



Bioethanol production from cashew (*Anacardium occidentale* L.) apple juice by batch fermentation using *Saccharomyces cerevisiae* E450

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ABSTRACT

Large amounts of cashew apples from Côte d'Ivoire are left over as waste in the plantations after nut separation, while it can be transformed into bioethanol. This study aimed at producing bioethanol from cashew apple juice by *Saccharomyces cerevisiae* E450 yeast. *S. cerevisiae* E450 was used as ferment at 10⁷ CFU/mL in anaerobic and aerobic conditions at temperatures of 30 °C and 33 °C. The fermentation was carried out in batch mode with sampling every 24 hours. The determination of ethanol and glycerol was performed by Gas Phase Chromatography and sugars (glucose, fructose and sucrose) by HPLC. The amounts of ethanol obtained at 30 °C and 33 °C respectively were of 65.10 and 73.17 g/L in anaerobic conditions and 62.05 and 75.79 g/L aerobic conditions. The fermentations carried out at 33 °C gave the highest ethanol concentrations with the maximum in aerobic which was 75.79 g/L. The fermentation carried out at 30 °C in anaerobic yields the lowest value of 62.05 g/L. This study showed the influence of temperature on the growth of cells and the synthesis of ethanol marked by the presence of oxygen which decreases fermentation time and thus improves productivity. It also revealed that cashew apple has great potential as a biofuel feedstock for bioethanol production.

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Keywords: Cashew apple juice, *Saccharomyces cerevisiae*, alcoholic fermentation, Bioethanol, Côte d'Ivoire.

INTRODUCTION

The 21st century is beset by challenges such as the decrease in fossil fuel resources, rapid rise in greenhouse gas emissions contributing to global warming, and the lack of capacity to respond to increasing energy demands (Deenanath et al., 2013). Therefore, it is necessary to develop alternative sources of energy from a renewable resources like biomass. Bioethanol is a biofuel produced from biomass via biochemical procedures

(Demirbas, 2008). Thus, in trying to reduce the impact of these global problems, the production of bioethanol from renewable resource is a remarkable alternative. It therefore represents a major environmental issue in addition to the socio-economic benefits. Bioethanol or ethyl alcohol can be used as an alternative to oil (Amigun et al., 2008) as is done in Brazil, the United States and Germany. The production of this biofuel requires pretreatment steps, including the

hydrolysis of biomass (cereals, lignocellulose and macroalgae) into fermentable monosaccharide sugars (Goh et al., 2010).

Fermentation is a traditional technology that can be used in plant foods to enhance the shelf-life, nutritional and organoleptic qualities and remove undesirable compounds (Hernandez et al., 2007). For fermentation, *S. cerevisiae* yeasts are the recommended microorganisms, for their ability to multiply anaerobically and easily convert sugars (Sanchez and Cardona, 2008). Biomass feedstock, namely wheat, barley, sorghum, rice, maize, potatoes, cassava and sugar cane, are widely available for bioethanol production and the processing of these raw materials were proven successfully (Linde et al., 2008; Balat and Balat, 2009). However, the present usage of these raw materials threatens their availability as a source of food (Deenanath et al., 2012). For example, following a severe drought on the North American continent, FAO called on the United States to limit ethanol production to redirect part of the maize to the food market (Riess, 2012). Alternatively, agricultural residues or industrial wastes such as Wheat, sugar cane bagasse, rice, barely and corn are the most favourable bioethanol production source in Iran (Najafi et al., 2009). These residues are largely lignocellulosic materials which require extensive and tedious pretreatment methods (Sun and Cheng, 2002). In Côte d'Ivoire, large amounts of cashew apples are wasted in the fields after nut separation, while it can contribute to the reduction of greenhouse gases by its transformation in bioethanol (Rocha et al., 2006; Preethi et al., 2019).

The cashew tree (*Anacardium occidentale* L.) belongs to the family Anacardiaceae. It is a native plant of Brazil. Cashew apple is the peduncle or pseudofruit of the cashew fruit, which is attached to the cashew nut, the real fruit (Rocha et al., 2006). Cashew apple contained a mixture of fermentable sugars of which glucose, fructose and sucrose (Layokun et al., 2006), minerals, vitamins, and some amino acids (Rabelo et al., 2009). Even though cashew apples processed

can be consumed as juice, and other foodstuffs, the cashew plant is grown in tropical countries mainly for its nuts (Honorato et al., 2007). In order to put the cashew apple to effective use, many studies have been carried out to assess the potential of its juice as carbon source in fermentation processes such as in production of mannitol (Fontes et al., 2009), biosurfactant (Rocha et al., 2006). Suganya and Dharshini (2011) summarized various added products from cashew apple such as juice, fenny, wine, dried cashew apple, syrup and jam.

In developing countries, bioenergy can be a source of both income and energy for people if produced and used locally (Riess, 2012) as well as for electricity production in Mauritania (Deenanath et al., 2012).

Côte d'Ivoire surpassed India for the first time last year as production leapt more than 24 percent over the previous season to 702,510 tonnes of nuts (RUSTERS, 2016). Unfortunately, almost all apple production is lost at the harvest site (Dedehou et al., 2015). The main reason for under-exploitation of the apple is the extreme astringency of its juice, which renders it unbearable (Abreu et al., 2001). The main nutritional component of CA is Vitamin C. It was found that cashew apples has a greater concentration of Vitamin C than other fruits such as oranges, grapes, mangoes, lemons, and pineapples (Dioha et al., 2011). In addition they contain phenolic compounds such as anarcadic acid, cardol and tannins as well as carotenoids which can act as potential antioxidants. However, strong radical scavenging activity proanthocyanidins (condensed tannins) are responsible for the typical astringency of some fruits (He et al., 2011) such as cashew apples.

For cashew wine production, the cashew apple juice is fermented using *S. cerevisiae* yeasts at temperatures between 28 °C and 30 °C and pH of 4.0. Alcohol content between 6% (v/v) and 10.6% (v/v) is possible (Araujo et al., 2011). This study focused on investigation of the cashew apple in order to produce bioethanol (ecological fuel) for the reduction of greenhouse gases or as petroleum alternative or additive.

MATERIALS AND METHODS

Sample collections

The samples composed of yellow and red cashew apples (Figure 1) were obtained from three regions namely Marahoué, Gbèkè and Zanzan of Côte d'Ivoire. The plantations were located in the village of the three regions. The cashew apples were harvested during the peak period of March-May 2015 of harvest season cashew production. In each of these areas, about 50 ripe cashew apples without injuries each variety were harvested. The batches consisted of cashew apples of the same variety, harvested in the same plantation. The subsamples were made from cashew apple batch of the same variety harvested in the same location and the sample itself was made from cashew apple subsamples of the same variety harvested in the same region. About 10 kg of each of the cashew apple varieties consisted the sample. The apples were safely transported in cool boxes to the laboratory for analysis.

The strains of *Saccharomyces cerevisiae* yeasts were the microorganisms used for fermentation. The strain *S. cerevisiae* E450, is a baker's yeast sold on local markets, purchased and maintained at -70 °C in 50% glycerol. In this study, the yeast was simply referred to E450.

Cashew apple juice extraction and preparation

Extraction and pretreatment of the juice was carried out according to the Pinheiro (2008) method. In order to obtain the juice, the cashew apples were collected, cut into small pieces and then crushed in a blender (BlenderLB20E, Torrington, USA, 2002). The ground material was filtered on a sterilized mousseline. 1% (w / v) of gelatin powder is added to the filtrate obtained and the whole is maintained at 4 °C for 24 hours. After centrifugation at 4000 g for 20 min, the supernatant was removed and added to 2.5 g/L of ammonium sulphate, sterilized at 121°C for 15 min and then stored at -80 °C for fermentation.

Culture media

Storage of strains

On the basis of work carried out by Riess (2012), the strains were subcultured in liquid medium YEPD (Yeast Extract Peptone Dextrose) composed of 40 g/L glucose, 15 g/L yeast extract and 10 g/L peptone. The reaction medium was stirred at 150 rpm (rotation per minute) for 24 hours using a mechanical stirrer (INFORS AG CH-4103, Bottingen) at 30 °C and 33 °C, respectively. These strains obtained were subcultured on agar slopes composed of YEPD medium supplemented with 20 g/L of agar and stored at 4°C for subsequent uses. These media, prior to use, were sterilized by autoclaving at 121 °C for 20 minutes

Pre-cultures

These precultures were carried out in YEPD medium (40 g/L glucose, 15 g/L yeast extract and 10 g/L soybean peptone). These precultures were inoculated from the agar slopes used to conserve the strain. These media, prior to use, were sterilized by autoclaving at 121°C for 20 minutes. The precultures were then placed in an orbital agitator (INFORS AG CH-4103, Bottingen) for 15 h with a stirring of 120 rpm at 30 °C for the fermentations carried out at 30 °C and 33 °C for the fermentations carried out at 33 °C.

Inoculation

A 20 ml aliquot of the pre-culture containing 10⁶ CFU/mL was inoculated into 500 ml Erlenmeyer flasks containing 250 mL of fermentation medium (CAJ).

Fermentation of cashew apple juice

A 20 mL aliquot of *Saccharomyces cerevisiae* pre-culture was inoculated into a 500 mL Erlenmeyer flask containing 250 mL of fermentation medium (CAJ, Red variety from the Zanzan Region). The fermentation was carried out at 30 °C and 33 °C in aerobic and anaerobic. For analysis, one (1) mL of the sample was carried out every 24 hours for 10 days at the rate of three (3) tests. These samples allowed to realize, counts and measurements of viability as well as the

determinations of ethanol and sugars (glucose, fructose and sucrose) were carried out respectively by GPC and HPLC on the supernatants obtained after centrifugation of the sample.

Yeast Viability

Yeast cell viability of the starter culture and during fermentation was determined by standard plate counts (Pattison et al., 1998). From the initial sample, 1 mL was aliquot into 9 mL of buffered peptone water (20 g/L) and tenfold serial dilution was performed. A volume of 100 μ L of the diluted suspension was spread plate onto malt extract agar (50 g/L). The agar plates were incubated at 30 °C for 24–48 hours and examined for the growth of colonies. Plates showing between 30–300 colonies were selected and counted. Viability was expressed by calculating the colony forming units per mL.

Determination of simple sugars

The determination of simple sugars such as glucose, fructose and sucrose in cashew apple was performed by HPLC (High Performance Liquid chromatography) according to the method of Kouamé et al. (2001). Twenty (20) μ l of the sample filtered through a Durapore hydrophilic membrane (millipore 0.5 μ m, Sartorius) were injected into the HPLC (Shimadzu Corporation, Japon). The separations of sugars are recorded with a refractometric detector. The column used was the supercosyl LC-NH₂ (5 μ m/0.46 x 25 cm; 120 Å). The mobile phase was constituted by acetonitrile-water mixture (75/25; v/v). The flow rate was 0.75 μ l/min.

Determination of ethanol in gas chromatography GC

Chromatograph: SHIMADZU GC 14A was used for the determination of ethanol and glycerol during fermentation. The column used is a pyrex glass capillary column 30 m X 0.32 mm) placed in an oven whose temperature is set at 120 °C. The mobile phase used is nitrogen circulating in constant mode at a flow rate of 0.5 mL.min⁻¹. A volume of 20

μ L of supernatant is injected and analyzed at the column outlet by a flame ionization detector (250 °C). The internal standards for ethanol and glycerol are respectively ethanol and glycerol reference

Determination of CO₂ volume

The volume of carbon dioxide (CO₂) was obtained according to the method of Guillou (1996). In this method, the yeasts ferment in a sealed flask connected by a tube to another sealed vial filled with water. The tube also comprises a drainage pipe which is discharged into a graduated cylinder. The production of carbon dioxide by the fermentation produces an overpressure in the water tube which causes the water to flow out of the drain tube. The measurement of the volume of water collected in the graduated cylinder makes it possible to quantify the volume of CO₂. After closing the tap at regular intervals every 2 hours the volume of gas in the test tube initially filled with water and returned to the water tank. The gas produced must then be characterized. This involves replacing the water in the receiving vial with lime water (obtained by dissolving the barium hydroxide in the distilled water until saturation). The barium carbonate precipitate indicates that the gas produced is carbon dioxide.

Statistical analysis

All analyzes were carried out in triplicates. Results were expressed by means of \pm SD. Statistical significance was established using two-way analysis of variance (ANOVA) models to estimate the effect of region and variety main effects and their interaction on some biochemical parameter contents of cashew apple from cashew at 5% level. Means were separated according to Duncan's multiple range analysis (P <0.05), with the help of the software STATISTICA 7 (Statsoft Inc, Tulsa-USA Headquarters) and XLSTAT-Pro 7.5.2 (Addinsoft Sarl, Paris-France).

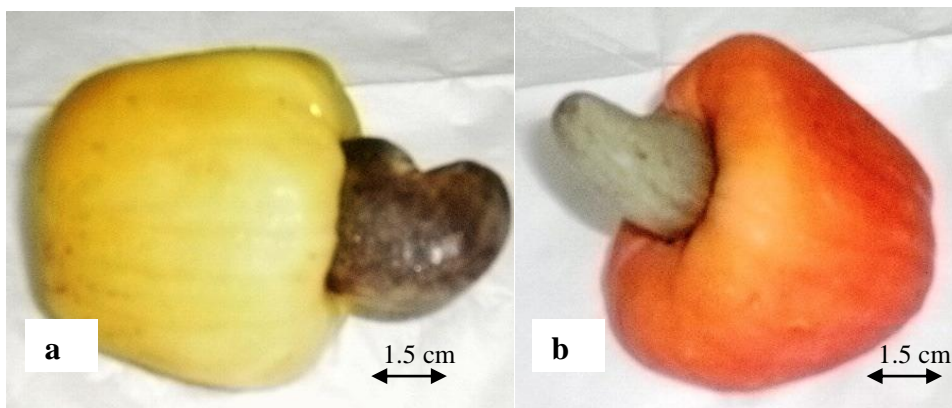


Figure 1: Cashew apple yellow (a) and red (b).

RESULTS

Effect of oxygen and temperature on the viability of *Saccharomyces cerevisiae*

The initial concentration of yeast is 10^7 CFU/mL. Both anaerobic and aerobic, the concentration of viable cells decreases after one day of fermentation at any temperature. After the first day, viable cell concentrations were $8.06 \pm 0.15 \cdot 10^6$ CFU / mL and $8.36 \pm 0.15 \cdot 10^6$ CFU/mL respectively for anaerobic temperatures of 30 °C and 33 °C, increased to reach maximum values of $8.95 \pm 0.13 \cdot 10^6$ CFU/mL and $9.56 \pm 0.12 \cdot 10^6$ CFU/mL 3rd day respectively for temperatures of 30 °C and 33 °C. They stabilize between the 4th and 6th days before entering a phase of decline after 6 days of fermentation. Viable cell concentrations decreased to the respective values of $7.04 \pm 0.13 \cdot 10^6$ CFU/ mL and $7.8 \pm 0.12 \cdot 10^6$ CFU/mL. In addition, aerobic concentrations of viable cells reached values of $8.71 \pm 0.11 \cdot 10^6$ CFU/ mL and $10.23 \pm 0.02 \cdot 10^6$ UFC/mL on day 5, respectively, for temperatures of 30 °C and 33 °C. These different observed values decreased to reach values of $9.87 \pm 0.05 \cdot 10^6$ CFU/mL and $8.48 \pm 0.04 \cdot 10^6$ CFU/mL respectively. Sustainable cell concentrations are higher in aerobic conditions than in anaerobiosis at any temperature. Moreover, at the temperature of 33 °C, the concentration values obtained are

the highest. Statistical analysis revealed significant differences in cell concentration ($p \leq 0.05$) both aerobically and anaerobically during fermentation irrespective of temperature (Table 1).

Evolution of sugars and metabolites in aerobic and anaerobic conditions at 33 °C during fermentation

The evolution curves of the sugars during the fermentation at 33 °C showed the same decreasing rate in both anaerobic and aerobic conditions (Figures 2 and 3). On the other hand, metabolites such as ethanol, glycerol and CO₂ were increasing in both anaerobic and aerobic conditions. CAJ was characterized by rates of 102.34 ± 1.02 g/L, 50.82 ± 0.40 g/L and 3.62 ± 0.43 g/L respectively for fructose, glucose and sucrose (Figure 4). In anaerobiosis, glucose and fructose concentrations decreased to 8.20 g/L (6th day) and 36.38 g/L (8th day) (Figure 1). The concentration of these sugars then gradually decreases to 31.83 g/L and 4.30 g/L on the 10th day of fermentation. As for the concentration of sucrose, it gradually decreases to 0.08 g/L on the 4th day of fermentation and then totally disappears from the reaction media. The concentrations of glycerol and CO₂ increase gradually to reach values of 6.23 g/L and 15.04 g/L on the 10th

day in anaerobiosis. In aerobic conditions they are 4.21 ± 0.04 g/L for glycerol and 7.07 g/L for CO_2 . However, the amount of CO_2 in aerobic is less than that recorded under anaerobic conditions. For glycerol, the values obtained at the end of the fermentation increase with the temperature whatever the conditions, with higher values under anaerobic conditions.

Effect of oxygen and temperature on ethanol production during fermentation

The concentration of ethanol was increased during fermentation both in

anaerobic and aerobic conditions, regardless of temperature. In anaerobiosis, it varied between 8.53 ± 0.47 g/L and 62.05 ± 0.05 g/L and between 9.7 ± 0.2 and 73.17 ± 0.03 g/L respectively for temperatures of 30°C and 33°C . The aerobic concentration of ethanol varies between 12.9 ± 0.91 g/L and 65.10 ± 0.01 g/L and between 19.27 ± 0.22 g/L and 75.79 ± 0.04 g/L respectively for temperatures of 30°C and 33°C . Thus, the highest concentrations are obtained with the temperature of 33°C both in anaerobic and aerobic conditions with a better yield ($p \leq 0.05$) in aerobic (Table 2).

Table 1 : Effect of oxygenation and temperature conditions on the viability of *Saccharomyces cerevisiae* cells (10^6 CFU / mL).

Days	Anaerobic		aerobic	
	T 30 °C	T 33 °C	T 30 °C	T 33 °C
0	10^b	10^b	10^b	10^b
1	8.06 ± 0.15^c	8.36 ± 0.15^m	8.71 ± 0.11^j	9.01 ± 0.14^h
2	8.18 ± 0.21^n	8.87 ± 0.21^i	9.07 ± 0.12^{gh}	9.82 ± 0.12^c
3	8.95 ± 0.13^h	9.560 ± 0.19^f	9.67 ± 0.14^e	10.18 ± 0.1^a
4	8.68 ± 0.45^k	9.18 ± 0.12^g	9.72 ± 0.13^d	10.22 ± 0.02^a
5	8.64 ± 0.48^k	9.186 ± 0.03^g	9.73 ± 0.09^d	10.23 ± 0.02^a
6	8.62 ± 0.09^k	9.20 ± 0.1^g	9.23 ± 0.02^g	9.76 ± 0.04^d
7	7.37 ± 0.22^q	8.11 ± 0.09^n	8.61 ± 0.09^k	9.02 ± 0.03^h
8	7.330 ± 0.32^q	7.92 ± 0.16^o	7.92 ± 0.06^o	8.60 ± 0.04^k
9	7.15 ± 0.19^r	7.9 ± 0.17^p	7.87 ± 0.05^p	8.49 ± 0.04^l
10	7.04 ± 0.13^s	7.8 ± 0.12^p	7.87 ± 0.05^p	8.48 ± 0.04^l

Values of the same line assigned to the same letter are not significantly different from each other on the basis of Duncan's multiple comparison test at the 5% threshold. These values are expressed as Mean \pm Standard Deviation ($n = 3$ determination).

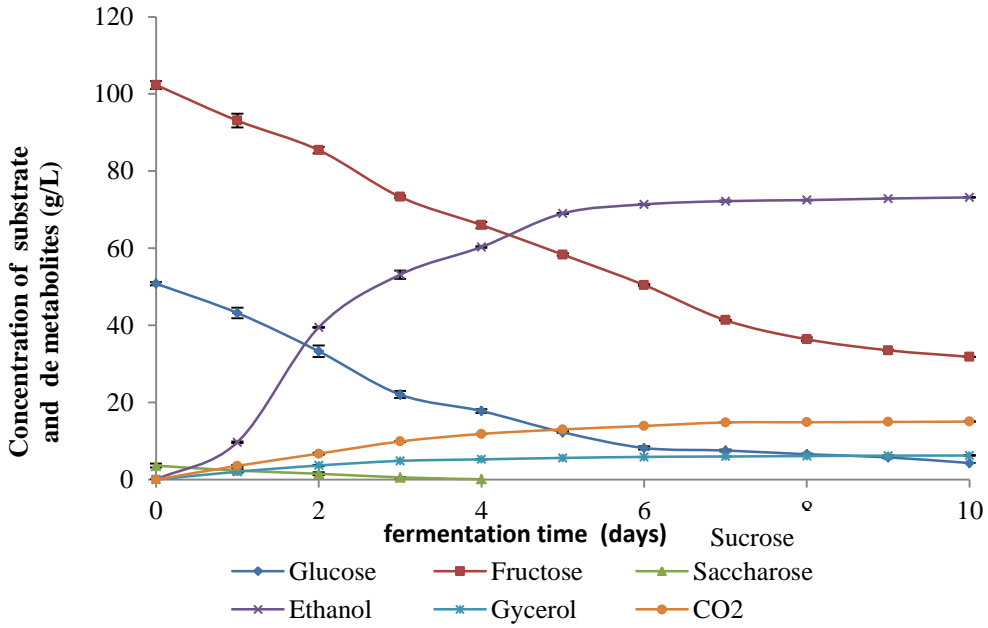


Figure 2: Evolution of sugars and metabolites in anaerobiosis at 33 °C during the fermentation of cashew apple juice.

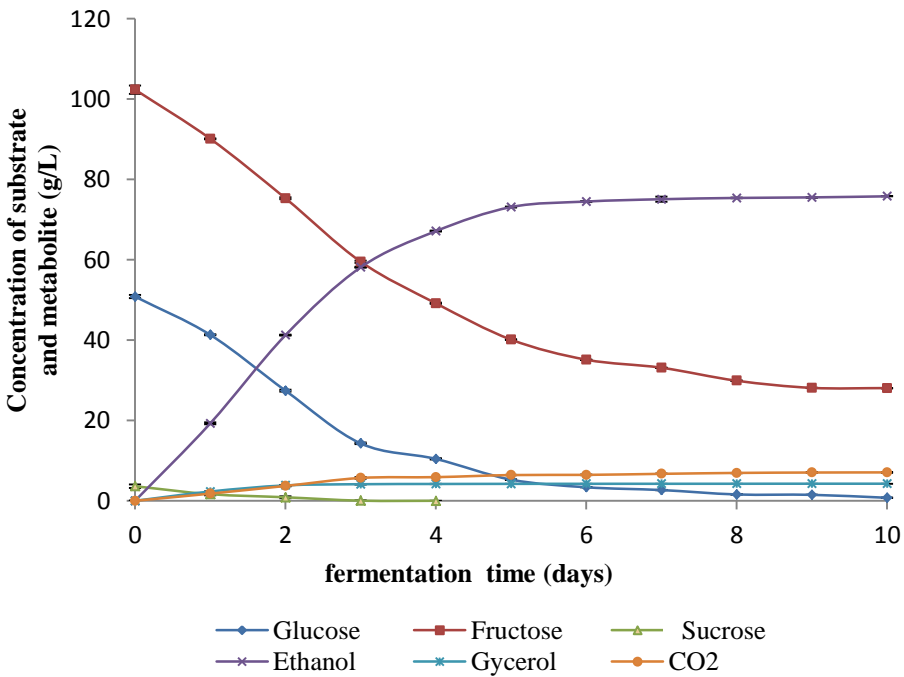


Figure 3: Evolution of substrates and metabolites aerobically at 33 °C during the fermentation of cashew apple juice.

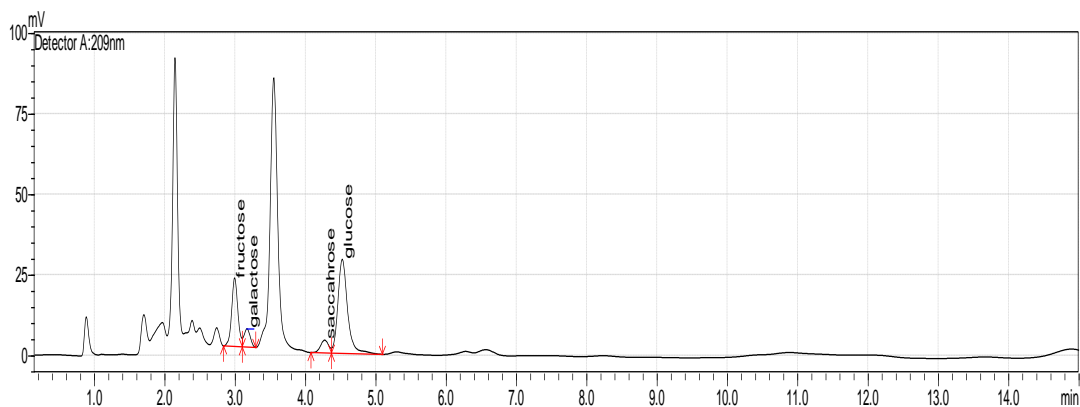


Figure 4: Chromatogram of cashew apple juice sugar (glucose, fructose, sucrose).

Table 2 : Effect of oxygen and temperature on the production of ethanol by the fermentation of apple juice.

Days	Anaerobic		aerobic	
	T 30 °C	T 33 °C	T 30 °C	T 33 °C
0	0	0	0	0
1	8.53 ± 0.47 ^a	9.7 ± 0.2 ^b	12.9 ± 0.91 ^μ	19.27 ± 0.22 ^z
2	27.22 ± 0.24 ^y	39.47 ± 0.14 ^w	30.22 ± 0.24 ^x	44.22 ± 0.13 ^t
3	41.23 ± 0.20 ^u	53.14 ± 1.09 ^q	46.2 ± 0.26 ^s	58.11 ± 0.12 ^{lm}
4	50.27 ± 0.25 ^r	62.32 ± 0.17 ⁿ	55.3 ± 0.23 ^o	67.12 ± 0.12 ^g
5	54.22 ± 0.13 ^p	69.42 ± 0.14 ^f	59.33 ± 0.15 ^l	73.11 ± 0.12 ^d
6	57.68 ± 0.05 ^m	71.66 ± 0.11 ^{ef}	63.33 ± 0.05 ^j	74.49 ± 0.10 ^c
7	59.68 ± 0.05 ^l	72.01 ± 0.14 ^e	64.38 ± 0.03 ^{ij}	75.07 ± 0.66 ^b
8	60.61 ± 0.07 ^{kl}	72.48 ± 0.06 ^{de}	64.79 ± 0.04 ^{ij}	75.38 ± 0.05 ^{ab}
9	61.91 ± 0.04 ^k	72.89 ± 0.02 ^{de}	64.88 ± 0.02 ^{ij}	75.52 ± 0.06 ^{ab}
10	62.05 ± 0.05 ^k	73.17 ± 0.03 ^d	65.10 ± 0.01 ⁱ	75.79 ± 0.04 ^a

The values of the same line assigned to the same letter are not significantly different from each other on the basis of Duncan's multiple comparison test at the 5% threshold. These values are expressed as Mean ± Standard Deviation (n = 3 determination).

DISCUSSION

Azam-Ali and Judge (2001) reported that only six percent of cashew apple production is exploited, and the rest gets rotten and wasted. Whereas, Cashew apple juice is able to supply energy and minerals instantly to the consumer (Preethi et al., 2019). According to Hernandez et al. (2007), fermentation is a traditional technology that can be used in plant foods to enhance the shelf-life, nutritional and organoleptic qualities and remove undesirable compounds. For cashew apple fermentation, the decrease of viable cells of *Saccharomyces cerevisiae* observed from the first day both in aerobic and anaerobic conditions (Table 1). This decrease is due to cell entry in a long phase of latency during which some cells could not survive (Deenanath et al., 2012). Sustainable cell concentrations are higher in aerobic conditions than in anaerobiosis at any temperature. These observations suggest that the decrease in viability is less marked by the presence of oxygen and by the increase in temperature. Indeed, in the presence of oxygen, a regime of micro-aeration is established and allows the yeast to be in breathable-fermentation metabolism favoring the production of ethanol and limiting the phenomena of stress of the cells that could be due to an oxygen limitation (Amillastre, 2012). Oxygen also promotes the production of unsaturated fatty acids and sterols, which allows the yeast to protect against osmotic pressure and increase ethanol and thus improve cell viability during fermentation (Lin et al., 2002). The synthesis of unsaturated lipids can only be carried out in the presence of oxygen. When the yeast is divided, the unsaturated lipids are divided between the daughter cell and the mother cell. In anaerobiosis, the membrane concentration of yeasts to unsaturated lipids decreases to a viability threshold below which the yeasts die (Duc et al., 2017). The dissolved oxygen requirements during the growth phase range from 4 to 40 ppm (Jacques et al., 2003). Oxygen must therefore be supplied when the yeast population increases.

As for temperature, it is the environmental factor that has the greatest influence on the physiology and activity of microorganisms (Amparo and Graham, 2006). It acts on both growth rate, ethanol production rate, CO₂ production, cell viability, composition and integrity of the plasma membrane (Aldiguier et al., 2004). Each microorganism has a growth limit temperature beyond which its survival is initiated. The growth limiting temperature of most yeasts is between 30 and 40 °C. It was evaluated at 30 to 33 °C for *Saccharomyces cerevisiae*. This, however, depends on the strains, medium and culture conditions. Growth at temperature above the boundary temperature leads to destruction of enzymes, alteration of the membrane (Guyot et al., 2005), and many other perturbations which kinetics and decrease fermentation performance and viability. The viability of yeasts is greatly affected by an increase in temperature (Torija et al., 2003). The temperature gradient applied, in addition to the temperature itself, has an impact on viability and a thermal shock will affect the viability of yeasts much more than a gradual change (Guyot et al., 2005). In addition to affecting the kinetics and viability of yeasts, elevation of temperature decreases the tolerance to other stresses; it amplifies the lethal effect of ethanol (Aldiguier et al., 2004) and osmotic stress (Beney and Gervais, 2001).

The observed decrease in sugars is due to their yeast metabolism in products from the 5th day (anaerobiosis) and the 3rd day (aerobic). *S. cerevisiae* yeast easily assimilates monosaccharides such as glucose and fructose and disaccharides such as maltose (Van Maris et al., 2007). High concentrations of sugar can cause osmotic stress in yeast cells (Gutt and Gutt, 2009). This is not the case in the present study because the yeast strain resisted the sugar concentration of the apple. Glucose and fructose are used at different rates with greater consumption of fructose. This difference is probably due to the low residual amount of glucose that has become insufficient for the biomass so that fructose is positioned as the preferential sugar causing a slowdown in production. In the presence of oxygen, it is the

carbonaceous substrate that will be determining in the orientation of its metabolism. The aerobic CO₂ content is lower than that found by Deenanath et al. (2013) which is 7.55%. This is due to the CO₂ losses of the fermentation device. On the other hand, in the absence of oxygen, much higher values are obtained. This is justified by the fact that the method of quantifying CO₂ is not specific to it, because there may be during the fermentation the formation of other volatile compounds such as acetaldehyde, ether and sulfuric acid (Raherimandimby, 2004).

The small amounts of glycerol found in aerobic conditions may. The small amounts of glycerol found under aerobic conditions can be explained by the fact that glycerol would oxidise the reduced coenzymes and balance the redox balance to the detriment of an energy dissipation. Glycerol production is therefore a response to stress (Torija et al., 2003). This is due to a greater activity of the enzyme glycerol 3-P dehydrogenase (Aldiguier et al., 2004).

Concerning the production of ethanol, it appears from the results that the cell is more sensitive to the effect of ethanol in anaerobic than in aerobic. This reveals that oxygen is a very decisive factor in improving the tolerance to ethanol. Therefore, at these concentrations, there is a slowdown in ethanol production and stabilization occurs. Unlike anaerobic fermentation, the production of bioethanol in the presence of oxygen promotes cell growth by the energy generated in the form of ATP during the complete degradation of the sugars. The production of ethanol in this case is consistent with the hypothesis that excessive glycolytic flux saturates the respiratory capacity. The surplus is redirected towards the production of ethanol (Vemo et al., 2017). The production of ethanol increases from the first day until stagnating towards the end of the fermentation and this, along with a decrease in sugars over time. This increase is due to the degradation of these sugars by yeast, in metabolites whose main is ethanol. The production of ethanol is higher in aerobic and stagnates two (2) days before that observed in anaerobiosis. This can be explained by the fact that in the presence of

oxygen there is a reduction in environmental stress (Deenanath et al., 2013), which creates more favorable conditions for ethanol production compared to the anaerobic environment. Thus, ethanol is produced more rapidly until it reaches a critical inhibitory concentration of its own production. In comparison with the work of Deenanath et al. (2013) under similar conditions significant differences in ethanol production at 30 °C. This discrepancy may be due to the strains but also to the fact that the fermentation has spread over 10 days. Moreover, the fact that the results at 33 °C are greater than those obtained by the latter (65.17 and 65.12 g/L) reinforces the significant influence of the temperature in alcoholic fermentation. Indeed the temperature of 33 °C gives better productions in ethanol. Its action is mainly on the maximum speed of production of ethanol. The increase in temperature between 3 and 10 °C following the strains favors the increase in the maximum production rate of ethanol above the optimum growth temperature, which can be explained by a maximum activity of alcohol dehydrogenase at 40 °C (Amparo and Graham, 2006). The study of the effect of temperature on the production of ethanol during fermentation shows that this factor has a significant influence on the production of ethanol. According to Kalyuzhin (2011), the optimum temperature for *S. cerevisiae* growth is 30–33 °C.

The yeast strain used is mesophilic, it is an advantage over thermophilic strains in that their use on an industrial scale will result in low energy consumption. Thermophilic strains require an increase in the temperature of the bioreactor requiring a significant amount of energy (Gomes et al., 2003). There is a relationship between ethanol production and cell viability. Thus, when the ethanol concentration increases, inhibition of cell growth is greater and the fall in viability is faster (Sandrasegarampillai and Vasanthy, 2012). This would explain the increase in higher ethanol production during the cell growth phase which subsequently approaches a stationary phase before the phase of decline. Increasing aeration would improve ethanol production rates and reduce the inhibitory

effect of ethanol on growth under ethanol production conditions (Thanawa et al., 2018). In addition, oxygen is required for yeast for the synthesis of certain compounds (unsaturated fatty acids, sterols). Oxygen improves the bioconversion of sugars by maintaining membrane integrity. The increase in the activity of alcohol dehydrogenase with the increase in temperature influences productivity so that the maximum final titer is observed for the conditions of these two (2) factors. This is fermentation carried out in aerobic conditions at 33 °C. The results of this investigation indicated that CAJ is an acceptable substrate, in conjunction with *Saccharomyces cerevisiae* E450 yeast for bioethanol production.

Conclusion

In spite of the high nutritional and therapeutic value of cashew apples, thousands of tons of CA are wasted every year. CAJ was used for the production of bioethanol with the baker's yeast strain *Saccharomyces cerevisiae* at different temperatures, in the presence and absence of oxygen. This revealed that the cashew apple has an interesting potential as a raw material for the production of bioethanol due to its biochemical composition. This study also showed the influence of temperature on the growth of cells and the synthesis of ethanol marked by the presence of oxygen which decreases fermentation time and thus improves productivity. Cashew apple juice is a raw material of choice for the production of bioethanol.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All authors contributed to the work and to the preparation of the manuscript.

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