

### **RESEARCH ARTICLE**

# STUDY OF THE PERFORMANCE OF FOUR STRAINS OF SACCHAROMYCES CEREVISIAE DURING ETHANOL PRODUCTION WITH CASHEW APPLE JUICE (ANACARDIUM OCCIDENTALE L.).

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#### Abstract

Cashew nut production is booming in Benin and picking apples are a valuable commodity with a high nutrient content. The main objective of this study is the valorization of cashew apples through the study of the performance of four strains of Saccharomyces cerevisiae during ethanol production from cashew apple juice. The alcoholic fermentation of cashew apple juice with an initial concentration of 157.5 g/L of reducing sugars and 1 g/L of yeast was conducted anaerobically at room temperature (30.8 °C) at pH 3.85-4.25 for 96 hours in polyethylenic triplicate bioreactors. During fermentation, it has been tested the performance of strains such as Saccharomyces cerevisiae var. bayanus, Saccharomyces cerevisiae Safale K-97, Saccharomyces cerevisiae Saflager W-34/70 and baker's yeast. After 48 hours of fermentation the maximum concentration of ethanol (104.3 g/L) is recorded with the yeast Saccharomyces cerevisiae var. bayanus with a residual sugar of 1.25 g/L. It is followed by Safale K-97, Saflager W-34/70 and baker's yeast, which have ethanol concentrations of 93.69; 73.95; 58.16 g/L respectively. In addition, the yeast Saccharomyces cerevisiae var. bayanus showed the highest efficiency (96.025 %), the highest productivity (1.604 g.L<sup>-1</sup>.h<sup>-1</sup>), the highest ethanol yield (0.491 g.g<sup>-1</sup>) and the highest rate substrate consumption (2.067 %.h<sup>-1</sup>).

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It appears from this study that the yeast *Saccharomyces cerevisiae* var. *bayanus* has the best bioconversion performance of fermentable sugars in ethanol. Therefore, it can be used as an effective strain in the perspective of intensive ethanol production.

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### Introduction

The decline in dependence on fossil fuels following its exhaustion, the increase in the global price of fuel, the increase in the population and the increase in global warming have increased in recent decades the interest of the use renewable raw materials to produce ethanol (Yu et Zhang, 2004, Demirbas et al., 2007, Demirbas, 2008). Ethanol is of undeniable economic importance because it is used in various sectors of industrial activities including the agri-food sector for the production of alcoholic beverages such as alcohol at 40 °GL, wine, beer, cider, vodka and gin, whiskey, brandy (Kaidi et Touzi, 2001). It is also used in the manufacture of solvents, detergents, disinfectants and chemical intermediates. Ethanol is also used in combination with gasoline to produce gaseous alcohol to fuel automobiles (Yu et Zhang 2004, Demirbas et al., 2007; Demirbas, 2008). It is a clean source of burning, renewable energy (Reddy et al., 2007). It is also an important raw material in the synthesis of aldehydes, ketones, carboxylic acid, carboxylic acid derivatives and hydroxyl groups which are important components of many pharmaceutical drugs (Solomon et al., 2008). The bestknown and most widely used means of producing ethanol is chemical production from petroleum products (Boulal et al., 2013). This process of producing ethanol is increasingly rejected because of the high cost of oil and its impact on the ecosystem. In Benin, ethanol is frequently used in various fields such as the food industry, the pharmaceutical industry, the cosmetics industry, in research laboratories and during traditional cults and festive ceremonies. The main raw material used for its production is palm wine, obtained most often by slaughtering and/or daily cutting of the apical bud (Kouchade et al., 2017). This practice is not only lethal for palms but also contributes to the destruction of the plant cover. Thus, it is imperative to diversify the sources of ethanol production, including the choice of cashew apple, a widely available agroresource. Cashew (Anacardium occidentale L.) is a tree native to South America (Olher 1967). Interest in this tree is focused on cashews which are the object of an international trade and which gives notoriety to the cashew nut (Lacroix, 2003). In 2015, the sub-region is ranked as the world's leading production area with more than 1,350,000 tonnes of raw nuts in front of Asia (India, Vietnam, Cambodia, Indonesia) which remains around 1,300,000 tonnes (Etéka, 2017). In Benin, cashew nuts are the second most important export crop after cotton (Yabi et al., 2018). In 2017, Benin's exports of cashew nuts are estimated at about 132 000 tonnes (Akomagni, 2017). In 2016, Benin is ranked sixth in the world with a production of 125,728 tonnes after Viet Nam, Nigeria, India, Côte d'Ivoire and Philippine (FAO, 2018). Unlike nuts, which are of great interest, the economic exploitation of the cashew apple remains underdeveloped and unreasonable. However, several studies have shown the richness of the cashew apple. It contains minerals, vitamin C, polyphenols and proteins (Adou et al., 2011, Adou et al., 2012a, Adou et al., 2012b, Kubo et al., 2006, Cavarlho et al., 2006). which give it antioxidant properties and make it an effective remedy for chronic dysentery in Cuba and Brazil (Kubo et al., 2006; Carvarlho et al., 2006). In India and Brazil, cashew apples are processed for the production of fruit juices or liqueurs (Cavalcante et al., 2003, Nanjundaswamy et al., 2001). According to Bando et Silva (2001), fruit growing is an alternative to the development and recovery of local economies. In addition, the anti-poverty strategy emphasizes the processing of our raw materials (Adou et al., 2012b). According to Holanda et al. (1998), the quantity of cashew apples abandoned each year represents a raw material potential that could be valorized by anaerobic bioconversion of glucose, fructose or sucrose into ethanol by the yeast Saccharomyces cerevisiae (Reddy et al., 2007). The valorization of these apples would be an important stake, not only in the field of forestry and the protection of the environment (reforestation, soil restoration, conservation of the land heritage in the cotton zone, etc.), but also on the socio-economic level (job creation, income-generating activities, socio-economic integration of women, improvement of the balance of payments, etc.) (Tokpa and Adoho,2006). It is in this context that the present study was initiated. Its main objective is the food valorization of cashew apples (Anacardium occidentale L.) through the study of the performance of four strains of Saccharomyces cerevisiae during the production of ethanol from cashew apple juice (Anacardium occidentale L.).

### **Material and Methods**

#### Vegetable material

The raw material used consisted of cashew apples collected in central Benin in the commune of Bantè (8 ° 25 '0' 'N and 1 ° 52' 60 " E). Interest in this commune is that it corresponds to the best cashew-producing region in Benin (Dédéou et *al.*, 2015, Gbohaïda et *al.*, 2015) and enjoys an interesting geographic position (Tandjiékpon et *al.* 2005).

#### Preparation of cashew apple juice

Once transported to the laboratory, the cashew apples were removed from their nuts and washed. The apples are washed by immersion in chlorinated water (50 ppm) for 15 minutes. Then, they were selected and washed with distilled water. Then, sliced and crushed using a blender (Blender LB20E, Torrington, USA, 2002). The resulting cashew apple puree was filtered using a filter of different mesh respectively 400; 300; 200; 150 and 50 micrometers.

The juice samples were sterilized by wet steam autoclaving at 110 °C for 10 min. The processed juice samples contain 157.5 g/L of reducing sugars (glucose + fructose) used in all experiments.

### Microorganisms and culture conditions

#### Ferments

Four active dry yeast strains are used as organic ferments. The total description of the strains tested is made in the table below:

Genus	Species	Variety	Nomenclature		Nomenclature Origin	
			Usual	adopted		
Saccharomyces	cerevisiae	cerevisiae	Bakery yeast	$S_1$	JOCK S.A. company located in Bordeaux, France	Used in breadmaking and available in supermarkets in Benin
Saccharomyces	cerevisiae	Bayanus	Bioferm Killer	$S_2$	Brouwland industrial company located in Beverlo, Belgium	Used in oenology and has a very high resistance to alcohol up to 16 % vol and contains the factor" Killer"
Saccharomyces	cerevisiae	cerevisiae	Safale K- 97	<b>S</b> <sub>3</sub>	Lesaffre industrial company located	High fermentation yeast adapted to the brewing of fine Belgian white beers
Saccharomyces	cerevisiae	cerevisiae	Saflager W-34/70	$S_4$	in Baroeul, France.	Used in the brewing industry

#### Table 1: Microorganisms used during alcoholic fermentation

#### **Pre-culture medium**

The pre-culture medium is a nutritious broth (buffered peptone water) composed of: Peptone (10,0 g/L) ; Sodium chloride (5.0 g/L); Disodium hydrogen phosphate (3.5 g/L); Potassium hydrogen phosphate (1.5 g/L); Final pH: 7.2  $\pm$  0.2.

#### Preparation of the inoculum

A mass of 1g of each yeast strain is introduced into 10 mL of nutrient broth (buffered peptone water). The mixture thus obtained is rapidly homogenized aerobically and then allowed to stand at 25 °C for 30 minutes. Then it is incubated at room temperature (30-32 °C) with rotary stirring for about half an hour in order to facilitate the revivification of yeast cells, necessary for a good fermentative activity.

#### **Pre-fermentation**

It allows the acclimatization of yeast cells to the substrate to be fermented. It was carried out by first mixing the inoculum with a volume corresponding to 10 % (v/v) of the total volume of the substrate to be fermented, equal to 100 mL of the must. The mixture is introduced into a fermenter and then incubated aerobically for 24 hours at room temperature (30-32  $^{\circ}$ C) on a TE 240 rotary shaker at 150 rpm. The fermenter used are sterile bottles with identical caps.

#### Fermentation

The remaining 90 % wort is added to each sample from the pre-fermentation. The total volume of the mixture in each bioreactor is then about 1000 mL. After rotary shaking, the study of the performance of four Saccharomyces cerevisiae strains was evaluated in the production of ethanol from cashew apple juice (*Anacardium occidentale* L.). Batch-controlled batch fermentation under anaerobic conditions was applied at laboratory temperature of 30.8 °C for 96 hours. The fermentation is conducted in 15 polyethylene bioreactors of capacity 3L and distributed in triplicate. The

control was carried out without the addition of selected yeast. To follow the evolution of the fermentation, Samples were taken every 24 hours and subjected immediately to physicochemical and biochemical analyzes.

#### Analytical methods

#### Cell biomass

Cell biomass was determined by adopting the dry mass method described by Parente et *al.* (2014). It consists of separating the cells from the medium, drying them and then weighing them.

#### **Concentration of reducing sugars**

The concentration of reducing sugars was determined by the colorimetric method with dinitrosalicylic acid (DNS) described by de Sousa et *al.* (2010).

#### pH and total titratable acidity

The pH and titratable acidity of the must was determined using the methods described by Adou et *al.* (2012b) and Massengo et *al.* (2014) respectively.

#### **Ethanol concentration**

The ethanol concentration was determined using an Assistent vinometer (4200, Germany) graduated from 0 to 25 % (v/v). The values obtained were converted to g/L using the method described by Parente et *al.* (2014).

#### **Refractometric solids content**

The content of refractometric dry extract was determined using a portable refractometer by the method of Soyer et *al*. (2003).

#### **Protein content**

The protein content was determined by the method described by Gornall et al. (1949).

#### Efficiency and Total hourly productivity

Efficiency, also known as the yield of alcoholic fermentation, gives quantitative values of the efficiency with which yeast converts reducing sugars into ethanol. Efficiency ( $\eta$ ; %) and total hourly productivity ( $\mu_P$ ; g.L<sup>-1</sup>.h<sup>-1</sup>) were determined according to the methods of Silva (2006) and Parente et *al*. (2014) respectively.

#### Ethanol yield and substrate conversion factor to cell biomass

The ethanol yield (Yp/s ;  $g.g^{-1}$ ) and the substrate conversion factor to cell biomass (Yx/s;  $g.g^{-1}$ ) were determined by the method described by Parente et *al.* (2014).

#### Limit attenuation and specific rate of sugar consumption

The limiting attenuation (Al ; %) and the specific rate of consumption of sugars ( $\mu_S$ ; %.h<sup>-1</sup>) were calculated from equations 1 and 2:

$$Al(\%) = \frac{(So - Sr)}{So} \times 100 \tag{1}$$

$$\mu_s = \frac{\frac{(so-sr)}{so} \times 100}{t} \tag{2}$$

 $S_0$ : Initial concentration of reducing sugars (g/L)  $S_r$ : Concentration of residual reducing sugars (g/L) t: Fermentation time (h)

#### Specific rate of cell growth

The specific rate of cell growth ( $\mu_x$ ; h<sup>-1</sup>) was determined using the method described by Stroppa et *al.* (2009)

#### Statistical analysis

The data was processed using Microsoft Excel 2007 and SPSS 16.0 software. The comparison of averages was made with the Turkey test with a significance level P<0.05.

### **Results and Discussion**

## Evolution of the concentration of reducing sugars and ethanol as a function of the duration of fermentation according to the four strains of *Saccharomyces cerevisiae*

The analysis of Figure 1 shows that the kinetics of alcoholic fermentation takes place in three phases. The first phase is from the  $1^{st}$  to the  $2^{nd}$  day and corresponds to a sudden decrease of the reducing sugars by the strains S1, S2, S3, S4 and the wild strains in each reaction medium. Indeed, the concentration of reducing sugars goes from 157.5 g/L to 39.46; 1.25; 12.39; 16.7; 32.3 g/L, a decrease of 118.04; 156.25; 145.11; 140.8; 127.2 g/L respectively for yeasts S1, S2, S3, S4 and wild-type strains. It follows from the above that yeast S2 has the highest consumption of reducing sugars (156.25 g/L) followed by yeasts S2 (145.11 g/L); S3 (140.8 g/L) and wild strains (127.2 g/L). Yeast S1 has the lowest consumption of reducing sugars (118.04 g/L). The sudden variation observed during the fermentation process corresponds to an increased consumption of the substrate due to prior adaptation of the yeast to the reducing sugars during the pre-fermentation phase. In parallel with the reduction of reducing sugars, there is a large increase in ethanol concentration. Indeed, the concentration of ethanol varies from 27.63 g/L to 58.16; 104.3; 93.69; 73.95; 45.21 g/L, an increase of 30.53; 77; 66.06; 46.32 and 17.58 g/L respectively for yeasts S1, S2, S3, S4 and wild-type strains. These analyzes show that the maximum concentration of ethanol (104.3 g/L) is obtained with the yeast S2. Thus the strain S2 is revealed as the most efficient yeast in terms of alcohol production and consumption of reducing sugars. The abrupt increase in ethanol concentration during the first 48 hours of fermentation would be due to a prior adaptation of these yeasts during the pre-fermentation phase and their biofermentative potential to biodegrade the substrate to produce ethanol. The second phase is from the 2<sup>nd</sup> to the 3<sup>rd</sup> day. This phase is characterized by a total stabilization of the concentration of reducing sugars to an average value of 39.46; 1.25; 12.39; 16.7; 32.3 g/L respectively for the yeasts S1, S2, S3, S4 and the wild strains. It remains constant until the end of the fermentation process. This indicates the cessation of the alcoholic fermentation reaction. This phenomenon is due to the different stresses experienced by the microorganisms involved in the fermentation (Novidzro et al., 2013). A similar phenomenon is observed in the kinetics of production of ethanol. Indeed, the concentration of ethanol remains constant at values of 58.16; 104.3; 93.69; 73.95 and 45.21 g/L respectively for yeast S1, S2, S3, S4 and wild-type strains. The third stage is from the 3<sup>rd</sup> to the 4<sup>th</sup> day and corresponds to a gradual decrease of the ethanol concentration up to 55.16; 100.58; 89.74; 70.16; 41.27 g/L is a decrease of 3; 3.32; 3.95; 3.79; 3.94 g/L respectively for yeasts S1, S2, S3, S4 and wild-type strains. This decreasing tendency observed after 72 hours of fermentation could be explained by an overpressure of yeast activity due to the high concentration of ethanol and the depletion of reducing sugars (Cai et Nip, 1990) or the assimilation ethanol as an energy source (Zaved et Foley, 1987).



Figure 1: Variation of the concentration of reducing sugars (g/L) and ethanol (g/L) as a function of time (days)

S1 :Bakery yeast	
S2:Saccharomyces cerevisiae var. bayanus	T:Witness
S3 : Saccharomyces cerevisiaeSafale K-97	SR : Concentration of reducing sugars
S4 :Saccharomyces cerevisiae Saflager W-34/70	<b>CE</b> :Ethanol concentration

## Evolution of cell biomass and dry extract as a function of the fermentation time according to the four strains of *Saccharomyces cerevisiae*

The results of the evolution of the cell biomass and of the dry extract as a function of the duration of fermentation are shown in figure 2. From the analysis of this figure, it appears that the kinetics of the cellular growth takes place in three phases. The first phase is from the 1<sup>st</sup> to the 2<sup>nd</sup> day and corresponds to a sudden decrease in the soluble dry matter of the reaction medium by the yeasts S1; S2; S3; S4 and wild strains. In fact, the content of soluble solids goes from 14 °Brix to 5; 5.2; 5; 5.4 and 7 °Brix is a decrease of 8.8; 8.8; 8.8; 8.6; 7 °Brix respectively for the yeasts S1; S2; S3; S4 and wild strains. The highest consumption of reducing sugars was recorded with S1 yeasts; S2 and S3. This sudden variation observed during the fermentation process corresponds to an increased consumption of fermentable sugars due to prior adaptation of the yeast to the reducing sugars during the pre-fermentation phase. The kinetic profile of the soluble solids is similar to that of reducing sugars. Along with the decrease in soluble solids, there is a large increase in cell biomass after 48 hours of fermentation: this is the exponential phase of cell growth. Indeed, the cellular biomass goes from 10 dg/L to 50; 40; 40; 40; 30 dg/L is an increase of 40; 30; 30; 30 and 20 dg/L respectively for the yeasts S1; S2; S3; S4 and wild strains. The sudden increase in cell biomass would be due to a prior adaptation of veast to reducing sugars during the pre-fermentation phase. The second phase is from the 2nd to the 3rd day. This phase shows a tendency towards stabilization of the soluble dry matter up to a value of 5 ° Brix for the yeasts S1, S2, S3, S4 and 6 °Brix for the wild strains. In addition to the soluble dry matter, there is a total stabilization of the cellular biomass at 40 dg for the yeasts S2, S3, S4 and 50 dg for the yeast S1 then 30 dg for the wild strains. This is the stationary phase of cell growth. This phenomenon is due to the high concentration of ethanol in the reaction medium (Cai et Nip, 1990). In addition to the soluble dry matter, there is a slight decrease in the cell biomass, which is explained by the presence in the reaction medium of  $CO_2$  and organic compounds (secondary metabolites) which inhibit the fermentative metabolism of Saccharomyces cerevisiae (Maiorella et al., 1983). This phenomenon is likely to induce the lethality of the microbial cells.



Figure 2:	Variation of	of cellular	biomass	(dg/L)	and s	oluble	drv matter	(°Brix)	as a fu	nction	of time	(days)
1 15ul c 2.	v un un on o	or containin	oronnabb	$(\mathbf{u}_{\mathbf{b}}, \mathbf{L})$	und b	oruore	ary matter	( DIIA)	ub u 10	netion	or time	(uu y b)

S1 : bakery yeast	
S2 :saccharomyces cerevisiae var. Bayanus	T:Witness
S3 : saccharomyces cerevisiaesafale k-97	ESR :Refractometric solids content
S4 :saccharomyces cerevisiaesaflager w-34/70	BM :Cell biomass

# Evolution of the pH and acidity according to the fermentation duration according to the four strains of Saccharomyces cerevisiae

The results of the evolution of the pH and the acidity as a function of the duration of fermentation are indicated in figure 3. It is apparent from the analysis of this figure that the pH slightly increases the first 24 hours of fermentation. Indeed, the pH goes from 4.32 to an average value of 4.41, an increase of 0.09. The slight increase in pH can be

explained by the production of ethanol which modifies the dissociation constants of the constituents and in particular the organic acids (Akin, 2008). From the 1<sup>st</sup> to the 2<sup>nd</sup> day, the pH dropped considerably to reach values of 3.81; 3.95; 3.76; 3.84 and 3.75 respectively for the yeasts S1, S2, S3, S4 and the wild strains. Then, it remains practically constant until the end of the alcoholic fermentation process. According to Akin (2008), the no less considerable decrease in pH at the second day of fermentation is linked to the consumption of nitrogen sources. In general, the pH dropped from the first to the fourth day of fermentation. In addition, there was a slight decrease in acidity on the first day of the fermentation process which rose from 0.58 % to 0.45 % or a decrease of 0.13 %. Then it oscillates on average from 0.45 to 0.64 % on the 4<sup>th</sup> day of fermentation. It should be noted that the curve is rising from the third to the fourth day, thus reflecting an increase in acidity. Indeed, the increase in the acidity is a consequence of the decrease in pH and results from the production in the reaction medium of organic acids such as lactic acid, acetic acid and succinic acid (Bortolini et al., 2001). There is also citramalic acid, dimethylglyceric acid which is present in a small proportion (Akin, 2008). The increase in acidity could also be due to the formation of carbon dioxide in the reaction medium. Indeed, the carbon dioxide  $(CO_2)$  can be dissolved in the reaction medium in the form of carbonic acid  $(H_2O, CO_2)$ , which is dissociated into hydrocarbon ions (HCO<sup>3-</sup>), carbonate (CO<sub>3</sub><sup>2-</sup>) and hydrogen ion (H<sup>+</sup>) (Garcia-Gonzalez et al., 2007). It is also verified that during the fermentation process the pH band (3.89-4.57) is sufficient enough to allow rapid alcoholic fermentation and inhibition of undesirable bacteria. Similar behaviors of pH and acidity were observed during the fermentation process by Bortolini et al. (2001), Andrate et al. (2003), Torres Neto et al. (2006). It is important to note that the variation in acidity during fermentation has a great influence on the stability and color of fermented beverages (Rizzon et al., 1994).





Figure 3: Variation in pH and acidity versus time (days)

S1 :Bakery yeast
S2: Saccharomyces cerevisiae var. bayanus
S3: Saccharomyces cerevisiae Safale K-97
S4: Saccharomyces cerevisiae Saflager W-34/70

## Evolution of the protein concentration as a function of the fermentation time according to the four strains of *Saccharomyces cerevisiae*

The results of the evolution of the concentration of proteins as a function of the duration of fermentation are shown in figure 4. It follows from the analysis of this figure that protein consumption is progressive until the end of the fermentation process alcoholic. Indeed, the protein content ranged from 12.80 g/L to 3.99; 6.34; 3.78; 4.75 and 6.80 g/L, a decrease of 8.81; 6.46; 9.02; 8.05 and 6 g/L respectively for yeast S1, S2, S3, S4 and wild-type strains. From the foregoing, it appears that yeasts have a strong ability to assimilate the proteins essential for their growth. According to Akin (2008), the variation in protein content during alcoholic fermentation is explained by the increased protein consumption by yeast as a nitrogen source.



Figure 4: \	Variation i	n protein	concentration	(g/L)	versus time	(days)
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S1 :Bakery yeast	
S2: Saccharomyces cerevisiae var. bayanus	S4: Saccharomyces cerevisiae Saflager W-34/70
S3 : Saccharomyces cerevisiae Safale K-97	T:Witness

## Values of kinetic parameters calculated to evaluate the performance of the four strains of Saccharomyces cerevisiae

Table 2: Kinetic	parameters	calculated	to evaluate	the pe	erformance	of yeasts	in the	production	of ethanol.

	Levures							
Paramètres	S1	<b>S</b> <sub>2</sub>	<b>S</b> 3	<b>S</b> 4	Т			
Al (%)	74,946 ± 0,511a	$99,206 \pm 0,301b$	$92,133 \pm 0,011c$	$89,524 \pm 0,670d$	79,492±0,512e			
$\mu_{s}$ (%. h <sup>-1</sup> )	$1,561 \pm 0,551a$	$2,067 \pm 0,333b$	$1,919 \pm 0,015b$	$1,865 \pm 0,676b$	$1,656 \pm 0,501a$			
$\mu_{x}(h^{-1})$	$0,034 \pm 0,765a$	$0,029 \pm 0,889a$	0,029±0,500a	0,029±0,700a	0,023±0,900b			
$\mu_{P}$ (g.L <sup>-1</sup> .h <sup>-1</sup> )	$0,636 \pm 2,315a$	$1,604 \pm 1,545b$	$1,376 \pm 3,168c$	$0,956 \pm 2,755d$	$0,366 \pm 1,899e$			
$Yx/s (g.g^{-1})$	$0,034 \pm 2,375a$	$0,019 \pm 2,913b$	$0,021 \pm 2,653c$	$0,021 \pm 2,621c$	$0,016 \pm 2,143$ c,a			
Yp/s (g.g <sup>-1</sup> )	$0,259 \pm 0,263a$	$0,491 \pm 0,123b$	$0,455 \pm 0,123b$	$0,329 \pm 0,243c$	$0,140 \pm 0,311$ d			
η (%)	50,615 ± 0,213a	$96,025 \pm 0,135b$	89,088 ± 0,132c	$64,379 \pm 0,235d$	$27,479 \pm 0,373e$			

Values with the same letter on the same line are not significantly different (p < 5%) from Turkey's multiple comparison tests.

S1 :Bakery yeast	S3: Saccharomyces cerevisiae Safale K-97
S2:Saccharomyces cerevisiae var. bayanus	S4 :Saccharomyces cerevisiae Saflager W-34/70
<b>η</b> : Efficiency	$\mu_{P}$ :Total hourly productivity
Y <sub>P/S</sub> : Ethanol yield	<b>Yx/s</b> : Substrate conversion factor to cell biomass
<i>Al</i> : Limit attenuation	$\mu_s$ : specific rate of sugar consumption

The analysis in this table shows that yeast S2 has the highest conversion efficiency of substruct to ethanol (96.025 %), the highest hourly productivity (1.604 g.L<sup>-1</sup>.h<sup>-1</sup>), the smallest factor substrate conversion to biomass (0.019 g.g<sup>-1</sup>), the highest yield of ethanol (0.491 g.g<sup>-1</sup>), the highest rate of substrate consumption (2.067 %.h<sup>-1</sup>). Thus the yeast S2 is revealed as the most successful strain. In fact yeast S<sub>2</sub> has a high tolerance to alcohol and has the phenotype "Killer", which allows it to have the greatest efficiency of conversion of the substrate ethanol and the best yield of ethanol. S<sub>3</sub> yeast also showed some interesting results. After the S<sub>2</sub> yeast, the S3 strain exhibited the highest conversion efficiency of the substrate to ethanol (89.088 %), the highest hourly total productivity (1.376 g.L<sup>-1</sup>.h<sup>-1</sup>), the highest yield of ethanol (0.455 g.g<sup>-1</sup>), the highest substrate consumption rate (1.919 %.h<sup>-1</sup>). In contrast, the control exhibited the lowest conversion efficiency of the substrate to ethanol (27.479 %), the lowest hourly productivity (0.366 g.L<sup>-1</sup>.h<sup>-1</sup>), the smallest substrate conversion factor to biomass (0.016 g.g<sup>-1</sup>), and the lowest yield of ethanol (0.140 g.g<sup>-1</sup>).

The selected yeast S<sub>1</sub>, for its part, proves to be the least effective of the four strains tested with a substrate conversion efficiency of 50.615 % ethanol and an ethanol yield of 0.259 g.g<sup>-1</sup>. But it is more effective in comparison with wild strains. In fact, the selected yeast S1 is a baker's yeast used in bread making. It allows resistance to alcohol. Thus the strains tested in the kinetics of alcoholic fermentation can be classified according to their performance as follows: S2> S3> S4> S1> T. The values obtained in this study are similar to those obtained by Pacheco et *al.* (2009), which found an effectiveness of 85.30 to 98.52 %, a 44.80 % to 96.50 % limit attenuation; a total hourly productivity of 3.30 to 6.31 g.L<sup>-1</sup>.h<sup>-1</sup> and an ethanol concentration ranging from 19.82 to 37.83 g.L<sup>-1</sup> when studying the alcoholic fermentation of apple bagasse of cashew nuts in Brazil.

### Conclusion

The yeast *Saccharomyces cerevisiae* var. *bayanus* had the highest concentration of ethanol with better ethanol yield and low substrate conversion to biomass. In addition, the calculated kinetic parameters showed that the best yields are obtained with the yeast *Saccharomyces cerevisiae* var. *bayanus*. Thus, it differs from other yeast strains by its high fermentative power. As a result, it can be used as a high performing strain for the purpose of intensive production of ethyl alcohol from cashew apple juice.

### Reference

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