Comparative Assessment for Forage Palm and *Agave sisalana* Biomass for Sustainable Biofuel Production

Valdeci Machado[1*], Evily Ribeiro Moura[1], Gabrielly de Moura Bezerra[1], Luciano Sergio Hocevar[1], Carine Tondo Alves[1], Marcos Machado da Rocha[1]

[1]*Federal University of Recôncavo of Bahia; Feira de Santana, Bahia, Brazil*

In recent years, interest in semiarid biomass has surged. This study examines the physical-chemical properties of *Opuntia fícus indica* Mill (*Palma forrageira*) and *Agave sisalana* Perrine (*Agave sisalana*). This study aimed to characterize these biomasses by analyzing key structural components: moisture content, ash content, cellulose, hemicellulose, and lignin. The results reveal that *Opuntia fícus indica* Mill contains 78.65% moisture, 3.65% ash, 54.11% cellulose, 18.60% hemicellulose, and 21.08% lignin. In contrast, *Agave sisalana* Perrine exhibits 82.36% moisture, 11.65% ash, 63.39% cellulose, 5.80% hemicellulose, and 18.67% lignin. Forage Palm, with its higher cellulose and hemicellulose content, may be better suited for biofuel production. Meanwhile, *Agave*'s elevated ash content warrants consideration when evaluating its combustion quality and energy yield. Balancing lignin levels in both biomasses is crucial for efficient utilization.

Keywords: Biomass. Biofuels. *Opuntia fícus indica* Mill. *Agave sisalana* Perrine.

The semiarid region of Brazil is home to a wide variety of plant species that have adapted to the harsh climate and soil conditions. Two noteworthy plants in this region are the forage palm (*Opuntia fícus indica* Mill) and *Agave sisalana* (*Agave Sisalana* Perrine), which have evolved to thrive in water-scarce environments with high temperatures, playing essential roles in the local economy and ecology [1]. There is increasing interest in sustainable energy sources, with bioethanol emerging as a promising option. Bioethanol is produced through the fermentation of materials rich in fermentable sugars or carbohydrates, leading to efforts focused on non-food biomass generation. This includes lignocellulosic biomass derived from forest residues, woody and herbaceous plants, non-food crops, municipal solid waste, and animal fat [2]. It is important to study the flora of the semiarid region to uncover its biomass potential.

Analyzing the physical-chemical composition of these resilient plants is crucial for developing technologies that support sustainable energy production and address the social and economic development needs specific to the semiarid region. This study is critical to diversifying Brazil's energy sources.

The forage palm, commonly used as livestock feed in the semiarid region, shows drought resistance and has high biomass productivity [1]. Apart from its role in local agriculture, this succulent plant holds significant promise for bioenergy production, contributing to the expansion of renewable energy sources in Brazil. Meanwhile, *Agave sisalana* Perrine, the predominant species cultivated in the Brazilian semiarid region, belongs to the Agavaceae family. Renowned for its robust fiber rich in cellulose and lignin, *Agave* has various industrial applications. Adapting to semiarid conditions further underscores its potential as a valuable biomass resource.

This study aimed to characterize some physical-chemical properties of Forage palm and *Agave sisalana* biomass, informing sustainable practices and contributing to diversifying Brazil's energy matrix.

Materials and Methods

This study evaluated the moisture, ash, lignin, cellulose, and hemicellulose of *Agave sisalana*

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Address for correspondence: Valdeci Machado.Av. Centenário, 697 - Sim. Zipcode: 44042-280. Feira de Santana, Bahia, Brazil. E-mail: valmachado@aluno.ufrb.edu.br.

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and forage palm. All experiments were conducted in triplicate at the Laboratory of Energies (LEN) at the Federal University of Recôncavo of Bahia (UFRB). Samples of *Agave* were collected in the city of Araci-BA at CETEP SISAL II, located at 11°20'20.6" S 38°57'45.4" W on April 5, 2024.Samples of *Opuntia* spp. were collected at CETENS-UFRB in Feira de Santana-BA at 12°15'14.0 "S 38°55'34.0 "W on May 10, 2024. The flour production involved 829.98 g of forage cactus and 956.88 g of *Agave*, dried at 60°C for 72 hours, ground, and sieved.

The moisture content was determined using a digital moisture balance (Belithermo G163L) on samples of forage palm and *Agave sisalana*, dried at 105 °C in the oven for 50 minutes in triplicate. In the second stage, ash content was determined by drying the biomasses at 105 °C in an oven for 24 hours, weighing them, and then placing them in a muffle furnace at 575 °C for 24 hours. After cooling, the ash content was obtained following the method proposed by Sluiter and colleagues (2005)[4].

The method used in this study for cellulose determination was proposed by Wright and Walles [5]. It involved preparing a 1 L solution containing 90 mL of nitric acid, 732 mL of glacial acetic acid, and distilled water. Three samples weighing 1 g of dried biomass each were prepared (Fraction A), and the material was transferred to a 50 mL flat-bottom flask. A 25 mL aliquot of the nitric acid-glacial acetic acid solution was added, and the mixture was refluxed for 25 minutes at 120 °C. After cooling, the cellulose residue was vacuum-filtered through filter paper and washed with 500 mL of hot water and 25 mL of ethanol, followed by drying in an oven at 100 ± 5 °C until constant weight (Fraction B). After cooling in a desiccator, the filter paper with the residue was weighed. The cellulose content was determined using Equation 1.

$$Celullose\ (\%) = \frac{Mass\ of\ Cellulose\ (B)\ (g)}{Mass\ of\ dry\ Biomass\ (A)\ (g)}\ x\ 100 \quad (1)$$

The lignin content was determined by adapting methodologies from Sluiter and colleagues [4] and Silwadi and colleagues [6] by the soluble lignin (ASL) method and by the insoluble lignin (AIL) method. Initially, 0.3 g of biomass was added to 3 mL of H_2SO_4 at 72% concentration, and the mixture was periodically agitated for 60 minutes at 30 °C in an ultrasonic water bath. The acid was then diluted with 84 mL of distilled water, and the sample was incubated at 120 °C for 60 minutes. After cooling, the sample was vacuum-filtered. Acid-soluble lignin was analyzed using a UV-visible spectrophotometer (Weblaborsp, WUV-M51) at 205 nm, and the values were converted to concentration using an absorptivity coefficient of 105 $L.g^{-1}.cm^{-1}$. The total of the ASL was determined using Equation 2.

$$ASL\ (\%) = \frac{Absorbance \times filtered\ volume \times dilution}{\varepsilon\ Absorbance \times mass\ of\ dry\ biomass \times path\ length\ of\ UV\ (cm)}\ x\ 100 \quad (2)$$

The insoluble fraction, Acid-Insoluble Lignin (AIL), was thoroughly washed with distilled water until excess acid was removed. It was then dried overnight in an oven at 105°C until reaching a constant mass (Fraction A). Equation 3 determined the total AIL content of the sample.

$$AIL\ (\%) = \frac{The\ mass\ of\ lignin\ insoluble\ (A)(g)}{The\ mass\ of\ dry\ biomass\ (g)}\ x\ 100 \quad (3)$$

The total lignin was determined by summing the AIL and ASL by Equation 4.

$$Total\ of\ Lignin\ (\%) = ASL\ (\%) + AIL\ (\%) \quad (4)$$

The hemicellulose content was estimated according to Kapoor and colleagues [7] by adding 10 mL of 1 M NaOH to 1.0 g of dried biomass. The sample was incubated in an autoclave at 120 °C for 60 minutes, then cooled, filtered, and reserved. The soluble fraction was neutralized with HCl, and the resulting precipitate (Fraction A) was separated by filtration and reserved. 95% ethanol (1:1) was added to the remaining solution to precipitate the remaining soluble hemicellulose (Fraction B). The precipitates obtained were filtered, washed with distilled water, and dried in an oven at 105 °C overnight until a constant mass was achieved. The estimated hemicellulose content was calculated using Equation 5.

$$Hemicellulose\ (\%) = \frac{Mass\ of\ the\ hemicellulose\ (A+B)}{Mass\ of\ the\ dry\ biomass\ (g)}\ x\ 100 \quad (5)$$

Results and Discussion

Chemical analysis of components such as moisture, ash, hemicellulose, and lignin is crucial for understanding prickly pear's structural and nutritional composition (*Opuntia ficus indica*) and *Agave* (*Agave* spp.). These parameters are essential for determining these plants' quality and potential use and understanding their biochemical and industrial properties. Following the completion of the laboratory study, the results obtained are presented in Table 1.

We observed that succulents like *Agave* and forage cacti have a high moisture content, indicating their ability to retain large amounts of liquid. This is due to their adaptation to long periods of drought, allowing them to survive in arid environments. This characteristic is particularly beneficial for processes such as alcoholic fermentation, as the high water retention in succulents means that less external water needs to be added during the fermentation process, making it more efficient and sustainable.

Additionally, the natural moisture of these plants can create a more favorable environment for the growth of yeast, which is essential for converting sugars into alcohol. Figures 1 and 2 visually present the values obtained through the methodologies described in the text in the context of analyzing the key constituents that enable alcoholic fermentation for bioethanol production.

Lignocellulosic biomass comprises cellulose (40-60%), hemicellulose (20-40%), and lignin (10-25%), which are intricately intertwined to form complex carbohydrates. These components play pivotal roles in bioethanol production through separate hydrolysis and fermentation. Lignin, a complex polymer with an amorphous structure composed of aromatic and aliphatic components, binds to lignocellulosic fibers, contributing to cell wall formation [8,9]. Characterized by a hydrophobic nature and a highly branched three-dimensional structure, lignin constitutes approximately 10-25% of plant composition [10]. It acts as an adhesive between cellulose and hemicellulose, essential structural elements of the cell wall. Removing lignin is essential to facilitate the conversion of cellulose and hemicellulose into sugars [11].

Figures 1 and 2 illustrate lignin contents of 18.67% in *Agave* spp. and 21.08% in *Opuntia* spp., indicating that *Opuntia* spp. has a higher percentage, making it less favorable than *Agave* spp. for bioethanol production. Pretreatment processes are necessary to degrade lignin for efficient sugar release, potentially increasing production costs or resulting in underutilizing biomass portions. Hemicellulose is a complex polysaccharide with a relatively low molecular weight, consisting of polymeric carbohydrates containing sugar units typically composed of five to six carbon atoms [10].

It can include sugars such as D-glucose, D-galactose, D-mannose, D-xylose, L-arabinose, D-glucuronic acid, and 4-O-methyl-D-glucuronic acid [12]. Structurally, hemicellulose bears more remarkable similarity to cellulose than to lignin. Rich in sugar composition, hemicellulose plays a crucial role in bioethanol production. *Agave* spp. Exhibits a lower hemicellulose content, which contrasts favorably with *Opuntia* spp., where hemicellulose content is more than three times higher. Pretreatment methods for hemicellulose breakdown are generally more efficient than those required for lignin [8].

Cellulose consists of dimeric units of anhydroglucose [$(C_6H_{10}O_5)$ n, where n is the number

Table 1. Chemical characterization results of *Agave* spp. and *Opuntia* spp.

	Moisture (%)	Ash (%)	Lignin (%)	Hemicellulose (%)	Cellulose (%)
Agave spp.	82.36 ± 0.82	11.65 ± 1.77	18.67 ± 1.71	5.80 ± 1.46	63.39 ± 12.82
Opuntia spp.	78.65 ± 3.10	3.65 ± 0.65	21.08 ± 3.17	18.60 ± 13.65	54.11 ± 7.95

Values represent mean ± standard deviation.

Figure 1. Chemical composition of the *Agave* spp.

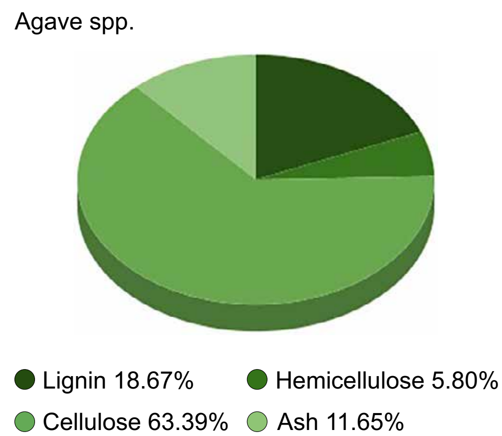
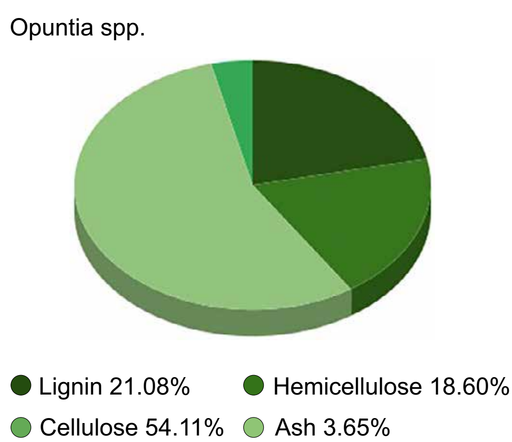


Figure 2. Chemical composition of the *Opuntia* spp.



of repeat units per chain], arranged in a linear chain. These dimers are linked by glycosidic bonds between carbon 1 (C1) of one dimer and carbon 4 (C4) of the adjacent dimer, forming a β 1-4 linkage [9,10,13]. In the absence of lignin, the length and packing of cellulose chains, which predominantly constitute the crystalline regions of cellulose, significantly influence material accessibility. The crystallinity of cellulose can affect processes like saccharification, where cellulose is converted into glucose. Tighter packing and longer cellulose chains reduce substrate accessibility, posing challenges for catalysts and solvents involved in the process [10,13]. Despite both plants containing high cellulose content, effective pretreatment initially targets lignin breakdown to facilitate access to cellulose and hemicellulose. Therefore, process feasibility hinges on pretreatments that effectively separate lignin from hemicellulose and cellulose. Ash content refers to the residue left after the decomposition and elimination of organic matter, achieved through calcination, which releases associated elements. This process enables comprehensive plant identification, with the ash content as a fundamental metric for subsequent experiments. It provides insights into the presence of inorganic materials within the plant, forming a basis for analyzing extractives and other compounds that may potentially hinder processes. Thus, ash content establishes a foundational understanding of the inorganic composition of the plant material.

Conclusion

Based on the results of the chemical characterization of *Agave* spp. and *Opuntia* spp., several important characteristics for their use as biomass sources can be highlighted. *Agave* spp. A significantly lower lignin content was observed compared to *Opuntia* spp., which may facilitate processes such as bioethanol production, where lignin removal is essential to improve the accessibility of cellulose and hemicellulose. On the other hand, *Opuntia* spp. showed a higher hemicellulose content, making it a potential source of sugars for fermentation.

The high moisture content found in both plants indicates a natural ability to retain water, which can be advantageous in processes like alcoholic fermentation, minimizing the need for external water addition.

Furthermore, ash content analysis provides important insights into the mineral composition of the plants, which is essential for understanding their nutritional potential and for processes involving impurity removal. Cellulose, despite being abundant in both plants, is closely associated with lignin and hemicellulose, the removal of which is crucial for optimizing biofuel production. Therefore, pretreatment strategies to effectively remove lignin and break down hemicellulose should be developed and optimized for efficient bioethanol production from these succulents. This maximizes biomass conversion into fermentable sugars and increases overall process efficiency, making it more economical and sustainable for future industrial applications.

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