ANA CLÁUDIA ALENCAR LOPES

NEW SWEET SORGHUM SPIRIT: YEAST INOCULUM SELECTION, CHEMICAL AND SENSORIAL CHARACTERIZATION

LAVRAS – MG

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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Microbiologia Agrícola, para a obtenção do título de Mestre.

Prof. Dr. Whasley Ferreira Duarte

Orientador

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NOVO DESTILADO DE SORGO SACARINO: SELEÇÃO DO INÓCULO, CARACTERIZAÇÃO QUÍMICA E SENSORIAL

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APROVADA em 17 de julho de 2018.

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RESUMO GERAL

O sorgo sacarino é uma planta capaz de armazenar altos níveis de açúcares solúveis em seu colmo e apresenta tecnologia de processamento similar à cana-de-açúcar, destacando-o como uma matéria prima promissora para utilização em destilarias na produção de aguardente. Este trabalho teve como objetivo a seleção de um inóculo e genótipo de sorgo sacarino apropriado para a produção de uma aguardente de sorgo baseando-se no perfil de compostos voláteis e aceitação sensorial do mesmo. Dois inóculos, somente a *Saccharomyces cerevisiae* e outro inóculo misto composto por *S. cerevisiae* e *Meyerozyma caribbica*, foram testados em uma etapa de triagem quanto a sua capacidade fermentativa. Somente os inóculos e genótipos que apresentaram o melhor desempenho fermentativo foram utilizados na etapa posterior de produção da aguardente. Os destilados de sorgo sacarino obtidos foram caracterizados quimicamente quanto à sua composição de compostos aromáticos por cromatografia gasosa e sensorialmente por 50 voluntários não treinados. Ambos inóculos e o genótipo BRS 506 foram selecionados durante a etapa de triagem devido à alta conversão de etanol, rendimento, eficiência e produtividade. Cinquenta e cinco compostos voláteis foram identificados em ambos destilados. A aguardente de sorgo sacarino produzido somente com *S. cerevisiae* resultou em 9249,35±77,75 µg/L de compostos voláteis, sendo em sua grande maioria ácidos, aldeídos e terpenos. Por outro lado, a aguardente produzida com inoculo misto apresentou em total 15536,00±0,79 µg/L, sendo em grande maioria ésteres e álcoois superiores. De modo geral, independentemente do inóculo utilizado, o sorgo sacarino mostrou-se um substrato promissor para a produção de aguardente. Ainda, a introdução da *M. caribbica* como co-inoculante resultou no enriquecimento do perfil de compostos voláteis e aceitação sensorial do destilado, principalmente em relação a intenção de compra dos voluntários.

Palavras-chave: Não-*Saccharomyces*. Inóculo misto. Compostos voláteis.

ABSTRACT

Sweet Sorghum is a crop able to store high levels of soluble sugars in its culm and presents processing technology similar to sugarcane, being a promising raw material for use in distilleries in the production of spirit. This study aimed to select an inoculum and sweet sorghum genotype suitable to produce a sweet sorghum distilled spirit considering its volatile compounds profile and sensorial acceptance. Two inocula, only *Saccharomyces cerevisiae* and another mixed inocula comprising the *S. cerevisiae* and *Meyerozyma caribbica*, were tested in a screening test according to their fermentative capacity. Only the inocula and sweet sorghum genotypes that presented the best performance were used in the next phase to produce the distilled beverage. The obtained sweet sorghum spirits were chemically characterized as to their volatile compounds profile by gas chromatography and sensorial characterized by 50 untrained volunteers. Both inocula and the BRS 506 genotype were selected during the screening due to their high ethanol conversion, yield, efficiency and productivity. Fifty-five volatile compounds were identified in both spirits. The sweet sorghum spirit produced with *S. cerevisiae* resulted in 9249.35±77.75 µg/L of volatiles, being most of them volatile acids, aldehydes and terpenes. On the other hand, the spirit fermented with the mixed inoculum presented a total of 15536.00±0.79 µg/L, being most of the esters and higher alcohols. Overall, regardless of the inocula, sweet sorghum proved to be a promising substrate for a distilled beverage. Even more, the inclusion of *M. caribbica* as a co-inoculant resulted in the enrichment of the volatile compounds profile and sensorial acceptance of the spirit, mainly in relation to the volunteers' intention of purchase.

Key words: Non-*Saccharomyces*. Mixed inoculum. Volatile compounds.

SUMÁRIO

1 INTRODUÇÃO

O sorgo sacarino (*Sorghum bicolor (L.) Moench*) é uma planta com alta adaptabilidade a condições ambientais adversas, como resistência a seca, calor e diferentes tipos de solo. Apresenta cultivo relativamente curto e facilitado por sementes, favorecendo o seu plantio mecanizado (REZENDE; RICHARDSON, 2017). A viabilidade técnica e econômica do sorgo sacarino chama a atenção para sua utilização na produção de aguardente, pois é uma planta com alta produtividade de biomassa, altas concentrações de açúcares em seu colmo e baixa necessidade de investimento. Ainda, destaca-se por suas semelhanças morfológicas e tecnologia de processamento similares à cana-de-açúcar (BARCELOS et al., 2016).

O sorgo apresenta ciclo de vida curto, variando entre 120 e 130 dias. Sua colheita ocorre principalmente nos meses de março e abril, período em que as destilarias estão ociosas devido à entressafra canavieira. O seu período de plantio e possibilidade da utilização do maquinário e instalações já presentes em destilarias, permitem que as indústrias antecipem e ampliem o período de moagem. Todas essas características contribuem para a possibilidade de utilização do sorgo sacarino como complemento à cana-de-açúcar e expansão da capacidade de indústrias de aguardente já estabelecidas ou sua utilização em regiões nas quais a cana-de-açúcar não se desenvolve bem (REGASSA & WORTMANN¸ 2014).

Excluindo os obstáculos agronômicos para utiliza-lo como matéria prima, como o controle de pragas e crescimento muito rápido das plantas, o principal gargalo na utilização do sorgo sacarino em processos fermentativos é avaliação de microrganismos fermentadores que se adaptam as características da matéria prima. Apesar de diferentes fatores interferirem na qualidade de um produto fermentado, a cepa da levedura utilizada e as condições de fermentação são os principais fatores para o rendimento de etanol e características organolépticas de bebidas (ANACLETO et al., 2017). Além do etanol e CO_2, os metabólitos secundários produzidos durante a fermentação são os principais responsáveis pelo aroma e sabor de bebidas fermentadas (FLAGFELDT et al., 2009).

O uso de inóculo selecionado é uma prática comum nas indústrias de cerveja e vinho, mas ainda não é totalmente estabelecido na produção de aguardente. A maioria das destilarias fazem o uso da fermentação espontânea, processo dependente da microbiota presente no ambiente e matéria prima (DA CONCEIÇÃO et al., 2014). A fermentação espontânea é um processo conduzido sem o controle da população de microrganismos presente durante a produção. Leveduras não-*Saccharomyces* estão presentes no início do processo e conforme aumenta-se a concentração de etanol a *Saccharomyces cerevisie* domina a fermentação. Essa

diversidade microbiana é responsável pelas características sensoriais do produto, mas também podem contribuir para o crescimento de contaminantes, inconsistência no aguardente e fermentações lentas ou interrompidas (BADOTTI et al., 2010).

A presença de leveduras não-*Saccharomyces* em processos fermentativos de forma controlada tem se mostrado capaz de aumentar a produção de compostos aromáticos de interesse, principalmente ésteres e álcoois superiores (OLIVEIRA et al., 2005). Em especial, trabalhos anteriores utilizando a co-inoculação da *Meyerozyma caribbica* e *S. cerevisiae* para a produção de cachaça mostraram que os teores de compostos aromáticos de interesse e a aceitação sensorial é consideravelmente maior do que quando comparado ao uso de somente a *S. cerevisiae* (AMORIM et al., 2016; DUARTE et al., 2013). Ainda, destaca-se a importância do estudo de matérias primas alternativas para a descentralização do consumo da cana-de-açúcar e desenvolvimento de inóculos alternativos para esse mercado.

Para o nosso conhecimento, este é o primeiro trabalho que relata a utilização do sorgo sacarino e inóculo misto para produção de uma aguardente de sorgo. Desta forma, este trabalho teve como objetivo selecionar o melhor inóculo e genótipo de sorgo sacarino para produção de uma aguardente de sorgo sacarino, caracterizar os destilados quimicamente e sensorialmente.

2 CONSIDERAÇÕES GERAIS

No presente trabalho foi realizada a seleção de um inóculo e genótipo de sorgo sacarino apropriado para a produção de uma aguardente de sorgo sacarino. Ambos inóculos, em especial o inóculo misto composto por *S. cerevisiae* e *M. caribbica*, foram capazes de fermentar o sorgo sacarino resultando em uma alta produção de etanol e compostos voláteis com características organolépticas desejáveis. A caracterização química e sensorial dos destilados de sorgo sacarino demonstraram que o mesmo é uma matéria-prima com alto potencial para utilização como substituto ou implemento à cana-de-açúcar na produção de aguardente.

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SEGUNDA PARTE – ARTIGO

ARTIGO 1 - New sweet sorghum spirit: yeast inoculum selection, chemical and sensorial characterization

Normas do periódico LWT - Food Science and Technology

Artigo submetido e sob revisão no periódico LWT - Food Science and Technology

New sweet sorghum spirit: yeast inoculum selection, chemical and sensorial characterization

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Abstract

This study aimed to select an appropriate inoculum and sweet sorghum genotype for the production of a novel spirit. An inoculum of *S. cerevisiae* and mix with *Saccharomyces cerevisiae* and *Meyerozyma caribbica* were tested for the fermentation of sweet sorghum genotypes BRS 506 and BRS 508. Both inocula and genotype BRS 506 were selected to produce the sweet sorghum spirit due to their high sugar conversion, ethanol yield, efficiency and productivity. Fifty-five volatile compounds were identified by GC-MS, most of them being esters and higher alcohols, which are desirable due to their fruity aromatic descriptors in distilled beverages. The sweet sorghum spirit produced with *S. cerevisiae* presented more volatile acids (1885.10 μg/L), aldehydes (66.39 μg/L) and terpenes (976.20 μg/L). In contrast, the spirit produced with mixed inocula showed 11604.25 μg/L of esters and 1942.95 μg/L of higher alcohols. The mixed inoculum improved the production of desirable volatile compounds, resulting in slightly greater acceptance in the sensorial analysis with a higher index of purchase intention. The sweet sorghum proved to be a good substrate to produce a distilled beverage.

Keywords: non-*Saccharomyces*, mixed inoculum, *Sorghum bicolor*, volatile compounds, distilled beverage

1 Introduction

Sweet sorghum (*Sorghum bicolor (L.) Moench)* is a crop with high biomass productivity, high sugar concentration (Barcelos, Santa Anna, & Pereira, 2016) and high adaptability to adverse environmental conditions. In addition, it presents relatively short crop period, which is facilitated using seeds, that also consequently, allows the use of mechanized planting (Rezende & Richardson, 2017).

Even more, sweet sorghum harvest period, enables its association with the sugarcane off-season, period when distilleries are stagnant, consequently, avoiding a drop in the production of distilled beverages (Bunhan, Jaisil, Sanitchon, Knoll, & Anderson, 2015). The technical and economic feasibility of sweet sorghum draws attention to its use as an alternative substrate for alcoholic fermentation.

Although the use of *S. cerevisiae* as a starter culture is currently a technology widely used in the production of alcoholic beverages such as cachaça and other spirits, the dominance of a single species results in the standardization of the final product between different seasons and reduces proliferation of contaminants. However, it decreases the variability of organoleptic characteristics of distilled beverages (Campos et al., 2010).

The presence of non-*Saccharomyces* in fermentative processes results in greater production of volatile compounds of interest (Oliveira, Rosa, Morgano, & Serra, 2005). Indeed, controlled mixed inoculations with *S. cerevisiae* and non-*Saccharomyces* has been shown as a possibility to increase the aroma complexity and sensorial characteristics of fermented beverages due to higher production of secondary metabolites (Whitener et al., 2017; Portugal, Alcarde, Bortoletto, & Silva, 2017). Previous studies using co-inoculation of *Meyerozyma caribbica* and *S. cerevisiae* to produce sugarcane spirit reported that the variety and quantity of volatile compounds of interest were considerably higher than when using pure *S. cerevisiae* (Amorim, Schwan, & Duarte, 2016; Duarte, Amorim, & Schwan, 2013). Here, to the best of

our knowledge, this is the first report on the use of sweet sorghum and mixed inoculum of different yeast to produce a distilled beverage. Hence, this study aimed to select the best combination of sweet sorghum genotype and yeast inoculum to a new spirit and characterize the produced beverage.

2 Material and methods

2.1 Sweet Sorghum harvesting and storage

Two genotypes of sweet sorghum, BRS 506 and BRS 508 were harvested after 150 days of planting at the farm Muquém, municipality of Lavras, MG – Brazil. The sorghum stalks were harvested manually, and its juice was extracted by mechanical milling. The obtained juice was decanted, filtered to remove solid particles and stored at -20 °C until its use (Bunphan et al., 2015).

2.2 Microorganisms and inoculum preparation

Previous studies using the mixed inoculation of *M. caribbica* and *S. cerevisiae CA11* in the cachaça production resulted in a high production of higher alcohols, esters and others desirable volatile compounds (Duarte et al., 2013). Therefore, these yeasts were chosen to be evaluated in the fermentation of sweet sorghum juice. The inocula were prepared with subsequent cultures in increasing volumes of YPD medium as described by Amorim et al. (2016), until obtaining populations of o 10^7 cells/mL *S. cerevisiae* and 10^8 cells/mL *M. caribbica.*

2.3 Screening of sweet sorghum genotypes and yeast inoculum

Both sweet sorghum genotypes, BRS 506 and BRS 508, were fermented with only the *S. cerevisiae*, and with mixed inoculum of *S. cerevisiae* and *M. caribbica*. The sweet sorghum

juices previously sterilized (121 °C, 15 min) were inoculated and the flasks were incubated at 28 °C without agitation, for 24 h (Bunphan et al., 2015). Fermentations were performed in duplicate. Sampling was collected at 0 and 24 h for the determination of glucose, fructose, sucrose and ethanol by HPLC. The data obtained from HPLC analysis were used to calculate ethanol yield ($Y_{p/s}$), ethanol conversion efficiency (Ef), sugar conversion (Conv) and ethanol volumetric productivity (Q_p) as described by Oliveira et al. (2005) and Duarte et al. (2010).

2.4 Sweet sorghum spirit production

Once defined the most efficient combination of inoculum and sweet sorghum genotype, a new fermentation was performed to produce the distilled beverage. The fermentative process was conducted in a fed-batch as described by Amorin et al. (2016) to avoid yeast cell stress due to high sugar concentration. Fermentations were carried out in duplicate until °Brix stabilization. After yeast cells decantation by gravity, the fermented juice was distilled according to Sampaio et al. (2013). The distillate was collected until reaching 42 °GL. The final product was stored in glass bottles until HS SPME GC-MS and sensorial analysis.

2.5 HPLC analysis

Glucose, fructose, sucrose and ethanol were quantified by HPLC. The analyses were performed in a Shimadzu chromatograph (Shimadzu Corp., Japan) equipped with an ion exclusion column Supelcogel 8H (7.8 mm X 30 cm - Supelco, Bellefonte, PA, USA) and refractive index detector (RID-10A). The column was operated at 30 °C and eluted with 5 mM sulfuric acid at 0.5 mL/min. Compounds identification was done by comparing the retention times of peaks in sample with those of pure standard injected under same conditions while quantification was performed by external calibration. All samples were evaluated in duplicate (Andrade, Melo, Genisheva, Schwan, & Duarte, 2017).

2.6 HS SPME GC-MS analysis

Vials of 15 mL were used to dilute 1 mL of sample in 4 mL of deionized water containing 0.25 g NaCl. Volatile compounds were extracted by solid phase micro extraction (SPME) at 60 °C for 25 min with a 50/30 µm DVB/Carboxen/PDMS Stable flex SPME (Supelco, Bellefonte, PA, USA) fiber in a manual holder (Amorim et al., 2016).

The volatile compounds were analysed using a GC-MS-QP2010 Plus (Shimadzu) with a Rtx-5MS (30 m x 0.25 mm x 0.25 µm) column. Thermal desorption in the injector was at 270 °C for 100 sec. The system was initially operated at 35 °C and increment of 4 °C/min until 240 °C, being helium the carrier gas at 1.78 mL/min. Injections were in splitless mode (30 s at 25 psi) opened for 100 s (Zacaroni, de Sales, Cardoso, Santiago, & Nelson, 2017). Compounds were identified using NIST library 2011, and their concentrations were expressed as equivalents with 4-nananol, used as an internal standard at a final concentration of 125µg/L (Amorim et al., 2016).

2.7 Sensory analysis

The obtained beverages were submitted to sensory analysis by 50 untrained volunteers. The samples were composed by a mixture of both duplicates in equal ratios. The beverages were evaluated according to their aroma, flavor and global impression using a hedonic scale from 1 to 9, being 1 extremely dislike and 9 extremely like. Also, samples were evaluated due to tasters' purchase intention in a scale from 1 to 5, being 1 certainly would not buy and 5 certainly would buy (Lutz, 2008).

2.8 Statistical analysis

Analyses of variance (ANAVA) and Scott-Knott test were performed using Sisvar 5.6 (Lavras, MG). The Principal Component Analysis was performed using the software Past 3.0 (Oslo, Norway).

3 Results and discussion

3.1 Sweet sorghum

The table 1 shows the content of sucrose, glucose and fructose of sweet sorghum juice. Genotype BRS 506 presented a proportion 95.87 % sucrose, 3.83 % glucose and 3.02 % fructose while genotype BRS 508 had 95.88 % glucose, 3.82 % glucose and 2.93 % fructose. Here, it was observed that the sugars composition from the analyzed genotypes presented high similarity to the sugarcane profile, with a high sucrose concentration, and the remaining divided into glucose and fructose, supporting the proposition of the great sweet sorghum potential to be used as substrate for alcoholic fermentation.

3.2 Sweet sorghum microfermentations

As shown in table 2, ethanol concentration, sugar consumption, $Y_{p/s}$, Ef and Q_p ranged according to the sweet sorghum genotype and inoculum used in the fermentation. To determine the total concentration of fermentable sugars in the substrate, sucrose concentration was mathematically converted to fructose and glucose.

Considering the overall mean of the fermentations, a significantly ($p<0.05$) higher sugar consumption (95.97%) was observed when the BRS 506 genotype was used (Table 2). Analyzing sugar consumption for each genotype separately, there was no significant difference between the used inocula for the BRS 506 genotype (Table 2). However, for the BRS 508 genotype, there was a significantly higher consumption of 94.40 % when the must was inoculated with the *S. cerevisiae*. This genotype with mixed inoculum was the one that

presented the lowest sugar consumption (89.36 %), and consequently, lower ethanol production (71.53 g/L) (Table 2). The above mentioned sugar consumption, regardless the genotype or inoculum, were higher than those reported by Duarte et al., (2013) using the same inocula, which demonstrates the potentiality of the yeasts for sweet sorghum fermentation.

There was higher ethanol production using the BRS 506 genotype, regardless the used inocula, with an overall mean for both inocula of 85.85 g/L, while BRS 508 genotype presented an overall mean of 76.70 g/L. When evaluating the inocula separately, BRS 506 with *S. cerevisiae* showed the highest ethanol concentration (87.48 g/L), while BRS 508 had the lowest ethanol concentrations for the mixed inoculum (71.53 g/L) (Table 2). It is interesting to note that compared to sugarcane, all fermentations, except the one using BRS 508 with mixed inoculum, showed higher ethanol concentrations than those reported by Amorin et al. (2016) and Duarte et al. (2013) using the same yeasts strains, highlighting the possibility to use sweet sorghum as an alternative substrate to produce distilled beverages. It was also observed that the obtained values were higher than those reported by Barcelos et al. (2016) and Bunphan et al. (2015) using sweet sorghum, whose ethanol concentrations were respectively 72.0 g/L and 59.8 g/L.

In relation to $Y_{p/s}$, there were no significant difference between both genotypes when considering the overall mean (Table 2). The only significant difference was found for the combination of BRS 508 with *S. cerevisiae* that presented $Y_{p/s}$ of 0.49 g/g (Table 2). The found $Y_{p/s}$ for BRS 506 and BRS 508 genotypes with *S. cerevisiae* or mixed inocula were higher than those reported when using sugarcane and different *S. cerevisiae* strains (Gomes, Silva, Marini, Oliveira, & Rosa, 2007; Marini et al., 2009; Silva et al., 2009). Furthermore, the sweet sorghum with *S. cerevisiae*, for both tested genotypes, showed higher $Y_{p/s}$ than sugarcane fermentation with the same yeast (Duarte et al., 2013). As this kinetic parameter is directly related to ethanol content, the values found for the different combinations of sweet sorghum genotypes and

inocula were in agreement with ethanol concentration reported above and consequently reinforce the viability of using sorghum for alcoholic fermentation.

The BRS 506 genotype showed Ef values statistically similar for both inocula. However, BRS 508 inoculated with *S. cerevisiae* presented Ef significantly higher (95.74 %) than its mixed inoculum (86.05 %) (Table 2). Similar to the $Y_{p/s}$ results, using sweet sorghum as substrate, *S. cerevisiae* showed higher efficiency when compared to sugarcane fermentation in the works of Gomes et al. (2007), Marini et al. (2009) and Silva et al. (2009).

The overall mean of Q_p for BRS 506 genotype (3.58 g/L h) was significantly higher than BRS 508 (3.20 g/L h) (Table 2). However, there was no significant difference when considering each inocula for each genotype. It is important to highlight that all Q_p values found here, were higher than those reported by Duarte et al. (2013) using the same inocula to ferment sugarcane juice.

Taking into account the above-mentioned results, the BRS 506 genotype with *S. cerevisiae* was selected for further tests. The BRS 506 genotype with mixed inoculum and BRS 508 genotype with *S. cerevisiae* presented similar results. However, it was possible to choose the BRS 506 with mixed inoculum due to its superior ethanol content. Therefore, the following tests were performed using the BRS 506 genotype with *S. cerevisiae* and mixed inocula.

3.3 Production and characterization of sweet sorghum spirit

3.3.1 Aromatic volatile compounds

Fifty-five volatile compounds were identified, being 24 esters, 11 alcohols, 9 terpenes, 6 acids, 3 aldehydes and 2 acetals. In general, the sweet sorghum spirit produced with mixed inoculum presented higher concentrations of esters, alcohols and acetals, while the one with *S. cerevisiae* showed higher concentrations of volatile acids, aldehydes and terpenes (Table 3). Compared to the volatile profiles found for sugar cane spirits in the works of Amorim et al.

(2016) and Duarte et al. (2013), the sweet sorghum spirit produced with mixed inoculum resulted in higher concentrations of esters, alcohols, and terpenes, in addition to decreasing the production of undesirable compounds, such as volatile acids and aldehydes. These results reinforce the fact that the use of *M. caribbica* in a mixed inoculum contributes to the production of desirable aromatic volatile compounds, even when using different substrate such as sweet sorghum, sugarcane (Amorim et al., 2016; Duarte et al., 2013) and grapes (Zuehlke, Glawe, & Edwards, 2015).

Among the 24 identified esters, 11 were ethyl esters, which are the main volatile compounds to provide floral and fruity aroma to distilled beverages. All of them were found at higher concentrations in the sweet sorghum spirit with mixed inoculum. As already reported in previous studies using the same inocula with sugarcane, the spirit produced with *M. caribbica* and *S. cerevisiae* tends to result approximately twice the ester content when compared to the spirit using only *S. cerevisiae* (Amorim et al., 2016; Duarte et al., 2013). A similar situation can be reported here when using this inoculum and sweet sorghum. The spirit produced with mixed inoculum presented 11604.25 µg/L of esters while the one with *S. cerevisiae* resulted in 4791.49 µg/L. The dominant esters in the sweet sorghum spirit with mixed inoculum, in decreasing order of concentration, were the ethyl decanoate (5990.04 µg/L; fruity, grape, woody), ethyl octanoate (2266.81 µg/L; fruity, sweet) and ethyl dodecanoate (1042.50 µg/L; fruity, sweet). The sweet sorghum spirit produced with *S. cerevisiae* also had these esters in more abundance; however, their concentrations were lower.

The 2-pheylethyl acetate (floral, sweet, honey) was detected at 92.18 µg/L and 22.23 µg/L in the sweet sorghum spirit produced with mixed and *S. cerevisiae* inocula, respectively. Other esters, such as isopentyl hexanoate, isobutyl octanoate, isoamyl decanoate, also responsible for floral aroma, were found at considerably higher concentrations in the sweet sorghum spirit with mixed inoculum (Table 3).

As the second most abundant class, higher alcohols can present positive or negative effects in the final product depending on their concentration (Scacco et al., 2012). The sweet sorghum spirit with mixed inoculum showed 1942.95 µg/L of higher alcohols, while the one produced with *S. cerevisiae* had 1523.46 µg/L (Table 3).

Isoamyl alcohols, 2-methy-1-butanol and 3-methyl-1-butanol, were the most abundant higher alcohols in both spirits. Despite the similar content of 3-methyl-1-butanol, the sweet sorghum spirit with mixed inoculum had approximately three times (508.21 µg/L) more 2-methyl-1-butanol than the one with *S. cerevisiae* (133.96 µg/L) (Table 3).

Phenylethyl alcohol was found at concentrations of 112.22 µg/L and 104.43 µg/L in the sweet sorghum spirit with mixed and *S. cerevisiae* inocula, respectively. Furthermore, the sweet sorghum spirit with mixed inoculum presented higher concentrations of 1-octanol, 1-decanol and 1-hexadecanol, all of them described with floral and fruity aromas. This higher alcohol profile is consistent with studies already described to spirits using sugarcane as the substrate. Higher concentrations of 2-methyl-1-butanol and 3-methyl-1-butanol are followed by aromatics alcohols like the 2-phenylethanol, all of them with considerable positive influence at the flavor and aroma in the final product (Capobiango, Oliveira, & Cardeal, 2012; Dato, Pizauro Júnior, & Mutton, 2005; Portugal et al., 2017).

In the case of terpenes, the sweet sorghum spirit produced with *S. cerevisiae* showed higher concentrations (976.20 µg/L) than the sweet sorghum spirit produced with mixed inoculum (751.98 µg/L). These volatile compounds are usually described as floral, herbal and citrus, which can change depending on the substrate due to the presence of different precursors (Whitener et al., 2017), but also depend on the yeast activity.

There was a higher farnesol, nerolidol and dinydrofarnesol production in the sweet sorghum spirit produced with *S. cerevisiae*, being their concentrations 389.07 µg/L, 335.99 µg/L and 147.46 µg/L, respectively (Table 3). The β-farnesene was detected only in the sweet

sorghum spirit with mixed inoculum (31.00 µg/L). All of these compounds are described with pleasant aromas and positive contribution to distilled beverages. This terpenoids profile is consistent with other studies that evaluated terpenoids production in wine and distilled beverages. In a distilled beverage made from steamed sorghum grains fermented with *S. cerevisiae* and four non-*Saccharomyces*, Wu, Zhu, Wang, & Xu (2015) found that nerolidol and farnesol were produced in much higher concentrations by *S. cerevisiae*.

The sweet sorghum spirit produced with *S. cerevisiae* showed 1885.10 µg/L of volatile acids, and the spirit with mixed inoculum had 1180.42 µg/L. The presence of volatile acids in distilled beverages is characterized in a negative way with rancidity notes when in concentrations higher than 20 µg/mL (Costa, Nicolli, Welke, Manfroi, & Zini, 2015).

Dodecanoic, decanoic and hexadecanoic acids were found in higher concentrations in the sweet sorghum spirit produced by *S. cerevisiae*. Even more, the decanoic acid, responsible for fatty and rancid notes, was present at 585.11 µg/L in the sweet sorghum spirit produced with *S. cerevisiae*, and only 9.55 µg/L in the one with mixed inoculum (Table 3).

Three aldehydes, nonanal, 2-nonenal and decanal, were found in the produced sweet sorghum spirits. Overall, they were at 66.39 µg/L in the sweet sorghum spirit produced with *S. cerevisiae* and 39.12 µg/L in the one with mixed inoculum. The lower aldehyde concentration in both spirits is possible due to the correct separation of the "head" and "heart" fractions during the distillation process.

Altogether, both sweet sorghum spirits showed a profile of volatile compounds similar to those reported for spirits using sugarcane juice. This similarity confirms the possibility of using sweet sorghum as an alternative substrate for sugarcane to produced distilled beverages.

The PCA analysis of volatile compounds showed that sweet sorghum spirit with mixed inoculum was more associated with esters while the one produced only with *S. cerevisiae* was grouped with aldehydes and volatile acids (Fig. 1). The sweet sorghum spirit produced with

mixed inoculum was correlated mainly with ethyl octanoate (7), ethyl dodecanoate (19) and ethyl hexadecanoate (23), which are compounds responsible for fruity aromas in distilled beverages. In contrast, the sweet sorghum spirit produced by *S. cerevisiae* was characterized by the presence of terpenes such as farnesol (41) and (E,E)-farnesol (43), and aldehydes the 2-nonenal (52) e decanal (53). While terpenes are associated with green and citrus aromas, volatile acids are responsible for undesirable aromas of rancid (Czerny et al., 2008). Considering the inocula used in this experiment, the sweet sorghum spirit produced with mixed inoculum had a higher diversity and concentration of volatile compounds than the spirit fermented with *S. cerevisiae,* which is consistent with previous studies using the same yeasts, but sugarcane as substrate. The inclusion *M. caribbica,* resulted in an increased production of desirable volatile compounds and reduction of undesirable compounds contributing positively to the sensorial characteristics of the beverage as showed below.

3.3.2 Sensorial analysis

The average scores for the sweet sorghum spirit produced with mixed inoculum in relation to aroma, flavor and global appearance were 6.02, 5.08 and 5.62, respectively, while the sweet sorghum spirit fermented only with *S. cerevisiae* scored 5.94, 4.98 and 5.68. Furthermore, the distilled beverages were evaluated according to the volunteers' intention of purchase, but in a scale ranging from 1 to 5. The sweet sorghum spirit produced with mixed inoculum scored 3.24 while the one fermented with *S. cerevisiae* scored 2.87.

Since the sweet sorghum spirit is a novel product and the volunteers were not trained, differences that were highly detected between both spirits considering their chemical profile were not perceived in the same proportion in the sensorial analysis. Considering the analyzed attributes, especially aroma and flavor, it was possible to note a slightly higher acceptance of the spirit with mixed inoculum. This agrees with the results obtained in the volatile compounds

analysis. However, the highest expression of preference by the volunteers regarding the sweet sorghum spirit produced with mixed inoculum was verified on the attribute intent of purchase, which presented a considerable higher score than the one produced only with *S. cerevisiae*.

4 Conclusions

The tested sweet sorghum genotypes presented sugar composition similar to sugarcane allowing their fermentation by the used yeast inocula. The evaluated inocula presented better fermentative performance when using the genotype BRS 506, being sweet sorghum spirit produced with *S. cerevisiae* characterized by a higher content of volatile acids, aldehydes and terpenes; while the one produced with mixed inoculum presented higher concentration and diversity of higher alcohols and esters. Consequently, the spirit produced by mixed inoculum showed greater acceptance and purchase intent in sensorial analysis. The obtained results confirmed the possibility of using sweet sorghum and mixed inoculum of *M. caribbica* and *S. cerevisiae* to produce a new spirit, which in addition to reaching new market niches, can also help in minimizing the costs of production in distilleries, for example, in the case of sugar cane.

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Conflict of interest

The authors declare no competing financial interest

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Figure Captions

Figure 1 – Principal component analysis (PCA) of volatile compounds in sweet sorghum spirits produced with *S. cerevisiae* and mixed inoculum by GC-MS

Figures

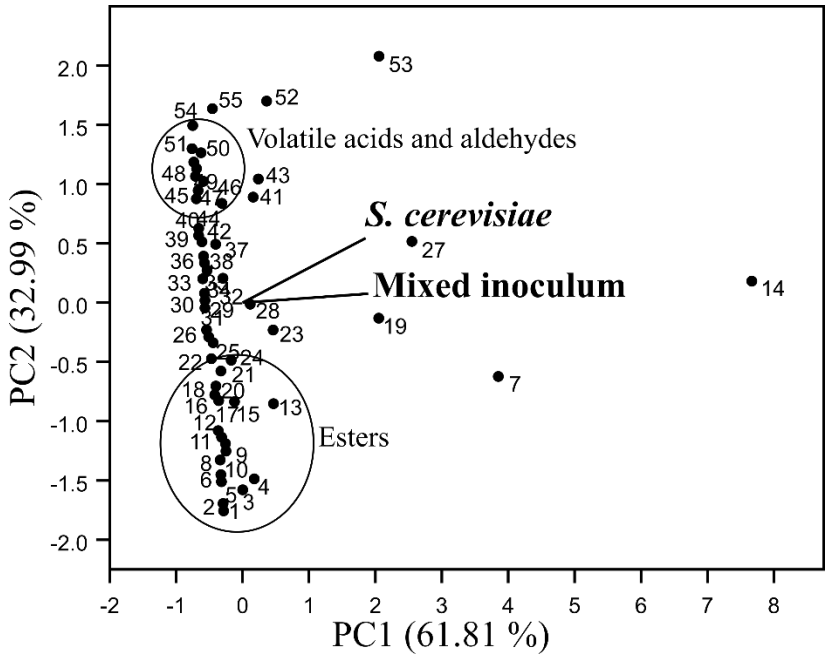

Figure 1

467 **Table 1 –** Sugar content of sweet sorghum juice of genotypes BRS 506 and BRS 508

Genotype	Sugars			Total sugars (g/L)
	Sucrose	Glucose	Fructose	
BRS 506	166.83[a] ± 3.32	7.04 [a] ± 1.33	5.53 [a] ± 0.13	183.22 [a] ± 4.81
BRS 508	164.06 [a] ± 10.20	6.85 [a] ± 0.90	5.27 [a] ± 0.17	180.09 [a] ± 9.82

468 Values followed by the same are not significantly different by the Scott-Knott test ($p>0.05$)

469
470 **Table 2** – Sugars, ethanol concentrations, and kinetics parameters of microfermentations of sweet sorghum
471 with mixed inoculation and pure *S. cerevisiae*
472

Genotype	Inoculum	Compounds		Kinetics parameters			
		Total sugars (g/L)	Ethanol (g/L)	Conv. (%)	$Y_{p/s}$ (g/g)	Ef (%)	Q_p (g/L h)
BRS 506	*S. cerevisiae*	181.16 ± 1.25	87.48 ± 3.48	97.36^{a} ± 1.32	0.50^{a} ± 0.01	97.23^{a} ± 1.88	3.64^{a} ±0.14
	Mixed	181.34 ± 7.71	84.23 ± 4.87	94.59^{a}± 2.56	0.49^{a} ± 0.02	96.30^{a} ± 4.08	3.51^{a} ± 0.20
	Mean	181.25 ± 4.51	85.85±3.93	95.97^{B}±2.30	0.49^{A}±0.01	96.77^{A}±2.65	3.58^{B}±0.16
BRS 508	*S. cerevisiae*	177.63 ± 1.83	81.87 ± 0.93	94.40^{b} ±0.08	0.49^{b} ± 0.00	95.74^{b} ± 0.19	3.41^{a}±0.04
	Mixed	182.25 ± 1.79	71.53 ± 6.09	89.36^{a} ± 1.48	0.44^{a} ± 0.03	86.05^{a} ± 5.06	2.98^{a} ± 0.25
	Mean	179.94±3.05	76.70±6.95	91.88^{A}±3.03	0.46^{A}±0.03	90.89^{A}±6.31	3.20^{A}±0.29

473 Values followed by the same are not significantly different by the Scott-Knott test (p>0.05); uppercase letter for the total mean of genotypes;
474 lowercase letter for the unfolding of inoculum within each genotype.

475 **Table 3**– Concentrations (μg/L) of volatile compounds in sweet sorghum distilled spirit with mixed inoculation and pure *S. cerevisiae*

No.	Compounds	LRI_{calc}	LRI_{lit}	*S. cerevisiae*	Mixed inoculum	Aromatic descriptors
	Esters					
1	Ethyl 2-methylbutanoate	849	850[c]	0.70±0.32	ND	Fermented apple[c], fruity[k]
2	Ethyl 3-methylbutanoate	853	853[e]	1.90±0.23	ND	Apple[d], fruity[k]
3	3-Methylbutyl acetate	877	877[c]	86.24±9.03	171.02±22.16	Banana[c], pear[d]
4	Ethyl hexanoate	1000	1000[c]	133.88±21.95	281.76±4.16	Sweet[a], fruity, green[k]
5	Heptyl acetate	1112	1113[g]	3.41±0.07	3.86±0.17	-
6	Ethyl 4-octanoate	1188	1188[f]	ND	14.49±1.78	-
7	Ethyl octanoate	1196	1201[g]	1189.59±10.41	2266.81±225.83	Fruity, sweet[a]
8	Isopentyl hexanoate	1248	1250[f]	2.53±0.18	20.53±0.24	Fruity[d]
9	2-Phenylethyl acetate	1254	1234[h]	22.23±1.90	92.18±7.40	Flowery[a],rose[d], honey[j]
10	Ethyl 3-nonenoate	1288	NI	25.75±1.37	86.69±6.10	-
11	Ethyl nonanoate	1293	1295[g]	16.13±3.53	51.22±4.72	Fruity[d]
12	Isobutyl octanoate	1345	1348[b]	7.11±0.73	23.29±2.53	-
13	Ethyl 9-decenoate	1385	1382[e]	270.97±24.79	416.34±29.60	Rose[a]
14	Ethyl decanoate	1397	1397[c]	1627.97±210.62	5990.04±494.85	Fruity, grape[a], woody[c]
15	3-Methylbutyl octanoate	1444	1447[g]	107.41±1.92	117.84±3.22	pineapple[d]
16	2-Methylbutyl octanoate	1446	1446[g]	21.51±1.31	47.07±4.43	-
17	Propyl decanoate	1488	1488[g]	ND	39.43±3.90	Floral, bitter[g]
18	Isobutyl decanoate	1543	1548[c]	24.33±1.06	13.07±0.51	Floral, bitter[g]
19	Ethyl dodecanoate	1594	1594[g]	844.19±34.06	1042.50±52.23	Fruity, sweet[a]
20	Isoamyl decanoate	1644	1644[e]	26.33±1.97	126.48±3.99	-
21	Ethyl tetradecanoate	1794	1796[c]	72.75±1.31	216.41±15.19	-
22	Isopropyl tetradecanoate	1824	1824[f]	ND	39.28±6.35	-
23	Ethyl hexadecanoate	-	1963[b]	284.71±61.93	501.10±29.83	Fruity, apple, wine-like[a]
24	Isopropyl hexadecanoate	-	2023[f]	21.84±2.51	42.83±0.46	-
	Total esters			**4791.49±43.89**	**11604.25±107.56**	-

476

No.	Compounds	LRI_{calc}	LRI_{lit}	*S. cerevisiae*	Mixed inoculum	Aromatic descriptors
	Alcohols					
25	3-Methyl-1-butanol	-	-	1113.17±26.64	1100.50±158.43	Fruity, sweet[k], solvent[d],
26	2-Methyl-1-butanol	-	-	133.96±15.11	508.21±19.76	Fruity, sweet[k], solvent[d],
27	3-Methyl-1-butanol formate	-	-	3.02±0.37	1.01±0.39	
28	3-Octen-1-ol	1063	NI	7.21±0.35	3.40±0.30	Mushroom[d]
29	1-Octanol	1076	1077[c]	5.71±0.23	10.07±1.33	Fruity, sweet[a]
30	Phenylethyl alcohol	1116	1119[f]	112.22±28.96	104.43±26.47	Honey[d], flowery[k]
31	1-Nonanol	1176	1174[e]	4.86±0.34	5.18±0.06	Raspberry[d],floral[j]
32	1-Decanol	1274	1283[g]	20.72±7.91	58.97±6.38	Bind cider, floral[c]
33	1-Dodecanol	1475	1479[c]	24.71±1.47	13.75±1.75	Floral[c]
34	1-Hexadecanol	1681	NI	17.41±0.68	26.72±1.62	-
35	1-Nonadecanol	1883	NI	80.47±11.55	110.69±18.58	-
	Total alcohols			**1523.46±10.86**	**1942.95±46.43**	-
	Terpenes and derivates					
36	Citronellol	1229	1231[b]	15.97±2.03	26.05±0.67	Citrus[c]
37	Citronellol acetate	1348	1352[b]	3.92±1.18	6.49±0.40	Rose dust[d]
38	β-Farnese	1451	1459[d]	ND	31.00±4.10	-
39	Nerolidol	1561	1566[g]	335.99±4.53	231.54±17.00	Apple, rose[a], floral[g]
40	Dihydrofarnesol	1688	1664[b]	147.46±4.33	124.70±13.18	-
41	Farnesol	1718	1725[c]	389.07±16.51	223.00±23.13	-
42	(E)-geranylgeraniol	1735	NI	10.01±0.50	11.75±1.35	-
43	(E,E)-Farnesol	1740	1741[b]	24.30±1.20	22.75±2.82	Lemon, floral, honey[h]
44	Farnesol acetate	1834	1834[f]	49.48±12.46	74.70±10.12	-
	Total terpenes			**976.20±5.93**	**751.98±8.21**	-

477

No.	Compounds	LRI_{calc}	LRI_{lit}	*S. cerevisiae*	Mixed inoculum	Aromatic descriptors
	Volatile acids					
45	Octanoic acid	1203	1199[c]	43.07±0.12	93.51±16.47	Rotten fruity[a], fatty[c,d], rancid[j]
46	3-nonenoic acid	1290	NI	ND	44.83±4.80	
47	Decanoic acid	1404	1391[e]	585.11±73.43	9.55±1.10	Fatty[a], rancid[c]
48	Dodecanoic acid	1585	NI	1079.76±153.92	897.81±1.01	Metalic[a], fatty[j]
49	Tetradecanoic acid	1773	NI	20.14±0.68	46.22±6.62	-
50	Hexadecanoic acid	-	-	157.02±8.53	88.90±24.38	
	Total volatile acids			**1885.10±62.96**	**1180.42±9.40**	-
	Aldehydes					
51	Nonanal	1107	1104[i]	21.82±1.31	4.84±0.16	Citrus, soapy[k]
52	2-Nonenal	1161	NI	27.52±0.13	23.84±1.60	Fatty, greenk
53	Decanal	1206	1207[f]	17.05±1.64	10.45±0.08	orange[d]
	Total aldehydes			**66.39±0.80**	**39.12±0.86**	-
	Acetals					
54	1,1-Diethoxyethane	-	726[c]	3.71±0.48	17.18±1.28	Fruity[c]
55	1,1-Diethyoxybutane	951	952[c]	ND	0.45±0.16	Fruity[c]
	Total acetals			**3.71±0.48**	**17.63±0.79**	-
	Total			**9249.35±77.75**	**15536±117.82**	

LRI, linear intention index; NI, LRI not identified; ND, not detected.
Data are presented as mean ± SD of duplicate analysis.
[a] Costa, Nicolli, Welke, Manfroi, & Zini (2018), [b] Cardeal & Marriott (2009), [c] Ledauphin et al. (2003), [d] Soares, Welke, Nicolli, & Zanus (2015), [e] Costa et al. (2015), [f] Dugo et al. (2014), [g] Alves et al. (2015), [h] Coelho, Coimbra, Nogueira, & Rocha (2009), [i] Zacaroni et al. (2017), [j] Whitener et al. (2017), [k] Czerny et al. (2008).