Food Research International 76 (2015) 787–795

Contents lists available at ScienceDirect

Food Research International

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

Microbiological and chemical parameters during cassava based-substrate fermentation using potential starter cultures of lactic acid bacteria and yeast

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ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form 19 July 2015
Accepted 24 July 2015
Available online 29 July 2015

Keywords:
Starter culture
Cassava
Bacteria
Yeasts
Indigenous fermented foods
Volatile compounds

ABSTRACT

Traditional Brazilian indigenous fermented foods and beverages are potential sources of new food products that promote health, but they are still produced by natural fermentation. In the present work, *Lactobacillus fermentum* CCMA 0215 isolated from the indigenous fermented cassava beverage *yakupa* was used as single or mixed starter culture with five different yeast strains (*Torulaspora delbrueckii* CCMA 0234 and CCMA 0235, *Pichia caribbica* CCMA 0198, and *Saccharomyces cerevisiae* CCMA 0232 and CCMA 0233) to ferment cassava. Fermentations using each yeast as single starter culture were also performed. The microbial population and metabolites produced during cassava fermentation were investigated. In all assays, the inoculated microorganisms fermented cassava, judged by lowering the pH from 6.0 to 4.0–5.0 within 24 h. Lactic acid bacteria (LAB) and yeast population increased during fermentation. Lactic acid was the main organic acid produced, reaching a maximum value of 4.5 g/L at 24 h in the co-culture with *L. fermentum* CCMA 0215 and *T. delbrueckii* CCMA 0234. Other organic acids, such as malic, tartaric, and succinic acids, were detected in low concentrations (less than 0.5 g/L). Ethanol and glycerol were produced in all assays inoculated with yeasts (single and co-cultured with LAB), reaching the maximum concentration of approximately 2.3 g/L and 0.6 g/L, respectively. Twenty-two volatile compounds were detected after 48 h of fermentation, varying widely between single and co-cultures. The compounds 2-phenylethyl alcohol, 1-butanol, 3-methyl (isoamyl alcohol), and acetoin were detected in single and co-cultures. This study demonstrated co-cultures of yeasts and LAB had the ability to improve the aroma profile of the final product and the safety of the product by lowering the pH.

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1. Introduction

Brazilian indigenous people are traditional producers of fermented foods and beverages using different substrates like corn, rice, and cassava. The production is still homemade, and the empirical knowledge is transmitted from one generation to the other inside the tribes. *Cauim*, *caxiri*, *yakupa*, and *calugi* beverages are prepared by women of the tribe and then left to ferment by natural fermentation without any microbial or environmental conditions controlled (Almeida, Rachid, & Schwan, 2007; Freire, Ramos, Almeida, Duarte, & Schwan, 2014; Miguel, Santos, Santos, Duarte, & Schwan, 2014; Ramos et al., 2010; Santos, Almeida, Pereira, & Schwan, 2012). The indigenous beverage *yakupa* is an acid cassava-fermented product, in which lactic acid bacteria (LAB) are responsible for the acidity and the pH decrease (Freire et al., 2014). Yeasts, mainly *Saccharomyces cerevisiae*, are also present during the *yakupa* fermentation (Freire et al., 2014). Yeasts have been described in African and Brazilian fermented foods and are related to alcohol production and other aroma compounds, stimulation of LAB growth, and improvement of nutritional value (Jespersen, 2003; Kamda et al., 2015; Olasupo, Odunfa, & Obayori, 2010).

Nowadays, consumer awareness towards healthy diets and changing eating habits has created a huge market demand for new foods with health benefits. In this context fermented products, particularly non-dairy beverages, are gaining popularity and acceptance because of their functional benefits (De Dea Lindner et al., 2013). New products have been launched based on fruit and cereals, and the incorporation of lactic acid bacteria (LAB) in food and beverages is a global trend. It is well known that fermented foods have important healthy roles in the human diet, and studies have demonstrated that regular intake of fermented foods can reduce the incidence of chronic diseases (Keszei, Schouten, Goldbohm, & van den Brandt, 2010; Larsson, Andersson, Johansson, & Wolk, 2008; Sonestedt et al., 2011). Further, the challenge to overcome the disadvantages associated with fermented dairy products, like lactose intolerance, allergy, and the impact in cholesterol levels (Prado, Parada, Pandey, & Soccol, 2008), has stimulated the improvement of traditional fermented foods prepared with different substrates like cassava. Many advantages are related to the use of starter cultures in the fermentative process, as the rapid acidification of the product and, thus, the inhibition of spoilage and pathogenic bacteria growth

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http://dx.doi.org/10.1016/j.foodres.2015.07.041
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produce foods with consistent quality (Holzapfel, 2002). Amoa-awua and Jakobsen (1995) and Amoa-awua, Frisvad, Sefa-dedeh, and Jakobsen (1997) have shown some important activities that occur when traditional inoculum is used to ferment cassava: the breaking down of the coarse texture of cassava dough, the souring of cassava dough, the reduction in the level of cyanogenic glucosides, and the synthesis of volatile aroma compounds.

Djeni et al. (2015) performed biochemical and microbial characterizations of the traditional cassava-fermented food attieké in Côte d'Ivoire, Africa, and found that the dominating LAB strains can be potential starter cultures for cassava fermentations. The *Lactobacillus plantarum* BFE 6710 and *Lactobacillus fermentum* BFE 6620 were also used as starter cultures for cassava fermentation for *gari* (a cassava-fermented food in Africa) production in a pilot study under controlled conditions (Huch née Kostinek et al., 2008). Although the application of LAB in dairy foods has been widely explored, cassava is an innovative matrix for the application of this microbial group. In this study, a *L. fermentum* CCMA 0215 isolated from *yakupa* was selected on the basis of some technological properties and used as starter cultures in single and co-culture with different yeast species to ferment a cassava substrate. Microbial and metabolite compound analysis were performed to provide sufficient knowledge about the final biochemical characteristics of the beverage in order to control and to standardize the cassava fermentation process.

2. Materials and methods

2.1. Microorganisms and culture conditions

The yeast *S. cerevisiae* CCMA 0232, *S. cerevisiae* CCMA 0233, *Torulaspora delbrueckii* CCMA 0234, *T. delbrueckii* CCMA 0235, *Pichia caribbica* CCMA 0198, and the LAB *L. fermentum* CCMA 0215 belonging to the Culture Collection of Agriculture Microbiology (CCMA) of Federal University of Lavras (Brazil). These strains were isolated from natural cassava fermentations (Freire et al., 2014; Ramos et al., 2015) (except *P. caribbica* CCMA 0198 that was isolated from coffee fermentation) and used as potential starter cultures for the fermentation of new cassava substrate. These strains were previously selected based on technological characteristics such as α-amylase secretion, acid production and growth in a cassava substrate (data not shown). The yeasts and LAB isolates were stored at −80 °C in YPD broth 10 g/L yeast extract (Merck, Darmstadt, Germany), 10 g/L peptone (Himedia, Mumbai, India), 20 g/L glucose (Merck, Darmstadt, Germany) and Man Rogosa Sharpe broth (MRS, Merck, Darmstadt, Germany), respectively, with 20% (v/v) glycerol. The strains were reactivated by streaking them onto YPD agar (yeasts) and MRS agar (LAB), and incubated for 48 h at 30 °C (yeasts) and 37 °C (LAB).

2.2. Preparation of cassava substrate a nd starter cultures for fermentation assays

The cassava substrate for the fermentation assays was prepared in a similar way as for the *yakupa* beverage (Freire et al., 2014), with some modifications. The cassava roots used in all experiments were purchased from the local market in Lavras, Minas Gerais, Brazil. Peeled cassava roots (0.5 kg) were cooked with 1000 mL of sterile distilled water for approximately 40 min, and then 1000 mL of sterile distilled water was added. This mixture was sieved and pasteurized at 90 °C for 20 min.

The preparation of inoculum for single and co-culture fermentation was performed as described by Santos, Libeck, and Schwan (2014). The yeast and LAB strains were each successively sub-culturing on YPD broth (pH 3.5 at 30 °C for 24 h) and MRS broth (37 °C for 48 h), respectively. The cells were inoculated in the cassava substrate with a population of 5 log CFU/mL for yeasts and 7 log CFU/mL for bacteria in both single and co-culture fermentations.

2.3. Single fermentations

The washed cells of yeasts and LAB were separately inoculated into 250 mL flasks containing 100 mL of the pasteurized cassava substrate and incubated at 37 °C for 48 h. The experiments were performed in three independent assays. For each repetition, two samples were taken at each time (duplicate).

2.4. Co-culture fermentations

Five co-culture fermentations were performed: (1) *S. cerevisiae* (CCMA 0232) and *L. fermentum* (CCMA 0215); (2) *S. cerevisiae* (CCMA 0233) and *L. fermentum* (CCMA 0215); (3) *T. delbrueckii* (CCMA 0234) and *L. fermentum* (CCMA 0215); (4) *T. delbrueckii* (CCMA 0235) and *L. fermentum* (CCMA 0215); and (5) *P. caribbica* (CCMA 0198) and *L. fermentum* (CCMA 0215). Inoculum addition was the same as described in Section 2.3.

2.5. Enumeration of microorganisms

Samples (1 mL) were taken from each fermentation flask. Serial tenfold dilutions were prepared in a solution of 0.9% NaCl (w/v) and 0.1% (w/v) bacto peptone (Difco). The total LAB, yeast, and Enterobacteriaceae populations were determined by plating in MRS (supplemented with 50 mg/L of nystatin), YPD (pH 3.5), and violet red bile agar (VRBG; Merck) media, respectively. The plates were incubated at 37 °C (LAB and Enterobacteriaceae) and 28 °C (yeasts) during 48 h, and the colony forming units (CFU) were enumerated in plates containing 30 to 300 colonies, and cell concentration was expressed as log CFU/mL. The analyses were performed in triplicate.

2.6. Analytical methods

2.6.1. Determination of pH

The pH levels of the fermenting cassava samples were measured on site with pH-Fix test strips (Macherey-Nagel GmbH and Co., Düren, Germany).

2.6.2. HPLC analysis

Organic acid (lactic acid, acetic acid, malic acid, succinic acid, and tartaric acid) and alcohol (ethanol and glycerol) analyses were performed as described by Duarte et al. (2010). A Shimadzu liquid chromatography system (Shimadzu Corp., Japan), equipped with a dual detection system consisting of a UV–Vis detector (SPD 10Ai) (for acid detection) and a refractive index detector (RID-10Ai) (for alcohol detection), was used. A Shimadzu ion exclusion column, Shim-pack SCR-101H (7.9 mm × 30 cm) was used at an operating temperature of 30 °C for ethanol and glycerol and 50 °C for acids. All samples were analyzed in duplicate.

2.6.3. GC–MS analysis

The volatile component profiles were determined at 0 and 48 h of fermentations according to the methods described by Oliveira, Faria, Sá, Barros, and Araújo (2006), with some modifications. First, samples were centrifuged twice, and then 7 mL was put into 10 mL culture tubes. A magnetic stir bar (22.2 mm × 4.8 mm) and 0.143 μg of 4-nonanol (internal standard) (Merck, Darmstadt, Germany) were added, and extraction was done by stirring the samples with 700 μL of chloroform (Merck, Darmstadt, Germany) for 15 min over agitation with a magnetic stirrer. After cooling at 0 °C for 10 min, the magnetic stir bar was removed and the organic phase was detached by centrifugation (RCF = 5118, 5 min, 4 °C). The extract was recovered into a vial using a Pasteur pipette. Then, the extract was dried with anhydrous sodium sulfate (Merck, Darmstadt, Germany) and picked up again into a new vial.

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