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PROJECT-BASED LEARNING: APPLICATION IN BIOCHEMICAL ENGINEERING

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ABSTRACT
The objective of this work was to apply the Project-Based Learning methodology in the teaching of the biochemical engineering discipline of the Food Engineering course. For this, the project focused on the development of an alcoholic beverage made from cupuaçu (*Theobroma grandiflorum),* divided into 4 basic stages: bibliographic research, bench-scale production (3L), pilot-scale production (30L) and microbiological and physicochemical analyses. Thus, it was possible to verify that the application of this active methodology in

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ISSN: 2358-2472

the discipline of Biochemical Engineering, provided the development of a complex product and passed on the knowledge in a practical way, enabling the improvement of the teaching-learning process. In addition, it was possible to identify a greater understanding of the concepts as well as the improvement of skills such as planning, commitment, collaboration and proactivity.

Keywords: Active methodologies. Quality education. Cupuaçu. Alcoholic fermentation.

Revista ARACÊ
ISSN: 2358-2472

INTRODUCTION

Active methodologies are teaching approaches in which the student plays an active role in the construction of his or her own knowledge (Prince, 2004). According to Mitre et al. (2008), they are described as "methodologies based on autonomy", while Souza et al. (2014) highlight that the main focus is on the student, who assumes co-responsibility for their learning, thus stimulating their autonomy in the educational process. According to Vieira (2015), the application of methodologies takes place through its fundamental component, which is the problem, in which a teacher becomes a facilitator, having the function of managing learning through cognitive problems and challenges, stimulating curiosity, exchanging knowledge (Macambira, 2011)

An active methodology of emphasis is project-based learning (PBL) which can be defined as an innovative method as opposed to the traditional teaching method and which has as characteristics, the selection of basic information, the performance of cooperative teamwork, definition of the driving issue (focus on students), feedback and review, research and innovation, the analysis of opportunities and reflection, the research process, the public presentation of the results and the appreciation of the student's voice and choice (BENDER, 2014; NOBRE et al, 2023).

This ABPj can be applied in complex disciplines, bringing benefits in the teaching-learning system, such as Biochemical Engineering, which according to Schmidell (2001) is the application of Chemical Engineering in the solution of problems that present in the implementation of biotechnological processes on a large scale and in their optimization.

One of the biotechnological products produced on a large scale is alcoholic beverages that are produced from various raw materials, especially from cereals, fruits and sugary products (Ward apud Silva, 2011). According to Decree No. 2,314, of September 4, 1997, fermented alcoholic beverages are beer, fermented fruit, cider, mead and fermented sugarcane. This decree defines that fermented fruit is a beverage with an alcohol content of fourteen percent volume, at twenty degrees Celsius, obtained from the alcoholic fermentation of the must of healthy, fresh and ripe fruit. (Brasil apud Silva, 2011).

The fermented fruit beverage is often called wine, however the legislation establishes the denomination of wine only for alcoholic beverages fermented from grapes (*Viris vinífera*) (BRASIL, 1988a). Wines that do not come from grapes must be labeled with the denomination fermented accompanied by the name of the fruit from which it originated, as examples: fermented pineapple, fermented orange, fermented cashew, fermented prickly pear, among others (Lopes; Silva, 2006).

Among the fruits, there is the cupuaçu (*Theobroma grandiflorum*), one of the most characteristic fruits of the Amazon, stands out for its unique flavor and aroma, being widely cultivated in Brazil and used both in the food industry and in cosmetics (Alves et al., 2014). Its fruit, a berry with a rigid skin and dark brown color, has an acidic, mucilaginous pulp with a pleasant flavor, usually yellow, cream or white, with an intense odor (Souza et al., 2011).

Cupuaçu pulp is the main product extracted from the fruit and has physicochemical, bioactive and sensory characteristics that make it ideal for the production of various food products, such as juices, ice creams, creams, bonbons, liqueurs, alcoholic fermented products and jams (OLIVEIRA; GENOVESE, 2013; PUGLIESE et al., 2013; OLIVEIRA PEROTE et al, 2024).

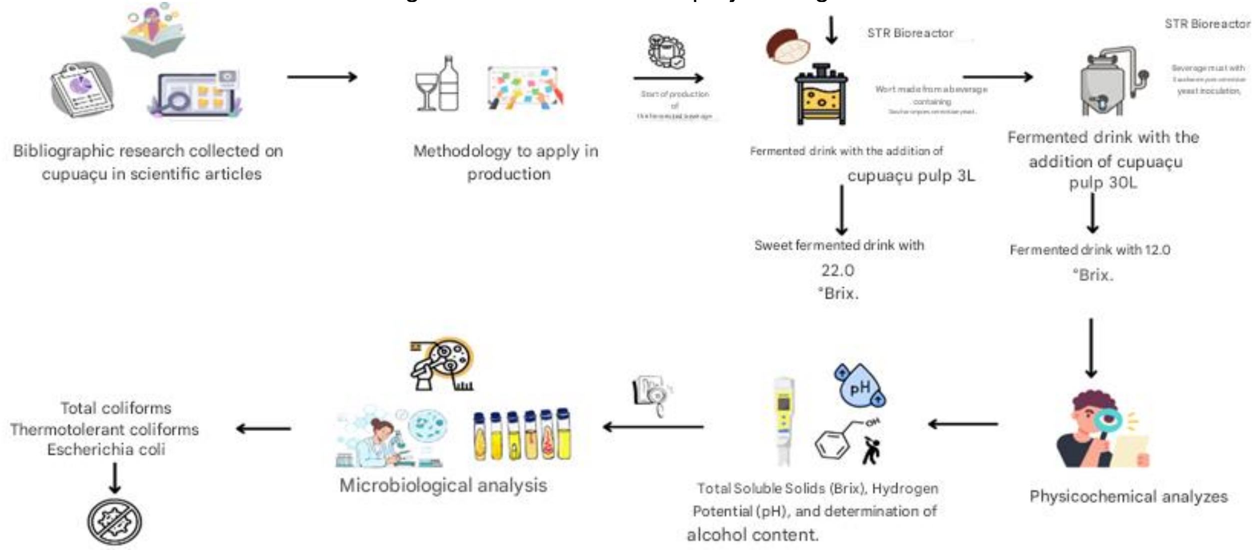
Thus, the objective of this work was to apply the ABPj methodologies in the teaching of the biochemical engineering discipline of the Food Engineering course.

METHODOLOGY

The project focused on the development of an alcoholic beverage made from cupuaçu (*Theobroma grandiflorum),* divided into 4 basic stages: (1) Bibliographic research, (2) Bench scale production (3L), (3) Pilot scale production (30L) and (4) Physicochemical and microbiological analyses. Stages 2, 3 and 4 were carried out at the Laboratory of Applied Thermodynamics (LABTERMO), located at the Faculty of Agrarian Sciences (FCA), Federal University of Amazonas (UFAM), Manaus Campus. See Figure 1.

Revista ARACÊ
ISSN: 2358-2472

Figure 1. Flowchart of the project stages.



Sources: Authors (2024).

LITERATURE RESEARCH

Initially, the students were divided into 3 groups to carry out research on alcoholic fermented fruits and on the technique of immobilization of cells and enzymes applied in the production of beverages.

The first team carried out the research according to the following criteria: 1) define a question for the study; 2) develop a search strategy; 3) establish inclusion and exclusion criteria and 4) perform screening to define the methodological quality of the retrieved articles. For the selection and exclusion of articles, the following criteria were applied: a) Keywords: Articles should contain at least one of the following keywords: Food, fermentation, amazon, fruit, drinks and amazon fruits; b) Search string: The search strategies were elaborated using Boolean operators (AND, OR and NOT); c) Year of publication: Only articles published between 2014 and 2024 were selected; d) Language: Only articles published in Portuguese and English; e) Country of origin: Only research carried out in Brazil was analyzed; f) Databases: The survey of studies was conducted in two databases: Scopus and Scielo; h) Selection and filtering: After the initial verification of the abstracts and a detailed reading, 15 articles were selected for their direct relevance to the objective of the proposed work.

The second team chose to review the literature on the potential of Amazonian fruits in fermented beverages, especially camu-camu, cupuaçu and passion fruit. The topic was included in ScienceDirect and Scielo, where the articles should contain the following keywords: Alcoholic fermentation, bioeconomy, potential of Amazonian fruits; in both

Revista ARACÊ
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Portuguese and English, the inclusion criteria covered articles published in the years 2018 to 2023, peer-reviewed studies, available in full text, and that directly addressed the use of Amazonian fruits in the production of fermented beverages. The exclusion criteria were applied to eliminate works that did not involve the aforementioned fruits or that dealt with other technological processes unrelated to fermentation.

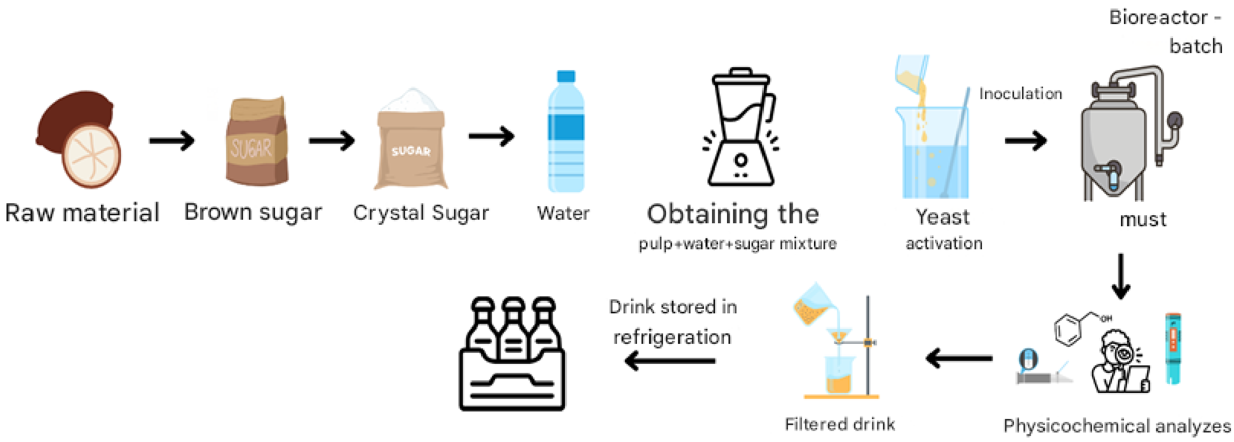
The third team performed a literature review on the use of immobilized cells and enzymes in beverage production using the databases Periódico Capes, Elsevier, Scielo, PubMed, USP BR and ScienceDirect. Bringing together articles on cell and enzyme immobilization supports and techniques for application in the production of fermented beverages. Using more recent publications and the following keywords: Immobilization, Enzyme, and Cell. Seven articles with the most relevant potential and year of publication between 2003 and 2024 were selected.

BENCH-SCALE PRODUCTION

After extensive bibliographic research and publication in the academic week of agronomy 2024 - UFAM, the production of an alcoholic fermented cupuaçu was made, with the aim of valuing the Amazonian product.

Figure 2 shows the flowchart of the production of a fermented alcoholic beverage made with cupuaçu pulp. The fermented product was developed according to adaptations of the methodologies of Lima (2021) and Araújo et al (2020).

Figure 2 - Flowchart of the production of cupuaçu fermentation.



Sources: Authors (2024).

The frozen pulp was purchased in the municipality of Manaus-AM. To start production, the bioreactor was asepsis carried out in the laboratory to avoid contamination.

Initially, the must was prepared with 25% pulp and 75% water. Next, the chaptalization of the must was performed up to 33° brix, using brown and refined sugar in equal proportions. The inoculum was prepared with the yeast Saccharomyces cerevisiae, activated at a concentration of 8 g/L. The must was transferred to a bioreactor without agitation, sanitized and equipped with a plastic hose for the CO_2 outlet. Fermentation was carried out in batch in a 3L bioreactor, kept at room temperature in the laboratory for 9 days, with periodic analyses for the kinetic study of the production.

PILOT SCALE PRODUCTION

The results of bench-scale production were presented at the 2024 Food Engineering academic week - UFAM, and it was decided to increase the production scale to 30L, adjusting parameters in order to increase the acceptability of the fermented produce (see figure 3).

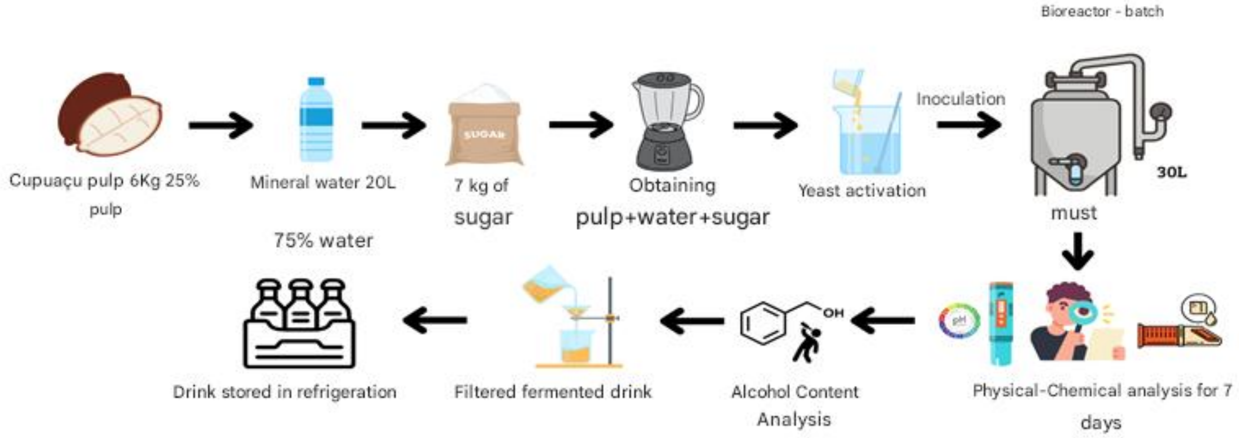
For the production of the fermented cupuaçu beverage on a pilot scale, in order to reduce the cost of the process, as well as increase the alcohol content, and based on the study by Santos and Santos (2024), it was decided to reduce the proportions of yeast and sugar used in the must. 6 kg of cupuaçu pulp, 20 liters of water (distributed throughout the process stages), 7 kg of sugar (sufficient to reach 25° Brix) and 80g of *Saccharomyces cerevisiae* yeast were used. The proportions were established ensuring the homogeneity and efficiency of the fermentation process.

Initially, part of the 20 liters of water was used to dilute the cupuaçu pulp, which, after the process, was filtered to remove solid residues and obtain a more uniform liquid. Another fraction of the water was used to dissolve the sugar, ensuring its total homogenization before being incorporated into the main solution. At the same time, a small amount of the same water was used to activate the yeast, hydrating the 80 g of *Saccharomyces cerevisiae* and ensuring its fermentative viability.

After these steps, all the components were assembled in the reactor. The mixture was then homogenized to ensure the uniform distribution of the yeast and the other ingredients, creating an ideal environment for the fermentation process.

Revista ARACÊ
ISSN: 2358-2472

Figure 3. Flowchart of the production of fermented cupuaçu on a Pilot scale.



Source: Authors (2024).

PHYSICOCHEMICAL AND MICROBIOLOGICAL ANALYSES

In this study, physicochemical analyses were carried out in triplicates during the fermentation period (kinetic study) evaluating the following parameters: Total Soluble Solids (°Brix), Hydrogen Potential and alcohol content (%v/v). These analyses were performed both at the bench and pilot scales.

The product developed on a pilot scale was analyzed in relation to total acidity, volatile acidity, fixed acidity, dry extract according to the Analytical Standards of the Adolf Lutz Institute (2008) and microbiological according to SCHWEIG (2018) and SANTOS (2020), however no regulation for alcoholic fermented beverages was seen in ANVISA's Normative Instruction (2022).

RESULTS AND DISCUSSION

The results as well as their respective discussions are described in items 3.1, 3.2 and 3.3.

LITERATURE RESEARCH

The first team selected 10 works that covered fruits such as Brazil nuts, taperebá, cocoa, cupuaçu, açaí, jabuticaba, peach palm and Araçá-boi. The products purchased include probiotic drinks, kombucha, yogurt and wine. The studies focused on the fermentation process and the characterization of physicochemical, sensory, and bioactive compounds. Probiotic viability, stability during storage, antioxidant compounds and sensory acceptability were evaluated. The most promising beverages include kombucha containing Amazonian fruits, yogurt containing cupuaçu, and alcoholic beverages made from cupuaçu, açaí, and araçá-boi, due to their sensory acceptability and functionality of bioactive compounds and antioxidants.

The second team had as a result of the bibliographic research the articles: biodiversity market and the camu-camu production chain (myrciaria dubia (h.b.k.) in the state of Amazonas (2018), Amazonian fruits: biotechnology and sustainability. (2020), Alcoholic fermented fruits (2020), Alcoholic fermented fruits: a review of the stages and parameters of production, physicochemical characteristics and bioactive potential of beverages (2022), Use of naturally occurring yeasts in the biotransformation of fermentable sugars from Amazonian fruits applied in the production of brandy (2021), Production of craft beer with the addition of ascorbic acid from Amazonian fruit (2019), Use of camu-camu (myrciaria dubia) for the production of fermented alcoholic beverages. acta amazônica (2003), Elaboration of caxiri beer-style craft beer with the addition of camu-camu (*myrciariadubia)* (2022), Batch production and sensory characterization of witbier craft beer using camu-camu in its composition (2018). Thus observing that camu-camu emerges as a fruit with enormous potential for innovation in the production of Amazonian fermented beverages, thanks to its high vitamin C content. Research and development play an essential role in overcoming challenges in cultivation, processing and conservation, as well as allowing the creation of differentiated beverages that serve a growing market for healthy and artisanal foods and beverages.

The third team viewed the following articles together with the literature search: Properties of hydrogel materials used for microbial cell trapping in the production of

Revista ARACÊ
ISSN: 2358-2472

fermented beverages (2024), Biopolymers and nanostructured materials to develop immobilized nanobiocatalytic systems based on pectinases for biotechnological applications (2021), Overview of the application of βgalactosidase "immobilized in nanoparticles" in dairy industries (2021), Use of immobilized cells of Bacillus subtilis and Saccharomyces cerevisiae in the simultaneous process of saccharification and continuous fermentation (2019), Production of alcohol-free beer using free and immobilized cells of Saccharomyces cerevisiae deficient in the tricarboxylic acid cycle (2010), Immobilization technologies and appropriate support materials in the production of alcoholic beverages: a review (2004) and Production of wine using immobilized yeast in quince biocatalyst temperatures between 30 and 0 °C (2003) and evaluated that cell and enzyme immobilization emerges as a promising technique in the production of fermented beverages, offering significant advantages in efficiency and quality. The diversity of supports and immobilization methods available allows the industry to adapt to market demands, optimizing processes and reducing costs.

Based on these researches, cost analyses and group discussions, it was decided to make an alcoholic fermented cupuaçu.

KINETIC STUDY

Table 1 shows the kinetic data of Total Soluble Solids (TSS), Alcohol Content (TA) and Hydrogen Potential (pH) from both the bench (3l) and pilot (30l) scales.

Table 1. Kinetic data of the alcoholic fermented cupuaçu.

	Bench Scale			Pilot Scale		
Time (days)	SST (°Brix)	T.A (%v/v)	ph	SST (°Brix)	T.A (%v/v)	ph
0	33	0	3,24	25	0	3,56
1	31,2	0,97	3,26	23	1,08	3,47
2	31	1,08	3,26	21	2,16	3,39
3	29	2,16	2,36	18	3,78	3,41
4	28	2,70	3,35	16	4,86	3,41
5	27	3,42	3,34	14	5,95	3,59
6	22	5,94	3,38	12	7,03	3,69
7	22	5,94	3,44	-	-	-

Source: Authors (2024).

The drop in TSS content (°Brix) occurred continuously and gradually over the days, showing a more linear behavior in pilot scale fermentation compared to the experiment carried out on bench. On the first day of fermentation, there was a reduction from 25 to 23 °Brix in the pilot scale, while in the laboratory experiment, the total soluble solids decreased from 33 to 31.2° Brix. This initial reduction is significant, which suggests that the yeasts quickly adapted to the wort in both experiments. However, pilot-scale performance indicates that the yeasts adapted more efficiently. Almeida (2020) reinforce this interpretation, highlighting that the reduction of total solids during fermentation is evidence of the conversion of sugar into alcohol, which occurs as microorganisms adjust to the environment and use sugars as an energy source.

This rapid adaptation is a positive aspect at both scales, as it demonstrates that the microorganisms began to metabolize the available sugars effectively early in the process. The TSS content gradually reduced over the days, until it reached approximately 22 and 12° Brix, in the pilot and bench scales, respectively, on the sixth day of fermentation. The bench-scale experiment was stabilized on the seventh day, because according to Brunelli (2015) fermentation is considered to be over with the stabilization of the soluble solids content (ºBrix) of the fermented product. This can be justified, according to Sroka and Tuszyński (2007), musts with higher initial concentrations of sugar can cause the inhibition of the fermentation process.

The final alcohol content was 5.94%v/v for the benchtop and 7.03% for the pilot scale, this difference can be attributed to the adjustment in the initial TSS for the pilot scale fermentation. These values are among the limits established by the Legislation that allows a content of 4 to 14% in volume (BRASIL, 2009). A similar result was found by Oliveira et al (2020) who obtained an alcohol content of 5.67 %v/v on the sixth day of fermentation of "cupuaçu wine".

The pH remained stable throughout the fermentation period, showing good process control. In the initial days, a reduction in pH values is observed for subsequent increase. The final values were 3.44 and 3.69 for the bench and pilot scales, respectively. These acid values are favorable to the process, since bacteria and other contaminants do not remain in low pH media and the generation of glycerol as a complete component of fermentation is reduced in acidic media (pH 3 to 4) (JEAN, 2010; LIMA et al, 2021).

Mathematical Modeling

In this topic, an essential part of Biochemical Engineering was founded, which is the understanding of the modeling of fermentative processes, which can be defined as the attempt to represent, through mathematical equations, associated the complex biochemical transformations that occur in the process and the speeds with which these transformations

Revista ARACÊ
ISSN: 2358-2472

are processed. According to Voleski and Votruba (1992), the formulation of a mathematical model must have a compromise between a reasonable degree of complexity and an economically desirable solution. Thus, in order to predict the dynamic and stationary behavior of this fermentation process, seeking to determine the economically optimal operating conditions of the system, the mathematical modeling was made in relation to the orders 0, 1 and 2 following the graphic method. For order models 1 and 2 of the product, the starting point (outliers) was excluded for better adjustments of the models. Tables 2 and 3 show the parameters of the substrate (TSS) and product (AT) modeling for the mentioned models.

Table 2 - Total Soluble Solids (°Brix) at bench and pilot scale

Workbench	Order 0	Order 1	Order 2
the	-0,053	-0,002	0,00008
b	32,40	3,488	0,030
R^2	0,926	0,934	0,936
Workbench	Order 0	Order 1	Order 2
the	-0,077	-0,004	0,000
b	23,31	3,265	0,035
R^2	0,985	0,962	0,921

Source: Authors (2024).

Table 3 - Alcohol content on bench and pilot scale

Workbench	Order 0	Order 1	Order 2
the	0,029	0,009	-0,003
b	0,32	0,054	0,860
R^2	0,926	0,793	0,609
Workbench	Order 0	Order 1	Order 2
the	0,043	0,013	-0,005
b	0,339	-0,142	0,916
R^2	0,979	0,965	0,858

Source: Authors (2024).

In the modeling of the substrates, it is observed that all models obtained good fits to the experimental data, with emphasis on the zero-order model on a pilot scale obtained a coefficient of determination equal to 0.985. This better fit of this model can be explained by the good adaptation of the yeasts to the wort, inferring that there were no adaptation or transition phases, and that possibly the fermentation had not yet finished in the time of 6 days.

As for the product (TA), it was observed that the zero-order models also obtained good adjustments mainly in the pilot scale, possibly due to the optimizations made during the scale change (reduction of the initial total soluble solids and the initial amount of yeasts).

Revista ARACÊ
ISSN: 2358-2472

MICROBIOLOGICAL AND PHYSICOCHEMICAL CHARACTERIZATION OF THE BEVERAGE

The table below shows the microbiological characterization of the cupuaçu alcoholic beverage carried out at the fish technology laboratory (LABTEC-UFAM), determination of total coliforms, thermotolerants and *escherichia coli*. It can be noted that the beverage did not present the presence of total, thermotolerant coliforms and *escherichia coli*, being within the standards, verifying that the production followed good production practices.

Table 4. Microbiological characterization of cupuaçu

Analysis	Data obtained
Total coliforms	Absent
Thermotolerant coliforms	Absent
Escherichia coli	Absent

Source: survey data (2024)

In addition, the physicochemical properties of the developed product (total titratable acidity, fixed acidity, volatile acidity, dry extract and specific mass) were determined, shown in Table 5. These analyses were performed in triplicate, according to the Adolfo Lutz Institute Method (2008).

Table 4. Microbiological characterization of cupuaçu

Parameters	Cupuaçu Fermented	Limits of Legislation*
Total Titratable Acidity (mEq/L)	42.88 ± 1.15	≥50.00 and ≤ 130.00
Fixed Acidity (mEq/L)	38.08 ± 2.00	≥30.00
Volatile Acidity (mEq/L)	6.88 ± 2.94	≥20.00
Dry Extract (M/V)	21 ± 0.5	≥7.00
Specific mass	0.97 ± 0.004	-

*Limits related to Ordinance No. 64, of April 23, 2008, which approves the technical regulation for the establishment of identity and quality standards for fermented fruits.

The data presented in the table above, referring to the fermented cupuaçu, were compared with the parameters established by the legislation, allowing the analysis of the fundamental aspects to ensure the stability and quality of the product. It is observed that the value of total acidity is below the limit provided for by legislation, which can influence the overall acidity of the product. On the other hand, the results for fixed acidity are within the required standards, reflecting the presence of fixed acids that confer sensory characteristics to the product. The volatile acidity is also below the established limit, which indicates that the drink has a low formation of volatile acids. The dry extract, in turn, suggests that the fermented product has a composition rich in solids, with values higher

ISSN: 2358-2472

than those required by the ordinance. This result contributes to a more full-bodied product with greater microbiological stability, factors that can positively influence its storage. The specific mass does not have a required standard, but the result obtained presents us with a denser drink, due to the presence of solids, as it is a fermented fruit, it is desirable that this drink is full-bodied, linked to the sensory of the product.

CONCLUSION

The application of the project-based teaching methodology in the discipline of Biochemical Engineering provided the development of a complex product and the transfer of knowledge in a practical way, enabling the improvement of the teaching-learning process. In addition, it was possible to identify a greater understanding of the concepts as well as the improvement of skills such as planning, commitment, collaboration and proactivity.

The developed product, "fermented cupuaçu", pointed out physicochemical and microbiological characteristics that meet the limits defined by the current legislation, evidencing positive, since the production, characterization and kinetics objectives were concluded, discovering the most appropriate musts that generated alcoholic fermented products within the manufacturing standards. It is possible to prove the technical feasibility of the production of an alcoholic beverage with the pulp, having it as an excellent alternative to contribute to the growth of the agroindustry, collaborating in the income of rural properties in the North region.

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Revista ARACÊ
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