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Interaction of *Saccharomyces cerevisiae* and *Lactococcus lactis* in the fermentation and quality of artisanal cachaça

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ABSTRACT. *Lactococcus lactis* and *Saccharomyces cerevisiae* in co-culture were evaluated during sugar cane fermentantion for cachaça production. The inocula containing *L. lactis* UFLA CA 312 and *S. cerevisiae* UFLA CA 11 were used in the population of approximately 10^5 CFU mL^{-1} and 10^8 CFU mL^{-1}, respectively. The sugar cane medium plus 1% of yeast extract (SCM) was efficient for growth of *L. lactis* UFLA CA 312 and *S. cerevisiae* UFLA CA 11 (letter 'b'-Tukey test). In flasks and vats fermentation the growth of UFLA CA 11 was not negatively influenced by *L. lactis* UFLA CA 312. However, after 19 hours of fermentation, bacterial population showed a slight decrease. Considering parameters higher alcohols and aldehydes, cachaça produced by pure culture of *S. cerevisiae* was similar to cachaça produced by mixed culture. Cachaça produced by mixed culture showed high values of volatile acidity (letter 'b'-Tukey test) being characterized by this parameters in the principal component analysis. High percentage of acceptance (81.10%) for the attribute aroma was observed in samples from cachaça produced by mixed culture.

Keywords: sugar cane spirit, mixed culture, lactic acid bacteria, starter culture.

Interação de *Saccharomyces cerevisiae* e *Lactococcus lactis* na fermentação e qualidade da cachaça artesanal

RESUMO. *Lactococcus lactis* e *Saccharomyces cerevisiae* foram avaliados em co-cultura durante fermentação para a produção de cachaça. *L. lactis* UFLA CA 312 e *S. cerevisiae* UFLA CA 11 foram utilizados com populações de aproximadamente 10^5 CFU mL^{-1} e 10^8 UFC mL^{-1}, respectivamente. O meio contendo caldo de cana de açúcar, adicionado de 1% de extrato de levedura (SCM) foi eficiente para o crescimento de *L. lactis* UFLA CA 312 e *S. cerevisiae* UFLA CA 11 (letra 'b' no teste de Tukey). Nas fermentações conduzidas em frascos e dornas o crescimento da levedura não foi influenciado negativamente pela bactéria lática. Entretanto, após 19h de fermentação, a população bacteriana apresentou pequeno decréscimo. Considerando os parâmetros alcoóis superiores e aldeídos, a cachaça produzida pela cultura pura de *S. cerevisiae* foi semelhante à cachaça produzida pela cultura mista. A cachaça produzida pela cultura mista apresentou elevados valores de acidez volátil (letra 'b' no teste Scott-Knott), sendo caracterizada por este parâmetro na análise de componente principal. Uma elevada porcentagem de aceitação (81,10%) para o atributo aroma foi observada para as amostras de cachaça produzidas com a cultura mista.

Palavras-chave: destilado, cultura mista, bactéria lática, cultura iniciadora.

Introduction

Cachaça is a typical and exclusive designation of sugar cane spirit produced in Brazil, with an alcoholic degree of 38 to 48% v v^{-1} at 20°C (BRASIL, 2005). The beverage is obtained by the distillation of fermented sugar cane juice and has singular sensorial features. Cachaça is the most traditional distilled beverage in Brazil, with an average of 11 L consumed per individual per year. The annual cachaça production in Brazil is estimated at 1.3 billion litres. The state of Minas Gerais has approximately 8,000 traditional distilleries producing a total of 230 million L of cachaça per year.

In the traditional process of fermentation the microbiota is complex and consists of yeast such as *Kluyveromyces marxianus*, *Pichia heimii*, *Hanseniaspora uvarum, Pichia subpelliculosa*, *Debaryomyces hansenii, Pichia methanolica, S. cerevisiae* and some lactic and acetic acid bacteria and bacteria belonging to enterobacteriaceae family (SCHWAN et al., 2001). Sensory attributes (aroma and taste) of cachaça are influenced by metabolites produced by yeast population during natural fermentation for cachaça production (NOVA et al., 2009). In Brazil, several studies have been performed with selected yeast because its use has advantages such as faster process

start-up, low contamination risk by spontaneous fermentation, more rapid and uniform fermentation rates, lower competition for essential nutrients, higher beverage yield, lower levels of residual sugars and the maintenance of beverage flavor properties (BERNARDI et al., 2008; CAMPOS et al., 2010; SILVA et al., 2006). The community of bacteria in the cachaça fermentation process is complex. Lactic acid bacteria (LAB) are the main group found during fermentation (GOMES et al., 2010), and species as *L. lactis*, *L. plantarum* and L. *fermentum* were abundantly identified. *L. lactis* was frequently found in sugar cane fermentations as contaminant during cachaça and other alcoholic fermentation (SKINNER; LEATHERS, 2004). According to Narendranath et al. (1997) LAB can cause reduction in ethanol yield by the consumption of carbohydrates to produce lactic acid. Although in wine the use of bacteria in malolactic fermentation contributes to improving the organoleptic quality (RIBÉREAU-GAYON et al., 2006), in cachaça production there are very few studies on the characterization of bacterial populations present in the fermentation vats and the relationship of these microorganisms with the production of secondary compounds responsible for the flavor of the beverage (DUARTE et al., 2011; GOMES et al., 2010). The aim of this study was to evaluate the influence of a mixed culture of *S. cerevisiae* and *L. lactis* in the quality of cachaça and additionally evaluate the feasibility of the use of sugar cane juice to obtain yeast and bacteria biomass.

Material and methods

Microorganisms

Saccharomyces cerevisiae UFLA CA 11 and *Lactococcus lactis* UFLA CA 312 were isolated from spontaneous fermentation of sugar cane juice and belong to the Microbial Physiology Laboratory Culture Collection at DBI/UFLA. Yeast and bacteria were cultured on YPD medium (g L^{-1}: dextrose, 20, bacteriological peptone, 20, yeast extract, 10), solidified with 1.5% of agar when required, prior to use in fermentation at 28 and 35°C, respectively, for 24 hours.

Evaluation of sugar cane juice as an alternative medium to multiply biomass of *S. cerevisiae* and *L. lactis*

S. cerevisiae UFLA CA 11 and *L. lactis* UFLA CA 312 were cultivated in two different culture media: YPD (reference medium) and culture medium containing autoclaved (121°C for 15 min.) sugar cane juice 5°Brix supplemented with 1% yeast extract (SCM). Sugar cane juice was previously autoclaved to eliminate indigenous microorganisms from sugar cane and from the equipment used to extract the juice. The experiment was performed in triplicate in flasks containing 250 mL of culture medium and incubated at 28°C (yeast) and 35°C (bacteria) at 30 rpm for 48 hours. The growth of yeast and bacteria was monitored by plating in YPD agar and TSA (g L^{-1}: casein peptone, 15, soy peptone, 5; sodium chloride, 5 and agar, 15), respectively. The plates were incubated at 28 and 37°C for 48 hours for yeasts and bacteria respectively.

Evaluation of the co-incubation of *S. cerevisiae* and *L. lactis* during alcoholic fermentation

The evaluation of the co-incubation of *S. cerevisiae* UFLA CA 11 and *L. lactis* UFLA CA 312 was performed in two steps. In a first step, the interaction was studied in flasks of 1600 mL and, in a second step, in batch fermentations in 20 L stainless steel vats containing sugar cane juice at 15°Brix (soluble solids content).

Flasks containing 1,600 mL of sugarcane juice 15°Brix were inoculated separately with (1) *L. lactis* UFLA CA 312 (10^5 CFU mL^{-1}), (2) *S. cerevisiae* UFLA CA 11 (10^8 CFU mL^{-1}) and (3) with mixed culture of *L. lactis* UFLA CA 312 and *S. cerevisiae* UFLA CA 11 (10^5 CFU mL^{-1} bacteria; 10^8 CFU mL^{-1} yeast).

Batch fermentations were carried out in stainless steel 20 L vats containing sugar cane (cultivar SP 801816) juice at 15°Brix (soluble solids content). Four batches (4 cycles of fermentation) were performed consecutively. The 2nd, 3rd and 4ht batches were performed using recycled inocula of *S. cerevisiae* UFLA CA 11, *L. lactis* UFLA CA 312 and *S. cerevisiae* UFLA CA 11 + *L. lactis* UFLA CA 312.

The fermentation temperature for cachaça production was approximately 30°C, and no stirring was performed during any stage of the fermentation. For each vat the initial inoculum was adjusted to obtain a final population of approximately 10^8 CFU mL^{-1} of yeast and 10^5 CFU mL^{-1} of bacteria in all fermentations. The maximal fermentation rate was determined by the maximum ethanol production. The fermentation was considered complete when the Brix levels stabilized. Fermentations were conducted in a simple batch system, and each batch fermentation was carried out at least four times. Samples were taken at the indicated points and analyzed microbiologically and chemically. The growth of yeast and bacteria was monitored by serial dilutions and plating in YPD added of ampicillin (500 μL L^{-1}) and MRS added of nystatin (4 mL L^{-1}).

The plates were incubated at 28 and 35°C for 48 hours for yeasts and bacteria count, respectively.

Distillation

Distillation was carried out in a copper still with a working capacity of 50 L, equipped with a condenser and gas heater. The temperature of the sugar cane wine was controlled between about 91 and 97°C to maintain the distillation rate at about 1 L h^{-1}. The head (first fraction of distillate), heart fraction (intermediate fraction of distillate) and the tail fraction (last fraction distillate) were collected separately as proposed by Campos et al. (2010). The 'heart fraction' (cachaça) was stored for thirty days in wooden (oak) barrels and maintained at room temperature in a cool place (approximately 20°C) for later sampling and sensory analysis.

Analytical methods

Moisture, dry matter, protein, ash, soluble solids, pH, acidity, reducing sugars (glucose), non-reducing sugars (sucrose), total sugars, calcium, phosphorus, potassium, magnesium, sulfur, nitrogen, copper, iron, zinc and sodium were performed in sugar cane juice according to AOAC (1990), Yemn and Willis (1954) and Miller (1959). Reducing sugar concentrations in sugar cane juice were determined by dinitrosalicylic acid (DNS) method (MILLER, 1959).

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), Brasil (1988) and the AOAC (1990). Higher alcohols (2-butanol, butanol, isobutanol, propanol, isoamyl alcohols, amyl alcohols and hexanol), were analyzed by gas chromatography (GC) using a Shimadzu model 17A, equipped with a flame ionization detector (FID) and using a capillary column of silica HP FFAP (30 m x 0.25 mm i.d. x 0.25 μm) (J and W Scientific, Folsom, USA). The conditions for GC analysis were those proposed by Duarte et al. (2011). The identification of volatile compounds was done by comparison of their retention times with those of standards. One sample, which contained the internal standard and the standard compounds at concentrations similar to those found in the cachaça, was also treated in the same way as the cachaça samples and the final calculations are described on the basis of the concentration of this reference solution (DUARTE et al., 2009). Evaluation of the different compounds was performed in triplicate.

Sensory evaluation

The final beverage was evaluated by 40 panellists, males and females, 22 to 50 years of age. The panellists were selected based on their preference for distilled beverages, interest, and availability. Randomized, samples of 10–15 mL were served in clear glasses with a volume of 20 mL. Distilled water was provided for rinsing of the palate during the testing. Evaluations took place in the mornings between 9 and 10 am and were conducted at room temperature (22–25°C) under white light. Cachaça samples were evaluated for taste, clarity, colour, and general acceptability according to the hedonic scale of nine categories: Dislike Extremely = 1, Dislike Much = 2; Dislike Moderately = 3; Dislike Slightly = 4, Neither Dislike nor Like = 5, Like Slightly = 6; Like Moderately = 7; Like Much = 8 , Like Extremely = 9 (DUARTE et al., 2011). The percentage of acceptance was calculated taking into account the number of tasters that has attributed grades (categories) 8 and 9 for the evaluated attributes.

Statistical analysis

The Tukey test was performed using the SISVAR 5.0 software. Data from fermented must and final beverages were compared by principal component analysis (PCA) using XLstat 7.5.2 (Addinsoft's, New York, NY, USA) software. Internal preference mapping was carried out on the hedonic ratings by the 50 volunteers.

Results and discussion

The efficiency of sugar cane juice 5°Brix with 1% of yeast extract (SCM) as an alternative low cost medium to obtain bacterial and yeast biomass (for use as inoculum in laboratorial scale) was observed by the comparison with the growth of *L. lactis* UFLA CA 312 and *S. cerevisiae* UFLA CA 11 (Figure 1).

When compared to YPD, SCM medium showed a significant increase (letter 'b'-Tukey test 5%) in the population of the yeast. The maximum yeast biomass production (10.8 log CFU mL^{-1}) was reached at 28 h of incubation (Figure 1A). This result is probably related to the composition of sugar cane juice (Table 1). Sugar cane juice was obtained by crushing sugar cane and contains glucose, sucrose and many vitamins and minerals suitable for *S. cerevisiae* growth (LIMTONG et al., 2007) even in high cell density (SAITOH et al., 2005). However, the supplementation with nitrogen provides improvements in the efficient of sugar cane juice as culture medium (LIMTONG et al., 2007).

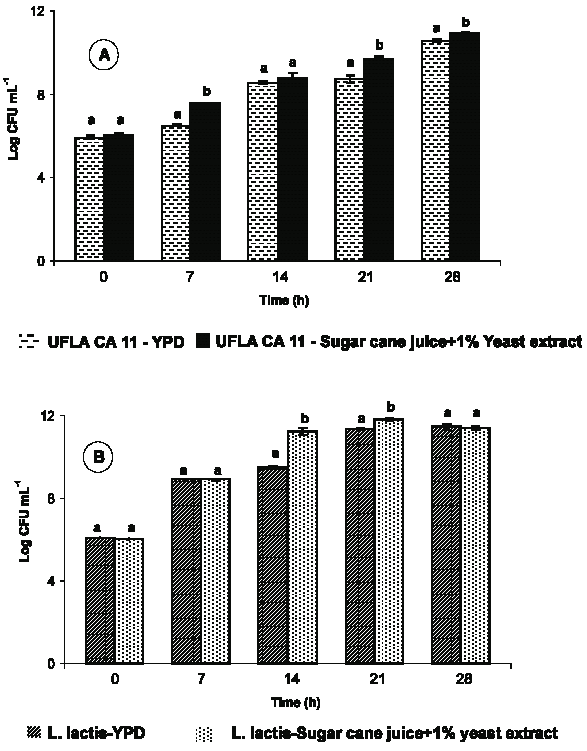

Figure 1. (A) *S. cerevisiae* growth in YPD and sugar cane juice 5ºBrix plus 1% yeast extract (SCM). (B) *L. lactis* growth in YPD and sugar cane juice plus 1% yeast extract. Values identified by the same letters are not significantly different at the 0.05 level (Tukey test).

Table 1. Physico-chemical analysis of sugar cane juice before and after sterilization.

Characteristics	Sugar cane juice Not sterilized	Sugar cane juice Sterilized
Moisture	79.85%	80.45%
Dry matter	20.15%	19.55%
Protein	0.44%	0.46%
Ash	0.49%	0.55%
Total soluble solids	25.00%	23.50%
pH	5.72	5.60
Reducing sugars (glucose)	1.79%	1.75%
Non reducing sugars (sucrose)	19.05%	19.40%
Total sugars	20.84%	22.15%
Calcium	0.06%	0.07%
Phosphorus	0.03%	0.03%
Potassium	0.04%	0.05%
Magnesium	0.01%	0.01%
Sulfur	0.10%	0.12%
Nitrogen	0.07%	0.08%
Copper	0.30 mg 100 g^{-1}	0.33 mg 100 g^{-1}
Iron	0.19 mg 100 g^{-1}	0.22 mg 100 g^{-1}
Zinc	2.54 mg 100 g^{-1}	2.61 mg 100 g^{-1}
Sodium	6.22 mg 100 g^{-1}	6.27 mg 100 g^{-1}

In Figure 1B, at times 14 and 21 hours of incubation, it was found higher efficiency (letter 'b'-Tukey test 5%) of the SCM medium for the growth of *L. lactis* UFLA CA 312. These results can be correlated with the results in the Table 1 where the characterization of sugar cane juice allowed the identification and quantification of nutrients such as magnesium, phosphorus, calcium, iron, zinc and sodium that are required for the growth of *L. lactis* (COCALGN-BOUSQUET et al., 1995; JENSEN; HAMMER, 1993; MØRETRØ et al., 1998; NOVAK et al., 1997). Our results are in good agreement with those found by Timbuntam et al. (2006). These authors showed that sugar cane juice added of 1% of yeast extract was an efficient culture medium for *Lactobacillus* sp. growth and lactic acid production.

Evaluation of the interaction of *S. cerevisiae* and *L. lactis* during alcoholic fermentation in flasks

As far as we know this work is the first report about the interaction of *L. lactis* and S. *cerevisiae* in the fermentation of sugar cane juice to produce cachaça. In fermentation inoculated with mixed culture of *L. lactis* UFLA CA 312 and *S. cerevisiae* UFLA CA 11, *L. lactis* did not negatively affect the growth of yeast UFLA CA 11 (Figure 2A), as observed by Nobre et al. (2007) in co-cultivation of *L. fermentum* and *S. cerevisiae*. After 19 hours of fermentation, *L. lactis* population decreased from 5 log CFU mL^{-1} to 3.3 log CFU mL^{-1} (Figure 2B). The reduction in bacterial growth may be due to the competition with yeast by essential nutrients. Since the yeast cell size is approximately 20-50 times greater than that of bacterial cells, on a cell basis a greater proportion of nutrients would be taken up by yeast cells.

Another possible reason for the reduction of bacterial growth is that alcohol produced by the yeast can exert inhibitory effects on the multiplication of lactobacilli (THOMAS et al., 2001). The reduction in Brix value during fermentation was similar in the growth of yeast or co-incubation of yeast and bacteria (Figure 2A). The decrease in Brix was minimal when *L. lactis* UFLA CA 312 was grown in pure culture (Figure 2B). The low consumption of sugars by *L. lactis* UFLA CA 312 in pure culture could not be measured by the use of refractometer. This fact may result from the sensitivity of the refractometer and because the Brix result is not only from the measurement of sugars such as glucose, fructose, and sucrose. Considering the profile of reducing sugars (data not shown), it was observed that the cultivation of *L. lactis* UFLA CA 312 in pure culture led to a consumption of approximately 1 g L^{-1} of reducing sugars (as glucose). The profile of reducing sugars for the mixed culture was similar to the one profile found for the pure culture of *S. cerevisiae* UFLA CA 11, showing that the use of *L. lactis* UFLA CA 312 in co-culture did not infer in the yeast activity.

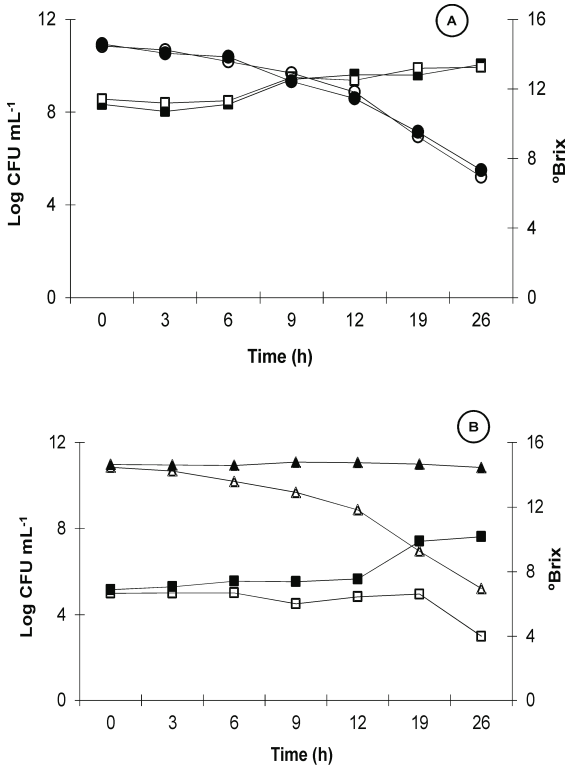

Figure 2. Brix value and growth of *S. cerevisiae* (A) and *L. lactis* (B) during alcoholic fermentation in flasks. (A) solid soluble consumption (Brix) for mixed culture of *S. cerevisiae* and *L. lactis* (open circle); solid soluble consumption for pure culture of *S. cerevisiae* (filled circle); yeast growth in mixed culture (open square); yeast growth in pure culture (filled square). (B) solid soluble consumption for mixed culture of *S. cerevisiae* and *L. lactis* (open triangles); solid soluble consumption for pure culture of *L. lactis* (filled triangles); *L. lactis* growth in mixed culture (open square); *L. lactis* growth in pure culture (filled square).

Fermentation in stainless steel vats

During vats fermentation samples were taken immediately after filling the vats with sugar cane juice to fermentation (Initial Time – T_i) and after removal of the fermented sugar cane juice (End Time – T_f). The yeasts and bacteria biomass found after beginning of fermentation were higher than expected from inocula. It might be due to sedimentation and difficult to homogenize during sampling. It was observed for pure culture of UFLA CA 11 an increase in the population between 1st and 4th batches, both for T_i and for T_f. In T_i, yeast population ranged from 10.58 log CFU mL^{-1} (1st batch) to 11.77 log CFU mL^{-1} (4^{th} batch). In T_f, the yeast population increased from 13.73 log CFU mL^{-1} in the first batch to 15.75 log CFU mL^{-1} in the fourth batch.

Among the population in T_f of a batch (e.g first batch) and the population in T_i of next batch (e.g second batch), the count in T_f was bigger than the count in T_i of next batch. This fact occurred due to the higher volume of sugar cane juice inside the vats on T_i (20 L). After removal of the fermented sugar cane juice (T_f), within the vat was 5 liters of sugar cane juice corresponding to 'pé-de-cuba' (inoculum for the next batch). This change in the ratio biomass/volume and the difficulty of homogenizing the sample due the yeast UFLA CA 11 flocculation led to obtaining a higher apparent population of *S. cerevisiae* UFLA CA 11 in T_f.

In mixed culture of *L. lactis* UFLA CA 312 + *S. cerevisiae* UFLA CA 11, the values for *S. cerevisiae* UFLA CA 11 population (Figure 3) were similar to those observed when the yeast was grown in pure culture. The effect of the ratio biomass $volume^{-1}$ (apparent high population) was also observed for *S. cerevisiae* UFLA CA 11 and *L. lactis* UFLA CA 312 populations during vats fermentation using the mixed inocullum.

No significant changes were found in the population of *S. cerevisiae* UFLA CA 11 and *L. lactis* UFLA CA 312 through the four batches fermentation. This result is different from that found for the population of *L. lactis* UFLA CA 312 during the fermentation in flask (Figure 2B) where at the end of fermentation was observed a decrease in the bacteria count. This change can result from the scale up or from the addition of new substrate after 24 hours of fermentation between consecutives batches.

Physico-chemical analyses

Chemical analyses were performed on cachaça samples after aging (30 days) in a 5 L oak barrel. The results for chemical analyses are the average of data from four successive batches fermentation and distillation (Table 2).

The relative density of the beverage produced in all fermentations was similar with values of 0.9502 and 0.9491 for cachaça produced by *S. cerevisae* UFLA CA11 and mixed culture (*L. lactis* with *S. cerevisiae*), respectively. The concentrations of aldehydes, esters, methanol, alcohol and volatile acids in the aged beverages were within the legal limits of Brazilian law (Table 2). The values for parameters copper, alcoholic degree and volatile acidity (as acetic acid) were different (Tukey test 5%) between cachaça produced by pure culture of *S. cerevisiae* UFLA CA 11 and mixed culture (*S. cerevisiae* UFLA CA 11 + *L. lactis* UFLA CA 312). Although the highest value for the volatile acidity (as acetic acid) was found to cachaça produced with mixed culture, this value was lower than those found by Campos et al. (2010) in a cachaça beverage with high quality.

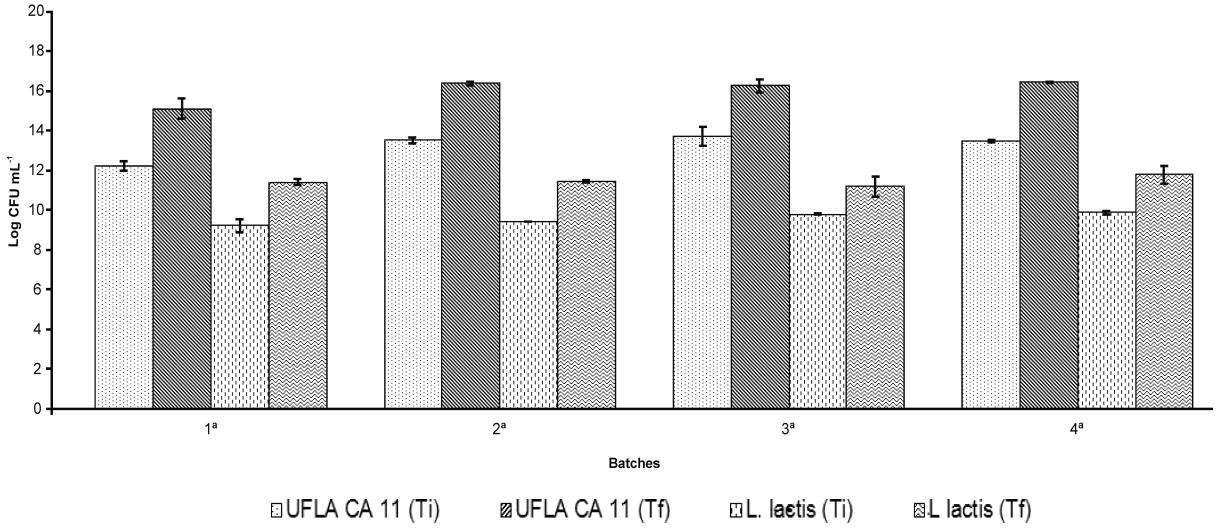

Figure 3. Growth of *S. cerevisiae* UFLA CA 11 and *L. lactis* UFLA CA 312 in mixed culture in the initial (T_i) and final time (T_f) during four successive batches fermentations. *S. cerevisiae* UFLA CA 11 in T_i ; *S. cerevisiae* UFLA CA 11 in T_f; *L. lactis* in T_i ; *L. lactis* in T_f .

The differences found for copper and alcoholic degree were probably caused by the separation of the volumes of the distillate fractions (head, heart and tail) during the distillation process or by the higher acidity of the beverage produced by the mixed culture.

Table 2. Results of physico-chemical analyses* of distilled beverages produced from fermentations inoculated with mixed culture and *S. cerevisiae* and the allowed limit of each parameter in accordance with Brasil (2005).

Parameters	Limit		Mixed culture	*S. cerevisiae*
	Min.	Max.		
Organoleptic characteristics	-	-	Normal	Normal
Relative density	-	-	0.9502[a]	0.9491[a]
Copper (mg L^{-1})	-	5	3.66[b]	2.96[a]
Dry extract (g L^{-1})	-	-	ND	ND
Alcoholic degree (GL)	38	48	40.25[a]	42.50[b]
Volatile acidity as acetic acid (mg 100 mL^{-1} anhydrous alcohol)	-	150	19.54[b]	15.73[a]
Higher alcohols (mg 100 mL^{-1} anhydrous alcohol)	-	360	307.41[a]	315.69[a]
Furfural (mg 100 mL^{-1} anhydrous alcohol)	-	5	ND	ND
Aldehydes (as acetic aldehyde) (mg 100 mL^{-1} anhydrous alcohol)	-	30	5.93[a]	6.13[a]
Esters (as ethyl acetate) (mg 100 mL^{-1} anhydrous alcohol)	-	200	36.47[a]	34.65[a]
Total secondary compounds (mg 100 mL^{-1} anhydrous alcohol)	200	650	369.34[a]	373.98[a]
Methanol (mg 100 mL^{-1} anhydrous alcohol)	-	20	0.02[a]	0.01[a]
Total sugars (g L^{-1} in sucrose)	> 6	≤ 30	ND	ND

ND not detected; min minimum; max maximum value; *average of four batches; Values identified by the same letters are not significantly different at the 0.05 level (Tukey test).

The results of physical-chemical properties were subjected to principal component analysis (PCA) to better understand the relationship between the inocula (*S. cerevisiae* or *S. cerevisiae* + *L. lactis*) used and the parameters analyzed. As can be seen in Figure 4 the first principal component (PC1), corresponding to 42.02% of total variance and allowed the differentiation between cachaça produced with the pure culture of *S. cerevisiae* UFLA CA 11 and the mixed culture of *S. cerevisiae* UFLA CA 11 + *L. lactis* UFLA CA 312 (for four batches).

The beverages produced with *S. cerevisiae* UFLA CA 11 were related to parameters secondary compounds, higher alcohols, aldehydes, and alcoholic degree (Figure 4), while the beverages produced with the mixed culture were characterized by methanol, esters, and volatile acidity (as acetic acid) (Figure 4).

According to Silva et al. (2006) acidity has a significant influence on the sensorial quality of cachaça.

The higher alcohols identified in three fractions (head, heart and tail) and their concentrations are presented in Table 3.

Table 3. Concentration (mg 100 mL^{-1} of anhydrous alcohol) of higher alcohols in three different fractions (head, heart and tail) of evaluated cachaça.

Higher alcohols	Fractions	Beverages	
		Yeast pure culture	Mixed culture
Propanol	Head	20.86±1.41	64.10±4.69
	Heart	32.37±0.98	39.37±1.90
	Tail	ND	18.40±0.94
Isobutanol	Head	16.70±0.54	51.50±2.83
	Heart	17.37±1.16	18.84±0.82
	Tail	ND	ND
Isoamyl alcohol	Head	282.47±5.72	810.21±15.84
	Heart	261.45±7.94	266.52±9.36
	Tail	124.50±3.96	319.10±4.80
	Tail		

The main objective of this separation in fractions is to ensure that the heart fraction has a low concentration of toxic and sensorial negative compounds, acceptable concentrations of ethanol, and compounds that are favorable to the aroma and flavor of the cachaça (RECHE et al., 2007).

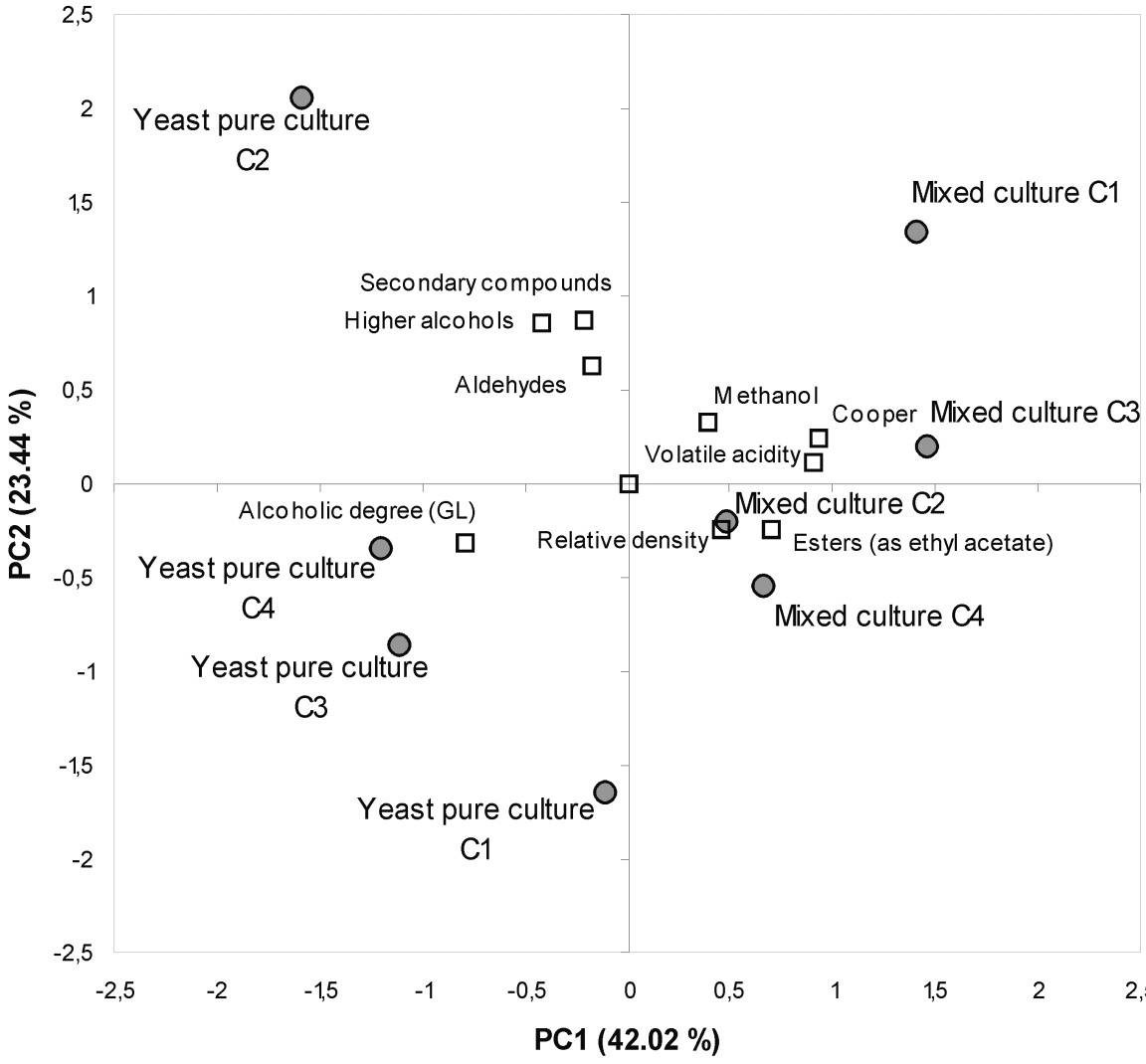

Figure 4. Principal component analysis for the physico-chemical analysis of the beverages produced from *S. cerevisiae* pure culture and mixed culture (*S. cerevisiae* + *L. latics*). C# corresponds to the consecutive batches.

The concentrations of propanol, isobutanol and isoamyl alcohol found in the heart fraction were slightly higher in the beverage produced with a mixed culture (Table 3). The beverage produced with mixed culture showed a higher proportion isoamyl alcohol: isobutanol (15:05). According to Campos et al. (2010) this ratio between higher alcohols is related to the quality of cachaça. The amount of propanol ranged from 32.37 to 39.37 mg mL^{-1} of anhydrous alcohol, in cachaça produced by *S. cerevisiae* UFLA CA 11 and mixed culture, respectively. The occurrence of low concentrations of propanol has been related to the cachaça with superior quality in sensory analysis (BOZA; HORII, 1998; LIMA et al., 2009).

Sensory evaluation

The final beverages were subjected to sensory analysis to assess its acceptance among the consumers. In the sensory analysis the attributes appearance, aroma, taste and overall were evaluated. For all attributes the acceptance was higher than 50% (Table 4).

Table 4. Percentage of acceptance of cachaças produced by *S. cerevisiae* pure culture and mixed culture (*S. cerevisiae* + *L. lactis*).

Attribute	UFLA CA 11	UFLA CA 11 + *L. lactis*
Appearance	75.70	81.10
Aroma	73.00	81.10
Taste	75.70	75.70
Overall	83.80	75.70

The results obtained in sensory analysis can be correlated with those obtained in the physical-chemical analysis. In Figure 4 the beverage produced with a mixed culture was characterized by the presence of methanol, copper, and volatile acidity, which can be a possible explanation for beverage is not related with the attribute taste in Figure 5.

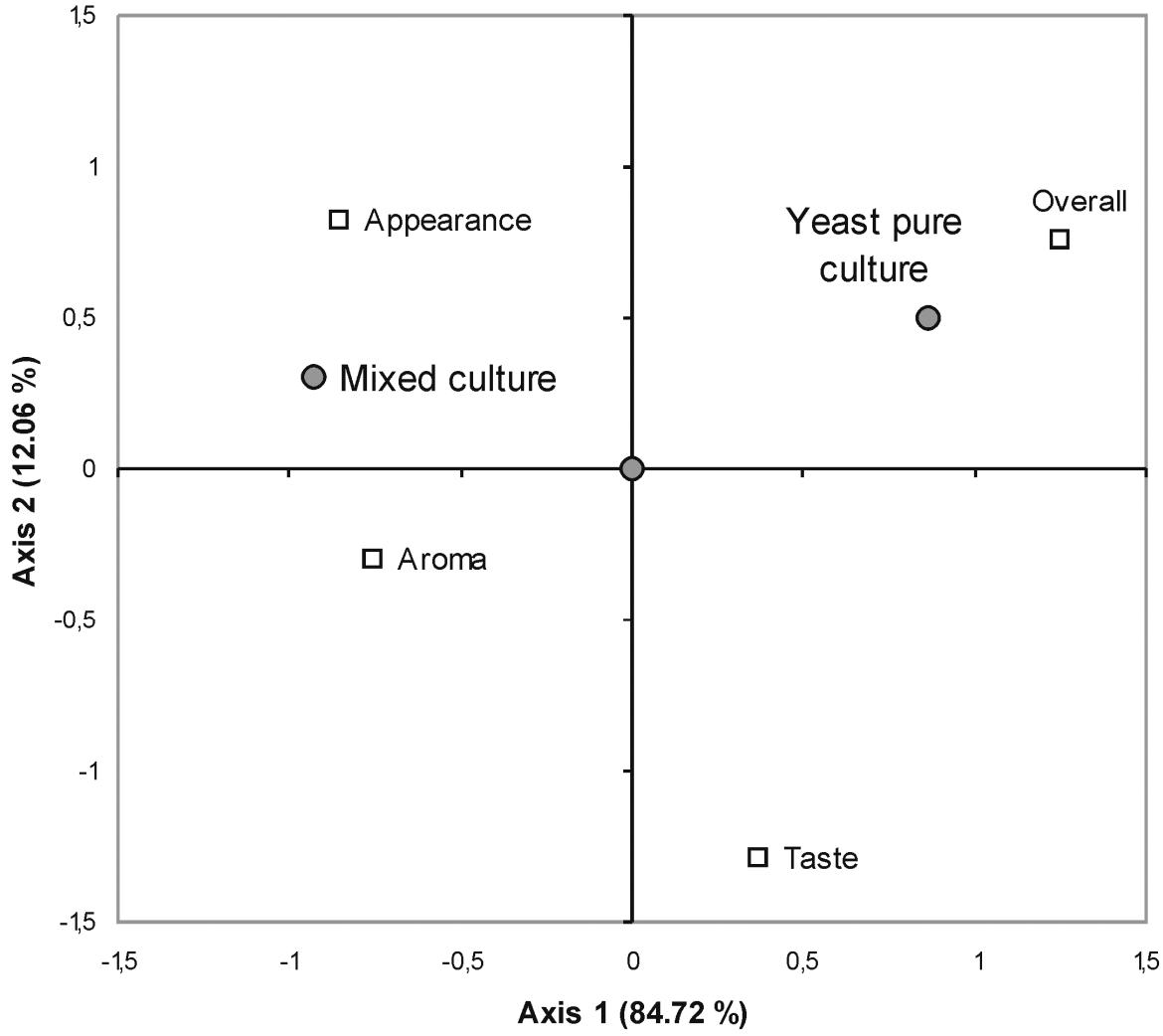

Figure 5. Internal preference mapping for the sensory analysis of the beverages Produced from *S. cerevisiae* UFLA CA 11 pure culture and mixed culture (*S. cerevisiae* UFLA CA 11 + *L. latics* UFLA CA 312).

However, beverage produced with a mixed culture was associated with attribute aroma (Figure 5) and esters (Figure 4).

The data from sensory analysis (percentage of acceptance), were subjected to principal components analysis (PCA) as internal preference mapping. In the first axis (corresponding to 84.72% of variance) the beverage produced with mixed culture was characterized by the attributes appearance and aroma, while beverage produced with *S. cerevisiae* UFLA CA 11 in pure culture was characterized mainly (axis 1) by attribute overall (Figure 5). According to Nurgel et al. (2002), specific compounds are responsible for the typical characteristics of smell and taste. The main source of these compounds is the metabolism of yeast during fermentation.

Conclusion

The results of this work showed that the SCM provided good results for the growth of *L. lactis* and *S. cerevisiae*. This culture medium can be used as low-cost way for multiplication of *L. lactis* and *S. cerevisiae* in laboratory scale. Our results indicate that, although the use of *L. lactis* and *S. cerevisiae* in co-culture led to an increase in the acidity of the beverage, the use of bacteria did not affect the yeast activity and improved the cachaça aroma. These results set a precedence for the new studies with *L. lactis* and other LAB in cachaça production.

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