

Full Length Research Paper

## Potential application of *Saccharomyces cerevisiae* strains for the fermentation of banana pulp

Alvarenga, Raquel Mendonca<sup>1\*</sup>, Carrara, Andrea Geocze<sup>1</sup>, Silva, Crislane Maria<sup>2</sup> and Oliveira, Evelyn Souza<sup>1</sup>

<sup>1</sup>Department Food Science, Faculty of Pharmacy, Federal University of Minas Gerais, Av. Presidente Antonio Carlos, 6627-Campus Pampulha, Belo Horizonte, 31270-901, Brazil.

<sup>2</sup>Laboratory of Microbiology and Bioprocess, Federal University of Tocantins, Avenida Ns 15 Al C No 14 S/N - Bloco IV. Palmas (TO), 77020-210, Brazil.

Accepted 15 March, 2011

This paper aimed at evaluating the fermentation behavior of selected *Saccharomyces cerevisiae* strains in banana pulp and they were compared with commercial yeast (baker's yeast) for subsequent production of distilled spirits. Five types of microorganisms were used: Four yeast strains obtained from accredited microbiology laboratories were isolated from domestic *cachaça* distilleries (UNICAMP-V1, UFMG-A905, UFMG-A1007 and UFMG-A1240) and commercial pressed yeast (COMMERCIAL-yeast). The fermentation parameters of the studied *S. cerevisiae* strains were significantly different. COMMERCIAL-yeast and UNICAMP-V1 strain presented higher ethanol yield and better yeast efficiency in converting total reducing sugars (TRS) into alcohol, with ethanol yields of 83.07 and 94.06%, and yeast efficiency of 90.75 and 96.41%, respectively for UNICAMP V1 and COMMERCIAL-yeast. The higher alcohol contents of 82.26 and 78.05 mg/100 ml anhydrous alcohol were obtained by the UNICAMP-V1 and COMMERCIAL-yeast, respectively. No significant differences in fermentative parameters were observed between COMMERCIAL-yeast and UNICAMP-V1 strain. The UFMG-A1240 strain showed the lowest ethanol yield and therefore not suitable for the production of distilled spirits made of bananas, despite being useful for the production of *cachaça*. Methanol contents did not significantly vary among the five strains tested, except for UFMG-A1007, which produced significantly higher quantities of 0.19 ml/100 ml anhydrous alcohol. However, higher alcohols contents varied significantly between the five strains tests, with the UFMG-A1007 and UFMG-A1240 strains producing the lowest quantities of higher alcohols (30.04 and 48.69 mg/100 ml anhydrous alcohol, respectively). In conclusion, the *S. cerevisiae* strains UNICAMP-V1 and the COMMERCIAL-yeast showed better fermentation behavior, did not produce high methanol and higher alcohols amounts, and therefore were recommended for the production of distilled spirits made of banana in pilot-scale plants.

**Key words:** Yeast, *Saccharomyces cerevisiae*, alcoholic fermentation, banana spirits.

### INTRODUCTION

The large amount of green bananas discarded due to fast post-harvesting ripening may reach 40 to 50% (Mascarenhas, 1999); however, these residues can

potentially be used for industrial purposes (Zhang et al., 2005). Bananas have a short shelf life due to its rapid senescence process, which causes the color of the peel to change from yellow to a 'muddy' brown, at the end of its shelf life. Wholesalers want to extend the banana shelf life between the ripening stage (more yellow than green) and final stage (yellow with light brown flecks) to increase its appeal to consumers, making them purchase higher quantities at one shopping occasion (Klieber et al., 2002). In Brazil, there are many fruits that can be used for the preparation of fermented-distilled beverages (spirits). The banana stands out among these fruits because of its

\*Corresponding author. E-mail: [raquelalvarenga@yahoo.com.br](mailto:raquelalvarenga@yahoo.com.br). Tel: 55 31 3409 6925. Fax: 55 31 3409 6743.

**Abbreviations:** TRS, Total reducing sugars; PDA, potato dextrose agar.

abundance and relatively high concentration of fermentable sugars (Silva, 2004). Banana is a highly available agricultural residue in Brazil, but seems to be underutilized as a potential growth medium for local yeast strains, despite its high content of carbohydrate and other essential nutrients that can support yeast growth. The use of bananas for the production of spirits, in addition to utilizing the surplus fruit and the stills during the periods between sugarcane harvesting seasons, may provide a new product to the market. Although still unfamiliar to the market, this product may have good prospects because of the growing recognition of the quality of Brazilian beverages, especially *cachaça* (Stanislau et al., 2006). According to Emaga et al. (2007), plantain peel is also rich in starch. During maturation, its soluble sugar content increases and starch content decreases due to the action of endogenous enzymes.

With the advancement of beverage technology, yeast strains have been selected according to the desirable characteristics of the process and the product. Ethanol yield and productivity, tolerance to ethanol and variations in temperature, resistance to high sugar concentrations, and the ability to flocculate and to produce or not certain aroma components are constant sources of interest in the choice of the yeast strain to be employed for the production of alcoholic beverages (Hammond, 1995). Geroyiannakia et al. (2007) studied two major toxic volatile compounds in grape *Varietal pomace* distillates and detected methanol and acetaldehyde contents to be much lower than the acceptable legal limits.

Several strains of indigenous yeasts capable of producing ethanol have been isolated from different local sources such as fermented foods and fermented pineapple juices, but in most of the studies, the preferred candidate for industrial production of ethanol has been *Saccharomyces cerevisiae* (Okunowo et al., 2005). This yeast has the ability to produce ethanol with no contamination by other products present in the substrate (Brooks, 2008). The use of selected yeast strains is an important factor in the industrial production of fermented-distilled beverages such as distilled spirits of high chemical and sensory quality. According to Bendoni et al. (1999), *S. cerevisiae* strains may also be used to improve wine flavor, as indicated by the higher isoamyl alcohol content of the transformants as compared to the parental strains. *S. cerevisiae* is a species of budding yeast. It is perhaps the most useful yeast owing to its use since ancient times in baking and brewing. Schuller et al. (2005) studied the biodiversity of the species *S. cerevisiae* in grapes collected in vineyards in Portugal to know their fermentation performance and characterization, and reported that commercial yeast strains were used by the wineries. Both specific *S. cerevisiae* strains and commercial yeast strains are capable of transforming sugar broth into alcohol, and adding essential sensory attributes, such as aroma and taste. Therefore, the choice of the appropriate yeast for fermentation of banana pulp is

essential for the development of techniques to produce distilled spirits from bananas. Jitjaroen (2007) produced banana wine from mashed bananas. He used 0.05% of the enzyme Distizym at 60°C for 5 h and two commercial yeast strains from beer (Siha 3 and G 74), and found that the main organic acids in the banana wine were malic acid (1.97 g l<sup>-1</sup>) and citric acid (1.42 g l<sup>-1</sup>). The prominent aroma components produced were acetic acid ethylester, acetic acid 2 and 3-methylbutylester, 2 and 3-methyl-1-butanol and phenethyl alcohol. The author also studied the production of wines from fermented musts of santol and ma-mao.

The study of the synthesis of secondary products during fermentation by these yeasts is very important, particularly in fruit spirits, because some toxic compounds in excess of the desirable and legal limits may be produced. The diversity of yeast species and strains may be monitored by determining the synthesis of secondary fermentation products, such as acetaldehyde, ethyl acetate and higher alcohols. During different stages of the spontaneous fermentation, acetaldehyde production was observed in some byproducts involved in wine bouquet (Romano et al., 1997). According to Zhang and Chen (2008), in studies of ethanol fermentation using *S. cerevisiae*, in addition to biomass and carbon dioxide, several byproducts are produced, including glycerol and organic acids (example, acetic acid, pyruvic acid and succinic acid). Also, the prevention of glycerol formation can be used to increase ethanol yield of *S. cerevisiae* without increasing the overall cost of the carbon source. Studies performed with fruit spirits made of mangos, pears, melon and banana showed a high production of compounds such as methanol and higher alcohols, indicating the importance of performing more detailed studies on the synthesis of such components in these beverages (Llobdanin, 2008; Lara, 2007; Hernández-Gómez, 2005). Hence, the aim of this paper was to evaluate the fermentation behavior of selected *S. cerevisiae* strains and compare them with commercial yeast (baker's yeast) in the fermentation of banana pulp for the production of distilled spirits.

## MATERIALS AND METHODS

### Source of bananas

The bananas used in this study were variety 'prata' (*Musa cavendishi*) obtained from local markets in Belo Horizonte, Minas Gerais, Brazil. This study used 2 L of bananas in last stage of maturation.

### Pre-treatment of banana samples

The bananas were crushed in compact depulper and the pulp was adjusted to 15° Brix (concentration of soluble solids) by the addition of distilled water. The pulp was then hydrolyzed using Pectinex Ultra SP enzyme (0.025%) for 2 h at 30°C. The enzyme Pectinex Ultra SP was obtained from Novozymes Company. The banana

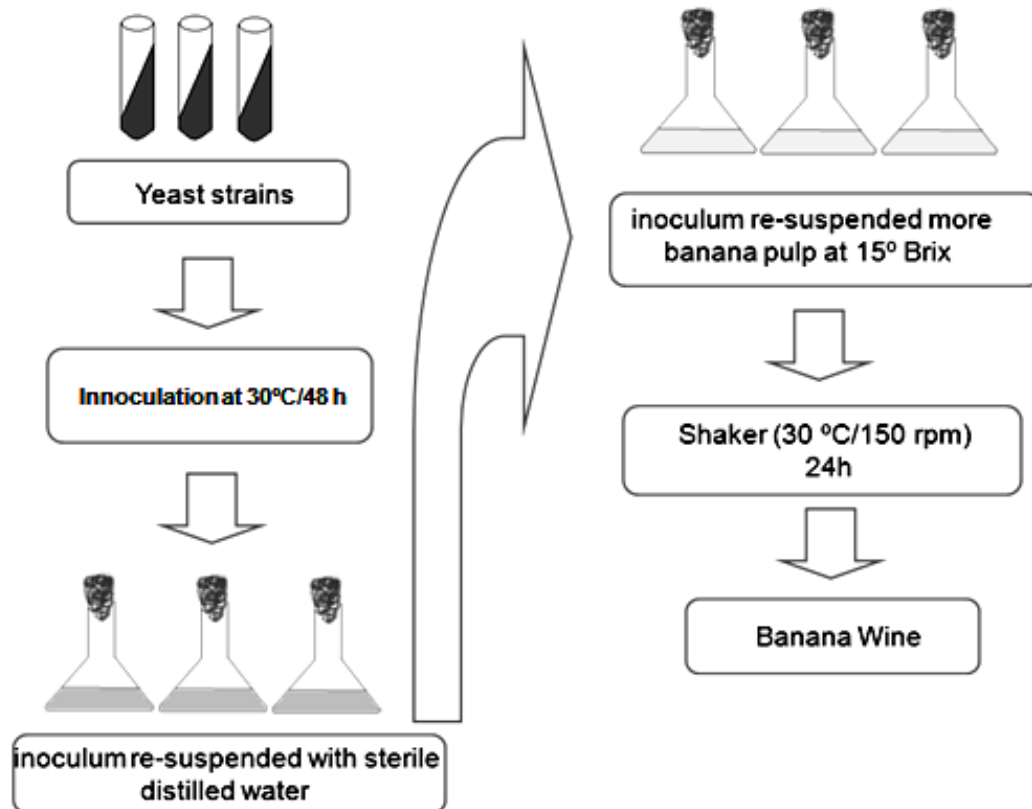


Figure 1. Schematic diagram of the experimental fermentation assays.

pulp was filtered through cottons bags, and 0.4 g/l ammonium sulfate was added. Finally, the pH of the pulp was adjusted to 5.0 by the addition of NaOH solution (0.1 N). The final product obtained was denominated banana pulp.

#### Microorganisms and inocula preparation

The strains were isolated from domestic *cachaça* distilleries by accredited microbiology laboratories: UNICAMP-V1, this yeast strain was obtained from the Industrial Microbiology and Biocatalysis Laboratory of the School of Pharmacy of the Federal University of Minas Gerais (UFMG); UFMGA-1240, UFMGA-1007 and UFMGA-A905 yeast strains were obtained from the Laboratory of Ecology and Microbiology of the Institute of Biological Sciences, UFMG and the commercial pressed yeast (COMMERCIAL-yeast) was obtained from the commercial bakery in Belo Horizonte–MG (Brazil).

All the yeast strains were maintained in potato dextrose agar at 4°C covered with a thin layer of sterilized mineral oil in order to avoid air contact. This practice prevents evaporation of liquid and mass losses. Cells of the selected strains and COMMERCIAL-yeast were inoculated for 48 h at 30 ± 1°C in potato agar for activation before performing the experiments.

#### Laboratory-scale fermentation

Each culture was previously grown in tubes containing agar for 48 h at 30 ± 1°C. Each culture was re-suspended in sterile water and

inoculated (volume corresponding to 10% of the volume of the culture medium, or 4g of dry yeast per liter of pulp) in 250 ml Erlenmeyer flasks containing 100 ml of sterile banana pulp (121 °C for 15 min, pH 5.0). Flasks were incubated for 24 h at 30 ± 1°C and 150 rev/min. Fermentation tests were conducted in triplicate. The schematic diagram of the experimental fermentation assays is shown in Figure 1.

All the analyses were performed in triplicate. The kinetic parameters of fermentation were calculated as a function of percent of ethanol yield and the efficiency of TRS conversion into ethanol.

#### Analytical methods

The final product obtained from fermentation was denominated banana wine. After fermentation, the banana wine was separated from cell biomass by centrifugation. The obtained supernatants (wine) were analyzed for density (with 10 ml pycnometer), pH (digital pHbmeter), ethanol content by the potassium dichromate method (Zimmermann, 1963), total reducing sugars (TRS) by the DNS (dinitrosalicylic acid) method (Miller, 1959), total acidity (ABNT, 1997), glycerol using the Laborlab triglyceride kit (MacGowan et al., 1983), methanol and higher alcohols (Brazil, 1986). Initial TRS concentration, density, acidity and pH were also determined in the banana pulp. Ethanol yield and efficiency of the conversion of TRS into ethanol by the yeast (%) were also determined.

The cell mass was washed twice with distilled water and dried in an oven at 60°C until constant weight was obtained in order to determine the dry mass. The obtained data were submitted to

analysis of variance followed by the Tukey's test (5%) for comparison of the mean values.

### Determination of total reducing sugars (TRS)

The DNS (3,5-dinitrosalicylic acid) method developed by Miller (1959) was used to determine TRS. This determination was preceded by acid hydrolysis to invert sucrose. A 2-ml aliquot of the sample was transferred to a 25-ml flask, and 2 ml of hydrochloric acid ( $2 \text{ mol L}^{-1}$ ) were added. The flask was heated in water bath at  $70^\circ\text{C}$  for 30 min and cooled. The contents were neutralized with 2 ml of  $2 \text{ mol L}^{-1}$  sodium hydroxide. The sample was transferred to a 100-ml volumetric flask, and the volume was completed with distilled water. When necessary, samples were further diluted to obtain concentrations within the linearity range of the calibration curve.

### Determination of total acidity

Total must and wine acidity was determined by titration with  $0.025 \text{ mol L}^{-1}$  sodium hydroxide, according to the ABNT (1997) method. Total acidity determination involved the titration of the acid present in the total sample with a base.

### Determination of glycerol

The Laborlab Liquiform Triglyceride commercial kit (using enzymatic colorimetric method) was employed for the determination of glycerol in the wine (MacGowan et al., 1983). Thirty-microliter aliquots of wine samples were transferred to test tubes, and 3 ml of the enzyme reagent was added to each tube. The tubes were homogenized and incubated for 15 min in a water bath at  $37^\circ\text{C}$ . The tubes were cooled, and the values of absorbance were measured at 505 nm on a spectrophotometer (FEMTO).

### Methanol and higher alcohol contents

Methanol and higher alcohol contents were determined according to the MAPA methods (Brazil, 1986). The total amount of higher alcohols was determined by spectrophotometric measurements performed under visible spectrum (540 nm). Such quantification was performed by comparison with a calibration curve constructed using a mixture of higher alcohols solubilized in ethanol/water (1:1) solvent. Methanol was quantified by spectrophotometric measurements carried out under visible spectrum (575 nm) and compared with the absorbance values established in a calibration curve constructed with standard ethanol/methanol solutions containing known amounts of methanol.

### Determination of ethanol and calculation of the kinetic fermentation parameters

Fermented media (banana wine) samples were steam-distilled in a Tecnal model *Te-012* microstill before ethanol concentration determination. Ethanol concentration was determined by spectrophotometer (FEMTO) at 600 nm using the potassium dichromate method (Zimmermann, 1963).

Ethanol yield was calculated as produced ethanol amount divided by the theoretical amount (calculated based on the quantity of sugar in the must) and expressed as percentage. The expected ethanol amount was calculated after fermentation stoichiometry, assuming that 1.0 g of total sugars produced 0.511 g of ethanol. Ethanol yield percentage was calculated according to the

following equation:

$$\text{Yield (\%)} = \frac{\text{Ethanol produced}}{\text{Theoretical quantity}} \times 100$$

The efficiency of TRS conversion into ethanol by the yeast (%) expresses the amount of produced ethanol relative to the theoretical quantity expected based on the sugar content of the must, and it was calculated accordingly with the following equation:

$$\text{Efficiency (\%)} = \frac{\text{Ethanol produced}}{(\text{TRS}_i - \text{TRS}_f) \times 0.511} \times 100$$

Where,  $\text{TRS}_i$  is the initial sugar content (before fermentation) and  $\text{TRS}_f$  is the final sugar content (after fermentation)

### Experimental procedure

The experimental procedure was developed in two stages:

1. Characterization of banana pulp and wine by different yeasts.
2. Determination of fermentation parameters (ethanol yield and efficiency of TRS conversion into ethanol), high alcohols and methanol contents in banana wine according to the different tested yeasts.

## RESULTS AND DISCUSSION

### Characterization of banana pulp and wine according to different yeasts

Banana pulp (hydrolyzed pulp) showed initial density of 1.02 g/ml and initial TRS of 125.05 g/l. The initial acidity of the hydrolyzed banana used for fermentation was 0.25 g of acetic acid per 100 ml, and that of the banana wine varied between 0.46 and 0.61 g of acetic acid/100 ml of sample. Lara (2007) obtained 0.28 g of acetic acid/100 ml of hydrolyzed banana pulp and approximately 0.49 g of acetic acid/100 ml of wine in the production of distilled banana spirits from the "prata" banana variety. The values obtained in the present study are similar to the findings of Guimaraes (2003), who used the "Nanicão" banana variety and observed acidity values of approximately 0.31g of acetic acid/100 ml of wine. Hernández-Gómez et al., (2005) obtained 0.54 g of acetic acid/100 ml in fermented melon juice. According to Paraggio and Fiore (2004), acetic acid is the main volatile acid in fermented beverages and it is recognized as one of the byproducts that has the most negative effects on the analytical profile of wine. Acetic acid appears to be formed early in fermentation due to bacterial infection (Romano et al., 1992).

Table 1 summarizes the mean final concentration values of total reducing sugars (TRS), ethanol, final acidity, pH and glycerol content of banana wine. Banana wine pH values ranged from 4.29 to 4.40. These values

**Table 1.** Mean values of the final total reducing sugars (TRS) concentration, ethanol content, final acidity, pH and glycerol content in banana wine.

Strain	TRS*	Ethanol**	Final acidity ***	pH	Glycerol *
COMMERCIAL-yeast	3.12 ± 0.17	7.84 ± 0.34	0.49 ± 0.04	4.30 ± 0.06	7.89 ± 1.15
UNICAMP V1	1.20 ± 0.08	6.47 ± 0.02	0.61 ± 0.01	4.34 ± 0.12	7.48 ± 0.67
UFMG A905	4.82 ± 0.17	5.64 ± 0.11	0.51 ± 0.02	4.35 ± 0.17	5.75 ± 0.56
UFMG A1007	4.10 ± 0.14	5.68 ± 0.21	0.50 ± 0.01	4.29 ± 0.05	6.71 ± 1.48
UFMG A1240	6.13 ± 0.03	5.34 ± 0.06	0.46 ± 0.01	4.40 ± 0.10	6.75 ± 0.25

\* g/l; \*\* %(v/v); \*\*\* g of acetic acid/100 ml. Mean ± standard deviation.

**Table 2.** Means of the fermentation parameters for selected yeasts and commercial pressed yeast.

Strain	Yeast efficiency (%)	Ethanol yield (%)
COMMERCIAL-yeast	96.41 <sup>a</sup>	94.06 <sup>a</sup>
UNICAMP V1	90.75 <sup>a</sup>	83.07 <sup>ab</sup>
UFMG A905	76.82 <sup>b</sup>	73.69 <sup>bc</sup>
UFMG A1007	76.53 <sup>b</sup>	73.90 <sup>bc</sup>
UFMG A1240	72.91 <sup>b</sup>	69.16 <sup>cd</sup>

Means followed by the same superscript are not significantly different by Tukey's test ( $P < 0.05$ ).

are normal and inhibit the growth of acetic bacteria in the substrate. The pH obtained by Hernández-Gómez et al., (2005) in different substrates of fermented melon juice varied between 4.4 and 4.9. Souflerous et al., (2004) obtained pH values between 4.1 and 5.7 in fermented blackberry fruit. The mean pH values obtained by Cortez et al. (2000) in fermented sugarcane juice were similar to that of this study. According to Caumeil and Le, (1983), this is the pH that usually characterizes wines. Souflerous and Bertrand (1987) reported pH values varying from 4.15 to 7.0 in tsipouro, while Lehtonen et al. (1999) obtained much lower pH values equal to 3.5, in brandy, and intermediate values of 3.95 and 3.89, in whiskey and rum, respectively.

Different strains produced ethanol concentrations ranging from 5.34 to 7.84% (v/v). This may be explained by differences in yeast tolerance to the ethanol produced. In orange juice fermented by different yeast strains, ethanol levels ranging from 3.19 and 6.8% were found by Okuwono et al. (2005). The authors explained this difference according to Staci et al. (2003), who suggested that the variation in alcohol levels may be due to differences in their optimal physical-chemical conditions (temperature or pH). It was reported that temperature affects gene expression in yeasts (Okunowo et al., 2005). Fernandez et al. (2008), using a heat-tolerant selected yeast strain in sugarcane juice found an ethanol level of 5.2% (v/v). The COMMERCIAL-yeast that produced the highest ethanol concentration was UNICAMP V1 yeast. The ethanol concentration provided by the UFMG A905 yeast (5.65% (v/v)) was lower than that obtained by Oliveira et al. (2004) and Silva et al. (2006), who applied

the same yeast strain in sugarcane juice (8.7% v/v) and synthetic medium (8.3% v/v), respectively.

Glycerol content is very important in alcohol fermentation, as it is related to the quality of alcoholic beverages. Parfait and Jouret (1975) reported that lactic bacteria can metabolize glycerol to produce acrolein, resulting in negative sensory product characteristics. Table 1 shows glycerol content in banana wines produced with different yeast strains and COMMERCIAL yeast. The results are consistent with those of Radler and Schutz (1982), who reported that the amount of glycerol produced in alcohol fermentation, depends on the yeast strain employed, with levels ranging between 4.2 and 10.4  $\text{gl}^{-1}$  of wine. Glycerol is one of the most extensively excreted products during alcoholic fermentation performed with *S. cerevisiae* (Jennings, 1984). According to Rankine and Bridson (1971), about 4 to 5% of the sugar metabolized by yeast is converted into glycerol and this compound in excess can reduce the yield of the beverage, as well as contribute to an increase in the acrolein content of distilled spirits, as in the case of fruit spirits. Acrolein or 2-propenal, is produced by glycerol dehydration during distillation; its presence in spirits is undesirable because of its strong pungent odor (Gutierrez, 1993).

#### Fermentation parameters, high alcohols and methanol contents

Fermentation parameter means obtained using selected yeasts and COMMERCIAL yeast is presented in the Table 2. Previous studies in the study laboratory

**Table 3.** Methanol and higher alcohol contents in banana wine.

Treatment (yeast)	Methanol content (ml/100 ml anhydrous ethanol)	Higher alcohol content (mg /100 ml anhydrous ethanol)
COMMERCIAL-yeast	0.174 <sup>a</sup>	78.052 <sup>b</sup>
UNICAMP V1	0.165 <sup>a</sup>	82.260 <sup>b</sup>
UFMG A905	0.177 <sup>a</sup>	226.191 <sup>c</sup>
UFMG A1007	0.189 <sup>b</sup>	30.043 <sup>a</sup>
UFMG A1240	0.173 <sup>a</sup>	48.689 <sup>a</sup>

Means followed by the same superscript are not significantly different by Tukey's test ( $P < 0.05$ ).

demonstrated the need of filtering the banana pulp and shaking the flasks during fermentation. Without these procedures, very low ethanol yields were obtained, ranging from 40 to 60% for A1240 UFMG, UNICAMP V1 and COMMERCIAL-yeast strains. Oliveira et al. (2004) compared different yeast strains for the production of *cachaça* spirit (distillate from sugarcane) obtained yields that ranged from 48.9 to 90.5% using a synthetic medium containing 150 g l<sup>-1</sup> of glucose as substrate. The author observed a 90.5% yield with synthetic medium and 77.1% with sugarcane broth when the strain UFMG A905 was employed. Silva et al. (2006), using the same yeast strain to ferment sugarcane juice, obtained yields of 84.93% with sugarcane juice and 82.48% with synthetic medium. The ethanol yields obtained by that same author, using UFMG A1240 strain, were 82.09% with synthetic medium and 80.15% with sugarcane juice. In kiwi wines, ethanol yield obtained using a *S. cerevisiae* strain ranged from 75.6 to 92.4, and yeast efficiency ranged from 38.6 to 47.2% (Bortolini et al., 2001). Among the yeast strains studied in this work, UNICAMP V1 was the most successful, with no significant difference in ethanol yield and the efficiency of conversion into ethanol as compared to the COMMERCIAL-yeast (Table 2). This result indicates that it is suitable for the fermentation of banana pulp. The efficiency of conversion into ethanol values (%) obtained in fermentations performed with UFMG A1007, UFMG A905 and UFMG A1240 yeast strains were lower than those obtained with UNICAMP V1 strain or with the COMMERCIAL-yeast.

The levels of methanol and total higher alcohols in banana wine are shown in Table 3. Methanol, a toxic alcohol, is produced during fermentation by the hydrolysis of naturally-occurring pectin in wort (Tomoyuki et al., 2000). The methanol levels produced during fermentation with UFMG A1007 strain (0.189 ml/100 ml anhydrous alcohol) were significantly higher than those produced by the other evaluated strains. In addition to producing more methanol, the fermentation with UFMG A1007 strain produced low ethanol yield and conversion efficiency values. In wines made of orange juice, methanol levels were very high when strains of the yeasts *S. cerevisiae*, *Saccharomyces carlsbergensis* and *Saccharomyces ellipsoideus* (Okunowo and Osuntoki, 2007) were used.

The production of methanol may be reduced by breaking down pectins by the addition of more pectinolytic enzymes prior to the fermentation process. High levels of methanol and higher alcohols found in the fermented beverages are very important because they will increase the levels of these compounds in spirits because the distillation process concentrates their levels even further. Hernández-Gómez et al., (2005) obtained methanol values in distilled melon varying between 610 and 4700 mg/l of anhydrous alcohol (equivalent to 0.75 ml/100 ml of anhydrous alcohol). Souflerous and Bertrand (1987) found values varying between 106 and 196 g/hL in blackberry fruit distillates (equivalent to 1.33 to 2.46 ml/100 ml of anhydrous alcohol). These values are excessively high according to Brazilian legislation, which establishes a maximum methanol value of 0.5 ml/100 ml of anhydrous in distilled spirits.

There was no difference between UFMG A1007 and UFMG A1240 strains as to the quantity of total higher alcohols produced, and they produced lower quantities of these compounds than the other strains. UFMG A905 was significantly different from the other strains, producing the highest quantity of higher alcohols. The quantity of higher alcohols produced by the strain UNICAMP V1 and COMMERCIAL-yeast was significantly lower than that obtained with the UFMG A905.

In studies on the production of banana spirits, several authors have obtained higher concentrations of higher alcohols than those allowed by the Brazilian legislation (Brazil, 2005). These authors include Guimaraes (2003) and Lara (2007), who obtained high concentrations (449.80 and 410.1 mg/100 ml of anhydrous alcohol, respectively) of the higher alcohols in the spirits produced. The excessively high alcohols concentrations found in banana spirits are comparable to those obtained in studies on spirits made of other fruits, such as mango, pineapple and cashew (Nunes and Silva, 2007). In the present study, levels close to 500 mg of higher alcohols per 100 ml of anhydrous alcohol were obtained in the banana spirits. Silva et al. (2009) found a concentration of higher alcohols of 434.01 mg/100 ml of anhydrous alcohol in a distilled beverage obtained from fermented banana peel; this concentration is higher than that allowed by the Brazilian legislation for this type of spirit

(maximum 360 mg/100 ml of anhydrous alcohol).

The results of the present study demonstrate the need and the importance of studying methods to adjust the amount of higher alcohols in fruit spirits to the legal limits. Among these methods, one possible approach would be to use yeast strains that produce lower quantities of higher alcohols during fermentation. The quality of the wine produced depends on the types and the source of the yeast strain employed in the fermentation process. This study shows significant differences in the fermentation parameters among *S. cerevisiae* strains. The COMMERCIAL-yeast and UNICAMP-V1 strain presented higher ethanol yield and better yeast efficiency in the conversion of total reducing sugars (TRS) into alcohol. Out of the other studied strains, UFMG A1240 and UFMG A1007 produced the lowest quantities of higher alcohols. UNICAMP V1 and COMMERCIAL-yeast, in addition to providing high ethanol yield (83.07 and 94.06%) and high conversion efficiency (90.75 and 96.41%), also produced low higher alcohols levels during fermentation (82.26 and 78.05 mg/100 ml anhydrous alcohol). Therefore, the *S. cerevisiae* strain UNICAMP-V1 and the COMMERCIAL-yeast showed better fermentative behavior and may be used in future studies in the production of distilled spirits from bananas in pilot-scale plants. Moreover, it would be interesting to investigate the amount of toxic compounds, including methanol and high alcohols in the distilled spirit made of banana wines.

## ACKNOWLEDGEMENT

The authors thank FAPEMIG for the financial support.

## REFERENCES

- ABNT Associação Brasileira de Normas Técnicas. (1997). NBR 13856: acidez titulável total, volátil total e fixa. São Paulo. p. 5.
- Bondoni B, Cavalieri D, Casalone E, Polsinelli M, Barberio C (1999). Trifluoroleucine resistance as a dominant molecular marker in transformation of strains of *Saccharomyces cerevisiae* isolated from wine. *FEMS Microbiol. Lett.* 180(2): 229-233.
- Bortolini F, Sant'anna ES, Torres RC (2001). Behaviour of Alcoholic and Acetic Fermentations of kiwi mashes (*Actinidia deliciosa*); Composition of mashes and production methods. *Ciência e Tecnologia de Alimentos.* 21(2): 236-243.
- BRAZIL (2005). Ministerio da Agricultura Pecuária e Abastecimento. Instrução normativa nº 13, de 29 de junho de 2005. *Diário Oficial da Republica Federativa do Brasil, Brasília, D.F. Secao 1.*
- BRAZIL (1986). Ministério da Agricultura Pecuária e Abastecimento. Portaria nº 76 de 26 de novembro de 1986. Dispõe sobre os métodos analíticos de bebidas e vinagre. *Diário Oficial da União, Brasília, 28 nov. de 1986. Seção 1, p. 2.*
- Brooks AA (2008). Ethanol production potential of local yeast strains isolated from ripe banana peels. *African J. of Biotechnol.* Vol. 7 (20) p.3749-3752.
- Caumeil M, Le Cognac (1983). *Revue Pour la Science, Edition Francaise de "Scientific Americain"* (France), pp. 48-57.
- Cortes SM, Gil ML, Fernandez E (2000). The influence of redistillation in the distribution of volatile components of marc spirit (aguardiente) and its repercussion on the aromatic quality. *Science des Aliments,* 22: 265-275.
- Emaga TH, Rado HA, Bernard W, Jean Tchango T, Michel P (2007). Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels. *Food Chem.* 103: 590-600.
- Fernandez T, Marcet M, Olivera W, Martín C (2008). Isolation and evaluation of thermotolerant strains of *Saccharomyces cerevisiae* for aguardiente and rum production. *Cienc. Technol. Aliment,* 6(1): 64-70.
- Geroyiannakia M, Komaitisa ME, Stavrakasa DE, Polysioub M, Athanasopoulou PE, Spanosa M (2007). Evaluation of acetaldehyde and methanol in greek traditional alcoholic beverages from varietal fermented grape pomaces (*Vitis vinifera* L.). *Food Control,* 18(8): 988-995.
- Guimaraes-Filho O (2003). Avaliação da produção artesanal da aguardente de banana utilizando *Saccharomyces cerevisiae* CA-1174. Doctoral Thesis, Federal University of Lavras, p. 82.
- Gutierrez LE (1993). Produção de alcoóis superiores por linhagens de *Saccharomyces* durante a fermentação alcoólica. *Scientia Agricola,* 50: 464-472.
- Hammond JRM (1995). Genetically-modified brewing yeasts for the 21st century. Progress to date. *Yeast,* 11(16): 1613-1627.
- Hernández-Gómez LF, Úbeda-Iranzo J, García-Romero E, Briones-Pérez A (2005). Comparative production of different melon distillates: chemical and sensory analyses. *Food Chem.* 90(1): 115-125.
- Jennings DH (1984). Polyol metabolism in fungi. *Adv. Microb. Physiol.* 25: 149-193.
- Jitjaroen W (2007). Influence of yeast strain and nutritive supplements on enological characteristics of tropical fruit wines. *Cuvillier Verlag Göttingen.* p 85.
- Klieber A, Bagnato N, Barrett R, Sedgley M (2002). Effect of post-ripening nitrogen atmosphere storage on banana shelf life, visual appearance and aroma. *Postharvest Biol. Technol.* 25: 15-24.
- Lara CA (2007). Produção de aguardente de banana: emprego de enzimas pectinolíticas e efeito de fontes de nitrogênio e quantidade de inóculo na formação de alcoóis superiores. Dissertation in Food Science, Federal University of Minas Gerais, p. 74.
- Lehtonen PJ, Keller LaDena A, Ali-Matila ET (1999). Multy method analysis of matured distilled alcoholic beverages for brand identification. *Zeitschrift fur Lebensmillel Untersuchung und Forschung A,* 208: 413-417.
- Llobdanin LG (2008). Potential of Blanquilla pear variety to produce pear spirits: Influence of the fermentation and distillation conditions in the final quality. Thesis Universitat Rovira i Virgili.Tarragona, p. 187.
- Macgowan MW, Artiss JD, Strandbergh DR, Zak B, (1983) A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin. Chem.* 29: 538-542.
- Mascarenhas GCC (1999). Banana: comercialização e mercados. *Informe Agropecuário,* 20(196): 97-108.
- Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analyt. Chem.* 31(3): 426-428.
- Nunes BRP, Silva FLH (2007). Estudo da destilação de fermentado de fruta: cinética do processo e caracterização físico-química do produto. In: Anais IV Congress of Scientific Initiation of the Universidad Federal de Campina Grande, (Brazil), p. 10.
- Okunowo WO, Osuntoki AA (2007). Quantitation of alcohols in orange wine fermented by four strains of yeast. *Afr. J. Biotechnol.* 1(6): 095-100.
- Okunowo WO, Okotore RO, Osuntoki AA (2005). The alcoholic fermentative efficiency of indigenous yeast strains of different origin on orange juice. *Afr. J. Biotechnol.* 4(11): 1290-1296.
- Oliveira ES, Rosa CA, Morgano, Marcelo A, Serra Gil E (2004). Fermentation characteristics as criteria for selection of cachaça yeast. *World J. Microbiol. Biotechnol.* 20(1): 19-24.
- Paraggio M, Fiore C (2004). Screening *Saccharomyces cerevisiae* wine strains for the production of acetic acid. *World J. Microbiol. Biotechnol.* 20: 743-747.
- Parfait A, Jouret C (1975). Formation of higher alcohols in rum. *Annales de Technologie Agricole,* 24: 421-436.
- Radler F, Schutz H (1982). Glycerol production of various strains of *Saccharomyces*. *Am. J. Enol. Viticulture,* 33(1): 36-40.
- Rankine BC, Bridson DA (1971). Glycerol in Australian wines and factors influencing its formation. *Am. J. Enol. Viticulture,* 22: 6-12.
- Romano P, Suzzi G, Domizio P, Fatichenti F (1997). Secondary

- products formation as a tool for discriminating non-Saccharomyces wine strains. *Antonie van Leeuwenhoek*, 71(3): 239-242.
- Romano P, Suzzi G, Comi G, Zironi R (1992). Higher alcohol and acetic acid production by apiculate wine yeast. *J. Appl. Bacteriol.* 73, 126-130.
- Schuller D, Alves H, Dequin S, Casal M (2005). Ecological survey of *Saccharomyces cerevisiae* strains from vineyards in the Vinho Verde Region of Portugal. *FEMS Microbiol. Ecol.* 51(2): 167-177.
- Silva CLC, Rosa CA, Oliveira ES (2006). Studies on the kinetic parameters for alcoholic fermentation by flocculant *Saccharomyces Cerevisiae* strains and non-hydrogen sulfide-producing strains. *World J. Microbiol. Biotechnol.* 22: 857-863.
- Silva EF (2004). Obtenção de Aguardente de Banana em Micro - Escala: Caracterização do Processo e do Produto. Dissertação, Mestrado em Ciência de Alimentos, Federal University of Paraíba, p. 112.
- Silva MBL, Chaves BP, Lelis VG, Alvarenga LM, Zuim DR, Silva PHA (2009). Qualidade Físico Químico e Sensorial de aguardentes de polpa de banana e banana integral submetidas à hidrólise enzimática. *Alim. Nutr.* 20(2): 217-221.
- Souflerous EH, Mygdalia AS, Natskouli P (2004). Characterization and safety evaluation of the traditional Greek fruit distillate "Mouro" by flavor compounds and mineral analysis. *Food Chem.* 86: 625-636.
- Souflerous E, Bertrand A (1987). Etude sur le "Tsipouro", eau-de-vie de marc traditionnelle de Grece, precurseur de l'ouzo. *Conn Vigne Vin*, 21(2): 93-111.
- Stanislau BJ, Ketzer DCM, Andrades GRR, Gobo AB (2006). Composição Química da Cachaça Produzida na região Noroeste do Rio Grande do Sul. *Ciência e Tecnologia de Alimentos*, 26(4): 793-798.
- Tomoyuki N, Tatsuro M, Hiroya Y, Yasuyoshi S, Nobuo K, Noboru T (2000). A Methylophilic Pathway Participates in Pectin Utilization by *Candida boidinii*. *Appl. Environ. Microbiol.* 66(10): 4253-4257.
- Zhang P, Roy LW, James NB, Bruce RH (2005). Banana starch: production, physicochemical properties, and digestibility-a review. *Carbohydrate Polymers*, 59(4): 443-458.
- Zhang A, Chen X (2008). Improve ethanol Yield through minimizing glycerol yield in ethanol fermentation of *Saccharomyces cerevisiae*. *Chin. J. Chem. Eng.* 16(4): 620-625.
- Zimmermann HW (1963) Studies on the dicromate method of alcohol determination. *Am. J. Enol. Viticulture*, 14: 205-213.