

Post-harvest degradation of *Agave* juice: challenges for industrial use

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Abstract: This study is part of an initiative to promote the development of ethanol production routes to support the *Agave* production chain in Brazil. One of the objectives was to assess the feasibility of using conventional industrial milling units, typically employed for sugarcane processing, for extracting *Agave* juice for potential ethanol production. As a proof of concept, 4 tons of *Agave* were transported from Conceição do Coité (BA) to an industrial processing facility in the state of Espírito Santo. Juice extraction was successfully carried out, and laboratory analyses were performed to evaluate its quality. Results indicated that the time between harvesting and processing significantly influenced juice composition, suggesting potential negative impacts on fermentability and ethanol yield.

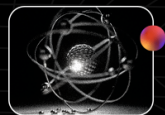
Keywords: *Agave* juice; postharvest degradation, juice analysis.

1. INTRODUCTION

A Proof of Concept (POC) is an experiment or project whose main objective is to validate the viability of an idea, technology, or solution before its large-scale implementation. In addition to validating the viability of an idea, the POC identifies flaws and opportunities for improvements in equipment, technology, and logistics before full development, avoiding wasting significant time and money on something that may not work. Therefore, the POC for experimental *Agave* juice extraction tests at an industrial unit aimed to provide the information necessary for designing an efficient handling and milling system, as well as assessing juice extraction yield. This information was crucial for developing engineering solutions to be applied to customize *Agave* juice handling and extraction equipment to optimize ethanol production.

In the mid-1970s, global events associated with the emission of substances that harm the environment, price pressures, and the prospect of depletion of non-renewable fossil fuel sources led Brazil to implement the National Alcohol Program (PROALCOOL) [1], [2–3]. This definitively introduced ethanol into the national energy matrix, making the country the world's leading producer of fuel ethanol [4–5]. In the 2015/2016 harvest, Brazilian ethanol production was estimated at 29.2 billion liters, ensuring the country's renewable energy profile [1–2].

Brazil plays an important role in the global scenario of technologies aimed at the production of renewable energy sources, due to the extensive land areas available for sugarcane, which, according to Ribeiro⁶, is the third most cultivated crop in the country, behind only soybeans and corn. With the increased demand for ethanol, the



number of alcohol distilleries throughout the country has been increasing [7].

An example of an initiative that fosters the sustainable, long-term development of ethanol production is the Brazil *Agave* Development (BRAVE) Innovation and Technology Program, developed in partnership with Unicamp University and Senai Cimatec. The program's main objective is to explore *Agave* as a source of clean and renewable energy, aiming to develop an agro-industrial chain based on the plant [8-9].

In this context, a series of results has already been generated at the laboratory scale. However, for the project to advance and achieve promising and relevant outcomes for technology development, identifying potential challenges related to scale-up, logistics, and *Agave* processing offers the opportunity to optimize extraction, filtration, and juice treatment technologies, among others. This is a fundamental step for the scale-up process.

The logistical challenges commonly faced during the development of the sugarcane ethanol industry are also likely to arise in the *Agave* processing industry. Factors such as harvesting timing, transportation distances, and handling procedures can significantly impact raw material quality and, consequently, the efficiency of the ethanol production process. Addressing these logistical issues early on is crucial to ensure the viability and scalability of *Agave*-based ethanol production.

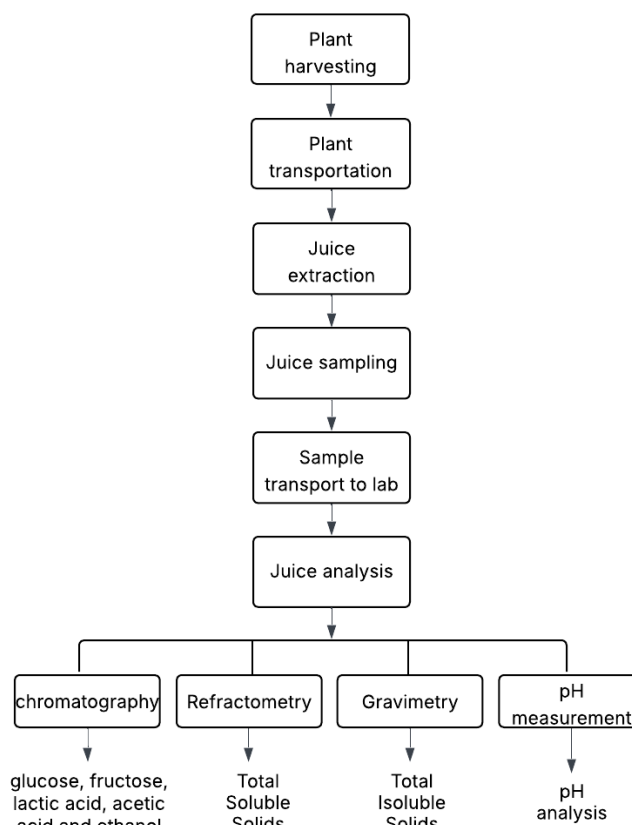
The present study aims to investigate these logistical challenges in detail, providing insights

and potential solutions to optimize the processing chain and improve overall ethanol yield.

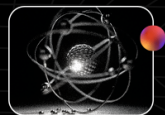
2. METHODOLOGY

The type of research in the present study has an exploratory approach based on qualitative and quantitative studies following bibliographic research procedure, documentary research, experimental research. To achieve the objectives of this proposal, an experimental plan was generated, as shown in Figure 1.

Figure 1. Flowchart describing the experimental steps to be performed



Source: Authorial (2025)



2.1. Plant Collection and Field Transportation

The plants from Conceição de Coité region, Bahia, were harvested between December 4, 2024, and December 5, 2024, loaded into a box truck on December 6, 2024, and transported to the city of Castelo, Espírito Santo, arriving on December 8, 2024. A total of 125 plants, weighing approximately 4,000 kg, were available for testing.

2.2. Milling of *Agave* plants

After receiving the samples at the facilities of AGUARDENTE CASA VELHA, located in the municipality of Castelo, Espírito Santo State, the tests were conducted in batches on December 11 and 12, 2024. A total of five batches were performed using a mixture of *Agave* plants as raw material. This was carried out in an industrial facility primarily dedicated to sugarcane processing for cachaça production.

2.3. Juice sampling

The grinding POC consisted of five batches of juice extraction. The Bioprocesses team of Senai Cimatec received six vials containing juice samples collected at various stages of the grinding test, as detailed in the caption to Figure 2. Juice samples were transported in a cooler box until they arrived at SENAI CIMATEC Park (Camaçari, BA).

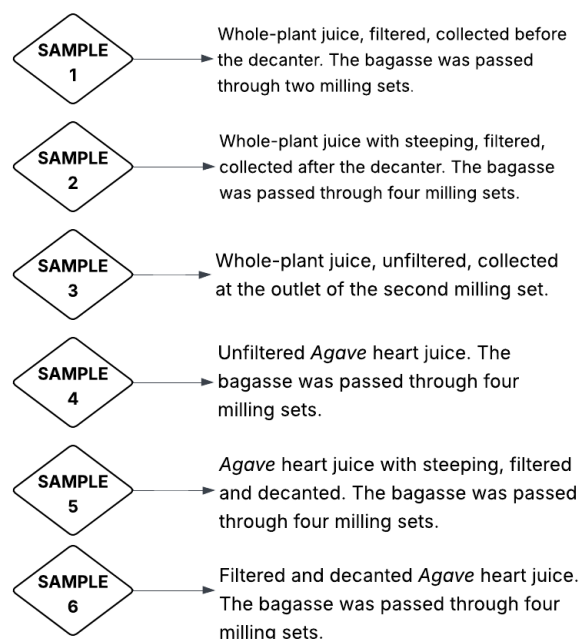


Figure 2. Juices collected during the grinding POC of the *Agave*.

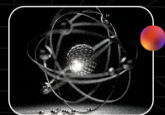
Source: Authorial (2024)

2.4. Juice Analysis

Upon receipt, the juices highlighted in Figure 2 were analyzed using the characterization techniques described in the flowchart (Figure 1).

2.4.1. HPLC Analysis

Before analysis, the samples were diluted with ultrapure water and filtered through a 0.22 μm membrane. The analysis was performed using an Agilent HPLC system equipped with a Hi-Plex H column. Sugari-Plex H column was used for sugar, ethanol and organic acid analysis, operating at 60°C with a flow rate of 0.6 mL/min. The injection volume was 10 μL , using 0.01 M sulfuric acid as the mobile phase.



2.4.2. Total Soluble Solids Analysis

The total soluble solids content was measured using a digital refractometer, with results expressed in degrees Brix ($^{\circ}\text{Brix}$). Samples were placed on the refractometer prism, and readings were taken at room temperature.

2.4.3. Total Insoluble Solids Analysis

The insoluble solids content was determined by gravimetric analysis. A known volume of the sample was filtered through a pre-weighed filter paper using vacuum filtration. The filter containing the insoluble residue was then dried in an oven at 105°C until a constant weight was achieved. The weight of the dried residue was measured, and the insoluble solids content was calculated as the percentage of the initial sample volume.

2.4.4. pH Analysis

For pH analysis, the samples were read with a pH meter to understand the alkalinity of the broth samples.

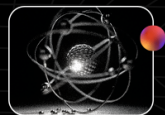
3. RESULTS AND DISCUSSION

The results, summarized in Table 1, reflect the influence of harvesting, transportation, and processing conditions on juice quality parameters, including sugar concentration, organic acids, soluble and insoluble solids, and pH. These findings are analyzed, with emphasis on their implications for fermentation potential, and overall ethanol yield.

A relevant aspect that caught our attention when analyzing the data in Table 1 is the high concentration of organic acids, especially lactic acid, which was present in concentrations of approximately 9 to 20 g/L. Acetic acid, on the other hand, was present in concentrations of approximately 2 to 5 g/L. It is likely that during the time elapsed until the plants were processed, biochemical reactions occurred caused by microbial activity (such as lactic acid-producing lactic acid bacteria) and spontaneous fermentation (approximately 2 g/L of identified ethanol), which occurs as the sugar-rich material is stored under ambient conditions. This result corroborates those already obtained in the laboratory, where it has been shown that logistics

Table 1: Results of the characterization of samples from the POC of *Agave* milling. The analyses were performed in triplicate. N.D. – not detected. *Estimated by peak area in the HPLC Analysis.

sample	$^{\circ}\text{BRIX}$	Soluble solids (%, m/m)	pH	Glucose (g/L)	Fructose (g/L)	Ethanol (g/L)	Acetic acid (g/L)	Latic acid (g/L)	*Inulin (g/L)
1	$15,8 \pm 0,1$	$13,6 \pm 0,8$	$4,26 \pm 0,01$	$0,3 \pm 0,0$	$14,8 \pm 0,5$	$2,6 \pm 0,0$	$2,4 \pm 0,0$	$18,8 \pm 0,5$	57,0
2	$11,7 \pm 0,2$	$14,9 \pm 0,0$	$4,13 \pm 0,01$	$0,2 \pm 0,0$	$8,0 \pm 0,1$	$2,2 \pm 0,0$	$3,9 \pm 0,0$	$18,1 \pm 0,1$	40,0
3	$17,8 \pm 0,7$	$14,2 \pm 0,7$	$4,20 \pm 0,01$	$0,3 \pm 0,0$	$4,5 \pm 0,1$	$1,7 \pm 0,5$	$4,0 \pm 0,0$	$18,2 \pm 0,1$	82,3
4	$12,6 \pm 0,1$	$14,2 \pm 0,8$	$4,26 \pm 0,01$	$0,2 \pm 0,0$	$3,5 \pm 0,3$	$2,1 \pm 0,2$	$5,0 \pm 0,0$	$20,0 \pm 0,0$	42,0
5	$16,7 \pm 0,1$	$13,4 \pm 1,9$	$3,97 \pm 0,03$	N.D.	$10,5 \pm 8,1$	$2,1 \pm 0,2$	$2,1 \pm 0,0$	$8,7 \pm 0,2$	90,8
6	$18,8 \pm 0,2$	$14,1 \pm 0,4$	$3,98 \pm 0,03$	$0,6 \pm 0,0$	$22,4 \pm 1,8$	$2,3 \pm 0,3$	$3,2 \pm 0,0$	$10,6 \pm 0,0$	88,1



and storage are sensitive issues for scaling up ethanol production from *Agave*. The presence of these organic acids in high concentrations in the raw material has the potential to negatively affect the quality of the *Saccharomyces cerevisiae* fermentation broth, as they tend to impair yeast cell viability, compromising ethanol yield. This issue was also highlighted by Ceballos-Schiavone¹⁰ in his study on the heat treatment of sugarcane juice to reduce bacterial contaminants (*Lactobacillus*) in ethanol production. The authors reported that the presence of contaminants during fermentation directly influences productivity and the final yield of the process by degrading sucrose and producing organic acids, which cause sugar loss and yeast toxicity.

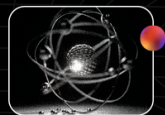
The °Brix values observed in Table 1 fall within an interesting range for ethanol production. Based on our previous observations, juices with °Brix values close to 20 can potentially yield between 60 and 70 g/L of ethanol under favorable fermentation conditions (data not shown in this article). This reinforces the relevance of °Brix as a quick reference parameter for assessing the fermentation potential of *Agave* juice.

Regarding the availability of total sugars (fructose, glucose, and inulin), which represent the theoretical potential of the juice for ethanol production, juices 5 and 6, resulting from the extraction of pine cone juice with re-passing of the bagasse through the milling system to simulate the use of four milling sets, stood out for

presenting total sugar concentrations of 101 and 111 g/L, respectively. Additionally, these juices had lower lactic acid concentrations compared to samples from the extraction of whole plant juice (samples 1, 2, and 3).

As previously verified in the laboratory by the Bioprocesses team, the juice extracted from *Agave* has a high concentration of insoluble solids. Gravimetric analysis revealed that these juices contained between 13 and 15% suspended solids (Table 1). Visual analysis revealed a significant presence of sand (not quantified). At this stage of the project, identifying a potential problem for scaling up offers the opportunity to adjust extraction, filtration, and juice treatment technologies before the process is scaled up. It is known that high concentrations of insoluble solids can lead to operational inefficiencies, such as increased energy consumption, the need for greater use of chemicals for juice treatment, and blockages in equipment (pumps, piping, and filters), which would result in additional costs and unexpected downtime. Furthermore, they can directly impact the quality of the ethanol produced. These solids can also impair fermentation by interfering with yeast activity. Furthermore, they can create environments conducive to the growth of undesirable microorganisms, further impairing the fermentation process and increasing the risk of product contamination.

The data presented in Table 1 show that there was no significant change in the pH of these broth samples. This is promising and expected, as



broth has a natural buffering capacity, composed of salts and organic substances, which tends to maintain a stable pH.

4. CONCLUSION

Even in favorable conditions, fermentation is often hindered by bacterial contamination, which reduces the efficiency of the process. Bacteria not only consume part of the production substrate but also generate toxic metabolites that inhibit yeast activity, promote flocculation, and result in lower ethanol yields. The acids produced in the fermentation medium can decrease yeast cell viability, and when combined with other stress factors, may stop yeast metabolism or even cause cell death. Therefore, preventing bacterial contamination must start early, ideally in the field, to avoid introducing large numbers of contaminants and their metabolites into the fermentation tank.

The results of this study indicate that *Agave* shows promising potential for first-generation ethanol production. However, to ensure success in the ethanol production chain, it is essential to minimize the time between plant harvesting and juice extraction. Longer delays lead to sugar degradation by microorganisms producing organic acids—especially lactic acid—thereby reducing the ethanol production potential.

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