First and Second Generation of Ethanol Production for Five Sweet Sorghum Cultivars during Soft Dough Grain

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Authors' contributions

This work was carried out in collaboration between all authors. The author EDD conducted the study, collected the analyzes in the field and did the statistical analysis of the data, wrote the protocol and wrote the first draft of the manuscript. The authors BRAA, JJG and JNT designed the study and monitored and supervised all of this study. The authors DCP and JELA assisted in literature searches, writing in the manuscript and discussing the data. The authors RSCM, RNAF and VCP helped in the search of the literature and in the translation of the same into English language. All authors read and approved the final manuscript.

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ABSTRACT

This study we aimed to identify how the harvest period of the stems from 5 sweet sorghum cultivars influences the production of sugar and ethanol under rainfed conditions in the municipality of Itambé, state of Pernambuco. Subsequently we evaluated the ethanol production from juice and bagasse of the different cultivars. The field experiment was evaluated in a factorial arrangement with two factors (5 cultivars and 3 harvest periods) and fours replications. The fermentation experiments, pretreatment and enzymatic hydrolysis were delineated in a completely randomized design in quadruplicate. Data obtained for all variables evaluated were submitted to an Analysis of Variance and the means compared by the Tukey test at 5% of probability. Results showed that the harvest period influenced the total soluble solids, and the harvest period of soft dough was chosen for assessments of ethanol production of first and second generation. Ethanol production from juice differed among cultivars with the best performance by cultivar SF 15. Significant differences were observed for the chemical composition of bagasses between cultivars, but there where no difference in efficiencies of enzymatic hydrolysis. The average conversion of cellulose in glucose was 64.87%. The cultivars of sweet sorghum biomass developed and adapted for the Northeastern region of Brazil showed potential for ethanol production from the juice and bagasse.

1. INTRODUCTION

In order to mitigate the environmental effects of fossil fuel use in the transportation sector, initiatives for the inclusion of biofuels in the energy matrix, especially ethanol, are increasing worldwide. About 100 billion liters of ethanol are currently produced in the world, and the major producers are the U.S. and Brazil [1]. Ethanol can be produced from different types of biomass, especially sweet crops (sugarcane and sugar beet) and starch (corn and wheat). In addition, research efforts have been focused to enable ethanol production from lignocellulosic biomass [2,3,4].

To increase ethanol production, energy crops evaluated in past decades have attracted the interest of researchers in various countries like USA [5], India [6] China [7] and Brazil [8]. Among energy crops, sweet sorghum has gained prominence for ethanol production, as it presents the possibility of full utilisation of biomass usually containing 37% juice, 8% grain, 36% bagasse and 19% leaves [9].

Sorghum main use is as fodder, but it can also be used for the production of energy, supplementing animal feed and fiber production [10]. Its main features are the efficient use of water and good development in different climate and soil conditions [11]. It is a crop widely cultivated due to its potential to produce ethanol, and several studies have shown its potential as a source of biomass with lower water requirements [12,13]. It is a grassy crop with C4 photosynthetic cycle cultivated in several countries, with high efficiency in the conversion of CO_2 into sugars via photosynthesis [14]. It is originated in Africa, being the fifth cereal most cultivated in the world.

Its productivity is highly variable and depends on growing and environmental conditions, but generally sweet sorghum yields 2.36 Mg ha^{-1} of grain and 42.15 Mg ha^{-1} of stalk [15], and the juice present in stalk, rich in sucrose, glucose and fructose, is the part of greatest interest for the production of first-generation ethanol [16]. The production of ethanol from the fermentation of sorghum juice is approximately 3451 L ha^{-1} [17], but can vary widely depending on the cultivation region and cultivar used [18].

Recently, research has been developed to increase yields of ethanol production by using other carbon sources present in sweet sorgum hydrolysates. For this, it is necessary to use yeasts that are capable of fermenting alternative carbon sources, such as xylose and cellobiose, because *Saccharomyces cerevisiae* cannot convert them to ethanol. In view of this, *Spathaspora passalidarum* and *Dekkera bruxelensis* are alternatives in the increase of ethanol production, from the assimilation and fermentation of xylose and cellobiose, respectively, which can make this process more economically feasible [19,20].

Much of the information about the use of sweet sorghum biomass for ethanol production concerns cultivars developed in countries like USA [21], China [22] and India [23]. In Brazil, despite efforts by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) and the Agronomic Institute of Pernambuco (IPA), there

are few cultivars developed for different climatic and soil conditions and lack of information about actual ethanol production for each cultivar. Previous study [24] demonstrated the potential of some sweet sorghum cultivars harvested at maturation stage in Pernambuco with theoretical values of ethanol per hactare between 681 and 3142 L from the juice fraction. In this context, the aim of this work was to assess the best harvest stage of stalks and subsequent ethanol production from stalk juice and bagasse using different cultivars with production history in Northeastern Brazil.

2. MATERIALS AND METHODS

2.1 Field Experiment

A field experiment was conducted in 2011 at the experimental station of the Agronomic Institute of Pernambuco (07 ° 24 'S and 35 ° 06' W), municipality of Itambé, Zona da Mata, northern state of Pernambuco, Brazil, to evaluate the agronomic performance of IPA 467, SF 11, BR 506, SF 15 and IPA 2502 cultivars. The area has annual average rainfall of 1200 mm and mean annual temperature of 25°C. The soil in the experimental area was characterized as clayey-sandy loam, containing 577, 102, 321 g.kg^{-1} of sand, silt and clay, respectively. Approximately 30 days from planting, 1.5 Mg ha^{-1} of lime was applied. Fertilization was also performed by applying 777 kg ha^{-1} of N (Urea), P (single superphosphate) and K (Potassium Chloride) 90-90-60 formulation. One month after planting, top-dressing fertilization was also performed by applying 60 kg N ha^{-1} in the form of urea.

Stalk harvest was carried out at three stages of plant development, boot stage (HS1), soft dough grain (HS2) and hard dough grain or maturation (HS3). Ten plants were cut close to the ground and had leaves and panicles removed in order to perform juice extraction from the clean stalk in sugarcane milling system, and each sample was passed three times in rolls. Identifying the best harvest time allowed determining ethanol production at optimum stage.

2.2 Characterization of Juices from Cultivars

After extraction and filtration, juices were characterized for the levels of total soluble solids (TSS) °Brix using portable refractometer Instruterm model RT-30ATC with scale from 0 to 32 °Brix, and total sugar concentration (TS) determined by the sum of sucrose, glucose and fructose contents by high-performance liquid chromatography (HPLC). Juices were also quantified for nitrogen levels (FAN-free amino nitrogen) using the ninhydrin method and for nutrients after digestion with sulfuric acid and hydrogen peroxide [25]. Total P (spectrophotometry) and K (flame photometry) were also determined in extracts generated.

2.3 Characterization of Bagasses from Cultivars

The characterization of bagasse samples originated from the juice extraction step was performed according to methodology of [26] to quantify NDF, ADF, carbohydrates (cellulose and hemicellulose) and lignin acid detergent. Moisture was determined using infrared analytical balance and ash by gravimetric method after calcination in muffle furnace at 600°C for 2 h.

2.4 Fermentation of Juices

Fermentations were carried out in simple batch system in Erlenmeyer flasks with total volume of 250 mL under a controlled temperature of 33°C in static condition for a period of 6 h. All assays were conducted in quadruplicate and arranged in a completely randomised design. Flasks were added of 100 ml of sterilized sorghum juice and inoculated with 10% (w/v) inoculum of *Saccharomyces cerevisiae* JP-1 yeast, previously grown in YPD medium (20 g L^{-1} glucose, 10 g L^{-1} peptone and 10 g L^{-1} yeast extract). In inoculums, cell viability and initial cell concentration were determined by counting method after staining with methylene blue in Neubauer chamber. In juice fermentation assays, aliquots were collected at time 0 h and 6 h to determine sugar consumption and fermentation products such as ethanol and glycerol. The kinetic parameters calculated at the end of fermentation were: sugar consumption (Ac), ethanol volumetric yield (Qp) and sugar conversion yield (sucrose, glucose and fructose) into ethanol (Y$_{p/s}$), according to equations 1 and 2.

$$Qp\ (g.L^{-1}h^{-1})\ = \frac{P}{T} \tag{1}$$

$$Y_{p/s} = \frac{P}{Ac} \tag{2}$$

Where: Q$_p$: volumetric yield; P: ethanol production; T: fermentation time; Y$_{p/s}$: ethanol

yield per sugar consumed; and Ac: sugar consumption.

2.5 Pretreatment and Enzymatic Hydrolysis of Sorghum Bagasses

Pretreatment was conducted in Erlenmeyer flasks with total volume of 250 mL where sorghum bagasse was added at a ratio of 4% total solids solid-liquid with hydrogen peroxide solution at concentration of 7.5% w/v in 100 mL of water. pH adjustment was carried out with 5M NaOH up to the value of 11.5. Flasks were incubated in orbital shaker table at 150 rpm and 25°C for 1 h [19].

Upon completion of the reaction, the two fractions derived from pretreatment were separated into liquid and the solid fraction by filtration and this step allowed quantifying the mass loss in the reaction (Equation 3). The solid fraction was washed with 1.5 L of distilled water in order to remove water-soluble solids. The solid fraction previously washed and dried in oven with air circulation at 45°C was submitted to enzymatic hydrolysis using commercial enzyme FibreZymeTM LDI (Table 1) at dose of 10 FPU g^{-1} of dry biomass without supplementation with β-glycosidases. The hydrolysis conditions were: total load of solids of 20 gL^{-1}, pH 4.8, stirring at 100 rpm, 50°C and time of 48 h. After 48 hours, aliquots were collected for the determination of glucose released using glucose oxidase enzyme KIT and total reducing sugars [27]. The cellulase activity was quantified in filter paper units according to methodology proposed by [28]. The β-glucosidase activity was determined based on cellobiose solution and expressed in units per mL according to [29]. The xylanase, CMCase and avicelase activities were also quantified. The enzymatic hydrolysis efficiency was expressed according to Equation 4 [30].

Table 1. Enzymatic activity of the commercial preparation FibreZymeTM LDI

Enzyme	Activity
FPase (Filtre Paper hydrolase)	11.5 FPU/mL
Xylanase	789 U/mL
B-glucosidase	10 CBU/mL
Avicelase	5.9 U/mL
CMCase (CarboxyMethyl Hydrolase)	175 U/mL

$$PM\,(\%) = \frac{Mi - Mf}{Mi} \, x\, 100 \tag{3}$$

$$EH\,(\%) = \frac{Cl}{M\,x\,W\,x\,1{,}11} x\, 100 \tag{4}$$

Where PM: weight loss; Mi: initial bagasse mass; Mf: final bagasse mass.

Where EH: enzymatic hydrolysis efficiency; C1: glucose concentration in the hydrolysate; M: mass of dry bagasse; W: cellulose content in pretreated bagasse; and 1.11 conversion factor of cellulose into glucose.

2.6 Fermentation of Hydrolysates

After enzymatic hydrolysis of the different types of bagasse, hydrolysates were submitted to fermentation with *Dekkera bruxellensis* GDB 248. Fermentation assays were performed in batch in 125 mL Erlenmeyer flasks added of 100 ml of hydrolysate with 2% w/v of *D. bruxellensis* cells previously grown in YPD for 24 h. The flasks were incubated at 32°C in static condition and 1 ml samples were collected at baseline and after 7 h. After centrifugation, the supernatant was filtered at 0.22 µm and used for analysis of fermentation metabolites by HPLC under the conditions described above. The kinetic parameters calculated at the end of fermentation were: ethanol volumetric yield (Qp) and sugar conversion yield (glucose and cellobiose) into ethanol, according to Equations 1 and 2.

2.7 Analytical Analyses

Carbohydrates and ethanol concentrations were measured by High Performance Liquid Chromatography (HPLC), using column Aminex HPX 87H+ (300 x 7.8 mm, Bio-Rad), with refractive index detector (RID), at 50°C and mobile phase 5 mM H_2SO_4, at flow rate of 0.6 mL/min.

2.8 Statistical Analyses

Field experiment was evaluated in factorial design with three harvest stages and five cultivars. The fermentation of juices extracted at the soft dough grain harvest stage was conducted in a completely randomized design. Pretreatment, enzymatic hydrolysis and fermentation of hydrolysates were also conducted in a completely randomized design. All determinations were performed in quadruplicate and the results for °Brix, N-FAN, P, K levels, pH, EC, initial sugars, mass loss, glucose content, total reducing sugars, efficiency of conversion of cellulose into glucose and

kinetic parameters of fermentation and ethanol concentration, ethanol volumetric yield and efficiency of conversion of sugars into ethanol were submitted to analysis of variance (ANOVA) and means were compared by the Tukey test at significance level of p ≤ 0.05 using the ASSISTAT software.

3. RESULTS AND DISCUSSION

3.1 Field Experiment

A field experiment was initially conducted to evaluate the best harvest stage for the different sweet sorghum cultivars under study. For this, variable °Brix was determined during three stalk harvest stages: booting, soft grain and maturation or hard grain. No significant interaction between harvest stage and cultivars was observed. The °Brix levels during soft grain and hard grain harvest stage were similar and significantly higher than in the booting harvest stage (Fig. 1). Therefore, earlier harvest in soft grain phase was chosen as the best to evaluate first- and second-generation ethanol production.

The choice of variable °Brix is related to the direct correlation between levels of total soluble solids (°Brix) and levels of total sugars in the sweet sorghum juice [31,24]. Cultivars reached the soft grain stage on different days: IPA 467 in

140 days, SF 11 in 130 days, BR 506 in 92 days, SF 15 in 140 days and IPA 2502 in 92 days.

3.2 Initial Characterization and Juice Fermentation

The initial characterization of juices from different sweet sorghum cultivars is shown in Table 2. All variables that characterized juices showed significant differences among sorghum cultivars evaluated. The levels of ° Brix ranged from 8.95 to 16.10 and the levels of total sugars (sucrose, glucose and fructose) ranged from 67.6 to 128.8 g L^{-1}. Generally, the levels of °Brix and total sugars are correlated, which allows the use of the determination of soluble solids in juices as important tool for sugar and alcohol facilities [32], [31,9]. Literature reports levels of °Brix in juice for stalk harvest from 15.5 to 16.5 as appropriate [17].

In addition to the levels of ° Brix and total sugars, nutrient composition, pH and electrical conductivity (EC) are important in the characterization of juices. The analysis of these variables allows inferring on the need for addition or dilution or even correction of juices to be fermented, because microorganisms need nutrients to convert soluble sugars into ethanol [33,34]. The levels of N-FAN nitrogen (free amino nitrogen) ranged from 193.4 to
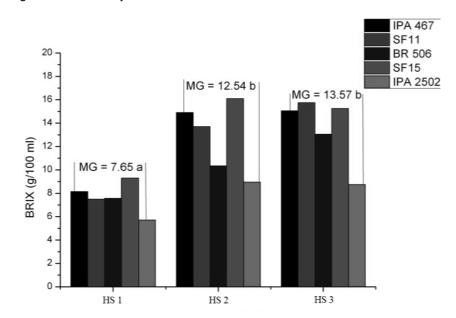

Fig. 1. Brix contents in sweet sorghum evaluated in the field experiment. Values for n = 4, MG: General average for data collection, 5% probability, CV (%) = 17.20. HS1: Harvest boot stage, HS2: Harvest soft grain stage, and HS3: Harvest hard dough grain stage

Table 2. Preliminary characterization of juices from distinct sweet sorghum cultivars obtained from a field experiment in Itambé, PE, Brazil

Cultivares	°Brix (g/100mL)	TS (g/L)	N-FAN (mg/L)	P (mg/L)	K (mg/L)	pH	EC(mS/cm)
IPA 467	14.65±3.56 ab	107.40 ± 36.64 ab	193.40 ± 65.03 b	74.90 ± 11.81 b	5403.29 ± 881.22 a	5.23 ± 0.06 bc	6.03 cd
SF 11	13.95± 1.18 ab	99.17 ± 10.31 ab	294.58 ± 19.31 ab	78.34 ± 19.22 b	4893.17 ± 1004.34 a	5.10 ± 0.05 c	7.24 ± 1.13 bc
BR 506	10.35 ± 1.87 bc	82.20 ± 23.26 ab	670.05 ± 121.05 a	161 ± 41.65 a	6205.21 ± 495.68 a	5.20 ± 0.04 bc	9.33 ± 0.49 a
SF 15	16.10 ± 1.14 a	128.80 ± 13.17 a	233.25 ± 18.31 ab	72.93 ± 12.38 b	2768.98 ± 1002.49 b	5.26 ± 0.03 b	5.19 ± 1.45 d
IPA 2502	8.95 ± 1.65 c	67.65 ± 17.29 b	655.24 ± 193.84 ab	127.05 ± 20.40 ab	5996.81 ± 669.61 a	5.42 ± 0.12 a	8.72 ± 0.75 ab

values for n=4. TS: Total sugar; N-FAN: Free amino Nitrogen; P: Phosphorus; K: Potassium; EC: Eletrical conductivity. Means with the same letter in the column are not significantly different (Tukey, P > 0.05)

Table 3. Kinetic parameters in the static fermentation of the juices from different cultivars of sweet sorghum at 33 °C, 6h using industrial yeast JP1

Cultivares	SC (%)	P (g/L)	Q_p(g.L.h)	$Y_{p/s}$	Vi (%)	Vf (%)	Ci (10^8)	Cf (10^8)
IPA 467	92.1b	42.37 abc	7.06 abc	0.45 a	97.03	95.54	4.46	4.63
SF 11	96.71ab	47.27 ab	7.88 ab	0.49 a	96.76	91.73	4.38	4.3
BR 506	98.11 ab	35.27 bc	5.88 bc	0.44 a	96.95	96.00	4.52	4.64
SF 15	95.64 ab	56.38 a	9.40 a	0.46 a	98.36	92.80	4.66	4.66
IPA 2502	99.42 a	28.31 c	4.71 c	0.42 a	95.86	98.64	4.9	4.82

Values for n=4. SC: Sugar consumed; P: ethanol; Q_p: Volumetric productivity; $Y_{p/s}$: Yield; Vi: Initial viability; Vf: Final viability; Ci: Initial concentration of cells; Cf: Final concentration of cells. Means with the same letter in the column are not significantly different (Tukey, P > 0.05)

670.05 mg l^{-1}. The phosphorus contents ranged from 72.93 to 161 mg l^{-1} and potassium levels from 2768.98 to 6205.21 mg l^{-1}. [35] observed similar concentrations of potassium, but different concentrations of nitrogen and phosphorus for four cultivars of sweet sorghum cultivated in Thailand. Differences in nutrient content in the juice can be explained by the harvest stage of the stems, where decreases levels of nutrients were observed since soft dough at maturation of the grain [36].

The pH values for all cultivars were similar and with overall average of 5.24, which is within optimal values for fermentation with yeasts of the genus *Saccharomyces* [37]. Significant differences were observed for electrical conductivity (EC), which is correlated with the salinity of juices and can interfere with the yeast metabolism and affect the fermentation yield. However, there are still few studies that relate EC with fermentation interferences.

The variability in the levels of °Brix, total sugars, nutrients and variables such as pH and electrical conductivity in juices from different sweet sorghum cultivars is associated with the genetic capacity of each cultivar and also with environmental conditions such as soil type, rainfall, incidence of solar radiation [38]. The analysis and characterization of these variables in cultivars with potential for ethanol production is critical to the viability of sweet sorghum cultivation.

The results of the kinetic parameters after 6 h of static fermentation with *Saccharomyces cerevisiae* of juices from different sweet sorghum cultivars are shown in Table 3.

The ability of yeast to consume sugars from juices, ethanol production, ethanol volumetric yield, fermentation yield, cell viability and cell concentration were used as parameters to evaluate cultivars. Significant differences were observed in the ability of yeasts to consume sugars present in the juice from different cultivars, from 92.71 to 99.42% of sugar consumption (Table 3). These differences are due to the quality of juices and are related to the concentration of nutrients and other elements such as vitamins. The ethanol concentration in the fermentation juice ranged from 28.31 to 56.38 gL^{-1}. [39] observed similar results when evaluating the production of ethanol from sweet and forage sorghum cultivars in a region of low

rainfall in Mexico, with ethanol content from 35.78 to 56.36 g L^{-1}.

Similar to the ethanol concentration, the volumetric yield showed differences between the cultivars (Table 3). The cultivar SF 15 was highlighted due to Qp. This parameter is important in determining the amount of ethanol per unit of time, which in the sugar and ethanol industry is between 6 to 12 hours for initial sugar concentrations of 120 gL^{-1}.

There were no differences for variable fermentation yield, which expresses the transformation efficiency of sugars consumed into products of interest such as ethanol (Table 3). Yp/s ranged from 0.42 to 0.49, which represents 82.35% to 96.07% conversion of sugars into ethanol from the maximum conversion coefficient of 0.51.

No growth of yeasts was observed during fermentation (Table 3). This is explained by the high initial inoculum value used of 10% m / v, representing an average of 4.58 x 10^8 cells.mL^{-1}. A decrease in cell viability was observed, which is the product of the concentration of products that inhibit the activity of yeasts.

3.3 Pretreatment, Enzymatic Hydrolysis and Fermentation of Hydrolysates

The chemical composition for the contents of cellulose, hemicellulose, and acid detergent lignin of the different types of fresh sweet sorghum bagasse and after pretreatment step are shown in Table 4.

The initial composition showed significant differences among cultivars. The highest cellulose levels were observed for IPA 2502 and IPA 467 cultivars. Cellulose concentrations ranged from 38.64 to 42.50%. After pretreatment, there was an increase in the cellulose levels for all bagasses from 58.11% to 91.42%. The hemicellulose content ranged from 27.99 to 33.34%, and after pre-treatment with H_2O_2, values decreased by 43.13%, 46.41%, 42.15%, 44.64% and 48.13% for the IPA 467, SF 11, BR 506, 15 SF and IPA 2502 cultivars, respectively compared to fresh bagasse, indicating solubilization of hemicellulose for the liquid fraction of the reaction. The acid detergent lignin (ADL) levels ranged from 2.63 to 4.02% and after reaction with H_2O_2, the values decreased, indicating delignification of bagasses. Reductions of 78.55%, 62.68%, 86.09%, 64.16% and 71.11% were observed for 467 IPA, SF 11, BR

506, SF 15 and IPA 2502 cultivars, respectively, compared to fresh bagasse.

Delignification processes using pretreatment with alkaline hydrogen peroxide and sodium hydroxide are quite variable in relation to the type of biomass being evaluated and pretreatment conditions. For sugarcane bagasse, for lignocellulosic biomass with higher lignin content to sweet sorghum, removal values of about 70% are reported [40,41] under conditions similar to those used in this study. For cotton biomass, reductions between 6.22% and 32% were observed using H_2O_2 at different temperatures and pressure [41]. Optimal lignin removal values of 65.66% were observed for rapeseed straw biomass with 5% H_2O_2 in alkaline medium in 1 h reaction at 50°C [42]. For sweet sorghum biomass [30] reported value of 78.84% delignification using 5% H_2O_2 for 24 h.

Pretreatment of lignocellulosic biomass is aimed to disrupt the organic matrix to allow the hydrolysis step of carbohydrates. For all types of bagasse evaluated in this study, pretreatment with H_2O_2 in alkaline medium resulted in an average mass loss of 34.55%, 38.15%, 32.03%, 41.5% and 36.22% for IPA 467, SF 15, BR 506, SF 15 and API 2502 cultivars, respectively. Mass loss is related to the solubilization of lignin, extractives, ash, and small fractions of carbohydrates into the liquid phase of the reaction.

Pretreatment with H_2O_2 is a process for delignification of lignocellulosic biomass [43] that allows achieving greater efficiency in the recovery of sugars in the enzymatic hydrolysis step [42], since the presence of lignin impairs the access of enzymes to the substrate. The efficiency of this type of pretreatment is dependent on the biomass being evaluated and the results indicate no significant difference in relation to the glucose production and significant difference to reducing sugars (Table 5) for the different types of sweet sorghum bagasses evaluated.

Table 4. Chemical composition of bagasse before and after alkaline H_2O_2 for differences sweet sorghum cultivars

Before H_2O_2 pretreatment			
Cultivares	Celulose (%)	Hemicelulose (%)	LDA (%)
IPA 467	42.46 ± 0.42 a	27.99 ± 0.46 b	4,01 ± 0.19 a
SF 11	38.64 ± 1.03 c	33.34 ± 1.13 a	4,02 ± 0.4 a
BR 506	40.7 ± 0.69 b	29.52 ± 0.71 b	2,66 ± 0.65 b
SF 15	38.73 ± 0.40 c	33.09 ± 0.96 a	3,07 ± 0.58 ab
IPA 2502	42.50 ± 1.03 a	33.33 ± 1.80 a	2,63 ± 0.4 b
After H_2O_2 pretreatment			
IPA 467	67.13 ± 0.48	15.92 ± 0.12	0.86 ± 0.15
SF 11	72.81 ± 2.23	17.87 ± 0.90	1.5 ± 0.3
BR 506	73.12 ± 3.02	17.08 ± 1.56	0.37 ± 0.1
SF 15	74.12 ± 1.83	18.36 ± 0.79	1.1 ± 0.2
IPA 2502	75.15 ± 1.54	17.29 ± 1.62	0.76 ± 0.4

Values for n=4 ± standard deviation. TS: Total sugar, LDA: Lignin detergent acid. Means with the same letter in the column are not significantly different (Tukey, P > 0.05)

Table 5. Efficiencies of enzymatic hydrolysis for different types of sweet sorghum bagasse, 2% substrate, 10 FPU/g dry bagasse, 50°C, pH = 4.8, 48h, before pretreatment with H_2O_2

Biomass	TS (g/L)	Glucose (g/L)	EH(%)
IPA 467	14.99 ± 0.83 ab	8.79 ± 0.66 a	61.44 ± 4.52 a
SF 11	13.60 ± 1.08 b	10.06 ± 2.09 a	66.11 ± 13.41 a
BR 506	15.17 ± 0.82 ab	9.31 ± 0.31 a	59.80 ± 2.60 a
SF 15	15.21 ± 1.22 ab	10.03 ± 0.63 a	64.69 ± 4.26 a
IPA 2502	16.04 ± 0.50 a	11.27 ± 2.41 a	72.30 ± 16.01 a

Values for n=4 ± standard deviation. Means with the same letter in the column are not significantly different (Tukey, P > 0.05). TS: Total sugar, EH: Efficiency conversion of cellulose hydrolysis in glucose

The efficiency of conversion of cellulose into glucose (EH) after 48 h was not affected by the type of sweet sorghum bagasse evaluated (Table 5). Values of 61.44%, 66.11%, 59.80%, 64.69% and 72.30% were observed for 467 IPA, SF 11, BR 506, SF 15 and IPA 2502 cultivars, respectively. EH depends on the chemical composition of the lignocellulosic substrate, type of pretreatment used, load of solids used in the hydrolysis, dose and type of enzyme, hydrolysis time and methodology used to calculate efficiency. For conditions similar to those used in this study, [29] evaluated the enzymatic hydrolysis of sweet sorghum bagasse pretreated with H_2O_2 and observed average EH of 62.46%.

In the comparison with other types of pretreatment used to treat sweet sorghum biomass for ethanol production, efficiency of conversion of cellulose into glucose similar to that shown in this study was observed. [44] used steam explosion to pretreat sorghum biomass and observed efficiencies between 50% and 90% in different process times (5 to 10 min) and temperatures (180 to 200°C) using enzymes Celuloclast 1.5 at dose of 20 FPU.gsubstrate^{-1} and Novozyme 188 at dose of 20 IU.gsubstrate^{-1}.

In another study, [4] evaluated four types of pretreatment for sweet sorghum biomass, like ionic liquids, steam explosion, dilute acid and lime. The enzymatic hydrolysis conditions were 10% of substrate, enzyme dose of 20 FPU.g substrate and time of 72 h. The maximum efficiency of conversion of cellulose into glucose was obtained for pretreatment with steam explosion with 72%, followed by dilute acid with 50%, ionic liquids and lime with 40%.

Since no significant difference was observed for enzymatic hydrolysis efficiencies for the different sorghum cultivars evaluated, we chose to ferment hydrolysate from SF15 cultivar, since this cultivar stood out in the juice fermentation analysis. After 7 h of fermentation with *D. bruxellensis*, ethanol production was 3.9 gL^{-1}, with volumetric yield of 0.56 gl^{-1}.h^{-1} and conversion of glucose into ethanol of 0.40. No significant changes in cell viability and growth of *D. bruxellensis* biomass were observed.

Fermentation of biomass hydrolysates usually leads to low ethanol concentrations in the medium, since the initial contents of pretreated biomass submitted to hydrolysis are low, between 2% and 10%, even maximising the enzymatic hydrolysis efficiency, reaching up to 90%. [37] fermented hydrolysates of sugarcane bagasse pre-treated with alkaline H_2O_2 under conditions similar to those of this study using *Saccharomyces cerevisiae* and obtained 2.5 g L^{-1} ethanol starting from hydrolysate with 6.5 g L^{-1} glucose and yield 0.38 g g^{-1}. In another study, [45] hydrolysates of *Leptochloa fusca* L. Kunth or Kallar grass was utilised as a substrate for ethanol production in simultaneous saccharification and fermentation process with *Kluyveromyces marxianus* and at optimum factor setting, the substrate conversion efficiency was 82%.

4. CONCLUSIONS

The results obtained demonstrated that the best stage for the harvest of sweet sorghum stalks to anticipate harvest is the phase in the soft grain stage, which can be extended until the physiological maturation of grains. Ethanol production from juice was influenced by cultivar, and SF 15 cultivar seems to be the most promising, which corroborates previous studies of our research group. Hydrogen peroxide is effective for the pretreatment of all types of bagasses from sorghum cultivars evaluated and allowed reasonable efficiencies in the enzymatic hydrolysis step of biomass. In addition, *Dekkera bruxellensis* showed potential to ferment biomass hydrolysates.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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