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Valorization of by-products from the agro-industrial sector in Côte d'Ivoire: Lactic Acid production from cashew apple juice supplemented with sugarcane molasses

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Abstract

Côte d'Ivoire, the global leader in cashew nut production, largely abandons cashew apples after harvest. Similarly, sugarcane molasses, a by-product of sugar production, is underutilized. This study proposes an innovative method to valorize these two agricultural residues by producing lactic acid using the bacterium *Lactobacillus casei* NRRLB-441. The approach combines cashew apple juice and sugarcane molasses to create an optimized culture medium, offering promising economic and ecological opportunities. To achieve this, an I-optimal mixture design was implemented, with cashew apple juice proportions ranging from 50% to 90%, and sugarcane molasses from 10% to 50%. An experimental matrix of 13 trials, based on the Scheffé model, was developed to determine optimal combinations and measure lactic acid yields. This approach aims to maximize the utilization of these agricultural residues while providing promising economic and environmental prospects. Biochemical and mineralogical analyses revealed that cashew apple juice and sugarcane molasses are rich in glucose (82.78 g/L), fructose (66.65 g/L), and minerals (calcium, magnesium, iron, sodium, and potassium). The results showed a significant lactic acid concentration (78.57 g/L) when cashew apple juice and sugarcane molasses are in equal proportions (50%), with a yield of 69.22% and a productivity of 1.09 g/L/h. These findings confirm the bio-convertibility of these agro-resources into other economically significant products such as lactic acid, citric acid, and bioethanol.

Keywords: Valorization; Cashew Apples; Supplemented; Sugarcane Molasses; Lactic Acid; Côte d'Ivoire

1. Introduction

The cashew tree (*Anacardium occidentale L.*), belonging to the Anacardiaceae family, is a tropical tree native to Brazil. It produces two types of fruit: the cashew nut, considered the "true fruit," composed of a kernel and a shell containing cashew balm, and the cashew apple, known as the "false fruit," which corresponds to the hypertrophied peduncle attached to the tree [1]. Today, Côte d'Ivoire is the world's largest producer of cashew nuts, with an estimated production of over 1.2 million tons in 2023, thereby generating more than 9 million tons of cashew apples [2]. Initially introduced to sub-Saharan Africa, particularly in Côte d'Ivoire, to combat drought and soil erosion, the cashew tree has become a major agricultural crop [3]. In Côte d'Ivoire, the cashew industry employs about 400,000 producers and represents the third-largest source of foreign exchange in the agricultural sector, after cocoa and rubber. This industry plays a crucial role in rural development and the country's economy. However, despite the economic importance of cashew nuts, cashew apples, which make up a significant part of the production, are largely underutilized. After

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harvesting the nuts, the cashew apples are left to decompose naturally in plantations, which constitutes a waste of potentially valuable resources [4].

Similarly, another agricultural by-product, sugarcane molasses (SCM), is also underutilized. Derived from the refining of sugarcane juice during sugar production, SCM is a viscous, dark-colored residue often discarded in the environment or marginally used as animal feed, despite its significant economic and ecological potential [5]. The valorization of cashew apples and sugarcane molasses represents a major challenge for Côte d'Ivoire, both economically and environmentally. Transforming these by-products into valuable resources could create new opportunities for rural populations, diversify the agricultural economy, and reduce the environmental impact of agricultural waste [6]. Conversely, the limited interest in these products results in the loss of high-potential economic and technological resources [7].

Moreover, prior studies on the valorization of cashew apples and sugarcane molasses in Côte d'Ivoire have mainly focused on simple physicochemical and biochemical characterizations, the production of juice and jam, or studies addressing only one of the products rather than their combined use [4], [8], [9]. This limited valorization of these agro-resources may have several negative impacts on sustainable development goals, particularly poverty and inequality. Poor management of these agro-resources can lead to income loss for farmers, thereby creating poverty and inequality [10]. Inadequate management of these products can also contribute to hunger and negatively impact food security. Cashew apples and molasses, being food sources, if underutilized, can exacerbate hunger and malnutrition, thus affecting food security [11]. Additionally, gender equality may also be negatively impacted by poor management of these agro-resources. Women, who are generally involved in the production and processing of agro-industrial products, can be particularly affected by insufficient valorization, exacerbating gender inequalities [12]. Furthermore, underutilization of these agro-resources can contribute to deforestation and other harmful agricultural practices, leading to climate change [10].

Therefore, developing innovative technologies to transform these products, particularly their combined use to provide additional nutrients in the culture medium, could enhance the production of economically valuable biomolecules like lactic acid.

Lactic acid is an organic compound used in numerous industries, including food, pharmaceuticals, cosmetics, and chemicals [13]. It is widely (70%) used in the food industry, notably in dairy, brewing, confectionery, and baking, as an acidifying agent, preservative, emulsifier, flavoring, and pH regulator [14]. Today, lactic acid finds new biotechnological applications, particularly in biodegradable polymers like polylactic acid [15], [16]. Lactic acid is produced industrially through two methods: chemical synthesis by hydrolysis of lactonitrile (10%), resulting in a racemic mixture (50% L(+)-lactic acid and 50% D(-)-lactic acid), or microbial fermentation (90%), which produces an optically pure product (L(+)-lactic acid) [17]. Microbial fermentation using *Lactobacillus casei* NRRLB-441 has been chosen in this study. Microbial fermentation is a more environmentally friendly process as it uses renewable resources and generates less toxic waste compared to chemical processes that may require harsh reagents and produce polluting by-products [18]. Other reasons for this choice include product purity, cost (utilizing low-cost raw materials like agricultural residues or industrial by-products) [19], adaptability, and scalability. The technology of microbial fermentation can be easily adapted and scaled using different types of microorganisms and substrates, enabling flexible and modular production based on needs [20]. Moreover, many microbial species, including lactic acid bacteria, certain fungi (*Rhizopus*), and even yeasts, can be used under acidic conditions to produce lactic acid from various biological substrates, such as agro-resources [21].

Agro-resources are the most suitable substrates for microbial lactic acid production as they are a low-cost, open-source "gold mine" of carbon, nitrogen, and minerals [22]. To reduce the high cost of industrial lactic acid production, the use of low-cost substrates, such as agro-resources, is a key parameter to consider before initiating the fermentation process. This study focuses on using cashew apple juice (CAJ) supplemented with sugarcane molasses (SCM) as a fermentable substrate for producing lactic acid through homolactic fermentation using *Lactobacillus casei* NRRLB-441.

2. Material and methods

2.1. Collection of cashew apple and sugarcane molasses samples

The cashew apples used in this study were collected in the North of Côte d'Ivoire, specifically in the Korhogo region. The apples were harvested directly from the cashew trees. Subsequently, the cashew nuts were carefully removed from the apples using linen thread to avoid damaging the apples. After collection, the apples were transported in plastic bins at ambient temperature (30°C) to the school factory of the Felix Houphouët-Boigny National Polytechnic Institute of

Yamoussoukro (INP-HB). Regarding the sugarcane molasses, it was provided by the SUCRE IVOIRE company of the SIFCA GROUP and delivered in a 1000 L IBC tank to the school factory of the Felix Houphouët-Boigny National Polytechnic Institute of Yamoussoukro.

A

B

Figure 1 Raw materials used for fermentation: cashew apples (A) and sugarcane molasses (B)

2.2. Substrate Treatment

The cashew apples were treated according to the method described by [23]. Once at the factory, the apples were thoroughly washed with 100 ppm active chlorine bleach. Subsequently, they were rinsed with tap water. After rinsing, the cashew apple juice (CAJ) was extracted using a hydraulic pulp press with a pressure power of 4.4 kW. The treatment of the CAJ consisted firstly of clarifying it with gelatin (1% v/v) to remove condensed tannins. Subsequently, the CAJ was filtered using a white cotton cloth with a mesh size of 1mm in diameter. Subsequently, the CAJ was centrifuged at 20,000 rpm for 10 minutes to remove impurities and particles still suspended in the juice. The supernatant was then collected to serve as a fermentation substrate.

The sugarcane molasses was treated according to the method described by [24]. This method involves treating the molasses with potassium hexacyanoferrate to complex and precipitate the excess amount of iron ions and other heavy metals. With an initial concentration of 45% w/v, the solution was diluted with 10% (w/v) distilled water to the desired sugar concentration (4.5% w/v), followed by the addition of 2g of potassium ferrocyanide, and then heated to 80°C for 60 minutes. After the precipitation of the ions, a decantation operation was carried out using a decanter, followed by filtration to separate the precipitate from the fermentation medium.

2.3. Microbial Strain

The bacterial strain used in this study is *Lactobacillus casei* NRRLB-441, obtained from the National Center for Agricultural Research of the United States, U.S. Department of Agriculture. The strain was placed in MRS (deMan, Rogosa, Sharpe) broth as a 50% v/v glycerol stock (5ml), and then stored at -20°C in sterile screw-cap tubes. It is a homofermentative lactic acid bacterium, i.e., it produces only L (+)-lactic acid. This bacterial strain was previously revived on MRS agar at 37°C for 48 hours. After 48 hours of incubation, the counting revealed 4.107 CFU/ml of cells.

2.4. Preparation of the Fermentation Medium

The fermentation medium was prepared according to an I-optimal mixture design. The Scheffé matrix was constructed to determine the proportions of cashew apple juice and sugarcane molasses. The matrix was constructed by setting the proportion bounds of cashew apple juice within the [50% - 90%] interval, while those of sugarcane molasses were set within the [10% - 50%] interval. The fermentation medium was 2 liters, consisting of a mixture of cashew apple juice and sugarcane molasses.

2.5. Preparation of the Bacterial Inoculum

The bacterial inoculum was prepared according to the method described by [25]. *Lactobacillus casei NRRLB-441* was revived on Petri dishes in Man, Rogosa and Sharpe (MRS) agar at 37°C for 48 hours. Subsequently, the activation of the organisms consisted of transferring two colonies into a 250 mL Erlenmeyer flask containing 100 mL of MRS broth. The pH of the culture medium was adjusted to 6.5 using phosphoric acid (H3PO4), and then incubated at 37°C for 24 hours.

2.6. Substrate Fermentation Process

The fermentation process consisted of autoclaving 2 liters of fermentation medium at 121°C for 15 minutes, then transferred into the tank of a LAMDA MINIFOR bioreactor, whose volume was 3 liters, with 1 liter of useful volume. Subsequently, 1% (v/v) of the previously prepared bacterial inoculum was transferred into the bioreactor. Subsequently, a homolactic fermentation in batch mode was triggered without any external nutrient input. The pH was automatically controlled by adding 5N sodium hydroxide (NaOH), with a range between 5.5 and 6.5. The fermentation medium was automatically stirred at a speed of 3 Hz (200 rpm) at 37°C for 72 hours of fermentation. Sampling was carried out throughout the fermentation with a 6-hour interval between two samples to determine the optical density, residual sugar concentrations (glucose and fructose) and lactic acid concentration [25].

2.7. Analytical Methods

After each sampling, the sample is immediately analyzed to determine the optical density (OD600) and cell growth (CFU/ml) by spreading on Petri dishes where MRS medium was poured, then incubated at 37°C for 48 hours. Sugar concentrations (glucose and fructose) and lactic acid were determined using a SHIMADZU LC-40D XR CL high-performance liquid chromatograph (HPLC) equipped with a CTO-40C CL column oven, an MR180 µL mixer (228-72652-44), a stainless steel tube (0.3×600 mm), and an SPD-40 CL UV-VIS spectrophotometric detector.

2.7.1. Proportion of Sugars Consumed During Fermentation (%)

The proportion of sugars consumed during fermentation (%) is a measure of the efficiency with which microorganisms utilize the sugars present in a fermentation medium for their growth and metabolism. It is expressed as a percentage and indicates the amount of sugars that has been converted relative to the initial sugar concentration. This metric is often used to evaluate fermentation kinetics, which refers to the rate at which sugars are consumed over time. It is represented by the following equation:

$$\mathbf{Pro_{SC}\ (\%) = [(Ci_S - Cf_S) / Ci_S] * (100)} \quad \text{.....(1)}$$

With,

Pro_{SC} : Proportion of sugars consumed (%)

C_{iS} : Initial sugar concentration (g/L)

C_{fS} : Final sugar concentration (g/L)

2.7.2. Monitoring Fermentation Parameters

Lactic Acid Yield (%)

The lactic acid production yield is a measure of the efficiency with which a process converts a raw material (usually sugar) into lactic acid. It refers to the amount of lactic acid produced per unit mass of sugar consumed. It is typically expressed in grams of lactic acid per gram of sugar (g/g), meaning that for each gram of sugar consumed, one gram of lactic acid is produced. But as part of this study, the yield will be expressed as a percentage. It is represented by the following relationship:

$$\mathbf{Y_{LA}\ (\%) = (C_{LAPro} / \Sigma C_{SC}) * (100)} \quad \text{.....(2)}$$

With,

Y_{LA}: Lactic acid production yield (%)

C_{LAPro}: Concentration of produced lactic acid (g/L)

ΣC_{SC}: Total concentration of sugars consumed (g/L)

Fermentation Productivity (g/L/h)

Lactic acid fermentation productivity measures the amount of lactic acid produced per unit of time and per unit of volume (or mass) of the fermentation medium. It reflects the rate at which microorganisms convert fermentable sugars into lactic acid. This parameter is critical for evaluating the efficiency and performance of a fermentation process, particularly in an industrial context. It is represented by the following equation:

$$P_F\ (g/L/h) = C_{LAPro} / T_f \quad \text{.....(3)}$$

With,
P_F : Fermentation productivity (g/L/h)
C_{LAPro} : Concentration of produced lactic acid (g/L)
T_f : Fermentation time (h)

2.8. Statistical analysis

The experiments were conducted three times in succession to ensure the reliability of the results. The obtained data were statistically analyzed using one-way ANOVA combined with Duncan's test via the STATISTICA software (version 7.0). A critical difference was determined with a significance threshold of 5% (P = 0.05) to identify statistically significant results.

3. Results and discussion

This study focused on producing lactic acid using cashew apple juice (CAJ) supplemented with sugarcane molasses (SCM) and utilizing *Lactobacillus casei* NRRLB-441 as the microbial source. To achieve this, the formulation of the fermentation medium required the application of an I-optimal mixture design to determine the proportions of CAJ and SCM in the blend. Since cashew apple juice and sugarcane molasses are naturally rich in minerals, their use did not require the addition of external minerals [1], [5]. Table 1 presents the Scheffé experimental matrix with 13 trials, including three center trials, as well as the results for lactic acid concentrations corresponding to the proportions of CAJ and SCM in each mixture.

Table 1 Scheffé Experimental Matrix with 13 Trials and the Results of Lactic Acid Concentrations

	Factor 1	**Factor 2**	**Responses**
Trials	CAJ (%)	SCM (%)	Lactic acid (g/L)
1	90	10	56.66
2	80	20	63.22
3	77	23	67.32
4	90	10	56.70
5	50	50	78.60
6	70	30	71.05
7	70	30	71.08
8	50	50	78.57
9	90	10	56.63
10	50	50	78.54
11	63	37	72.54
12	70	30	71.01
13	60	40	77.06

CAJ (%): Cashew apple juice in percentage ; SCM (%) : Sugarcane molasses in percentage

After experimentation, we observe that the highest lactic acid concentrations were recorded in trials 5 (78.60 g/L), 8 (78.57 g/L), and 10 (78.54 g/L), when the substrates were in equal proportions of 50%. Meanwhile, the lowest concentrations were observed in trials 1 (56.66 g/L), 4 (56.70 g/L), and 9 (56.63 g/L), when the proportions of JPC and MCS were 90% and 10%, respectively. The results show that the lactic acid concentrations in the different trials increase with the proportions of sugarcane molasses in the fermentation medium. This could be explained by several reasons. First, molasses, being rich in fermentable sugars, provides additional nutrients for the growth of *Lactobacillus casei* NRRLB-441 [25]. Additionally, the presence of molasses could further stimulate microbial activity, resulting in increased lactic acid content. Other reasons, such as the sugars present in sugarcane molasses and the pH (5) of the molasses, may have created a favorable environment for the metabolic activities of *Lactobacillus casei* NRRLB-441, which evidently had a positive impact on lactic acid concentrations [25]. The influence of sugars present in the fermentation medium on lactic acid concentration has already been mentioned in a study conducted by [26] on cashew apple juice for lactic acid production by *Lactobacillus casei* NRRLB-442.

Figure 2 shows the gradual decrease in the concentration of glucose and fructose as they are consumed by the bacterium *Lactobacillus casei* NRRLB-441. As for Figure 3, it illustrates the proportions of glucose and fructose consumed during fermentation. Figure 3 highlights the shares of glucose and fructose consumed at different fermentation times, as well as the bacterium *Lactobacillus casei NRRLB-441's* preference for glucose over fructose. Between 6 and 12 hours of fermentation (Figures 1 and 2), there is an almost identical consumption of both sugars. This is because, at the beginning of fermentation, the bacterium has not yet developed a preference for one sugar over the other, leading to nearly equal consumption of glucose and fructose. Similar observations were made by Vidra *et al*. [27] in studies conducted on sugarcane molasses. Other reasons may explain the balanced consumption of the two sugars at the start of fermentation, as glucose and fructose share a common metabolic pathway-glycolysis. Glucose is converted into pyruvate through glycolysis, producing ATP and NADH in the process. Subsequently, pyruvate is converted into lactic acid through lactic fermentation. As for fructose, it is first converted into fructose-1-phosphate by fructokinase, then into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, which enter glycolysis.

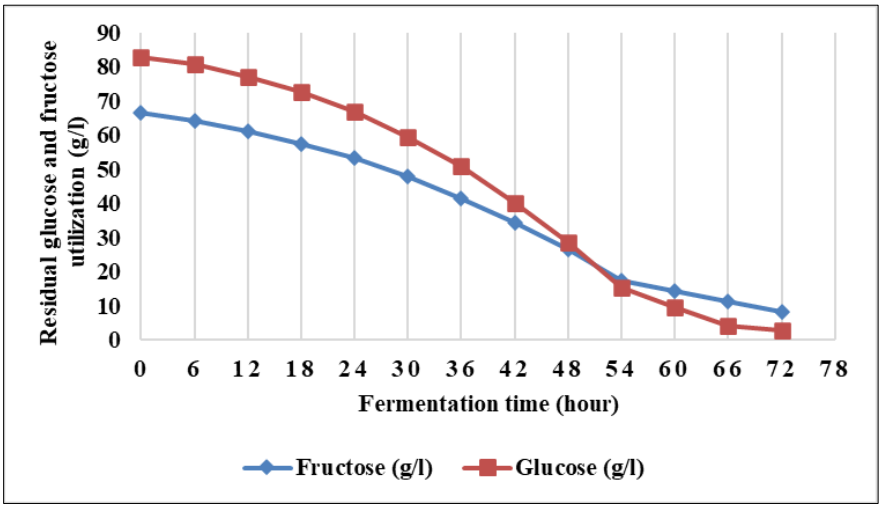

Figure 2 Glucose and fructose consumption during homolactic fermentation by *Lactobacillus casei* NRRLB-441

These interactions account for the initial similar consumption of both sugars. All these combined factors clearly explain why glucose and fructose are consumed similarly by *Lactobacillus casei* NRRLB-441 at the beginning of fermentation. Later (between 24 and 54 hours of fermentation), the consumption of glucose by the bacterium becomes significantly higher (57.37%) compared to fructose (40.07%). Consequently, a faster consumption of glucose is observed after 24 hours of fermentation. This sugar consumption pattern (glucose and fructose) has also been demonstrated by other authors [27] in research on sugarcane molasses for lactic acid production. The metabolic preference of *Lactobacillus casei* NRRLB-441 for glucose over fructose between 24 and 54 hours of fermentation is attributed to the bacterium's possession of specific enzymes that are more efficient at metabolizing glucose compared to fructose. For example, hexokinase catalyzes the phosphorylation of glucose into glucose-6-phosphate, a critical step in glucose metabolism. The presence of fructokinase in *Lactobacillus casei* NRRLB-441 does not allow for faster fructose phosphorylation because this enzyme is less effective than hexokinase. After 72 hours of fermentation, glucose consumption reaches 79.93 g/L, corresponding to a consumption rate of 96.56%. Meanwhile, the fructose consumed amounts to 58.24 g/L, representing 87.38% consumption.

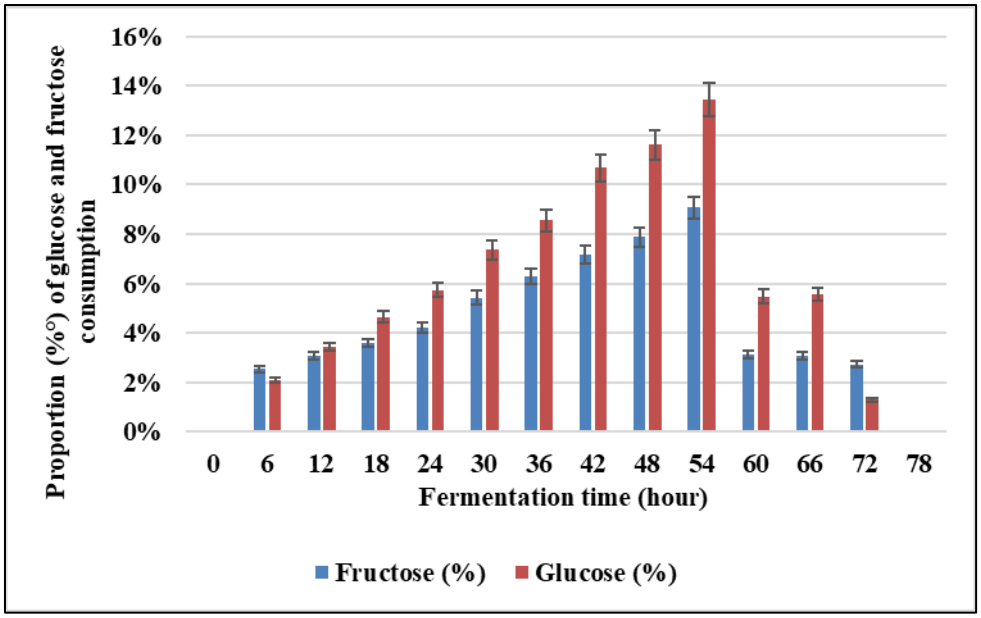

Figure 3 Proportions of glucose fructose consumed and during homolactic fermentation by *Lactobacillus casei* NRRLB-441

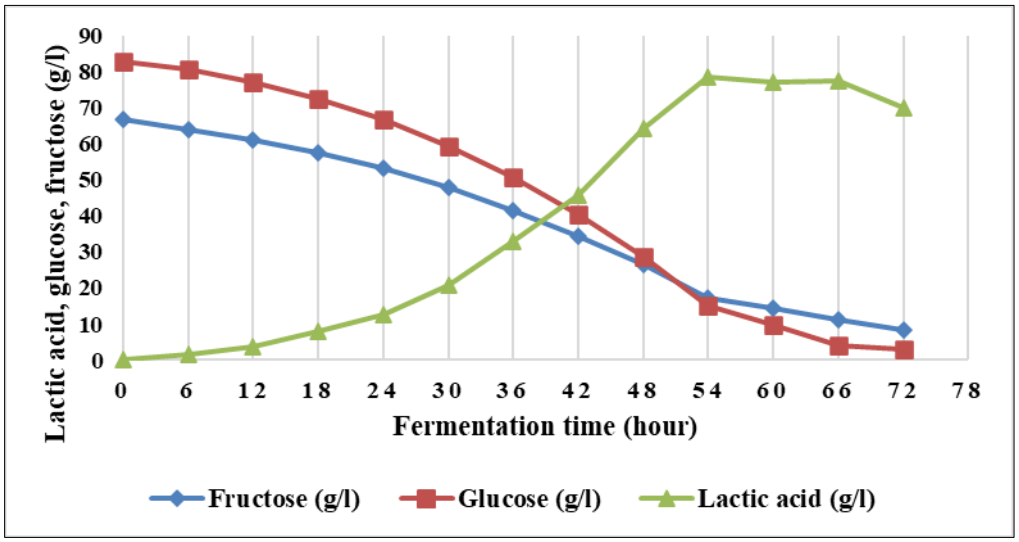

Figure 4 Lactic acid production during the fermentation of cashew apple juice supplemented with cane molasses

Figure 4 shows the production of lactic acid during the use of reducing sugars (glucose and fructose) in the fermentation medium. After 72 hours of fermentation, the maximum lactic acid concentration produced is 78.57 g/L, when each substrate in the fermentation medium was present at a proportion of 50%. This concentration is reached after 54 hours of fermentation, with a productivity of 1.09 g/L/h and a yield of 56.86%. After 54 hours of fermentation, the lactic acid concentration in the medium decreases until the end of the fermentation process. This decrease in lactic acid concentration in the medium could be explained by metabolic saturation due to incomplete consumption of reducing sugars (glucose and fructose); it could also be due to an accumulation of intermediate metabolites, as well as a possible osmotic stress caused by unfermented sugars in the culture medium. Similar observations were made by Paramasivam *et al.* [28] during studies on cashew apple juice for lactic acid production using *Lactobacillus casei* B-442. In their case, the fermentation duration was 72 hours, and the maximum lactic acid concentration (59.3 g/L) was achieved after 54 hours of fermentation.

Figure 5 illustrates the relationship between Optical Density (OD600) and cell growth (CFU/ml). The results show a harmonious relationship between Optical Density (OD600) and cell growth. At the start of fermentation, an Optical Density (OD600) of 3.5 was recorded, which increases during fermentation, reaching a maximum value of 25.12 after

54 hours of fermentation. Subsequently, it decreases to a value of 15.5 at the end of the fermentation process. It is important to note that throughout the fermentation process, Optical Density (OD600) correlates with cell growth. Monitoring the evolution of Optical Density helps track cell health and the quality of the fermentation process. Abnormalities in the evolution of OD may indicate issues such as contamination or a lack of nutrients. In summary, the evolution of OD during fermentation is a key indicator of cell growth and the efficiency of the fermentation process.

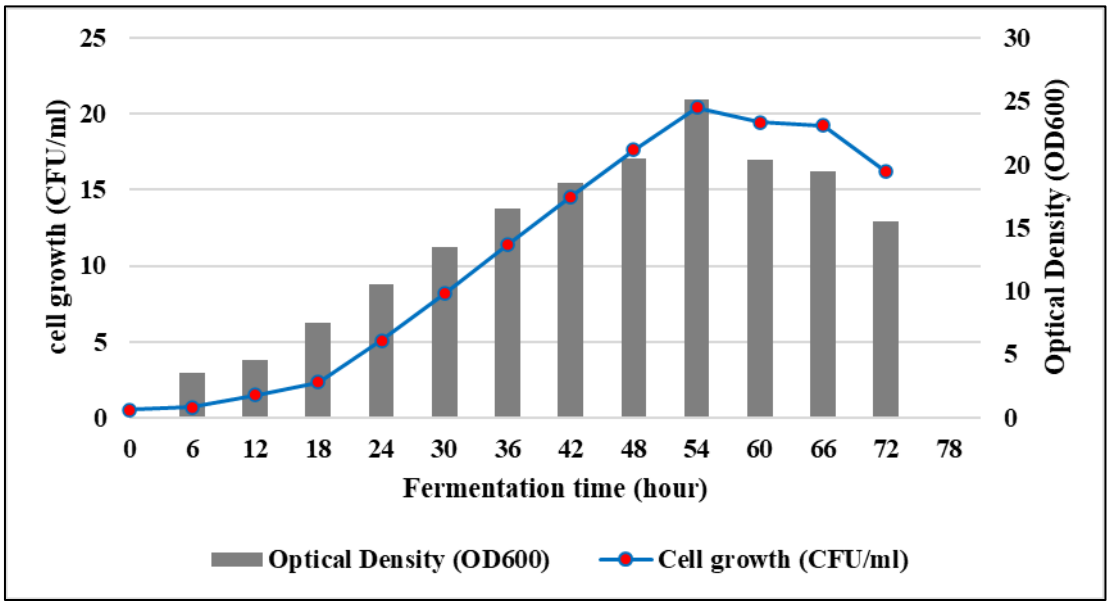

Figure 5 Relationship between Optical Density and Cell Growth during Fermentation

4. Conclusion

The aim of this study was to produce lactic acid from cashew apple juice supplemented with cane molasses using *Lactobacillus casei* NRRLB-441 during homolactic fermentation. The choice of substrates was motivated by their abundant availability and low cost on one hand, and their bioconversion by *Lactobacillus casei* NRRLB-441 on the other. The results showed that the lactic acid concentration was highest when the fermentation medium consisted of 50% of each substrate. *Lactobacillus casei* NRRLB-441 demonstrated a preference for using glucose over fructose. It is therefore important to note that cashew apple juice and cane molasses represent a reliable alternative for the production of high-value-added products.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest concerning this study.

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