

## ANALYSIS OF THE SECONDARY COMPOUNDS PRODUCED BY *SACCHAROMYCES CEREVISIAE* AND WILD YEAST STRAINS DURING THE PRODUCTION OF “CACHAÇA”

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### ABSTRACT

The aim of this study is to compare the composition of “cachaças” produced in 10 fermentation cycles by *Saccharomyces cerevisiae* (*Sc*) and wild yeast strains [*Pichia silvicola* (*Ps*), *Pichia anomala* 1 (*Pa1*), *Pichia anomala* 2 (*Pa2*) and *Dekkera bruxelensis* (*Db*)], isolated from distilleries in Jaboticabal – SP, Brazil. The secondary components of the heart fraction were determined by gas chromatography. The levels of secondary components were influenced by the wine pH, which varied among yeast strains. *S. cerevisiae* showed slightly more secondary components, whereas wild strains produced more higher alcohols. Wild yeast strains were shown to be adequate for the production of a high quality “cachaça”.

**Key words:** “cachaça”, distillate composition, aldehyde, higher alcohols, secondary components, yeast

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### INTRODUCTION

The sugar cane spirit named “cachaça” is a typical Brazilian drink that is gaining a bigger share in the international market of distilled beverages. The Brazilian production in 2002 season was around 1.3 billion liters. According to the Brazilian Legislation (4,5), sugar cane “cachaça” is a beverage that contains 38 to 48% (v/v) alcohol and is obtained by distillation of fermented sugar cane juice.

The alcoholic fermentation of sugar cane juice produces ethyl alcohol as its major product and many other secondary components, such as aldehydes, methanol, higher alcohols, acids and esters. The nature and quality of these components depend on the raw material, fermentation, distillation and ageing. The Brazilian Legislation (4,5) establishes that the secondary components of “cachaça”, resulting from the sum of aldehydes, volatile acids, esters, furfural and higher alcohols, should be between 0.2 and 0.65 g .100 mL<sup>-1</sup> of anhydrous alcohol (a. a.).

A good balance among volatile compounds is essential to produce a high quality sugar cane spirit or “cachaça” (1). Thus, many studies evaluated the influence of yeast strains in the

formation of flavour compounds in different alcoholic beverages such as wine, beer, whisky, cognac and rum (6,12,13,15,16,25). These studies suggest that the differences in the flavour components are attributed mainly to the yeast strains.

Recently, it has been observed that there is a natural substitution of the inoculum cell population by contaminant wild yeasts during the process of fermentation (3,10,20,23). These contaminating yeasts are often shown to be resistant to adverse conditions, such as a high temperature of the medium, substrate and nutrient concentrations, growth rate, and fermentation time, among others. Nevertheless, there is little information about the importance of wild yeasts and their contribution to the quality and aroma of the “cachaça”. Besides, it is not yet known if the utilization of non-conventional yeast strains might or not improve the quality of the “cachaça”, resulting in a beverage with more stable contents of secondary components. Considering the importance of the wild strains in “cachaça” production, this study determined the main components of the “cachaças” produced by four wild yeast strains, using *S. cerevisiae* as control.

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## MATERIALS AND METHODS

Cotton-filtered sugar cane juice was obtained from a distillery in the region of Jaboticabal, SP, during the season 1999/2000.

The yeasts used in the fermentation process belong to the collection of the Laboratories of Sugar and Alcohol Technology and Fermentation Microbiology - FCAV/UNESP – Brazil. The yeasts were selected based on their unique results in the biochemical tests used for identification and characterization, done as described by Barnett *et al.* (2) and Kreger van Rij (11). The strains were *Pichia silvicola* (Ps), *Pichia anomala* 1 (Pa1), *Pichia anomala* 2 (Pa2) and *Dekkera bruxelensis* (Db). *Saccharomyces cerevisiae* (Sc) was used as a standard.

The strains were reactivated for 12 h in 250mL of sterile YEPD medium (2% yeast extract, 1% peptone and 2% dextrose), incubated at 30° and 100 rpm. The produced mass was centrifuged and the cells were multiplied in vats (splitting process) using must at 6°Brix, until enough start was obtained.

The fermentation was performed in two replications using 5 L of must inoculated with 30 g cells.L<sup>-1</sup>. The must was prepared from sugar cane juice at 30°C, 15°Brix and pH 3.5. The must was added to the vat hourly during 3 h. The fermentation was carried out in stainless steel vats, using the batch process and 10 fermentation cycles. Each cycle lasted from 17 to 20 h, and the inoculum was decanted and recovered in the end of each cycle. The end of fermentation took place when the substrate indicated 0° Brix. Cells were then separated and submitted to microbial inoculum treatment (pH adjustment to 3.5 and shaking for two hours) and reused in the new fermentation process. Both wine total acidity and pH were determined as described in Copersucar (9).

The wine was submitted to distillation in a glass apparatus, with a 3-liter carter head with a rectifying column with meandered copper wire. The average distillation time was around 1 hour, which resulted in 2.2 L of wine. The fractions were separated during distillation considering proportions of 10% for the head, 80% for the heart and 10% for the tail portion. The heart fraction was recovered and individually analysed. The secondary components (acetaldehyde, ethyl acetate, propanol, isobutanol) of the heart fraction were determined by gas chromatography (17).

## RESULTS AND DISCUSSION

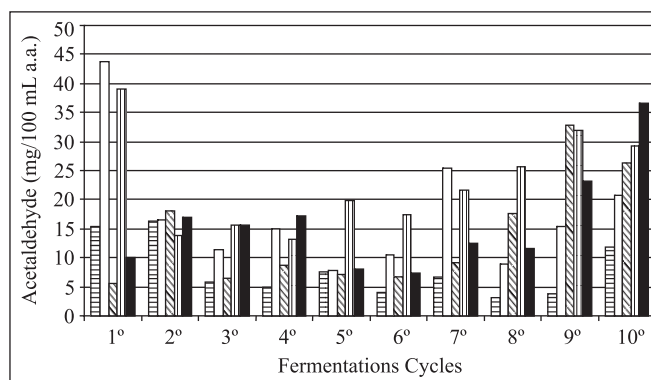
The main components of the wine or distillate were ethanol and water, besides a series of volatile substances that distil together and comprise a smaller portion of the wine. These volatiles substances together with the components that are present in higher proportions give peculiar flavour characteristics to the “cachaça”. The nature and composition of these components depend on the characteristics of the raw material, and on the fermentation and distillation processes.

According to Soumalainen and Lehtonen (25), the type of inoculum and the fermentation conditions may interfere with the formation of aromatic substances, and may have influence on the production of other substances such as sulphur and phenolic compounds.

During the production of “cachaça”, some conditions should be observed in order to obtain a fine quality product, with good aroma and flavour. The spirit should have harmonious proportions of non-alcoholic components, as established by the Brazilian Legislation (4,5).

The content of acetaldehyde, which is the main aldehyde found in “cachaça”, ranged from 3.07 to 43.76 mg. 100 mL<sup>-1</sup> of anhydrous alcohol (Fig. 1). The legislation allows a maximum value of 30 mg .100 mL<sup>-1</sup> a.a. The highest values were seen in strain Pa1, which were almost 50% above the legal acetaldehyde limit in the 1<sup>st</sup> cycle. The strains Db and Sc also showed an aldehyde production higher than the permitted values in the 1<sup>st</sup> and 10<sup>th</sup> cycles, respectively. Acetaldehyde levels were within the admissible range in the other cycles. Aldehydes are important for aroma and taste of alcoholic beverages (18), but high concentrations may be toxic to humans, since these are considered to be responsible for the disagreeable effects after drinking too much. It is thought that acetaldehyde-rich “cachaças” are originated in distilleries that do not separate the head fraction products during the distillation process (7).

Acetaldehyde production by strains Pa1 and Db, in the first fermentation cycle; Pa2 and Db in the ninth, and Sc in the tenth, suggest that the levels of acetaldehyde depend on the yeast strain used as inoculum, and also on the conditions in which the fermentation processes and distillation have been carried out. This is supported by the fact that the fractions were separated as recommended in order to obtain a good quality beverage.



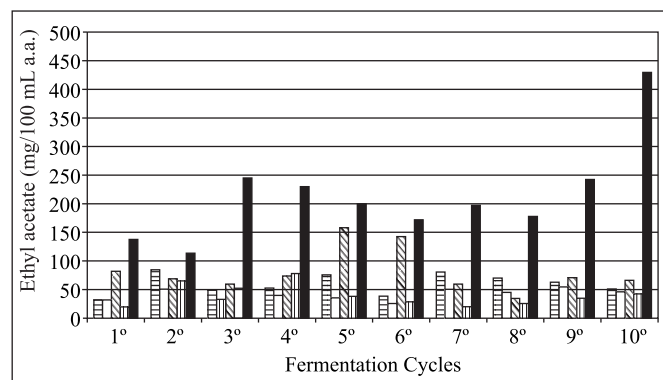
**Figure 1.** Acetaldehyde levels (mg.100 mL<sup>-1</sup> a.a.) of the “cachaças” produced in 10 fermenting cycles by *Pichia silvicola* (Ps), *Pichia anomala* 1 (Pa1), *Pichia anomala* 2 (Pa2), *Dekkera bruxelensis* (Db) and *Saccharomyces cerevisiae* (Sc), included as a reference.

The values of ethyl acetate produced by Sc strain in the 3<sup>rd</sup>, 4<sup>th</sup>, 9<sup>th</sup> and 10<sup>th</sup> cycles (Fig. 2) were above the maximum limit permitted by the legislation, 200 mg.100 mL<sup>-1</sup> anhydrous alcohol. The other strains resulted in values below this limit. Soles *et al.* (24) have also reported that the highest ester levels were produced by *S. cerevisiae* when compared to other examined yeasts.

Among the wild yeasts producing acceptable ethyl acetate levels, Pa2 produced the highest levels. The mean value of wine acidity was also higher for this strain, followed by the standard strain, (5.01 and 4.78 g. H<sub>2</sub>SO<sub>4</sub>.L<sup>-1</sup>, respectively). The other strains resulted in wine acidity values of 4.37 (Db), 3.39 (Ps) and 3.33 g.H<sub>2</sub>SO<sub>4</sub>.L<sup>-1</sup> (Pa1). The higher acidity probably contributed to the higher Sc and Pa2 production of ethyl acetate in “cachaças” when compared to the other strains. Similar results have been already described and it was concluded that a higher wine acidity was correlated to the higher concentration of ethyl acetate in the distillate that is produced (8).

The concentration of hydrogen ions in the medium correlated inversely with the ethyl acetate concentration present in the final distillate, i.e. the higher the fermentation pH, the lower the ethyl acetate concentration. The mean pH of the wines produced by Sc and Pa2 were 3.24 and 3.31, respectively, while Ps, Pa1 and Db showed values of 3.40, 3.38 and 3.27, respectively.

Methanol may be produced during some steps of “cachaça” production. It is an undesirable component on the final product, due to its high toxicity for humans. No methanol was detected in the samples of the spirits produced by *S. cerevisiae*. Some samples of *P. silvicola* and *P. anomala* 1 showed distillates with minimal quantities of methanol, varying from 0 to 5 mg.100 mL<sup>-1</sup>. These are acceptable levels for “cachaça” because they are not detrimental to the production process and are far below the maximum levels that are permitted (200 mg.100 mL<sup>-1</sup> a.a.).



**Figure 2.** Ethyl acetate levels (mg.100 mL<sup>-1</sup> a.a.) of the “cachaças” produced in 10 fermentation cycles by *Pichia silvicola* (Ps), *Pichia anomala* 1 (Pa1), *Pichia anomala* 2 (Pa2), *Dekkera bruxelensis* (Db) and *Saccharomyces cerevisiae* (Sc), included as a reference.

Methanol production is associated with the acidic degradation of pectine, a polysaccharide that is present in very low quantities in sugar cane bagasse (14). Probably, a great part of the bagasse fragments that came with the must was eliminated, and so was pectin, resulting in a low methanol distillate production.

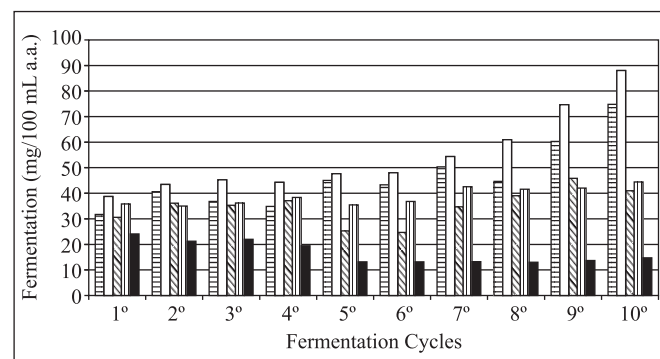
The analysis of higher alcohols revealed that Sc produced the lowest propanol levels (Fig. 3) and wild yeasts Pa1 and Ps showed the highest levels. Db and Pa2 also showed higher values than Sc (Figs. 3 to 6). Rankine (21) reported similar results, showing an increase in the quantity of higher alcohols produced by the yeasts when the wine pH was higher. In the present study, the pH values of the wines produced by Ps and Pa1 were 3.4 and 3.38, respectively, whereas pH was 3.24 for the wine produced by Sc. Propanol concentrations was shown to be proportional to the increase in the pH of the produced wines.

The highest levels of isobutanol were produced by the strains Ps and Pa1 in most of the fermentation cycles, followed by Pa2 and Db (Fig. 4), while Sc produced the lowest values. As seen for propanol, wine pH significantly influenced isobutanol levels.

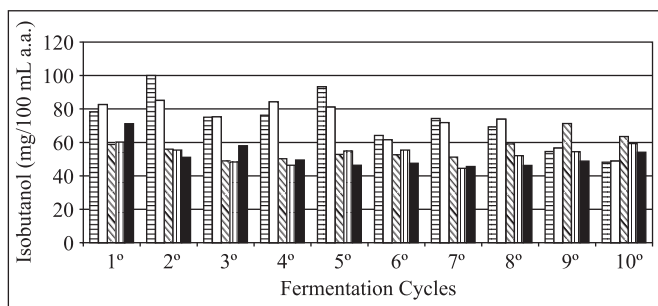
The profile of isoamylic alcohol levels (Fig. 5) was similar to those seen in other higher alcohols. Mean values were the lowest in Sc, whereas Pa1 and Ps showed the highest ones, followed by Db and Pa2.

Similar to what was observed for propanol and isobutanol, the pH of the wine also influenced isoamylic alcohol levels. There was an increase in the pH of the wine produced in Ps and Pa1 fermentations, which resulted in a higher concentration of isoamylic alcohol, corroborating results from Rankine (21).

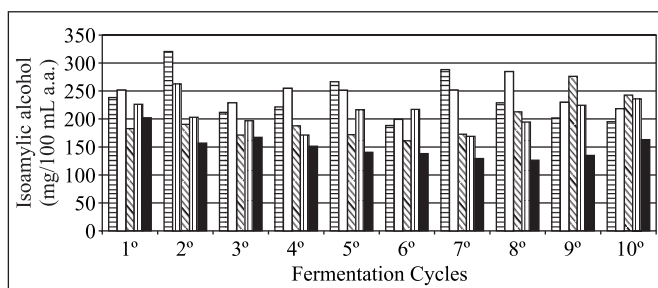
The increase in the hydrogen ions of the wine increased the concentration of propylic, isobutylic and isoamylic alcohol in the distillates. According to Soumalainen and Lehtonen (25), the levels of propanol, isobutanol and isoamylic alcohol produced during the fermentation of grape must varied considerably



**Figure 3.** Propanol content (mg.100 mL<sup>-1</sup> a.a.) of the “cachaças” produced in 10 fermentation cycles by *Pichia silvicola* (Ps), *Pichia anomala* 1 (Pa1), *Pichia anomala* 2 (Pa2), *Dekkera bruxelensis* (Db) and *Saccharomyces cerevisiae* (Sc), included as a reference.



**Figure 4.** Isobutanol content ( $\text{mg}\cdot 100\text{ mL}^{-1}$  a.a.) of the “cachaças” produced in 10 fermentation cycles by *Pichia silvicola* (Ps), *Pichia anomala* 1 (Pa1), *Pichia anomala* 2 (Pa2), *Dekkera bruxelensis* (Db) and *Saccharomyces cerevisiae* (Sc), included as a reference.



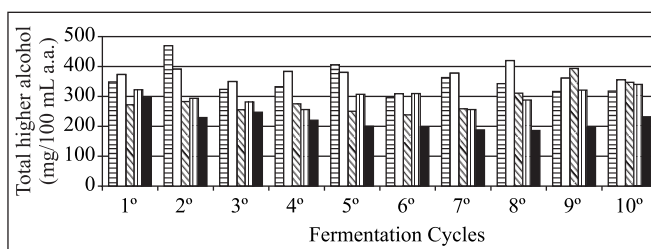
**Figure 5.** Isoamylic alcohol content ( $\text{mg}\cdot 100\text{ mL}^{-1}$  a.a.) of the “cachaças” produced in 10 fermentation cycles by *Pichia silvicola* (Ps), *Pichia anomala* 1 (Pa1), *Pichia anomala* 2 (Pa2), *Dekkera bruxelensis* (Db) and *Saccharomyces cerevisiae* (Sc), included as a reference.

depending on the yeast strain that was used. These results are similar to the obtained in this study, since yeast strains produced different quantities of secondary compounds.

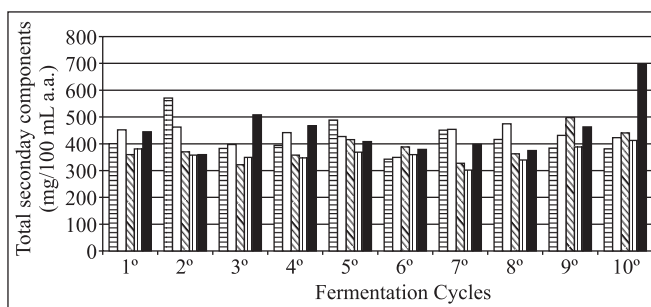
Considering the total of higher alcohols in Fig. 6, the wild strains showed the highest and Sc the lowest levels. Other researchers (2,12) have also reported a lower production of higher alcohols by *S. cerevisiae*, which had the highest levels of ethyl acetate, suggesting that strains that produced more quantities of higher alcohols also had lower ester levels. This profile was also seen for wild strains and Sc in the present study.

The total of secondary components (Fig. 7) ranged from 301.81 to 697.9  $\text{mg}\cdot 100\text{ mL}^{-1}$  a.a. The high level of ethyl acetate that was produced by Sc in the 10<sup>th</sup> cycle resulted in a higher sum of secondary components when compared with the other strains.

The wild yeasts produced acceptable concentrations of all reported compounds. Longo *et al.* (12) studied the production of higher alcohols, acetaldehyde, ethyl acetate and other components and their findings corroborate the results described in the present study.



**Figure 6.** Total of higher alcohols ( $\text{mg}\cdot 100\text{ mL}^{-1}$  a.a.) of the “cachaças” produced in 10 fermentation cycles by *Pichia silvicola* (Ps), *Pichia anomala* 1 (Pa1), *Pichia anomala* 2 (Pa2), *Dekkera bruxelensis* (Db) and *Saccharomyces cerevisiae* (Sc), included as a reference.



**Figure 7.** Sum of secondary components ( $\text{mg}\cdot 100\text{ mL}^{-1}$  a.a.) of the “cachaças” produced in 10 fermentation cycles by *Pichia silvicola* (Ps), *Pichia anomala* 1 (Pa1), *Pichia anomala* 2 (Pa2), *Dekkera bruxelensis* (Db) and *Saccharomyces cerevisiae* (Sc), included as a reference.

The quality and the type of inoculum that is used are very important, since different microorganisms will produce many secondary components in different proportions (22). Thus, inoculum is sometimes considered more important than the raw material used in the fermentation process (19,22).

Since the levels of secondary components were within the range that is permitted by law, the selected wild yeasts (*P. silvicola*, Ps; *P. anomala* 1, Pa1; *P. anomala* 2, Pa2; *D. bruxelensis*, Db) and *S. cerevisiae* (Sc) may be used to produce “cachaça”.

## RESUMO

### Análise dos componentes secundários produzidos por *Saccharomyces cerevisiae* e leveduras selvagens durante a produção de cachaça

O presente trabalho visou estabelecer uma comparação entre composição de cachaças produzidas por *Saccharomyces cerevisiae* (Sc) e estirpes de leveduras selvagens [*Pichia silvicola* (Ps), *Pichia anomala* 1 (Pa1), *Pichia anomala* 2 (Pa2)

e *Dekkera bruxelensis* (Db)], isoladas em destilarias da região de Jaboticabal-SP. Os componentes secundários da fração denominada coração foram determinados por cromatografia gasosa. Os níveis dos componentes secundários foram influenciados pelo pH dos respectivos vinhos, os quais dependem da estirpe de levedura empregada no processo fermentativo. A *Saccharomyces cerevisiae* apresentou valores ligeiramente superiores de componentes secundários, enquanto as estirpes selvagens produziram maiores teores de álcoois superiores. As estirpes selvagens de leveduras mostraram-se adequadas para obtenção de uma cachaça de boa qualidade.

**Palavras-chave:** cachaça, composição do destilado, aldeídos, álcoois superiores, componentes secundários

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