Food Research International 49 (2012) 524–532

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Physico-chemical and microbiological characterization of corn and rice 'calugi' produced by Brazilian Amerindian people

Maria Gabriela da Cruz Pedrozo Miguel [a,b], Marianna Rabelo Rios Martins Santos [b], Whasley Ferreira Duarte [b], Euziclei Gonzaga de Almeida [b], Rosane Freitas Schwan [b,*]

[a] Food Sciences Department, Federal University of Lavras, 37200-000 Lavras, MG, Brazil
[b] Biology Department, Federal University of Lavras, 37200-000 Lavras, MG, Brazil

ARTICLE INFO

Article history:
Received 27 June 2012
Accepted 11 August 2012

Keywords:
Corn
Rice
Fermented foods
Bacteria
Yeasts
Calugi

ABSTRACT

'Calugi' is a fermented porridge that is produced from corn and rice by Javaé Amerindians. Samples of this porridge were chemically and microbiologically characterized. The microbial population was composed of yeasts, aerobic mesophilic bacteria (AMB), lactic acid bacteria (LAB), acetic acid bacteria (AAB) and some enterobacteria. The population of lactic acid bacteria (LAB), acetic acid bacteria (AAB) and yeasts increased slightly during 'calugi' fermentation. During the initial fermentation period (12 and 24 h), counts of the bacterial population (LAB, AMB and enterobacteria) and yeast increased. After 48 h of fermentation, the population of mesophilic bacteria was 5.06 log CFU/mL; lactic acid bacteria (LAB), 4.69 log CFU/mL; yeast, 4.37 log CFU/mL; enterobacteria, 3.29 log CFU/mL and acid acetic bacteria (AAB), 3.14 log CFU/mL. During the fermentation process, *Lactobacillus plantarum*, *Streptococcus salivarius*, *Streptococcus parasanguis*, *Weissella confusa*, *Enterobacter cloacae*, *Bacillus cereus* and *Bacillus* sp. and the yeasts *Saccharomyces cerevisiae*, *Pichia fermentans* and *Candida* sp. were detected by PCR-DGGE. The LAB were dominant during the process and were likely most responsible for the reduction in pH value, which permitted yeast growth. Carbohydrates (70.48 g/L — maltose), alcohols (1.70 g/L — glycerol) and acids (4.56 g/L — acid lactic) were identified by HPLC. Fifteen volatile compounds were identified and quantified by GC-FID. From the fermentation, acetaldehyde, 1-1-diethoxyethane, isobutyl acetate, ethyl acetate, furfuryl alcohol and nonanoic acid were identified, with maximum concentrations of 457.02 μg/L, 73.96 μg/L, 54.03 μg/L, 24.82 μg/L, 755.82 μg/L, 61.85 μg/L, respectively.

1. Introduction

In several countries, foods and beverages are largely produced from cereals, and the preparation of many indigenous or traditional fermented foods and beverages remains a home art (Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003).

Spontaneous food fermentations are performed by proto-cooperation between yeast, lactic acid bacteria (LAB), acetic acid bacteria (AAB) and fungi (Blandino et al., 2003). The symbiosis between bacteria and yeasts has been reported in the fermentation of traditional beverages such as hawaijar (Jeyaram et al., 2008), doenjang (Kim et al., 2009), kefir (Miguel, Cardoso, Lago, & Schwan, 2010), cauim (Ramos et al., 2010) and caxiri (Santos, Almeida, Melo, & Schwan, 2012).

Studies of a Brazilian beverage produced from cereals such as rice, cassava, peanuts, and cotton seed demonstrated that both bacteria and yeast were present during fermentation. Recently, Santos et al. (2012) reported the presence of *Bacillus subtilis*, *Bacillus pumilus*, *Lysinibacillus fusiformis*, *Lactobacillus fermentum*, *Pediococcus acidilactici*, *Enterobacter* sp., *Saccharomyces cerevisiae*, *Rhodotorula mucilaginosa*, *Pichia guilliermondii* and *Pichia membranifaciens* during caxiri fermentation. In cotton seed and rice fermentations (Ramos, Almeida, Freire, & Schwan, 2011), *Lactobacillus vermiforme*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *B. subtilis* and *Bacillus cereus* and the yeasts *Candida parapsilosis*, *Candida orthopsilosis* and *R. mucilaginosa* were identified. Almeida, Rachid, and Schwan (2007) and Schwan, Almeida, Souza-Dias, and Jespersen (2007) identified *L. plantarum*, *Lactobacillus pentosus*, *Enterobacter cloacae*, *B. cereus*, *Bacillus circulans*, *B. pumilus* and the yeasts *S. cerevisiae*, *C. parapsilosis*, *Candida intermedia* and *P. guilliermondii*, among other microorganisms, in the beverage cauim.

Microbial activity produces many different chemical compounds, such as the organic acids commonly found in fermented products, as a result of hydrolysis and biochemical metabolism. The quantitative determination of organic acids such as lactic acid, acetic acid, malic acid and propionic acid in fermented foods is important for

* Corresponding author at: Departamento de Biologia, Universidade Federal de Lavras, 37.200-000 Lavras-MG, Brazil. Tel.: +55 35 38291614; fax: +55 35 38291100.
E-mail addresses: gabis_bio@posgrad.ufla.br (M.G.C.P. Miguel), mariannarabelo@yahoo.com.br (M.R.R.M. Santos), whasleyfd@yahoo.com.br (W.F. Duarte), euziclei@yahoo.com.br (E.G. de Almeida), rschwan@dbi.ufla.br (R.F. Schwan).

0963-9969
http://dx.doi.org/10.1016/j.foodres.2012.08.012

technical, nutritional, sensorial and microbial reasons (Shukla et al., 2010).

The beverage 'calugi', which is made from different substrates such as rice, cassava and corn, is traditionally produced and consumed by Javaé Brazilian Indians. This non-alcoholic beverage is consumed by adults and children. However, to the best of our knowledge, the physicochemical parameters, volatile composition and microbiota during the fermentation of 'calugi' have not been determined. Thus, the aims of this work were to identify the microorganisms involved in the fermentation of 'calugi' by PCR-DGGE and identify the metabolites present in the fermentation process by gas chromatography-flame ionization detection (GC-FID) and high-performance liquid chromatography (HPLC).

2. Materials and methods

2.1. Beverage preparation and sampling

A 'calugi' of corn and rice was prepared by the local Amerindian Javaé, who live next to the Formoso do Araguaia mountain (Tocantins, Brazil). To prepare the porridge (Fig. 1), approximately 700 g of dried corn (*Zea mays*), 3 kg of rice and 100 g of sweet potato (*Ipomoea batatas* L.) were used. The corn was soaked in water for 30 min before maceration in a rustic wooden pestle. The resulting corn flour was mixed with 3 L of water and sieved to remove the peel. The corn and rice were mixed with 7 L of water and cooked for 2 h; the mixture was stirred every 10 min. The sweet potato was peeled and washed before use. Approximately 40 min after the end of cooking (when the porridge had cooled), the inoculum (mastication juice of the sweet potato) was added. Then, the mixture was homogenized and allowed to ferment at ambient temperature (approximately 30 °C). Three fermentations were performed in the same conditions described above.

2.2. Sampling

Twenty milliliters of cooked and fermenting substrate was collected at 12 h intervals and added to a sterile bottle containing 180 mL of saline peptone solution (0.1% peptone, 0.5% NaCl, and 0.03% $Na_2H_2PO_4$). The samples were maintained at 4–6 °C until analysis (approximately 4 h).

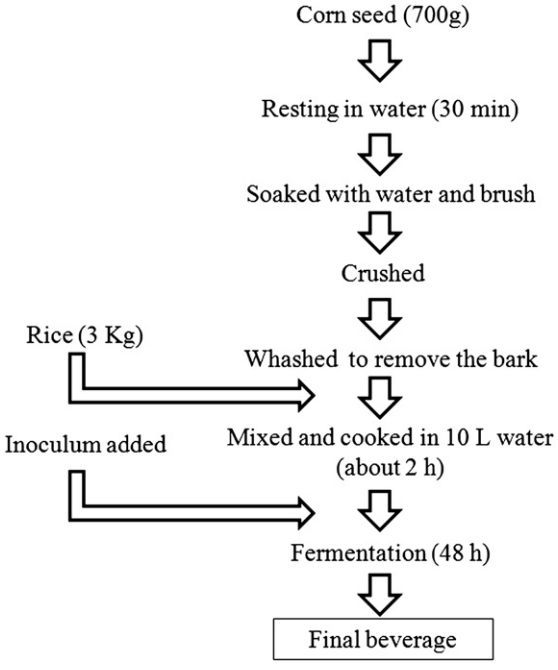

Fig. 1. Flow diagram for the manufacture of the beverage from corn and rice.

2.3. Isolation and counting

Bacteria and yeasts were enumerated on five different culture media, namely MRS (De Man Rogosa Sharpe, Merck, Darmstadt, Germany), GYC [50 g/L glucose (Merck, Darmstadt, Germany), 10 g/L yeast extract (Merck, Darmstadt, Germany), 30 g/L calcium carbonate (Merck, Darmstadt, Germany), pH 5.6], nutrient agar medium, VRBG agar (violet red bile with glucose) (Oxoid, Hampshire, England) and YPD agar [10 g/L yeast extract (Merck, Darmstadt, Germany), 10 g/L peptone (Himedia, Mumbai, India), 20 g/L glucose (Merck, Darmstadt, Germany), 20 g/L agar (Merck, Darmstadt, Germany)]. MRS agar containing 0.1% cysteine-HCl was used to enumerate LAB under anaerobic conditions. AAB were enumerated on GYC agar containing 0.1% cycloheximide to inhibit yeast growth and 50 mg/L penicillin (Sigma, St. Louis, USA) to inhibit LAB growth. Nutrient agar medium (Merck, Darmstadt, Germany) was used as a general medium for the growth of mesophilic bacteria. The VRBG medium was used for enterobacteria. For yeast enumeration, YPD agar containing 100 mg/L chloramphenicol (Sigma, St. Louis, USA) and 50 mg/L chlortetracycline (Sigma, St. Louis, USA) to inhibit bacterial growth was used. After spreading, the MRS plates were incubated in acrylic anaerobic jars at 30 °C for 3–4 days. YPD and nutrient agar plates were maintained at 30 °C for 2–5 days. VRBG plates were maintained at 36 °C for 3–4 days. GYC plates were maintained at 25 °C for 5–8 days. After the incubation period, the colony-forming units (CFUs) were enumerated.

2.4. PCR analysis

The total DNA was extracted from samples acquired at different times during the fermentation process using the protocol DNA Purification from Tissues [QIAamp DNA Mini Kit (Qiagen, Hilden, Germany)] in accordance with the manufacturer's instructions. The final samples were stored at −20 °C until further use.

The DNA from the bacterial community was amplified with the primers 338fgc (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG-3′) (the GC clamp is underlined) and 518r (5′-ATT ACC GCG GCTGCT GG-3′), which span the V3 region of the 16S rRNA gene (Ovreas, Forney, Daae, & Torsvik, 1997). A fragment of the D1-region of the 26S rRNA gene was amplified with the eukaryotic universal primers NL1GC (5′ CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA TAT CAA TAA GCG GAG GAA AAG-3′) (the GC clamp is underlined) and LS2 (5′-ATT CCC AAA CAA CTC GAC TC-3′), which amplified a fragment of approximately 250 bp (Cocolin, Bisson, & Mills, 2000). All reactions were performed in a 25 μL volume containing 0.625 U Taq DNA polymerase (Promega, Milan, Italy), 2.5 mL 10× buffer, 0.1 mM dNTP, 0.2 mM of each primer, 1.5 mM $MgCl_2$ and 1 mL of extracted DNA. The amplification was performed according to Ramos et al. (2010). Aliquots (2 mL) of the amplified products were analyzed by electrophoresis on 1% agarose gels before the DGGE analysis.

2.5. PCR-DGGE analysis

The PCR products were separated in polyacrylamide gels [8% (w/v) acrylamide:bisacrylamide (37.5:1)] in 1× TAE buffer with a DCode system apparatus (BioRad Universal Dcode Mutation Detection System, Richmond, CA, USA); solutions containing 35–70% denaturant [100% denaturant corresponds to 7 M urea and 40% (v/v) formamide] were used for the bacterial community, and solutions containing 30–60% denaturant were used for the yeast community. The gels were run at 60 °C for 6 h at a constant voltage of 120 V. After electrophoresis, the gels were stained with SYBR-Green I solution (Molecular Probes,

Eugene, UK) (1:10.000 v/v) for 30 min, and the images were visualized and photographed with a transluminator (LPix®).

Various DGGE bands were excised from the acrylamide gels. The DNA fragments were purified with a QIAEX II gel extraction kit (Qiagen, Chatsworth, CA, USA) and re-amplified with the primer 338fgc and 518r for bacteria and NL1 and LS2 for yeast. The PCR products were purified and sequenced by Macrogen Inc. (Seoul, South Korea), and the obtained sequences were compared with those available in the GenBank database with the BLAST algorithm (National Center for Biotechnology Information, Maryland, USA).

2.6. Chemical analysis of the fermented 'calugi' beverage

The samples were characterized by pH, total soluble solids (°Brix), protein, fat (AOAC, 2000), total sugars (Dische, 1962) and starch content (Areas & Lajolo, 1987).

2.7. Substrates and metabolites

Before GC-FID analysis, volatile compounds were extracted according to Duarte et al. (2010). The extract containing the volatile compounds was analyzed in a Shimadzu GC Model 17A equipped with a flame ionization detector (FID) and a capillary column of silica DB Wax (30 m × 0.25 mm i.d. × 0.25 μm) (J & W Scientific, Folsom, Calif., USA). The temperature program began with 5 min at 50 °C, followed by a gradient of 50 °C to 190 °C at 3 °C/min; the temperature was then maintained at 190 °C for 10 min. The injector and detector temperature were kept at 230 °C and 240 °C, respectively. The carrier gas (N_2) was used at a flow rate of 1.2 mL/min. Injections of 1 μL were made in the split mode (1:10). Volatile compounds were identified by comparing the retention times of the compounds in the samples with the retention times of standard compounds injected under the same conditions. Volatile compounds were semi-quantified with 4-nonanol at a final concentration of 312 μg/L as an internal standard. All samples were examined in duplicate.

Carbohydrate (glucose, sucrose, maltose and fructose), organic acid (acetic acid, lactic acid, malic acid, citric acid, propionic acid and succinic acid) and alcohol (ethanol and glycerol) analyses were performed with a Shimadzu liquid chromatography system (Shimadzu Corp., Japan) equipped with a dual detection system consisting of a UV–vis detector (SPD 10A*i*) and a refractive index detector (RID-10A*i*). A Shimadzu ion exclusion column Shim-pack SCR-101 H (7.9 mm × 30 cm) was used at an operating temperature of 30 °C for ethanol and glycerol and 50 °C for acids. Perchloric acid (100 mM) was used as the eluent at a flow rate of 0.6 mL/min. Acids were detected via UV absorbance (210 nm), while alcohols were detected via RID. Carbohydrates were analyzed on a Supelcosil LC-NH_2 column (4.6 mm × 25 cm) operating at 30 °C with acetonitrile:water (75:25) as the mobile phase at a flow rate of 1 mL/min. The sugars were detected via RID. Individual compounds were identified by comparing their retention times with the retention times of certified standards, and concentrations were determined according to external calibration methodology. All samples were analyzed in duplicate.

2.8. Statistical analysis

Principal component analyses were performed with the software XLSTAT 7.5.2 (Addinsoft, New York, N.Y., USA) for group data from microbial counts and metabolites produced during corn and rice 'calugi' fermentation.

3. Results and discussion

3.1. Microbial counts

The populations of LAB, AAB, enterobacteria and yeasts increased slightly during 'calugi' fermentation (Fig. 2). After 48 h of fermentation, the counts of these four groups were 4.69 log CFU/mL (LAB), 4.37 log CFU/mL (yeast), 3.29 log CFU/mL (enterobacteria) and 3.14 log CFU/mL (AAB). LAB, AAB and yeast have been reported to be the most common and important groups of microorganisms in the fermentation of indigenous cereal-based foods produced with sorghum, wheat, maize and rice (Blandino et al., 2003). These groups of microorganisms are responsible for the production of lactic acid, acetic acid and alcohol.

The population of mesophilic bacteria ranged from 4.9 log CFU/mL to 5.06 log CFU/mL. Although mesophilic bacteria are commonly found in different cereal-based fermented foods and beverages as well as roots (Almeida et al., 2007; Foma, Destain, Mobinzo, Kayisu, & Thonart, 2012; Gadaga, Mutukumiraa, Narvhus, & Feresuc, 1999; Ramos et al., 2011), the population found in 'calugi' was lower than that reported by Ramos et al. (2010) for a cauim beverage produced with peanut and rice. This difference could be due to the inoculum source and the substrate used for the beverage preparation.

The LAB were detected throughout the fermentation process. At the beginning of the fermentation, the LAB population was approximately 2.01 log CFU/mL; after 24 h of fermentation, the LAB population reached 4.25 log CFU/mL and remained at this level until the

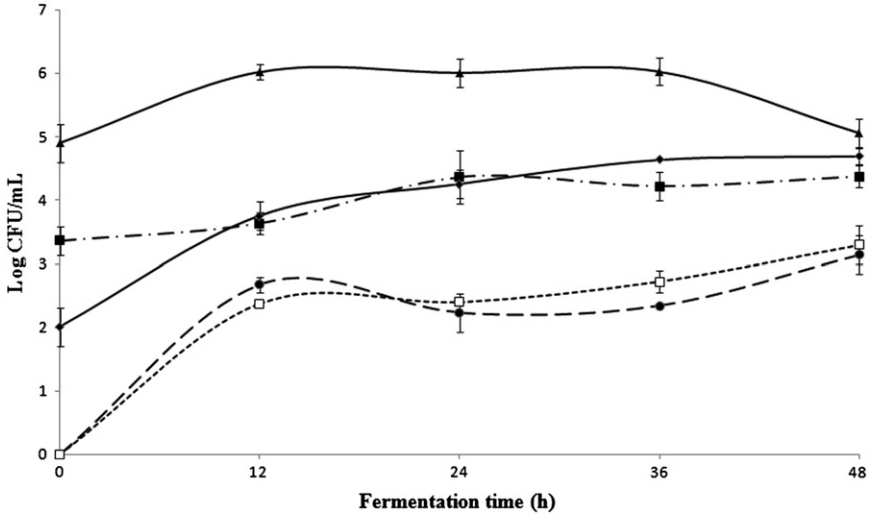

Fig. 2. Numbers (log CFU/mL) of total mesophilic bacteria (▲), acetic acid bacteria (●), lactic acid bacteria (♦), *Enterobacter* (□) and yeast (■) during the 'calugi' fermentation.

end of the process (Fig. 2). According to McDonald, Fleming, and Hassan (1990), LAB are acid-tolerant and often dominate the fermentation of vegetables and cereals, mainly due to their ability to transport and metabolize different carbohydrates. The LAB behavior observed in this work is in good agreement with results reported by Ramos et al. (2011) for cauim produced with cotton seed and rice or with peanut and rice (Ramos et al., 2010). According to these authors, a symbiotic association occurs between the coexisting mesophilic bacteria, LAB and yeast during fermentation to produce the studied beverages.

The yeast population exhibited moderate variations throughout the fermentation process, with values ranging between 3.36 and 4.36 log CFU/mL (Fig. 2). The growth of yeasts in fermented foods is favored by the acidification of the environment by bacteria, and the growth of bacteria is stimulated by the presence of yeasts, which can provide growth factors such as vitamins and soluble nitrogen compounds (Nout & Sarkar, 1999).

Enterobacteria were not detected at 0 h of fermentation (Fig. 2). At 12 h to 48 h of fermentation, the enterobacterial population ranged from 2.37 log CFU/mL to 3.29 log CFU/mL. Similar results were obtained by Almeida et al. (2007) and Madoroba et al. (2011) in their studies of fermented cereal-based foods and beverages. The trend in enterobacterial counts was parallel to that observed for the AAB population throughout the time course of the fermentation (Fig. 2). Moderate variations in the AAB population were observed, which ranged from 2.67 CFU/mL (12 h fermentation) to 3.14 CFU/mL (48 h fermentation). In their studies of ogi, a fermented food produced in Nigeria, Oyarekua (2011) have also reported a low count of enterobacteria and other gram-negative bacteria, which indicates that their growth may have been inhibited by the presence of lactic acid, resulting in a decrease in the population and in the pH value.

3.2. DGGE analysis of the bacterial and yeast communities

The results of the culture-independent methods (PCR-DDGE) demonstrated that the bacterial population was dominant over the yeast population throughout the fermentation process. In most of cereal-based products, fermentation does not occur spontaneously and requires a mixed culture of yeasts, fungi and bacteria. These groups of microorganisms can act simultaneously in a cooperative manner, and thus, the dominance varies during fermentation (Blandino et al., 2003). The predominance of bacteria in this study may be due to the secretion of compounds such as organic acids, which may inhibit the growth of some bacteria and yeasts in the fermenting medium. The succession of bacteria and yeasts is determined by the sensitivities of the microorganisms to the very acidic conditions that develop during the fermentation process (Ampe, Sirvent, & Zakhia, 2001). Figs. 3 and 4 show the results of the DGGE analysis of the bacterial and yeast communities, respectively. Changes in the bacterial community were evident after 12 h of fermentation (Fig. 3). Sequencing of the bands indicated the presence of *L. plantarum* (98% similarity), *Streptococcus salivarius* (99%), *Streptococcus parasanguis*

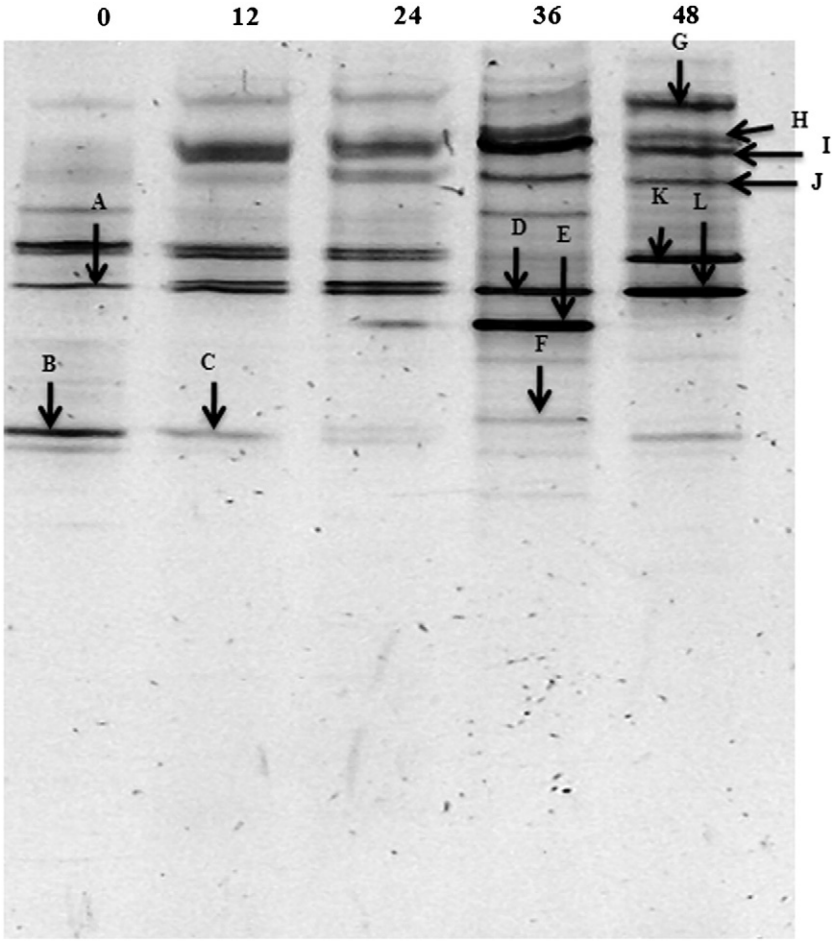

Fig. 3. Denaturing gradient gel electrophoresis profiles of the V3 regions of the bacterial 16S rRNA gene amplified from the 'calugi' beverage throughout the 48 h of fermentation. A = *Streptococcus salivarius* (GU425986.1), B = *Uncultured bacteria* (AB241206.1), C = *Uncultured bacteria* (FN780500.1), D = *Streptococcus salivarius* (CP002888.1), E = *Enterobacter cloacae* (HM438951.1), F = *Bacillus cereus* (HQ333011.1), G = *Lactobacillus plantarum* (EF426261.1), H = *Uncultured bacteria* (FN775581.1), I = *Streptococcus parasanguis* (AM157421.1), J = *Weissella confusa* (HM032145.1), K = *Bacillus* sp. (AY176766) and L = *Streptococcus salivarius* (CP002888.1).

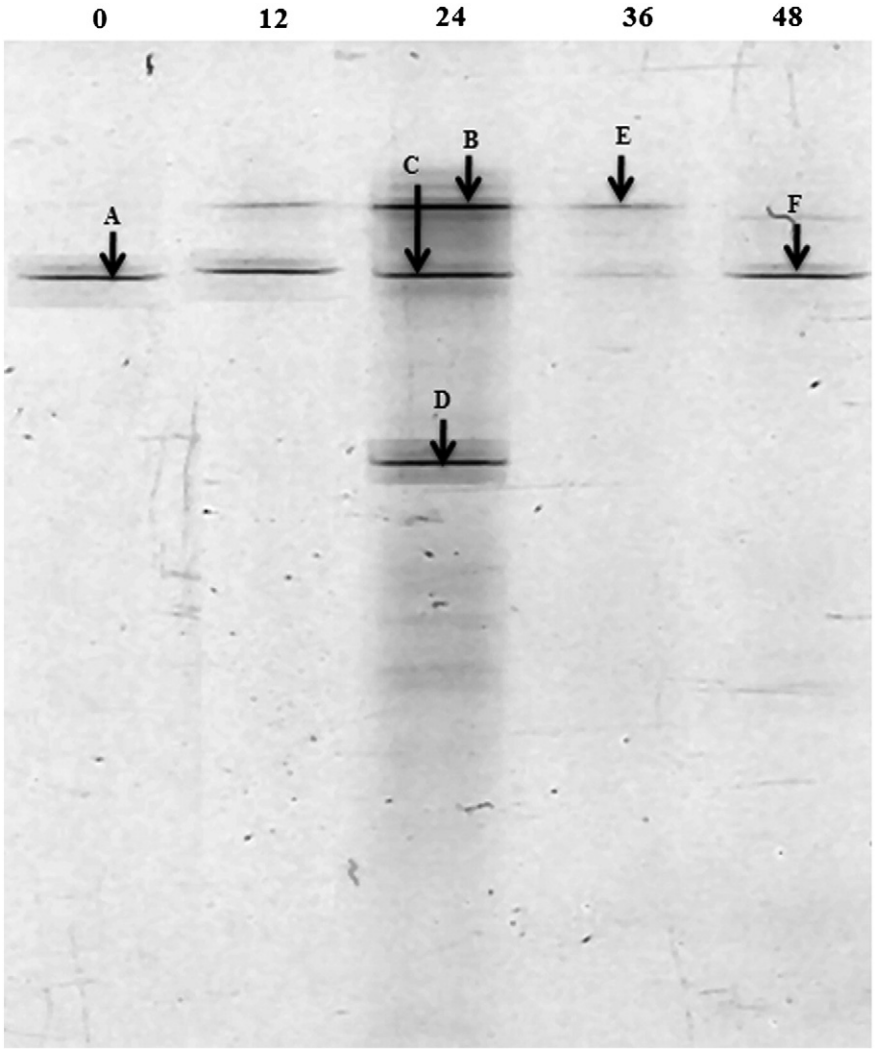

Fig. 4. Denaturing gradient gel electrophoresis profiles of the NS3 regions of the yeast 18S rRNA gene amplified from the 'calugi' beverage throughout the 48 h of fermentation. A = *Saccharomyces cerevisiae* (EU649673.1), B = *Pichia fermentans* (FJ770542.1), C = *Saccharomyces cerevisiae* (GM981616), D = *Candida* sp. (GI190714325), E = *Pichia fermentans* (DQ665310.1) and F = *Saccharomyces cerevisiae* (EU649673.1).

(100%), *Weissella confusa* (98%), *E. cloacae* (100%), *B. cereus* (98%) and *Bacillus* sp. (97%) (Fig. 3) and the yeasts *S. cerevisiae* (99%), *Pichia fermentans* (100%) and *Candida* sp. (98%) (Fig. 4).

The LAB are required to mediate the fermentation process in most fermented foods; species of the genera *Leuconostoc*, *Lactobacillus*, *Streptococcus*, *Pediococcus* and *Micrococcus* are the most common in the fermentation of food and beverages produced using cereals such as sorghum, wheat, maize and rice (Blandino et al., 2003). They also frequently predominate in fermented foods due to their ability to tolerate low pH; species of this group produce a variety of antimicrobial substances and create conditions that are unfavorable for the growth of pathogens and toxigenic and spoilage organisms. The species *L. plantarum* and *W. confusa* were identified by sequencing of DNA from excised bands of DGGE technique in other cereal-based foods such as a rice and peanut beverage (Ramos et al., 2010), cotton seeds and rice (Ramos et al., 2011), cassava dough (Miambi, Guyot, & Ampe, 2003) and togwa (Mugula, Narvhus, & Sørhaug, 2003).

S. salivarius was the predominant species throughout the fermentation of the 'calugi' beverage, indicating that this bacterium is most likely derived from the chewed sweet potato inoculum. This species is a prominent member of the oral microbiota of humans and fermented foods such as yogurt and cheese (Delorme, 2008; Talon, Walter, Viallon, & Berdagué, 2002) and is closely related to *Streptococcus thermophilus* (Bentley, Leigh, & Collins, 1991).

In this study, *Bacillus* species belonging to group cereus were detected during fermentation process. These species have been detected in other indigenous fermentation food in Brazil and Africa, such as caxiri, cotton seed and rice beverage, agbelima and others (Amoa-Awua & Jakobsen, 1995; Ramos et al., 2010; Ramos et al., 2011; Santos et al., 2012). Members of the *Bacillus* genus are typically found in soils and may have harmful effects when detected high populations. These bacteria might be beneficial when secreting a wide range of degradative enzymes, including amylases and proteases, which may be important in the fermentation process (Amoa-Awua & Jakobsen, 1995; Rusul & Yaacob, 1995).

S. cerevisiae was the only yeast detected in the 'calugi' beverage in samples T0 and T12 (Fig. 4). The yeast population changed after 24 h of fermentation, at which time *S. cerevisiae*, *P. fermentans* and *Candida* sp. were detected. After 36 and 48 h of fermentation, *P. fermentans* and *S. cerevisiae* were detected, respectively (Fig. 4). The *S. cerevisiae* has also been reported by others authors in fermented non-alcoholic caium beverages produced by Brazilian Amerindians (Ramos et al., 2010, 2011) and is considered the most common yeast in cereal-based beverages (Blandino et al., 2003). The main role of *S. cerevisiae* in the

Table 1
Physico-chemical characterization of 'calugi'.

Characteristics	Fermentation time (h)				
	0	12	24	36	48
Fat	9.44 ± 0.09	8.74 ± 0.14	9.06 ± 0.58	8.98 ± 0.69	8.16 ± 0.18
Total sugars (g/100 g)	5.76 ± 0.36	4.64 ± 0.00	4.44 ± 0.00	4.2 ± 0.00	5.93 ± 0.09
Protein (%)	1.58 ± 0.05	1.34 ± 0.06	1.51 ± 0.06	1.63 ± 0.03	1.53 ± 0.06
Starch (%)	4.6 ± 0.01	4.36 ± 0.05	4.35 ± 0.00	4.3 ± 0.06	4.21 ± 0.01

fermentation of foods and beverages is the conversion of carbohydrates to alcohols and other aroma components such as esters, organic acids and carbonyl compounds, which are responsible for the aroma that characterizes the final product (Torner, Martínez-Anaya, Antuña, & Benedito de Barber, 1992).

3.3. Beverage analysis

3.3.1. Chemical characterization

The pH value decreased from 6.0 (T0) to 3.5 after 48 h of fermentation. The production of organic acids in fermented food commonly reduces the pH to values below 4.0, which ensures the microbiological safety of the product because some pathogens do not survive at this pH (Blandino et al., 2003). The total soluble solids exhibited a slight decrease from 11 °Brix (0 h fermentation) to 10 °Brix (48 h fermentation).

The starch content (4.6% at 0 h of fermentation) decreased during the fermentation period to 4.21% at 48 h of fermentation (Table 1). This reduction in the starch content may be due to its use as a carbon source by the microorganisms present in the beverage, such as *Bacillus*, *Enterobacter*, *Lactobacillus* and *Candida* (Mugula et al., 2003). The fat and protein contents decreased to 8.16% and 7.53%, respectively, after 48 h of 'calugi' fermentation (Table 1). The slight reduction in the fat content in this study is likely due to the lipolytic activity of the bacteria and/or yeast genera that are present during the 'calugi' fermentation process, such as *Saccharomyces*, *Candida* and *Bacillus* (Arpigny & Jaeger, 1999). The slight decrease in soluble protein levels can be correlated with the presence of proteolytic organisms such as *L. plantarum*, *Bacillus*, *E. cloacae* and *S. cerevisiae*. *L. plantarum*, which have been isolated from cassava fermentation and can secrete proteases, thereby contributing to the hydrolysis of protein (Holzapfel, Schillinger, Du Toit, & Dicks, 1997). After 48 h of fermentation, the total sugar content increased (5.93 g/100 g) (Table 1), most likely due to starch degradation (Mugula et al., 2003).

3.3.2. Chromatographic analysis

The results of the HPLC analysis are presented in Table 1. The carbohydrates identified included glucose, fructose, sucrose and maltose. A decrease in all carbohydrates was observed after 12 h of fermentation. At T0, the sample concentrations of glucose, fructose, sucrose and maltose were 1.35 g/L, 0.50 g/L, 1.70 g/L and 58.77 g/L, respectively. Sucrose was not detected after 12 h of fermentation, most likely due to hydrolysis, releasing glucose and fructose, catalyzed by enzymes from microorganisms such as *S. cerevisiae* and *Bacillus* (Uzunova, Vassileva, Ivanova, Spasova, & Tonkova, 2002). Glucose can originate not only from sucrose but also from the conversion of maltose by the action of amylolytic enzymes of *Bacillus* ssp. and *L. plantarum* (Almeida et al., 2007; Panda, Parmanick, & Ray, 2006). The maltose concentration increased at the end of the fermentation process (88.02 g/L). In our study, species of the genera *Bacillus*, *Lactobacillus* and *Saccharomyces*, which are capable of hydrolyzing starch, were identified. Maltose is continuously produced from the hydrolysis of starch by the enzymatic action of amylases commonly produced by some microorganisms (Almeida et al., 2007).

The alcohols identified by HPLC were ethanol and glycerol (Table 2). The ethanol concentration was 0.19 g/L (after 12 h of fermentation) and increased to 0.31 g/L at 48 h of fermentation. This compound can

Table 2
Concentrations of organic compounds in the fermented 'calugi' beverage, as determined by HPLC and GC.

Compounds	Fermentation time (h)				
	T0	T12	T24	T36	T48
HPLC analyses (g/L)					
Glucose	1.35 ± 0.4	1.08 ± 0.3	0.77 ± 0.0	0.50 ± 0.0	0.32 ± 0.0
Fructose	0.50 ± 0.1	0.67 ± 0.2	0.35 ± 0.0	0.21 ± 0.0	0.13 ± 0.0
Sucrose	1.70 ± 0.3	nd	nd	nd	nd
Maltose	58.77 ± 1.4	66.88 ± 9.1	30.63 ± 3.1	72.82 ± 7.9	88.02 ± 15.7
Glycerol	0.05 ± 0.0	1.04 ± 0.1	1.43 ± 0.3	1.53 ± 0.1	1.70 ± 0.0
Ethanol	nd	0.19 ± 0.0	0.27 ± 0.0	0.33 ± 0.0	0.31 ± 0.0
Acetic acid	0.55 ± 0.0	0.06 ± 0.0	0.10 ± 0.0	0.12 ± 0.0	0.19 ± 0.0
Lactic acid	0.10 ± 0.0	1.21 ± 0.1	1.93 ± 0.5	2.00 ± 0.3	2.00 ± 0.2
Citric acid	0.05 ± 0.1	nd	nd	nd	nd
Malic acid	0.09 ± 0.2	0.04 ± 0.0	0.03 ± 0.1	0.03 ± 0.0	0.12 ± 0.1
Succinic acid	0.50 ± 0.0	0.12 ± 0.2	0.10 ± 0.0	0.08 ± 0.1	0.08 ± 0.0
Propionic acid	0.08 ± 0.0	0.07 ± 0.0	0.07 ± 0.0	0.06 ± 0.0	0.07 ± 0.0
GC analyses (μg/L)					
Acetaldehyde	69.46 ± 8.5	53.71 ± 9.7	42.62 ± 9.8	36.32 ± 5.9	457.02 ± 63.7
1,1-Diethoxyethane	14.43 ± 0.2	21.03 ± 0.9	973.96 ± 8.2	62.36 ± 5	17.49 ± 4.2
Ethyl acetate	6.14 ± 1.6	nd	14.79 ± 10.6	nd	24.82 ± 2.8
Isobutyl acetate	42.96 ± 3.9	30.27 ± 7.7	31.53 ± 1.8	37.49 ± 15.8	54.03 ± 9.5
Furfural	nd	nd	nd	187.75 ± 5.12	70.64 ± 0.2
Furfuryl alcohol	nd	87.80 ± 3.93	29.71 ± 9.0	755.82 ± 6.6	705.26 ± 46.0
b-Citronellol	nd	nd	39.66 ± 7.34	165.64 ± 0.3	nd
Hexanoic acid	nd	nd	nd	nd	30.52 ± 0.9
Guaiacol	nd	nd	nd	25.10 ± 7.0	Nd
2-Ethyl caproic acid	nd	nd	69.15 ± 4.4	39.05 ± 5.2	nd
Heptanoic acid	nd	nd	81.27 ± 5.2	77.88 ± 1.7	67.65 ± 4.78
Diethyl malate	nd	nd	33.91 ± 0.6	190.91 ± 2.8	nd
Octanoic acid	nd	88.07 ± 22.0	58.07 ± 11.1	nd	nd
Nonanoic acid	nd	52.04 ± 6.0	22.55 ± 10.3	61.85 ± 7.7	nd
Decanoic acid	113.88 ± 5.1	134.48 ± 1.0	nd	nd	nd

nd — not detected; ± standard deviation.

result from the heterofermentative and/or alcoholic fermentation of sugars by LAB and yeasts, which were present throughout the 'calugi' fermentation. However, the low ethanol content could be due to the predominance of LAB and the lower yeast population (Fig. 2). In addition to ethanol, glycerol is one of the main products of fermentation by yeasts (Swiegrs, Bartowsky, Henschke, & Pretorius, 2005). The glycerol content of the 'calugi' beverage was 0.05 g/L after 12 h of fermentation and 1.70 g/L at the end of the fermentative process (48 h). This increase in the glycerol content coincided with the increase in the yeast population (Fig. 2).

Acetic acid, lactic acid, citric acid, malic acid, succinic acid and propionic acid were measured in the 'calugi' and during the fermentation process (Table 2). Organic acids occur in fermented products as a result of hydrolysis, biochemical metabolism and microbial activity. The quantitative determination of organic acids in fermented foods is important for nutritional, sensorial and microbial reasons (Blandino et al., 2003). The concentrations of acetic acid, citric acid and succinic acid decreased after 12 h of fermentation to 0.19 g/L (acetic acid) and 0.08 g/L (succinic acid) at 48 h of fermentation. Citric acid could not be detected after 12 h of fermentation and may have been metabolized to lactic acid and acetic acid. Citric acid can be metabolized by several genera of LAB, resulting in the production of acetic acid, lactic acid and diacetyl (Bartowsky et al., 2004). The acetic acid concentration ranged from 0.55 g/L (0 h) to 0.19 g/L (48 h). This variation can be attributed to the acetic acid bacteria that were identified during the 'calugi' fermentation, as shown in Fig. 2. The production of succinic acid was most likely due to the presence of heterofermentative LAB, which are able to grow during the fermentation of the beverage and produce succinic acid by fermentation of sugars (Swiegrs et al., 2005).

The other acids identified in the 'calugi' by HPLC were malic acid, propionic acid and lactic acid. The highest concentration of malic acid measured in the 'calugi' beverage was 0.12 g/L (48 h of fermentation). According to Duarte, Dias, Pereira, Gervásio, and Schwan (2009), high levels of malic acid negatively influence the sensory quality of the beverage. Propionic acid concentrations remained constant throughout the fermentation process (0.07 g/L). The lactic acid concentration increased after 12 h of fermentation, reaching a concentration of 2.00 g/L at the end of the process. The increase in lactic acid occurred when there was an increase in the population of lactic acid bacteria (Table 2 and Fig. 2). The LAB may produce several organic acids, including lactic acid, acetic acid and propionic acid, which are responsible for specific tastes in fermented products. In addition, these organic acids may interact with other substances such as alcohols and aldehydes, which produce additional flavor compounds during the fermentation process (Liu, Han, & Zhou, 2011).

Fifteen volatile compounds were identified and quantified with GC-FID. These compounds included aldehydes, acids, acetates, terpenes, alcohols and esters (Table 2). The aldehydes identified and quantified were acetaldehyde and furfural. The highest concentration of acetaldehyde was 457.02 μg/L (48 h of fermentation). According to Blandino et al. (2003), acetaldehyde is commonly formed during the fermentation process in cereal-based (corn, rice, sorghum and others) beverages and food. The highest concentration of furfural, 187.75 μg/L, was detected after 36 h of fermentation. Furfural is virtually ubiquitous in food and is formed from the acid hydrolysis or heating of polysaccharides that contain pentose and hexose fragments (Lake et al., 2001). This compound is common in foods including cocoa, coffee, alcoholic beverages, fruits, vegetables and bread (Adams et al., 1997).

The acids were the largest group of compounds identified in 'calugi' and included six compounds: hexanoic acid, 2-ethyl caproic acid, heptanoic acid, octanoic acid, nonanoic acid and decanoic acid. Different acids were detected at different fermentation times, octanoic acid and decanoic acid were the most abundant acids during the process, with concentrations of 88.07 μg/L and 134.48 μg/L, respectively, at 12 h of fermentation (Table 2).

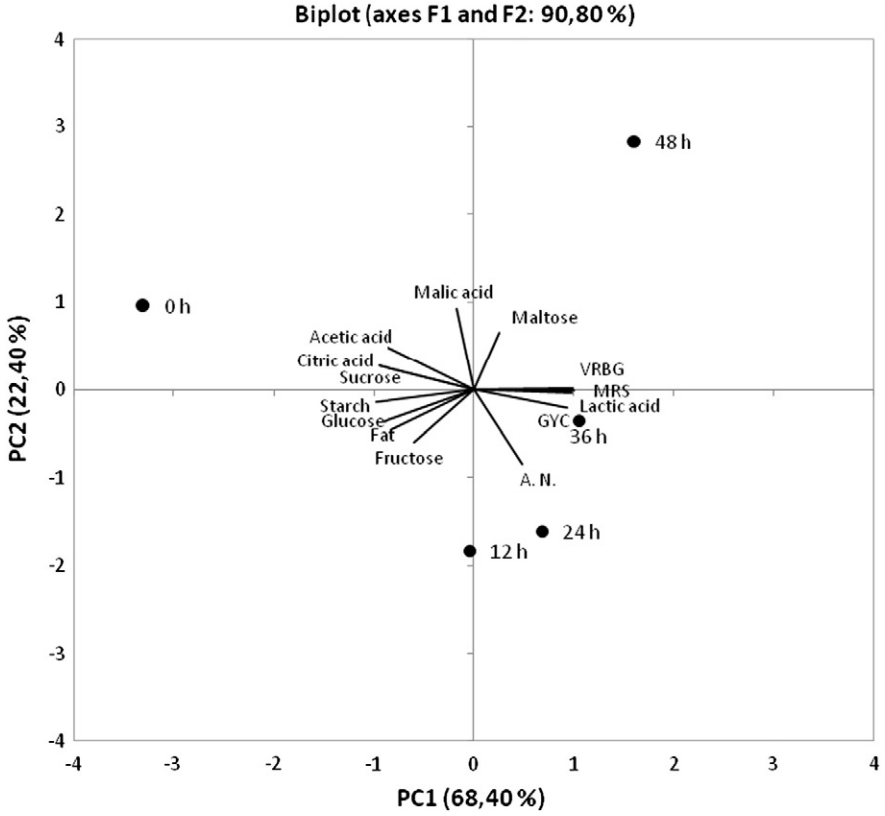

Fig. 5. Principal component analysis (PCA) of the count and physical parameters during the fermentation of the corn and rice 'calugi' beverage.

The acetates ethyl acetate and isobutyl acetate were present at concentrations of 24.82 μg/L (48 h of fermentation) and 54.03 μg/L (48 h of fermentation). Acetates result from the reaction of acetyl-CoA with higher alcohols, which are formed from the degradation of amino acids or carbohydrates (Perestrelo, Fernandes, Albuquerque, Marques, & Camara, 2006). According to Dajanta, Apichartsrangkoon, and Chukeatirote (2011), ethyl acetate and isobutyl acetate were identified in the spontaneous fermentation of thua nao (a Thai fermented soy product) and are correlated with fruit aromas such as apple, banana and pineapple.

Two terpenes, *b*-citronellol and guaiacol, were found at concentrations of 165.64 μg/L and 25.10 μg/L, respectively, at 36 h of fermentation. The terpenes can be liberated by α-glycosidases from yeasts during the fermentation process (King & Dickinson, 2003) and are considered a positive quality factor because they contribute to the aroma of the beverages (floral nuances) and can be used to differentiate beverages (Calleja & Falqué, 2005).

Furfuryl alcohol was the only alcohol identified in the 'calugi' beverage, with a maximum concentration of 755.82 μg/L after 36 h of fermentation. This alcohol has also been reported in soy sauce (Lee & Kwok, 1987), miso (Ku, Chen, & Chiou, 2000) and Korean doenjang (Park, Gil, & Park, 2003) as important contributors of flavor.

The 1,1-diethoxyethane was detected throughout the fermentation process, with a concentration of 73.96 μg/L after 24 h of fermentation. Diethyl malate was the only ester identified in this work and was produced after 24 h fermentation with a concentration of 190.91 μg/L after 36 h of fermentation. This study is the first to report the presence of 1,1-diethoxyethane and diethyl malate in the fermentation of corn and rice.

3.4. Multivariate analyses of count and metabolites

The results obtained for the microbial counts and metabolites produced in the 'calugi' fermentation shown in Fig. 2 and Table 2 were submitted to PCA to identify the compounds that may be directly related to a specific microbial group (Fig. 5). The first (PC 1) and second (PC 2) principal components explain 90.8% and 22.4%, respectively, of the total variance. On the positive side of PC 1, the 24 h, 36 h and 48 h time points of the 'calugi' fermentation were correlated with the count in MRS medium, which exhibited the highest population of LAB and lactic acid, showing that these three variables are directly correlated, as expected. On the negative side of PC 1 and the positive side of PC 2, the time T0 sample of the 'calugi' fermentation was correlated with the substrate (starch, glucose, sucrose and fructose) subsequently metabolized during the fermentation process.

4. Conclusions

Lactobacillus and *Saccharomyces* were the predominant microorganisms in the 'calugi' fermentative process. Our results indicated that the methods used permitted an appropriate characterization of the microbiological and metabolic profile during the spontaneous fermentation of corn and rice to produce 'calugi'. The identification of the compounds produced during fermentation processes can facilitate a better understanding of population dynamics and identify the compounds responsible for the flavor and aroma of fermented foods. This is the first study to report the microbiological and physicochemical characterization of 'calugi' porridge.

Acknowledgments

The authors thank Ms. Claudia Cristina Auler do Amaral Santos for her help during sampling, and are also grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil (CNPq), the Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial and scholarship support.

References

Adams, T. B., Doull, J., Goodman, J. I., Munro, I. C., Newberne, P., Portoghese, P. S., et al. (1997). The FEMA GRAS assessment of furfural used as a flavor ingredient. *Food and Chemical Toxicology*, *35*, 739–751.

Almeida, E. G., Rachid, C. C. T. C., & Schwan, R. F. (2007). Microbial population present in fermented beverage 'cauim' produced by Brazilian Amerindians. *International Journal of Food Microbiology*, *120*, 146–151.

Amoa-Awua, W. K. A., & Jakobsen, M. (1995). The role of *Bacillus* species in the fermentation of cassava. *Journal of Applied Bacteriology*, *79*, 250–256.

Ampe, F., Sirvent, A., & Zakhia, N. (2001). Dynamics of the microbial community responsible for traditional sour cassava starch fermentation studied by denaturing gradient gel electrophoresis and quantitative rRNA hybridization. *International Journal of Food Microbiology*, *65*, 45–54.

AOAC (2000). *Official methods of analyses of the association of official analytical chemists. Vol 2* (pp. 915–922). (Seventeenth Ed.). Gaithersburg: Association of Official Analytical Chemists.

Areas, A. G., & Lajolo, F. M. (1987). Determinação do amido e açúcares em bananas. *Ciência e Cultura*, *30*.

Arpigny, J. L., & Jaeger, K. -E. (1999). Bacterial lipolytic enzymes: Classification and properties. *Biochemical Journal*, *343*, 177–183.

Bartowsky, E. J., Dillon, S. J., Henschke, P. A., Markides, A. J., Dumont, A., Pretorius, I. S., et al. (2004). The potential of *Saccharomyces cerevisiae* wine yeast to improve red wine colour. *Australian and New Zealand Grapegrower and Winemaker*, *490*, 83–85.

Bentley, R. W., Leigh, J. A., & Collins, M. D. (1991). Intrageneric structure of *Streptococcus* based on comparative analysis of small-subunit rRNA sequences. *International Journal of Systematic Bacteriology*, *41*, 487–494.

Blandino, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero, D., & Webb, C. (2003). Review: Cereal-based fermented foods and beverages. *Food Research International*, *36*, 527–543.

Calleja, A., & Falqué, E. (2005). Volatile composition of Mencía wines. *Food Chemistry*, *90*, 357–363.

Cocolin, L., Bisson, L. F., & Mills, D. A. (2000). Direct profiling of the yeast dynamics in wine fermentations. *FEMS Microbiology Letters*, *189*, 81–87.

Dajanta, K., Apichartsrangkoon, A., & Chukeatirote, E. (2011). Volatile profiles of thua nao, a Thai fermented soy product. *Food Chemistry*, *125*, 464–470.

Delorme, C. (2008). Safety assessment of dairy microorganisms: *Streptococcus thermophilus*. *International Journal of Food Microbiology*, *126*, 274–277.

Dische, Z. (1962). General color reactions. In R. L. Whistler & M.L. Wolfran (Eds.), *Carbohydrates chemistry*, *1*. (pp. 477–512). New York: Academic Press.

Duarte, W. F., Dias, D. R., Oliveira, J. M., Teixeira, J. A., Silva, J. B. A., & Schwan, R. F. (2010). Characterization of different fruit wines made from cacao, cupuassu, gabiroba, jabuticaba and umbu. *LWT — Food Science and Technology*, *43*, 1564–1572.

Duarte, W. F., Dias, D. R., Pereira, G. V. M., Gervásio, I. M., & Schwan, R. F. (2009). Indigenous and inoculated yeast fermentation of gabiroba (*Campomanesia pubescens*) pulp for fruit wine production. *Journal of Industrial Microbiology and Biotechnology*, *36*, 557–569.

Foma, R. K., Destain, J., Mobinzo, P. K., Kayisu, K., & Thonart, P. (2012). Study of physicochemical parameters and spontaneous fermentation during traditional production of munkoyo, an indigenous beverage produced in Democratic Republic of Congo. *Food Control*, *25*, 334–341.

Gadaga, T. H., Mutukumiraa, A. N., Narvhus, J. A., & Feresuc, S. B. (1999). A review of traditional fermented foods and beverages of Zimbabwe. *International Journal of Food Microbiology*, *53*, 1–11.

Holzapfel, W., Schillinger, U., Du Toit, M., & Dicks, L. (1997). Systematics of probiotic lactic acid bacteria with reference to modern phenotypic and genomic methods. *Microbiology and Therapy*, *10*, 1–10.

Jeyaram, K., Mohebdro Singh, W., Prenarani, T., Ranjitadevi, A., Selina Chanu, K., Talukdar, N. C., et al. (2008). Molecular identification of dominant microflora associated with "Hawaijar" — A traditional fermented soybean (*Glycine max* (L.)) food of Manipur, India. *International Journal of Food Microbiology*, *122*, 259–268.

Kim, T. W., Lee, J. H., Kim, S. E., Park, M. H., Chang, H. C., & Kim, H. Y. (2009). Analysis of microbial communities in doenjang, a Korean fermented soybean paste, using nested PCR-denaturing gradient gel electrophoresis. *International Journal of Food Microbiology*, *131*, 265–271.

King, A. J., & Dickinson, J. R. (2003). Biotransformation of hop aroma terpenoids by ale and lager yeasts. *FEMS Yeast Research*, *3*, 53–62.

Ku, K. L., Chen, T. P., & Chiou, R. Y. (2000). Apparatus used for small-scale volatile extraction from ethanol-supplemented low-salt miso and GC–MS characterization of the extracted flavors. *Journal of Agricultural and Food Chemistry*, *48*, 3507–3511.

Lake, B. G., Edwards, A. J., Price, R. J., Phillips, B. J., Renwick, A. B., Beamand, J. A., et al. (2001). Lack of effect of furfural on unscheduled DNA synthesis in the in vivo rat and mouse hepatocyte DNA repair assays and in precision-cut human liver slices. *Food and Chemical Toxicology*, *39*, 999–1011.

Lee, M. H., & Kwok, K. F. (1987). Studies on the flavor components of soy sauce. *Journal of the Chinese Agricultural Chemical Society*, *25*, 101–111.

Liu, S., Han, Y., & Zhou, Z. (2011). Lactic acid bacteria in traditional fermented Chinese foods. *Food Research International*, *44*, 643–651.

Madoroba, E., Steenkamp, E. T., Theron, J., Scheirlinck, I., Cloete, T. E., & Huys, G. (2011). Diversity and dynamics of bacterial populations during spontaneous sorghum fermentations used to produce ting, a South African food. *Systematic and Applied Microbiology*, *34*, 227–234.

McDonald, L. C., Fleming, H. P., & Hassan, H. M. (1990). Acid tolerance of *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. *Applied and Environmental Microbiology*, *56*, 2120–2124.

Miambi, E., Guyot, J. P., & Ampe, F. (2003). Identification, isolation and quantification of representative bacteria from fermented cassava dough using an integrated approach of culture-dependent and culture-independent methods. *International Journal Food Microbiology*, *82*, 111–120.

Miguel, M. G. C. P., Cardoso, P. G., Lago, L. A., & Schwan, R. F. (2010). Diversity of bacteria present in Milk kefir grains using culture-dependent and culture-independent methods. *Food Research International*, *43*, 1523–1528.

Mugula, J. K., Narvhus, J. A., & Sørhaug, T. (2003). Use of starter cultures of lactic acid bacteria and yeasts in the preparation of togwa, a Tanzanian fermented food. *International Journal of Food Microbiology*, *83*, 307–318.

Nout, M. J. R., & Sarkar, P. K. (1999). Lactic acid fermentation in tropical climates. *Antonie Van Leeuwenhoek*, *76*, 395–401.

Ovreas, L., Forney, L., Daae, F. L., & Torsvik, V. (1997). Distribution of bacterioplankton in meromicticlake Saelenvannet, as determined by denaturing gradient electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Applied and Environmental Microbiology*, *63*, 3367–3393.

Oyarekua, M. A. (2011). Evaluation of the nutritional and microbiological status of co-fermented cereals/cowpea 'OGI'. *Agriculture and Biology Journal of North America*, *2*, 61–73.

Panda, S. H., Parmanick, M., & Ray, R. C. (2006). Lactic acid fermentation of sweet potato (*Ipomoea batatas* L.) into pickles. *Journal of Food Processing and Preservation*, *31*, 83–101.

Park, H. K., Gil, B., & Park, J. K. (2003). Characteristic flavor compounds of commercial soybean paste. *Food Science and Biotechnology*, *12*, 377–607.

Perestrelo, R., Fernandes, A., Albuquerque, F. F., Marques, J. C., & Camara, J. S. (2006). Analytical characterization of the aroma of Tinta Negra Mole red wine: Identification of the main odorants compounds. *Analytica Chimica Acta*, *563*, 154–164.

Ramos, C. L., Almeida, E. G., Freire, A. L., & Schwan, R. F. (2011). Diversity of bacteria and yeast in the naturally fermented cotton seed and rice beverage produced by Brazilian Amerindians. *Food Microbiology*, *28*, 1380–1386.

Ramos, C. L., Almeida, E. G., Pereira, G. V. M., Cardoso, P. G., Dias, E. S., & Schwan, R. F. (2010). Determination of dynamic characteristics of microbiota in a fermented beverage produced by Brazilian Amerindians using culture-dependent and culture-independent methods. *International Journal of Food Microbiology*, *140*, 225–231.

Rusul, G., & Yaacob, N. H. (1995). Prevalence of *Bacillus cereus* in selected foods and detection of enterotoxin using TECRA-VIA and BCET-RPLA. *International Journal of Food Microbiology*, *25*, 131–139.

Santos, C. C. A. A., Almeida, E. G., Melo, V. P., & Schwan, R. F. (2012). Microbiological and physicochemical characterisation of caxiri, an alcoholic beverage produced by the indigenous Juruna people of Brazil. *International Journal of Food Microbiology*, *156*, 112–121.

Schwan, R. F., Almeida, E. G., Souza-Dias, M. A., & Jespersen, L. (2007). Yeast diversity in rice–cassava fermentations produced by the indigenous Tapirapé people of Brazil. *FEMS Yeast Research*, *7*, 966–972.

Shukla, S., Choi, T. B., Park, H. -K., Kim, M., Lee, I. K., & Kim, J. -K. (2010). Determination of non-volatile and volatile organic acids in Korean traditional fermented soybean paste (Doenjang). *Food and Chemical Toxicology*, *48*, 2005–2010.

Swiegrs, J. H., Bartowsky, E. J., Henschke, P. A., & Pretorius, I. S. (2005). Yeast and bacterial modulation of wine aroma and flavor. *Australian Journal of Grape and Wine Research*, *11*, 139–173.

Talon, R., Walter, D., Viallon, C., & Berdagué, J. L. (2002). Prediction of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* populations in yoghurt by Curie point pyrolysis-mass spectrometry. *Journal of Microbiological Methods*, *48*, 271–279.

Torner, M. J., Martínez-Anaya, M. A., Antuña, B., & Benedito de Barber, C. (1992). Headspace flavour compounds produced by yeasts and lactobacilli during fermentation of preferments and bread doughs. *International Journal of Food Microbiology*, *15*, 145–152.

Uzunova, K., Vassileva, A., Ivanova, V., Spasova, D., & Tonkova, A. (2002). Thermostable exo-inulinase production by semicontinuous cultivation of membrane-immobilized Bacillus sp. 11 cells. *Process Biochemistry*, *37*, 863–868.