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ORIGINAL ARTICLE

Features of *Saccharomyces cerevisiae* as a culture starter for the production of the distilled sugar cane beverage, cachaça in Brazil

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Abstract

Aims: To evaluate the dominance and persistence of strains of *Saccharomyces cerevisiae* during the process of sugar cane fermentation for the production of cachaça and to analyse the microbial compounds produced in each fermentative process.

Methods and Results: Three *S. cerevisiae* strains were evaluated during seven consecutive 24-h fermentation batches using recycled inocula. The UFLA CA 116 strain had the largest population of viable organisms, and the maximum population was achieved in the fourth batch after 96 h of fermentation. The UFLA CA 1162 and UFLA CA 1183 strains grew more slowly, and the maximum population was reached in the seventh batch. Molecular characterization of isolated yeast cells using PFGE (pulse field gel electrophoresis) revealed that more than 86% of the isolates corresponded to the initially inoculated yeast strain. The concentration of aldehydes, esters, methanol, alcohol and volatile acids in the final-aged beverages were within the legal limits.

Conclusions: Cachaça produced by select yeast strains exhibits analytical differences. UFLA CA 1162 and UFLA CA 116 *S. cerevisiae* isolates can be considered the ideal strains for the artisanal production of cachaça in Brazil.

Significance and Impact of the Study: The use of select yeast strains can improve the quality and productivity of cachaça production. Our findings are important for the appropriate monitoring of yeast during sugar cane fermentation. In addition, we demonstrate that UFLA CA 116 and UFLA CA 1162, the ideal yeast strains for cachaça production, are maintained at a high population density. The persistence of these yeast strains in the fermentation of sugar cane juice promotes environmental conditions that prevent or decrease bacterial contamination. Thus, the use of select yeast strains for the production of cachaça is a viable economic alternative to standardize the production of this beverage.

Introduction

Cachaça is a rum-like spirit that is fermented and distilled from sugar cane and has an alcohol content of *c.* 38–48% (v/v) at 20°C. This beverage also contains high levels of alcohols, ethyl esters, aldehydes and organic acids which are responsible for the distinct flavours of the final beverage (Cardoso *et al.* 2004). Cachaça is the second most consumed alcoholic beverage in Brazil and the third most consumed worldwide. In addition, cachaça is produced by *c.* 30 000 producers at an annual volume of *c.* 1·3 billion litres.

The process of fermenting sugar cane juice for the production of cachaça starts with yeast naturally derived

from cane juice and dilution waters, which are the raw materials of cachaça. The natural microbial starter culture is usually prepared by a method known as 'fermento caipira' (wild micro-organisms). Currently, there is no standardization method for the production of the starter culture (Schwan *et al.* 2001). Consequently, each cachaça-producing region and unit has variations in the production, yield and quality of the beverage.

Yeast colonizes the fruit to a minor extent and constantly increases in number after the onset of fermentation. Modern industrial processes recommend the addition of cultured *Saccharomyces cerevisiae* strains to speed up the fermentative process, increase the levels of the desired metabolites and prevent the production of deleterious components by microbial contaminants. The use of pure yeast cultures, generally in the form of active dry yeast, provides a useful tool for standardizing the beverage production (Fleet and Heard 1993). Use of indigenous yeast strains isolated from the local production area can ensure the adequate control of alcoholic fermentation and can preserve the positive contributions of indigenous yeast.

It is known that the use of select *S. cerevisiae* strains for the production of cachaça can quicken the process and guarantee the quality of the beverage produced (Bernardi *et al.* 2008). Thus, the objective of this study was to assess the persistence and dominance of three select *S. cerevisiae* strains and to identify the main microbial compounds produced during sugar cane fermentation.

Materials and methods

Yeast strains and culture media

The three *S. cerevisiae* strains used in this study were previously isolated from sugar cane fermentations. These strains were selected because of their general fermentative behaviour in the presence of different concentrations of glucose, fructose and sucrose, their tolerance to ethanol, their production of acetic acid and glycerol and their flocculation ability (data not shown). The yeast strains belong to the Microbial Physiology Laboratory Culture Collection at DBI/UFLA and are coded as UFLA CA 116, UFLA CA 1162 and UFLA CA 1183. These strains were cultured for 48 h at 30°C on YPD medium (2% dextrose, 2% bacteriological peptone and 1% yeast extract). When required, YPD medium was solidified with 1·5% agar prior to use in fermentation.

Fermentation conditions

Batch fermentations were carried out in stainless steel 20-l vats containing sugar cane juice (cultivar SP 801816) at 16° Brix (soluble solids content). The fermentation temperature for the production of cachaça was *c.* 30°C, and no stirring was performed during any stage of the fermentation process. Inocula of the different *S. cerevisiae* strains were prepared as follows: one colony from a fresh YPD plate was inoculated into 200 ml of YPD broth and grown at 30°C until a cell density of *c.* 10^8 CFU ml^{-1} was reached. The cells were counted, and an equal number of cells per strain were resuspended in sugar cane juice. Each vat was then inoculated with 10 ml of this suspension, corresponding to a final density of 1×10^8 CFU ml^{-1}, unless otherwise stated. Determination of the maximal fermentation rate was based on the maximum amount of ethanol produced and the level of decrease in sugar content. The fermentation process was considered complete when Brix levels stabilized. Fermentations were conducted using a simple batch system. In addition, each batch fermentation was carried out at least four times. Seven consecutive fermentation batches of 24 h were performed using recycled inocula of *S. cerevisiae*. Samples were taken at the indicated time points and analysed microbiologically and chemically.

Microbiological analysis

For counting and isolation of yeast cells, 100 μl of the appropriate culture dilutions was plated in triplicate on DRBC (Dicloran Rose Bengal Cloramphenicol) plates (0·5% w/v soy peptone, 1% w/v glucose, 0·1% w/v potassium dihydrogen phosphate, 0·05% w/v magnesium sulfate, 0·0002% dicloran, 0·0025% w/v rose-bengal, 1·5% w/v agar). The plates were incubated at 30°C until colonies appeared (1–3 days); at this time, the number of CFU ml^{-1} of culture was determined. For each sample, randomly chosen representative colonies were purified and characterized according to standard methods (Kurtzman and Fell 1998; Barnett *et al.* 2000). Cell viability was measured using methylene blue. The budding index, defined as the fraction of cells with visible buds, was determined for *c.* 300 cells using a microscope; this measure has been shown to be a good indicator of the rate of cell proliferation. The dry cell weight of 10 ml of each culture was determined using 0·45-μm membrane filters and a microwave oven (180 W, 15 min).

Electrophoretic karyotype

Analysis of yeast chromosome polymorphisms was performed as described by Bernardi *et al.* (2008). After electrophoresis, gels were stained with 1% ethidium bromide for 1 h and rinsed twice with Milli-Q water (Purelab Ultra Elga, High Wycombe, UK) for 15 min. The gels were visualized under UV transillumination and documented using a

Polaroid camera. The *S. cerevisiae* strain YNN 295 was used as the reference strain.

Distillation

Distillation of each fermentation reaction was carried out in a copper still with a working capacity of 50 l and equipped with a condenser and gas heater. The temperature of the sugar cane wine was kept at 91–97°C to maintain a distillation rate of *c.* 1 l h^{-1}. The head fraction was collected separately and standardized to a volume corresponding to *c.* 10% of the total volume of cachaça, thus equalizing the distillation time of the head components. The heart fraction was collected when the concentration of ethanol was of *c.* 40% v/v. The distilled beverages were stored in oak barrels at *c.* 20°C for later sampling and sensory analysis.

Analytical methods

Analyses of pH, density, ethanol content and concentration of volatile acids, higher alcohols, aldehydes, esters, methanol and secondary metabolites were performed according to the methodology proposed by Fernandes *et al.* (2007), Brazil (1988) and the AOAC (1990).

After treatment of the samples with hydroxylamine hydrochloride and diquinolyl, the presence of copper ions was determined by spectrophotometry using a Shimadzu UV/VIS spectrophotometer (Shimadzu UV-1601 PC). The amounts of copper were determined by comparing the absorbance values of the cachaça samples against the absorbance values obtained from a copper standard calibration curve at 546 nm (Fernandes *et al.* 2007).

The levels of higher alcohols (1-propanol, isobutanol, 1-butanol, isoamyl alcohol, amyl alcohol and hexanol), acetaldehyde, methanol, ethanol, acetic acid and esters (ethyl acetate and methyl acetate) present in the samples were analysed by gas chromatography (GC). For these analyses, we used a Shimadzu model 17A gas chromatograph equipped with a flame ionization detector and a capillary HP-FFAP (high polarity–free fatty acid phase) silica column (30 m × 0·25 mm i.d. × 0·25 μm; J&W Scientific Agilent, Santa Clara, CA) (Duarte *et al.* 2009). Prior to injection into the GC, 100 μl of each sample (non-distilled) was diluted 20-fold in Milli-Q water and filtered using a nitrate–cellulose membrane of 0·20-μm pore size. Operating conditions were as follows: the oven temperature was maintained at 60°C for 3 min, then increased to 75°C at a rate of 2°C min^{-1}, kept at 100°C for 3 min, then increased to 184°C at a rate of 3°C min^{-1} and maintained for 30 min and then increased again to 220°C in 15 min. The injector and detector temperatures were kept at 240°C, and the carrier gas (N_2) flow rate was maintained at 1·2 ml min^{-1}. Volatile compounds were identified by comparing their retention times to those of standards. One sample containing both an internal standard and the standard compounds at concentrations similar to those found in the final beverage was treated in the same way as all other samples; all final calculations are described on the basis of this reference solution. Evaluation of the various compounds was performed in triplicate. The coefficient of variation was <5% in each case.

Sensory evaluation

The final beverage was evaluated by 50 male and female panellists between the ages of 21–55. The panellists were selected for participation on the basis of their preference for consuming distilled beverages and availability. Randomized samples of 5–10 ml were served in clear glasses marked with three-digit random numbers and covered with plastic Petri dishes. Distilled water was provided for rinsing of the palate during the evaluation. Evaluations took place between 9:00 and 10:00 AM and were conducted at room temperature (22–25°C) under white light.

The cachaça was evaluated for taste, clarity, colour and general acceptability according to the hedonic scale. This scale is based on the comparison, punctuation and classification of foods and beverages of the same class or origin according to their qualities and defects. A card containing six parameters (visual examination, smell intensity, smell quality, taste intensity, taste quality, harmony) was provided to each participant. Each parameter was evaluated using a nine-category scale: Dislike Extremely = 1, Dislike Much = 2, Dislike Moderately = 3, Dislike Slightly = 4, Neither Like nor Dislike = 5, Like Slightly = 6, Like Moderately = 7, Like Much = 8, Like Extremely = 9. The sensory analysis was performed in two sessions, each lasting 1 h. The spirit was evaluated in duplicate in each session, and the mean score for each attribute was computed.

Statistical analysis

Statistical analysis was performed using STATISTICA® software ver. 7.6 (Statsoft Inc., Tulsa, OK). Data from fermented must and final beverages were compared by principal component analysis (PCA) using The UNSCRAMBLER® 9.7 (CAMO, Oslo, Norway) software.

Results and discussion

Yeast fermentation

To assess the fermentation capacity of the three *S. cerevisiae* strains UFLA CA 116, UFLA CA 1162 and UFLA CA 1183, sugar cane juice was inoculated in triplicate with

10^8 yeast cells ml^{-1} and allowed to ferment at room temperature (*c.* 28°C) under semi-anaerobic, nonsterile conditions. Samples were taken at 6-h intervals to determine the viability of the yeast cells and to detect bacterial contamination microscopically. Based on the viable cell counts (data not shown) and the total population of yeast in the fermenting sugar cane juice (Table 1), it was determined that, of the three yeast strains used, UFLA CA 116 was superior in persistence and dominance during the seven successive batches. The UFLA CA 116 strain had the largest viable microbial population (data not shown). In addition, UFLA CA 116 reached its maximum abundance in the fourth batch after 96 h of fermentation ($4{\cdot}5–5{\cdot}1 \times 10^8$ CFU ml^{-1}). In contrast, UFLA CA 1162 and UFLA CA 1183 exhibited slower growth, and the maximum population ($3{\cdot}1–18{\cdot}9 \times 10^7$ CFU ml^{-1} and $7{\cdot}95 \times 10^7$ CFU ml^{-1}, respectively) was not reached until the seventh batch.

Over the course of the seven batches (Table 1), it was determined that the population of UFLA CA 116 was, on average, nine times greater than the population of UFLA CA 1162 and 12 times greater than the population of UFLA CA 1183. The strain UFLA CA 1162 continued to grow until the seventh batch, representing 82% of the total microbiota present in the fermenting must. UFLA CA 1162 and UFLA CA 1183 isolates represented 96% and 94%, respectively, of the total yeast population in the fermenting sugar cane juice. Despite the greater representation of the latter two isolates in their respective batch cultures, these strains were 9–12 times less abundant than UFLA CA 116. This variation in growth capacity may have been reflected in the productivity of the cultures. The ethanol productivity from fermentation of seven batches, measured as litres of wine per litres of cachaça, was of 16–30% for UFLA CA 116 (data not shown), 15–22% for UFLA CA 1162 and 10% for UFLA CA 1183 when compared with 'fermento caipira'. The smaller ethanol yield by UFLA CA 1183 was because of the fact that this strain did not persist throughout the fermentation process (Table 1) and because of the high degree of bacterial contamination ($\geq 10^6$ CFU ml^{-1}) in the fermenting sugar cane juice, which decreased the viability of the yeast.

Eighteen different yeast morphotypes, sampled from each of the seven batches, were characterized from the fermenting sugar cane juice cultures, totalling 140 isolates. Eleven morphotypes were isolated (numbered from 1 to 11; data not shown) from the fermentation with the UFLA CA 1162 starter strain, four morphotypes (12–15) were isolated from fermentation with UFLA CA 116, and three morphotypes were isolated (16–18) from fermentation with the UFLA CA 1183 strain. Molecular characterization of yeast cells isolated from all cultures was carried out using PFGE. From these results, it was possible to verify that even when there were other morphotypes present in the culture medium, more than 86% of the isolates obtained corresponded to the inoculated starter yeast. Chromosome profiling was performed for all morphotypes isolated during fermentation with UFLA CA 1162 and UFLA CA 116 (Fig. 1). Isolates 1–4 and 12–14 presented a different molecular profile than the UFLA CA 1162 strain (Fig. 1a). Isolates 10 and 11 shared the same molecular profile, which differed from the electrophoresis profile of the inoculated isolate, and were considered non-*Saccharomyces*. The electrophoretic profile of the isolates obtained from fermentation with UFLA CA 116 is shown in Fig. 1b. UFLA CA 116 persisted and dominated during the seven batches analysed. As shown in Fig. 1b, lines 8–14 show the same karyotyping profile as UFLA CA 116, demonstrating the dominance of the selected yeast strain. It has already been reported in the literature that there is a high degree of genetic polymorphism in *S. cerevisiae* during the cachaça production process (Pataro *et al.* 2000). In our study, the yeast inocula were persistent and dominant throughout the process, and their population was significantly greater than non-*Saccharomyces* strains and bacteria (data not shown). The isolates identified as *S. cerevisiae* did not show the genetic differences observed by Gomes *et al.* (2009). Contaminating bacteria present in high populations ($>10^6$ CFU ml^{-1}) can damage the quality of the beverage by increasing the acidity of the sugar cane juice. Other groups that study alcohol fermentation for the production of alcohol fuel (Basso *et al.* 2008) have experienced difficulty with the persistence and dominance of non-select

Table 1 Yeast and total populations of microbiota ($\times 10^7$ CFU ml^{-1}) in seven successive batches of fermentation for the production for cachaça

Batches/time of fermentation (h)	UFLA CA 116	Total microbiota	UFLA CA 1162	Total microbiota	UFLA CA 1183	Total microbiota
1/24	64·5	108·0	19·1	19·9	1·2	1·4
2/48	95·0	144·0	16·5	17·2	8·6	9·3
3/72	123·0	180·0	21·2	22·4	23·4	25·3
4/96	206·0	254·0	22·6	23·3	24·3	25·3
5/120	268·0	306·0	21·2	21·7	16·0	16·6
6/144	252·0	266·0	22·7	23·2	6·9	7·2
7/168	257·0	280·0	21·5	23·1	5·1	5·4

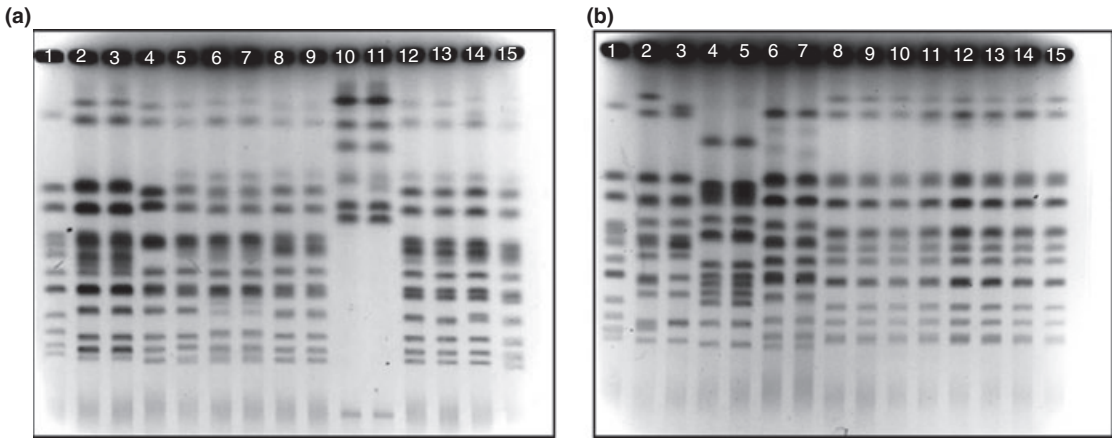

Figure 1 Electrophoretic karyotypes (profiles) of yeasts isolates from sugar cane fermentation reactions inoculated with (a) UFLA CA 1162 and (b) UFLA CA 116. (a) Lines: (1) *Saccharomyces cerevisiae* YNN 295; (2) Profile I (isolate 1); (3) Profile I (isolate 2); (4) Profile II (isolate 3); (5) UFLA CA 1162; (6–9) Profile III (UFLA CA 1162); (10–11) Profile IV (isolates 9 and 10) and (12–15) Profile V (isolates 21, 25, 26 and 29). (b) Lines: (1) *S. cerevisiae* YNN 295; (2) Profile I (isolate 16); (3) Profile II (isolate 17); (4 and 5) Profile III (isolates 18 and 30); (6 and 7) Profile IV (isolates 47 and 48); (8–14) Profile V (isolates 31, 33, 34, 35, 38, 41 and 46) and (15) Profile V UFLA CA 116.

strains of *S. cerevisiae* in fermentation. In the manufacture of alcoholic beverages, persistence of *S. cerevisiae* enables not only the production of ethanol, but also that of secondary compounds such as glycerol, esters, alcohols and other compounds responsible for the aroma that characterizes the final product (Lurton *et al.* 1995). The quantitative variations in these secondary compounds are because of the particular yeast strain used in the beverage production process (Oliveira *et al.* 2005). In fermented alcohol beverages, the naturally present yeast population can contribute either positively or negatively to the final characteristics of the beverage (Souza Liberal *et al.* 2007). The persistence of desirable yeast strains in the fermenting sugar cane juice also creates environmental conditions that prevent or decrease bacterial contamination. Measures to prevent contamination during the artisanal manufacture of cachaça, such as the use of antiseptics, are not permitted by legislation. Thus, the use of select yeast strains for the production of cachaça is a viable economic alternative to standardize the production of this beverage. Spontaneous fermentation has been observed during the production of cachaça when the population of *S. cerevisiae* decreases at the end of the process. This phenomenon was not observed with the yeast strains used in our study; *S. cerevisiae* was present and dominant through the last batch, corresponding to 168 h of fermentation.

Sensory analysis of the beverage

After chemical analysis, the beverage was subjected to sensory analysis to assess its acceptance among consumers. Table 2 shows the percentage of acceptance, based on the 9-point hedonic scale, by 50 untrained tasters. For all attributes assessed, the distilled beverage produced by UFLA CA 116 showed greater acceptance when compared to the beverages produced by the other two strains (Table 2). From the distribution of the individual scores for each point on the hedonic scale for the different attributes, we found that the majority of panellists choose the spirit produced by the UFLA CA 116 strain. The differences in sensory analysis found for these distilled beverages may be the result of the different compositions of the final products.

Chemical analysis during fermentation and after distillation

Chemical analyses were performed on cachaça samples after fermentation with the three tested isolates and

Table 2 Frequency and average scores for the attributes of sensorial analysis

Attribute	Yeast	(1–4) Rejected (%)	(6–9) Accepted (%)
Appearance	UFLA CA 116	4	96·0
	UFLA CA 1162	2·7	82·3
	UFLA CA 1183	6·5	77·9
Aroma	UFLA CA 116	2	98·0
	UFLA CA 1162	5·2	94·8
	UFLA CA 1183	11·9	88·1
Taste	UFLA CA 116	14·5	85·5
	UFLA CA 1162	28·7	71·3
	UFLA CA 1183	36·5	63·5
Overall	UFLA CA 116	7·6	92·4
	UFLA CA 1162	15·7	84·3
	UFLA CA 1183	28·5	71·5

1 = dislike extremely; 9 = like extremely.

Table 3 Results of physico-chemical analyses of distilled beverages produced from fermentation reactions inoculated with the UFLA CA 116, UFLA CA 1162 and UFLA CA 1183 yeast strains and the allowed limit of each parameter in accordance with Brasil (2005)

Parameters	Limit	UFLA CA 116	UFLA CA 1162	UFLA CA 1183
Relative density (g cm^{-3})	Min.	0·94	0·95	0·95
	Max.	0·95	0·95	0·955
	Ageing	0·95	0·94	0·95
Copper (mg l^{-1})	Min.	1·19	2·06	1·96
	Max. = 5·0	2·07	2·46	2·30
	Ageing	1·6	1·32	0·9
Dry extract (g l^{-1})	Min.	0·03	0·01	0·09
	Max.	0·16	0·16	0·4
	Ageing	1·2	0·52	1·2
Alcoholic degree (GL)	Min. = 38·0	41	40·0	39
	Max. = 48·0	45	42·0	42
	Ageing	40	43·0	42
Volatile acidity as acetic acid (mg 100 ml^{-1} anhydrous alcohol)	Min.	27·73	45·22	44·57
	Max. = 150·0	47·74	73·57	64·0
	Ageing	65·0	82·64	97·5
Higher alcohols (mg 100 ml^{-1} anhydrous alcohol)	Min.	120·66	226·02	159·42
	Max. = 360·0	200·71	309·86	246·03
	Ageing	249·11	286·93	246·3
Aldehydes (as acetic aldehyde) (mg 100 ml^{-1} anhydrous alcohol)	Min.	6·10	6·29	5·86
	Max. = 30·0	7·84	10·35	7·35
	Ageing	11·44	11·71	9·8
Esters (as ethyl acetate) (mg 100 ml^{-1} anhydrous alcohol)	Min.	22·37	13·39	23·96
	Max. = 200	25·74	19·61	26·78
	Ageing	29·76	23·41	28·67
Secondary compounds (mg 100 ml^{-1} anhydrous alcohol)	Min. = 200	193·72	229·55	255·81
	Max.	279·70	317·46	323·28
	Ageing	289·00	304·69	302·56
Methanol (ml 100 ml^{-1} anhydrous alcohol)	Min.	0·006	0·014	0·006
	Max. = 0·25	0·026	0·026	0·02
	Ageing	0·003	0·012	0·003

Ageing, amount of time the beverage was aged in oak barrels (mature in 30 days); Min, minimum value; max, maximum value; nd, nondetectable.

during the 30-day ageing period in 5-l oak barrels. Table 3 provides the data obtained from these analyses. The values express the mean of the measurements obtained during distillation in successive batches. The relative density of the beverage produced by all three isolates was similar (0·95 g cm^{-3}). The concentrations of aldehydes, esters, methanol, alcohol and volatile acids in the aged beverages were within the legal limits. The highest alcohol concentration (309·86 mg 100 ml^{-1}) in the beverage produced by UFLA CA 1162 exceeded the stipulated legal limit, but after the ageing period, the concentration of alcohol decreased to 286·93 mg 100 ml^{-1}, which is within the limit permitted (300 mg 100 ml^{-1}).

There was no difference in the qualitative chemical composition of the cachaça produced by the three yeast strains tested, but the concentrations of the compounds analysed were significantly different in almost all seven batches (Scott–Knott test; see Supporting Information Tables S1 and S2).

Among the compounds analysed, we found that isoamyl alcohol was the main metabolite produced during sugar cane fermentation by all three yeast strains (Table 4). Fermentation by UFLA CA 116 resulted in a sugar cane juice with relatively high concentrations of ethanol (8·8% vv^{-1}), isobutanol (60·7 mg 100 ml^{-1}), ethyl acetate (12·0 mg 100 ml^{-1}) and isoamyl alcohol (188·5 mg 100 ml^{-1}); however, after distillation, ethyl acetate (43·8 mg 100 ml^{-1}), 1-hexanol (32·5 mg 100 ml^{-1}) and isoamyl alcohol (169·2 mg 100 ml^{-1}) dominated in the beverage. The sugar cane juice produced by UFLA CA 1162 fermentation contained mostly isoamyl alcohol (145·4 mg 100 ml^{-1}), amyl alcohol (68·1 mg 100 ml^{-1}), hexanol (59·2 mg 100 ml^{-1}), isobutyl alcohol (50·6 mg 100 ml^{-1}), acetaldehyde (23·3 mg 100 ml^{-1}) and acetic acid (0·40 mg 100 ml^{-1}); after distillation, the beverage was composed primarily of isoamyl alcohol (111·4 mg 100 ml^{-1}), hexanol (55·7 mg 100 ml^{-1}) and propanol (47·30 mg 100 ml^{-1}). After fermentation, the wine produced from fermentation by UFLA CA 1183 presented

Table 4 Average levels of organic compounds obtained after fermentation and distillation with the three different yeast strains used for cachaça production

Compounds (mg 100 ml^{-1})	LOQ* (mg 100 ml^{-1})	UFLA CA 116		UFLA CA 1162		UFLA CA 1183	
		Fermented	Distilled	Fermented	Distilled	Fermented	Distilled
Acetaldehyde	0·01	15·6 ± 2·2	0·03 ± 0·01	23·3 ± 1·5	0·4 ± 0·07	17·2 ± 1·7	0·03 ± 0·01
Acetic acid	0·01	0·02 ± 0·001	≤10^{-4}	0·40 ± 0·05	≤10^{-4}	0·08 ± 0·002	≤10^{-4}
Amyl alcohol	0·2	36·0 ± 1·4	10·2 ± 1·0	68·1 ± 1·6	4·2 ± 0·7	37·5 ± 1·7	4·5 ± 1·6
Ethanol (g 100 ml^{-1})	2·0	8·8 ± 0·5	40·1 ± 0·2	7·3 ± 0·9	40·6 ± 0·6	8·3 ± 0·5	40·5 ± 0·3
Ethyl acetate	0·8	12·0 ± 1·3	43·8 ± 3·1	5·6 ± 0·7	23·2 ± 2·1	6·3 ± 0·6	34·0 ± 1·8
1-hexanol	0·6	43·8 ± 0·7	32·5 ± 2·1	59·2 ± 3·4	55·7 ± 2·2	59·6 ± 2·6	20·7 ± 1·6
Isoamyl alcohol	0·5	188·5 ± 2·8	169·2 ± 4·2	145·4 ± 3·4	111·4 ± 2·2	194·8 ± 2·6	146·8 ± 3·7
Isobutyl alcohol	0·4	60·7 ± 2·0	19·7 ± 1·8	50·6 ± 2·0	23·2 ± 0·4	50·5 ± 2·2	29·8 ± 1 .4
Methanol	0·02	<0·02	<0·02	<0·02	0·17 ± 0·03	0·16 ± 0·01	0·68 ± 0·004
1-propanol	0·2	32·9 ± 2·4	19·0 ± 3·4	40·0 ± 2·7	25·8·0 ± 1·9	52·3 ± 0·6	26·9 ± 1·4
Isoamyl : isobutyl	–	3·1	8·5	2·9	4·8	3·9	4·9
Propanol : isobutyl	–	0·6	1·0	0·8	1·7	1·0	0·9

*Limit of quantification.

greater concentrations of isoamyl alcohol (194·8 mg 100 ml^{-1}) and similar concentrations of 1-hexanol, 1-propanol and isobutyl alcohol. Souza *et al.* (2006) reported that higher alcohols such as amyl, isoamyl and isobutyl alcohols and their esters are responsible for the formation of the essential components of the cachaça aroma. The beverage produced by the UFLA CA 116 strain had a greater isoamyl alcohol : isobutanol ratio (8·5) and a lower propanol : isopropanol (1·0) ratio when compared to the beverage produced by the other two

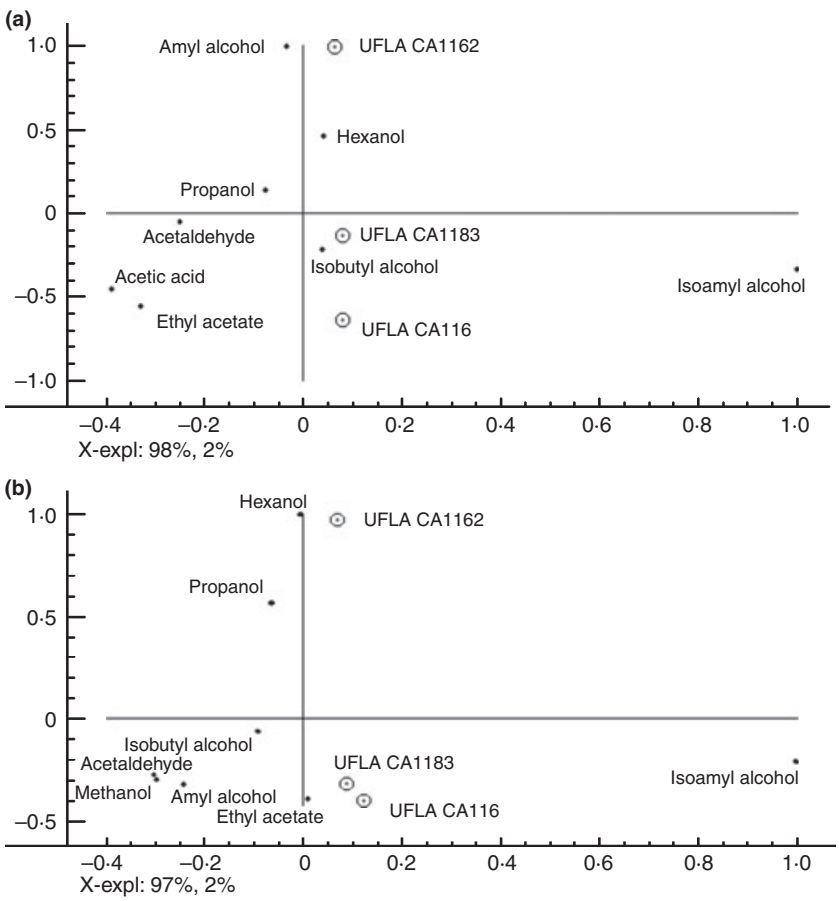

Figure 2 Principal component analysis of organic compounds found during the production of cachaça using three different strains of *Saccharomyces cerevisiae*. (a) Data obtained from fermented sugar cane juice; (b) Data obtained after distillation.

strains (Table 4). According to Fernandes *et al.* (2007) and Lima *et al.* (2009), this ratio between higher alcohols is related to the quality of the cachaça.

At the end of each batch fermentation and after distillation, ten compounds were identified by GC, including acetaldehyde, ethyl acetate, methanol, 1-propanol, isobutanol, isoamyl alcohol, amyl alcohol, 1-hexanol, acetic acid and ethanol (data not shown). According to our data, there was no significant difference in the ethanol content when recycling UFLA CA 116 during the seven consecutive batches fermented. The other metabolites produced during fermentation exhibited different concentrations between batches.

PCA (Fig. 2) was carried out with the organic compounds produced during fermentation and after distillation. Figure 2 shows the similarities and differences between the fermentation processes by the three different yeast strains. The organic compounds clustered into two groups, which were related to the type of yeast inoculum. The qualitative chemical compositions of the beverages produced by UFLA CA 116 and UFLA CA 1183 were rather similar (Fig. 2). The fermenting must and spirit produced by UFLA CA 116 were characterized mainly by the presence of isoamyl alcohol, ethyl acetate and isobutyl alcohol. Hexanol, acetaldehyde, amyl alcohol and 1-propanol characterized the spirits produced by UFLA CA 1162 and UFLA CA 1183. A high proportion of superior alcohols, such as amyl and isoamyl alcohol (300 mg 100 ml^{-1}), small quantities of ethyl acetate (<200 mg 100 ml^{-1}) and acetic acid (<150 mg 100 ml^{-1}), the presence of secondary compounds (>200 mg 100 ml^{-1}) and the absence of n-propanol and methanol conferred a characteristic bouquet to the beverage. All of these compounds were detected at levels in accordance with Brazilian legislation using the three different yeast strains. The cachaça produced by fermentation with the UFLA CA116 strain contained optimal quantities of the ideal compounds for a good-quality beverage including an ideal ethanol level (40·1%), high levels of amyl (10·2 mg 100 ml^{-1}) and isoamyl (169·2 mg 100 ml^{-1}) alcohols, no acetic acid or methanol and low levels of 1-propanol (19·0 mg 100 ml^{-1}) and acetaldehyde (0·03 mg 100 ml^{-1}). Ethyl acetate was detected only after distillation at a superior value (43·8 mg 100 ml^{-1}) compared to the other yeast strains.

The distillation process affects the flavour of alcoholic beverages, especially with respect to the volatile compounds present in the beverage. As observed by Singer (1966), determining the ratio between compounds such as propanol : isobutanol and isoamyl alcohol : isobutanol is a way of monitoring the distillation process; the best beverages will have the highest volatile compound ratio. However, a sensorial test should be carried out to confirm the acceptability of the beverage among a panel of untrained tasters. As the production of cachaça is a commercial activity, factors such as quality and productivity should be considered. Here, we show that UFLA CA 1162 and UFLA CA 116 are the most appropriate yeast strains for the production of cachaça by fermentation. In conclusion, cachaça developed on a laboratory scale with select yeast strains exhibited clear analytical differences, and these strains may be ideal starter cultures for the artisanal production of cachaça in Brazil.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Analysis of compounds (Scott–Knott test, $P \leq 0{\cdot}05$) produced during fermentation using three selected yeast inocula in seven consecutive batches.

Table S2 Analysis of average concentrations (Scott–Knott test, $P \leq 0{\cdot}05$) of compounds produced during distillation using three selected yeast inocula in seven consecutive batches.

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