

# The clarification of sugarcane juice and the use of CA-11 yeast produces better quality cachaça<sup>1</sup>

Clarificação do caldo de cana e o uso do fermento CA-11 produz cachaça de melhor qualidade

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**ABSTRACT** - Cachaça is the second most consumed alcoholic beverage in Brazil, obtained by distilling wine from fermented sugarcane must, and has an alcohol content of 38 to 48% (v/v) at 20 °C. The quality of the drink is affected by the raw material used, the treatment of the juice, the type of yeast and the distillation process. The aim of this study was to evaluate the performance of two types of yeast (selected and pressed) and the prior physical and chemical treatment of the sugarcane juice on the quality of the cachaça. The experimental design was completely randomised with 9 replications. The primary treatment was represented by the must (obtained from clarified and non-clarified juice) and the secondary treatment by the types of yeast (CA-11 and pressed biological). The microbiological behaviour of the yeast during fermentation was evaluated together with the chemical composition of the wine and cachaça. The use of selected strains and the prior treatment of the juice resulted in better performance of the fermenting yeasts, producing distillates of suitable physical and chemical standards and quality.

**Key words:** Quality beverage. Liming. Selected yeast. *Saccharomyces cerevisiae*. Fermentation process.

**RESUMO** - A cachaça é a segunda bebida alcoólica mais consumida no Brasil, obtida pelo destilado do vinho a partir do mosto fermentado de cana-de-açúcar com teor alcoólico de 38 a 48% (v/v) a 20 °C. A qualidade da bebida é afetada pela matéria-prima utilizada, tratamento do caldo, pelo tipo de fermento e processo de destilação. Objetivou-se avaliar o desempenho de dois tipos de fermento (selecionado e prensado) e o prévio tratamento físico-químico do caldo de cana para a qualidade da cachaça. O delineamento experimental foi inteiramente casualizado com 9 repetições. O tratamento primário representado pelo mosto (obtido de caldo clarificado e não clarificado) e o secundário pelos tipos de fermento (CA-11 e prensado biológico). Avaliou-se o comportamento microbiológico da levedura em fermentação, a composição química do vinho e da cachaça. A utilização de cepas selecionadas e o prévio tratamento do caldo possibilitou um melhor desempenho das leveduras fermentadoras, resultando em destilados com padrões físico-químicos adequados e de qualidade.

**Palavras-chave:** Bebida de qualidade. Caleagem. Levedura selecionada. *Saccharomyces cerevisiae*. Processo fermentativo.

DOI: 10.5935/1806-6690.20200067

Editor do artigo: professor Alek Sandro Dutra - alekdutra@ufc.br

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Received for publication in 13/11/2019; approved in 28/05/2020

<sup>1</sup>Parte de Dissertação do segundo autor, apresentado ao curso de Pós-Graduação em Microbiologia Agropecuária - FCAV/UNESP Jaboticabal

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## INTRODUCTION

Cachaça is a typical beverage, exclusive to Brazil, obtained by distilling wine fermented from the must of sugarcane juice, and has an alcohol content of 38% to 48% by volume at 20 °C, (BRASIL, 2005).

The country has 40 thousand manufacturers producing 1.6 billion litres of cachaça annually, of which approximately 11 million litres are exported each year (ASSOCIAÇÃO BRASILEIRA DE BEBIDAS, 2018). With the growth of the market and the possibility of exporting the beverage, it is necessary for the manufacturing process to be based on carefully determined practices, in order to obtain a standardised product. Among the factors that affect the quality of the beverage are the raw materials and fermentation conditions, both of which have a strong effect on the chemical composition of the distillate (CARDOSO, 2013).

The cachaça must also meet the quality requirements provided for in the technical regulations of Normative Instruction No. 13, of 29 June 2005 (BRASIL, 2005), which establishes limits on substances that can be harmful to human health; among these, methanol, aldehydes and the congener coefficient, with emphasis on toxic metabolites such as ethyl carbamate and acrolein (AZEVEDO *et al.*, 2007).

Although this sector enjoys high productivity, with eleven thousand producers and four thousand brands of cachaça (INSTITUTO BRASILEIRO DA CACHAÇA, 2019), there are paradigms that are still inherent in the process that significantly interfere in the quality of the beverage, resulting in a product that does not meet current legislation. Among these, the use of yeasts sold to the baking industry should be highlighted; these remain in the process for a short time, being easily replaced by contaminating yeasts found in the raw material, which can negatively affect the composition of the distillate (VIANA *et al.*, 2020).

In this context, the use of selected yeast strains is recommended, such as *Saccharomyces cerevisiae* CA-11, which remains in the production process during the harvest, and affords rapid fermentation and easy separation of the wine, in addition to producing beverages with superior levels of acidity and alcohol that characterise the product (MONTIJO *et al.*, 2014; TEIXEIRA *et al.*, 2019). These strains also more adaptive and produce quality cachaça even when the raw material is highly compromised with insect pests (ALMEIDA *et al.*, 2020). Ribeiro *et al.* (2017), further demonstrated that compared to native yeasts, CA-11 also promotes cachaça with better chemical characteristics.

In addition to selecting the yeast, the process of clarifying the juice should be highlighted, a basic

constituent of good manufacturing practice, where heating and changing the pH of the sugarcane juice results in coagulation and flocculation of undesirable compounds, such as earth, plant residue, acids and proteins, etc. These compounds are considered the precursors of methanol, ethyl carbamate, acrolein and acids, among others in the beverage (TEIXEIRA *et al.*, 2019). It should also be considered that treating the juice affords a significant reduction in the amount of bacteria and yeasts that accompany the sugarcane from the field (COSTA *et al.*, 2014) and that can contaminate fermentation, resulting in a lack of conformity in the beverage (TEIXEIRA *et al.*, 2019).

As such, the aim of this study was to evaluate the fermentation of must prepared from clarified sugarcane juice, using CA-11 (selected) and Fleischmann (pressed biological) yeasts, and their effect on the composition of the cachaça.

## MATERIAL AND METHODS

The experiment was carried out during the 2014/2015 harvest at the Laboratory for Sugar and Alcohol Technology and Fermentation Microbiology of the Department of Technology at FCAV/UNESP, Jaboticabal Campus, in the state of São Paulo (SP).

The SP83-2847 variety of sugarcane, obtained from a production unit in the region of Jaboticabal, SP, was harvested by hand without previously burning the straw, and then topped and immediately processed. The juice was extracted by milling, and filtered to remove impurities and residue, and was then characterised for Brix, pH, Reducing Sugars (RS), Total Reducing Sugars (TRS), Total Acidity (TA) and Total Phenolic Compounds (TPC) (CENTRO DE TECNOLOGIA CANAVIEIRA, 2009).

### Preparation of the Must

Must 1 (non-clarified) was standardised at 16 °Brix with the temperature adjusted to 32 °C. To obtain Must 2 (clarified), the juice was standardised at 16 °Brix and pH 6.0 (with the addition of calcium hydroxide 6°Be), heated until boiling, and transferred to a flat-bottomed stainless steel decanter, where it was left to rest for one hour; the supernatant was then siphoned.

Considering the optimal fermentation conditions for baker's yeast, the pH of the musts was adjusted to 4.5 by adding 10N H<sub>2</sub>SO<sub>4</sub>; however, the pH was not corrected for the must used with the CA-11 yeast. The chemical and technological characteristics of the musts were determined by analysing the pH, TA, TRS, TPC

and Total Nitrogen (CENTRO DE TECNOLOGIA CANAVIEIRA, 2009).

### Preparation of the Yeast

Fleischmann commercial baker's yeast and CA-11 selected yeast were used. For the CA-11, 30 g of yeast were diluted in 600 mL of drinking water. For the baker's yeast, a ratio of 400 g of pressed yeast to 1 litre of 0.5% glucose solution was used. In both cases, the first feed was carried out after 30 minutes using 3 L of juice at 10 °Brix, when the Brix had reduced to 2, another 6 L of juice was added. This process was repeated until the biomass required to form the yeast starter was obtained.

### Fermentation Process

Fermentation was carried out in stainless steel vats with a conical base and a working capacity of 6 L, in a fed-batch system with the yeast recovered by sedimentation. To prepare the yeast starter, the baker's yeast and the CA-11 were diluted in 1 L of 0.5% NaCl solution so that the final concentration of the fermentation must was approximately  $10^6$  colony forming units per mL (CFU/mL), with a Cell Viability greater than 85%. Two feeds were carried out, one of 2.0 L and the other of 3.0 L of must at 16 °Brix, at intervals of 30 minutes and 1.5 hours respectively. The end of fermentation was determined when the Brix value of the wine was less than or equal to 1, or when the maximum limit of 20 hours fermentation was reached. After completion, 2/3 of the vat was siphoned through the side opening to be used as the wine.

Aliquots were removed 40 minutes after the second feed and at the end of fermentation to analyse Cell Viability, Bud Viability and the Budding Index, using the methodology described by Lee, Robinson and Wong (1981).

### Characterisation of the Wine

The wines were characterised for Brix, pH, TA, Glycerol (CENTRO DE TECNOLOGIA CANAVIEIRA, 2009) and Alcohol Content.

To determine this last parameter, the wines were distilled in a Tecnal TE-012 alcohol micro-distiller, adding 60 mL of wine and recovering 20 mL of distillate, in which the ethanol was quantified by automated density meter (Anton Paar DMA-48).

### Distillation of the Wine and Characterisation of the Cachaça

The wines were distilled in a simple still with a copper boiler, dome and neck. The still was equipped with

a thermometer and a gas heating system, which gives a more standardised distillation process from the point of view of heating speed, temperature and vapour pressure. The distillate was divided into three fractions, 10% head, 80% heart and 10% tail.

The ethyl carbamate in the distillate was analysed as per the methodology proposed by Anjos *et al.* (2011). The equipment used was a Shimadzu SPD-M20A high performance liquid chromatograph equipped with two high-pressure pumps, a DGU-20A3 degasser, CBM-20 interface and SIL-10AF automatic injector. In addition, the °GL, congener coefficient, acrolein and methanol were analysed by gas chromatography using the GC 3900 system.

### Experimental Design and Data Analysis

The experimental design was of subdivided plots, with nine replications. The main treatments consisted of two musts (clarified and non-clarified), and the secondary treatments of two yeasts (Fleischmann and CA-11). The results were submitted to analysis of variance by F-test with the mean values compared by Tukey's test (5%), using the ASSISTTA v7.7 beta software (SILVA; AZEVEDO, 2016).

## RESULT AND DISCUSSION

### Characterisation of the juice and musts

The first step was to characterise the raw material used in the trial. It was found that the juice obtained from the SP83-2847 variety had a high concentration of sugars, with a Brix of 20.8% and TRS of 16.45%. The pH was determined to be 5.0, with a TA of 1.28 g/L H<sub>2</sub>SO<sub>4</sub> and TPC of 363 mg/L. Ripoli and Ripoli (2009), explain that sugarcane at the maturation stage shows a TRS greater than 14% and a pH greater than 5.0. As such, it was inferred that the raw material was suitable for harvesting and processing.

The must was then prepared from the juice. The results for Brix, TRS, pH, TA, TPC and Nitrogen are shown in Table 1.

It is assumed that must is any solution that presents an ideal concentration of sugars, acidity, pH, temperature and nutrients for the proper metabolic development of the yeast during the fermentation process (LIMA; BASSO; AMORIM, 2001). It should also be noted that industrial fermentation requires a substrate containing 14 to 22% sugars, a pH of 4.5, Nitrogen between 60-70 mg/L (STEINDL, 2010) and TPC less than 450 mg/L (RAVANELI *et al.*, 2011).

**Table 1** - Mean values for Brix, Total Reducing Sugars (TRS), pH, Total Acidity (TA), Total Phenolic Compounds (TPC) and Nitrogen in the musts obtained from original and clarified juice

Must	Brix (°)	TRS (%)	pH	TA (g/L H <sub>2</sub> SO <sub>4</sub> )	TPC (mg/L)	Nitrogen (%)
Non-Clarified	16.0	12.59	4.5 B	1.59 A	363 A	21.46 A
Clarified	16.3	12.83	6.0 A	1.00 B	251 B	20.88 B
F-test	2.94 <sup>ns</sup>	0.32 <sup>ns</sup>	475.37**	5.64*	29.49 **	7.53**
MSD	0.41	0.89	0.14 <sup>ns</sup>	0.52	43.78	2.98
CV	2.55	7.07	2.68	40.43	14.25	11.26

Mean values followed by similar letters do not differ by Tukey's test at 0.05 probability; ns - Not significant; \*Significant at a level of 0.05; \*\*Significant at a level of 0.01. MSD - Minimum Significant Difference. CV - Coefficient of Variation

It was found that the process of clarifying the sugarcane juice resulted in a must with better technological characteristics for the fermentation process, since significant amounts of TA and TPC were removed. These results are similar to those obtained by Costa *et al.* (2014), who, evaluating the composition of clarified juice, determined similar behaviour to that seen in this study. However, Teixeira *et al.* (2019), when conducting the same procedure, did not obtain any significant reduction in TA. It should also be noted that although there was a reduction in the nitrogen content of the must, the values were still within the specification range proposed by Steindl (2010).

### Fermentation Process

Following inoculation of the musts with the CA-11 and Fleischmann yeasts, Cell Viability, Bud Viability and the Budding Index were determined in the yeasts at the start and end of the fermentation process (Table 2). There were no significant differences in budding rate between the treatments under study, with this parameter varying from 8-13% at the start, and from 20-24% at the end of the fermentation process. Such yeast behaviour should be emphasised, since yeast budding during fermentation cycles is essential to maintain high yields during the harvest, and always results in new cells that can promote high ethanol production (LIMA; BASSO; AMORIM, 2001; MONTIJO *et al.*, 2014).

**Table 2** - Mean values for Cell Viability, Bud Viability and Budding Index in Fleischmann and CA-11 yeasts, at the start and end of the fermentation of musts obtained from original and clarified juice

Must (M)	Budding (%)		Bud Viability (%)		Cell Viability (%)	
	Start	End	Start	End	Start	End
Clarified	8.16	20.83	91.62	93.98 A	86.21	80.87
Non-Clarified	13.08	24.85	91.42	88.38 B	84.78	80.18
F-test (M)	1.51 <sup>ns</sup>	0.93 <sup>ns</sup>	0.01 <sup>ns</sup>	3.51**	0.14 <sup>ns</sup>	0.02 <sup>ns</sup>
MSD	8.16	8.46	12.32	6.09	7.78	9.17
CV	113.06	54.54	19.82	9.84	13.40	16.77
Yeast (Y)	Budding (%)		Bud Viability (%)		Cell Viability (%)	
Fleischmann	9.58	22.04	89.69	84.54 B	83.12	79.68
CA-11	11.66	23.64	93.35	97.82 A	87.87	81.37
F-test (Y)	0.26 <sup>ns</sup>	0.14 <sup>ns</sup>	0.36 <sup>ns</sup>	19.74**	1.54 <sup>ns</sup>	0.14 <sup>ns</sup>
MSD	8.16	8.46	12.32	6.09	7.78	9.17
CV	113.06	54.54	19.82	9.84	13.40	16.77
M x Y	0.36 <sup>ns</sup>	0.01 <sup>ns</sup>	4.97 <sup>ns</sup>	4.16*	1.05 <sup>ns</sup>	4.77 <sup>ns</sup>

Mean values followed by similar letters do not differ by Tukey's test at 0.05 probability; ns - Not significant; \*Significant at a level of 0.05; \*\*Significant at a level of 0.01. MSD - Minimum Significant Difference. CV - Coefficient of Variation. M x Y - Interaction between musts and yeasts

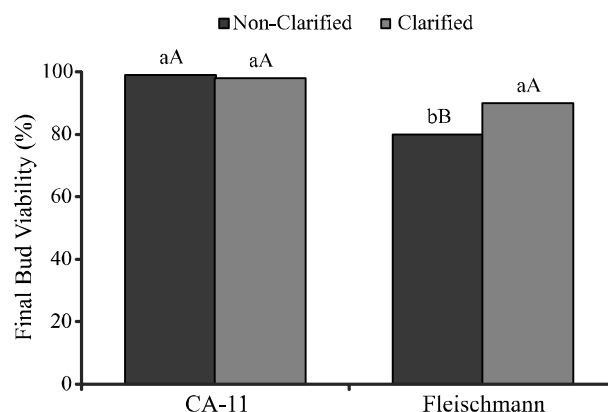
Based on the two musts used, it was also found that there were no significant differences between the studied treatments for yeast cell viability at the start and end of the fermentation process.

When evaluating the number of live buds, it was seen that at the start of the process, fermentation of the different musts did not result in any significant differences between the yeasts. However, during fermentation, bud viability decreased considerably when using baker's yeast with non-clarified juice, a fact that resulted in a reduction of 14% in this parameter (Figure 1). The highest concentration of TA in this raw material probably reflected negatively on the yeast, since acids are inhibitors of fermenting yeasts (OLIVEIRA FILHO; BORTOLETTO; ALCRSDE, 2016). This effect may have been stronger at the highest levels of TPC, which also inhibit the yeast during the fermentation process (RAVANELI *et al.*, 2011). However, it should be noted that the CA-11 selected yeast was not affected by the adverse conditions resulting from the lower quality must.

### Characterisation of the Wine

The results for TA, pH, Brix, TRRS, Alcohol Content and Glycerol of the wines are shown in Table 3. The fermentation of must obtained from the clarified juice resulted in lower values for TA and a higher pH. This difference is probably due to the must itself prior to

**Figure 1** - Breakdown of the interaction between musts (clarified and non-clarified) and yeasts (CA-11 and Fleischmann) for Bud Viability at the end of fermentation. Uppercase letters compare musts and lowercase letters compare yeasts.



inoculation by the yeasts, since at the start the non-clarified substrate had 0.59 g/L more H<sub>2</sub>SO<sub>4</sub> than did the clarified substrate. It can also be seen that the substrates had no effect on the Brix, TRRS, Alcohol Content or Glycerol of the wine.

Comparing the quality of the wine obtained by fermentation between yeast strains, it was determined that CA-11 results in better quality material than does

**Table 3** - Mean values for Total Acidity (TA), pH, Brix, Total Residual Reducing Sugars (TRRS), Alcohol Content, Glycerol and Fermentation Efficiency (EF), of wines originating from the fermentation of musts obtained from original and clarified juice, by Fleischmann and CA-11 yeasts

	AT (g/L H <sub>2</sub> SO <sub>4</sub> )	pH	Brix (°)	TRRS (%)	Alcohol Content (% v/v)	Glycerol (%)
<b>Must (M)</b>						
Clarified	2.63 B	3.7 A	0.7	0.11	7.09	0.59
Non-Clarified	3.00 A	3.5 B	0.7	0.07	6.61	0.74
F-test (M)	4.80*	5.03*	0.01 <sup>ns</sup>	4.19 <sup>ns</sup>	1.57 <sup>ns</sup>	3.30 <sup>ns</sup>
MSD	0.34	0.15	0.38	0.04	0.81	0.17
CV	17.84	6.18	74.66	65.66	16.84	38.66
<b>Yeast (Y)</b>						
Fleischmann	3.08 A	3.5 B	0.7	0.06	6.58 B	0.79 A
CA-11	2.54 B	3.7 A	0.8	0.11	7.13 A	0.54 B
F-test (Y)	10.27**	5.72*	0.25 <sup>ns</sup>	3.03 <sup>ns</sup>	5.14*	8.11**
MSD	0.34	0.15	0.38	0.05	0.51	1.17
CV	17.84	6.18	74.66	88.52	10.55	38.66
M x Y	0.01 <sup>ns</sup>	0.85 <sup>ns</sup>	0.25 <sup>ns</sup>	0.86 <sup>ns</sup>	0.20 <sup>ns</sup>	1.66 <sup>ns</sup>

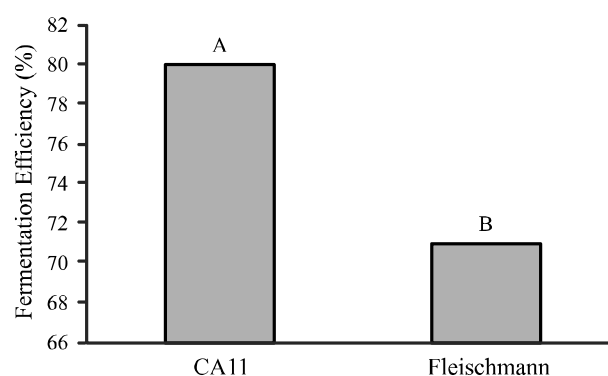
Mean values followed by similar letters do not differ by Tukey's test at 0.05 probability; ns - Not significant; \*Significant at a level of 0.05; \*\*Significant at a level of 0.01. MSD - Minimum Significant Difference. CV - Coefficient of Variation. M x Y - Interaction between musts and yeasts

Fleischmann, since it had lower values for TA and Glycerol, and a higher alcohol content. It is important to note that the alcoholic fermentation process of the yeast *Saccharomyces cerevisiae* consists of the metabolism of carbohydrates and inorganic compounds, which are then converted into TAP, new cells, acids, glycerol, ethanol and other metabolites.

It can be seen that the more the yeast produces acids and glycerol, the smaller the amount of ethanol it generates. It can also be said that glycerol is always produced by yeast, however under adverse conditions, such as a higher concentration of acids and salts in the substrate, the microorganism diverts the metabolic pathway for producing this biomolecule, as it regulates the metabolism, adapting the yeast to the substrate (TEIXEIRA *et al.*, 2019). Thus, CA-11 yeast can be considered as affording greater fermentation efficiency, since a minimally adverse substrate has no effect on its metabolism (Figure 2).

Montijo *et al.* (2014) and Teixeira *et al.* (2019), evaluating the composition of wine obtained by fermentation with CA-11 yeast, obtained a pH of 3.4 and 3.7, TA of 2.6 and 3.53 g/L H<sub>2</sub>SO<sub>4</sub>, Glycerol of 0.59 and 0.94% and Alcohol Content of 6.5 and 8% respectively.

**Figure 2** - Fermentation efficiency of CA-11 and Fleischmann yeasts. Uppercase letters compare yeasts



### Composition of the Cachaça

The wines obtained from the nine replications were blended and distilled in a copper still to give the various cachaças, which were then characterised. The results are shown in Table 4.

**Table 4** - Composition of the cachaças resulting from the fermentation of musts obtained from original and clarified juice by Fleischmann and CA-11 yeasts

Components Analysed	Legislation		Fleischman		CA-11	
	Min.	Max.	Clarified	Non-Clarified	Clarified	Non-Clarified
Alcoholic Content <sup>1</sup>	38	48	42.32	39.77	40.30	42.11
Total Aldehydes <sup>2</sup>		30	14.44	25.77	14.11	43.50
Total Esters <sup>2</sup>		200	12.47	16.19	9.15	20.23
Methanol <sup>2</sup>		20	1.00	3.49	0.97	1.86
Acrolein <sup>2</sup>		5	n.d.	n.d.	n.d.	n.d.
Ethyl Carbamate <sup>3</sup>		210	9.01	29.39	25.67	25.47
Volatile Acidity <sup>2</sup>		150	34.0	36.20	44.7	34.2
Congener coefficient	200	650	346.28	410.82	278.85	364.97
Electrical Conductivity <sup>4</sup>			15.0	17.00	20.0	17.0
Turbidity <sup>5</sup>			0.48	0.33	0.60	0.26
pH			4.8	4.9	4.8	4.7
<b>Higher alcohols<sup>2</sup></b>						
Sec-Butyl Alcohol		10	n.d.	3.87	0.76	0.87
N-propanol			40.50	50.38	72.80	57.65
Isobutanol			72.44	79.18	42.49	55.39
n-butanol		3	2.92	1.54	2.11	3.01
Isoamyls			194.65	229.75	125.36	141.88
Sum of Higher Alcohols		360	307.60	359.31	240.64	254.91

<sup>1</sup>GL V/V; <sup>2</sup>mg/100mL anhydrous alcohol; <sup>3</sup>µg/L; <sup>4</sup>µs at 2 °C; <sup>5</sup>NTU; n.d. - not detected

When comparing the beverages originating from treating the juice, it was found that clarifying the extracted juice resulted in a distillate with better chemical and technological characteristics, since for most of the parameters under evaluation the values were lower compared to the non-clarified juice; e.g. methanol, with half the value of the conventional treatment. It should be noted that methanol is formed from the hydrolysis of pectin, found in residue resulting from the extraction process during fermentation (ZACRSONI *et al.*, 2011). As such, treating the juice caused a marked removal of these products. High levels of methanol can affect the respiratory system, and cause blindness and death (CRSDOSO, 2013).

Another important point is the significant reduction in ethyl carbamate when using juice treated and fermented with Fleischmann yeast. Various authors report the high presence of this contaminant in sugarcane spirits. Masson *et al.* (2014), evaluating samples of sugarcane spirit produced in the state of Minas Gerais, obtained values ranging from 23 µg/L to 930 µg/L. The removal of nitrogen by the clarification process probably hindered greater production of this biomolecule, since the element is considered a precursor to the formation of carbamate in cachaça (GALINRSO; FRANCO, 2011).

It was found that cachaça derived from clarified juice had a lower concentration of higher alcohols. Teixeira *et al.* (2019), when evaluating a similar process, saw comparable behaviour regarding higher alcohols. High levels of these alcohols are the result of the conditions under which the fermentation process takes place, since excess sludge during alcoholic fermentation causes an increase of up to 50% in the higher-alcohol content (SORSES; SILVA; SCHWAN, 2011). It can therefore be inferred that the sludge found in the fermentation vats is, in most cases, related to earth and residue present in the must, and that treating the juice contributes positively to the removal of these elements.

When comparing the effect of yeasts on beverage quality, it was found that the use of CA-11 selected yeast promoted better quality cachaça compared to baker's yeast. In this sense, the congener coefficient and higher-alcohol content should be highlighted. The formation of higher alcohols is greater when the biological activity of the yeast is weak (MOREIRA; NETTO; MRSIA, 2012) causing a delay in the fermentation process, a fact that may be associated with the baker's yeast being used in a production system for which was not designed.

## CONCLUSIONS

1. Compounds that interfere in alcoholic fermentation, such as total acids and phenolic compounds, are removed by the juice clarification process;
2. The CA-11 yeast is robust enough for the fermentation of clarified and non-clarified juice, with a greater number of live buds, lower levels of secondary metabolites and, consequently, a higher alcohol content in the wine by the end of the process, compared to the Fleischmann yeast.
3. The cachaça produced from juice previously clarified and fermented with CA-11 yeast shows better quality distillates whose chemical composition is within the limits established by Brazilian legislation.

## ACKNOWLEDGEMENTS

The authors would like to thank the Programa de Microbiologia Agropecuária, and the teams at the Laboratório de Açúcar e Álcool and the Laboratório de Microbiologia das Fermentações of FCAV/UNESP Jaboticabal in São Paulo. Thanks also go to the Frutal Unit of the Universidade do Estado de Minas Gerais.

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