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Yeast recycling and the chemical and sensory quality of cachaça

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Abstract

Why was the work done: Cachaça fermentation often involves recycling the yeast for subsequent fermentations. This practice, common in the brewing and ethanol/biofuel industries, has received little technical attention in the production of cachaça. This work was conducted to address the gap in knowledge and assess the impact of recycling yeast on the quality of cachaça.
What are the main findings: Two commercial yeast strains - DistilaMax® RM ('RM') and CanaMax® ('CNX') - were used. Using sugarcane must, 14 consecutive recycled fermentations were performed. Cachaça (after distillation) was analysed for volatile compounds (GC-MS), ethyl carbamate (GC-FID) and sensorial analysis (Rate All That Apply) with evaluation using PCA.
Why is the work important: The sum of volatile compounds (coefficient of congeners) in cachaça produced with the CNX yeast showed greater consistency across all fermentation cycles compared to cachaça made with RM. Yeast CNX demonstrated superior robustness and adaptation to the local climate. However, the sensory quality of cachaça produced with RM was more favourably received by a sensory panel. Fermentation was successful with both yeasts through 14 cycles, producing cachaça that complied with Brazilian legislation. Sensory consistency was maintained up to the seventh cycle, but bacterial contamination was observed from the tenth cycle onwards.
Why is the work important: Recycling yeast cells across cachaça fermentations is a reliable technique, ensuring compliance with Brazilian regulations. The study highlights that using selected yeasts with recycling produced a standard final product, and potentially consistent batches of cachaça throughout the annual harvest.

Keywords

Saccharomyces cerevisiae; recycling; fermentation; cachaça; distilled spirit; rate all that apply; acceptance test; physicochemical characteristics.

Introduction

Cachaça is a Brazilian spirit produced by the distillation of fermented sugarcane juice with an alcohol content of 38 to 48% (v/v). The ethanol/water matrix is characterised by a complex mixt of flavour and aroma compounds including higher alcohols, ethyl esters, aldehydes, and organic acids (Nonato et al. 2001; Souza et al. 2006; Duarte et al. 2011). The physicochemical and sensory profiles of cachaça depend on several factors including manufacturing practice, distillation, ageing and fermentation (Bortoletto et al. 2018).

Traditionally, cachaça production is through the spontaneous or natural fermentation of sugarcane juice, with *Saccharomyces cerevisiae* being the predominant yeast (Oliveira et al. 2008). Spontaneous fermentation occurs by inoculation of sugarcane juice with microorganisms from the local environment (Portugal et al. 2016).As the contribution of *S. cerevisiae* in the microbiota is low, it is necessary to use a 'yeast starter' to increase the contribution of this yeast. In natural fermentation, the influence of yeast can be complex as different species or strains confer distinctive features to the flavour and aroma profile of spirits (Portugal et al. 2017). The natural process results in a more random fermentation that reflects the quality and quantity of microorganisms present in the juice (Campos et al. 2010).

However, in modern industrial fermentations, selected *S. cerevisiae* strains are used to accelerate the process, increase the content of the desirable metabolites, and limit the production of unwanted compounds. The use of pure yeast cultures prevents a major source of variation, and is recommended to standardise the production of cachaça (Fleet and Heard 1993). Further, selected *S. cerevisiae* strains speed up fermentation, improve the quality of the cachaça (Bernardi et al. 2008) with low acetic acid production and improved stress tolerance (Badotti et al. 2010).

In the bioethanol and brewing industries, the recycling of yeast cells from fermentation to fermentation is a common practice. This supports a consistent yeast addition at the start of fermentation and improves yield while reducing operational costs for yeast and water (Lopes et al. 2016).

In the brewing industry (Jenkins et al. 2003), yeast quality is managed to ensure optimal fermentation. Proper handling and storage of yeast, along with stringent hygienic practices, are implemented to avoid unwanted microbial contamination that could compromise the quality of the final product. The process involving the recovery of yeast through flocculation (or centrifugation) and its reuse (recycling) in subsequent fermentations, facilitates efficient production in brewing (Kalayu 2019) and the ethanol industry (Lopes et al. 2016). However, the application of yeast recycling to cachaça production presents challenges, as the alcohol yield, and sensory and chemical quality of the spirit must be carefully managed.

This study addresses the empirical nature of yeast cell recycling in cachaça production, aiming to characterise and improve the process. The work reported here compares two commercial yeast strains DistilaMax® RM ('RM')and CanaMax® ('CNX'). RM has been used in other sugarcane based distillates but not cachaça whereas CNX is an established strain used in the production of cachaça. This research provides insight into the recycling yeast between fermentations and the subsequent chemical and sensory quality of cachaça. Data is reported for optimising yeast recycling practices, enhancing product standardisation, and improving the quality of cachaça production.

Materials and methods

Cachaça production

Cachaça was produced by using two strains of *S. cerevisiae* - DistilaMax® RM (RM), and CanaMax® (CNX) - from Lallemand Biofuels & Distilled Spirits (Milwaukee, WI, United States). The manufacturer's instructions were followed with the dried yeasts reactivated by hydrating with filtered water at 33°C for 15 minutes. The yeasts were inoculated into sugarcane juice (18° Brix) as viable dry mass, with 0.6 g/L (2 x 10^{10} CFU/g) of RM and 0.5 g/L (1 x 10^{10} CFU/g) of CNX, respectively. In subsequent fermentations (recycling), yeast was not removed or added; the entire yeast mass was carried over to the next fermentation. The yeast was recovered from the supernatant by gravity decantation. A closed 35 L vessel (60 mm x 42 mm x 42 mm) with

an attached airlock and 30% headspace was used for fermentation without forced mixing. Fermentation temperature was 30°C, and fermentation was complete at 0° Brix. Yeast was recycled 14 times, with seven cachaça samples taken after one, three, five, seven, ten, 12 and 14 cycles.

Distillation was performed (in triplicate) in a copper still (50 cm height × 28 cm diameter + 28 cm length of the extension/cooler). The useful volume was 10 L with an average flow rate of 180 mL/min. A direct gas flame, which is widely used in Brazil, was used to heat the copper still. The fermented musts were distilled. The head and tail fractions were not recovered and were discarded with the residue. The distilled spirit or heart fraction accounted for 11% of the total volume, with approximately 50% (w/v). The heart fraction was separated using an alcoholometer and stored in glass bottles for a month, shielded from light and heat. This was diluted with distilled water to 40% (v/v) and used in the sensory analysis.

Chemical analysis

The heart fractionsof the distillate were analysed to quantify ethanol, volatile congeners, and ethyl carbamate. Aldehydes, esters, methanol, acetic acid, and higher alcohols (propyl alcohol, isobutanol, and isoamyl alcohol) were analysed using gas chromatography with flame ionisation detection (Bortoletto and Alcarde 2013). Aliquots (1 µL) were injected automatically into the chromatographic system (Shimadzu, QP-2010 PLUS, Tokyo, Japan) equipped with a Stabilwax-DA column (crossbond carbowax polyethylene glycol, 30 m × 0.18 mm × 0.18 µm film thickness). The analyses were performed with a 1:20 split ratio. Nitrogen was used as the carrier gas (flow rate of 1.5 mL/min, total flow of 27 mL/min at a pressure of 252.4 kPa). The temperature of the injector and detector was 240°C. The programme for the oven temperature was 40°C for 4 min, followed by an increase to 120°C at a rate of 20°C/min, held for 1 min, increased to 180°C at a rate of 30°C/min, and maintained for 4 min.

The analysis of ethyl carbamate was performed using a Shimadzu QP-2010 Plus gas chromatographic system coupled with a mass spectrometer, employing electron impact ionisation with an energy of 70 eV and a chromatography capillary HP-FFAP column with polar phase (esterified polyethylene glycol, 50 m × 0.20 mm × 0.33 µm stationary phase film thickness). The injector and the detector were set at (respectively) 230 and 220°C. The oven was programmed as follows: 90°C for 1 min, increasing to 150°C at a rate of 10°C/min, followed by an increase to 230°C at a rate of 30°C/min, and held for 2 min. An aliquot (1 µL) was injected in duplicate using splitless injection. Helium was the carrier gas at a flow rate of 1.2 mL/min. Selected ion monitoring acquisition was applied to monitor the m/z 62 ions for ethyl carbamate (Bortoletto et al. 2015).

Sensory analysis

The sensory study was approved by the Human Research Ethics Committee of the Escola Superior de Agricultura 'Luiz de Queiroz' (21491119.0.0000.5395). To compare the sensory characteristics of the cachaça samples, three samples were selected for each yeast strain from the 1st (beginning), 7th (middle), and 14th (end) of the 14 cycles of fermentation.

A panel of 15 cachaça producers (21 - 55 years old), with extensive technical expertise evaluated the cachaça samples. The panel underwent a two-day training course in the sensory quality of cachaça, during which the assessors were introduced to sensory science, received instructions and reference materials, and were exposed to the focus samples. Additionally, they were screened for sensory acuity for basic tastes. The selection of the team was based on the study by Giacalone and Hedelund (2016).

The analyses were performed in a single session following the procedures of the International Organization for Standardization (ISO 8589 2007). The panel assessed the six samples of cachaça obtained from the two yeast strains (RM and CNX). Each sample was adjusted with deionised water to to 40% (v/v). Samples (10 mL) of each sample (1st, 7th and 14th recycle for each yeast strain) were randomly given to tasters following the Williams Latin Square design (Williams 1949) in transparent cups marked with random three-digit numbers and covered with Petri dishes. Water was provided for rinsing the panellist's palate between tests.

Evaluation took place between 10 and 11 am, at room temperature (22–25°C), and under white light.

Using the rate-all-that-apply (RATA) test (Àres et al. 2014), the panellists were instructed to assess the samples using a 5-point scale using established sensory terms. In all, 15-18 terms were used covering various sensory considerations (appearance, aroma, flavour/taste, mouthfeel). The terms 'low', 'medium' and 'high' allowed the tasters to rate the intensity of selected terms on an ordinal scale.

The attributes for aroma included: fruity, floral, sweet, herbal/vegetable and for taste: acidic, bitter, sweet, salty and umami. Tactile sensations included: spicy, pungent, astringent, alcoholic, peppery, sweet. The 'peppery' and 'sweet' tactile sensations were respectively interpreted as 'burning' and 'velvety'. All terms were selected from the attributes of the sensory wheel of cachaça (Bortoletto 2023). For intensity of the sensory attributes, the references were based on Caetano et al (2021).

For the acceptance test, the same panel of trained tasters used a 9-point structured hedonic scale to evaluate the cachaça samples in terms of aroma, flavour, and overall acceptability. This evaluation allowed the panellists to rate how much they liked or disliked each sample.

Statistical analysis

The chemical analyses underwent multiple factor analysis (MFA), as the data represent groups of variables of different magnitudes and units. Five groups were selected of active variables to form the factors related to chemical parameters. In addition, two groups of supplementary qualitative variables were used reflecting recycling and yeast strain. The groups of active variables included ethanol content, fermentation yield (%), coefficient of congeners (acetaldehyde, ethyl acetate, and higher alcohols - propyl alcohol, isobutanol, and isoamyl alcohol), acetic acid, furfural), methanol, ethyl lactate, and ethyl carbamate.

The results of the chemical analyses and the acceptance test were analysed using analysis of variance (ANOVA) at a significance level of 5%, using SPSS software version 20.0. The results of RATA test were subjected to principal component analysis (PCA). The data were analysed using the software RStudio (R version 3.4.3) and packages RcmdrMisc, FactoMineR, SensoMineR, and factoextra (Lê et al. 2008).

Results and discussion

Cachaça production

After 14 consecutive fermentation cycles using sugarcane must, samples were analysed for volatile compounds (GC-MS), ethyl carbamate (GC-FID), and sensory analysis (rate-all-that-apply). The ethanol yield from fermentation is typically 90–92% (Guerra et al. 2001) and, in this study, the majority of fermentations achieved this, with the exception of the 10th and 12th cycles using yeast RM (Figure 1).

Ethanol and volatile compounds

Table 1 shows the ethanol content of cachaça after 14 recycles with two yeast strains (RM and CNX). After distillation and before standardisation, the ethanol content ranged from 47.3 to 53.8% (w/v). This was consistent during yeast recycling with CNX, but lower values were obtained after the 10th and 12th recycles using RM.

The cachaça samples from fermentation with yeast RM had higher total ester levels, particularly ethyl lactate and ethyl acetate (Table 1). Ethyl acetate as the predominant ester in cachaça, is formed during fermentation and after distillation by esterification of alcohols and acids (Serafim et al. 2013). While ethyl acetate at concentrations above 0.15 – 0.20 g/L can contribute a solvent-like flavour (Erten and Tanguler 2010), at lower concentrations it contributes a sweetish, fruity flavour (Amorim et al. 2016). The levels of ethyl acetate in cachaça from yeast RM ranged from 0.04 to 0.23 g/L, whereas cachaça produced with yeast CNX ranged from 0.03 to 0.07 g/L (Table 1). In all samples, the maximum acceptable value for ethyl acetate was not exceeded.

The levels of ethyl lactate (Table 1) ranged from 2.7 to 333 mg/L, suggesting the presence of lactic acid bacteria from the 10th recycle of yeast RM onwards. Ethyl lactate is the second most significant ester in cachaça (Nacimento et al. 2008).

Table 1.

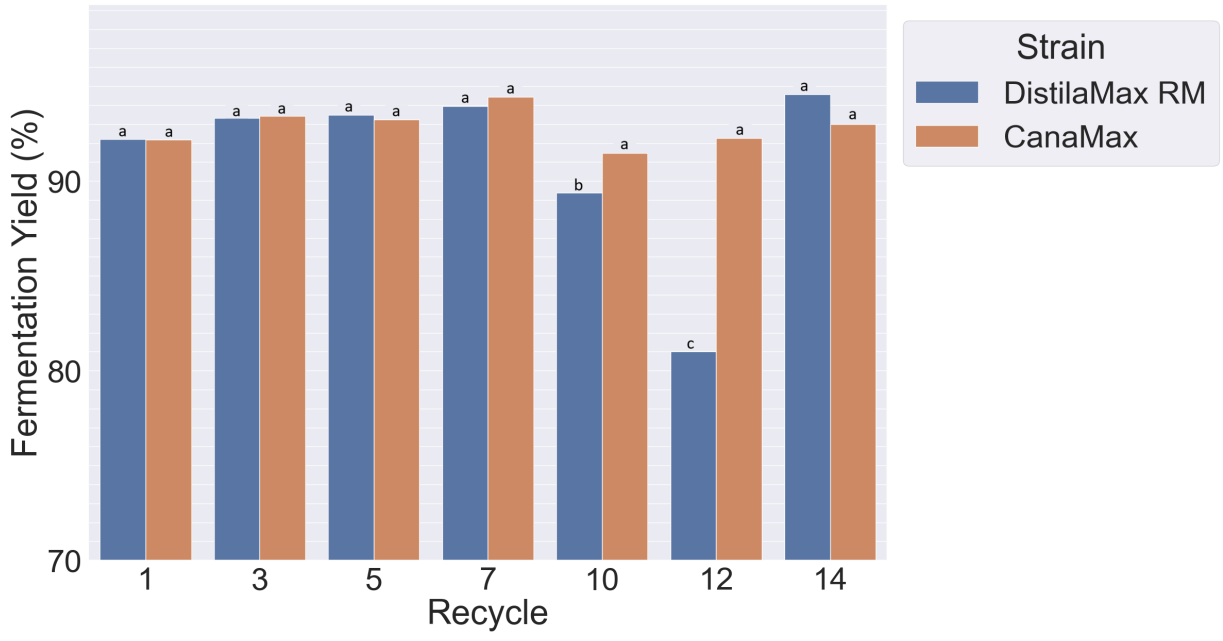
Concentration of volatile congeners (g/L), ethyl carbamate (µg/L) and ethyl lactate (mg/L) in cachaça samples from the 1st, 3rd, 5th, 7th, 10th, 12th, and 14th recycles using yeast strains RM and CNX.

Recycle	ETN	ACT	EA	MET	PRO	ISB	ETC	ISA	ACA	FUR	HGA	CON	ELC
1RM	52.38 ^{a}	0.10 ^{a}	0.16 ^{b}	< DL*	0.16 ^{b}	0.40 ^{a}	6.0 ^{d}	1.27 ^{a}	0.13 ^{e}	< DL*	0.56 ^{a}	2.22 ^{b}	6.96 ^{e}
3RM	52.58 ^{a}	0.04 ^{b}	0.10 ^{c}	< DL*	0.22 ^{a}	0.33 ^{a}	7.1 ^{d}	1.22 ^{a}	0.08 ^{e}	0.01 ^{a}	0.55 ^{a}	2.00 ^{b}	2.73 ^{e}
5RM	53.57 ^{a}	0.05 ^{b}	0.06 ^{d}	< DL*	0.29 ^{a}	0.20 ^{b}	11.6 ^{d}	0.74 ^{b}	0.10 ^{e}	0.01 ^{a}	0.49 ^{b}	1.45 ^{c}	2.99 ^{e}
7RM	53.49 ^{a}	0.02 ^{c}	0.04 ^{e}	< DL*	0.28 ^{a}	0.18 ^{b}	8.4 ^{d}	0.65 ^{b}	0.18 ^{e}	0.01 ^{a}	0.46 ^{b}	1.36 ^{c}	28.29 ^{d}
10RM	49.57 ^{b}	0.02 ^{c}	0.13 ^{b}	< DL*	0.10 ^{b}	0.08 ^{c}	87.9 ^{b}	0.30 ^{c}	1.15 ^{b}	< DL*	0.18 ^{c}	1.78 ^{b}	248.39 ^{b}
12RM	47.32 ^{c}	0.02 ^{c}	0.23 ^{a}	< DL*	0.10 ^{b}	0.07 ^{c}	135.8 ^{a}	0.35 ^{c}	2.07 ^{a}	< DL*	0.17 ^{c}	2.84 ^{a}	333.36 ^{a}
14RM	51.16 ^{a}	0.02 ^{c}	0.06 ^{d}	< DL*	0.20 ^{a}	0.06 ^{c}	48.2 ^{c}	0.47 ^{c}	0.58 ^{c}	0.02 ^{a}	0.26 ^{c}	1.41 ^{c}	239.37 ^{b}
1CNX	53.80 ^{a}	0.05 ^{b}	0.07 ^{d}	< DL*	0.12 ^{b}	0.23 ^{b}	9.3 ^{d}	1.08 ^{a}	0.33 ^{d}	< DL*	0.35 ^{b}	1.88 ^{b}	8.55 ^{e}
3CNX	52.21 ^{a}	0.03 ^{c}	0.04 ^{e}	0.01 ^{a}	0.16 ^{b}	0.23 ^{b}	16.0 ^{d}	0.90 ^{b}	0.12 ^{e}	0.01 ^{a}	0.39 ^{b}	1.50 ^{c}	2.81 ^{e}
5CNX	52.07 ^{a}	0.03 ^{c}	0.03 ^{e}	< DL*	0.16 ^{b}	0.21 ^{b}	35.7 ^{c}	0.67 ^{b}	0.14 ^{e}	0.01 ^{a}	0.37 ^{b}	1.25 ^{c}	3.54 ^{e}
7CNX	51.41 ^{a}	0.06 ^{b}	0.06 ^{d}	< DL*	0.21 ^{a}	0.14 ^{b}	40.5 ^{c}	0.53 ^{c}	0.12 ^{e}	0.01 ^{a}	0.35 ^{c}	1.13 ^{c}	2.72 ^{e}
10CNX	51.63 ^{a}	0.04 ^{b}	0.07 ^{d}	0,01 ^{a}	0.19 ^{a}	0.17 ^{b}	51.3 ^{c}	0.68 ^{b}	0.13 ^{e}	0.02 ^{a}	0.36 ^{b}	1.31 ^{c}	22.30 ^{d}
12CNX	51.17 ^{a}	0.03 ^{c}	0.04 ^{e}	0,01 ^{a}	0.17 ^{a}	0.15 ^{b}	54.7 ^{c}	0.79 ^{b}	0.13 ^{e}	0.02 ^{a}	0.32 ^{b}	1.34 ^{c}	46.25 ^{c}
14CNX	51.43 ^{a}	0.02 ^{c}	0.03 ^{e}	0,01 ^{a}	0.19 ^{a}	0.13 ^{b}	82.5 ^{b}	0.79 ^{b}	0.20 ^{d}	0.02 ^{a}	0.32 ^{b}	1.39 ^{c}	67.47 ^{c}

Ethanol (ETN); acetaldehyde (ACT); ethyl acetate (EA); methanol (MET); propyl alcohol (PRO); isobutanol (ISB); ethyl carbamate (ETC); isoamyl alcohol (ISA); acetic acid (ACA); furfural (FUR); higher alcohols (HGA); coefficient of congeners (CON); ethyl lactate (ELC). * DL = Detection limit. The mean data with different superscript lowercase letters are significantly different ($p < 0.05$) according to the analysis of variance test.

Figure 1.

Fermentation yield (%) after recycling with yeast strains RM and CNX.



Lactic acid bacteria (*Lactobacillus* spp.) are commonly associated with sugarcane (Bortoletto et al. 2015). The 10th-14th recycled fermentations with yeast RM and 12th and 14th with yeast CNX suggest the presence of lactic acid bacteria. Nonetheless, the levels of ethyl lactate in this study remained below the levels reported in previous studies (Nascimento et al. 2008; Maia et al. 2020). Although good manufacturing practices used by commercial distilleries were replicated in this project, Bortoletto et al (2018) reported that the practice that limits the presence of bacteria in the must is pasteurisation of the sugarcane juice. This process was not employed in this study.

The level of acetaldehyde decreased during the recycling of both yeast strains (Table 1). Acetaldehyde is the major aldehyde in cachaça and at moderate levels imparts a fruity, herbaceous (green leaves) character in non-aged cachaça (Amorim et al. 2016; Serafim et al. 2013). In contrast, higher levels of acetaldehyde can produce a pungent, irritating aroma, which may be associated with undesirable side effects or possible health risks (Lachenmeier and Sohnius 2008). The accumulation of acetaldehyde in the liver is associated with hangover symptoms, such as headache and nausea (Lachenmeier and Sohnius 2008). As acetaldehyde is produced by yeast and acetic bacteria, it is important to adhere to good practice and control during fermentation. Furthermore, effective separation of the head fraction during distillation is essential to prevent excessive levels of acetaldehyde (Dato et al. 2005). Distillation in these trials removed 1.5% of the head fraction which avoided undesirable side effects, maintaining the concentration of acetaldehyde in cachaça at 20-60 mg/L which is below the upper limit allowed by Brazilian legislation (Brazil 2022).

The concentration of acetic acid ranged from 0.08 to 2.07 g/L in cachaça fermentations with yeast RM and from 0.12 to 0.20 g/L using CNX. The levels increased in the final cycles of fermentation with yeast RM, although the acetic acid level in the distillates was below the maximum legal limit (Brazil 2022) until the 7th cycle. In the 10th and 12th cycles, the content of acetic acid exceeded the legal limit but in the 14th cycle was within the legal standard. The acetic acid content in cachaça produced with yeast CNX was within the legal limits across all fermentations.

Acetic acid contributes to the acidity, burning and pungent sensations in cachaça and its presence can lead to sensory rejection (Odello et al. 2009). Excessive levels are associated with spoilage by lactic or acetic bacteria during or after fermentation (Bortoletto et al. 2018). Indeed, high levels of volatile acidity in distilled spirits are linked to the presence of bacteria during fermentation (Bortoletto and Alcarde 2013) with elevated levels of acetic acid and ethyl lactate in fermentation linked to bacterial contamination (Nascimento et al. 2008). Overall, acetic acid is an undesirable compound and is associated with cachaça of lower quality.

With the higher alcohols (Table 1), the level of isobutanol - associated with a bitter taste (Duarte et al. 2010) - increased during yeast recycling for both yeast strains. Conversely, the production of propyl alcohol decreased with recycling. The presence of propyl alcohol is linked to an 'alcoholic aroma', and its production reflects the strain of *S. cerevisiae* used (Czerny et al. 2008). Isoamyl alcohol was the most abundant higher alcohol although its concentration decreased across the fermentation cycles with both strains. Isoamyl alcohol is associated with 'banana' and 'sweet' descriptors, which enhance the taste and aroma of the spirits. Higher alcohols are formed by yeast during fermentation from aromatic and branched-chain amino acids (catabolic) or from pyruvic acid (anabolic). The levels of these compounds is also related to the amino nitrogen content in the medium. When nitrogen is limited, yeast synthesises amino acids, increasing the formation of higher alcohols (Vidal et al. 2013). The levels of higher alcohols reflect good manufacturing practice (Bortoletto et al. 2018), with distillation shortly after the end of fermentation essential for reducing their level.

All cachaça samples were within the range for the coefficient of congeners - the sum of volatile compounds including esters, aldehydes, furfural, higher alcohols, and volatile acidity (Brazil 2022). Cachaça derived from distillation after fermentation using yeast RM exhibited a higher coefficient of congeners in all cycles compared to yeast CNX. Excluding the 12th cycle, the coefficient of congeners for RM ranged from 1.36 to 2.22 g/L. However, with the 12th cycle, acetic acid was 2.84 g/L, which was likely to be due to bacterial contamination.

Cachaça using yeast CNX showed a gradual increase in the content of ethyl carbamate throughout the yeast cycles (Table 1). This compound is potentially carcinogenic and can be present in beverages produced by fermentation (Alcarde et al. 2012). Its presence in food and beverages is a concern and should be kept within the limit (210 µg/L) (Brazil 2022).

Cachaça using yeast RM maintained constant levels of ethyl carbamate throughout the first seven cycles, with an increase only in the later cycles. This increase was influenced by the acidity of the medium as well as by the presence of bacteria. Previous studies have shownthat some yeast strains keep the levels of ethyl carbamate low (Portugal et al. 2017; Duarte et al. 2010). This suggests that in this work in the later fermentation cycles with RM, there was bacterial contamination which could have increased the content of ethyl carbamate (Bortoletto et al. 2015).

Multiple factor analysis

The analyses were subjected to multiple factor analysis (MFA) to understand the role of sets of variables in contributing to variance within the samples. The analytical data sets underwent Principal Component Analysis (PCA) and showed clustering of the cachaça samples around on the yeast strains used in their production.

In the multiple factor analysis both active and supplementary variables were reported (Figure 2). Supplementary variables including fermentation yield, alcohol content, level of higher alcohols, and coefficient of congeners were related to dimension 1 of the multiple factor analysis. The active variables represented on the horizontal axis, corresponding to dimension 1 (38.2% of the total variance) were ethyl lactate, acetic acid, ethyl carbamate, and acidity. These congeners are associated with bacterial activity. Figure 2 shows that the variability caused by these congeners is related to the coefficient of congeners as well as to the fermentation yield.

The yield vector is opposite to that of the congeners indicating bacterial presence and suggesting an impact on yield. These samples had lower yields and lower alcohol content. This is illustrated in Figure 2, where the vectors for ethyl lactate, ethyl carbamate, and acetic acid are opposite that for alcohol content. The increase in ethyl lactate suggests the presence of lactic acid bacteria, microorganisms that adversely affect ethanol production.

Dimension 2 in Figure 2, represented on the vertical

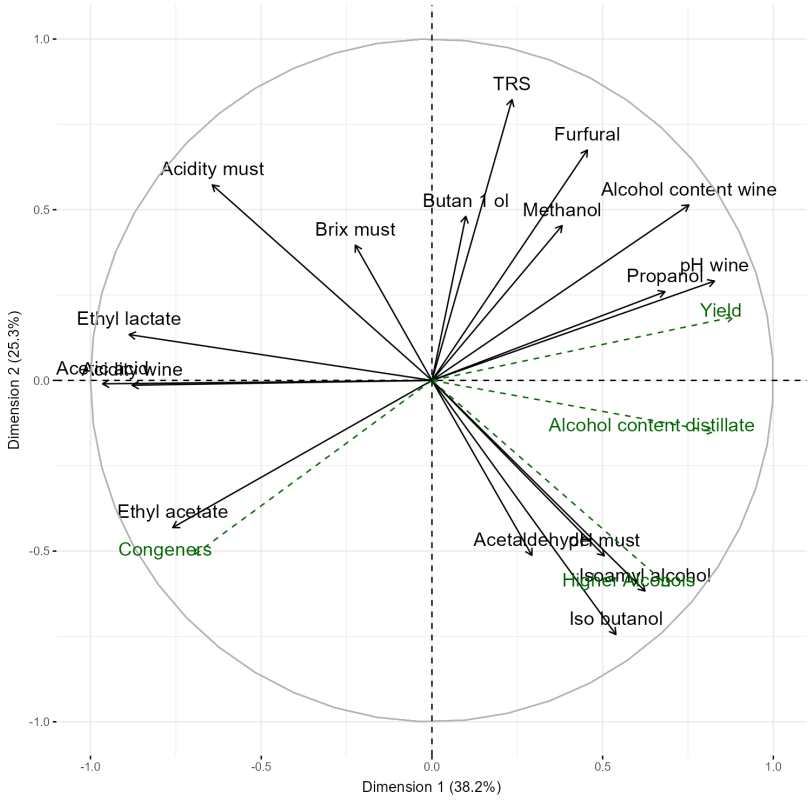


Figure 2.

Contribution of supplementary (green) **and active** (black) **quantitative variables to dimensions 1 and 2 of the multiple factor analysis.**

axis, the active variables alcohol content and pH are shown together with the supplementary variable for the coefficient of congeners. This dimension concentrates the variability related to the parameters of the 'wine' (fermented sugarcane juice). The pH of the wine is opposed to the vectors for acetic acid and ethyl acetate, indicating that as pH increases the total content of esters and acidity decrease. The alcohol content of the wine (dimension 2), is opposite to the congeners, indicating that higher levels of congeners are correlated with a lower alcoholic content.

Figure 3 shows the cachaça samples projected on the first two dimensions generated by the MFA. Higher values of the variables are observed in cachaça from the 10th and 12th cycles from yeast RM in relation to dimension 1. This is associated with a higher concentration of acetic acid, ethyl lactate, and ethyl carbamate which are likely to be associated with microbial spoilage. With the 12th fermentation cycle using RM, the coefficient of congeners was higher in the cachaça (Table 1).

The cachaça samples produced from the 1st and 3rd fermentation cycles using RM exhibited a greater distance to the other samples due to higher values of acetaldehyde, isobutanol, and isoamyl alcohol

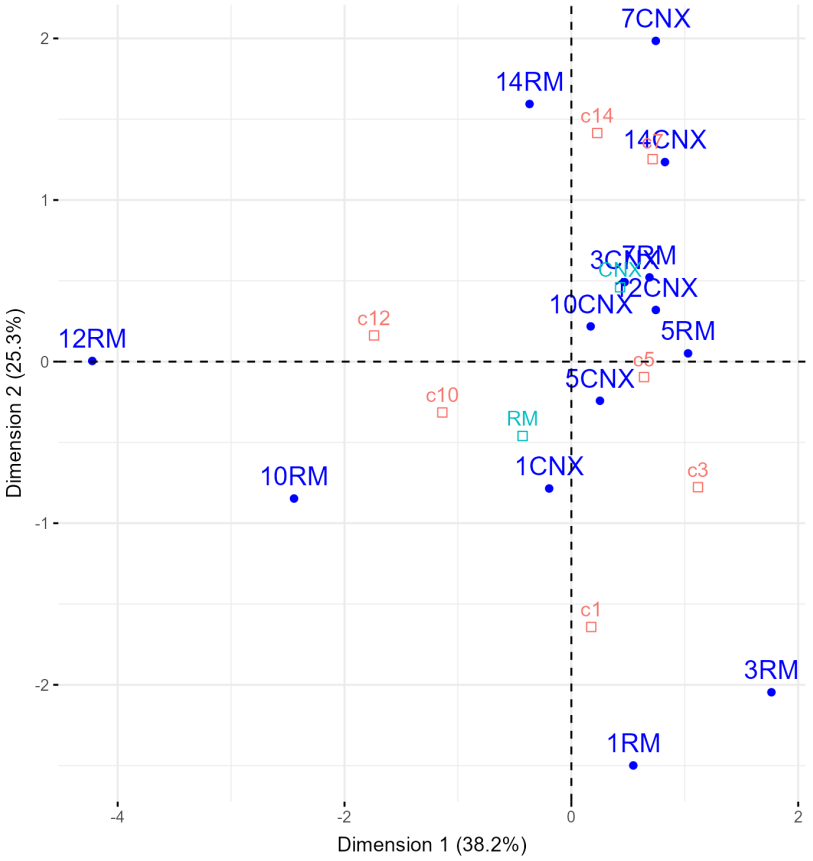
On the vertical axis (dimension 2), a greater multivariate proximity is observed from cachaça produced using yeast CNX. This proximity reflects the consistency and similarity of these cachaça samples across most variables compared to those from yeast RM. This is important insight for yeast recycling, contributing to a more stable final product.

Sensory analysis

Figures 4 and 5 show the positioning of cachaça from the 1st, 7th, and 14th fermentation cycles using yeast RM and CNX, according to their similarities and differences in dimensions 1 and 2 (Figure 4), and 1 and 3 (Figure 5). The sample from the 1st cycle using RM is associated with dimension 1, with strong aroma, aftertaste, floral and fruity sensations

Figure 3.

Cachaça produced using yeasts RM and CNX and their respective recycles (1st, 3rd, 5th, 7th, 10th, 12th, and 14th) in dimensions 1 and 2 of the multiple factor analysis.



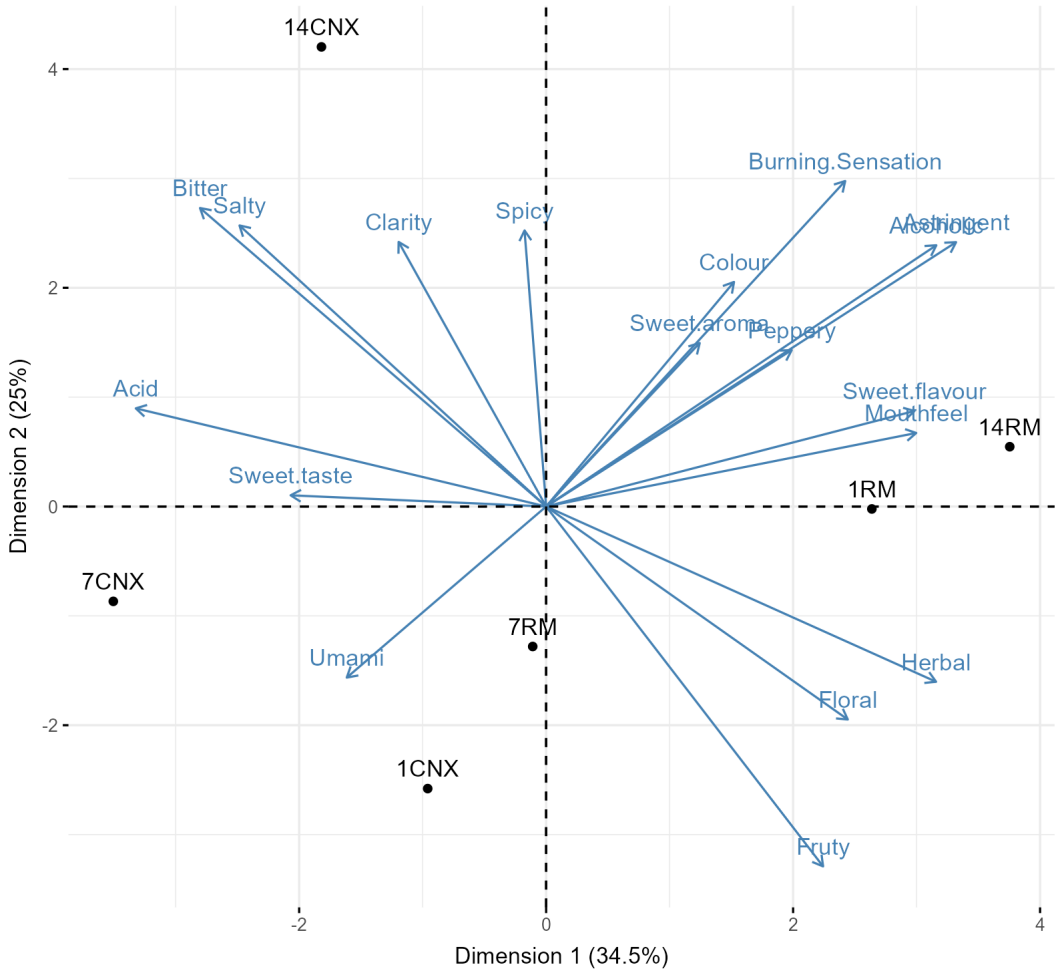
(Figure 4). The distilled spirit from the 14th cycle using RM yeast showed a more pronounced astringent and alcoholic sensation compared to the 1st fermentation. The sample from the 7th cycle using RM was more distinct and closely related to dimension 3, with a strong sweet taste, intense sweet aroma, and a slight spicy sensation (Figure 5).

The cachaça distilled from fermentation with yeast CNX showed distinct sensory features. While contributing a consistent chemical composition across all recycles, the sensory character varied. The sample from the 1st cycle using CNX was associated with dimension 3 with a spicy flavour and a slight sweet aroma (Figure 5). The cachaça samples from the 7th and 14th cycles using CNX were more closely associated with dimension 1. The sample from the 7th cycle was characterised by a strong acidic and sweet flavour (Figure 4), while the sample from the 14th recycle exhibited bitter, salty, and acidic tastes (Figure 5).

Characteristics such as sweet taste and aroma contribute to acceptability of a product by consumers. In contrast, flavours such as bitter, salty, and acidic taste, together with astringent and alcoholic sensations are less well received. The present results align with a previous study (Odello et al. 2009), which demonstrated that sweet taste improves the sensory quality of cachaça, while acidity, alcoholic flavour, and bitter taste are considered less desirable.

Figure 4.

Cachaça produced using yeasts RM and CNX and their respective recycles (1st, 7th, and 14th) in dimensions 1 and 2 of the principal component analysis.



Drawing parallels between the physicochemical and sensory analyses, the cachaça samples obtained using CNX yeast demonstrated better overall quality. The acceptance test indicated positive results for the cachaça samples produced using RM yeast highlighting their sensory attributes.

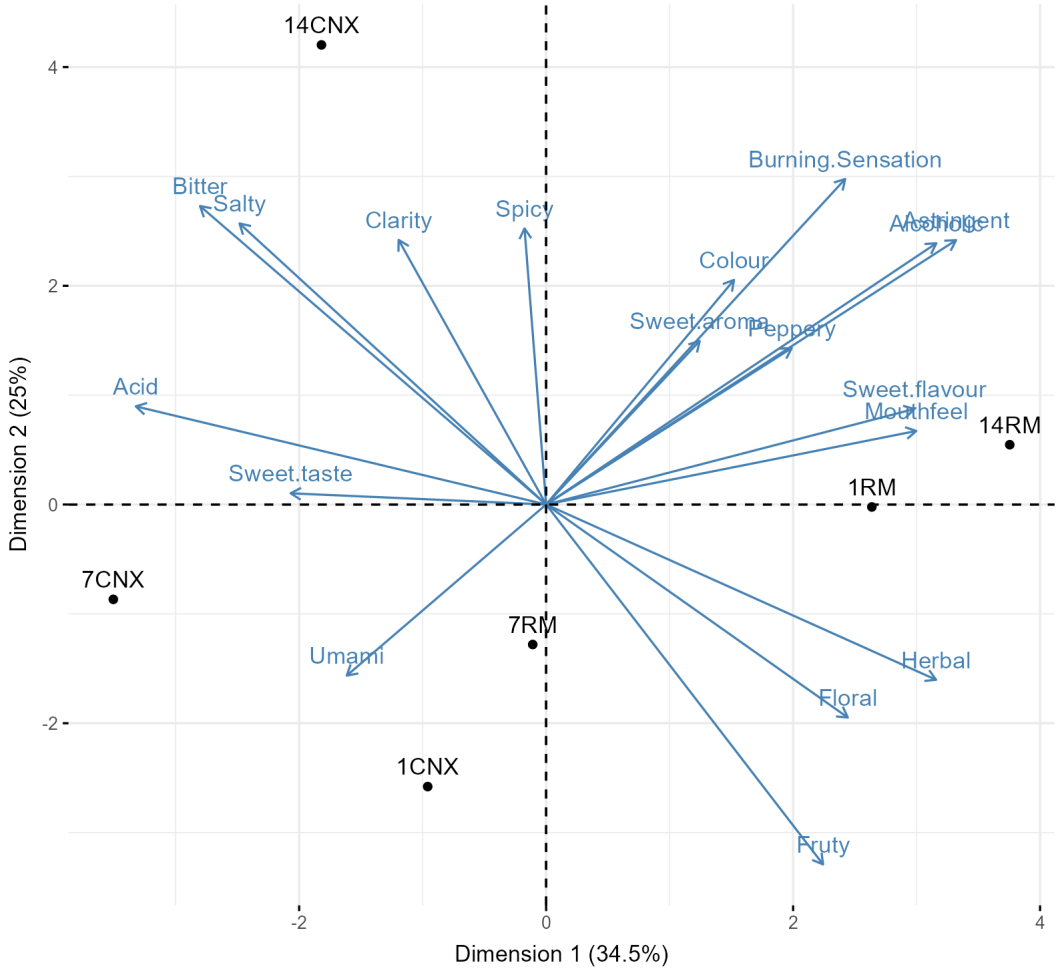
Yeast CNX produced a more consistent cachaça in terms of congener content across the fermentation cycles. In contrast, yeast RM did not produce consistent cachaça samples, with notable variations in the 10th and 12th recycles affecting the yield. Nonetheless, this yeast strain generated cachaça samples with well regarded characteristics, including the most highly accepted sample among those tested.

Recycling yeast in cachaça fermentations has proven to be a reliable technique, providing producers with a consistent method. The resulting distillates meet the stringent standards set by Brazilian legislation. Additionally, this research shows that when this approach is combined with selected yeast strains, it results in a standardised final product, potentially ensuring uniformity across batches throughout the annual harvest.

Oliveira et al (2023) highlighted the significance and impact of microbial contamination during the fermentation of cachaça and in ethanol production. There is a wide range of contaminating microorganisms, with those from the *Lactobacillaceae* family being prevalent.

Figure 5.

Cachaça produced using yeasts RM and CNX and their respective recycles (1st, 7th, and 14th) in dimensions 1 and 3 of the principal component analysis.



Both bacterial and yeast contaminants can disrupt fermentation and affect the final product, necessitating stringent control measures. Research has highlighted the physiological traits and diverse applications of contaminating yeast and bacteria. This understanding is important to improve fermentation involving *S. cerevisiae* and identifying factors that influence this process.

Different yeast strains affect the chemical and sensory profiles of cachaça, with yeast CNX demonstrating consistent results across recycling and yeast RM producing cachaça with well regarded sensory attributes despite some variability. These findings are useful for both scientific understanding and industrial practice, as they underscore the importance of yeast strain selection to optimise both quality and consistency in cachaça production. Effective control of microbial contamination during fermentation is also highlighted, reflecting the significant impact on product quality. Future studies should focus on the impact of microbiological contamination on cachaça fermentation whether they be positive or negative. Additionally, research should explore and identify useful fermentation practices, for example the acid washing of yeast. This is used manage bacterial contamination in both the brewing and the ethanol industries. Understanding these factors will be important for optimising fermentation processes and improving the overall quality of cachaça.

Conclusions

The two strains of *S. cerevisiae* used in this work impacted the physicochemical profile of cachaça. Yeast RM resulted in wine with higher levels of total esters, including ethyl lactate and ethyl acetate. Yeast CNX did not produce significant amounts of congeners across the fermentation cycles. These differences in chemical composition impacted sensory quality, with yeast RM yielding the most preferred samples. Both yeast strains demonstrated effective adaptation to the fermentation process with sensory analysis of the distillate showing satisfactory acceptance scores for the cachaça spirit.

Author contributions

Luisa C. Carvalho: formal analysis, methodology, project administration, writing (original draft).
Giovanni C. Silvello: data curation.
Mariana C. Castro: formal analysis.
Maryse Bolzon: conceptualisation.
Robert Piggot: conceptualisation.
Elena Fossati: formal analysis, writing (review and editing).
André R. Alcarde: conceptualisation, funding acquisition, methodology, supervision, writing (review and editing).

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Conflict of interest

The authors declare that there are no conflicts of interest.

References

Alcarde AR, De Souza LM, Bortoletto AM. 2012. Ethyl carbamate kinetics in double distillation of sugarcane spirit. *J Inst Brew* 118:27-31. https://doi.org/10.1002/jib.14

Amorim JC, Schwan RF, Duarte WF. 2016. Sugarcane spirit (cachaça): Effects of mixed inoculum of yeasts on the sensory and chemical characteristics. *Food Res Int* 85:76–83. https://doi.org/10.1016/j.foodres.2016.04.014

Àres G, Bruzzone F, Vidal L, Cadena RS, Giménez A, Pineau B, Hunter DC, Paisley AG, Jaeger SR. 2014. Evaluation of a rating-based variant of check-all-that-apply questions: Rate-all-that-apply (RATA). *Food Qual Prefer* 36:87–95. https://doi.org/10.1016/j.foodqual.2014.03.006

Badotti F, Belloch C, Rosa CA, Barrio E, Querol A. 2010. Physiological and molecular characterisation of *Saccharomyces cerevisiae* cachaça strains isolated from different geographic regions in Brazil. *World J Microbiol Biotechnol* 26:579–587. https://doi.org/10.1007/s11274-009-0206-0

Bernardi TL, Pereira GVM, Cardoso PG, Dias ES, Schwan RF. 2008. *Saccharomyces cerevisiae* strains associated with the production of cachaça: identification and characterization by traditional and molecular methods (PCR, PFGE and mtDNA-RFLP). *World J Microbiol Biotechnol* 24:2705–2712. https://doi.org/10.1007/s11274-008-9799-y

Bortoletto AM. 2023. Rum and cachaça, p 61-74. *In* Hill A, Jack F (eds), *Distilled Spirits*. Academic Press.

Bortoletto AM, Alcarde AR. 2013. Congeners in sugarcane spirits aged in casks of different woods. *Food Chem* 139:695–701. https://doi.org/10.1016/j.foodchem.2012.12.053

Bortoletto AM, Silvello GC, Alcarde AR. 2015. Chemical and microbiological quality of sugarcane juice influences the concentration of ethyl carbamate and volatile congeners in cachaça. *J Inst Brew* 121:251–256. https://doi.org/10.1002/jib.213

Bortoletto AM, Silvello GC, Alcarde AR. 2018. Good manufacturing practices, hazard analysis and critical control point plan proposal for distilleries of cachaça. *Sci Agric* 75: 432-443. http://dx.doi.org/10.1590/1678-992X-2017-0040

Brazil. 2022. MAPA Ordinance No. 539/2022. Establishes the Identity and Quality Standards for sugarcane brandy and cachaça. https://infoalimentario.com/2023/01/18/brazil-mapa-ordinance-no-539-2022-establishes-the-identity-and-quality-standards-for-sugarcane-brandy-and-cachaca/

Caetano DL, Lima CM, Sanson AL, Silva DF, Hassemer GS, Verruck S, Silva GA, Afonso RJCF, Coutrim MX, Gregório SR. 2021. Descriptive screening and lexicon development of non-aged artisanal cachaça sensorial profile using principal component analysis and Kohonen artificial neural networks. *J Sens Stud* 36:12645. https://doi.org/10.1111/joss.12645

Campos CR, Silva CF, Dias DR, Basso LC, Amorim HV, Schwan RF. 2010. Features of *Saccharomyces cerevisiae* as a culture starter for the production of the distilled sugarcane beverage, cachaça in Brazil. *J Appl Microbiol* 108:1871–1879. https://doi.org/10.1111/j.1365-2672.2009.04587.x

Czerny M, Christlbauer M, Christlbauer M, Fischer A, Granvogl M, Hammer M, Hartl C, Hernandez NM, Schieberle P. 2008. Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. *Eur Food Res Technol* 228:265–273. https://doi.org/10.1007/s00217-008-0931-x

Dato MCF, Pizauro Júnior JM, Mutton MJR. 2005. Analysis of the secondary compounds produced by *Saccharomyces cerevisiae* and wild yeast strains during the production of 'cachaça'. *Braz J Microbiol* 36:70–74. https://doi.org/10.1590/S1517-83822005000100014

Duarte WF, Dias DR, Oliveira JM, Vilanova M, Teixeira JÁ, Silva JBA, Schwan RF. 2010. Raspberry (*Rubus idaeus* L.) wine: Yeast selection, sensory evaluation and instrumental analysis of volatile and other compounds. *Food Res Int* 43:2303–2314. https://doi.org/10.1016/j.foodres.2010.08.003

Duarte WF, Sousa MVF, Dias DR, Schwan RF. 2011. Effect of co-inoculation of *Saccharomyces cerevisiae* and *Lactobacillus fermentum* on the quality of the distilled sugarcane beverage cachaça. *J Food Sci* 76:C1307–C1318. https://doi.org/10.1111/j.1750-3841.2011.02412.x

Erten H, Tanguler H. 2010. Influence of *Williopsis saturnus* yeasts in combination with *Saccharomyces cerevisiae* on wine fermentation. *Lett Appl Microbiol* 50: 474–479. https://doi.org/10.1111/j.1472-765X.2010.02822.x

Fleet GH, Heard GM. 1993. Yeast-growth during fermentation. *In* Fleet GH (ed), *Wine Microbiology and Biotechnology*, Harwood Academic Publishers, Chur, Switzerland.

Giacalone D, Hedelund PI. 2016. Rate-all-that-apply (RATA) with semi-trained assessors: An investigation of the method reproducibility at assessor-, attribute- and panel-level. *Food Qual Prefer* 51: 65–71. https://doi.org/10.1016/j.foodqual.2016.02.017

Guerra JB, Araújo RA, Pataro C, Franco GR, Moreira ES, Mendonça-Hagler LC, Rosa CA. 2001. Genetic diversity of *Saccharomyces cerevisiae* strains during the 24 h fermentative cycle for the production of the artisanal Brazilian cachaça. *Lett Appl Microbiol* 33:106–111. https://doi.org/10.1046/j.1472-765x.2001.00959.x

International Organization for Standardization. ISO 8589:2007. 2007. Sensory Analysis: General guidance for the design of test rooms. International Organization for Standardization: Geneva, Switzerland. https://www.iso.org/standard/36385.html#:~:text=ISO%208589%3A2007%20provides%20general,those%20that%20are%20merely%20desirable.

Jenkins CL, Kennedy AI, Hodgson JA, Thurston P, Smart KA. 2003. Impact of serial repitching on lager brewing yeast quality. *J Am Soc Brew Chem* 61:1–9. https://doi.org/10.1094/ASBCJ-61-0001

Kalayu G. 2019. Serial re-pitching: its effect on yeast physiology, fermentation performance, and product quality. *Ann Microbiol* 69:787-796. https://doi.org/10.1007/s13213-019-01493-4

Lachenmeier DW, Sohnius EM. 2008. The role of acetaldehyde outside ethanol metabolism in the carcinogenicity of alcoholic beverages: Evidence from a large chemical survey. *Food Chem Toxicol* 46:2903–2911. https://doi.org/10.1016/j.fct.2008.05.034

Lê S, Josse J, Husson F. 2008. FactoMineR: an R package for multivariate analysis. *J Stat Softw* 25:1-18. https://doi.org/10.18637/jss.v025.i01

Lopes ML, Paulillo SCL, Godoy A, Cherubin RA, Lorenzi MS, Giometti FHC, Bernardino CD, Amorim Neto HB, Amorim HV. 2016. Ethanol production in Brazil: a bridge between science and industry. *Braz J Microbiol* 47:64–76. https://doi.org/10.1016/j.bjm.2016.10.003

Maia AB, Marinho LS, Nelson DL. 2020. Advance in the characterization of alambic cachaça: ethyl lactate. *Res Soc Dev* 9:e297997116. https://doi.org/10.33448/rsd-v9i9.7116

Nascimento ESP, Cardoso DR, Franco DW. 2008. Quantitative ester analysis in cachaça and distilled spirits by gas chromatography-mass spectrometry (GC-MS). *J Agric Food Chem* 56:5488–5493. https://doi.org/10.1021/jf800551d

Nonato E, Carazza F, Silva FC, Carvalho CR, Cardeal ZL. 2001. A headspace solid-phase microextraction method for the determination of some secondary compounds of Brazilian sugarcane spirits by gas chromatography. *J Agric Food Chem* 49:3533–3539. https://doi.org/10.1021/jf000896r

Odello L, Braceschi GP, Seixas FRF, Silva AA, Galinaro CA, Franco DW. 2009. Sensory evaluation of cachaça. *Quim Nova* 32:1839–1844. https://doi.org/10.1590/S0100-40422009000700029

Oliveira ACD, Oliveira CAF, Kamimura ES. 2023. Microbial contamination in the ethanol and cachaça fermentation process: impacts and applications. *Food Sci Technol* 43:1-8. https://doi.org/10.5327/fst.80422

Oliveira VA, Vicente MA, Fietto LG, Castro IM, Coutrim MX, Schüller D, Alves H, Casal M, Santos JO, Araújo LD, Silva PHA, Brandão RL. 2008. Biochemical and molecular characterization of *Saccharomyces cerevisiae* strains obtained from sugarcane juice fermentations and their impact in cachaça production. *Appl Environ Microbiol* 74:693–701. https://doi.org/10.1128/AEM.01729-07

Portugal CB, Alcarde AR, Bortoletto AM, Silva AP. 2016. The role of spontaneous fermentation for the production of cachaça: a study of case. *Eur Food Res Technol* 242:1587-1597. https://doi.org/10.1007/s00217-016-2659-3

Portugal CB, Silva AP, Bortoletto AM, Alcarde AR. 2017. How native yeasts may influence the chemical profile of the Brazilian spirit, cachaça? *Food Res Int* 91:18–25. https://doi.org/10.1016/j.foodres.2016.11.022

Serafim FAT, Seixas FRF, Silva AA, Galinaro CA, Nascimento ESP, Buchviser SF, Odello L, Franco DW. 2013. Correlation between chemical composition and sensory properties of Brazilian sugarcane spirits (*Cachaças*). *J Braz Chem Soc* 24:973–982. https://doi.org/10.5935/0103-5053.20130125

Souza MDCA, Vásquez P, Del Mastro NL, Acree TE, Lavin EH. 2006. Characterization of cachaça and rum aroma. *J Agric Food Chem* 54:485–488. https://doi.org/10.1021/jf0511190

Vidal EE, Billerbeck GM, Simões DA, Schuler A, François JM, Morais Junior MA. 2013. Influence of nitrogen supply on the production of higher alcohols/esters and expression of flavour-related genes in cachaça fermentation. *Food Chem* 138:701–708. https://doi.org/10.1016/j.foodchem.2012.10.147

Williams EJ. 1949. Experimental designs for the estimation of residual effects of treatments. *Aust J Agric Res* 2:149-168. https://doi.org/10.1071/CH9490149