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Production of bioethanol from stems of *Sorghum saccharatum* monitoring kinetic parameters of fermentation

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The objective of this study was to evaluate the performance of three stumps of *Saccharomyces cerevisiae* in the ethanol bioconversion of the diluted stems of sweet sorghum (*Sorghum saccharatum* L.) and to monitor the kinetic parameters of fermentation. Therefore, different initial concentrations of three stems from *S. cerevisiae* are used to ferment the juice. The juice obtained was formulated from one kilogram (1 kg) of stems of *S. saccharatum* and 0.5 L of distilled water. The monitoring of the kinetic fermentation parameters of the musts revealed that the best production yields (28.31±0.07 and 24.23±0.07 mL/kg) from bioethanol are obtained with the fermented mashes in the presence of the *Angel brand super alcohol* (3 and 5 g/L). This study showed that the stem of *S. saccharatum* L. constitutes a good fermentable biomass that could be valorized through the production of first-generation biofuel.

Key words: Sorghum, *Saccharomyces cerevisiae*, fermentation, bioethanol.

INTRODUCTION

The control of renewable energies has become today a major concern for developing countries in order to get the planet out of the multiple nuisances related to the massive use of fossil fuels. These renewable energies are a credible alternative to fossil energy (Vinson, 2016)). Their development is a huge challenge in terms of diversification and energy security (Leclerc et al., 2014). In Africa in general, and in Benin in particular, the biofuels sector is still in its infancy due to persistent problems related to hunger, health, youth unemployment and low income. As the policy of developing countries is generally to eradicate hunger, endemic diseases and ensure the best social coverage of population, it seems inappropriate, if not impossible, to produce bioethanol in t

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and low income. As the policy of developing countries is generally to eradicate hunger, endemic diseases and ensure the best social coverage of population, it seems inappropriate, if not impossible, to produce bioethanol in a country where people are dying from hunger.

Bioethanol production may be poorly perceived. In order to find solutions to the problems of hunger, livestock fodder and energy distribution, with the aim of reducing greenhouse gases, decision-makers had thought in 2012 about the species of cereals capable to provide leaves, bagasse and sugars (Ren et al., 2012). Among these renewable biomasses is the species *Sorghum saccharatum* (L) which is a plant with multiple potential uses. Sweet sorghum is considered a promising energy crop for biofuel production (Carrillo et al., 2014; Yin et al., 2013). The grains of sweet sorghum contain various high value-added molecules (tannins, anthocyanins, kafirines, aconitic acid, etc.) that are used in agribusiness (Temple et al., 2017). Because of the diverse uses of sorghum grain in human nutrition in sub-Saharan Africa, they are better appreciated (FAOSTAT, 2016; Pierre Gary, 2012). Sorghum stalks are lignocellulosic biomass in energy production through anaerobic digestion, thermometry, ethanol fuel production, composite plastics and building block manufacturing (Chantereau et al., 2013). The leaves and bagasses are used for fodder with high nutritional value in different forms (green, hay or ensilage). (Braconnier et al., 2014). Due to its plasticity and great diversity of forms, sorghum can be integrated into many growing systems in tropical and temperate zones (Damasceno et al., 2014; Vinutha et al., 2014). It is a cover plant for soil conservation and remobilization of minerals in cropping systems. Although it is also suitable for drier and less fertile environments, it remains an essential food crop for people living in the arid and semi-arid tropics (BSI, 2018; (Vinson, 2016); Temple et al., 2017; Carrillo et al., 2014). Its ability to provide many ecosystem services makes it a culture of the future (Temple et al., 2017). As a result, average sorghum production has risen (in the last five years) to 60 million tons a year worldwide. The 2015-2016 seasons had given global sorghum production of 60.16 million tons. This production reached 64.20 million tons for the 2016-2017 crop season (COMMODAFRICA, 2016). Intensification of sorghum cultivation would contribute to solving the problems of climate change, health, food insecurity and energy distribution (Visser et al., 2005). The varieties in use of sorghum fit the various food stakes, energy and environmental challenges and will enable rural populations to improve their standard of living thanks to a better valorization of agricultural production and the creation of new local commercial sectors generating jobs. Its intensive culture could help to fight against the immigration of young people. Sweet sorghum that has the potential to provide seeds, leaves, stalks and sugars could better increase household income by keeping rural people in villages and rural areas. Sweet sorghum could generate investment and compete with cereals in the market (Chantereau et al., 2013; Damasceno et al., 2014; Vinutha et al., 2014; Temple et al., 2017). With more than 23% of world sorghum production, Africa could lead new and effective policies by contributing to development goals by 2025 in renewable energy production (COMMODAFRICA, 2016). In the development perspective, it is good to consider the production of bioethanol from this lignocellulosic sweet biomass which is grown in small quantities for its grains. It is in this line of thought that the choice of sweet sorghum (*S. saccharatum* L.) took place. Thus, the objective of this work is to value the species.

MATERIALS AND METHODS

The stem of sweet sorghum (*S. saccharatum* L.) used as plant material in the framework of this study was collected in Ségbana in the Department of Alibori (North Benin) and had been directly transported fresh to the laboratory where it had been kept at -10°C in the freezer.

Three yeast strains of *Saccharomyces cerevisiae*, mainly industrial, from the Chinese company "Angel Yeast Co., Ltd" and marketed were used in alcoholic fermentation. It includes:

i) *Angel brand Thermal-tolerant alcohol*
ii) *Angel brand super alcohol*
iii) *Angel super alcohol*.

Experimental procedure

Raw material preparation

One kilogram of stems of *S. saccharatum* had been chopped, crushed into fine particles and pressed with a mechanical press fitted with a filter in the presence of 0.5 L of distilled water. The resulting juice was sterilized at 121°C for 15 minutes (Sidney, 1984).

Preparation of yeast suspensions

The dry and active yeast strains were revived at 37±1°C in peptone water for 30 min. The three revived *S. cerevisiae* strains were used as juice ferments for sweet sorghum stems.

Fermentation

The sterilized juice, cooled with *S. saccharatum* and distributed in the fermenters, was inoculated with *S. cerevisiae* strains at different concentrations (from 1 to 5 g/L). The juice wort consisting of juice without ferment was used to evaluate the incidence of the yeasts used. The alcoholic fermentation was run in batch mode for seven (7) days at room temperature (25°C).

Monitoring of fermentation parameters

Parameters such as degree brix, relative density and pH of the musts were followed from the beginning to the end of the alcoholic fermentation:

i) The total soluble content (Brix, expressed in °Bx) of the different samples was determined by direct measurement using a Palm Abbe 201 MISCO digital portable refractometer.
ii) The pH of the juice is determined using an OHAUS ST10 digital pH-meter.
iii) The relative density at 20°C of the juices was determined according to a standard method of the Association of Official Analytical Chemists (2003) and described by Novidzro (2013).

Distillation of the must

At the end of the fermentation, the extraction of the ethanol was carried out by distillation of the musts using a QUICKFIT/FC3/13 column of vigreux distiller of 85 cm in length and 4.45 cm in diameter. During the distillation, the temperature was maintained at 79°C at the head of the vigreux column until the alcohol from the must of the heating flask was exhausted (Sidney, 1984; Novidzro et al., 2013).

Attenuation limit

This gives an estimate of the level of sugars consumed or likely to be converted into alcohol during fermentation (Novidzro, 2013).

$$\mathrm{Al} = \frac{\text{Brix iniatial} - \text{brix final}}{\text{Brix initial}} \times 100 \quad (1)$$

Determination of bioethanol yield of production

The average yield of bioethanol production of a sweet sorghum must is determined from its alcoholic strength by volume (TAV), its volume and the mass of stems used as plant material. He had given the amount of alcohol obtained as a function of the mass of sweet sorghum stems used. It is expressed by the formula:

$$R_m = \frac{V_0 V_d T_{AV}}{100MV} \quad (2)$$

M: Mass of stems of sweet sorghum entered in the formulation of the juice (kg)
R_m: Average yield of production
T_{AV}: Alcoholic volume title or alcoholic degree (% v/v)
V: Volume of fermented juice (mL)
V_0: Volume of distilled water used in juice formulation (mL)
V_d: Volume of distillate obtained (mL).

Statistical analyzes

The tests had been repeated three times and the data had been processed using the Microsoft Excel 2010 software. The statistical analysis of the data and the comparison of the independent averages were made at the 5% threshold by SPSS 16.0.

RESULTS AND DISCUSSION

Fermentation time

Table 1 presented the average fermentation time of the musts of stems of *S. saccharatum* (L). In the presence of *Angel brand super alcohol* and *Angel super alcohol,* the average fermentation time of musts was 48±1 h. The fermented mash containing *Thermal-tolerant alcohol* had 72±2 h. Compared to the must wort, the must sown *to Angel brand super alcohol* (and *Super Angel yeast*) then *Thermal-tolerant alcohol*, respectively had their fermentation time reduced by 72 and 48 h.

The average fermentation time of juice from pretreatment of sweet sorghum stalks is 48 h (Chen, 2012; Matsakas and Christakopoulos, 2013a;Gubicza et al., 2016). At very high density with or without urea supplement and then in the presence of *S. cerevisiae* KL17, according to Appiah-Nkansah et al. (2018), the fermentation time of flour and sweet sorghum juice is 72 h. When a sweetened biomass juice is enriched with urea, phosphorus, etc., as nutrients for yeasts, its fermentation time can be reduced. The average duration of a good fermentation of the juice of a lignocellulosic biomass in the presence of *S. cerevisiae* as ferment is between 48 and 72 h (Soro et al., 2012; Gbohaïda et al., 2015). Our results are in agreement with those of the authors despite different methods used during our study. This work has shown that the fermentation time of a sweet sorghum stem juice is between 48 and 72 h (Table 1).

pH of the must

Figure 1 showed evolution of the pH of the musts of *S. saccharatum* from the beginning to the end of the alcoholic fermentation. Overall, the curves showed the same pace. Two major phases are observed in the pH variation.

A decay phase was observed, which lasted 48 h, during which the pH of all the musts usually dropped. The mashes with *Thermal-tolerant alcohol* (Figure 1A) and *Angel super alcohol* (Figure 1C) which initially had their pH equal 4 had dropped to 3.65 in 48 h of fermentation. After 48 h also, the pH of the musts containing *Angel brand super alcohol* was 3.6. During this period, the juice must have a pH of 3.6. Compared to the control must, this decay phase showed that the acidification of the fermentation medium was not linked to the yeasts used but rather to the nature of the juice of sweet sorghum stems.

A constant linearization phase is observed in the vicinity of the pH equal to 3.5. The linear phase is between the 2nd and the 7th day of the alcoholic fermentation. In the constant linearization phase, the pH of the musts did not significantly vary, except for the pH of the must with *Angel super alcohol* of initial concentrations 1, 3 and 4 g/L of Figure 1C. In particular, the rise in pH is between the 4th and 7th day of fermentation of must containing *Angel super alcohol* at initial concentrations 1, 3 and 4 g/L.

In the works of Gubicza et al. (2016) on the pretreated stems of sweet sorghum, the pH of the musts is maintained at 6.3 during the fermentation of the juice for a better yield and at 37°C. The increase in acidity could

Table 1. Fermentation time from stem of *Sorghum saccharatum* (in hours).

Must	Initial yeast concentration (g/L)					
	0	1	2	3	4	5
Thermal-tolerant alcohol	120±3[a]	72±2[a]	72±2[a]	72±2[a]	72±2[a]	72±2[a]
Angel brand super alcohol	120±3[a]	48±1[b]	48±1[b]	48±1[b]	48±1[b]	48±1[b]
Angel super alcohol	120±3[a]	48±1[b]	48±1[b]	48±1[b]	48±1[b]	48±1[b]

Values with the same letter in the same column are not significantly different (p <5%) according to ANOVA and Tukey multiple comparison tests.

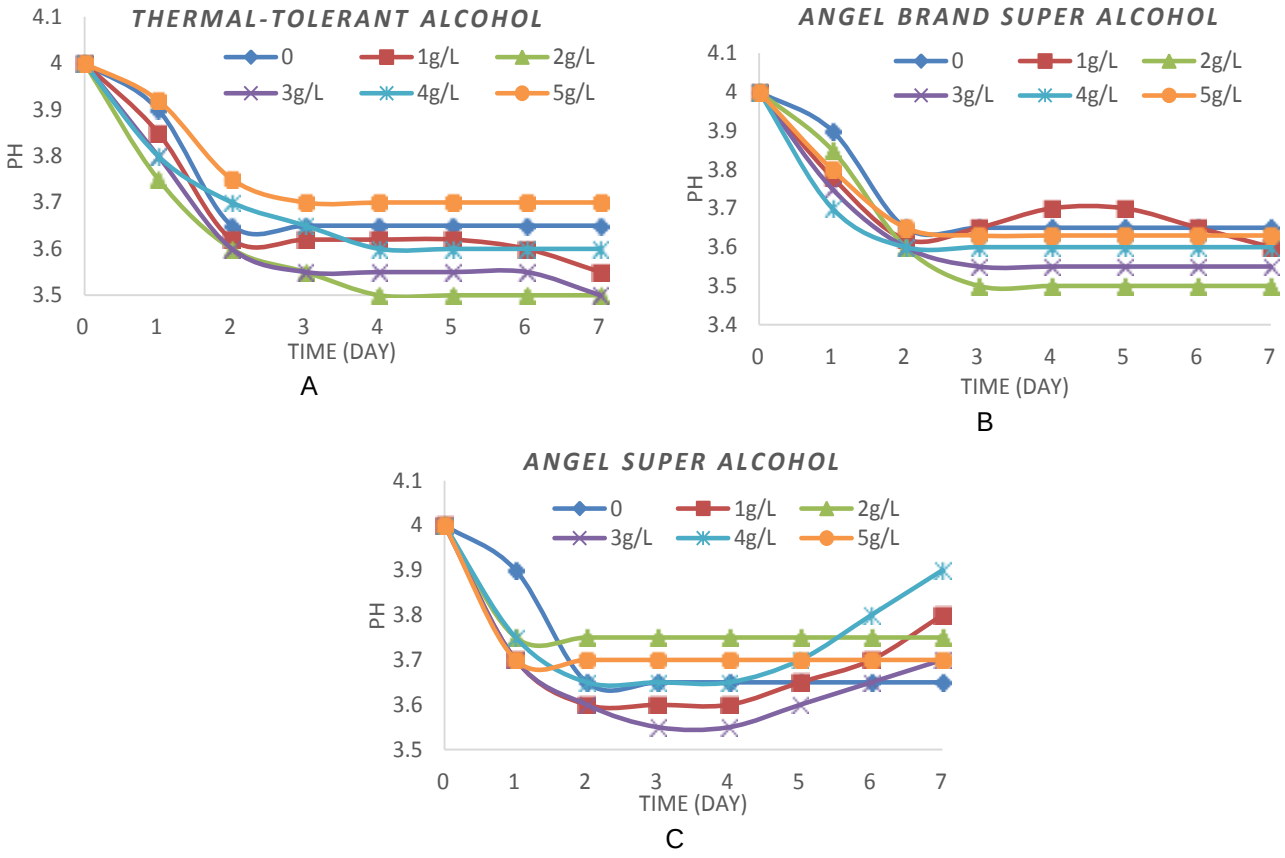

Figure 1. Variation of the pH of the different musts of *Sorghum saccharatum* during the alcoholic fermentation in the presence of (A) *Thermal-tolerant alcohol* (B) *Angel brand super alcohol* and (C) *Angel super alcohol* of *Saccharomyces cerevisiae.*

be due to the production of carbon dioxide or acidic compounds by yeasts during fermentation. The carbon dioxide (CO_2) can be dissolved in the liquid medium in the form of carbonic acid (H_2CO_3), which is dissociated into bicarbonate ions (HCO_3^-), carbonates (CO_3^{2-}) and hydrogen (H^+) (Burgot, 2011; Gbohaïda et al., 2016).

Density

The weight loss was observed (Figure 2) in the musts of *S. saccharatum* in the presence of yeast strains used at different initial concentrations. Figure 2 showed the variation of the relative density of musts during fermentation. Generally, the initial density (1.05) had dropped to 0.98. This lower density obtained after 7 days of fermentation was derived from juices containing *S. cerevisiae* at concentrations 3, 4 and 5 g/L.

Two phases were observed in the variation of the relative density. The phase of sudden loss of juice weight was observed in the first 3 days of fermentation. There had been a rapid drop in the mass of all musts. During this phase, the initial relative density of 1.05 musts decreased to 1.01. It was marked by a decrease in the

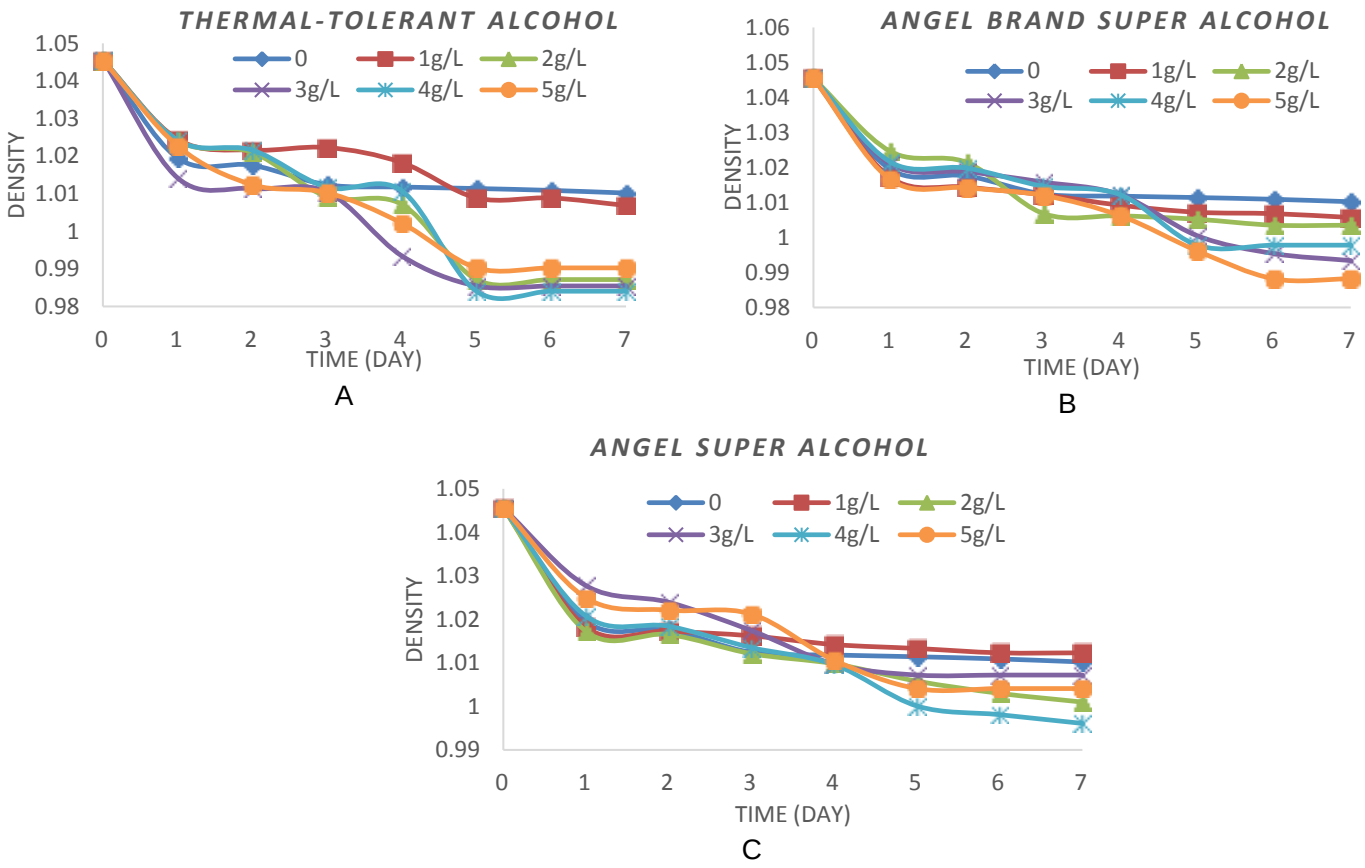

Figure 2. Variation of the density of the different musts of *Sorghum saccharatum* during the alcoholic fermentation in the presence of (A) *Thermal-tolerant alcohol* (B) *Angel brand super alcohol* and (C) *Angel super alcohol* of *Saccharomyces cerevisiae.*

density of musts. The slowing phase was observed between the 2nd and the 7th day of fermentation. The slower weight loss of the samples started from the 1.01 density to 0.98. This phase was considered as the kinetic relaxation time of alcoholic fermentation. The work of Soro (2012), Novidzro et al. (2013) and from Gbohaïda et al. (2015) showed that the drop in the relative density of a must is closely related to the decrease in the level of soluble matter in the fermentation medium. This connection evoked by these authors was obtained in our results between Figures 2 and 3. All this showed that the loss of weight of all musts could mark the end of alcoholic fermentation.

Figure 3 provides information on the evolution of the brix degree of musts. This evolution was in agreement with the observations made in Figure 2. In 48 h of fermentation, a rapid decrease (by more than 60%) was observed in the brix level of the stem strains *Angel brand super alcohol* and *Angel super alcohol* (Figures 2B and 2C). This decrease is greater for all the concentrations of strains used. This taught us about the depletion of the total soluble solids contained in the fermentation medium. Figures 3B and 3C showed that the highest consumption of sugars by *Angel brand super alcohol* and *Angel super alcohol* strains lasted only 48 h. Stopping the consumption of sugars marked the end of the alcoholic fermentation. The *Thermal-tolerant alcohol* must and the control respectively revealed their sugar consumption time which was 72 and 120 h (Figure 3A).

Brix degree of the must

Figure 3 presented information on the evolution of the Brix degree of the musts. This evolution was in agreement with the observations made in Figure 2. In 48 h of fermentation, a rapid decrease (by more than 60%) was observed in the brix level of the stem strains *Angel brand super alcohol* and *Angel super alcohol* (Figures 2B and 2C). This decrease is greater for all the concentrations of strains used. This taught us about the depletion of the total soluble solids contained in the fermentation medium. Figures 3B and 3C showed that the highest consumption of sugars by *Angel brand super alcohol* and *Angel super alcohol* strains lasted only 48 h. Stopping the consumption of sugars marked the end of the alcoholic fermentation. The *Thermal-tolerant alcohol* must and the control respectively revealed their sugar

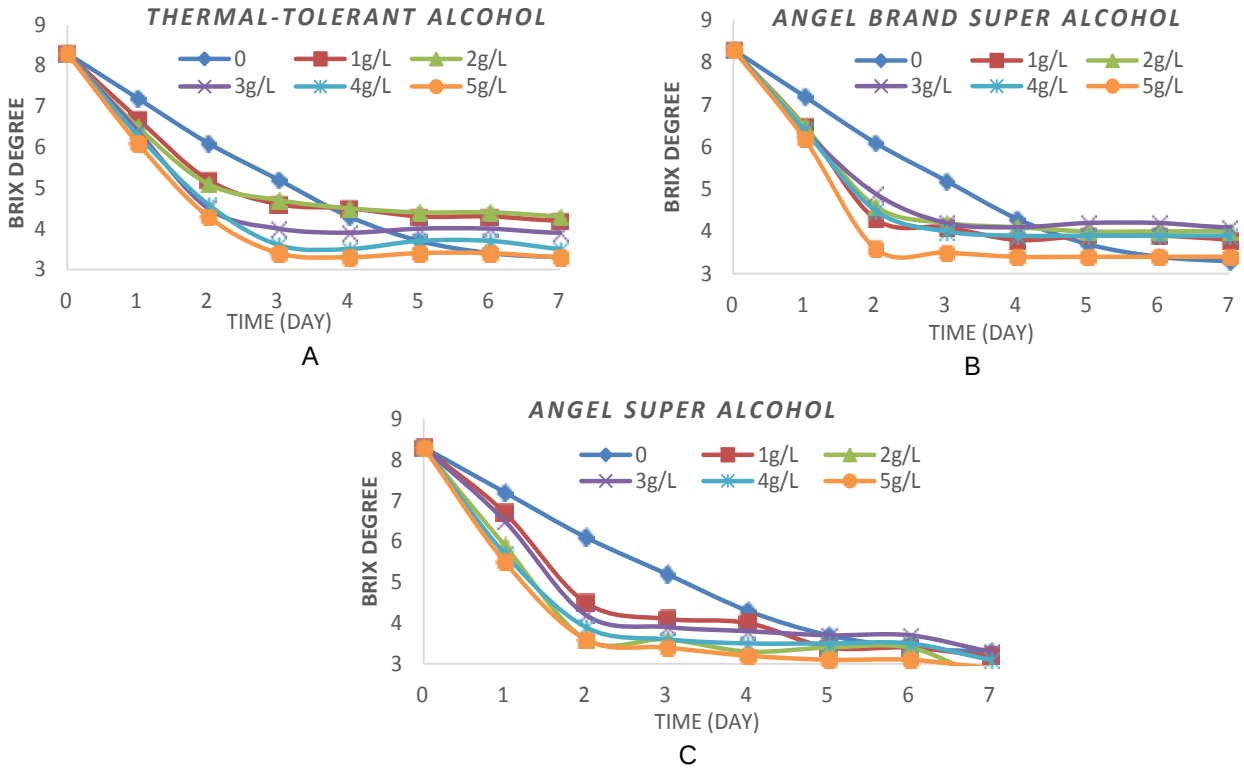

Figure 3. Variation of the brix degree of the different musts of *Sorghum saccharatum* during the alcoholic fermentation in the presence of (A) *Thermal-tolerant alcohol* (B) *Angel brand super alcohol* and (C) *Angel super alcohol* of *Saccharomyces cerevisiae.*

Table 2. Attenuation limit (%).

Must	Initial yeast concentration (g/L)					
	0	1	2	3	4	5
Thermal-tolerant alcohol	60.2±0.4^{a}	50.4±0.7^{a}	50.6±0.4^{c}	53.1±0.2^{a}	59.1±0.3^{a}	60.2±0.4^{a}
Angel brand super alcohol	60.2±0.4^{a}	56.6±0.2^{b}	53.1±0.3^{b}	53.1±0.4^{a}	53.1±0.3^{b}	59.1±0.4^{a}
Angel super alcohol	60.2±0.4^{a}	61.4±0.5^{c}	65.1±0.4^{c}	65.9±0.5^{b}	66.2±0.6^{c}	66.2±0.5^{b}

Values with the same letter in the same column are not significantly different (p <5%) according to ANOVA and Tukey multiple comparison tests.

consumption time which was 72 and 120 h (Figure 3A).

Attenuation limit

Table 2 showed the limiting attenuation of all fermented musts. The musts containing the *Angel super alcohol* strain have a higher sugar consumption rate. Their consumption rate is between 61.2 and 66.2%. In the presence of *Angel brand super alcohol*, the musts have a rate of attenuation between 53.1 and 59.1%. The rate of limit attenuation of musts to *Angel super alcohol* and musts to *Thermal-tolerant alcohol* increases with the increase of their initial concentrations. Generally, the yeast sugar consumption rate is between 50.4 and 66.2%. These levels reveal the consumption capacity of yeast sugars used in this study.

In the case of the alcoholic fermentation of the juice (extracted at a ratio of 1.5 L of distilled water per 1 kg of *S. saccharatum* stems), enriched with 2 g/L urea or not, in the presence of *Saccharomyces carlsbergensis* at different initial concentrations (0, 1, 2 and 3 g/L), the rate of the attenuation limit is between 72.1 and 74.4% (Mossi et al., 2017). These results may be at odds because of the different initial rates of soluble materials, yeasts used, and urea enrichment of the juice.

Average production of ethanol

Table 3 gives the average values of ethanol obtained from fermented musts of *S. saccharatum* in the presence

Table 3. Average production of ethanol (in mL/kg of stem of the *Sorghum saccharatum*).

Musts	Initial yeast concentrations					
	0	1 g/L	2 g/L	3 g/L	4 g/L	5 g/L
Thermal-tolerant alcohol	5.64±0.06[a]	12.87±0.06[a]	14.65±0.08[a]	14.60±0.08[a]	19.55±0.07[a]	12.14±0.06[a]
Angel brand super alcohol	5.64±0.06[a]	9.53±0.07[b]	18.71±0.09[b]	28.31±0.07[b]	13.97±0.07[b]	24.23±0.07[b]
Angel super alcohol	5.64±0.06[a]	13.00±0.07[c]	8.83±0.07[c]	10.21±0.09[c]	8.85±0.05[c]	7.55±0.05[c]

Values with the same letter in the same column are not significantly different (p <5%) according to ANOVA and Tukey multiple comparison tests

of *S. cerevisiae* yeasts. The most important production is obtained with the yeast *Angel brand super alcohol* and is 28.31±0.14 mL.kg^{-1} with the must of concentration 3 g.L^{-1}. 5.64 mL.kg^{-1} was collected by spontaneous fermentation of the musts of *Sorghum*. Against all odds, the production of alcohol from yeast *Angel super alcohol* has generally remained lower than that of *Thermal-tolerant alcohol* and *Angel brand super alcohol*. This could be explained by more complex biological processes not studied in this work.

The sweet sorghum bagasse pretreated with steam explosion in the presence of 7.5 FPU/g of cellulase solids gives a theoretical ethanol yield of 29.4 mL/kg after fermentation (Shen et al., 2012). Wang et al. (2013) found an ethanol yield of 49.43 mL/kg from the bagasse (10% solid) of sweet sorghum pretreated with sulfuric acid (H_2SO_4) with an enzymatic load of 29 FP/g. Kim et al. (2014), in their work report the highest concentration of ethanol (96.9 g/L) in the presence of wild-type yeast strain used (*S. cerevisiae* KL17) that is able to consume both glucose and galactose. According to Yu et al. (2014), when the work is carried out under the optimal conditions of simultaneous saccharification and co-fermentation, 49.48 mL/kg of ethanol is obtained. The result of the work of Appiah-Nkansah et al. (2018) gives 20.25% (v/v) in the production of ethanol from flour and very high density sweet sorghum juice with or without supplementation of urea by simultaneous saccharification. This productivity can reach 96% (fermentative efficiency) when the dissolved solids are at least 33% (w/v). (Matsakas and Christakopoulos, 2013b).

The research results are sometimes in agreement with those of the aforementioned authors because of the different methods of formulation and fermentation of the juice, the physicochemical composition of the plant material and the yeasts used. Some authors had pre-treated their plant material before the alcoholic fermentation by steam explosion, enzymatic hydrolysis, while others had simultaneously pretreated their material with acid and enzymatic hydrolysis.

Conclusion

The results of the present study reveal on the one hand, the importance of *S. cerevisiae* strains in the alcoholic fermentation of *S. saccharatum* (L) stems. This work shows the fermentable bioethanol potential of sweet sorghum. The monitoring of the parameters made it possible to evaluate the pH, density, brix degree, limit attenuation and bioethanol production of the musts. It appears from this work that the *Angel brand super alcohol*, in concentrations (3 to 5 g/L) in the musts, gives better ethanol production. The intensive production of *S. saccharatum* could be a credible strategy in the fight against hunger, animal fodder and bioethanol production (FAOSTAT, 2015; Leclerc et al., 2014). In general, these results have made it possible to note that sweet sorghum could be promising. It could serve the people and allow attention to be paid to the intensive cultivation of sweet sorghum in order to provide solutions for human food, cattle fodder and bioethanol production.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interest.

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