

## Bacaba beverage produced by Umutina Brazilian Amerindians: Microbiological and chemical characterization

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### Abstract

Bacaba chicha is a beverage prepared by the indigenous Umutina people from the bacaba fruit (*Oenocarpus bacaba*), a purple berry that is rich in fat and carbohydrates, as well as a source of phenolic compounds. In this study, samples of bacaba chicha beverage were collected, and the microbial community was assessed using culture-dependent and -independent techniques. The nutritional composition and metabolite profiles were analyzed, and species belonging to lactic acid bacteria (LAB) and yeasts were detected. The LAB group detected by culture-dependent analysis included *Enterococcus hormaechei* and *Leuconostoc lactis*. Polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) detected additional *Propionibacterium avidum*, *Acetobacter* spp., and uncultured bacteria. *Pichia caribbica* and *Pichia guilliermondii* were detected in a culture-dependent method, and *Pichia caribbica* was confirmed by PCR-DGGE analysis. The pH value of the beverage was 6.2. The nutritional composition was as follows:  $16.47 \pm 0.73$  g 100 mL<sup>-1</sup> dry matter,  $2.2 \pm 0.0$  g 100 mL<sup>-1</sup> fat,  $3.36 \pm 0.44$  g 100 mL<sup>-1</sup> protein, and  $10.87 \pm 0.26$  g 100 mL<sup>-1</sup> carbohydrate. The metabolites detected were 2.69 g L<sup>-1</sup> succinic acid, 0.9 g L<sup>-1</sup> acetic acid, 0.49 g L<sup>-1</sup> citric acid, 0.52 g L<sup>-1</sup> ethanol, and 0.4 g L<sup>-1</sup> glycerol. This is the first study to identify microbial diversity in bacaba chicha spontaneous fermentation. This study is also the starting step in the immaterial record of this Brazilian indigenous beverage prepared from bacaba fruit.

**Key words:** non-alcoholic beverage, indigenous beverage, lactic acid bacteria, yeasts, bacaba.

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### Introduction

Bacaba (*Oenocarpus bacaba* Mart.), a native palm found in the Brazilian Amazon and Cerrado (Brazilian Savannah) biomes, produces edible purple berries that ripen between December and April. The fruits are collected in the extractive system by indigenous and river communities and are used for feeding as a natural juice or processed into fermented beverages, jelly, and ice cream.

The Umutina indigenous people, who live in the city of Barra do Bugres in the state of Mato Grosso, Brazil, traditionally collect bacaba fruit in the forest and use this substrate to make a beverage called bacaba chicha. The beverage is also known as bacaba wine or bacaba milk, as the crushed bacaba almonds, prepared with water, produce a beverage that is creamy/light brown in color and has a

pleasant taste, similar to açai (*Euterpe oleracea*) (ISA, 2009).

Chicha can be prepared using several cereals and fruits obtained by spontaneous fermentation, or drunk just after preparation (non-fermented) (Vallejo *et al.*, 2013). The Umutina people make chicha using bacaba, pumpkin, rice, corn, sweet corn, and maniva.

The mesocarp of bacaba is rich in fat, carbohydrates, and total dietary fibers (39.3 g 100 g<sup>-1</sup> fresh weight). It is also a good source of unsaturated fatty acids, with a profile similar to that of olive oil. The total soluble solid of bacaba fruits is 7.89°B and pH 5.3-4.8, with a gross energy value of  $606.3 \pm 12.8$  kcal 100g<sup>-1</sup> fresh weight. In addition, bacaba is a promising source of phenolic compounds; it has a high anthocyanin content and significant antioxidant capacity (Abadio Finco *et al.*, 2012).

In spontaneous cereal-based fermentations is usually observed an association between lactic acid bacteria (LAB) and yeast, since yeast growth is favored by the acidification. The bacterial species are belonging to the genera *Lactobacillus*, *Bacillus*, *Leuconostoc*, *Enterococcus*, *Streptomyces*, *Enterobacter*, *Acinetobacter*, *Escherichia*, *Cronobacter*, *Klebsiella*, *Bifidobacterium* and *Propionibacterium*. The yeasts species are from the genera *Candida*, *Saccharomyces*, *Rhodotorula*, and *Pichia* (Almeida *et al.*, 2007; Schwan *et al.*, 2007; Ramos *et al.*, 2010; Miguel *et al.*, 2012; Santos *et al.*, 2012; Freire *et al.*, 2013).

Considering the nutritional profile and the potentially functional properties of bacaba, its consumption must be stimulated between local communities, as the fruits are widely available and help low-income populations achieve rich and healthful nutrition. Because there is a tendency in these populations to consider industrial preparations superior to Brazilian foods, it is important to demonstrate the value of Brazilian produce to ensure an ideal intake of healthy food (Abadio Finco *et al.*, 2012). There is no industrial production of bacaba chicha beverage in Brazil.

This is the first study to identify the microbial diversity in bacaba chicha spontaneous fermentation and to document the processing techniques and the biochemical composition of this beverage. Such information will support the selection of starter cultures and the future production, in laboratories and on an industrial scale, of this traditional beverage of Brazilian Amerindians. This study is important for promoting the appreciation of and safeguarding this Brazilian indigenous beverage as an immaterial cultural heritage.

## Materials and Methods

### Bacaba chicha beverage production and sample collection

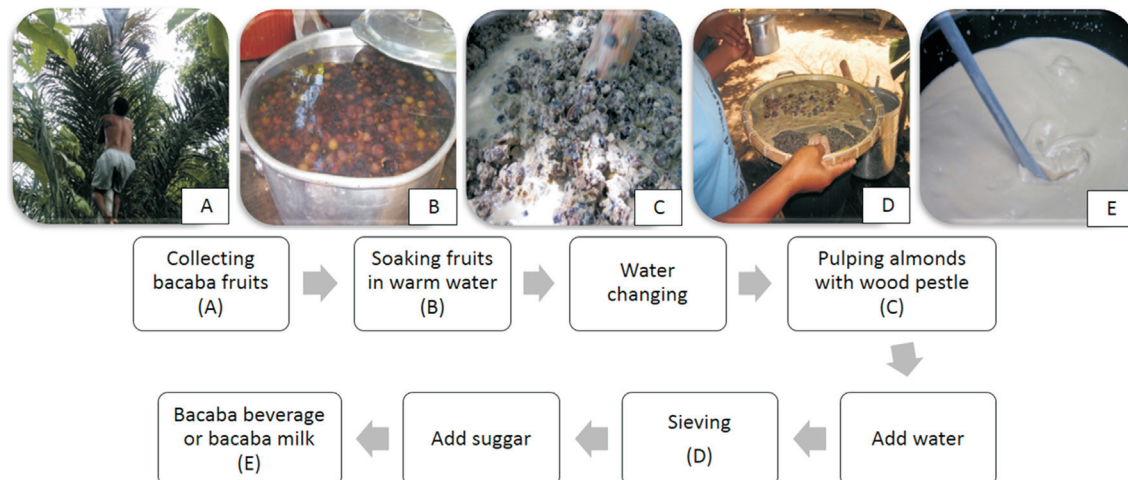
The bacaba fruits were collected in the indigenous Umutina land a day before beverage preparation. The fruits were washed with boiled water. Figure 1 depicts the production of the bacaba beverage. The first step in the preparation of the beverage entailed selecting the fruit, adding enough boiled water to cover the fruit, and letting it stand for half an hour. The water was then changed and the fruit was immediately soaked with wood pestle. The skins were crushed, after which more water was added and a sieve was used to remove the skins and seeds. Sugar cane was added and the substrates were mixed. The beverage can be consumed immediately or after allowing it to ferment for a few hours. The time between harvesting the fruits and preparing the beverage was approximately 30 h.

Samples of the ready bacaba beverage were taken in duplicate. Sequential tenfold dilutions in 0.1% peptone (0.1% peptone; HiMedia, Mumbai, India and 0.5% NaCl; Merck, Darmstadt, Germany) were prepared to quantify the microbial groups. Undiluted samples (50 mL) were taken in duplicate for pH and soluble solid determination, high-pressure liquid chromatography (HPLC) analysis, DNA extraction, and centesimal composition.

### Culture-dependent microbiological analyses

#### Enumeration and isolation of microorganisms

After mixing the sample in a Stomacher® 80 Biomaster, for 60 s, tenfold dilutions in saline peptone water ( $10^{-2}$ - $10^{-6}$ ) were prepared and spread in duplicate on nutrient agar (Merck, Darmstadt, Germany) to perform an aerobic mesophilic bacterial count; on de Man, Rogosa & Sharpe (MRS) agar (Merck, Darmstadt, Germany) containing 0.1% nystatin (Sigma, St. Louis, EUA) for LAB; violet



**Figure 1** - Photographs and flow diagram representing the bacaba chicha beverage preparation.

red bile glucose (VRBG) agar (Oxoid, Hampshire, England) for Gram-negative bacteria; and yeast extract peptone glucose (YEPG) agar (Merck, Darmstadt, Germany), pH 3.5 for yeast count. The plates were incubated aerobically at 28 °C for 48 h and 5 days for bacteria and yeasts, respectively. Based on macroscopic observations, the square root of the total colonies was randomly chosen. The isolates were morphologically identified, purified, and preserved in YEPG broth with 20% glycerol at -20 °C for further identification.

#### *Phenotypic and genotypic characterization of isolates*

The purified isolates were examined by cell morphology, and Gram stain, catalase, oxidase, motility, and sporulation tests were performed as recommended in *Bergey's Manual of Determinative Bacteriology* (Holt *et al.*, 1994) and *The Prokaryotes* (Hammes and Hertel, 2003). The isolates were grouped according to their features and subjected to further biochemical testing.

The Gram-negative strains were identified using Bac tray kits I, II (oxidase negative), and III (oxidase positive) (Laborclin, Paraná, Brazil), according to the manufacturer's instructions. The Bac tray software for *Enterobacter* identification (Laborclin) was used to interpret the results.

Gram-positive bacteria were identified according Magalhães *et al.* (2010). Gram-positive bacteria were divided in spore-formers and non-spore-formers. Gram-positive, non-spore-forming, catalase-negative and oxidase-negative rods and cocci were presumptively classified as LAB and thus were classified as obligately homofermentative, facultatively heterofermentative, and obligatory heterofermentative by their ability to produce CO<sub>2</sub> from glucose. Biochemical characterization was performed by measuring urease activity, as well as with the gelatinase test, the Triple Sugar and Iron (TSI) test, and the Voges-Proskauer (VP) test. Each isolate was also tested for the ability to ferment carbohydrates.

#### *REP-PCR analyses*

Following the preliminary phenotypic characterization, molecular biology-based grouping of the isolates was performed using repetitive extragenic palindromic (REP)-PCR. The bacterial DNA from pure cultures was extracted using a 20 µL aliquot of ultra-pure water added to the pellet. DNA was quantified to 60 ng. The suspension was then subjected to a 90 °C/15 min thermocycling program and REP-PCR according de Melo Pereira *et al.* (2012). The extracts were used for PCR without further processing. Rep-PCR (GTG5-primer) was carried. Amplification products were separated by electrophoresis on a 1.8% (w/v) agarose gel at 70 V for 4 h and stained with SYBR Green (Invitrogen, Foster City, CA, USA). DNA fragments were visualized by UV transillumination, and images were captured using a Polaroid camera. A ladder marker (GeneRuler 100 bp DNA Ladder Plus) was used as a size reference. The

REP-PCR profiles were normalized, and a cluster analysis was performed using BioNumerics<sup>®</sup> v.6.6 software (Applied Maths, Belgium). The dendrogram was calculated on the basis of Dice's coefficient of similarity with the unweighted pair group method with the arithmetic averages (UPGMA) clustering algorithm. Based on the phenotypic and genotypic grouping, representative isolates were selected and subjected to sequencing according de Melo Pereira *et al.* (2012).

#### *Culture independent analysis: PCR-DGGE*

Total DNA was extracted from the samples of bacaba chicha beverage using a QIAamp DNA kit (Qiagen, EUA) according to the manufacturer's instructions. To conduct the DGGE analyses, the PCR products from the microbial community were analyzed using a Bio-Rad DCode universal mutation detection system (Bio-Rad, Richmond, CA). Table 1 presents information regarding the primers and PCR-DGGE conditions. Aliquots (2.0 µL) of the amplification products were analyzed by electrophoresis on 0.8% agarose gels before they were subjected to DGGE according de Melo Pereira *et al.* (2012). The gels were visualized via UV transillumination. Individual bands in the DGGE profiles were excised and amplified to provide a template for sequencing. The conditions for amplification were the same as those described for DGGE analysis, using the same primer without the GC clamp. The new PCR products were purified using a QIAEX<sup>®</sup> III purification kit (Qiagen, Chatsworth, CA), following the manufacturer's protocol. The PCR products were sequenced by UNESP, Jaboticabal, São Paulo. The sequences were then compared to the GenBank database using the BLAST algorithm (NCBI).

#### *Physicochemical, metabolite, and nutritional composition*

The pH was measured according to the methodology proposed by the Association of Official Analytical Chemists (AOAC) International, at room temperature, using a digital pH meter (Micronal B474 model, São Paulo, Brazil) (AOAC, 2000).

Moisture, dry matter, fat, and ash content were determined according to the methodology proposed by AOAC (2000). Total nitrogen content was determined using the Kjeldahl method, and crude protein content was calculated using the conversion factor 6.25. The concentration of carbohydrates was determined as 100 - (% moisture + % protein + % fat + % ash content), and energy value was calculated using the Atwater method (Wisker and Feldeheim, 1990).

The concentrations of alcohols (ethanol, glycerol, and methanol), organic acids (oxalic, citric, tartaric, malic, succinic, propionic, butyric, acetic, and lactic), and carbohydrates (glucose, sucrose, fructose, maltose, and raffinose) were determined by HPLC according Duarte *et al.* (2010) using a Jasco chromatograph equipped with a refractive index (RI) detector (Jasco 830-RI, Madrid, Spain) and UV-

**Table 1** - DGGE-PCR primers used to detect the microorganisms in bacaba chicha beverage.

Primer	Sequence (5'-3')	Community	Target	PCR conditions	References
338fGC	GCA CGC GGG GAC TCC TAC GGG AGG CAG CAG	Bacteria	V3 region of the 16S rRNA gene	1	a
518r	ATT ACC GCG GCT GCT GG				
27fGC	AGA GTT TGA TCC TGG CTC AG	Bacteria	16S rRNA gene	2	
1512r	ACG GCT ACC TTG TTA CGA CT				
NS3fGC	GCA AGT CTG GTG CCA GCA GCC	Yeast	18S region of the rDNA	3	b
YM951r	TTG GCA AAT GCT TTC GC				
ITS1fGC	TCC GTA GGT GAA CCT GCG G	Yeast	ITS region	4	
ITS4r	TCC TCC GCT TAT TGA TAT GC				

GC clamp CGC CCG CCG CGC GCG GCG GGC GGG GCG GG; f forward primer, r reverse primer.

PCR Condition 1 - denatured for 5 min at 95 °C; 30 cycles; denaturing at 92 °C / 1 min; annealing at 55 °C / 1 min and extension at 72 °C / 1 min; final extension at 72 °C / 10 min.

PCR Condition 2 - denatured for 5 min at 94 °C; 30 cycles; denaturing at 94 °C / 40 s; annealing at 52 °C / 1 min and extension at 72 °C / 1 min; final extension at 72 °C / 7 min.

PCR Condition 3 - denatured for 5 min at 95 °C; 35 cycles; denaturing at 95 °C / 1 min; annealing at 50 °C / 1 min and extension at 72 °C / 1 min; final extension at 72 °C / 7 min.

PCR Condition 4 - denatured for 5 min at 95 °C; 30 cycles; denaturing at 95 °C / 30 s; annealing at 52 °C / 30 s and extension at 72 °C / 1 min. final extension at 72 °C / 10 min.

a - Øvreås *et al.*, 1997.

b - Haruta *et al.*, 2006.

Visible detector (Jasco 870-UV-visible). A Chrompack column (300 mm x 6.5 mm) at 60 °C, using 5 mM sulphuric acid as the eluent, at a flow rate of 0.5 mL min<sup>-1</sup> and a sample volume of 20 µL was used.

## Results and Discussion

### Microbial identification by culture-dependent/independent methods

The bacaba chicha beverage contained similar mesophilic bacteria, LAB, and a yeast population in quantities of 4.8, 4.9, and 4.8 log cfu mL<sup>-1</sup>, respectively. The Gram-negative bacteria population was lower, at 3.3 log cfu mL<sup>-1</sup>.

The 543 bacterial and 279 yeast isolates were biochemically classified and grouped by REP-PCR using BioNumerics (Figure 2 and 3). Considering the patterns obtained, 51 bacterial and 26 yeast isolates were submitted to DNA-sequencing analysis. Gram-positive bacteria dominated the microbiota. The sequencing of 42 Gram-positive isolates revealed two genera belonging to the LAB group-*Enterococcus* spp. (n = 39) and *Leuconostoc* spp. (n = 3). Phylogenetic analysis of the 16S rRNA gene showed that these isolates were closely related to *Enterococcus durans*, the dominant bacteria, *E. hirae*, and *Leuconostoc lactis* (99% similarity). *Enterococcus* spp. are found in human feces and appear in soil, surface water, plants, and vegetables. This genera is found in dairy product fermentation and fermented sausages and olives (Ong *et al.*, 2012). LAB metabolism contributes to flavor promotion and beverage preservation. *Enterococcus* is reported to be responsible for contributing to flavor development, probably through proteolysis, lipolysis, and citrate breakdown (Foulquié Moreno *et al.*, 2006; Shori, 2012).

Representative Gram-negative bacteria isolated from the bacaba beverage were tested and grouped according to morphological and biochemical features. The sequencing of representative isolates by REP-PCR identified four isolates as belonging to the genus *Enterobacter*, with high similarity to *E. hormaechei*, and three isolates were identified as *Pantoea dispersa*.

*Enterobacter*, a member of the Enterobacteriaceae family, is a ubiquitous bacterium that adapts to a wide variety of environments and can be isolated from different sources and foods, such as infant formula, cereal products, milk powder, environmental samples (Shaker *et al.*, 2007), Chinese liquor (Zheng *et al.*, 2012), grapes (Barata *et al.*, 2012), and sobia, a wheat and malt beverage (Gassem, 2002). *E. hormaechei* has been isolated from inyu, a Taiwanese soy sauce (Wei *et al.*, 2013). It is not possible to determine whether the presence of *Enterobacter* in bacaba fruits is due to contamination from the environment or as a constituent of endophytic populations, as reported in strawberries (de Melo Pereira *et al.*, 2012).

The yeast population was composed mainly of *Pichia caribbica* (n = 19), and in lower numbers, *Pichia guilliermondii* (n = 8). Yeasts are frequently isolated from fruit surfaces, with populations ranging from 2 to 6 log cfu/g<sup>-1</sup> on apples, grapes, strawberries, and masau fruit (Nyanga *et al.*, 2007). *Pichia* spp. typically form films on liquid media and are known to be important in producing indigenous foods in various parts of the world. *Pichia* is frequently found on grape surfaces, growing during the initial stage of wine fermentation (Urso *et al.*, 2008; Stringini *et al.*, 2009; Chavan *et al.*, 2009; Li *et al.*, 2010) and in rice wine (Lv *et al.*, 2012). *Pichia* spp. are normally present at the beginning of wine fermentation, mainly in damaged rip-



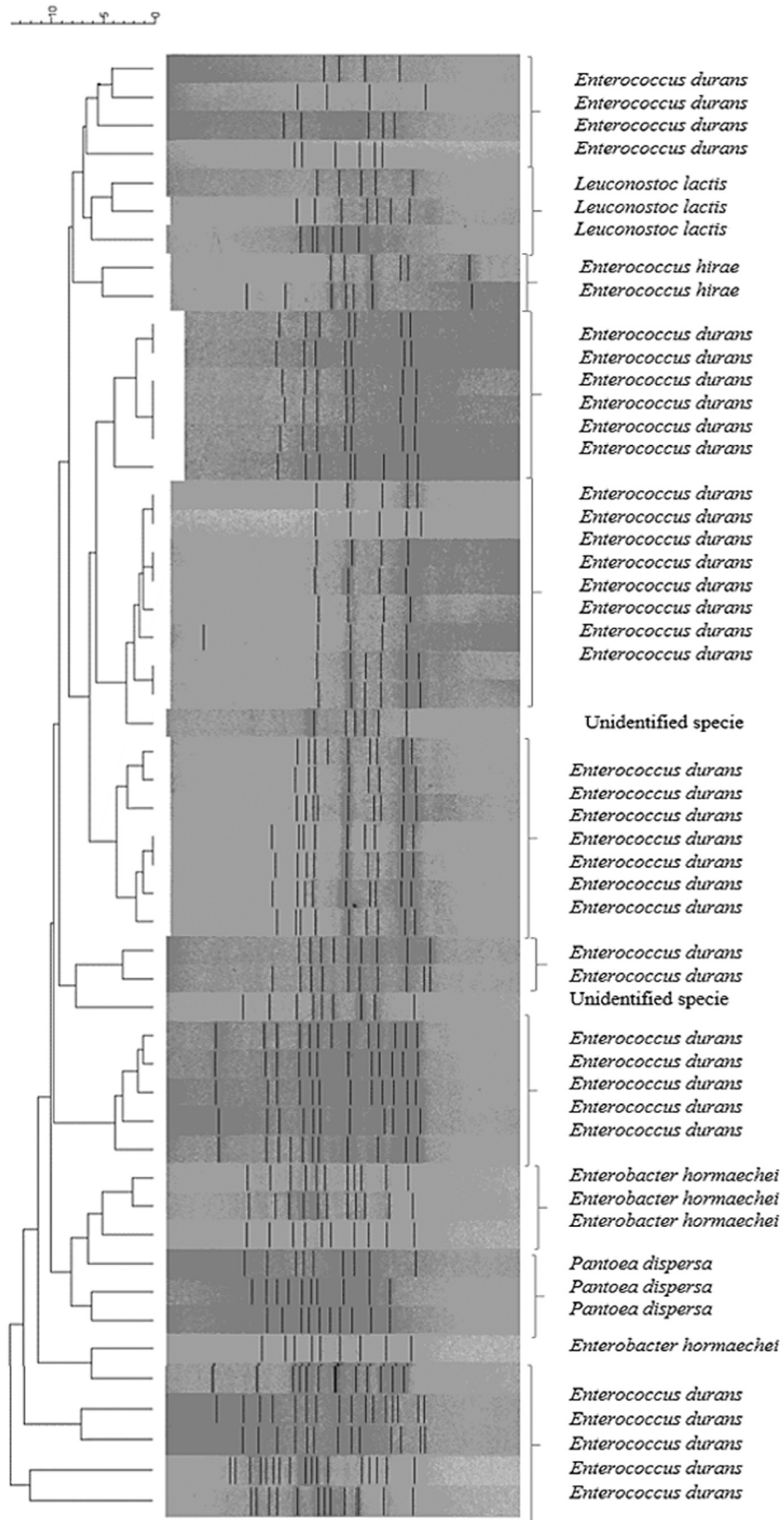


Figure 2 - Dendrogram generated after cluster analysis of Rep-PCR bacterial DNA fingerprints in bacaba chicha beverage.

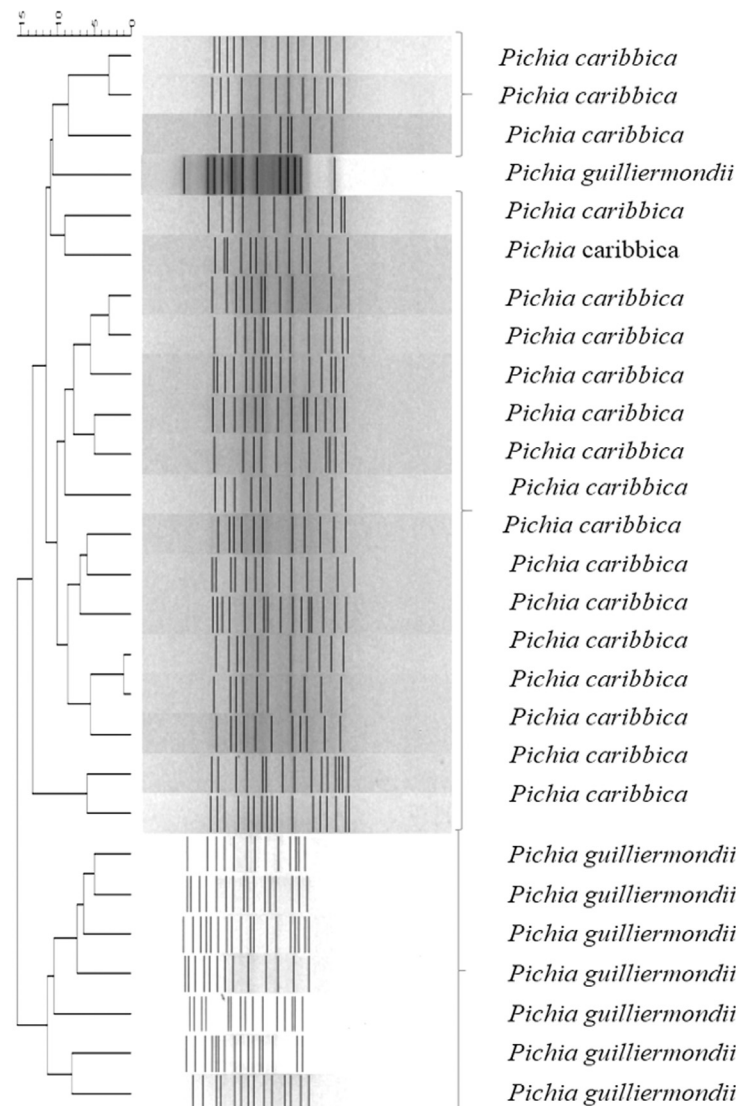
ened fruits, when the sugar is more available (Barata *et al.*, 2012). Chanprasartsuk *et al.* (2010) reported *Hanseniaspora uvarum* and *P. guilliermondii* as the main species isolated from freshly crushed pineapple juice and natural fermented wine from Thailand and Australia, but *Saccharomyces* yeasts were not found.

The association of yeast and LAB bacteria found in bacaba preparations has also been described in several other fermented foods produced from cassava, rice, peanuts, and fruit (Caplice and Fitzgerald, 1999; Gassem, 2002; Almeida *et al.*, 2007; Schwan *et al.*, 2007; Wilfrid Padonou *et al.*, 2009; Ramos *et al.*, 2010; Chadare *et al.*, 2010; Miguel *et al.*, 2012; Freire *et al.*, 2013). Stringini *et al.* (2009) verified this association in a West African palm wine, and Nyanga *et al.* (2007) found an association between LAB and yeast in masau fruit and fermented fruit pulp.

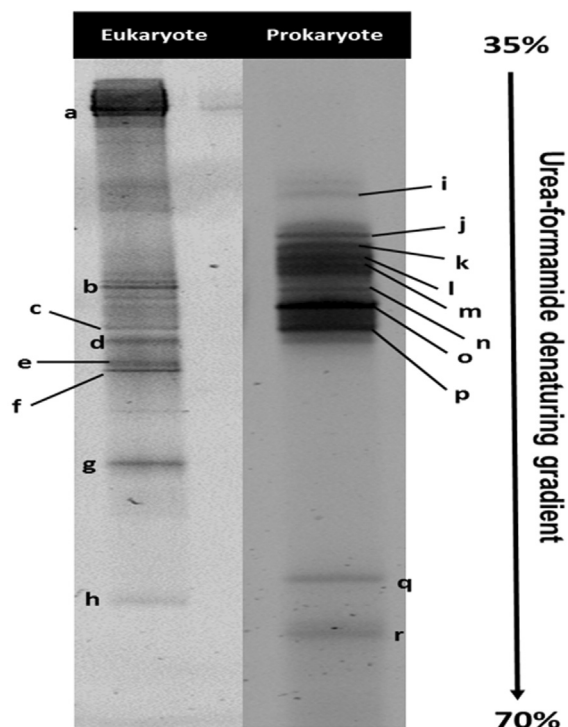
LAB contributes to beverage fermentation by acidification (McDonald *et al.*, 1990), and flavor development is a result of LAB and yeast activity. Yeasts can produce volatile compounds and metabolites that improve the flavor properties of the final product and enhance LAB growth by the release of nutritive compounds (Tofalo *et al.*, 2012).

Figure 4 shows the DGGE profile obtained after amplification of the V3 region of bacterial 16S rRNA and 18S and ITS region of fungal rDNA, obtained directly from the samples. The bands showed the presence of *Enterococcus* spp. and *Enterobacter* spp., as detected by REP-PCR, and uncultured bacteria. The DGGE assessment revealed the further presence of *Propionibacterium avidum* and *Acetobacter* spp.

PCR-DGGE was not able to detect the *Leuconostoc* species. It should be pointed out that the cell numbers of those species fell below the detection limit of PCR-DGGE ( $10^3$  cfu mL<sup>-1</sup>) (Cocolin *et al.*, 2001). The PCR-DGGE pro-



**Figure 3** - Dendrogram generated after cluster analysis of Rep-PCR yeast DNA fingerprints in bacaba chicha beverage.



Identical profiles were obtained for the following primers pairs: Eukaryote (NS3fGC/YM951r and ITS1fGC/ITS4r) Prokaryote (338fGC/518r and 27fGC/1512r)

**Figure 4** - PCR-DGGE profiles of the Eukaryote and Prokaryote communities in bacaba chicha beverage. Eukaryote: The closest relatives of the fragments sequenced, based on a search of GenBank (> 99% similarity), were bands a - uncultured Eukaryote; b - *Pichia caribbica*; c - uncultured *Malassezia*; d - uncultured Eukaryote; e - not sequenced; f - uncultured Eukaryote; g - not sequenced; h - not sequenced. Prokaryote: The closest relatives of the fragments sequenced, based on a search of GenBank (> 97% similarity), were bands: i - not sequenced; j - not sequenced; k - *Propionibacterium avidum*; l - *Enterococcus* spp.; m - uncultured bacteria; n - *Enterobacter* spp.; o - *Acetobacter* spp.; p - uncultured bacteria; q - not sequenced; r - uncultured bacteria.

file of fungal DNA extracted directly from the samples was composed mainly of *Pichia caribbica* and others uncultured eukaryotes.

Conventional microbiological methods, which require selective enrichments and sub-culturing, are dependent on the ability of a microorganism to grow on the medium under the culturing conditions applied, on the number of isolates, and on the selection methodology. Thus, those methods might fail to detect some microorganisms, excluding members of the microbial community (Jany and Barbier, 2008; Magalhães *et al.*, 2010). Culture-independent approaches have the advantage of being able to reveal microorganisms that are difficult or impossible to culture (Quigley *et al.*, 2011).

#### Chemical composition of bacaba chicha beverage

The pH value of the final beverage was 6.2. The organic acids that were present were probably the result of yeast and bacteria metabolism during the preparation time,

when the fruit rested in warm water. Organic acid concentration was low, and therefore not enough to reduce the pH.

From a nutritional point of view, the bacaba fruit mesocarp has a moisture content of 58.0 g 100 g<sup>-1</sup>, fat content of 30.2 g 100 g<sup>-1</sup>, ash content of 1.2 g 100 g<sup>-1</sup>, protein content of 4.6 g 100 g<sup>-1</sup>, and 606.3 kcal 100 g<sup>-1</sup> (Escrive *et al.*, 1999). The bacaba beverage exhibited an energy value of 76.7 kcal 100 mL<sup>-1</sup>. The macronutrient composition consisted of 16.47 ± 0.73 g 100 mL<sup>-1</sup> dry matter content, 2.2 ± 0.0 g 100 mL<sup>-1</sup> fat content, 3.36 ± 0.44 g 100 mL<sup>-1</sup> protein content, and 10.87 ± 0.26 g 100 mL<sup>-1</sup> carbohydrate content (Table 2). The high carbohydrate content reflects the added sugar. The soluble protein content was considerably higher than that reported in caxiri (1.0%) and corn and rice calugi (1.53%) (Miguel *et al.*, 2012; Santos *et al.*, 2012).

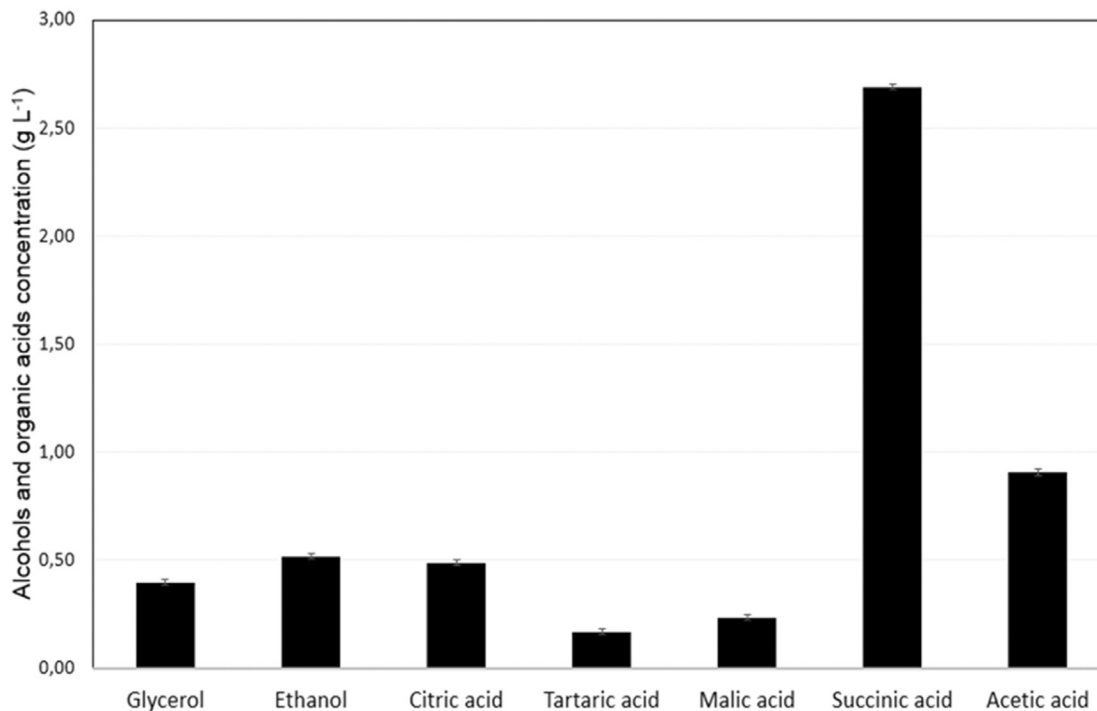
The indigenous Huitoto, Andoque, Yukuna, Muinane, and Miraña tribes that settled in the Amazon region of Colombia consume the bacaba fruit mesocarp in the form of chicha or cahuana, contributing a substantial amount of fat and carbohydrate to their diet (Escrive *et al.*, 1999).

A spontaneous fermentation occurred when the fruit rested in warm water, it underwent three types of spontaneous fermentation, confirmed by HPLC results: alcoholic, succinic, and acetic (Figure 5). The metabolic compounds verified in the bacaba beverage occurred because the long postharvest period stimulates spontaneous fermentation. Considering that the first preparation step consists of placing the fruit in warm water for half an hour, the increased water content, the availability of nutrients, and the temperature created ideal conditions for the natural microorganisms present on the fruit surface and environment to grow. A specific microbiota produced metabolic compounds and modifying the taste and smell of the beverage. These microbial communities vary in composition and diversity according to a range of environmental factors, including pH, sugars, moisture availability, fruit growing conditions, and transport and storage procedures (Nyanga *et al.*, 2007; Aguiar *et al.*, 2013; Leff and Fierer, 2013). The spontaneous postharvest fermentation is also favored by intrinsic

**Table 2** - Nutritional composition of bacaba chicha beverage.

Nutrient	Composition (g 100mL <sup>-1</sup> ± DP)
Moisture	83.58 ± 0.70
Dry matter	16.47 ± 0.73
Ash	0.18 ± 0.0
Fat	2.20 ± 0.0
Fiber	1.68 ± 0.13
Protein (N x 6.25)	3.36 ± 0.44
Carbohydrate	10.87 ± 0.26
Energy (kcal 100mL <sup>-1</sup> )	76.70

Values are means ± SD for triplicate analys.



**Figure 5** - Organic acids and alcohols production by natural microbiota in bacaba chicha beverage.

and extrinsic factors such as pH (3-5), sugar content, and the respiratory activity of the fruit, which turn the environment anaerobic and prone to fermentation (Aguiar *et al.*, 2013).

The concentration of succinic acid (2.69 g L<sup>-1</sup>), acetic acid (0.9 g L<sup>-1</sup>), and citric acid (0.49 g L<sup>-1</sup>) was due to the microbiota metabolism (LAB and yeast) during the first step of the bacaba beverage production. Acetic acid in higher concentrations is generally considered an undesirable metabolite in fruit wines (Barata *et al.*, 2012; Duarte *et al.*, 2010). Malic and tartaric acid were present in lower concentrations, but other acids (lactic, oxalic, propionic, and butyric) were not detected by HPLC. The sugars present in the beverage and the respective concentrations (g L<sup>-1</sup>) were fructose (3.62 ± 0.3), glucose (4.0 ± 0.9), and sucrose (88.4 ± 1.0). The sucrose was added during the preparation of the beverage. Maltose and raffinose were not found.

Ethanol was present at a low level (0.52 g L<sup>-1</sup>) and the glycerol content was 0.4 g L<sup>-1</sup>; they are the main products of fermentative yeasts. High temperatures (19-31 °C) increase ethanol production (Zajsek and Gorsek, 2010). The ambient temperature in the Umutina region during the beverage production ranged from 30 ° to 35 °C. Methanol was not identified. Due to the low ethanol content, the bacaba beverage was classified as a non-alcoholic beverage.

## Conclusion

This study represents the first attempt to examine the bacaba chicha beverage. The microbiological results indi-

cated that the better microbial community description could be reached when using culture-dependent and culture-independent methods together. The study of the chemical composition and the metabolite changes helped in understanding the nutritional importance of the bacaba beverage to the Umutina people and in validating its consumption, as bacaba fruit is widely available and has high nutritional value. This study is important for promoting the appreciation of and safeguarding this Brazilian indigenous beverage as an immaterial cultural heritage.

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