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Evaluation of potential ethanol production and nutrients for four varieties of sweet sorghum during maturation

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ABSTRACT

Sweet sorghum was investigated to an alternate feedstock for fuel ethanol production. juices from 4 sorghum varieties (BRS 506, BRS 508, BRS 509, BRS 511 and BRS); all developed by Embrapa (Brazilian Agricultural Research Corporation) Maize and Sorghum) were evaluated for sugar, starch and nutrient contents and theoretical ethanol yields. The levels of nitrogen, phosphorus, calcium, magnesium, starch and sugars (glucose, fructose and sucrose) were measured weekly over a period of 70 days. Fermentations were performed using yeast *Saccharomyces cerevisiae*. BRS 508, BRS 509 and BRS 511 showed potential to be useful for industrial applications for maturities exceeding 30 days. BRS 511 showed the highest sugar production, with levels higher than 140 g/L during the majority of the experiment and reaching a maximum of 191 g/L. All varieties showed similar behaviors with respect to nutrient content, which was characterized by a decrease in nutrient concentrations over the period analyzed. Juice from BRS 508 was successfully fermented within 8 h with a productivity (9.0 g/L h) and yield (90.5% of theoretical) similar to those observed for sugar cane juice.

1. Introduction

The worldwide demand for renewable fuels has expanded rapidly in recent years as a strategy to reduce greenhouse gas emissions [1]. Moreover, uncertainty about the future availability of non-renewable resources and geopolitical tensions in oil-producing regions have increased worldwide interest in biofuels because they are the most viable substitutes for oil on a large scale [2]. Biofuels have a promising future as the global demand for this type of energy grows due to its sustainability, and they present an opportunity to improve the agricultural economy [3,4].

Ethanol can be produced from various sources of biomass such as starch crops (maize and cereal grains), beets (sugar cane, beets, and sweet sorghum saccharide) and cellulosic material (wood and crop residues) [5]. In Brazil, ethanol production is primarily from the fermentation syrup of cane sugar. However, the production of ethanol from sugar cane has limitations, especially in areas of low rainfall, which highlights the need for the addition of other raw materials for ethanol production [6]. Among the various renewable raw materials available for ethanol production, special attention has been given to sorghum because the juice extracted from sorghum stems consists sucrose, glucose, and fructose and can therefore be easily fermented to ethanol [7].

Sorghum (*Sorghum bicolor* (L.) Moench) is a tropical grass that is part of the monocot family Poaceae; sorghum is grown in different regions of the world and has a high efficient photosynthetic. The plant originated in Africa and is the fifth-most cultivated cereal in the world. Its main features lie in its efficient use of water (1/3 that of cane sugar and 1/2 that of corn) and robust adaptation to various types of climates and soils [8,9]. Several studies have shown that the varieties with succulent modify font of sugars are promising to supplement the harvest of sugar cane as feedstock for ethanol production [10,11].

Sorghum is a highly promising alternative bioenergy crop from an agronomic and industrial viewpoint, in accordance to the objectives proposed in the National Plan on Agroenergy (2006–2011).

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Sweet sorghum should be suitable for production in Brazil as well as other countries including the United States, China, and India. Sweet sorghum has the following advantages: fast lifecycle (ranging from 90 to 120 days), fully mechanized crop planting and harvesting (planting seeds at 5–7 kg/ha and mechanical harvesting), succulent stems with directly fermentable sugars (40–60 ton/ha), ability to use bagasse as a source of energy (important for industrialization), electricity cogeneration, second-generation ethanol or fodder for animals, favorable energy balance, drought tolerance and ability to grow in infertile conditions [1,2,12–15].

Industrially, sweet sorghum can be treated in a similar fashion as sugar cane, including the use of commercial yeast for fermentation. The sorghum grains, which are rich in starch and lignocelluloses residues, and byproducts of distillation can also be used in animal feed and power generation [16,17].

There are many cultivars of sorghum distributed worldwide that provide a diverse genetic base for developing regional-specific and highly productive plants. Despite these advantages, there is a limited amount of experience in the industrial production of ethanol from sweet sorghum [2]. In the literature, there are few reports concerning the composition of nutrients in the sorghum syrup during the maturation time of the plant.

In the literature there are few data on the composition of nutrients in the juice of sorghum during the time of maturity of the plant. Aiming to address this shortcoming, this paper aims to present the monitoring of the concentration of the major nutrients (N, Ca, P, Mg), starch and sugars (glucose, fructose and sucrose) during the maturation period of four varieties of sweet sorghum. Moreover, in this paper are presented the results of ethanol production, total sugar concentration and cell growth during the alcoholic fermentation. The results obtained in the alcoholic fermentation of sorghum are compared with the results of alcoholic fermentation of sugar cane, in the same process conditions.

2. Materials and methods

2.1. Raw material

As raw materials for the present work were grown varieties of sweet sorghum BR 508, BR 509 and BR 511, from seeds provided by the National Research Center for Maize and Sorghum, Embrapa Maize and Sorghum, located in Sete Lagoas – Brazil. These varieties of sorghum were grown in soil classified as Oxisol sandy texture in an area owned by the Federal Institute of Triangulo Mineiro, in Ituiutaba – MG, Brazil. These sorghum varieties were cultivated on January 2012. At planting, were grown plots constituted by 20 rows of 5 m, with spacing of 0.70 m for each variety with two replications, saving after roughing up 10 plants per meter of ridge.

A correction of soil was carried before planting according to the method of neutralization of Al^{3+} and of elevation of Ca^{2+} and Mg^{2+} levels, with dolomitic limestone to raise the pH to a value close to 5.0. As fertilization of planting were applied 300 kg ha^{-1} of formulated 04-14-08 and 200 kg ha^{-1} of urea. Other cultural practices were similar to those used in the culture to the region. At 20 days after emergence was made cover fertilization with 200 kg ha^{-1} urea.

The first harvest of sorghum for evaluation of sugars and nutrients occurred 77 days after planting and the end of the harvest occurred at 148 days after planting. This experiment took place during the period of rain, which was from January to March 2012.

The syrup for analysis was obtained, the sweet sorghum stalks of each variety, without leaves and without panicles were subjected to an extraction process using a simple cane mill system. This cane mill extracts the syrup using pressure. The syrup from this extraction was used in analyzes of chemical characterization and fermentation for each variety.

2.2. Construction of the maturation curve

Maturation curves were constructed to evaluate the sequence of physicochemical changes that occurred during the days of maturation of different varieties of sorghum as well as to monitor the formation of sugars in the stalks of sweet sorghum to find the optimal harvest season.

Samples were collected weekly from 4 varieties of sweet sorghum for 3 months during the maturation period to monitor sugar and nutrient composition. Stems were harvested manually after cutting the leaves and stem panicles to make the juice extraction system for simple milling of sugar cane. The syrup was applied to the entire stem of the sorghum (bottom, middle, and upper) and then frozen at −4 °C.

2.3. Chemical characterization of juice

Sugar (sucrose, glucose and fructose) was quantified from previously filtered samples by High Performance Liquid Chromatography – HPLC (Shimadzu Model LC-20A Prominence) equipped with a Supelcogel Ca column using a mobile phase of deionized water flowing at a rate of 0.5 ml/min and a temperature of 80 °C. The injection volume was 20 uL. All the juice characterization tests were conducted in triplicate. Analyses of nitrogen, phosphorus, calcium and magnesium in the juice of sweet sorghum were performed according to the methodology proposed by Malavolta [18]. The concentration of starch in the syrup of sweet sorghum was determined according to the procedure described by Miller [19].

2.4. Fermentation assays

The syrup obtained after extraction process was filtered to remove impurities and added to the fermentor. Fermentations were performed at 35 °C and the initial pH of the fermentation juice was adjusted to 4.8. The fermentation was monitored for a period of 8 h and all assays were conducted in triplicate. The total volume of the fermenter was 2 L and was filled with a working volume of 1.50 L. The cell concentration used in fermentations was 30 g/L. The yeast strain *Saccharomyces cerevisiae* Y940 was used for fermentations and was provided courtesy of Mauri (Mauri Ind. Com Ltd. Brazil). The yeasts were hydrated in sweet sorghum juice for 10 min prior to inoculation.

Fermentations were monitored over time for concentrations of sucrose, glucose, fructose, glycerol and ethanol was determined using HPLC. Briefly, aliquots of fermentation syrup were centrifuged at 8000 rpm for 10 min and the supernatants were collected and injected into the chromatograph. The viable counts were performed using the Neubauer chamber and methylene blue dye.

Kinetic parameters were calculated for the fermentation yield in grams of ethanol per grams of total reducing sugars ($Y_{P/ART}$) after 8 h by Equation (1), and productivity was calculated as grams of ethanol per grams of total reducing sugars after 8 h of fermentation according to Equation (2).

$$Y_{P/ART} = \frac{EC_F}{(TS_I - TS_F) \times 0.511} \times 100 \quad (1)$$

$$P_{ethanol} = \frac{EC_F}{t} \quad (2)$$

where:

$Y_{P/ART}$ = ethanol yield produced in relation to total consumed sugar (%);
EC_F = final ethanol concentration (g/L);

TS_F = total residual sugar concentration (g/L);
TS_I = total initial sugar concentration (g/L);
$P_{ethanol}$ = productivity of ethanol;
t = fermentation time (h).

3. Results and discussion

The economical and sustainable production of ethanol from sweet sorghum requires minimum a level of sugar and total sugar content in the juice (AT). An AT of 140 g/L is desirable because to yeast can completely convert this concentration of sugar into ethanol within 8 h. AT concentrations lower than 140 g/L can result in lower efficiencies compared to sugar cane and increased production costs for ethanol production.

Fig. 1 shows the profile of accumulation of total sugar (AT), which is the sum of sucrose, glucose and fructose, during the maturation period. This figure shows the sequence of physicochemical changes that occurred during maturation of different varieties of sorghum as well as the formation of sugars in the stalks of sweet sorghum, which can be used to better characterize the optimal harvest season for the time studied.

From this maturation curve, it is also possible to assess the industrial use period (PUI), which comprises the cultivating period for which AT is at or above 140 g/L. Industrially, it is recommended that PUI be at least 30 days to enable good planning and processing of raw material.

It can be observed from Fig. 1 that BRS 506 had a PUI less than 30 days. BRS 511, which had the highest AT levels that peaked at 191 g/L, had a PUI of 44 days.

BRS 509 had AT levels that remained above 140 g/L for 42 days (93–135 days) with a maximum of 165 g/L. BRS 508 maintained an AT concentration greater than 140 g/L for 42 days (102–144 days) and reached a maximum value of 162 g/L. BRS 506 had the lowest maximum AT levels (max 154 g/L) and only sustained AT levels greater than 140 g/L for 28 days (105–133 days). Although BRS 511 had the highest peak AT value, its maximum production dropped faster than the other varieties. As shown, BRS 508, BRS 509 and BRS 511 had longer durations of elevated AT (i.e., longer PUI) that would make them amenable to industrial production.

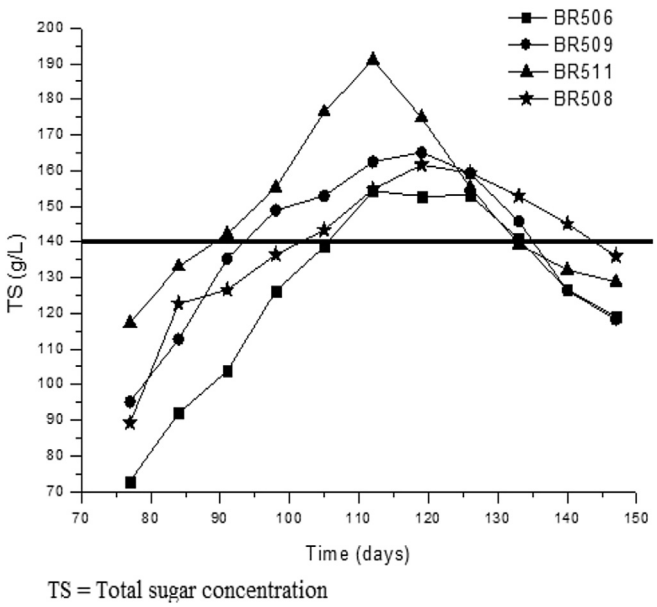

Fig. 1. Profile of accumulation of total sugar (AT) during the maturation period.

The ratio of sugar sucrose, glucose and fructose, have shown that the species 511 and 508 presented highest sucrose concentrations (over 70%) and smallest amounts of fructose (less than 12%). After 120 days of planting, sucrose, glucose and fructose concentrations decreased for all species studied. However, the percentage of sucrose increased with respect to glucose and fructose percentage, showing that these sugars are preferably consumed by the plant.

According to Prasad et al. [17], the type of sorghum and environmental conditions are the main factors that influence the optimal maturation time. Therefore, it is necessary to continuously monitor the maturation of each type of plant in the desired environmental cultivation conditions. Similarly, Almodares [20] indicated that the concentrations of non-structural carbohydrates of sweet sorghum are also affected by temperature, time of day, spacing and fertilization.

Accordingly, the maximum amounts of sugars found in the 4 varieties of sorghum analyzed in this study were higher than the amounts reported by Almodares [20] and other authors who reported values ranging from 85 to 120 g/L for the strains Río, M81E, Della, Tato and Thor that were cultivated in China [21,22]. Guigou [2] and Zhao [23] observed maximum sugar values very close to the maximum AT varieties analyzed in this work. According to Guigou [2], the Topper variety (grown in Uruguay) presented, at average, 190 g/L of AT.

In all the four varieties tested, the main sugar component was sucrose, followed by glucose and fructose. The same trend in concentrations of sugars was observed by Guigou [2], Prasad et al. [17], Gnansounou et al. [21], Zhao [23], Wang et al. [24] and Krishnaveni et al. [25]. These results differ from those reported by Gomez [26], who found that glucose was the most abundant sugar.

The levels of phosphorus, calcium, magnesium and nitrogen in the sorghum juice during maturation can be viewed in Figs. 2–5, respectively. For all varieties analyzed in this study, the levels of all nutrients decreased over the period of study. The change in composition can be understood by understanding changes in the physiology of sweet sorghum as it matures.

From panicle initiation until the beginning of flowering is a phase of rapid development that is characterized by an accumulation of dry matter and nutrients. It is also the stage during which floral differentiation occurs (between 30 and 40 days); in this case, the plant stops producing vegetative parts, stems and leaves and

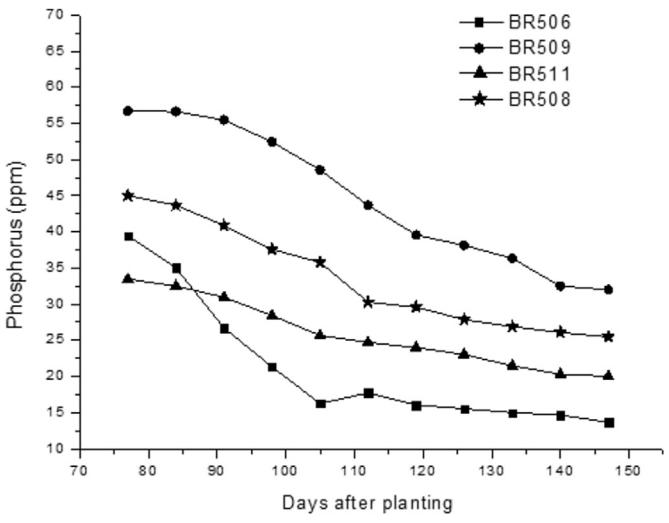

Fig. 2. Concentrations of phosphorus during the maturation period of sweet sorghum varieties BRS 506 (■), BRS 508 (★), BRS 509 (●) and BRS 511 (▲).

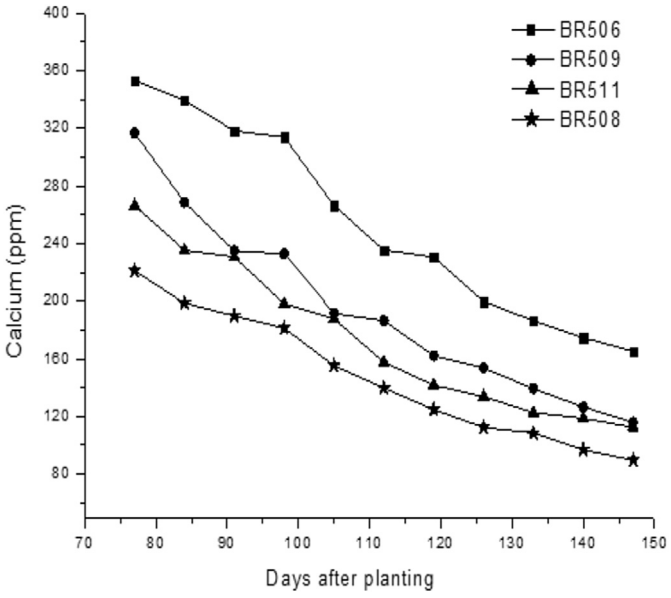

Fig. 3. Concentrations of calcium during the maturation period of sweet sorghum varieties BRS 506 (■), BRS 508 (★), BRS 509 (●) and BRS 511 (▲).

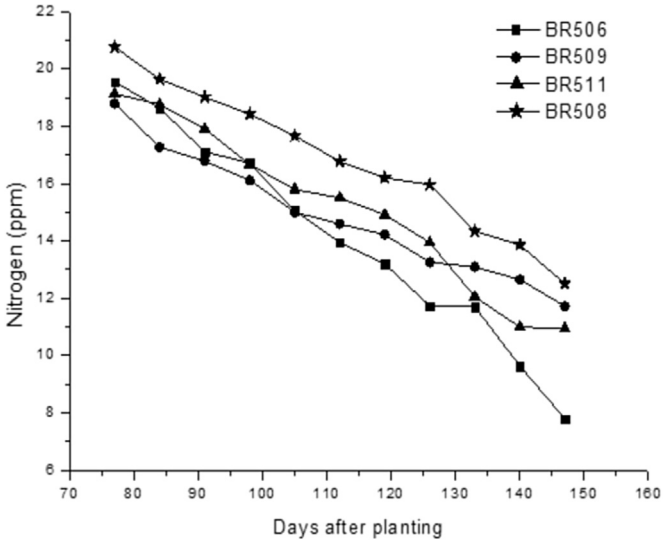

Fig. 5. Concentrations of nitrogen during the maturation period of sweet sorghum varieties BRS 506 (■), BRS 508 (★), BRS 509 (●) and BRS 511 (▲).

instead starts the formation of the reproductive part, the panicle. The stem rapidly elongates in a process we call booting, which takes approximately 50–55 days to complete. The panicle emerges at the end of this rapid growth period, and flowering occurs between 60 and 80 days after plant emergence for most cultivars [27].

From flowering to physiological maturity, nutrients rapidly translocate from stems to panicles. This process can be clearly visualized in Figs. 2–5, where nutrients significantly decline 85 days after planting [27].

With respect to phosphorus found in the juice of sweet sorghum varieties analyzed, cultivar BR 509 showed the highest concentration of phosphorus. All varieties showed similar trends in phosphorus concentration which decreased with growth. At the end of the growth phase and early maturation phosphorus levels found were on average 57.23 ppm. In the late stage of maturation the phosphorus content was 36.12 ppm. The variety BR 506 showed the lowest levels of phosphorus throughout the period and also lower concentration of sugar and PUI.

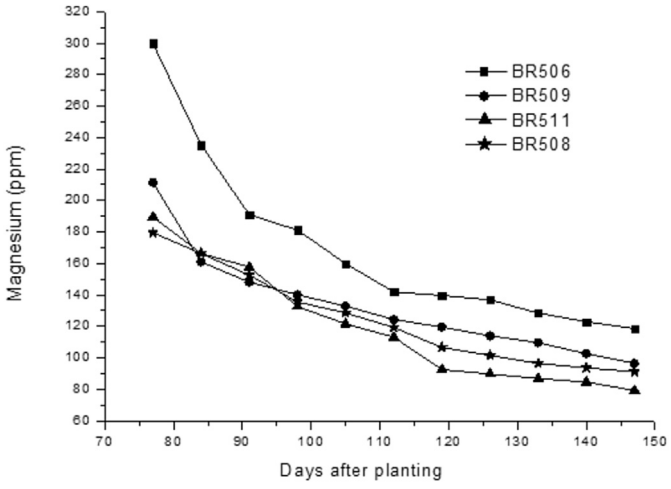

Fig. 4. Concentrations of magnesium during the maturation period of sweet sorghum varieties for BRS 506 (■), BRS 508 (★), BRS 509 (●) and BRS 511 (▲).

The levels of phosphorus in the fermentation juice of sweet sorghum are very important; according to Amorim [28], phosphorus is absorbed by yeast during fermentation and affects energy transfer in the yeast cell. This element is considered essential for the absorption of carbohydrates and their subsequent conversion into ethanol. To achieve high yield transformation of total reducing sugars into alcohol, the wort intended for fermentation should have between 50 and 100 ppm phosphorus.

Han et al. [29] observed that the accumulation of phosphorus in the juice of sweet sorghum decreases during maturation. This same behavior was observed for the 5 different varieties of sorghum analyzed in this work.

Figs. 3 and 4 show the calcium and magnesium concentrations for the different varieties of sorghum during their maturation periods.

Calcium and magnesium were highest in the cultivar BR 506. At the end of their growth period, the calcium contents averaged 359 ppm; however, by the late stage of maturation, the calcium content ad decreased to 165 ppm. Other varieties showed similar trends 110 days after planting.

Magnesium was the highest at 300 ppm in BR 506 in the early stage of maturation; however, by later stages of maturation, magnesium had dropped to 118 ppm. Other varieties had similar levels of magnesium, as shown in Fig. 4. It is noteworthy that the 506 variety showed higher concentrations of calcium and magnesium and lower concentrations of phosphorus and a lower AT and PUI.

According to a report by Laopaiboon et al. [30], the syrup variety of sorghum KKU 40, cultivated in Thailand, showed a content of 20 ppm phosphorous, 166 ppm calcium, and 194 ppm magnesium. Yu [31] et al. found concentrations of 50 ppm phosphorus, 93 ppm calcium and 84 ppm magnesium in their work with the juice of sweet sorghum harvested in Pequin. All varieties evaluated here had concentrations greater than the KKU 40 variety.

It should be noted that the absorption of nutrients by plants is limited by several factors. According to studies by Franco et al. [32], plant type, climate, crop cycle, soil type and amount of fertilizer applied are important factors that influence the mineral composition of the plant.

Fig. 5 shows the nitrogen concentration in the juice during the study period. Nitrogen declined the most in BRS 506, which also had lower concentrations of phosphorus, nitrogen and sugar in the

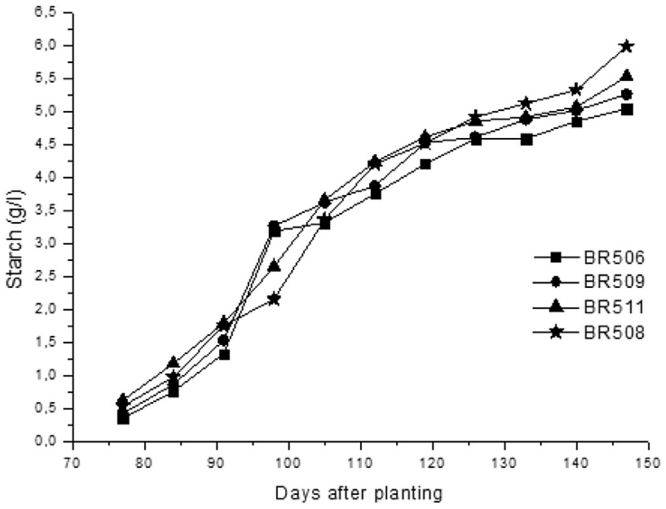

Fig. 6. Concentrations of starch during the maturation period of sweet sorghum varieties BRS 506 (■), BRS 508 (★), BRS 509 (●) e BRS 511 (▲).

final period, as well as a shorter PUI. Laopaiboon et al. [30] found nitrogen concentrations of 18.4 ppm in the juice of sorghum KKU 40. Unfortunately, there were no previous reports that monitored the nutrient content of the juice of sweet sorghum stalks in the plant during the maturation period. Therefore, the results presented in this paper in relation to the concentration of phosphorus, calcium, magnesium and nitrogen as a function of time during the maturation of the plant are hardly found in the literature.

Fig. 6 shows starch concentrations in the different varieties of sorghum during the maturation period.

Based on the results shown in Fig. 6, we observed that there were no significant differences between the starch contents of different sorghum varieties analyzed in this work and all had similar accumulation profiles during the maturation period.

In the reproductive phase, when grains begin to form (60–80 days), they are filled with sugar. This sugar is derived from the stem and leaves. Grains become heavier due to the conversion of sugars (sucrose) to starch, and as the conversion occurs, the grain, which was initially mostly aqueous, becomes pasty and ultimately hard. In the stems of the sorghum varieties studied, the starch concentration steadily increases throughout the maturation period, which shows that the plant may use starch as a form of carbohydrate reserve [27].

Comparing the results obtained in this study with the results presented by Andrzejewski [33], the amount of starch in the juice of sweet sorghum is higher than in the juice of sugar cane.

According to Eggleston et al. [34], the starch content is higher in immature sugar cane than in mature sugar cane. The authors found that during the harvest there was a decrease in starch content in all samples and systems; they attributed this to the increased maturity of the cane sugar. Sweet sorghum shows a significant increase in starch content during the ripening period; by the end of the maturation period, there was little variation in these levels, likely due to the stability of the stems after maturation. Similar studies of increasing starch concentration during the maturation of sorghum were not found in the literature.

According to Anyangwa et al. [35], starch is a polysaccharide of natural cane sugar, and its concentration depends on several factors such as growing conditions, plant variety, soil cultivation, and harvesting method. Their study showed that the maturation index is considered the most important factor in determining starch quality.

Eggleston et al. [36] studied the starch content of four varieties of sugar cane in Australia and concluded that the amount and content of starch is dependent on the variety of sugar cane. The authors found that the starch content in sugar cane did not significantly change during the last three months of maturity.

Given the high starch content in sweet sorghum juice, it should be co-utilized for ethanol production (Starch would be saccharified and fermented along with the juice).

According to a study conducted by Barcelos [37], due to the large amount of starch present in the juice of sweet sorghum, the addition of amylase enzymes hydrolyzes the starch and increases the final glucose concentration to 40%. This approach is one of the possible alternatives to minimize problems caused by starch in the syrup.

3.1. Alcoholic fermentation of the juice of sweet sorghum

For comparison, fermentations were made with the juice of 4 varieties of sorghum and sugar cane. The fermentations were conducted in batches using a fermenter syrup with sorghum and sugar cane concentrations of AT 155.3 g/L and 158.2 g/L, respectively. These fermentations were not enriched with any nutrients. Preliminary tests with BRS 506 enriched with nutrients (KH_2PO_4 (5.0 g/L), NH_4Cl (1.5 g/L), $MgSO_4.7H_2O$ (1.0 g/L), KCl (1.0 g/L)) showed that introducing nutrients caused an increase in the productivity in fermentation.

Table 1 shows the initial and average residual concentrations of total sugars, living cells/ml, average concentration of ethanol produced, yield and productivity. The fermentation time was 8 h, which was based on results from preliminary tests.

In all experiments, the concentrations of total sugar and initial cells were close. There was an increase in cell number due to the high initial concentration of yeast. Table 1 shows that BRS 508 had the most similar performance and productivity to sugar cane juice. According to Viegas [38], a conventional continuous fermentation generally produces a yield of 87% of theoretical and a productivity of 7.9 g ethanol/L h when sugar cane juice is used as a raw material. All varieties in this study had similar productivity and yield to those obtained in other plants. However, the yield of the product relative to the substrate was lower ($Y_{P/S}$ = 0.46 for BRS 508 and 0.463 for

Table 1
Conditions and results of the alcoholic fermentation.

Cultivars	TS_I (g/L)	TS_F (g/L)	CV_I/mL	CV_F/mL	EC_F (g/L)	$Y_{P/ART}$ (%)	$P_{ethanol}$ (g/L h)
BRS 506	157.4	4.9 ± 0.06	1.7×10^8	1.45×10^9	68.6 ± 0.50	88.4 ± 0.28	8.6 ± 0.48
BRS 508	158.2	1.9 ± 0.06	9.7×10^7	1.3×10^9	72.3 ± 0.42	90.5 ± 0.58	9.0 ± 0,40
BRS 509	155.3	2.8 ± 0.21	1.1×10^8	1.3×10^9	67.9 ± 0.25	87.1 ± 0.87	8.5 ± 0.24
BRS 511	156.5	4.6 ± 0.15	9.5×10^7	1.2×10^9	67.8 ± 04	87.3 ± 0.7	8.5 ± 0.46
Sugar cane juice	157.9	0.5 ± 0.20	1.7×10^8	1.4×10^9	73.2 ± 0.47	91.0 ± 0.71	9.2 ± 0.47

TS_I = Total initial sugar concentration (g/L); TS_F = average total residual sugar concentration (g/L); CV_I = initial concentration of living cells; CV_F = final concentration of living cells; EC_F = average residual ethanol concentration (g/L); $Y_{P/ART}$ = average ethanol yield produced in relation to total consumed sugar (%); $P_{ethanol}$ = average ethanol productivity.

sugar cane juice) compared to the theoretical yield of 0.511 [39,40]. It is noteworthy that the species BR 508 showed a higher percentage of sucrose than the BR 506 and BR 509 and a higher percentage of glucose than the BR 511. This fact may have contributed to the increased productivity of species BR 508.

The pH is an extremely important variable in the fermentation process because it controls dissolution of nutrients and enzymatic activity and prevents proliferation of microorganisms that contaminate the fermentation [41]. For all fermentations, reductions in pH were observed (average 4.2).

Fig. 7 shows the concentration profiles of sucrose, ethanol production and cell growth as a function of time for the fermentation of BR 508 variety of sorghum and sugar cane. This figure shows a comparison of the fermentation with sugar cane and with sorghum juice at the same process conditions. It is noted in this figure that the behavior of the fermentation of sorghum and sugar cane syrup was similar for total sugar consumption, ethanol production and cell growth. These results indicate that it is possible to ferment syrup of sweet sorghum in industrial plants already installed for the fermentation of sugar cane syrup. Sugar cane industrial plants are generally out of service during a period of the year, and they could be used for sweet sorghum fermentation at these times. Furthermore, ethanol production can be increased by the hydrolysis of the starch that is in sorghum syrup.

The ethanol concentration after 8 h of fermentation for all varieties was higher than reported by Gomez [26] in the fermentation juice of sweet sorghum BRS 506 (with an average of ethanol concentration of 59.07 g/L) and other varieties tested by this author. Similar results were found by Yu [31], Lima [42], Ratnavathi [43], and Rank [44] in the fermentation of different varieties of sweet sorghum.

4. Conclusion

During the maturation of the sweet sorghum varieties in this study, it was found that there was an increase in the concentrations of total sugar and starch and a reduction in the concentration of phosphorus, calcium, magnesium and nitrogen in the juice of sorghum stalks. BRS 508, 509 and 511 showed the longest time for which AT remained high in the plant, i.e., a larger PUI, which facilitates effective harvest of the plant. The alcoholic fermentation of the juice of sweet sorghum showed satisfactory results for the performance and productivity for the four varieties studied, with an emphasis on 508, whose values were similar to those obtained for sugar cane juice under the same operating conditions. The sorghum syrup is a promising alternative substrate for ethanol production in existing industrial installations.

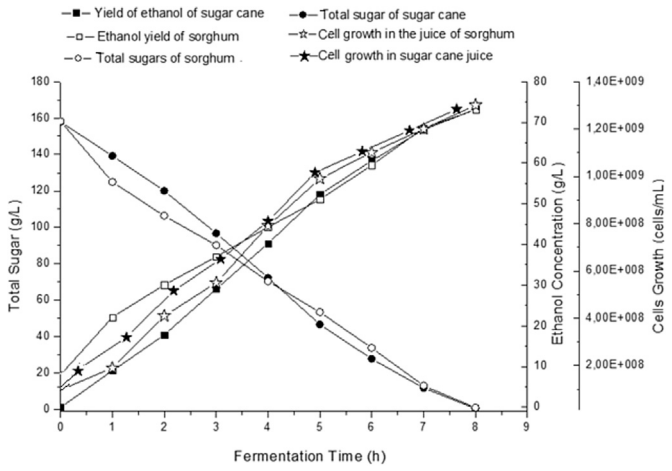

Fig. 7. Evolution of Concentrations of ethanol from sorghum juice (▫) and the juice of sugar cane (■), consumption of total sugars of sorghum juice (○) and sugar cane juice (●), cell growth in sorghum juice (☆) and sugar cane juice (★) in function of time of fermentation.

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