BIOSCIENCE JOURNAL

SWEET SORGHUM: BROTH CLARIFICATION WITH ENZYMATIC TREATMENT INCREASES THE QUALITY OF THE FERMENTATION WORT FOR ETHANOL PRODUCTION

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How to cite: FERREIRA, O.E., et al. Sweet sorghum: broth clarification with enzymatic treatment increases the quality of the fermentation wort for ethanol production. *Bioscience Journal*. 2021, **37**, e37094. https://doi.org/10.14393/BJ-v37n0a2021-54172

Abstract

Sweet sorghum is currently being evaluated throughout the world as a raw material for biofuel production because its stem juices are rich in sugars that can be directly fermented to ethanol. In this work, the fermentative efficiency of three sweet sorghum genotypes was evaluated, aiming at ethanol production, harvested in two seasons, clean and whole stems, and the treatment of the juice and broth with amylolytic enzymes in order to use the present starch to increase the production of ethanol. The experiment was carried out in the 2013/2014 harvest, in the municipality of Jaboticabal, São Paulo, Brasil, located at 21°14'05''S and 48°17'09''W. The experimental design was completely randomized, with sub-subdivided plots and four replications. The primary treatments were the sweet sorghum genotypes (CV147, CV198, and BRS508), the secondary treatments, the type of harvest (whole stems and clean stems); the tertiary the two sampling times (102 and 116 days after sowing - d.a.s) and the quaternary the application of enzymes. In the fermentation process, the yeast PE-2 was used, at the end, the wine was recovered and characterized. Fermentation efficiency and liters of ethanol per ton of sorghum were calculated. The clarification of the juice with enzymatic treatment increases the quality of the fermentation broth and makes it possible to obtain wines with lower levels of RRTs and Brix. Fermentation efficiency is not affected by the genotype; however, it is influenced by the time of harvest and the technological quality of the juice. The use of amylolytic enzymes makes it possible to obtain wines with lower levels of RRTS and Brix. The best period of industrialization was at 102 d.a.s., and the processing of whole stalks resulted in less ethanol production.

Keywords: Alpha-Amylase. Amyloglucosidase. Bioenergy. Biofuels. Fermentation.

1. Introduction

The worldwide demand for renewable fuels has expanded rapidly in recent years. Moreover, with the agreement of the COP-21, the signatory countries are looking for alternatives to reduce carbon dioxide emissions to the atmosphere at their borders. Thus, new technologies for renewable fuel production have been studied for large-scale implementation. Countries like Brazil, the United States, India, and South Africa have used ethanol as a viable substitute for gasoline in automotive vehicles, using sugarcane and maize as major raw materials. However, these same countries also evaluate the potential of sweet sorghum (*Sorghum bicolor* (L.) Moench) as a complement to ethanol production.

In the specific case of Brazil, the sweet sorghum has been studied in plantations in sugarcane renovation areas during late spring and early summer. As a result of the crop's short lifecycle (90-120 days), it can be harvested during the sugarcane off-season, supplying feedstock, and increasing the ethanol production period in the country without a larger area planted for energy purposes (Lozano et al. 2018).

From an agronomic viewpoint, the crop has a similar potential to some varieties of sugarcane grown under irregular conditions (Costa et al. 2018), as well as a high content of glucose, fructose, sucrose, and fiber in its stalks (Barcelos et al. 2016). Industrially, the sweet sorghum has a fully mechanized crop planting and harvesting similar to those used by the sugarcane sugar mills, and its viability is similar to the sugarcane (Durán et al. 2018), including the use of commercial yeast for fermentation, ability to use bagasse as a source of energy, electricity cogeneration, second-generation ethanol, and a favorable energy balance (Dar et al. 2018).

Throughout the maturation stage, the sucrose stored in the stalks during the plant vegetative period is translocated to the grains transforming into starch. Thereby, this process occurs to add more resistance to the plant in its reproductive process allowing the grain to keep longer in dormancy in the soil (Taiz and Zeiger 2017). In addition, this biomolecule may also be present in the leaves (Silva et al. 2016). Thus, this behavior is not interesting for industrial processes because the starch is a sugar that cannot be fermented by the *Saccharomyces cerevisiae* yeast, resulting in a decrease in industrial yields (Bai et al. 2008). Considering this issue, authors such as Silva et al. (2016), Macedo et al. (2017), and Lozano et al. (2018) search for minimizing the problem by removing the panicle in the field or by applying flowering inhibitor. However, these are expensive techniques and may become the sweet sorghum production an unfeasible process.

Brazilian companies that started to cultivate sweet sorghum without this academic information observed low yields in ethanol production. In the country, there were cases of mills that obtained 1000 L of ethanol per hectare of sweet sorghum (May et. al. 2012), while research show values of 2500 to 3000 L per hectare (Lozano et al. 2018).

Thus, this divergence of values may be due not only to improper crop management in the field but also to the use of cultivars unsuitable for climatic and edaphic conditions or inappropriate harvesting times; as well as in the non-development of industrial technologies that support the processing of the raw material with high yields. Therefore, there is a lack of recommendations and cropping guidelines for sweet sorghum due to insufficient research.

In this context, the aim of the study was to evaluate the effects of enzymatic treatment of the juice on the fermentation broth quality with three genotypes of sweet sorghum (CV147, CV198, and BRS508, harvested with and without leaves), harvested in two seasons.

2. Material and Methods

Experimental design

The experimental design was a split-split plot arrangement with four replicates in a randomized complete block design, and the following treatments were studied: Primary - harvest systems (whole and clean stalks); Secondary - harvest times (102 and 116 d.a.s). The tertiary treatment resulted from the application of the α-amylase enzyme. This design was applied for each sorghum genotype studied (CV147, CV198, and BRS508).

For the wort clarification/preparation stage, the quaternary treatment was carried out, which was the enzymatic treatment (with and without enzyme). The four treatments were used to obtain the wine and calculations of the yield and fermentative efficiency of the treatments.

Experimental conditions

Sorghum was planted at 21°14’05’’S and 48°17’09’’W at the 2012/2013 harvest. The spacing used for sowing was 45 cm between rows. Each plot in the field was constituted by 12 lines of 11 meters each, with 59.4 m² of total area, being considered for the useful area, the 8 central lines, with 9 meters of length, totaling 32.4 m².

The planting was done manually with a rate of 15 seeds per meter, covered with soil layers from 2 to 3 cm. At 15 d.a.s, the pruning was carried out, which results in about 120.000 plants ha^{-1}. The planting fertilization was 36-126-72 kg ha^{-1} of N-P_2O_5-K_2O, using the 8-28-16 formula. During the experimental period, the phytosanitary treatment was done to keep the maintenance of the crop's sanity. No irrigation was done and weed control was by a combination of pre-emergent herbicide application and post-emergent tilling. For tilling pests' control at 22 d.a.s, 250 mL ha^{-1} of Thiamethoxam + Lambda-cyhalothrin was applied.

Harvest and juice extraction

At the sampling times, two harvesting systems were performed: 15 whole plants (stalks with panicles and leaves) and 15 stalks (without panicles and leaves). The juice was extracted according to the Tanimoto hydraulic press method (1964). The filtered juice was analyzed for Brix, pH, Reducing Sugars (RS), Total Reducing Sugars (TRS), Total Acidity (CTC 2005); phenolic compounds (Folin and Ciocalteau 1927), starch content (Chavan et al. 1991) and starch content (Chavan et al. 2016) and fiber (Consecana 2006).

Juice clarification

The extracted juice was clarified with pH adjusted to values close to 7.0 with calcium hydroxide Ca $(OH)_2$ (6° Be). After liming, the juice was heated to a boiling point and transferred to 1000 mL beakers containing 2 $mg.L^{-1}$ of Flomex 9076 polyelectrolyte to accelerate the sedimentation of impurities (Costa et al. 2018). Subsequently, the juice was cooled and maintained at 90°C for the application of the alpha-amylase enzyme (Novozym 50188) in the dosage of 0.020 $mg.L^{-1}$ of processed sorghum, remaining in clarifier for 60 minutes for hydrolysis of the starch. After the retention time, the juice was siphoned and determined by the same analyzes described in the 2.3 item. To obtain the broths, Brix (15.5 to 16 ° ± 0.1), pH (corrected with sulfuric acid to 4.5 ± 0.3), and the temperature (32ºC) of the clarified broth were standardized.

Conducting the fermentation process

Aliquots of 250 mL of the must were subjected to enzymatic treatment with an application of amyloglucosidase (Novozym 50189) in the dosage of 0.04 $mg.L^{-1}$ of processed sorghum for saccharification of the starch, and subsequent inoculation of the selected yeast (PE-2) at a concentration of 10^6 Colony Forming Units per mL (CFU mL^{-1}). The inoculated vials were kept at 32 ± 1 °C during the fermentation process. The conduction was carried out in batch with the recovery of the yeast by centrifugation. The process was monitored by densimetry, the end being established when the Brix had values ≤ 1.0 or when the reading stabilized in the interval of 30 minutes. In wine, Brix, pH, total acidity (CTC 2005), Total Residual Reducing Sugar (RRTS) by DNS, Glycerol (Copersucar 2001), and alcohol content by densimetry were determined. Next, the fermentative efficiency and liters of ethanol obtained per ton of processed sorghum were calculated (Fernandes 2006).

Statistical analyzes

The results were submitted to analysis of variance by F test and the means were compared by Tukey test (5%) using the AgroStat software as described by Barbosa and Maldonado Junior (2015).

3. Results and Discussion

The first step was to determine the juice from the three sweet sorghum genotypes harvested with and without panicles, in two sample times and submitted to enzymatic treatment. Table 1 shows the average values obtained for Brix, TRS, RS, pH, and total acidity of the juice extracted for the studied genotypes, harvested with and without panicle in 2 sampling periods, submitted to enzymatic treatment. Evaluating the harvesting systems, it was found that the full stalk harvest did not influence the values of Brix, RS, TRS, Phenol, and Starch. These results indicate that harvesting the entire plant does not result in a decrease in the technological quality of the juice.

The cultivars showed different values of Brix, especially BRS508, which contained 1.1% more soluble solids than the others. The harvest season influenced the Brix values, with an increase in this parameter with the harvest performed at 116 a.s., with values in the order of 16.7 Brix. Rutto et al. (2013) evaluating sweet sorghum genotypes described values between 13.9 and 18.7 Brix.

Considering the levels of RS and TRS, there was no interaction between genotypes and the type of harvest. However, these parameters were directly influenced by the sampling period. At 102 a.s., the genotypes harvested with or without panicles showed a high concentration of RA and low levels of TRS. After 14 days of cultivation in the field, there was a reduction in RA levels and a consequent increase in TRS levels. Teixeira (1999) observed that the TRS content in the stalks was higher when the plants reached their physiological maturity, about 120 days after planting, a behavior similar to that observed in this work at 116 a.s.

For pH, there was no interaction between genotypes and harvest time. The management system directly influenced this parameter, and the highest values were obtained with integral stems. Similar values were obtained by Giacomini et al. (2013) that in the characterization of the sorghum broth, determined pH values between 4.6 and 5.1.

Table 1. Physico-chemical characterization of the juice for the CV147, CV198, and BRS508 genotypes of sweet sorghum, harvested with and without panicles, at 102 d.a.s. and 116 d.a.s. Jaboticabal-SP/2013-2014 Harvest.

	Brix (%)	TRS (%)	RS (%)	Ph	Total Acidity ($g.L^{-1}$ H_2SO_4)
Genotype (A)					
CV147	15.7^{B}	11.4^{A}	3.20^{A}	4.8^{A}	1.61^{B}
CV198	15.8^{B}	12.0^{A}	2.59^{A}	4.8^{A}	1.60^{B}
BRS508	16.9^{A}	12.0^{A}	2.43^{A}	4.8^{A}	1.84^{A}
F Test (A)	16.83**	3.86^{ns}	4.81^{ns}	1.00^{ns}	13.46^{**}
SMD	0.92	0.76	0.80	0.15	0.15
CV (%)	5.33	5.97	27.02	2.91	8.55
Treatment (B)					
Clean stalks	16.1^{A}	12.0^{A}	2.76^{A}	4.7^{B}	1.67^{A}
Whole stalks	15.8^{A}	11.7^{A}	2.72^{A}	4.9^{A}	1.70^{A}
F Test (B)	1.17^{ns}	0.88^{ns}	0.01^{ns}	11.89^{**}	0.20^{ns}
SMD	0.64^{A}	0.61^{A}	0.64^{A}	0.14	0.13
CV (%)	6.14	7.95	35.83	4.43	12.21
Seasons (C)					
102 d.a.s.	15.3^{B}	11.4^{B}	3.43^{A}	4.8^{A}	1.60^{B}
116 d.a.s	16.7^{A}	12.3^{A}	2.05^{B}	4.8^{A}	1.77^{A}
F Test (C)	29.23^{**}	8.91^{**}	31.92^{**}	0.06^{ns}	6.42^{*}
SMD	0.54	0.65	0.51	0.11	0.14
CV (%)	5.63	9.13	30.73	3.78	13.87
F blocks Test	2.47^{ns}	8.40^{*}	0.49^{ns}	5.92^{*}	17.63^{**}
Inter. AxB	0.32^{ns}	3.17^{ns}	1.36ns	3.56^{ns}	4.65^{*}
Inter. AxC	0.29^{ns}	0.58^{ns}	3.44ns	1.77^{ns}	4.50^{*}
Inter. BxC	2.35^{ns}	0.44^{ns}	0.02ns	2.62^{ns}	6.57^{*}
Inter. AxBxC	0.39^{ns}	1.64^{ns}	1.23ns	2.13^{ns}	2.41^{ns}

**significant at the 1% probability level (p<0.01); * significant at the 5% probability level (0.01=<p<0.05); ns not significant (p>=0.05). Different capitals in the same column differ by Tukey test. S.M.D = significant minimum deviation. C.V. = coefficient of variation.

From the analysis of the total acid content present in the sweet sorghum stems, it was observed that these are directly related to the processed genotype. The BRS508 genotype showed 0.24 g L^{-1} more acids than the CV198 and CV147 genotypes. Freita et al. (2014) evaluating these biomolecules in 3 different genotypes of sweet sorghum, determined contents between 1.54 and 1.76 g L^{-1}, values similar to those obtained in this research Table 2.

Considering the processing of whole stalks and clean stems, it was observed that the addition of leaves and panicles did not result in the addition of these total acids in the extracted juice. In this context, it should also be noted that the longer cultivation of sorghum promotes an increase in acids in the juice. The plant probably synthesizes these molecules to carry out the translocation of sucrose from the stems to the grains.

There was no difference for fiber between genotypes, the values were between 13.79 and 14.54%, being influenced by the system and time of harvest, it was observed that at 116 a.s. there was an increase in fiber in the stalks. This fact can be associated with water loss (weight) of the stalk in addition to the increase of grains in the panicle (Table 2). According to Guiying et al. (2000). The processing of whole stalks resulted in a significant increase in the fiber content, of the panicle, which has fibrous characteristics.

Evaluating the interaction of phenol contents, it was observed that there was no difference between the genotypes harvested whole or cleaned. However, the total phenolic compounds present in the broth were affected by the harvest season, being higher at 116 d.a.s. According to Ravaneli et al. (2011) and Awika et al. (2004), raw materials with phenol levels greater than 500 mg L^{-1}, directly affect the fermentation process by inhibiting yeast activity and significantly reducing the ethanol content produced. In this sense, the second harvest season was unfavorable to industrial processing.

Table 2. The Fiber, Phenol, and Starch contents in sweet sorghum broth throughout the harvest seasons, harvested with and without panicles, at 102 d.a.s. and 116 d.a.s. for CV147, CV198, and BRS508 genotypes. Jaboticabal-SP/2013-2014 Harvest.

	Fiber (%)	Phenol (mg.L^{-1})	Starch (mg.L^{-1})
Genotype (A)			
CV147	13.79^{A}	801^{A}	453^{A}
CV198	14.54^{A}	616^{A}	474^{A}
BRS508	14.39^{A}	792^{A}	545^{A}
F Test (A)	3.08^{ns}	0.43^{ns}	0.89^{ns}
SMD	0.98	684.62	623.37
CV (%)	6.39	85.64	25.47
Treatment (B)			
Clean stalks	12.70^{B}	796^{A}	494^{A}
Whole stalks	15.78^{A}	677^{A}	487^{A}
F Test (B)	210.33^{**}	1.13^{ns}	0.95^{ns}
SMD	0.48	253.19	246.00
CV (%)	5.18	52.61	15.74
Seasons (C)			
102 d.a.s.	13.41^{B}	412^{B}	429^{A}
116 d.a.s	15.07^{A}	1061^{A}	553^{A}
F Test (C)	63.40^{**}	24.29^{**}	0.40^{ns}
SMD	0.43	276.60	307.46
CV (%)	5.05	61.92	27.42
F blocks Test	1.36^{ns}	1.42^{ns}	0.52^{ns}
Inter. AxB	5.38^{*}	0.56^{ns}	0.28^{ns}
Inter. AxC	0.57^{ns}	2.83^{ns}	0.32^{ns}
Inter. BxC	8.07^{*}	8.46^{**}	0.49^{ns}
Inter. AxBxC	0.49^{ns}	3.07^{ns}	0.54^{ns}

**significant at the 1% probability level ($p<0.01$); * significant at the 5% probability level ($0.01=<p<0.05$); ns not significant ($p>=0.05$). Different capitals in the same column differ by Tukey test. S.M.D = significant minimum deviation. C.V. = coefficient of variation.

Analyzing the starch contents, it is observed that they were similar for the three genotypes, with no significant differences for stem management systems and harvest times. According to Guiying et al. (2000), the levels of starch in the plant are influenced by the stage of development and characteristics of the genotype studied, and the higher the level of maturation the greater the amount of starch stored in the grain.

Such results show that the panicle, despite containing considerable levels of starch, may not contribute to the increase of this in the broth, due to the difficulty of extraction through the system used to extract the stem juice. The use of mills even with previous preparation of stalks in chippers and defibrators does not allow the total breakdown of the grains in a meaningful way that allows the availability of starch. The sorghum starch is formed and stored in protein matrix capsules, so for availability, it would be necessary to use a system that allows the grinding of the grain and the breaking of the cuticle that surrounds the grain (Icrisat 2009).

After the clarification of the broth, the phenol contents were reduced considerably when compared to the values of the original broth. Similar behavior was observed for the starch content in the clarified broth for the three genotypes, which showed a drastic reduction in starch content. The highest values occurred at 116 d.a.s. indicating that the plant was in the final maturation process, translocating the reserves to the grains (Table 3).

Table 3. Effect of the clarification process on the contents of Phenol and Starch, submitted or not to treatment with alpha-amylase, for genotypes CV147, CV198, and BRS508 of sweet sorghum, harvested with and without panicles at 102 d.a.s. and 116 d.a.s. Jaboticabal-SP/2013-2014 Harvest.

	Starch ($mg.L^{-1}$)		
	CV147	CV198	BRS508
Harvest (A)	0.98^{ns}	0.52^{ns}	17.15^{*}
Clean stalks	539.64^{A}	580.32^{A}	723.17^{A}
Whole stalks	455.70^{A}	517.33^{A}	437.47^{B}
SMD	269.72	277.91	219.46
CV (%)	59.00	55.13	41.17
Treatments (B)	3.18^{ns}	3.31^{ns}	2.93^{ns}
Original juice	801.40^{A}	616.18^{A}	792.88^{A}
Without alpha amylase	572.72^{A}	907.93^{A}	702.34^{A}
With alpha amylase	118.89^{B}	122.36^{B}	245.73^{B}
SMD	440.76	312.16	369.53
CV (%)	93.97	60.35	67.56
Seasons (C)	8.82^{**}	22.99^{**}	8.95^{**}
102 d.a.s.	304.74^{B}	401.43^{B}	446.43^{B}
116 d.a.s	690.60^{A}	696.22^{A}	714.20^{A}
SMD	219.17	212.73	184.16
CV (%)	72.64	63.94	52.35
F blocks Test	3.18^{ns}	3.31^{ns}	2.93^{ns}
Inter. AxB	0.98^{ns}	0.52^{ns}	17.15^{*}
Inter. AxC	0.03^{ns}	0.05^{ns}	10.48^{**}
Inter. BxC	9.66^{**}	4.59^{*}	0.26^{ns}
Inter. AxBxC	0.32^{ns}	1.62^{ns}	11.01^{**}

**significant at the 1% probability level ($p<0.01$); * significant at the 5% probability level ($0.01=<p<0.05$); ns not significant ($p>=0.05$). Different capitals in the same column differ by Tukey test. S.M.D = significant minimum deviation. C.V. = coefficient of variation.

It appears that the behavior of the three genotypes was similar when treated with the alpha-amylase enzyme with a drastic reduction in starch contents. For genotypes CV147 and BRS508, the harvesting of clean stems resulted in an increase in the starch content in the clarified broth, when compared to treatments with whole stems. Considering the action of the enzyme on these biomolecules, it was found that from 102 to 116 of, when the alpha-amylase enzyme was used, for the clarified broth the levels of this biomolecule significantly reduced to around 245.73 $mg.L^{-1}$ for BRS508, and 118mg.L-1 and 120 mg.L-1 for CV147 and CV198 genotypes respectively (Table 3). The reduction of starch contents is desirable since, when present in the wort, it contributes to increasing the viscosity in the juice, causing a decrease in the yield of alcoholic fermentation (Stupiello 2010). The garnet panicles of sweet sorghum are rich in starch and require adjustments for the harvesting operation. According to the structure of the plant, if the elimination of panicles does not occur effectively and it is not possible to separate the mass of grains from the mass of

stalks, adjustments in industrial processes would be necessary to reduce the undesirable compounds present in the panicle, including starch and phenol.

For the CV147 and CV198 genotypes, the interaction between seasons and enzymatic treatment was observed, with the second season having the highest starch values. It is noteworthy that regardless of the harvest time for both genotypes, there was a significant reduction in the contents of this biomolecule when subjected to the clarification process with alpha-amylase.

Table 4 shows the average values obtained for Brix, RRTS, pH, acidity, glycerol, and alcohol content of the wine. It is observed that none of the evaluated parameters were affected by the evaluated genotypes. When the alpha-amylase enzyme was used, for the two periods evaluated, the starch content decreased significantly by approximately 80%, which may have favored the fermentation process since in the treatments where the enzyme was applied, lower values were obtained RRTS and Brix (Table 4).

Table 4. The wine composition, for CV147, CV198, and BRS508 genotypes of sweet sorghum submitted or not to enzymatic treatment, harvested with and without panicles at 102 d.a.s. and 116 d.a.s. Jaboticabal-SP/2013-2014 Harvest.

Harvest (A)	Brix (%)	RRTS (%)	pH	Total Acidity ($g.L^{-1}$ H_2SO_4)	Glycerol (%)	Alcohol content (%)	Fermentation efficiency (%)
CV147 Clean stalks	2.6^{A}	0.35^{A}	4.0^{A}	1.93^{A}	1.84^{A}	6.7^{A}	85^{A}
CV147 Whole stalks	2.7^{A}	0.29^{A}	4.1^{A}	1.96^{A}	1.82^{A}	6.7^{A}	85^{A}
CV198 Clean stalks	2.7^{A}	0.33^{A}	3.7^{A}	2.11^{A}	1.56^{A}	6.9^{A}	86^{A}
CV198 Whole stalks	3.1^{A}	0.35^{A}	3.9^{A}	2.04^{A}	1.58^{A}	7.0^{A}	86^{A}
BRS508 Clean stalks	2.8^{A}	0.30^{A}	3.9^{A}	1.98^{A}	1.79^{A}	7.1^{A}	85^{A}
BRS508 Whole stalks	3.0^{A}	0.32^{A}	4.0^{A}	2.01^{A}	1.60^{A}	6.7^{A}	82^{A}
F Test (A)	1.79^{ns}	0.40^{ns}	1.16^{ns}	0.94^{ns}	1.63^{ns}	2.76^{ns}	0.81^{ns}
SMD	0.59	0.18	0.53	0.30	0.48	0.5	8.46
CV (%)	18.27	47.91	11.67	13.04	24.62	6.74	8.65
Treatment (B)							
Without alpha amylase	3.0^{A}	0.35^{A}	4.0^{A}	1.98^{A}	1.73^{A}	6.9^{A}	84^{A}
With alpha amylase	2.6^{B}	0.30^{B}	3.9^{A}	2.03^{A}	1.67^{A}	6.8^{A}	85^{A}
F Test (B)	35.64^{**}	6.97^{*}	2.34^{ns}	2.32^{ns}	0.35^{ns}	1.46^{ns}	0.86^{ns}
SMD	0.12	0.03	0.09	0.07	0.21	0.16	2.07
CV (%)	10.29	27.62	5.62	9.07	29.61	5.59	5.67
Seasons (C)							
102 d.a.s.	2.7^{A}	0.35^{A}	4.0^{A}	2.23^{A}	2.07^{A}	7.0^{A}	89^{A}
116 d.a.s	2.9^{A}	0.30^{B}	3.9^{A}	1.78^{B}	1.33^{B}	6.7^{B}	80^{B}
SMD	3.23^{ns}	5.13^{*}	0.25^{ns}	39.91^{**}	44.15^{**}	8.65^{**}	46.25^{**}
CV (%)	0.23	0.05	0.20	0.14	0.22	0.24	2.73
F blocks Test	20.21	37.69	12.57	17.32	32.08	8.60	7.73
Inter. AxB	5.65^{**}	3.62^{*}	9.75^{**}	1.77^{ns}	0.22^{ns}	3.73^{*}	4.31^{*}
Inter. AxC	0.92^{ns}	0.81^{ns}	0.49^{ns}	3.25^{*}	0.99^{ns}	2.91^{*}	3.14^{*}
Inter. BxC	2.21^{ns}	0.90^{ns}	1.01^{ns}	1.09^{ns}	0.87^{ns}	0.27^{ns}	4.92^{**}
Inter. AxBxC	0.59^{ns}	1.06^{ns}	1.21^{ns}	0.07^{ns}	0.01^{ns}	0.59^{ns}	1.45^{ns}
	0.08^{ns}	0.36^{ns}	0.10^{ns}	0.23^{ns}	0.42^{ns}	0.88^{ns}	1.65^{ns}

**significant at the 1% probability level (p<0.01); * significant at the 5% probability level (0.01=<p<0.05); ns not significant (p>=0.05). Different capitals in the same column differ by Tukey test. S.M.D = significant minimum deviation. C.V. = coefficient of variation.

The reduction of starch contents is desirable since, when present in the wort, it contributes to increasing the viscosity in the juice. Causing a drop in the yield of alcoholic fermentation Stupiello (2010). In this sense, the enzymatic saccharification step becomes important to eliminate the starch from the stem, thus avoiding problems with wine viscosity that would cause centrifugation difficulties. For this biomolecule, the highest and lowest values were observed in BRS 508 without panicle and panicle, for CV147 and CV198 the levels of this parameter were not influenced by the harvesting system.

Evaluating the action of the enzymatic treatment on the technological characteristics of the wine, it was found that only for Brix and RRTS there were significant differences, indicating that the application of the enzymes alpha-amylase and beta amyloglucosidase resulted in a significant decrease of 0.4% and 0.05% of Brix and RRTS respectively (Table 4). It should be noted that even with the enzymatic treatment there was no total consumption of sugar by yeast for ethanol production. These compounds are probably soluble solids in fermentescible (dextrins) by *Saccharomyces cerevisiae* (PE-2).

Comparing the wines obtained at different harvest times, it was found that at 116 d.a.s there were lower values of RRTS, glycerol, total acidity, and alcohol content. For the fermentative efficiency of the three genotypes studied at 116 AD, it was the time with the lowest fermentative efficiency values close to 80%. This fact may be due to the greater amount of phenols in the broth in the second season, which may have inhibited yeast activity. At 102 d.a.s, fermentation efficiency rates were observed close to 89% (Table 4), this value is similar to those obtained by Ratnavathi et al. (2010) and Tahmina and Capareda (2011), who fermenting sweet sorghum broth obtained efficiencies close to 90%.

The presence of panicles, as well as the enzymatic treatment, promoted an increase in this parameter for genotypes CV147 and BRS508, unlike CV198, which, when subjected to treatment with amylase and glucosidase, resulted in lower rates of fermentative efficiency. An interaction was observed between the genotype, enzymatic treatment, and type of harvest, the CV147 genotype was the one with the highest fermentative efficiency, the presence of panicle did not affect the fermentative efficiency and the enzymatic treatment contributed to its increase. For CV198 genotypes, the presence of panicle and enzyme treatment resulted in less fermentative efficiency. BRS508 was the genotype that showed less fermentative efficiency.

Considering the harvesting system with and without panicles, it was found that for the 3 genotypes, the processing of clean stems resulted in better values of fermentative efficiency in the two evaluated seasons, with the exception of the CV147 genotype. In general, the yield for the treatment with leaves was approximately 11-17% lower than for whole stems, it is noteworthy that this treatment was the one that presented the highest concentration of phenol (Table 2). Such results are superior to those determined by Freita et al. (2014), who, evaluating the processing of sweet sorghum with and without panicle, obtained values of loss of efficiency when processing with leaves of up to 10.75%.

The is Figure 1 shows the average values obtained by genotype for the number of liters of ethanol produced per ton of sweet sorghum processed with and without panicle, and submitted to enzymatic treatment, in two sampling periods. It is observed that the performance of enzymatic treatment promoted greater ethanol production only for the BRS508 genotype when processed without panicle, in the two harvest seasons. This phenomenon may be associated with the highest starch content of this genotype in the broth, in addition to the fact that this genotype had the highest Brix and TRS values (Table 1).

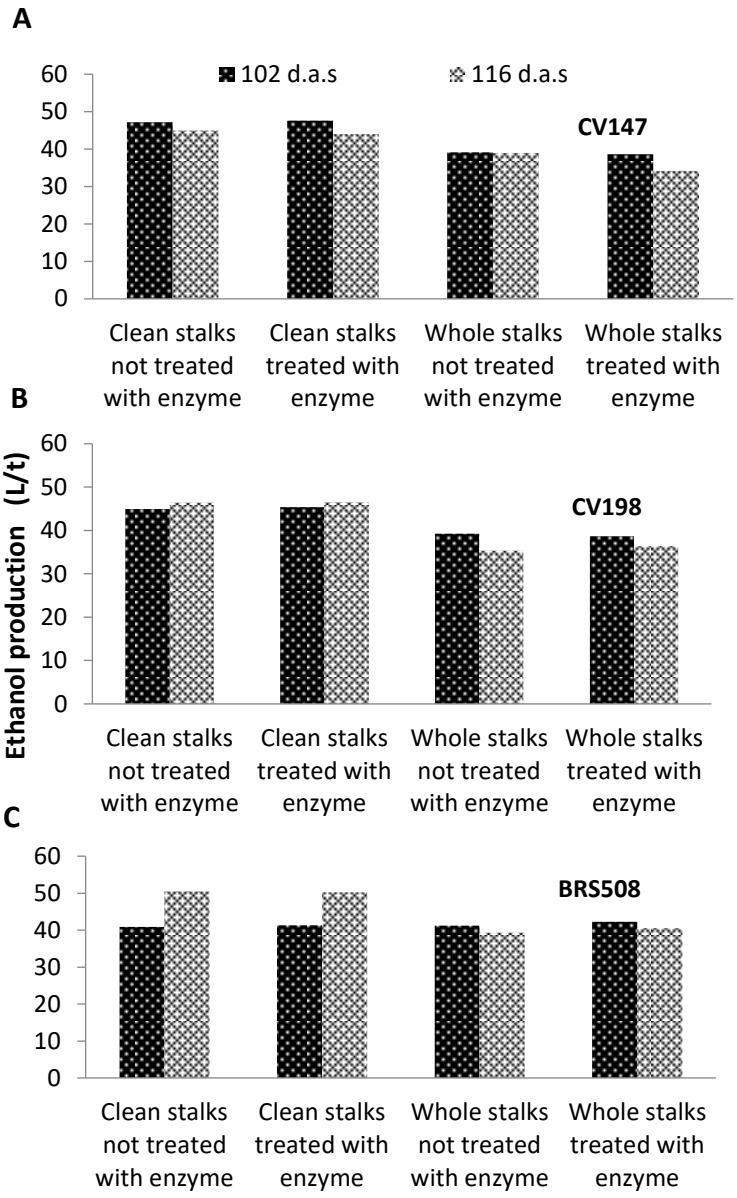

Figure 1. Ethanol production (L t-1), for the sweet sorghum genotypes: A – CV147, B – CV198 and C – BRS508, harvested with and without panicles at 102 a.s. and 116 a.s. Jaboticabal-SP/2013-2014 Harvest.

It is observed that BRS508 was the genotype that presented the highest ethanol productivity in liters per ton of processed sorghum, mainly at 116 d.a.s., without panicle. For CV147 and CV198 at 102 d.a.s, the highest ethanol yields were obtained, 45 and 47 $L.t^{-1}$ respectively, when processed without panicle. The presence of panicle reduced the productivity for the three genotypes evaluated. The reduction in productivity in ethanol/ton resulting from the processing of whole stalks (with leaves and panicles), has already been reported by Ferreira (2014) and Freita (2014).

Considering the ethanol production, it was found that the yields determined in this work are similar to those obtained by Masson (2013) and Ferreira (2014), which determined values between 40 and 53 $L.t^{-1}$, for fermentation of sorghum broth saccharine. In this sense, Embrapa (2012) mentions that this raw material has a production potential of 35 to 40 L t1. It is observed that the performance of enzymatic treatment promoted greater ethanol production only for the BRS508 genotype when processed without panicle, in the two harvest seasons. This phenomenon may be associated with the highest starch content of this genotype in the broth, in addition to the fact that this genotype had the highest values of Brix and TRS (Table 1). Thus, when the enzyme amyloglucosidase was applied before the fermentation process was carried out, there was greater hydrolysis of this molecule, making more sugars available for the yeast to ferment, promoting higher levels of ethanol per ton.

On average, panicle stalk processing reduced ethanol productivity by 10 L for each ton processed, for BRS508 and CV198, and by 7 L for CV147. This reduction in productivity may be a result of the bagasse of this material having higher fiber contents, which may have prevented or hindered the extraction of the juice, reducing the number of liters of juice extracted for the three genotypes in the two evaluated seasons (Figure 1). This behavior was also observed by Almodares and Hadi (2009), who determined that higher levels of fiber in the raw material reduced the amount of sugar extracted.

4. Conclusions

The clarification of the juice with enzymatic treatment increases the quality of the fermentation broth and makes it possible to obtain wines with lower levels of RRTS and Brix. Fermentation efficiency is not affected by the genotype; however, it is influenced by the time of harvest and the technological quality of the juice. The best period of industrialization is at 102 d.a.s., and the processing of whole stalks results in less ethanol production per ton of sorghum.

Authors' Contributions: FERREIRA, O.E.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; COSTA, G.H.G.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; SILVA, A.F.: conception and design, acquisition of data, drafting the article, and critical review of important intellectual content; MONTIJO, N.A.: conception and design, acquisition of data, drafting the article, and critical review of important intellectual content; MUTTON, M.A.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; MUTTON, M.J.R.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

Acknowledgments: The authors would like to thank the funding for the realization of this study provided by the Brazilian agency FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais - Brasil).

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Received: 26 April 2020 | **Accepted:** 11 March 2021 | **Published:** 29 December 2021