Sugar Tech (Nov-Dec 2019) 21(6):966–975
https://doi.org/10.1007/s12355-019-00732-x

RESEARCH ARTICLE

Production and Characterization of a New Sweet Sorghum Distilled Beverage

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Received: 8 March 2019 / Accepted: 20 May 2019 / Published online: 28 May 2019

Abstract Sweet sorghum is a culture that has received increasing attention in the last years. In many countries, genetic breeding programs have been developed seeking increases in the production of juice and sugars for alcoholic fermentation. In our study, *S. cerevisiae* and *M. caribbica* were evaluated to produce a distilled beverage from sweet sorghum, which was chemically and sensorially characterized. 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. The produced novel sorghum spirit was chemically and sensorially characterized. Fifty-five volatile compounds were identified by GC–MS, most of them belonging to the groups of 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 (9431.86 µg/L), aldehydes (331.93 µg/L) and terpenes (4881 µg/L). In contrast, the spirit produced with mixed inoculum showed 58,021.27 µg/L of esters and 9717.07 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. Based on our results, the sweet sorghum proved to be a good substrate for alcoholic fermentation to produce a spirit, which may represent an interesting alternative in the market of distilled beverages.

Keywords *Sorghum bicolor* · Volatile compounds · Distilled beverage · *Meyerozyma caribbica* · Non-*Saccharomyces*

Introduction

Sweet sorghum (*Sorghum bicolor* (L.) *Moench*) is a crop with high biomass productivity, high sugar concentration and high adaptability to environmental conditions (Eggleston et al. 2013). In addition, it presents relatively short crop period, which is facilitated using seeds, that also consequently, allows the use of mechanized planting (Rezende and Richardson 2017). Its culm has considerably high sucrose content, ranging from 53 to 85%, being glucose and fructose the remaining soluble carbohydrates (Barcelos et al. 2016). Furthermore, 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 (Bunphan et al. 2015). The technical and economic feasibility of sweet sorghum draws attention to its use as an alternative substrate for alcoholic fermentation.

Although the science of the production of distillates such as cachaça, tequila and rum, among others, is well established, the search for new substrates and the development of new beverages is a constantly growing area. Among the main points studied in this scenario, the fermentation is one of the most outstanding. Concerning the fermentation, 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, once the dominance of a

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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). An alternative to avoid this reduction is to use non-*Saccharomyces* yeasts. The presence of non-*Saccharomyces* in fermentative processes results in greater production of volatile compounds of interest (Oliveira et al. 2005). Indeed, controlled mixed inoculations with *S. cerevisiae* and non-*Saccharomyces* have been shown as a possibility to increase the aroma complexity and sensorial characteristics of fermented beverages due to higher production of secondary metabolites (Hu et al. 2016; Whitener et al. 2017). Previous studies using mixed inoculum of *Meyerozyma caribbica* and *S. cerevisiae* to produce sugarcane spirit reported that the variety and quantity of desirable volatile compounds were considerably higher than those found for pure *S. cerevisiae* (Duarte et al. 2013; Amorim et al. 2016). The main aims of this work were to produce and characterize, chemically and sensorially, a distilled beverage using the best combination of sweet sorghum genotype and yeast inoculum. To the best of our knowledge, this is the first report on the use of sweet sorghum and mixed inoculum of yeasts to produce a distilled beverage.

Materials and Methods

Sweet Sorghum Harvesting and Storage

The genotypes of sweet sorghum, BRS 506 and BRS 508 (Embrapa Maize and Sorghum—Brazil), were harvested after 150 days of planting at Muquém experimental farm (University of Lavras—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).

Microorganisms and Inoculum Preparation

The yeasts *Meyerozyma caribbica* and *Saccharomyces cerevisiae* were used to produce the beverage. The inocula were prepared with subsequent cultures in increasing volumes of YPD medium until obtaining populations of 10^7 cells/mL *S. cerevisiae* and 10^8 cells/mL *M. caribbica* (Amorim et al. 2016).

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 according to the topic 2.5. 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) (Oliveira et al. 2005; Duarte et al. 2010b).

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 to avoid yeast cell stress due to high sugar concentration (Amorim et al. 2016). Fermentations were carried out in duplicate until °Brix stabilization. After yeast cells decantation by gravity, the fermented juice was distilled (Duarte et al. 2011). The first 10% (head) of the expected volume of the distillate were collected and discarded aiming to eliminate undesirable volatile compounds such as methanol and acetaldehyde (Campos et al. 2010). The following 80% of the distillate were collected until the sweet sorghum spirit reached 42°GL. The final product was stored at room temperature in glass bottles until HS-SPME GC–MS and sensorial analysis.

The obtained sweet sorghum spirits were submitted to HS-SPME GC–MS and sensorial analysis.

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 × 30 cm—Supelco, Bellefonte, PA, USA) and refractive index detector (RID-10A). The column was operated at 30 °C with an isocratic system, where 5 mM sulfuric acid was used as the mobile phase at a flow of 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 et al. 2017).

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 microextraction (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 analyzed using a GC–MS-QP2010 Plus (Shimadzu) with an Rtx-5MS (30 m × 0.25 mm × 0.25 µm) column. Thermal desorption in the injector was at 270 °C for 100 s. 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 et al. 2017). Compounds were identified using NIST library 2011, and their concentrations were expressed as equivalents with 4-nonanol, used as an internal standard at a final concentration of 125 µg/L (Duarte et al. 2010a).

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 according to tasters' purchase intention in a scale from 1 to 5, being 1 certainly would not buy and 5 certainly would buy (Lutz 2008).

Statistical Analysis

Analyses of variance (ANOVA) 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) to correlate the volatile aromatic compounds profile with the used yeasts inocula.

Results and Discussion

Sweet Sorghum

Table 1 shows the content of sucrose, glucose and fructose of sweet sorghum juice. Genotype BRS 506 presented a proportion of 95.87% sucrose, 3.83% glucose and 3.02% fructose, while genotype BRS 508 had 95.88% sucrose, 3.82% glucose and 2.93% fructose. The sugar composition of sweet sorghum may range from 53 to 85% of sucrose, 9 to 3% of glucose and 6 to 21% of fructose (Ndaba et al. 2014), while sugarcane usually presents 90% sucrose, 5% glucose and 5% fructose (Zabed et al. 2014). Here, it was observed that the sugar 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.

Sweet Sorghum Microfermentations

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 other desirable volatile compounds (Duarte et al. 2013). Therefore, these yeasts were chosen to be evaluated in the fermentation of sweet sorghum juice. 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. Measuring the parameters cited above is important once the fermentative efficiency of a process to obtain alcoholic beverages is determined by the consumption of sugars and ethanol production. Hence, a fermentation process is considered efficient when the consumed sugars are used, in their majority, to produce ethanol and metabolites of interest instead of biomass (Barcelos et al. 2016).

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

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	$166.83^{a} \pm 3.32$	$7.04^{a} \pm 1.33$	$5.53^{a} \pm 0.13$	$183.22^{a} \pm 4.81$
BRS 508	$164.06^{a} \pm 10.20$	$6.85^{a} \pm 0.90$	$5.27^{a} \pm 0.17$	$180.09^{a} \pm 9.82$

Values followed by the same letters in the superscript are not significantly different by the Scott–Knott test ($p > 0.05$)

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

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

Values followed by the same letters in the superscript are not significantly different by the Scott–Knott test ($p > 0.05$); uppercase letter for the total mean of genotypes; lowercase letter for the unfolding of inoculum within each genotype

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 of 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 of 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 when using the same yeast strains and sugarcane (Duarte et al. 2013; Amorim et al. 2016), 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 using sweet sorghum, with achieved ethanol concentrations of 72.0 g/L (Barcelos et al. 2016) and 59.8 g/L (Bunphan et al. 2015).

In relation to $Y_{p/s}$, there were no significant differences 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 was higher than those reported when using sugarcane and different *S. cerevisiae* strains (Gomes et al. 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. 2010a). 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 results of $Y_{p/s}$, *S. cerevisiae* showed higher efficiency when using sweet sorghum as substrate compared to sugarcane (Gomes et al. 2007; Marini et al. 2009; Silva et al. 2009).

The overall mean of Q_p for BRS 506 genotype (3.58 g/L h) was significantly higher than for BRS 508 (3.20 g/L h) (Table 2). However, there was no significant difference when considering each inoculum for each genotype. It is important to highlight that all Q_p values found here were higher than those reported using the same inocula to ferment sugarcane juice (Duarte et al. 2013). In contrast, the values were considerably lower than fermentations using sugarcane and other *S. cerevisiae* strains, ranging from 5.88 to 6.40 g/L h (Marini et al. 2009; Silva et al. 2009; Campos et al. 2010).

Considering 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.

Production and Characterization of Sweet Sorghum Spirit

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 (Duarte et al. 2013; Amorim et al. 2016) 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 substrates such as sweet sorghum, sugarcane (Duarte et al. 2013; Amorim et al. 2016) and grapes (Zuehlke et al. 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. The ester concentrations are influenced by several factors related to the fermentation conditions, such as temperature and aeration, but another main factor is the yeast strain used in the process (Portugal et al. 2016). Non-S*accharomyces* yeasts are able to promote the esterification of several alcohols, like ethanol, isoamyl alcohols and 2-phenylethanol, and are consequently responsible for increasing ester content in distilled beverages. As already reported in previous studies using the same inocula with sugarcane, the spirit produced with *M. caribbica* and *S. cerevisiae* tends to result in ester content approximately twice, as higher as the ester content of the spirit using only *S. cerevisiae* (Duarte et al. 2013; Amorim et al. 2016). A similar situation can be reported here when using this inoculum and sweet sorghum. The spirit produced with mixed inoculum presented 52,021.27 μg/L of esters, while the one with *S. cerevisiae* resulted in 23,957.45 μg/L. The dominant esters in the sweet sorghum spirit with mixed inoculum, in decreasing order of concentration, were the ethyl decanoate (29,950.20 μg/L; fruity, grape, woody), ethyl octanoate (11,334.04 μg/L; fruity, sweet) and ethyl dodecanoate (5212.50 μg/L; fruity, sweet). The sweet sorghum spirit produced with *S. cerevisiae* also had these esters in abundance; however, their concentrations were lower. The 2-phenylethyl acetate (floral, sweet, honey) was detected at 460.90 μg/L and 111.17 μg/L in the sweet sorghum spirit produced with mixed and *S. cerevisiae* inocula, respectively. Other esters, such as isopentyl hexanoate, isobutyl octanoate and isoamyl decanoate, responsible for floral aroma, were found at considerably higher concentrations in the sweet sorghum spirit with mixed inoculum (Table 3).

The second most abundant class of compounds was higher alcohols. These compounds can bring positive or negative effects in the final product depending on their concentration (Czerny et al. 2008). The sweet sorghum spirit with mixed inoculum showed 9717.07 μg/L of higher alcohols, while the one produced with *S. cerevisiae* had 7616.35 μg/L (Table 3). Both concentrations were lower than that (300 mg/L) considered to have a negative effect on the quality of the beverage. Isoamyl alcohols, 2-methyl-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 (2541.07 μg/L) more 2-methyl-1-butanol than the one with *S. cerevisiae* (669.82 μg/L) (Table 3). Even when detected in high concentrations, 3-methyl-1-butanol is one of the main higher alcohols in fermented beverages, and precursor of the 3-methyl-1-butanol acetate, both compounds with fruity and sweetish characteristics (Czerny et al. 2008; Portugal et al. 2016). Phenylethyl alcohol was found at concentrations of 524.15 μg/L and 560.13 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 using sugarcane as 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 in the final product (Dato et al. 2005; Capobiango et al. 2012). Beyond having an important role in the sensorial characteristics of distilled beverages, higher alcohols are involved in the formation of other desirable secondary compounds, mainly esters (Portugal et al. 2016).

In the case of terpenes, the sweet sorghum spirit produced with *S. cerevisiae* showed higher concentrations (4881.02 μg/L) than the sweet sorghum spirit produced with mixed inoculum (3759.89 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 depending on the yeast activity. A previous study with these same yeasts obtained a slightly higher terpenes concentration in sugarcane spirit fermented with *M. caribbica* and *S. cerevisiae* (Duarte et al. 2013; Amorim et al. 2016). The *M. Caribbica* used in both experiments is

Table 3 Concentrations (μg/L) of volatile compounds in sweet sorghum spirit produced with mixed inoculum and pure *S. cerevisiae*

No.	Compounds	LRI_{calc}	LRI_{lit}	*S. cerevisiae*	Mixed inoculum	Aromatic descriptors
Esters						
1	Ethyl 2-methylbutanoate	849	850[c]	3.52 ± 1.59	ND	Fermented apple[c], fruity[k]
2	Ethyl 3-methylbutanoate	853	853[e]	9.50 ± 1.17	ND	Apple[d], fruity[k]
3	3-Methylbutyl acetate	877	877[c]	431.20 ± 45.13	855.12 ± 110.78	Banana[c], pear[d]
4	Ethyl hexanoate	1000	1000[c]	669.40 ± 109.74	1408.79 ± 20.81	Sweet[a], fruity, green[k]
5	Heptyl acetate	1112	1113[g]	17.06 ± .37	19.32 ± 0.86	–
6	Ethyl 4-octanoate	1188	1188[f]	ND	72.45 ± 8.88	–
7	Ethyl octanoate	1196	1201[g]	5947.94 ± 52.05	11,334.04 ± 1129.17	Fruity, sweet[a]
8	Isopentyl hexanoate	1248	1250[f]	12.64 ± 0.88	102.67 ± 1.22	Fruity[d]
9	2-Phenylethyl acetate	1254	1234[h]	111.17 ± 9.51	460.90 ± 37.02	Flowery[a], rose[d], honey[j]
10	Ethyl 3-nonenoate	1288	–	128.73 ± 6.86	433.45 ± 30.48	–
11	Ethyl nonanoate	1293	1295[g]	80.67 ± 17.63	256.10 ± 23.61	Fruity[d]
12	Isobutyl octanoate	1345	1348[b]	35.55 ± 3.64	116.43 ± 12.64	–
13	Ethyl 9-decenoate	1385	1382[e]	1354.85 ± 126.96	2081.72 ± 148.00	Rose[a]
14	Ethyl decanoate	1397	1397[c]	8139.87 ± 1053.10	29,950.20 ± 2474.25	Fruity, grape[a], woody[c]
15	3-Methylbutyl octanoate	1444	1447[g]	537.06 ± 9.58	589.22 ± 16.08	Pineapple[d]
16	2-Methylbutyl octanoate	1446	1446[g]	107.57 ± 6.55	235.33 ± 22.15	–
17	Propyl decanoate	1488	1488[g]	ND	197.17 ± 19.49	Floral, bitter[g]
18	Isobutyl decanoate	1543	1548[c]	121.65 ± 5.32	65.35 ± 2.54	Floral, bitter[g]
19	Ethyl dodecanoate	1594	1594[g]	4220.96 ± 170.32	5212.50 ± 261.15	Fruity, sweet[a]
20	Isoamyl decanoate	1644	1644[e]	131.63 ± 6.84	632.42 ± 9.95	–
21	Ethyl tetradecanoate	1794	1796[c]	363.76 ± 6.57	1082.07 ± 75.93	–
22	Isopropyl tetradecanoate	1824	1824[f]	ND	196.38 ± 31.73	–
23	Ethyl hexadecanoate	–	1963[b]	1423.54 ± 309.63	2505.49 ± 149.15	Fruity, apple, wine-like[a]
24	Isopropyl hexadecanoate	–	2023[f]	109.19 ± 12.54	214.16 ± 2.30	–
	Total esters			23,957.45 ± 219.44	58,021.27 ± 537.81	
Alcohols						
25	3-Methyl-1-butanol	–	–	5565.85 ± 133.21	5502.50 ± 792.15	Fruity, sweet[k], solvent[d],
26	2-Methyl-1-butanol	–	–	669.82 ± 75.54	2541.07 ± 98.82	Fruity, sweet[k], solvent[d],
27	3-Methyl-1-butanol formate	–	–	15.12 ± 1.73	5.07 ± 1.94	
28	3-Octen-1-ol	1063	–	36.04 ± 1.76	16.99 ± 1.20	Mushroom[d]
29	1-Octanol	1076	1077[c]	28.54 ± 1.14	50.32 ± 6.63	Fruity, sweet[a]
30	Phenylethyl alcohol	1116	1119[f]	560.13 ± 21.12	524.15 ± 11.78	Honey[d], flowery[k]
31	1-Nonanol	1176	1174[e]	24.29 ± 1.69	25.90 ± 0.32	Raspberry[d], floral[j]
32	1-Decanol	1274	1283[g]	103.61 ± 39.56	294.85 ± 31.88	Bind cider, floral[c]
33	1-Dodecanol	1475	1479[c]	123.55 ± 7.37	68.77 ± 8.76	Floral[c]
34	1-Hexadecanol	1681	–	87.03 ± 3.40	133.62 ± 8.10	–
35	1-Nonadecanol	1883	–	402.37 ± 57.74	553.45 ± 92.92	–
	Total alcohols			7616.35 ± 42.54	9717.07 ± 233.69	–
Terpenes and derivates						
36	Citronellol	1229	1231[b]	79.87 ± 10.13	130.23 ± 3.36	Citrus[c]
37	Citronellol acetate	1348	1352[b]	19.61 ± 5.88	32.46 ± 2.02	Rose dust[d]
38	β-Farnese	1451	1459[d]	ND	155.01 ± 20.49	–
39	Nerolidol	1561	1566[g]	1679.93 ± 22.67	1157.72 ± 85.01	Apple, rose[a], floral[g]
40	Dihydrofarnesol	1688	1664[b]	737.29 ± 21.67	623.50 ± 65.88	–
41	Farnesol	1718	1725[c]	1945.34 ± 82.57	1114.98 ± 115.65	–
42	(E)-geranylgeraniol	1735	–	50.06 ± 2.51	58.74 ± 6.75	–
43	(E,E)-Farnesol	1740	1741[b]	121.51 ± 6.00	113.73 ± 14.09	Lemon, floral, honey[h]

Table 3 continued

No.	Compounds	LRI_{calc}	LRI_{lit}	*S. cerevisiae*	Mixed inoculum	Aromatic descriptors
44	Farnesol acetate	1834	1834[f]	247.40 ± 62.32	373.51 ± 50.58	–
	Total terpenes			4881.02 ± 29.65	3759.89 ± 41.04	
Volatile acids						
45	Octanoic acid	1203	1199[c]	215.35 ± 0.59	466.59 ± 34.14	Rotten fruity[a], fatty[c,d], rancid[j]
46	3-Nonenoic acid	1290	–	ND	222.15 ± 24.01	
47	Decanoic acid	1404	1391[e]	2927.14 ± 161.00	47.76 ± 5.52	Fatty[a], rancid[c]
48	Dodecanoic acid	1585	–	5403.58 ± 151.15	4489.05 ± 5.07	Metalic[a], fatty[j]
49	Tetradecanoic acid	1773	–	100.69 ± 3.38	231.08 ± 33.08	–
50	Hexadecanoic acid	–	–	785.10 ± 42.63	442.64 ± 25.43	
	Total volatile acids			9431.86 ± 76.35	5899.26 ± 12.96	
Aldehydes						
51	Nonanal	1107	1104[i]	109.08 ± 6.56	24.18 ± 0.81	Citrus, soapy[k]
52	2-Nonenal	1161	–	137.61 ± 0.65	119.19 ± 8.01	Fatty, green[k]
53	Decanal	1206	1207[f]	85.23 ± 8.21	52.23 ± 0.40	Orange[d]
	Total aldehydes			331.93 ± 3.98	195.61 ± 4.82	
Acetals						
54	1,1-Diethoxyethane	–	726[c]	190.46 ± 7.07	85.88 ± 6.39	Fruity[c]
55	1,1-Diethoxybutane	951	952[c]	ND	2.23 ± 0.80	Fruity[c]
	Total acetals			190.46 ± 7.07	88.11 ± 3.95	
	Total					

Data are presented as mean ± SD of duplicate analysis

LRI linear retention index, *ND* not detected

[a]Costa et al. (2018)

[b]Cardeal and Mariott (2009)

[c]Ledauphin et al. (2003)

[d]Soares et al. (2015)

[e]Costa et al. (2015)

[f]Dugo et al. (2014)

[g]Alvez et al. (2015)

[h]Coelho et al. (2009)

[i]Zacaroni et al. (2017)

[j]Whitener et al. (2017)

[k]Czerny et al. (2008)

a great β-glucosidase producer (Duarte et al. 2013); thereby, it was also expected that the sweet sorghum spirit would present higher terpenes concentration than the one produced with pure *S. cerevisiae*; however, the opposite was detected. The β-glucosidase activity can be affected by several factors related to the must, such as ethanol and glucose concentration. In fact, the evaluation of β-glucosidase activity of non-*Saccharomyces* in wine production showed that as the glucose and ethanol concentration increase, from 52 up to 77%, the glycolytic activity can be lost (Hu et al. 2016). When the *M. caribbica* and *S. cerevisiae* were tested to produce sugarcane spirit, the initial °Brix of the must was standardized to 16 °Brix, and fed-batch was carried out without exceeding this value. However, to produce the sweet sorghum distilled spirit there was no dilution of the must, which initially was 18 °Brix. This higher initial sugar concentration, consequently higher ethanol production, may have affected β-glucosidase activity of the *M. caribbica*; another explanation may be the fact that sweet sorghum has a low concentration of this compound. Although there was this lower terpenes concentration in the sweet sorghum spirit produced with mixed inoculum, the terpenes concentration in both sweet sorghum spirits were still higher than those already related in sugarcane spirits with the same yeasts (Duarte et al. 2013; Amorim et al. 2016).

There was a higher production of farnesol, nerolidol and dihydrofarnesol in the sweet sorghum spirit produced with *S. cerevisiae*, with concentrations of 1942.34 µg/L, 1679.93 µg/L and 737.29 µg/L, respectively (Table 3).

The β-farnesene was detected only in the sweet sorghum spirit with mixed inoculum (155.01 µg/L). All 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,* it was found that nerolidol and farnesol were produced in much higher concentrations by *S. cerevisiae* (Wu et al. 2015).

The sweet sorghum spirit produced with *S. cerevisiae* showed 9431.86 µg/L of volatile acids, and the spirit with mixed inoculum had 5899.26 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 et al. 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 2927.14 µg/L in the sweet sorghum spirit produced with *S. cerevisiae*, and only 47.76 µg/L in the one with mixed inoculum (Table 3). Probably, due to the high variety of esters that were detected when compared to the volatile acids, most of the acids that were produced during fermentation were esterified after the distillation, leaving 6 volatile acids detected in both spirits.

Three aldehydes, nonanal, 2-nonenal and decanal, were found in the produced sweet sorghum spirits (Table 3). Overall, they were at 331.93 µg/L in the sweet sorghum spirit produced with *S. cerevisiae* and 195.61 µg/L in the one with mixed inoculum. Although aldehydes are usually detected in wine and distilled beverages, this class of compounds is accountable for negatively affecting the flavor and aroma of beverages (Capobiango et al. 2012). Even more, they can affect the central nervous system when consumed in excess, causing headaches (Dato et al. 2005). All aldehydes were detected at higher concentrations in the sweet sorghum spirit fermented only with the pure *S. cerevisiae*, emphasizing once more that the co-inoculation with non-*Saccharomyces* influences directly the profile of volatile compounds.

Only two acetals, 1,1-diethoxyethane and 1,1-diethoxybutane, were detected in sweet sorghum spirits, both accountable for fruity aroma (Table 3). The 1,1-diethoxyethane was found at 190.46 µg/L in the sweet sorghum spirit with pure *S. cerevisiae*, and 85.88 µg/L in the sweet sorghum spirit with mixed inoculum. On the other hand, 1,1-diethoxybutane was detected only in the sweet sorghum spirit with mixed inoculum (Table 3). 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 produce distilled beverages.

The PCA 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 such as 2-nonenal (52) and 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 work, 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, thus contributing positively to the sensorial characteristics of the beverage as shown below.

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.*

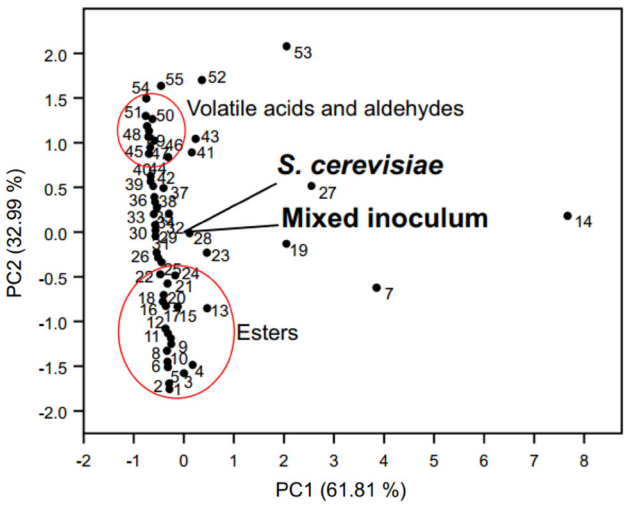

Fig. 1 Principal component analysis (PCA) of volatile compounds in sweet sorghum spirits produced with *S. cerevisiae* and mixed inoculum by GC–MS

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 considerably higher score than the one produced only with *S. cerevisiae*.

Conclusions

The yeast inoculum influence the quality of the distillate, being sweet sorghum spirit produced with *S. cerevisiae* characterized by a higher content of volatile acids, aldehydes and terpenes. The beverage 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 showed the potential of using sweet sorghum and mixed inoculum of *M. caribbica* and *S. cerevisiae* to produce a new spirit, which can help in minimizing the costs of production in distilleries, for example, in the case of sugarcane spirit.

Acknowledgements This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001. The authors also would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil (CNPq) and Fundação de Amparo à Pesquisa de MG (FAPEMIG) for financial support.

Author's Contributions Ana Cláudia Alencar Lopes conducted the planning, laboratory experiments, and organization of data and writing under the supervision and guidance of Whasley Ferreira Duarte. Jose Airton carried out the planting and harvesting of all sweet sorghum used in the study. Zlatina Genisheva assisted in the writing and revision of the final draft.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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