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Technological quality of sweet sorghum processed without panicles for ethanol production

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Abstract

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is recognized as a promising biomass energy crop for meeting the increasing demand for bioenergy feedstocks, because of its high adaptability to different soil and climates, in addition to the large accumulation of sugars in their stalks. In order to be successfully deployed for the production of sugar and biofuels, agronomic systems should be developed. The effects of inhibiting the development of panicles (by removing the top of the stalks at anthesis period) and harvest times on the technological quality of sweet sorghum were evaluated in this research. The experiment was arranged in a split-split-plot as a completely randomized statistical design, with four replications. Main treatments corresponded to sweet sorghum cultivars (CV147, CV198 and BRS508), sub-plots were the stalks management (integral and panicle removed) and tertiary were the harvest times (102 and 116 DAP). To evaluate the technological quality of sorghum juice, the following analyzes were conducted: total soluble solids content, pH, acidity, reducing sugars, total reducing sugars, starch and phenolic compounds. According to the results, compared with the quality standards for juice use, results showed that the cultivar BRS508 was more suitable for ethanol production. Higher sorghum quality was verified at 116 days after planting. The panicle removal promoted lower starch and phenolic compounds content in the juice.

Keywords: Bioenergy; Biomass; Raw material; *Sorghum bicolor*; Sucroenergetic sector.
Abbreviations: BRIX_Total Soluble Solids Content; DAP_Days after planting; IAA_indol acetic acid; RS_Reducing Sugars; TRS_ Total Reducing Sugars.

Introduction

The use of renewable energy sources is growing in the world, and the search for alternative biomass for fuel production, such as ethanol, has been intensified. Ethanol is one of the most important viable alternatives to replace gasoline, because of its low carbon dioxide emission rate. With the increase of ethanol consumption as a result of increasing flex fleet vehicles and the increase in admixture with gasoline, there is a need to increase the production of biofuels. To meet this demand, different renewable raw materials have been studied, in order to complement the production of sugarcane ethanol.

The three crops with the highest energy potential for fuel production are sugarcane, sugar beet and sweet sorghum. Sugarcane and sweet sorghum are tropical crops that store fermentable sugars in the stalks, besides solid residue (bagasse), that can use for energy production, fuel (second generation ethanol), animal feed or organic fertilization (Monti and Venturi, 2009; Ratnavathi et al, 2010).

Sweet sorghum is a C4 grass and presents high photosynthetic efficiency, a short vegetative cycle (90 to 120 days), high sugar and biomass yields, drought tolerance and growth capacity under several environmental conditions. In Brazil, its cultivation can be done in sugarcane replant fields, increasing the fuel production per area. Furthermore, the implements used for sorghum harvest, loading and transportation are the same used in the sugarcane industry (Almodares and Hadi, 2009).

The industrial processing of sweet sorghum extraction is conducted similarly to the sugarcane form, requiring small operational adjustments. Ethanol is also produced by fermentation technology, similarly to sugarcane, and can use the same infrastructure and machinery as in the sugar industry (Srinivasa et al., 2009). However, differently from sugarcane, the end of the vegetative cycle in sweet sorghum is characterized by the sucrose translocation from stalks to panicle grains (anthesis stage). In this process, sucrose is converted to starch, a sugar unfermentable by *Saccharomyces cerevisiae* yeast. Thus, the removal of the top of the emerging panicle before the grain filling can positively affect the sugar yield because the photoassimilates that would be used for the grain formation can be stored as fermentable sugars in stalks (Erickson et al., 2012).

Despite these advantages of sweet sorghum use in the fuel industry, relatively little is known about the ethanol production on large scale (Guigou et al., 2011). This use viability depends on practices that optimize its energy yield; thus, the knowledge of the chemical-technological characteristics and its adjustment to the fermentation process are extremely important.

This research evaluated the effect of panicle growth inhibition, by removing the top of the stalks at anthesis stage of sweet sorghum to characterize the technological quality of sweet sorghum cultivars for the production of ethanol in two harvest seasons.

Results and Discussion

Characterization of Brix and pH

Tables 1 and 2 show the results obtained for Brix of the extracted juice from the sweet sorghum cultivars, in different management system and two harvest times. The cultivar BRS508 presented the highest value when compared to others; the soluble solids concentration were between 13.8 and 17.0, and are in agreement with the results obtained by Rutto et al. (2013), who verified values between 13.8 and 18.7%.

Furthermore, the removal of the panicles promoted a decrease in Brix content for the cultivars CV147 and CV198 (Table 1). Rajendran et al. (2000) also observed this behavior when inhibiting the growth of panicles in sweet sorghum. This effect was attributed to the use of sugars in the plant metabolism, because under normal conditions there is sugar translocation from stalk to panicle, and it is stored in the grains as starch. The removal of panicles prevents this plant metabolism, but stalks generating and new panicle formation were observed, and probably resulted in consumption of these sugars.

Erickson et al (2012) and Broadhead (1973) also observed that the growth inhibition of sweet sorghum panicles resulted in an increase in Brix. According to these authors, the high increase in sugars accumulated in stalks was attributed to changes in the sugar distribution pattern. From the panicle removal time, the sugars are concentrated mainly in the stalks. Nothing has been reported on the occurrence of new panicle growth.

Considering the effects of the harvest times x stalk managements on the Brix values (Table 2), at 102 DAP, the stalks of both treatments (with or without panicles) presented similar Brix content. However, after 14 days we observed that the integral stalks accumulate more soluble solids, increasing Brix values by 1.8%, differently from with the panicle removed, which presented a decrease in relation to the first harvest time. Probably in this time the shoot and new panicle were in development, using the photoassimilates stored in the stalks.

Evaluating the pH (Table 3), there was a difference among the cultivars in the integral stalks; the higher values were observed to the CV147, followed by CV198 and BRS508. When panicles were removal, there was a significant reduction in the pH, mainly in cultivars CV147 and CV198. This behavior can be associated with the higher metabolic activity conditions presented by the plants (due to shooting and panicle formation). In this case, pH is reduced, in function of conditions favorable to acids and growth regulator enzyme action, such as IAA (Taiz and Zeiger, 2010) and acid invertase, that present a decisive role in the accumulate sucrose regulation (Yang et al., 2013).

Considering the harvest times observed, in all cultivars and management systems there was a pH decrease of 0.1 during 14 days. Similar results were found by Lingle et al (2013), who also verified the pH reduction during the harvest times (90, 115 and 140 DAP).

Total Acidity

In relation to juice total acidity (Table 4), the cultivar BRS508 showed the highest acidity when compared to the others. Also, the stalk processing at 116 DAP resulted in the highest amount of these molecules in relation to 102 DAP. These results are directly related to those obtained for pH, because we observed pH values inversely proportional to the total acids. Lingle et al (2013) also observed this relationship of pH reduction and acidity increase during the harvest times. The reduction in pH and increase in the acidity in mature sorghum can be unfavorable to the fermentation process, since for the pH correction to 6.0 (necessary for juice clarification and organic acid neutralization) a higher amount of calcium hydroxide is necessary, and the calcium concentration increase in the juice can reduce the yeast fermentation yield (Lingle et al, 2013).

The inhibition of panicle growth resulted in lower overall acidity. High acidity values are related to factors detrimental to the raw material quality, which will reflect in the fermentation process and ethanol yield (Narendranath et al., 2001). The acidity values were higher than 0.8 mg L^{-1} of H_2SO_4, the maximum value considered adequate for sugarcane juice (Ripoli and Ripoli, 2004) that causes no fermentation losses. The acids amount present in sorghum is higher than in sugarcane, however, there are no available results demonstrating if these acid concentrations in sorghum can influence the yeast yield during the fermentation process, thus, new experiments are necessary to explain this hypothesis.

Reducing Sugars and Starch Concentration

In relation to reducing sugars content in the extracted juice (Table 4), the cultivar CV147 showed the highest values. These results corroborate Channappagoudar et al. (2007), who determined concentrations between 1.8 to 7.1, according to the processing of different cultivars and harvest times. Is important mention that these values are higher than those obtained in the sugarcane juice, where mature cultivars presented a maximum RS concentration around 1%. According to Billa et al. (1997), the sweet sorghum glucose content is near 3%, higher than that found in sugarcane juice.

Considering the management systems, a no significant difference was observed for this parameter. However, at 116 DAP, there was a decrease in the RS for all cultivars, processing with or without panicle, when compared to the 102 DAP harvest. This behavior can be associated with the translocation of metabolites to the panicle in integral stalks; in those with panicle removed, the RS reduction can be associated with shoot emission and new panicle emission, as previously described.

For total reducing sugars (TRS), mean values were about 11.67% for all treatments (Table 4); however, the maximum concentration of 13.05% was obtained for cultivar BRS508 harvested at 116 DAP (Table 5). McBee and Miller (1993) observed a lower amount of TRS in sorghum juice harvested in the initial growth stage, and a significant increase in panicle extension.

Considering that starch is a characteristic sorghum sugar, found in low concentration in juice, and intensely in panicle grains, it was quantified in the extracted juice of all treatments (Table 4); where the mean value was 430 mg L^{-1} for the cultivars, lower than that verified by Andrzejewski et al (2013), that observed values about 1476 mg L^{-1}. The panicle removal caused a decrease of 23.5% in starch content in the extracted juice. This behavior occurred due to the

Table 1. Interaction between sweet sorghum cultivars (CV147, CV198 and BRS508) and stalk management systems (integral and panicle removed) for juice Brix. Jaboticabal/SP, Brazil. Season 2013/2014.

Brix (%)	Stalks Management	
Cultivars	Integral	Panicle Removed
CV147	15.2Ba	13.8Bb
CV198	15.7Aba	14.8Bb
BRS508	16.6Aa	17.0Aa

Upper case letters compare column means. Lower case letters compare line means. Means followed by the same letter are not different by Tukey test at 5% of probability.

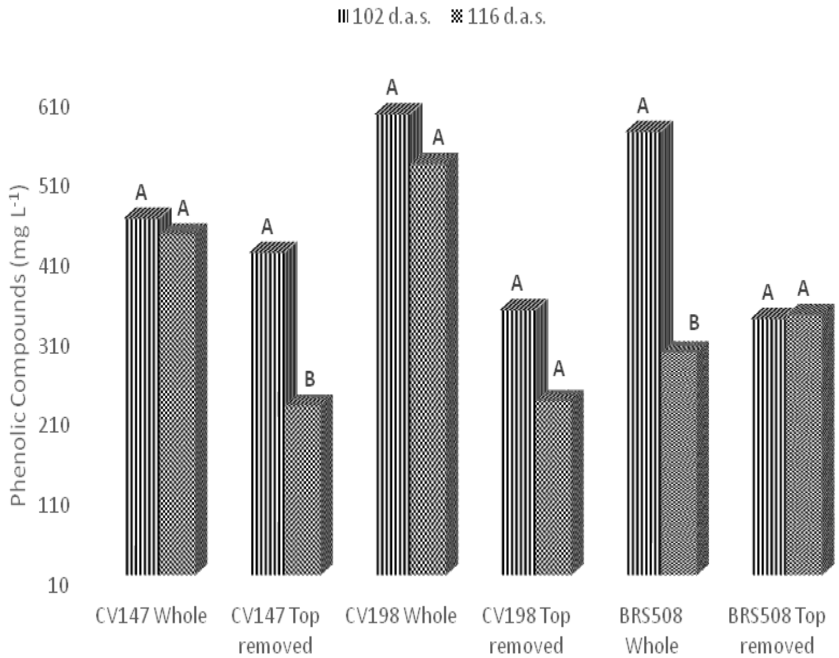

Fig 1. Graphic representation for juice phenolic compounds (mg L^{-1}) for the genotypes CV147, CV198 and BRS508, in function of the stalk management systems (whole and panicle removed) and harvest times (102 and 116 DAP). Jaboticabal/SP, Brazil. Season 2013/2014.

Table 2. Interaction between stalk management systems (integral and panicle removed) and harvest times (102 and 116 DAP) for juice Brix. Jaboticabal/SP, Brazil. Season 2013/2014.

Brix	Harvest times (DAP)	
Stalks management	102	116
Integral	14.9Ab	16.7Aa
Panicle Removed	15.5Aa	14.9Ba

Upper case letters compare column means. Lower case letters compare line means. Means followed by the same letter are not different by Tukey test at 5% of probability.

Table 3. Interaction between sweet sorghum cultivars (CV147, CV198 and BRS508) and stalk management systems (integral and panicle removed) for juice pH. Jaboticabal/SP, Brazil. Season 2013/2014.

pH	Stalk management	
Cultivars	Integral	Panicle Removed
CV147	5.05Aa	4.87Ab
CV198	4.95Ba	4.82Ab
BRS508	4.87Ca	4.84Aa

Upper case letters compare column means. Lower case letters compare line means. Means followed by the same letter are not different by Tukey test at 5% of probability.

Table 4. ANOVA results for pH, Total Acidity, Reducing Sugars (RS), Total Reducing Sugars (TRS) and Starch in the extracted juice of three sweet sorghum cultivars (CV147, CV198 and BRS508), two stalk management systems (integral and panicle removed) and two harvest times (102 and 116 DAP). Jaboticabal/SP, Brazil. Season 2013/2014.

Treatments	pH	Total Acidity	RS	TRS	Starch
Cultivars (C)		g L^{-1}	%	%	mg L^{-1}
CV147	4.9A	1.42B	3.59A	11.61A	415.2A
CV198	4.8B	1.57AB	2.52B	11.23A	360.2A
BRS508	4.8B	1.83A	1.85B	12.18A	515.9A
F test	38.0**	7.43*	28.14**	1.43ns	0.28ns
LSD	0.03	0.33	0.718	1.72	578
C.V	0.71	18.90	24.94	13.64	32.77
Stalk Management (M)					
Integral	4.9A	1.70A	2.73A	11.74A	487.8A
Panicle Removed	4.8B	1.50B	2.57A	11.61A	373.1B
F test	43.1**	8.61*	0.59ns	0.17ns	6.27*
LSD	0.03	0.15	0.44	0.71	107.9
C.V	1.20	14.25	25.48	9.36	12.12
Harvest times - DAP (E)					
102	4.9A	1.47B	3.02A	11.49A	335.3A
116	4.8B	1.73A	2.27B	11.86A	525.6A
F test	9.4**	17.96**	13.23**	1.52ns	0.32
LSD	0.07	0.131	0.433	0.63	321
C.V	2.38	13.50	26.92	8.91	25.59
F test CxM	6.30*	0.93ns	0.76	1.81ns	1.7 ns
F test CxE	0.80ns	1.53ns	0.69ns	5.97*	0.20ns
F test MxE	0.02ns	1.61ns	0.42ns	1.77ns	0.15ns
F test CxMxE	0.16ns	0.675ns	0.35ns	1.87ns	0.29ns

**significant at 1% of probability ($p<0.01$); * significant at 5% of probability ($0.01=<p<0.05$); ns - not significant ($p>=0.05$). Upper case letters compare column means. Means followed by the same letters do not differ according Tukey test at 5% of probability. L.S.D = Least Significant Difference. C.V = Coefficient of Variation.

Table 5. Interaction between sweet sorghum cultivars (CV147, CV198 and BRS508) and harvest times (102 and 116 DAP) for juice Total Reducing Sugars. Jaboticabal/SP, Brazil. Season 2013/2014.

TRS (%)	Harvest times (DAP)	
Cultivars	102	116
CV147	11.55Aa	11.67ABa
CV198	11.61Aa	10.85Ba
BRS508	11.31Ba	13.05Aa

Upper case letters compare column means. Lower case letters compare line means. Means followed by the same letter are not different by Tukey test at 5% of probability.

emasculation process, since in the maturation stage there is translocation of the stored sugars from stalks to panicle grains, and their conversion int starch (Almodares and Hadi, 2009). The reduction in starch content in juice is a benefit to industrial processing because this sugar is unfermentable and increases the juice viscosity, negatively affecting clarification (Costa et al, 2014).

Phenolic compounds

Fig 1 presents the interaction between the stalk management systems of three sweet sorghum cultivars, in two harvest times for juice phenolic compounds. Generally, the mean values were about 434 mg L^{-1}, and the cultivars CV147 (panicle removed) and BRS508 (integral), presented a significant decrease from 102 to 116 DAP. The amount of phenolic compounds varies according to the genotype, and the literature shows results that indicate a variation between 100 mg L^{-1} and 6000 mg L^{-1} (Dicko et al., 2006). The main phenolic groups present in sorghum are simple phenols, hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, chaconnes, hydroxycoumarin, lignin and polyflavans, highlighting the absence of tannic acid and hydrolysed tannins in sweet sorghum juice (Dicko et al., 2006). The phenolic compounds negatively affect the ethanol production process, because they act as yeast inhibitors during fermentation, resulting in significant ethanol yield losses and alterations in distillate composition (Ravaneli et.al., 2011).

Materials and Methods

Plant materials

The sweet sorghum cultivars were planted in Jaboticabal, São Paulo, Brazil, (21º14’05’’S and 48º17’09’’W) in the 2012/2013 season, at 0.45m between rows. The fertilization with 36-126-72 kg ha^{-1} of N-P2O5-K2O was conducted at planting. The experiment lasted five months. Planting was conducted in January 2013 and the harvest in April 2013.
The planting was done on January 3, 2013, through a manual distribution of 15 seeds per meter in a furrow, which were covered with 2-3cm soil. At 15 DAP, seedlings were thinned, maintaining six plants per meter, to obtain a final stand of 120,000 plants ha^{-1}.

During the experimental period, phytosanitary treatment was conducted to assure the crop health. For fall armyworm control, 250 mL ha^{-1} of Engeo Pleno (Thiamethoxam / Lambda cyalothrin) was applied at 22 DAP. At 70 DAP, the stage of panicle development when the flag leaf was visible, the manual removal of the last internode in the appropriate plots was performed, using pruning hooks and scissors, to inhibit panicle flowering. In control plots, the panicle was kept intact.

Treatments and experimental design

The experiment was arranged in a split-split-splot as a completely randomized statistical design, with four replications. Main treatments corresponded to three sweet sorghum varieties (CV147, CV198 and BRS508); secondary corresponded to the stalk managements: with the panicle removed at the anthesis stage and integral stalks (control without panicle removal). Tertiary treatments were the two harvest times (102 and 116 DAP). Each plot was consisted of 12 lines, 11 m in length. The total area was 59.4 m², and the useful plot area was made up of eight central lines, 9 m in length, totaling 32.4 m².

Traits measured

In each harvest time, 15 integral stalks (with panicle) and 15 stalks with panicle removed were harvested. The juice was extracted according to the hydraulic press method (Tanimoto, 1964) and characterized as to Brix, pH, Reducing Sugars, Total Reducing Sugars, Total Acidity (ICUMSA, 2013), Total Phenolic Compounds (Folin and Ciocalteau, 1927) and Starch content (Chavan et al., 1991).

Statistical analysis

Results were submitted to ANOVA by the F-test and means were compared by the Tukey test at 5% of probability.

Conclusion

The panicle removal reduced the starch and phenolic compounds in sorghum juice, but for other characteristics, such as Brix and TRS the removal of panicles negatively affected the technological quality of sweet sorghum. The cultivar BRS508 showed the best chemical-technological characteristics for industrial processing. The adequate harvest time for sweet sorghum processing was 116 DAP.

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