**Research Article** 

### BIOTECHNOLOGICAL AND ENVIRONMENTAL EDUCATION POTENTIAL OF *Inga edulis* Martius: SEEDS COLLECTED IN THE AMAZON, BRAZIL

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#### **ARTICLE HIGLIGHTS**

- Inga edulis offer potential for biotechnological and environmental applications.
- High protein and carbohydrate content in seeds support diverse agro-industrial uses.
- *I. edulis* seeds enhance environmental education via forest nurseries schools.
- Biotechnological potential of *I. edulis* seeds extends to ecosystem services.

#### **Article Information**

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#### ABSTRACT

Inga edulis Martius is a native species of the Amazon Forest with great potential for urban afforestation in the cities of the Brazilian Amazon and widely used for the recovery of degraded areas. It is commonly cultivated by the Amazonian population for its edible fruit, quality wood, and excellent agroforestry components. This study aimed to select the matrices and obtain information about the behavior of the seeds in terms of drying, biometry, purity and germination, and chemical composition. Carbohydrates were analyzed by using High-Performance Liquid Chromatography, proteins by using Elemental Analysis, and inorganic composition by using Inductively Coupled Plasma-Optical Emission Spectroscopy. The seeds collected in the environmental education action were recognized about potentialities of use for biotechnological products and environmental protection. The exploitation of this species' seeds is appealing due to their inorganic composition (N 20.4 g/ kg, P 1.71 g/kg, and others) and organic content (20.1% protein and 58% carbohydrates), as well as their potential for forest integration. The seeds exhibited a quality suitable for cultivation in nurseries, including in school settings. Inga edulis is recognized in the literature for its various uses, although these are not directly related to seed exploitation. I. edulis seedlings in the schools contribute to environmental education as a sustainable practice with biotechnological potential for the Amazon region.

Keywords: agroforestry, biotechnology, germination, Inga edulis, seeds

#### **INTRODUCTION**

The Brazilian fruit industry holds a prominent position nationally and internationally, owing to the country's vast variety of exotic species. In Brazil, only a few native fruit species have commercial significance, with some having regional or local importance. In contrast, many unexplored or underexplored species possess potential importance and can pose a challenge to sustainable commercialization (Batista *et al.* 2019). The Ingá-cipó (*Inga edulis*), belonging to the Fabaceae family, is a tree species also known as Angá, Ingá, Ingá-comum, Ingá-de-macaco, Ingáde-metro, Ingá-doce, Ingá-macarrão, Ingá-rabo-demico, Ingá-timbó, Ingá-vermelho, Ingá-verdadeiro, and Ingazeiro (Cruz 2021).

In Brazil, *I. edulis* is found in the states of Acre, Amazonas, Amapá, Bahia, Espírito Santo, Mato Grosso, Minas Gerais, Pará, Paraná, Paraíba, Pernambuco, Rio de Janeiro, Rondônia, Roraima, Santa Catarina, and São Paulo (The Brazil Flora Group 2021). It can also be found in Peru (Rollo *et al.* 2020), Ecuador (Abril-Saltos *et al.* 2018), Argentina (Correa *et al.* 2021), and others.

This species can be found in almost all Central and South American countries, occurring in nearly all regions of Brazil, providing ecosystem services and attracting floral visitors. Additionally, I. edulis contains bioactive compounds with antioxidant and antiproliferative activities (Tauchen et al. 2016; Lima et al. 2020). According to Lima et al. (2017) and Gomes et al. (2023), the I. edulis is of medium to tall stature, with rapid growth, reaching heights of 15-20 m in open areas and up to 40 m in the forest. The flowering and fruiting stage start at three years of age in open areas. The inflorescences are axillary, sometimes terminal, grouped, with 4 to 5 spikes in the leaf axils. The flowers are white, attractive, and fragrant. The fruit is a long pod of variable size, indehiscent, green, cylindrical, thick, with multiple longitudinal sutures, and can reach up to 2-m long. Seeds of I. edulis are black, with a variable number per fruit, ellipsoid in shape, smooth and glabrous, measuring 2-3 cm in length and 1-1.5 cm in width, covered with a white, soft, fibrous, fluffy, succulent, sweet, and edible aril. I. edulis aged 3-4 years can produce 20,000 to 100,000 flowers and 200 to 800 fruits. One of the most important characteristics of this species is its ability to tolerate acidic soils.

According to Urruth *et al.* (2022), the need for tropical forest conservation and the strengthening of environmental policies to mitigate anthropogenic land use promote a need for environmental management with native species. Therefore, there is a need for high-quality seeds and seedlings. Desiccation tolerance is one of the most essential properties of the seeds of *I. edulis*. Desiccation tolerance is a necessary phenomenon in the plant's life cycle and serves as an adaptation strategy that ensures seed survival during storage under stressful environmental conditions, thus ensuring species dissemination.

Regarding flowers, *Inga* species and Amazonian legumes, in general, are practically unexplored in characterizing volatile compounds, which are important for pollinating fragrance (Marinho *et al.* 2014) and their biological activities. This feature potentially opens doors for biotechnological exploration. Similarly, the leaves of *I. edulis* Martius can also be used due to biological activity of their composition, such as anti-inflammatory properties. Polyphenols have this effect and can also be associated with treating cardiovascular, neurodegenerative, and cancer-related diseases (Silva *et al.* 2007). Results from more than 15 years of research have already identified gallic acid, catechins, quercetins, and other phenolic compounds in *I. edulis* leaves, as well as flavonoids in the roots (Dias *et al.* 2010).

Due to its native status and benefits to the ecosystem, the *I. edulis* tree is suitable for reforestation and restoration of degraded areas impacted by various human activities in its native regions, such as the Amazon (Martins *et al.* 2022). Consequently, it is understood that this species is important. Among the potential uses in its natural habitat, the flowers, leaves, and seeds of *I. edulis* can be investigated to develop new products. Therefore, there is a search to recognize the potential use of seeds for developing bioproducts or biofuels based on their composition and variability and the potentials described in the literature for the seeds of this species or similar ones.

Producing a species with high seed productivity and natives to a region incentivizes agro-industrial exploration (Rodrigues *et al.* 2021). This approach avoids the introduction of exotic species to produce food or bioinputs and diversifies products, creating new market opportunities. Promoting the planting of species like *I. edulis* in the Amazon region is essential for biodiversity conservation, ensuring ecosystems' healthy functioning and local communities' well-being. This approach also prevents agricultural movements from introducing exotic species associated with ecological imbalance.

The *Inga* tree is a species that thrives in Brazil and can have low production costs, integrated with forest conservation. It warrants a study of the biotechnological potential and utilization of its seeds for homogeneous batches for commercial-scale production (Rodrigues *et al.* 2021). Incorporating *I. edulis* into agroforestry systems can increase agricultural biodiversity and improve the resilience of productive systems. This contributes to food security and agriculture sustainability (Kittur *et al.* 2024). Additionally, *I. edulis* can act as a shade tree for coffee cultivation,



Figure 1 Research diagram of *I. edulis* exploitation to biotechnological and environmental education approach

leading to higher productivity and contributing to carbon stocks (Solis *et al.* 2020), assisting in soil recovery, and helping to maintain local biodiversity (Domínguez-Núñez 2022).

Likewise, there is a recognition of the potential for seedling production to encourage the establishment of forest nurseries in schools, promoting environmental education and reforestation with a native species of exploitable biotechnological potential integrated with the environment.

#### MATERIALS AND METHODS

The research was conducted according to a diagram presented in Figure 1. Samples from five different matrices were separated, with 50 seeds (ten seeds from each matrix) used for characterization. These seeds underwent procedures for biometric observation (drying, weighing, and measuring), moisture content determination, and purity analysis. Some collected and analyzed seeds were stored and preserved in a dry chamber to form germplasms in the Plant Germplasm Bank of the Federal Institute of Amazonas (IFAM).

Analyses were conducted to identify the seed composition's main inorganic and organic components. Selected seeds were planted to produce seedlings. These seedlings were donated or used to establish an experimental forest nursery at the school. The formation of the plant germplasm bank and the establishment of the nursery contributed to the teaching and learning process, aiming to contextualize and assimilate the potential of the species under study.

#### Seed Sampling and Treatment

Fruits from five different matrices were collected in the first semester of 2022 according to the coordinates shown in the map (Fig. 2). The collection method involved manual and ladderassisted techniques, as some matrices were large. Students from two schools involved in the research participated in this stage.

Seeds were extracted from the fruits manually. Subsequently, the pulp surrounding the seeds was removed, and the seeds were washed under running water. Then, the seeds were placed on cotton fabric and stored individually in a cold chamber (5-7 °C) until biometric measurements were taken.



Figure 2 Location map of the collected I. edulis matrices Source: Georeferencing Laboratory at UNISC.

In the first biometric assessment, the seeds were weighed with their impurities. They were then sun-dried for 8 hours to 12 hours and reweighed. Afterward, they were dried in a laboratory oven at 40-45 °C for 60 minutes. Their size was measured using calipers and a measuring tape. The flesh was removed after submerging the fruits in water for 12 hours to 24 hours. The softened pulp was macerated and separated through a sieve. The seeds, still in the sieve, were washed under running water.

In a water tank, residual materials, empty and deteriorated seeds floating, and fruit remnants were removed through flotation, while seeds in good condition sank. After this process, the seeds were ready for drying (Scremin Dias *et al.* 2006). Seeds were stored in a dry chamber to preserve and form the germplasm bank. Seeds with fungi during this storage stage were discarded.

The purity level (P) was calculated for batches of 50 seeds and presented in relation to viable seeds (%). The moisture content (%) of the 50 seeds was calculated in relation to moisture loss in an oven.

Physiological analysis of the seeds was carried out using a binocular microscope (Physis) to observe the structure, consisting of Internal Seed Coat, External Seed Coat, Hilum, Hypocotyl, Embryonic Region, Endosperm, and Radicle. The seeds were also assessed using a UVB Transilluminator (Loccus do Brasil, LTB-20X20 STi).

#### **Chemical Analysis of Seeds**

Representative seed samples from five matrices (M1 to M5), stored in a dry chamber, were ground in a knife mill and analyzed for inorganic composition, macro and micronutrients, structural composition of carbohydrates, and elemental analysis of C and N.

The inorganic composition was determined based on ash content obtained by using gravimetry method conducted in a muffle furnace at 575 °C for 12 hours (de Souza *et al.* 2020). The ash content (%) was reported based on dry biomass. Inorganic macro and micronutrient contents in the ground dried seeds (mixed matrices) were determined using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES/Perkin Elmer, Optima 8300 model). Carbohydrate content and the monosaccharide profile were determined using the concentrated acid hydrolysis method recommended by the National Renewable Energy Laboratory of the United States (NREL-USA) (Sluiter et al. 2010). Weighed samples were treated with 72% H<sub>2</sub>SO<sub>4</sub> solution (4%, v:v) and autoclaved for one hour, followed by the analysis of monosaccharide composition in the hydrolysate using High-Performance Liquid Chromatography (HPLC/Shimadzu, QP2010 plus). An Elemental Analyzer (Flash EA1112, Thermo Electron Corporation, Milan, Italy) was used to determine the elemental composition of C and N. Protein content was calculated by multiplying the N (%) by a factor of 6.25.

# Establishment of the Experimental Forest Nurseries

The Experimental Forest Nurseries were established at two schools. The first occurred at CETI (Centro Educacional de Tempo Integral) Áurea Pinheiro Braga, located on Brasil Avenue, s/n – Compensa III, CEP: 69036-660, Manaus/ AM (geographical coordinates: 3.0932137,-60.0596746,15). The second school was IV CMPM (Colégio da Polícia Militar), located on Açaizeiros Avenue, s/n – Gilberto Mestrinho, CEP: 69086-485, Manaus/AM (geographical coordinates: -3.0756539,-59.9277989,15).

At both schools, participating students were between 10 and 17 years old. Activities included presenting aspects of the cultivation of *I. edulis*, its main characteristics, seed storage conditions, seedling preparation, planting, transportation, and seedling donation.

### **Statistical Analysis**

The experimental results of the physical evaluation of seeds were obtained in at least three replicates and analyzed for variance (ANOVA) with a confidence level of 95% using GraphPad Prism 10.3.1 software. Differences among the data means were tested using Tukey's multiple test.

# Prospects for Biotechnological Utilization of *I. edulis*

A bibliometric analysis was conducted to assess the biotechnological potential of *I. edulis* seeds. A discussion was held based on experimental results and the potential of similar seeds found in the literature. The bibliometric analysis was conducted based on documents selected from the databases available in the Capes Journals Portal for the last five years. For some terms, it was necessary to investigate documents from the last 20 years. The documents were analyzed using Vosviewer 1.6.18 software. The bibliometric search was conducted using the terms: "Inga," "Inga edulis," "seed," "sugar," "antioxidant," and "carbohydrate."

### **RESULTS AND DISCUSSION**

### Seed Quality

Seed quality is an essential factor in recognizing its potential utilization. Seeds not viable for germination are also seeds with low utilization potential. On average, the purity level of the seeds was  $42.5\pm11.72\%$  (Table 1). This information is also crucial for establishing experimental nurseries in schools in the Amazon region, as it is the viable seeds that students would be planting. The moisture content parameter of seeds in this research was higher than 46%, consistent with Rodrigues *et al.* (2021), indicating that the seeds had a high water content.

Parameters	M1	M2	M3	M4	M5
Width (cm)	$0.87 \pm 0.08^{a}$	0.96±0.1 <sup>b</sup>	0.99±0.12°	$1.27 \pm 0.18^{d}$	1.12±0.18 <sup>c</sup>
Length (cm)	3.67±0.16ª	4.1±0.49 <sup>b</sup>	3.79±0.24ª	4.54±0.17 <sup>b</sup>	4.1±0.32 <sup>b</sup>
Diameter (cm)	3.9±0.13ª	4.37±0.33 <sup>b</sup>	4.54±0.42 <sup>b</sup>	5.84±0.42°	4.51±0.32 <sup>b</sup>
Purity (%)	53.80±12.73ª	39.63±8.80 <sup>b</sup>	37.52±9.33 <sup>b</sup>	39.89±16.77 <sup>b</sup>	41.67±10.99 <sup>b</sup>
Moisture content (%)	46.20	60.37	62.48	60.11	58.33

Table 1 Seeds biometric measurements, purity, and moisture content of seeds collected

Notes: Values are mean $\pm$ SD for n = 10. Different superscripts on the same line indicate significant differences (P < 0.05); M1 to M5 = matrices.

After analyzing the physiology and morphology of seeds from five matrices, a portion of viable seeds was sent for seedlings production. Despite the first matrix showing higher purity and lower physiological water loss (P < 0.05), it was found that the fourth matrix (M4) yielded the best results in terms of formation and potential use as seed plantation. According to Mata (2009) and Santos *et al.* (2016), germination is associated with morphological and physiological changes that occur during seed maturation. Seed vigor, which is crucial for successful seedling emergence, is influenced by seed size; thus, larger seeds generally exhibit greater vigor.

Seed germination allowed for identifying structures, such as endosperm, embryo, hilum, hypocotyl, cotyledon, and radicle. Seeds collected in this research exhibited the necessary structures for proper functioning, growth, and development during germination and seedlings production. Subsequently, these seedlings were cultivated and donated to establish experimental forest nurseries in schools in the Amazon region.

#### **Establishment of Experimental Nurseries**

In the activities carried out in schools in the Amazon region, students observed the time of root protrusion and seedling formation. Most of the seeds germinated within three days, and there were differences among the matrices. Despite having higher purity, even the Matrix 1 was not recommended for planting. Matrix 4 contributed to most of the seedlings as it produced more seeds with complete structures.

In addition to learning about seedling and preservation, students also learned how to prepare substrate for seedling production in 1-kg bags using a special substrate (NPK and limestone or chicken manure and burnt rice husk). Subsequently, this special substrate was mixed with vermiculite – a mineral that retains water in the substrate and aids in aeration. A drainage layer was added to the bottom of the bags using the husks from the fruits of *I. edulis*. The produced seedlings are shown in Figure 3.



Figure 3 Seedlings of Inga edulis Martius

# Seed Analysis for the Assessment of Biotechnological Potential

#### Inorganic Composition

The ash content was determined to assess the inorganic composition. Ash in a plant sample is the inorganic residue from the combustion of organic matter lost through volatilization or combustion (Veloso *et al.* 2004). The ash content of *I. edulis* ranged from 28.3% to 32.3%, with an average of  $29.58\pm1.60\%$ . It showed lower values than the same species (44%) from another region, as reported by Aguiar (2021).

In the analysis of macro and micronutrients in biomass, it was observed that there were elements that serve as nutrients for both human and animal consumption. The main elements found in *I. edulis* seeds as well as Ni, Cd, Na, Pb, and Si were also analyzed, and no concentrations above the detection limit were found (Table 2).

The elements contained in the seeds are relevant for nutrient replenishment in the soil, either naturally or through the formulation of biotechnological products. *I. edulis* is among the species of nitrogen-fixing trees and may even have the ability to enhance the production of neighboring trees (Nichols & Carpenter 2006).

The elements analyzed are present in various seeds of Amazonian fruit trees. However, their concentrations vary according to the species and edaphoclimatic conditions. In the case of *I. edulis* seeds, the values observed across the five matrices indicated a potential for these seeds to be utilized in biotechnological applications, owing to the presence of these elements in their biomass. Nitrogen, phosphorus, potassium, calcium,

Table 2 Ash composition obtained from the combustion of *I. edulis* seed samples

			1		
Elements	Unity	Values	Elements	Unity	Values
Nitrogen	g/kg	20.40	Boron	mg/kg	5.48
Phosphorus	g/kg	1.71	Copper	mg/kg	9.72
Potassium	g/kg	8.67	Iron	mg/kg	47.61
Calcium	g/kg	3.01	Manganese	mg/kg	32.90
Magnesium	g/kg	1.39	Zinc	mg/kg	35.14
Sulfur	g/kg	2.46	Molybdenum	mg/kg	1.74

Table 3 The carbohydrate content of the analyzed I. edulis matrices

Matrices —	Gluc	Glucose		Arabinose		<b>D</b> (1)
	Average	SD	Average	SD	Carbonydrate (%)	Protein (%)
M1	55.26ª	0.73	2.83ª	0.20	58.10	17.5
M2	58.18 <sup>b</sup>	0.62	2.83ª	0.36	61.01	17.8
M3	56.00ª	0.19	2.78ª	0.36	58.79	20.1
M4	59.35°	0.22	2.82ª	0.15	62.16	16.6
M5	57.09 <sup>d</sup>	0.15	2.59 <sup>b</sup>	0.17	59.68	15.9
Average	57.18	0.38	2.77	0.25	59.95	17.6

Notes: Different superscripts in the same column indicate significant differences (P < 0.05).

magnesium, sulfur, and the micronutrients boron, copper, iron, manganese, zinc, and molybdenum are essential for metabolism and are crucial in the tissue composition in organisms that contribute to biomass growth (Andrews *et al.* 2024). These trace elements can be particularly important in processes involving microorganisms, such as anaerobic digestion, where molybdenum, nickel, and iron may be vital for maintaining process stability and influencing biogas production (Yu *et al.* 2016).

Another example of the importance of *Inga* seeds biomass composition lies in their potential as a biofertilizer. Plants, like rapeseed (Eggert & von Wirén 2016), require these elements, as these nutrients can affect the behavior of microorganisms, enzymatic activities, and metabolic byproducts (Soltan *et al.* 2019).

#### **Organic Composition**

The organic macromolecules commonly present in seeds are proteins and carbohydrates. In the analyzed samples, the average protein content found was 17.6%. This value is close to forage legumes, considered a source of plant protein, such as beans with up to 28% protein content (Naeem *et al.* 2022).

Proteins are complex molecules of carbon, hydrogen, oxygen, nitrogen, and other elements. They can be analyzed by CHNS elemental analysis

due to their composition of amino acids linked together by peptide bonds (Ordónez 2004). The presence of proteins in seeds is paramount for the organism that consumes this bioproduct because proteins provide essential amino acids that the human body cannot synthesize (Leone et al. 2016). Additionally, proteins can be important for utilizing the seed biomass in food composition due to their texture (Ribeiro & Seravalli 2007). The protein fraction varies with the plant's maturity (Pinheiro et al. 2005). It is associated with enzymatic activity responsible for metabolism (e.g., lipases and lipoxygenases, enzymes involved in lipid biosynthesis) and biochemical cycles that function to form structural components of cells and complex organisms (Damodaran 2017). Like other seeds, it can be a source of plant protein, even if it is not the main component of the seed (Ullmann et al. 2023).

Carbohydrates, on the other hand, are organic compounds produced in the photosynthetic cells of plants and are widely distributed, being present in both animal and plant tissues (Ordóņez 2005). Glucose, fructose, and sucrose, which are responsible for the sweet taste in various foods (Ribeiro & Seravalli 2007), belong to this group. In the analyzed samples, carbohydrate content averaged 59.95%, making it an excellent source of carbohydrates, rich in glucose polysaccharides (Table 3).



Figure 4 Research profile on *I. edulis* in the last five years (2019-2023) according to databases available in the Capes Periodicals Portal

## Potential Biotechnological Use of *I. edulis* Seeds

#### **Bibliometric Approach**

When conducting a literature review for the last five years (2019-2023) , 23 research articles with "*I. edulis*" in their titles were found. These documents indicate potential plant uses (Fig 4).

Among the studies shown in Figure 4, there is a commitment to studying Amazonian species to obtain compounds with antifungal activity. Dib *et al.* (2019) purified and characterized a trypsin inhibitor from *I. edulis* seeds with potential application against *Candida* spp., including *Candida buinensis* and *Candida tropicalis*.

Lima *et al.* (2020) obtained extracts with high antioxidant activity from the seeds due to the content of anthocyanins. They isolated an anthocyanin and a mixture of three anthocyanins. Lima *et al.* (2022) identified several compounds with biological activity, including 16 compounds among terpenes, phenolic acids, flavonoids, and anthocyanins. These authors highlighted the high content of phenolic compounds, which are important antioxidants and inhibit rheumatoid arthritis.

Seed applications related to using polysaccharides have not been observed in journal databases for the last 20 years, which is surprising considering that the seeds contain 59.95% carbohydrates. Given the production profile of *I. edulis*, there is great potential for using polysaccharides, although it is not documented.

Many products extracted from seeds that could receive biotechnological use can be investigated for their chemoprotective and anticancer properties. Those products could become innovative, active formulations, principles in pharmaceutical cosmetics, and food. The products present preventive and inhibitory functions on the growth of cancer cells, with anti-inflammatory, gastroprotective, antioxidant, antimicrobial, and other effects, as shown for the seeds of Syzygium cumini Skeels (Kumar et al. 2022), Luffa operculata (Silva et al. 2022), Eugenia stipitata Mc Vaugh, and Myrtaceae (Neri-Numa et al. 2013). According to Dib et al. (2019), seeds provide plant survival and perpetuation activities associated with chitinases, glucanases, lectins, and thionins.

According to Cotabarren *et al.* (2020), *I. edulis* seeds contain a serine protease inhibitor. A serine protease inhibitor implies a powerful tool to regulate critical biological processes, prevent damage caused by excessive protease activity, and develop therapies for various diseases. This inhibition process has applications in biotechnology, such as in the production of food products, where proteases need to be regulated to control foods' texture and other properties (Tavano *et al.* 2018). It can also have

applications as a bioinsecticide (Abd El-latif 2014) and prevent immune responses by regulating proteases (Carvalho *et al.* 2023).

According to de Moura Martins *et al.* (2020), some species of *Inga* have already been studied for their phytochemical composition. The biological activity of interest to the pharmaceutical industry has been published for *Inga marginata* Willd (Álvarez *et al.* 1998), *Inga goldmanii, Inga umbellifera* (Lokvam & Kursar 2005), and *Inga laurina* Kunitz (Macedo *et al.* 2011). *I. edulis* contains small peptides that act as inhibitors of proteolytic activity (serine), which can be a practical approach to deactivate proteases associated with various human diseases, such as arthritis, pancreatitis, hepatitis, cancer, AIDS, thrombosis, emphysema, hypertension, and muscular dystrophy (Cotabarren *et al.* 2020).

Starch stands out among the metabolites stored in *I. edulis* seeds, followed by proteins (Rodrigues *et al.* 2021). Starch and proteins are important for *I. edulis* seeds' biotechnological application. Extracts from *I. edulis* seeds inhibited 85% of multiple myeloma cell proliferation, demonstrating the significant potential of these seeds for developing new therapeutics (Ferro *et al.* 2022).

Another aspect to highlight is that some uses of *I. edulis* are not directly related to the seed itself. *I. edulis* stands out for its economic potential in reforestation, phytotherapy, energy production, and food. Its bark, pulp, and seeds are used in indigenous natural medicine and the landscaping of some cities (Pritchard *et al.* 1995; Bilia *et al.* 2003; Caramori *et al.* 2009). The scientific and technological exploration of the potential of these seeds is quite limited, with only folk knowledge about them. Research is crucial to driving biotechnological development.

This is corroborated by the high germination rate and extensive seed and viable fruit production throughout the year, as well as the fact that the populations of the Amazon region highly appreciate its edible fruits. This is why its cultivation is widespread in agroforestry backyards of local communities. In addition to consuming its pulp, in indigenous communities (Brazil and Ecuador), roasted seeds are used for food (Lojka *et al.* 2010; Kinupp *et al.* 2021).

#### Experimental Approach

Our research found more than 58% glucose content in these carbohydrates, indicating a high starch content, which would be relevant for fermentation processes. Glucose content facilitates ethanol production using yeast (Bahlawan *et al.* 2022). Additionally, pentoses (xylose and arabinose) can be converted into ethanol using a *Zimomonas mobilis* strain, as Khounani *et al.* (2019) demonstrated for hydrolyzed monosaccharides from safflower seeds.

In biotechnological processes, biotransformation involves complex processes that utilize various elements. In the seeds of *I. edulis*, iron was found at 47.61 mg/kg, which aids enzymes that drive metabolic reactions associated with biotechnological processes. Zinc (35.14 g/kg) plays a role in DNA synthesis and glucose metabolism, while magnesium is crucial for photosynthesis (Ahmed *et al.* 2024). These elements may be necessary depending on the biotransformation process employed, allowing *Inga* seed biomass containing polysaccharides and proteins to serve as a substrate for new products.

Biotechnological processes that assist agriculture involve other elements found in the seeds, such as N, P, Ca, B, Cu, S, Mn, and Mo, which may be important for biofertilization. Additionally, molecules from the polysaccharides hydrolysis and proteins can have biostimulant functions (Ugolini *et al.* 2015).

Protein content in the samples ranged from 15.9% to 20.1%, which may have biotechnological applications, as recognized by Dib *et al.* (2019), given that the proteins of *I. edulis* exhibit antimicrobial activity.

The proteins and carbohydrates found in *I. edulis* seeds, when hydrolyzed into extracts by chemical, enzymatic, or biological agents, such as fungi or bacteria, generate monosaccharides and amino acids, respectively (do Prado *et al.* 2021). These compounds play a crucial role as biostimulants in germination, developing new products that can be produced from seed cake.

In a biotechnological development approach utilizing the biomass from *I. edulis* seeds, there

is potential for separating proteins followed by carbohydrates. The proteins possess added value for health-related products, and subsequently, the carbohydrates can be used to develop bioinputs, biofuels, and other products.

#### Environmental Approach

Seeds collecting, seedlings production, and trees planting activities provide a significant environmental approach in schools and the involved community. These activities allow students and the community to understand the natural and artificial environment. Furthermore, the activities encourage acquiring knowledge, values, behaviors, and practical skills, enabling active participation in the prevention and solution of environmental management challenges (Dias & Salgado 2023).

Pedagogical practices carried out in schools with the establishment of experimental forest nurseries have proven to be suitable for environmental education. They seek to instill new social values, knowledge, skills, attitudes, and competencies for sustainable, student-centered environmental practices.

The changes in natural environments in the Amazon, including those highlighted in the media from 2019 to 2022, have been intense, degrading biodiversity-rich areas and other natural resources. There is a need to involve students in the production of forest nurseries because of the risk of water shortages, wildfires, the advance of extensive livestock farming, and predatory and irregular mineral exploitation. These issues contribute to the extinction of flora and fauna in the Amazon rainforest (Luis Val & Wood 2022).

In the activities conducted in this research, an interaction was promoted between students from the technical course in Environmental Management at IFAM and the school community in the Amazon region. The activity aimed to create a sense of belonging and scientific understanding of such a meaningful action. This included the preservation of native species from the region and the empowerment of students to plant, consequently helping to address the environmental issues in the Amazon. Furthermore, the popularization of *I. edulis* planting can help in soil recovery due to the leaf litter formed and its potential to survive in degraded soils, fixing nutrients (Nichols & Carpenter 2006).

Our environmental education work with *I. edulis* becomes more relevant when we understand that this species has been chosen for planting in degraded soils due to its high survival rate, good canopy development in the early years after planting, and ease of cultivation in nurseries, making it suitable for agroforestry systems (Fernandez Barrancos *et al.* 2022).

The concern for environmental education related to Amazonian fruit trees stems from the importance of raising awareness about biodiversity conservation and the significant role these trees play in addressing the socio-economic needs of socially marginalized communities and food insecurity (Vieira & Panagopoulos 2024).

During school activities, the educational focus was on utilizing these fruits for children's nutrition as an alternative to traditional fruits. Nurseries are responsible for highlighting the importance of these fruits for nutrition and the care required for their seeds to ensure successful planting. Cultivating and ensuring food security in urban environments using native local species is a beneficial practice that is gradually forgotten as urbanization progresses. Environmental education is crucial in revitalizing the connection with forest food species (Albrecht & Wiek 2021).

Therefore, this activity was based on the premise that the school environment is one of the first steps in raising environmental awareness among future citizens, engaging students with a healthier coexistence with nature (de Sousa *et al.* 2011). We also consider that activities involving students can promote initiatives beyond the school environment. Certain species with potential for agroforestry can be studied for conscious biotechnological exploitation, balancing scientific and technological progress with ethical values and environmental concerns to promote human wellbeing and global sustainability.

#### **CONCLUSION**

In the biotechnological context, this study unveiled the composition of the primary components of I. edulis seeds, with matrix M4 showing the most favorable results in terms of physiological development. Results of this study highlighted the potential for further research into using this species, especially considering its richness in carbohydrates and proteins. This discovery brings innovation to integrated forest production without causing destruction. It was also found that the fruit-bearing *I. edulis* is a suitable alternative for promoting environmental education in schools, engaging a larger number of people in preserving the natural resources of the Amazon biome. These results demonstrate the importance of valuing and sustainably utilizing the region's resources and promoting practices that encourage conservation and environmental awareness.

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