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ORIGINAL ARTICLE

Assessment of chemical and sensory quality of sugarcane alcoholic fermented beverage

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Abstract This study aimed to verify the technological feasibility, chemical quality and sensory acceptance of alcoholic fermented beverage obtained from sugarcane juice. A completely randomized design was applied. Sugar and alcohol content, phenolic (HPLC–MS) and volatile (GS–MS) compounds, pH, density, dry matter and acidity of the fermented beverage of sugarcane were quantified, as well as the acceptance of the product was carried out. The complete fermentation of sugarcane lasted 7 days, and it was obtained an alcohol content of 8.0% v/v. Titrable acidity of the beverage was of 67.31 meq L^{-1}, pH 4.03, soluble solids of 5 °Brix, reducing sugar of 0.07 g glucose 100 g^{-1}, density of 0.991 g cm^{-3}, reduced dry matter of 14.15 g L^{-1}, sulfates lower than 0.7 g K_2SO_4 L^{-1}. Various phenolic compounds, among which, gallic acid (10.97%), catechin (1.73%), chlorogenic acid (3.52%), caffeic acid (1.49%), vanillic acid (0.28%), *p*-coumaric acid (0.24%), ferulic acid (6.63%), *m*-coumaric acid (0.36%), and *o*-coumaric acid (0.04%). Amongst aromatic compounds, were found mainly esters with fruity aromas (ethyl ester hexanoic acid and ethyl ester octanoic acid). The sugarcane juice can be commercialized as an alternative wine, as it presented adequate features to an alcoholic fermented beverage and was sensory accepted by consumers.

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Introduction

Palatable wines can be made from many fruits. Wine can be fermented with yeast, which occurs naturally in grapes. In countries where grapes are not produced, other fruits are used for wine making. Apples and citrus fruits with sufficient fermentable sugars are crushed, and the fermentable juices are either pressed out for fermentation or the entire mass is fermented. Some soft fruits from both temperate and tropical regions can be used to produce wines, whose pigment stabilities and flavor profiles match those of many grape wines, but suffer from the lack of intensive research and development given to grape wines.

Sugarcane, which is grown on approximately 24 million hectares in 102 countries in tropical and subtropical zones of both Northern and Southern hemisphere countries (AFRIS (Animal Feed Resources Information System of FAO) 2015), has long been an important product of the Brazilian economy. Sugarcane provides sugar and alcohol and its cultivation originates from Southeastern Asia (Farah 2013). Brazil is the biggest worldwide producer of sugarcane, and is also the biggest exporter of sugar, according to the United States Department of Agriculture (2017). Brazilian sugarcane production is projected to be 645 million metric tons for the 2017/2018 marketing year. Sugarcane juice was chosen as the substrate for wine making in this study because of its abundant supply and consumption in Brazil.

Sugarcane juice is a common indigenous drink, economical, and widely consumed in countries such as India (Kalpana et al. 2013) and Brazil. It is rich in carbohydrates

and several electrolytes. This juice showed to be effective as a sports and rehydration drink during exercise (Kalpana et al. 2013). The main component of sugarcane juice is sucrose, which is a highly suitable carbon source for microbial growth and, thus, can be directly used as a fermentation medium (Nualsria et al. 2016).

Sugarcane juice is obtained by pressing the fibrous stems of the grass which are rich in sugar (James and Ngarmsak 2010), and appeals to many people, mainly due to its peculiar taste. In addition, it is an excellent medium for fermentation in the development of alcoholic beverages (Kulkarni et al. 2011), especially distilled liquors such as cachaça and rum. However, there are few reports on the fermentation of sugarcane juice for preparation of fermented beverages similar to wine, as well as on the chemical and sensory quality of such products.

The challenge in the production of wine, or similar fermented beverages, is to obtain a high-quality product, although the processes involved in its production are relatively simple (Ough et al. 1988). The wine fermentation process is a combination of complex interactions involving a variety of materials, yeast, and specific techniques (Tzeng et al. 2010).

Aromas and fruity flavors of wine or other alcoholic beverages are derived mainly from the raw materials used in the fermentation process, although some aromas are also produced during it. In addition, they greatly contribute to the sensory quality of the wine. Most of the volatile compounds from grapes are also known to be constituents of many other fruits (Feng et al. 2015). However, little is known of the volatile compounds of fermented alcoholic beverages made from sugarcane juice.

It has been recently demonstrated that moderate consumption of wine is associated with a lower prevalence of metabolic syndrome in an elderly population with high cardiovascular risk (Tresserra-Rimbau et al. 2015) because of its high content of antioxidants and phenolic compounds.

Studies of aromatic compounds in fermented sugarcane alcoholic beverages are scarce in the literature and, to the best of our knowledge, there are no reports concerning the phenolic profile of this type of beverage. In this context, the aim of this study was to assess the technological feasibility, the chemical quality, and the sensory palatability of an alcoholic fermented beverage made from sugarcane juice.

Materials and methods

Raw materials

The stalks of sugarcane variety RB 867515 were collected in the experimental area of the Agronomy School of the Federal University of Goiás, located in Goiânia, GO, Brazil. Immediately after harvest, they were transported to the laboratory, washed and crushed in a Wiley mill (Botini, Low electric B120, São Paulo, Brazil). The sugarcane juice obtained was filtered to remove suspended solids. Secondary ingredients used were sucrose (Cristal, Goiânia, Goiás), dry selected yeast (*Saccharomyces cerevisiae*) (Blastosel Delta, Perdomini, Italy) and potassium metabisulfite (Pall Filtration and Separations S.p.a., San Martino Buon Albergo, Italy).

Physico-chemical characteristics of sugarcane juice

Moisture, ash, protein, lipids, total dietary fiber, carbohydrates, energetic value, sulfuric acidity, pH and soluble solids were determined according to the methods of the Association of Analytical Chemists (AOAC 2016). Samples were analyzed in triplicates.

Fermentation conditions

The fermentation was carried out in three original repetitions. The total soluble solids content of the sugarcane juice was standardized to 20 °Brix and the pH to 5.5. Potassium metabisulfite (0.1 g L^{-1}) was added to the broth 2 h before inoculation. The inoculum was activated by solubilization of 10 g of yeast in 100 mL of water (40 °C), kept at rest for 10 min and then manually stirred. This procedure was repeated twice more, and then the yeast was added to the wort at a concentration of 10 g L^{-1}, as instructed by the manufacturer. The fermentation step, following inoculation, was performed in a glass container (1 L), fitted with a hydraulic bung outlet for carbon dioxide. The vessel was kept under controlled temperature (28 °C) in an incubator (Tecnal, TE-421, Piracicaba, Brazil), until the soluble solid content reached 5 °Brix, or became constant. After the fermentation, the medium was filtered over a polypropylene filter (5–15 μm) and then bottled in glass bottles, previously cleaned and sterilized, which were kept in refrigerated chambers under controlled temperature of 12 °C. Daily racking was held until it was no longer observed decanted waste at the bottom of the bottles.

Monitoring of fermentation parameters

During the fermentation process, which lasted 7 days, the soluble solids content and pH were analyzed according to the methods proposed by AOAC (2016), and measured the temperature.

Physico-chemical analyses of the beverage

Reducing and non-reducing sugars, titrable and volatile acidity, alcoholic strength, density, total and reduced dry

extract, ash, ratio alcohol/reduced dry extract, sulfates, chlorides and proteins were analyzed. All parameters were determined according to the methods proposed by AOAC (2016).

Total phenolic compounds

Total phenolic compounds (TPC) were determined according to the modification proposed by Kiralp and Toppare (2006), of the colorimetric method described by Singleton and Rossi.

Individual phenolic compounds by high performance liquid chromatography

The chromatographic analysis of phenolic compounds were performed as described by Peña-Neira et al. (2000), with modifications. Samples (20 µL) were subjected to separation by reverse phase chromatography at 20 °C (Shimadzu, GCMA-QP2010 Plus, Tokyo, Japan), using a Nova-Pak C_{18} column. DAD (photodiode array detector) was established from 210 to 360 nm. A gradient was adapted to a flow of 1 mL min^{-1} for 0–55 min and 1.2 mL min^{-1} for 55–90 min as follows: 100–20% A from 0 to 55 min, 20–10% A from 55 to 57 min and 10–0% A from 57 to 90 min. The identification of compounds was performed by comparison of their spectras (210–360 nm) and retention times with standards. Quantitative determination was performed using the method of external standards with commercial standards. Calibration curves were obtained by injection of standard solutions under the same conditions of the analyzed compounds. Standards (Supelco, Pennsylvania, USA) of gallic acid, catechin, caffeic acid, chlorogenic acid, vanillin, ferulic acid, *p*-coumaric acid, *o*-coumaric acid, *m*-coumaric acid, quercetin, *trans*-cinnamic acid, and rutin were used.

Antioxidant activity

The antioxidant capacity was evaluated using the method of DPPH (2,2-difenil-1-picrilhidrazila) free radical scavenging, according to Pérez-Jiménez and Saura-Calixto (2006), with modifications. The extract was prepared from 5 mL of sample. An aliquot of 3.9 mL of the DPPH (24 mg L^{-1}) methanolic solution was added to 100 µL of the extract. Three dilutions (1:1; 1:4 and 1:5) were performed in triplicate, and from each one was taken an aliquot of 100 µL and added 3.9 mL of the DPPH solution. After 100 min in the absence of light, the absorbance of the samples was read at 515 nm in a spectrophotometer Cary 50 Probe UV–Vis (Varian, Santa Clara, USA).

Volatile profile by gas chromatography

The volatile compounds were extracted by Solid Phase Micro Extraction (SPME). Volatile compounds were determined by gas chromatography. Samples analysis were performed on a GC–MS (Shimadzu, CG-17A, Kyoto, Japan) (Gas Chromatography coupled with Mass Spectrometer), and mass detector (Shimadzu, QP5050A, Kyoto, Japan), according to the methodology proposed by Blanco et al. (2013). 50 µL of an internal standard solution (5 g L^{-1} of 4-methyl-2-pentanol in 50% ethanol) were added to 5 mL of wine. An aliquot of 2 µL of this mixture was injected (split 1:30) into a CP-WAX 57CB fused silica capillary column of 50 m × 0.25 mm and 0.2 µm film thickness (Chrompack, Middelburg, Netherlands). Instrumental conditions were as follows: injector temperature of 275 °C, detector temperature of 300 °C, hydrogen as carrier gas at 3.3 mL min^{-1}, and nitrogen as make-up gas at 30 mL min^{-1}. The flow rates of detector gas hydrogen and air were 40 and 400 mL min^{-1}, respectively. The temperature program was as follows: 50 °C for 5 min, increased to 200 °C at a rate of 4 °C min^{-1} and held at 200 °C for 15 min. Mass spectra were compared with those of the literature and a computerized Willey 8, NIST10 and NIST11 database.

Microbiological control

Analyses of coliforms at 45 °C, molds and yeasts and *Salmonella* sp. were performed according to the American Public Health Association (2015). For each sample, two portions of 25 g were weighted aseptically, one portion for *Salmonella* spp. analysis and the other for the remaining analyses. The 25 g were placed in a sterile pack, added of 225 mL of buffered peptone water and peptone water 1%, respectively. The samples were disintegrated on Bagmixer® (Interscience, France). The serial decimal dilutions were prepared from these solutions.

Sensory analysis

Sensory acceptance of the sugarcane juice fermented beverage was performed with 60 untrained men and women, above 18 years old. The sample was maintained cooled at 10–12 °C and 25 mL was served in a single session in a disposable transparent cup. The cups were labeled with random three-digit numbers. The panelists evaluated the beverage using a structured hedonic scale of 9 points, to the parameters of appearance, color, taste, odor and overall impression. This project was submitted to the Ethics Research Committee (CEP) and approved under the protocol 793.530.

Results and discussion

Characteristics of raw material

The Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) established that the comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment (WHO 1991).

The moisture content of the sugarcane juice was considerably high, 85.13 ± 0.19 g per 100 g. This value is in accordance with the Organisation for Economic Co-operation and Development (OECD 2011) which states that the extracted juice has about 85% of water. This indicates that sugarcane juice can be a good source for fermentation because it facilitates the homogenization for the preparation of fermented beverages.

The nutritional value of sugarcane juice is linked to its high sugar content (40–50 g per 100 g in dry matter) (Silva et al. 2016) since its protein and lipid content is extremely low, 0.17 ± 0.0 and 0.14 ± 0.01 g per 100 g, respectively, as found in this study. OECD (2011) considers these nutrients negligible in the sugarcane juice.

The ash content, which was found to be 0.96 ± 0.01 g per 100 g, is considered low, once it fits to the lower limit level reported by OECD (2011) (0.9–4.8 g per 100 g in dry matter), indicating that the sample was poor in minerals. Calcium, potassium and phosphorus are the main minerals found in sugarcane juice (AFRIS (Animal Feed Resources Information System of FAO) 2015). The amount of minerals present in the sugarcane juice can be linked to the harvest process. One factor that could confound this analysis may be soil dust attached to the cane while it was processed (Oliveira et al. 2016) and through the filtration process, which could have retained some mineral particles. Rakkiyappan et al. (2003) reported an average ash content for mid-late sugarcane clones of 0.398 g per 100 g.

According to the Animal Feed Resources Information System of FAO (2015), the juice of sugarcane has about 78 kcal, 21.14 g per 100 g of carbohydrates, and 0.0 g per 100 g total fiber. These values are 23, 31% higher, respectively for energy and carbohydrates count than the averages obtained in this study (60.04 kcal, 14.53 ± 0.18 g per 100 g of carbohydrates). However the fiber content found in this study was very low (0.03 ± 0.0 g per 100 g of total fiber). Variations in the chemical composition of a food or beverage can occur for various reasons such as differences between varieties, cultivation conditions, harvest period, and industrial operations such as extraction and filtration (Oliveira et al. 2016).

In the sugar and alcohol industry, the measurement of titrable acidity is the most common way of determining acidity and is expressed in grams of sulfuric acid per liter of broth. The average value of sulfuric acid for sugarcane broth in this study was low, 0.40 ± 0.01 g H_2SO_4 L^{-1}. Sulfuric acid values below 0.8 g H_2SO_4 L^{-1} can be estimated as an ideal quality parameter for sugarcane juice. Low acidity is desirable because it improves the efficiency of ethanol production. It increases the metabolism of yeast and the fermentation yield during the ethanol production process (Oliva-Neto and Yokoya 2001). Determination of the pH of *in natura* sugarcane juice allows us to ascertain its natural state. The obtained pH of 5.46 ± 0.05 indicates that the sugarcane broth was in good condition for intake, since, according to Hamerski et al. (2012), pH values under 4.2 are evidence of deterioration. pH variation can be related to the period between the harvest and processing stage of the crop, which is necessary to consider to prevent chemical alterations in the juice.

The minimum soluble solid content for fermentation is 14 °Brix according to Brazilian legislation. Therefore, the product was suitable for the fermentation and production of alcoholic fermented sugarcane beverage, since the soluble solids content was found to be 20.0 ± 0.00 °Brix, without the need to concentrate the broth or add other fermentable materials. The soluble solids content of sugarcane juice observed in this work was under the range reported by Rhein et al. (2016) of 22.62–24.32 °Brix. Variation in soluble solids content may occur because of climate factors, soil type, harvesting period, or variety of the crop as well as the way the crop was harvested because the sugar is mainly found in the base of stalks. Therefore, the closer the kirn was to the base, the higher the solids concentration (Wu and Birch 2007) and the more suitable the crop will be for fermentation process due to its sugar content available.

The composition of sugarcane juice may vary according with the variety, age and health of the sugarcane, to the environment, agricultural planning (maturity, harvest period, handling, transportation and storage), pests and diseases (OECD 2011).

Monitoring of fermentation

The average yield for the fermentation in this study was approximately 76%, meaning that for each 1000 mL of must (20 °Brix), 760 mL of fermented beverage was obtained. There was no significant difference between the three fermentation replicates. The substrate consumption of sugarcane juice fermentation lasted for a period of approximately 7 days.

The substrate content of the fermented sugarcane juice decreased significantly until the fifteenth hour, when it stabilized. Kumoro et al. (2012), while studying the alcoholic fermentation of jackfruit, noted that in the first 5 or 6 days, there was a rapid consumption of substrates and

that the soluble solids stabilized after this period. This pattern was observed in the fermentation process analyzed in this study.

Changes in soluble solids content reported by Tzeng et al. (2010) during the fermentation of sugarcane wine showed a high consumption rate of total sugars, which were reduced from 26.0 to 9.6 °Brix in 16 days. In this study, the soluble solids content was reduced from 20 to 5 °Brix in 15 h. A fast utilization rate of total sugars was observed. However, in the study of Tzeng et al. (2010) of fermentation by *S. cerevisiae* of sugarcane, the consumption of soluble solids occurred more gradually and stabilized at 9.6 °Brix. This variation between experiments could be explained by the cultivars, harvesting, and climate as well as by the fact that the initial concentration of soluble solids in the study by Tzeng et al. (2010) was 26 °Brix.

A sharp rise in temperature was observed in the first 12 h of fermentation, which can be explained by the predominance of simple-sugars in sugarcane juice and a decrease afterward, when stabilized from the second day onward.

In the process of fermentation, a general decrease in pH was observed. The initial pH of sugarcane juice was 5.5. The most rapid reduction in pH occurred after 3 days of fermentation, reaching an average pH of 4.03, and then it remained constant throughout the fermentation period. This was similar to the report by Dellacassa et al. (2017), who observed a pH of 4.0 for the pineapple (*Ananas comosus* L. Merr.) fermented beverage from Angola. However, this is in contrast to the results obtained by the increase in the total acidity during the fermentation process and, the consequent reduction in pH likely resulted from the production of organic acids such as lactic acid, acetic acid, and succinic acid.

Physicochemical properties of the beverage

The variables analyzed in the fermentation were compared to those of wines since there is no legislation covering all properties of alcoholic fermentation of fruit in Brazil. In the USA, there is only one document relating to both beverages (wine and fruit wine). However, this does not encompass all parameters studied here (GPO (U.S. Government Publishing Office) 2011). This document stipulates that the term "wine," when used without qualification, includes every kind (class and type) of product produced on bonded wine premises from grapes, other fruits (including berries), or other suitable agricultural products (such as sugarcane) and containing up to 24% alcohol by volume.

Titrable acidity is an important factor in the final quality of a fermented alcoholic beverage, and the values found for fermented sugarcane juice (Table 1) are within the standard values established by the Brazilian regulation, 50.0–130.0 meq L^{-1} or 0.0032–0.0083 g citric acid L^{-1}.

Another important factor in determining the final quality of fermented beverages is the volatile acidity, which indicates the presence of acetic acid and is undesirable for alcoholic fermentation since, in addition to modifying the flavor and aroma, it indicates contamination by acetic bacteria (Masson et al. 2012). Brazilian law requires a

Table 1 Physicochemical characterization of sugarcane alcoholic fermented beverage in three repetitions (mean ± standard deviation)

Parameter	A	B	C
Titrable acidity (meq L^{-1})	$67.64^{a1} \pm 0.4$	$66.65^{a1} \pm 0.4$	$67.64^{a1} \pm 0.4$
Volatile acidity (meq L^{-1})	$1.5782^{a1} \pm 0.0$	$1.5782^{a1} \pm 0.0$	$1.5782^{a1} \pm 0.0$
pH	$4.01^{a1} \pm 0.0$	$4.03^{a2} \pm 0.0$	$4.05^{a3} \pm 0.0$
Reducing sugar (g GLU 100 g^{-1})	$0.08^{a1} \pm 0.02$	$0.07^{a1} \pm 0.01$	$0.06^{a1} \pm 0.01$
Total sugar (g GLU 100 g^{-1})	$0.08^{a1} \pm 0.005$	$0.07^{a1} \pm 0.002$	$0.06^{a1} \pm 0.0$
Density (g cm^{-3})	$0.9911^{a1} \pm 0.0$	$0.991^{a1} \pm 0.0$	$0.991^{a1} \pm 0.0$
DM (g L^{-1})	$11.69^{a1} \pm 0.4$	$12.00^{a1} \pm 0.4$	$12.85^{a1} \pm 0.4$
RDM (g L^{-1})	$13.65^{a1} \pm 0.1$	$13.97^{a1} \pm 0.9$	$14.82^{a1} \pm 0.03$
Alcohol/RDM	$4.69^{a2} \pm 0.4$	$4.59^{a2} \pm 0.4$	$4.32^{a1} \pm 0.4$
Sulfate (g K_2SO_4 L^{-1})	<0.7	<0.7	<0.7
Chloride (g L^{-1})	<0.2	<0.2	<0.2
Protein (%)	$0.0050^{a1} \pm 0.0$	$^{a1}\pm0.0$	$^{a1}\pm0.0$

Values identified by the same letter, in the line, are not significantly different at the 0.05 level (Scott–Knott test)

GLU glucose, *DM* dry matter, *RDM* reduced dry matter

threshold for volatile acidity of 20.0 meq L^{-1} or 0.00128 g citric acid L^{-1}. In the United States, white wines produced from juice of 28 °Brix or more, volatile acidity can be 1.5 g L^{-1} (Zoecklein et al. 1995). Higher volatile acidity indicates that the contact with oxygen was greater than desired to produce alcohol through anaerobic fermentation. This study found low volatile acidity values for all treatments (Table 1), which indicates that appropriate procedures were carried out in the wine making process as well as there was a good control of temperature and anaerobic conditions.

The pH and acidity determination relevance are connected. The acidity translates, overall, to the gustative characteristics of the wine, while the pH acts on the wine stability (Leonardelli 2016).

The pH of the fermented sugarcane juice (Table 1) varied within the range established for wines in Brazil, between 2.9 and 4.0. However, according to Leonardelli (2016) the ideal pH for table wines should be in the range of 3.1–3.3, although Missouri white wines have pH around 3.5. The differences between samples are likely related to the composition of sugarcane juice as well as the reactions that occur during processing. A relatively low pH assures the freshness characteristics of the wine (Asquieri et al. 2004). According to Fang and Dalmasso (1993), wines with pH 3.4 present better resistance to bacterial infection than those with pH 3.8. Chen and Liu (2016) obtained lychee wines with pH between 3.66 and 3.85, after 20 days of fermentation. While Berenguer et al. (2016) obtained pH ranging from 3.4 to 3.58 for pomegranate wine fermented with three different yeast strains.

The fermented beverage presented reducing sugar content (Table 1) below the reported values for lychee wine (0.12 g glucose 100 mL^{-1} and 0.18 g fructose 100 mL^{-1}) (Chen and Liu 2016). The sugarcane beverage can be characterized as dry, since dry wine should contain less than 4.0 g L^{-1} total residual sugars (The National Archives 2011).

Existing differences amongst reducing sugar content of the fermented beverages are related to the fact that fermentable sugars such as glucose and fructose originate from the fruit/crop metabolism, specifically by the enzymatic hydrolysis of the sucrose (Clemens et al. 2016). The fermentation of these sugars promotes the transformation of the fruit to an alcoholic beverage.

Regarding density, dry wines, that is, with lower sugar content, have values lower than 1 g cm^{-3} (Mouchrek Filho et al. 2002), as was found in the fermented sugarcane juice (Table 1). Berenguer et al. (2016) obtained density values from 952.30 to 997.60 mg L^{-1} for the three pomegranate wines analysed.

The low alcohol content of fermented sugarcane juice (8 °GL) is related to the low residual sugar content present at the end of the fermentation (5 °Brix). These sugars called as non-fermentable sugars such as dextrin do not contribute to fermentation and produce residual sweetness in addition to contributing to a higher final density, as was observed in the fermented sugarcane juice (Table 1).

The Brazilian classifications for alcoholic fermented beverages concerning alcohol degree are “light wines” (7–10 °GL) and “table wines” (10–13 °GL). It was determined that fermented sugarcane juice can be classified as a light wine.

The sugarcane fermented beverage showed dry extract/matter content (Table 1) lower than the value observed for white wine (20.13 g L^{-1}) (Coldea et al. 2014). This difference could be explained by the possible caramelization of sugars in the drying process, which was performed in order to quantify the dry extract present in the beverage and to prevent the complete evaporation of the samples.

The amount of reduced dry matter (RDM) determines the body of the wine. If it is under 20 g L^{-1}, it is considered light, and if it is above 25 g L^{-1}, it is considered full-bodied, according with the Brazilian legislation. Thus, the fermented sugarcane beverage can be considered light. The average value found for RDM in this study was 14.15 g L^{-1}, which is lower than that found for jabuticaba sweet wine, 23.26 g L^{-1} (Asquieri et al. 2004). Brazilian legislation does not establish a minimum threshold for RDM, but does establish a maximum for the alcohol in weight/reduced dry extract ratio for common red wines, which is 4.8. This ratio was found to be within the limit (Table 1).

Sulfates appear as a result of sulfur dioxide oxidation and are added to some wines to reduce the pH to stabilize the beverage (Asquieri et al. 2004). The content of potassium sulfates in this study was lower than 0.7 g K_2SO_4 - L^{-1}, which is in accordance with the Brazilian legislation (maximum of 1.0 g L^{-1} for table wines).

With regard to the chloride content (upper limit of 0.20 g L^{-1}), fermented sugarcane juice falls within the allowable limit (Table 1). However, the methodology used for this determination was only qualitative. According to Coli et al. (2015), this indicates that the beverage was not over fermented, but did allow for adequate extraction of wort constituents.

Protein content was observed to be present in small quantities in the sugarcane juice (Table 1), possibly because it was hydrolyzed during the fermentation process. The near absence of protein in fermented sugarcane juice appears to be an advantage because, according to Salazar et al. (2006), in grape wine, proteins are classified among the compounds causing wine turbidity, along with some glycoproteins and polysaccharides, and may be related to the formation of cases, which also cause turbidity.

Bioactive compounds and antioxidant activity

The quantification of phenolic compounds is an estimate of the content of all compounds belonging to subclasses of phenolic compounds present in a sample (Table 2). Phenolic compounds identified in the beverage were gallic acid, catechin, chlorogenic acid, caffeic acid, vanillin, *p*-coumaric acid, ferulic acid, *m*-coumaric, and *o*-coumaric (Table 3). There was no significant difference detected between phenolic compounds among replicates.

In wine, there are two groups of phenolic acids: hydroxybenzoic acids and hydroxycinnamic acids (Cabrita et al. 2008). Hydroxybenzoic acids include gallic acid, which was found in high concentration in this study, followed by chlorogenic and ferulic acids.

Gallic acid, a type of phenolic acid, occurs in plants in the form of free acids, esters, catechin derivatives, and hydrolysable tannins (Naczk and Shahidi 2006). Gallic acid and its derivatives show great biological potential, presenting efficacy as an antimicrobial, antioxidant, and antidiabetic agent. The amount of gallic acid in a wine is generally between 10.0 and 100.0 mg L^{-1}, meaning that the content of gallic acid in sugarcane wine is comparable to regular wine (Tian et al. 2009).

Tian et al. (2009) explored the phenolic compounds of dry wine from *Vitis vinifera* L. cv Vidal and found similar components, amongst others, to the wine produced in this study, such as gallic acid, chlorogenic acid, vanillic acid, caffeic acid, *p*-coumaric acid, and ferulic acid.

Ortega et al. (2003) investigated the phenolic compounds of sherry white wine (Oloroso wine) and found the values of 7.93 mg L^{-1} for gallic acid and 1.62 mg L^{-1} for ferulic acid, which were, respectively, 26.64 and 80.36% lower than the results obtained in this study. Additionally, Ortega et al. (2003) reported values of 2.22 mg L^{-1} for *p*-coumaric acid, 5.28 mg L^{-1} for caffeic acid, and 23.7 mg L^{-1} for catechin, which were 92.8, 67.6, and 96.3% higher, respectively, than the levels in sugarcane wine.

Tannins are polyphenolic biomolecules, which aid in the viscosity of fermented beverages and were found in levels ranging from 38.29 to 44.51%. Antioxidant activity for fermented sugarcane juice ranged from 11.85 to 14.57% (Table 2).

Volatile compounds

In fermented sugarcane juice, 11 volatile compounds were identified, mainly esters (Table 4). These are formed, predominantly, by sugars under aerobic conditions and by amino acids under anaerobic conditions. Higher alcohols are important precursors for the formation of esters and are related to pleasant aromas (Le et al. 2012). The concentration of total alcohols in this study did not exceed this threshold; therefore, these compounds likely contributed in a positive way to the wine aroma.

The major compounds of this class found in the fermented beverage (Table 4) were 3-methyl-1-butanol (isoamyl alcohol) and 2-methyl-1-butanol (active amyl alcohol). These compounds were also found by Tzeng et al. (2010) in fermented sugarcane beverages.

Isobutanol (2-methyl-1-propanol) is also naturally produced during the fermentation of carbohydrates, comprising 6% of the total volatile profile of the beverage. 1-octanol contributes to a fruity aroma in beverages (Fan and Qian 2005) and positively contributes to their flavor, making them more harmonious, increasing sweetness and improving the aftertaste (Zhang et al. 2011).

Benzene ethanol is a compound derived from L-phenylalanine through *S. cerevisiae* metabolism and is the only higher alcohol known to have a floral aroma (Hazelwood et al. 2008). It constituted 2.0% of the total volatile profile of the beverage. It was also detected in cherry wine (52.0 mg L^{-1}) and in *Pinot Noir* wine (47.7–53.8 mg L^{-1}) (Chuenchomrat et al. 2008).

Acetal (1,1-diethoxyethane) is a volatile compound found in wines and produced during fermentation. It plays an important role in the aromatic composition and is responsible for a sweet "cookie" flavor (Jewison et al. 2012). This volatile compound was the fourth more relevant found for sugarcane wine.

Ethyl octanoate and ethyl hexanoate are volatile esters found in alcoholic beverages and are produced during fermentation by yeast. Ethyl esters are formed by the

Table 2 Biochemical characterization of sugarcane alcoholic fermented beverage in three repetitions (mean ± standard deviation)

Parameter	A	B	C
TPC (mg GAE 100 mL^{-1})	$62.22^{a1} \pm 0.20$	$57.44^{a1} \pm 5.40$	$57.45^{a1} \pm 0.90$
Tannins (mg TAE 100 mL^{-1})	$38.29^{a1} \pm 1.60$	$43.31^{a1} \pm 3.00$	$44.51^{a1} \pm 7.60$
EC_{50} (mg L^{-1})	$52{,}052.67^{a2} \pm 1.80$	$34{,}198.96^{a1} \pm 2.60$	$47{,}125.00^{a2} \pm 5.40$
RSA (%)	$13.05^{a1} \pm 1.20$	$14.57^{a1} \pm 0.60$	$11.85^{a1} \pm 0.55$

Values identified by the same letter, in the line, are not significantly different at the 0.05 level (Scott–Knott test)

TPC total phenolic compounds, *RSA* radical scavenging activity

Table 3 Individual phenolic compounds of sugarcane alcoholic fermented beverage in three repetitions (mean ± standard deviation): A, B and C

Peak number	Compound (mg L^{-1})	A	B	C
1	Gallic acid	$10.81^{a} \pm 0.43$	$10.66^{a} \pm 0.40$	$11.46^{a} \pm 0.42$
2	Catechin	$0.87^{a} \pm 0.87$	$2.62^{a} \pm 0.87$	$1.61^{a} \pm 0.87$
3	Chlorogenic acid	$4.45^{a} \pm 0.99$	$2.49^{a} \pm 0.99$	$3.61^{a} \pm 0.99$
4	Caffeic acid	$1.71^{a} \pm 0.41$	$1.02^{a} \pm 0.41$	$1.75^{a} \pm 0.41$
5	Vanillin	$0.22^{a} \pm 8.83$	$0.43^{a} \pm 8.83$	$0.20^{a} \pm 8.83$
6	*p*-Coumaric acid	$0.16^{a} \pm 0.11$	$0.20^{a} \pm 0.11$	$0.37^{a} \pm 0.11$
7	Ferulic acid	$8.25^{a} \pm 2.26$	$4.05^{a} \pm 2.26$	$7.60^{a} \pm 2.26$
8	*m*-Coumaric acid	$0.39^{a} \pm 0.05$	$0.38^{a} \pm 0.05$	$0.30^{a} \pm 0.05$
9	*o*-Coumaric acid	$0.09^{a} \pm 0.05$	$0.01^{a} \pm 0.00$	$0.01^{a} \pm 0.00$

Values identified by the same letters, in the line, are not significantly different at the 0.05 level (Scott–Knott test)

Table 4 Volatile compounds of sugarcane alcoholic fermented beverage

Peak number	RT	Area (%)	Component	Aroma
1	2.22	6 ± 0.04	2-Methyl-1-propanol	Sweet
2	3.33	10.77 ± 0.20	1,1-Diethoxy-ethane	Bicuity
3	3.51	47.23 ± 1.20	3-Methyl-1-butanol	Malt
4	3.57	15.05 ± 0.05	2-Methyl-1-butanol	Wine
5	11.00	2.08 ± 0.34	Ethyl ester hexanoic acid	Flowery, fruity
6	13.73	0.69 ± 0.09	1-Octanol (fatty alcohol)	Fruity
7	15.23	1.83 ± 0.05	Benzeneethanol	Sweet
8	18.22	16.22 ± 0.32	Ethyl ester octanoic acid	Sweet
9	26.57	0.07 ± 0.06	n.d.	–
10	26.83	0.03 ± 0.41	*s*-Propyl ester octanethioic acid	–
11	26.89	0.03 ± 0.57	Betahistine	–

Data are presented as mean ± standard deviation of three repetitions

n.d not detected, *RT* retention time

reaction between ethanol and a fatty acid and have sweet, pleasant aromas. Ethyl hexanoate confers fruity aromas, such as those of pineapple, apple peel, and strawberry (Saerens et al. 2010). The concentration of these esters decreases with storage time because of spontaneous hydrolysis.

Microbiological control

Wine contains yeasts and bacteria, which may be either harmful or beneficial to the final product. The analyses of fermented sugarcane juice were negative for *Coliform* and *Salmonella*. For yeast, all samples presented values below 10 colony forming units (CFUs) g^{-1}.

Sensory analysis

All attributes received scores above 6, except taste (5.15), which characterizes the fermented beverage as acceptable. The score for the aroma of fermented sugarcane juice could be explained by the fact that consumers are not acquainted with this type of beverage, although all testers were consumers of alcoholic beverages, including wine. However, 66.7% of the participants scored the appearance attribute higher than 6.0 and 61.66% gave the same score for the color attribute. More than 50% of the consumers scored the beverage 6.0 or higher for taste and 71.67% for aroma.

Conclusion

The major volatile compounds of the fermented sugarcane beverage were 3-methyl-1-butanol and 2-methyl-1-butanol, followed by 2-methyl-1-propanol. However, other volatile compounds were found and they positively contributed to the flavor and aroma of the beverage such as 1-octanol, benzene ethanol, 1,1-diethoxyethane, ethyl octanoate, and ethyl hexanoate.

Many phenolic compounds were identified, with gallic acid being the predominant one, in addition to chlorogenic acid, vanillic acid, caffeic acid, *p*-coumaric acid, and

ferulic acid, and they were found in comparable concentrations to other wine varieties.

The results suggest that sugarcane juice can be used as an alternative to produce wine, once it is optimized to have appropriate characteristics for an alcoholic fermented beverage, such as an alcohol content of 8% (v/v) and a general sensorial acceptance by consumers. Therefore, fermented sugarcane juice may eventually find a place in the agroindustrial market.

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