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Physico-Chemical and Sensorial Evaluation of Sugarcane Spirits Produced Using Distillation Residue

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ABSTRACT

The objective of the present study was to analyze the use of vinasse from cachaça as an ingredient of the fermentation medium for the spirit production. The fermentations were conducted out in three successive batches using a Saccharomyces cerevisiae *isolate. In the first batch, the sugarcane broth was only diluted with distilled water. In the second and third batches, the fermentations were carried out using the cane broth diluted with vinasse from the distillation of the sugarcane wines of the first and second batches, respectively at a concentration of 10% (v/v). The spirits were submitted to the physicochemical and sensorial analyses. The results showed that vinasse addition did not affect the fermentation, distillation and chemical-sensorial quality of the beverage. Therefore, the vinasse addition could be an alternative use for that residue.*

Key words: vinasse, ethanol, sugarcane spirits, physico-chemical composition, sensory analysis, *Saccharomyces cerevisiae*

INTRODUCTION

Brazil produces about 1.3 billion liters of sugarcane spirits, being the second most consumed alcoholic beverage in Brazil and the third in the world. It is produced by more than 30,000 manufacturers throughout the country (Campos et al. 2010). By Brazilian legislation, "cachaça" is the typical and exclusive denomination for the cane liquor produced in Brazil, with alcohol content from 38 to 48% in volume at 20ºC obtained by the distillation of the fermented must of the sugar cane broth (Brasil 2005).

In the production of sugarcane spirits, residues are generated, such as cane bagasse, ferment and vinasse; among these, the last one poses serious environmental damages. Vinasse is the main residue generated during the distillation process of ethanol and sugarcane spirits production. The production of sugarcane spirits and bioethanol generates from 4-14 L of residue per L of product (Cardoso 2006; Shojaosadati et al. 1999). This distillery efluent is characterized by its high organic matter content, of a dark brown color, acidic nature (low pH), and high concentration of solids (Satyawali and Balakrishnan 2008). Vinasse is constituted mainly of water, organic matter and potassium. Other elements such as N,P,Ca, Mg, S, Na and Cl are present; however, in lower amounts. Among the organic compounds, the most important are the organic acids and carbohydrates (Doelsch et al. 2009). The chemical composition varies considerably and is determined by the raw material used, the alcoholic fermentation method and system, the yeast and also the apparatus used in the distillation (Gloria and Orlando Filho 1983). This pollutant, with high concentrations of soluble material and non-volatile fermentation by-

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products, including high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) values (Navarro et al. 2000; Silva et al. 2011), has long been discharged into the environment and, therefore, the study of methods for its suitable disposal is of great importance.

One form of use for the residue is called fertigation, in other words, its application in soil fertilization, especially for sugarcane crops. This is the most used method in Brazil, but it presents limitations because of contamination to underground waters (Goldemberg et al. 2008). This work aimed to study an alternative use for the vinasse.

MATERIAL AND METHODS

Microorganism and its propagation

The yeast *Saccharomyces cerevisiae* (UFLA Sc15) was provided by the Microorganism Physiology and Molecular Genetics Laboratory, Biology Department, Federal University of Lavras. The stock culture was stored in YEPG broth with glycerol and later frozen.

The culture was propagated in cane broth and yeast extract medium, following the methodology based on Silva et al. (2009) and Marini et al. (2009). The cells count values were >10^8 viable cells /mL.

Fermentation

The fermentations were conducted in 20-liter stainless steel vats. The first batch was carried out with cane broth diluted to 15º Brix using the distilled water. The total must volume prepared was 11 liters. This was sterilized in an autoclave at 121ºC/25min and after cooling, 10% inoculum (v/v) was added and incubated at 30ºC for 48 h. After the stabilization of the total soluble solids content, the separation of yeasts was carried out through the centrifugation and later the sugarcane wine was distilled. The resulting vinasse was mixed with the cane broth and water to a concentration of 10% (v/v) to carry out the second batch. The same was done in the third batch using the vinasse generated from the distillation of the second batch.

Distillation

The volume of distillate consisted of 15% of the clarified fermented broth volume fed in the still. Three fractions were collected during the distillation, being 10% of the distilled volume for the first fraction, 80% for the second and 10% for third fractions, according to the methodology used for producing the sugarcane spirits (Pereira et al. 2003; Oliveira et al. 2005; Cardoso 2006; Silva et al. 2009). The compounds more volatile and dangerous, such as methanol and acetaldehyde, distilled first (head fraction) and the less volatile, such as higher alcohols, distilled later (tail fraction). The beverage cachaça was the intermediate fraction (heart fraction), which was rich in ethanol and contained lower quantities of the secondary compounds that distilled together with the water–ethanol mixture, providing flavor and color (Rosa et al. 2009). The samples were later stored for a rest period of three months.

Origin and analysis of vinasse

The vinasses used in the experiments were generated from the distillation of the sugarcane wines and were analyzed for the pH, carbon, nitrogen, phosphorus, potassium, calcium and magnesium concentrations according to Orlando Filho (1983) and sulfate according to the methodology proposed by Silva (1999).

Analysis of total soluble solids and ethanol

Total soluble solids contents were determined by the refractometry using a portable Instrutherm RT-30ACT refractometer. Those analyses were carried out at 0, 4, 8, 20, 24, 28, 32, 44 and 48 h and the results expressed in ºBRIX. At the end of fermentation, the ethanol content in the wine was determined in the three batches in a Universal 3750 Ebulliometer. The results were expressed in % (v/v).

Physico-chemical analyses of the sugarcane spirits

The analyses of relative density, copper, alcohol level, volatile acidity in acetic acid, higher alcohols, aldehydes in acetic aldehyde, esters in ethyl acetate and methyl alcohol were conducted according to the Analysis Methods for fermented, distilled, non-alcoholic beverages and vinegar established by the Normative Instruction No. 2.314 of September 4, 1997, of the Ministry of Agriculture, Livestock and Provisioning (Brasil 1997).

Sensorial analysis
The sensorial analysis in relation to the flavor, appearance, aroma and global aspect of the produced sugarcane spirits was done using an acceptance test. Forty non-trained judges evaluated the samples in individual booths. The results were analyzed through the variance analysis (ANOVA) and the Tukey test by Statistica v.8.

RESULTS AND DISCUSSION

Analysis of vinasse
The results of the vinasse analyses are shown in Table 1. Second batch vinasse was generated from the residue of the first distillation and in turn the third batch vinasse from the second distillation.

Table 1 showed that the compositions of the two vinasses were similar, having a variation only in the organic matter level. Cortez et al. (1998) analyzed various samples of vinasse obtained from the cane broth fermentation, which presented pH values between 3.7 and 4.6, total nitrogen between 0.150 and 0.700, P_2O_5 between 0.010 and 0.210, K_2O between 1.20 and 2.10, CaO between 0.13 and 1.54, MgO from 0.20 to 0.490, SO_4 from 0.60 to 0.76, organic matter around 19.5 (all the values in Kg/m³). Comparing with the results obtained in this study, it could be noticed that various components presented similar concentrations and some, such as organic matter, presented different values that could be explained by the different process conditions and cane varieties, among other aspects.

Table 1 - Results of chemical analysis of the vinasse.

Components (Kg/m³)	2^{nd}. Batch	3^{rd}. Batch
N	0.2	0.2
P_2O_5	0.2	0.2
K_2O	0.9	0.9
CaO	0.31	0.31
MgO	0.29	0.29
SO_4	0.33	0.33
Organic Matter	47.56	53.33
pH	3.4	3.4

Fermentation
Results on the total soluble solid contents during the fermentation showed similarity in three batches, with the total soluble solid contents decreasing from 13 to 15 to approximately 4.5° Brix (Fig.1).

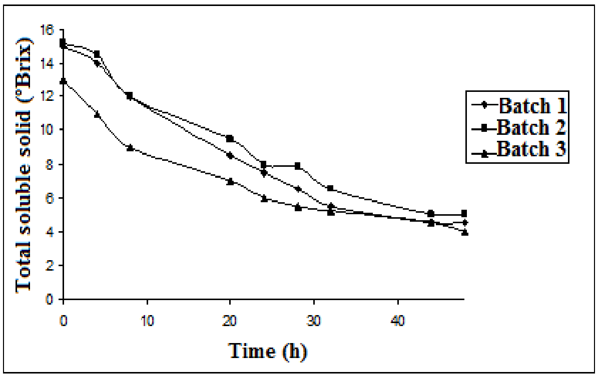

Figure 1 - Total Soluble Solids (° Brix) content during fermentation of sugarcane broth diluted with vinasse.

Carvalho et al. (2008) conducted fermentations using sugarcane broth at 15 °Brix, only diluted with water, and a commercially bakery yeast (*S. cerevisiae)* and obtained a final total soluble solids content of value 4.0 °Brix. Soares et al. (2011) studied several strains of *Sacchomyces cerevisiae* (UFLA CA116, UFLA CA1162, BG-1 and CAT1) in the fermentation of sugarcane broth diluted to16 º Brix. At the end of the fermentation, the total soluble solids content was on average 8.0 °Brix, which was higher than the results obtained in this work. The dilution of sugarcane juice is performed, since the high sugar concentration causes cellular stress in the beginning of the fermentation (Schwan et al. 2001). The average alcohol level in the sugarcane wine was 7.4°G.L. at the end of the fermentation (Table 2).

Fermented sugarcane broths for cachaça production have usually from 5 to 9% (v/v) ethanol (Lima 2001). Carvalho et al. (2008) observed a final alcoholic content of 6% (v/v) using the fermentation medium at 15°Brix and a bakery yeast (*S. cerevisiae*). The ethanol concentration during the fermentation increased gradually and reached approximately 8% (v/v) in cachaça production (Morais et al.1997)

Table 2 - Alcohol content (%v/v) in the three sugarcane wines.

Alcohol content (%v/v)	Batch 1	Batch 2	Batch 3
Initial	0	0	0
Final	7.6± 0.1	7.2± 0.1	7.2± 0.1

Physico-chemical analyses of sugarcane spirits

The results obtained for physicochemical analysis of sugarcane spirits are presented in Table 3. The components of the sugarcane spirits were within the limits stipulated by the Brazilian legislation (Brasil 2005), except for the superior alcohols for the three sugarcane spirits and the alcohol level for the spirit produced in the first distillation.

In relation to the higher alcohols levels, the vinasse addition did not affect its concentration, because the first batch spirit was produced without vinasse addition and presented the highest value. The other two batches spirits contained higher alcohols above the maximum limit established by the Brazilian legislation, although the values found surpassed the limit by at most 15%. Gomes et al. (2007), testing the indigenous yeast stains obtained a concentration of higher alcohols passing the maximum limits laid by the law (360 mg 100 ml^{-1} anhydrous alcohol) in almost all the samples analyzed at the beginning of the experiment, without the reuse of the yeast sediment. These spirit samples were collected after the distillation process, seven days after the beginning of each experiment. Sugarcane spirits produced with the reuse of the yeast sediment exhibited higher alcohol levels according to the Brazilian legislation (Brasil 2005).

The high concentration of superior alcohols could be related to the care taken in the cutting of the cane, as well as to the waiting time for the grinding and fermentation. The cane broth used was bought from a producer in the municipal district of Lavras-MG.

A cane storage period above 48 h, before grinding, could also be responsible for the elevation of the higher alcohol levels. The tip of the cane also should not be used, because it possessed a high level of amino acids that would be degraded to higher alcohols (Cardoso 2006). The production of higher alcohols also seemed to be a characteristic of the yeasts in general, and the amounts produced varied with the fermentation conditions and also with the genus, species and, probably, with the strain used (Giudici et al. 1990). There was no methanol in the produced spirits. It could be explained by the separation of the head fraction, containing the more volatile compounds during the distillation. Methanol level in the cachaça also concerns distillers because of severe intoxication consequences related to its ingestion (Lamiable et al. 2004).

Table 3 - Physico -chemical analyses of sugarcane spirits.

Items analyzed	Batch 1	Batch 2	Batch 3	Minimum	Maximum
Copper (mg/L)	0.048	0.17	0.08	- x -	5.0
Alcohol level 20°C (% V/V)	32.44	38.29	39.38	38.0	54.0
Volatile acidity in acetic acid *	70.05	100.89	71.17	- x -	150.0
Superior Alcohols *	408.15	392.13	395.42	- x -	360.0
Aldehydes in acetic aldehyde *	12.13	13.51	- x -	- x -	30.0
Esters in ethyl acetate *	35.65	33.66	29.34	- x -	200.0
Methyl alcohol *	0.00	0.00	0.00	- x -	20.0

* in mg/100mL anhydrous alcohol

Sensory Analysis

The results of the sensory analysis are presented in Table 4. Evidently there was no significant difference among the samples regarding the appearance, aroma and flavour. However, regarding the overall impression, there was a statistically significant difference between the samples 2 and 3. Comparing the first batch cachaça (without vinasse addition) with the second batch and third batch cachaça (with vinasse addition) showed that overall impression scores were not statistically different between each pair. Thus, the vinasse addition did not influence the sensory acceptance of the produced beverages.

Silva et al. (2009), using different the strains of selected yeasts, produced four spirits, which were sensorially evaluated with results varying from 4.90 to 6.48 for aroma, 4.9 to 6.13 for flavour and 5 to 6.16 for the overall impression. Oliveira et al. (2005), using different *S. cerevisiae* isolates, produced various sugarcane spirits and obtained values for the samples varying between 6.27 and 6.93 for aroma, 5.78 to 6.56 for flavor and 6.10 to 6.59 for overall impression. Comparing these results with those introduced in Table 4, it could be concluded that the scores were very similar and only the attribute flavour was lower than the values found by the authors that could be associated to the high level of higher alcohols in the produced beverages.

Table 4 - Mean scores given to the three cachaças produced after the acceptance tests for the attributes appearance, aroma, flavour and overall impression.

Treatments	Appearance	Aroma	Flavor	Overall Aspect
Batch 1	7.76^{a}	6.76^{a}	5.26^{a}	6.6^{ab}
Batch 2	7.76^{a}	6.46^{a}	5^{a}	6.1^{a}
Batch 3	7.73^{a}	6.8^{a}	4.53^{a}	7^{b}

* Means followed by same letter in same column are significantly similar by the Tukey test ($p \leq 0.05$).

CONCLUSION

The results showed that the vinasse additions did not affect the fermentation, distillation and physicochemical and sensorial quality. Therefore, the addition of vinasse 10% (v/v) to the sugarcane broth could be an alternative use for this residue.

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