academicJournals

Vol. 11(7), pp. 287-295, 21 February, 2017
DOI: 10.5897/AJMR2016.8415
Article Number: B09ECC362919
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Post-treatment of cachaca (Brazilian sugarcane spirit) with charcoal made from cane bagasse

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Received 19 December, 2016; Accepted 13 February, 2017

The objective of this study was to ferment sugarcane juice using two types of yeast and evaluate the quality of the distillate after beverage filtration in a column filled with ground charcoal derived from cane bagasse. The experimental design was randomized block (split plots, 2 x 2) with three replicates. The primary treatment involved two types of yeast: CA-11 and baker's yeast. Secondary treatment involved filtration or not of the beverage in the charcoal column. The following procedures were adopted: percolation of all samples to a single column and passage of the distillate from each repetition (blocks) using three similar columns. Selected yeast had similar cachaça production as compared to the baker's yeast. However, higher acidity occurred in the distillate using the selected yeast, which contributed to the increased transfer of copper to the beverage. In the charcoal filtration that used one column per block, the copper was removed from the beverage, and the acidity was decreased, whereas the alcohol content, fixed acidity, pH, and turbidity of the cachaça did not change.

Key words: Spirits, yeast strains, fermentation, distillation, filtration columns.

INTRODUCTION

Cachaça (Brazilian sugar cane spirit) is a distillate produced in Brazil and appreciated throughout the world (Borges et al., 2014). This distillate is obtained by the fermentation of sugarcane juice and needs to have an alcohol content between 38 and 48% $(v/v)^{-1}$ (Brazil, 2005). It is the third most consumed distillate in the world after vodka and soju (Kunigk et al., 2011). Despite the high yield and the import from Brazil to more than 60 countries, the export is still insignificant, corresponding to a little more than 1% of the total production. One of the reasons for the limited export includes the variation in quality and copper content higher than 2 mg L^{-1} (Lima et al., 2009).

After the alcoholic fermentation of sugarcane juice, distillation is performed mainly in copper stills (Alves et al., 2011) to reduce the acidity and the content of aldehydes and sulfur compounds in beverages (Pereira et al., 2012). However, during distillation, cachaça may

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be contaminated with metals ions removed from the distiller.

Moreover, some compounds produced by yeasts during fermentation, particularly organic acids, may promote the transfer of copper from the stills to the beverage (Boza and Horii, 2000). During the fermentation process, several yeast types can be used, including baker's yeast (pressed), which is readily available and fast-growing, and selected yeast, which is adapted to the production of cachaça. Although, these strains belong to the same species (*Saccharomyces cerevisiae*), they produce wines with different ethanol, acidity and secondary compounds concentration (Basso et al., 2011). One of the yeast strains selected for cachaça production is CA-11.

Alcarde et al. (2012) found that strain CA-11 provided the most appropriate chemical composition to the distillate compared with the other strains studied (Y-904, BG-1, PE-2, SA-1 and CAT-1). Action of CA-11 is different from other strains during fermentation. Montijo et al. (2014) did not correct the pH of the must and inoculated CA-11 directly into the must because, according to the authors, the acids produced during fermentation lowered the pH of the must. The lowering of the pH during fermentation is necessary to promote the increased use of sugars by the yeasts rather than by contaminating bacteria (Basso et al., 2011). However, during distillation, the improper sanitation of the distiller and the high concentration of acid in wine favors the transfer of the verdigris [$CuCO_3.Cu(OH_2)$] to the beverage (Boza and Horii, 2000).

The cachaça samples obtained in different Brazilian states have a high percentage of copper. The copper content of the beverages analyzed in southern Minas Gerais was higher than that recommended in 16.67% of the samples, and the content in one evaluated sample was as high as 9 mg L^{-1} (Zacaroni et al., 2011). Marinho et al. (2009) observed that the copper content in the cachaça produced in Goiás was as high as 8.80 mg L^{-1}. The average levels of copper in the beverages produced in Rio Grande do Sul ranged between 2.95 and 10.22 mg L^{-1}, and depending on the region, up to 60% of the samples were contaminated (Garbin et al., 2005) by exceeding the copper level limit of 5 mg L^{-1} established by Brazilian legislation (Brazil, 2005).

Several methods can be used to remove organic and ionic contaminants from cachaça. For copper removal, the main techniques include adsorption on activated carbon and ion exchange resins (Lima et al., 2006; Kunigk et al., 2011), adsorption in activated carbon/iron oxide composites (Lima et al., 2009), and double distillation (Alcarde et al., 2012), among others. However, the efficiency of the removal of metals and other undesirable components could be improved by converting the bagasse into charcoal, and use this for cachaça filtration.

Charcoal has a high adsorption capacity. Several studies have evaluated the efficacy of charcoal as an adsorbent, including its use for the retention of organic compounds such as trihalomethanes, nitrates, chloride ions, sulfide ions, metals (Pb, Cu, and Fe), and microorganisms such as fecal coliforms and total coliforms (Ta-Chung et al., 2008). Zhao et al. (2008) evaluated the adsorption of 2,4-dichlorophenol to charcoal (Xu and Shi, 2002) and the elimination of nitrates (Mizuta et al., 2004) and four types of phthalate esters in water.

Charcoal from sugar maple wood (*Acer saccharum*) has been used to remove fusel oil from whiskey by filtration using the charcoal mellowing process (Labbe et al., 2006). The charcoal produced from sugarcane bagasse also has adsorption capacity. Teixeira et al. (2016) used bagasse charcoal for filtration and the removal of solids from vinasse, which was subsequently used as dilution water for the preparation of the must of molasses, a waste product of sugar production.

However, the use of sugarcane bagasse charcoal for the filtration of alcoholic beverages has been little explored. The possibility of using charcoal for the removal of toxic components present in the beverage is interesting and is an alternative use of bagasse.

In this context, this study aimed to compare the quality of cachaça obtained after distillation of must fermented using baker's and selected yeasts and to investigate the final quality of the beverage after filtration using a column filled with ground charcoal produced from ground sugarcane bagasse.

MATERIALS AND METHODS

Experimental design

The experimental design was randomized block (split-plot) with three replicates. The primary treatment involved the use of two types of yeast for the production of cachaça: selected yeast and baker's yeast. Secondary treatment was performed after fermentation and distillation, with the filtration or not of the cachaça in a column containing ground charcoal produced from sugarcane bagasse.

The experiment was conducted in blocks to distinguish the different days of fermentation (March 21, 22, and 29, 2016). The sugarcane juice was extracted from sugarcane variety IAC95-5000, with 11 months, and obtained from the first cut.

Fermentation: Preparation, treatment and analysis

Soluble solids content (°Brix), pH, acidity and total reducing sugars (TRS) were determined in the must used in fermentation, as established in the *Centro de Tecnologia Canavieiro* CTC (2011). For fermentations, the following yeast strains were used: baker's *Saccharomyces cerevisiae* (Levasaf®, Argentina) and selected yeast CA-11 (LNF®, Brazil).

The original total cell count and cell viability were determined for each type of yeast with Ringer's solution in a Neubauer chamber, as detailed by Lee et al. (1981). Subsequently, based on the cell viability, the amount of yeast to be added in order to reach a concentration of 10^7 CFU mL^{-1} of yeast cells during fermentation

Figure 1. Fifteen-liter still used for the distillation of the fermented must.

Figure 2. Charcoal column used for filtration of the cachaça obtained from fermented must produced with the selected and compressed yeast.

was calculated.

Initially, yeast was diluted in must (2500 mL) that was used in the fermentation process. The feeds with 2500 mL of must, with temperature of 32°C, were timed every 15 min until the Fed Batch fermentation vat reached a volume of 10,000 mL. Fifteen minutes after the last feed, samples were collected for another evaluation of initial cell viability. Fermentation was completed after 24 h (Oliveira et al., 2008), when soluble solids (°Brix) was less than 1 (one). Then, wine samples were collected for the assessment of final cell viability and execution of the analyses.

Analysis of wine samples

Sulfuric acidity (g H_2SO_4 L^{-1}), alcohol content (% vv^{-1}), glycerol (10^{-3}%, wv^{-1}), total residual reducing sugars (TRRS - %), and pH were analyzed according to the CTC (2011). The amount of alcohol produced in wine was calculated by taking into account the amount of wine produced multiplied by the alcohol content. The fermentation efficiency (%) was calculated by dividing the amount of alcohol produced by the theoretical amount of alcohol produced, as detailed by Fernandes (2006).

Distillation

The wine was distilled in copper stills (D&R Alambiques®, Belo Horizonte, Brazil) (Figure 1), and three fractions were obtained: the first fraction (10%) with mainly head compounds (aldehydes and esters), a major fraction (80%) that is considered the heart (cachaça), and the last fraction (10%), that is, the tails compounds (phurphural and others not desirable components) (Bruno, 2012). The last fraction was not separated and remained in the vinasse because the distiller was switched off after the separation of the heart fraction. Distillation was initially conducted in the wine produced with the selected yeast, followed by the wine produced with the baker's yeast. The order of the type of yeast was changed in the other replicates. Before each distillation, the equipment was cleaned with water only. The fractions removed from the equipment were maintained at room temperature in glass bottles covered with aluminum foil. The amount of material to be separated in the heart fraction was calculated to obtain a beverage with an ethanol content of approximately 40% at the end of the distillation.

Charcoal production and treatment of cachaça

Charcoal was produced using sugarcane bagasse. The bagasse was carbonized at 250°C for 1 h, and then at 400°C for 1 h in an adapted (Machado et al., 2014) muffle (Tecnal®, Piracicaba, Brazil). The process was repeated until a sufficient amount of charcoal was obtained for making the filtration columns. The yield of charcoal production was approximately 30%.

The glass column used was 35 cm in height and 5 cm in diameter, with 500 mL of useful volume with markings (Figure 2). The charcoal was ground and sieved through a 35-mesh sieve (0.5 mm) and placed in the column. Cotton was added (35 mL – this grading was used as a reference for the mounting of other columns) to keep the charcoal particles in place inside the column during filtration. A filter layer was made with the ground and sieved charcoal (95 mL), and cotton (25 mL) was placed above this layer. A layer of crushed charcoal (55 mL), which corresponded to the prefilter layer, was stacked above this cotton layer. Another layer of cotton (30 mL) was placed above the prefilter layer to prevent the spreading of the crushed charcoal layer during the addition of the beverage. The total final volume of the filter layer was 240 mL and this procedure was repeated for every column filtration build.

The column was packed using commercial cachaça (51®, Pirassununga, Sao Paulo, Brazil) to settle the filtering layers of charcoal and cotton. For beverage filtration, 260 mL of each replicate was passed through the entire column and then discarded. Another fraction of 260 mL was filtered; this fraction was considered the analyte. This procedure was followed for each sample. Filtration was performed in the dark at room temperature (approximately 25°C). The residence time for percolation of 260 mL of cachaça in the column was approximately 15 min.

An aliquot of the heart fraction was filtered with charcoal two months after storage in the bottle. Two ways of filtration were conducted as follows: a) use of a single charcoal column for passage of all three replicates for both yeasts and b) use of one

Table 1. Average and standard error of pH, soluble solids (°Brix), acidity (g H_2SO_4 L^{-1}), and TRS (%) from three replicates of the cane juice used as must for the production of cachaça.

Analysis of the cane juice	Mean and standard error	Recommended*
pH	5.25±0.07	4.5 to 5.5
°Brix (%)	16.93±0.96	≥ 18
Acidity (grams of H_2SO_4 L^{-1})	2.31±0.27	≤ 0.8
TRS (%)	14.33±0.79	≈ 18

*Amorim et al. (1996).

filtration column per block (replicate). The order of passage of the type of yeast was switched for each block. In the first block, the selected yeast was used first, followed by the baker's yeast. Then, another column was made and changed: cachaça from bakers filtered first and yeast selected after. And the ultimate column was prepared for third repetition, and passed spirits from selects followed by baker's yeast.

The variables pH, alcohol content, turbidity (MS Tecnopon, TB1000, Brazil), total acidity, fixed acidity, and acetic acid were measured in the distillate (treated or not) according to the protocols of the Adolfo Lutz Institute (2008). The copper content was determined only in samples that were passed through the different columns per block. The copper content was determined using atomic absorption spectroscopy in an AAnalyst800 spectrometer (Perkin Elmer®, USA) with an air-acetylene flame and the standard addition method. The following analytical conditions were used: wavelength of 324.8 nm, air flow rate of 17.0 L min^{-1}, acetylene flow rate of 2.2 L min^{-1}, and burner slot width of 0.7 nm.

The copper content of one of the samples, which had concentrations below the detection limit of the flame, was determined using atomic absorption spectroscopy with a graphite furnace and the standard addition method. For this purpose, an AAnalyst800 spectrometer (Perkin Elmer, USA) and the following analytical conditions were used: wavelength of 324.8 nm, pyrolysis temperature of 1200°C, atomization temperature of 2200°C, and chemical modifier consisting of 5 µg of Pd and 5 µg of Mg. The use of a chemical modifier allows the use of high temperatures to remove the matrix, prevents the loss of the analytes, decreases the volatility of the analytes, and permits the separation of the analyte from the matrix.

Statistical analysis

The quality of the must, fermentation, and wine were compared by evaluating the differences between the means with standard errors. The results obtained from only filtration or in three blocks columns were subjected to analysis of variance using the F-test and Tukey test ($p \leq 0.05$), as detailed by Barbosa and Maldonado Jr. (2015).

RESULTS AND DISCUSSION

Fermentation

The analysis of the must (Table 1) indicated that the sugarcane variety used was not yet at peak maturation because the soluble solids and TRS values were lower than expected for the production of cachaça and the acidity was higher than 0.8 g H_2SO_4 L^{-1} (Amorim et al., 1996). This result was expected because sugarcane was harvested purposely before the beginning of the harvest season (from May to November for sugarcane in Brazil) (Oliveira et al., 2008). In March, performing the fermentation and producing high-quality distillates would be more difficult. However, this condition was ideal to test the method of removal of impurities from the beverage using charcoal. The sugar content and pH of the cane juice were not adjusted to simulate what, in practice, occurs during the production of cachaça using a nonstandard must. However, the must was the same for both types of yeast in each block (replicate).

Table 2. Average and standard error of three replicates for the initial and final cell viability (%) in the fermented must using selected and pressed yeast for production of cachaça.

Yeast type	Cell viability	
	Initial (%)	Final (%)
Selected	87.84±1.89	68.04±7.17
Baker's	79.79±5.87	38.30±14.21

The high acidity of the must significantly decreased the yeast cell viability during the fermentation process (Table 2). The original viability of the baker's yeast was lower than that of the selected yeast. The cell viability of the baker's yeast was below the recommended value of 85% (Amorim et al., 1996). Previous calculations were performed to avoid the shortage of yeast in fermentation because the original cell viability of the baker's yeast was low (77.61%), whereas the viability of the selected yeast was 81.65%. For this reason, no shortage of yeast occurred because of addition of sufficient cells to have 10^7 viability cells for both strains.

Viability remained low at the beginning of fermentation (Table 2). At the end of fermentation, the decrease in cell viability of both types of yeasts was higher which could prevent the reuse of the yeast in new fermentation cycles. In these cases, the addition of a new batch of yeast would be required. Furthermore, a higher precipitation of the selected yeast was observed after fermentation, which made the separation of the wine from the yeast easier. The same result was obtained by

Table 3. Average and standard error of three replicates regarding pH, alcohol content (% v v^{-1}), glycerol (10^{-3}% w v^{-1}), acidity (H_2SO_4 g L^{-1}), total residual reducing sugar TRRS (%), and fermentation efficiency (%) in the fermented must obtained with the selected and pressed yeast for cachaça production.

Yeast type	pH	Alcohol content	Glycerol	Acidity	TRRS	Fermentation efficiency
Selected	3.99±0.04	9.57±0.26	0.83±0.07	3.12±0.16	0.63±0.19	93.26±5.77
Baker's	3.99±0.04	9.41±0.34	0.89±0.05	3.19±0.28	0.62±0.33	91.44±3.14

Table 4. Average and standard error of three replicates regarding alcohol content (% v v^{-1}) in the first fraction, major fraction, and vinasse + last fraction, and volume (mL) of ethanol present in the stills (adjusted to 40% alcohol content) for the production of cachaça using two types of yeast.

Yeast type	Alcohol content			Total volume	Volume adjusted to 40%
	First fraction	Heart fraction	Vinasse + last fraction		
Selected	47.80±1.61	44.23±0.44	2.41±0.19	1416.67±83.33	1567.50±101.28
Baker's	45.49±4.81	46.17±2.44	1.66±0.60	1333.33±66.67	1542.75±120.16

Table 5. Analysis of variance for total acidity (g of CH_3COOH 100 mL^{-1} of sample), fixed acidity (g of CH_3COOH 100 mL^{-1} of sample), volatile acidity (mg of CH_3COOH 100 mL^{-1} of anhydrous ethanol), pH, alcohol content (% v v^{-1}), and turbidity (NTU) for cachaça produced using two yeast strains and filtered or not in a single charcoal column for all replicates.

Causes of variation	Total acidity	Fixed acidity	Volatile acidity	pH	Alcohol content	Turbidity
Blocks	1.09^{ns}	2.07^{ns}	0.05^{ns}	3.41^{ns}	3.92^{ns}	6.45^{ns}
Yeast (L)	46.35*	2.42^{ns}	18.86*	10.78^{ns}	2.89^{ns}	0.04^{ns}
Selected	35.90±3.44 ^{A}	4.81±1.09^{A}	69.90±6.44^{A}	5.15±0.08^{A}	44.49±0.51 ^{A}	1.33±0.24 ^{A}
Baker's	17.95±1.70^{B}	2.90±0.83 ^{A}	32.70±4.33 ^{B}	5.43±0.07^{A}	46.19±0.61 ^{A}	1.31±0.28 ^{A}
CV of the fraction	16.96	55.13	28.92	2.79	3.82	13.85
Charcoal (T)	1.14^{ns}	10.42*	7.99*	3.16^{ns}	0.59^{ns}	4.07^{ns}
With treatment	25.07±2.86^{A}	5.69±1.14 ^{A}	42.84±6.40 ^{B}	5.20±0.11^{A}	45.48±0.52 ^{A}	0.95±0.26 ^{A}
Without treatment	28.78±2.28^{A}	2.02±0.78 ^{B}	59.77±4.37 ^{A}	5.39±0.02^{A}	45.20±0.60 ^{A}	1.69±0.26 ^{A}
Interaction (L x T)	2.24^{ns}	1.67^{ns}	6.82^{ns}	5.04^{ns}	0.39^{ns}	0.18^{ns}
CV of the sub-fraction	22.39	51.08	20.22	3.50	1.43	48.49

**Significant at a probability of 1% using the F-test; ns: not significant; CV: coefficient of variation (%). The same letter in each column indicates the absence of a significant difference using Tukey test (p ≤ 0.05).

Alcarde et al. (2012). Yeast flocculation is desired during the production of cachaça (Basso et al., 2008) because poor separation of the wine from the yeast may increase the amount of yeast present in the distiller, which in turn increases the concentration of fatty acids in the distillate (Serafim et al., 2011).

A slight decrease was observed in the glycerol content in the wine produced with the selected yeast as compared to the wine produced with the baker's yeast (Table 3). Furthermore, using standard error, pH, alcohol content, acidity, TRRS and fermentation efficiency was similar for both strains.

The alcohol content of the first (initial 10% that was separated from the beverage) and heart fraction was similar for both strains (Table 4). However, the last fraction + vinasse have more alcohol content for select yeast. This result lead us to affirm that more alcohol could be recovered and it was lost. Total volume of the produced distillate (heart fraction) was higher in select yeast; however, if the alcohol content was standardized to 40% (Table 4), the volume of the heart fraction was similar between strains. Baker's and selected yeast had close performance for cachaça production.

Filtration of all replicates using a single charcoal column

After the distillate filtration of all replicates using a single column, the volatile acidity was 2.13-fold greater for the selected yeast, although the acidity obtained using the two types of yeast was within the values recommended by Brazilian legislation (up to 150 mg of acetic acid per 100 mL of anhydrous alcohol) (Brazil, 2005) (Table 5).

The yeast strain has been shown to affect the chemical composition of the distillate (Alcarde et al., 2012).

Table 6. Analysis of variance for total acidity (g of CH_3COOH 100 mL^{-1} of sample), fixed acidity (g of CH_3COOH 100 mL^{-1} of sample), volatile acidity (mg of CH_3COOH 100 mL^{-1} of anhydrous ethanol), pH, alcohol content (% v v^{-1}), and turbidity (NTU) in cachaça produced with two types of yeast and filtered or not in a single charcoal column per replicate.

Variation causes	Total acidity	Fixed acidity	Volatile acidity	pH	Alcohol content	Turbidity	Copper content
Blocks	9.18^{ns}	11.59^{ns}	3.59^{ns}	0.04^{ns}	2.17^{ns}	4.88^{ns}	0.43^{ns}
Yeast (L)	259.89**	3.28^{ns}	95.50*	3.01^{ns}	1.64^{ns}	3.35^{ns}	7.81^{ns}
Selected	$32.39±1.44^{A}$	$4.57±1.46^{A}$	$62.90±3.36^{A}$	$5.14±0.12^{A}$	$44.25±0.61^{A}$	$1.51±0.29^{A}$	$12.66±1.04^{A}$
Baker's	$15.03±1.57^{B}$	$3.31±1.21^{A}$	$25.44±2.33^{B}$	$5.54±0.19^{A}$	$45.82±0.84^{A}$	$1.91±0.48^{A}$	$7.11±1.13^{A}$
CV of the fraction	7.87	30.57	15.02	7.37	4.75	22.16	34.82
Charcoal (T)	24.15**	4.35^{ns}	74.15**	2.03^{ns}	0.28^{ns}	0.01^{ns}	83.76**
With treatment	$18.64±1.21^{B}$	$5.86±1.36^{A}$	$28.58±1.51^{B}$	$5.49±0.21^{A}$	$44.88±0.83^{A}$	$1.74±0.40^{A}$	$3.82±1.21^{B}$
Without treatment	$28.78±1.80^{A}$	$2.02±1.32^{A}$	$59.77±4.19^{A}$	$5.20±0.10^{A}$	$45.20±0.62^{A}$	$1.69±0.37^{A}$	$15.95±0.96^{A}$
Interaction (L x T)	7.90*	0.20^{ns}	18.03*	0.37^{ns}	0.35^{ns}	0.23^{ns}	34.85**
CV of the subfraction	15.07	80.97	14.20	6.64	2.31	55.76	23.24

**Significant at a probability of 1% using the F-test; ns: not significant; CV: coefficient of variation (%). The same letter in each column indicates absence of significant difference using Tukey test ($p \leq 0.05$).

Depending on the acids produced during fermentation, the concentration of these acids can be higher in the first or last fraction. Accordingly, the concentration of acetic acid, succinic acid, glycolic acid, citramalic acid and lactic acid was much higher in the last fraction, whereas capric acid, lauric acid, myristic acid, and palmitic acid were more abundant in the initial fractions (Serafim et al., 2011). Alcarde et al. (2012) studied the yeast strain CA-11 in double distilled cachaça and found that volatile acidity in the first, heart and last fractions was 14.20, 23.67 and 124.51 mg 100 mL^{-1}, respectively, indicating that the volatile acid content was higher in the last fraction.

The balance of the types of acids, alcohol content of the wine, and other secondary compounds from fermentation are essential to define when they will be removed from the wine during the distillation process. The baker's yeast could produce a higher percentage of fatty acids than the selected yeast considering that the separation between the yeast fraction and the wine was more difficult, and therefore, more yeast was added to the distiller. Because of the higher boiling point of fatty acids, the removal of organic acids may have changed for the beverage obtained with the baker's yeast, resulting in lower concentrations of these compounds in the heart fraction.

The total acidity was similar between the beverages in a single filtration column using charcoal; however, it was significantly different for the fixed and volatile acidity (Table 5). The fixed acidity increased 2.82-fold after filtering with charcoal whereas the volatile acidity decreased by 40% in the filtered distillate. The increase in fixed acidity may be due to the presence of iron and aluminum in the charcoal because bagasse ashes have 6.87% of Fe_2O_3 and 7.48% of Al_2O_3 on average (Rodriguez-Diaz et al., 2015). Metal ions with high charge and small volume, such as Fe^{3+} and Al^{3+}, in aqueous medium, in the form of $[Fe(H_2O)_6]^{3+}$ (pKa = 3.5 x 10^{-3}) and $[Al(H_2O)_6]^{3+}$ (pKa = 1.4 x 10^{-5}), respectively, can act as Lewis acids and produce acidic solutions (Atkins and Jones, 2006).

Passage of the distillate using one column per block

Differences in fixed acidity were not significant between the treatments subjected or not to filtration with charcoal (Table 6). However, there was an interaction among the yeast type and charcoal filtration for total acidity (Figure 3A) and volatile acidity (Figure 3B).

The difference in total acidity after filtration with charcoal was more pronounced for the beverage from selected yeast (Figure 3). The acidity for the selected yeast decreased significantly (by 65.3%) after filtration, and the values obtained with the selected yeast were closer to those obtained with the baker's yeast. No significant difference in total acidity after filtration with charcoal was observed for the baker's yeast. The total acid content of the beverage produced with this type of yeast was low before and after filtration.

Volatile acidity is a parameter used in Brazil to assess the quality of cachaça decrease in beverages produced with each type of yeast after filtration (Figure 3B). This reduction reached 2.8 and 1.9-fold using the selected and baker's yeast, respectively, after filtration. Moreover, the volatile acidity value of the beverage produced with the selected yeast after filtration (39.61mg) was similar to that obtained using baker's yeast (33.35 mg of acetic acid) before filtration.

The copper content found in the unfiltered beverages was very high (Table 6). The cachaça samples with excess copper present levels between 8 and 10 mg L^{-1}

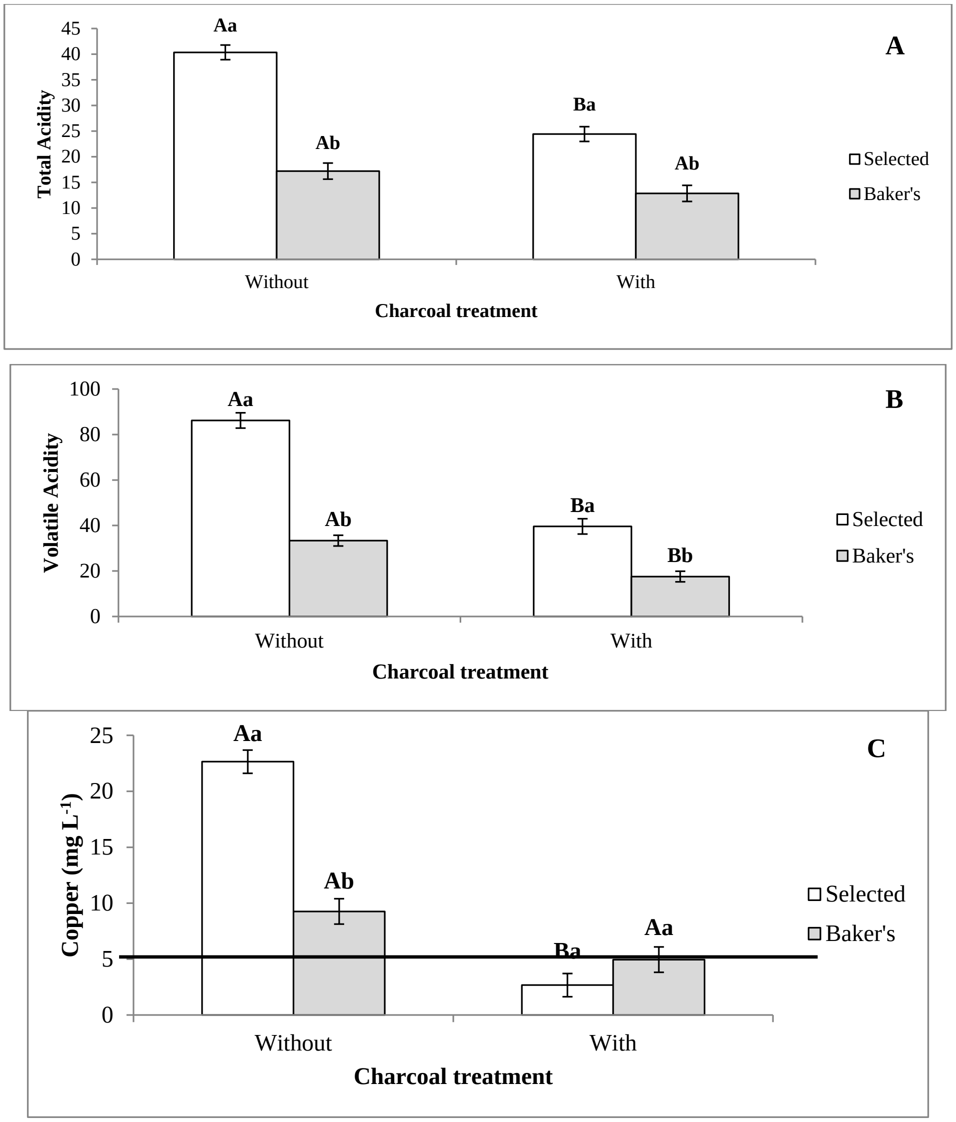

Figure 3. Filtration using a single charcoal column per replicate of cachaça produced. A. Total acidity (g CH_3COOH 100 mL^{-1} of sample); B. Volatilty acidity (mg of CH_3COOH 100 mL^{-1} anhydrous ethanol); and C. Copper content (mg L^{-1}). Bold black line indicates the maximum copper level allowed by Brazilian legislation (5 mg L^{-1}). Bars are the standard error. Capital letter compares filtration in each type of yeast and lower case letters compare strains in each kind of filtration. Same letters did not differ by Tukey test ($p \leq 0.05$).

(Lima et al., 2006) or more. Negri et al. (2015) found copper levels of up to 28 mg L^{-1} in the evaluated distillates. The copper content in samples prepared with must fermented with the selected yeast was up to 25.16 mg L^{-1} (Figure 4B). This result might be because the still used was made of copper and the low cleaning efficiency of the still, which increased the levels of copper above those permitted by Brazilian legislation. For the baker's yeast, the maximum level found was 10.68 mg L^{-1}. The acids help to drag the verdigris to the beverage (Boza and Horii, 2000). The increase of the metal in the beverage might have occurred because the selected yeast produced a higher amount of total acids in the distillate (Table 6).

After filtration, the copper concentration (15.95 mg L^{-1}) decreased 4.17-fold to 3.82 mg L^{-1} (Table 6). However, the effect of the interaction indicated the cachaça produced with the selected yeast, which contained higher copper levels due to discharge from the still, the decrease was even higher (8.45-fold) (Figure 3C). This effect occurred because of the order of passage of the beverage replicates inside the charcoal column (Figure 4). For the baker's yeast, copper level decreased from 8.885 to 0.248 mg L^{-1} in the filtered sample (Figure 4B).

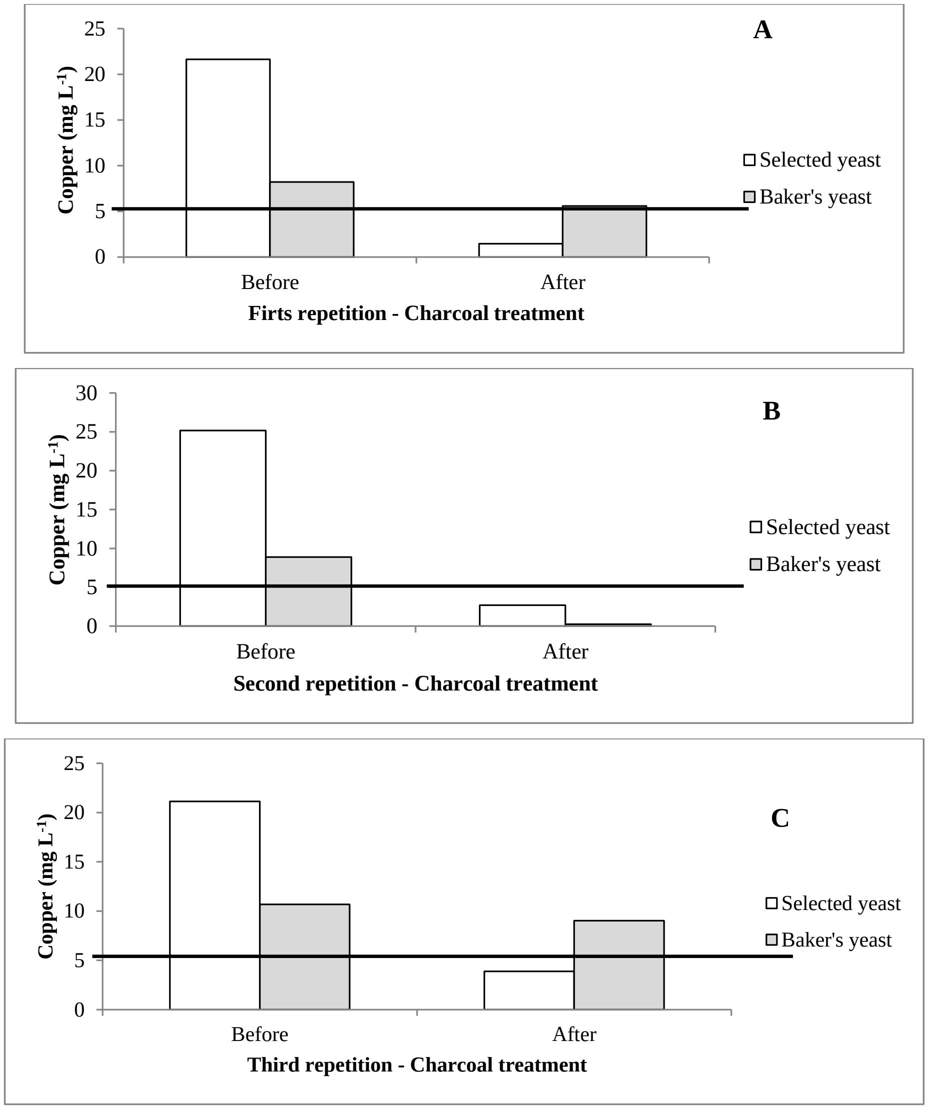

Figure 4. Filtration of cachaça using a single charcoal column per replicate. A. Filtration was performed in the beverage produced with the selected yeast and then in the beverage produced with the baker's yeast. B. In the second replicate, filtration was performed in the beverage produced with the baker's yeast, followed by selected yeast. C. In the third replicate, filtration was performed in the beverage produced with the selected yeast and then with baker's yeast. The bold black line indicates the maximum value allowed by the Brazilian legislation (5 mg L^{-1}).

The charcoal column removed high amounts of metal but presented problems of saturation (Figure 4). Therefore, the correct procedure would be mounting one column per sample to decrease the amount of copper in the cachaça to internationally acceptable levels.

No changes in the amount of ethanol in the beverages or in pH and turbidity were observed, as possibility of dragging of the charcoal. Bagasse would be discarded or used for burning or heating of the still during distillation. This residue is easily accessible to producers, and they only need to build the oven, grind and sieve the charcoal, and construct the column for cachaça filtration. It was verified that efficiency of conversion of sugarcane bagasse into charcoal was 30%. The cost of these materials is low when compared with other copper-removal technologies. Moreover, the charcoal used in filtering can be reused as fertilizer because it contains the nutrients required for new plantations of sugarcane.

Conclusions

Selected yeast had similar cachaça production as compared to the Baker's yeast. The beverage produced by fermentation using the selected yeast, contain a higher content of volatile acids in the distillate. The copper content in the beverage, in the experimental conditions used, was very high, with a maximum level of 25.16 mg L^{-1}. However, the charcoal obtained from sugarcane bagasse, from which sugarcane juice was extracted, can remove significant amounts of copper from the beverage to levels considered acceptable in the consumer market.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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