Chapter 49

Biotechnological potential of açaí (Euterpe oleracea Mart.) seeds for biofuel production

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1 INTRODUCTION

The economic progress associated with the consumption habits of society, in general, has presented increasing generation and accumulation of solid waste. This is because, in most cases, there is no proper reuse of waste in the production process. Given this scenario, the concept of Circular Economy (CE) arises, seeking to transform the linear production model, based on the concept of extract, produce, consume and dispose, into a circular model, in which materials must return to the production cycle (Siqueira & Moraes, 2009).

CE is a model that has been gaining space in the corporate world, allied with strategies for sustainable development. The term CE can be defined as:

> "a restorative or regenerative system by intention and design that can be achieved by eliminating waste through superior design of materials, products, systems, and within that, business models" (DINCĂ et al., 2022)

A new understanding for global problems, which are not only reduced to environmental degradation, but the integration of strategies such as optimization of production processes, recycling and new business opportunities, migrating to a long-term economy and with a systemic vision (Foundation, 2014).

Throughout the 21st century, anthropogenic intervention has increased environmental damage and thus, changing production and consumption patterns to minimize resource depletion has become a priority, making the linear model based on extracting materials, transforming them into products, and then discarding them as waste, without any reuse initiative, unfeasible (Arauzo-Carod et al.,2022; Bourdin et al.,2022). In a circular system, the order is to rely less on pure raw materials, prioritizing inputs with more durable properties, reuse materials, and recycle waste (Accorsi et al.,2020).

The transformations required to move from linear economy to CE also demand a new regional and urban governance (Arauzo-Carod et al., 2022; Obersteg et al., 2019) and consequently promote a balance of business expectations with the public interest. With regard to the public interest, public institutions can be employed of legislation and policy, supporting infrastructure, and social awareness. As for industry, collaborative business models, product design, supply chain, and information and communication technology (Calzolari et al., 2021). Thus, CE and Sustainability goals should be supported by public policies that have a regional dimension, environmental management, and entrepreneurship support activities (Johansson & Henriksson, 2020; Polverini & Miretti, 2019).

The food production sector can be seen as one of the most important for the application of sustainable practices, due to its socio-environmental impact (León Bravo et al., 2021). In search of solutions based on CE requirements, several authors report that on average, 25 to 30% of waste is generated in fruit and vegetable processing (Ayoub et al., 2016; Sagar et al., 2018). Agroindustrial wastes are rich sources of lignocellulosic materials that can be biodegraded and converted into new materials, evidencing a key role in the desired evolution. In this way, food companies can work on identifying niches within value chains with greater potential to improve the environment and increase the social impacts of production while including economic viability in production and consumption chains (Fidelis et al., 2019; Velenturf et al., 2019).

Environmental awareness is growing among countries. At the same time, new legislations are being proposed due to the need for social responsibility, causing organizations to seek new economic models that adapt to the current situation.

Today, one of the greatest environmental challenges faced on a global level is the mitigation of the impacts generated by the acceleration of global warming and consequent climate change. Fuel Independence fossil fuels and the need for sustainable technologies is becoming light to meet the growing energy demand, combining sustainability and economic growth (Nizami et al., 2017).

Therefore, the need for sustainable technologies in the constant energy/fuel supply has become essential to implement circular economies in several countries (Calzolari et al., 2021; Kumar et al., 2019; Sadef et al., 2016).

1.2 LIGNOCELLULOSIC BIOMASS

Lignocellulosic biomass is a material of plant origin that has high energy potential, and can be used in many areas, such as, for example, in burning for direct heat generation or transformation into biofuels (Aditiya et al., 2016; Antunes et al., 2019; Dos Santos et al., 2016; Gupta & Verma, 2015). Thus, lignocellulosic biomass generated in different sectors (agriculture, forestry, industrial) acts as a precursor in the production of second generation (2G) bioethanol (Antunes et al., 2019).

Lignocellulosic materials have been used by mankind since ancient times to obtain energy. The burning of wood was the main source of thermal and light energy used for millennia, helping in the preparation of food, as well as in defense against predators. During the Industrial Revolution, the main source of energy became coal of mineral origin, which played a fundamental role in the establishment of the first industrial matrices in England and, successively, around the world. From the 20th century on, with the arrival of automobiles, oil took the central role as a raw material for obtaining fuels and energy, causing environmental, economic, and political problems (Rodrigues et al., 2017).

According to Fernando and collaborators (2006) producing products from agricultural commodities, such as biomass, and using it as an input in the production of various products in a similar way to an oil refinery (fossil fuels) is a promising technique, and its main objective is to transform biological materials into products usable in industries using a combination of biotechnological technologies and processes.

Besides being a favorable segment because it is renewable and low cost, the production of lignocellulosic biomass presents several other benefits to offset the use of fossil fuels, such as increasing energy security in regions without abundant reserves of fossil fuels and avoiding competition with food crops (Field et al., 2008). Energy and chemicals produced from such resources are known to be universally used, however, the burning of fossil fuels exacerbates environmental challenges such as global warming, climate change, and pollution (Singh et al., 2020).

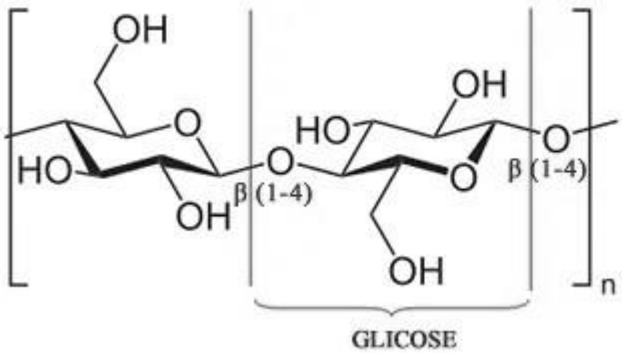
The continued depletion of fossil energy reserves and greenhouse gas emissions are critical concerns in the current scenario. On a global basis, the transportation sector has increased CO2 emissions by 24% and is expected to grow to 1.3 billion vehicles by 2030 and 2 billion by 2050 (IEA, 2020). If the available biomass is used efficiently to produce biofuels and other various bio-based compounds, it would be possible to promote the circular economy and decrease the global environmental burden in the future. In addition, the emergence of biomass-based industries provides several employment opportunities, as the use of biomass involves a number of logistical processes such as bulk collection, transportation, and pretreatment (Bajpai, 2020).

Lignocellulosic biomass generally comprises three types of biopolymers: cellulose, six-carbon glucose polymers; hemicellulose, five-carbon sugar polymers; and lignin, phenol polymers (Santiago & Rodrigues, 2017).

1.2.1 Cellulose

Cellulose is the most abundant natural polymer, insoluble in water and the major component of the cell wall of plant fibers (40 to 90% by mass of fiber) responsible for fiber strength, due to its high degree of polymerization and molecular orientation (Albinante et al., 2013). Chemically linear, it is composed of glucose disaccharide with β-1,4-glycosidic bonds, with intramolecular hydrogen bonds (bonds between glucose units of the same molecule) and intermolecular (between units of glucose from adjacent molecules), Figure 1 shows a portion of a cellulose polymer. The orientations of the bonds, as well as the amount of hydrogen bonds present in cellulose molecules, will define its shape: amorphous or crystalline Kassaye et al., 2016).

Figure 1: Part of a cellulose polymer



Source: Adapted from Raele et al., 2014

Intermolecular bonds are responsible for stiffness; and intramolecular bonds are responsible for the formation of fibrils, highly ordered structures that associate to form cellulose fibers. The fibrils present from regions with a high degree of crystallinity, in which the glycan chains are firmly linked in parallel, to regions with a lower degree of ordering, called amorphous regions. In the crystalline region, the fibers have higher tensile strength than in the amorphous region, where the fiber has its greatest flexibility (Vásquez et al., 2007).

The set of linear chains of cellulose molecules forms a crystalline structure that is highly inert and inaccessible to chemical reagents. Consequently, cellulose molecules have a higher resistance to be hydrolyzed by acids or enzymes. Thus, cellulose hydrolysis is considered a critical step in biofuel production, in the conversion of complex organic polymers such as proteins, lipids, and carbohydrates into simpler molecules. These molecules can undergo acidogenesis (conversion of long chain fatty acids to volatile fatty acids and sugars) and acetogenesis (conversion of volatile fatty acids to acetic acid, carbon dioxide [CO2] and/or hydrogen) (Hlavsová et al., 2014).

1.2.2 Hemicellulose

The term hemicellulose is a collective term that represents a family of polysaccharides, whose units include mainly, aldopentoses, such as xyloses and arabinoses, and aldohexoses, such as glucose, mannose and galactose. The variety of linkages and branching, as well as the presence of different monomeric units, contributes to the complexity of the hemicellulose structure and its different conformations (Carvalho et al., 2010).

Hemicellulose presents a variety of amorphous molecules, and unlike cellulose (Table 1), it does not present crystallinity, mainly due to the branching and the presence of acetyl groups connected to the main chain. Thus, they have a low degree of polymerization, and can be hydrolyzed under mild conditions. And their units are formed by carbon chains with a hydroxyl group, except those that can be in the form of carbonyl or hemiacetal bond (Albinante et al., 2013).

Table 1. Differences between hemicellulose and cellulose.

Component	Feature
Cellulose	Glucose units joined together, with a high degree of polymerization (1000 to 1500 glucose units and capable of forming a fibrous arrangement. It has amorphous and crystalline regions and is insoluble in alkaline solvents. Finally, it is slowly attacked by hot diluted inorganic acid.
Hemicellulose	Units of different pentoses and hexoses linked together, with a low degree of polymerization (60 to 300 sugar units) and does not form a fibrous arrangement. The hemicellulose has only amorphous regions and is soluble in a basic medium. Finally, it is attacked rapidly by hot diluted inorganic acid.

Source: Adapted from Pereira et al. (2008).

1.2.3 Lignin

The complex structure of lignin is formed by more recalcitrant components and, therefore, presents a greater barrier for lignocellulosic biomass to develop into an economically viable energy feedstock. Lignin is responsible for the union of plant cells and allows mechanical, chemical and biological protection to plants. Therefore, it is necessary that the lignocellulosic material undergoes treatment, allowing the lignin to be separated, enabling the hydrolysis of cellulose and hemicellulose (Abnisa et al., 2011; Yaman, 2004).

Lignin is the second largest component by mass of plant fiber, formed by an aromatic, amorphous system with high molar mass and phenylpropane units, connected to each other by a variety of carbon-oxygen and carbon-carbon bonds. Such bonds confer a stiffness, impermeability, and resistance to microbiological and mechanical attacks on plant tissues (Albinante et al., 2013).

In particular, lignin is notoriously resistant to oxidative, hydrolytic and biological degradation (Sheng et al., 2021). The complex and lignins alone can be broken down and their fraction separated by treatment with strong sulfuric acid, in which lignins are insoluble (Yaman, 2004). Thus, existing lignin dissolution methods involve aggressive pyrolytic and solvothermal processes aided by corrosive acids or alkaline reagents (Gabhane et al., 2015).

Lignin is a major barrier to the use of lignocellulosic biomass in the process of biological conversion into bioenergy. In general, softwoods have the highest amount of lignin, followed by hardwoods and grasses. Since hardwood generally contains less lignin than softwood, it makes it economically more viable to apply in the anaerobic digestion process for energy conversion. Agricultural residues generally contain lower amounts of lignin (10-20% lignin, 20-30% hemicellulose, and 40-50% cellulose) and are therefore more suitable as biomass feedstocks (Den et al., 2018).

1.3 LIGNOCELLULOSIC BIOFUELS / 2G ETHANOL PRODUCTION

Biofuels, as products obtained from renewable and sustainable sources with similar properties to fossil fuels are called, have significantly better CO2 emission rates in their life cycle assessment, and lower pollutant gas rates in their combustion (Lin & Lu, 2021).

Renewable energy sources in Brazil today represent about 46.1% of the national energy matrix. This rate is three times higher than the world average of 14.2%, which keeps Brazil as a reference in the development and use of alternative technologies to the use of fossil fuels. Between the years 2018 and 2019 alone, the use of ethanol and bagasse increased by 0.6% and biodiesel by 0.3% (Brazil, 2020). The trend is that this percentage will increase each year with the optimization of processes for the production of second generation biofuels, biofuels obtained from lignocellulosic biomass (Ripa et al., 2021). Besides being a renewable and clean source of energy generation, it is the only one that can supply a quarter of the demand in global transportation, which predominantly relies on fossil derivatives (Souza et al., 2017).

Among the various possibilities for biofuel production, second-generation ethanol obtained from lignocellulosic biomass has stood out in recent years. Mainly because it allows the reuse of agroindustrial waste and does not compete with the agricultural production of food (Su et al., 2020).

The process of obtaining second generation (2G) ethanol is carried out from a series of steps. The first is the pre-treatment, which consists of separating the lignin and cellulose, modifying the cellulose fiber structure, and solubilizing the hemicellulosic compounds. The effective consolidation of this step occurs in order to avoid degradation or loss of carbohydrates and the preparation of the fiber for the next step, saccharification. This step, usually enzymatic, aims to obtain fermentable sugars from the cellulosic material. In the third step the fermentation of the juice obtained by microorganisms that assimilate the compounds present occurs. And finally, the steps of separation and purification of the ethanol (Costa et al., 2019).

Some challenges accompany the development of this technology, due to the diversity of potential raw materials for its application and consequent adaptation of the processes, besides the recalcitrant characteristics of lignocellulosic biomass.

1.3.1 Pre-treatment

The use of lignocellulosic biomass, presents challenges due to the structural complexity of lignocellulosic biomass, such as the high lignin content, the crystalline nature of cellulose, and the propensity of lignin. Thus, physical, chemical, and biological pretreatment methods are applied to remove lignin and hemicellulose (Den et al., 2018). The pretreatment step aims, to alter the macroscopic, submicroscopic and microscopic structures of the biomass by removing the lignin-cellulose-hemicellulose complex, facilitating the access of hydrolytic enzymes that convert cellulose and hemicellulose into fermentable sugars (Zabed et al., 2016).

Sugarcane bagasse, for example, is prepared to be used as a raw material in enzymatic hydrolysis. The residues that can affect the production process and reduce the production yield are separated at this stage, ensuring a better quality of the final product. The pretreatment consists in destructuring the fibers of the lignocellulosic biomass (bagasse), because the bagasse is not homogeneous and presents variations in composition and morphological structure (Santos et al., 2012).

Pretreatment can be physical (mechanical reduction and microwave) or chemical (using acids or bases, or organic solvents). The type of pretreatment promotes the efficiency of enzymatic hydrolysis, where the type of treatment can have various effects and yields (Chemmés et al., 2013). In this step, it is essential to separate as much lignin as possible from cellulose and hemicellulose, for better yields in subsequent steps (Santos et al., 2012).

1.3.2 Enzymatic hydrolysis

This step is one of the key steps in the ethanol production process 2nd, since the efficiency of enzymatic hydrolysis is a criterion for evaluating the feasibility of conversion and bioprocess selection (Kucharska et al. 2018).

Saccharification is essential before fermentation. In this step, the polymers undergo enzymatic hydrolysis. This is where the hydrogen bonds and glycosidic bonds in the hemicellulose and cellulose fractions are broken, reducing them to their constituent sugars to form monomers of pentoses and hexoses (Taherzadeh & Karimi, 2007; Mussatto et al., 2010). The availability of polymers for enzymes is an individual characteristic, depending on the conditions, methods under which the biomass was processed and its characteristics. The suitability of the pH and temperature operating range is determined according to the enzyme cocktail suitable for the characteristics of the biomass (Kucharska et al., 2020).

However, enzymes can only be used once in these processes, because they are water soluble and thus end up being discarded at the end, which makes the production route more expensive (Collares, 2014). The temperature of this step is around 40-50 ºC, and presents low formation of degradation byproducts, which represents the high costs of production are the enzymes (Rabelo et al., 2008).

By making the sugar monomers available to the microorganisms after saccharification, they reactivate the enzymatic process and the desired fermentation product is obtained. In this context, there is the possibility of separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and simultaneous saccharification and co-fermentation (SSCF) (Zhu et al., 2014; Dong et al., 2016).

The SHF process is characterized by the steps of cellulase production; cellulose hydrolysis; sterilization of the fermentable sugar and fermentation. It can have hexose and pentose fermentation performed separately. Some nutrients may be lost and unwanted compounds formed in the sterilization process, through the Maillard reaction. SSF is represented by the processes of cellulose hydrolysis and the fermentation of the products of cellulose hydrolysis in a single step. This process prevents the formation of inhibitors, enabling synergism with the microorganism fermenter (Zhu et al., 2014).

The presence of fermentative microorganisms together with cellulite enzymes reduces the accumulation of sugar in the fermenter. Thus, as a main advantage, higher hydrolysis rates and higher conversion percentages are ensured compared to the SHF process. SSF processes generally have a shorter operating time, using fewer enzymes to achieve an increased ethanol yield, resulting in lower cost and reduced risk of contamination by other microorganisms (Lorenzi & Andrade, 2019). The main disadvantage, on the other hand, is associated with the optimal conditions of pH and temperature, being suitable the use of thermotolerant microorganisms (Rabelo et al., 2008). Another important factor is that the SSF process ensures the inhibition of cellulase by glucose resulting from hydrolysis, however, does not prevent the inhibition caused by cellobiose, in which, industrial yeasts, which ferment glucose, cannot ferment cellobiose (Kucharska et al., 2020).

There is also SSCF, in which the fermentation of products from the hydrolysis of cellulose and hemicellulose are performed simultaneously. This process relies on the development of a combination of microorganisms that ferment both sugars (Lorenzi & Andrade, 2019). To this end, there is no separation between the hemicellulosic hydrolysate and cellulose after pre-treatment. While the cellulose undergoes the saccharification process, the sugars originating from the hemicellulose are fermented (Zhu et al., 2014).

1.3.3 Fermentation

In the fermentation step followed by the distillation step. The glucose obtained in the previous step is transformed into ethyl alcohol through the action of specific organisms. For this, yeasts, such as Saccharomyces cervisiae, are used because of the desirable and efficient characteristics they possess for an industrial fermentation of sugars (Ostergaard et al., 2000). Thus, at the end of these steps, 2G ethanol is produced.

According to Silva and Couto (2007), alcoholic fermentation is the transformation of sugars into ethyl alcohol and CO2 by the action of a certain group of organisms. In the case of 2G ethanol, glucose is obtained from the previous step, by enzymatic hydrolysis. After fermentation, a fermented wine is obtained that already has ethanol in its composition, but needs to be separated (Murakami et al., 2016). According to Nova Cana (2006), this mixture must be separated using distillation. In this process, the liquid is placed in distillation columns in which it is heated until it evaporates. This vapor goes through a condensation stage, returning to the liquid state, obtaining ethanol. This results in hydrous alcohol, used as fuel ethanol, with an alcohol content of about 96% (Murakami et al., 2016).

1.4 AÇAÍ - EUTERPE OLERACEA MART.

The açaí (Euterpe oleracea), native to the Brazilian Amazon, is a palm tree of great cultural, economic and social importance in the northern region, with high agronomic, technological and nutritional potential (Albiero et al., 2012; Rocha, 2004; Yuyama et al., 2011). About 90% of the fruit is stone, the remaining 10% consists of pulp and fiber (Rossetto et al., 2020).

The açaí seed is an oleaginous seed formed by a small solid endosperm attached to a tegument rich in sugars. Several authors have characterized the kernel as containing cellulose (40.29 to 53.2%), hemicellulose (1.26 to 14%), lignin (4 to 22%), lipids (0.61 to 3.56%), total fiber (29 to 63%),

total ash (0.15 to 1.7%), variable moisture (10.15%), protein (5 to 7%) and fixed carbon (0.83%) (Rodríguez-zúñiga et al., 2008; Qiu et.al., 2012; Tamiris et al., 2015; Cordeiro et al., 2019; Serrão et al., 2021). However, recent studies have shown that unlike what was previously reported, the fruit is predominantly composed of the polysaccharide mannan - which when hydrolyzed we obtain mannose - and not cellulose. This difference can be explained by the use of indirect methodologies for composition determination, which instead of quantifying sugars by chromatographic methods, quantify total fiber contents (Monteiro et al., 2019). De Lima and colleagues (2019), characterized açaí (Euterpe oleracea Mart.) kernel by direct methods and reported mannose (75%) as the predominant sugar, in addition to glucose (6%), galactose (2%), arabinose (0.8%) and xylose (0.6%).

With a dark coloration, ranging from purple to black, the rounded fruit is born in clusters and most often in places with wetter or flooded soil. The açaí palm can reach more than 20 meters in height and produce about 4 bunches per year. Açaí is rich in protein, vegetable fat, vitamin B1, C and E, minerals, and fiber. The high amount of fat present corresponds largely to monounsaturated (60%) and polyunsaturated (13%) fats that help reduce bad cholesterol (LDL) and improve good cholesterol (HDL) (Silva et al., 2020). Due to one of its main products, the fruit has gained importance for its antioxidant properties, its high energy content, and also, due to the presence of the so-called "good fat" in its composition. And, currently, it is employed in pharmaceutical, cosmetic and food industries (Silva et al., 2020).

The planting is done on terra firme, with the objective of facilitating the management and obtaining production for marketing, however, its abundance is concentrated in floodplain areas (Galeão, 2017). The state of Pará is one of the largest producers in Brazil, as it concentrates large amounts of açaize trees in total area and many islands that are major producers (Silva et al., 2020).

The commercialization of the açaí fruit is widespread on the national and international scene, mainly due to its nutritional properties and for being a food with numerous functions in the human diet (Silva et al., 2020). In the last ten years, with the expansion of the market, there have been drastic changes in the production system, migrating from a poorly productive extractive (4.2 tons per hectare) to well-managed systems and irrigated crops that can reach 15 tons per hectare, with the possibility of further growth with scientific and technological advances (Santos et al., 2012; Silva et al., 2015).

1.4.1 Biotechnological potential

The residual biomass of açaí may be a potential source for a wide range of applications, mainly biotechnological, such as solid-state fermentation processes and production of fermentable sugars (mannose), as a raw material for food, pharmaceutical and other industries (De Lima et al., 2019).

Biotechnology applied to the food industry promotes new ways of using fruits to generate innovative products and help in the discovery of new bioactive molecules, proteins, and microorganisms, enabling the application in the development of new products, especially in the development of techniques for the full utilization of fruits and the use of their residues (Almeida & Santos, 2020).

Several studies are being done for bioenergy and biofuels production from açaí (Euterpe oleracea Mart.) residues, using thermochemical processes (Virmond et al., 2012), pyrolysis (Alves et al., 2021), heterogeneous magnetic acid catalysts (Araújo et al., 2021), obtaining bio-oil (Rocha de Castro et al., 2021) and second generation ethanol (De Andrade Cordeiro et al., 2019).

The interest in using this residue is due to its social and environmental representativeness (Monteiro et al., 2019). The seeds of the açaí (Euterpe oleracea Mart.) are one of the main residual biomasses of the Amazon region. Besides being produced in large quantities, the fruit pulp removed for commercialization is small. Its residue characterized by seeds covered by lignocellulosic fibers represents about 80% of the total fruit (Alves et al., 2021). Despite its reuse as fertilizer or as fuel in boilers, these sectors are unable to use the entire volume generated. A large part of the waste does not receive the proper final destination, which leads to major sanitary problems and water and soil pollution (Bufalino et al., 2018; Queiroz et al., 2020). According to Araújo and collaborators (2021), in 2019 in the state of Pará alone, approximately 107kt of seed were generated.

The characteristics of the açaí seed, predominantly composed of cellulose (40.29%), hemicellulose, and lignin, point to it as a promising lignocellulosic biomass for second-generation ethanol generation (De Andrade Cordeiro et al., 2019).

If we take into consideration the characterization according to Monteiro and collaborators (2019), where the fruit is predominantly composed of mannans, it is worth noting that the enzymatic hydrolysis of this polysaccharide has been scientifically explored from the use of residues from coffee extraction (Kenny et al., 2010), palm kernel oil extraction (Fan et al., 2014) and açaí (Cordeiro et al., 2019). To date, there is no information about the use of these processes on an industrial scale, but the enzymatic hydrolysis of lignocellulose is a consolidated process in the 2G ethanol industry (Saville et al., 2016). Although different products can be obtained from lignocellulosic biomass, the cellulosic biofuel market has been the main driver for the development of enzymatic hydrolysis (Da Silva et al., 2020).

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