 1128 and 1099 cm^{-1} peaks band indicates C-O-C asymmetric stretching in cellulose and hemicellulose.

The 1010, 1060 and 1164 cm^{-1} peaks (not showed in Figure 3) are characteristic of the C-O, C=C and C-C-O groups stretching of cellulose, hemicellulose and lignin (Sills et al., 2012). The peaks absorptivity values in acid treated material showed higher values than the untreated and delignified ones. It has happened due to hemicellulose removal during acid treatment, showing lignin's structure in hemicellulose absence. It is possible to verify drastically reduced absorptivity after delignification, which exhibits lower values than the untreated material and H₂SO₄ treated ones.

Finally, the 937, 871 and 804 cm^{-1} peaks, observed in all materials, could be assigned to the C-O-C stretching in the β -glucosides bonds (Ciolacu et al., 2011).

4. Conclusions

It was possible to conclude the used tools to untreated and treated açai seed microstructure evaluation allowed a better understanding of the material and the treatment effects on the material modification point of view. SEM imaging showed more effective lignocellulosic material disruption after H₂SO₄ and delignification treatment, which also showed the lowest crystallinity after lignin and hemicellulose removal. Whereas FTIR allowed to verify important chemical alterations in the analysed treated materials.

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