EXTRACTION OF LIGNIN AND OTHER PHENOLIC COMPOUNDS FROM BABASSU MESOCARP WITH EUTECTIC SOLVENTS

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ABSTRACT

Babassu coconut (*Orbignya phalerata* or *Attalea speciosa*) is an important fruit in Brazil's economy. In the extraction of the oil from the kernel, a large amount of mesocarp biomass as a by-product is generated. This biomass is rich in lignocellulosic material and phenolic compounds, presenting an opportunity for valorization. Due to its advantages, eutectic solvents have emerged as a promising green alternative for the extraction of lignin and phenolic compounds in substitution of conventional methods. This study investigates the extraction of lignin and phenolic compounds from babassu mesocarp biomass using eutectic solvents. The biomass was treated with two different eutectic solvents, choline chloride:acetic (CCAA) acid and L-proline: acetic acid (PROAA), at various temperatures (70°C, 100°C, and 130°C). CCAA was more effective in lignin extraction than PROAA, particularly at higher temperatures. UV/Vis spectrophotometry and HPLC-MS analysis indicated the presence of lignin and phenolic compounds such as epicatechin gallate and procyanidin B dimer. FTIR spectroscopy confirmed successful delignification in treated samples. The study concludes that eutectic solvents, especially CCAA, effectively extract lignin from babassu mesocarp, presenting a viable method for biomass valorization.

Keywords: Babassu mesocarp. Biomass valorization. Eutectic solvents.

1 INTRODUCTION

Babassu coconut (*Orbignya phalerata* or *Attalea speciosa*) is a valuable oleaginous fruit, typical of the South American Amazon region, especially in Brazil's northern states of Maranhão, Tocantis and Piauí, occupying about 196 thousand km² of territory. The kernel, from which is extracted the babassu oil, only represents just 7% of the total weight of the fruit. While the other parts, like the endocarp, mesocarp, and epicarp (59, 23 and 11%, respectively) of the total weight of the fruit, are considered residual biomass of low added value[1]. After processing to extract oil, large quantities of residual mesocarp biomass are generated and this by-product normally finds reuse as an ingredient in animal feed or is consumed as flour in the human diet. However, the babassu mesocarp is considered a lignocellulosic biomass and is rich in phenolic compounds, including lignin[2].

Lignocellulosic biomass is considered one of the most important natural sources of organic carbon, presenting as an alternative renewable energy resource and feedstock for platform chemicals production. Most existing processes for lignocellulosic biomass suffer from low selectivity during pre-treatment, often neglecting lignin. This fraction holds significant biotechnological potential, as an abundant source of aromatics and could be converted into a range of value-added compounds[3].

Eutectic solvents can be defined as mixtures of two or more components, where one acts as a hydrogen bond acceptor and the other as a hydrogen bond donor, forming a eutectic mixture with a lower melting point than the pure components. Their remarkable properties, including the ability to solubilize lignin, recyclability, low vapor pressure, and relatively low toxicity pose the eutectic solvents as a promising alternative pre-treatment method to valorize lignocellulosic biomasses[4]. Furthermore, several studies have also been applying DES in the extraction of other phenolic compounds in a variety of biomasses in alternative to the extraction with conventional organic solvents, such as ethanol, methanol, and acetone[5].

Therefore, this work aims to test two different eutectic solvents in the extraction of lignin and other phenolic compounds from babassu mesocarp biomass.

2 MATERIAL & METHODS

The residual biomass from babassu mesocarp was acquired in the local market at Centro Luiz Gonzaga de Tradições Nordestinas (Rio de Janeiro, Brazil). The biomass was washed with distilled water, dried for 24 h, crushed, and sieved (28–35 mesh).

Two eutectic solvents, choline chloride:acetic acid (CCAA) and L-proline:acetic acid (PROAA), both at molar ratio 1:2, were prepared by heating the mixture of components at 80°C until a homogeneous liquid was formed. The treatment with the ES was conducted by mixing 0.25g of the biomass with 2.5g of each solvent in glass tubes. These tubes were heated for 2 hours at three different temperatures, 70°C, 100°C and 130°C, to test the effect of temperature in lignin extraction. After the treatment, the samples were centrifugated at 5000 rpm for 10 min to separate the liquid and solid fractions.

Raw and treated babassu mesocarp were analyzed by ATR-FTIR spectroscopy from 400 – 4000 cm^{-1} in IRTracer 100 (Shimadzu®).

The liquid fractions obtained in dissolution experiments were analyzed using UV/VIS spectrophotometry with a SpectraMax E2 (Thermo Scientific®) spectrophotometer. Absorbance within the 200 to 700 nm spectral range was measured with 2 nm of spectral resolution. The liquid fraction was diluted in 1,4-dioxane and the lignin concentration was measured by spectrophotometry, with the absorbance measured at 280 nm. Alkali lignin was used as a standard to construct a calibration curve.

40 mL of distilled water were added to the liquid fraction and the tubes were placed at 4°C, to precipitate the lignin dissolved. After 24 hours, the samples were centrifugated at 10000 rpm for 10 min. The solid obtained were washed with distilled water, centrifugated again and freeze-dried. The supernatant was collected and liquid-liquid extraction was performed with the following solvents in series: cyclohexane, ethyl acetate and n-butanol.

Each fraction was collected, diluted in methanol and analyzed by HPLC-MS using a Dionex Ultimate 3000 HPLC system (Thermo Scientific with a C18 column at 40°C at a flow rate of 0.5 mL/min. The solvents used as the mobile phase were water with 0.1% formic acid (A) and methanol (B). The gradient flow was as follows: 0-10min: 98% A; 10-35min: linear gradient from 2 to 50% B; 35-43min: 50% B; 43-45min: linear gradient 2-50% B. The eluate was injected in Ion Trap 3D LCQ mass spectrometer (Thermo Scientific) with an electrospray ionization probe operating in negative ionization mode, scanning the ions in the m/z range from 100 to 1000.

3 RESULTS & DISCUSSION

The characterization of raw and treated babassu mesocarp confirms the delignification as the main peak associated with lignin aromatic ring stretching (~1520 cm^{-1}) are present in raw biomass and absent in treated biomass (Figure 1). The UV/Vis spectrum also indicates the dissolution of the lignin in the eutectic solvents, due to the presence of a peak around 280 nm (Figure 2), corresponding to the phenolic absorption of the lignin[6].

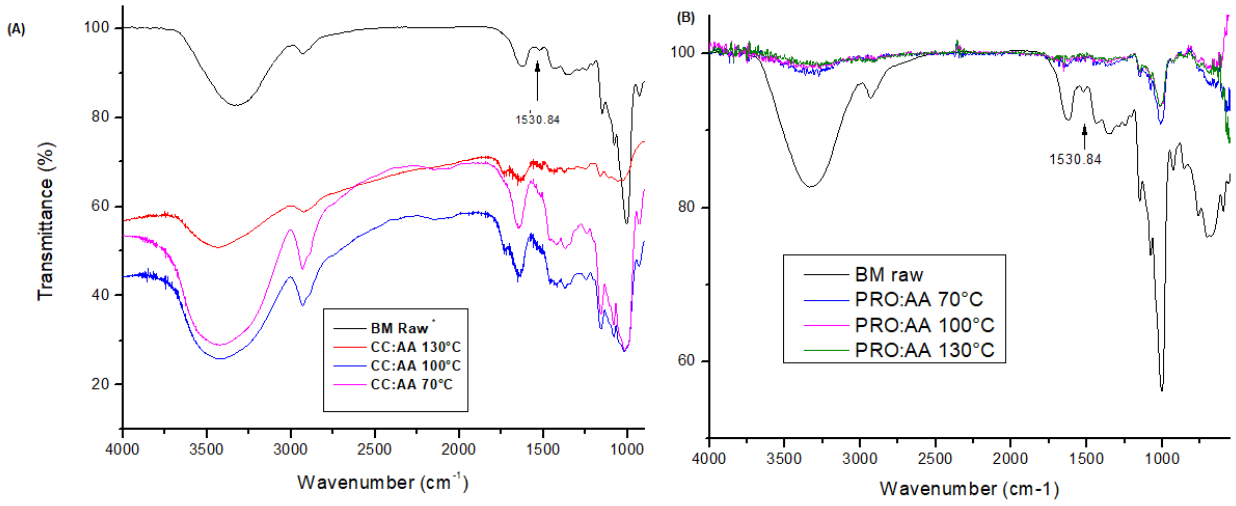

Figure 1 FTIR spectrum of raw babassu mesocarp and treated with (A) CCAA and (B) PROAA at different temperatures

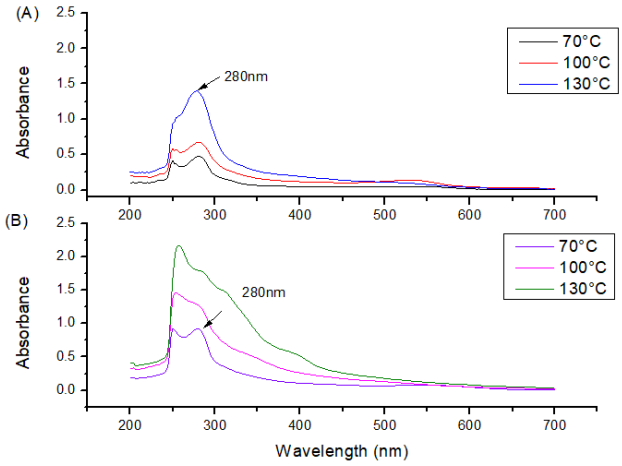

Figure 2 UV/Vis spectrum of liquid fractions of (A) CCAA and (B) PROAA treatment.

Several factors influence the extraction of lignin with eutectic solvents. Table 1 summarizes the yield of lignin recovered from the babassu mesocarp biomass in the treatment with CCAA and PROAA at different temperatures (70, 100 and 130°C). Temperature and lignin yield normally have a positive correlation, as higher temperatures promote higher lignin extraction[7]. From the results, it can be observed that the treatments with CCAA the increase in temperature had a strong positive influence on the dissolution of lignin, in contrast to the treatment with PROAA, where the increase in temperature did not increase dissolved lignin. In the case of PROAA, the viscosity of the solvent could have hindered the dissolution as it impacts the mass transfer rate and lowers the interaction between the solvent and the biomass matrix.

Table 1 Lignin concentration in liquid fractions after treatment and lignin recovery after precipitation

Eutectic solvent	Extraction temperature (°C)	Lignin concentration (mg/mL)	Lignin recovery (mg/g biomass)
CCAA	70	2.35±0.27	8.99±0.32
	100	9.78±0.41	44.12±0.74
	130	10.51±0.31	34.01±0.12
PROAA	70	3.09±0.44	18.91±0.22
	100	2.33±0.15	24.37±0.76
	130	2.76±0.37	16.20±0.33

In addition to lignin, other phenolic compounds were found in the extract. After the liquid-liquid extraction, the analysis in HPLC-MS demonstrated the presence of several peaks detected at 280 nm absorbance (Figure 3). From all these peaks, only 2 compounds were successfully identified in CCAA extracts: epicatechin gallate (26.92 min) and procyanidin B dimer (21.01 min). The m/z relation of precursor ion (441 and 577, respectively) and the fragments generated in electrospray ionization were used to identify in comparison to literature results[8].

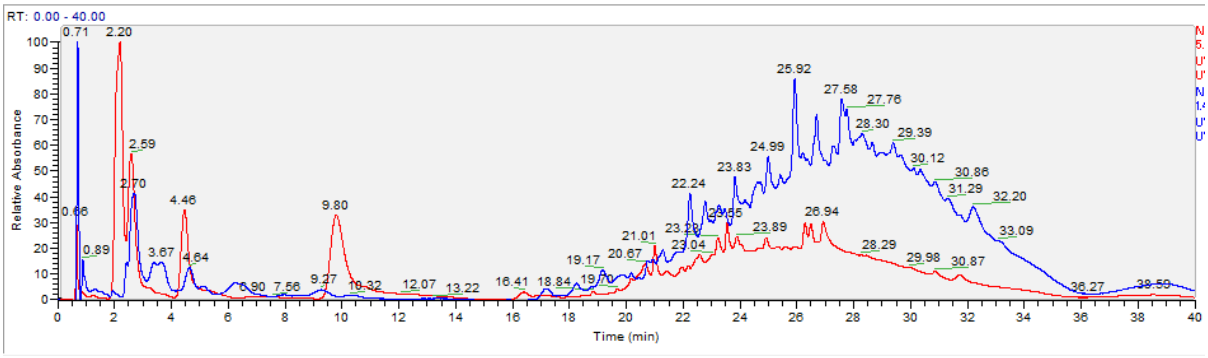

Figure 3 Chromatograms of ethyl acetate extract obtained from CCAA (red) and PROAA (blue) treatment at 100°C

4 CONCLUSION

Both eutectic solvents tested could promote some dissolution of the lignin of babassu mesocarp biomass. CCAA reached higher dissolution than PRO:AA, with the best conditions for dissolution at 100°C and 130 °C. The recovery of the lignin with antisolvent addition was higher in samples treated at 100°C for both eutectic solvents. Furthermore, HPLC-MS analysis demonstrated other phenolic compounds were extracted, with the presence of several peaks with absorption at 280 nm. Among these, two molecules could be identified due to the fragmentation profile of precursor ions, epicatechin gallate and procyanidin B dimer.

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