

## Characterization of the Jambolan (*Syzygium cumini* L.) Fruit Wine Processing

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Yeasts were isolated and the alcohol genic features were tested for their direct use in the wine processing of jambolan. In addition, changes in the total phenolic compounds during the maceration-fermentation process were investigated. Yeasts were selected from the spontaneous fermentation of the jambolan pulp, and the feasibility and fermentative production of its alcohol was tested. Out of the group of yeasts selected, the one that stood out was subjected to DNA extraction and sequencing. The yeast was identified as *Saccharomyces cerevisiae*, and the fermentation tests came back as 80.6% and the ethanol production yield was 8.35%. The chemical composition of raw materials was analyzed by spectrophotometrics and high performance liquid chromatography (HPLC) methods. The overall results also indicated that the evolution during the maceration-fermentation process of phenolic compound concentrations was influenced by the varietal factor. The concentration of phenolic compounds increased 30%, while the concentration of tannins increased 27.4% in the final product.

*Keywords:* Jambolan wine; *Saccharomyces cerevisiae*; Bioactive compounds; Chemical monitoring

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### INTRODUCTION

Fruit production is one of the most prominent sectors of the Brazilian agribusiness. Through a wide variety of cultures, produced across the country and in different climates, fruit growing achieves significant results and generates opportunities for Brazilian small businesses (SEBRAE 2015). Along with the large volume produced is a high percentage of losses related to the lack of standards during classification and, particularly, to their unsuitable handling during transportation, storage, and marketing (Lana 2016).

This fact highlights the need for simple and inexpensive processes that can provide producers with profitable alternatives for better use of their fruits. Therefore, the use of ripe fruits or their juices for wine production, which could be an alternative use of the large volume of fruit produced in its peak season, is considered to be an attractive way of utilizing surplus and over-ripe fruits (Araújo *et al.* 2011; Jagtap and Bapat 2015).

The fermentation process for the preparation of the beverage depends on the conditions of fermentation, the composition of the fruit juice, and the action of yeasts to convert the sugars into alcohol and secondary metabolites that impact the organoleptic

properties of wine (Dias *et al.* 2007; Ferreira Duarte *et al.* 2009; Lu *et al.* 2017). The occurrence and growth of yeasts during alcoholic fermentation can be affected by the initial population, the use of starting cultures, the chemical composition of the grape “must” (including residues of fungicides or pesticides), the temperature of fermentation, and the interactions between the different species and strains of micro-organisms (Fleet 1993; Bisson 1999; Fleet 2001; Ciani *et al.* 2016). Grape “must” is freshly pressed juice from the entirety of the grape.

During the production of biomass and the alcoholic fermentation, yeast cells are subjected to various stress conditions. The osmotic stress imposed by the high concentration of sugar in the grape must, the production of ethanol during fermentation, and the use of antimicrobials are the most important of such stress conditions (Bauer and Pretorius 2000). The use of selected strains favors a quicker start to the process, and the risks of contamination posed by spontaneous fermentation can be avoided, which favors less competition for essential nutrients, and a higher yield and quality of the resulting product (Fleet *et al.* 1984; Sanni and Lonner 1993).

The use of selected starter culture is widely spread in winemaking (Ciani *et al.* 2016). The use of starter strains of *S. cerevisiae* is an important strategy for maintaining the quality and assuring the reproducibility of wine features. Although the use of any selected yeast is a viable alternative, the use of yeasts from the process itself is the future expectation for the biotechnological processes. Regarding fruit wine spontaneous fermentation, the microbial ecology seems to be similar to that for wine fermentation (Dias *et al.* 2017). Based on this fact, in this study, yeasts were isolated and the alcoholic features were tested for the use of the wine jambolan extract. The use of strains isolated from specific regions could be even more interesting because of the high adaptation to their own climatic conditions, in addition to the fact that they are related to the use of the inoculum of yeast isolated from the process itself. Even more, these strains are usually associated with particular wine characteristics that frequently identify specific wines and regions (Guimaraes *et al.* 2006; Andrietta *et al.* 2007).

Jambolan or jamun (*Syzygium cumini* (L.) Skeels) is a member of the family Myrtaceae and is a large perennial tree inherent to the Indian subcontinent (Akhtar *et al.* 2016) commonly known in Brazil as *jamelão*. The fruit is very attractive for its purple colour with good taste that combines sweetness with a mildly sour and astringent flavour (Sehwag and Das 2014). The quality of jambolan is greatly deteriorated between its harvesting and consumption so there is a considerable wastage of this fruit. To prevent this wastage and to make the jamun available year-round, it is practicable to convert the fruit into value-added products (Shahnawaz *et al.* 2012; Akhtar *et al.* 2016). The main use of jambolan is in its leaves, which are used in the preparation of teas recommended for diabetic patients because they are rich in tannins and saponins (Morton 1987; Santiago *et al.* 2016). In India and in the Philippines, jambolan is often used in the processing of fermented beverages, spirits, and vinegar (Banerjee and Dasgupta 2005).

Antioxidant activity is one of the most studied biological activities of the jambolan. The jambolan fruit has been studied for its richness in bioactive phenolic compounds, mainly anthocyanins, that give an intense purple coloration to the low-hanging fruit, which enables the transfer of these constituents to products that use it as a raw material (Coelho *et al.* 2016; Santiago *et al.* 2016). According to Carneiro (1986), jambolan presents pH values in the neighborhood of 4.3, and reducing sugars at 4.4 g.100 g<sup>-1</sup>. Ripe jamun have almost 83% water with practically 14% solids containing a mixture of fermentable sugar (Akhtar *et al.* 2016). The Jamun pulp contains significant amounts

of fermentable sugar, which may be used for alcoholic fermentation (Chowdhury and Ray 2007). Therefore, it may be considered as a raw material for environmental alcohol fermentation processes, due to its high contents of total soluble solids, an important substrate for yeasts during fermentation.

In winemaking, during spontaneous fermentation of the must, there is a succession of yeast species. *S. cerevisiae* grows during this period and persists until the end of fermentation because of its high ethanol tolerance (Dias *et al.* 2017). Thus, the objectives of this work are to isolate and characterize strains of *S. cerevisiae* associated with the jambolan fruit extract that could be used as a tool to improve the quality of the wine and the reproducibility of the wine obtained. This would allow the creation of a strong identity that could facilitate its entry into the market of alcoholic beverages. This study also assesses and compares the effect of processing in the concentration of total phenolics and antioxidant activity.

## EXPERIMENTAL

### Materials

#### *Samples*

The jambolan must made from the selected fruits was prepared according to Dias *et al.* (2007). The fruits were collected daily from individual shrubs located in the campus of the State University of Feira de Santana (UEFS) in Bahia, Brazil. The fruits were selected, washed with a 2.5% w/w sodium hypochlorite solution, and mechanically depulped (NPC Equipamentos<sup>®</sup>, Itabuna, Brazil) for the must preparation. The fruit pulp was chaptalized at 22 °Brix by the addition of inverted sugar and potassium metabisulfite (Labsynth, Diadema, Brazil) added at a rate of 0.05 g.L<sup>-1</sup> to avoid contamination by wild yeasts and other microorganisms; the fruit pH was maintained at 3.5.

The over-ripe fruits with no physical contaminants, such as twigs, leaves, and insects, were manually depulped for the extraction of seeds, but they were not sanitized in the sodium hypochlorite solution. This pulp was only used for spontaneous fermentation.

### Methods

#### *Spontaneous fermentation and yeast selection*

Yeast strains were obtained *via* isolation and selection during spontaneous fermentation processes and screened for ethanol production capacity under laboratory conditions. To isolate fermentative yeasts, the must of jambolan fruit was placed in a TE 420 incubator (TECNAL Equipment for Laboratories<sup>®</sup>, Piracicaba, Brazil) with constant shaking at 120 rpm at 26 °C. After 48 h, the must was maintained for 15 days in the same incubator at the same temperature, but without shaking. From this must, serial dilutions were then prepared in 0.1% peptone water and seeded onto the surface of sabouraud dextrose agar (HIMEDIA<sup>®</sup>) plates. At least three colonies of each morphotype were randomly collected and purified after several sub-culturing steps onto sabouraud dextrose agar. Three isolates (from each sample) sharing the same morphology were tested in relation to their fermentative efficiency of ethanol production.

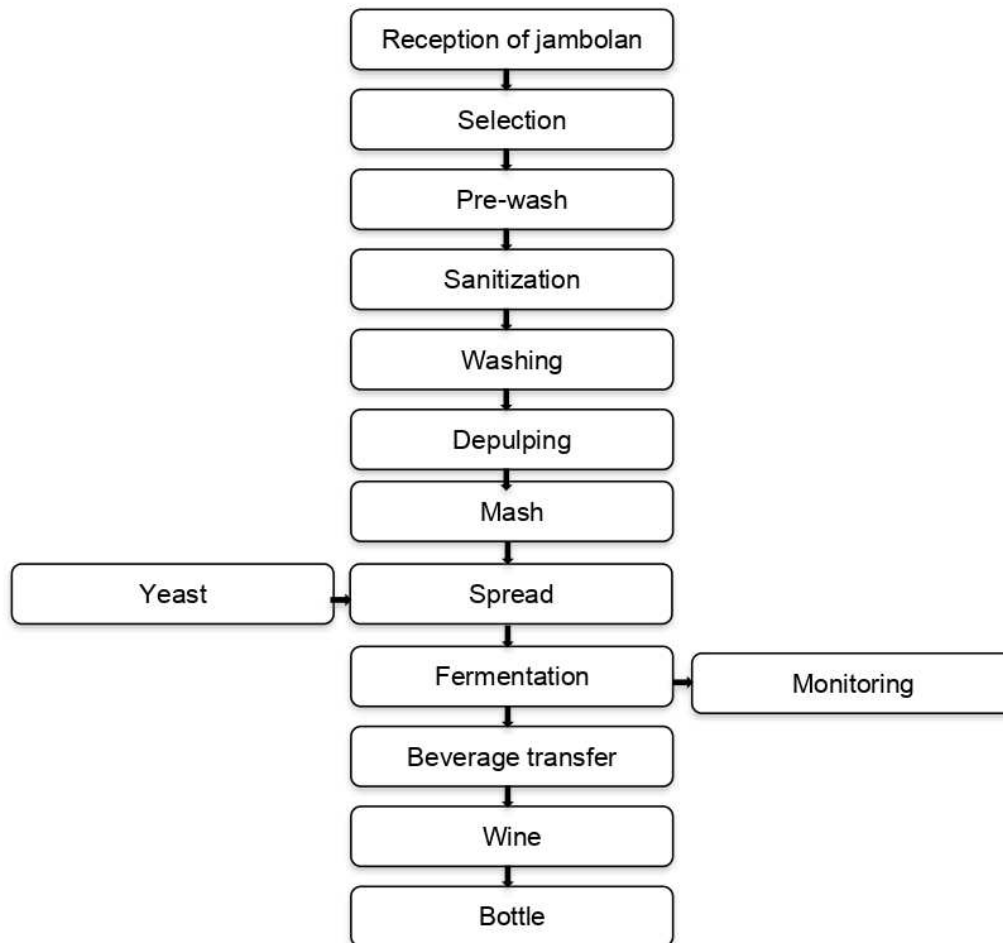
The fermentation efficiency was evaluated from the total yeast count (CFU.mL<sup>-1</sup>) and substrate consumption during fermentation. Cell viability (viable and non-viable cells) was determined using the International Coloring Method with methylene blue (Sigma Aldrich<sup>®</sup>, São Paulo, Brazil), according to the committee of the American Society

of Brewing Chemists (ASBC 1996). The methodology proposed by the Ministry of Agriculture (MAPA 1986) was used to determine the alcohol production at the end of fermentation. During the yeast selection process, the microbiological analyses were restricted to colony-forming unit counts at 28 °C for 48 h to 72 h, by a serial dilution method.

The cryopreservation was carried into a GYMP medium (2% glucose, 0.5% yeast extract, 1% malt extract, and 0.2% sodium phosphate) with sterile 15% glycerol and was stored at –85 °C. The isolates were deposited in the Culture Collection of Microorganisms of Bahia (CCMB/UEFS, Bahia, Brazil).

#### *Fermentation for beverage production using the selected yeast strain*

The process of production of the fermented alcoholic jambolan beverage is described in the flowchart in Fig. 1.



**Fig. 1.** Flow chart of process of wine production of jambolan with *S. cerevisiae* lineage CCMB 520

Fermentation was performed in 6-L conical flasks using 75% of their capacity. Rubber tubing was attached to the flasks and immersed in water to enable the removal of CO<sub>2</sub>, but avoiding contamination by the air. To achieve the initial population of 10<sup>8</sup> cells/mL, the spread in shacker was set at 30° C with agitation to 220 rpm. The media

were incubated in fermentation B.O.D., 24 °C for 15 days. Fermentation was performed in triplicate using 5% of an active culture of the most efficient wild yeast in the must chaptalized with inverted sugar. Samples of must were taken every three days to follow fermentation process and the quality of the fermented product, using physicochemical analyses (pH, soluble solids expressed in °Brix, and chemical characterization) and the total yeast count (CFU.mL<sup>-1</sup>). After stabilization of the total soluble solids (°Brix), the beverage was subjected to the first transfer, as described by Duarte *et al.* (2010), where the yeast and impurities were removed by natural decantation. At the end of fermentation, jambolan fermented musts were transferred to bottles with a capacity of 750 mL and stored at 5 °C for sedimentation of the biomass. After 24 h, the beverages were transferred without aeration to new bottles, followed by two transfers with duration of 15 days, beverages were then filtered using cellulose filters and stored at 5 °C in glass bottles filled completely to avoid oxygen entrance.

#### *Yield and productivity*

Both the alcohol yield (%) and the ethanol volumetric productivity ( $Q_p$ ) expressed in g.(Lh)<sup>-1</sup> for the production of a fermented alcoholic beverage from jambolan fruit were calculated according to Bortolini *et al.* (2001) and Lopes and Silva (2006).

#### *Yeast identification*

The total DNA was extracted using the methods described by Brandão *et al.* (2011). The D1/D2 variable domains of the large subunit of the rRNA gene were amplified and sequenced as previously described by Lachance *et al.* (1999). The sequences obtained were compared with those included in the GenBank database using the Basic Local Alignment Search Tool (BLAST, at <http://www.ncbi.nlm.nih.gov>) (Altschul *et al.* 1997).

#### *Chemical characterization*

The chemical composition of raw materials was analyzed by methods recommended in the fruit-vegetable industry “Official Methods of Analysis – AOAC International (2005). The pulp before fermentation was analyzed for its total soluble solids (°Brix), reducing sugars, acidity, and pH. Samples were analyzed in triplicate for each wine and data were expressed as the mean ± standard deviation (SD).

The total phenolic compound contents were determined using the conventional procedure developed by Folin-Ciocalteu (Georgé *et al.* 2005), and they are expressed in terms of gallic acid. The tannin contents were determined by the casein powder precipitation method (Abdel-Hameed 2009). The total anthocyanin contents were measured by the colorimetric method of differential pH (Wrolstada *et al.* 2005). The antioxidant activity was measured as a function of the sequestering activity of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Co., São Paulo, Brazil) (Liu and Yao 2007). These analyses were performed in an ELISA microplate reader POLARIS® (Marca Medica Comercio e Serviços LTDA, Uberlândia, Brazil). An ethanolic solution of gallic acid (VETEC®, São Paulo, Brazil) was used as the positive control. The value for  $EC_{50}$  was defined as the final concentration in mg.mL<sup>-1</sup> of the dry extract present in the cuvette, required to decrease the initial DPPH concentration by 50% (Hidalgo *et al.* 1994; Pyrzyńska and Pekal 2013).

*Chromatographic analyses of the jambolan wine*

Sugars (glucose, fructose, and sucrose) (Sigma Co., São Paulo, Brazil) and organic acids (malic, lactic, and acetic) (Sigma Co., São Paulo, Brazil) were determined using high-performance liquid chromatography (HPLC) (Kelebek *et al.* 2009). Chromatographic analyses were performed in the EZChrom Elite HPLC system, consisting of a VRW Hitachi L-2130 pump equipped with a VRW Hitachi L-2200 automatic injector and a VRW Hitachi L-2490 refractive index detector (RI), a VRW Hitachi L-2455 diode arrangement detector (DAD), and a VRW Hitachi L-2300 oven (Hitachi Medical Corp, Tokyo, Japan), as well as an HPX-87H Aminex® column 300 mm × 7.8 mm i.d./9 µm (Bio-Rad, Hercules, CA, USA). The injection volume was 20 µL and the mobile phase used isocratic flow composed of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) 0.01 N. The mobile phase flow rate was 0.6 mL/min at room temperature with a total race time of 15 min and 210 nm of wavelength.

**RESULTS AND DISCUSSION**

Wine quality is primarily determined by the fruit quality, mainly its chemical composition (Dias *et al.* 2017). The fresh pulp showed a concentration of total soluble solids of 12.78 ± 0.02 °Brix, similar behavior was described by Coelho *et al.* (2016), and smaller than found by VenuGopal and Anu-Appaiah (2017). However, it was necessary to add sucrose to the must due to the fact that the fermented beverage obtained presented a higher alcohol content. It was verified that the pH range (3.58 and 3.92) throughout the fermentation process was enough to enable a quick alcoholic fermentation and inhibit unwanted bacteria. Thus, pH correction was not necessary. A similar behavior in the pH was described by Almeida *et al.* (2006), Andrade *et al.* (2003), and Coelho *et al.* (2016). The composition of jambolan fruits is given in Table 1.

**Table 1.** Composition of Jambolan Pulp

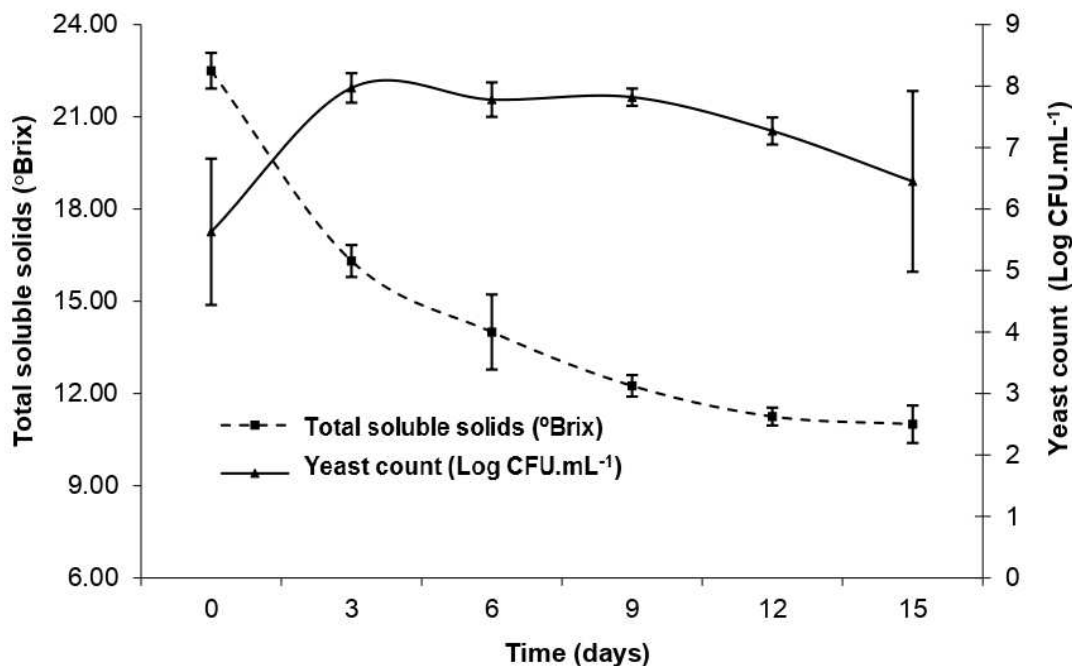
Composition	Value
Total soluble solids	12.78 ± 0.02 °Brix
pH	3.75 ± 0.17
Total monomeric anthocyanins	173.69 ± 43.2 mg.100g <sup>-1</sup>
Total phenolics	423.09 ± 43.12 mg.100g <sup>-1</sup>
Total tannins	291.16 ± 25.60 mg.100g <sup>-1</sup>
EC <sub>50</sub>	163.96 ± 6.10 µg.mL <sup>-1</sup>
Percentage of free radial abduction	74.95 ± 0.64 %

Data are presented as mean ± SD of triplicate analysis.

Spontaneous fermentations were performed with the jambolan pulp for the isolation of yeasts prevalent in the processes. The most efficient yeast in the fermentation process of the jambolan pulp was the most resistant one to high concentrations of ethanol, when compared with the other strains. This strain was identified as *Saccharomyces cerevisiae* according to its sequence of the D1/D2 domains of the rRNA gene. This strain was deposited in the collection of micro-organism cultures of Bahia as CCMB 520.

During the process of alcoholic fermentation, the kinetic profile of concentrations of total soluble solids (substrate) and cellular (biomass) was observed. The averages of the experiments performed in triplicate are shown in Fig. 2, which shows that the kinetic

behavior in two experiments showed a very good reproducibility of the data cell concentration (Log CFU.mL<sup>-1</sup>) and total soluble solids (TSS).



**Fig. 2.** Consumption of total soluble solids (°Brix) and yeast count (LogUFC.mL<sup>-1</sup>) during the alcoholic fermentation of jambolan with *S. cerevisiae* strain CCMB 520 (24 °C for 15 days)

As shown in Fig. 2, there was a gradual reduction of soluble solids during the fermentation period, which reached a steady level of 10 °Brix after 15 days of fermentation due to the consumption of the substrate by microorganisms. Consequently, there was an increase in the concentration of ethanol that reached  $8.35 \pm 0.75$  v/v.

The results indicated the efficiency of the yeast of the CCMB 520 *S. cerevisiae* species used, with good alcoholic outcome, and reduction in the number of viable cells at the end of the process. However, the total soluble solids content at the end of the process, probably due to the presence of non-fermentable sugars in the fruit pulp (Corazza 2001), influenced the assessment of the density of the wine content obtained (1.031 g.L<sup>-1</sup>). Its reading was characteristic of alcoholic fermented compounds with higher sugar levels and lower alcoholics levels, and density exceeding 1.0 (Vogt 1972).

The ethanol yield factor ( $Y_p/s$ ) was 0.44 g.g<sup>-1</sup>, which means a 44% yield of alcoholic fermentation or in others words that 44 g of ethanol were obtained from 100 g of fermented sugar. These results are consistent with those found in the literature, because according to Hashizume (1983), even in optimum conditions of work, the highest yield in alcoholic fermentation does not exceed 48%, and in the industrial process, the value reached is even lower (Ilha *et al.* 2008). The fermentation efficiency or efficiency of substrate to ethanol conversion was 87.84 %, with volumetric productivity ( $Q_p$ ) of 0.36 g.(L.h)<sup>-1</sup>, demonstrating that *S. cerevisiae* CCMB 520 presents a good ethanol production.

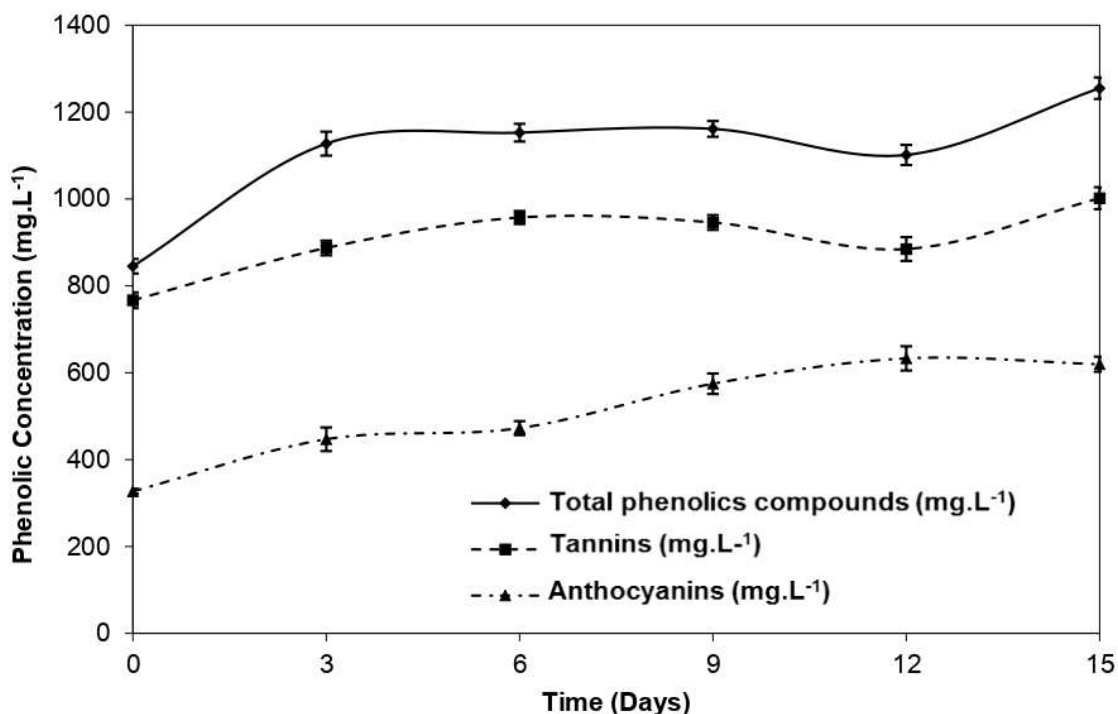
Lower values of ethanol yield factor (41.53%) and efficiency (81.27%) were reported after 84 hours in the mead production by Ilha *et al.* (2008). In strawberry wine production, Andrade *et al.* (2014) reported alcohol yield (42.09%), efficiency (82.38%)

and a lower value of productivity ( $0.1336 \text{ g} \cdot (\text{L} \cdot \text{h})^{-1}$ ) related to the long fermentation time (720 h).

#### Characterization of jambolan wine

During the fermentation process, the chemical evolution of the main bioactive process variables was rated: total phenolics, tannins, and anthocyanins, and total time of fermentation. The must was composed of unfiltered fruit pulp, which included the peel or pulp of the fruit. Similar to the fermentation of red wine, where the main feature lies in the fact that the must ferments in contact with most of the bunch, it was possible to ensure the obtaining of an alcoholic beverage fermented from features considered excellent by the progressive dissolution of dye materials and bioactive compounds in solid parts.

Different from that observed by Zardo *et al.* (2008) in apple juice wine, the wine processing of jambolan increased phenolic concentration (total phenolics, tannins, and anthocyanins) as shown in Fig. 3.



**Fig. 3.** Evolution of phenolic concentration in the course of the fermentation with *S. cerevisiae* strain CCMB 520

Red wine is traditionally derived from crushing and destemming grapes, with the must obtained remaining in contact with skins and seeds for a greater or lesser period. In this method, the dissolution of the coloring substances is aided by the presence of alcohol produced during fermentation (Lorenzo *et al.* 2016). Thus, in the jambolan wine, the color comes in a first instance from the extraction of anthocyanin pigments present in the fruit peel, during fermentation. As noted by Lorenzo *et al.* (2016), during the initial stages of winemaking, the increasing extraction of

polyphenols and anthocyanins is due to the maceration of fruit skin occurring in the must and to the formation of ethanol during fermentation, which enhances the solubilization of these polyphenolic compounds. There was an increase in the concentration of anthocyanins from  $326.9 \pm 0.13 \text{ mg.L}^{-1}$  in the must to  $667.96 \pm 0.01 \text{ mg.L}^{-1}$  in the fermented alcohol. The concentration of phenolic compounds increased 38.4% in the fermented alcohol, while the tannin concentration increased 27.7%. As described by VenuGopal and Anu-Appaiah (2017), the fermentation process has led to a greater extraction of phenolic compounds. The physico-chemical composition of the must and jambolan wine can be observed in Table 2.

**Table 2.** Chemical Composition of Must and Jambolan Wine Processed with *S. cerevisiae* strain CCMB 520

Parameters	Must	Jambolan wine	Chowdhury and Ray (2007)
Total soluble solids ( $^{\circ}$ Brix)	$23.0 \pm 0.41$	$11.5 \pm 0.62$	$2.8 \pm 0.00$
Reducing sugar ( $\text{g} \cdot (100 \text{ mL})^{-1}$ )	$16.17 \pm 0.26$	$2.13 \pm 0.78$	$0.49 \pm 0.04$
Sucrose ( $\text{g} \cdot \text{L}^{-1}$ )	-	$28.60 \pm 1.43$	-
Glucose ( $\text{g} \cdot \text{L}^{-1}$ )	-	$4.50 \pm 0.28$	-
Fructose ( $\text{g} \cdot \text{L}^{-1}$ )	-	-	-
pH	$3.66 \pm 0.04$	$3.1 \pm 0.12$	$3.3 \pm 0.07$
Titrateable acidity ( $\text{mEq} \cdot \text{L}^{-1}$ )	$158.31 \pm 4.67$	$56.04 \pm 0.26$	-
Total phenolics compounds ( $\text{mg} \cdot \text{L}^{-1}$ )	$845.09 \pm 0.22$	$1,371.00 \pm 0.02$	$2,200.0 \pm 0.03$
Anthocyanins ( $\text{mg} \cdot \text{L}^{-1}$ )	$326.9 \pm 0.13$	$667.96 \pm 0.01$	$600.0 \pm 4.50$
Tannins ( $\text{mg} \cdot \text{L}^{-1}$ )	$766.75 \pm 0.18$	$1,061.16 \pm 0.02$	$140.0 \pm 0.75$
Ethanol production (%)	-	$8.35 \pm 0.75$	$6.0 \pm 0.25$
$EC_{50}$ ( $\mu\text{g} \cdot \text{mL}^{-1}$ )	$174.44 \pm 0.22$	$186.12 \pm 7.03$	-
% SRL	$78.40 \pm 0.64$	$86.85 \pm 0.88$	-

- Not informed

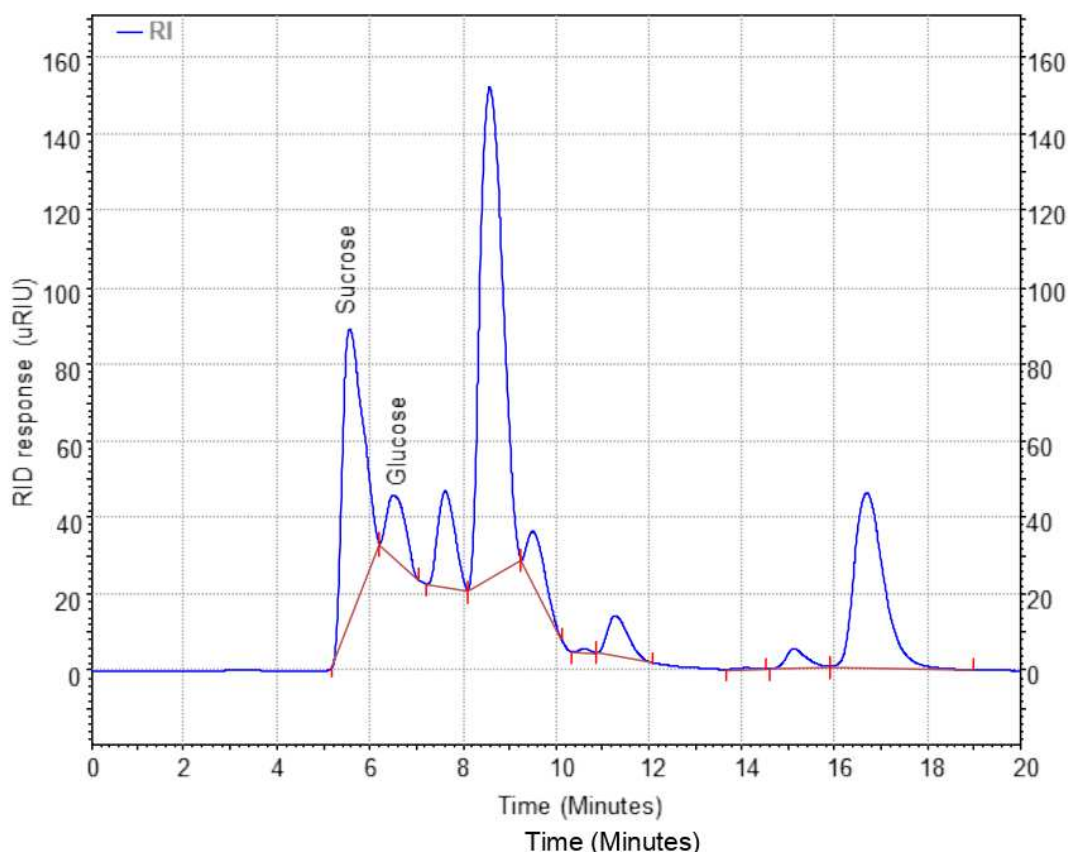
Data are presented as mean  $\pm$  SD of triplicate analysis.

Tannins provide astringency and bitterness to wine by forming aggregates in solution with salivary proteins, and have demonstrated antimicrobial activity, including activity against oral pathogens (Riihinen *et al.* 2014). A high concentration of tannins was identified in the sample ( $1061.16 \text{ mg} \cdot \text{L}^{-1}$ ).

The results of the quantitative assessment of the evolution of the percentage of sequestration of DPPH free radical (% SRL) and pulp fermented beverage samples in different concentrations showed that all samples had sequestered DPPH radical activity. The total antioxidant capacity was quantified according to the method of free radical sequestration with 1,1-diphenyl-2-picrilhidrazil (DPPH). The  $EC_{50}$  value corresponds to the sample needed to reduce 50% of the initial concentration of DPPH radical (Rufino *et al.* 2007). That figure is inversely proportional to the antioxidant capacity. The higher the DPPH consumption of a sample, the lower the  $EC_{50}$  figure and the higher its antioxidant activity (Banerjee and Dasgupta 2005; Kuskoski *et al.* 2006). In this study, the  $EC_{50}$  value found for the pulp of jambolan ( $163.96 \pm 6.10 \mu\text{g} \cdot \text{mL}^{-1}$ ) was greater than that presented by Benherlal and Arumughan (2007) in the case of the ethanolic extract of jambolan ( $158.0 \pm 5.0 \mu\text{g} \cdot \text{mL}^{-1}$ ). Comparing the results of the antioxidant activity of must and wine, one can assume that the metabolism of the jambolan during fermentation

favors the formation of substances that increase the ability of antioxidant activity of the product.

Chowdhury and Ray (2007) also studied the jambolan, but with a wine fermentation process that was different from the one performed in this work, where the pulp was then crushed with water, at room temperature of  $32 \pm 2$  °C during 6 days, pH 4.5, and 17 °Brix. The concentration of phenolic content at  $2200 \pm 0.01$  mg.L<sup>-1</sup> was higher than the one found in this work. However, the concentration of tannins,  $14 \pm 0.75$  mg.L<sup>-1</sup>, was inferior. Lower values,  $1.01 \pm 0.03$  mg.L<sup>-1</sup>, were also observed by VenuGopal and Anu-Appaiah (2017) in jambolan wine. Fruit quality is affected by both environmental and plant factors (Joshi *et al.* 2017), thus these differences can be related to planting, climate, and soil characteristics of the raw material.



**Fig. 4.** Chromatogram of compounds present in the jambolan wine processed with *S. cerevisiae* lineage CCMB 520, analyzed by HPLC, refractive index detector (RI). The x- and y- axes represent the retention time (min) and refractive index detector response (uRIU), respectively

The values obtained for phenolic concentration and total monomeric anthocyanins and tannins, in jambolan wine, are similar to those found by other authors studying red wine. The red wine studied by Paixão *et al.* (2007) found 1936.00 mg.L<sup>-1</sup> of phenolic compounds and Rizzon *et al.* (2000) found 288.00 mg.L<sup>-1</sup> and 800.00 mg.L<sup>-1</sup> of total anthocyanins and tannins, respectively.

The titratable acidity of the fermented alcoholic beverage of jambolan is presented according to the limits recommended by the Brazilian legislation for wines, *i.e.*, between 55 mEq.L<sup>-1</sup> and 130 mEq.L<sup>-1</sup> (MAPA 1988). As noted by Rizzon and Miele (2003), by analyzing the Merlot wine, the analytical results of titratable acidity of the jambolan

wine confirmed reduced values in relation to the must ( $158.31 \text{ mEq.L}^{-1}$ ).

As expected, the content of reducing sugars ( $\text{g.}(100 \text{ mL})^{-1}$ ) was reduced from the initial value from  $16.17 \pm 0.26$  to  $2.13 \pm 0.78$  in wine. The same occurred with the concentration of total soluble solids, determined in °Brix, which declined from  $23.0 \pm 0.41$  in must to  $11.5 \pm 0.62$  in wine.

As an example, the authors reported the chromatographic profile of the sample acquired at 210 nm. Figure 4 displays the chromatogram with the composition of the organic jambolan wine and its respective retention times, obtained from injections in HPLC/RI. The compounds identified consist of sucrose (5.62 min) and glucose (6.61 min). In the final product, residual reducing sugar fructose was not identified, as well as organic acids (malic, lactic, and acetic). The remaining peaks present in the sample were not identified; peaks in the diode arrangement detector (DAD) were not identified either.

## CONCLUSIONS

1. In addition to representing an innovative alternative to fruit wine, the use of isolated fruit yeasts proved to be perfectly feasible, allowing for a percentage reduction of losses in the harvest period, and opportunities for small businesses.
2. For the conditions of this work, the use of the fermentations of *Saccharomyces cerevisiae* CCMB 520, obtained by spontaneous fermentation of the jambolan fruit, was proven to be efficient in converting the substrate into ethanol.
3. The income and productivity parameters obtained confirmed that the yeast presented good performance ( $8.35 \pm 0.75\%$  ethanol production,  $0.36 \text{ g.}(L.h)^{-1}$ ).
4. Similar to red wine, phenolic concentrations that included condensed tannins, monomer anthocyanins, and the antioxidant activity, tended to increase throughout the fermentation process.

## ACKNOWLEDGEMENTS

The authors thank the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

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Article submitted: April 9, 2017; Peer review completed: May 22, 2017; Revised version received and accepted: August 2, 2017; Published: August 8, 2017.  
DOI: 10.15376/biores.12.4.7069-7083