UNIVERSIDADE FEDERAL DE LAVRAS

ANA LUIZA FREIRE

SELEÇÃO DE CULTURAS PARA PRODUÇÃO DE ALIMENTOS INDÍGENAS COM PROPRIEDADES FUNCIONAIS

LAVRAS - MG

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Orientadora

Dra. Cíntia Lacerda Ramos
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SELECTION OF CULTURES FOR THE PRODUCTION OF INDIGENOUS FOODS WITH FUNCTIONAL PROPERTIES

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, área de concentração em Microbiologia Agrícola, para a obtenção do Título de Doutor.

APROVADA em 24 de novembro de 2016.

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LAVRAS - MG

2017

À minha família e ao amor da minha, e a todos os amigos, mestres e colegas que me acompanharam sempre e estiveram envolvidos, direta ou indiretamente, na realização deste trabalho

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“Sejam quais forem os resultados com êxito ou não, o importante é que no final cada um possa dizer: ‘fiz o que pude’.”

Louis Pasteur

RESUMO GERAL

Atualmente, a maioria dos alimentos probióticos, presentes no mercado alimentício, é à base de leite ou derivados. Entretanto o surgimento de diversas alergias e intolerâncias a esse tipo de matéria-prima tem estimulado o desenvolvimento de novos produtos, a partir de cereais e outros vegetais, que poderão suprir a crescente demanda por novos produtos funcionais não lácteos. O presente trabalho teve como objetivo desenvolver bebidas fermentadas não lácteas utilizando como cultura bactérias do ácido lático (BAL) com potencial probiótico e levedura. Para o desenvolvimento de uma bebida à base de mandioca, as linhagens *Lactobacillus plantarum* CCMA 0743, *Torulaspora delbrueckii* CCMA 0235 e o probiótico comercial *L. acidophilus* LACA 4 foram utilizados como culturas iniciadoras em culturas simples e co-cultivo. As populações de bactérias atingiram cerca de 8,0 log UFC / mL ao final das fermentações. Maiores concentrações de amido residual foram detectadas, nos cultivos simples de bactérias (10.6%), indicando que o co-cultivo com a levedura poderia auxiliar na quebra do amido. O ácido lático foi o principal ácido orgânico detectado (> 1.6 g/L) e o etanol foi inferior a 0,5%, consistindo em uma bebida não alcoólica. Os cultivos contendo a levedura apresentaram a maior atividade antioxidante. A análise sensorial das bebidas indicou o potencial, para sua comercialização, uma vez que a nota média dada pelos consumidores para cada atributo foi em torno de 5, ou seja, não desgostaram e nem gostaram. Uma nova bebida fermentada de cereais, a partir de milho e arroz, foi desenvolvida, utilizando uma cultura mista de *L. plantarum* CCMA 0743, *T. delbrueckii* CCMA 0235 e *L. acidophilus* LACA 4. Duas concentrações de prebiótico, 2 e 5% de FOS (fruto-oligossacarídeo) foram testadas. O crescimento do probiótico *L. acidophilus* LACA 4 foi favorecido pelo prebiótico, na concentração de 5% e, após o tempo de armazenamento (28 dias a 4°C), manteve a população de 10^7 UFC / mL. Os ácidos, láctico e acético, foram os principais ácidos orgânicos detectados, em torno de 3,7 g / L e 0,5 g / L, respectivamente. A concentração de etanol foi inferior a 0,5% consistindo em uma bebida não alcoólica. Um total de 55 compostos voláteis, incluindo ácidos, álcoois, aldeídos, ésteres, cetonas, pirazinas e outros, foi detectado por Cromatografia Gasosa acoplada ao Espectrômetro de Massas (GC-MS). A análise sensorial indicou uma boa aceitação das bebidas pela maioria dos consumidores (≥50%), cujas notas variaram de 6 a 9, indicando seu potencial para comercialização.

Palavras-chave: Probiótico. Bactéria do ácido lático. Levedura. Mandioca. Milho. Arroz.

GENERAL ABSTRACT

Currently, most probiotic foods present in the food market are based on milk or derivatives. However, the emergence of many allergies and intolerances to this type of raw material have stimulated the development of new products based on cereals and other plants, allowing the provision of the increasing demand for functional non-dairy products. This work aimed at developing non-dairy fermented beverages using Lactic Acid Bacteria (LAB) as culture, with probiotic and yeast potential. For developing a cassava-based beverage, the lines *Lactobacillus plantarum* CCMA 0743, *Torulaspora delbrueckii* CCMA 0235 and the commercial probiotic *L. acudiohilus* LACA 4, were used as starting cultures in simple and co-cultivation cultures. The bacterial populations reached near 8.0 log CFU/mL at the end of fermentation. Higher concentrations of residual starch were detected in the simple bacteria cultivations (10.6%), indicating that the co-cultivation with yeast might aid in breaking the starch. The lactic acid was the main organic acid detected (>1.6g/L), with ethanol being inferior to 0.5%, constituting a non-alcoholic beverage. The cultivations containing yeast presented higher antioxidant activity. Sensorial analysis of the beverages indicated the potential for its commercialization, given that the average grade given by consumers for each attribute was around 5, that is, they did not dislike or like the product. A new cereal fermented beverage, based on corn and rice was developed using a mixed culture of *L. plantarum* CCMA 0743, *T. delbrueckii* CCMA 0235 and *L. acidophilus* LACA 4. Two concentrations of prebiotic, 2 and 5% of FOS (frutooligosaccharide), were tested. The growth of probiotic *L. acidophilus* LACA 4 was favored by the prebiotic at concentrations of 5% and, after time in storage (28 days at 4^oC), it maintained a population of 10^7 CFU/mL. The lactic and acetic acids were the main organic acids detected, around 3.7 g/L and 0.5g/L, respectively. The concentration of ethanol was inferior to 0.5%, indicating a non-alcoholic beverage. A total of 55 volatile compounds, including acids, alcohols, aldehydes, esters, ketones, pyrazines and others, were detected by Gas Chromatography/Mass Spectrometry (GC/MS). The sensorial analysis indicated good acceptance of the beverages by most consumers (≥50%), of which grades ranged from 6 to 9, indicating its potential for commercialization.

Keywords: Probiotic. Lactic acid bacteria. Yeast. Cassava. Corn. Rice.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

Há alguns anos o conceito de alimento deixou de ser abordado, simplesmente, do ponto de vista nutricional, cuja finalidade era servir de fonte de energia e nutrientes à formação e manutenção de células e tecidos e passou a ser encarado como fonte de componentes capazes de estimular, de maneira positiva, determinados sistemas biológicos, sendo considerados alimentos funcionais.

Dentre a categoria de alimentos funcionais, incluem-se os alimentos probióticos, ou seja, alimentos que contêm em sua composição microrganismos vivos que conferem benefícios à saúde do hospedeiro. Tradicionalmente eles têm sido encontrados em alimentos fermentados à base de leite, como iogurtes e leites fermentados. No entanto a crescente demanda por produtos não lácteos, contendo esses microrganismos benéficos à saúde, tem sido observada.

Alimentos fermentados tradicionais constituem-se em rica fonte de microrganismos que podem apresentar propriedades tecnológicas e probióticas interessantes para serem utilizados como culturas em processos fermentativos. A produção de alimentos fermentados tradicionais, via fermentação natural, é muito comum, principalmente, em países Africanos, Asiáticos e da América do Sul. No Brasil, os povos indígenas têm amplo conhecimento a respeito da produção e consumo de bebidas e alimentos fermentados produzidos, a partir de substratos amiláceos, como mandioca, arroz e milho. O uso de bactérias do ácido lático (BAL), para fermentação e produção de diversos tipos de alimentos, é bastante antigo e, em alimentos tradicionais à base de cereais e mandioca, a atuação de diferentes espécies de BAL tem um papel importante no processo fermentativo.

O mercado de alimentos funcionais, hoje, é, basicamente, dependente de produtos lácteos. Tendo em vista o crescente surgimento de problemas alérgicos e de intolerância à lactose, associado aos altos níveis de colesterol na população e doenças cardíacas relacionadas, a demanda por novos produtos não lácteos que associem saúde e bem-estar à nutrição, tem aumentado. O presente trabalho teve como objetivo a obtenção de culturas iniciadoras mistas de BAL e leveduras, para a fermentação e produção de alimentos à base de cereais e tubérculos (como milho, arroz e mandioca), com base em alimentos indígenas tradicionais do Brasil, que apresentem potenciais características funcionais.

2 REFERENCIAL TEÓRICO

2.1 Alimentos e bebidas fermentados tradicionais

A fermentação é uma das formas mais antigas de preservação dos alimentos no mundo, além de promover melhora da qualidade nutricional e das características sensoriais (MARSH et al., 2014). Desde os primórdios da civilização, alimentos fermentados à base de leite, carnes, vegetais e cereais têm sido descritos. As primeiras evidências, datadas de 7000 anos A.C, já demonstram a fermentação de arroz, mel e bebidas de frutas na China (MCGOVERN et al., 2004), a produção de kombucha há 220 anos A.C. (DUFRESNE; FARNWORTH, 2000) e a utilização de kefir para fermentação de leite há 3500 anos na Ásia (YANG et al., 2014). Desde então, diversos alimentos fermentados indígenas têm sido produzidos e consumidos, estando intimamente, ligados à cultura e à tradição dos povos de pequenas comunidades rurais e vilarejos, em muitas regiões da África, Ásia, Europa, Oriente Médio e América do Sul (ALOYS; ANGELINE, 2009; MARSH et al., 2014).

Na África, os alimentos tradicionais podem ser produzidos, a partir de cereais fermentados e não fermentados, como sorgo, milho e milheto, além de incluírem produtos à base de mandioca e sementes de leguminosas, de carne, leite e bebidas alcóolicas (Tabela 1). Para os africanos, a fermentação de cereais e raízes tuberosas constitui uma importante forma de melhora das características de aroma e sabor, de aumento do valor nutricional e da digestibilidade dessas matérias-primas, além de ser uma maneira barata de preservação dos alimentos (FRANZ et al., 2014).

Tabela 1 - Alimentos fermentados tradicionais produzidos a partir de cereais, raízes amiláceas e proteínas de origem animal, em diversas regiões da África.

Product	Fermentable substrate	Microorganisms reported to be involved in the fermentation
African, non-alcoholic cereal based foods		
Mahewu (magou)	Maize, sorghum or millet	*L. delbrueckii subsp. bulgaricus; L. delbrueckii subsp. delbrueckii; Leuconostoc spp.;* heterofermentative lactobacilli
Ogi	Maize, sorghum or millet	*Ped. pentosaceus, L. fermentum, L. plantarum,* yeast *(Saccharomyces cerevisiae, Candida kruseii)*
Koko and Kenkey	Maize, sorghum or millet	*W. confusa, L. fermentum, L. salivarius, L. vaccinostercus, L. pantheris, Pediococcus spp.* and yeast
Uji	Maize, sorghum or millet	*L. plantarum, L. paracasei, L. fermentum, L. buchneri, Ped. acidilactici, Ped. pentosaceus*
Kisra	Sorghum	LAB
Hussuwa	Sorghum	*L. fermentum, Ped. acidilactici, Ent. faecium (*minor proportions)
Injera	Sorghum	*Candida guillermondi*
Ting	Sorghum	*L. fermentum, L. plantarum, L. rhamnosus*
Obusera	Millet	LAB
Mawe	Maize	*Lact. lactis, Ped. pentosaceus, L. plantarum*
Kunu-zaki	Millet, sorghum	*L. fermentum, P. pentosaceus, W. confusa, Ent. Faecalis*
Bogobe	Sorghum	Unknown
Potopoto	Maize	*L. gasseri, L. plantarum/paraplantarum, L. acidophilus, L. delbrueckii, L. reuteri, L. casei, Bacillus spp., Enterococcus spp.*
Dégué	Millet	*L. gasseri, L. fermentum, L. brevis, L. casei, Enterococcus spp.*
African fermented starchy root products		
Ben saalga	Millet	*L. plantarum* and other LAB
Gari	Cassava	*L. plantarum, L. fallax, L. fermentum (predominating) W. paramesenteroides, L. brevis, Leuc. pseudomesenteroides (minor proportions), Strep. lactis, Geotrichum candidum, Corynebacterium manihot* (also reported)
Lafun	Cassava	*L. fermentum, L. plantarum, W. confusa, yeast (Saccharomyces cerevisiae, Pichia scutulata, Klyveromyces marxianus, Hanseniaspora guillermondii), and Bacillus spp.*
Fufu	Cassava	*Ped. pentosaceus, L. fermentum, L. plantarum*
Kivunde	Cassava	*L. plantarum,* other LAB, yeast
Chikawngue	Cassava	LAB, yeast
Kocho	Ensette or Abyssinian banana	LAB, yeast
Agbelima	Cassava	*L. plantarum, L. brevis, L. fermentum, Leuc. mesenteroides, also Bacillus spp., Candida tropicalis, Geotrichum candidum, Penicillium spp.*
African fermented animal proteins		
Nono (milk curd)	Milk	LAB
Maziwalala	Milk	*"Strep." (Lact.) lactis, Strep. thermophilus*
Leban (sour milk)	Milk	*Lactic streptococci (lactococci), Leuc. lactis, Leuc. mesenteroides subsp. cremoris*
Wara	Milk	*Lactococcus lactis, Lactobacillus spp*
Guedj	Fish	*Lact. lactis*

Fonte: Modificado e adaptado de Franz et al. (2014).

Os cereais constituem a base da alimentação, em muitos países no mundo e uma grande variedade de produtos obtidos, via processamento e fermentação desses substratos, já são conhecidos. Na África e Ásia, os alimentos obtidos, a partir da fermentação de cereais, são divididos em duas categorias bem distintas: as massas cozidas (semi)-sólidas e mingaus e as bebidas líquidas. Estas últimas, por sua vez, dividem-se em caldos não alcoólicos, cervejas, vinhos e destilados (NOUT, 2009). Na categoria de massas cozidas, há exemplos como *ogi, agidi e fura,* preparados com base em milho, sorgo ou milheto (ACHI; UKWURU, 2015), o *mawè* (HOUNHOUIGAN et al., 1993, 1999) e o *kenkey* (AMOA-AWUA et al., 2007; ANNAN et al., 2003) feitos a partir de milho, e o *idli* (KOH; SINGH, 2009) e *mifen* (LU et al., 2008) de arroz. Na categoria de bebidas alcoólicas, o *burukutu, otika* (ACHI; UKWURU, 2015)*, tchoukoutou* (KAYODE et al., 2007; KAYODE; HOUNHOUIGAN; NOUT, 2007) e *jiu* (WANG; SHI; GONG, 2008) feitos de sorgo, o *jnard* (AIDOO; NOUT; SARKAR, 2006) de milheto. Já as bebidas não alcoólicas incluem o *uji* (ONYANGO et al., 2004) feito de sorgo, *kunun-zaki* (ACHI; UKWURU, 2015) e *ben-saalga* (MOUQUET-RIVIER et al., 2008; SONGRE-OUATTARA et al., 2008) de milheto.

Na África, América do Sul e Sudeste asiático, tubérculos como mandioca, batata-doce e inhame constituem importantes alimentos de primeira necessidade, para a população e são amplamente utilizados como matérias-primas na produção de alimentos e bebidas fermentados tradicionais. A partir da mandioca, diversos alimentos fermentados são produzidos e incluem: *gari, fufu, lafun, agbelima, attieke, placali, kivunde* e *abacha,* produzidos na África; *tapai,* produzido na Indonésia (Ásia); *polvilho azedo,* produzido na América Latina, especialmente, no Brazil e Colômbia; e *parakari,* que é uma bebida fermentada de mandioca produzida por povos indígenas da Guiana (RAY; SIVAKUMAR, 2009).

De acordo com Sõukand et al. (2015), a respeito de alimentos e bebidas fermentados tradicionais, na Europa, especificamente, em países da Europa oriental, foi registrada uma rica diversidade biocultural, no uso de frutas ricas em taninos e compostos fenólicos de espécies da família *Rosaceae,* no preparo de diversos alimentos e bebidas fermentadas. Nestes países, a produção e o consumo de alimentos fermentados tradicionais, ainda, desempenham um papel crucial, na culinária popular e tal conhecimento precisa ser avaliado e, rapidamente, documentado.

No Brasil, os povos indígenas utilizam diversos tipos de substratos para produção de alimentos e bebidas fermentados. Schwan et al. (2016), em sua revisão, descrevem que os alimentos à base de mandioca são os mais comuns entre as tribos indígenas brasileiras e incluem a farinha de puba, o cauim, caxiri, yakupa e tarubá. Entretanto outros substratos, também, podem ser utilizados, como milho, arroz, semente de algodão e frutas. A produção desses alimentos associa fatores nutricionais, medicinais e até mesmo religiosos e a maioria deles é obtida, artesanalmente, em pequena escala, sendo, muitas vezes, desconhecida fora de suas tribos, tornando difícil seu estudo.

A farinha de puba ou carimã é um alimento de origem indígena, obtida, após um processo de pubagem da mandioca, no qual as raízes ficam imersas em água, por um período de 3 a 7 dias, antes de ser processada e secada ao sol para obtenção da farinha. No processo de pubagem, ocorre a fermentação natural do substrato que leva à acidificação, amolecimento, detoxificação (degradação de compostos cianogênicos), formação de metabólitos voláteis e não voláteis e remoção de fitato. Atualmente a farinha de puba serve de alimento básico à população de muitas regiões, no Brasil, principalmente, nas regiões Norte e Nordeste (SCHWAN et al., 2016).

Os índios Tapirapé, no Mato Grosso (MT), produzem o *cauim*, uma bebida fermentada não alcoólica produzida, a partir de mandioca, arroz, milho, amendoim entre outras matérias-primas. O *cauim* é comumente utilizado na alimentação de crianças até dois anos de idade e de seus pais. A forma de produção da bebida, ainda, é bastante rudimentar e envolve, basicamente, o cozimento e a fermentação do substrato, a partir da adição da saliva obtida da mastigação da batata-doce (ALMEIDA; RACHID; SCHWAN, 2007; RAMOS et al., 2010, 2011; SCHWAN et al., 2016).

Os povos indígenas Juruna, habitantes do Parque Indígena do Xingu (MT), produzem duas bebidas fermentadas de mandioca, o *caxiri* que é uma bebida alcoólica produzida, durante rituais festivos e o *yakupa* que é bebida não alcoólica consumida diariamente. Apesar de apresentarem formas de produção distintas, em ambos os processos, é necessário realizar, primeiramente, a pubagem da mandioca, ou seja, a mandioca da variedade “brava” é deixada imersa em água, por alguns dias, para que ocorra a detoxificação das raízes que contêm altos teores de cianeto (FREIRE et al., 2013; MIGUEL et al., 2015; SANTOS et al., 2012; SCHWAN et al., 2016).

O *calugi* é um tipo de mingau fermentado, produzido pelos povos Javaé (Tocantins), a partir de milho, mandioca e arroz, adicionado da saliva obtida da mastigação da batata-doce, como no *cauim*. O *calugi*, então, consiste de um alimento obtido da mistura destes substratos processados, não alcoólico, consumido, diariamente, por crianças e adultos da tribo (MIGUEL et al., 2012, 2014; SCHWAN et al., 2016).

Povos indígenas do estado do Amazonas produzem o *tarubá,* uma bebida fermentada de mandioca, que, diferentemente, das outras bebidas, é obtida, via fermentação em estado sólido da massa de mandioca, que, posteriormente, é diluída em água para o consumo (RAMOS et al., 2015; SCHWAN et al., 2016).

A grande diversidade de alimentos e bebidas fermentados tradicionais produzida, em diversas regiões do mundo, acaba por ampliar as fontes de obtenção de novos alimentos naturais que podem ser explorados, quanto às suas qualidades e benefícios à saúde e serem, então, utilizados como modelos para inovação (ACHI; UKWURU, 2015).

2.2 Fermentação de cereais

No contexto dos alimentos e bebidas tradicionais fermentados, o termo “fermentação” refere-se ao efeito transformador dos microrganismos e de seus produtos (principalmente, ácidos orgânicos, álcoois, enzimas e CO_2) sobre os substratos utilizados no preparo dos alimentos (SÕUKAND et al., 2015).

Os cereais são considerados excelentes substratos, para a fermentação microbiana, uma vez que são ricos em carboidratos, minerais, vitaminas, esteróis e outros fatores de crescimento, que possibilitam o crescimento dos microrganismos. Entretanto alguns fatores como composição química, métodos de processamento, capacidade de crescimento e de fermentação do microrganismo influenciam, diretamente, no processo e, portanto devem ser considerados, quando da sua utilização como substratos (ACHI; UKWURU, 2015). Grande parte dos alimentos fermentados tradicionais à base de cereais é obtida, via fermentação lática, ocorrendo, na maioria deles, de forma espontânea, por ação de um conjunto de microrganismos, naturalmente presentes, como bactérias, fungos e leveduras (BLANDINO et al., 2003).

Durante o processo fermentativo, os grãos de cereais sofrem uma série de modificações, que resultam em alterações bioquímicas dentro do endosperma de reserva (rico em carboidratos), no qual enzimas hidrolíticas vão atuar para liberação dos açúcares fermentescíveis. Grãos de cereais maduros apresentam baixo conteúdo de açúcares livres, para fermentação, no entanto, ainda, são capazes de promover o início do processo. A quebra do amido, em açúcares simples fermentescível como maltose e glicose, durante o processo, ocorre pela ação de enzimas endógenas, pela adição de malte ou, principalmente, pela atividade enzimática de microrganismos, como fungos filamentosos e bactérias do ácido lático (ACHI; UKWURU, 2015).

De modo geral, algumas mudanças bioquímicas importantes ocorrem, durante o processo fermentativo natural de cereais, como: o consumo de carboidratos, inclusive, de poli e oligossacarídeos não digeríveis; síntese de alguns aminoácidos e aumento da disponibilidade de vitaminas do complexo B; redução do pH e degradação enzimática de fitato, levando ao aumento no conteúdo de ferro, zinco e cálcio; além de mudanças que levam à melhora na vida útil, na textura, no sabor e aroma do produto final (BLANDINO et al., 2003).

Diversos métodos e tecnologias de processamento dos cereais são aplicados, na tentativa de melhorar a qualidade nutricional desses alimentos, como a suplementação com grãos de leguminosas, para aumentar o valor proteico; o cozimento, para aumentar a disponibilidade do amido e, especialmente, a fermentação que, além de aumentar a qualidade sensorial, elimina substancias tóxicas antinutricionais (taninos, ácido fítico, polifenóis), aumentando, assim, a qualidade nutricional (ACHI; UKWURU, 2015).

2.3 Fermentação da mandioca

Raízes tropicais e tubérculos, como a mandioca, batata-doce e o inhame, são considerados alimentos de primeira necessidade, em diversos países da América do Sul, África e Sudeste Asiático. De acordo com os dados obtidos pela Food and Agriculture Organization of the United Nations (FAO), a produção mundial de mandioca, em 2014, foi maior que 270 milhões de toneladas, visto que mais de 146 milhões de toneladas foram produzidas, na África, mais de 77 milhões de toneladas, no Sudeste Asiático e mais de 32 milhões de toneladas na América. O Brasil é um dos maiores produtores mundiais, com uma produção total de mais 23 milhões de toneladas em 2014 (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO, 2014).

Apesar de ser um alimento básico na dieta de muitos países, a mandioca é considerada um alimento inferior, uma vez que suas raízes apresentam baixo teor de proteínas, de minerais essenciais e vitaminas, além de ser altamente perecível, possuir altas concentrações de glicosídeos cianogênicos (CG) em algumas variedades e de taninos. Dessa forma, muitos produtos e alimentos à base de mandioca têm sido obtidos, a partir da fermentação desse substrato, principalmente via fermentação, em estado sólido (RAY; SIVAKUMAR, 2009).

A fermentação é um importante meio de processamento da mandioca, capaz de melhorar características como palatabilidade, textura e valor nutricional, além de reduzir os níveis de cianeto, para concentrações seguras de consumo (< 30mg HCN/Kg) e prolongar o tempo de vida útil do alimento fermentado (RAY; SIVAKUMAR, 2009). Além da fermentação, outros métodos de processamento das raízes de mandioca já são muito bem conhecidos, como fervura, torrefação e secagem, e a utilização de um ou vários métodos em conjunto vai depender da variedade de mandioca utilizada (HOLLEMAN; ATTEN, 1956).

O método mais popular de processamento é a fermentação, principalmente, nas variedades com alto teor de glicosídeos cianogênicos (KOSTINEK et al., 2005). A fermentação lática, em conjunto com outras formas de processamento, tem papel importante na detoxificação das raízes de mandioca e, consequentemente, na segurança do produto final. Isso se deve à capacidade de produção de enzimas linamarases por algumas bactérias do ácido lático isoladas desses alimentos, que hidrolisam os componentes tóxicos presentes na mandioca (OYEWOLE, 1997).

O processo de pubagem da mandioca consiste de um tipo de fermentação submersa, natural das raízes, que são deixadas de molho em água por até 7 dias. Tal processo tem origem indígena e é feito de forma muito similar entre os povos indígenas brasileiros e do Oeste africano, para obtenção de diferentes alimentos fermentados. Importantes modificações ocorrem, durante a pubagem, em decorrência da sucessão microbiológica que ocorre entre BAL (particularmente *Lactobacillus* and *Leuconostoc* sp.), *Enterobacteriaceae*, *Corynebacterium* sp., *Bacillus* sp., *Clostridium* sp., leveduras e fungos filamentosos. Tais modificações incluem a detoxificação das raízes e eliminação de compostos antinutricionais, além da produção de ácidos orgânicos, que são responsáveis pela segurança do produto final e o desenvolvimento de características sensoriais como flavour e textura (SCHWAN et al., 2016).

2.4 Microbiota de alimentos e bebidas fermentados tradicionais

As fermentações naturais, ainda, são muito comuns no preparo de alimentos e bebidas fermentadas tradicionais. Esse tipo de processo é resultado das atividades competitivas de uma série de microrganismos contaminantes, ocorrendo típica sucessão microbiana, na qual as bactérias dominam os estágios iniciais do processo seguido pelas leveduras, que são favorecidas por substratos ricos em carboidratos fermentescíveis (HOLZAPFEL, 2002).

A diversidade de microrganismos, nos alimentos fermentados, desempenha alguns papéis importantes nestes produtos, como preservação, aumento do valor nutricional e sensorial. A produção de ácidos orgânicos (ácido lático e acético), álcool, bacteriocinas e bacteriolisinas contribuem para a preservação desses produtos. O aumento e a biodisponibilização de compostos bioativos, como vitaminas, antioxidantes, enzimas e aminoácidos, contribuem para a funcionalidade destes produtos. E, finalmente, a combinação das atividades oxidativas de bactérias, associadas às atividades proteolíticas e lipolíticas de leveduras, resultam na composição das características sensoriais do produto final (SCHWAN; RAMOS, 2015).

A fermentação lática de cereais e mandioca é, predominantemente, dominada por bactérias do ácido lático (BAL), mas, em muitos casos, envolve, também, espécies de leveduras embora em populações inferiores. Tais microrganismos desempenham papel importante, na fermentação destes substratos, promovendo melhoras nas características de sabor, aroma, textura e acidez, além de aumentar a digestibilidade e o valor nutricional desses produtos (ACHI; UKWURU, 2015; NOUT, 2009; RAY; SIVAKUMAR, 2009). A comum ocorrência de BAL, nos alimentos, aliada à sua longa utilização histórica, contribui para a sua aceitação como GRAS, sendo segura para consumo humano. As BAL contribuem não apenas para a preservação dos alimentos, mas também para a promoção do sabor. Em substratos ricos em carboidratos, essas bactérias crescem, frequentemente, em associação com leveduras, que, normalmente, estão presentes em populações mais baixas. Trabalhos têm descrito as BAL como componentes importantes, em diversos alimentos fermentados, como iogurte e leites fermentados e alimentos indígenas fermentados à base de cereais (SCHWAN; RAMOS, 2015).

Os gêneros de BAL mais comuns, nas fermentações de cereais, são *Leuconostoc, Lactobacillus, Streptococcus e Pediococcus.* Nesses produtos, o papel preservativo das BAL tem sido atribuído à produção de ácidos orgânicos que, ao reduzirem o pH abaixo de 4,0, inibem a sobrevivência de microrganismos deteriorantes; além da produção de peróxido de hidrogênio e antibióticos que, também, apresentam efeito antimicrobiano (BLANDINO et al., 2003).

Em alimentos fermentados à base milho e consumidos na África, como *mawé* (massa não cozida fermentada de milho), a fermentação natural é dominada por BAL heterofermentativas como *Lactobacillus fermentum, L. brevis, L. curvatus, L. buchneri, Weissella confusa* e pediococci e leveduras como *Candida krusei, C. kefyr, C. glabrata* e *Saccharomyces cerevisiae,* tipicamente presentes em fermentações láticas tradicionais. Em alimentos utilizados, na alimentação complementar de lactentes, como *ogi* e *uji*, a fermentação é dominada por *L. plantarum, L. fermentum, L. cellobiosus, L. buchneri, Pediococcus acidilactici* e *Ped. pentosaceus,* e as espécies dominantes de leveduras são *S. cerevisiae*, *Rhodotorula graminis, C. krusei, C. tropicalis, Geotrichum candidum* e *Geotrichum fermentum*. A fermentação do *kenkey* é dominada pelas espécies *L. plantarum, L.*

fermentum, L. brevis, L. reuteri e *Ped. pentosaceus* e pelas espécies de leveduras *C. krusei* e *S. cerevisiae* (FRANZ et al., 2014).

Em diferentes alimentos e bebidas fermentados à base de mandioca, muitas espécies de BAL e algumas leveduras, também, já foram identificadas e os principais gêneros e espécies relatados, em alguns destes produtos, estão demonstrados na Tabela 2.

Tabela 2 - Bactérias do ácido lático, comumente identificadas em alimentos e bebidas fermentados à base de mandioca, consumidos em países africanos e por povos indígenas no Brasil.

Alimento	**Microrganismos**	**Origem**	**Referência**
Gari	*L. plantarum, L. fallax, L. fermentum, W. paramesenteroides, L. brevis, Leuc. pseudomesenteroides, Strep. lactis, Geotrichum candidum, Corynebacterium manihot*	Nigéria e outros países da África ocidental.	Kostinek et al. (2005, 2007), Oguntoyinbo (2008) e Oguntoyinbo e Dodd (2010).
Fufu	*Ped. pentosaceus, L. fermentum, L. plantarum*	Nigéria e no Congo.	Oyewole (2001).
Lafun	*L. fermentum, L. plantarum, W. confusa,* leveduras *(S. cerevisiae, P. scutulata, Klyveromyces marxianus, Hanseniaspora guillermondii)*	África.	Padonou et al. (2009).
Agbelina	*L. plantarum, L. brevis, L. fermentum, Leuc. mesenteroides, Bacillus spp., Candida tropicalis, Geotrichum candidum*	Gana, Togo e Benin.	Mante, Sakyi-Dawson e Amoa-Awua (2003).
Cauim	*L. pentosus, L. plantarum, L. fermentum, L. paracasei* e *L. brevis, S. cerevisiae, Candida parapsilosis, C. orthopsilosis, Clavispora lusitaniae* e *Rhodotorula mucilaginosa*	Brasil.	Almeida, Rachid e Schwan (2007) e Ramos et al. (2010, 2011).
Yakupa	*L. fermentum, L. plantarum, W. cibaria* e *W. confusa, S. cerevisiae* e *Pichia kudriavzevii*	Brasil.	Freire et al. (2014).
Tarubá	*L. plantarum, Bacillus sp., Chitinophaga terrae,* leveduras (*Pichia exigua, Candida rugosa, T. delbrueckii, Candida tropicalis, P. kudriavzevii, Wickerhamomyces anomalus, Candida ethanolica*)	Brasil.	Ramos et al. (2015).

A partir da identificação da microbiota, envolvida nos diferentes alimentos fermentados tradicionais, é possível inferir que esses alimentos são fontes potenciais de microrganismos com características específicas, que podem ser associadas aos atributos de probióticos. Dessa forma, estudos relacionados à seleção e manutenção de culturas iniciadoras probióticas devem ser intensificados, buscando melhorar os benefícios à saúde fornecidos por estes alimentos.

2.4.1 Bactérias do ácido lático (BAL)

As BAL correspondem a um grupo relacionado de bactérias, cujo maior produto metabólico obtido, via fermentação de carboidratos, é o ácido lático (REDDY et al., 2008). Estão classificadas no filo Firmicutes, incluindo, aproximadamente, 20 gêneros e o gênero *Lactobacillus* compreende cerca de 80 espécies descritas. São, tipicamente, Gram-positivas, não esporulantes, catalase-negativa, anaeróbios aerotolerantes, cocos ou bacilos, ácido-tolerantes e com um complexo requerimento nutricional por aminoácidos e vitaminas (AXELSSON, 2004). Quanto ao padrão fermentativo, podem ser classificadas em homofermentativas, as quais produzem, principalmente, ácido lático, a partir de glicose e heterofermentativas, que produzem somente, além de ácido lático, etanol e dióxido de carbono (CO_2) e, menos comum, ácido acético (REDDY et al., 2008).

Bactérias do ácido lático amilolíticas (ALAB) apresentam habilidade de hidrolisar, parcialmente, o amido pela atividade de suas α-amilases (RODRIGUEZ-SANOJA et al., 2000). Sua ocorrência em alimentos fermentados amiláceos é determinada pelo modo de processamento da matéria-prima utilizada (GUYOT; CALDERON; MORLON-GUYOT, 2000). Em razão dessa característica, as ALAB têm a capacidade de fermentar diferentes tipos de substratos amiláceos e, por isso, têm sido isoladas de diferentes alimentos à base de cereais e mandioca. Estirpes de *Lactobacillus plantarum* e *L. fermentum* foram isoladas de vários alimentos fermentados amiláceos tradicionais da Nigéria (SANNI; MORLON-GUYOT; GUYOT, 2002) e de alimentos à base de milho, ogi e mawè, tradicionais de Benin (AGATI et al., 1988).

Uma vez que os cereais e a mandioca consistem, principalmente, de amido, o uso de ALAB pode ser vantajoso e, por isso, tem sido utilizado na fermentação de alimentos amiláceos. Nguyen et al. (2007) investigaram o efeito da utilização da estirpe amilolítica *L. plantarum* A6, em combinação com métodos de gelatinização, na produção de alimentos à base de arroz e soja, para o desenvolvimento de um novo processo biotecnológico, para obtenção de alimentos com alto valor energético usado na alimentação de crianças.

Essa mesma linhagem foi utilizada por Songré-Ouattara et al. (2008), no preparo do *ben-saalga*, um alimento àa base de milheto, tradicionalmente, consumido em Burkina Faso. Os autores compararam três métodos, a inoculação com culturas iniciadoras de ALAB, a fermentação natural e o uso de uma cultura mãe (back slopping) e observaram que a estirpe *L. plantarum* A6 pode ser uma eficiente cultura iniciadora, nesse tipo de alimento, podendo substituir o uso do malte nessas fermentações.

Dessa forma, estirpes de ALAB isoladas de alimentos à base de cereais e mandioca podem ser, potencialmente, aplicadas como cultura iniciadora nessas fermentações, possibilitando a conversão da biomassa amilácea em ácido lático com uma única etapa de fermentação (REDDY et al., 2008).

2.5 Alimentos funcionais

O termo funcional surgiu pela primeira vez, no Japão, na década de 80 e faz parte de uma nova concepção dos alimentos. Alimentos funcionais são definidos como alimentos que, além de apresentarem função nutricional básica, contêm quantidades adequadas de componentes, biologicamente, ativos, que atuam, positivamente, sobre a saúde do consumidor (ACHI; UKWURU, 2015).

Segundo Roberfroid (2002), alimentos funcionais apresentam as seguintes características:

a) são alimentos convencionais e consumidos na dieta normal/usual;
b) são compostos por componentes naturais, algumas vezes, em elevada concentração ou presentes em alimentos que, normalmente, não os supririam;
c) apresentam efeitos positivos além do valor básico nutritivo, que pode aumentar o bem-estar e a saúde e/ou reduzir o risco de ocorrência de doenças, promovendo benefícios à saúde além de aumentar a qualidade de vida, incluindo os desempenhos físico, psicológico e comportamental;
d) a alegação da propriedade funcional deve ter embasamento científico;
e) pode ser um alimento no qual a bioatividade de um ou mais componentes tenha sido modificada.

A legislação brasileira, por meio da Agência Nacional de Vigilância Sanitária (ANVISA), não apresenta uma definição de alimento funcional, mas define como alegação de propriedade funcional: “aquela relativa ao papel metabólico ou fisiológico que o nutriente ou não nutriente tem no crescimento, desenvolvimento, manutenção e outras funções normais do organismo humano”. Alimentos com alegações de propriedades funcionais e ou de saúde são regulamentados pelas Resoluções 18/99, 17/99 e 19/99 da ANVISA, que estabelecem as diretrizes básicas, para uso de alegações de propriedades funcionais, à avaliação de risco e segurança e os procedimentos para registro, respectivamente (BRASIL, 1999).

Dentre as classes de alimentos funcionais podem-se citar: os probióticos e prebióticos, os alimentos sulfurados e nitrogenados, vitaminas antioxidantes, compostos fenólicos, ácidos graxos poli-insaturados e as fibras.

Recentemente, alimentos fermentados, a partir de cereais e seus ingredientes, têm sido considerados alimentos funcionais, uma vez que fornecem fibras dietéticas (β-glucano e arabinoxilano), proteínas, energia, minerais (selênio), vitaminas (vitamina E, folato) e antioxidantes (ácidos fenólicos), essenciais para a saúde humana. β-glucano é uma fibra solúvel em água, capaz de promover o atraso, no esvaziamento gástrico, aumentando o tempo de trânsito gastrointestinal e a viscosidade luminal. Além das fibras, os cereais, também, possuem carboidratos de amido resistente, como galacto e fruto-oligossacarídeos, que são açúcares fermentescíveis, tornando-os ótimos substratos para o crescimento de microrganismos probióticos (ACHI; UKWURU, 2015).

Entretanto a maioria dos alimentos e bebidas fermentados tradicionais, especialmente, os produtos não lácteos, ainda, é pouco estudada, em relação aos seus benefícios à saúde, apresentando apenas alegações não fundamentadas ligando-os a efeitos positivos sobre a saúde humana. Nesse sentido, estudos mais aprofundados e específicos, que busquem provas científicas credíveis, devem ser realizados sob a forma de triagens randomizadas, controladas e replicáveis (MARSH et al., 2014).

2.5.1 Probióticos

Probióticos são definidos como “microrganismos vivos não patogênicos que, quando administrados em quantidades adequadas, confere benefícios à saúde do hospedeiro” (WORLD HEALTH ORGANIZATION - WHO, 2006). De uma maneira geral, concentrações entre 8 e 9 log de bactérias probióticas por mL ou g de alimento são recomendadas, para que se obtenham os efeitos benéficos à saúde. Alguns desses efeitos incluem a melhora em casos de diarreias infantis, diarreias causadas pelo uso de antibióticos, colites, infecções po *Heliobacter pylori*, doença inflamatória intestinal, câncer, infecções urogenital feminina, além de efeitos benéficos em casos de intolerância à lactose, redução nos níveis de colesterol, aumento da utilização dos nutrientes, entre outros (ANGMO et al., 2016).

As bactérias do ácido lático são microrganismos desejáveis, na microbiota do trato-gastrointestinal e, por isso, são os probióticos mais estudados nas últimas décadas. Os gêneros de BAL, comumente utilizados como probióticos para consumo humano, são *Bifidobacterium* e *Lactobacillus* (MARGOLLES; MAYO; RUAS-MADIEDO, 2009), os quais são reconhecidos como GRAS (“Generally Recognized As Safe”) pela organização United States

Food and Drug Administration (FDA). Outras BAL como *Lactococcus lactis, Streptococcus thermophilus* e muitas espécies de *Leuconostoc* e *Pediococcus* apresentam o status de seguros QPS (“Qualified Presumption of Safety”) como proposto pela European Food Safety Authority (EFSA) (EUROPEAN FOOD SAFETY AUTHORITY - EFSA, 2007).

As cepas microbianas candidatas a probióticos devem satisfazer alguns critérios como os relacionados à segurança, produção ou fabricação, administração, sobrevivência e colonização no trato-gastrointestinal do hospedeiro. Por meio de testes experimentais *in vitro*, é possível realizar a seleção das cepas que atendam a estes critérios e, a partir de testes *in vivo*, é que se confirmam os reais benefícios à saúde (ANGMO et al., 2016).

2.5.2 Prebióticos e simbióticos

Os prebióticos são definidos como “ingredientes seletivamente fermentáveis que resultam em mudanças específicas na composição e/ou atividade da microbiota do trato-gastrointestinal (TGI), conferindo, assim, benefícios à saúde do hospedeiro” (MEETING OF THE INTERNATIONAL SCIENTIFIC ASSOCIATION OF PROBIOTICS AND PREBIOTICS - ISAPP, 2008). Para que os prebióticos atinjam as porções mais distais do TGI, é importante que não sejam digeridos por enzimas humanas ou apenas sejam parcialmente digeridos (MARTINEZ; BEDANI; SAAD, 2015). Desta forma, eles são capazes de influenciar o crescimento das populações microbianas e a produção de ácidos graxos de cadeia curta (SCFAS), alterando, assim, a funcionalidade intestinal (PATURI et al., 2015).

Os principais benefícios relacionados ao consumo de prebióticos são: aumento da microbiota desejável no cólon intestinal, redução do risco de câncer, melhora no controle da glicemia, aumento da absorção de cálcio e outros minerais, aumento de vitaminas do complexo B e folato, aumento das fezes, redução do valor calórico e aumento da resposta imunológica do hospedeiro (ROLIM, 2015).

Os prebióticos mais estudados e conhecidos, atualmente, são os fruto-oligossacarídeos (FOS), galacto-oligossacarídeos (GOS) e a inulina, os quais são, seletivamente, utilizados por bifidobactérias e lactobacilii (PATURI et al., 2015). De forma similar aos probióticos, o consumo de prebióticos deve ser diário para que ocorram efeitos benéficos. Segundo a legislação brasileira, a dose diária recomendada de prebióticos (inulina, FOS e lactulose) por porção servida de alimento deve ser de 3g para alimentos sólidos e 1,5g para os líquidos (BRASIL, 2008).

Um produto simbiótico é a combinação de um ou mais microrganismos probióticos com um prebiótico. Tal combinação pode levar à adaptação prévia do probiótico ao substrato prebiótico, promovendo, assim, uma interação positiva *in vivo*. Em alguns casos, isso pode levar a uma vantagem competitiva para o probiótico, se consumido junto com a fibra prebiótica (MARTINEZ; BEDANI; SAAD, 2015).

2.5.3 Bactérias do ácido lático funcionais em alimentos

Alimentos fermentados tradicionais, normalmente, obtidos por fermentações naturais, constituem grandes reservatórios de novas cepas de BAL com potencial probiótico (KUMARI et al., 2016; RAMOS et al., 2013; SONAR; HALAMI, 2014), como demonstrado na Tabela 3.

Alguns estudos têm demonstrado que bebidas fermentadas por BAL à base de cereais, assim como outros tipos de alimentos fermentados (leite, carnes e vegetais), apresentaram melhoras na digestibilidade de proteínas (HOLZAPFEL, 1997), na biodisponibilidade de minerais e outros micronutrientes (AGARRY; NKAMA; AKOMA, 2010; GREFFEUILLE et al., 2011), além de ter prolongando o tempo de vida útil do alimento (ANGELOV et al., 2006; GUPTA; COX; ABU-GHANNAM, 2010) e promovido melhoras nas características sensoriais do produto (NIONELLI et al., 2014; PEYER; ZANNINI; ARENDT, 2016).

Tabela 3 - Alimentos fermentados tradicionais com potencial probiótico.

(Continua)

Produto	**Microrganismos probióticos**	**Substratos**
Adai	LAB	Cereais, legumes
Agbelima	*Lb. plantarum, Lb. brevis, Lb. fermentum, Leuc. mesenteroides*	Mandioca
Atole	LAB	Milho
Bem-saalga	LAB	Milheto
Boza	*Lb. plantarum, Lb. brevis, Lb. rhamnosus, Lb. fermentum, Leuc. mesenteroides*, subsp. *dextranium*	Cereais
Dosa	*Leuc. mesenteroides, Lb. fermentum, Sacch. cerevisiae*	Arroz, Bengal gram
Idli	*Leuc. mesenteroides*, LAB, yeast	Cerais e legumes
Ilambazi lokubilisa	LAB	Milho
Kecap	LAB	Trigo, soja
Kenkey	*Lb. casei, Lb. lactis, Lb. plantarum, Lb. brevis, Lb. acidophilus, Lb. fermentum*, yeast	Milho
Kimchi	*Lb. plantarum, Lb. brevis, Leuc. mesenteroides, Lb. curvatus, Lb. sake*	Vegetais
Kishk	LAB	Cereais, leite
Kisra	*Lactobacillus sp., Lb. brevis*	Sorgo
Koko	*Lb. fermentum, Lb. salivarius*	Milheto
Mahewu	*Lb. brevis, Lb. bulgaricus*	Milho
Mawe	*Lb. fermentum, Lb. brevis, Lb. salivarius, Sacch. cerevisiae*	Milho
Ogi	*Lb. plantarum, Lb. fermentum, Leuc. mesenteroides, Sacch. cerevisiae*	Milho
Sauerkraut	*Leuc. mesenteroides, Lactococcus lactis*, LAB	Repolho

Tabela 3 - Alimentos fermentados tradicionais com potencial probiótico.

(Conclusão)

Produto	Microrganismos probióticos	Substratos
Som-fyg	LAB	Peixe
Tarhana	*Streptococcus thermophilus, Lb. bulgaricus, Lb. plantarum*	Farelo de trigo parboilizado, iogurte
Tempeh	*LAB, Lb. plantarum*	Soja
Uji	LAB	Milho, sorgo, mandioca, milheto

Fonte: Rivera-Espinoza e Gallardo-Navarro (2010).

Além das aplicações como probióticos, muitas BAL têm sido utilizadas com outras funções nos alimentos como preservação e segurança e aumento do valor nutricional. Pela produção de compostos antimicrobianos, dentre eles as bacteriocinas, muitas estirpes de BAL (*L. lactis* subsp. *lactis, Enterococcus sp., Lb. curvatus, Lb. sakei, P. acidilactici, E. faecium, Lb. plantarum, L. lactis*) constituem alternativa ao uso de aditivos químicos nos alimentos (HOLZAPFEL; GEISEN; SCHILLINGER, 1995; LUCKE, 2000). As bacteriocinas são peptídeos de baixo peso molecular ou proteínas com ação antibacteriana, que produzidas *in situ*, na matriz alimentar, podem aumentar a competitividade da estirpe produtora, prevenindo a deterioração do alimento (LEROY; VUYST, 2004).

Muitos estudos com produtos lácteos (BENKERROUM et al., 2002), linguiças fermentadas (CALLEWAERT; HUGAS; DE VUYST, 2000), vegetais fermentados (HARRIS, 1998; HARRIS; FLEMING; KLAENHAMMER, 1992; RUIZ-BARBA et al., 1994) têm indicado que estirpes iniciadoras de BAL, ao produzirem suas bacteriocinas nos alimentos, acabam inibindo o crescimento de microrganismos deteriorantes e de muitas bactérias patogênicas como, por exemplo, *Clostridium botulinum, Staphylococcus aureus* e *Listeria monocytogenes* (NETTLES; BAREFOOT, 1993).

Com relação ao aumento do valor nutricional dos alimentos, o uso de culturas funcionais de BAL leva à redução de fatores tóxicos ou antinutricionais pela remoção de inibidores de proteinase, ácido fítico e taninos, evitando a má digestão e aumentando a biodisponibilidade de minerais (HOLZAPFEL, 1997, 2002); remoção de toxinas como glicosídeos cianogênicos presentes na mandioca (HOLZAPFEL, 2002; KIMARYO et al., 2000), assim como aminas biogênicas em alimentos fermentados tradicionais (HOLZAPFEL, 2002).

Outra vantagem nutricional é a produção de vitaminas do complexo B como, por exemplo, folato e riboflavina, que aumentam a qualidade do produto final. Espécies de *Lb.*

delbrueckii subsp. *bulgaricus, L. lactis, S. thermophilus* têm sido relatadas como produtoras de folato em produtos lácteos (HUGENHOLTZ; KLEEREBEZEM, 1999; WOUTERS et al., 2002). A deficiência dessas vitaminas pode levar a defeitos, no tubo neural, abortos espontâneos e anemia megaloblática, no caso do ácido fólico, e a falhas no crescimento, inflamação na pele ou perda de visão no caso da riboflavina.

Dessa forma, culturas iniciadoras de BAL que combinem diferentes características funcionais poderiam ser utilizadas, no desenvolvimento de novos alimentos ou alimentos de maior qualidade, a partir de matérias-primas locais, focando nas necessidades nutricionais e de saúde (TURPIN; HUMBLOT; GUYOT, 2011).

2.5.4 Alimentos funcionais preparados a partir de matérias-primas não lácteas

Atualmente a demanda por alimentos funcionais, saudáveis e diversificados tem crescido entre os consumidores, que buscam, na alimentação, uma forma de satisfazer condições relacionadas à nutrição, como intolerâncias e alergias, mas também capazes de satisfazer escolhas relacionadas ao estilo de vida, como vegetarianismo e veganismo (PEYER; ZANNINI; ARENDT, 2016).

Segundo dados obtidos pela Euromonitor International, o mercado de alimentos e bebidas com alegações de saúde e bem-estar movimentou cerca de 750 bilhões de dólares, em 2013, sendo 264 bilhões de dólares representados apenas pelo mercado de produtos funcionais. A perspectiva é que, globalmente, produtos de saúde e bem-estar tenham um crescimento superior aos produtos convencionais dos setores de alimentos e bebidas. A América-Latina, por exemplo, tem hoje 17% do mercado total de alimentos e bebidas funcionais (ROLIM, 2015).

Entre os alimentos contendo probióticos, o iogurte e o leite fermentado são os mais comuns. No entanto, em razão da grande quantidade de pessoas intolerantes à lactose, alérgicas à proteína do leite, com hipercolesterolemia ou que optaram pelo veganismo, o mercado de bebidas e alimentos à base de plantas vem crescendo (CRUZ et al., 2010; GRANATO et al., 2010).

As bebidas feitas, a partir de plantas ou substratos não lácteos, fermentadas ou não e utilizadas como potenciais substitutos aos leites correspondem, hoje, ao segmento de novos produtos alimentares que mais cresce dentro do mercado de bebidas funcionais no mundo. A previsão é de que o mercado de bebidas à base de plantas cresça 15% de 2013 a 2018 devendo atingir um valor de 14 bilhões de dólares (SETHI; TYAGI; ANURAG, 2016).

Bebidas de cereais têm sido amplamente avaliadas como alimentos funcionais e probióticos, uma vez que apresentam, naturalmente, propriedades nutricionais e promotoras de saúde, como fibras solúveis e fitoestrógenos. Produtos à base de cereais fermentados têm sido desenvolvidos, ao longo da história da humanidade, no entanto estudos que empregam culturas iniciadoras de BAL e que se concentram na avaliação dos atributos sensoriais e/ou de sabor de bebidas fermentadas base de cereais são mais recentes (PEYER; ZANNINI; ARENDT, 2016) (TABELA 4).

Tabela 4 – Estudos empregando bactérias do ácido lático como culturas iniciadoras no desenvolvimento de novas bebidas à base de cereais.

Tópico de estudo	**Substrato**	**Bactéria do ácido lático**	**Referência**
Bebida similar a iogurte	Aveia	*Lb. plantarum* LP01, LP06, LP09, LP32, LP39, LP40, LP48, LP51; *Lb. casei* LC10, LC11, LC03; *Lb. paracasei* LPC02, LPC16	Nionelli et al. (2014).
Perfil de voláteis e estabilidade de flavour	Malte de cevada	*Lb. brevis* R2D; *W. cibaria* PS2; *Lb. plantarum* FST1.7; *Lb. reuteri* R29	Peyer, Zannini e Arendt (2015).
Análise de voláteis de apartir de cepas probióticas	Aveia, trigo, cevada	*Lb. plantarum* NCIMB 8826	Salmerón et al. (2009).
Análise de voláteis de preparações probióticas	Aveia, cevada e malte de cevada	*Lb. acidophilus* NCIMB 8821; *Lb. plantarum* NCIMB 8826; *Lb. reuteri* NCIMB 11951	Salmerón, Thomas e Pandiella (2014).
Aceitação de bebidas probióticas de cereais	Aveia e malte	*Lb. acidophilus* NCIMB 8821; *Lb. plantarum* NCIMB 8826; *Lb. reuteri* NCIMB 11951	Salmerón, Thomas e Pandiella (2015).
Modulações de texturas em novas bebidas	Malte de cevada	*W. cibaria* MG1	Zannini et al. (2013).

Fonte: Adaptado de Peyer, Zannini e Arendt (2016).

Cepas de *L. reuteri, L. acidophilus* e *Bifidobacterium* apresentaram bom crescimento em substratos à base de aveia, a qual é considerada um substrato funcional por ser rica em fibras (MÅRTENSON; ÖSTE; HOLST, 2002). Salmerón, Thomas e Pandiella (2015) avaliaram as características físico-químicas e aceitabilidade de diferentes bebidas à base de cereais (farinha de aveia, malte e cevada) fermentadas por cepas de *L. acidophilus* NCIMB 8821, *L. plantarum* NCIMB 8826, e *L. reuteri* NCIMB 11951 isoladas de humanos.

Costa et al. (2016) avaliaram os parâmetros reológicos de uma nova bebida desenvolvida, a partir de extrato de arroz (farelo de arroz e grãos partidos na proporção de 8:92) fermentado (FRE) por culturas probióticas comerciais de *Streptococcus thermophilus*, *Bifidobacterias* sp. e *Lactobacillus acidophilus*, adicionado de amido de milho em diferentes concentrações. Os autores observaram um comportamento reológico semelhante aos iogurtes e bebidas lácticas, descrito na literatura, indicando que tais produtos podem ser comparáveis aos iogurtes tradicionais em relação à viscosidade.

Estudos utilizando sucos de frutas como substrato, para a obtenção de produtos contendo BAL probióticas, têm sido realizados, os quais apresentam um apelo a serem alimentos saudáveis e refrescantes, além de agradarem todos os grupos de consumidores, de diferentes faixas etárias (SHEEHAN; ROSS; FITZGERALD, 2007; TUORILA; CARDELLO, 2002; YOON; WOODAMS; HANG, 2004). Prado et al. (2015) desenvolveram uma bebida funcional à base de água de coco, a partir da fermentação com *L. plantarum* AC-1, a qual apresentou importantes características probióticas como resistência aos sais biliares, tolerância a baixo pH, à presença de NaCl, além de atividade inibitória contra patogênicos. A análise sensorial do produto demonstrou maior preferência e aceitabilidade pelas bebidas adoçadas com sacarose e flavorizantes artificiais.

Yoon, Woodams e Hang (2004) avaliaram a produção de uma bebida probiótica, a partir de suco de tomate, utilizando cepas de *L. acidophilus, L. plantarum, L. casei* e *L. delbrueckii* como culturas iniciadoras. Os autores relataram que as quatro cepas foram capazes de crescer rapidamente no substrato, atingindo uma população maior que 10^8 ufc/mL, após 48 h de fermentação e de produzir ácido lático, além de manterem viáveis, após quatro semanas de armazenamento à temperatura de 4 °C, o que as tornam potencias candidatas a cepas probióticas.

O milho é uma das mais importantes fontes de alimentos, para milhões de pessoas, especialmente, na América Latina e África (YOUSIF; EL TINAY, 2000). Helland, Wicklund e Narvhus (2004) relataram a sobrevivência de probióticos (*L. reuteri, L. acidophilus e L. rhamnosus*) em meio fermentado de milho. Estudando compostos voláteis produzidos, durante a fermentação de mingau á base de milho, Gonzalez et al. (1994) relataram o acúmulo de diacetil e acetaldeído. No geral, a fermentação do milho induz a produção de compostos de aroma frutado em alimentos mexicanos.

Bebidas fermentadas de arroz, também, são bastante comuns, principalmente, em países Asiáticos. Ghosh et al. (2015) estudaram o papel de *L. fermentum* KKL1 na fermentação da bebida de arroz haria. Os autores relatam que o produto fermentado com esta cepa probiótica apresentou melhoras na sua composição nutricional (vitaminas e minerais), digestibilidade e potencialidades terapêuticas.

Muitos estudos têm demonstrado que bebida à base de soja é um ótimo meio para o crescimento de bactérias probióticas como *L. casei* (GARRO et al., 1999), *L. helveticus* (MURTI et al., 1993), *L. fermenti* (CHUMCHUERE; ROBINSON, 1999), *L. fermentum* (GARRO; DE VALDEZ; DE GIORI, 2001, 2004), *L. reuteri* (TZORTZIS et al., 2004), e *L. acidophilus* (WANG et al., 2003, 2006; WANG; YU; CHOU, 2002). Santos, Libeck e Schwan (2014) desenvolveram uma nova bebida funcional fermentada de leite de soja e amendoim com diferentes cepas de BAL e leveduras em co-cultivo. Os autores relatam que o co-cultivo entre a cepa comercial probiótica *L. acidophilus* LACA 4 com *Pediococcus. acidilactici* (UFLA BFFCX 27.1) e *Saccharomyces cerevisiae* (UFLA YFFBM 18.03) aumentou a viabilidade da cepa probiótica.

Bernat et al. (2014) desenvolveram uma nova bebida simbiótica, a partir de meio de amêndoas, utilizando as cepas probióticas *L. reuteri* ATCC 55730 e *S. thermophilus* CECT 986 e o prebiótico inulina.

3 CONCLUSÕES E CONSIDERAÇÕES FINAIS

Com base nas informações obtidas, na revisão de literatura apresentada, foi possível conhecer um pouco da diversidade de alimentos não “convencionais” que são produzidos, no mundo todo, por diferentes povos e culturas, utilizando diferentes matérias-primas, formas de processamento e microrganismos. Destacam-se como matérias-primas mais comumente utilizadas, os cereais como milho, sorgo, milheto e arroz e os vegetais tuberosos, principalmente, a mandioca. A fermentação desses alimentos ocorre, principalmente, por bactérias do ácido lático (BAL) e, também, por leveduras.

Tais alimentos constituem, dessa forma, potenciais fontes de novos microrganismos e até mesmo novos produtos, para a indústria de alimentos, principalmente, para o mercado de alimentos funcionais. Os estudos de isolamento e identificação de novas linhagens de BAL, assim como a avaliação de suas propriedades, potencialmente probióticas, aumentam as possibilidades de obtenção de novas linhagens microbianas que podem ser aplicadas ao desenvolvimento de produtos com propriedades funcionais e, principalmente, que sejam produzidos, a partir de matérias-primas não lácteas.

O mercado de alimentos probióticos, atualmente, é dominado por alimentos à base de leite e derivados. No entanto diversos estudos, para o desenvolvimento de alimentos, a partir de bebidas de vegetais, como soja, amêndoas e cereais, têm sido realizados, para suprir a crescente demanda por novos produtos não alergênicos e livres de colesterol. Nesse contexto, a seleção de culturas microbianas adequadas, para a fermentação de tais substratos, é uma etapa crucial para o desenvolvimento desses alimentos. Microrganismos provenientes de fermentações naturais de alimentos tradicionais apresentam características adaptativas interessantes, para o processo fermentativo, como rápida acidificação do meio, rápido crescimento, produção de substâncias antimicrobianas, produção de enzimas e metabólitos específicos, entre outras. Tais propriedades tornam essas linhagens bem adaptadas aos meios de origem, mas também podem ser, potencialmente, aplicadas na fermentação de novos substratos.

REFERÊNCIAS

ACHI, O. K.; UKWURU, M. Cereal-based fermented foods of Africa as functional foods. **International Journal of Microbiology and Application**, New York, v. 2, n. 4, p. 71-83, 2015.

AGARRY, O. O.; NKAMA, I.; AKOMA, O. Production of Kunun-zaki (A Nigerian fermented cereal beverage) using starter culture. **International Research Journal of Microbiology**, Sapele, v. 1, n. 2, p. 18-25, 2010.

AGATI, V. et al. Isolation and characterization of new amylolytic strains of *Lactobacillus fermentum* from fermented maize doughs (mawe and ogi) from Benin. **Journal of Applied Microbiology**, Oxford, v. 85, n. 3, p. 512-520, 1998.

AIDOO, K. E.; NOUT, M. J. R.; SARKAR, P. K. Occurrence and function of yeasts in Asian indigenous fermented foods. **FEMS Yeast Research**, New York, v. 6, p. 30-39, 2006.

ALMEIDA, E.; RACHID, C.; SCHWAN, R. Microbial population present in fermented beverage ‘cauim’ produced by Brazilian Amerindians. **International Journal of Food Microbiology**, Amsterdam, v. 120, n. 1/2, p. 146-151, 2007.

ALOYS, N.; ANGELINE, N. Traditional fermented foods and beverages in Burundi. **Food Research International**, Barking, v. 42, n. 5, p. 588-594, 2009.

AMOA-AWUA, W. K. et al. The effect of applying GMP and HACCP to traditional food processing at a semi-commercial kenkey production plant in Ghana. **Food Control**, Guildford, v. 18, n. 11, p. 1449-1457, 2007.

ANGELOV, A. et al. Development of a new oat-based probiotic drink. **International Journal of Food Microbiology**, Amsterdam, v. 112, n. 1, p. 75-80, 2006.

ANGMO, K. et al. Probiotic characterization of lactic acid bacteria isolated from fermented foods and beverage of Ladakh. **LWT - Food Science and Technology**, Trivandrum, v. 66, p. 428-435, Mar. 2016.

ANNAN, N. T. et al. Influence of starter culture combinations of *Lactobacillus fermentum, Saccharomyces cerevisiae* and *Candida krusei* on aroma in Ghanaian maize dough fermentation. **European Food Research and Technology**, Berlin, v. 216, p. 377-384, 2003.

AXELSSON, L. Lactic acid bacteria: classification and physiology. In: SALMINEN, S.; WRIGHT, A. von; OUWEHAND, A. (Ed.). **Lactic acid bacteria:** microbiological and functional aspects. 3rd ed. rev. New York: M. Dekker, 2004. p. 1-66.

BENKERROUM, N. et al. Biocontrol of *Listeria monocytogenes* in a model cultured milk (lben) by in situ bacteriocin production from *Lactococcus lactis ssp. lactis*. **International Journal of Dairy Technology**, Huntingdon, v. 55, n. 3, p. 145-151, 2002.

BERNAT, N. et al. Vegetable milks and their fermented derivative products. **International Journal of Food Studies**, Lisboa, v. 3, n. 1, p. 93-124, Apr. 2014.

BLANDINO, A. et al. Cereal-based fermented foods and beverages. **Food Research International**, Barking, v. 36, n. 6, p. 527-543, 2003.

BRASIL. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. **Lista de alegações de propriedade funcional aprovadas**. Brasília, DF, 2008. Disponível em: <http://www.anvisa.gov.br/alimentos/comissoes/tecno_lista_alega.htm>. Acesso em: 11 out. 2016.

BRASIL. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. **Resolução nº 18**, de 30 de abril de 1999. Regulamento Técnico que Estabelece as Diretrizes Básicas para Análise e Comprovação de Propriedades Funcionais e ou de Saúde Alegadas em Rotulagem de Alimentos. Brasília, DF, 1999. Disponível em: <http://www.saude.rj.gov.br/comum/code/MostrarArquivo.php?C=MjI0OQ%2C%2C>. Acesso em: 10 out. 2016.

CALLEWAERT, R.; HUGAS, M.; DE VUYST, L. Competitiveness and bacteriocin production of Enterococci in the production of Spanish-style dry fermented sausages. **International Journal of Food Microbiology**, Amsterdam, v. 57, p. 33-42, 2000.

CHUMCHUERE, S.; ROBINSON, R. K. Selection of starter cultures for the fermentation of soya milk. **Food Microbiology**, London, v. 16, p. 129-137, 1999.

COSTA, K. K. F. D. et al. Rheological properties of fermented rice extract with probiotic bacteria and different concentrations of waxy maize starch. **LWT - Food Science and Technology**, Trivandrum, v. 72, p. 71-77, Oct. 2016.

CRUZ, A. G. et al. Processing optimization of probiotic yogurt containing glucose oxidase using response surface methodology. **Journal of Dairy Science**, Champaign, v. 93, n. 11, p. 5059-5068, 2010.

DUFRESNE, C.; FARNWORTH, E. Tea, Kombucha, and health: a review. **Food Research International**, Barking, v. 33, n. 6, p. 409-421, 2000.

EUROPEAN FOOD SAFETY AUTHORITY. Introduction of a Qualified Presumption of Safety (QPS) approach for as- sessment of selected microorganisms referred to EFSA: opinion of the Scientific Committee. **The EFSA Journal**, Parma, n. 587, p. 2-16, 2007.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. **Crops**. Rome, 2014. Disponível em: <http://faostat.fao.org/beta/en/#data/QC>. Acesso em: 13 nov. 2016.

FRANZ, C. M. A. P. et al. African fermented foods and probiotics. **International Journal of Food Microbiology**, Amsterdam, v. 190, p. 84-96, Nov. 2014.

FREIRE, A. L. et al. Study of the physicochemical parameters and spontaneous fermentation during the traditional production of yakupa, an indigenous beverage produced by Brazilian Amerindians. **World Journal of Microbiology and Biotechnology**, Oxford, v. 30, n. 2, p. 567-577, 2013.

GARRO, M. S.; DE VALDEZ, G. F.; DE GIORI, G. S. Application of conductimetry for evaluation of lactic starter culture in soymilk. **Journal of Food Science**, Chicago, v. 67, p. 1175-1178, 2001.

GARRO, M. S.; DE VALDEZ, G. F.; DE GIORI, G. S. Temperature effect on the biological activity of *Bifidobacterium longum* CRL 849 and *Lactobacillus fermentum* CRL 251 in pure and mixed cultures grown in soymilk. **Food Microbiology**, London, v. 21, p. 511-518, 2004.

GARRO, M. S. et al. Starter culture activity in refrigerated fermented soymilk. **Journal of Food Protection**, Des Moines, v. 62, n. 7, p. 808-810, 1999.

GHOSH, K. et al. Microbial, saccharifying and antioxidant properties of an Indian rice based fermented beverage. **Food Chemistry**, London, v. 168, p. 196-202, Feb. 2015.

GONZALEZ, S. et al. Acetaldehyde production by strains used as probiotic in fermented milk. **Journal of Food Protection**, Des Moines, v. 57, p. 436-440, 1994.

GRANATO, D. et al. Probiotic dairy products as functional foods. **Comprehensive Reviews in Food Science and Food Safety**, Chicago, v. 9, n. 5, p. 455-470, 2010.

GREFFEUILLE, V. et al. Changes in iron, zinc and chelating agents during traditional African processing of maize: effect of iron contamination on bioaccessibility. **Food Chemistry**, London, v. 126, n. 4, p. 1800-1807, 2011.

GUPTA, S.; COX, S.; ABU-GHANNAM, N. Process optimization for the development of a functional beverage based on lactic acid fermentation of oats. **Biochemical Engineering Journal**, Amsterdam, v. 52, n. 2, p. 199-204, 2010.

GUYOT, J.; CALDERON, M.; MORLON-GUYOT, J. Effect of pH control on lactic acid fermentation of starch by *Lactobacillus manihotivorans* LMG 18010T. **Journal of Applied Microbiology**, Oxford, v. 88, n. 1, p. 176-182, 2001.

HARRIS, L. J. The microbiology of vegetable fermentations. In: ______. **Microbiology of fermented foods**. New York: Springer, 1998. p. 45-72.

HARRIS, L. J.; FLEMING, H. P.; KLAENHAMMER, T. R. Novel paired starter culture system for sauerkraut, consisting of a nisin-resistant Leuconostoc mesenteroides strain and a nisin-producing Lactococcus lactis strain. **Applied and Environmental Microbiology**, Washington, v. 58, p. 1484-1489, 1992.

HELLAND, M. H.; WICKLUND, T.; NARVHUS, J. A. Growth and metabolism of selected strains of probiotic bacteria, in maize porridge with added malted barley. **International Journal of Food Microbiology**, Amsterdam, v. 91, p. 305-313, 2004.

HOLLEMAN, W. J.; ATTEN, A. **Processing of Cassava and Cassava products in rural industries**. Rome: FAO Press, 1956. 115 p.

HOLZAPFEL, W. H. Appropriate starter culture technologies for small-scale fermentation in developing countries. **International Journal of Food Microbiology**, Amsterdam, v. 75, p. 197-212, 2002.

HOLZAPFEL, W. H. Use of starter cultures in fermentation on a household scale. **Food Control**, Guildford, v. 8, p. 241-258, 1997.

HOLZAPFEL, W. H.; GEISEN, R.; SCHILLINGER, U. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. **International Journal of Food Microbiology**, Amsterdam, v. 24, n. 3, p. 343-362, 1995.

HOUNHOUIGAN, D. J. et al. Characterization and frequency distribution of species of lactic acid bacteria involved in the processing of mawe, a fermented maize dough from Benin. **International Journal of Food Microbiology**, Amsterdam, v. 18, n. 4, p. 279-287, 1993.

HOUNHOUIGAN, D. J. et al. Use of starter cultures of lactobacilli and yeast in the fermentation of mawe, an African maize product. **Tropical Science**, London, v. 39, p. 220-226, 1999.

HUGENHOLTZ, J.; KLEEREBEZEM, M. Metabolic engineering of lactic acid bacteria: overview of the approaches and results of pathway rerouting involved in food fermentations. **Current Opinion in Biotechnology**, London, v. 10, n. 5, p. 492-497, 1999.

KAYODE, A. P. P. et al. Household production of sorghum beer in Benin: technological and socio-economical aspects. **International Journal of Consumer Studies**, New York, v. 31, p. 258-264, 2007b.

KAYODE, A. P. P.; HOUNHOUIGAN, D. J.; NOUT, M. J. R. Impact of brewing process operations on phytate, phenolic compounds and in-vitro solubility of iron and zinc in opaque sorghum beer. **Food Science and Technology - LWT**, Trivandrum, v. 40, p. 834-841, 2007.

KIMARYO, V. M. et al. The use of a starter culture in the fermentation of cassava for the production of “kivunde”, a traditional Tanzanian food product. **International Journal of Food Microbiology**, Amsterdam, v. 56, n. 2, p. 179-190, 2000.

KOH, B. K.; SINGH, V. Cooking behavior of rice and black gram in the preparation of idli, a traditional fermented product of indian origin, by viscography. **Journal of Texture Studies**, Westport, v. 40, p. 36-50, 2009.

KOSTINEK, M. et al. Characterisation and biochemical properties of predominant lactic acid bacteria from fermenting cassava for selection as starter cultures. **International Journal of Food Microbiology**, Amsterdam, v. 114, p. 342-351, 2007.

KOSTINEK, M. et al. Diversity and technological properties of predominant lactic acid bacteria from fermented cassava used for the preparation of Gari, a traditional African food. **Systematic and Applied Microbiology**, Stuttgart, v. 28, n. 6, p. 527-540, 2005.

KUMARI, A. et al. Probiotic attributes of indigenous *Lactobacillus*. **Journal of Food Science and Technology**, Trivandrum, v. 53, n. 5, p. 2463-2475, May 2016.

LEROY, F.; VUYST, L. Lactic acid bacteria as functional starter cultures for the food fermentation industry. **Trends in Food Science & Technology**, Cambridge, v. 15, p. 67-78, 2004.

LU, Z. H. et al. Isolation, characterization and identification of lactic acid bacteria and yeasts from sour Mifen, a traditional fermented rice noodle from China. **Journal of Applied Microbiology**, Oxford, v. 105, p. 893-903, 2008.

LUCKE, F. K. Utilization of microbes to process and preserve meats. **Meat Science**, Barking, v. 56, p. 105-115, 2000.

MANTE, E. S.; SAKYI-DAWSON, E.; AMOA-AWUA, W. K. Antimicrobial interactions of microbial species involved in the fermentation of cassava dough into agbelina with particular reference to the inhibitory effect of lactic acid bacteria on enteric pathogens. **International Journal of Food Microbiology**, Amsterdam, v. 89, p. 41-50, 2003.

MARGOLLES, A.; MAYO, B.; RUAS-MADIEDO, P. Screening, identification, and characterization of *Lactobacillus* and *Bifidobacterium* strains. In: NOMOTO, K.; SALMINEN, S.; LEE, Y. K. (Ed.). **Handbook of probiotics and prebiotics**. New Jersey: J. Willey, 2009. p. 4-43.

MARSH, A. J. et al. Fermented beverages with health-promoting potential: past and future perspectives. **Trends in Food Science & Technology**, Cambridge, v. 38, n. 2, p. 113-124, 2014.

MÅRTENSSON, O.; ÖSTE, R.; HOLST, O. The effect of yoghurt culture on the survival of probiotic bacteria in oat-based, non-dairy products. **Food Research International**, Barking, v. 35, n. 8, p. 775-784, 2002.

MARTINEZ, R.; BEDANI, R.; SAAD, S. Scientific evidence for health effects attributed to the consumption of probiotics and prebiotics: an update for current perspectives and future challenges. **British Journal of Nutrition**, Cambridge, v. 114, n. 12, p. 1993-2015, 2015.

MCGOVERN, P. E. et al. Fermented beverages of pre-and proto-historic China. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, v. 101, n. 51, p. 17593-17598, 2004.

MEETING OF THE INTERNATIONAL SCIENTIFIC ASSOCIATION OF PROBIOTICS AND PREBIOTICS, 6., 2008, London. **Proceedings…** London, 2008. 1 CD-ROM.

MIGUEL, M. G. C. P. et al. Bacterial dynamics and chemical changes during the spontaneous production of the fermented porridge (Calugi) from cassava and corn. **African Journal of Microbiology Research**, Nairobi, v. 8, n. 9, p. 839-849, Feb. 2014.

MIGUEL, M. G. C. P. et al. Physico-chemical and microbiological characterization of corn and rice “calugi” produced by Brazilian Amerindian people. **Food Research International**, Barking, v. 49, n. 1, p. 524-532, Nov. 2012.

MIGUEL, M. G. C. P. et al. Physicochemical and microbiological description of Caxiri-a cassava and corn alcoholic beverage. **International Journal of Food Science & Technology**, Oxford, v. 50, n. 12, p. 2537-2544, 2015.

MOUQUET-RIVIER, C. et al. Consumption pattern, biochemical composition and nutritional value of fermented pearl millet gruels in Burkina Faso. **International Journal of Food Sciences and Nutrition**, Hants, v. 59, n. 7/8, p. 716-729, 2008.

MURTI, T. W. et al. Croissance des lactobacilles dans l'extrait de soja: effects sur la viscosité, les composés volatils et la protéolyse. **Sciences des Aliments**, Paris, v. 13, n. 3, p. 491-500, 1993.

NETTLES, C. G.; BAREFOOT, S. F. Biochemical and genetic characteristics of bacteriocins of food-associated lactic acid bacteria. **Journal of Food Protection**, Des Moines, v. 56, n. 4, p. 338-356, 1993.

NGUYEN, T. et al. Effect of fermentation by amylolytic lactic acid bacteria, in process combinations, on characteristics of rice/soybean slurries: a new method for preparing high energy density complementary foods for young children. **Food Chemistry**, London, v. 100, n. 2, p. 623-631, 2007.

NIONELLI, L. et al. Exploitation of Albanian wheat cultivars: characterization of the flours and lactic acid bacteria microbiota, and selection of starters for sourdough fermentation. **Food Microbiology**, London, v. 44, p. 96-107, 2014.

NOUT, M. J. R. Rich nutrition from the poorest: cereal fermentations in Africa and Asia. **Food Microbiology**, London, v. 26, p. 685-692, 2009.

OGUNTOYINBO, F. A. Evaluation of diversity of *Candida* species isolated from fermented cassava during traditional small scale gari production in Nigeria. **Food Control**, Guildford, v. 19, p. 465-469, 2008.

OGUNTOYINBO, F. A.; DODD, C. E. R. Bacterial dynamics during the spontaneous fermentation of cassava dough in gari production. **Food Control**, Guildford, v. 21, n. 3, p. 306-312, Mar. 2010.

ONYANGO, C. et al. Proximate composition and digestibility of fermented and extruded uji from maize-finger millet blend. **Food and Science Technology - LWT**, Trivandrum, v. 37, p. 827-832, 2004.

OYEWOLE, O. B. Characteristics and significance of yeasts involvement in cassava fermentation for ‘fufu’ production. **International Journal of Food Microbiology**, Amsterdam, v. 65, p. 213-218, 2001.

OYEWOLE, O. B. Lactic fermented foods in Africa and their benefits. **Food Control**, Guildford, v. 8, p. 289-297, 1997.

PADONOU, S. W. et al. The microbiota of Lafun, an African traditional cassava food product. **International Journal of Food Microbiology**, Amsterdam, v. 133, p. 22-30, 2009.

PATURI, G. et al. Differential effects of probiotics, prebiotics, and synbiotics on gut microbiota and gene expression in rats. **Journal of Functional Foods**, New York, v. 13, p. 204-213, Mar. 2015.

PEYER, L. C.; ZANNINI, E.; ARENDT, E. K. Lactic acid bacteria as sensory biomodulators for fermented cereal-based beverages. **Trends in Food Science & Technology**, Cambridge, v. 54, p. 17-25, Aug. 2016.

PRADO, F. C. et al. Development and evaluation of a fermented coconut water beverage with potential health benefits. **Journal of Functional Foods**, New York, v. 12, p. 489-497, Jan. 2015.

RAMOS, C. L. et al. 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**, Amsterdam, v. 140, n. 2/3, p. 225-231, 2010.

RAMOS, C. L. et al. Diversity of bacteria and yeast in the naturally fermented cotton seed and rice beverage produced by Brazilian Amerindians. **Food Microbiology**, London, v. 28, n. 7, p. 1380-1386, 2011.

RAMOS, C. L. et al. Microbiological and chemical characteristics of tarubá, an indigenous beverage produced from solid cassava fermentation. **Food Microbiology**, London, v. 49, p. 182-188, Aug. 2015.

RAMOS, C. L. et al. Strain-specific probiotics properties of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. **Food Microbiology**, London, v. 36, n. 1, p. 22-29, 2013.

RAY, R. S. P.; SIVAKUMAR, P. S. Traditional and novel fermented foods and beverages from tropical root and tuber crops: review. **International Journal of Food Science & Technology**, Oxford, v. 44, n. 6, p. 1073-1087, 2009.

REDDY, G. et al. Amylolytic bacterial lactic acid fermentation: a review. **Biotechnology Advances**, New York, v. 26, p. 22-34, 2008.

RIVERA-ESPINOZA, Y.; GALLARDO-NAVARRO, Y. Non-dairy probiotic products. **Food Microbiology**, London, v. 27, n. 1, p. 1-11, 2010.

ROBERFROID, M. Functional food concept and its application to prebiotics. **Digestive and Liver Disease**, New York, v. 34, p. 105-110, 2002.

RODRIGUEZ SANOJA, R. et al. Comparative characterization of complete and truncated forms of Lactobacillus amylovorus alpha -Amylase and Role of the C-Terminal Direct Repeats in Raw-Starch Binding. **Applied and Environmental Microbiology**, Washington, v. 66, n. 8, p. 3350-3356, 2000.

ROLIM, P. M. Development of prebiotic food products and health benefits. **Food Science and Technology**, Trivandrum, v. 35, n. 1, p. 3-10, 2015.

RUIZ-BARBA, J. L. et al. Use of Lactobacillus plantarum LPCO10, a bacteriocin producer, as a starter culture in Spanish-style green olive fermentations. **Applied and Environmental Microbiology**, Washington, v. 60, p. 2059-2064, 1994.

SALMERÓN, I. et al. Volatile compounds produced by the probiotic strain *Lactobacillus plantarum* NCIMB 8826 in cereal-based substrates. **Food Chemistry**, London, v. 117, n. 2, p. 265-271, 2009.

SALMERÓN, I.; THOMAS, K.; PANDIELLA, S. S. Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. **Journal of Functional Foods**, New York, v. 15, p. 106-115, May 2015.

SALMERÓN, I.; THOMAS, K.; PANDIELLA, S. S. Effect of substrate composition and inoculum on the fermentation kinetics and flavour compound profiles of potentially non-dairy probiotic formulations. **LWT - Food Science and Technology**, Trivandrum, v. 55, n. 1, p. 240-247, 2014.

SANNI, A.; MORLON-GUYOT, J.; GUYOT, J. New efficient amylase-producing strains of *Lactobacillus plantarum* and *L. fermentum* isolated from different Nigerian traditional fermented foods. **International Journal of Food Microbiology**, Amsterdam, v. 72, n. 1/2, p. 53-62, 2002.

SANTOS, C. C. A. A. et al. Microbiological and physicochemical characterisation of caxiri, an alcoholic beverage produced by the indigenous Juruna people of Brazil. **International Journal of Food Microbiology**, Amsterdam, v. 156, n. 2, p. 112-121, 2012.

SANTOS, C. C. A. A.; LIBECK, B. S.; SCHWAN, R. F. Co-culture fermentation of peanut-soy milk for the development of a novel functional beverage. **International Journal of Food Microbiology**, Amsterdam, v. 186, p. 32-41, Sept. 2014.

SCHWAN, R. F. et al. Brazilian indigenous food. In: PENNA, A. L. B.; NERO, L. A.; TODOROV, S. D. (Ed.). **Fermented foods of Latin America:** from traditional knowledge to innovative applications. Amsterdam: CRC; Taylor and Francis, 2016. v. 1, p. 214-226.

SCHWAN, R. F.; RAMOS, C. L. Role of microbes and their diversity in fermented foods. In: RAVISHANKAR RAI, V.; BAI, J. A. (Ed.). **Beneficial microbes in fermented and functional foods**. Boca Raton: CRC; Taylor and Francis, 2015. v. 1, p. 507-530.

SETHI, S.; TYAGI, S. K.; ANURAG, R. K. Plant-based milk alternatives an emerging segment of functional beverages: a review. **Journal of Food Science and Technology**, Trivandrum, v. 53, n. 9, p. 3408-3423, 2016.

SHEEHAN, V. M.; ROSS, P.; FITZGERALD, G. F. Assessing the acid tolerance and the technological robustness of probiotic cultures for fortification in fruit juices. **Innovative Food Science & Emerging Technologies**, London, v. 8, n. 2, p. 279-284, 2007.

SONAR, N. R.; HALAMI, P. M. Phenotypic identification and technological attributes of native lactic acid bacteria present in fermented bamboo shoot products from North-East India. **Journal of Food Science and Technology**, Trivandrum, v. 51, n. 12, p. 4143-4148, 2014.

SONGRÉ-OUATTARA, L. et al. Enzyme activities of lactic acid bacteria from a pearl millet fermented gruel (ben-saalga) of functional interest in nutrition. **International Journal of Food Microbiology**, Amsterdam, v. 128, n. 2, p. 395-400, 2008.

SÕUKAND, R. et al. An ethnobotanical perspective on traditional fermented plant foods and beverages in Eastern Europe. **Journal of Ethnopharmacology**, Lausanne, v. 170, p. 284-296, July 2015.

TUORILA, H.; CARDELLO, A. V. Consumer responses to an off-flavor in juice in the presence of specific health claims. **Food Quality and Preference**, Barking, v. 13, n. 7, p. 561-569, 2002.

TURPIN, W.; HUMBLOT, C.; GUYOT, J. P. Genetic screening of functional properties of lactic acid bacteria in a fermented pearl millet slurry and in the metagenome of fermented starchy foods. **Applied and Environmental Microbiology**, Washington, v. 77, p. 8722-8734, 2001.

TZORTZIS, G. et al. In vitro evaluation of the fermentation properties of galactooligosaccharides synthesized by a-galactosidase from *Lactobacillus reuteri*. **Applied Microbiology and Biotechnology**, Berlin, v. 64, p. 106-111, 2004.

WANG, C. L.; SHI, D. J.; GONG, G. L. Microorganisms in Daqu: a starter culture of Chinese Maotai-flavor liquor. **World Journal of Microbiology and Biotechnology**, Oxford, v. 24, p. 2183-2190, 2008.

WANG, Y. C. et al. Antioxidatives activities of soymilk fermented with lactic acid bacteria and bifidobacteria. **Food Microbiology**, London, v. 23, p. 128-135, 2006.

WANG, Y. C. et al. Sugar and acid content in soymilk fermented with lactic acid bacteria alone or simultaneously with bifidobacteria. **Food Microbiology**, London, v. 20, p. 333-338, 2003.

WANG, Y. C.; YU, R. C.; CHOU, C. C. Growth and survival of bifidobacteria and lactic acid bacteria during the fermentation and storage of cultured soymilk drinks. **Food Microbiology**, London, v. 19, p. 501-508, 2002.

WORLD HEALTH ORGANIZATION. Food and Agriculture Organization of the United Nations. **Probiotics in foods:** health and nutritional properties and guidelines for evaluation. Rome, 2006. 50 p. (FAO Food and Nutrition Paper, 85).

WOUTERS, J. T. M. et al. Microbes from raw milk for fermented dairy products. **International Dairy Journal**, Barking, v. 12, n. 2, p. 91-109, 2002.

YANG, Y. et al. Proteomics evidence for kefir dairy in Early Bronze Age China. **Journal of Archaeological Science**, New York, v. 45, p. 178-186, May 2014.

YOON, K. Y.; WOODAMS, E. E.; HANG, Y. D. Probiotication of tomato juice by lactic acid bacteria. **Journal of Microbiology**, Seoul, v. 42, n. 4, p. 315-318, 2004.

YOUSIF, N. E.; EL TINAY, A. H. Effect of fermentation on protein fractions and in vitro protein digestibility of maize. **Food Chemistry**, London, v. 70, p. 181-184, 2000.

ZANNINI, E. et al. Barley malt wort fermentation by exopolysaccharide-forming *Weissella cibaria* MG1 for the production of a novel beverage. **Journal of Applied Microbiology**, Oxford, v. 115, n. 6, p. 1379-1387, 2013.

SEGUNDA PARTE - ARTIGOS

ARTIGO 1 - *NONDAIRY CASSAVA-BASED BEVERAGE PRODUCED BY CULTURES OF LACTIC ACID BACTERIA AND YEAST*

ARTIGO FORMATADO DE ACORDO COM AS NORMAS DO PERIÓDICO INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY

Abstract

Traditional fermented foods are rich sources of microorganisms with probiotic characteristics. The purpose of this work was to select a potential probiotic LAB to be used as starter culture for cassava and rice beverage development. Initially, eighty-seven LAB strains were screened by the following tests: α-amylase secretion, acid production, survival on pH 2.0 and bile tolerance. *L. plantarum* CCMA 0743 strain was the only isolate that presented the ability to survival and growth in the low pH value and in the presence of bile salts and it was selected to be used as starter culture in cassava and rice fermentations. In addition, it was used to perform co-culture assays with the yeast *T. delbrueckii* CCMA 0235. The commercial probiotic strain *L. acidophilus* LACA 4 was also tested as starter culture in single and co-culture assays with the yeast strain. At end of fermentation there was no significant difference ($P < 0.05$) in bacteria population amongst the single and co-cultures assays, and cell viability was around 8.0 Log (cfu/mL) and 7.7 Log (cfu/mL), respectively. Yeast population showed not any significant difference ($P < 0.05$) in single and co-culture assays after 48 h of fermentation. However, the cell growth in single culture was lower than in co-cultures during all fermentation time, and showed the lowest value after 48 h, 5.0 Log (cfu/mL). Chemical compounds were analyzed by High Performance Liquid Chromatography (HPLC) and maltose was detected at the beginning of fermentation (0.1287 g/L), being the main carbohydrate derived from starch hydrolysis. Starch was measured and higher residual contents were noted in the assays fermented by single LAB stains (10.6 % (w/w)) than in co-cultures assays with *T. delbrueckii*. Samples fermented by *L. plantarum* in single and co-culture assays registered the highest amount of lactic acid (1.61 g/L and 1.74 g/L, respectively). Other organic acids (malic, succinic and tartaric) were detected in very low concentrations. Ethanol concentrations in all assays (around 0.03 % and 0.04 % (w/v)) were lower than 0.5% (w/v) in all cultures. The pH rapidly decreased from 6.87 at the beginning of fermentation to 4.0 in the assays with *L. plantarum* (single and co-culture) and to 4.5 in the assays with single yeast and co-cultured with *L. acidophilus*, after 48 h of fermentation. The results from the acceptability test show that most of the sensorial attribute scores were not significantly different ($P > 0.05$) between the samples. In general, the judges neither dislike nor like (score 5) the samples. Based on the results obtained in this work, new cassava and rice –based fermented beverage is a promising product with functional property and beneficial to health of consumer.

Keywords: cassava, rice, *Lactobacillus plantarum, Lactobacillus acidophilus, Torulaspora delbrueckii,* functional food.

1. Introduction

Consumers worldwide are becoming increasingly aware of the relationship between diet and health, and the market for so-called functional foods has been growing in recent years (Carrillo et al., 2013). Experts estimate that among functional foods, probiotic foods comprise 60–70% of the total market (Tripathi and Giri, 2014). Probiotics are live microorganisms, which when administered in adequate amounts in the diet, deliver health benefits to the host (Vidhyasagar and Jeevaratnam, 2013).

Lactic acid bacteria (LAB), including the genera *Lactobacillus* and *Bifidobacterium*, are commonly used in probiotic preparations. Naturally found in traditional fermented foods, *Lactobacillus* species are technologically suitable for food applications, since they are more resistant to low pH and have adaptation to milk and other food substrates (Tripathi and Giri 2014). Although the LAB are commonly associated with dairy products, this group of microorganisms also plays a role in other food systems, and this versatility incites scientists to search for new applications to obtain novel products for a continuously growing functional food market (Molina et al., 2012).

Traditional fermented foods are rich sources of microorganisms with probiotic characteristics and have been tested as starter cultures for production of many functional beverages, as a traditionally fermented millet alcoholic beverage in Korea (Oh and Jung, 2015), fermented cereal beverages like oat, barley, and malt (Salmerón et al., 2015), and a rice-based fermented beverage (Ghosh et al., 2015). In addition, vegetable-derived substrates are potential sources for the development of new functional foods and beverages, such as a fermented coconut water beverage (Prado et al., 2015), extracts of soy and quinoa (Bianchi et al., 2014), peanut-soy milk (Santos et al., 2014), soybean (Molina et al. 2012), and cassava (Freire et al., 2015), among others.

Cassava (*Manihot esculenta* Crantz) plays an important role in the global food diet, mainly in developing countries. Over 100 countries are producing cassava, and Brazil is the second-largest producer in the world, with a production amount of 21.4 million tons in 2013 (IBGE, 2013). Brazilian indigenous people are traditional producers of cassava fermented foods and beverages, such as *cauim*, *caxiri*, *yakupa*, and *tarubá*, and the microbiota present during the natural substrate fermentation has been already studied (Almeida et al., 2007; Freire et al., 2014; Ramos et al., 2010, 2015; Santos et al., 2012). Furthermore, rice (*Oryza sativa*) is a cereal produced and consumed worldwide; it plays important roles in dietary health, containing nutritional compounds such as phenolic compounds, tocopherols, tocotrienols, and others (Iqbal et al., 2005). In this context, cassava and rice are potential substrates for the development of functional foods fermented by potential probiotics autochthonous LAB strains. In addition, cassava is a nondairy product, free from cholesterol, lactose, and gluten, safe for vegetarians and people who are lactose-intolerant or who have celiac disease.

The purpose of this work was to develop a nondairy beverage based on Brazilian indigenous beverages by selecting a potential probiotic LAB strain isolated from different Brazilian indigenous foods to be used as starter culture in cassava and rice substrates. The fermentative performance of starter cultures was monitored by the microbial dynamics and metabolite compound evaluations during the fermentation. Antioxidant activity, minerals, and acceptance of the beverage were evaluated. This knowledge will enable future pilot-scale fermentations with adequate starter cultures for the development of a nondairy functional cassava and rice fermented beverage.

2. Materials and Methods

2.1. Screening of LAB strains for starter culture selection

2.1.1. Microorganisms

Eighty-seven LAB strains, which were previously isolated from different Brazilian indigenous foods (Almeida et al., 2007; Freire et al., 2014; Miguel et al., 2012, 2014; Puerari et al., 2015; Ramos et al., 2010, 2011; Santos et al., 2012;;) were initially employed in this study and belong to the Culture Collection of Agriculture Microbiology (CCMA) of the Federal University of Lavras, Brazil. The strains were stored at -80 °C with 20% (v/v) glycerol and cultured in Man Rogosa Sharpe (MRS) (Merck, Darmstadt, Germany) broth at 37 °C for 48 h. The LAB strains were tested for technological and probiotic characteristics.

2.1.2. α-Amylase secretion assay

For this assay, modified MRS (Merck, Darmstadt, Germany) agar plates containing 0.2% (w/v) soluble starch as a carbon source (without glucose) were inoculated with the LAB strains. After incubation, the plates were flooded with iodine solution (1% iodine [w/v]; 2% potassium iodide [w/v]); the existence of a clear zone around the colonies (Kostinek et al., 2005) indicated enzyme secretion. The assay was performed in triplicate. The selected strains were used for further tests.

2.1.3. Acid production assay

Acid production test was carried out according to Kostinek et al. (2005). LAB strains were inoculated in MRS broth (Merck, Darmstadt, Germany) adjusted to pH 6.5, and the culture pH was measured after 6, 24, and 48 h of incubation. The assay was performed in triplicate.

2.1.4. Survival at pH 2.0

The LAB strains were subjected to a pH 2.0 tolerance assay in order to select the resistant isolates as described by Ramos et al. (2013). Cell cultures (optical density of 0.2 at 600 nm) corresponding to approximately 10^8 cell/mL were centrifuged and re-suspended in MRS broth (Merck, Darmstadt, Germany) with pH adjusted to 2.0 using 1N HCl and incubated for 3 h at 37 °C. After this step, the strains were subsequent inoculated on neutral pH MRS agar plates (48 h at 37 °C) to observe their growth. The assay was performed in triplicate.

2.1.5. Bile tolerance assay

The method described by Guo et al. (2009) was utilized to study the effect of bile on the growth rate of acid-tolerant LAB isolates. Tolerance to bile was evaluated based on the time required to increase the absorbance at 620 nm by 0.3 units in MRS broth with and without 0.3% oxgall. The difference in time (hours) to obtain 0.3 units between the measurements of the culture media with and without bile was considered as the adaptation time (AT) of the cells to adapt to media containing bile. The experiments were performed in triplicate.

2.2. Cassava and rice fermentation media

The fermentation media for controlled assays was prepared based on the Brazilian indigenous beverage *cauim*, with some modifications. The cassava roots and the rice were purchased from the local market in Lavras, Minas Gerais, Brazil. Peeled cassava roots (4 kg) were ground in an industrial blender to obtain cassava dough. After that, the dough was left for 30 min at 60 °C to obtain cassava flour. Approximately 0.6 kg of cassava flour and 0.3 kg of rice were cooked together in 9 L of sterile distilled water for approximately 15 minutes after boiling (Almeida et al., 2007). The mixture was sieved and the heat treatment was carried out by heating at 90 °C/20 min, followed by immediate cooling to 4 °C (Santos et al., 2014).

2.3. Starter cultures

Three different starter cultures were tested for the development of the beverage—the selected strain *Lactobacillus plantarum* CCMA 0743, which demonstrated potential characteristics for study as a probiotic and technological proprieties during screening tests; the yeast strain *Torulaspora delbrueckii* CCMA 0235 isolated from tarubá, a Brazilian indigenous cassava food; and the commercial probiotic culture *L. acidophilus* LACA 4 acquired from Danisco (Yo Mix, Deutschland).

The inoculum preparation was performed as described by Santos et al. (2014). The yeast strain was subcultured on YPD [10 g L^{-1} yeast extract (Merck, Darmstadt, Germany), 10 g L^{-1} soy peptone (Himedia, Mumbai, India), 20 g L^{-1} glucose (Merck, Darmstadt, Germany), and 20 g L^{-1} agar (Merck, Darmstadt, Germany)].

2.4. Single and co-culture fermentations

The washed cells of the LAB strains and yeast were inoculated into 500 mL Erlenmeyer flasks containing 400 mL of cassava and rice substrate, and incubated at 37 °C for 48 h. Microbial cells were inoculated in the substrate with a population of 5 log CFU/mL for yeast and 7 log CFU/mL for bacteria, in both single and co-culture fermentations.

For the single fermentation assays, each microorganism was inoculated in the substrate separately, while co-culture fermentations were performed as follows: (1) *L. plantarum* CCMA 0743 and *T. delbrueckii* CCMA 0235; (2) *L. acidophilus* LACA 4 and *T. delbrueckii* CCMA 0235. The experiments were performed in three independent assays.

2.5. Enumeration of microorganisms, pH, and starchy content determinations

Samples (1 mL) were taken from each fermentation flask. The total LAB, yeast, and Enterobacteriaceae populations were determined by plating in MRS agar (supplemented with 50 mg/L of nystatin), Dichloran Rose Bengal Chloramphenicol (DRBC) agar, and violet red bile agar (VRBG; Merck) media, respectively. Plates were incubated at 37 °C (LAB and Enterobacteriaceae) and 28 °C (yeast) for 48 h, and the colony-forming units (CFU) were enumerated. The pH levels of the fermenting cassava and rice samples were measured on site with pH-Fix test strips (Macherey-Nagel

GmbH and Co, Düren, Germany). The final starchy contents in the samples (after 48 h of fermentation) were determined following the methodology described by Hall (2009). Briefly, samples were submitted to enzymatic hydrolysis to break the starchy molecules into glucose. The glucose was measured according to the methodology of Nelson (1944) for determination of reducing sugars. A starch standard (0.1g) was used to convert the glucose content in a percentage of starch in the samples. The analyses were performed in triplicate.

2.6. HPLC analysis

Organic acids (lactic, acetic, malic, succinic, and tartaric acid), alcohol (ethanol and glycerol), and carbohydrates (glucose, fructose, and maltose) analysis was 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 acids) and a refractive index detector (RID-10Ai) (for alcohol and carbohydrates) was used. A Shimadzu ion exclusion column, Shim-pack SCR-101 H (7.9 mm x 30 cm) was used at an operating temperature of 30 °C for alcohol and carbohydrates and 50 °C for acids. All samples were analyzed in duplicate.

2.7. Determination of potential antioxidant activity of beverages

The radical scavenging activity (RSA) of fermented beverage samples was evaluated using the radicals 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Brand-Williams et al., 1995) and 2,2'-azino-bis(3 ethylbenzothiazoline- 6-sulphonic acid) (ABTS) (Re et al., 1999), with minor modifications. Briefly, 3.9 mL of DPPH solution (0.06 mM in methanol) was added to 0.1 mL of properly diluted samples, followed by incubation for 80 min in the dark, and the absorbance was measured at 515 nm. Similarly, 0.1 mL of methanol was used instead of the sample for the blank. RSA was calculated using Eq. (1). For ABTS test 30 µL of properly diluted samples was added to 3 mL ABTS$^{+\cdot}$ radical and incubated for 6 min in the dark. The absorbance was measured at 734 nm. Similarly, 30 µL of ethanol was used instead of the sample for the blank. RSA was calculated using Eq. (1).

Eq. (1): $\% \text{ RSA} = \left(\frac{Ab-As}{Ab}\right) x\ 100$

Where *Ab* is the absorbance of blank and *As* is the absorbance of sample.

A standard Trolox curve was prepared with known Trolox concentrations, and the reactions occurred in the same way as described above for each radical assay. The antioxidant capacity of samples was expressed as Trolox equivalent antioxidant capacity (μM Trolox/L).

Antioxidant activity by the coupled oxidation of beta-carotene and linoleic acid was performed as described by Marco (1968), with modifications. Aliquots of 5 mL of β-carotene/linoleic acid emulsion were mixed with 500 μL of the samples. The absorbance was measured at 470 nm, at the initial time and after 120 min of incubation at 50 °C for the oxidation reaction. The antioxidant activity (AA) was expressed by the percentage of protection compared to control after 120 min of incubation, using Eq. (2).

Eq. (2): $\% \text{ AA} = \left[\left(\frac{(Abi-Abf)-(Asi-Asf)}{(Abi-Abf)}\right) x\ 100\right]$

Where, *Abi* is the initial absorbance of blank, *Abf* is the final absorbance of blank, *Asi* is the initial absorbance of sample, and *Asf* is the final absorbance of sample.

2.8. Mineral contents

The samples were acid-digested using a mixture of nitric and perchloric acid (2:1) (v/v). The amount of minerals in the digested samples was determined by atomic absorption spectrophotometry and flame photometry methods, following the method described by Malavolta et al. (1997).

2.9. Sensorial analysis

Sensory analysis of fermented cassava and rice beverage was performed using consumer acceptance test. Fifty untrained panelists were selected based on their consumption of nonalcoholic fermented beverages, aged between 18 and 55 years, and consisted of students and workers at the Federal University of Lavras. Each panelist received the five different samples. Randomized 15 mL samples were served in clear 50 mL glasses at between 4–10 °C. The consumers rinsed their mouths with water between tastings. The consumers evaluated the samples for appearance, color, flavor, taste, texture, and general acceptability according to the hedonic scale of nine categories ranging from dislike extremely (1) to like extremely (9).

2.10. Statistical analysis

The data were subject to analysis of variance (ANOVA), and differences in values were considered significant when the P value was less than 0.05. The statistical analyses were performed using the Sisvar 5.3 software.

3. Results

3.1. LAB screening for starter cultures selection

Among the 87 LAB isolates, 18 showed amylolytic activity and were selected for further assays (Table 1). Acid production was evaluated after 24 h of incubation, and the strains were able to acidify the medium rapidly, reaching pH values of around 4.0. The potential probiotic of the strains was analyzed by their tolerance to pH 2.0 and to bile salts, simulating the gastrointestinal tract environment. Only the strains *Leuconostoc lactis* CCMA 0415, *L. plantarum* CCMA 0743, *L. fermentum* CCMA 0751, CCMA 0753, CCMA 0212, CCMA 0208 were able to survive after 3 h of exposure to pH 2.0 (Table 1). Regarding the bile tolerance test, only four LAB isolates, *L. fermentum* CCMA 0745, *L. plantarum* CCMA 0743, CCMA 0744 and CCMA 0746 were able to tolerate bile salts at 0.3% (w/v) (oxgall). The isolates *L. plantarum* CCMA 0744 and CCMA 0746 had the shortest adaptation time (approximately 2 and 8 h, respectively). However, the *L. plantarum* CCMA 0743 strain was the only isolate that presented the two characteristics—ability to survive and grow in the low pH value and in the presence of bile salts (Table 1).

Table 1. Tolerance of selected amylolitic LAB strains to pH 2.0, bile salts, and their capability of acidification (pH after 24 h). AT = adaptation time obtained by the difference in time (hours) to obtain 0.3 units between the measurements of the culture media with and without bile.

Isolate	**Source**	**α-Amilase activity**	**Acid Production (pH)**	**Survival on pH 2.0**	**AT (h)**
Leuconostoc lactis CCMA 0415	Chicha	+	4.5	+	Nd
Lactobacillus plantarum CCMA 0743	Cauim	+	4.0	+	11.24±0.39^{d}
Enterococcus hirae CCMA 0742	Cauim	+	4.0	-	Nd
Lactobacillus plantarum CCMA 0744	Cauim	+	4.0	-	2.33±0.31^{a}
Pediococcus acidilactici CCMA 0748	Cauim	+	4.0	-	Nd
Lactobacillus fermentum CCMA 0745	Cauim	+	4.0	-	10.45±0.01^{c}
Pediococcus acidilactici CCMA 0749	Cauim	+	4.5	-	Nd
Lactobacillus plantarum CCMA 0746	Cauim	+	4.0	-	8.58±0.02^{b}
Enterococcus hirae CCMA 0747	Cauim	+	4.5	-	Nd
Lactobacillus fermentum CCMA 0201	Yakupa	+	4.5	-	Nd
Lactobacillus fermentum CCMA 0212	Yakupa	+	4.5	+	Nd
Lactobacillus fermentum CCMA 0211	Yakupa	+	4.5	-	Nd
Lactobacillus fermentum CCMA 0208	Yakupa	+	4.5	+	Nd
Lactobacillus fermentum CCMA 0215	Yakupa	+	4.5	-	Nd
Enterococcus faecium CCMA 0750	Calugi	+	4.5	-	Nd
Lactobacillus fermentum CCMA 0751	Calugi	+	4.5	+	Nd
Lactobacillus fermentum CCMA 0752	Calugi	+	4.5	-	Nd
Lactobacillus fermentum CCMA 0753	Calugi	+	4.5	+	Nd

Presented values are means of triplicate determinations; ± indicates standard deviations from the mean. Mean values (± standard deviation) within the same column followed by different superscript letters differ significantly ($P < 0.05$) by Scott–Knott test. Nd = not determined

Based on these results, the *L. plantarum* CCMA 0743 strain was selected to be used as the starter culture in cassava and rice fermentations.

3.2. Microbial growth performance during cassava and rice fermentation

In Fig. 1 is presented the microbial growth profile in single and co-cultures. The two lactobacilli strains cultured in the cassava and rice-based substrate were able to grow after 48 h of fermentation, reaching populations from 7.59 log CFU/mL (*L. acidophilus* LACA 4 co-cultured with *T. delbrueckii* CCMA 0235) to 8.05 log CFU/mL (*L. plantarum* CCMA 0743 single-cultured) (Fig. 1A). At the end of fermentation, there was no significant difference ($P < 0.05$) in bacteria populations among the single and co-culture assays (8.0 log CFU/mL and 7.7 log CFU/mL, respectively). Yeast population did not show any significant difference ($P < 0.05$) (around 6.7 log CFU/mL) among co-culture assays after 48 h of fermentation (Fig. 1B). However, the growth in the single culture was lower ($P < 0.05$) than in co-cultures during all fermentation times, and showed the lowest value (5.0 log CFU/mL) after 48 h. No Enterobacteriaceae growth was detected during fermentation process by plating in VRBG medium, which confirmed the substrate pasteurization effectiveness (data not shown).

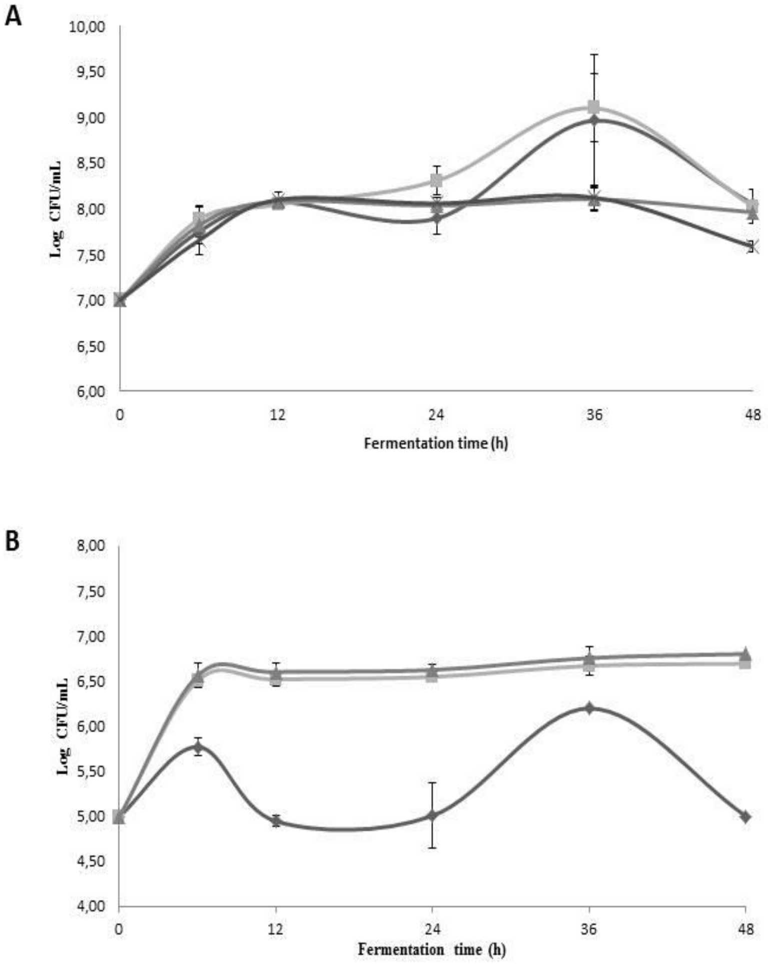

Fig. 1. Microbial populations during single and co-culture fermentations of cassava and rice. **(A)** Population of the starter cultures *L. plantarum* CCMA 0743 and *L. acidophilus* LACA 4. Symbols: (♦) *L. plantarum* CCMA 0743; (■) *L. acidophilus* LACA 4; (▲) *L. plantarum* CCMA 0743) + *T. delbrueckii* CCMA 0235; (x) *L. acidophilus* LACA 4 + *T. delbrueckii* CCMA 0235. **(B)** Population of the starter culture *T. delbrueckii* CCMA 0235. Symbols: (♦) *T. delbrueckii* CCMA 0235; (■) *T. delbrueckii* CCMA 0235 + *L. plantarum* CCMA 0743; (▲) *T. delbrueckii* CCMA 0235 + *L. acidophilus* LACA 4.

3.3. Chemical compounds and pH analysis

Results derived from the analysis of carbohydrates, organic acids, and alcohols by high-performance liquid chromatography (HPLC) and starchy contents are shown in Table 2. The amounts of these compounds and the pH values were quantified at the initial (0 h) and final (48 h) time of fermentation. At the beginning of fermentation, the starchy contents were around 13%. Higher residual starch contents were noted in the assays fermented by single LAB strains (around 10.6%) than in co-culture assays with *T. delbrueckii* (lower than 6%). Maltose is one of the main carbohydrates derived from starchy hydrolysis, and it was detected at the beginning of fermentation (0.13 g/L) and at the end of single-culture assays (0.1 g/L for *L. plantarum* and *T. delbrueckii* and 0.34 g/L for *L. acidophilus*). Maltose was not detected in the co-culture assays. No glucose was detected in the fermentations and a low concentration of fructose was detected only at the beginning (0.13 g/L). Glucose is a single carbohydrate derived from maltose hydrolysis, and the microorganisms consume it rapidly as a carbon and energy source. This may explain why this sugar was not detected during the fermentation.

The cassava and rice beverages fermented with *L. plantarum* in single and co-culture assays registered the highest amounts of lactic acid (1.61 g/L and 1.74 g/L, respectively), while the beverage inoculated with *L. acidophilus* and *T. delbrueckii* (co-culture) registered 0.83 g/L of lactic acid. A very low concentration (0.01 g/L) of malic acid was detected only at beginning of fermentation. Succinic and tartaric acids (< 0.3 g/L) were detected only at end of fermentation. The pH of the cassava and rice beverage rapidly decreased from 6.87 at the beginning of fermentation to 4.0 in the assays with *L. plantarum* (single and co-culture) and to 4.5 in the assays with single yeast and co-cultured with *L. acidophilus*, after 48 h of fermentation. These results clearly demonstrated the microbial activity during fermentation by consuming and producing chemical compounds and changing the pH.

Table 2. Concentration of starch, carbohydrates, organic acids and alcohols in cassava and rice based beverages fermented with different starter cultures.

Time (h)	Isolates	% Starch (w/w)	Fructose (g/L)	Glucose (g/L)	Maltose (g/L)	Lactic acid (g/L)	Malic acid (g/L)	Succinic acid (g/L)	Tartaric acid (g/L)	Glicerol (g/L)	Etanol (g/L)
0		13.23	0.13	Nd	0.13	0.43	0.01	Nd	Nd	0.55	Nd
48	*L. plantarum* CCMA 0743	10.63	Nd	Nd	0.10	1.61	Nd	0.01	0.10	0.51	Nd
	L. acidophilus LACA 4	10.65	Nd	Nd	0.34	1.25	Nd	0.02	0.07	0.39	Nd
	L. plantarum CCMA 0743 +*T. delbrueckii* CCMA 0235	5.62	Nd	Nd	Nd	1.74	Nd	Nd	0.16	0.59	0.32
	L. acidophilus LACA 4 + *T. delbrueckii* CCMA 0235	0.31	Nd	Nd	Nd	0.83	Nd	0.01	0.23	0.29	0.41
	T. delbrueckii CCMA 0235	4.47	Nd	Nd	0.11	0.21	Nd	0.03	Nd	0.07	0.36

Nd= not detected

3.4. Antioxidant activity and mineral analysis of cassava-rice beverages

The RSA of the different fermented beverages was evaluated by the $ABTS^{+\cdot}$ and DPPH radical assays, and by oxidation of beta-carotene/linoleic acid method. All analyzed samples showed no significant differences in scavenging ability against $ABTS^{+}$ radical cation (Table 3). Otherwise, the scavenging ability against the DPPH radical of the beverage fermented with the co-culture *L. acidophilus* and *T. delbrueckii* was significantly lower than the others (0.68 ± 0.27%). Regarding to antioxidant activity obtained by the oxidation of beta carotene/linoleic acid method, we noted significant differences among the beverages (Table 3). The highest potential antioxidant was observed for the beverage fermented with single *T. delbrueckii* (31.99 ± 0.10%).

Mineral contents were evaluated and are shown in Table 4. Calcium (Ca), sodium (Na), and magnesium (Mg) were the main minerals detected in the different beverages and were related to the cassava and rice substrate. However, potassium (K), manganese (Mn), and zinc (Zn) were not detected in the substrate but were present in the beverages, e.g., K (5 mg/100 g) was observed in the beverage fermented with *T. delbrueckii*; Mn (1.75 mg/100 g) in the beverage with co-culture of *T. delbrueckii* and *L. acidophilus*; and Zn in the beverages with single cultures of *T. delbrueckii* and *L. plantarum*, showing that the microbial activity may affect the mineral content.

Table 3. Radical scavenging activity and antioxidant activity of different fermented beverages measured by DPPH, ABTS and beta-carotene/linoleic acid methods.

Fermented beverages	**DPPH scavenging activity (%)**	**ABTS scavenging activity (%)**	***AA (%)**
T. delbrueckii	3.56±0.75^{a}	10.15±0.47^{a}	31.99±0.10^{c}
L.plantarum	4.01±0.37^{a}	5.51±0.99^{a}	21.48±0.35^{b}
L.acidophilus	4.82±0.62^{a}	6.49±0.73^{a}	20.59±0.21^{b}
L.plantarum + *T.delbrueckii*	5.18±0.46^{a}	10.27±0.54^{a}	10.16±0.12^{a}
L.acidophilus + *T.delbrueckii*	0.68±0.27^{b}	11.49±0.56^{a}	17.02±0.30^{b}

*Antioxidant activity obtained by beta-carotene/linoleic acid system.

Presented values are means of triplicate determinations; ± indicates standard deviations from the mean. Mean values (± standard deviation) within the same column followed by different superscript letters differ significantly ($P < 0.05$) by Scott–Knott test.

Table 4. Mineral contents detected in the different fermented beverages

Samples	**Mineral content (mg/100g)**						
	K	Ca	Mg	Mn	Zn	Fe	Na
T. delbrueckii	5.00±0.32	30.00±1.75	10.00±0.12	Nd	0.10±0.02	0.73±0.01	13.66±0.56
L. plantarum	Nd	26.67±1.13	10.00±0.01	Nd	0.08±0.01	1.01±0.03	13.58±0.41
L. acidophilus	Nd	30.00±0.98	10.00±0.20	Nd	Nd	0.90±0.02	14.46±0.50
T. delbrueckii + *L. plantarum*	Nd	30.00±2.20	10.00±0,10	Nd	Nd	0.93±0.01	14.89±0.22
T. delbrueckii + *L. acidophilus*	Nd	26.67±1.14	10.00±0.35	1.75±0.07	0.01±0.01	0.74±0.01	13.80±0.45
Substrate	Nd	30.00±0.90	10.00±0.02	Nd	Nd	1.09±0.03	15.48±0.20

Nd = not detected

3.5. Acceptance of functional cassava and rice beverages

According to the results for acceptability, the consumers neither dislike nor like (score 5) the samples (Table 5). Appearance, color, overall impression, taste, and texture did not differ significantly ($P > 0.05$) among the samples. However, regarding to flavor attribute, the beverage fermented with *L. acidophilus* and *T. delbrueckii* in co-culture showed the lowest ($P < 0.05$) average score (4.20). Figure 2 shows these results by demonstrating that the flavor and taste average scores were more variable between the samples than the scores from others attributes (appearance, color, overall impression, and texture). In this study, co-culture of *L. acidophilus* and *T. delbrueckii* showed the lowest scores for flavor attribute, while single *T. delbrueckii* and co-culture with *L. plantarum* samples showed the highest average scores for these attributes, respectively (Fig. 2 and Table 5).

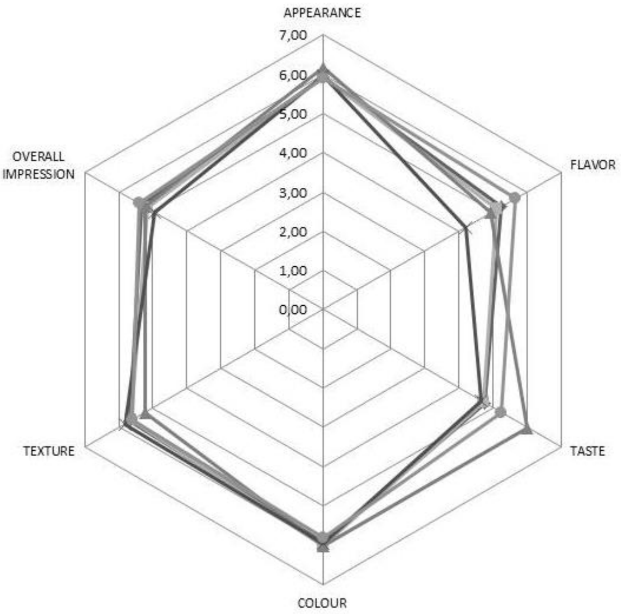

Fig. 2. Hedonic scale for the sensory attributes of beverages fermented with different starter cultures (♦) *L. plantarum* CCMA 0743, (■) *L. acidophilus* LACA 4, (▲) *L. plantarum* CCMA 0743 + *T. delbrueckii* CCMA 0235, (x) *L. acidophilus* LACA 4 + *T. delbrueckii* CCMA 0235, and (●) *T. delbrueckii* CCMA 0235.

Table 5. Acceptance of cassava and rice -based beverages fermented with three different starter cultures in single and co-culture.

Beverages	**Sensory Attributes**					
	Appearance	**Flavor**	**Color**	**Overall impression**	**Taste**	**Texture**
T. delbrueckii CCMA 0235	5.91^{a}	5.68^{a}	5.81^{a}	5.41^{a}	5.26^{a}	5.60^{a}
L. acidophilus LACA 4	5.94^{a}	5.11^{a}	6.01^{a}	5.17^{a}	4.74^{a}	5.65^{a}
L. plantarum CCMA 0743	5.94^{a}	5.26^{a}	5.94^{a}	5.32^{a}	4.77^{a}	5.68^{a}
T. delbrueckii CCMA 0235+ *L. acidophilus* LACA 4	6.05^{a}	4.20^{b}	5.97^{a}	4.94^{a}	4.65^{a}	5.83^{a}
T. delbrueckii CCMA 0235+ *L. plantarum* CCMA 0743	6.17^{a}	4.94^{a}	6.01^{a}	5.23^{a}	4.71^{a}	5.98^{a}

Values with a different letter in the same column are significantly different ($P < 0.05$) according to Scott-Knott test. Acceptability was evaluated using a structured hedonic scale of 9 points, from 1 (dislike very much) to 9 (like very much).

4. Discussion

4.1. Screening of LAB for starter culture selection

Among 87 LAB strains previously isolated from different indigenous fermented products, 18 were selected based on their amylolytic activity. LAB that are capable of converting starchy biomass to lactic acid by producing extracellular amylolytic enzymes are called amylolytic lactic acid bacteria (ALAB) (Kanpiengjai et al., 2015).

The 18 selected strains were subjected to pH 2.0 and bile tolerance. The most critical characteristics of probiotic strains are acid and bile salt resistance; without these, they could not reach the human intestine, where they are expected to exert their health-promoting effects (Erkkilä and Petäjä, 2000). Amongst the 18 LAB strains, only *L. plantarum* CCMA 0743 was able to tolerate pH 2.0 and grow in the presence of 0.3% bile salts. Based on these results, *L. plantarum* CCMA 0743 was selected as starter culture. The safety of selected strains is an important criterion for starter culture selection and should be taken into account. *L. plantarum* is generally regarded as safe (GRAS status) according to The American Food and Drug Administration. Further, this strain showed rapid acidification ability (decreasing the pH from 6.0 to 4.0 in less than 24 h) and susceptibility to the antibiotics ampicillin and chloramphenicol (data not shown). The rapid acidification is important for improving safety by inactivating pathogens and spoilage microorganisms via acid production, and the antibiotic susceptibility is essential to limit the transmission of antibiotic-resistant genes to unrelated pathogenic or opportunistic microorganisms (Ammor and Mayo, 2007).

In addition to *L. plantarum* CCMA 0743, the yeast *T. delbrueckii* CCMA 0235 and the commercial probiotic culture *L. acidophilus* LACA 4 were also used as starter culture in co-cultures and single cultures for cassava and rice fermentation to develop a nondairy fermented beverage.

4.2. Beverage production and chemical analysis

The growth of *L. plantarum* CCMA 0743 and *L. acidophilus* LACA 4 was enhanced significantly over the fermentation time until 36 h (Fig. 1). After this time (36 h), the viable bacteria cell concentration in the fermented products (single and co-culture with *T. delbrueckii* CCMA 0235 was above (> 7.5 log CFU/mL) the minimum dose recommended for a probiotic product to confer a therapeutic effect (6 log CFU/mL based on a 100 mL daily dose) (Sanders and Huis in't Veld, 1999). Therefore, 36 h of fermentation was adequate time for cassava and rice fermentation.

Previous study has shown the effects of the interaction between LAB and yeasts during cassava fermentation. The yeast strain *T. delbrueckii* CCMA 0235 was first used to ferment cassava in co-culture with *L. fermentum* CCMA 0215 strain and was demonstrated to be important for the aroma and flavor profile of the final product (Freire et al., 2015). Narvhus and Gadaga (2003) have reported about fermented milk and described that the co-cultured organisms may compete for growth nutrients, produce metabolic products that inhibit each other's growth, and can influence each other's metabolism, leading to different profiles of aroma and flavor compounds in the final product (). Otherwise, the growth of yeasts in fermented foods is favored by acidification of the environment created by LAB and yeasts, which can provide growth factors such as vitamins and soluble nitrogen compounds, stimulating the growth of LAB (Nout and Sarkar, 1999). The results clearly show these interactions; the yeast population in co-culture fermentation was favored by the reduction of the pH values to 4.0–4.5. On the other hand, a competition for nutrients between LAB and yeast was observed at 36 h of fermentation. Regarding the influence of these interactions on the chemical profile of the fermented products, our results confirmed the differences between single and co-culture fermentations. Comparing the single LAB assays, the residual content of starch in co-culture assays were two times lower with *L. plantarum* CCMA 0743 (5.62%), and almost no residual starch was observed with *L. acidophilus* LACA 4 (0.31%). This can be explained by the amylolytic activity previously described for *T. delbrueckii* CCMA 0235 (Ramos et al. 2015), and which can be confirmed by the low residual starchy content (4.47%) found in single fermentation with this strain. Lactic acid was the main organic acid found in beverages, and these results are in accordance with other authors who used LAB as starter culture for nondairy foods fermentation (Freire et al., 2015; Salmerón et al., 2015; Santos et al., 2014). Lactic acid has been related to the flavor attribute of mild sour in fermented beverages when in concentrations above its threshold value of 0.93 g/L (D'Arcy et al., 1997; Stroehle et al., 2006). In this study, the cassava and rice beverages showed concentrations of lactic acid above its threshold value, except for the beverage fermented with *L. acidophilus* LACA 4 and *T. delbrueckii* CCMA 0235. This may be due to consumption of lactic acid as carbon and energy sources by *T. delbrueckii* strains (Casal and Leão 1995).

Organic acids production may be related to the beverage’s pH decrease, reaching pH values below 4.5. This is very important, because it has been reported that pH of around 3.5–4.5 in food formulations aids the pH increase of the gastrointestinal tract, thus enhancing the stability and benefits of the probiotic strains consumed (Kailasapathy and Chin, 2000).

Glycerol and ethanol are common compounds found in co-culture fermentation of LAB and yeast. Ethanol production was the result of yeast metabolism and thus, it was detected only in fermentation assays of *T. delbrueckii* CCMA 0235. Ethanol concentrations in all assays (around 0.03% and 0.04%) were lower than 0.5% in all cultures, so the final product cannot be considered an alcoholic beverage (Brasil, 2009). Our results are in accordance with Freire et al. (2015), who used the non-*Saccharomyces* yeast, e.g., *T. delbrueckii* CCMA 0235 and *Pichia caribbica* to ferment cassava with LAB and showed low ethanol production. These yeasts are commonly used in wine fermentation, and although they did not produce high amounts of ethanol, they have a positive effect on the taste and aroma of the final beverage (Fleet, 2003; Lappe-Oliveras et al., 2008). Glycerol is a metabolite typically produced by yeasts coupled to their growth, but it is highly produced when in stressful conditions (Walker, 1998), such as during nutrient competition or in the presence of toxic metabolites. Lower glycerol concentration (around 0.07 g/L) was detected in single yeast fermentation than in co-cultures (0.56 g/L with *L. plantarum* and 0.29 g/L with *L. acidophilus*).

4.3. Antioxidant activity, minerals, and acceptance of cassava and rice beverages

A standardized method for determining the antioxidant properties of foods and beverages has not been established; therefore, using at least two or more methods in combination is recommended to provide comprehensive information on the total antioxidant capacity of a food product. In this study, the RSA of the different fermented beverages was evaluated by the $ABTS^{+\cdot}$ and DPPH radical assays, and by oxidation of the beta-carotene/linoleic acid method. In general, the different beverages showed similar antioxidant activity by the different methods, except for beta-carotene/linoleic acid system, in which the single culture *T. delbrueckii* CCMA 0235 showed the highest value ($P < 0.05$) followed by the LAB single cultures and co-culture of *T. delbrueckii* CCMA 0235 with *L. acidophilus* LACA 4. In accordance with our results, other studies have demonstrated that antioxidant activity of yeasts seems to be higher than that of lactic acid bacteria (Amaretti et al., 2013; Gil-Rodríguez et al., 2015). Furthermore, the co-culture of *T. delbrueckii* CCMA 0235 with *L. plantarum* CCMA 0743 showed the highest antioxidant activity ($P < 0.05$) by DPPH assay. It seems that *T. delbrueckii* CCMA 0235 may positively influence the product's antioxidant activity. Yeasts and yeast extracts are important agents related to antioxidant activity of food products, mainly due to the high content of (1-3)-β-D-glucan and other β-glucans found in the cell wall, which have an essential antioxidant role, beside other substances such as superoxide dismutase, glutathione peroxidase, catalase, carotenoids, resveratrol, and octacosanol (Chen et al., 2010; Gil-Rodríguez et al., 2015). The protective and immunomodulation roles of (1-3)-β-D-glucan in living organisms have been related to its ability to exert free radical scavenging properties (Kogan et al., 2005).

Minerals are required by our body, and are essential inorganic elements necessary for the regulation of metabolic processes and structural function as the main components of bones and teeth (Frazier, 2009). In this study, important minerals such as calcium, magnesium, sodium, and iron were detected in all beverages as well as in the nonfermented substrate. Calcium salts provide skeletal rigidity, and calcium ions play a role in many if not most metabolic processes (FAO/WHO, 2001). It is especially necessary for optimal health, growth, and development of infants and young children. The expression of the amount of calcium per 100 g of the samples as percentages of the recommended calcium intake for infants and children < 3 years (300 and 500 mg per day) was ≤ 10.0% (FAO/WHO, 2001), which was almost three times higher than the calcium content found for Akamu (≤ 3.2%), a fermented maize infant complementary food from Nigeria (Obinna-Echem et al., 2015), and 10 times higher than our results. Sodium plays an important role in maintaining water balance and the functioning of the nerves and muscles; iron is essential for the formation of blood cells and is an integral part of important enzyme systems in various tissues, and its deficiency results in anemia; and magnesium as a cofactor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes (Berdanier and Berdanier, 2015; Department of Health, 2012; FAO/WHO, 2001). The content of other minerals, such as zinc, potassium, and manganese, varied in the different beverages.

Interestingly, zinc and manganese were not found in the substrate but were detected in beverages fermented with single LAB cultures and the co-culture of yeast and *L. acidophilus*, respectively. It is known that fermentation has great importance in the improvement of the mineral content of cereal foods, because the degradation of anti-nutritional phytate during fermentation by bacteria led to the availability of calcium, magnesium, phosphorus, zinc, and iron (Greffeuille et al., 2011; Oyarekua, 2011; Sanni et al., 1999).

The beverages were submitted to sensorial analysis to evaluate their acceptability by the consumers. The results showed that in general, the judges neither dislike nor like the different beverages. This is an important result, considering that the beverage had no flavoring additive and absence of sweet taste, normally present in fermented foods such as yogurt and fermented milks. The fermented rice and cassava beverage is an alternative to fermented dairy products and the lactose-intolerant consumer may consume them. Furthermore, it uses low-cost raw-materials that could be produced and consumed in many development countries.

5. Conclusions

A nondairy cassava and rice fermented beverage is a promising product with potential functional properties and would be beneficial to consumer health. Fermentation with the indigenous LAB strain *L. plantarum* CCMA 0743 as well as the commercial probiotic *L. acidophilus* LACA 4 confers potential health benefits to this fermented product; additionally, rapid and adequate acidification and production of organic acids improve safety, avoiding pathogen growth. In addition, co-culture with the yeast strain *T. delbrueckii* CCMA 0235 can improve the hydrolysis of starch, increases the antioxidant activity of beverages, and further stimulates LAB growth during fermentation. This study was the first that produced a functional fermented beverage using rice and cassava as a media of fermentation using these selected starter cultures.

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References

Almeida, E.G., Rachid, C.C., Schwan, R.F., 2007. Microbial population present in fermented beverage ‘cauim’ produced by Brazilian Amerindians. Int. J. Food Microbiol. 120, 146-151.

Amaretti, A., Di Nunzio, M., Pompei, A., Raimondi, S., Rossi, M., Bordoni, A., 2013. Antioxidant properties of potentially probiotic bacteria: in vitro and in vivo activities. Appl. Microbiol. Biotechnol. 97, 809-817.

Ammor, M.S., Mayo, B., 2007. Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: An update. Meat Sci. 76, 138–146.

Berdanier, C.D., Berdanier, L.A., 2015. Advanced nutrition: macronutrients, micronutrients, and metabolism, second ed. CRC Press, London.

Bianchi, F., Rossi, E.A., Gomes, R.G., Sivieri, K., 2014. Potentially synbiotic fermented beverage with aqueous extracts of quinoa (*Chenopodium quinoa* Willd) and soy. Food Sci. Technol. Int. 0, 1-13.

Brand-Williams, W., Cuvelier, M.E., Berset, C.L.W.T., 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28, 25-30.

Brasil (2009). Decreto n° 6.871, de 4 de junho de 2009. Regulamenta a Lei no 8.918, de 14 de julho de 1994, que dispõe sobre a padronização, a classificação, o registro, a inspeção, a produção e a fiscalização de bebidas. Legislação Bebidas. Presidência da República. http://www.planalto.gov.br/ccivil_03/_Ato2007-2010/2009/Decreto/D6871.htm/ Accessed 30.06.16.

Carrillo, E., Prado-Gascó, V., Fiszman, S., Varela, P., 2013. Why buying functional foods? Understanding spending behaviour through structural equation modelling. Food Res. Int. 50, 361–368.

Casal, M., Leão, C., 1995 Utilization of short-chain monocarboxylic acids by the yeast *Torulaspora delbrueckii*: Specificity of the transport systems and their regulation. Biochim. Biophys. Acta 1267, 122-130.

Chen, L.S., Ma, Y., Maubois, J.L., Chen, L.J., Liu, Q.H., Guo, J.P., 2010. Identification of yeasts from raw milk and selection for some specific antioxidant properties. Int. J. Dairy Technol. 63, 47-54.

D'Arcy, B.R., Rintoul, G.B., Rowland, C.Y., Blackman, A.J., 1997. Composition of Australian honey extractives. 1. Norisoprenoids, monoterpenes, and other natural volatiles from blue gum (*Eucalyptus leucoxylon*) and yellow box (*Eucalyptus melliodora*) honeys. J. Agric. Food Chem. 45, 1834–1843.

Department of Health, 2012. Manual of nutrition, 12th ed. TSO, London.

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 Sci. Technol. 43, 1564–1572.

Erkkilä, S., Petäjä, E., 2000. Screening of commercial meat starter cultures at low pH and in the presence of bile salts for potential probiotic use. Meat Sci. 55, 297-300.

FAO/WHO, 2001. Human vitamin and mineral requirements: Report of a joint FAO/WHO expert consultation. Bangkok, Thailand. Rome: FAO.

Fleet, G., 2003. Yeast interactions and wine flavour. Int. J. Food Microbiol. 86, 11–22.

Frazier, R. A., 2009. Food Chemistry. In Campbell-Platt, G. (Ed.), Food Science and Technology. Wiley-Blackwell, Oxford, pp. 5-31.

Freire, A.L., Ramos, C.L., de Almeida, E.G., Duarte, W.F., Schwan, R.F., 2014. Study of the physicochemical parameters and spontaneous fermentation during the traditional production of yakupa, an indigenous beverage produced by Brazilian Amerindians. World J. Microbiol. Biotechnol. 30, 567-577.

Freire, A.L., Ramos, C.L., Schwan, R.F., 2015. Microbiological and chemical parameters during cassava based-substrate fermentation using potential starter cultures of lactic acid bacteria and yeast. Food Res. Int. 76, 787–795.

Ghosh, K., Ray, M., Adak, A., Halder, S.K., Das, A., Jana, A., Mondal, K.C., 2015. Role of probiotic *Lactobacillus fermentum* KKL1 in the preparation of a rice based fermented beverage. Bioresour. Technol. 188, 161-168.

Greffeuille, V., Polycarpe Kayodè, A.P., Icard-Vernière, C., Gnimadi, M., Rochette, I., Mouquet-Rivier, C., 2011. Changes in iron, zinc and chelating agents during traditional African processing of maize: effect of iron contamination on bioaccessibility. Food Chem**.** 126, 1800-1807.

Gil-Rodríguez, A.M., Carrascosa, A.V., Requena, T., 2015. Yeasts in foods and beverages: in vitro characterisation of probiotic traits. LWT-Food Sci. Technol. 64, 1156-1162.

Guo, Z., Wang, J., Yan, L., Chen, W., Liu, X.M., Zhang, H.P., 2009. In vitro comparison of probiotic properties of *Lactobacillus casei* Zhang, a potential new probiotic, with selected probiotic strains. LWT-Food Sci.Technol. 42, 1640-1646.

Hall, M.B., 2009. Determination of starch, including maltooligosaccharides, in animal feeds: comparison of methods and a method recommended for AOAC collaborative study. J. AOAC Int. 92, 42-49.

IBGE (Instituto Brasileiro de Geografia e Estatística), 2013. Levantamento Sistemático da Produção Agrícola. IBGE, Rio de Janeiro, pp. 1-86.

Iqbal, S., Bhanger, M.I., Anwar, F., 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. Food Chem. 2, 265-272.

Kailasapathy, K., Chin, J., 2000. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunol. Cell Biol. 78, 80–88.

Kanpiengjai, A., Lumyong, S., Nguyen, T., Haltrich, D., Khanongnuch, C., 2015. Characterization of a maltose-forming α-amylase from an amylolytic lactic acid bacterium *Lactobacillus plantarum* S21. J. Mol. Catal. B: Enzym. 120, 1–8.

Kogan, G., Staško, A., Bauerová, K., Polovka, M., Šoltés, L., Brezová, V., Mihalová, D., 2005. Antioxidant properties of yeast (1→ 3)-β-D-glucan studied by electron paramagnetic resonance spectroscopy and its activity in the adjuvant arthritis. Carbohydr. Polym. 61, 18-28.

Kostinek, M., Specht, I., Edward, V.A., Schillinger, U., Hertel, C., Holzapfel, W.H., Franz, C.M., 2005. Diversity and technological properties of predominant lactic acid bacteria from fermented cassava used for the preparation of Gari, a traditional African food. Syst. Appl. Microbiol. 28, 527-540.

Lappe-Oliveras, P., Moreno-Terrazas, R., Arrizón-Gaviño, J., Herrera-Suárez, T., García Mendoza, A., & Gschaedler-Mathis, A., 2008. Yeasts associated with the production of Mexican alcoholic non-distilled and distilled Agave beverages. FEMS Yeast Res. 8, 1037–1052.

Malavolta, E., Vitti, G.C., Oliveira, S.A., 1997. Avaliação do estado nutricional das plantas: princípios e aplicações, segunda ed. Associação Brasileira para Pesquisa do Fosfato, Piracicaba.

Marco, G.J., 1968. A rapid method for evaluation of antioxidants. J. Am.Oil Chem. Soc. 45, 594-598.

Miguel, M.G.D.C.P., Santos, M.R.R.M., Duarte, W.F., de Almeida, E.G., Schwan, R.F., 2012. Physico-chemical and microbiological characterization of corn and rice ‘calugi’produced by Brazilian Amerindian people. Food Res. Int. 49, 524-532.

Miguel, M.G.C.P., Santos, C.C.A.A., Santos, M.R.R.M., Duarte, W.F., Schwan, R.F., 2014. Bacterial dynamics and chemical changes during the spontaneous production of the fermented porridge (Calugi) from cassava and corn. Afr. J. Microbiol., 8, 839-849.

Molina, V., Médici, M., de Valdez, G.F., Taranto, M.P., 2012. Soybean-based functional food with vitamin B_{12}-producing lactic acid bacteria. J. Funct. Foods 4, 831-836.

Narvhus, J.A., Gadaga, T.H., 2003. The role of interaction between yeasts and lactic acid bacteria in African fermented milks: a review. Int. J. Food Microbiol. 86, 51–60.

Nelson, N., 1944. A fotometric adaptaion of Somogyi method for the determination of glucose. J. Biol. Chem. 153, 375-80.

Nout, M.J.R., Sarkar, P.K., 1999. Lactic acid fermentation in tropical climates. Anton. Leeuw. 76, 395–401.

Obinna-Echem, P.C., Beal, J., Kuri, V., 2015. Effect of processing method on the mineral content of Nigerian fermented maize infant complementary food–Akamu. LWT-Food Sci. Technol. 61, 145-151.

Oh, Y. J., Jung, D.S., 2015. Evaluation of probiotic properties of *Lactobacillus* and *Pediococcus* strains isolated from Omegisool, a traditionally fermented millet alcoholic beverage in Korea. LWT-Food Sci. Techno. 63, 437-444.

Oyarekua, M.A., 2011. Evaluation of the nutritional and microbiological status of co-fermented cereals/cowpea ‘OGI’. Agr. Biol. J. N. Am., 2, 61-73.

Prado, F.C., Lindner, J.D.D., Inaba, J., Thomaz-Soccol, V., Brar, S.K., Soccol, C.R., 2015. Development and evaluation of a fermented coconut water beverage with potential health benefits. J. Funct. Foods 12, 489-497.

Puerari, C., Magalhães-Guedes, K.T., Schwan, R.F., 2015. Physicochemical and microbiological characterization of chicha, a rice-based fermented beverage produced by Umutina Brazilian Amerindians. Food microbiol. 46, 210-217.

Ramos, C.L., de Almeida, E.G., Pereira, G.V., 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. Int. J. Food Microbiol. 140, 225-231.

Ramos, C.L., de 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 Microbiol. 28, 1380-1386.

Ramos, C.L., Thorsen, L., Schwan, R.F., Jespersen, L., 2013. Strain-specific probiotics properties of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. Food Microbiol. 36, 22-29.

Ramos, C.L., de Sousa, E.S., Ribeiro, J., Almeida, T.M., Santos, C.C.A.D.A., Abegg, M.A., Schwan, R.F., 2015. Microbiological and chemical characteristics of tarubá, an indigenous beverage produced from solid cassava fermentation. Food Microbiol. 49, 182-188.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231-1237.

Salmerón, I., Thomas, K., Pandiella, S.S., 2015. Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. J. Funct. Foods 15, 106–115.

Sanders, M., Huis in't Veld, J., 1999. Bringing a probiotic-containing functional food to the market: microbiological, product, regulatory and labeling issues. Anton. Leeuw. 76, 293-315.

Sanni, A.I., Onilude, A.A., Ibidapo, O.T., 1999. Biochemical composition of infant weaning food fabricated from fermented blends of cereal and soybean. Food Chem. 65, 35-39.

Santos, C.C.A.A., Almeida, E.G., Pereira, G.V.M., Schwan, R.F., 2012. Microbiological and physicochemical characterization of caxiri, an alcoholic beverage produced by the indigenous Juruna people of Brazil. Int. J. Food Microbiol. 156, 112–121.

Santos, C.C.A.A., Libeck, B.S., Schwan, R.F. 2014. Co-culture fermentation of peanut– soy milk for the development of a novel functional beverage. Int. J. Food Microbiol. 186, 32–41.

Stroehle, L., Zweytick, G., Berghofer, E., 2006. Sauerkraut fermentation with L(+)-lactic acid producing bacteria. Ernaehrung, 30, 293–303.

Tripathi, M.K., Giri, S.K., 2014. Probiotic functional foods: Survival of probiotics during processing and storage. J. Funct. Foods 9, 225–241.

Vidhyasagar, V., Jeevaratnam, K., 2013. Evaluation of *Pediococcus pentosaceus* strains isolated from Idly batter for probiotic properties in vitro. J. Funct. Foods 5, 235–243.

Walker, G.M., 1998. Yeast physiology and biotechnology. JohnWiley & Sons Ltd, England.

ARTIGO 2 - *EFFECT OF SYNBIOTIC INTERACTION OF FRUCTOOLIGOSACCHARIDE AND PROBIOTIC ON THE FERMENTATION KINETIC AND CHEMICAL PROFILE OF MAIZE-BASED BEVERAGES*

ARTIGO FORMATADO DE ACORDO COM AS NORMAS DO PERIÓDICO FOOD MICROBIOLOGY

Abstract

Today the development of new non-dairy probiotic beverages could be a key for functional food market. This work aimed to develop new cereal fermented beverages from maize and rice, based on Brazilian indigenous beverage calugi. The indigenous strains *Lactobacillus plantarum* CCMA 0743 and *Torulaspora delbrueckii* CCMA 0235, and the commercial probiotic strain, *L. acidophilus* LACA 4, were used as a mixed starter culture. Two prebiotic concentrations, 2% and 5% of FOS (fructooligosaccharide), were tested in the fermentation assays. The growth of probiotic *L. acidophilus* LACA 4 was favored by 5% FOS, and after 28 days under refrigeration (storage period) its population kept above 10^7 cfu/mL. Lactic and acetic acid were the main organic acids detected, around 3.7 g/L and 0.5 g/L, respectively. Ethanol was lower than 0.5% (w/v) consisting in a non-alcoholic beverages. Total of 55 volatile compounds, including acids, alcohols, aldehydes, esters, ketones, pyrazines and others, were detected by GC–MS, and were important for final flavor of beverages. The acceptance test of the final beverages demonstrated that more than 50% of consumers liked slightly or until liked extremely (notes 6 to 9). Besides, the averages scores of beverages containing the prebiotic FOS were significantly higher ($P > 0.05$) than those without it. So that, a potential symbiotic cereal beverages were successfully obtained from a mix starter culture of LAB and yeast. New studies should be conducted in order to enable the commercial production of the beverages.

1. Introduction

The importance of fermentation as a cheap means of preservation, improving nutritional quality and enhancing sensory characteristics of foods and beverages, was independently discovered by societies worldwide (Marsh et al., 2014). Nowadays traditional fermented foods still play a major role in the diet of numerous civilizations and the world dietary culture can be classified based on staple cereal used as, cooked-rice eaters of Eastern food culture, wheat/barley-based breads/loaves of Western and Australian food culture, and sorghum/ maize porridges of African and South American food culture (Franz et al., 2014; Tamang and Samuel, 2010).

Cereal grains are considered to be one of the most important sources of protein, carbohydrates, vitamins, minerals and fiber for people all over the world (Rivera-Espinoza and Gallardo-Navarro, 2010). Some cereals, including maize, have high content of soluble non-starch polysaccharides such as beta glucan which has a health promoting role, and are popular substrates used for the production of many traditional fermented foods (Achi and Ukwuru, 2015). Examples of maize-based food in Africa, include *ogi* (Nigeria/West Africa), *kenkey* (Ghana), *mawé* (Benin) and *uji* (Kenya); in Mexico and Guatemala maize is used to produce *pozol*, while *masa agria* is produced in Colombia; *chicha de jora* is produced in Andean regions and *calugi* in Brazil (Franz et al., 2014; Tamang and Samuel, 2010; Chaves-lopez et al., 2016; Ray and Montet, 2015 ; Miguel et al., 2012, 2014).

Calugi is produced by indigenous people Javaé, located in the state of Tocantins (northern Brazil). This non-alcoholic porridge is consumed by adults and children. It is still produced by natural fermentation and the traditional corn and rice's *calugi* is obtained by mixing the corn flour with water, sieving and cooking the mixture. Traditionally, the mastication juice of sweet potato is added to the mixture, and after that fermentation occurs at room temperature (approximately 30 °C) for two days. During the fermentation process, lactic acid bacteria (LAB) are dominant and the microbial species *Lactobacillus plantarum*, *Weissella confuse, Streptococcus salivarius, S. parasanguis*, and *Bacillus* sp. and the yeasts *Saccharomyces cerevisiae*, *Pichia fermentans* and *Candida* sp., were commonly found (Miguel et al., 2012).

It is already known natural fermentation can improves nutritional value and sensorial characteristics of food; however fluctuation in these parameters of quality is the major problem in final products (Roger et al., 2015). The use of selected starter cultures can solve many of these problems and traditional fermented foods could be considered an important source of microorganism, involving mixed cultures of yeast, lactic acid bacteria (LAB) and fungi (Blandino et al., 2003), some of them showing probiotic characteristics (Rivera-Espinoza and Gallardo-Navarro, 2010). Probiotics have been traditionally added to yogurt and other fermented dairy products, however, non-dairy probiotic foods have been attracting more attention in recent years (Marsh et al., 2014). Due to their lack of dairy allergens, low cholesterol content and vegan friendly status, and also because different substrates can provide different combinations of antioxidants, dietary fibre, minerals and vitamins, this market is projected to have an annual growth rate of 15% between 2013 and 2018 (Marsh et al., 2014).

Prebiotic was defined by Gibson and Roberfroid (1995) as non-digestible oligosaccharides, especially fructooligosaccharides (FOS) and inulin, able to selectively stimulate the growth and/or activity of endogenous gut microbiota, when suffering fermentation, getting this way a primary role in the intestinal physiology. Furthermore, they are related to functions as decrease cancer risk, improve glycemic control, increase calcium and other mineral absorption, increase folate and B vitamins, increase faeces, reduce caloric value and increase immune response (Rolim, 2015). The symbiotic, combination of probiotics and prebiotics, may enhance the beneficial effect of each of them, however there is lack of studies on development of symbiotic and their in vivo effectiveness has not been intensively studied to date.

Therefore, the purpose of the present study was to produce potential probiotic maize-based beverages fermented by a mixed starter culture of lactic acid bacteria (LAB) and yeast, and analyze the influences of prebiotic (FOS) on the microbial growth and survival during fermentation and storage period under refrigeration. Furthermore, chemical and sensorial analyses were performed to evaluate the metabolites and volatile profiles, and the acceptance of the final beverages by consumers, respectively.

2. Material and Methods

2.1 Starter culture microorganisms and inoculum preparation

The starter culture used in this work was composed by two indigenous strains, *Lactobacillus plantarum* CCMA 0743 and *Torulaspora delbrueckii* CCMA 0235, both belonging to the Culture Collection of Agriculture Microbiology (CCMA) of the Federal University of Lavras, Brazil, and by the commercial probiotic *L. acidophilus* LACA 4, from Danisco (Denmark). The strains were cultured in Man Rogosa Sharpe (MRS) (Merck, Darmstadt, Germany) for bacteria, and YPD [10 g L^{-1} yeast extract (Merck, Darmstadt, Germany), 10 g L^{-1} soy peptone (Himedia, Mumbai, India), 20 g L^{-1} glucose (Merck, Darmstadt, Germany) for yeast, and stored at -80 °C with 20% (v/v) glycerol. For inoculum preparation the yeast strain was successively subcultured on YPD broth while LAB strains in MRS (Merck, Darmstadt, Germany) broth according to Santos et al. (2014).

2.2 Fermentation medium for development of maize beverages

The medium for controlled fermentation assays was prepared based on the Brazilian indigenous beverage ‘calugi’ (Miguel et al., 2012; 2014), with some modifications. Two different mediums, with only maize (M) and with maize mixed rice (MR) were prepared as describe in the flowchart (Figure 1). Approximately 550 g of dried maize (*Zea mays*) and 500 kg of rice flour were used. First, maize grains were soaked in water for 30 min and then macerated until obtain a kind of flour. The resulting maize flour was mixed with 3 L of water and sieved to remove the peel. The rice was ground to flour and added to this mixture with more 3 L of water and cooking was carried out for 1 h; after that, properly starter culture and prebiotic were added, and allowed to ferment at 37 °C for 24 h. The maize and rice were purchased from the local market in Lavras, Minas Gerais, Brazil.

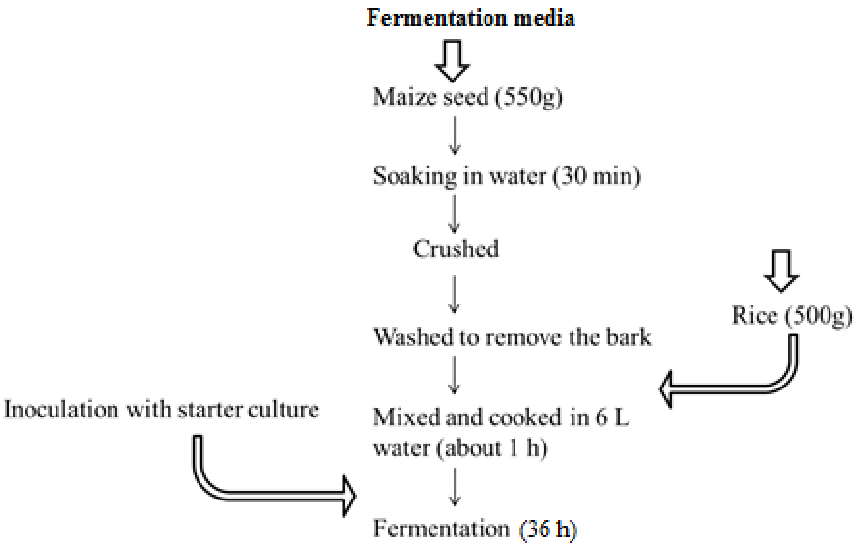

Figure 1. Flow diagram for the manufacture of maize and maize and rice-based beverages.

2.3 Fermentation assays and sampling

For both, M and MR media, three treatments were performed using different prebiotic concentrations (Mishra and Mishra, 2013). They were: (1) without prebiotic, (2) with 2% (w/v) and (3) with 5% (w/v) of fructooligosaccharides (FOS - Sigma). The prebiotic was directed added to the media before inoculation with the starter culture. The microorganisms were inoculated (mixed starter culture) in the fermentation media with populations of 5 log CFU/mL for *T. delbrueckii* CCMA 0235, 6 log CFU/mL for *L. plantarum* CCMA 0743 and 7 log CFU/mL for *L. acidophilus* LACA 4. A control assay was performed for each media without inoculation and prebiotic.

The fermentation assays occurred into 500 mL Erlenmeyer flasks containing 400 mL of the different substrates, and were performed at 37 °C for 36 h. All assays were performed in triplicate. After the fermentation time, the beverages were kept under refrigeration at 4 °C for 28 days. Samples (2 mL) were taken at 0, 6, 12 and 24 h of fermentation and at 14 and 28 days of refrigeration for subsequent analysis.

2.4 Chemical analysis

2.4.1 Determination of pH

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

2.4.2 Determination of starchy content

The initial and final starchy contents of the samples were determined following the methodology described by Hall (2009), with minor modifications. Samples were submitted to hydrolysis with the enzymes α-amylase ca 20000 liquefon U/g (thermotolerant Termamyl 120 L, NovoNordisk) and amyloglucosidase 100 U/mL (Product E-AMGDF, Megazyme International Ireland, Ltd., Bray, Co. Wicklow, Ireland) to break the starchy molecules into glucose. After that, the glucose was measured according to the methodology of Nelson (1944) for determination of reducing sugars. A starch standard (0.1g) was used to convert the glucose content in a percentage of starch in the samples. The analyses were performed in triplicate.

2.4.3 Substrates and metabolites analysis by HPLC

The analyses of organic acids, alcohols and carbohydrates were carried out using a high performance liquid chromatography system (HPLC) (Shimadzu, model LC-10Ai, Shimadzu Corp., Japan), equipped with a dual detection system consisting of a UV–vis detector (SPD- 10Ai) and a refractive index detector (RID-10Ai), according to the methodology proposed by Duarte et al. (2010). A Shimadzu ion exclusion column (Shim-pack SCR-101H, 7.9mm×30 cm) was used for alcohols and organic acids determination. For carbohydrates, the Supelcosil LC-NH2 column (4.6 mm× 25 cm) was used, according to methodology described by Santos, Libeck and Schwan (2014). The compounds were identified based on the retention time of standards, and their concentrations were determined using the external calibration method. All samples were examined in triplicate.

2.4.4 Volatile compounds extraction and GC-MS analysis

The volatile compounds of beverages samples were extracted using the solid-phase micro-extraction technique in the headspace (SPME-HS) as described by Menezes et al. (2016), with minor modifications. Two milliliters of the samples was placed in a 15 mL sample vial to which 10 µL of 4-nonanol (internal standard at 125 mg/L) was added. A 50/30 µm divinylbenzene/ carboxene/polydimethylsiloxane (DVB/CAR/PDMS) fiber provided by Supelco (Bellefonte, PA, USA) was used to extract the volatile compounds. This fiber was balanced for 15 min at 60 °C and then exposed to the samples in 15 mL vials for 30 min at the same temperature.

The volatile compounds were analyzed by gas chromatography– mass spectrometry (GC–MS) (Shimadzu, Model GCMS-QP2010 SE, Tokyo, Japan) equipped with an RTX-5MS (30 m × 0.25 mm id × 0.25 µm film thickness). The oven temperature was set at 40 °C for 5 min, increased until it reached 200 °C (at a rate of 10 °C/min), and finally maintained at 200 °C for 30 min. The carrier gas was high purity helium, at 0.7 ml/min. The splitless injection mode was used at 240 °C (0.5 min). The selective mass detector was a quadrupole, with an electronic impact ionization system at 70 eV and at 260 °C. Volatile compounds were tentatively identified using GC/MS Solution software (Version 2.6). Linear retention indices relative to a mixture of n-alkanes were calculated according to the Kovats retention index (KI). Volatile compounds were tentatively identified by probability-based matching of their mass spectra with those obtained from a database (NIST 14 GC Method/Retention Index Database) and by matching the KI of the compounds with values from the literature. Quantitative data of the identified compounds were obtained by interpolation of the relative areas versus the internal standard area using calibration graphs built for pure reference compounds.

2.5 DNA extraction and qPCR analysis

The microbial population during the fermentation period and storage at 4 °C was monitored by Real-Time Quantitative PCR. The total DNA from the samples was extracted with a PureLink® Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA 92008 USA) in accordance with the manufacturer's instructions for DNA purification. The DNA was stored at -20 °C for further use.

Specific primers for the LAB species, *L. plantarum* and *L. acidophilus*, and for the yeast specie *T. delbrueckii* used in this study, were previously described in the literature and are shown in Table 1. The specificity of each primer pair was confirmed by searching in GenBank using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Real-time PCR was carried out using the Rotor-Gene Q System (Quiagen, Hombrechtikon, ZH, Switzerland) following methodology described by Batista et al. (2015), with minor modifications. Each reaction comprised 12.5 µL Rotor-Gene SYBR Green PCR Master Mix (Qiagen, Stockach, Konstanz, Germany), 0.7 mM of each primer (Invitrogen, São Paulo, SP, Brazil) and 1 mL template DNA extracted from beverages samples for a total volume of 20 µL. All analyses were performed in triplicate. For standard curves LAB species were cultivated in MRS agar at 37 °C for 48 h, while the yeast specie was cultivated in YPD agar at 30 °C for 24 h. Their genomic DNA was extracted using the PureLink® Genomic DNA Mini Kit and serially diluted (1:10) from 10^8-10^7 down to 10 CFU/mL. Each point on the calibration curve was measured in triplicate.

2.6 Sensory analysis

Sensory analysis of the beverages were performed using a consumer acceptance test, according to the hedonic scale of nine categories ranging from dislike extremely (1) to like extremely (9) (Stone and Sidel,1985). The consumers evaluated the samples for appearance, color, aroma, taste, texture, and general acceptability.

The tests were performed in closed cabins with white illumination at the Sensory Analysis Laboratory, Federal University of Lavras (Lavras, MG). The samples were labeled with three random digits on a white surface. These samples had a monadic form and followed a balanced order of presentation (Walkeling and Macfie, 1995). Fifty untrained panelists were selected based on their consumption of non-alcoholic fermented beverages, aged between 18 and 55 years, and consisted of students and workers at the Federal University of Lavras. Randomized 15 mL samples were served in clear 50 mL glasses at between 4–10 °C. The consumers rinsed their mouths with water between tastings.

Table 1. Specific primers used for qPCR analysis and qPCR parameters of standard curves obtained from 10-fold dilution of LAB and yeast strains DNA by qPCR.

Species	Primers			qPCR Parameters			References
	Name	Sequence	Product Size (pb)	R^2	Slop	Efficiency (%)	
L. plantarum	*pln*EF fw	5’ CTATTTCAGGTGGCGTTTTC 3’	93	0.99	-3.466	94	(Cho et al., 2010)
	*pln*EF rev	5’ GTGGATGAATCCTCGGACAG 3’					
L. acidophilus	F_acid_IS	5’GAAAGAGCCCAAACC AAGTGATT 3’	85	0.99	-3.243	100	(Haarman and Knol, 2006)
	R_acid_IS	5’CTTCCCAGATAATTCAACTAT CGCTTA 3’					
T. delbrueckii	Tods L2	5’CAAAGTCATCCAAGCCAGC3’	104	0.99	-3.799	83	(Zott et al., 2010)
	Tods R2	5’TTCTCAAACAATCATGTTTGGTAG3’					

3. Results and Discussion

3.1 Microbial growth during fermentation and storage period of beverages

For maize fermentations with different prebiotic concentrations, a mixed starter culture of LAB (*Lactobacillus plantarum* CCMA 0743 and *L. acidophilus* LACA 4) and yeast (*Torulaspora delbrueckii* CCMA 0235) were used. The results for microbial dynamics during M, and MR fermentations in the different assays with prebiotic (FOS) are demonstrated in Figure 2. As shown in Fig. 2A, in MR beverage *L. plantarum* CCMA 0743 populations remained stable throughout the fermentations, and after the end (24 h) population did not differ significantly ($P < 0.05$) between the assays with no prebiotic (6.3 log CFU/mL), with 2% FOS (6.4 log CFU/mL) and 5% FOS (6.4 log CFU/mL). Similar results occurred for this strain in M beverage (around 6.2 log CFU/mL), as shown in Fig. 2C. The *L. acidophilus* LACA 4 populations in MR beverage presented a slight decreased after the first 6 h of fermentation, but recovering after 12 h in the assays with FOS and maintaining until 24 h (7.1 log CFU/mL). Its population in 5% FOS assay differed significantly ($P < 0.05$) from the assay without prebiotic after 24 h (6.9 log CFU/mL). However no significant difference was observed in M beverage for *L. acidophilus* LACA 4 populations on the different assays at the end of fermentation. Its population remained stable throughout the 24 h (around 7.0 log CFU/mL).

As already known FOS is a prebiotic capable of stimulate the growth and activity of probiotic bacteria (Oliveira et al., 2011). In our work, the growth of probiotic *L. acidophilus* LACA 4 was favored by 5% FOS (MR beverages), and after storage time it was above the minimum recommended level (10^7 cfu/mL). This value is related to the effectively health functionalities of probiotics in the organism (Sanz and Dalmau, 2008). Similar results were also found by Mishra and Mishra (2013), in which the activity and growth of *L. acidophilus* in fermented soy milk was enhanced with 2% FOS.

The yeast population in M beverage increased 1 log CFU in the assays with 2% FOS (6.3 log CFU/mL) and 5% FOS (6.2 log CFU/mL) after 24 h of fermentation, and were significantly higher ($P < 0.05$) than in the assay without prebiotic (Fig. 2D). In MR beverage (Fig. 2B), although yeast populations showed a slight increase after 12 h in the assays with prebiotic, it decreased at the end of fermentation. However, *T. delbrueckii* population was significantly higher in 5% FOS assay (5.7 log CFU/mL) than in the assay without prebiotic (5.4 log CFU/mL). Interestingly, it seems that *T. delbrueckii* was more favored by the FOS than BAL, since it was able to increase its population during storage period. This result may be related to a probable fructanase activity of this yeast. As well known the hydrolysis of fructans (fructose polymers synthesized in nature from sucrose) is carried out by microbial fructosidases, and a high fructanase activity was observed for *Torulaspora delbrueckii* by Arrizon et al. (2012) when studying yeasts isolated from fermenting musts of Mezcal. The authors also observed a high biomass production by this yeast coupled with high fructanase activity on sucrose and inulin.

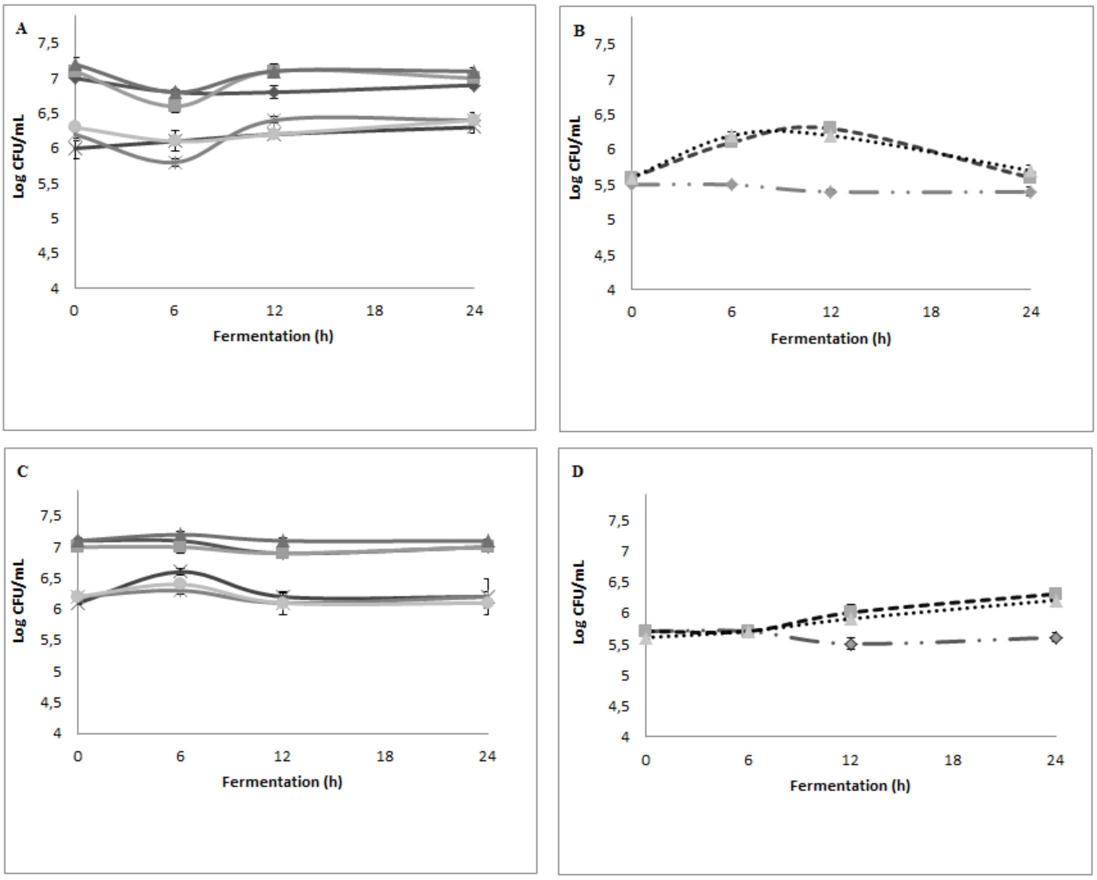

Fig. 2. Microbial populations during MR fermentation assays **(A and B)** and during M fermentation assays **(C and D).** Continuous lines correspond to LAB populations, while dotted lines correspond to yeast population. Symbols: (♦) *L. acidophilus* LACA 4 and *T. delbrueckii* CCMA 0235 with no prebiotic FOS; (■) *L. acidophilus* LACA 4 and *T. delbrueckii* CCMA 0235 with 2% FOS; (▲) *L. acidophilus* LACA 4 and *T. delbrueckii* CCMA 0235) with 5% FOS; (×) *L. plantarum* CCMA 0743 with no prebiotic FOS; (●) *L. plantarum* CCMA 0743 with 2% FOS; (●) *L. plantarum* CCMA 0743 with 5% FOS.

Regarding the maintenance of the microbial populations in the final products during storage period (28 days) at 4°C, the results are shown in Fig. 3. As demonstrated in Fig. 3A and 3B, for both fermentation medium, the LAB and yeast populations presented similar results. For LAB, the populations did not differ significantly ($P < 0.05$) in the assays after 28 days under refrigeration (around 7.0 log CFU/mL for *L. acidophilus* LACA 4, and 6.3 log CFU/mL for *L. plantarum* CCMA 0743). Furthermore, their population remained constant from the end of fermentation (24 h) until the end of the storage time, and they did not differ significantly ($P < 0.05$). For the yeast *T. delbrueckii* CCMA 0235, however, the populations in the assays with 2% and 5% of FOS were higher (around 6.7 and 6.4 log CFU/mL, respectively) than in the assay with no prebiotic (around 5.6 log CFU/mL), after 28 days. In this case, the yeast populations in the assays with FOS increased from the end of fermentation (24 h) until the end of the storage time, and were higher than in the assay without prebiotic.

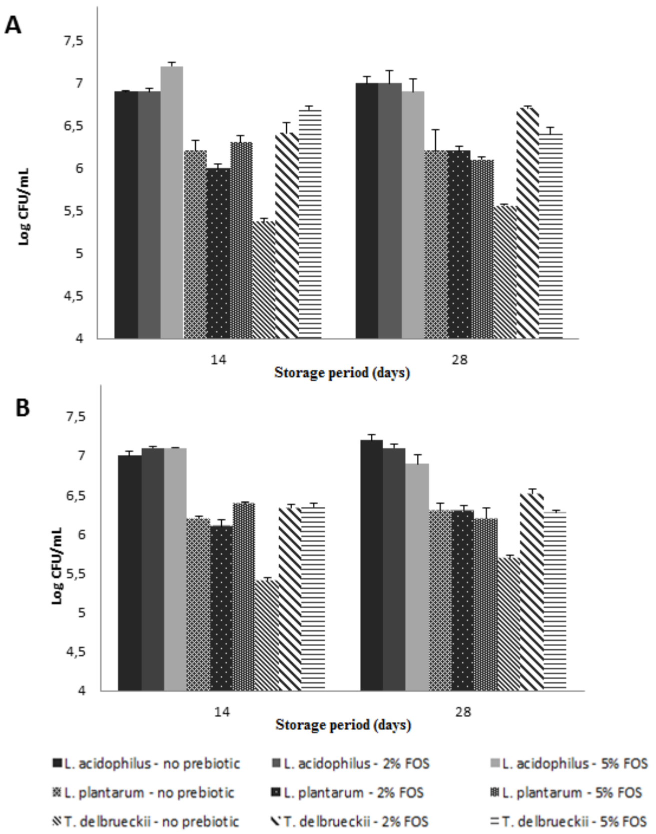

Fig. 3 . Microbial populations of MR beverage **(A)** and M beverage **(B)** during storage period at 4°C.

Our investigation revealed that prebiotic FOS had a positive influence on the populations of the potential probiotic starter culture during the fermentation of M and MR beverages, especially during the storage period of the beverages. The results were in accordance with other studies about the development of new symbiotic almond milk product fermented with probiotic *L. reuteri* (Bernat et al., 2015), and with new symbiotic soy yoghurt development using *L. acidophilus* NCDC11 (LA) and *Streptococcus salivarius* subsp. *thermophilus* NCDC118 (ST) as starter cultures (Mishra and Mishra, 2015).

3.2 Chemical parameters

The pH value and the carbohydrates contents during the fermentation assays and storage period were evaluated and are demonstrated in Table 2. After 6 h of fermentation, there was a drop of pH from 6.4 to 4.0 and from 6.6 to 4.0 in MR and M samples, respectively. The pH continued to drop throughout the fermentations in the assays with 2% and 5% of FOS reaching 3.5 after 24/36 h and 3.0 after 28 days under refrigeration. In fermented products pH is significant to determine microbiological stability against foodborne pathogens, since acidity (pH below 3.8) could create a harsh environment for pathogenic microorganisms (Salmerón et al., 2014). Our results are in agreement with the results previously reported for fermented maize porridge with added malted barley (Helland, Wicklund, Narvhus, 2004) and for non-dairy probiotic formulations with oat, barley and malt (Salmerón et al., 2014).

Starch was the main carbohydrate present at the beginning of fermentation of MR and M beverages, with concentrations of 7.24% and 9.84%, respectively. After 36 h of fermentation, the starch contents of different assays remained relatively constant in M samples, and were similar to the control assay (around 9.8%). On the other hand, in MR samples, the starch contents reduced from 7.24%, reaching 3.62% in the assay without prebiotic, 4.72% in the assay with 2% FOS and 6.52 in the assay with 5% FOS, after 36 h of fermentation. The strains *L. plantarum* CCMA 0743 and *T. delbrueckii* CCMA 0235 were isolated from cassava fermentations, and have been previously described as amylase producer (Ramos et al., 2015; Almeida et al., 2007). The starch reduction observed during fermentation might be resulted from microbial amylase activity. Indeed the selection of appropriate starter strains is the key to accurately reproduce the desirable characteristics of traditional health-promoting beverages for mass production. For instance, amylolytic microbes for digestion of starch could be considered desirable for fermented cereal production, given that they have adapted to their respective environments over thousands of years (Marsh et al., 2014).

Fructose, glucose, sucrose and maltose were detected at the beginning of fermentation in both substrates, but after 24 h of fermentation none of these sugars were detected in the assays without prebiotic (Table 2), indicating that they were rapid consumed by the starter culture. Similar results were observed in *kutukutu* (fermented maize paste consumed in Africa) fermentation, in which the decrease of pH was due to hydrolysis of carbohydrates during the fermentation which was followed by the production of organic acids (Roger et al., 2015). Otherwise, in the assays with prebiotic these sugars were detected throughout the fermentation time and also during storage period. Except maltose in MR samples and glucose in M samples which were not detected in the assays with FOS after 12 h of fermentation. FOS chains are formed by 2 to 10 units of fructose and were result from inulin hydrolysis (Roberfroid, 2005). This could explain the residual fructose and sucrose contents in the assays with prebiotic, since in our study, *T. delbrueckii* seems to have fructanase activity.

Table 2. Analysis of carbohydrates contents and pH changes during fermentation of M and MR beverages. ND=Not detected

				Compound concentration (g/L)									
Fermentation time (hour)	**Samples**	**pH value**		**Fructose**		**Glucose**		**Sucrose**		**Maltose**		**% Starch (w/v)**	
		MR	**M**	**MR**	**M**	**MR**	**M**	**MR**	**M**	**MR**	**M**	**MR**	**M**
0		6.4	6.6	0.311±0.00	0.222±0.00	0.916±0.05	0.526±0.38	1.394±0.05	1.012±0.11	0.197±0.01	0.141±0.08	7.24±0.0	9.84±0.8
6	Control	6.0	6.0	0.503±0.27	0.183±0.02	0.751±0.34	0.745±0.04	1.161±0.02	1.348±0.11	0.104±0.06	0.404±0.37		
	No prebiotic	4.0	4.0	0.025±0.01	0.070±0.00	0.049±0.00	0.077±0.01	ND	ND	ND	0.095±0.01		
	2% FOS	4.0	4.0	3.725±0.33	5.578±0.61	0.113±0.00	ND	2.143±0.39	2.910±0.23	0.003±0.00	0.028±0.01		
	5% FOS	4.0	4.0	6.888±1.04	7.512±0.66	0.107±0.02	0.093±0.04	5.301±0.35	6.666±0.08	0.039±0.03	0.030±0.01		
12	Control	6.0	6.0	0.151±0.02	0.163±000	0.489±0.02	0.608±0.05	0.483±0.05	1.095±0.10	0.117±0.12	0.093±0.00		
	No prebiotic	4.0	4.0	0.014±0.02	ND	0.036±0.00	ND	ND	ND	ND	ND		
	2% FOS	4.0	4.0	6.001±0.56	6.240±0.89	0.106±0.01	ND	2.442±0.05	2.392±0.01	ND	ND		
	5% FOS	4.0	4.0	5.240±0.24	8.453±0.02	0.139±0.08	ND	5.292±0.12	6.994±0.86	ND	ND		
24	Control	6.0	6.0	0.342±0.01	0.206±001	1.059±0.03	0.710±0.02	1.282±0.04	1.332±0.06	0.249±0.08	0.139±0.03		
	No prebiotic	4.0	4.0	ND	ND	ND	ND	ND	ND	ND	ND		
	2% FOS	3.5	3.5	5.153±1.22	4.761±1.58	0.064±0.01	ND	1.452±0.07	1.776±0.18	ND	0.026±0.02		
	5% FOS	3.5	3.5	1.133±0.09	9.263±0.00	0.126±0.02	ND	5.273±0.18	6.511±0.30	ND	ND		
36	Control	6.0	6.0	0.207±0.08	0.114±0.03	0.674±0.07	0.370±0.09	0.745±0.20	0.842±0.21	0.159±0.04	0.144±0.03	4.97±0.0	9.8±0.8
	No prebiotic	4.0	4.0	ND	ND	0.042±0.00	ND	ND	ND	ND	ND	3.62±1.5	10.4±0.0
	2% FOS	3.5	3.5	3.389±0.13	1.801±1.02	0.065±0.00	ND	0.579±0.46	4.332±0.12	ND	0.037±0.01	4.72±0.9	10.4±0.0
	5% FOS	3.5	3.5	1.286±0.15	1.147±0.04	0.161±0.03	ND	5.197±0.27	4.127±0.23	ND	0.019±0.03	6.52±0.6	9.9±0.0
Storage period (days)													
14	Control	5.5	5.0	0.116±0.16	0.055±0.08	0.489±0.69	0.581±0.27	0.459±0.65	1.156±0.13	0.074±0.10	0.153±0.03		
	No prebiotic	4.0	4.0	ND	ND	ND	ND	ND	ND	ND	ND		
	2% FOS	3.0	3.0	6.922±0.07	4.068±0.06	0.110±0.00	ND	1.197±0.57	1.902±0.11	ND	0.100±0.01		
	5% FOS	3.0	3.0	2.316±0.19	1.790±0.46	0.157±0.03	0.011±0.02	5.121±0.01	8.225±1.39	ND	0.014±002		
28	Control	5.5	5.0	0.295±0.06	0.036±0.00	1.107±0.11	0.381±0.01	1.507±0.12	1.066±0.05	0.198±0.02	0.156±0.03		
	No prebiotic	4.0	4.0	ND	ND	ND	ND	ND	ND	ND	ND		
	2% FOS	3.0	3.0	6.699±0.31	4.270±0.46	0.063±0.01	ND	0.546±0.04	1.473±0.15	ND	0.096±0.02		
	5% FOS	3.0	3.0	2.835±0.00	1.878±0.01	0.070±0.02	ND	5.547±0.02	7.716±0.08	ND	0.075±0.01		

Regarding organic acids and alcohols detected in the samples during fermentation, the results are in Figure 4 and 5 for MR and M beverage, respectively. For the two fermented substrates, the organic acids and alcohols profiles were very similar, being lactic acid the main metabolite produced by LAB. It reached the maximum values of 3.7 g/L in the assays with 2% and 5% FOS in MR samples, and 3.6 g/L in the assay with 5% FOS in M sample, at end of fermentation. Acetic acid concentration increased in the first 12 h, after that it remained constant until the end of fermentation. In MR beverage, the assays with prebiotic reached the highest acetic acid concentration at 12 h, approximately 0.5g/L, while in M beverage similar acetic acid concentration was detected in all assays (including the assay without prebiotic), reaching the maximum concentration (around 0.4 g/L) after 24 h of fermentation. Our results demonstrated a similar situation that occurs in sourdough fermentations, in which cereal flours and water are fermented with a mix of yeasts and LAB strains, and the non-volatiles lactic and acetic acid are important flavour compounds produced (Salimur, Paterson, Piggott, 2006). Lactic acid has a mild acidic note that can be perceptible in concentrations above the taste threshold of 20 mg/l in water, in lower concentrations it is odourless (Hartwig and McDaniel, 1995). On the other hand, acetic acid is released in lower concentrations compared to lactic acid, but because of its lower taste threshold (15 mg/L in water) and higher volatility, it can become perceptible as pungent sour with a “cider-vinegar” aroma above a concentration of 100 mg/L (Burdock, 2016).

Citric acid was detected in the beginning of fermentation (around 0.2 g/L) but it was promptly consumed at the first 12 h in all assays. Similarly result occurred for propionic acid, which was detected at initial concentration of approximately 0.1 g/L and was depleted in the first 6 h, except in the assay without prebiotic of M samples. Malic acid was also detected in the beginning of fermentation (around 0.2 g/L) and its concentration varied in the assays throughout the fermentation. It is well known LAB strains have the ability to metabolize malic (malolactic fermentation) and citric acids, leading to a great number and diversity of flavor changes. Malolatic fermentation is very important in wine production and its main reaction is the conversion of malic acid in lactic acid, which gives the wine a smoother taste. On the other hand, citric acid breakdown leads to production of acetic acid, increasing the volatile acidity of the wine, and diacetyl (Moreno-Arribas and Polo, 2008).

Isobutiric acid was detected in low concentration (around 0.05 g/L) after 12 h (MR) and 24 h (M) of fermentation, only in the assay with no prebiotic. Ethanol and glycerol were detected in all assays, reaching the highest concentrations in the assays with prebiotic. In the assays with 5% FOS, ethanol reached maximum concentrations of 0.9 g/L and 1.2 g/L in MR and M samples, respectively. Thus, the alcoholic concentrations of both MR (0.09%) and M (0.12%) beverages are below 0.5% (v/v), being considered non-alcoholic beverages accordance with Brazilian legislation (Brasil, 2009). Slight alcoholic beverages are very common in indigenous lactic fermentations, in which there are mix of LAB and yeasts acting on the substrate (Freire et al., 2014; Ramos et al., 2011). Low ethanol concentration is a good condition for *T. delbrueckii'*s growth, as this yeast has a low ethanol tolerance (Bely et al., 2008). The maximum concentrations of glycerol were 1.3 g/L in the assay with 2% FOS and 1.0 g/L in the assay with 5% FOS L in MR and M beverage, respectively.

Comparing our results with maize and rice naturally fermented calugi (Miguel et al., 2012), we can observed a similar profiles of organic acids and alcohols, although concentrations of some compounds were higher in our work than in calugi. For instance, in our study lactic acid concentration were almost 2 times higher than in *calugi*. It maybe be an consequence of the high inoculated LAB population and the absence of contaminants, when using starter culture in controlled processes.

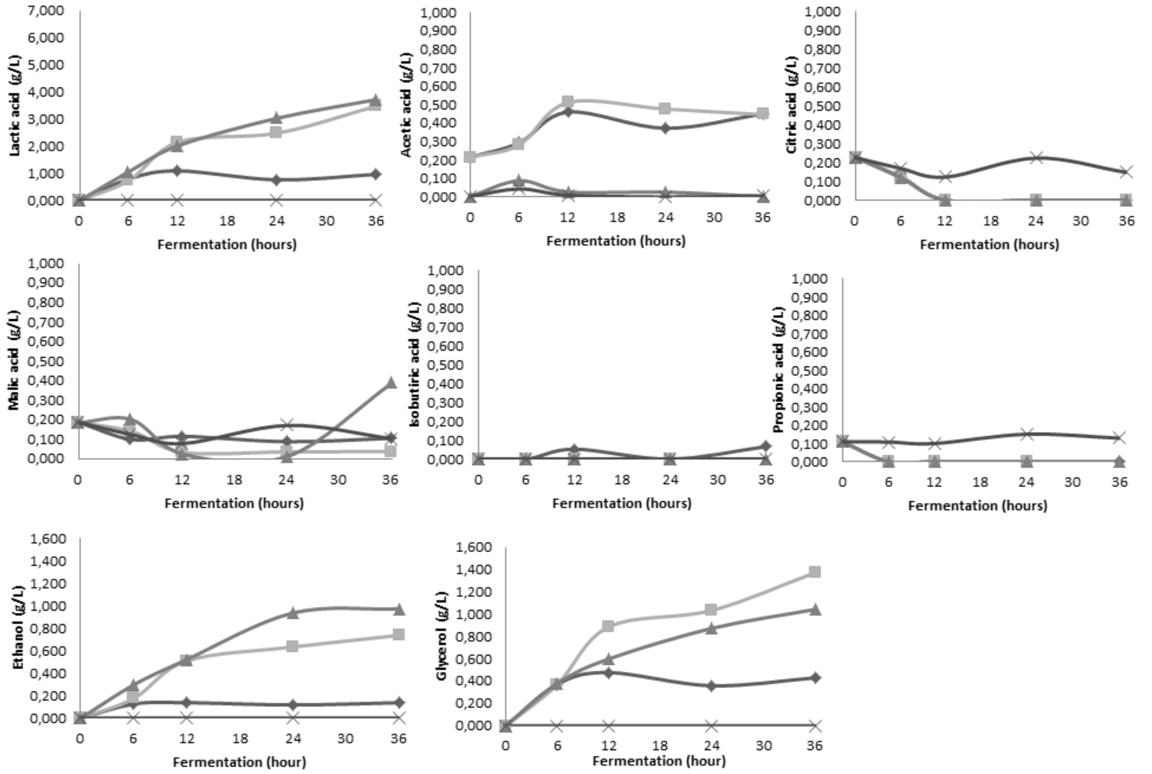

Fig. 4 HPLC analysis of organic acids and alcohols of MR-based calugi samples during 36 h of fermentation. Symbols: (♦) No prebiotic assay; (■) 2% FOS assay; (▲) 5% FOS assay; (×) control.

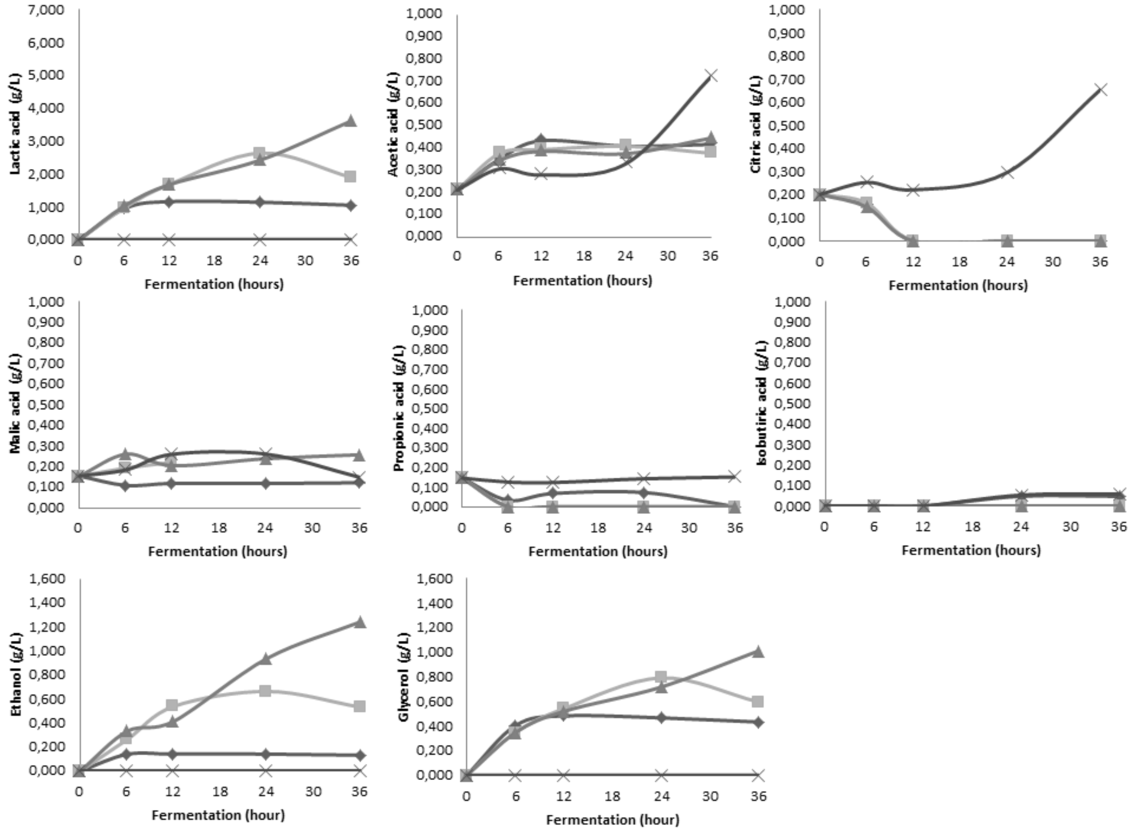

Fig. 5 HPLC analysis of organic acids and alcohols of M-based calugi samples during 36 h of fermentation. Symbols: (♦) No prebiotic assay; (■) 2% FOS assay; (▲) 5% FOS assay; (×) control.

In complex matrices, like foods or beverages, flavour is usually the result of the presence of many volatile and nonvolatile components possessing diverse chemical and physicochemical properties (Longo and Sanromán, 2006). Whereas the nonvolatile compounds contribute mainly to the taste, the volatile ones influence both taste and aroma. The volatile fraction comprises principally some carboxylic acids, alcohols, aldehydes, ketones, and esters (Lasekan, and Lasekan, 2012). In our work total of 55 volatile compounds, including acids, alcohols, aldehydes, esters, ketones, pyrazines and others, were detected by GC–MS in M and MR beverages, and their concentrations are shown in Table 3. Alcohols group were the most abundant with 21 compounds, followed by acids with 7, aldehydes, esters and ketones, with 5 each one, terpenes with 4, pyrazines with 3 and other 4 compounds. Their concentrations varied among the used substrate and prebiotic concentrations. Important flavor compounds, for instance esters and alcohols compounds were detected in the fermented samples. Esters are commonly used flavouring agents, very appreciated for the fruity aromas they provide. Ethyl acetate, for instance, has and odour note of pineapple with a flavor threshold in water of 5.0 mg/L (Liu, Holland, Crow, 2004). In our work, ethyl acetate was the main ester quantified and it presence was more related to fermented M beverages, which concentrations were above it threshold. Comparing to other works, the concentrations we found for ethyl acetate in M fermented beverages (5.96 to 10.58 mg/L) are higher than those reported for cereals probiotic beverages inoculated with *L. acidophilus* and *L. plantarum* (0.09 to 0.114 mg/L) (Salmerón et al., 2014) and for those reported for kefir (0.004 to 0.06 mg/kg) (Beshkova et al., 2003). Ethyl or methyl esters of short-chain fatty acids, octanoic acid, ethyl ester, decanoic acid, ethyl ester and butanoic acid, 2-methyl-, were also detected in the beverages. These compounds generally bring about fruity flavours, and are related to cheese production (Liu, Holland, Crow, 2004).

Yeasts are related to produce long-chain and complex alcohols that together with their derived esters have interesting flavor properties. One of the most relevant aroma-related alcohols is phenylethyl alcohol, which possesses a rose-like smell (Longo and Sanromán, 2006). In our investigation, this compound was identified in high concentration in M baverages samples fermented with 2% FOS (17.90 mg/L) and 5% FOS (14.19 mg/L). Both concentrations are above the aroma threshold value of 3 to 5 mg/L (Burdock, 2016).

Aldehydes and ketones, when present in low concentrations,may be associated with pleasant smells and flavors in fermented foods. Hexanal was the main aldehyde found in our work, and it has also been identified in several cereal based fermented products such as *kenkey* (Annan et al., 2003) and sorghum malt beverage (Lasekan, Lasekan, Idowu, 1997). This compound also show strong antimicrobial properties against pathogen microorganisms at low concentrations (Fadida, Selilat-Weiss, Poverenov, 2015). The ketone 2-heptanone was identified in our work, with higher concentrations in M beverages samples. These methyl ketones are aromas employed in a wide range of flavouring applications, especially those related to blue cheese and fruit flavours (Hagedorn and Kaphammer, 1994).

Terpenes are widespread in nature, mainly in plants as constituents of essential oils (Longo and Sanromán, 2006). It has been reported that linalol and its degradation product α-terpineol have potent anti-microbial activity against periodontopathic and cariogenic bacteria (Park et al., 2012). In our work α-terpineol and D-Limonene (which has a pleasant, lemon-like odor) were the most common terpene compounds detected in the beverages, and their concentrations varied from 0.030 to 0.584 g/L and 0.021 to 1.239 g/L, respectively.

Table 3. Concentration of volatile compounds obtained by GC–MS analysis at the beginning (0h) and at the final fermentation time (36h) of different assays.

Compounds	Characteristics	MR- 0h (mg/L)	MR- 0 (mg/L)	MR- 2% (mg/L)	MR- 5% (mg/L)	MR- C (mg/L)	M- 0h (mg/L)	M- 0 (mg/L)	M- 2% (mg/L)	M- 5% (mg/L)	M- C (mg/L)
Acids											
Heptanoic acid	Rancid odor	1.701	1.304	0.282	0.202	1.718	1.242	0.465	2.140	1.250	ND
Octanoic acid	Mildly unpleasant odor, and a burning, rancid taste	0.982	1.074	0.428	0.336	0.893	0.716	0.845	3.343	2.376	0.295
Isobutyric acid	Strong penetrating odor of rancid butter	ND	0.773	0.352	0.331	ND	ND	0.717	5.215	3.587	ND
Nonanoic acid	-	ND	0.335	0.066	0.030	ND	ND	0.274	0.535	0.513	ND
Decanoic acid	Fatty, unpleasant, rancid odor	1.294	0.249	0.391	0.234	ND	ND	0.322	0.835	0.681	ND
Benzoic acid	Odorless or exhibits a faint balsamic odor and a sweet-sour to acrid taste	ND	0.469	0.081	0.045	ND	ND	0.340	0.448	0.144	ND
Hexanoic acid	-	0.699	ND	ND	ND	ND	0.254	0.280	ND	ND	ND
Alcohols											
1-Butanol, 2-methyl-	Cooked, roasted aroma with fruity or alcoholic undernotes	35.156	2.377	0.097	0.149	1.086	13.402	ND	1.213	0.523	9.990
1-Pentanol (Amyl alcohol)	Fusel-like sweet and pleasant odor and burning taste	2.254	0.345	0.039	0.027	0.764	0.935	ND	0.276	0.135	0.617
1-Hexanol	Flavoring ingredient: fruity odor and aromatic flavor	0.529	0.302	0.026	0.014	ND	0.247	0.811	0.128	0.037	0.090
1-Octanol	Fresh, orange-rose odor, that is quite sweet with an oily, sweet, slightly herbaceous taste	2.244	0.927	0.150	0.125	1.454	1.404	0.852	1.447	0.788	0.801
1-Decanol	Floral odor resembling orange flowers and a slight, characteristic fatty taste	0.383	0.136	0.041	0.051	0.280	0.392	0.186	0.448	0.378	0.225
Phenylethyl Alcohol	Rose-like odor	0.398	0.853	1.556	1.994	5.585	2.139	1.587	17.903	14.197	2.067
1-Dodecanol	Characteristic fatty, waxy flavor; unpleasant at high concentrations but delicate and floral on dilution	ND	0.119	0.020	ND	0.258	0.237	0.164	0.146	0.068	0.088
3-Nonen-1-ol, (Z)-	-	ND	ND	ND	ND	ND	ND	ND	0.209	0.146	ND
1-Propanol, 3-(methylthio)-	Powerful sweet soup or meat-like odor and flavor in high dilution	1.165	0.701	ND	0.046	3.457	0.917	0.521	ND	ND	0.772
1-Propanol, 2-methyl-	Flavoring ingredient; disagreeable odor	ND	ND	0.535	0.549	ND	ND	ND	6.667	7.082	ND
1-Butanol	Flavoring agent; odor similar to amyl alcohol, and a dry,	ND	0.214	ND	0.035	ND	ND	ND	0.342	0.176	ND

Table 3. Concentration of volatile compounds obtained by GC–MS analysis at the beginning (0h) and at the final fermentation time (36h) of different assays (continued).

Compounds	Characteristics	MR- T0 (mg/L)	MR- 0 (mg/L)	MR- 2% (mg/L)	MR- 5% (mg/L)	MR- C (mg/L)	M- T0 (mg/L)	M- 0 (mg/L)	M- 2% (mg/L)	M- 5% (mg/L)	M- C (mg/L)
Alcohols											
Isoamyl alcohol	Pungent odor and repulsive taste; only in high dilution becoming pleasant, fruity-winey	ND	4.963	6.417	7.204	1.545	2.958	ND	67.411	63.510	1.221
Linalool	Typical floral odor, free from camphoraceous and terpenic notes	ND	ND	0.049	0.044	ND	0.192	0.092	0.646	0.534	ND
Geraniol	Rose-like odor	ND	ND	0.019	0.041	ND	ND	ND	0.233	0.324	ND
1-Heptanol	Fragrant, woody, heavy, oily, faint, aromatic, fatty odor, and a pungent, spicy taste	1.035	4.174	0.478	0.391	1.026	0.907	2.367	2.351	1.635	3.671
10-Undecen-1-ol	Fatty odor reminiscent of lemon with a fatty, burning taste	ND	ND	0.015	0.013	ND	ND	ND	0.209	0.146	ND
Benzyl alcohol	Characteristic pleasant, fruity odor and a slightly pungent, sweet taste	ND	0.114	0.040	0.024	ND	ND	ND	0.398	0.103	ND
1-Octen-3-ol	Powerful, sweet, earthy odor with strong herbaceous note reminiscent of lavender-lavandin, rose and hay. Sweet, herbaceous taste	ND	ND	ND	ND	4.149	ND	0.681	ND	ND	ND
1-Dodecanol	Characteristic fatty odor; fatty, waxy flavor	ND	0.085	0.019	ND	0.230	ND	0.112	0.355	0.057	ND
1-Butanol, 3-methyl-	Fusel oil, whiskey-characteristic, pungent odor	ND	ND	ND	ND	ND	ND	0.611	ND	ND	ND
2-Methoxy-4-vinylphenol	-	1.115	0.200	0.033	0.025	0.979	0.790	0.321	0.235	0.141	10.255
Aldehydes											
Hexanal	Flavoring ingredient. Fatty, green, grassy, powerful, penetrating characteristic fruity odor and taste	16.061	0.937	0.510	0.251	8.060	18.471	2.792	3.129	0.543	4.459
Heptanal	Very strong, fatty, harsh, pungent odor, and na unpleasant, fatty taste	4.029	ND	ND	ND	9.763	1.797	ND	ND	ND	ND
2-Heptenal, (Z)-	Flavoring ingredient : green, fatty odor	ND	ND	0.026	0.016	0.455	ND	0.205	0.180	0.055	0.227
Benzaldehyde	Powerful sweet odor reminiscent of freshly crushed bitter almonds	1.531	0.254	0.056	0.044	1.236	1.213	0.406	0.672	0.320	0.734
2-Undecenal	Powerful fresh aldehydic odor	ND	0.059	0.025	0.012	0.335	0.135	0.081	0.216	0.107	0.070
Esters											
Ethyl Acetate	Fresh, ether, fruity	ND	ND	ND	2.137	7.479	19.742	5.968	9.000	10.582	9.431

Table 3. Concentration of volatile compounds obtained by GC–MS analysis at the beginning (0h) and at the final fermentation time (36h) of different assays.

Compounds	**Characteristics**	**MR- T0 (mg/L)**	**MR- 0 (mg/L)**	**MR- 2% (mg/L)**	**MR- 5% (mg/L)**	**MR- C (mg/L)**	**M- T0 (mg/L)**	**M- 0 (mg/L)**	**M- 2% (mg/L)**	**M- 5% (mg/L)**	**M- C (mg/L)**
Octanoic acid, ethyl ester	Flavoring ingredient: pleasant, fruity, floral odor	0.919	ND	0.097	0.065	1.307	1.165	ND	0.710	0.257	0.448
Hexyl formate	fruity, apple-like or unripe-plum odor and a corresponding sweet taste	1.972	2.638	0.274	0.236	1.137	0.948	2.692	2.744	1.089	3.137
Decanoic acid, ethyl ester	Fruity odor; oily, brandy-like odor	1.132	ND	0.168	0.164	0.959	0.486	ND	0.987	0.463	ND
Esters											
Butanoic acid, 2-methyl-	Fruity odor	ND	0.515	0.111	0.116	ND	ND	ND	1.364	0.969	ND
Ketones											
2-Heptanone	Fruity, spicy, cinnamon, banana, slightly spicy odor	7.642	1.422	0.076	0.034	13.712	5.853	0.909	0.759	0.364	1.756
trans-.beta.-Ionone	-	0.489	ND	ND	ND	0.532	0.305	ND	ND	ND	ND
4-Hydroxy-3-methylacetophenone	-	0.200	0.033	0.025	0.979	0.790	0.321	0.235	0.142	10.255	
Isophorone	Slight minty odor	7.861	0.670	0.075	0.044	2.817	1.866	0.395	0.544	0.351	0.746
2-Octanone	Floral and bitter, green, fruity (unripe apple) odor, and bitter, camphoraceous taste	ND	ND	ND	ND	3.388	ND	0.403	ND	ND	ND
Pyrazines											
2-Methylpyrazine	Nutty, roasted odor	5.805	0.785	0.214	0.112	ND	4.682	ND	0.848	0.478	2.019
2-Ethylpyrazine	Musty, nutty, peanut, woody, roasted, green, sweet	ND	ND	0.135	ND	ND	ND	ND	ND	ND	ND
Pyrazine, 2-ethyl-6-methyl-	Roasted-sweet odor	1.979	ND	0.135	ND	ND	1.486	ND	ND	0.110	0.376

Table 3. Concentration of volatile compounds obtained by GC–MS analysis at the beginning (0h) and at the final fermentation time (36h) of different assays.

Compounds	**Characteristics**	**MR- T0 (mg/L)**	**MR- 0 (mg/L)**	**MR- 2% (mg/L)**	**MR- 5% (mg/L)**	**MR- C (mg/L)**	**M- T0 (mg/L)**	**M- 0 (mg/L)**	**M- 2% (mg/L)**	**M- 5% (mg/L)**	**M- C (mg/L)**
Terpenes											
alpha-Terpinene	Woody, terpene, lemon odor with a lemony flavor	ND	ND	ND	ND	7.888	ND	0.234	ND	ND	ND
(-)-Terpinen-4-ol	Flavoring ingredient: taste: sweet, citrus green with a tropical fruit character	ND	0.088	ND	ND	ND	0.306	ND	ND	ND	ND
.alpha.-Terpineol	-	0.584	0.103	0.030	0.052	ND	0.358	ND	0.292	0.289	ND
D-Limonene	Pleasant, lemon-like odor	ND	ND	ND	0.021	ND	1.239	1.147	ND	0.235	0.667
Others											
2-Pentylfuran	Fruity odor	3.668	0.616	0.263	0.105	7.847	3.457	ND	1.600	0.580	1.633
2(3H)-Furanone, dihydro-5-pentyl-	strong odor of reminiscent coconut and a fatty, peculiar taste	ND	1.954	0.473	0.326	1.905	0.785	2.388	2.895	1.544	0.383
Acetoin	bland, woody, yogurt odor with a fatty creamy butter taste. Flavor ingredient in butter, milk, yogurt and strawberry flavors	ND	ND	ND	0.023	ND	ND	ND	ND	ND	6.605
Phenol, 4-ethyl-	Powerful woody-phenolic, yet somewhat sweet odor	ND	5.845	0.713	0.471	2.426	1.105	10.384	10.105	5.210	2.182

ND= Not detected. MR= maize and rice-based calugi. M=maize-based calugi. 0= no prebiotic; 2%= FOS concentration; 5%= FOS concentration; C= control.

3.3. Sensory evaluation of beverages

The acceptability of MR and M beverages were evaluated by consumers according to the hedonic scale of nine categories, ranging from dislike extremely (1) to like extremely (9), and results are shown in Table 4. The scores for taste, aroma, overall impression, texture, appearance and color did not differ significantly ($P > 0.05$) among the samples in M beverages. On the other hand, in MR, the 5% FOS assay received the highest score for taste (6.13) and aroma (6.26) attributes and were significantly different ($P < 0.05$) from the control. Furthermore, the beverages produced with 2% and 5% FOS showed the highest ($P < 0.05$) average score (6.23 and 6.53, respectively) for the overall impression attribute.

Table 4. Consumer's acceptance of final beverages produced from maize and maize and rice.

Calugi beverages	**Sensory Attributes**					
	Taste	**Aroma**	**Overall impression**	**Texture**	**Appearance**	**Color**
M - No prebiotic	5.64^{a}	5.60^{a}	6.02^{a}	6.58^{a}	6.80^{a}	6.96^{a}
M - 2% FOS	5.88^{a}	6.02^{a}	6.36^{a}	6.62^{a}	7.08^{a}	7.00^{a}
M - 5% FOS	6.08^{a}	6.12^{a}	6.46^{a}	6.84^{a}	7.00^{a}	7.12^{a}
M - Control	5.80^{a}	5.96^{a}	6.18^{a}	6.66^{a}	6.82^{a}	7.10^{a}
MR - No prebiotic	5.53^{ab}	5.81^{ab}	5.91^{ab}	6.26^{a}	6.43^{a}	6.68^{a}
MR - 2% FOS	5.74^{ab}	6.08^{ab}	6.23^{b}	6.58^{a}	6.47^{a}	6.75^{a}
MR - 5% FOS	6.13^{b}	6.26^{b}	6.53^{b}	6.75^{a}	6.62^{a}	6.83^{a}
MR – Control	5.08^{a}	5.42^{a}	5.40^{a}	6.38^{a}	6.57^{a}	6.79^{a}

Values with different letters in the same column are significantly different ($P < 0.05$) according to Tukey test. M= maize-based calugi; MR= maize and rice-based calugi

The figures 6 and 7 show the attributes notes in relation to the number of consumers, for M and MR beverages, respectively. The results demonstrated that most of consumers (≥ 50%) have had a good acceptation of the beverages, since for all attributes, their notes varied from 6 to 9, which indicate that consumers liked slightly or until liked extremely. Interestingly, prebiotic-containing beverages had the highest percentage of consumers (60 to 92%) who scored 6 to 9 for all attributes, compared to non-prebiotic and control beverages.

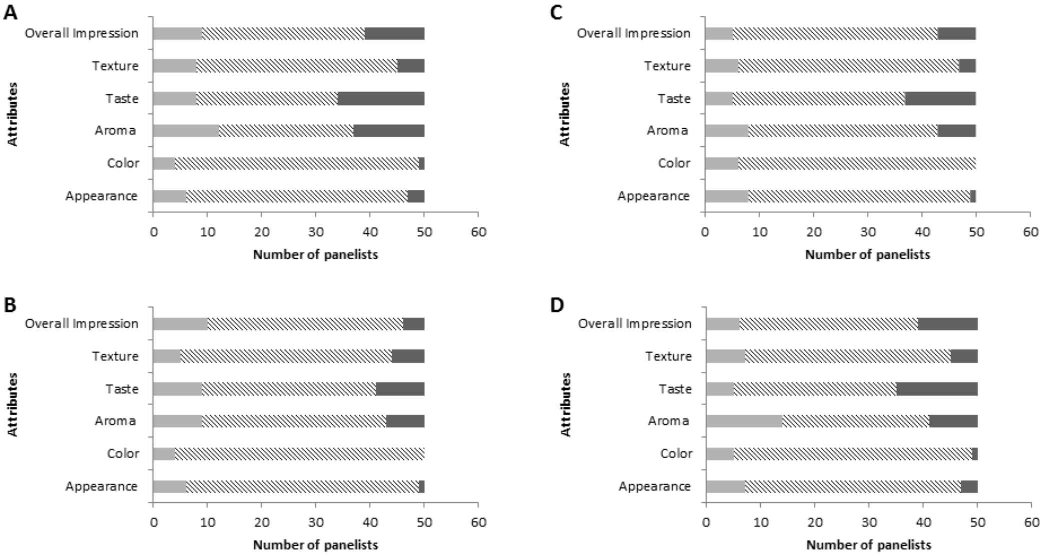

Fig. 6 Acceptance test notes of M beverages added with FOS. Figures A, B, C and D represent the beverages with FOS's concentration of 0, 2%, 5% and the control, respectively. Dark gray bars indicate notes from 1 to 4; Light gray bars indicate note 5; and streaked bars indicate notes from 6 to 9.

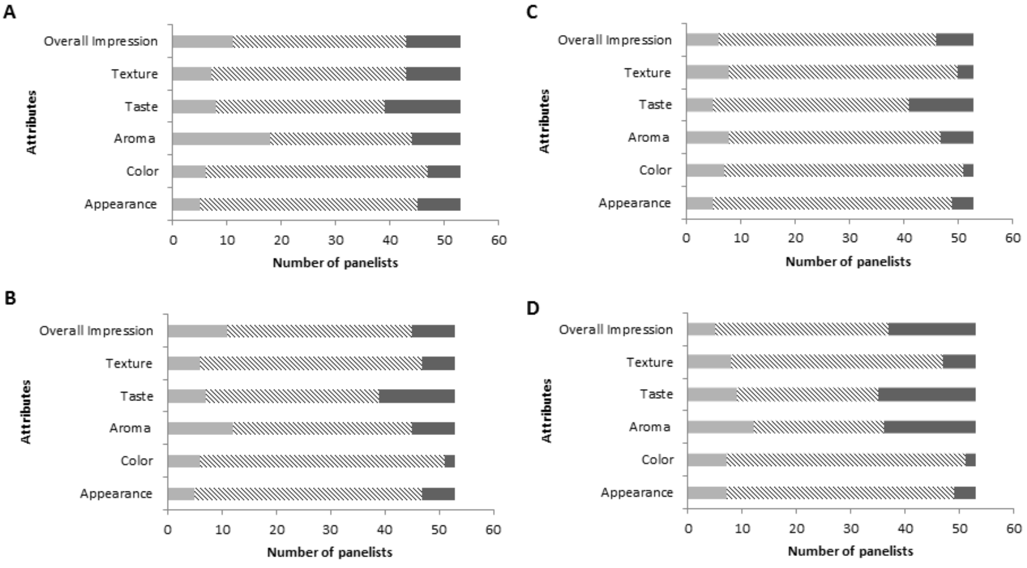

Fig. 7 Acceptance test notes of MR beverages added with FOS. Figures A, B, C and D represent the beverages with FOS's concentration of 0, 2%, 5% and the control, respectively. Dark gray bars indicate notes from 1 to 4; Light gray bars indicate note 5; and streaked bars indicate notes from 6 to 9.

The slight preference for M beverages may be due to its flavor compound set, which includes carbohydrates, organic acids, alcohols and volatile compounds. As described above, compounds like ethyl acetate and 2-heptanone, that have pleasant odour, were identified in higher concentrations in M beverages. However the scores for sensorial attributes did not differ significantly ($P > 0.05$) among the samples. In this case, prebiotic did not interfered in the sensory acceptance of the beverages. Similar results were found by Gonzalez et al. (2011) studying the effect of a prebiotic (FOS) on the sensory properties and consumer acceptability of peach-flavored drinkable yogurts. As in our work, the beverages containing the prebiotic were not significantly different from their comparable controls indicating that a prebiotic can be added without impacting acceptance. However, in the case of MR beverages, it seems that prebiotic had a positive influence on the attributes of taste, aroma and overall impression.

Analyzing the acceptability results we could confirm the potential of M and MR symbiotic beverages have to be commercialized and consumed. Indeed, cereals have a huge potential as vehicles for functional compounds such as antioxidants, dietary fiber, minerals, prebiotics, vitamins, and also probiotics (Nionelli et al., 2014). Examples of commercial products are ProvivaR (Skane Dairy, Sweden), the first oat-based probiotic food beverage where the active probiotic component is *L. plantarum* 299v (Prado et al., 2008); and Whole Grain Probiotic LiquidR (Grainfields, Australia), a refreshing, effervescent liquid containing both LAB (*L. acidophilus, L. delbrueckii*) and yeasts (*Saccharomyces cerevisiae* var. *boulardii* and *S. cerevisiae*) as well as vitamins, amino acids, and enzymes (Soccol et al., 2012).

4. Conclusions

Potential functional beverages were obtained by inoculation of maize and maize mixed rice media with a mixed starter culture of LAB and yeast. The prebiotic (FOS) was important to keep viable probiotic's population ($\geq 10^7$ CFU/mL) during fermentation and refrigeration period of 28 days, and besides it presence in fermentation media favored the growth of the yeast. The chemical compounds identified such as carbohydrates, organic acids, alcohols, and volatiles compounds merged to provide the unique taste and aroma of the beverages, mainly because of the presence of lactic and acetic acids and some esters. Beverage’s acceptability by consumers was more than 50% of the panelists (scores between 6 and 9), indicating the potential for commercial production of these symbiotic maize-based beverages. Besides, beverages with the prebiotic received scores significantly higher than the controls without FOS. For the future, further studies focusing in appropriate scale up production is necessary to evaluate some parameters such as identification and quantification of promising bioactive compounds, the formulation based on traditional fermented beverages, and the stability of the starter culture.

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References

Achi, O.K., Ukwuru, M., 2015. Cereal-Based Fermented Foods of Africa as Functional Foods. Int. J. Microbiol. Appl. 2, 71–83.

Almeida, E.G., Rachid, C.C.T.C., Schwan, R.F., 2007. Microbial population present in fermented beverage “cauim” produced by Brazilian Amerindians. Int. J. Food Microbiol. 120, 146–151. doi:10.1016/j.ijfoodmicro.2007.06.020

Annan, N. T., Poll, L., Sefa-Dedeh, S., Plahar, W. A., Jakobsen, M., 2003. Volatile compounds produced by *Lactobacillus fermentum, Saccharomyces cerevisiae* and *Candida krusei* in single starter culture fermentations of Ghanaian maize dough. J. Appl. Microbiol., 94, 462-474.

Arrizon, J., Morel, S., Gschaedler, A., Monsan, P., 2012. Bioresource Technology Fructanase and fructosyltransferase activity of non- Saccharomyces yeasts isolated from fermenting musts of Mezcal 110, 560–565. doi:10.1016/j.biortech.2012.01.112

Batista, N.N., Ramos, C.L., Ribeiro, D.D., Pinheiro, A.C.M., Schwan, R.F., 2015. Dynamic behavior of Saccharomyces cerevisiae, Pichia kluyveri and Hanseniaspora uvarum during spontaneous and inoculated cocoa fermentations and their effect on sensory characteristics of chocolate. LWT - Food Sci. Technol. 63, 221–227. doi:10.1016/j.lwt.2015.03.051

Bely, M., Stoeckle, P., Masneuf-Pomarède, I., Dubourdieu, D., 2008. Impact of mixed *Torulaspora delbrueckii-Saccharomyces cerevisiae* culture on high-sugar fermentation. Int. J. Food Microbiol. 122, 312–320.

Bernat, N., Chafer, M., Chiralt, A., Gonzalez-Martinez, C., 2015. Development of a non-dairy probiotic fermented product based on almond milk and inulin. Food Sci. Technol. Int. 21, 440–453. doi:10.1177/1082013214543705

Beshkova, D., Simova, E., Frengova, G., Simov, Z., 1998. Production of flavour compounds by yogurt starter cultures. J. Ind. Microbiol. Biotechnol., 20, 180-186.

Blandino, A., Al-Aseeri, M.E., Pandiella, S.S., Cantero, D., Webb, C., 2003. Cereal-based fermented foods and beverages. Food Res. Int. 36, 527–543. doi:10.1016/S0963-9969(03)00009-7

Brasil (2009). DECRETO No 6.871, DE 4 DE JUNHO DE 2009. Regulamenta a Lei no 8.918, de 14 de julho de 1994, que dispõe sobre a padronização, a classificação, o registro, a inspeção, a produção e a fiscalização de bebidas. Legislação Bebidas. Presidência da República (Retrieved from:http://www.planalto.gov.br/ccivil_03/_Ato20072010/ 2009/Decreto/D6871.htm2010/2009/Decreto/D6871.htm.).

Burdock, G. A., 2016. Fenaroli's handbook of flavor ingredients. CRC press.

Chaves-lopez, C., Serio, A., Delgado-ospina, J., Audisio, M.C., Grande-Tovar, C.D., Paparella, A., 2016. Exploring the Bacterial Microbiota of Colombian Fermented Maize Dough “ Masa Agria ”

(Maiz Añejo). Front. Microbiol. 7, 1–12. doi:10.3389/fmicb.2016.01168

Cho, G.S., Hanak, A., Huch, M., Holzapfel, W.H., Franz, C.M.A.P., 2010. Investigation into the Potential of Bacteriocinogenic Lactobacillus plantarum BFE 5092 for Biopreservation of Raw Turkey Meat. Probiotics Antimicrob. Proteins 2, 241–249. doi:10.1007/s12602-010-9053-4

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 Sci. Technol. 43, 1564–1572.

Fadida, T., Selilat-Weiss, A., Poverenov, E., 2015. N-hexylimine-chitosan, a biodegradable and covalently stabilized source of volatile, antimicrobial hexanal. Next generation controlled-release system. Food Hydrocolloids, 48, 213-219.

Franz, C.M.A.P., Huch, M., Mathara, J.M., Abriouel, H., Benomar, N., Reid, G., Galvez, A., Holzapfel, W.H., 2014. African fermented foods and probiotics. Int. J. Food Microbiol. 190, 84–96. doi:10.1016/j.ijfoodmicro.2014.08.033

Freire, A.L., Ramos, C.L., de Almeida, E.G., Duarte, W.F., Schwan, R.F., 2014. Study of the physicochemical parameters and spontaneous fermentation during the traditional production of yakupa, an indigenous beverage produced by Brazilian Amerindians. World J. Microbiol. Biotechnol. 30, 567–577. doi:10.1007/s11274-013-1476-0

Freire, A.L., Ramos, C.L., Schwan, R.F., 2015. Microbiological and chemical parameters during cassava based-substrate fermentation using potential starter cultures of lactic acid bacteria and yeast. Food Res. Int. 76, 787–795.

Gabriela, M., Miguel, P., Cristina, C., Santos, A., 2014. Bacterial dynamics and chemical changes during the spontaneous production of the fermented porridge (Calugi) from cassava and corn 8, 839–849. doi:10.5897/AJMR2013.6240

Gibson G. R., and Roberfroid, M. B., 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. nutr, 125, 1401-1412.

Gonzalez, N. J., Adhikari, K., Sancho-Madriz, M. F., 2011. Sensory characteristics of peach-flavoured yoghurt drinks containing prebiotics and synbiotics. LWT - Food Sci. Technol., 44, 158-163.

Haarman, M., Knol, J., 2006. Quantitative Real-Time PCR Analysis of Fecal Lactobacillus Species in Infants Receiving a Prebiotic Infant Formula Quantitative Real-Time PCR Analysis of Fecal Lactobacillus Species in Infants Receiving a Prebiotic Infant Formula. Appl. Environ. Microbiol. 72, 2359–2365. doi:10.1128/AEM.72.4.2359

Hagedorn, S., Kaphammer, B., 1994. Microbial biocatalysis in the generation of flavor and fragrance chemicals, Annu. Rev. Microbiol. 48, 773–800.

Hall, M.B., 2009. Determination of starch, including maltooligosaccharides, in animal feeds: comparison of methods and a method recommended for AOAC collaborative study. J. AOAC Int. 92, 42-49.

Hartwig, P., and McDaniel, M.I.N.A., 1995. Flavor characteristics of lactic, malic, citric, and acetic acids at various pH levels. J. Food Sci., 60, 384-388.

Lasekan O.O, Lasekan W.O, Idowu M.A, 1997. Flavor volatiles of “malt beverage” from roasted Sorghum. Food Chem., 58, 341-344.

Liu, S.Q., Holland, R., Crow, V.L., 2004. Esters and their biosyn-thesis in fermented dairy products: A review, Int. Dairy J., 14, 923–945.

Longo, M.A., and Sanromán, M.A, 2006. Production of Food Aroma Compounds, Food Technol. Biotechnol., 44, 335–353.

Marsh, A.J., Hill, C., Ross, R.P., Cotter, P.D., 2014. Fermented beverages with health-promoting potential: Past and future perspectives. Trends Food Sci. Technol. 38, 113–124. doi:10.1016/j.tifs.2014.05.002

Menezes, A.G.T., Batista, N.N., Ramos, C.L., de Andrade e Silva, A.R., Efraim, P., Pinheiro, A.C.M., Schwan, R.F., 2016. Investigation of chocolate produced from four different Brazilian varieties of cocoa (Theobroma cacao L.) inoculated with *Saccharomyces cerevisiae*. Food Res. Int. 81, 83–90. doi:10.1016/j.foodres.2015.12.036

Miguel, M.G. da C.P., Santos, M.R.R.M., Duarte, W.F., de Almeida, E.G., Schwan, R.F., 2012. Physico-chemical and microbiological characterization of corn and rice “calugi” produced by Brazilian Amerindian people. Food Res. Int. 49, 524–532. doi:10.1016/j.foodres.2012.08.012

Mishra Pandey, S., Mishra, H.N., 2015. Optimization of the prebiotic & probiotic concentration and incubation temperature for the preparation of synbiotic soy yoghurt using response surface methodology. LWT - Food Sci. Technol. 62, 458–467. doi:10.1016/j.lwt.2014.12.003

Mishra, S., and Mishra, H. N., 2013. Effect of synbiotic interaction of fructooligosaccharide and probiotics on the acidification profile, textural and rheological characteristics of fermented soy milk. Food and Bioprocess Technol., 6, 3166-3176.Nelson, N., 1944. A fotometric adaptaion of Somogyi method for the determination of glucose. J. Biol. Chem. 153, 375-80.

Moreno-Arribas, M.V., and Polo, M.C., 2008. Wine Chemistry and Biochemistry. Springer Science+Business Media, New York, New York.

Nionelli, L., Coda, R., Curiel, J.A., Poutanen, K., Gobbetti, M., Rizzello, C.G., 2014. Manufacture and characterization of a yogurt-like beverage made with oat flakes fermented by selected lactic acid bacteria. Int J Food Microbiol, 185, 17–26.

Oliveira, S. P. R., Florence, R. C. A., Perego, P., Oliveira, N. M., & Converti, A., 2011. Use of lactulose as prebiotic and its influence on the growth, acidification profile and viable counts of different probiotics in fermented skim milk. Int. J. Food Microbiol., 145, 22-27.

Park, S. N., Lim, Y. K., Freire, M. O., Cho, E., Jin, D., Kook, J. K., 2012. Antimicrobial effect of linalool and a-terpineol against periodontopathic and cariogenic bacteria. Anaerobe, 18, 369-372.

Paterson, A., and Piggott, J. R., 2006. Flavour in sourdough breads: a review. Trends Food Sci. Technol., 17, 557-566.

Prado, F.C., Parada, J.L., Pandey, A., Soccol, C.R., 2008. Trends in non-dairy probiotic beverages. Food. Res. Int., 41, 111–23.

Ramos, C.L., de Almeida, E.G., Freire, A.L., Freitas Schwan, R., 2011. Diversity of bacteria and yeast in the naturally fermented cotton seed and rice beverage produced by Brazilian Amerindians. Food Microbiol. 28, 1380–1386.

Ramos, C.L., Sousa, E.S.O. de, Ribeiro, J., Almeida, T.M.M., Santos, C.C.A. do A., Abegg, M.A., Schwan, R.F., 2015. Microbiological and chemical characteristics of tarubá, an indigenous beverage produced from solid cassava fermentation. Food Microbiol. 49, 182–188. doi:10.1016/j.fm.2015.02.005

Ray, R. C., and Montet D., 2015. Microorganisms and fermentation of traditional foods. CRC Press, 2014.

Rivera-Espinoza, Y., Gallardo-Navarro, Y., 2010. Non-dairy probiotic products. Food Microbiol. 27, 1–11. doi:10.1016/j.fm.2008.06.008

Roberfroid, M. B., 2005. Introducing inulin-type fructans. The British Journal of Nutrition, 93, 13-25. Roger, T., Léopold, T.N., Carl, M., Funtong, M., 2015. Nutritional Properties and Antinutritional Factors of Corn Paste (Kutukutu) Fermented by Different Strains of Lactic Acid Bacteria.

Roger, T., Léopold, T.N., Carl, M., Funtong, M., 2015. Nutritional Properties and Antinutritional Factors of Corn Paste (Kutukutu) Fermented by Different Strains of Lactic Acid Bacteria 2015.

Rolim, P.M., 2015. Development of prebiotic food products and health benefits. Food Sci. Technol. 35, 3–10. doi:10.1590/1678-457X.6546

Salmerón, I., Thomas, K., Pandiella, S.S., 2014. Effect of substrate composition and inoculum on the fermentation kinetics and flavour compound profiles of potentially non-dairy probiotic formulations. LWT - Food Sci. Technol. 55, 240–247. doi:10.1016/j.lwt.2013.07.008

Santos, C.C.A. do A., Libeck, B. da S., Schwan, R.F., 2014. Co-culture fermentation of peanut-soy milk for the development of a novel functional beverage. Int. J. Food Microbiol. 186, 32–41. doi:10.1016/j.ijfoodmicro.2014.06.011

Sanz Y and Dalmau J., 2008. Los probió ticos en el marco de la nueva normativa europea que regula los alimentos fun cionales. Acta Pediátrica Espanõla 66, 27–31.

Soccol, C.R., De Dea Lindner, J., Yamaguishi, C.T., Spier, M.R., Porto de Souza Vandenberghe, L., Soccol, V.T., 2012. Probiotic nondairy beverages. In: Hui YH, editor. Handbook of plant-based fermented food and beverage technology. Florence, S.C.: Taylor & Francis Group. p. 707–28.

Stone, H., and Sidel, J. L., 1985. Sensory evaluation practices. Orlando, Florida: Academic Press.

Tamang, J. P., Samuel, D., 2010. Dietary Cultures and Antiquity of Fermented Foods and Beverages, pp. 1-40, In: Tamang, J. P., Kailasapathy, K. (Ed.), Fermented Foods and Beverages of the World, CRC Press, New York, USA.

Wakeling, I. N., and MacFie, H. J., 1995. Designing consumer trials balanced for first and higher orders of carry-over effect when only a subset of k samples from t may be tested. Food Quality and Preference, 6(4), 299-308.

Ying, S., Lasekan, O., Naidu, K.R.M., Lasekan, S., 2012. Headspace solid-phase microextraction gas chromatography-mass spectrometry and gas chromatography-olfactometry analysis of volatile compounds in pineapple breads. Molecules, 17, 13795-13812.

Zott, K., Claisse, O., Lucas, P., Coulon, J., Lonvaud-Funel, A., Masneuf-Pomarede, I., 2010. Characterization of the yeast ecosystem in grape must and wine using real-time PCR. Food Microbiol. 27, 559–567. doi:10.1016/j.fm.2010.01.006