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ELVIRA DE LOURDES CHAVES MACÊDO

POTENCIAL BIOTECNOLÓGICO DE LEVEDURAS ISOLADAS DE FRUTAS DA CAATINGA FERMENTADAS

João Pessoa
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Tese apresentada ao Programa de Pós-Graduação em Ciências da Nutrição do Centro de Ciências da Saúde da Universidade Federal da Paraíba em cumprimento ao requisito para obtenção do título de Doutor em Ciências da Nutrição.

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RESUMO

Frutas são fontes de leveduras com propriedades biotecnológicas nas quais pesquisas têm se focado visando as características que podem agregar aos produtos fermentados. Diversas frutas abundantemente produzidas no Bioma Caatinga têm sido exploradas como fonte de compostos bioativos, entretanto têm sido escassamente exploradas como fonte de leveduras com potencial biotecnológico, ou mesmo como matrizes para o desenvolvimento de formulações derivadas da fermentação por leveduras. O presente trabalho teve como objetivo a avaliação do potencial biotecnológico de leveduras isoladas a partir graviola e umbu-cajá naturalmente fermentadas. Leveduras foram isoladas a partir de graviola e umbu-cajá fermentadas naturalmente. Os isolados foram identificados via MALDI-TOF e as leveduras *Hanseniaspora opuntiae* 125, *Issatchenkia terricola* 129 e *Hanseniaspora opuntiae* 148 foram selecionadas para fermentação de polpas de graviola e umbu-cajá com base em seu *score* de identificação e histórico de aplicação em alimentos. As polpas fermentadas foram caracterizadas quanto a aspectos físico-químicos (pH, sólidos solúveis e acidez titulável), perfil de açúcares, ácidos orgânicos, compostos voláteis e fenólicos (perfil e bioacessibilidade). As polpas sofreram mudanças na composição quando submetidas à fermentação pelas leveduras isoladas e as cepas *H. opuntiae* 125 e *I. terricola* 129 mantiveram a viabilidade na amostra de graviola mesmo após condições gastrointestinais simuladas (≥ 6 log UFC/g). A fermentação das polpas de frutas com as leveduras influenciou na quantidade e bioacessibilidade dos fenólicos evidenciando a ocorrência de biotransformação destes compostos. De modo geral, a fermentação com as leveduras aumentou a quantidade de compostos fenólicos bioacessíveis, mas o aumento variou com a cepa e fruta avaliada. A cepa *I. terricola* 129 foi escolhida para testes *in vitro* de modulação da microbiota intestinal de adultos de meia-idade hipertensos devido aos efeitos produzidos nas polpas durante a fermentação e por sua baixa produção de etanol. A abundância relativa dos grupos bacterianos foi avaliada por meio de fluorescência de hibridização *in situ* acoplada a citometria de fluxo multiparamétrica durante fermentação colônica *in vitro* por 48 horas. Durante a fermentação foram mensurados valores de pH e alíquotas foram retiradas para análises de açúcares, ácidos orgânicos e compostos fenólicos. A polpa de graviola fermentada com *I. terricola* 129 melhorou a microbiota por aumentar a abundância relativa de *Lactobacillus* spp. e *Bifidobacterium* spp. e reduzir a abundância relativa de *Eubacterium rectale/Clostridium coccoides* e *Clostridium histolyticum*, resultando no maior índice prebiótico entre os produtos testados. Ao mesmo tempo, observou-se o consumo de ramnose e ácido gálico e maiores teores de ácidos acético e propiônico e procianidina B2. Os resultados evidenciam as alterações decorrentes da fermentação de polpas de frutas da Caatinga com leveduras isoladas de frutas e indicam potencial benefícios da polpa fermentada de graviola com *I. terricola* 129 na microbiota colônica de adultos hipertensos de meia-idade.

Palavras-chave: compostos fenólicos, bioacessibilidade, microbiota intestinal, *Annona muricata* L., *Spondias* spp.

ABSTRACT

Fruits are sources of yeast with biotechnological properties on which research has focused on the characteristics they can add to fermented products. Several fruits abundantly produced in the Caatinga Biome have been explored as a source of bioactive compounds, however they have been scarcely explored as a source of yeast with biotechnological potential, or even as matrices for the development of formulations derived from yeast fermentation. The present work aimed to evaluate the biotechnological potential of yeasts isolated from naturally fermented soursop and umbu-cajá. Yeasts were isolated from naturally fermented soursop and umbu-cajá. The isolates were identified via MALDI-TOF and the yeasts *Hanseniaspora opuntiae* 125, *Issatchenkia terricola* 129 and *Hanseniaspora opuntiae* 148 were selected for fermentation of soursop and umbu-cajá pulps based on their identification score and history of application in food. The fermented pulps were characterized regarding physical-chemical aspects (pH, soluble solids, and titratable acidity), sugar profile, organic acids, volatile and phenolic compounds (profile and bioaccessibility). The pulps showed changes in composition when subjected to fermentation by the isolated yeasts and the strains *H. opuntiae* 125 and *I. terricola* 129 maintained viability in the soursop sample even after simulated gastrointestinal conditions (≥ 6 log CFU/g). The fermentation of fruit pulps with yeast influenced the quantity and bioaccessibility of phenolics, demonstrating the occurrence of biotransformation of these compounds. In general, fermentation with yeast increased the amount of bioaccessible phenolic compounds, but the increase varied depending on the strain and fruit evaluated. The *I. terricola* 129 strain was chosen for *in vitro* tests to modulate the intestinal microbiota of hypertensive middle-aged adults due to the produced effects on the pulp during fermentation and its low ethanol production. The relative abundance of bacterial groups was evaluated using fluorescence *in situ* hybridization coupled to multiparametric flow cytometry during *in vitro* colonic fermentation for 48 hours. During fermentation, pH values were measured, and aliquots were taken for analysis of sugars, organic acids, and phenolic compounds. Soursop pulp fermented with *I. terricola* 129 improved the microbiota by increasing the relative abundance of *Lactobacillus* spp. and *Bifidobacterium* spp. and reduce the relative abundance of *Eubacterium rectale/Clostridium coccoides* and *Clostridium histolyticum*, resulting in the highest prebiotic index among the products tested. At the same time, the consumption of rhamnose and gallic acid and higher levels of acetic and propionic acids and procyanidin B2 were observed. The results highlight the changes resulting from the fermentation of fruit pulps from the Caatinga with yeasts isolated from fruits and indicate potential benefits of fermented soursop pulp with *I. terricola* 129 on the colonic microbiota of middle-aged hypertensive adults.

Keywors: phenolics, bioaccessibility, intestinal microbiota, *Annona muricata* L., *Spondias spp*.

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LISTA DE ABREVIATURAS E SIGLAS

Bac 303	*Bacteroides* spp./*Prevotella* spp.
BAL	Bactéria lática
Bif 164	*Bifidobacterium* spp.
CFM	Citometria de Fluxo Multiparamétrica
Chis 150	*Clostridium histolyticum*
CLAE	Cromatografia Líquida de Alta Eficiência
ECA	Enzima conversora de angiotensina I
Erec 482	*Eubacterium rectale*/*Clostridium coccoides*
FISH	Fluorescence *in situ* hybridization
Lab 158	*Lactobacillus* spp./*Enterococcus* spp.
MRS	de Man, Rogosa e Sharpe
SCFAs	*Short-chain fatty acids*
TGI	Trato gastrointestinal
YPD	*Yeast Peptone Dextrose*

SUMÁRIO

1 INTRODUÇÃO

Embora o Brasil possua ampla variedade de frutas em sua mata nativa, a maior parte das frutas que são comercializadas localmente tem origem europeia, africana ou asiática. O Bioma Caatinga, que se encontra situado na Região Nordeste do Brasil, possui uma grande variedade de frutas que são pouco estudadas e continuam desconhecidas pela maioria dos brasileiros.

Diversas frutas nativas ou cultivadas na Caatinga são consumidas apenas localmente e permanecem pouco exploradas industrialmente devido a elevada perecibilidade, porém, têm chamado a atenção da comunidade científica em razão da sua composição nutricional e conteúdo de compostos bioativos (ALBUQUERQUE *et al*., 2016; DUTRA *et al*., 2017).

Estudos têm sido realizados, entretanto, ainda há escassez de pesquisas relacionadas às espécies de frutas consumidas localmente, tais como, a graviola e o umbu-cajá, principalmente no que diz respeito à sua exploração como matéria-prima para aplicação biotecnológica e desenvolvimento de novos produtos.

A graviola (*Annona muricata* L.). é uma das frutas produzidas no Bioma Caatinga que tem se destacado e despertado o interesse de pesquisadores e consumidores devido ao seu valor nutricional. Pertencente à família *Annonaceae* a espécie é originária do Caribe e nativa dos trópicos americanos, apresentando-se como uma árvore frutífera cujo fruto é apreciado por sua polpa levemente ácida, aromática e suculenta (CEBALLOS; GIRALDO; ORREGO, 2012), além de sua rica composição em compostos bioativos tais como polifenóis, e elevado conteúdo de fibra alimentar e carboidratos (SIQUEIRA *et al*., 2015; AGU e OKOLIE, 2017; CHANG *et al*., 2018). Devido à sua composição possui propriedades antioxidantes, anti-inflamatórias e hipoglicemiantes, sendo seus compostos bioativos prevalentes as acetogeninas, os alcaloides e compostos fenólicos tais como os derivados dos ácidos cinâmico e *p*-cumárico que podem contribuir para os efeitos benéficos da graviola na saúde (JIMÉNEZ *et al*., 2014; CORIA-TÉLLEZ *et al*., 2016).

Dentre as frutas nativas abundantes na Caatinga, o umbu-cajá (*Spondias* spp.) também possui elevada concentração de compostos fenólicos (ZERAIK *et al*., 2016). A espécie pertence à família *Anacardiaceae* e caracteriza-se como um híbrido natural entre umbu (*Spondias tuberosa)* e o cajá (*Spondias monbin)* (GONDIM *et al*., 2013), sendo ainda apontada como híbrida entre as espécies *Spondias tuberosa* e *Spondias dulcis* (cajá-manga) (NOBRE *et al*., 2018).

Obtidos principalmente de forma extrativista, os frutos da umbu-cajazeira são encontrados em plantios desorganizados, distribuídos pela região Nordeste, sendo comercializados por pequenos produtores e utilizados principalmente na forma processada como polpas, sucos, néctares e sorvetes (LIMA *et al*., 2002; GONDIM *et al*., 2013; SANTOS *et al*., 2021). Devido ao seu teor de polifenóis totais, o consumo de seus frutos pode contribuir para o aporte de antioxidantes na dieta (MOREIRA *et al*., 2012; DUTRA *et al*., 2017).

Assim, devido às propriedades sensoriais atrativas e o aumento do reconhecimento do valor nutricional e terapêutico das frutas tropicais e exóticas, sua procura tem se elevado nos mercados nacional e internacional (RUFINO *et al*., 2010; ALBUQUERQUE *et al*., 2016). Além disso, as frutas têm sido amplamente recomendadas para a prevenção de doenças cardiovasculares tais como a hipertensão, diabetes *mellitus* tipo 2 e alguns tipos de câncer (LIMA *et al.*, 2018), sendo que determinados fenólicos presentes nos alimentos têm se mostrado promissores devido a seus efeitos hipotensores, especialmente daqueles com capacidade de inibir a enzima conversora de angiotensina I (ECA) (ALU'DATT *et al*., 2019).

Algumas frutas da Caatinga têm sido especialmente exploradas no que diz respeito aos compostos bioativos, entretanto têm sido escassamente exploradas como fonte de leveduras com potencial biotecnológico, ou mesmo como matrizes para o desenvolvimento de formulações derivadas da fermentação por leveduras.

Diferentes espécies de leveduras com capacidade metabólica distintas estão presentes na microbiota autóctone de frutas estando envolvidas na fermentação espontânea destes substratos. Leveduras dos gêneros *Aureobasidium*, *Candida*, *Meyerozyma* e *Saccharomyces* já foram isoladas de abacaxi (AMORIM; PICCOLI; DUARTE, 2018), de azeitonas pretas (BONATSOU; PARAMITHIOTIS; PANAGOU, 2018) e diversas outras matrizes alimentares têm sido exploradas na busca por novas cepas.

Por serem normalmente resistentes a antibióticos e não transferirem esses genes a outros microrganismos, as leveduras podem trazer vantagens nos processos fermentativos em relação às bactérias láticas (RAI; PANDEY; SAHOO, 2019). Elas também podem levar a melhora da saúde e bem-estar do hospedeiro devido a sua capacidade de interagir com a matriz e produzir substâncias antioxidantes, tais como, carotenoides, ácidos cítrico e ascórbico, tocoferóis, entre outras que são capazes de combater o estresse oxidativo (ARROYO-LÓPEZ *et al*., 2012).

Nesse contexto, o conhecimento sobre os microrganismos envolvidos nos processos de fermentação natural é de grande importância, pois essas culturas ao serem isoladas podem ser aplicadas em processos de obtenção de produtos funcionais, tendo em vista os benefícios que podem ser obtidos por meio de seu metabolismo fermentativo (RESENDE *et al*., 2018). Ademais, a dinâmica populacional e os compostos responsáveis pelo sabor e aroma desses alimentos fermentados podem ser mais bem compreendidos quando se identificam os compostos produzidos durante esses processos (PUERARI; MAGALHAES-GUEDES; SCHWAN, 2015).

Desse modo, o presente trabalho teve como objetivos: i) o isolamento de leveduras a partir dos frutos graviola e umbu-cajá naturalmente fermentados; ii) a avaliação do potencial biotecnológico de cepas selecionadas para a produção de polpas fermentadas; iii) a caracterização das polpas fermentadas obtidas quanto aos aspectos físico-químicos (pH, sólidos solúveis e acidez titulável), perfil de açúcares, ácidos orgânicos, compostos voláteis e compostos fenólicos (perfil e bioacessibilidade); e a avaliação *in vitro* dos efeitos das polpas de frutas fermentadas na modulação da microbiota intestinal humana de adultos hipertensos de meia-idade.

2 REFERENCIAL TEÓRICO

2.1 Revisão de literatura

A abordagem inicial da Tese foi embasada na bibliografia existente e se refere à utilização de frutas naturalmente fermentadas como fontes de leveduras e bactérias láticas com potencial para aplicação biotecnológica na formulação de produtos alimentícios. Os estudos apontam direcionamentos sobre a exploração de microrganismos de valor agregado, isolados a partir de fontes não convencionais, o que foi compilado e descrito na revisão de literatura publicada no periódico *Trends in Food Science & Technology*.

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Understanding the potential of fruits, flowers, and ethnic beverages as valuable sources of techno-functional and probiotics strains: Current scenario and main challenges

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ABSTRACT

Background: Fruit, vegetables, flowers, and ethnic beverages may be a source of microbial species with techno-functional and health-promoting properties.
Scope and approach: This review explored the added-value microorganisms isolated from unconventional sources and their techno-functional and probiotic properties. Fruits (strawberry, guava, apple, peach, grape, and papaya), vegetables (peppers, corn, zucchini, lettuce, cucumber, coffee beans, and olives), flowers (narcissus, pink rose, red rose, yellow rose, and sunflower), and ethnic fermented beverages (tchapalo, tarubá, cauim, chicha, caxiri, kombucha, and water kefir) are source of lactic acid bacteria (*Lactobacillus* and amended genera, *Leuconostoc, Enterococcus, Pediococcus, Fructobacillus,* and *Weissella*) and yeasts (*Saccharomyces, Candida, Pichia, Rhodotorula, Torulaspora, Cryptococcus, Hansenula,* and *Debaromyces*).
Key findings and conclusions: Strains isolated from unconventional sources showed antimicrobial capacity, production of bioactive metabolites, and technological properties, suggesting their utilization as biopreservatives in food products or against phytopathogens, and for improving the nutritional value and sensory characteristics of food products. Their utilization as starter cultures in fermented foods may decrease the fermentation time and improve the products' characteristics. Some strains showed probiotic potential, presenting important adhesion and auto and co-aggregation properties, cell surface hydrophobicity, safety, and resistance to the gastrointestinal tract. These probiotic cultures showed anti-hypertensive, antilipidemic, immunomodulatory, and anti-diabetic properties in *in vitro* assessments or animal models. However, clinical studies are necessary to demonstrate the health effects in humans. In conclusion, cultures isolated from unconventional sources have a high potential for use in processing and functionalization of foods and can be alternative tools for developing vegan probiotic products.

1. Introduction

More attention has been devoted to fruit, raw vegetables, and flowers as source of microbial species with techno-functional and health-promoting properties in the last decade. Besides, naturally fermented foods have been explored as niches for the isolation of bacteria and yeast strains with potential for biotechnological application and/or functionalization of foods (Menezes et al., 2020; Pei et al., 2020; Semjonovs et al., 2017).

The microbial composition of fruits, raw vegetables, and naturally fermented beverages depends on the intrinsic (carbohydrate and protein contents and pH values) and extrinsic (climatic conditions and harvesting) parameters (Fessard & Remize, 2019; Garcia et al., 2016). Furthermore, it may be associated with pollinators that visit flowers and fruits, birds fed with them, or insects (Rodríguez et al., 2019). Some of these strains can antagonize other microorganisms and show

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biotechnological applications (Costa et al., 2018; Fessard et al., 2017; Linares-Morales et al., 2020), while others can present important health effects associated with their probiotic properties, such as microbiota modulation (Costa et al., 2019), hypocholesterolemic, anti-hypertensive, and hypoglicemic properties (Verón et al., 2019), and immunomodulatory effects (Fakruddin et al., 2017).

In general, the microbial population of fruits and raw vegetables is around 10^5 - 10^7 cfu/g. Yeasts are the dominant group (10^2–10^6 CFU/g), and *Saccharomyces, Candida, Pichia, Rhodotorula, Torulaspora, Cryptococcus, Hansenula,* and *Debaromyces* are the most commonly found genera (Barros et al., 2019; Di Cagno et al., 2013; Leff & Fierer, 2013; Martins et al., 2019; Riesute et al., 2021). Otherwise, Lactic Acid Bacteria (LAB) comprises between 10^2 - 10^4 cfu/g (Di Cagno et al., 2013; Leff & Fierer, 2013), and the most found genera are *Lactobacillus, Leuconostoc, Enterococcus, Pediococcus, Fructobacillus,* and *Weissella* (Fessard et al., 2017; Rodríguez et al., 2019).

Yeasts have stood out for their favorable performance in biotechnological applications, such as fermentation and metabolite production (Karim et al., 2020; Semjonovs et al., 2017; Taheur et al., 2020). In addition, recent studies have demonstrated the probiotic potential of several yeast strains isolated from fruits and vegetables (Hsiung et al., 2020; Menezes et al., 2020). At the same time, LAB can have probiotic properties and can produce organic acids (mainly lactic acid) and other compounds contributing to increase the shelf life of food products and improving their physicochemical and sensory characteristics (Costa et al., 2018; Di Cagno et al., 2016; Oliveira, Araújo, et al., 2020). Various LAB strains have been isolated from fruit and vegetables, such as *Lactiplantibacillus plantarum CM-3* (former *Lactobacillus plantarum* CM-3) from strawberry (*Fragaria* × *ananassa* Duch. Cv. "Hongyan") (Chen et al., 2020), *Leuconostoc mesenteroides, Enterococcus mundtii* and *Enterococcus faecium* from fruits (guava, green apple, and orange) and vegetables (corn and chilaca and jalapeno peppers) (Linares-Morales et al., 2020), *Weissella paramesenteroides* FX1, FX2, FX5, FX9, FX12 from sapota, cherry, banana, orange, and plum smashed fruits (Pabari et al., 2020), *L. plantarum* 49, *Lacticaseibacillus paracasei* 108 (former *Lactobacillus. paracasei* 108) and *L. plantarum* 201 from fruits (mango and acerola) and fruit by-products (mango, acerola, and soursop) (Garcia et al., 2016), *L. mesenteroides* (OP4, OP9, OP21, OP18 and OP23) from fresh fruits of *Opuntia ficus-indica* (L.) Mill. (Sanguigna genotype) (Di Cagno et al., 2016), and *L. plantarum* LPBR01 from coffee fruits (Pereira et al., 2016).

Flowers have been attracting attention due to the presence of bacteria of the genus *Fructobacillus* that comprise species with fructophilic characteristics, i.e., preference for D-fructose over D-glucose, producing mannitol and acetate instead of ethanol (Behare et al., 2020; Maeno et al., 2019; Patil et al., 2020; Sakandar et al., 2019). Mannitol is a low-calorie polyol widely used in the food industry, suggesting that these strains can serve as tools for biotechnological application (Behare et al., 2020; Maeno et al., 2019). Recent studies have isolated fructophilic strains from various flowers and fruits such as *F. fructosus* MCC 3996 from nectar of *Butea monosperma* flower (Patil et al., 2020), and *F. pseudoficulneus* JNGBKS, JNGBKS3, *F. fructosus* JNGBKS2, JNGBKS4, and *F. durionis* JNGBKS5 from fresh fruits (apple, banana, Chinese peach, plum, cantaloupe, kiwi fruit, and lychee) and flowers (narcissus, pink rose, red rose, yellow rose, and sunflower) (Sakandar et al., 2019).

Traditional fermented foods obtained from spontaneous fermentation, are exploited as rich microbial niches, including fermented cereal-based beverages such as rice, corn, wheat, rye, cassava, or sorghum. Among these beverages stand out *tchapalo*, *tarubá*, *cauim*, *chicha*, *caxiri*, and fermented drinks based on teas and fruit juices such as *kombucha* and water *kefir*, which are produced by *back-slopping* from a consortium of bacteria and yeasts (Aka et al., 2020; Freire et al., 2017; Grijalva-Vallejos et al., 2020; Pei et al., 2020; Ramos et al., 2015; Taheur et al., 2020). In naturally fermented beverages, the main microbial groups comprise LAB, yeasts, and acetic acid bacteria, simultaneously found or as predominant microbial groups during the fermentative process (Grijalva-Vallejos et al., 2020; Ramos et al., 2015). Several strains of yeasts and LAB with techno-functional properties have been isolated from ethnic beverages over the last years, such as *Limosilactobacillus fermentum* S6 (former *Lactobacillus fermentum* S6) and *Pediococcus acidilactici* S7 from *tchapalo* (Aka et al., 2020), *S. cerevisiae* EYS5 and *Torulaspora delbrueckii* EGT1 from *chicha* (Grijalva-Vallejos et al., 2020), *L. plantarum* SLG10, *P. occidentalis, Candida sorboxylosa, Hanseniaspora opuntiae*, *Komagataeibacter rhaeticus* P 1463, and *K. hansenii* B22 from *kombucha* (Pei et al., 2020; Semjonovs et al., 2017; Taheur et al., 2020), *L. plantarum* CCMA0743 and *T. delbrueckii* CCMA0235 from *cauim* (Freire et al., 2017), and *L. plantarum*, *Levilactobacillus brevis* (former *Lactobacillus brevis*), *L. mesenteroides, Bacillus subtilis*, and *T. delbrueckii* from *tarubá* (Ramos et al., 2015).

Previous reviews have mainly explored the isolation of autochthonous microorganisms from vegetables, fruits, or fermented foods and beverages and their use as starters, without evidencing the production of metabolites, application of these cultures as biopreservatives or in the inhibition of phytopathogens (Pessôa et al., 2019; Torres et al., 2020; Pérez-Armendáriz & Cardoso-Ugarte, 2020). Furthermore, the *in vitro* and *in vivo* probiotic effects of these isolated strains have not been extensively reported. None of the reviews discussed the flower microbiota as a source of added-value species. Therefore, this review aimed to explore the added-value microorganisms isolated from unconventional sources (fruit, raw vegetables, ethnic fermented beverages, and flowers) and their associated techno-functional and potentially probiotic properties. The main challenges in characterizing new probiotic strains and functionalizing foods with these new strains are discussed. A focused discussion of the main reported effects of these strains as biotechnological tools and the known *in vitro/in vivo* health-promoting effects is provided. Furthermore, the antagonistic effects, the active microbial metabolites, and the effects on products' technological and sensory aspects are presented. Finally, the field's future perspectives close this review, making available information to help researchers in further studies.

2. Probiotic potential of strains isolated from unconventional sources

2.1. Selection of probiotic cultures from unconventional sources

Probiotics are viable microorganisms that confer health effects to the individuals when consumed in adequate amounts (Hill et al., 2014). The strains mostly used as probiotics are LAB, represented by species of *Lactobacillus* (*L. acidophilus*), *Lactiplantibacillus* (*L. plantarum*), *Ligilactobacillus* (*L. salivarius*), *Lacticaseibacillus* (*L. rhamnosus* and *L. casei*), and *Limosilactobacillus* (*L. reuteri* and *L. fermentum*). Furthermore, strains of the *Bifidobacterium* genus (*B. longum, B. animalis, B. bifidum, B. lactis, B. breve,* and *B. infantis*) and yeasts (*Saccharomyces cerevisiae*) are commonly used (Pimentel et al., 2021).

Dairy products are the main probiotic products available in the market. However, there is a demand for the development of non-dairy probiotic products based on cereals, fruits, and vegetables because of lifestyle choices (e.g., vegetarianism and veganism) and the increased rates of milk allergy and lactose intolerance in the population (Vera-Pingitore et al., 2016). However, most commercial probiotic strains are derived from human gastrointestinal tract or dairy products (Pimentel et al., 2021). Probiotic cultures used in plant-based fermented products should preferentially be isolated from plant materials because plant-derived substrates present significant technological and physiological challenges to which strains of the intestinal origin or dairy products might not be adapted (Gupta & Abu-Ghannam, 2012). Fig. 1 presents the main probiotic properties observed for microorganisms isolated from fruit, vegetables, and ethnic fermented beverages.

Table 1 presents the main studies with potentially probiotic strains isolated from unconventional sources and their main functional features. The selection of probiotic strains considers several aspects,

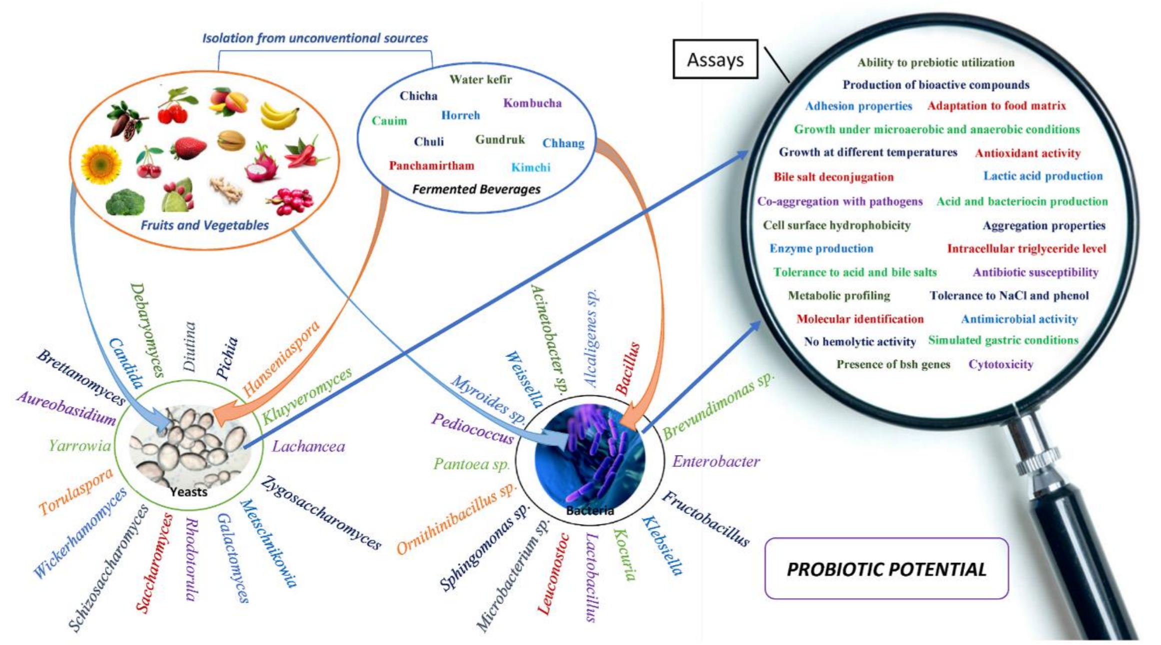

Fig. 1. Main genera of yeast and bacterial isolates and assays for probiotic potential.

including safety for the consumer (antibiotic susceptibility, hemolytic activity, and mucin degradation), and physiological functionalities (acid and bile salt tolerance, bile salt deconjugation, cell surface hydrophobicity, auto-aggregation, co-aggregation with pathogens, antagonistic activity against pathogens, and capability of surviving during exposure to gastrointestinal conditions). Furthermore, the technological aspects (proteolytic and lipolytic activity, tolerance to NaCl, and EPS and bioactive compounds production) may also be considered (Albuquerque et al., 2018; Torres et al., 2020). Thus, the potentially probiotic strain should consider all these aspects.

The growth temperature is a primary characteristic for selecting probiotic strains since they must remain viable in the temperature found in the host. Di Cagno et al. (2020) evaluated ten yeast strains belonging to different species and previously isolated from plant matrices. Only *Saccharomyces cerevisiae* DDNd10, *Pichia kudriavzevii* DCNa1, and *Wickerhamomyces subpelliculosus* DFNb6 were able to propagate at the temperature of 37 °C, which is the common temperature found in the human body. Other previous studies also reported isolates from fruits and vegetables with growth at 37 °C, which may be a differential for human applications (Fakruddin et al., 2017; Oliveira et al., 2017). The results suggest that it is important to evaluate the resistance of probiotic cultures of vegetal origin to the target host's temperatures, as the adaptability to the host temperature may improve its survival and health-related aspects (Gil-Rodríguez et al., 2015).

High viability and survival to adverse conditions are required for probiotics since the microbial population, and its metabolism directly relates to the effects on the environment or the host. Therefore, probiotic cultures should survive the food environment (Fiocco et al., 2020). Furthermore, the ability to survive in the upper digestive tract and reach the large intestine demands tolerance to acidity and bile. Thus, the microorganism's survival to simulated gastrointestinal conditions is predictive of its survival in the gastrointestinal tract (Xu et al., 2020). Studies have shown that microorganisms isolated from fruits, vegetables, and flowers were able to withstand the simulated gastrointestinal conditions, such as *W. paramesenteroides* isolated from citrus fruits (Pabari et al., 2020), *Saccharomyces cerevisiae* IFST062013 isolated from fruit (unspecified) (Fakruddin et al., 2017), *L. fermentum* 296 isolated from fruit by-products (Albuquerque et al., 2018), *W. koreensis* FK121 and *Lactobacillus crispatus* G19 isolated from south Indian fermented koozh and guerkins (fermented cucumber), *L. pentosus* 129, *L. brevis* 59, and *L. fermentum* 111 isolated from fruit by-products (Garcia et al., 2016), *L. buchneri* SS50.4, *L. plantarum* SBR64.7, *L. fermentum* SS50.10 isolated from silage, artisanal salami, and cocoa beans (Leandro et al., 2020), and *F. fructosus* MCC 3996 isolated from nectar of flowers (*B. monosperma*) (Patil et al., 2020). The results suggest that acid and bile salts tolerance are, generally, strain-specific and some strains isolated from fruits and fruits by-products may not withstand the harsh condition of the gastrointestinal tract (Albuquerque et al., 2018; Garcia et al., 2016). Furthermore, it seems that strains isolated from sources with high acidity may better tolerate the stomach's acidic conditions (Cao et al., 2019).

Co-aggregation and auto-aggregation are important probiotic characteristics, as they allow the entrapment of bacteria in an aggregated form, increasing the stability of microbial strains in the gastrointestinal tract (Oliveira et al., 2017). Studies have described important auto-aggregation performances among yeasts (Bonatsou et al., 2018; Di Cagno et al., 2020; Hsiung et al., 2020; Menezes et al., 2020) and bacterial strains (Fernández-Pacheco et al., 2020; Leandro et al., 2020; Patil et al., 2020) isolated from fruits, flowers, and traditional fermented foods. Albuquerque et al. (2018) reported that LAB isolated from fruit by-products (*L. plantarum* 53, *L. fermentum* 60 and 296, and *L. paracasei* 106) showed high auto-aggregation capacity, resulting in a higher barrier effect, preventing the colonization by pathogens, and increasing the competition for sites with pathogens. The authors also reported important co-aggregation properties of the probiotics against pathogens (*Listeria monocytogenes* and *Escherichia coli*). The auto and co-aggregation capacities were stated as strain-specific and dependent on the pathogen and incubation time (Albuquerque et al., 2018; Anandharaj et al., 2015; Patil et al., 2020).

Gut colonization by microorganisms is usually preceded by adhesion

Table 1
Relevant studies approaching potential probiotics, isolation sources and main functional features of strain isolated from fruits, flowers, and traditional fermented foods/beverages.

Strains	Isolation source	Performed test	Findings	References
Pichia kudriavzevii DCNa1 and *Wickerhamomyces subpelliculosus* DFNb6	Different fruits	Simulated gastrointestinal tract (GIT) conditions Cell surface hydrophobicity Aggregation properties Biofilm formation Antioxidant activity Hemolytic activity Antibiotic susceptibility	The cells remain viable in high concentration, as well as after TGI simulated conditions. All strains presented high hydrophobicity and faster auto-aggregation, higher biofilm formation and antioxidant activity at different levels. None of the strains was hemolytic and both presented sensitivity to fluconazole and nystatin.	Di Cagno et al. (2020)
Thirteen yeast strains	Pistachio fruits (*Pistacia vera*)	Simulated GIT conditions Antioxidant activity Aggregation properties Cell surface hydrophobicity Biofilm formation Molecular identification	Six from thirteen strains had a high survival rate to the GIT conditions Different auto-aggregation and hydrophobicity rates. *Diutina rugosa* 14 followed by *Diutina rugosa* 8 were the best wild yeast as potential probiotic and *Hanseniaspora guilliermondii* 6 and *Aureobasidium proteae* presented notable biocontrol and antioxidant capabilities.	Fernández-Pacheco et al. (2020)
Lachancea dasiensis JYC2615MN648701, *Candida metapsilosis* JYC2616, *Rhodotorula mucilaginosa* JYC2617, *Saccharomyces cerevisiae* JYC2618, JYC2619, *Brettanomyces bruxellensis* JYC2620, JYC2621 and *S. boulardii* I-3799	Fermented food and beverages in Taiwan	Tolerance to acid and bile salts Cell surface hydrophobicity Aggregation properties Antioxidant activity Enzymatic activity Molecular identification	The strains were acid tolerant and had good auto-aggregation and cell-surface hydrophobicity. The strains grew well under the bile salt and acid conditions. The antioxidant capacities varied, and majority had a good performance regarding cell surface hydrophobicity and β-galactosidase activity.	Hsiung et al. (2020)
Weissella confusa MD1 and *Weissella cibaria* MD2	Fermented batter	Tolerance to acid and bile salts Simulated GIT conditions Tolerance to phenol Hemolytic activity Cell surface hydrophobicity Aggregation properties Antioxidant activity Adhesion properties Antibiotic susceptibility	The strains had high survivability in gastrointestinal conditions, tolerance to lysozyme and phenol, adhesion in intestinal epithelial cells (HT-29). They also showed high bile salt hydrolase activity towards sodium taurocholate and bile salt mixture. The intact cells showed strong antioxidant activity and inhibition of linoleic acid peroxidation, and biofilm formation. Both strains showed sensitivity towards conventionally used antibiotics, no hemolytic activity and exhibited strong auto-aggregation property and co-aggregation with *Listeria monocytogenes*.	Lakra et al. (2020)
Lactiplantibacillus plantarum SBR64.7 and S180.7 (former *Lactobacillus plantarum* SBR64.7 and S180.7), *Lentilactobacillus buchneri* SS50.4 (former *Lactobacillus buchneri* SS50.4), *Limosilactobacillus fermentum* SS50.10 (former *Lactobacillus fermentum* SS50.10)	Forage plants, cocoa beans fermented	Tolerance to acid and bile salts Simulated GIT conditions Cell surface hydrophobicity Antibiotic susceptibility Aggregation properties Adhesion properties Bile salt deconjugation Enzymatic activity Biogenic amine production Hemolytic activity Molecular identification	Eighty-two isolates were screened, nineteen strains genotyping. The species L. *plantarum* was prevalent. The strains presented tolerance to acid and capacity to grow in bile salts, antimicrobial activity, susceptibility to antibiotics, adhesion capacity and negative virulence factors. No biogenic amine production The strain L. *plantarum* SBR64.71 was the most promising for probiotic use.	Leandro et al. (2020)
Nineteen LAB strains	*Theobroma cacao* fermented fruit juice	Tolerance to acid and bile salts Acid and bacteriocin production Antibiotic susceptibility Molecular identification	All the tested LAB isolates showed at least 50% cells survival at pH 2 after incubation up to 3 h. They were also susceptible to antibiotics amoxicillin (30 μg), erythromycin (15 μg), chloramphenicol (30 μg) and imipenem (10 μg).	Mabeku et al. (2020)
Weissella paramesenteroides FX1, FX2, FX5, FX9, FX12	Sapota, cherry, banana, orange, and plum smashed fruits	Survival at pH 2.5 and salt 1.0% Enzymatic activity Hemolytic activity Adhesion properties Antimicrobial activity	Ability to survive acid pH and sodium taurocholate. Suitable adhesion to mucin, ability to utilize low molecular weight galactooligosaccharides (GOS) and fructooligosaccharides (FOS), organic	Pabari et al. (2020)

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Table 1 (*continued*)

Strains	Isolation source	Performed test	Findings	References
		Ability to prebiotic utilization Short-chain fatty acids (SCFAs) production Biogenic amine production Molecular identification	acids and SCFAs production. Biofilm formation, antimicrobial effect, and not harbor any virulent traits. The strains FX5 and FX9 were the best performance. No biogenic amine production	
Fructobacillus fructosus MCC 3996	Nectar of *Butea monosperma* flower	Tolerance to acid and bile salts Adhesion properties Hemolytic activity Antibiotic susceptibility	Survival rate 67–68% at pH 2 and 3 and 52 to 8% from bile, besides 81% at synthetic gastric juice. Resistance to antibiotics amoxiclav, carbenicillin, cefotaxime, ciprofloxacin, colistin, co-trimoxazole, co-trimazine, gatifloxacin, nitrofurantoin, norfloxacin, oxacillin and streptomycin. GRAS *status*.	Patil et al. (2020)
Levilactobacillus brevis B13-2 (former *Lactobacillus brevis* B13-2)	Chinese cabbage kimchi	Tolerance to acid and bile salts Simulated GIT conditions Antioxidant activity Enzymatic activity	*L. brevis* B13-2 exhibited tolerance against artificial gastric acid and bile salts and did not produce β-glucuronidase. High DPPH radical scavenging activity, Heat-	Song et al. (2020)
Latilactobacillus sakei AM2, AM8, AM32, ADM14, ADM19, YRM13, YRM20, RGM12, GP32, GP48, GN57, YG19 (former *Lactobacillus sakei* AM2, AM8, AM32, ADM14, ADM19, YRM13, YRM20, RGM12, GP32, GP48, GN57, YG19), *L. plantarum* S-2AM31 and *Leuconostoc pseudomesenteroides* S-YRWM27, S-YRWM28	Kimchi	Tolerance to acid and bile salts Intracellular triglyceride level Antibiotic susceptibility Enzymatic activity Molecular identification	Two hundred and twenty-five lactic acid bacteria were isolated, fifteen strains were tolerant to acid, and bile salts. The strains showed excellent intestinal cell adhesion, most of them did not produce β-glucuronidase. The susceptibility to ten tested antibiotics was varied between the strains. *L. sakei* ADM14 had the potential to be used as a probiotic.	Won et al. (2020)
Multiple LAB strains	Fermented broccoli, cherry, ginger, white radish, and white-fleshed pitaya juice	Tolerance to acid and bile salts Simulated GIT conditions Adhesion properties Antibiotic susceptibility Molecular identification	In broccoli and ginger juices, the genus *Lactobacillus* occupied the dominant position (79.0 and 30.3%, respectively); in cherry and radish juices, *Weissella* occupied the dominant position (78.3 and 83.2%); and in pitaya juice, *Streptococcus* and *Lactococcus* occupied the dominant positions (52.2 and 37.0%). The antibiotic resistance profile, adhesion properties and survival rates at simulated gastrointestinal transit were distinct among all the isolates indicating a high level of diversity.	Xu et al. (2020)
Sixteen strains*:* 13 *Lactiplantibacillus pentosus* (former *Lactobacillus pentosus* and) 3 *L. plantarum* Lpl15	Table olives biofilms	Tolerance to acid and bile salts Antibiotic susceptibility Lactic acid production Aggregation properties Simulated GIT conditions Presence of *bsh* genes Hemolytic activity Enzymatic activity Molecular identification	Five hundred and fifty-four lactic acid bacteria were isolated, sixteen of which had predominant genotypes and were identified. All sixteen strains showed different levels of survival to simulated GIT, resistance to pH and bile, susceptibility to antibiotics, and ability to auto and co-aggregation. β-hemolysis activity was detected for all *L. plantarum* strains, but not for any of the *L. pentosus* Most strains do not exhibit α-glucosidase.	Benítez-Cabello et al. (2019)
L. plantarum (25 different strains), *Enterococcus casseliflavus* S4b	De'ang pickled tea	Tolerance to acid and bile salts Simulated GIT conditions Antioxidant activity Adherence capacity Antibiotic susceptibility Molecular identification	Twenty-six LAB strains were isolated. Eighteen strains showed a higher tolerance to simulated GIT The strains showed adhesive abilities and were auto-aggregative dependent on species and even strains. Two L. *plantarum* strains, ST and STDA10 exhibited good probiotic properties and showed a good ability of scavenging DPPH and ABTS+. All tested LAB strains were resistant to kanamycin, streptomycin, gentamicin, and vancomycin and sensitive to tetracycline and chloramphenicol. Ten out of the eighteen strains are resistant to ampicillin, and the remaining strains are sensitive to ampicillin.	Cao et al. (2019)

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Table 1 (*continued*)

Strains	Isolation source	Performed test	Findings	References
			Four out of the eighteen strains showed resistance to erythromycin.	
L. plantarum P24-1, P24-2 P24-3, P24-4, P24-, P24-6, P24-8 and *Pediococcus acidilactici* A1-2, A2-1	Pulque and aguamiel	Simulated GIT conditions Antibiotic susceptibility Molecular identification	All the strains were susceptible to amoxicillin, amikacin, chloramphenicol, gentamicin, levofloxacin, spectinomycin, tetracycline, except to vancomycin.	Cervantes-Elizarrarás et al., (2019)
Twenty-one LAB isolates	Fresh vegetables (cauliflower, gherkins, cluster beans, fenugreek, cow pea, bitter gourd, french beans, tomato, ridged gourd, cucumber, and bottle gourd)	Tolerance to acid and bile salts Simulated GIT conditions Antioxidant activity Antibiotic susceptibility	Two hundred and sixty-six LAB were isolated, of which twenty-one had a potential probiotic. At different levels, the strains showed tolerance to bile salt, acidic pH and pancreatin. They had the ability to survive in artificial intestinal condition, presented resistance to antibiotics and showed antioxidant activity.	Junnarkar et al., (2019)
Saccharomyces cerevisiae (KU200270, KU200280, and KU200284)	Cucumber jangajji	Tolerance to acid and bile salts Adherence capacity Antibiotic susceptibility Molecular identification	High resistance to artificial gastric and bile juices High epithelial cell adhesion	Lee et al. (2019)
103 bacterial isolates most of which were *Bacillus* sp., *Enterobacter* sp., *Acinetobacter* sp., *Pantoea* sp., *Microbacterium* sp., *Klebsiella* sp., *Sphingomonas* sp.,*Ornithinibacillus* sp., *Myroides* sp., *Kocuria* sp., *Alcaligenes* sp., and *Brevundimonas* sp.	*Panchamirtham*, an Indian ethnic fermented fruit mix	Tolerance to acid and bile salts Biofilm formation Hemolytic activity Enzymatic activity Exopolysaccharides (EPSs) production Cytotoxicity Antibiotic susceptibility Molecular identification	The bacterial isolates exhibited pH, bile salt tolerance, produced biofilm and exopolysaccharide, but exhibited no hemolytic activity. The strains *E. xiangfangensis* M5S2B6, *B. safensis* M5S2B8, *E. hormaechei* subsp. *oharae* M6S1B2, *B. velezensis* M4S1B1 and *P. terrae* M7S2B1 displayed essential characteristics of potential probiotics and may be used as starter culture to produce quality panchamirtham.	Maheshwari et al. (2019)
A hundred and sixteen yeasts isolated	Brazilian indigenous fermented food, cocoa fermentation	Simulated GIT conditions Cell surface hydrophobicity Aggregation properties Antioxidant activity Phytate hydrolysis	Thirty-six strains were tolerant to gastrointestinal conditions evaluated by tolerance to pH 2.0, bile salts (0.3% w/v), and 37 °C. Fifteen isolates had a similar or higher percentage of hydrophobicity, auto-aggregation, and coaggregation with *E. coli* than the reference strain *S. boulardii.*	Menezes et al. (2019)
Fructobacillus pseudoficulneus JNGBKS, JNGBKS3, *F. fructosus* JNGBKS2, JNGBKS4, *F. durionis* JNGBKS5, *Apilactobacillus kunkeei* JNGBKS6, JNGBKS7, JNGBKS8 (former *Lactobacillus kunkeei* JNGBKS6, JNGBKS7, JNGBKS8)	Fresh fruits (apple, banana, Chinese peach, plum, cantaloupe, kiwi fruit, and lychee) flowers (narcissus, pink rose, red rose, yellow rose, and sunflower) and honey, and rose petals jam	Tolerance to acid and bile salts Simulated GIT conditions Aggregation properties Antimicrobial activity Cholesterol assimilation Antibiotic susceptibility Molecular identification	All the strain exhibited some degree of auto-aggregation, inhibition of *E. coli, S. typhimurium*, and *S. aureus* and presented resistance to ciprofloxacin, ceftriaxone, gentamicin, novobiocin, vancomycin antibiotics. *L. kunkeei*, *F. durionis* JNGBKS presented cholesterol assimilation.	Sakandar et al. (2019)
L. plantarum, Enterococcus faecalis, Lactiplantibacillus paraplantarum (former *Lactobacillus paraplantarum*), *and Weissella paramesenteroides*	Leaves of papaya, cassava, sugarcane, yam, and taro	Growth at different temperatures Tolerance to acid and bile salts Simulated GIT conditions Antibiotic susceptibility Molecular identification	Exhibited strong survival properties, tolerance to simulated intestinal juices, susceptible to streptomycin, penicillin, chloramphenicol, ampicillin, and tetracycline.	Samedi and Charles (2019)
L. pentosus CHIG, NAG1 *L. fermentum* PRS1	Fermented foods, fruits, and vegetables	Tolerance to acid and bile salts Simulated GIT conditions Tolerance to NaCl and phenol Adhesion properties Gamma amino butyric acid (GABA) production Antibiotic susceptibility Biogenic amine production Enzymatic activity	Twenty-eight *Lactobacillus* strains were isolated; six strains were selected to evaluate. The strains survive and grow in simulated oro-gastrointestinal tract, showed adhesion to mucin, biofilm formation, bile salt hydrolase and GABA production, also had β-galactosidase activities. The strains do not degrade mucin, do not produce DNAse, and are non-hemolytic activity.	Shekh et al. (2020)

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Table 1 (*continued*)

Strains	Isolation source	Performed test	Findings	References
LAB Strains: RG2A, RG3A, RG3B, RG6A, RG6B, RG6C, RG7A, RG8B, C9C, C11A, C11C, C11D, *Pediococcus pentosaceus* RG4A, RG7B, RG8A, RG8C and C11C, *L. plantarum* RG8A	Cardinal and Red Globe grape fruits	Mucin degradation Antimicrobial activity Tolerance to acid and bile salts Simulated GIT conditions Cell surface hydrophobicity Molecular identification	Eighteen isolates. Six strains had an identification of species. Strains showed probiotic potential and *P. pentosaceus* RG7B was more promising.	Taroub et al. (2019)
Fifty yeasts strains	Pineapple (*Ananas comosus* (L.) *Merril*) peel, fresh juice, and spontaneous fermentation of pineapple	Tolerance to acid and bile salts Simulated GIT conditions Aggregation properties Hydrophobicity properties Antibiotic susceptibility Molecular identification	A hundred and fifty yeasts were isolated. Fifty of which were evaluated, thirteen strains were not able to grow at pH 2.0 but maintained high populations (>90%). Five strains resisted pepsin e four resisted to bile salt. All isolates showed high level of auto-aggregation and five of them, had a hydrophobicity property and resistance to ampicillin, chloramphenicol, erythromycin, G penicillin, streptomycin, and tetracycline.	Amorin, Piccoli & Duarte, (2018)
Forty-nine yeast strains	Kalamata table olive fermentation	Simulated GIT conditions Aggregation properties Hemolytic activity	Forty-two out of the forty-nine yeast strains presented a survival rate higher than 50%, and 24 strains showed survival higher than 70% at the end of the digestions. The majority strains showed hydrophobicity higher than 75%, while the auto-aggregation ability ranged between 72 and 91%. None of the strains showed hemolytic activity.	Bonatsou et al. (2018)
L. fermentum 56, 60, 250, 263, 139, 141 and 296, *L. plantarum* 53, *Lacticaseibacillus paracasei* 106 (former *Lactobacillus paracasei* 106)	Different fruit processing byproducts	Tolerance to acid and bile salts Simulated GIT conditions Cell surface hydrophobicity Hemolytic activity Mucin degradation Bile salt deconjugation Antibiotic susceptibility	The resistance to antibiotics varied among the strains with potentially transferable resistance to tetracycline. Had no resistance to ampicillin and chloramphenicol, as well as presented no hemolytic and mucinolytic activity or ability to bile deconjugation.	Albuquerque et al. (2018)
Liquorilactobacillus mali K8 (former *Lactobacillus mali* K8)	Water kefir grains	Antibiotic resistance Hemolytic activity Adaptation to food matrix	*L. mali* K8 had tolerance to pH 2.5, and resisted bile salts, pepsin and pancreatin, No hemolytic activity Susceptibility to the standard antibiotics	Koh et al. (2018)
L. plantarum 3701, 3725, 3739, 3735, 25234, 25294, 2519, 3736, 3711, *Lacticaseibacillus casei* 3734 (former *Lactobacillus casei* 3734)	Mature *Cornus officinalis* fruits	Tolerance to acid and bile salts Cell surface hydrophobicity Antioxidant activity Antibiotic susceptibility Molecular identification	The strains possess desirable probiotic properties, similar or superior to the strain *Lacticaseibacillus rhamnosus* GG (former *Lactobacillus rhamnosus* GG) used as reference.	Tang et al. (2018)
Thirty different strains comprise *Lactobacillus (fermentum, plantarum,* and *brevis) Weissella cibaria, Enterococcus (faecium* and *faecalis*), *Leuconostoc* (*citreum* and *mesenteroides* subsp. *mesenteroides*) and *Pediococcus pentosaceus*	Horreh, a traditional Iranian fermented food	Tolerance to acid and bile salts Antibacterial activity Antibiotic susceptibility Molecular identification	A hundred and forty isolates were identified as LAB and thirty of which were evaluated. *L. plantarum* and *L. mesenteroides* subsp. *mesenteroides* isolates were able to grow at pH 2.5 and 3.5. Some of L. *fermentum* and *L. plantarum* isolates showed quite high hydrophobicity and antimicrobial activity. All strains were sensitive to ampicillin, chloramphenicol, cycloheximide, erythromycin, neomycin, streptomycin, tetracycline, and rifampicin, but resistant to vancomycin.	Vasiee et al. (2018)
Leuconostoc mesenteroides E14, M67	Silage and honey	Simulated GIT conditions Antibiotic susceptibility Cell surface hydrophobicity Exopolysaccharides (EPS) production	The strains E14 and M67 showed resistance to gastrointestinal tract. The antibiogram presented resistance and sensitivity variables to several antibiotics. Both strains had a low EPS production and low rate of hydrophobicity.	Zarour et al. (2018)

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Table 1 (*continued*)

Strains	Isolation source	Performed test	Findings	References
Saccharomyces cerevisiae IFST062013	Fruit (unspecified)	Simulated GIT conditions Antioxidant activity Production of bioactive compounds Tolerance to stress Antibiotic susceptibility	The strain was tolerant to a wide range of temperature and pH, high concentration of bile salt, NaCl, and presents resistance to gastric juice, α-amylase, trypsin, and lysozyme. The strain produced killer toxin, vitamin B12, glutathione, siderophore, enzymes (amylase, protease, lipase, cellulase), biofilm, and strong antioxidant activity. Also, it showed resistance to tetracycline, ampicillin, gentamicin, penicillin, polymyxin B and nalidixic acid.	Fakruddin et al. (2017)
L. plantarum CCMA0743, *Torulaspora delbrueckii* CCMA0235	Cauim (blended of cassava and rice)	Tolerance to acid and bile salts Antioxidant activity Lactic acid production Enzymatic activity	The beverage in co-culture improved the products digestibility by reducing starch, for single and co-culture The assays containing yeast showed the highest antioxidant activity (around 10% by DPPH and ABTS methods).	Freire et al. (2017)
Pediococcus acidilactici Ch-2	Chuli (a fermented apricot product)	Tolerance to acid and bile salts Simulated GIT conditions Aggregation properties Metabolic profiling	The strain Ch-2 was resistance to low pH, bile salts and simulated gastric and intestinal conditions. Also, susceptible to selected eleven antibiotics, unable to produce gelatinase and DNase and non-hemolytic nature. The strain produced squalene – a rare and therapeutic anticancer compound.	Gupta and Sharma (2017)
L. plantarum E1/18, E5/6, S0/3, S0/7 and S3/16	Fermented stinky bean (Sa Taw Dong)	Tolerance to acid and bile salts Simulated GIT conditions Cell surface hydrophobicity Growth under microaerobic and anaerobic conditions Hydrolase activity Antimicrobial activity Antibiotic susceptibility Molecular identification	The strain was susceptible to almost all antibiotics tested (ampicillin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline, chloramphenicol, vancomycin, and ciprofloxacin). It had neither hemolytic activity nor virulence factor genes.	Saelim et al. (2017)
Candida tropicalis 1A and 3A; *Debaryomyces hansenii* 8A; *Galactomyces reessii* 33A and 34A; *Pichia guilliermondii* 25A; *P. manshurica* 2A; *P.membranifaciens* 29A and 3B; *Rhodotorula glutinis* 27A; *R. graminis* 20A; *Saccharomyces cerevisiae* 15A and 15B; *Candida boidinii* 32A and 37A and *C. norvegica* 7A.	Negrinha de Freixo cv. olives	Simulated GIT conditions Aggregation properties Antioxidant activity Enzymatic activity Capacity to grow at 37 °C	*S. cerevisiae* strains showed high ability to grow at 37 °C, lipase activity. The strain 15A showed higher auto- aggregation percentage. *P. guilliermondii* and *C. norvegica* exhibited the ability to survive human gastrointestinal tract digestion. *S. cerevisiae* strains showed high antioxidant capacity.	Oliveira et al. (2017)
L. casei 24, *L. fermentum* 38, 47, 62 and *L. plantarum* 81,90, 96, 100 and 105	Fermentation Process of "Cupuaçu" *Theobroma grandiflorum*	Simulated GIT conditions Aggregation properties	The strains produced diffusible inhibitory compounds and promoted co-aggregation *with S. Typhimurium* ATCC 6538.	Ornellas et al. (2017)
L. brevis HAC06, HAC08, HAC09, *L. plantarum* HAC01, HAC02, HAC03, HAC07 and *Latilactobacillus sakei* HAC04, HAC05, HAC10, HAC011 (former *Lactobacillus sakei* HAC04, HAC05, HAC10, HAC011)	White kimchi	Simulated GIT conditions Tolerance to phenol Bile salt deconjugation Hemolytic activity Biogenic amine production Antibiotic susceptibility Molecular identification	The strains were resistant to adverse conditions and were able to grow in the cabbage juice in which addition of 2% salt favorably influenced. None of the strains showed hemolysis or gelatin hydrolysis activity. *L. brevis* strains produced precursors of biogenic amines, and no other strain showed this ability. The susceptibility to erythromycin, gentamicin, ampicillin, tetracycline, chloramphenicol, streptomycin, ciprofloxacin was variable among strains.	Park et al. (2016)
Four strains: *L. plantarum* S-811, S-TF2, *Fructobacillus fructosus* S-22 and S-TF7	Fresh and spoiled cactus (*Opuntia ficus-indica*) pears of the green cultivar	Tolerance to acid and bile salts Simulated GIT conditions Cell surface hydrophobicity Aggregation properties Enzymatic activity Antioxidant capacity Adaptation to food matrix	Seventeen isolated of LAB. Four strains potentially probiotic selected. The strains showed starter potential in cactus pear juice fermentation. All showed resistance to bile salts and most of them were resistant to pH 3, but not to a pH 2 gastric juice. Five strains (S-02, S-22, S-24, S-TF3 and S-TF7) showed hydrophobicity values above 40%. Related to auto-aggregation, had values	Verón et al. (2017)

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Table 1 (*continued*)

Strains	Isolation source	Performed test	Findings	References
		Screening of ferulic acid Production of bioactive compounds Molecular identification	between 5.1 and 62.5%. 3 isolates, S-811, S-TF1 and S-TF2, showed feruloyl esterase activity, related to antioxidant activity.	
L. brevis 59 *L. pentosus* 129 *L. paracasei* 108 *L. plantarum* 49 *L. fermentum* 111	Byproducts of fruit pulp process *Malpighia glabra* L., *Mangifera indica* L., *Annona muricata* L., *and Fragaria vesca* L.	Tolerance to acid and bile salts Ability to growth on two different cultivation media Molecular identification	The strains showed tolerance to different acidic conditions and bile salt concentrations. Suitable growth in two laboratory and edible growth media, and variable susceptibility to different antibiotics.	Garcia et al. (2016)
L. plantarum F22	Traditional inoculum Phab used in chhang beverage	Acid production Tolerance to acid and bile salts Cell surface hydrophobicity Aggregation properties Bacteriocin production Antibiotic susceptibility Molecular identification	The strain was resistant to bile salt, able to survive in simulated low gastric pH. It showed broad antagonism against a wide range of foodborne/spoilage causing bacteria. It had a strong auto-aggregation, hydrophobicity, and sensitivity to most of the clinical antibiotics.	Handa and Sharma (2016)
L. plantarum DGK-17	Kimchi	Tolerance to acid and bile salts Survival to GIT conditions Aggregation properties Adhesion properties	Nutritionally improved DGK-17 was able to survive under low pH and high bile salt conditions, simulated gastric juice environment. Auto-aggregation, co-aggregation and adhesion indicated that DGK-17 prevents colonization of pathogenic bacteria.	Khan and Kang (2016)
L. plantarum 277, 281, 286, 289, 291 and *L. fermentum* 260, 265, 266	Brazilian cocoa fermentation	Simulated GIT conditions Cell surface hydrophobicity Heat tolerance Antibiotic susceptibility Molecular identification	The strains showed variation on tolerance to heat shock with reduction in viability ranged from 1.9 to 3.4 log orders. The resistance to GIT simulation varied among strains and *L. plantarum* 286 showed the most promising result, followed by *L. plantarum* 289. *L. plantarum* 281 displayed higher hydrophobicity (16.9%). All strains showed sensitivity to both amoxicillin and chloramphenicol but resistance to both vancomycin and nalidixic acid.	Santos et al. (2016)
L. casei Q11, *L. sakei* Q82, *L. plantarum* Q823, Q825 and *Leuconostoc lactis* Q615	Different quinoa varieties and amaranth seeds	Tolerance to acid and bile salts Simulated GIT conditions Antibiotic susceptibility	Eighteen LAB isolated were selected based on the genomic profiling, five presented no resistance to gentamicin, kanamycin, streptomycin, erythromycin and clindamycin and were selected for their potential probiotic. The strains were tolerant to lysozyme, bile salts, and had similar adhesion. *L. plantarum* Q823 was selected for *in vitro* and *in vivo* studies.	Vera-Pingitore et al. (2016)
Genera *Candida* (2 strains), *Kluyveromyces* (4 strains), *Lachancea* (1strain), *Metschnikowia* (5 strains), *Pichia* (10 strains), *Saccharomyces* (68 strains), *Schizosaccharomyces* (5 strains), *Torulaspora* (23 strains), *Wickerhamomyces* (9 strains), *Yarrowia* (2 strains) and Zygosaccharomyces (1 strain)	Strains from different sources of which 66 strains were from grape must	Capacity to grow at 37 °C Survival the GIT conditions Antioxidant activity Molecular identification	A hundred and thirty yeast strains isolated from food. About 50% of the yeast developed at 37% with growth kinetic parameters higher at 37 °C than at 25 °C. A total of 95% of the strains survive to the exposition to conditions simulating to the GIT. The strains exhibit auto-aggregation phenotype, antioxidant activity and better growth capacity at 37 °C than at 25 °C. Good tolerance to GIT stress conditions, and high auto-aggregation percentage.	Gil-Rodríguez et al. (2015)
Levilactobacillus spicheri G2 (former *Lactobacillus spicheri* G2)	Gundruk a non-salted, fermented acidic vegetable	Acid and bacteriocin production Aggregation properties Adhesion to solvents Tolerance to acid and bile salts Antibiotic susceptibility	The strain produced lactic acid, bacteriocin and showed 53.65% of auto-aggregation capacity. Isolate showed some degree of bile salt tolerance and presented sensitive to 14 clinical antibiotics.	Gautam and Sharma (2015)
L. plantarum 0103, 0123, 0140, 0147, 0157, 0611, 0612, 0825, 1002; *L. brevis* 0808, 08771, (21%), *L. rhamnosus* 0504, *Lactobacillus*	Malaysian fermented *Bambangan* (*Mangifera pajang)*	Tolerance to acid and bile salts Aggregation properties Enzymatic activity	Based on acid tolerance thirteen LAB strains were selected. Around 36% of the isolated LAB strains displayed a high survival rate at pH 3.0	Ng et al. (2015)

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Table 1 (*continued*)

Strains	Isolation source	Performed test	Findings	References
delbrueckii, *L. paracasei* and *Pediococcus pentosaceus* 1001		High temperature and osmotic pressure Antimicrobial activity Adhesion to solvents Molecular identification	with at least 4 log CFU/mL after 24 h at 2.0% bile salt. Five *L. plantarum* strains showed at least 70% viability at 60 °C/10 min while one *L. brevis* and three *L. plantarum* strains were tolerant to 6% sodium chloride. They presented aggregation activity (>20%) in most of the LAB strains. The maximum β-galactosidase activity was found in four *L. plantarum* strains.	

to intestinal mucosa leading to biofilm formation, and this feature disfavors the binding of enteropathogens (Pabari et al., 2020). Moreover, the biofilms formed around these bacteria increase their survival to adverse conditions. Within the used approaches, *in vitro* adhesion to the Caco-2 cell is the most frequent assay to evaluate that property. Yeast and bacteria from fruits, vegetables and traditional fermented foods have been showed important adhesion properties (Bonatsou et al., 2018; Leandro et al., 2020; Menezes et al., 2019; Vera-Pingitore et al., 2016), which was associated to the production of enzymes and EPS that promote interactions between probiotics and host-specific receptors and act as a capsule bound to the cell surface protecting against toxic agents and stressing conditions encountered during the gastrointestinal tract passage or by food preservation technologies (García-Ruiz et al., 2014). The adhesion properties are dependent on the source of the microorganisms, and some strains with low adhesion capacity may still present important health effects. Therefore, this characteristic may not be primordial for probiotic selection (Santos et al., 2016).

To provide health benefits to hosts by improving the nutrient content, a probiotic should have the ability to produce related enzymes (Fakruddin et al., 2017). Fakruddin et al. (2017) reported that *Saccharomyces cerevisiae* IFST062013 isolated from fruit could produce enzymes, such as lipase, cellulase, and protease, but it could not produce amylase, DNase, and gelatinase. The production of enzymes (amylase, protease, lipase, cellulase, and galactosidase) is a positive characteristic for probiotics (Benitez-Cabello et al., 2019; Fakruddin et al., 2017; Shekh et al., 2019; Song et al., 2020; Won et al., 2020; Xu et al., 2020), while the absence of production of gelatinase and DNase suggests their safety to be used by human as most of the pathogenic microorganisms produce these enzymes as part of their pathogenesis. In a general view, the microorganisms isolated from fruits and vegetables produce beneficial enzymes.

The food industries may carefully evaluate new probiotic species' efficacy and safety before incorporating them into food products (Maheshiwari et al., 2019). Probiotic strains can be resistant to antibiotics, remaining in the environment where they are inserted and allowing their utilization in patients undergoing treatments with antibiotics. However, they should not transmit this resistance to other microorganisms to be considered safe for human and animal consumption (Fakruddin et al., 2017). Previous studies reported that LAB isolated from fruits and vegetables were sensitive to all the evaluated antibiotics (Leandro et al., 2020; Sakandar et al., 2019). Other studies found Lactobacillus strains with resistance to antibiotics, such as gentamicin, cefuroxime, kanamycin, vancomycin, and erythromycin, but it was natural or intrinsic and non-transmissible (Anandharaj et al., 2015; Pabari et al., 2020; Tang et al., 2018). However, a previous study showed that strains isolated from fruit by-products (*L. plantarum* 53, and *L. fermentum* 60) showed potentially transferable resistance to antibiotics (tetracycline) (Albuquerque et al., 2018). This characteristic is of paramount importance to guarantee the safety of potentially probiotic cultures, but many studies obtained strains with resistance to antibiotics and did not evaluate their possible transmittance of genes (Fakruddin et al., 2017; Garcia et al., 2016; Gautam & Sharma, 2015). Therefore, the antibiotic resistance and, mainly, the possible transmittance of genes should be evaluated for each potentially probiotic strain.

Safety aspects should also consider the production of biogenic amines. Biogenic amines are recognized as undesirable metabolic products of some starter cultures or probiotic strains that could be found in fermented foods. The consumption of high concentrations of biogenic amines may result in risk health effects for consumers, such as headaches, neurological disorders, tachycardia, hypotension, among others (Ku et al., 2020). Previous studies have reported no biogenic amine production by probiotics (*Lactiplantibacillus plantarum* SBR64.7 and S180.7*, Lentilactobacillus buchneri* SS50.4*, Limosilactobacillus fermentum* SS50.10) isolated from silage, cocoa beans, and artisanal salami (Leandro et al., 2020), and *Weissella paramesenteroides* isolated from fruits (Pabari et al., 2020). However, Park et al. (2016) observed that all *L. brevis* strains isolated from Korean white kimchi could produce biogenic amines (tyramine and/or histamine). Therefore, the biogenic amine production should be evaluated for each potentially probiotic strain.

Probiotic cultures could not present hemolytic activity or the ability to degrade mucin. Hemolysins are toxins that can cause erythrocytes lysis, while the production of enzymes capable of degrading mucin is a virulence factor in enteropathogens. Mucins are important as they cover the epithelial cells of the intestine and prevent mucosal penetration and translocation by pathogens (Albuquerque et al., 2018). Several LAB isolated from fruit by-products (*L. plantarum* 53, *L. fermentum* 56, 60, 250, 263, 139, 141 and 296, and *L. paracasei* 106) had no hemolytic activity or ability to degrade mucin. Lack of ability to degrade mucin was observed for *L. plantarum*, *P. acidilactici*, *L. fermentum*, *L. zeae*, and *L. buchneri* isolated from cocoa beans, artisanal salami, and silage (Leandro et al., 2020), *Weisella* strains isolated from fruits (banana, orange, cherry, sapota, and plum (Pabari et al., 2020), and *F. fructosus* MCC 3996 isolated from nectar of flowers (*B. monosperma*) (Patil et al., 2020). However, six bacteria isolated from panchamirtham (Indian ethnic fermented fruit mix) showed β-hemolytic activity (Maheshwari et al., 2019). Therefore, the hemolytic activity should be evaluated for each potentially probiotic strain.

The reducing power measures the antioxidant activity, nitric oxide and hydroxyl radical scavenging activity, cell cytotoxicity and toxicity, and metal ion chelating activity. Previous studies reported increased antioxidant activity when microbial isolates of fruits, vegetables, and traditional fermented foods were used (Cao et al., 2019; Di Cagno et al., 2020; Fakruddin et al., 2017; Gil-Rodríguez et al., 2015; Hsiung et al., 2020; Junnarkar et al., 2019; Lakra et al., 2020; Menezes et al., 2019; Oliveira et al., 2017; Song et al., 2020; Tang et al., 2018). The antioxidant activity was associated with the type of matrix and was strain-specific (Cao et al., 2019; Di Cagno et al., 2020). Yeasts may show higher antioxidant activity than LAB, which has been associated with the high content of (1/3)-β-D glucan and other β-glucans found in their cell wall, among other cellular compounds (Menezes et al., 2020).

The utilization of prebiotic compounds by probiotic cultures is an important feature in the development of synbiotic products. Probiotics may utilize prebiotic compounds and increase their viability and/or activity in the product. The utilization of prebiotic compounds seems to be strain-dependent, as *Weissella* strains were able to grow in the presence of FOS and GOS, but not on XOS (Pabari et al., 2020). However, future studies are needed to better evaluate prebiotic compounds' utilization by probiotic cultures isolated from unconventional sources.

2.2. Health effects of probiotics strains from unconventional sources

Fig. 2 presents the main health effects associated with probiotics isolated from unconventional sources. The main studies assessing the *in vitro* and/or *in vivo* (human and animal model) physiological effects of the probiotic strains isolated from unconventional sources are provided in Table 2. The beneficial effects of probiotics on the host are related to one or more mechanisms, such as modulation of the intestinal microbiota, block of pathogen adhesion sites, modulation of the host immune responses, and competition by nutrients (Vera-Pingitore et al., 2016).

2.2.1. Hypocholesterolemic, anti-hypertensive and hypoglycemic effects

In vitro tests have demonstrated that probiotic cultures from unconventional sources could assimilate cholesterol. *Weissella cibaria* MD2 and *Weissella confusa* MD1 isolated from *Mudakathan dosai* (fermented rice with leaves of herbaceous vine) could assimilate 67 and 78% of the cholesterol medium (Lakra et al., 2020), while *L. pentosus* and *L. plantarum* isolated from table olives biofilm assimilated 13.09–38.42% (Benitez-Cabello et al., 2019). The capacity of cholesterol assimilation was strain-dependent (Benitez-Cabello et al., 2019).

In an *in vivo* test, the consumption of *L. fermentum* 296 isolated from fruit (10^9 CFU/mL, 4 weeks) by *Wistar* rats subjected to a high-fat diet resulted in alleviation of hyperlipidemia and blood pressure but no impact on glucose tolerance and insulin resistance (Cavalcante et al., 2019). At the same time, the consumption of *L. fermentum* 263, *L. fermentum* 139, and *L. fermentum* 296 isolated from fruit by-products (10^9 CFU/mL, 1:1:1. 8 weeks) by *Wistar* rats subjected to a dyslipidemic diet resulted in reductions of total cholesterol, increases in HDL-cholesterol, and improvements in blood pressure, but no impact on insulin resistance (Oliveira, Cavalcante, et al., 2020). On the other hand, the consumption of cactus pear juice fermented with *L. plantarum* S-811 isolated from cactus pear (10^9 CFU/mL, 7 weeks) by obese mice resulted in improvements in insulin resistance, hyperlipemia, and hyperglycemia (Verón et al., 2019). Finally, the consumption of *L. plantarum* 49 and *L. plantarum* isolated from fruits (10^9 CFU/mL, 28 days) by male healthy *Wistar* rats resulted in a reduction in blood glucose levels and total cholesterol. The effect of long-lasting glycemic control was dependent on the probiotic strain, with *L. plantarum* 49 presenting a better performance (Costa et al., 2019). The results suggest that the hypocholesterolemic and anti-hypertensive effects were frequently observed after probiotic consumption, while the hypoglycemic properties were dependent on the studied group (healthy, dyslipidemic, obese), probiotic strain, and vehiculation form (probiotic strain isolated or in a food product). The reduction of cholesterol may be associated with deconjugation of bile salts, resulting in more demand of cholesterol for *de novo* synthesis of bile salts and the reduction of its solubility and absorption in the lumen of the intestine (Saelim et al., 2017). Furthermore, the anti-hypertensive properties were related to decreases in systolic arterial pressure and sympathetic hyperactivity (Cavalcante et al., 2019). The anti-diabetic properties may be influenced by changes in the microbiota and production of short-chain fatty acids (Costa et al., 2019).

2.2.2. Immunomodulatory and anti-inflammatory properties and antitumor activity

In vitro tests have also demonstrated that probiotic cultures from unconventional sources show immunomodulatory properties. *Levilactobacillus brevis* B13-2 (former *Lactobacillus brevis* B13-2) isolated from kimchi induced the expression of some cytokines (IL-1β, TNF-α, and IL-6) and iNOS, indicating immunomodulatory activity (Song et al., 2020).

In an *in vivo* test, the consumption of *S. cerevisiae* IFST 062013 isolated from fruits (10^9 CFU/mL, single dose) by male swiss albino mice resulted in stimulation of T-lymphocyte specific proliferative response, potentiation of cell-mediated and humoral immunity, and antitumor activity. Furthermore, it induced anti and pro-inflammatory mediators and maintained the balance of Th1 and Th2 cytokines, improving host immunity (Fakruddin et al., 2017). The consumption of *L. plantarum* 81 and *L. plantarum* 90 isolated from cupuaçu fermentation (10^8 CFU/mL,

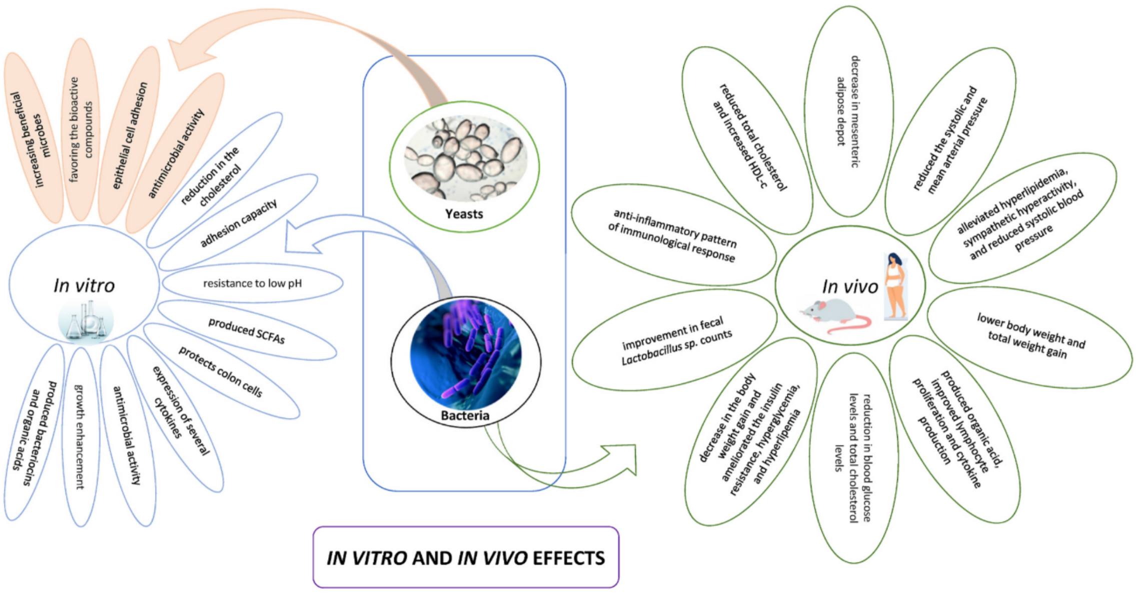

Fig. 2. Most relevant *in vivo* and *in vitro* effects.

Table 2
Main studies assessing the effects of probiotic isolated from unconventional sources.

Strains	Type of study	Isolation Source	Assay	Main Effects	References
Hypocholesterolemic, anti-hypertensive and hypoglycemic effects					
Weissella confusa MD1 and *Weissella cibaria* MD2	*In vitro*	Fermented batter	Cholesterol assimilation	High reduction in the cholesterol levels (>67%)	Lakra et al. (2020)
Sixteen strains: 13 *L. pentosus* and 3 *L. plantarum*	*In vitro*	Table olives biofilms	Cholesterol assimilation	All the strains showed the ability to reduce the cholesterol, but with differences among them.	Benítez-Cabello et al. (2020)
L. plantarum E1/18, E5/6, S0/3, S0/7 and S3/16	*In vitro*	Fermented stinky bean (Sa Taw Dong)	Cholesterol assimilation	All five strains exhibited the ability to assimilate cholesterol. The strains E5/6 and S0/7 were more effective.	Saelim et al. (2017)
L. fermentum 296	*In vivo*	Fruit pulp processing byproducts	Male *Wistar* rats Control (CTL):commercial diet and saline solution; (HF) received a High-fat commercial diet and saline solution (HF + Lf 296) treated with High Fat (HF) diet + *L. fermentum* 296 in a solution of about 1x10^9 CFU/mL. Administration was performed daily for 4 weeks by oral gavage. Body weight, biochemical measurements, glucose and insulin tolerance tests, arterial pressure and heart rate records, spectral analysis of systolic arterial pressure and cardiac interval, baroreflex sensitivity, sympathetic-vagal balance and sympathetic vascular tone were evaluated in each group.	The administration of *L. fermentum* 296 for 4 weeks alleviated hyperlipidemia, sympathetic hyperactivity, and reduced systolic blood pressure in HF rats without affecting baroreflex sensibility.	Cavalcante et al. (2019)
L. fermentum 139, 263 and 296	*In vivo*	Fruit by-products	Wistar rats (n = 14) were fed with a control diet (CTL = 7) or a dyslipidemic diet (DLP = 7) during pregnancy and lactation. After weaning, male and female offspring received a standard diet up to 90 days of life. Rats were allocated groups: CTL group + saline solution (n = 14); DLP group + saline solution (n = 14) DLP group receiving a probiotic cocktail (n = 14). A CLT or probiotic formulation containing *L. fermentum* 139, 263 and 296 at 1 × 10^9 CFU/mL) was administered daily by oral gavage/8 weeks. The lipid and glucose profile, insulin tolerance test, malondialdehyde (MDA) as indicator of oxidative stress, arterial blood pressure, peripheral chemoreflex, sympathetic vascular tone were evaluated.	The strains of *L. fermentum* reduced total cholesterol and increased HDL-c, but did not affect the insulin resistance induced by maternal dyslipidemia in male and female offspring and reduced the systolic and mean arterial pressure, only in male. The group that received the probiotic showed improvement in blood pressure and sympathetic tone, without affecting baroreflex modulation. The probiotic intervention did not cause significant changes in the body weight, triglycerides, VLDL, TBARS, OGTT, UTT.	Oliveira et al. (2020)
L. plantarum S-811	*In vivo*	Cactus Pear (*Opuntia Ficus-Indica*) Juice Fermented	Obese e lean mice were randomly in two groups (n = 24): lean group (L) that was fed a standard diet (SD); an obese group (Ob) that was fed a high-fat diet (HFD). So, the animals were subdivided in four groups (n = 6) received: 1) water *ad libitum.* 2) a daily dose of *L. plantarum* S-811 as a suspension in water; 3) pasteurized pear juice; 4) Cactus pear juice fermented by	Administration of fermented juice to obese mice ameliorated the insulin resistance, hyperglycemia, and hyperlipemia that characterize obesity. *O. ficus-indica* fruits can reduce the percentage of body fat, total cholesterol, and glycaemia.	Verón et al. (2019)

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Table 2 (*continued*)

Strains	Type of study	Isolation Source	Assay	Main Effects	References
			L. plantarum S-811 at 1.2 × 10^9 CFU/mL. The intervention was 7 weeks. Plasma leptin, insulin, glucose, cholesterol, triglycerides, glucose tolerance, homeostatic model assessment (HOMA-IR) and intestinal cytokines were measured.		
L. plantarum 49, and *L. plantarum* 201	*In vivo*	Pulp of *Mangifera indica* L., or from industrial fruit pulp processing byproducts of *Malphigia glabra* L., *M. indica* L., *Annona muricata* L. and *Fragaria ananassa* L.	Sixteen L. *plantarum* strains were used as growth promoting effects models in *Drosophila melanogaster*. Two strains were selected to observe the safety aspects and beneficial effects of strains using Wistar rats (n = 48)/21 days. The animals were randomly distributed into three groups (n = 16): group Lp49, which received 9 log CFU/mL of strain *L. plantarum* 49; group Lp201, which received 9 log CFU/mL of strain and control group, which received PBS daily by gavage/4 weeks. The murinometric parameters, levels of aspartate transaminase (AST), alanine transaminase (ALT) and lipids profile were measured. To evaluate the strains' safety, histopathological analysis was performed to observe translocation.	Daily administration of *L. plantarum* 49 and *L. plantarum* 201 did not affect food intake or morphometric parameters. Both strains were associated with reduction in blood glucose levels and total cholesterol levels. The maintenance of the effects varied with the strain and time of ingestion. The strains were detected in the intestine and did not cause alteration or translocate to spleen, kidneys, or liver during the experimental or wash-out period.	Costa et al. (2019)
Immunomodulatory and anti-inflammatory properties, and antitumor activity					
Lactiplantibacillus pentosus B13-2 (former *Lactobacillus pentosus* B13-2)	*In vitro*	Chinese cabbage kimchi	Expression of cytokines related to immunomodulation	Induction of the expression of several cytokines (TNF-α, IL-1β, and IL-6) and iNOS by activation of RAW 264.7 murine macrophages.	Song et al. (2020)
Saccharomyces cerevisiae IFST062013	*In vivo*	Fruit (unspecified)	Dose of 150 μL (~10^9 CFU) were administered orally on albino mice to safety and immuno-modulatory activity evaluation.	The isolate produced organic acid, improved lymphocyte proliferation and cytokine production in treated mice.	Fakruddin et al. (2017)
L. casei 24, *L. fermentum* 38, 47, 62 and *L. plantarum* 81,90, 96, 100 and 105.	*In vivo*	Fermentation Process of "Cupuaçu" *Theobroma grandiflorum*	Germ-free Swiss mice of both sexes with 6 weeks-old, received a single dose of 0,1 mL of *Lactobacillus* suspension containing about 8.0 log of CFU by intragastric intubation. The intestinal colonization was evaluated in fresh faeces. *Ex vivo* antagonism in germ-free mice, cumulative mortality, translocation to liver and spleen, histopathological examination of liver and ileum and mRNA cytokine gene expression during an experimental infection with 0,1 mL suspension *S. Typhimurium* containing 5.0 log CFU to enteropathogen challenge.	The strains showed higher survival after enteropathogen challenge, lower hepatic translocation of enteropathogen, lower histopathological lesions in ileum and liver and anti-inflammatory pattern of immunological response. *L. plantarum* 81 and *L. plantarum* 90 were selected as potential probiotics.	Ornellas et al. (2017)
Adipogenesis inhibition					
Latilactobacillus sakei AM2, AM8, AM32, ADM14, ADM19, YRM13, YRM20, RGM12, GP32, GP48, GN57, YG19 (former *Lactobacillus sakei* AM2, AM8, AM32, ADM14, ADM19, YRM13, YRM20, RGM12, GP32, GP48, GN57, YG19), *L. plantarum* S-2AM31 and	*In vitro*	Kimchi	Cell culture and adipocyte differentiation test	Various strains presented distinct inhibitory effects under lipid accumulation through inhibition of lipid transcription factors and gene expression. *L. sakei ADM14* was very efficient to inhibit adipogenesis in adipocytes.	Won et al. (2020)

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Table 2 (*continued*)

Strains	Type of study	Isolation Source	Assay	Main Effects	References
Leuconostoc pseudomesenteroides S-YRWM27, S-YRWM28					
L. plantarum S-811	*In vivo*	Cactus Pear (*Opuntia Ficus-Indica*) Juice Fermented	Obese e lean mice were randomly in two groups (n = 24): lean group (L) that was fed a standard diet (SD); an obese group (Ob) that was fed a high-fat diet (HFD). So, the animals were subdivided in four groups (n = 6) received: 1) water *ad libitum.* 2) a daily dose of *L. plantarum* S-811 as a suspension in water; 3) pasteurized pear juice; 4) Cactus pear juice fermented by *L. plantarum* S-811 at 1.2 × 10^9 CFU/mL. The intervention was 7 weeks. Body weight gain, adipose tissue weight, plasma leptin, insulin, glucose, cholesterol, triglycerides, glucose tolerance, homeostatic model assessment (HOMA-IR) and intestinal cytokines were measured.	Administration of fermented juice to obese mice caused a significant decrease in the body weight gain. *O. ficus-indica* fruits can reduce the percentage of body fat.	Verón et al. (2019)
L. brevis HAC06, HAC08, HAC09, *L. plantarum* HAC01, HAC02, HAC03, HAC07 and *L. sakei* HAC04, HAC05, HAC10, HAC011.	*In vivo*	White kimchi	Seven-week-old C57BL/6J male mice were housed and provided with filtered water and rodent diet, *ad libitum* for 10 weeks. After adaptation, each group (n = 5) received 1 × 10^8 CFU viable cells of a strain mixture (50:50 of *L. plantarum* HAC01 and *L. sakei* HAC13 suspension were given to the mice once/day for 10 more weeks. Sterile suspension was administered to the control groups. The L. *rhamnosus* GG, was used as reference strain and administered in the same way.	The strain mixture showed a noticeable decrease in mesenteric adipose depot as compared to LGG and the positive control. The intervention resulted in a significantly lower body weight and total weight gain for eight weeks compared to the high-fat control group.	Park et al. (2016)
Antimicrobial activity					
Lactiplantibacillus plantarum SBR64.7 and S180.7 (former *Lactobacillus plantarum* SBR64.7 and S180.7), *Lentilactobacillus buchneri* SS50.4 (former *Lactobacillus buchneri* SS50.4), *Limosilactobacillus fermentum* SS50.10 (former *Lactobacillus fermentum* SS50.10)	*In vitro*	Forage plants, cocoa beans fermented	Antimicrobial activity	Inhibition of pathogenic and spoilage microorganisms against *Staphylococcus* sp. (ATCC® 27,626), Shigella sp. (ATCC® 23,354), Salmonella sp. (ATCC® 700,623), Escherichia coli (ATCC® 25,922), Pseudomonas sp. (ATCC® 19,151), Proteus mirabilis (ATCC® 21,100), and Klebsiella sp. (ATCC® 700,834) and all strains had a high percentage of CaCo2 cell adhesion.	Leandro et al. (2020)
Weissella confusa MD1 and *Weissella cibaria* MD2	*In vitro*	Fermented batter	Antimicrobial activity	The strains exhibited antimicrobial activity against foodborne pathogens (*Escherichia coli, Salmonella enterica, Listeria monocytogenes, Salmonella typhi and Staphylococcus aureus*).	Lakra et al. (2020)
P. pentosaceus RG7B and C11C, and *L. plantarum* RG7B	*In vitro*	Cardinal and Red Globe grape fruits	Antimicrobial activity	*P. pentosaceus* RG7B and C11C and *L. plantarum* RG8A showed antifungal activities against *Aspergillus niger aggrégats* and *A. carbonarius. Pediococcus pentosaceus* RG7B	Taroub et al. (2019)
Weissella paramesenteroides FX5, FX9	*In vitro*	Sapota, cherry, banana, orange, and plum smashed fruits	Antimicrobial activity	The strains inhibited *E. coli* and *S. aureus.*	Pabari et al. (2020)
Twenty-one LAB isolates	*In vitro*	Fresh vegetables (cauliflower, gherkins, cluster beans,	Antimicrobial activity	The cellular extract produced bacteriocin and bacteriocin like	Junnarkar et al. (2019)

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Table 2 (*continued*)

Strains	Type of study	Isolation Source	Assay	Main Effects	References
		fenugreek, cow pea, bitter gourd, french beans, tomato, ridged gourd, cucumber, and bottle gourd)		substances. It had antibacterial potential against selected plant and human pathogens.	
Fructobacillus pseudoficulneus JNGBKS, JNGBKS3, *F. fructosus* JNGBKS2, JNGBKS4, *F. durionis* JNGBKS5, and *Apilactobacillus kunkeei* JNGBKS6, JNGBKS7, JNGBKS8 (former *Lactobacillus kunkeei* JNGBKS6, JNGBKS7, JNGBKS8)	*In vitro*	Fresh fruits (apple, banana, Chinese peach, plum, cantaloupe, kiwi fruit, and lychee) flowers (narcissus, pink rose, red rose, yellow rose, and sunflower) and honey, and rose petals jam	Antimicrobial activity	The strains inhibited *E. coli, S. typhimurium*, and *S. aureus, L. kunkeei* strains	Sakandar et al. (2019)
Fructobacillus fructosus MCC 3996	*In vitro*	Nectar of *Butea monosperma* flower	Antimicrobial activity	Antagonistic activity against *B. pumilus* (NCIM 2327), *E. coli* (NCIM 2109), *S. typhimurium* (NCIM 2501), *S. aureus* (NCIM 2079), *Proteus vulgaris* (NCIM 2172) and *P. aeruginosa* (NCIM 2036).	Patil et al. (2020)
L. plantarum, Enterococcus faecalis, Lactiplantibacillus paraplantarum (former *Lactobacillus paraplantarum*), and *Weissella paramesenteroides*	*In vitro*	Leaves of papaya, cassava, sugarcane, yam, and taro	Antimicrobial activity	Exhibited antimicrobial activity against Gram-positive *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus*, and the Gram-negative *Escherichia coli* pathogen.	Samedi and Charles (2019)
L. plantarum (twenty-five different strains), and *Enterococcus casseliflavus* S4b	*In vitro*	De'ang pickled tea	Antimicrobial activity	The strains had antimicrobial activity against *Salmonella* Typhimurium, *Escherichia coli* and eight out of the eighteen strains suppressed growth of *Shigella flexneri.*	Cao et al. (2019)
L. plantarum P24-1, P24-2, P24-3, P24-4, P24-4, P24-5, P24-6, P24-8 and *Pediococcus acidilactici* A1-2, A2-1	*In vitro*	Pulque and aguamiel	Antimicrobial activity	Sixty percent of the isolates exhibited antimicrobial effect against *Escherichia coli, Staphylococcus aureus.* The growth of *H. pylori* ATCC 43504 was suppressed by all the LAB.	Cervantes-Elizarrarás et al., (2019)
A hundred and three bacterial isolates most of which were *Bacillus* sp., *Enterobacter* sp., *acinetobacter* sp., *Pantoea* sp., *Microbacterium* sp., *Klebsiella* sp., *sphingomonas* sp., *Ornithinibacillus* sp., *Myroides* sp., *Kocuria* sp., *Alcaligenes* sp., and *Brevundimonas* sp.	*In vitro*	*Panchamirtham*, an Indian ethnic fermented fruit mix	Antimicrobial activity	Seventeen isolates exhibited antimicrobial activity against human pathogens: *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.*	Maheshwari et al. (2019)
Nineteen LAB strains	*In vitro*	*Theobroma cacao* fermented fruit juice	Antimicrobial activity	The strain showed inhibitory effect against 8 *H. pylori* clinical strains (Hp0011, Hp0012, Hp0013, Hp0014, Hp0015, Hp0016, Hp00116 and HP00117). Antagonistic effect was observed in 65.52% of isolated LAB. The LAB19 produced bacteriocins to control *H. pylori* and that LAB4′, LAB8, LAB11′, LAB12, LAB13′, LAB15, LAB16 and LAB17 were through organic acids. The overall inhibitory activity was two to three-fold reduced when CFSs were used instead of LAB isolates themselves.	Mabeku et al. (2020)
Multiple lactic acid bacteria strains	*In vitro*	Fermented broccoli, cherry, ginger, white radish, and white-fleshed pitaya juice	Antimicrobial activity	*L. plantarum* 445 exhibited the highest antagonistic activity against *E. coli*, *S. aureus*, and *L. monocytogenes* EGD-e, L. Already, *L. plantarum* 430 exhibited the highest antagonistic activities against *S. Enteritidis* and *S. Typhimurium*.	Xu et al. (2020)

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Strains	Type of study	Isolation Source	Assay	Main Effects	References
L. pentosus CHIG, NAG1, and *L. fermentum* PRS1.	*In vitro*	Fermented foods, fruits, and vegetables	Antimicrobial activity	Exhibited inhibition against food spoilage organisms and intestinal pathogens as *Escherichia coli, Enterobacter aerogenes, Salmonella typhi* MTCC 98, *Serratia marcescens* MTCC 97, *Shigella* sp., *Pseudomonas aeruginosa* MTCC2587, *Proteus vulgaris, Klebsiella pneumoniae, Yersinia enterocolitica* MTCC4858, *Enterococcus faecalis, Micrococcus luteus, Bacillus* sp, *Listeria monocytogenes* MTCC1143, *Staphylococcus aureus* MTCC1144, *Aspergillus niger* MTCC3496, *Aspergillus flavus* MTCC2798, *Rhizoctonia solani* MTCC633, *Penicillium roqueforti* MTCC933 and *Candida* strains.	Shekh et al. (2020)
L. fermentum 56, 60, 250, 263, 139,141 and 296, *L. plantarum* 53, *Lacticaseibacillus paracasei* 106 (former *Lactobacillus paracasei* 106)	*In vitro*	Different fruit processing byproducts	Antagonistic activity	Inhibition of *S. aureus* INCQS 00015, *S. typhimurium* INCQS 00150, *S. enteritidis* INCQS 00258, *L. monocytogenes* INCQS 00266, and *E. coli* INCQS 00219.	Albuquerque et al. (2018)
L. fermentum (DUR18), *L. plantarum* (DUR2, DUR5, DUR8), *Limosilactobacillus reuteri* DUR12 (former *Lactobacillus reuteri* DUR12), *Lactobacillus crispatus* (DUR4), *L. pentosus* (DUR20).	*In vitro*	*Tempoyak* (Malaysian fermented condiment)	Antimicrobial activity	The strains had inhibitory activities against the tested enteric pathogens (*Staphylococcus aureus, Listeria monocytogenes, Salmonella Typhimurium, E. coli and Pseudomonas aeruginosa*) with some variations between the strains and inhibition zone from 3.0 to 16.8 mm.	Khalil et al. (2018)
L. plantarum 3701, 3725, 3739, 3735, 25234, 25294, 2519, 3736, 3711, *L. casei* 3734	*In vitro*	Mature *Cornus officinalis* fruits	Antimicrobial activity	The strains present inhibition capacity to *E. coli ATCC* 25922, *Salmonella enteritidis* ATCC 13076 and *S. typhimurium* ATCC 14028, *Listeria monocytogenes* EGD-e and *S.aureus* ATCC 29213.	Tang et al. (2018)
Thirty different strains comprise *Lactobacillus (fermentum, plantarum,* and *brevis*), *Weissella cibaria, Enterococcus* (*faecium* and *faecalis*), *Leuconostoc* (*citreum* and *mesenteroides* subsp. *mesenteroides*) and *Pediococcus pentosaceus*.	*In vitro*	Horreh, a traditional Iranian fermented food	Antimicrobial activity	The strains had an important antimicrobial activity, *L. innocua* and *S. aureus* were the most sensitive indicator bacteria against the selected LAB species, while *E. coli* and *B. cereus* showed greater resistance.	Vasiee et al. (2018)
Leuconostoc mesenteroides E14, M67	*In vitro*	Fermented Silage (oat and barley) and honey	Antimicrobial activity	Both strains had an inhibitory effect against *Staphylococcus aureus* and *Escherichia coli*	Zarour et al. (2018)
Saccharomyces cerevisiae IFST062013	*In vitro*	Fruit (unspecified)	Antimicrobial activity	Showed moderate antimicrobial activity against bacteria and fungi.	Fakruddin et al. (2017)
L. plantarum CCMA0743, *Torulaspora delbrueckii* CCMA0235	*In vitro*	Cauim (blended of cassava and rice)	Antimicrobial activity	The strains inhibited *Salmonella enterica* subsp. *enterica serovar Typhimurium* ATCC 6538, *Escherichia coli* ATCC 11229, and *Listeria monocytogenes* ATCC 15313.	Freire et al. (2017)
Pediococcus acidilactici Ch-2	*In vitro*	Chuli (a fermented apricot product)	Antimicrobial activity	The strain was able to produce bacteriocin and lactic acid against serious food borne and spoilage microorganisms.	Gupta and Sharma (2017)
Saccharomyces cerevisiae 15A and 15B, *Candida norvegica* 7A	*In vitro*	Negrinha de Freixo cv. olives	Antimicrobial activity	*C. norvegica* had antimicrobial activity against *C. neoformans.*	Oliveira et al. (2017)
Lacticaseibacillus casei 24 (former *Lactobacillus casei* 24), *L. fermentum* 38, 47, 62, and *L. plantarum* 81,90, 96, 100 and 105	*In vitro*	Fermentation Process of “Cupuaçu” *Theobroma grandiflorum*	Antimicrobial activity	The strains produced diffusible inhibitory compounds	Ornellas et al. (2017)

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Strains	Type of study	Isolation Source	Assay	Main Effects	References
Four strains: *L. plantarum* S-811 and S-TF2, *Fructobacillus fructosus* S-22, and *F. fructosus* S-TF7 from seventeen isolated of LAB	*In vitro*	Fresh and spoiled cactus (*Opuntia ficus-indica*) pears of the green cultivar	Antimicrobial activity	Only S-22, S-811, S-TF1, S-TF2 and S-TF7 isolates showed some antibacterial effect against juice contaminant *Bacillus* sp.	Verón et al. (2017)
Sixteen strains*:* 13 *L. pentosus* and 3 *L. plantarum*	*In vitro*	Table olives biofilms	Antimicrobial activity	The genotypes assayed were able to produce the inhibition of the food-borne pathogens as E. *coli* and *L. monocytogenes*	Benítez-Cabello et al. (2020)
Levilactobacillus brevis 59 (former *Lactobacillus brevis* 59), *L. pentosus* 129, *L. paracasei* 108, *L. plantarum* 49, and *L. fermentum* 111	*In vitro*	Byproducts of fruit pulp process *Malpighia glabra* L., *Mangifera indica* L., *Annona muricata* L., *and Fragaria vesca* L.	Antimicrobial activity	The strains showed capacity to inhibit *Staphylococcus aureus, Salmonella Typhimurium, Salmonella Enteritidis, Listeria monocytogenes and Escherichia coli.*	Garcia et al. (2016)
L. plantarum F22	*In vitro*	Traditional inoculum Phab used in chhang beverage	Antimicrobial activity	The strain showed antagonistic effect against *Staphylococcus aureus* IGMC, *Enterococcus faecalis* MTCC 2729, *Listeria monocytogenes* MTCC 839, *Clostridium perfringens* MTCC 1739, *Leucononstoc mesenteroids*, MTCC 107, and *Bacillus cereus* CRI.	Handa and Sharma (2016)
L. plantarum E1/18, E5/6, S0/3, S0/7 and S3/16	*In vitro*	Fermented stinky bean (Sa Taw Dong)	Antimicrobial activity	The strain inhibited *Bacillus cereus* MST5040, *E. coli* DMST4212, *Listeria monocytogenes* DMST17303, *Staphylococcus aureus* DMST8840 and *Candida sorphophila* SM01 and was able to inhibit the growth of coliform during the fermentation.	Saelim et al. (2017)
L. plantarum DGK-17	*In vitro*	Kimchi	Antimicrobial activity	Nutritionally improved DGK-17 showed antimicrobial activity against several pathogenic microorganisms with enhanced antimicrobial activity and bactericidal effect against *Pseudomonas aeruginosa* 03K711 and *Klebsiella pneumoniae* 05K279. Also, DGK-17 protects colon cells against P. aeruginosa (03K711) and *K. pneumoniae* (05K279).	Khan and Kang (2016)
Endophytic *Bacillus* sp CaB5	*In vitro*	Surface of young fruits of *Capsicum annuum*	Antimicrobial activity Screening for presence of potential biosynthetic gene clusters, the ability to influence in plant growth.	Identification of non-ribosomal peptide synthases, PKS Type I, iturin, surfactin, DAPG and gacA genes in the isolate CaB 5 from *Capsicum annuum.* The strain proved growth enhancement on *Vigna radiata.*	Jasim et al. (2016)
L. plantarum (277, 281, 286, 289, 291) *L. fermentum* (260, 265, 266)	*In vitro*	Brazilian cocoa fermentation	Antimicrobial activity	All strains showed varying degrees of pathogen inhibition in the four tests evaluated.	Santos et al. (2016)
Levilactobacillus spicheri G2 (former *Lactobacillus spicheri* G2).	*In vitro*	Gundruk a non-salted, fermented acidic vegetable	Antimicrobial activity	The strain showed antagonistic spectrum against foodborne pathogens/food spoilage bacteria as *Listeria monocytogenes* MTCC 839, *Leuconostoc mesenteroides* MTCC 107, *Enterococcus faecalis* MTCC 2729, *Lactobacillus plantarum* CRI, *Bacillus cereus* CRI, *Clostridium perfringens* MTCC 1739, *Pectobacterium carotovorum* MTCC 1428, *Escherichiacoli* IGMC, *Staphylococcus aureus* IGMC and *Aeromonas hydrophila* IGMC.	Gautam and Sharma (2015)
Strains: *Candida* (2 strains), *Kluyveromyces* (4 strains), *Lachancea* (1strain), Metschnikowia (5 strains), *Pichia*	*In vitro*	Strains from different sources of which 66 strains were from grape must	Antimicrobial activity Killer phenotype	Only *S. bayanus* IFI-702, *S. cerevisiae* IFI-716 and CYC-SGA, *M. pulcherrima* IFI-1242 and *W. subpelliculosa* IFI-1253	Gil-Rodríguez et al. (2015)

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Strains	Type of study	Isolation Source	Assay	Main Effects	References
(10 strains), *Saccharomyces* (68 strains), *Schizosaccharomyces* (5 strains), *Torulaspora* (23 strains), *Wickerhamomyces* (9 strains), *Yarrowia* (2 strains) and Zygosaccharomyces (1 strain)				(8.5% of the 59 strains studied) displayed killer activity in at least one of the conditions tested. Overall, the killer phenotype was not widely distributed amongst the yeasts tested, as it only appeared in 8.5% of the strains. Interestingly, all positive yeasts to killer exhibited activity against strains belonging to other species, and sometimes, even to different genera.	
L. plantarum 0103, 0123, 0140, 0147, 0157, 0611, 0612, 0825, 1002; *L. brevis* 0808, 08771, (21%), *L. rhamnosus* 0504, *Lactobacillus delbrueckii*, *L. paracasei* and *Pediococcus pentosaceus* 1001	*In vitro*	Malaysian fermented *Bambangan* (*Mangifera pajang*)	Antimicrobial activity	The strains showed antibacterial activity to pathogens *Staphylococcus aureus* ATCC 25,923, *Listeria monocytogenes* ATCC 13,932, *Salmonella Typhimurium* ATCC 13,311, *S. Enteritidis* ATCC 13,076 and *Yersinia enterocolitica* ATCC 23,715.	Ng et al. (2015)
L. plantarum KCC-24	*In vitro*	Italian rye-grass (*L. multiflorum*) forage	Antimicrobial activity	The strain showed antifungal activity against strains of *Aspergillus fumigatus*, *Penicillium chrysogenum*, *P. roqueforti*, *Botrytis elliptica*, *Fusarium oxysporum*	Vijayakumar et al. (2015)
Pichia kudriavzevii DCNa1 and *Wickerhamomyces subpelliculosus* DFNb6	*In vitro*	Different fruits	Culture-based bacteriological analyses of faecal batches Chemical detection of acids and volatile compounds	Yeasts modulated the intestinal microbiota increasing beneficial microbes.	Di Cagno et al. (2020)
L. fermentum 296	*In vivo*	Fruit pulp processing byproducts	Male *Wistar* rats divide in 3 groups: Control (CTL) that received a commercial diet and saline solution; (HF) received a High-fat commercial diet and saline solution and (HF + Lf 296) treated with High Fat (HF) diet + *L. fermentum* 296 in a solution of about 1x10^9 CFU/mL. Administration was performed daily for 4 weeks by oral gavage. Fecal microbiota	The administration of *L. fermentum* 296 for 4 weeks recovered fecal *Lactobacillus* sp. counts	Cavalcante et al. (2019)
L. fermentum 139, 263 and 296.	*In vivo*	Fruit by-products	Wistar rats (n = 14) were fed with a control diet (CTL = 7) or a dyslipidemic diet (DLP = 7) during pregnancy and lactation. After weaning, male and female offspring received a standard diet up to 90 days of life. Rats were allocated groups: CTL group + saline solution (n = 14); DLP group + saline solution (n = 14) and DLP group receiving a probiotic cocktail (n = 14). A CLT or probiotic formulation containing *L. fermentum* 139, 263 and 296 at 1 × 10^9 CFU/mL) was administered daily by oral gavage/8 weeks. Microbial count on fecal samples were evaluated.	The group that received the probiotic showed improvement in fecal *Lactobacillus* sp. counts The microbial count varied among male and female offspring and the probiotic intervention for eight weeks resulted in increase in fecal counts of *Lactobacillus* spp. in the DLP male and female offspring and decreased the fecal counts of *Enterobacteriaceae* when compared to the CTL and DLP groups.	Oliveira et al. (2020)
L. plantarum 49, and *L. plantarum* 201	*In vivo*	Pulp of *Mangifera indica* L., or from industrial fruit pulp processing byproducts of *Malphigia glabra* L., *M. indica* L., *Annona muricata* L. and *Fragaria ananassa* L.	Sixteen L. *plantarum* strains were used as growth promoting effects models in *Drosophila melanogaster*. Two strains were selected to observe the safety aspects and beneficial effects of strains using Wistar rats (n = 48)/21 days. The animals were randomly distributed into three groups (n	The strains were detected in the intestine.	Costa et al. (2019)

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Strains	Type of study	Isolation Source	Assay	Main Effects	References
			= 16): group Lp49, which received 9 log CFU/mL of strain *L. plantarum* 49; group Lp201, which received 9 log CFU/mL of strain and control group, which received PBS daily by gavage/4 weeks.		
			Fecal microbiota		
L. plantarum Q823	*In vivo*	Different quinoa varieties and amaranth seeds	Seven health individual consumed 20 mL of the quinoa-based beverage containing 9.19 Log10 CFU/mL once a day for 7 day and for the subsequent period (washout) for another 7 days Fecal samples were analyzed to observe colonization.	Fecal counts of 5–7 Log10 CFU/g from the third day onwards. The counts started to decrease after the final dose. *L. plantarum* Q823 was still present in all subjects four days after the end of administration and remained present after seven days in three of the subjects.	Vera-Pingitore et al. (2016)

single dose) by mice resulted in decreases in the production of IFN-γ and IL-6 (pro-inflammatory cytokines) and increases in the production of regulatory cytokine IL-10. Therefore, an anti-inflammatory activity was observed (Ornellas et al., 2017). Probiotic cultures can metabolize non-digestible compounds and produce short-chain fatty acids, which contribute to immunity (Song et al., 2020).

2.2.3. Adipogenesis inhibition and body weight control

In vitro tests have demonstrated that probiotic cultures from unconventional sources could inhibit adipogenesis in adipocytes. *Latilactobacillus sakei* ADM14 (former *Lactobacillus sakei* ADM14) isolated from kimchi decreased the intracellular content of triglycerides on 3T3-L1 adipocytes and reduced the expression of adipogenic marker genes (aP2, PPARγ, FAS, C/EBPα, and CD36), resulting in inhibition of adipogenesis (Won et al., 2020). In an *in vivo* test, the consumption of cactus pear juice fermented with *L. plantarum* S-811 isolated from cactus pear (10^9 CFU/mL, 7 weeks) by obese mice resulted in decreases in body weight gain (Verón et al., 2019). In another study, the consumption of *L. sakei* HAC10 and *L. plantarum* HAC01 isolated from Kimchi (Korean traditional fermented food, 10^8 CFU/mL, 10 weeks) by obese mice resulted in lower total weight gain and body weight (Park et al., 2016). EPS produced by probiotic cultures can have inhibitory effects on adipogenesis and body weight gain (Won et al., 2020).

2.2.4. Antimicrobial activity and improvement of intestinal microbiota

The production of antimicrobial compounds, such as organic acids, short-chain fatty acids, and bacteriocins is a characteristic of probiotics (Lakra et al., 2020; Leandro et al., 2020). The inhibition of the classic foodborne pathogens *(E. coli, S. aureus,* and *L. monocytogenes, S. Enteritidis and S. Typhimurium)* by probiotic cultures is advantageous, is considered as an appropriate alternative to antibiotic treatment, and could increase the shelf life of food products (Xu et al., 2020). Antimicrobial properties against classical foodborne pathogens have been reported in several studies with LAB isolated from unconventional sources, such as forage plants, artisanal salami, and cocoa beans (Leandro et al., 2020), fruits (Pabari et al., 2020; Taroub et al., 2019), vegetables (Junnarkar et al., 2019), flowers (Patil et al., 2020; Sakandar et al., 2019), plant leaves (Samedi & Charles, 2019), and fermented foods (Cao et al., 2019; Cervantes-Elizarrarás et al., 2019; Maheshiwari et al., 2019). In another study, LAB strains isolated from *Theobroma cacao* fermented fruit juice were able to inhibit *Helicobacter pylori* by producing bacteriocins or organic acids. This is important, as *Helicobacter pylori* is recognized as an infectious agent, causing gastritis, ulcers, and tumors (Mabeku et al., 2020). In a general view, the antimicrobial effect of probiotic cultures was more pronounced for Gram-negative bacteria than Gram-positive bacteria (Xu et al., 2020).

Probiotic cultures may modulate the intestinal microbiota. Anaerobes are the most found microorganisms in the small intestine, but, in cases of dysbiosis they can be replaced by other microorganisms, mainly hemolytic bacteria (*Staphylococcus* spp.). This can result in inflammation in the intestine and/or dysfunction of the gut epithelial cells (Di Cagno et al., 2020). Probiotic cultures isolated from fruits, vegetables, flowers, and traditional fermented beverages and included in food products may improve the intestinal microbiota. *Wickerhamomyces subpelliculosus* DFNb6 and *Pichia kudriavzevii* DCNa1 were used as starter cultures in cornelian cherry beverage. *In vitro* tests showed that the functional beverage improved the number of total anaerobes bacteria and decreased the number of *Staphylococcus* spp., with better results than FOS, a recognized prebiotic compound (Di Cagno et al., 2020).

In *in vivo* tests, the consumption of *L. fermentum* 296 isolated from fruit (Cavalcante et al., 2019), *L. fermentum* 263, *L. fermentum* 139, and *L. fermentum* 296 isolated from fruit by-products (Oliveira, Cavalcante, et al., 2020), and *L. plantarum* 49 and *L. plantarum* isolated from fruits (Costa et al., 2019) by rats increased the counts of fecal *Lactobacillus* spp. and decreased *Enterobacteriaceae* counts (Oliveira, Cavalcante, et al., 2020). In a clinical trial, the consumption of a probiotic beverage based on quinoa (*L. plantarum* Q823 isolated from quinoa and amaranth seeds, 20 mL, 10^9 CFU/mL, 7 days) by healthy female individuals resulted in the survival of the probiotic culture to the gastrointestinal tract and persistence for 5–7 days after the period of washout (Vera-Pingitore et al., 2016). The results indicate that probiotic cultures could survive and colonize the gastrointestinal tract of both animals and humans (Costa et al., 2019; Vera-Pingitore et al., 2016). However, further studies are needed, as clinical trials only evaluated females and with health status.

3. Techno-functional potential of strains isolated from unconventional sources

3.1. Fruits, vegetables, and flowers

Fruits, vegetables, and flowers are considered carriers of a wide variety of microorganisms, belonging to the group of bacteria, yeasts, and molds. The diversity and concentration of the microorganisms are associated with several factors such as water quality, environmental conditions, maturation stage, among others (Fessard & Remize, 2019; Rodríguez et al., 2019). In recent years, a series of studies have discovered new microorganisms isolated from fruits and vegetables with high potential for use in food processing, to improve their nutritional and sensory quality, or acting as natural biopreservatives (Barros et al., 2019; Costa et al., 2018; Di Cagno et al., 2016; Linares-Morales et al., 2020). However, studies involving the isolation of microorganisms from flowers are still scarce (Patil et al., 2020; Sakandar et al., 2019). Fig. 3 presents the main techno-functional properties of microorganisms isolated from fruit, vegetables, flowers, and ethnic fermented beverages.

Table 3 presents the main studies that evaluated the techno-functional properties of strains isolated from fruits, vegetables, and flowers. *Lactobacillus, Leuconostoc, Weissella, Enterococcus,* and *Pediococcus* are the LAB genera most frequently isolated from fruits, vegetables, and flowers (Di Cagno et al., 2016). However, LAB represent a minority part of the indigenous microbiota of these products, and several yeast strains have also been isolated, such as *Saccharomyces boulardii, Saccharomyces cerevisiae, Pichia anomala, Rhodotorula mucilaginosa, Candida* spp., and *Ochrobactrum* spp. (Barros et al., 2019; Habiba et al., 2019; Souza et al., 2017).

The LAB and yeast isolated from fruit and vegetables have shown the ability to inactivate pathogenic and contaminant microorganisms (Martins et al., 2019; Peng et al., 2020; Pereira et al., 2015; Souza et al., 2017). In *in vitro* tests, the growth of foodborne pathogens (*Listeria monocytogenes)* and contaminants (*Fusarium oxysporum)* was inhibited by *Leuconostoc mesenteroides, Enterococcus mundtii* and *Enterococcus faecium* isolated from pepper (chilaca and jalapeno), guava, green apple, corn, and orange (Linares-Morales et al., 2020). *Saccharomyces cerevisiae* IFST 062013 (9 log cfu/mL) isolated from fruits exhibited moderate antibacterial (mainly against Gram Negative bacteria) and antifungal (*Aspergillus, Penicillium* and *Rhizopus*) activities when compared to controls (doxycycline and fluconazole, respectively) (Fakruddin et al., 2017). *Pichia anomala* CCMA0148, *Saccharomyces cerevisiae* CCMA0159 and *Rhodotorula mucilaginosa* (4–7 log cfu/mL) isolated from coffee and cocoa beans exhibited high potential as biocontrol agents, inhibiting the growth of the toxigenic fungi *Aspergillus carbonarius* (CCDCA 10608 and CCDCA 10408) and *Aspergillus ochraceus* (CCDCA 10612) after cultivation at 28 °C for seven days, with a reduction of up to 53% in the mycelial growth (*A. ochraceus* CCDCA10612). Furthermore, there was less production of ochratoxin (OTA) (Souza et al., 2017), which is important as OTA is recognized as a substance with hepatotoxic, nephrotoxic, immunosuppressive, and teratogenic effects, and it can cause kidney and liver tumors (Souza et al., 2017). The results demonstrate the potential of using fruit and vegetable-derived strains as bioprotective agents, based on *in vitro* tests, which open opportunities to increase food products' shelf life.

The increase of shelf life of perishable products using biopreservatives is of industrial interest, mainly due to consumers' demand for products with no synthetic preservatives. *L. plantarum* 49, *L. paracasei* 108 and *L. plantarum* 201 isolated from fruits (mango and acerola) and industrial fruit pulp processing by-products (mango, acerola, and soursop) were able to antagonize the growth of food-related bacteria *in vitro* (5 log cfu/mL, *Staphylococcus aureus* ATCC 952806, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 1915, *Salmonella Enteritidis* PT4 and *S. Typhimurium* PT4). Furthermore, these strains showed a high capacity to produce organic acids and were effective as biopreservatives in Minas Frescal cheese and ground chicken breast (Costa et al., 2018). The results suggest that LAB and yeasts' antimicrobial effect isolated from fruits and vegetables are strain-specific and may be a potentially replacer for synthetic preservatives (Souza et al., 2017).

Fruit and vegetables are very perishable products, resulting in physiological deterioration and changes in the nutritional and quality characteristics due to consumption of internal water and use of reserve substances by microorganisms. In products sensitive to fungal infection, it is demanded alternatives that may act against phytopathogens, which opens opportunities for LAB and yeasts isolated from fruits and vegetables (Habiba et al., 2019). The endophytic strain *L. plantarum* CM-3 isolated from strawberry (*Fragaria* × *ananassa* Duch. Cv. "Hongyan") exhibited inhibitory activity against *Botrytis cinerea,* which is a fungus recognized for causing grey mold and a severely damage disease in strawberries. This microorganism decreased mycelial growth (*in vitro*), and inhibited spore germination and the incidence and diameter of the lesions (*in vivo*) (Chen et al., 2020). *Saccharomyces boulardii* and *Saccharomyces cerevisiae* (7 log cfu/mL) obtained from the surfaces of fruits (mango, orange, grapefruit, and lemon) and fresh vegetables (green pepper and tomato) exhibited antagonistic activity against phytopathogens, being the maximum activity observed against *Penicillium digitatum.* In the *in vivo* tests, the yeast delayed natural postharvest infection by *P. digitatum* in kinnow fruits, preserving their quality (lower loss of soluble solids and weight and higher total phenolic content)

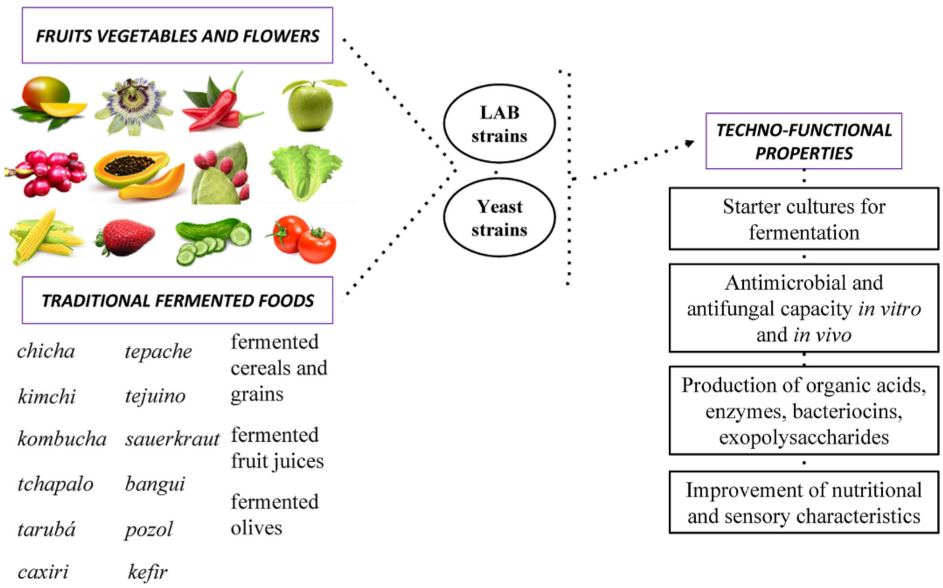

Fig. 3. Unconventional sources of microbial isolation and their techno-functional properties.

Table 3
Main studies that evaluated the technological properties of strains isolated from fruits and vegetables.

Microorganisms	Isolation source	Experiments	Analysis performed	Insightful findings	References
LAB strains *Lactiplantibacillus plantarum CM-3* (former *Lactobacillus plantarum* CM-3) (selected)	Strawberry fruit (*Fragaria* × *ananassa* Duch. cv. "Hongyan")	It isolated endophytic LAB strains and evaluated their potential in relation to *in vitro* and *in vivo* biocontrol against *Botrytis cinerea*.	Evaluation of the inhibitory effect of isolates against *B. cinerea in vitro.* Identification and characterization of the most potent antagonist Analysis of mycelial growth, spore germination and elongation of the germ tube Biocontrol efficacy of CM-3 against *B. cinerea in vivo* (Storage at 20 °C for 6 d) Evaluation of colonization of the selected antagonist in strawberry wounds Concentration: *L. plantarum* CM-3: (6, 7, 8 and, 9 log cfu/mL^{-1}) *B. cinerea:* (1 × 10^5 spores mL^{-1})	*L. plantarum* CM-3 demonstrated the highest potential for *in vitro and in vivo* control of *B. cinerea* *L. plantarum* CM-3 decreased the mycelial growth of *B. cinerea in vitro* (between 55.27% and 79.80%) Inhibition of spore germination in the presence of living cell suspensions and reduction of decay incidence and lesion diameter of grey mold decay in *in vivo* biocontrol assays Concentrations of 8 and, 9 log cfu/mL^{-1}, decreased the incidence of grey mold in 48.23% and 75% respectively; Cell-free filtrates of the strain failed to provide any protection against *B. Cinerea*	Chen et al. (2020)
LAB strains	Fresh fruits and vegetables Chilaca chili, Guava, Green apple, Jalapeño chili, Corn, Orange, Zucchini, Peach, Red apple, pear, Green tomato, Pomegranate, Lettuce, Mandarin, Cucumber, Bell pepper, Grape, Soybean sprout, Nopal flower (Obtained from Farmlands, Street markets, and Supermarkets in Chihuahua City, Mexico)	It isolated strains of LAB from different fruits and vegetables and evaluated the antimicrobial activity and the enzymatic and adhesion activity of these isolates.	Isolation of Lactic Acid Bacteria (LAB) Antimicrobial Activity (against Gram-positive, Gram-negative, LAB, mold, and yeast strains) Protease, lipase, amylase and citrate metabolism Adhesion Capacity Biofilm Formation Molecular Identification	76 LAB isolates were obtained. Largest amounts of LAB were isolated from chilaca chilis (10), guava (8), green apple, jalapeno chilis, corn (6) and orange (5). LAB strains of *Enterococcus faecium* PIM4, *Enterococcus mundtii* ELO8, and *Leuconostoc mesenteroides* (PIM5, CAL14) strongly inhibited the growth of *L. monocytogenes* *Enterococcus mundtii* (JAV15, TOV9) strongly inhibited the growth of *F. oxysporum.* Seven isolates hydrolyzed starch, 46 proteins, 14 lipids, and 36 metabolized citrate. LAB isolates with the best activities were molecularly identified as *Leuconostoc mesenteroides, Enterococcus mundtii* and *Enterococcus faecium.*	Linares-Morales et al. (2020)
L. plantarum 53, *Lacticaseibacillus paracasei 106 (former Lactobacillus paracasei 106), Limosilactobacillus fermentum* 56 *(former Lactobacillus fermentum* 56) and *Lacticaseibacillus casei* L-26 (former *Lactobacillus casei* L-26)	Pulp fruit of *Malphigia glabra* L. and *Mangifera indica* L. or Byproducts of *M. glabra* L., *M. indica* L. and *Annona muricata* L.	It evaluated the effects of mixed culture of four potentially probiotic *Lactobacillus* isolates on the physicochemical characteristics, contents of bioactive compounds and antioxidant activity of acerola and guava industrial by-products submitted to a submerged fermentation process for 120 h.	Enumeration of viable counts (zero - right after homogenization and after 8, 24, 48, 72 and 120 h of fermentation). Physico-chemical analysis (color, pH, titratable acidity and total soluble solids). Determination of bioactive compounds Analysis of antioxidant activity by FRAP and ABTS. Determination of the content of ascorbic acid, carotenoids, total flavonoids and total phenolics. Concentration: LAB: (10 log cfu/mL) Mix of strains (1:1:1:1 ratio).	The viable counts of LAB strains after the 120-h fermentation of the by-products were ≥5.5 log cfu/mL The fermentation increased the luminosity, titratable acidity, total flavonoids, total phenolics and antioxidant activity and decreased the content of total soluble solids and the pH in the two by-products tested. Ascorbic acid content decreased in the fermentation of the acerola by-product and increased in the guava. There were no significant changes in the carotenoid content. Different phenolic compounds (mainly Kaempferol and quercetin) were found during the fermentation of the by-products.	Oliveira et al. (2020)
Yeasts strains	Fresh fruits and seeds of: Achachairu (*Garcinia humilis*), Araçá-boi (*Eugenia*	It isolated endophytic strains of yeasts present in the fruits of achachairu, araçá-boi and bacaba, and evaluated their potential	Identification of microorganisms Fermentation Determination of total phenolic compounds	Main genera identified were *Candida, Rhodotorula and Ochrobactrum.* The largest number of strains detected was in araçá-boi (3),	Barros et al. (2019)

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Table 3 (*continued*)

Microorganisms	Isolation source	Experiments	Analysis performed	Insightful findings	References
	stipitata) and Aacaba (*Oenocarpus bacaba)* fruits	in the production of bioactive compounds and their antioxidant activity.	Identification and quantification of phenolic and flavonoid compounds by UHPLC-QqQ-MS/MS Analysis of antioxidant activity. Concentration: Yeasts: (7 log cfu/mL)	followed by bacaba (2) and achachairu (1). *Rhodotorula glutinis* produced the highest amount of total phenolics among all 3 fruit residues and several species of isolated microorganisms. The use of bacaba waste as a medium resulted in a higher production of total phenolics. Higher concentrations were found for chlorogenic acid (460.00%), catechin (94.75%) and rutin (27.53%). The antioxidant activity increased [ORAC (118.62%), FRAP (90.32%) and ABTS (39.25%)] in fermented products.	
LAB strains	Pickled white cabbage (*Brassica oleracea* var. capitata), Papaya (*Carica papaya*) and Tomatoes (*Lycopersicon esculentum*)	Identified and characterized LAB strains isolated from fruits and vegetables, as well as evaluated the ability of isolates to produce exopolysaccharides (EPS), the influence of growth temperature and tolerance to acid, salt, bile salts or hydrogen peroxide.	Ability to produce exopolysaccharides (EPS) Identification of species and typing Analysis of tolerance to acid, oxidative and osmotic stress and bile salts Analysis of growth parameters	77 isolated strains belonging to the species *Lactobacillus plantarum* (3), *Lactobacillus* spp., (3), *Lactococcus lactis* (13), *Leuconostoc pseudomesenteroides* (25), *Leuconostoc lactis* (1), *Leuconostoc mesenteroides* (7), *Leuconostoc citreum* (14), *Weissella cibaria* (4), *Weissella confusa* (4), other *Weissella* species (2) and *Fructobacillus tropaeoli* (1). Several isolates from *Weissella* and *Lactobacillus* were particularly tolerant to acid and osmotic stress. *Lc. pseudomesenteroides* 60 was highly tolerant to oxidative stress. Weissella 30, 64 and 58, *Leuconostoc* 60 and 12b, *Lactobacillus* 75 and *Fructobacillus* 77 were the isolates that have the best characteristics for their use as starters or as preservatives.	Fessard and Remize (2019)
Isolates of *Saccharomyces boulardii* e *Saccharomyces cerevisiae*	Fresh fruit and vegetables surfaces: Mango (*Mangifera indica* L.) Green chili (*Capsicum annuum* L.) Orange (*Citrus sinensis* (L.) Osbeck cv. Valencia) Grape fruit (*Citrus paradise* Macfad.) Lemon (*Citrus limon* (L.) Burm.) and Tomato (*Lycopersicon esculentum* Mill.)	Isolation and identification of epiphytic yeasts from healthy fruit and vegetables, and their role in delaying the natural postharvest *P. digitatum* rot and physiochemical properties in kinnow at room temperature to 15 days.	Isolation and identification of epiphytic yeast In vitro antifungal assay against *Penicillium digitatum* Evaluation of yeast in delaying natural postharvest infection of *P. digitatum* in kinnow fruits Evaluation of yeast in delaying post-harvest decomposition in kinnow Physiochemical Analysis Concentration: Yeasts: (7 log cfu/mL) *P. digitatum* spores: (6 log cfu/mL)	25 strains of epiphytic yeasts were isolated, 20 of these showed maximum *in vitro* activity against *P. digitatum.* Eight selected isolates showed less infection of *P. digitatum* with minimal loss of quality compared to the control and positive control (1% K sorbate). HAB-31 and HAB-53 isolates showed better characteristics. Decreased weight loss (18.39% and 18.87%, respectively), soluble solids and improved phenolic content. No effects on firmness.	Habiba et al. (2019)
S. cerevisiae CCMA 0200 (former UFLA CA11) *T. delbrueckii* CCMA 0684	Sugar cane *Coffea arabica* L. var. Bourbon5	Evaluated the performance of yeasts *Saccharomyces cerevisiae* CCMA 0200 e *Torulaspora delbrueckii* CCMA 0684 as a starter in coffee varieties processed by wet method fermentation and the impact on sensory quality and compound profile	Microorganisms quantification Analysis of acids and sugars Volatile compounds Sensory analysis Concentration: Yeasts: (7 log cfu/mL)	*T. delbrueckii* CCMA 0684 showed better adaptation to the process (72 h of drying). Eighteen volatile compounds were detected in green coffee and 75 in roasted coffee. 2-Furanmethanol propanoate and 2-ethyl-3,5-dimethylpyrazine were identified only in the inoculated treatments. All treatments received scores greater than 80 in the sensory analysis. *T. delbrueckii* CCMA 0684 exhibited better results in	Martins et al. (2019)

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Table 3 (*continued*)

Microorganisms	Isolation source	Experiments	Analysis performed	Insightful findings	References
				sensory analysis, being preferable for use as a starter.	
LAB strains	Ripe wild fruits of guava (pink and yellow varieties), Papaya, Passion fruit, Custard apple, Medlar, Mulberry, Fig, Khaki, and Flowers of Medlar, Passion fruit, Custard apple, Papaya	Evaluated the lactic microbiota of these tropical fruits and wild flowers and their technological properties of metabolite production.	Microbiological Analyses and Isolation of LAB Analysis of metabolic properties (mannitol and organic acids production) Analysis of aroma compounds (diacetyl production) Analysis of technological properties (Acidifying capacity and growth rate bacterial, pectinolytic activity, cinnamoyl esterase activity, biogenic amine production)	Bacteria of the genera *Enterococcus, Fructobacillus, Lactobacillus, Lactococcus, Leuconostoc* and *Weissella* were observed, and 21 different species were identified Most of the strains found were capable of producing mannitol, organic acids and aromatic compounds; as well, some had activities of cinnamoyl esterase, pectinase and esterase.	Rodríguez et al. (2019)
Sixteen LAB strains	Pulp fruit of *Malphigia glabra* L. and *Mangifera indica* L. or Byproducts of *M. glabra* L., *M. indica* L. and *Annona muricata* L.	Evaluated the antagonistic activity of sixteen LAB strains derived from fruits against food-related bacteria and the effects on the survival of *L. monocytogenes* and *S. Enteritidis* PT4 in fresh cheese and ground chicken breast	Antagonistic activity assays Assays with cell-free culture supernatants Analysis of organic acids production Bioconservation analyzes in ground chicken and cheese "Minas Frescal" Analysis of production of organic acids in the matrices. Concentration: LAB strains and indicators: (5 log cfu/mL)	All LAB strains tested were able to antagonize the growth of food-related bacteria *in vitro*. The highest production of organic acids was detected for *L. plantarum* 49, *L. paracasei* 108 and *L. plantarum* 201. *L. plantarum* 49 and *L. paracasei* 108 decreased *L. monocytogenes* counts in cheese. *L. paracasei* 108 decreased the count of *S. Enteritidis* PT4 in chicken breast.	Costa et al. (2018)
Saccharomyces cerevisiae IFST 062013	Fruit (non-specified)	Evaluated the antimicrobial and antioxidant activities of whole cells, culture supernatant and cell lysate of the isolated *Saccharomyces cerevisiae* IFST 062013	Analysis of antibacterial and anti-fungal activity Analysis of antioxidant activity. Concentration: *Saccharomyces cerevisiae* IFST 062013: (9 log cfu/mL)	*Saccharomyces cerevisiae* IFST 062013 showed moderate antibacterial and antifungal activity when compared to the control (doxycycline and fluconazole) Cell lysate showed more effective antibacterial and antifungal effects. Antibacterial effect of the isolate was better against gram negative pathogens *Saccharomyces cerevisiae* IFST 062013 showed strong antioxidant activity, reducing power, sequestering activity of nitric oxide and hydroxyl radical, significant cytotoxicity of artemia and acute toxicity and chelating activity of metal ions.	Fakruddin et al. (2017)
Twenty-eight LAB strains	Tomatoes (*Lycopersicon esculantum*), Papaya (*Carica papaya*) and Sliced cabbage (*Brassica oleacera* var. capitata)	Evaluated the production of exopolysaccharides (EPS) and the acidification kinetics of LAB isolated from fruit and its potential as a starter for fermentation in pineapple juice (40 mL), teas (green and black) (40 mL) or fruit puree (papaya or mango) (40 g) with views for technological use.	Microbiological analyses and isolation of LAB Analysis of EPS production and acidification kinetics Growth in apple juice Production of fermented Physico-chemical analyzes and viability Determination of sugar and phenolic content Sensory analysis Analysis of antioxidant activity Concentration: LAB: (5 log cfu/mL)	34 strains of the genera *Lactobacillus, Leuconostoc, Weissella, Lactococcus and Fructobacillus* were isolated. Among these, 13 species were represented. EPS formation was observed for all strains of *Leuconostoc* spp., *W. confusa* and *W. cibaria*. The kinetic parameters of acidification showed different behavior within the group of strains. Fermented juices were sensorially appreciated. *W. cibaria* 64 produced little grew in apple or pineapple juice, however, significantly increased the phenolic content and antioxidant activity. *Lc. pseudomesenteroides* 12b increased antioxidant activity in fermented papaya pulp, however, decreased phenolic content in infusion of green tea and mango pulp.	Fessard et al. (2017)

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Table 3 (*continued*)

Microorganisms	Isolation source	Experiments	Analysis performed	Insightful findings	References
LAB strains	Potato tocosh peruvian (freshly harvested potatoes, one-month and eight-months production)	Evaluated the diversity of LAB present in tocosh, in addition to the safety aspects and the biotechnological potential of these isolates for future commercial applications	Microbiological analyses and isolation of LAB (both culture-dependent and -independent approaches) Analysis of exopolysaccharides (EPS) production Analysis of phytate degradation activity and amylolytic activity Analysis of group B vitamin production and biogenic amines production Analysis of antimicrobial activity	The genus *Lactobacillus* was predominant in LAB populations at any stage of tocosh fermentation *Latilactobacillus sakei* (former *Lactobacillus sakei*) and *Lc. mesenteroides* were present in all tocosh samples. Of the twenty-four LAB species detected, six were recovered by culture. *Lb. sakei* and *Ln. mesenteroides* strains exhibited the ability to break down amylase and phytate, as well as the production of EPS and B vitamins (riboflavin and folate) Tocosh LAB species showed antibacterial activities, as well as the ability to produce biogenic amines.	Jiménez et al. (2017)
Thirty-two yeasts strains	Coffee and Cocoa beans	Evaluated the antagonistic potential of yeast isolates against *Aspergillus carbonarius* (CCDCA 10608 and CCDCA 10408) and *Aspergillus ochraceus* (CCDCA 10612) after cultivation at 28 °C for seven days.	Toxigenic fungi biocontrol *in vitro* assays Evaluation of vegetative growth Evaluation of *Aspergillus* spore production Antagonistic activity of different water activity over ochratoxin (OTA) production Analysis of antagonistic activity by potential yeast strain at cellular level. Concentration: Yeasts: (4–7 log cfu/mL) *Aspergillus:* (10^5 spores mL^{-1})	Yeasts showed greater inhibitory effects (53%) on the mycelial growth of the isolate *A. ochraceus* (CCDCA10612). *Pichia anomala* CCMA0148 and *Saccharomyces cerevisiae* CCMA0159 provided the greatest growth inhibition of all fungal strains. *Rhodotorula mucilaginosa* was effective in inhibiting the production of OTA by the three isolates of *Aspergillus.* *S. cerevisiae* CCMA0159 and *Pichia anomala* CCMA0148 showed high potential as biocontrol agents under the conditions tested	Souza et al. (2017)
Leuconostoc mesenteroides strains	Fresh fruits of *Opuntia ficus-indica* (L.) Mill., (genotype Sanguigna)	It evaluated the technological potential of autochthonous lactic acid bacteria isolated from prickly pear (*Opuntia ficus-indica* L.) fruits as a starter for fermentation, as well as improvement in shelf life, rheological, sensory and functional characteristics in prickly pear fruit puree	Isolation, identification and starter selection Fermentation of prickly pear fruit puree Microbiological analysis Quality parameters Analysis of carbohydrates, organic acids, volatile components, and free amino acids Antimicrobial assays Analysis of determination of total phenols and DPPH radical scavenging activity Vitamin C and betalains analysis Functionality analysis using Caco-2/Tc7 cells Sensory analysis Concentration: (4%, v/v) (7 log cfu/g^{-1})	Strains showed ability to degrade mucilage *in vitro.* *Leuc. mesenteroides* OP4, OP9, OP21, OP18 and OP23 showed the best growth and acidification performance, and synthesis of exopolysaccharides (EPS) during fermentation. Strains improved their shelf life, color parameters, darkening index, sensory attributes, antimicrobial activity, vitamin C and betaine levels. The fruit puree initiated significantly inhibited the inflammatory state of Caco-2 cells in addition to improving the immunomodulatory activity of the fruit puree.	Di Cagno et al. (2016)
LAB strains	Coffee fruits	Evaluated the potential use of lactic acid bacteria (LAB) isolated from coffee fruits and selected to conduct the moist fermentation process of coffee beans in order to improve the quality attributes of the beverages produced with these beans	Microbiological analyses for LAB selection Fermentation assays Analysis of determination of sugars and organic acids by HPLC Headspace solid phase micro extraction and gas chromatography analysis Coffee sensorial quality evaluation Concentration: (7 log cfu/g^{-1})	*L. plantarum* LPBR01 showed better performance as a starter for the fermentation of coffee beans. *L. plantarum* LPBR01 had no significant influence on yeast growth. *L. plantarum* LPBR01 improved the production of volatile aromatic compounds during the fermentation process. Beverages produced with coffee beans inoculated with *L. plantarum* LPBR01 presented better aroma, flavor, acidity, body, balance, aftertaste, and	Pereira et al. (2016)

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Table 3 (*continued*)

Microorganisms	Isolation source	Experiments	Analysis performed	Insightful findings	References
				overall quality in the quality test compared with the conventional process.	
Lactiplantibacillus pentosus B281 (former *Lactobacillus. pentosus* B281) *L. plantarum* B282	Industrially fermented olives	It evaluated the viability of potentially probiotic LAB during the shelf life of fermented green olives cv. Halkidiki stored (4 and 20 °C for 12 months) in modified atmosphere (70% N2–30% CO2), as well as the quality attributes.	Microbiological analyses Physicochemical analyses (pH, water activity, colour, acidity, salt content and firmness) Sensory Analyses Concentration: (~7–8 log cfu/mL)	Strains showed good survival rates. The initial and final LAB population was 5.6–5.9 and 5.8–6.4 log CFU/g respectively. Both strains were recovered in high percentages (64.7% for B281 and 94.1% for B282) after 6 months and in lower survival rates after one year (35.0% for B281 and 13.3% for B282) at 4 °C. Lower survival rates were observed after one year. Olives showed better acceptability after 6 months than 12 months of storage at both temperatures.	Argyri et al. (2015)
Pichia fermentans YC5.2	Spontaneous coffee fermentation	Evaluated the use of *P. fermentans* YC5.2 as an initial culture in controlled fermentations of coffee beans of varieties Mundo Novo and Catuaí Vermelho with or without the addition of 2% (w/v) sucrose during wet processing, and its impact on the metabolite profile. Concentration: (7 log cfu/mL)	Fermentation assays Microbiological analyses Metabolite target analysis Determination of volatile compounds and sugar composition Quality evaluation	Sucrose did not interfere with the growth or frequency of P. fermentans YC5.2 *P. fermentans* YC5.2 increased the production of specific volatile aroma compounds (e.g., ethanol, acetaldehyde, ethyl acetate and isoamyl acetate) and decreased the production of lactic acid during the fermentation proce.ss. The strain improved the quality of the coffee by intensifying the 'vanilla' flavor and 'floral' aromas	Pereira et al. (2015)

compared to control and positive control (potassium sorbate at 1%) (Habiba et al., 2019). Chemical fungicides are the cheapest approach to minimize postharvest spoilage of fruits; however, they can result in the development of chemical residues, pathogens resistance, phytotoxicity to the environment or other microorganisms, and public health issues (Chen et al., 2020). Therefore, the studies suggest that LAB and yeasts isolated from fruits and vegetables could be used as potential alternatives to synthetic fungicides, being eco-friendly, safe, and renewable, and resulting in lower postharvest losses and products with improved quality parameters.

The food industry is interested in low-cost processes for obtaining ingredients and compounds of interest. LAB and yeasts can contribute with food quality due to their fermentative activity, producing various desirable compounds, such as organic acids, enzymes, bacteriocins, and EPS (Peng et al., 2020). *Enterococcus, Fructobacillus, Lactobacillus, Lactococcus, Leuconostoc* and *Weissella* were isolated from ripe fruits (pink and yellow guava, papaya, passion fruit, pinecone, loquat, blackberry, fig, khaki) and wildflowers (flower loquat, passion fruit flower, pinecone, papaya). Most of the strains showed good production capacity for mannitol, organic acids, and aromatic compounds (Rodríguez et al., 2019). *Lactilactobacillus sakei* (former *Lactobacillus sakei)* and *Leuconostoc mesenteroides* isolated from Peruvian tocosh potato demonstrated ability to metabolize starch and phytate, and produce EPS, as well as synthesizing B vitamins (riboflavin and folate). Therefore, these LAB could be used to improve the nutritional value of food products by increasing the concentrations of important compounds (vitamins) and decreasing antinutrients (phytate) (Jiménez et al., 2017).

The utilization of starter cultures of vegetal origin is important for vegan products, as most of the starter cultures commercially available are of dairy origin (Pimentel et al., 2021). *Leuconostoc mesenteroides, Enterococcus mundtii* and *Enterococcus faecium* isolated from pepper (chilaca and jalapeno), guava, green apple, corn, and orange presented important characteristics to be used as starter cultures in food products, such as the capacity to hydrolyze starch, proteins, lipids and metabolize citrate (Linares-Morales et al., 2020). *Enterococcus, Fructobacillus, Lactobacillus, Lactococcus, Leuconostoc*, and *Weissella* isolated from ripe fruits and wildflowers presented enzymatic activity related to cinnamoyl esterase, pectinase, and esterase enzymes (Rodríguez et al., 2019). Therefore, these cultures may be used for producing enzymes. The production of enzymes by microorganisms is more desired than those from other sources because they can show improved catalytic activities, increased yields, and be produced using culture media of low costs (Linares-Morales et al., 2020).

The utilization of LAB from fruit and vegetables in food products processing may improve their sensory properties and increase bioactive compounds' content. *Lactobacillus, Leuconostoc, Weissella, Lactococcus,* and *Fructobacillus* isolated from tomato, papaya, and cabbage were able to form EPS and showed good acidification properties. Therefore, they were used as starter cultures (5 log cfu/mL) in fermentation of apple juice, pineapple juice, and papaya pulp, improving the products' antioxidant activity and sensory profile (Fessard et al., 2017). *Candida, Rhodotorula* and *Ochrobactrum* isolated from Brazilian fruits (araçá-boi, bacaba and achachairu) proved to be effective as starter cultures (7 log cfu/mL) in fermentation of the residue of these fruits, increasing total phenolic contents and antioxidant activity (Barros et al., 2019). A mix (1:1:1:1 ratio) of the potentially probiotic strains *L. plantarum* 53, *L. paracasei* 106, *L. fermentum* 56, and *Lacticaseibacillus casei* L-26 (former *Lactobacillus casei* L-26) (10 log cfu/mL) isolated from fruits (mango and acerola) and fruit by-products (mango, acerola, and soursop) positively affected the physicochemical characteristics of agro-industrial by-products of acerola (*Malpighia emarginata* DC) and guava (*Psidium guajava* L.) during submerged fermentation for 120 h. They improved brightness, titratable acidity, flavonoid content, bioactive compounds content (ascorbic acid and phenolic compounds) and antioxidant activity (Oliveira, Araújo, et al., 2020). Finally, *Leuconostoc mesenteroides* (OP4, OP9, OP21, OP18 and OP23) isolated from fresh fruits of *Opuntia ficus-indica* (L.) Mill. (Sanguigna genotype) showed *in vitro* ability to degrade mucilage, and excellent performance of acidification and synthesis of EPS during fermentation of mashed figs from India (7 log cfu/g). Therefore, they increased shelf life, improved color parameters, darkening index, sensory attributes, increased antimicrobial activity, and vitamin C and betaine (Di Cagno et al., 2016). Overall, the studies demonstrate that the utilization of LAB and yeasts isolated from fruit and vegetables as starter cultures may enhance safety, shelf life, texture, sensory properties, and health-promoting characteristics of the products.

Coffee fruit is commonly processed by fermentation using the indigenous species, eliminating the mucilage of the beans and forming volatile compounds (Pereira et al., 2015).

It is of interest for the industry to obtain consistent quality products, which may be achieved by controlling the fermentation step (Pereira et al., 2015). LAB and yeasts isolated from fruit and vegetables may be used in the processing of coffee. *L. plantarum* LPBR01 (7 log cfu/g) isolated from coffee fruits were used as starter culture for the wet fermentation of coffee beans, resulting in improved production of volatile aromatic compounds during fermentation process. The produced beverages showed better aroma, flavor, acidity, body, balance, residual flavor, and overall quality compared to that obtained by the spontaneous fermentation (Pereira et al., 2016). *Saccharomyces cerevisiae* CCMA 0200 isolated from sugar cane and *Torulaspora delbrueckii* CCMA 0684 isolated from *Coffea arabica* L. var. Bourbon5 were used in the wet fermentation of coffee varieties (Mundo Novo and Catuaí Vermelho). It was observed improvements on volatile compounds' profile, resulting in better sensory quality of the coffee beverages (Martins et al., 2019). In another study, *Pichia fermentans* YC5.2 (7 log cfu/mL) isolated from spontaneous coffee fermentation increased the production of aroma-specific volatile compounds (e.g. ethanol, acetaldehyde, ethyl acetate and isoamyl acetate) and decreased lactic acid production during the coffee bean fermentation process, resulting in a coffee beverage with improved sensory quality due to the more intense "vanilla" and "floral" aromas (Pereira et al., 2015). The results indicate that the use of starter cultures isolated from fruits and vegetables can reduce the fermentation time and improve the aroma and flavor of the coffee beverages, resulting in a product with a distinctive flavor and added value (Martins et al., 2019; Pereira et al., 2016).

3.2. Ethnic fermented beverages

Fermented foods are good sources for the isolation of LAB and yeasts with tecno-functional properties (Menezes et al., 2020; Taheur et al., 2020). Fermented dairy products, such as fermented milks, yogurts, and cheeses, have been the main source of microorganisms over the years, as they are products produced on a large scale and with great commercial appeal (Rezac et al., 2018). However, several studies have shown that the behavior and efficacy of a strain is related to its source, which has driven the interest in isolation of microorganisms from unconventional sources, allowing their application in most diverse products and technological processes (Maidana et al., 2020; Wuyts et al., 2020). In this context, the microbiota of plant origin's fermented food still represents a source to be explored and with great potential for new biotechnological applications. Table 4 shows the main studies that evaluated the techno-functional properties of strains isolated from ethnic fermented beverages and other fermented foods.

Olives represent one of the most consumed fermented vegetables around the world. They are produced through fermentation by their native microbiota, which improves the sensory characteristics of the products. In this way, studies have investigated the potential of LAB and yeasts isolated from table olives for application and improvement of the fermentation process (Benítez-Cabello et al., 2020; Iorizzo et al., 2016). *L. plantarum* (B3 and B11) and *Lactiplantibacillus pentosus* (B4) (former *Lactobacillus pentosus* (B4)) isolated from fermented green olives cv. "Nocellara del Belice" have shown positive *in vitro* results, such as high cell viability (even without glucose) for up to 15 days, good adaptability to high concentrations of salt (up to 10% NaCl), low temperature (7 °C) and low pH (about 3.6), good tolerance to phenolic content of common olives (0.3% of total phenol), and high degradability of oleuropein and β-glucosidase activity (Iorizzo et al., 2016). In another study, the yeast strain *Wickerhamomyces anomalous* Y12 and a combination of LAB strains (*L. pentosus* LPG1, *L. pentosus* Lp13, *L. plantarum*) resulted in lower pH values and higher acid production and sugar consumption, LAB multiplication, biofilm formation, and production of auto-inducer-2 (AI-2) in Spanish green olives. Furthermore, the sensorial analysis did not show differences between the inoculated treatments and uninoculated (spontaneous) control (Benítez-Cabello et al., 2020). *L. pentosus* B281 and *L. plantarum* B282 (~7–8 log cfu/mL) isolated from industrial fermented olives showed good viability (64.7% and 94.1%, respectively) after 6 months and 1 year (35.0% and 13.3% respectively) in fermented green olives (cv. Halkidiki) stored (4 and 20 °C) in modified atmosphere (70% N2–30% CO2). There were no significant changes in pH, acidity, or salt content, but the olives treated with the strains showed better results for color and firmness, reflecting better sensory acceptance (Argyri et al., 2015). The results suggest that microorganisms isolated from olives may be used as starter cultures in their fermentation, resulting in an accelerated fermentation process and products with similar or improved characteristics compared to those obtained by spontaneous fermentation.

LAB and yeasts with technological potential have also been isolated from different traditional fermented foods and beverages worldwide. The main technological properties reported were efficacy as starter cultures, antimicrobial and antifungal activity, capacity for degradation of toxins, and production of desirable compounds (Aka et al., 2020; Pei et al., 2020; Ramos et al., 2015; Taheur et al., 2020). *Limosilactobacillus fermentum* S6 (former *Lactobacillus fermentum* S6) and *Pediococcus acidilactici* S7 were isolated from fermentation of *tchapalo* (traditional Ivoirian beverage produced from fermented sorghum) and showed good ability to act as starter cultures in the production of sorghum-based beverages and to produce bacteriocins (Aka et al., 2020). *Torulaspora delbrueckii*, *Pichia exigua* and *Bacillus* spp. isolated from tarubá (traditional fermented beverage from cassava) demonstrated ability to degrade starch, proteolytic activity and thermotolerance. These characteristics are desired for cassava fermented foods production; therefore, the isolated microorganisms could be used as starter culture (Ramos et al., 2015). A diversity of microorganisms was isolated from *chichas* (indigenous Andean beer) produced with rice, oats, grapes, and a mixture of seven varieties of corn. Most microorganisms showed good tolerance to stress conditions (concentrations of ethanol, low and high temperature and osmotic stress). *Cryptococcus* sp. showed the highest amylase activity, while *Saccharomyces cerevisiae* EYS5 presented the highest invertase enzyme activity and tolerance to dehydration. The results suggested that the isolated yeasts could be used as dry starters to produce *chichas* or in other alcoholic fermentations (Grijalva-Vallejos et al., 2020). Therefore, interesting results have been reported for the isolation of microorganisms from ethnic beverages and application as starter cultures in the production of the same beverages, aiming to replace spontaneous fermentation. This is important, as a previous study has observed that species of opportunistic pathogens (*Candida tropicalis*, *Candida inconspicua*, and *Kluyveromyces marxianus*) could secrete hydrolytic enzymes (that contributed to their pathogenicity) in ethnic fermented beverages such as *tchapalo* and *bangui*. Therefore, these results open discussion about safety of traditional spontaneously fermented beverages (Egue et al., 2018).

LAB and yeasts from fermented beverages or their metabolites may show antimicrobial activities. *L. plantarum* SLG10 isolated from *kombucha* (traditional fermented beverage based on sugared tea) produced a bacteriocin that was thermostable (storage at 37 °C for 14 days or 2 months at 4 °C), tolerant to a pH range of 2.0–7.0, and sensitive to most proteases (except trypsin and pepsin). Furthermore, it showed good antimicrobial activity against Gram-positive and Gram-negative

Table 4
Main studies that evaluated the techno-functional properties of strains isolated from traditional fermented foods/beverages.

Strain	Isolation source	Experiments	Analysis performed	Insightful findings	References
LAB strains	*Tchapalo* traditional Ivoirian fermented beverage produced from sorghum	Identified and characterized LAB involved in the tchapalo fermentation process, in order to use them as potential starter cultures, as well as the production of bacteriocins by these bacteria	Tchapalo Processing LAB isolation and enumeration Analysis of antimicrobial activity Identification of LAB and analysis of genomic and general phylogenetic characteristics Detection of antibiotic resistance genes and potential virulence factors	Dominant LAB species in tchapalo fermentation were *L. fermentum* (64%), followed by *Pediococcus acidilactici* (14%). Of the 465 isolates, 27 were capable of producing bacteriocins. No antibiotic resistance genes or any potential virulence factors have been detected. *L. fermentum* S6 and *P. acidilactici* S7, and two potential bacteriocin producers, *Weissella confusa* AB3E41 and *Enterococcus faecium* AT1E22, were selected for characterization. *L. fermentum* S6 and *P. acidilactici* S7 proved to be good candidates for starter cultures	Aka et al. (2020)
Lactiplantibacillus pentosus LPG1 (former *Lactobacillus pentosus* LPG1), *L. pentosus* Lp13, *Lactiplantibacillus plantarum* Lp115 (former *Lactobacillus plantarum* Lpl15), and *Wickerhanomyces anomalous* Y12	Fermented table olives biofilms	Evaluated the use of strains (alone or in combination) as multifunctional starters for Manzanilla Spanish-style green table olive fermentations, as well as its impact on physical-chemical, microbiological and sensory parameters.	Inoculum treatment Physicochemical analysis. Microbiological analysis Observation of the presence of biofilm in the fruits Self-inducing bioassay-2 Genotyping of the LAB population and metataxonomic analysis Sensory analysis Concentration: LAB: (~7 log cfu/mL) Y12: (~5 log cfu/mL)	*Wickerhanomyces anomalous* Y12 followed by a combination of the LAB strains showed the best results (faster drop in pH, higher acid production, sugar consumption, LAB growth, biofilm formation and AI-2 production mainly in the first weeks). LPG1 strain and, particularly Lp13, were excellent biofilms former Besides *Lactobacillus*, high proportions of sequences from other genera such as *Marinilactobacillus, Alkalibacterium, Halolactobacillus* and low levels of *Halomonas* and *Aerococcus* were observed. There were no differences between the inoculated treatments and the non-inoculated (spontaneous) control in the sensory analysis.	Benítez-Cabello et al. (2020)
Yeasts strains	Ecuadorian *Chichas* (indigenous Andean beer) produced with: Rice (RC), Oats (OC), Grape (GC) and A mixture of seven varieties of corn (yamor, YC)	It isolated, identified and characterized yeast strains in order to select initiators for application in the fermentation process of *Chicha* or other fermented foods, and biochemical and molecular aspects during fermentation were also evaluated.	Molecular yeast identification Stress tolerance analysis Detection of amylase activity Growth in molasses and tolerance to dehydration Analysis of fermentative capacity Determination of invertase activity	Eleven yeast genera and 16 species were identified Stress tolerance varied between species One *Cryptococcus* sp. isolate had the highest amylase activity. Ecuadorian strain of *S. cerevisiae* EYS5 exhibited excellent fermentative performance, invertase activity (1.33 times greater than the control) and showed high viability after dehydration. *T. delbrueckii* EGT1 also showed high potential as non-*Saccharomyces* yeast for alcoholic fermentations.	Grijalva-Vallejos et al. (2020)
Thirty-four LAB strains	*Kimchi*	It selected LAB strains isolated from kimchi with anti-listeria capacity and investigated the cell surface properties (self-aggregation and	Identification of selected anti-listerial LAB isolates Analysis of cell surface aggregation and hydrophobicity activity Inhibition evaluation of	Six BAL identified as *L. plantarum* (I.60, M.2 and M.21), *L. curvatus* (B.67), *L. sakei* (D.7) and *Leuconostoc mesenteroides* (J.27) have shown anti-	Hossain et al. (2020)

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Table 4 (*continued*)

Strain	Isolation source	Experiments	Analysis performed	Insightful findings	References
		hydrophobicity) as well as the inhibitory effect of isolates against biofilm formation of *L. monocytogenes* in lettuce and MBEC™ biofilm device.	*Listeria monocytogenes* biofilm activity *in vitro*, in stainless steel coupons (SS), in lettuce and in the MBEC ™ biofilm device. Characterization analyzes of biofilm formation Concentration: LAB: (8 log cfu/mL) *L. monocytogenes*: (5 log cfu/mL)	listerial capability. All isolates exhibited remarkable auto-aggregation and hydrophobicity potential. LAB strains suppressed *L. monocytogenes* biofilms by up to 2.17 log cfu/cm^2, 1.62 log cfu/cm^2 and 1.09 log cfu/peg in SS, lettuce and MBEC ™, respectively. *L. curvatus* B.67 isolate was the one with the highest inhibition efficiency.	
13 yeast strains: 12 *S. cerevisiae* *P. kluyveri*	*Kefir* and *Caxiri* (indigenous beverage) Cocoa fermentation	It evaluated the antimicrobial effect of potentially probiotic yeast strains, previously isolated from fermented foods, on adhesion of foodborne pathogens to Caco-2 cells.	Co-aggregation evaluation Analysis of Scanning electron microscopy (SEM) of yeasts co-aggregated with bacterial pathogens Analysis of inhibition of pathogenic bacteria adhesion to Caco-2 Analysis of Antimicrobial activity Concentration: Yeast: (7 log cfu/mL) *L. monocytogenes, EPEC e S. Enteritidis*: (9 log cfu/mL)	All strains were able to co-aggregate with the pathogens tested, however, this activity was dependent on the strain. All yeasts were effective in reducing adhesion and bacterial infection (over 50%) in caco-2 cells. As observed for *P. kluyveri* (CCMA 0615) for EPEC in an exclusion test; *S. cerevisiae* (CCMA 0731, CCMA 0732) and *P. kluyveri* (CCMA 0615) for *L. monocytogenes* in exclusion and competition tests; and *S. cerevisiae* (CCMA 0731) in exclusion and *S. cerevisiae* (CCMA 0731, CCMA 0732), *P. kluyveri* (CCMA 0615) in a competition trial for *S. Enteritidis*. Yeast antimicrobial compounds have not been produced	Menezes et al. (2020)
L. plantarum 332, *Lactiplantibacillus paraplantarum G2114 (former Lactobacillus paraplantarum G2114)*, *Levilactobacillus brevis* R413 (former *Lactobacillus brevis* R413), *Latilactobacillus curvatus* 154 (former *Lactobacillus* curvatus 154), *Weissella hellenica* 152, *W. cibaria* G44, *Pediococcus pentosaceus* 2211, *P. acidilactici* 45AN, *Leuconostoc mesenteroides* 153, and *Lactococcus lactis* 37BN	Spontaneously fermented curly kale	It selected LAB isolates as starter cultures for the fermentation of kale juice, evaluating the functional and technological characteristics of the fermented juice from multistrain formulations.	Curly kale juice fermentation Technological and functional potential analysis of fermented curly kale juices (pH and total acidity, determination of viable cells, neutral sugar and protein content, antioxidant activity (ABTS), gentisic and salicylic acid (HPLC) content) Antibacterial activity test PCA analysis for starter culture selection Analysis of technological and functional characteristics of fermented juice with starter culture Analysis of the total phenolic content (TPC), Cell viability in cold storage (4 °C for 4 weeks) Concentration: LAB: (~7.5 log cfu/mL) (5% v/v) *Salmonella enterica, Staphylococcus aureus* and *Escherichia coli*: Briefly, 0.5 mL of inoculum overnight ($OD_{600} = 1.0$)	*L. paraplantarum* G2114, *Lb. plantarum* 332 and *Pediococcus pentosaceus* 2211 showed better performance in the evaluation of functional and technological properties by PCA and were selected as starter cultures. Kale juice fermented by the combined use of these strains showed rapid acidification with a high number of viable cells, increased antioxidant activity and antimicrobial effects against some pathogenic bacteria tested (mainly Gram negative).	Michalak et al. (2020)
L. plantarum SLG10	Traditional *kombucha* of South China	It isolated an antimicrobial strain of *Lactobacillus* that produces bacteriocin from	Isolation and identification of bacteriocinogenic LAB Evaluation of bacteriocin	SLG10 strain was selected because it is the only strain capable of acting on both	Pei et al. (2020)

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Table 4 (*continued*)

Strain	Isolation source	Experiments	Analysis performed	Insightful findings	References
		kombucha, in addition to tracking and purifying the bacteriocin produced, demonstrating its mechanisms of action.	production by the SLG10 strain Bacteriocin purification analyzes Structural characterization analyzes of bacteriocin Bacteriocin stability analysis Antimicrobial activity of bacteriocin SLG10 Analysis of inhibition of biofilm formation Concentration: Indicator bacteria: (6 log cfu/mL) *S. aureus* CICC 10384: (8 log cfu/mL)	Gram-positive and Gram-negative bacteria (*S. aureus* CICC10384 and *E. coli* CICC10302). The maximum production of bacteriocin SLG10 was recorded after 30 h of growth in MRS broth. Bacteriocin SLG10 showed thermostability after storage at 37 °C for 14 days or even after 2 months at 4 °C, pH tolerance characteristics in the range of 2.0–7.0, and was sensitive to most proteases, but not to trypsin or pepsin. Bacteriocin SLG10 proved to be bactericidal, increased the permeability of the cell membrane, causing the release of potassium ion and was also able to inhibit the formation of biofilms.	
Six-teen strains: LAB (7) and Yeasts (9)	*Kombucha* (pellicle and fermented tea)	It evaluated the capacity of bacteria and/or yeasts isolated from *Kombucha* to biodegrade Aflatoxin B1 (AFB1), as well, as the cytotoxicity of the by-products in Hep2 cells and in brine shrimp (*Artemia salina*).	Microbiological analysis Ability of kombucha for degrading and adsorbing mycotoxin from contaminated tea Evaluation of AFB biodegradation properties of strains isolated from kombucha Identification of active strains degrading AFB1 Mycotoxins contents analysis (HPLC). Cytotoxicity test for AFB1 on Hep2 cells and brine shrimp. Concentration: LAB: (~9 log cfu/mL) Yeasts: (~7 log cfu/mL)	*Kombucha* showed a degradation capacity of 97% of AFB1 in black tea after 7 days of fermentation. *In vitro* degradation tests showed that LAB degraded 20–45% of AFB1, while isolated yeasts showed no degradation capacity. In black tea, yeasts showed better degradation potential of AFB1 (37%). *Pichia occidentalis, Candida sorboxylosa* and *Hanseniaspora opuntiae* have been identified as yeasts effective in degrading AFB1 *P. occidentalis* (59%) showed the greatest degradation capacity of AFB1 when grown in black tea. Biodegraded products were less toxic than pure AFB1, both in the cytotoxicity test in Hep2 cells and in brine shrimp.	Taheur et al. (2020)
LAB strains	Fruits and vegetables: Ilama (*Annona macroprophyllata*), papaya (*Carica papaya* L.), mango (*Mangifera indica* L. var. Ataulfo), cocoa (*Theobroma cacao* L.), cabbage (*Brassica oleracea* var. Capitata), melon (*Cucumis melo* L.), sapodilla (*Pouteria sapota* L.) and sabila (*Aloe vera* L.) Fermented foods: *Pozol* (traditional drink made from corn and ground cocoa mass) *Tepache* (fermented drink made with fimxi), *Tejuino* (fermented corn drink) and *Sauerkraut* (fermented cabbage).	It isolated and identified LAB with antifungal activity against *Colletotrichum gloeosporioides* from vegetables and fermented foods.	Isolation of LAB Antifungal assays Identification of LAB Concentration: LAB: not specified *C. gloeosporioides* Penz: (1×10^6 spores/mL.)	54 strains of LAB were isolated, of these, 50 were from fermented foods. 18% of the isolates showed strong antifungal activity against *C. gloeosporioides,* inhibiting both spore germination and mycelial growth. The inhibition of spore germination varied between 50 and 100% (inhibition range between 30 and 40 mm) All isolates presented were of the genus *Lactobacillus* The isolated strains *L. pentosus* TEJ10, *L. paracasei* TEP6 and *L. plantarum* TEP15 showed the greatest inhibition of spore germination of the phytopathogen. All strains inhibited the growth of the mycelium of *C. gloeosporioides,* surpassing the	Barrios-Roblero et al. (2019)

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Table 4 (*continued*)

Strain	Isolation source	Experiments	Analysis performed	Insightful findings	References
				positive controls (natamycin and mancozebe)	
L. plantarum LUHS135 and *Lacticaseibacillus paracasei* LUHS244 (former *Lactobacillus. paracasei* LUHS244)	Spontaneously fermented Rye flour	It isolated strains of LAB from spontaneous fermentation of rye flour and evaluated their technological potential regarding carbohydrate metabolism, gas production, low pH survival, growth at different temperatures, antimicrobial properties, antibiotic resistance, mycotoxin reducing properties, as well as application in encapsulated dairy by-products.	Purification, isolation, identification and characterization analyzes Analysis of antimicrobial activity and antibiotic resistance Analysis of mycotoxin reducing properties Evaluation of growth and encapsulation in whey substrate Concentration: LAB: (~7–8 log cfu/mL)	*L. plantarum* LUHS135 and *L. paracasei* LUHS244 were able to metabolize a wide spectrum of carbohydrates, showed tolerance to acidity (viable cells: 7.69 and 7.55 $\log^{10}$ cfu/mL $^{-1}$) and susceptibility to antibiotics (gentamycin, tetracycline, erythromycin, amoxicillin and trimethoprim). *L. plantarum* LUHS135 and *L. paracasei* LUHS24 inhibited the growth of a wide range of pathogenic bacteria and showed no gas production. The highest antimicrobial activity was found for *L. plantarum* LUHS135 against *B. cereus*	Bartkiene et al. (2018)
Candida strains .	*Tchapalo* and *bangui* (or palm wine) traditional beverages	It evaluated the diversity of *Candida* species in popular alcoholic drinks from Côte d'Ivoire, as well as the enzymatic activity of these strains.	Microbiological Analysis Molecular identification tests of yeast isolates Analysis of enzyme activity	A total number of 136 yeasts (64 of tchapalo 72 of bangui) were identified, belonging to seven species: *Candida tropicalis, Candida inconspicua, Candida rugosa, Saccharomyces cerevisiae, Kluyveromyces marxianus, Hanseniaspora guilliermondii, Trichosporon asahii* (7 log cfu/mL). Three species of *Candida (C. tropicalis, C. inconspicua* and *C. rugosa*). were isolated from beverages. *C. rugosa* was specific to *bangui* while *T. asahii* was specific to *tchapalo*. Four species were found to be opportunistic pathogens (*C. tropicalis, C. inconspicua* and *K. marxianus*). The pathogens evaluated showed a variable amount of secreted hydrolytic enzymes, such as esterase, esterase lipase, valine and cystine arylamidase, alpha chymotrypsin, alkaline phosphatase and naphthol phosphohydrolase.	Egue et al. (2018)
Komagataeibacter strains	*Kombucha* beverages	Evaluated four different commercial *Kombucha* for isolating strains with the potential to produce bacterial cellulose (BC) under static cultivation conditions using different sugars and sugar-alcohol mannitol as carbon sources, as well such as apple juice and cheese whey as low-cost substrates. In addition, it also evaluated the physical and mechanical properties of the cellulose produced.	Isolation analysis and identification of cellulose-producing strains Characterization of BC (FT-IR) BC production and quantification tests Determination of sugars and acidic organics Determination of the degree of cellulose polymerization Evaluation of the mechanical properties of BC	The best cellulose producers were the B17 and B22 isolates belonging to the genus *Komagataeibacter* (former *Gluconacetobacter*) and classified as *K. rhaeticus P 1463* and *K. hansenii* B22, respectively. *K. rhaeticus* P 1463 was more effective with BC yield of ~25% in Hestrin and Schramm (HS) medium and ~37% in fermented apple juice for 5 days. Synthesized BC showed good physical and mechanical properties	Semjonovs et al. (2017)
L. plantarum (B3, B11) and *L. pentosus* (B4)	Fermented Green Olives cv. "*Nocellara del Belice*"	It evaluated the technological potential *in vitro* of *Lactobacillus* strains previously isolated from fermented olives in terms of multiplication,	Determination of bacterial viability and fermentative metabolism Determination of the content of glucose, total phenolics, Oleuropein and	All strains showed high cell viability even with no glucose up to 15 days. Acidification capacity (degradation of all glucose in 24 h, final pH: ~4.1),	Iorizzo et al. (2016)

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Table 4 (*continued*)

Strain	Isolation source	Experiments	Analysis performed	Insightful findings	References
		acidification capacity, enzymatic activities and oleuropein degradation capacity under different conditions.	hydroxytyrosol Analysis of enzyme activity Analysis of oleuropein-degrading capability and β-Glucosidase activity	*L. plantarum* B3 and B11 exhibited good adaptability to high concentrations of salt (up to 10% NaCl), low temperature (7 °C) and pH (about 3.6). Good tolerance to the phenol content of common olives (0.3% of total phenol) and high degradability of oleuropein. Lesser action of degradation of oleuropein was observed in a medium rich in nutrients All strains evaluated produced β-glucosidase activity, but without esterase	
Komagataeibacter intermedius FST213-1	Fermented fruit juices of pineapple, apple and guava	It isolated and identified a new strain producing bacterial cellulose (BC) in fermented fruit juices, still evaluated the production of BC at different pHs and characterized the BC produced.	Preparation of fermented fruit juices Isolation of BC producing strain Identification tests of BC producing strain Material property analysis	The isolated strain was identified as *Komagataeibacter intermedius* FST213-1. *K. intermedius* FST213-1 was able to produce BC at pH values between 4 and 9, exhibiting maximum BC production (1.2 g/L) at pH 8 in short term cultivation (4 days). BC produced from *K. intermedius* FST213-1 exhibited higher water content (99.5%), lower thermostability (315 °C), lower crystallinity (79.3%) and similar mechanical properties compared to the producer BC, *Gluconacetobacter xylinus* 23769.	Lin et al. (2016)
LAB and yeasts strains	*Tarubá* (traditional fermented beverage produced from cassava)	Identified and characterized the microbiota present during solid state fermentation in the final stage of *tarubá* production, as well as the chemical characteristics throughout and at the end of the fermentation of beverage.	Microbial population and characterization (by culture-dependent and -independent methods) Analysis of amylolytic and proteolytic activity Thermotolerance analysis Analysis of chemical parameters Determination of sugars, organic acids and ethanol by HPLC	LAB and *Bacillus* species such as *L. plantarum, L. brevis, Leuconostoc mesenteroides* and *Bacillus subtilis* were predominant during fermentation. *Torulaspora delbrueckii* species was the dominant yeast during fermentation. Amylolytic, proteolytic and thermotolerance properties were observed for some isolates. *T. delbrueckii, P. exigua* and *Bacillus* spp. exhibited all of these properties.	Ramos et al. (2015)

bacteria (*S. aureus* CICC10384 and E. coli CICC10302) and inhibited the formation of biofilms. Therefore, this bacteriocin could be used as an antimicrobial compound in the food industry (Pei et al., 2020). *Lactiplantibacillus pentosus* TEJ10 (former *Lactobacillus pentosus* TEJ10), *L. paracasei* TEP6 and *L. plantarum* TEP15 isolated from fermented foods/beverages *(pozol, tepache, tejuino* and *sauerkraut*) showed *in vitro* antifungal activity against phytopathogenic strain *Colletotrichum gloeosporioides* Penz, being able to inhibit both spore germination (between 50 and 100%) and mycelial growth (surpassing the positive controls natamycin and mancozebe) (Barrios-Roblero et al., 2019). LAB isolated from "chicha" (a traditional maize-based fermented beverage) and "tocosh" fermented potatoes exhibited antimicrobial capacity against fungi (*A. oryzae* and *M. guilliermondii)* and food pathogens (*E. coli* O157:H7, *L. innocua*, and *Salmonella* Typhi) (Yépez et al., 2017). Therefore, the antimicrobial activity of LAB and yeasts isolated from fermented foods may increase the plant resistance to pathogens and food products' shelf life.

The capacity of microorganisms isolated from traditional beverages for degradation of toxins has also been reported. Kombucha culture and isolates (*Pichia occidentalis, Candida sorboxylosa and Hanseniaspora opuntiae)* could degrade aflatoxin B1 in black tea, decreasing its toxicity in both Hep2 and artemia cells. Aflatoxin B1 is recognized as the most harmful mycotoxin and its presence in tea makes it unsuitable for consumption due to the risks to human health. Therefore, kombucha and its strains can be applied in feed and food industries to detoxify aflatoxin B1 (Taheur et al., 2020).

LAB and yeasts from fermented beverages may also be used to produce desirable compounds. Four different commercial *kombuchas* and the isolated strains (*Komagataeibacter rhaeticus* P 1463 and *Komagataeibacter hansenii* B22) showed a strong capacity to produce cellulose with good physical and mechanical properties both *in vitro* (~25%) and in fermented apple juice for 5 days (~37%) (Semjonovs et al., 2017).

Komagataeibacter intermedius FST213-1 isolated from fermented fruit juices (pineapple, apple, and guava) exhibited ability to produce bacterial cellulose (BC) (at pH between 4 and 9) with desirable technological properties (water content capacity (99.5%), low thermostability (315 °C), less crystallinity (79.3%) and good mechanical properties) (Lin et al., 2016). Bacterial cellulose has many advantages compared to plant cellulose, such as higher crystallinity, mechanical strength, biocompatibility, biodegradability, and purity. It has many applications, such as biosensor, paper production, and tissue engineering (Lin et al., 2016).

LAB and yeast from fermented foods may also be used to improve food products' nutritional value (Fekri et al., 2020). Yépez et al. (2019) observed that LAB (*L. plantarum* M5MA1-B2) isolated from Andean improved riboflavin content in kefir-like cereal-based beverages (obtained from maize, oat, and barley flours).

Previous studies also reported the isolation of microorganisms from fermented foods that presented multiple functions. *L. plantarum* and *L. paracasei* LUHS244 isolated from spontaneous fermentation of rye flour were able to metabolize a wide spectrum of carbohydrates, inhibited the growth of a wide range of pathogenic bacteria, and reduced mycotoxins (3% of each inoculum) in *in vitro* tests. In addition, they showed good stability and multiplication when encapsulated in whey (enriched with 2.5% glucose, 2.0% yeast extract and 0.5% sucrose) (Bartkiene et al., 2018). *Lactiplantibacillus paraplantarum* G2114 (former *Lactobacillus paraplantarum* G2114), *L. plantarum* 332 and *Pediococcus pentosaceus* 2211 isolated from spontaneously fermented kale showed rapid acidification, and high viability, antioxidant, and antimicrobial activity (against Gram positive and Gram-negative bacteria). These characteristics were maintained in fermented cabbage juice during storage (4 °C) for 4 weeks when the combination of these strains was used as starter cultures (~7.5 log cfu/mL) (5% v/v) for fermentation (Michalak et al., 2020).

4. Conclusions and future perspectives

This was the first review to explore the added-value microorganisms isolated from unconventional sources (fruit, raw vegetables, ethnic fermented beverages, and flowers) and their associated techno-functional and potentially probiotic properties. Fruits (strawberry, guava, apple, peach, grape, and papaya), vegetables (peppers, corn, zucchini, lettuce, cucumber, coffee beans, and olives), flowers (narcissus, pink rose, red rose, yellow rose, and sunflower), and ethnic fermented beverages (*tchapalo*, *tarubá*, *cauim*, *chicha*, *caxiri*, *kombucha*, and water *kefir)* presented lactic acid bacteria (*Lactobacillus*, *Leuconostoc*, *Enterococcus*, *Pediococcus*, *Fructobacillus*, and *Weissella*) and yeasts (*Saccharomyces, Candida, Pichia, Rhodotorula, Torulaspora, Cryptococcus, Hansenula,* and *Debaromyces*) with techno-functional properties, such as antimicrobial capacity, production of compounds of interest, and technological properties as starter cultures. Furthermore, they showed properties to be considered probiotic cultures, such as safety (non-hemolytic activity and mucin degradation), good resistance to adverse conditions (acid and bile salt tolerance, cell surface hydrophobicity, auto-aggregation, co-aggregation with pathogens, antagonistic activity against pathogens, and capability of surviving during exposure to gastrointestinal conditions), and technological aspects (proteolytic and lipolytic activity, and EPS and bioactive compounds production). However, the resistance to antibiotics should be carefully evaluated, mainly by demonstrating that no transference to pathogenic microorganisms will occur. This is of paramount importance, and many studies observed the antibiotic resistance but considered as an intrinsic characteristic of the cultures, with no further assessment.

The probiotic cultures presented anti-hypertensive, antilipidemic, immunomodulatory, and anti-diabetic properties and could reduce weight gain. However, the health effects were studied using mainly rats, denoting that clinical studies are necessary to prove humans' health effects. Furthermore, they were concentrated in LAB isolated from fruits and fermented beverages and dislypidemic/obese or healthy individuals, suggesting that studies covering new probiotic species (yeasts and other LAB) from other sources (flowers, vegetables, and ethnic beverages) and different types of hosts and diets (diabetics, healthy, elderly) are also needed. This highlights the range of study opportunities in the area. The probiotic efficacy was strain-specific, emphasizing the importance of having a catalog of probiotic strains with information about their health effects, which would assist the industries in developing new functional products aiming specific human health promotion (Oliveira, Cavalcante, et al., 2020). Furthermore, it is important to carry out the complete phenotypic and genotypic identification of the isolated probiotic cultures and not only demonstrate the health effects (Mabeku et al., 2020).

In conclusion, fruits, vegetables, flowers, and ethnic fermented beverages are important sources of techno-functional and probiotic cultures with high potential for use in food processing to increase the nutritional and sensory quality, acting as natural biopreservatives, and promote health effects. The utilization of these cultures may enhance the development of vegan probiotic products and non-dairy fermented beverages, allowing processing at lower times and resulting in products with more consistent characteristics. Furthermore, it opens opportunities to isolate important microorganisms from available sources in each country, such as ethnic beverages or traditionally fermented foods.

Declaration of competing interest

Authors declare no conflict of interest.

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References

Aka, S., Dridi, B., Bolotin, A., Yapo, E. A., Koussemon-Camara, M., Bonfoh, B., & Renault, P. (2020). Characterization of lactic acid bacteria isolated from a traditional Ivoirian beer process to develop starter cultures for safe sorghum-based beverages. *International Journal of Food Microbiology, 322*, Article 108547. https://doi.org/10.1016/j.ijfoodmicro.2020.108547

Albuquerque, T. M. R., Garcia, E. F., Araújo, A. O., Magnani, M., Saarela, M., & Souza, E. L. (2018). In vitro characterization of *Lactobacillus* strains isolated from fruit processing by-products as potential probiotics. *Probiotics and Antimicrobial Proteins, 10*(4), 704–716. https://doi.org/10.1007/s12602-017-9318-2

Amorim, J. C., Piccoli, R. H., & Duarte, W. F. (2018). Probiotic potential of yeasts isolated from pineapple and their use in the elaboration of potentially functional fermented beverages. *Food Research International, 107*, 518–527. https://doi.org/10.1016/j.foodres.2018.02.054

Anandharaj, M., Sivasankari, B., Santhanakaruppu, R., Manimaran, M., Rani, R. P., & Sivakumar, S. (2015). Determining the probiotic potential of cholesterol-reducing *Lactobacillus* and *Weissella* strains isolated from *gherkins* (fermented cucumber) and south Indian fermented *koozh*. *Research in Microbiology, 166*(5), 428–439. https://doi.org/10.1016/j.resmic.2015.03.002

Argyri, A. A., Nisiotou, A. A., Pramateftaki, P., Doulgeraki, A. I., Panagou, E. Z., & Tassou, C. C. (2015). Preservation of green table olives fermented with lactic acid bacteria with probiotic potential under modified atmosphere packaging. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 62*(1), 783–790. https://doi.org/10.1016/j.lwt.2014.11.046

Barrios-Roblero, C., Rosas-Quijano, R., Salvador-Figueroa, M., Gálvez-López, D., & Vázquez-Ovando, A. (2019). Antifungal lactic acid bacteria isolated from fermented beverages with activity against *Colletotrichum gloeosporioides*. *Food Bioscience, 29*, 47–54. https://doi.org/10.1016/j.fbio.2019.03.008

Barros, R. G. C., Oliveira, C. S. de, Oliveira, L. T. S., Pereira, U. C., Silva, T. O. M., Denadai, M., & Narain, N. (2019). Enhancement of phenolic antioxidants production in submerged cultures of endophytic microorganisms isolated from achachairu (*Garcinia humilis*), araçá-boi (*Eugenia stipitata*) and bacaba (*Oenocarpus bacaba*) fruits. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 111*, 370–377. https://doi.org/10.1016/j.lwt.2019.05.046

Bartkiene, E., Zavistanaviciute, P., Lele, V., Ruzauskas, M., Bartkevics, V., Bernatoniene, J., Gallo, P., Tenore, G. C., & Santini, A. (2018). *Lactobacillus plantarum* LUHS135 and *paracasei* LUHS244 as functional starter cultures for the food fermentation industry: Characterisation, mycotoxin-reducing properties, optimisation of biomass growth and sustainable encapsulation by using dairy by-product. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 93*, 649–658. https://doi.org/10.1016/j.lwt.2018.04.017

Behare, P. V., Mazhar, S., Pennone, V., & McAuliffe, O. (2020). Evaluation of lactic acid bacteria strains isolated from fructose-rich environments for their mannitol-production and milk-gelation abilities. *Journal of Dairy Science, 103*(12), 11138–11151. https://doi.org/10.3168/jds.2020-19120

Benítez-Cabello, A., Calero-Delgado, B., Rodríguez-Gómez, F., Bautista-Gallego, J., Garrido-Fernández, A., Jiménez-Díaz, R., & Arroyo-López, F. N. (2020). The use of multifunctional yeast-*lactobacilli* starter cultures improves fermentation performance of Spanish-style green table olives. *Food Microbiology, 91*, Article 103497. https://doi.org/10.1016/j.fm.2020.103497

Benítez-Cabello, A., Calero-Delgado, B., Rodríguez-Gómez, F., Garrido-Fernández, A., Jiménez-Díaz, R., & Arroyo-López, F. N. (2019). Biodiversity and multifunctional features of lactic acid bacteria isolated from table olive biofilms. *Frontiers in Microbiology, 10*, 836. https://doi.org/10.3389/fmicb.2019.00836

Bonatsou, S., Karamouza, M., Zoumpopoulou, G., Mavrogonatou, E., Kletsas, D., Papadimitriou, K., Tsakalidou, E., Nychas, G. J. E., & Panagou, E. (2018). Evaluating the probiotic potential and technological characteristics of yeasts implicated in cv. Kalamata natural black olive fermentation. *International Journal of Food Microbiology, 271*, 48–59. https://doi.org/10.1016/j.ijfoodmicro.2018.02.018

Cao, Z., Pan, H., Li, S., Shi, C., Wang, S., Wang, F., Ye, P., Jia, J., Ge, C., Lin, Q., & Zhao, Z. (2019). *In vitro* evaluation of probiotic potential of lactic acid bacteria isolated from yunnan *de'ang* pickled tea. *Probiotics and Antimicrobial Proteins, 11*(1), 103–112. https://doi.org/10.1007/s12602-018-9395-x

Cavalcante, R. G. S., Albuquerque, T. M. R. de, Freire, M. O. de L., Ferreira, G. A. H., Santos, L. A. C. dos, Magnani, M., Cruz, J. C., Braga, V. A., Souza, E. L. de, & Alves, J. L. de B. (2019). The probiotic *Lactobacillus fermentum* 296 attenuates cardiometabolic disorders in high fat diet-treated rats. *Nutrition, Metabolism, and Cardiovascular Diseases, 29*(12), 1408–1417. https://doi.org/10.1016/j.numecd.2019.08.003

Cervantes-Elizarrarás, A., Cruz-Cansino, N., Ramírez-Moreno, E., Vega-Sánchez, V., Velázquez-Guadarrama, N., Zafra-Rojas, Q., & Piloni-Martini, J. (2019). In vitro probiotic potential of lactic acid bacteria isolated from *aguamiel* and *pulque* and antibacterial activity against pathogens. *Applied Sciences, 9*(3), 601. https://doi.org/10.3390/app9030601

Chen, C., Cao, Z., Li, J., Tao, C., Feng, Y., & Han, Y. (2020). A novel endophytic strain of *Lactobacillus plantarum* CM-3 with antagonistic activity against *Botrytis cinerea* on strawberry fruit. *Biological Control, 148*(19), Article 104306. https://doi.org/10.1016/j.biocontrol.2020.104306

Costa, W. K. A., Brandão, L. R., Martino, M. E., Garcia, E. F., Alves, A. F., Souza, E. L. de, Aquino, J. S., Saarela, M., Leulier, F., Vidal, H., & Magnani, M. (2019). Qualification of tropical fruit-derived Lactobacillus plantarum strains as potential probiotics acting on blood glucose and total cholesterol levels in wistar rats. *Food Research International, 124*, 109–117. https://doi.org/10.1016/j.foodres.2018.08.035

Costa, W. K. A., Souza, G. T. de, Brandão, L. R., Lima, R. C. de, Garcia, E. F., Lima, M. dos S., Souza, E. L. de, Saarela, M., & Magnani, M. (2018). Exploiting antagonistic activity of fruit-derived *Lactobacillus* to control pathogenic bacteria in fresh cheese and chicken meat. *Food Research International, 108*, 172–182. https://doi.org/10.1016/j.foodres.2018.03.045

Di Cagno, R., Coda, R., De Angelis, M., & Gobbetti, M. (2013). Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiology, 33*(1), 1–10. https://doi.org/10.1016/j.fm.2012.09.003

Di Cagno, R., Filannino, P., Cantatore, V., Polo, A., Celano, G., Martinovic, A., Cavoski, I., & Gobbetti, M. (2020). Design of potential probiotic yeast starters tailored for making a cornelian cherry (*Cornus mas* L.) functional beverage. *International Journal of Food Microbiology, 323*, Article 108591. https://doi.org/10.1016/j.ijfoodmicro.2020.108591

Di Cagno, R., Filannino, P., Vincentini, O., Lanera, A., Cavoski, I., & Gobbetti, M. (2016). Exploitation of *Leuconostoc mesenteroides* strains to improve shelf life, rheological, sensory and functional features of prickly pear (*Opuntia ficus-indica* L.) fruit puree. *Food Microbiology, 59*, 176–189. https://doi.org/10.1016/j.fm.2016.06.009

Egue, L. A. N., N'guessan, F. K., Aka-Gbezo, S., Bouatenin, J. K. M., & Koussemon-Camara, M. (2018). *Candida* species in *tchapalo* and *bangui*, two traditional alcoholic beverages from côte d'Ivoire. *Fungal Biology, 122*(5), 283–292. https://doi.org/10.1016/j.funbio.2018.01.002

Fakruddin, M., Hossain, M. N., & Ahmed, M. M. (2017). Antimicrobial and antioxidant activities of *Saccharomyces cerevisiae* IFST062013, a potential probiotic. *BMC Complementary and Alternative Medicine, 17*(1), 1–11. https://doi.org/10.1186/s12906-017-1591-9

Fekri, A., Torbati, M., Khosrowshahi, A. Y., Shamloo, H. B., & Azadmard-Damirchi, S. (2020). Functional effects of phytate-degrading, probiotic lactic acid bacteria and yeast strains isolated from Iranian traditional sourdough on the technological and nutritional properties of whole wheat bread. *Food Chemistry, 306*, Article 125620. https://doi.org/10.1016/j.foodchem.2019.125620

Fernández-Pacheco, P., García-Béjar, B., Jiménez-del Castillo, M., Carreño-Domínguez, J., Pérez, A. B., & Arévalo-Villena, M. (2020). Potential probiotic and food protection role of wild yeasts isolated from pistachio fruits (*Pistacia vera*). *Journal of the Science of Food and Agriculture*. https://doi.org/10.1002/jsfa.10839

Fessard, A., Bourdon, E., Payet, B., & Remize, F. (2017). Identification, stress tolerance, and antioxidant activity of lactic acid bacteria isolated from tropically grown fruits and leaves. *Canadian Journal of Microbiology, 62*(7), 550–561. https://doi.org/10.1139/cjm-2015-0624

Fessard, A., & Remize, F. (2019). Genetic and technological characterization of lactic acid bacteria isolated from tropically grown fruits and vegetables. *International Journal of Food Microbiology, 301*, 61–72. https://doi.org/10.1016/j.ijfoodmicro.2019.05.003

Fiocco, D., Longo, A., Arena, M. P., Russo, P., Spano, G., & Capozzi, V. (2020). How probiotics face food stress: They get by with a little help. *Critical Reviews in Food Science and Nutrition, 60*(9), 1552–1580. https://doi.org/10.1080/10408398.2019.1580673

Freire, A. L., Ramos, C. L., Souza, P. N. da C., Cardoso, M. G. B., & Schwan, R. F. (2017). Non-dairy beverage produced by controlled fermentation with potential probiotic starter cultures of lactic acid bacteria and yeast. *International Journal of Food Microbiology, 248*, 39–46. https://doi.org/10.1016/j.ijfoodmicro.2017.02.011

García-Ruiz, A., Llano, D. G., Esteban-Fernández, A., Requena, T., Bartolomé, B., & Moreno-Arribas, M. V. (2014). Assessment of probiotic properties in lactic acid bacteria isolated from wine. *Food Microbiology, 44*, 220–225. https://doi.org/10.1016/j.fm.2014.06.015

Garcia, E. F., Luciano, W. A., Xavier, D. E., Costa, W. C. A. da, Oliveira, K. de S., Franco, O. L., Júnior, M. A. de M., Lucena, B., Picão, R. C., Magnani, M., Saarela, M., & Souza, E. L. (2016). Identification of lactic acid bacteria in fruit pulp processing byproducts and potential probiotic properties of selected Lactobacillus strains. *Frontiers in Microbiology, 7*, 1–11. https://doi.org/10.3389/fmicb.2016.01371

Gautam, N., & Sharma, N. (2015). Evaluation of probiotic potential of new bacterial strain, *Lactobacillus spicheri* G2 isolated from *gundruk*. *Proceedings of the National Academy of Sciences, India - Section B: Biological Sciences, 85*(4), 979–986. https://doi.org/10.1007/s40011-014-0458-9

Gil-Rodríguez, A. M., Carrascosa, A. V., & Requena, T. (2015). Yeasts in foods and beverages: In vitro characterisation of probiotic traits. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 64*(2), 1156–1162. https://doi.org/10.1016/j.lwt.2015.07.042

Grijalva-Vallejos, N., Aranda, A., & Matallana, E. (2020). Evaluation of yeasts from Ecuadorian *chicha* by their performance as starters for alcoholic fermentations in the food industry. *International Journal of Food Microbiology, 317*, Article 108462. https://doi.org/10.1016/j.ijfoodmicro.2019.108462

Gupta, S., & Abu-Ghannam, N. (2012). Probiotic fermentation of plant based products: Possibilities and opportunities. *Critical Reviews in Food Science and Nutrition, 52*(2), 183–199. https://doi.org/10.1080/10408398.2010.499779

Gupta, A., & Sharma, N. (2017). Characterization of potential probiotic lactic acid bacteria *Pediococcus acidilactici* Ch-2 isolated from *Chuli* a traditional apricot product of Himalayan region for the production of novel bioactive compounds with special therapeutic properties. *Journal of Foodservice: Microbiology, Safety & Hygiene, 2*(1), 1–11. https://doi.org/10.4172/2476-2059.1000119

Habibama, R. N., Ali, S. A., Hasan, K. A., Sultana, V., Ara, J., & Ehteshamul-Haque, S. (2019). Evaluation of biocontrol potential of epiphytic yeast against postharvest *Penicillium digitatum* rot of stored kinnow fruit (*Citrus reticulata*) and their effect on its physiochemical properties. *Postharvest Biology and Technology, 148*, 38–48. https://doi.org/10.1016/j.postharvbio.2018.10.007

Handa, S., & Sharma, N. (2016). In vitro study of probiotic properties of *Lactobacillus plantarum* F22 isolated from chhang–A traditional fermented beverage of Himachal Pradesh, India. *Journal of Genetic Engineering and Biotechnology, 14*(1), 91–97. https://doi.org/10.1016/j.jgeb.2016.08.001

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology, 11*(8), 506. https://doi.org/10.1038/nrgastro.2014.66

Hossain, M. I., Mizan, M. F. R., Ashrafudoulla, M., Nahar, S., Joo, H. J., Jahid, I. K., Park, S. H., Kim, K. S., & Ha, S.-D. (2020). Inhibitory effects of probiotic potential lactic acid bacteria isolated from *kimchi* against *Listeria monocytogenes* biofilm on lettuce, stainless-steel surfaces, and MBECTM biofilm device. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 118*, Article 108864. https://doi.org/10.1016/j.lwt.2019.108864

Hsiung, R. T., Fang, W. T., LePage, B. A., Hsu, S. A., Hsu, C. H., & Chou, J. Y. (2020). *In vitro* properties of potential probiotic indigenous yeasts originating from fermented food and beverages in taiwan. *Probiotics and Antimicrobial Proteins*, 1–12. https://doi.org/10.1007/s12602-020-09661-8

Iorizzo, M., Lombardi, S. J., Macciola, V., Testa, B., Lustrato, G., Lopez, F., & De Leonardis, A. (2016). Technological potential of *Lactobacillus* strains isolated from fermented green olives: *In vitro* studies with emphasis on oleuropein-degrading capability, 2016 *Science World Journal*, 1–11. https://doi.org/10.1155/2016/1917592.

Jasim, B., Mathew, J., & Radhakrishnan, E. K. (2016). Identification of a novel endophytic *Bacillus sp.* from *Capsicum annuum* with highly efficient and broad spectrum plant probiotic effect. *Journal of Applied Microbiology, 121*(4), 1079–1094. https://doi.org/10.1111/jam.13214

Jiménez, E., Yépez, A., Pérez-Cataluña, A., Vásquez, E. R., Dávila, D. Z., Vignolo, G., & Aznar, R. (2017). Exploring diversity and biotechnological potential of lactic acid bacteria from *tocosh* - traditional Peruvian fermented potatoes - by high throughput sequencing (HTS) and culturing. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 87*, 567–574. https://doi.org/10.1016/j.lwt.2017.09.033

Junnarkar, M., Pawar, S., Gaikwad, M. A., Jass, J., & Nawani, N. (2019). Probiotic potential of lactic acid bacteria from fresh vegetables: Application in food preservation. *Indian Journal of Experimental Biology, 57*, 825–838.

Karim, A., Gerliani, N., & Aïder, M. (2020). *Kluyveromyces marxianus*: An emerging yeast cell factory for applications in food and biotechnology. *International Journal of Food Microbiology, 333*, Article 108818. https://doi.org/10.1016/j.ijfoodmicro.2020.108818

Khalil, E. S., Manap, A., Yazid, M., Mustafa, S., Alhelli, A. M., & Shokryazdan, P. (2018). Probiotic properties of exopolysaccharide-producing *Lactobacillus* strains isolated from *tempoyak*. *Molecules, 23*(2), 398. https://doi.org/10.3390/molecules23020398

Khan, I., & Kang, S. C. (2016). Probiotic potential of nutritionally improved *Lactobacillus plantarum* DGK-17 isolated from *Kimchi*–A traditional Korean fermented food. *Food Control, 60*, 88–94. https://doi.org/10.1016/j.foodcont.2015.07.010

Koh, W. Y., Utra, U., Ahmad, R., Rather, I. A., & Park, Y. H. (2018). Evaluation of probiotic potential and anti-hyperglycemic properties of a novel *Lactobacillus* strain isolated from water kefir grains. *Food Science and Biotechnology, 27*(5), 1369–1376. https://doi.org/10.1007/s10068-018-0360-y

Ku, S., Yang, S., Lee, H. H., Choe, D., Johnston, T. V., Ji, G. E., & Park, M. S. (2020). Biosafety assessment of Bifidobacterium animalis subsp. lactis AD011 used for human consumption as a probiotic microorganism. *Food Control, 117*, Article 106985. https://doi.org/10.1016/j.foodcont.2019.106985

Lakra, A. K., Domdi, L., Hanjon, G., Tilwani, Y. M., & Arul, V. (2020). Some probiotic potential of *Weissella confusa* MD1 and *Weissella cibaria* MD2 isolated from fermented batter. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 125*, Article 109261. https://doi.org/10.1016/j.lwt.2020.109261

Leandro, E. dos S., Ginani, V. C., Alencar, E. R., Pereira, O. G., Rose, E. C. P., Vale, H. M. M. do, Pratesi, R., Hecht, M. M., Cavalcanti, M. H., & Tavares, C. S. O. (2020). Isolation, identification, and screening of lactic acid bacteria with probiotic potential in silage of different species of forage plants, cocoa beans, and artisanal salami. *Probiotics and Antimicrobial Proteins*, 1–14. https://doi.org/10.1007/s12602-020-09679-y

Lee, N. K., Hong, J. Y., Yi, S. H., Hong, S. P., Lee, J. E., & Paik, H. D. (2019). Bioactive compounds of probiotic *Saccharomyces cerevisiae* strains isolated from cucumber jangajji. *Journal of Functional Foods, 58*, 324–329. https://doi.org/10.1016/j.jff.2019.04.059

Leff, J. W., & Fierer, N. (2013). Bacterial communities associated with the surfaces of fresh fruits and vegetables. Edited by gabriele berg. *PloS One, 8*(3), Article e59310. https://doi.org/10.1371/journal.pone.0059310

Linares-Morales, J. R., Cuellar-Nevárez, G. E., Rivera-Chavira, B. E., Gutiérrez-Méndez, N., Pérez-Vega, S. B., & Nevárez-Moorillón, G. V. (2020). Selection of lactic acid bacteria isolated from fresh fruits and vegetables based on their antimicrobial and enzymatic activities. *Foods, 9*(10). https://doi.org/10.3390/foods9101399

Lin, S. P., Huang, Y. H., Hsu, K. D., Lai, Y. J., Chen, Y. K., & Cheng, K. C. (2016). Isolation and identification of cellulose-producing strain *Komagataeibacter intermedius* from fermented fruit juice. *Carbohydrate Polymers, 151*, 827–833. https://doi.org/10.1016/j.carbpol.2016.06.032

Mabeku, L. B. K., Ngue, S., Nguemo, I. B., & Leundji, H. (2020). Potential of selected lactic acid bacteria from *Theobroma cacao* fermented fruit juice and cell-free supernatants from cultures as inhibitors of *Helicobacter pylori* and as good probiotic. *BMC Research Notes, 13*(1), 64. https://doi.org/10.1186/s13104-020-4923-7

Maeno, S., Kajikawa, A., Dicks, L., & Endo, A. (2019). Introduction of bifunctional alcohol/acetaldehyde dehydrogenase gene (AdhE) in *Fructobacillus fructosus* settled its fructophilic characteristics. *Research in Microbiology, 170*(1), 35–42. https://doi.org/10.1016/j.resmic.2018.09.004

Maheshwari, S. U., Amutha, S., Anandham, R., Hemalatha, G., Senthil, N., Kwon, S. W., & Sivakumar, N. (2019). Characterization of potential probiotic bacteria from '*panchamirtham*'; A southern Indian ethinic fermented fruit mix. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 116*, Article 108540. https://doi.org/10.1016/j.lwt.2019.108540

Maidana, S. D., Ficoseco, C. A., Bassi, D., Cocconcelli, P. S., Puglisi, E., Savoy, G., Vignolo, G., & Fontana, C. (2020). Biodiversity and technological-functional potential of lactic acid bacteria isolated from spontaneously fermented chia sourdough. *International Journal of Food Microbiology, 316*, Article 108425. https://doi.org/10.1016/j.ijfoodmicro.2019.108425

Martins, P. M. M., Ribeiro, L. S., Miguel, M. G. da C. P., Evangelista, S. R., & Schwan, R. F. (2019). Production of coffee (*Coffea arabica*) inoculated with yeasts: Impact on quality. *Journal of the Science of Food and Agriculture, 99*(13), 5638–5645. https://doi.org/10.1002/jsfa.9820

Menezes, A., Tavares, G., Melo, D. de S., Ramos, C. L., Moreira, S. I., Alves, E., & Schwan, R. F. (2020). Yeasts isolated from Brazilian fermented foods in the protection against infection by pathogenic food bacteria. *Microbial Pathogenesis, 140*, Article 103969. https://doi.org/10.1016/j.micpath.2020.103969

Michalak, M., Kubik-Komar, A., Waśko, A., & Polak-Berecka, M. (2020). Starter culture for curly kale juice fermentation selected using principal component analysis. *Food Bioscience, 35*, Article 100602. https://doi.org/10.1016/j.fbio.2020.100602

Ng, S. Y., Koon, S. S., Padam, B. S., & Chye, F. Y. (2015). Evaluation of probiotic potential of lactic acid bacteria isolated from traditional Malaysian fermented *bambangan* (*Mangifera pajang*). *CyTA - Journal of Food, 13*(4), 1–10. https://doi.org/10.1080/19476337.2015.1020342

Oliveira, S. D., Araújo, C. M., Borges, G. da S. C., Lima, M. dos S., Viera, V. B., Garcia, E. F., Souza, E. L. de, & Oliveira, M. E. G. de (2020). Improvement in physicochemical characteristics, bioactive compounds and antioxidant activity of acerola (*Malpighia emarginata* D.C.) and guava (*Psidium guajava* L.) fruit by-products fermented with potentially probiotic lactobacilli. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 134*, Article 110200. https://doi.org/10.1016/j.lwt.2020.110200

Oliveira, Y., Cavalcante, R. G. S., Neto, M. P. C., Magnani, M., Braga, V. A., Souza, E. L., & Alves, J. L. B. (2020). Oral administration of *Lactobacillus fermentum* post-weaning improves the lipid profile and autonomic dysfunction in rat offspring exposed to maternal dyslipidemia. *Food and Function, 11*(6), 5581–5594. https://doi.org/10.1039/d0fo00514b

Oliveira, T., Ramalhosa, E., Nunes, L., Pereira, J. A., Colla, E., & Pereira, E. L. (2017). Probiotic potential of indigenous yeasts isolated during the fermentation of table olives from northeast of Portugal. *Innovative Food Science & Emerging Technologies, 44*, 167–172. https://doi.org/10.1016/j.ifset.2017.06.003

Ornellas, R. M. S., Santos, T. T., Arcucio, L. B., Sandes, S. H. C., Oliveira, M. M., Dias, C. V., de C. Silva, S., Uetanabaro, A. P. T., Vinderola, G., & Nicoli, J. R. (2017). Selection of lactic acid bacteria with probiotic potential isolated from the fermentation process of 'cupuaçu' (*Theobroma grandiflorum*). In *Advances in experimental medicine and biology* (Vol. 973, pp. 1–16). https://doi.org/10.1007/5584_2017_5

Pabari, K., Pithva, S., Kothari, C., Purama, R. K., Kondepudi, K. K., Vyas, B. R. M., Kothari, R., & Ambalam, P. (2020). Evaluation of probiotic properties and prebiotic utilization potential of *Weissella paramesenteroides* isolated from fruits. *Probiotics and Antimicrobial Proteins, 12*(3), 1126–1138. https://doi.org/10.1007/s12602-019-09630-w

Park, S., Ji, Y., Park, H., Lee, K., Park, H., Beck, B. R., Shin, H., & Holzapfel, W. H. (2016). Evaluation of functional properties of *lactobacilli* isolated from Korean white *kimchi*. *Food Control, 69*, 5–12. https://doi.org/10.1016/j.foodcont.2016.04.037

Patil, M., Jadhav, A., & Patil, U. (2020). Functional characterization and *in vitro* screening of *Fructobacillus fructosus* MCC 3996 isolated from *Butea monosperma* flower for probiotic potential. *Letters in Applied Microbiology, 70*(4), 331–339. https://doi.org/10.1111/lam.13280

Pei, J., Jin, W., El-Aty, A. M. A., Baranenko, D. A., Gou, X., Zhang, H., Geng, J., Jiang, L., Chen, D., & Yue, T. (2020). Isolation, purification, and structural identification of a new bacteriocin made by *Lactobacillus plantarum* found in conventional *kombucha*. *Food Control, 110*, Article 106923. https://doi.org/10.1016/j.foodcont.2019.106923

Peng, K., Koubaa, M., Bals, O., & Vorobiev, E. (2020). Recent insights in the impact of emerging technologies on lactic acid bacteria: A review. *Food Research International, 137*, Article 109544. https://doi.org/10.1016/j.foodres.2020.109544

Pereira, G. V. de M., Neto, D. P. de C., Medeiros, A. B. P., Soccol, V. T., Neto, E., Woiciechowski, A. L., & Soccol, C. R. (2016). Potential of lactic acid bacteria to improve the fermentation and quality of coffee during on-farm processing. *International Journal of Food Science and Technology, 51*(7), 1689–1695. https://doi.org/10.1111/ijfs.13142

Pereira, G. V. de M., Neto, E., Soccol, V. T., Medeiros, A. B. P., Woiciechowski, A. L., & Soccol, C. R. (2015). Conducting starter culture-controlled fermentations of coffee beans during on-farm wet processing: Growth, metabolic analyses and sensorial effects. *Food Research International, 75*, 348–356. https://doi.org/10.1016/j.foodres.2015.06.027

Pérez-Armendáriz, B., & Cardoso-Ugarte, G. A. (2020). Traditional fermented beverages in Mexico: Biotechnological, nutritional, and functional approaches. *Food Research International, 136*, Article 109307. https://doi.org/10.1016/j.foodres.2020.109307

Pessôa, M. G., Vespermann, K. A. C., Paulino, B. N., Barcelos, M. C. S., Pastore, G. M., & Molina, G. (2019). Newly isolated microorganisms with potential application in biotechnology. *Biotechnology Advances, 37*(2), 319–339. https://doi.org/10.1016/j.biotechadv.2019.01.007

Pimentel, T. C., da Costa, Barão, C. E., Rosset, M., & Magnani, M. (2021). Vegan probiotic products: A modern tendency or the newest challenge in functional foods. *Food Research International, 140*, 110033.

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 Microbiology, 49*, 182–188. https://doi.org/10.1016/j.fm.2015.02.005

Rezac, S., Kok, C. R., Heermann, M., & Hutkins, R. (2018). Fermented foods as a dietary source of live organisms. *Frontiers in Microbiology, 9*, 1785. https://doi.org/10.3389/fmicb.2018.01785

Riesute, R., Salomskiene, J., Moreno, D. S., & Gustiene, S. (2021). Effect of yeasts on food quality and safety and possibilities of their inhibition. *Trends in Food Science & Technology, 108*, 1–10. https://doi.org/10.1016/j.tifs.2020.11.022

Rodríguez, L. G. R., Mohamed, F., Bleckwedel, J., Medina, R., De Vuyst, L., Hebert, E. M., & Mozzi, F. (2019). Diversity and functional properties of lactic acid bacteria isolated from wild fruits and flowers present in northern Argentina. *Frontiers in Microbiology, 10*, 1091. https://doi.org/10.3389/fmicb.2019.01091

Saelim, K., Jampaphaeng, K., & Maneerat, S. (2017). Functional properties of *Lactobacillus plantarum* S0/7 isolated fermented stinky bean (*sa taw dong*) and its use as a starter culture. *Journal of Functional Foods, 38*, 370–377. https://doi.org/10.1016/j.jff.2017.09.035

Sakandar, H. A., Kubow, S., & Sadiq, F. A. (2019). Isolation and *in-vitro* probiotic characterization of fructophilic lactic acid bacteria from Chinese fruits and flowers. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 104*, 70–75. https://doi.org/10.1016/j.lwt.2019.01.038

Samedi, L., & Charles, A. L. (2019). Isolation and characterization of potential probiotic *Lactobacilli* from leaves of food plants for possible additives in pellet feeding. *Annals of Agricultural Science, 64*(1), 55–62. https://doi.org/10.1016/j.aoas.2019.05.004

Santos, T. T., Ornellas, R. M. S., Arcucio, L. B., Oliveira, M. M., Nicoli, J. R., Dias, C. V., Uetanabaro, A. P. T., & Vinderola, G. (2016). Characterization of *lactobacilli* strains derived from cocoa fermentation in the south of bahia for the development of probiotic cultures. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 73*, 259–266. https://doi.org/10.1016/j.lwt.2016.06.003

Semjonovs, P., Ruklisha, M., Paegle, L., Saka, M., Treimane, R., Skute, M., Rozenberga, L., Vikele, L., Sabovics, M., & Cleenwerck, I. (2017). Cellulose synthesis by *Komagataeibacter rhaeticus* strain P 1463 isolated from *kombucha*. *Applied Microbiology and Biotechnology, 101*(3), 1003–1012. https://doi.org/10.1007/s00253-016-7761-8

Shekh, S. L., Boricha, A. A., Chavda, J. G., & Vyas, B. R. M. (2020). Probiotic potential of lyophilized *Lactobacillus plantarum* GP. *Annals of Microbiology, 70*(1), 16. https://doi.org/10.1186/s13213-020-01556-x

Song, M. W., Chung, Y., Kim, K. T., Hong, W. S., Chang, H. J., & Paik, H. D. (2020). Probiotic characteristics of *Lactobacillus brevis* B13-2 isolated from *kimchi* and investigation of antioxidant and immune-modulating abilities of its heat-killed cells.

Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 128, Article 109452. https://doi.org/10.1016/j.lwt.2020.109452

Souza, M. L., Passamani, F. R. F., Ávila, C. L. da S., Batista, L. R., Schwan, R. F., & Silva, C. F. (2017). Uso de Leveduras Selvagens Como Agente de Biocontrole Contra Fungos Toxigênicos e Produção de OTA. *Acta Scientiarum. Agronomy, 39*(3), 349–358. https://doi.org/10.4025/actasciagron.v39i3.32659

Taheur, F. B., Mansour, C., Jeddou, K. B., Machreki, Y., Kouidhi, B., Abdulhakim, J. A., & Chaieb, K. (2020). Aflatoxin B1 degradation by microorganisms isolated from *kombucha* culture. *Toxicon, 179*, 76–83. https://doi.org/10.1016/j.toxicon.2020.03.004

Tang, H., Qian, B., Xia, B., Zhuan, Y., Yao, Y., Gan, R., & Zhang, J. (2018). Screening of lactic acid bacteria isolated from fermented *cornus officinalis* fruits for probiotic potential. *Journal of Food Safety, 38*(6), e12565. https://doi.org/10.1111/jfs.12565

Taroub, B., Salma, L., Manel, Z., Ouzari, H. I., Hamdi, Z., & Moktar, H. (2019). Isolation of lactic acid bacteria from grape fruit: Antifungal activities, probiotic properties, and *in vitro* detoxification of ochratoxin A. *Annals of Microbiology, 69*(1), 17–27. https://doi.org/10.1007/s13213-018-1359-6

Torres, S., Verón, H., Contreras, L., & Isla, M. I. (2020). An overview of plant-autochthonous microorganisms and fermented vegetable foods. *Food Science and Human Wellness, 9*(2), 112–123. https://doi.org/10.1016/j.fshw.2020.02.006

Vasiee, A., Behbahani, B. A., Yazdi, F. T., Mortazavi, S. A., & Noorbakhsh, H. (2018). Diversity and probiotic potential of lactic acid bacteria isolated from horreh, a traditional Iranian fermented food. *Probiotics and Antimicrobial Proteins, 10*(2), 258–268. https://doi.org/10.1007/s12602-017-9282-x

Vera-Pingitore, E., Jimenez, M. E., Dallagnol, A., Belfiore, C., Fontana, C., Fontana, P., von Wright, A., Vignolo, G., & Plumed-Ferrer, C. (2016). Screening and characterization of potential probiotic and starter bacteria for plant fermentations. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 71*, 288–294. https://doi.org/10.1016/j.lwt.2016.03.046

Verón, H. E., Cano, P. G., Fabersani, E., Sanz, Y., Isla, M. I., Espinar, M. T. F., Ponce, J. V. G., & Torres, S. (2019). Cactus pear (*Opuntia ficus-indica*) juice fermented with autochthonous *Lactobacillus plantarum* S-811. *Food and Function, 10*(2), 1085–1097. https://doi.org/10.1039/c8fo01591k

Verón, H. E., Di Risio, H. D., Isla, M. I., & Torres, S. (2017). Isolation and selection of potential probiotic lactic acid bacteria from *Opuntia ficus-indica* fruits that grow in Northwest Argentina. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 84*, 231–240. https://doi.org/10.1016/j.lwt.2017.05.058

Vijayakumar, M., Ilavenil, S., Kim, D. H., Arasu, M. V., Priya, K., & Choi, K. C. (2015). In-vitro assessment of the probiotic potential of *Lactobacillus plantarum* KCC-24 isolated from Italian rye-grass (*Lolium multiflorum*) forage. *Anaerobe, 32*, 90–97. https://doi.org/10.1016/j.anaerobe.2015.01.003

Won, S.-M., Chen, S., Lee, S. Y., Lee, K. E., Park, K. W., & Yoon, J.-H. (2020). *Lactobacillus sakei* ADM14 induces anti-obesity effects and changes in gut microbiome in high-fat diet-induced obese mice. *Nutrients, 12*(12), 3703. https://doi.org/10.3390/nu12123703

Wuyts, S., Beeck, W. V., Allonsius, C. N., van den Broek, M. F. L., & Lebeer, S. (2020). Applications of plant-based fermented foods and their microbes. *Current Opinion in Biotechnology, 61*, 45–52. https://doi.org/10.1016/j.copbio.2019.09.023

Xu, Y., Zhou, T., Tang, H., Li, X., Chen, Y., Zhang, L., & Zhang, J. (2020). Probiotic potential and amylolytic properties of lactic acid bacteria isolated from Chinese fermented cereal foods. *Food Control, 111*, Article 107057. https://doi.org/10.1016/j.foodcont.2019.107057

Yépez, A., Luz, C., Meca, G., Vignolo, G., Mañes, J., & Aznar, R. (2017). Biopreservation potential of lactic acid bacteria from Andean fermented food of vegetal origin. *Food Control, 78*, 393–400. https://doi.org/10.1016/j.foodcont.2017.03.009

Yépez, A., Russo, P., Spano, G., Khomenko, I., Biasioli, F., Capozzi, V., & Aznar, R. (2019). In situ riboflavin fortification of different kefir-like cereal-based beverages using selected Andean LAB strains. *Food Microbiology, 77*, 61–68. https://doi.org/10.1016/j.fm.2018.08.008

Zarour, K., Prieto, A., Pérez-Ramos, A., Kihal, M., & López, P. (2018). Analysis of technological and probiotic properties of Algerian *L. mesenteroides* strains isolated from dairy and non-dairy products. *Journal of Functional Foods, 49*, 351–361. https://doi.org/10.1016/j.jff.2018.09.001

2.2 Tópicos complementares

2.2.1 Fermentação natural

O processo de fermentação é constituído por diversos mecanismos tais como a metabolização de compostos presentes no substrato, ação de enzimas e lise de células de leveduras (CIOCH-SKONECZNY *et al*., 2021). Embora seja um dos meios mais antigos para o processamento de alimentos, a fermentação é utilizada até os dias atuais em grande variedade de alimentos produzidos por grandes empresas (PEREIRA *et al.*, 2020).

Alimentos fermentados podem ser preparados a partir de matrizes alimentares cruas ou cozidas e adquirem suas características de aroma e sabor a partir do metabolismo microbiano (KAMDA *et al*., 2015). As técnicas mais antigas para a produção de alimentos fermentados baseavam-se na fermentação natural utilizando a microbiota autóctone proveniente da matéria-prima ou do meio ambiente (de utensílios, manipuladores e local de processamento), sendo eles representados por variadas bactérias, leveduras e fungos filamentosos (RESENDE *et al*., 2018; PEREIRA *et al.*, 2020). Nesse sentido, diversos estudos têm apontado a coexistência de leveduras e bactérias láticas (BAL) nesse tipo de fermentação (PUERARI; MAGALHAES-GUEDES; SCHWAN, 2015; FREIRE *et al*., 2017).

As diversas atividades metabólicas dos grupos de microrganismos, sejam eles de leveduras ou BAL, envolvidos no processo de fermentação são determinantes para as características e estabilidade do produto fermentado (BONATSOU *et al*, 2018). Mudanças na disponibilidade de nutrientes e oxigênio, assim como variações de pH, temperatura e ácidos orgânicos podem afetar a fermentação natural, alterando a dinâmica de populações de microrganismos envolvidos no processo e causando modificações na qualidade do produto fermentado final (GREPPI *et al*., 2013; RIBEIRO *et al*., 2018).

Durante o processo de fermentação natural, algumas espécies de leveduras podem desaparecer devido ao aumento na concentração de ácido lático proveniente do metabolismo de BAL, já outras espécies apresentam resistência, podendo se manter em altas contagens mesmo após acidificação do meio (GREPPI *et al*., 2013). Além disso, alguns microrganismos conseguem coexistir durante um processo de fermentação sem causar interferência umas às outras. No entanto, ao modificar as características do processo de fermentação, alguns microrganismos tornam-se dominantes inibindo outros por meio de seus metabólitos (RESENDE *et al*., 2018).

Na literatura podem ser encontrados diversos estudos a respeito dos processos naturais de fermentação em frutas e dos microrganismos isolados a partir desses processos. Durante a fermentação espontânea de mosto de uvas tintas e brancas, Cioch-Skoneczny *et al*. (2020) foi capaz de isolar cepas de *Hanseniaspora* e *Saccharomyces*, além de *Metschnikowia pulcherrima*, *Candida railenensis*, *Torulaspora delbrueckii* e *Wickerhamomyces onychis*, que foram capazes de provocar alterações químicas desejáveis nos vinhos produzidos. No estudo a respeito do *Khadi*, uma bebida do Botswana feita de frutos de uma espécie de groselha selvagem (*Grewia flava*), puderam ser identificadas bactérias e leveduras *Saccharomyces* e não-*Saccharomyces* nos fermentados (MOTLHANKA *et al*., 2020). Lu *et al*. (2021) avaliaram a sucessão de leveduras e bactérias durante a fermentação natural de caqui concluindo que leveduras dos gêneros *Saccharomyces*, *Hanseniaspora*, *Rhodotorula*, e bactérias dos gêneros *Leuconostoc* e *Lactobacillus* foram capazes de desempenhar importante papel no perfil de voláteis dos vinhos produzidos. Além disso, Ogodo *et al*. (2018) observaram que grande parte das bactérias e fungos deteriorantes desapareceram após três semanas de fermentação, enquanto BAL e leveduras benéficas permaneceram até o final, sendo essa uma característica desejável no que diz respeito ao perfil de segurança desses fermentados.

Leveduras das espécies *Aureobasidium pullulans*, *Dothichiza pithyophila*, *Dioszegia zsoltii*, *Hanseniaspora uvarum* e outras dos gêneros *Metschnikowia*, *Cryptococcus* e *Rhodotorula* foram identificados durante a produção de uma bebida fermentada, muito apreciada no sul da Europa, feita a partir de frutos de medronheiro (*Arbutus unedo* L.), sendo possível observar que ao final do processo de fermentação as espécies de *Saccharomyces cerevisiae* e *Pichia membranaefaciens* dominaram o processo, substituindo as cepas anteriormente presentes no mosto caracterizado então por alto teor de etanol (ESPÍRITO SANTO *et al*., 2012).

Assim sendo, o conhecimento sobre os microrganismos envolvidos em processos de fermentação natural é de grande importância, pois essas culturas ao serem isoladas podem ser aplicadas em processos para a obtenção de produtos funcionais, tendo em vista os benefícios que podem ser obtidos por meio de seu metabolismo fermentativo (RESENDE *et al*., 2018). Além disso, a dinâmica populacional e os compostos responsáveis pelo sabor e aroma desses alimentos fermentados podem ser mais adequadamente compreendidos quando se identificam os compostos produzidos durante esses processos (PUERARI; MAGALHAES-GUEDES; SCHWAN, 2015). Portanto, a seleção de cepas para a produção de produtos fermentados, utilizando cepas endógenas provenientes desse tipo de fermentação, pode tornar possível a obtenção de produtos de maior qualidade e segurança (EMER *et al*. 2021).

2.2.2 Leveduras em produtos fermentados de fruta

As leveduras são microrganismos eucarióticos unicelulares e podem ser encontradas de modo natural na superfície de vegetais (EINSON *et al*., 2018), sendo capazes de transformar carboidratos em etanol, água, CO_2, além de produzirem outros compostos tais como aminoácidos, purinas, vitaminas e álcoois superiores derivados do metabolismo secundário (ARROYO-LÓPEZ *et al*., 2012; CORDENTE *et al*., 2019).

Representadas pelos filos dos *Ascomicetos* e *Basidiomicetos*, as leveduras são um grupo heterogêneo de microrganismos que se multiplicam por brotamento ou fissão e que possuem um potencial biotecnológico aplicável na indústria de alimentos (DE VUYST *et al*., 2016; RAI; PANDEY; SAHOO, 2019).

O emprego de leveduras na indústria de alimentos se deve principalmente às mudanças nos aspectos sensoriais e nutricionais que elas provocam nos produtos fermentados. Entretanto, grande parte dos estudos com frutas fermentadas é realizado utilizando BAL (GUERGOLETTO *et al.*, 2020, PALACHUM *et al.*, 2020) e estudos com frutas naturalmente fermentadas por leveduras ainda são escassos (NYANGA *et al.*, 2013, FARINAZZO *et al.*, 2020). A utilização de leveduras em detrimento das BAL em processos fermentativos pode ser vantajosa devido ao fato de que, embora leveduras sejam normalmente resistentes a antibióticos elas não transferem esses genes a outros microrganismos (RAI; PANDEY; SAHOO, 2019). As leveduras podem ainda levar a melhora da saúde e bem-estar do hospedeiro devido a sua capacidade de produzir substâncias antioxidantes tais como carotenoides, ácidos cítrico e ascórbico, tocoferóis, entre outras que são capazes de combater o estresse oxidativo (ARROYO-LÓPEZ *et al*., 2012). Devido a isso, também têm sido amplamente empregadas na indústria farmacêutica para a produção de vitaminais, particularmente as do complexo B (DE VUYST *et al*., 2016).

Além disso, as leveduras podem contribuir para a segurança e qualidade de alimentos e bebidas exibindo atividade antagonista frente a microrganismos deteriorantes devido a produção de acetato de etila e outros metabólitos, tais como as glicoproteínas, o que pode ser conveniente tendo em vista que podem assim reduzir a necessidade de conservantes para a estabilização do produto (ARROYO-LÓPEZ *et al*., 2012; CODA *et al*., 2013; DE VUYST *et al*., 2016).

Leveduras de origem autóctone podem fermentar o substrato com maior intensidade por provavelmente estarem mais bem adaptadas às condições da matriz alimentar durante a fermentação (APONTE *et al*., 2020), entretanto, o modo como elas se multiplicam e se sucedem durante o processo deve ser bem conhecido (GREPPI *et al*., 2013). A população de variadas espécies de leveduras observada durante a fermentação espontânea de alimentos e bebidas é complexa e sua sucessão depende de vários fatores intrínsecos e extrínsecos relacionados à matriz alimentar, tais como tolerância a fatores de estresse microbiano como etanol e ácidos orgânicos, além de interações com outros microrganismos (JOHANSEN *et al*., 2019).

Bebidas fermentadas por leveduras podem apresentar altas concentrações de glicose e frutose em sua composição, baixas concentrações de ácido acético e alto valor de fenólicos totais, apresentando boa aceitação sensorial (AMORIM; PICCOLI; DUARTE, 2018). Frutas como o abacaxi foram utilizadas para a produção de bebida fermentada com compostos voláteis distintos provenientes do metabolismo de leveduras e consideradas de qualidade e sabor agradável aos consumidores (DELLACASSA et al, 2017). Hu *et al*. (2020) também observaram a melhora da qualidade sensorial do vinho de Ponkan obtido a partir da co-fermentação de cepas selecionadas de leveduras não-*Saccharomyces* com *Saccharomyces cerevisiae*, que foram capazes de levar a menor produção de etanol e maior teor de compostos voláteis aromáticos.

Os compostos voláteis ocorrem naturalmente em frutas e em bebidas fermentadas e têm uma origem muito diversa podendo ser provenientes da ação de microrganismos durante a fermentação ou envelhecimento e oxidação (JANUSZEK; SATORA, 2021). Estes compostos são responsáveis por boa parte das características sensoriais de um produto fermentado. No que diz respeito a essas características, por meio das fermentações, os vegetais são transformados de modo que passam a fornecer diferentes sabores e texturas a depender do resultado desejado (EINSON *et al*., 2018). Na produção de vinhos, por exemplo, pesquisadores têm tido a percepção de que a limitada variedade de cepas comerciais autorizadas para uso tem levado a produção de vinhos muito uniformes entre si, sem diversidade de aroma e sabor. Por essa razão, muitos estudos têm tido como objetivo a busca por meios para aumentar a diversidade de características sensoriais que levem a diferenciação entre os fermentados e adicione valor comercial a estes produtos (PUERTAS *et al*., 2018). Para tal, os estudos têm demonstrado que o uso de leveduras nativas, provenientes de fermentação natural, na produção de vinhos tem demonstrado a capacidade de melhorar tanto a qualidade como também as características sensoriais do produto (MEDINA *et al*., 2013; XU et al., 2020). A cepa de *Saccharomyces cerevisiae* selecionada a partir de uvas da variedade Pedro-Ximenes, parcialmente fermentadas, foi utilizada para a fermentação de vinho misto de *blueberry* e uvas e os resultados demonstraram maiores valores de fenólicos totais e atividade antioxidante nos vinhos obtidos a partir da fermentação espontânea desse mosto misto (MARTÍN-GÓMEZ *et al*., 2021). Foram encontradas maiores concentrações de compostos fenólicos em vinhos *Cabernet Sauvignon* fermentados espontaneamente em comparação com os vinhos inoculados (UZKUÇ; BAYHAN; TOKLUCU, 2022). O estudo de Postigo *et al*. (2022) mostrou que as cepas não-*Saccharomyces* também têm potencial para a produção de bebida com baixo teor alcoólico, tendo em vista que, tendem a produzir menos álcool que leveduras do gênero *Saccharomyces*.

Alguns estudos têm apontado que leveduras do grupo não-*Saccharomyces* predominam nos estágios inicial e intermediário durante processos de fermentação espontânea, enquanto as *Saccharomyces* predominam nos estágios posteriores (ESPÍRITO SANTO *et al*., 2012; LI; HU; XU, 2019). Skotniczny *et al.* (2020) observaram maior quantidade de leveduras das espécies *Hanseniaspora uvarum*, *Metschnikowia spp*. e *Pichia kudriavzevii* no início da fermentação natural de purê de ameixa, no entanto, nas fases intermediária e final houve predominância da espécie *S. cerevisiae*. Também foram observadas a sucessão de diferentes grupos de leveduras em mostos de uvas tintas ‘Rondo’ e ‘Regent’, com prevalência de cepas de *Hanseniaspora uvarum* e *Candida railenensis*, substituídas por cepas de *Saccharomyces cerevisiae* no decorrer do processo fermentativo (CIOCH-SKONECZNY *et al*., 2018).

O uso de cepas de leveduras não-*Saccharomyces* como iniciadoras de fermentação em vinhos apresentam a vantagem de produzir vinhos com maior complexidade e diversidade de sabor e aroma em relação aos que utilizam cepas comerciais de *Saccharomyces* (MEDINA *et al*., 2013). Nesse sentido, as co-inoculações com leveduras não-*Saccharomyces* têm sido cada vez mais utilizadas com o intuito de produzir vinhos de alta qualidade. Leveduras não-*Saccharomyces* das espécies *H. uvarum*, *Lachancea thermotolerans* e *Metschnikowia pulcherrima*, isoladas de vinho do Porto, produzidos a partir de fermentação natural, demonstraram alto desempenho de crescimento em condições de estresse peculiares a que os vinhos são expostos (MATEUS *et al*., 2020). Vaquero *et al*. (2021) observaram que a co-inoculação de *L. thermotolerans* e outras espécies não-*Saccharomyces* tornou a acidificação mais eficaz, protegendo a coloração destes vinhos e trazendo um perfil aromático mais complexo em vinhos produzidos em regiões quentes do planeta. Em outro estudo, observou-se que a inoculação com *M. pulcherrima* resultou em vinhos rosés com perfil de ésteres melhorado, redução de acetaldeído e aumento de antocianinas e taninos (MUÑOZ-REDONDO *et al*., 2021).

Outras características, que são importantes do ponto de vista da saúde, podem ser observadas durante os processos de fermentação por leveduras. Zullo e Ciafardini (2019) observaram que as leveduras *Wickerhamomyces anomalus* reduziram em cerca de 50% o colesterol presente em meio de fermentação (*in vitro*). Além disso, foi demonstrado que o extrato metanólico do sobrenadante de leveduras da espécie *Pichia kudriavzevii* inibiu a proliferação de células cancerígenas do cólon humano (HT-29 e Caco-2) induzindo a apoptose (SABER *et al*., 2017), trazendo diversos benefícios que necessitam de estudos mais aprofundados para melhor compreensão.

2.2.3 Frutas da caatinga com potencial para aplicação biotecnológica

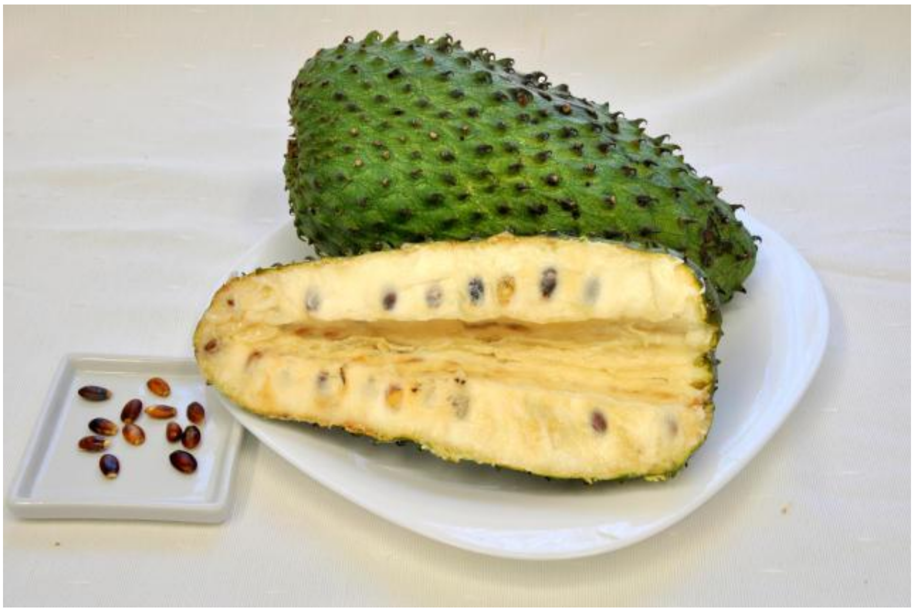
Diversas frutas consideradas exóticas e ainda desconhecidas do público em geral possuem grande potencial para tornarem-se parte da alimentação cotidiana devido às suas propriedades funcionais (VIDIGAL *et al*., 2011). O Brasil possui grande número de espécies frutíferas nativas e exóticas subexploradas, com potencial de interesse para a indústria (SCHULZ *et al*., 2020). Sendo o país detentor de uma região geográfica com condições climáticas adequadas, o cultivo dessas frutas nativas possui um excelente potencial agroindustrial (PAZ *et al*., 2015). Muitas dessas frutas brasileiras ainda desconhecidas ou subexploradas não estão sendo produzidas ou consumidas provavelmente devido ao desconhecimento dos consumidores, principalmente daqueles residentes em áreas urbanas (BERNI *et al*., 2019).

Essas frutas são mais consumidas nos interiores do Brasil, em especial no bioma Caatinga, que possui ampla variedade de frutas exóticas. Devido às suas propriedades sensoriais atrativas e ao aumento do reconhecimento do valor nutricional e terapêutico, o consumo das frutas exóticas e tropicais está crescendo nos mercados nacional e internacional (RUFINO *et al*., 2010; ALBUQUERQUE *et al*., 2016). Esse novo mercado tem sido o responsável pela demanda de estudos sobre os possíveis benefícios à saúde associados ao consumo de frutas exóticas e tropicais, fazendo com que ganhem cada vez mais importância dentro da alimentação humana (ALBUQUERQUE *et al*., 2016).

As frutas, além de suas características de sabor e frescor, fornecem minerais (potássio, zinco, cobre, magnésio, cálcio, entre outros), vitaminas (especialmente vitamina C), fibras e outros compostos (flavonoides, fenólicos, carotenoides), sendo amplamente recomendadas para prevenção de doenças cardiovasculares, diabetes *mellitus* tipo 2 e alguns tipos de câncer (LIMA *et al*., 2018).

Além disso, frutas do bioma Caatinga podem ser fontes de microrganismos com potencial de aplicação em processos biotecnológicos (ASSIS *et al*., 2021). Dentre as espécies frutíferas escassamente estudadas como fonte de microrganismos com potencial biotecnológico destaca-se a graviola (*Annona muricata* L.) (Figura 1).

Figura 1 – Graviola *in natura*



Fonte: Embrapa. Disponível em: < https://www.embrapa.br/bme_images/m/101160040m.jpg>. Acesso em: 25 out. 2022.

Pertencente à família *Annonaceae,* é originária do Caribe e nativa dos trópicos americanos. Nos últimos anos, a graviola tem recebido considerável atenção dos consumidores devido ao seu valor nutricional. Estudos têm relatado que a graviola é fonte de carboidratos, micronutrientes e minerais (AGU e OKOLIE, 2017; CHANG *et al*., 2018).

A graviola é uma fruta tropical que apresenta alto valor comercial no Brasil, com grandes perspectivas econômicas para comercialização e exportação, apresentando características sensoriais e valor nutritivo significativo, sendo destinada à produção de polpas, sucos e néctares (LEMOS, 2014; WATANABE *et al*., 2014).

Além de possuir excelentes características sensoriais, a graviola é rica em compostos bioativos. Coria-Téllez *et al.* (2018) mencionaram que 212 compostos bioativos foram observados na graviola, dos quais acetogeninas, alcaloides e fenóis são prevalentes. Foram encontrados na polpa de graviola 37 compostos voláteis, principalmente ésteres aromáticos e alifáticos (CHEONG *et al.*, 2011). Jiménez *et al*. (2014) observaram a prevalência de derivados dos ácidos cinâmico e *p*-cumárico com outros compostos que podem contribuir para os efeitos benéficos da graviola na saúde.

Ainda no contexto de espécies subexploradas desse bioma, o umbu-cajá é outra espécie que tem se destacado. Pertencente à família *Anacardiaceae*, o umbu-cajá (Figura 2) originou-se a partir do cruzamento natural entre umbu (*Spondias tuberosa)* e cajá (*Spondias mombin*) no Nordeste do Brasil (CARVALHO; SOARES FILHO; RITZINGER, 2010). Por ser característica da região semiárida ela é uma espécie tolerante à seca sendo a árvore de aspecto similar ao umbuzeiro (NARAIN *et al*., 2007). As espécies do gênero *Spondias* (família *Anacardiaceae*) podem ser encontradas nas regiões tropicais de quatro continentes (Américas, África, Ásia e Oceania). Os frutos são de formato, redondo, ovalado ou piriforme, de acidez elevada, aroma agradável, possuindo polpa suculenta, doce e fibrosa e apresentando também sabor amargo (VIANA *et al*., 2015; PEREIRA *et al*., 2021). O umbu-cajá tem como característica relevante as elevadas concentrações de compostos bioativos, dentre eles vitamina C, taninos e flavonóides (LIMA *et al*., 2002).

Figura 2 – Umbu-cajá *in natura*

Fonte: Empaer. Disponível em: <https://empaer.pb.gov.br/noticias/extensionista-rural-representa-empaer-em-simposio-sobre-umbu-caja-em-alagoas>. Acesso em: 25 out. 2022.

O conhecimento científico sobre essas frutas aliadas à sua exploração comercial pode estimular o desenvolvimento sustentável, melhores hábitos alimentares, proteção contra a biopirataria, além de inovação nos sistemas alimentares (BERNI *et al*., 2019).

2.2.4 Efeitos dos compostos fenólicos sobre a saúde dos indivíduos

Os compostos fenólicos estão entre as moléculas que exercem efeito sobre a saúde dos indivíduos e têm sido amplamente estudadas. Eles são metabólitos secundários das plantas e podem influenciar vários processos que modulam a saúde de órgãos e sistemas do corpo humano (DOMÍNGUEZ-AVILA *et al*., 2021). Diversos estudos têm sido realizados para melhor compreender as transformações que a microbiota intestinal ocasiona em tipos específicos de polifenóis derivados de frutas e vegetais e identificar quais os microrganismos responsáveis pelas transformações ocorridas (SELMA; ESPÍN; TOMAS-BARBERAN, 2009).

Dentre os compostos bioativos transformados pela microbiota intestinal está a procianidina B2 que tem sido relatada por seus efeitos inibidores do estresse oxidativo, redução da pressão arterial e melhora da função renal em ratos (DING *et al*., 2018; YAO *et al*., 2021), além de reduzir a calcificação vascular devido à sua potente ação antioxidante (Liang *et al*., 2021). O ácido gálico, por sua vez, também é um metabólito secundário dos polifenóis e é bem conhecido por sua característica antioxidante, possuindo ainda ação anti-hiperlipidêmica, cardioprotetora, anti-hiperglicêmica e anticarcinogênica (ZANWAR *et al*., 2014; ASHRAFIZADEH *et al*., 2021). A catequina também tem sido reconhecida por seu efeito anti-hipertensivo por promover a inibição da ECA (HE, 2017). Lapi *et al*. (2020) relataram que a administração de catequina em ratos foi capaz de induzir a recuperação endotelial e reduzir a pressão arterial.

Os diferentes efeitos dos compostos fenólicos sobre a saúde de cada indivíduo resultam variabilidade interindividual da microbiota intestinal que serão determinantes para a absorção desses compostos através do processo fermentativo que promovem (SELMA; ESPÍN; TOMAS-BARBERAN, 2009; DOMÍNGUEZ-AVILA *et al*., 2021). O cólon é o principal local de ação dos compostos fenólicos, podendo levar a efeitos qualitativos e quantitativos na microbiota intestinal, induzindo assim efeitos promotores da saúde, tanto através da ação desses microrganismos, como também promovendo ações anti-inflamatória, antioxidante e antiproliferativa (DOMÍNGUEZ-AVILA *et al*., 2021).

Apesar da numerosa quantidade de compostos fenólicos presentes na dieta, o número de metabólitos ao final do processo é menor, podendo os benefícios de seu consumo serem atribuídos aos metabólitos capazes de modular a microbiota intestinal (SELMA; ESPÍN; TOMAS-BARBERAN, 2009).

2.2.5 Modulação da microbiota intestinal na hipertensão arterial

A hipertensão arterial é uma doença crônica não-transmissível, caracterizada por pressão arterial sistólica igual ou superior a 140 mmHg e pressão arterial diastólica igual ou superior a 90 mmHg sem o uso de medicação anti-hipertensiva e avaliados por um profissional treinado (BARROSO *et al*., 2021). Ela tem sido dada coma a principal causa de doenças cardiovasculares e morte prematura em todo o mundo, e sua prevalência tem aumentado especialmente nos países de baixa e média renda (MILLS; STEFANESCU; HE, 2020).

Os processos inflamatórios crônicos têm sido associados à danos na barreira intestinal. Por essa razão, os efeitos dos compostos fenólicos na permeabilidade intestinal têm sido relacionados com suas propriedades anti-inflamatórias (SELMA; ESPÍN; TOMAS-BARBERAN, 2009).

Alterações positivas na microbiota colônica demonstraram atuar prevenindo ou atenuando problemas relacionados à saúde como a obesidade (CHENG *et al*., 2018), doenças inflamatórias intestinais (SCHAUBECK *et al*., 2016) e outros distúrbios tais como a doença hepática não-alcoólica (XUE *et al*., 2017), além da hipertensão arterial (VERHAAR et al., 2020), melhorando assim o bem-estar dos indivíduos.

Estudos também têm demonstrado que os microrganismos do grupo *Lactobacillus* são capazes de prevenir a disbiose e estresse oxidativo tratando a disfunção endotelial que ocorre na hipertensão arterial (ROBLES-VERA *et al*., 2018; PALMU; LAHTI; NIIRANEN, 2021).

Efeitos similares aos do *Lactobacillus* também têm sido relatados em relação às populações de *Bifidobacterium*, com melhora do perfil da microbiota intestinal, assim como na redução do estresse oxidativo, prevenindo inclusive lesões da aorta e tratando a disfunção endotelial, principalmente devido à elevação da produção de acetato causada por esse grupo de microrganismos (ROBLES-VERA *et al*., 2018; LU *et al*., 2022).

Bactérias do gênero *Bacteroides* têm sido encontradas em quantidades elevadas em indivíduos hipertensos (CALDERÓN-PÉREZ *et al*., 2020; CALDERÓN-PÉREZ *et al*., 2021). O grupo de bactérias *Eubacterium rectale* - *Clostridium coccoides* está relacionado a riscos elevados para eventos cardio e cerebrovasculares em pacientes com hipertensão refratária (JIAO *et al*., 2022), portanto, reduções nas populações desse grupo são desejáveis.

Considerando o exposto, o presente trabalho visou isolar leveduras a partir dos frutos graviola e umbu-cajá naturalmente fermentados e avaliá-las quanto ao potencial biotecnológico na produção de polpas fermentadas, caracterizando-as quanto aos aspectos físico-químicos (pH, sólidos solúveis e acidez titulável), perfil de açúcares, ácidos orgânicos, compostos voláteis e fenólicos (perfil e bioacessibilidade), além de avaliar *in vitro* seus efeitos na modulação da microbiota intestinal humana de adultos hipertensos de meia-idade.

3 MATERIAIS E MÉTODOS

3.1 Tipo de estudo

Trata-se de uma pesquisa experimental em laboratório (in vitro) realizada no Laboratório de Processos Microbianos da Universidade Federal da Paraíba (UFPB) em parceria com pesquisadores e laboratórios da Universidade Federal de Lavras (UFLA) e do Instituto Federal do Sertão Pernambucano (IFSertãoPE).

3.2 Amostras

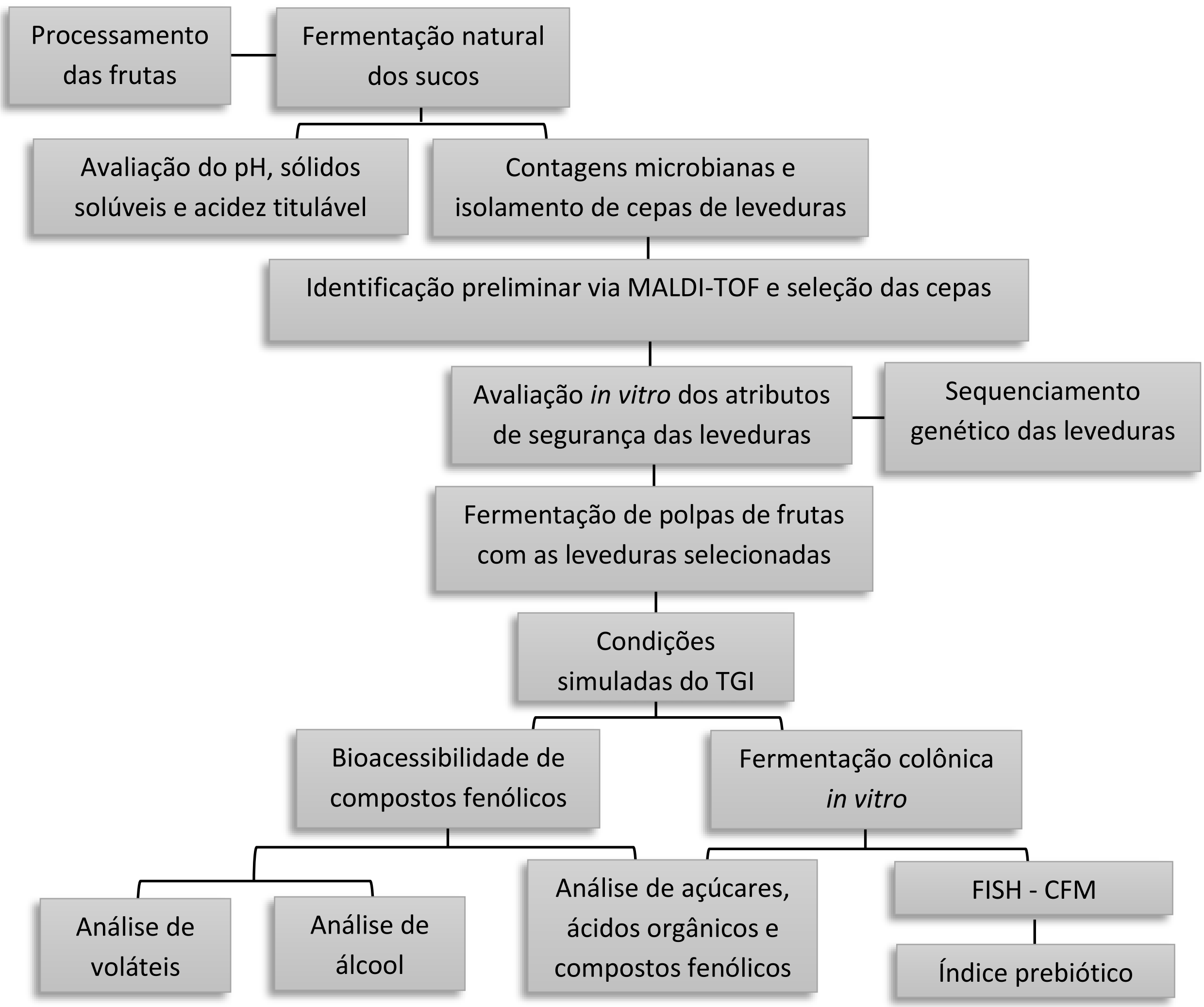
Os frutos de graviola (*Annona muricata* L.) e umbu-cajá (*Spondias spp.*) em estádio de maturação em que comumente são consumidos foram adquiridos em propriedades locais. Os frutos foram selecionados e padronizados com base na uniformidade de tamanho e forma, firmeza, cor e ausência de danos mecânicos e infecções visíveis. Em seguida, os frutos foram transportados em caixas refrigeradas (5 ± 1°C) e armazenados a 4 ± 1°C até o processamento dos sucos que foi realizado no mesmo dia.

3.3 Delineamento experimental

As frutas foram adquiridas, processadas e os sucos resultantes foram utilizados na execução da fermentação natural. Antes e durante o processo de fermentação as características físico-químicas dos sucos foram avaliadas, procedeu-se as contagens microbianas e o isolamento de leveduras a partir dos sucos fermentados. As leveduras isoladas foram submetidas à identificação micro e macro morfológicas e posteriormente identificadas através da técnica MALDI-TOF MS. Após a identificação foram selecionadas cepas para ensaios de segurança *in vitro* e em seguida utilizadas na fermentação de polpas de frutas das quais foram isoladas. As polpas foram analisadas para a determinação de açúcares, ácidos orgânicos, compostos fenólicos e álcool antes e após o processo de fermentação. Também foram analisados compostos voláteis na polpa fermentada e não fermentada. As polpas fermentadas foram submetidas a condições simuladas do trato gastrointestinal e foi determinada a bioacessibilidade dos fenólicos. As cepas selecionadas foram submetidas à sequenciamento genético e a cepa *Issatckenkia terricola* isolada a partir da polpa de umbu-cajá foi utilizada para os ensaios de fermentação das polpas de graviola e umbu-cajá com inóculo fecal *in vitro*. A identificação da abundância relativa de populações microbianas específicas foi realizada utilizando a técnica de fluorescência de hibridização *in situ* (FISH) acoplada a citometria de fluxo multiparamétrica (CFM). Durante a fermentação colônica foram retiradas alíquotas para a avaliação do pH, ácidos orgânicos, compostos fenólicos e açúcares. A partir dos dados da citometria foi realizada a determinação do índice prebiótico para cada processo de fermentação.

A Figura 3 expõe o desenho de execução do estudo.

Figura 3 – Desenho de execução do estudo do potencial biotecnológico de leveduras isoladas de frutas da caatinga fermentadas



Fonte: Autoral, 2022. Legenda: MALDI-TOF - *Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry*; TGI - Trato gastrointestinal; FISH - Hibridização por fluorescência *in vitro*; CFM - Citometria de fluxo multiparamétrica.

3.4 Processamento das amostras e fermentação

A fermentação foi realizada conforme metodologia descrita por Amorim; Piccoli; Duarte (2018) com algumas modificações. Os frutos foram higienizados com água destilada estéril e descascados assepticamente em câmara de fluxo laminar. As cascas e sementes de graviola foram desprezadas e do umbu-cajá foram desprezadas apenas as sementes.

A polpa fresca de cada fruta foi triturada separadamente com o auxílio de almofariz e pistilo e passadas por uma peneira de inox de uso doméstico com abertura de malha (*mesh*) 18 para remover o excesso de fibra. Essas etapas foram realizadas em condições assépticas. Aproximadamente 400 mL de suco integral foram obtidos de cada tipo de fruta. As polpas foram mantidas à temperatura ambiente (25 ± 1 ºC) por 48 h para fermentação natural.

Os valores de pH e sólidos solúveis totais (TSS) foram medidos nos tempos 0, 24 e 48 h de fermentação natural. O pH foi avaliado com um medidor de pH (Quimis, São Paulo, Brasil) e o TSS foi determinado com um refratômetro manual (Vodex, modelo VX032SG). A acidez titulável (AT) foi medida por titulação (0,1 N NaOH) e expressa como % de ácido cítrico. Todas as medições seguiram os procedimentos da AOAC (AOAC, 2016).

3.5 Contagens microbianas e isolamento de leveduras

As contagens e isolamentos foram realizados com base em metodologia proposta por (FREIRE *et al.*, 2017), com algumas modificações. Alíquotas (1 mL) foram coletadas de cada frasco de fermentação nos tempos 0, 24 e 48h para a realização das contagens de levedura e BAL e posterior isolamento de leveduras. Diluições seriadas de (10^{-1} a 10^{-6}) foram realizadas e o plaqueamento de alíquotas de 0,1 mL de cada diluição foram realizadas em meios específicos para leveduras e BAL (bactérias láticas).

Para a contagem e isolamento de leveduras foram utilizadas placas de Petri contendo o meio de cultura *Yeast Peptone Dextrose* (YPD) formulado com 1% de extrato de levedura (p/v), 2% de peptona (p/v), 1,5% ágar (p/v) (Acumedia, Michigan, USA), 2% de D-glicose (p/v) (Dinâmica Química Contemporânea, São Paulo, Brazil), e pH 3,5, suplementado com 100 mg/L de cloranfenicol (Sigma-Aldrich, St. Louis, USA) para a inibição do crescimento bacteriano.

Para a contagem de BAL cada diluição foi inoculada em placas de Petri contendo ágar De Man Rogosa Sharpe (MRS) (HiMedia, Mumbai, Índia) suplementado com 50 mg/L de nistatina 85+% (Acros Organics, New Jersey, USA) para inibição do crescimento de leveduras.

Essas etapas foram realizadas sob condições assépticas. As placas foram incubadas a 30°C por 72 h para leveduras e a 37°C por 48 h sob anaerobiose para BAL (Anaerogen, Oxoid Ltda., Wade Road, UK) com O_2 <1%. Os resultados foram reportados como log de unidades formadoras de colônia por mililitro (log UFC/mL).

As unidades formadoras de colônias (UFC) foram enumeradas em placas contendo de 30 a 300 colônias, e a concentração de células foi expressa como log UFG/mL (SANTOS, LIBECK; SCHWAN, 2014) (Figura 4). As leveduras isoladas foram submetidas a análises macroscópicas e micro morfológicas e as características de tamanho da colônia, tipo, estrutura de borda, cor, textura, aparência, elevação, brilho e forma foram observadas. Assim, a purificação das colônias de leveduras foi feita com base na morfologia presente nas placas e a raiz quadrada do total de isolados de cada morfotipo de colônia foi calculada para subsequente purificação (SENGUN *et al*., 2009).

Figura 4 – Placa de petri contendo leveduras

Fonte: Autoral, 2022.

Coloração simples com azul de metileno a partir de uma preparação a fresco e testes de verificação da capacidade de fermentação de carboidratos (maltose, glicose e sacarose) foram utilizados para a seleção dos isolados submetidos à identificação. Posteriormente, as culturas puras foram mantidas e subcultivadas até a identificação preliminar via MALDI-TOF MS e testes seguintes.

Todos os isolados foram armazenados em meio específico contendo 20% de glicerol e armazenados a - 80 °C (MIGUEL *et al*., 2017; RESENDE *et al*., 2018).

3.6 Identificação preliminar via MALDI-TOF MS e seleção das cepas

As leveduras selecionadas foram submetidas a identificação via *Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry* (MALDI-TOF MS) (CARVALHO *et al.*, 2017). As medições foram realizadas com um ultrafleXtreme MALDI-TOF MS (Bruker Daltonics, Bremen, Alemanha) equipado com um laser de granada de ítrio alumínio dopado com neodímio de 1000 Hz.

Culturas de leveduras foram cultivadas por 18 h em placas de Petri utilizando meio de cultura específico, conforme descrito acima. Aproximadamente 1 µg de biomassa da placa de cultura foi adicionado a um microtubo contendo 6µl de ácido fórmico a 25% em água (v/v). As amostras foram agitadas por 60 segundos em vórtice e, em seguida, centrifugadas por 60 segundos a 4000 g e temperatura ambiente.

O sobrenadante de cada amostra (1 µl) foi colocado na placa de aço inoxidável MALDI-TOF MS (aço polido alvo MSP 96; Bruker Daltonics). Quando a amostra estava quase seca na placa de aço inoxidável MALDI-TOF MS, 1 µl da solução de matriz de ácido a-ciano-4-hidroxicinâmico (Fluka) saturado em uma solução orgânica foi adicionado e misturado suavemente. Posteriormente, as amostras secas ao ar foram analisadas por MALDI-TOF MS.

Cada amostra de MALDI-TOF MS foi analisada em triplicata para testar a reprodutibilidade. Antes das análises, a calibração foi realizada com um padrão de teste (Bruker Daltonics) contendo um extrato de *Escherichia coli* DH5 alfa previamente preparado. Os espectros de massa foram processados com o pacote de software MALDI Biotyper 3.0 (Bruker Daltonics) para agrupamento estatístico e identificação microbiana.

O MALDI Biotyper System utiliza a espectrometria de massa MALDI-TOF para a interpretação dos resultados. As pontuações de identificação foram determinadas da seguinte forma (+++) representa a identificação da espécie altamente provável (pontuações 2.300 a 3.000); (++) representa a identificação segura do gênero, provável identificação da espécie (pontuações 2.000 a 2.299); (+) representa a provável identificação do gênero (escores 1.700 a 1.999); e (-), que representa a identificação não confiável (pontuações de 0,000 a 1,699).

As cepas foram escolhidas segundo seus *scores* de identificação e suas potencialidades de aplicação de acordo com a literatura consultada para serem aplicadas em testes de fermentação (LUAN *et al*., 2018; SHI *et al*, 2019).

3.7 Avaliação *in vitro* dos atributos de segurança das leveduras

A segurança dos isolados foi investigada avaliando a hemólise, atividade de DNAse e a hidrólise de gelatina, conforme descrito por Fonseca *et al*. (2021a), com algumas modificações. Para todos os testes, a cepa *Staphylococcus aureus* ATCC 25923 incubadas a 37°C por 48 h foi utilizada como controle positivo.

3.7.1 Hemólise

A atividade hemolítica foi determinada inoculando as cepas em placas de ágar sangue contendo 5% de sangue de ovelha após 48 h de incubação a 37°C. A ausência de efeito nas placas sanguíneas (γ-hemólise) foi considerada não hemolítica. As zonas verdes ao redor das colônias (α-hemólise) foram consideradas como atividade hemolítica parcial, e as cepas com áreas claras de hidrólise resultantes da lise das células sanguíneas ao redor das colônias foram classificadas como cepas hemolíticas (β-hemólise).

3.7.2 Atividade de DNAse

Para o teste de DNAse, as cepas foram semeadas no meio de ágar teste DNAse (Difco, EUA) e as placas foram incubadas a 30°C por 48 h. Após este tempo, uma solução de HCl 1 M foi adicionada à placa. Uma zona clara ao redor das colônias após a incubação foi considerada positiva para a produção de DNAse.

3.7.3 Hidrólise de gelatina

A produção de gelatinase por cepas foi analisada usando ágar triptona-neopeptonedextrose (TND) (17,0 g triptona, 3,0 g neopeptona, 2,5 g dextrose, 5,0 g NaCl, 2,5 g K_2HPO_4, 15 g ágar e 1 L de água destilada) contendo 0,4% de gelatina. As culturas leveduras foram inoculadas pontualmente em placas contendo o meio e incubadas a 30°C durante 48 horas. A produção da enzima foi visualizada pela formação de um halo ao redor da colônia após a adição de uma solução saturada de sulfato de amônio para confirmar a hidrólise da gelatina.

3.8 Aplicação tecnológica das leveduras na fermentação de polpas de frutas

3.8.1 Fermentação das polpas de frutas

As polpas de graviola e umbu-cajá foram obtidas de agricultores locais em estágio de maturação comercial. As frutas foram processadas sem adição de água ou conservantes nas plantas industriais da Fazenda Mangai (graviola) e Pé de Fruta (umbu-cajá) para obtenção das polpas.

As cepas selecionadas para o experimento, *Hanseniaspora opuntiae* 125, *Issatchenkia terricola* 129 e *Hanseniaspora opuntiae* 148 foram inoculadas em 5 mL do meio de cultura YPD (mesma composição relatada na Seção 3.5, sem a adição de ágar) e incubadas a 30 ± 1 °C por 24 h. Após o período de incubação, as culturas de células de levedura foram centrifugadas (3500 g, 15 min, 4°C), lavadas duas vezes com água peptonada 0,1% estéril (HiMedia, Mumbai, Índia) e ressuspensas em 5 mL da mesma solução (FREIRE *et al*., 2017). Em seguida, 0,4 µL do inóculo (6 log UFC/mL) foi adicionado a 40 mL de polpa previamente pasteurizada a 80 ºC por 5 min e resfriada em banho de gelo (FONSECA *et al*., 2021b).

As microfermentações foram realizadas em tubos de 50 mL com 40 mL de polpa de fruta em incubadora BOD (Caltech Indústria e Comércio LTDA, São Paulo, Brasil) a 14 °C por 72 h de fermentação estática. Polpas sem adição de cepa de levedura foram utilizadas como controle.

Os valores de pH foram avaliados nos tempos 0, 24, 48 e 72 h de fermentação conforme metodologia descrita anteriormente na Seção 3.4. Após o processo de fermentação, as amostras e controles foram submetidos à contagem de leveduras seguindo as metodologias relatadas na Seção 3.5. Após o processo de fermentação as amostras e seus controles não fermentados foram submetidos a condições simuladas do trato gastrointestinal (TGI).

3.8.2 Condições simuladas do TGI e bioacessibilidade de compostos fenólicos

A metodologia descrita por Minekus *et al*. (2014) foi utilizada para a realização da digestão *in vitro* das polpas de graviola e umbu-cajá fermentadas.

Para simular a fase oral o fluido salivar simulado (SSF) (3,5 mL) foi homogeneizado com as polpas fermentadas (5 g) e a mistura foi incubada a 37 ± 1°C por 2 min sob agitação (90 rpm) em uma incubadora de agitação orbital (Thoth Equipamentos, modelo 6420, Brasil) (Figura 4).

Figura 5 – Frascos contendo amostras em incubadora orbital

Fonte: Autoral, 2022.

O SSF consistiu em uma solução de 975 µL de água e 25 µL de 0,3 M $CaCl_2$ contendo 0,5 mL de 1500 U/mL de α-amilase (Sigma-Aldrich, St. Louis, EUA). O fluido gástrico simulado (SGF) (7,5 mL) foi adicionado em seguida para simular a digestão gástrica. A SGF consistiu numa mistura de 0,695 µL de água, 5 µL de 0.3 M $CaCl_2$ e 1,6 mL de 2000 U/mL de pepsina suína (Sigma- Aldrich, St. Louis, EUA). O pH foi ajustado (pH 3.0) usando HCl 1 M e a mistura foi incubada por 2 h a 37 ± 1°C com agitação. Ao final desse tempo a mistura gástrica (20 mL) foi adicionada com fluido intestinal simulado (SIF) (11 mL) e 2,5 mL de bile fresca (Sigma-Aldrich, St. Louis, EUA, 160 mM de bile fresca), 5,0 mL de solução de pancreatina 800 U/mL (Sigma-Aldrich, St. Louis, EUA) em solução SIF, 1,31 mL de água e 40 µL de $CaCl_2$ 0,3 M foram adicionados para simular a fase intestinal. O pH foi ajustado (pH 7,0) com NaOH 1 M e a mistura foi incubada a 37 ± 2°C por 2 h com agitação (90 rpm). Conforme a necessidade os valores de pH foram controlados e ajustados em cada etapa da digestão *in vitro*.

Os conteúdos finais das digestões foram dialisados de acordo com modificações nos procedimentos descritos por Guergoletto *et al*. (2016). Alíquotas foram colocadas em tubos de celulose regenerada para diálise (14 KDa cut-off, Sigma-Aldrich, St. Louis, EUA) por 18 h contra NaCl 0,01 M a 5 ± 0,5 °C. Os fluidos foram trocados e após mais 2 h o processo foi encerrado.

A bioacessibilidade dos fenólicos foi calculada de acordo com a Eq. (1) (RODRÍGUEZ-ROQUE *et al*., 2013). Além disso, contagens para monitorar a viabilidade das leveduras foram realizadas antes da digestão *in vitro*, após a fase gástrica e ao final da digestão conforme descrito anteriormente na Seção 3.5.

$$\text{Bioacessibilidade (\%)} = \left(\frac{BC\ intestinal}{BC\ amostra} \times 100\right) \qquad \text{Eq. (1)}$$

Onde BC intestinal refere-se à concentração de compostos fenólicos no final da digestão *in vitro* (fração bioacessível), e BC amostra refere-se à concentração fenólica do fruto antes da digestão *in vitro*.

3.8.3 Análise de açúcares, ácidos orgânicos, compostos fenólicos e álcool

As polpas de graviola e umbu-cajá fermentadas, as frações bioacessíveis provenientes da simulação do TGI e seus controles não-fermentados foram centrifugadas (3500 rpm/15 min) e o sobrenadante foi filtrado em um filtro Millex-HA de 0,45 µm (Millipore Co., Bedford, MA), conforme metodologia descrita por Coelho *et al.* (2018).

Para a determinação de açúcares e ácidos orgânicos os filtrados foram analisados por Cromatografia Líquida de Alta Eficiência (CLAE), utilizando-se cromatografia Agilent (modelo 1260 Infinity LC, Agilent Technologies, EUA) acoplada a detector de arranjo de diodos (DAD) e detector de índice de refração (DIR). As condições analíticas foram coluna Agilent Hi-Plex H (7,7 × 300 mm, 8 µm) e H2SO4 4 M em água ultrapura como uma fase móvel (taxa de fluxo 0,5 mL / min).

Para quantificar os compostos fenólicos, 20 µL do extrato foram injetados no cromatógrafo conforme descrito por Padilha *et al.* (2017). A separação cromatográfica dos compostos fenólicos foi realizada utilizando uma coluna Zorbax Eclipse Plus RP-C18 (100 × 4,6 mm, 3,5 µm) e a pré-coluna Zorbax C18 (12,6 × 4,6 mm, 5 µm). A temperatura da coluna foi ajustada para 35°C e as fases móveis foram compostas por água acidificada (pH 2) com ácido fosfórico

0,1 mM/L (fase A) e metanol acidificado com ácido fosfórico 0,5% (fase B). A taxa de fluxo foi mantida a 0.8 mL/min. As aquisições de dados do DAD foram processadas usando o software OpenLAB CDS ChemStation EditionTM (Agilent Technologies).

Os picos de açúcares, ácidos orgânicos e compostos fenólicos foram identificados pela comparação de seus tempos de retenção com os de padrões externos (Sigma Aldrich, St. Louis, EUA). Os seguintes padrões foram utilizados para a análise dos compostos fenólicos: cis-resveratrol e trans-resveratrol da Cayman Chemical Company (Ann Arbor, MI, EUA); ácidos clorogênico, gálico, siríngico, p-cumárico, cafeico e transcaftárico, catequina, epicatequina, hesperidina, naringenina, procianidina B1 e B2, delfinidina 3-glucosídeo, cianidina 3,5-diglucosídeo, cianidina 3-glucosídeo, malvidina 3,5-diglucosídeo, malvidina 3-glucosídeo, pelargonidina 3,5-diglucosídeo da Sigma-Aldrich (St. Louis, EUA); e procianidina A2, kaempferol 3-glicosídeo, quercetina 3-glicosídeo, quercetina 3-rutinosídeo (rutina), miricetina, epicatequina galato, epigalocatequina galato, pelargonidina 3-glicosídeo, petunidina 3-glicosídeo, e peonidina 3-O -glicosídeos da Extrasyntese (Genay, França).

Para identificação dos ácidos orgânicos e açúcares os padrões utilizados foram glicose e frutose (Sigma-Aldrich, St. Louis, EUA); maltose e ramnose (Chem Service, West Chester, EUA); e ácidos cítrico, tartárico, málico, succínico, lático, fórmico, acético, propiônico e butírico (Química Vetec, Rio de Janeiro, Brasil). A quantificação foi feita por meio de curvas de calibração do padrão externo de acordo com os métodos validados descritos por Coelho *et al*. (2018) para açúcares e ácidos orgânicos, e Padilha *et al*. (2017) para fenólicos.

Todos os compostos analisados apresentaram curvas de calibração com R2 $\geq$ 0,998. A pureza espectral de pico foi verificada usando a ferramenta Limiar para garantir a precisão da identificação em comparação com o padrão externo.

Etanol foi analisado de acordo com Duarte *et al*. (2009). As análises foram realizadas com um sistema de CLAE (Shimadzu, modelo LC-10Ai, Shimadzu Corp., Tóquio, Japão), equipado com um sistema de detecção duplo composto por um detector de ultravioleta (SPD-10AI) e um detector de índice de refração (10A). Uma coluna de troca catiônica Shimadzu (Shim-pack SCR-101H, 7,9 mm × 30 cm) foi operada a 30 ° C utilizando ácido perclórico 100 mM (70%) como eluente, a uma taxa de fluxo de 0,6 mL/min e um volume de injeção de 20 μL.

O etanol foi detectado por índice de refração e foi identificado comparando seus tempos de retenção com os de padrões certificados, tendo sido quantificado por meio da aplicação de curva de calibração obtida com o composto padrão (Sigma-Aldrich, Steinheim, Alemanha).

3.8.4 Análise de compostos voláteis

A concentração de compostos orgânicos voláteis nas polpas antes e depois de fermentadas foram determinados por microextração em fase sólida em modo headspace (HS-SPME) utilizando um cromatógrafo gasoso acoplado a espectrometria de massa (GCMS) Shimadzu QP-2010 SE de acordo com os métodos de Fonseca *et al*. (2021b).

Foi utilizada uma coluna Carbowax 20 M (película de 30 m × 0,25 mm ID × 0,25 µm). A alíquota da amostra (3,0 mL) foi adicionada a um frasco de 20 mL e equilibrada por 15 min a 60°C.

Os compostos voláteis foram capturados usando um suporte automático SPME (Supelco, Bellafonte, PA, EUA) com uma fibra longa (2 cm) DVB/CAR/PDMS (50/30 µm). A fibra SPME foi introduzida na amostra por 30 min a uma profundidade constante. A temperatura foi mantida em 60ºC. Em seguida, os voláteis foram dessorvidos diretamente no forro do cromatógrafo a gás e mantidos a 230ºC por 2 min. O hélio foi usado como gás de arraste a uma taxa de fluxo de 1,0 mL/min. A temperatura foi de 60ºC/5 min, aumentada para 230ºC a 10ºC/min e mantida nesta temperatura por 15 min.

Uma série de alcanos (C10-C40) (Sigma-Aldrich, St. Louis, EUA) foi usada para determinar o índice linear de retenção (LRI) para cada composto. Os dados foram analisados utilizando o software GCMSsolution (versão 4.4, Shimadzu Corporation, Japão) e o banco de dados NIST NIST/EPA/NIH 2014.

A identificação química de cada volátil foi realizada comparando os espectros de MS com o conjunto de dados. O 4-nonanol (Sigma-Aldrich, St. Louis, EUA) na concentração final de 6,250 ng/mL foi utilizado como padrão interno para quantificar cada pico de acordo com a concentração relativa (ng/g).

3.9 Sequenciamento genético das cepas selecionadas

As cepas utilizadas na fermentação das polpas de graviola e umbu-cajá e preliminarmente identificadas via MALDI-TOF MS como *Hanseniaspora opuntiae* (*Hanseniaspora opuntiae* 125), *Issatckenkia terricola* (*Issatckenkia terricola* 129) e *Hanseniaspora opuntiae* (*Hanseniaspora opuntiae* 148) foram submetidas ao sequenciamento genético para a confirmação das identificações.

O *DNA* de cada isolado foi extraído utilizando o *QIAamp DNA Mini Kit*, seguindo o protocolo "*DNA Purification from Tissues*" (Qiagen, Hilden, Germany). A região ITS foi amplificada utilizando os primers ITS1 e ITS4 (MANTER e VIVANCO, 2007).

Os produtos da PCR foram enviados para sequenciamento na empresa ACTgene. As sequências foram comparadas com o banco de dados *GenBank* usando o programa *Basic Local Alignment Tool* (*BLAST*) (*National Center for Biotechnology Information*, Bethesda, MD) para a identificação dos isolados.

3.10 Avaliação dos efeitos das polpas fermentadas na fermentação colônica *in vitro*

3.10.1 Inóculo fecal humano de adultos hipertensos de meia-idade

O preparo do inóculo foi realizado a partir de amostras fecais frescas doadas por quatro voluntários adultos hipertensos (dois homens e duas mulheres, com idades entre 45 e 59 anos) após a aprovação do Comitê Institucional de Ética em Pesquisa com Seres Humanos (Universidade Federal da Paraíba, João Pessoa, Paraíba, Brasil, parecer n° 5.315.511).

Os critérios de inclusão estabelecidos foram pessoas sem doença gastrointestinal ou do cólon, que seguissem dieta onívora regular, sem uso de alimentos probióticos ou prebióticos concentrados e que não tivessem utilizado antibióticos durante os seis meses anteriores ao estudo. Além disso, eles foram diagnosticados com hipertensão, uma doença crônica não transmissível caracterizada por pressão arterial sistólica igual ou superior a 140 mm Hg e pressão arterial diastólica igual ou superior a 90 mm Hg sem o uso de medicação anti-hipertensiva e avaliados por um profissional treinado (Barroso *et al*., 2021).

Os doadores receberam instruções específicas para a coleta das amostras, além de um kit higiênico de coleta/ armazenamento adequado contendo máscara, luvas e frasco estéril. Após coletados, os frascos com as fezes foram dispostos em embalagem com sistema gerador de anaerobiose (AnaeroGen, Oxoid, Basingstoke Inglaterra) e encaminhadas ao laboratório. Em seguida, as amostras fecais foram misturadas em igual proporção (1:1:1:1) e diluídas na proporção de 1:10 com solução salina fisiológica modificada autoclavada (NaCl 8,5 g L^{-1}, cisteína-HCl 0,5 g L^{-1}, Sigma-Aldrich, St. Louis, EUA) para obter uma suspensão fecal.

A suspensão fecal foi homogeneizada sob agitação (200 rpm por 2 min) e filtrada utilizando gaze de tripla camada para a remoção de partículas maiores, sendo em seguida armazenadas em frascos estéreis, sob condições anaeróbias, a 37 ± 1ºC (AnaeroGen, Oxoid, Basingstoke Inglaterra) (HU *et al*., 2013; ANDRADE *et al*., 2020; MENEZES *et al*., 2021).

3.10.2 Preparação de meios de cultura e fermentação colônica

O meio nutriente basal para a fermentação colônica foi composto de 4,5 g L^{-1} de NaCl, 4,5 g L^{-1} de KCl, 1,5 g L^{-1} de $NaHCO_3$, 0,69 g L^{-1} de $MgSO_4$, 0,8 g L^{-1} de L-cisteína, 0,5 g L^{-1} de KH_2PO_4, 0,5 g L^{-1} de K_2HPO_4, 0,4 g L^{-1} de sal biliar, 0,08 g L^{-1} $CaCl_2$, 0,005 g L^{-1} de $FeSO_4$, 1 mL L^{-1} de Tween 80. Como indicador anaeróbio foi adicionado ao meio 4 mL L^{-1} de solução de resazurina (0.025%, v/v) e água destilada foi utilizada para a diluição dos componentes. O pH do meio basal foi ajustado para 6.8 com a adição de HCl (1M) (BIANCHI *et al*., 2010; ANDRADE *et al*., 2020).

Para a fermentação fecal foram utilizadas culturas descontínuas de 40 mL compostas por 40% de meio de crescimento (v/v), 40% do inóculo fecal humano (v/v) e 20% da polpa fermentada e digerida de graviola ou umbu-cajá (v/v). Como controles do experimento outras culturas descontínuas foram elaboradas com 20% de suspensão de *Saccharomyces boulardii* CNCM I-745 (Floratil®, Merck) (com contagem padronizada conforme a contagem de 10^6 UFC/mL, correspondente à contagem de *Issatchenkia terricola* 129 na polpa fermentada digerida e dialisada) (v/v), ou sem adição de substrato/ cepa para comparação. Os tratamentos citados foram denominados respectivamente como: SOUR-IT129, UMB-IT129, SB(CNCM I-745) e Controle. Em seguida ao preparo as culturas foram incubadas sob anaerobiose (AnaeroGen) por 48 horas a 37 ± 1 °C (HU *et al*., 2013; MENEZES *et al*., 2021).

Alíquotas das culturas descontínuas correspondentes aos tratamentos SOUR-IT129, UMB-IT129, SB(CNCM I-745) e Controle nos tempos 0, 24 e 48 horas foram coletadas para aferição do pH que foi realizada utilizando um pHmetro Testo 206-pH1 (Testo AG, Lenzkirch, Germany) e para as análises de açúcares, ácidos orgânicos e compostos fenólicos conforme a metodologia descrita na Seção 3.8.3.

3.10.3 Abundância relativa de grupos bacterianos avaliados por fluorescência de hibridização *in situ* acoplada com citometria de fluxo multiparamétrica

Os percentuais de grupos microbianos selecionados que se sucederam durante as 48 horas de fermentação foram analisados por meio da técnica de FISH com sondas oligonucleotídicas selecionadas e projetadas para atingir regiões específicas do gene 16S rRNA desses microrganismos. Essa técnica foi combinada a CFM com o objetivo de avaliar a capacidade das polpas fermentadas em modular a microbiota intestinal humana durante a fermentação colônica *in vitro* (CONTERNO *et al*., 2019; MENEZES *et al*., 2021).

As sondas utilizadas no experimento foram: Bif 164, específica para *Bifidobacterium*; Lab 158, específica para *Lactobacillus spp*. – *Enterococcus spp*.; Bac 303, específica para *Bacteroides spp*. – *Prevotella spp*.; Erec 482, específica para *Eubacterium rectale* - *Clostridium coccoides*; e Chis 150, específica para *Clostridium histolyticum*.

A seleção dos grupos bacterianos foi baseada na sua representatividade na microbiota fecal e sua associação com respostas metabólicas positivas ou negativas (MEDEIROS *et al*., 2021). Além disso, estes grupos têm sido utilizados como marcadores em estudos anteriores que avaliam o impacto da administração de ingredientes funcionais na microbiota intestinal (ALBUQUERQUE *et al*., 2021; MASSA *et al*., 2022; MEDEIROS *et al*., 2021; MENEZES *et al*., 2021).

Para a execução do experimento as sondas foram marcadas com o corante fluorescente Cy3 (Sigma-Aldrich) (RODRIGUES *et al*., 2016; MENEZES *et al*., 2021) e o marcador SYBR Green (Molecular Probes, Invitrogen, Carlsbad, CA, EUA) foi utilizado para a marcação da fita dupla de DNA para enumeração da população total de bactérias de cada grupo avaliado (CONTERNO *et al*., 2019).

A estabilização da estrutura celular das culturas foi realizada nos tempos 0, 24 e 48 horas utilizando alíquotas de 375 µL que foram fixadas a 4 ºC (overnight) com 1125 µL de solução de paraformaldeído filtrada (4%, p/v). Em seguida as alíquotas foram centrifugadas (10000 × g, 5 minutos, 4 °C), lavadas duas vezes com PBS 1 M (10000 × g, 5 minutos, 4 °C), ressuspensas em 300 µL de PBS:etanol 99% (1:1 v/v), filtradas com filtro de membrana com poro de tamanho 0,45 µm (Whatman®) e armazenadas a -20 ºC.

A hibridização *in situ* foi realizada por meio da diluição de 10 µL da suspensão de células fixadas em 190 µL de PBS 1X (Gibco, Gaithersburg, EUA; pH 7,2) seguida de centrifugação a 4000 × g por 15 minutos a 4 °C e descarte do sobrenadante. A células foram então ressuspensas em 200 µL de tampão Tris-EDTA (100 mM Tris-HCl e 50 mM EDTA; pH 8,0) e centrifugadas sob as mesmas condições anteriormente citadas.

As amostras foram tratadas com lisozima (1 mg/mL) diluída em 200 µL de Tris-EDTA e incubadas por 10 minutos em temperatura ambiente (25 ± 0,5 °C) em local escuro para promover a permeabilização das células que então receberam as sondas Lab 158 e Bif 164, e foram centrifugadas (4000 × g, 15 minutos, 4 °C).

As amostras foram então ressuspensas em 45 µL de tampão de hibridização composto por 0,9 M de NaCl, 20 mM de Tris-HCl (pH 7.5) e 0.1% de dodecil sulfato de sódio (DSS) (p/v) e foram adicionadas 5 µL de sonda oligonucleotídica fluorescente (50 ng/µL) sendo mantidas no escuro e na temperatura de hibridização apropriada para cada sonda (45°C para Bac 303 ou 50 °C para as demais sondas).

Após o período de 4 horas as amostras foram centrifugadas (4000 × g, 15 minutos, 25 °C), ressuspensas em 200 µL de tampão de hibridização sem adição de DSS e mantidas no escuro por 30 minutos sob temperatura de lavagem apropriada para cada sonda (45 ou 50 °C, conforme citado anteriormente) para a retirada das sondas não ligadas.

As amostras foram novamente centrifugadas (4000 × g, 15 minutos, 25 °C), ressuspensas em 200 µL de PBS 1X e 20 µL de SYBR Green (1:1000 solução estoque diluída em dimetil sulfóxido ≥99,9%, Sigma-Aldrich), incubadas por 10 minutos no escuro sob temperatura ambiente (25 ± 0,5 °C), centrifugadas (4000 × g, 15 minutos, 25 °C) e ressuspensas com 200 µL de PBS 1X.

Para cada amostra preparada também foi elaborada uma amostra em branco (sem a sonda oligonucleotídica e sem SYBR Green), e uma amostra marcada somente com SYBR Green utilizando o mesmo método das amostras hibridizadas, com o objetivo de definir um limiar de detecção para o citômetro de fluxo (BD Accuri C6, New Jersey, EUA) permitindo revelar a potencial autofluorescência das amostras e excluindo falsos positivos.

O princípio da CFM consiste na passagem dos sinais fluorescentes das células individuais através de uma zona de laser, sendo coletados como sinais logarítmicos (medidas da área de pulso) pelos canais FL1 (SYBR Green) e FL2 (Lab 158, Bif 164, Bac 303, Chis 150 e Erec 482). A configuração foi realizada de modo que as amostras em baixo fluxo, com nível limite para dispersão direta (FSC) ajustado para 30 000 e com total de 10 000 eventos coletados para cada amostra.

O software BD Accuri C6 (Becton Dickinson and Company) foi utilizado para o registro dos citogramas de emissão de fluorescência. Os resultados foram expressos como abundância (porcentagem relativa) de células de grupo bacteriano hibridizadas por cada sonda Cy3 específica (registrados como eventos fluorescentes) comparados ao total de bactérias enumeradas com a coloração SYBR Green (CONTERNO *et al*., 2019).

3.10.4 Determinação do índice prebiótico

A determinação do índice prebiótico foi feita após o cálculo da abundância relativa (porcentagem) de cada grupo bacteriano medido com base nos resultados do FISH-CFM (ALBUQUERQUE *et al*., 2021) para o qual se utilizou a seguinte equação:

Índice prebiótico = %Lab + %Bif – %Bac – %Chis – %Erec   Eq. (2)

onde %Lab = (*Lactobacillus spp*./*Enterococcus spp*. abundância relativa em 24 ou 48 h) – (abundância relativa desse grupo bacteriano no tempo zero); %Bif = (abundância relativa de *Bifidobacterium spp*. em 24 ou 48 h) – (abundância relativa desse grupo bacteriano no tempo zero); %Bac = (abundância relativa de *Bacteroides spp*./*Prevotella spp*. em 24 ou 48 h) – (abundância relativa desse grupo bacteriano no tempo zero); %Chis = (abundância relativa de *C. histolyticum* em 24 ou 48 h) – (abundância relativa desse grupo bacteriano no tempo zero); e %Erec = (relative abundância de *E. rectale*/*C. coccoides* em 24 ou 48 h) – (abundância relativa desse grupo bacteriano no tempo zero).

Resultados positivos para o índice prebiótico indicam uma modulação benéfica global da microbiota intestinal, enquanto resultados negativos indicam uma modulação geral indesejável (ALBUQUERQUE *et al*., 2021, GUNATHILAKEA *et al*., 2018).

3.11 Análises estatísticas

As análises foram realizadas em triplicata e em três experimentos distintos. Os resultados foram expressos como média ± desvio padrão. Os dados foram analisados por meio de análise de variância (ANAVA) e teste de Tukey ou teste-t de Student considerando $p < 0.05$. Os dados obtidos também foram submetidos à análise de componentes principais (ACP) utilizando a correlação de Pearson. As análises estatísticas foram realizadas com o auxílio do software XLSTAT® 2021.4.1 (Addinsoft™, Paris, França).

4 RESULTADOS

Os resultados da tese são apresentados na forma de dois artigos originais, dispostos em APÊNDICES. O primeiro artigo intitulado como "*Yeasts from fermented Brazilian fruits as biotechnological tools for increasing phenolics bioaccessibility and improving the volatile profile in derived pulps*" foi aceito para publicação na revista Food Chemistry, cujo fator de impacto é de 9.231 (2022). O segundo artigo intitulado *"Effects of fermented soursop and umbu-cajá pulps on the colonic microbiota of middle-aged hypertensive adults"* foi aceito para publicação na revista Food Bioscience, cujo fator de impacto é de 5.318 (2022).

No apêndice A estão descritos os resultados do primeiro artigo original desta tese, e trata-se do uso de leveduras provenientes da fermentação de frutas da caatinga brasileira na fermentação de polpa de graviola e umbu-cajá, bem como sua caracterização microbiológica, química (açúcares, ácidos orgânicos, álcool, compostos voláteis e fenólicos) e seus efeitos sobre a bioacessibilidade de compostos fenólicos. Frutos do Bioma Caatinga têm sido pouco explorados como fonte de leveduras biotecnológicas. Este estudo isolou leveduras de frutos da Caatinga fermentados naturalmente e avaliou *Hanseniaspora opuntiae* 125, *Issatchenkia terricola* 129 e *Hanseniaspora opuntiae* 148 na fermentação de polpas de graviola e umbu-cajá. Todas as cepas foram capazes de fermentar as polpas (72 h), aumentando ($p < 0,05$) o ácido acético, a concentração de fenólicos e a bioacessibilidade, e mantendo contagens acima de 7 log UFC/mL após fermentação e/ou digestão *in vitro*. *H. opuntiae* 125 apresentou as maiores contagens (8,43 – 8,76 log UFC/mL; $p < 0,05$) nas polpas e, maior produção de ácidos orgânicos, maior sobrevivência à digestão e maior bioacessibilidade de vários compostos fenólicos ($p < 0,05$) no umbu- polpa de caixa. *I. terricola* 129 e *H. opuntiae* 148 apresentaram maior atividade metabólica, concentração e bioacessibilidade de fenólicos específicos nas polpas de umbu-cajá e graviola, respectivamente ($p < 0,05$). Os voláteis variaram ($p < 0,05$) com a cepa de levedura. De maneira geral, o desempenho biotecnológico da levedura para fermentação da polpa foi melhor em sua fonte de fruta.

No apêndice B é apresentado o segundo artigo que teve por objetivo avaliar os efeitos das polpas fermentadas de graviola e umbu-cajá na modulação na microbiota intestinal humana de adultos hipertensos de meia-idade abordando também a evolução do pH e o perfil de metabólitos (açúcares, ácidos orgânicos e compostos fenólicos) durante o processo de fermentação colônica *in vitro*. Os efeitos das polpas de graviola e umbu-cajá fermentadas pela levedura *Issatchenkia terricola* 129 sobre a microbiota colônica de adultos hipertensos de meia-idade foram avaliados durante 48 h de fermentação *in vitro*. A abundância relativa de grupos bacterianos distintos, valores de pH e teores de açúcares, ácidos orgânicos e compostos fenólicos foram avaliados em 0, 24 e 48 h de fermentação. *Saccharomyces boulardii* CNCM I-745, uma levedura com reconhecidas propriedades probióticas, foi utilizada para fins de comparação. Durante a fermentação colônica, maltose, glicose e frutose foram transformadas, resultando em valores de pH reduzidos e uma menor abundância relativa de *Bacteroides* spp./ *Prevotella* spp. Os efeitos sobre a microbiota colônica foram menos pronunciados para *S. boulardii* seguido pela polpa de umbu-cajá com *I. terricola* 129. A polpa de graviola fermentada com *I. terricola* 129 melhorou a microbiota por aumentar a abundância relativa de *Lactobacillus* spp. e *Bifidobacterium* spp. e reduzir a abundância relativa de *Eubacterium rectale*/*Clostridium coccoides* e *Clostridium histolyticum,* resultando no maior índice prebiótico entre os produtos testados. Ao mesmo tempo, observou-se o consumo de ramnose e ácido gálico e maiores teores de ácidos acético e propiônico e procianidina B2.

Assim, nossos achados demonstram pela primeira vez que fenólicos derivados de frutas da Caatinga brasileira podem ser biotransformados por leveduras isoladas de fermentação natural transformando-os em fenólicos mais bioacessíveis. Além disso, as alterações positivas ocorridas na abundância dos grupos da microbiota intestinal indicam efeitos benéficos ao público hipertenso de meia-idade.

REFERÊNCIAS

AGU, K. C.; OKOLIE, P. N. Proximate composition, phytochemical analysis, and *in vitro* antioxidant potentials of extracts of *Annona muricata* (Soursop). **Food Science & Nutrition**, v. 5, n. 5, p. 1029-1036, 2017. DOI 10.1002/fsn3.498

ALBUQUERQUE, T. G. *et al.* Nutritional and phytochemical composition of *Annona cherimola* Mill. fruits and by-products: Potential health benefit. **Food Chemistry**, [*s. l.*], v. 193, p. 187-195, 2016. DOI 10.1016/j.foodchem.2014.06.044

ALBUQUERQUE, T. M. R. de *et al*. Effects of digested flours from four different sweet potato (*Ipomoea batatas* L.) root varieties on the composition and metabolic activity of human colonic microbiota *in vitro*. **Journal of Food Science**, v. 86, n. 8, p. 3707-3719, 2021. DOI 10.1111/1750-3841.15852

ALU'DATT, M. H. *et al*. Contents, profiles and bioactive properties of free and bound phenolics extracted from selected fruits of the *Oleaceae* and *Solanaceae* families. **LWT - Food Science and Technology**, v. 109, p. 367-377, jul. 2019. DOI 10.1016/j.lwt.2019.04.051

AMORIM, J. C.; PICCOLI, R. H.; DUARTE, W. F. Probiotic potential of yeasts isolated from pineapple and their use in the elaboration of potentially functional fermented beverages. **Food Research International**, v. 107, p. 518-527, 2018. DOI 10.1016/j.foodres.2018.02.054

ANDRADE, R. M. S. de *et al*. Potential prebiotic effect of fruit and vegetable byproducts flour using *in vitro* gastrointestinal digestion. **Food Research International**, v. 137, p. 109354, 2020. DOI 10.1016/j.foodres.2020.109354

AOAC. (2016). Official Methods of Analysis. 20th ed. **AOAC** Intl., Gaithersburg, MD (20th ed.). Gaithersburg, MD: AOAC Intl.

APONTE, M. *et al*. Dominance of S. cerevisiae commercial starter strains during *Greco di Tufo* and *aglianico* wine fermentations and evaluation of oenological performances of some indigenous/residential strains. **Foods**, v. 9, n. 11, p. 1549, 2020. DOI 10.3390/foods9111549

ARROYO-LÓPEZ, F. N. *et al*. Potential benefits of the application of yeast starters in table olive processing. **Frontiers in Microbiology**, v. 3, n. 161, p. 1-4, 2012. DOI 10.3389/fmicb.2012.00161

ASHRAFIZADEH, M. *et al*. Gallic acid for cancer therapy: Molecular mechanisms and boosting efficacy by nanoscopical delivery. **Food and Chemical Toxicology**, v. 157, p. 112576, nov. 2021. DOI 10.1016/j.fct.2021.112576

ASSIS, B. B. T. de *et al*. Biotransformation of the Brazilian Caatinga fruit-derived phenolics by *Lactobacillus acidophilus* La-5 and *Lacticaseibacillus casei* 01 impacts bioaccessibility and antioxidant activity. **Food Research International**, v. 146, p. 110435, 2021. DOI 10.1016/j.foodres.2021.110435

BARROSO, W. K. S. *et al*. **Brazilian guidelines of hypertension–2020**. Arquivos brasileiros de cardiologia, v. 116, p. 516-658, 2021. DOI 10.36660/abc.20201238

BERNI, P. *et al*. Non-conventional Tropical Fruits: Characterization, antioxidant Potential and carotenoid bioaccessibility. **Plant Foods for Human Nutrition**, v. 74, n. 1, p. 141-148, 2019. DOI 10.1007/s11130-018-0710-1

BONATSOU, S. *et al*. Evaluating the probiotic potential and technological characteristics of yeasts implicated in cv. *Kalamata* natural black olive fermentation. **International Journal of Food Microbiology**, [*s. l.*], v. 271, p. 48-59, 2018. DOI 10.1016/j.ijfoodmicro.2018.02.018

BONATSOU, S.; PARAMITHIOTIS, S.; PANAGOU, E. Z. Evolution of yeast consortia during the fermentation of *Kalamata* natural black olives upon two initial acidification treatments. **Frontiers in microbiology**, [*s. l.*], v. 8, p. 2673, 2018. DOI 10.3389/fmicb.2017.02673

CALDERÓN-PÉREZ, L. *et al*. Gut metagenomic and short chain fatty acids signature in hypertension: a cross-sectional study. Scientific Reports, 10(1), 1-16, 2020. DOI 10.1038/s41598-020-63475-w

CALDERÓN-PÉREZ, L. *et al*. Interplay between dietary phenolic compound intake and the human gut microbiome in hypertension: A cross-sectional study. **Food Chemistry**, 344, 128567, 2021. DOI 10.1016/j.foodchem.2020.128567

CARVALHO, B. F. *et al*. Fermentation profile and identification of lactic acid bacteria and yeasts of rehydrated corn kernel silage. **International Journal of Applied Microbiology**, v. 122, p. 589-600, 2017. DOI 10.1111/jam.13371

CARVALHO, R. da S.; SOARES FILHO, W. dos S.; RITZINGER, R. Umbu-cajá como repositório natural de parasitoide nativo de moscas-das-frutas. **Pesquisa Agropecuária Brasileira**, v. 45, p. 1222-1225, 2010. DOI 10.1590/S0100-204X2010001000024

CEBALLOS, A. M.; GIRALDO, G.; ORREGO, C. E. Effect of freezing rate on quality parameters of freeze dried soursop fruit pulp. **Journal of Food Engineering**, v. 111, n. 2, p. 360-365, jul. 2012. DOI 10.1016/j.jfoodeng.2012.02.010

CHANG, L. S. *et al*. Storage stability, color kinetics and morphology of spray-dried soursop (*Annona muricata* L.) powder: effect of anticaking agents. **International Journal of Food Properties**, v. 21, n. 1, p. 1937-1954, 2018. DOI 10.1080/10942912.2018.1510836

CHENG, M. *et al*. A metagenomics approach to the intestinal microbiome structure and function in high fat diet-induced obesity mice fed with oolong tea polyphenols. **Food & Function**, v. 9, n. 2, p. 1079-1087, 2018. DOI 10.1039/C7FO01570D

CHEONG, K. W. *et al*. Optimization of equilibrium headspace analysis of volatile flavor compounds of Malaysian soursop (*Annona muricata*): Comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC× GC-TOFMS). **Food Chemistry**, v. 125, n. 4, p. 1481-1489, abr. 2011. DOI 10.1016/j.foodchem.2010.10.067

CIOCH-SKONECZNY, M. *et al.* Biodiversity of yeasts isolated during spontaneous fermentation of cool climate grape musts. **Archives of Microbiology**, v. 203, n. 1, p. 153-162, 2021. DOI 10.1007/s00203-020-02014-7

CIOCH-SKONECZNY, M. *et al*. Determination of the oenological properties of yeast strains isolated from spontaneously fermented grape musts obtained from cool climate grape varieties. **European Food Research and Technology**, v. 246, p. 2299-2307, jul. 2020. DOI 0.1007/s00217-020-03574-0

CIOCH-SKONECZNY, M. *et al*. Quantitative and qualitative composition of yeast microbiota in spontaneously fermented grape musts obtained from cool climate grape varieties *'Rondo'* and *'Regent'*. **FEMS Yeast Research**, v. 18, n. 8, p. foy089, dez. 2018. DOI 10.1093/femsyr/foy089

CODA, R. *et al*. Antifungal activity of *Meyerozyma guilliermondii*: Identification of active compounds synthesized during dough fermentation and their effect on long-term storage of wheat bread. **Food Microbiology**, [*s. l.*], v. 33, n. 2; p. 243-251, abr. 2013. DOI 10.1016/j.fm.2012.09.023

COELHO, E. M., *et al.* Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: Method validation and characterization of products from northeast Brazil. **Journal of Food Composition and Analysis**, v. 66, n. 1, p. 160–167, mar. 2018. DOI 10.1016/j.jfca.2017.12.017

CORDENTE, A. G. *et al*. Harnessing yeast metabolism of aromatic amino acids for fermented beverage bioflavouring and bioproduction. **Applied Microbiology Biotechnology**, 103, n. 8, p. 4325–4336, abr. 2019. DOI 10.1007/s00253-019-09840-w

CORIA-TÉLLEZ, A. V. *et al*. *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. **Arabian Journal of Chemistry**, v. 11, n. 5, p. 662-691, jul. 2018. DOI 10.1016/j.arabjc.2016.01.004

CORIA-TÉLLEZ, A. V.; MONTALVO-GONZÁLEZ, E.; OBLEDO-VÁZQUEZ, E. N. Soursop (*Annona muricata*). **Fruit and Vegetable Phytochemicals: Chemistry and Human Health**, 2nd Edition, p. 1243-1252, 2017. DOI 10.1002/9781119158042.ch66

DE VUYST, L. *et al*. Yeast diversity of sourdoughs and associated metabolic properties and functionalities. **International Journal of Food Microbiology**, [*s. l.*], v. 165, p. 26-34, dez. 2016. DOI 10.1016/j.ijfoodmicro.2016.07.018

DELLACASSA, E. *et al*. Pineapple (*Ananas comosus* L. Merr.) wine production in Angola: Characterisation of volatile aroma compounds and yeast native flora. **International Journal of Food Microbiology**, v. 241, p. 161-167, 2017. DOI 10.1016/j.ijfoodmicro.2016.10.014

DING, H. *et al*. Role of NADPH oxidase pathway in renal protection induced by procyanidin B2: In L-NAME induced rat hypertension model. **Journal of Functional Foods**, v. 47, p. 405-415, ago. 2018. DOI 10.1016/j.jff.2018.04.005

DOMÍNGUEZ-AVILA, J. A. *et al*. Phenolic compounds promote diversity of gut microbiota and maintain colonic health. **Digestive Diseases and Sciences**, v. 66, n. 10, p. 3270-3289, 2021. DOI 10.1007/s10620-020-06676-7

DUARTE, W. F *et al*. Indigenous and inoculated yeast fermentation of gabiroba (*Campomanesia pubescens*) pulp for fruit wine production, **Journal of Industrial Microbiology and Biotechnology**, 36, 557–569. 2009. DOI 10.1007/s10295-009-0526-y

DUTRA, R. L. T. *et al*. Bioaccessibility and antioxidant activity of phenolic compounds in frozen pulps of Brazilian exotic fruits exposed to simulated gastrointestinal conditions. **Food Research International**, v. 100, p. 650-657, 2017. DOI 10.1016/j.foodres.2017.07.047

EINSON, J. E. *et al*. A Vegetable Fermentation Facility Hosts Distinct Microbiomes Reflecting the Production Environment. **Applied and Environmental Microbiology**, v. 84, n. 22, p. e01680-18, 2018. DOI 10.1128/AEM.01680-18

EMER, C. D. *et al*. Biogenic amines and the importance of starter cultures for malolactic fermentation. **Australian Journal of Grape and Wine Research**, v. 27, n. 1, p. 26-33, 2021. DOI 10.1111/ajgw.12462

ESPÍRITO SANTO, D. *et al*. Yeast diversity in the Mediterranean strawberry tree (*Arbutus unedo* L.) fruits' fermentations. **Food Research International**, v. 47, n. 1, p. 45-50, 2012. DOI 10.1016/j.foodres.2012.01.009

FONSECA, H. C. *et al*. Probiotic properties of *lactobacilli* and their ability to inhibit the adhesion of enteropathogenic bacteria to Caco-2 and HT-29 cells. **Probiotics and antimicrobial proteins**, v. 13, n. 1, p. 102-112, 2021a. DOI 10.1007/s12602-020-09659-2

FONSECA, H. C. *et al.* Sensory and flavor-aroma profiles of passion fruit juice fermented by potentially probiotic *Lactiplantibacillus plantarum* CCMA 0743 strain. **Food Research International**, p. 110710, 2021b. DOI 10.1016/j.foodres.2021.110710

FREIRE, A. L. *et al*. Nondairy beverage produced by controlled fermentation with potential probiotic starter cultures of lactic acid bacteria and yeast. **International Journal of Food Microbiology**, v. 248, n. 2; p. 39-46, 2017. DOI 10.1016/j.ijfoodmicro.2017.02.011

GONDIM, P. J. S. *et al*. Qualidade de frutos de acessos de umbu-cajazeira (*Spondias* sp.). **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 17, p. 1217-1221, 2013. DOI 10.1590/S1415-43662013001100013

GREPPI, A. *et al*. Yeast dynamics during spontaneous fermentation of *mawè* and *tchoukoutou*, two traditional products from Benin. **International Journal of Food Microbiology**, [*s. l.*], v. 165, n. 2, p. 200-207, jul. 2013. DOI 10.1016/j.ijfoodmicro.2013.05.004

HE, J. Bioactivity-guided fractionation of pine needle reveals catechin as an anti-hypertension agent via inhibiting angiotensin-converting enzyme. **Scientific Reports**, v. 7, n. 1, p. 1-9, 2017. DOI 10.1038/s41598-017-07748-x

HU, J. *et al*. *In vitro* fermentation of polysaccharide from the seeds of *Plantago asiatica* L. by human fecal microbiota. **Food Hydrocolloids**, v. 33, n. 2, p. 384-392, 2013. DOI 10.1016/j.foodhyd.2013.04.006

HU, L. *et al*. The sensory quality improvement of citrus wine through co-fermentations with selected non-*Saccharomyces* yeast strains and *Saccharomyces cerevisiae*. **Microorganisms**, v. 8, n. 3, p. 323, 2020. DOI 10.3390/microorganisms8030323

JANUSZEK, M.; SATORA, P. How Different Fermentation Type Affects Volatile Composition of Plum Jerkums. **Applied Sciences**, v. 11, n. 10, p. 4658, 2021. DOI 10.3390/app11104658

JIAO J. *et al*. A Preliminary Study on the Value of Intestinal Flora in Predicting Major Adverse Cardiovascular and Cerebrovascular Events in Patients with Refractory Hypertension. **Computational and Mathematical Methods in Medicine**, 2022. DOI 10.1155/2022/7723105

JIMÉNEZ, V. M. *et al*. Identification of phenolic compounds in soursop (*Annona muricata*) pulp by high-performance liquid chromatography with diode array and electrospray ionization mass spectrometric detection. **Food Research International**, v. 65, p. 42-46, 2014. DOI 10.1016/j.foodres.2014.05.051

JOHANSEN, P. G. *et al*. Occurrence and importance of yeasts in indigenous fermented food and beverages produced in sub-Saharan Africa. **Frontiers in Microbiology**, v. 10, p. 1789, 2019. DOI 10.3389/fmicb.2019.01789

KAMDA, A. G. S. *et al*. *In vitro* determination of volatile compound development during starter culture-controlled fermentation of *Cucurbitaceae* cotyledons. **International Journal of Food Microbiology**, v. 192, n. 2; p. 58-65, 2016. DOI 10.1016/j.ijfoodmicro.2014.09.030

LAPI, D. *et al*. The pomace extract taurisolo protects rat brain from ischemia-reperfusion injury. **Frontiers in Cellular Neuroscience**, v. 14, p. 3, jan. 2020. DOI 10.3389/fncel.2020.00003

LEMOS, E. E. P. de. A produção de anonáceas no Brasil. **Revista Brasileira de Fruticultura**, v. 36, p. 77-85, 2014. DOI 10.1590/S0100-29452014000500009

LI, J.; HU, W.; XU, Y. Diversity and dynamics of yeasts during vidal blanc icewine fermentation: A strategy of the combination of culture-dependent and high-throughput sequencing approaches. **Frontiers in Microbiology**, v. 10, p. 1588, jul. 2019. DOI 10.3389/fmicb.2019.01588

LIANG, Y. *et al*. Procyanidin B2 reduces vascular calcification through inactivation of ERK1/2-RUNX2 pathway. **Antioxidants**, v. 10, n. 6, p. 916, jun. 2021. DOI 10.3390/antiox10060916

LIMA, E. D. P. de A. *et al*. Caracterização física e química dos frutos da umbu-cajazeira (*Spondias spp*) em cinco estádios de maturação, da polpa congelada e néctar. **Revista Brasileira de Fruticultura**, v. 24, p. 338-343, 2002. DOI:10.1590/S0100-29452002000200013

LIMA, L. L. de A. *et al.* Néctar misto de umbu (*Spondias tuberosa* Arr. Câmera) e mangaba (*Hancornia Speciosa* Gomes): elaboração e avaliação da qualidade. **Brazilian Journal of Food Technology**, v. 21, 2018. DOI 10.1590/1981-6723.03417

LU, W. *et al*. *Bifidobacterium longum* CCFM752 prevented hypertension and aortic lesion, improved antioxidative ability, and regulated gut microbiome in spontaneously hypertensive rats. **Food & Function**, p 6373-6386, 2022. DOI 10.1039/D1FO04446J

LU, Y. *et al*. Comparative study of microbial communities and volatile profiles during the inoculated and spontaneous fermentation of persimmon wine. **Process Biochemistry**, v. 100, p. 49-58, 2021. DOI 10.1016/j.procbio.2020.09.023

LUAN, Yu *et al*. Effects of different pre-fermentation cold maceration time on aroma compounds of *Saccharomyces cerevisiae* co-fermentation with *Hanseniaspora opuntiae* or *Pichia kudriavzevii*. **LWT - Food Science and Technology**, v. 92, p. 177-186, 2018. DOI 10.1016/j.lwt.2018.02.004

MANTER, D. K.; VIVANCO, J. M. Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. **Journal of Microbiological Methods**, v. 71, n. 1, p. 7-14, out. 2007. DOI 10.1016/j.mimet.2007.06.016

MARTÍN-GÓMEZ, J. *et al*. Phenolic compounds, antioxidant activity and color in the fermentation of mixed blueberry and grape juice with different yeasts. **LWT - Food Science and Technology**, v. 146, p. 111661, jul. 2021. DOI 10.1016/j.lwt.2021.111661

MASSA, N. M. L. *et al*. *In vitro* colonic fermentation and potential prebiotic properties of pre-digested jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg) by-products. **Food Chemistry**, v. 388, p. 133003, 2022. DOI 10.1016/j.foodchem.2022.133003

MATEUS, D. *et al*. Identification and characterization of non-saccharomyces species isolated from port wine spontaneous fermentations. **Foods**, v. 9, n. 2, p. 120, 2020. DOI 10.3390/foods9020120

MEDEIROS, V. P. B. de *et al*. Freshwater microalgae biomasses exert a prebiotic effect on human colonic microbiota. **Algal Research**, v. 60, p. 102547, 2021. DOI 10.1016/j.algal.2021.102547

MEDINA, K. *et al*. Increased flavour diversity of *Chardonnay* wines by spontaneous fermentation and co-fermentation with *Hanseniaspora vineae*. **Food Chemistry**, v. 141, n. 3, p. 2513-2521, 2013. DOI 10.1016/j.foodchem.2013.04.056

MENEZES, F. N. D. D. *et al*. Acerola (*Malpighia glabra* L.) and guava (*Psidium guayaba* L.) industrial processing by-products stimulate probiotic *Lactobacillus* and *Bifidobacterium* growth and induce beneficial changes in colonic microbiota. **Journal of Applied Microbiology**, v. 130, n. 4, p. 1323-1336, 2021. DOI 10.1111/jam.14824

MIGUEL, M. G. da C. P. *et al*. Cocoa fermentation: Microbial identification by MALDI-TOF MS, and sensory evaluation of produced chocolate. **LWT - Food Science and Technology**, v. 77, p. 362-369, 2017. DOI 10.1016/j.lwt.2016.11.076

MILLS, K. T.; STEFANESCU, A.; HE, J. The global epidemiology of hypertension. **Nature Reviews Nephrology**, v. 16, n. 4, p. 223-237, 2020. DOI 10.1038/s41581-019-0244-2

MINEKUS, Mans *et al*. A standardised static *in vitro* digestion method suitable for food–an international consensus. **Food & function**, v. 5, n. 6, p. 1113-1124, 2014. DOI 10.1039/c3fo60702j

MOREIRA, A. *et al*. Polyphenols in Caja-Umbuzeiro Fruit: Extraction Process Efficiency and Antioxidant Potential. **The Natural Products Journal**, v. 2, n. 2, p. 139-148, 2012. DOI 10.2174/2210315511202020139

MOTLHANKA, K. *et al*. Fermentative microbes of khadi, a traditional alcoholic beverage of botswana. **Fermentation**, v. 6, n. 2, p. 51, 2020. DOI 10.3390/fermentation6020051

MUÑOZ-REDONDO, J. M. *et al*. Impact of sequential inoculation with the non-Saccharomyces *T. delbrueckii* and *M. pulcherrima* combined with *Saccharomyces cerevisiae* strains on chemicals and sensory profile of rosé wines. **Journal of Agricultural and Food Chemistry**, v. 69, n. 5, p. 1598-1609, 2021. DOI 10.1021/acs.jafc.0c06970

NARAIN, N.; GALVAO, M. de S; MADRUGA, M. S. Volatile compounds captured through purge and trap technique in caja-umbu (*Spondias* sp.) fruits during maturation. **Food Chemistry**, v. 102, n. 3, p. 726-731, 2007. DOI 10.1016/j.foodchem.2006.06.003

NOBRE, L. L. de M. *et al*. Phylogenomic and single nucleotide polymorphism analyses revealed the hybrid origin of *Spondias bahiensis* (family *Anacardiaceae*): de novo genome sequencing and comparative genomics. **Genetics and molecular biology**, v. 41, p. 878-883, 2018. DOI 10.1590/1678-4685-GMB-2017-0256

OGODO, A. C. *et al*. Production and evaluation of fruit wine from *Mangifera indica* (cv. Peter). **Applied Microbiology Open Access**, v. 4, n. 144, p. 2, 2018. DOI 10.4172/2471-9315.1000144

PADILHA, C. V. S., *et al*. Rapid determination of flavonoids and phenolic acids in grape juices and wines by RP-HPLC/DAD: Method validation and characterization of commercial products of the new Brazilian varieties of grape. **Food Chemistry**, 228, 106–115. 2017. DOI 10.1016/j.foodchem.2017.01.137

PAZ, M. *et al*. Brazilian fruit pulps as functional foods and additives: Evaluation of bioactive compounds. **Food Chemistry**, v. 172, p. 462-468, 2015. DOI 10.1016/j.foodchem.2014.09.102

PEREIRA, F. R. A. *et al*. Biometry in Umbu fruits from the semi-arid region of Paraiba. **Revista Brasileira de Fruticultura**, v. 43, n. 6, 2021.

PEREIRA, G. V. de M. *et al*. A review of selection criteria for starter culture development in the food fermentation industry. **Food Reviews International**, v. 36, n. 2, p. 135-167, 2020. DOI 10.1080/87559129.2019.1630636

POSTIGO, V. *et al*. Impact of Non-*Saccharomyces* Wine Yeast Strains on Improving Healthy Characteristics and the Sensory Profile of Beer in Sequential Fermentation. **Foods**, v. 11, n. 14, p. 2029, jul. 2022. DOI 10.3390/foods11142029

PUERARI, C.; MAGALHÃES-GUEDES, K. T.; SCHWAN, R. F. Physicochemical and microbiological characterization of *chicha*, a rice-based fermented beverage produced by Umutina Brazilian Amerindians. **Food Microbiology**, v. 46, p. 210-217, 2015. DOI 10.1016/j.fm.2014.08.009

PUERTAS, B. *et al*. The influence of yeast on chemical composition and sensory properties of dry white wines. **Food Chemistry**, v. 253, p. 227-235, 2018. DOI 10.1016/j.foodchem.2018.01.039

RAI, A. K.; PANDEY, A.; SAHOO, D. Biotechnological potential of yeasts in functional food industry. **Trends in Food Science & Technology**, v. 83, p. 129-137, 2019. DOI 10.1016/j.tifs.2018.11.016

RESENDE, L. V. *et al*. Microbial community and physicochemical dynamics during the production of *'Chicha'*, a traditional beverage of Indigenous people of Brazil. **World Journal of Microbiology and Biotechnology**, v. 34, n. 3, p. 1-11, 2018. DOI 10.1007/s11274-018-2429-4

RIBEIRO, L. S. *et al*. Microbiological and chemical-sensory characteristics of three coffee varieties processed by wet fermentation. **Annals of Microbiology**, v. 68, p. 705-716, 2018. DOI 10.1007/s13213-018-1377-4

ROBLES-VERA, I. *et al*. The probiotic Lactobacillus fermentum prevents dysbiosis and vascular oxidative stress in rats with hypertension induced by chronic nitric oxide blockade. Molecular nutrition & food research, v. 62, n. 19, p. 1800298, 2018. DOI 10.1002/mnfr.201800298

RODRIGUES, D. *et al*. *In vitro* fermentation and prebiotic potential of selected extracts from seaweeds and mushrooms. **LWT - Food Science and Technology**, v. 73, p. 131-139, 2016. DOI 10.1016/j.lwt.2016.06.004

RODRÍGUEZ-ROQUE, M. J. *et al*. Changes in vitamin C, phenolic, and carotenoid profiles throughout in vitro gastrointestinal digestion of a blended fruit juice. **Journal of Agricultural and Food Chemistry**, v. 61, n. 8, p. 1859-1867, 2013. DOI 10.1021/jf3044204

RUFINO, M. DO S. M. *et al*. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. **Food Chemistry**, v. 121, n. 4, p. 996-1002, 2010. DOI 10.1016/j.foodchem.2010.01.037

SABER, A. *et al*. Secretion metabolites of probiotic yeast, *Pichia kudriavzevii* AS-12, induces apoptosis pathways in human colorectal cancer cell lines. **Nutrition Research**, v. 41, p. 36-46, 2017. DOI 10.1016/j.nutres.2017.04.001

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**, v. 186, p. 32-41, 2014. DOI 10.1016/j.ijfoodmicro.2014.06.011

SANTOS, R. A. R. dos *et al*. Process optimization for elaboration of cajá-umbu (Spondias spp.) fruit jelly: The effect of pulp and pectin contents on sensory attributes and volatile constituents. **International Journal of Gastronomy and Food Science**, v. 24, p. 100315, 2021. DOI 10.1016/j.ijgfs.2021.100315

SCHAUBECK, M. *et al*. Dysbiotic gut microbiota causes transmissible Crohn's disease-like ileitis independent of failure in antimicrobial defence. **Gut**, v. 65, n. 2, p. 225-237, 2016. DOI 10.1136/gutjnl-2015-309333

SCHULZ, M. *et al*. Composition and potential health effects of dark-colored underutilized Brazilian fruits–A review. **Food Research International**, v. 137, p. 109744, nov. 2020. DOI 10.1016/j.foodres.2020.109744

SELMA, M. V.; ESPIN, J. C.; TOMAS-BARBERAN, F. A. Interaction between phenolics and gut microbiota: role in human health. **Journal of Agricultural and Food Chemistry**, v. 57, n. 15, p. 6485-6501, 2009. DOI 10.1021/jf902107d

SENGUN, I.Y. *et al*. Identification of lactic acid bacteria isolated from *Tarhana*, a traditional Turkish fermented food. **International Journal of Food Microbiology**, v. 135, p. 105–111, 2009. DOI 10.1016/j.ijfoodmicro.2009.07.033

SHI, W. *et al.* Effect of *Issatchenkia terricola* and *Pichia kudriavzevii* on wine flavor and quality through simultaneous and sequential co-fermentation with *Saccharomyces cerevisiae*. **LWT - Food Science and Technology**, v. 116, p. 108477, 2019. DOI 10.1016/j.lwt.2019.108477

SIQUEIRA, A. de M. O. *et al*. Dietary fibre content, phenolic compounds and antioxidant activity in soursops (*Annona muricata* L.). **Revista Brasileira de Fruticultura**, v. 37, n. 4, p. 1020-1026, out.-dez., 2015. DOI 10.1590/0100-2945-211/14

SKOTNICZNY, M. *et al*. Growth dynamics and diversity of yeasts during spontaneous plum mash fermentation of different varieties. **Foods**, v. 9, n. 8, p. 1054, 2020. DOI 10.3390/foods9081054

UZKUÇ, N. M. Ç.; BAYHAN, A.; TOKLUCU, A. K. Phenolics and color components of young Cabernet Sauvignon wines: effect of spontaneous fermentation and bottle storage. **European Food Research and Technology**, v. 248, n. 2, p. 393-401, nov. 2022.

VAQUERO, C. *et al*. Biocompatibility in ternary fermentations with *Lachancea thermotolerans*, other non-*Saccharomyces* and *Saccharomyces cerevisiae* to control pH and improve the sensory profile of wines from warm areas. **Frontiers in Microbiology**, v. 12, p. 656262, 2021. DOI 10.3389/fmicb.2021.656262

VERHAAR, B. J. H. *et al*. Gut microbiota in hypertension and atherosclerosis: a review. **Nutrients**, v. 12, n. 10, p. 2982, 2020. DOI 10.3390/nu12102982

VIDIGAL, M. C. T. R. *et al*. Effect of a health claim on consumer acceptance of exotic Brazilian fruit juices: Açaí (*Euterpe oleracea* Mart.), Camu-camu (*Myrciaria dubia*), Cajá (*Spondias lutea* L.) and Umbu (*Spondias tuberosa* Arruda). **Food Research International**, v. 44, p. 1988-1996, 2011. DOI 10.1016/j.foodres.2010.11.028

WATANABE, H. S. *et al*. Annona's marketing profile at brazilian terminal markets. **Revista Brasileira de Fruticultura**, v. 36, p. 65-70, 2014. DOI 10.1590/S0100-29452014000500007

XU, Weina *et al*. Organic cultivation of grape affects yeast succession and wine sensory quality during spontaneous fermentation. **LWT - Food Science and Technology**, v. 120, p. 108894, fev. 2020. DOI 10.1016/j.lwt.2019.108894

XUE, L. *et al*. Probiotics may delay the progression of nonalcoholic fatty liver disease by restoring the gut microbiota structure and improving intestinal endotoxemia. **Scientific Reports**, v. 7, n. 1, p. 1-13, 2017. DOI 10.1038/srep45176

YAO, Y. *et al*. Polyphenol-rich extract from litchi chinensis seeds alleviates hypertension-induced renal damage in rats. **Journal of Agricultural and Food Chemistry**, v. 69, n. 7, p. 2138-2148, jan. 2021. DOI 10.1021/acs.jafc.0c07046

ZANWAR, A. A. *et al*. Role of gallic acid in cardiovascular disorders. In: **Polyphenols in human health and disease**. Academic Press, 2014. p. 1045-1047. DOI 10.1016/B978-0-12-398456-2.00080-3

ZERAIK, M. L. *et al*. Antioxidants, quinone reductase inducers and acetylcholinesterase inhibitors from *Spondias tuberosa* fruits. **Journal of Functional Foods**, v. 21, p. 396-405, ma. 2016. DOI 10.1016/j.jff.2015.12.009

ZULLO, B. A.; CIAFARDINI, G. Evaluation of physiological properties of yeast strains isolated from olive oil and their *in vitro* probiotic trait. **Food Microbiology**, v. 78, p. 179-187, 2019. DOI 10.1016/j.fm.2018.10.016

APÊNDICES

APÊNDICE A – ARTIGO I

“Yeasts from fermented Brazilian fruits as biotechnological tools for increasing phenolics bioaccessibility and improving the volatile profile in derived pulps”

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Yeasts from fermented Brazilian fruits as biotechnological tools for increasing phenolics bioaccessibility and improving the volatile profile in derived pulps

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ABSTRACT

Caatinga Biome fruits have been scarcely explored as a source of biotechnological yeasts. This study isolated yeasts from naturally fermented Caatinga fruits and evaluated Hanseniaspora opuntiae 125, Issatchenkia terricola 129, and Hanseniaspora opuntiae 148 on fermentation of soursop and umbu-cajá pulps. All strains were able to ferment the pulps (72 h), increasing ($p < 0.05$) acetic acid, phenolics concentration and bioaccessibility, and maintaining counts above 7 log CFU/mL after fermentation and/or *in vitro* digestion. H. opuntiae 125 showed the highest counts (8.43–8.76 log CFU/mL; $p < 0.05$) in pulps and, higher organic acids production, increased survival to digestion, and higher bioaccessibility of various phenolics ($p < 0.05$) in the umbu-cajá pulp. I. terricola 129 and H. opuntiae 148 showed higher metabolic activity, concentration and bioaccessibility of specific phenolics in umbu-cajá and soursop pulps, respectively ($p < 0.05$). Volatiles varied ($p < 0.05$) with the yeast strain. Generally, the yeast biotechnological performance for pulp fermentation was better on its fruit source.

1. Introduction

The Caatinga Biome is in the Northeast region of Brazil. It has a high diversity of fruits, and two of the most underexplored fruit are soursop (*Annona muricata* L.) and umbu-cajá (*Spondias spp.*). Soursop is a tropical and exotic fruit with unique flavor and aroma characteristics, white flesh, sour taste, and creamy texture (Ho et al., 2021). Umbu-cajá (*Spondias spp)* is a hybrid form of umbu (*Spondias tuberosa* Arruda Camara) and cajá (*Spondias mombin* L.). In addition, it has a juicy, sweet, and fibrous pulp and shows a bitter aftertaste (Pereira, Pereira, Pessoa, & Vasconcelos, 2021). Both umbu-cajá and soursop fruits have an interesting nutritional composition and have attracted attention because of the concentration of bioactive compounds, primarily polyphenols (Ho et al., 2021). However, due to their high perishability, they are mainly consumed locally.

The fermentation of fruit pulps may result in products with improved aromatic profile and sensory characteristics, and extended shelf life (Sabidi, Koh, Shukor, Sharifudin, & Sew, 2020). Furthermore, many microorganisms may biotransform phenolic compounds during fermentation, resulting in greater bioactivity (Morais, Borges, Lima, Martín-Belloso, & Magnani, 2019). The fermentation is commonly performed using commercial starter cultures, mainly from dairy or animal-derived products (Pimentel, Costa, Barão, Rosset, & Magnani, 2021). The utilization of starter cultures derived from fruits may raise the development of vegan and nondairy fermented products, reduce the processing times, and improve the characteristics of the products (Pimentel, Oliveira, et al., 2021).

Fruits from the Caatinga biome may be sources of microorganisms with potential application in biotechnological processes (Assis et al., 2021). Among them, yeasts have been highlighted due to their higher viability in fruits than lactic acid bacteria (LAB) and good performance in fermentation processes (Pimentel, Oliveira, et al., 2021). Furthermore, yeasts usually are resistant to antibiotics and do not transfer resistance genes to other microorganisms (Rai, Pandey, & Sahoo, 2019).

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In addition, they can also lead to improved host health and well-being by producing bioactive substances such as citric acid, carotenoids, tocopherols, and ascorbic acid, among others, that can combat oxidative stress (Arroyo-López et al., 2012).

Most studies with fermented fruit pulps were performed using LAB (Guergoletto et al., 2020, Palachum, Choorit, Manurakchinakorn, & Chisti, 2020). However, fermented fruit pulps processed with yeast are still scarce (Nyanga, Nout, Smid, Boekhout, & Zwietering, 2013, Farinazzo et al., 2020). Furthermore, no previous study isolated yeasts from Caatinga biome fruits and applied them in the fermentation of fruit pulps. Therefore, this study aimed to isolate yeasts from naturally fermented soursop and umbu-cajá fruits and use them to produce fermented pulps of these fruits.

2. Material and methods

2.1. Fruits for natural fermentation

Soursop (*Annona muricata* L.) and umbu-cajá (*Spondias spp.*) in the commercial maturity stage were obtained from local farms (protected identity), as they are commonly used to get frozen pulps regionally sold. First, fruits were standardized and selected considering the uniformity of shape and size, color, firmness, and lack of mechanical damage. Then, the fruits were transported in refrigerated boxes (5 ± 1 °C) and processed for juice production and yeast isolation.

Fermentation was carried out following the methodology described by Amorim, Piccoli, and Duarte (2018), with minor modifications. First, the fruits were cleaned with sterile distilled water and peeled in a safety cabinet under aseptic conditions. Soursop peels and seeds and umbu-cajá seeds were discarded. Next, the fresh pulp of each fruit was crushed separately with a mortar and pestle and passed through an 18-mesh stainless steel household sieve to remove excess fiber. These steps were performed under aseptic conditions. Approximately 400 mL of juice were obtained from each fruit type. Pulps were maintained at room temperature (25 ± 1 °C) for 48 h for natural fermentation.

pH values and total soluble solids (TSS) were measured at times 0, 24, and 48 h of natural fermentation. pH was evaluated using a pH meter (Quimis, São Paulo, Brazil), and TSS was determined using a manual refractometer (Vodex, model VX032SG). Titratable acidity (TA) was measured by titration (0.1 N NaOH) and expressed as % citric acid. All measurements followed the AOAC procedures (AOAC, 2016).

2.2. Microbial counts and yeast isolation from naturally fermented fruits

The microbial counts and isolation were performed following the methodology proposed by Freire, Ramos, Souza, Cardoso, and Schwan (2017), with minor modifications. Sampling (1 mL) was performed at times 0, 24, and 48 h in each fermentation flask to perform the yeast and LAB counts and subsequent yeast isolation. Serial dilutions were performed (10^{-1}-10^{-6}) and plated on yeast extract-peptone-dextrose agar (YEPD) (2 % peptone (w/v), 1 % yeast extract (w/v), 1.5 % agar (w/v) (Acumedia, Michigan, USA), 2 % D-glucose (w/v) (Dinâmica Química Contemporânea, São Paulo, Brazil), pH 3.5, 100 mg/L of chloramphenicol (Sigma-Aldrich, St. Louis, USA) (to bacterial growth inhibition) for counting yeasts or De Man, Rogosa, and Sharpe agar (MRS) (HiMedia, Mumbai, India) supplemented with 50 mg/L of nystatin 85+% (Acros Organics, New Jersey, USA) (to inhibit yeast growth) for counting LAB. The incubation was performed for 72 h at 30 °C for yeasts and 48 h at 37 °C and under anaerobiosis ($O_2 < 1$ %) for LAB (Anaerogen, Oxoid Ltd., UK). Results were reported as log colony-forming unit per milliliter (log CFU/mL).

The purification of yeast colonies was based on the morphology present on the plates, and the square root of the total number of isolates was calculated for subsequent purification using each colony morphotype (Sengun, Nielsen, Karapinar, & Jakobsen, 2009). The isolated yeasts were submitted to macroscopic and micromorphological analysis, and the characteristics of colony type, size, border structure, texture, color, elevation, appearance, shape, and shine were observed. Simple staining with methylene blue from fresh preparation and fermentation tests with carbohydrates were used to identify the isolates. Afterward, pure cultures were maintained and sub-cultured until identification via Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). Finally, the isolates were frozen and stored (-80 °C) in the corresponding medium containing 20 % glycerol (Freire et al., 2017).

2.3. Identification of isolated yeasts by MALDI-TOF MS and strains selection

The yeasts were identified by MALDI-TOF MS (Carvalho et al., 2017). The analyzes were performed in an ultrafleXtreme MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) containing a 1000 Hz neodymium-doped aluminum yttrium garnet laser. Yeast cultures were grown for 18 h at 28 °C on YEPD. Approximately 1 μg of culture biomass was added to a tube (500 μL) containing 6 μL of formic acid (25 % v/v). The samples were vortexed (60 s) and centrifuged (4000 g, 60 s, room temperature). Each sample supernatant (1 μL) was placed on the stainless steel MALDI-TOF MS plate (Bruker Daltonics, MSP 96 target polished steel). After sample drying, 1 μL of the matrix solution (α-cyano-4-hydroxycinnamic acid (Fluka), saturated in an organic solution, was added and lightly mixed. Afterward, the samples were analyzed by MALDI-TOF MS. Each sample was analyzed in triplicates aiming reproducibility test. A standard (Bruker Daltonics) test containing *Escherichia coli* DH5 alpha extract was used for calibration. Mass spectra were evaluated using the MALDI Biotyper 3.0 software package (Bruker Daltonics) for microbial identification and statistical clustering.

The MALDI Biotyper System uses MALDI-TOF mass spectrometry for the interpretation of results. The identification scores were determined as follows (+++) represents the highly probable species identification (scores 2.300 to 3.000); (++) represents the secure identification of the genus, probable identification of the species (scores 2.000 to 2.299); (+) represents the likely identification of the genus (scores 1.700 to 1.999); and (-), which represents the unreliable identification (scores 0.000 to 1.699).

Among the identified isolates, those selected for further assays were chosen based on their identification scores and potential application in fermentation tests (Pimentel, Oliveira, et al., 2021). The selected isolates were subjected to safety tests, including hemolytic activity, gelatin hydrolysis, and DNAse activity (Singh et al., 2012). Hemolytic activity was measured after strain inoculation onto blood agar plates with 5 % sheep blood and incubation at 37 °C for 48 h. The strains' gelatinase production was determined using tryptone-neopeptonedextrose (TND) agar (3.0 g neopeptone, 17.0 g tryptone, 5.0 g NaCl, 2.5 g dextrose, 15 g agar, 2.5 g K_2HPO_4, and 1 L distilled water) and 0.4 % gelatin. Yeast cultures were spotted onto plates and incubated at 30 °C for 48 h. The DNAse test was performed by seeding the strains on the DNAse test agar medium (Difco, USA) and incubating the plates at 30 °C for 48 h. Then, an HCl solution (1 M) was added. The *Staphylococcus aureus* ATCC 25,923 strain was used as a positive control for all tests and incubated at 37 °C for 48 h.

2.4. Use of selected yeast strains in fruit pulps fermentation

Soursop and umbu-cajá pulps were obtained from local farmers in the commercial maturity stage (protected identity, Paraíba State, Brazil). Fruits were processed without the addition of water or preservatives at the industrial plants of Fazenda Mangai (soursop) and Pé de Fruta (umbu-cajá) to obtain the pulps.

The strains selected for the experiment, *Hanseniaspora opuntiae* 125, *Issatchenkia terricola* 129, and *Hanseniaspora opuntiae* 148 were inoculated in 5 mL of YEPD broth (same composition reported in section 2.2) and incubated at 30 ± 1 °C for 24 h. After the incubation period, yeast cells were harvested by centrifugation (3500 g, 15 min, 4 °C), washed twice with sterile 0.1 % peptone water (HiMedia, Mumbai, India), and resuspended in 5 mL of the same solution (Freire et al., 2017). Then, 0.4 μL of the inoculum (6 log CFU/mL) was added to 40 mL of pulp previously pasteurized at 80 °C for 5 min and cooled in an ice bath (Fonseca, Melo, Ramos, Dias, & Schwan, 2021). Microfermentations were performed in 50 mL tubes with 40 mL of fruit pulp in a BOD incubator (Caltech Indústria e Comércio LTDA, São Paulo, Brazil) at 14 °C for 72 h of static fermentation. Pulps without the addition of yeast strain were used as controls.

The pH values were evaluated at times 0, 24, 48, and 72 h of fermentation as previously described above. After the fermentation process, the samples and controls were subjected to yeast counts following the methodologies reported in section 2.2.

2.5. Fermented fruit pulps in vitro digestion and bioaccessibility of phenolic compounds

The *in vitro* digestion was carried out following the methodology described by Minekus et al. (2014). Fermented pulps (5 g) were added with simulated salivary fluid (SSF) (3.5 mL) to simulate the oral phase. SSF comprised 0.5 mL of 1500 U/mL α-amylase (Sigma-Aldrich, St. Louis, USA) in a solution of 975 μL of water and 25 μL of 0.3 M $CaCl_2$. The mixed sample was incubated at 37 ± 1 °C for 2 min under agitation (90 rpm) in an orbital shaking incubator (Thoth Equipamentos, model 6420, Brazil). Then, simulated gastric fluid (SGF) (7.5 mL) was added to simulate the gastric digestion, which was a mixture of 0.695 μL of water, 5 μL of 0.3 M $CaCl_2$ and 1.6 mL of 2000 U/mL porcine pepsin (Sigma-Aldrich, St. Louis, USA). The pH was adjusted (pH 3.0) using 1 M HCl, and the mixture was incubated for 2 h at 37 ± 1 °C with agitation. Finally, the gastric mixture (20 mL) was added with simulated intestinal fluid (SIF) (11 mL), 2.5 mL of fresh bile (Sigma-Aldrich, St. Louis, USA, 160 mM in fresh bile), 5.0 mL of 800 U/mL pancreatin solution (Sigma-Aldrich, St. Louis, USA) in SIF solution, 1.31 mL of water, and 40 μL of 0.3 M $CaCl_2$, to simulate the intestinal phase. The pH was adjusted (pH 7.0) with 1 M NaOH, and the mixture was incubated at 37 ± 2 °C for 2 h with agitation (90 rpm). The pH values were controlled during the *in vitro* digestion and adjusted when needed for each corresponding digestive step.

The bioaccessibility of phenolics was determined according to Eq. (1) (Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2013). In addition, counts to monitor yeast viability were performed before the *in vitro* digestion, after the gastric phase, and at the end of the digestion.

$$Bioaccessibility(\%) = (intestinal\,BC/BC\,sample) \times 100 \tag{1}$$

Where intestinal BC refers to the phenolic compound concentration at the end of the *in vitro* digestion (bioaccessible fraction), and the BC sample refers to the fruit phenolic concentration before *in vitro* digestion.

2.6. Organic acids, sugars, phenolic compounds, and alcohol in fermented fruit pulps

Fermented soursop and umbu-cajá pulps, their bioaccessible fractions, and unfermented controls were centrifuged (15 min; 3500 g) (Centrifuge SL-701; Solab, São Paulo, Brazil), and the supernatant was filtered using Millex-HA filter (0.45 μm, Millipore, Bedford, USA).

The organic acids and sugars were determined by High-Performance Liquid Chromatography (HPLC) using an Agilent chromatography (model 1260 Infinity LC, Agilent Technologies, USA) coupled with a detector index of refraction (DIR) and a diode array detector (DAD). An Agilent Hi-Plex H column (7.7 × 300 mm, 8 μm) and 4 M H_2SO_4 in ultrapure water as the mobile phase (flow rate 0.5 mL/min) were used.

The phenolic compounds were determined by HPLC using the Zorbax precolumn (C18; 12.6 × 4.6 mm, 5 μm) and a Zorbax column (Eclipse Plus RP-C18; 100 × 4.6 mm, 3.5 μm). The mobile phases comprised acidified water (pH 2 using 0.1 mM/L phosphoric acid; A phase) and acidified methanol using 0.5 % phosphoric acid (B phase). The injection volume was 20 μL of the sample, previously diluted in phase A, and filtered through 0.45 μm membrane. The gradient used for separation was 0–5 min: 5 % B; 5–14 min: 23 % B; 14–30 min: 50 % B; 30–33 min: 80 % B. The column temperature was kept at 35 °C. The flow rate was maintained at 0.8 mL/min.

Data acquisitions were processed using OpenLAB CDS ChemStation EditionTM software (Agilent Technologies). Peaks of organic acids, sugars, and phenolic compounds were identified by comparing their retention times and external standards. The standards (purity level of ≥98 %) used for the analysis of the phenolic compounds were chlorogenic, gallic, syringic, p-coumaric, caffeic and *trans*-caftaric acids, catechin, epicatechin, hesperidin, naringenin, procyanidin B1 and B2, delphinidin 3-glucoside, cyanidin 3,5-diglucoside, cyanidin 3-glucoside, malvidin 3,5-diglucoside, malvidin 3-glucoside, pelargonidin 3,5-diglucoside from Sigma-Aldrich (St. Louis, USA). Procyanidin A2, kaempferol 3-glucoside, quercetin 3-glucoside, quercetin 3-rutinoside (rutin), myricetin, epicatechin gallate, epigallocatechin gallate, pelargonidin 3-glucoside, petunidin 3-glucoside, malvidin 3-O-glucoside and peonidin 3-O-glucoside were from Extrasyntese (Genay, France). cis-Resveratrol and *trans*-resveratrol were from Cayman Chemical Company (Ann Arbor, MI, USA). The standards used for organic acids and sugars were glucose and fructose (Sigma-Aldrich, St. Louis, USA); maltose and rhamnose (Chem Service, West Chester, USA); and tartaric, malic, lactic, citric, and acetic acids (Química Vetec, Rio de Janeiro, Brazil). The solutions were prepared using ultrapure water obtained through the Milli-Q system (Millipore, Bedford, USA). Quantification was performed using external standard calibration curves following validated methods (Coelho et al., 2018, Padilha et al., 2017, for sugar and organic acids and phenolics, respectively). The calibration curves showed $R^2 \geq 0.998$ for all compounds. The spectral purity of the peaks was verified with the Threshold tool to ensure identification accuracy compared to the external standard.

Ethanol was analyzed according to procedures proposed by Duarte et al. (2010). An HPLC system (Shimadzu, model LC-10Ai, Shimadzu Corp., Tokyo, Japan) equipped with a dual detection system was used (refractive index detector (RI; 10A) and ultraviolet detector (SPD-10AI). The Shimadzu cation exchange column (Shim-pack SCR-101H, 7.9 mm × 30 cm) was operated at 30 °C using 100 mM perchloric acid (70 %) eluent. The flow rate was set as 0.6 mL/min, and the injection volume was 20 μL. The quantification of ethanol was performed by applying calibration curves obtained using the standard compound (Sigma-Aldrich, Steinheim, Germany).

2.7. Fermented fruit pulps volatile profile

The volatile organic compounds were determined by solid-phase microextraction in headspace mode (HS-SPME) using a gas chromatograph coupled to mass spectrometry (GCMS) Shimadzu QP-2010 SE according to the methods of Fonseca et al. (2021). A Carbowax 20 M column (30 m × 0.25 mm ID × 0.25 μm film) was used. The sample aliquot (3.0 mL) was added to a 20 mL vial and equilibrated for 15 min at 60 °C. The volatile compounds were captured using an automatic SPME holder (Supelco, Bellafonte, PA, USA) with a DVB/CAR/PDMS (50/30 μm) long fiber (2 cm). The SPME fiber was exposed to the upper space of the sample for 30 min at a constant depth. The temperature was maintained at 60 °C. Then, the volatiles were desorbed directly in the gas chromatograph liner and kept at 230 °C for 2 min. Helium at a 1.0 mL/min flow rate was used as carrier gas. The temperature was 60 °C/5 min, increased to 230 °C at 10 °C/min, and kept at this temperature for 15 min. An alkanes series (C10-C40) (Sigma-Aldrich, St. Louis, USA) was used to determine the linear index of retention (LRI) for each compound. Data were analyzed using the GCMSsolution software (version 4.4, Shimadzu Corporation, Japan) and the NIST NIST/EPA/NIH 2014 database. Chemical identification of each volatile was performed by comparing the MS spectra with the dataset. The 4-nonanol (Sigma-Aldrich, St. Louis, USA) at a final concentration of 6.250 ng/mL was used as an internal standard to quantify each peak accordingly as relative concentration (ng/g).

2.8. Statistical analysis

The experiments were repeated three times. The analyzes were performed in triplicates. Data were analyzed using analysis of variance (ANOVA) and Tukey test or Student's *t*-test considering $p < 0.05$. Principal Component Analysis (PCA) was performed using the data of volatile compound groups to show which ones predominated in each formulation. The matrices consisted of 4 columns and 7 lines for each pulp studied. PCA was also done using phenolic compounds (content and bioaccessibility) to differentiate between soursop and umbu-cajá pulps. The matrix consisted of 8 lines (4 soursop and 4 umbu-cajá pulp formulations) and 35 columns. Finally, a PCA was performed with phenolic compounds (content and bioaccessibility) and yeast counts for each fruit pulp. The matrix consisted of 4 lines (pulp formulations) and 35 columns for umbu-cajá, and 27 columns for soursop. Analyzes were performed using XLSTAT® 2021.4.1 software (Addinsoft ™, Paris, France).

3. Results and discussion

3.1. Natural fermentation of fruits, isolation of yeasts, and subsequent identification

Soursop juice showed TSS values of 18.40 ± 0.20, pH values of 3.88 ± 0.05, TA of 0.77 ± 0.03 %citric acid, and counts of 6.24 log CFU/mL for yeast and 4.07 log CFU/mL for LAB. At the same time, umbu-cajá juice had TSS values of 9.73 ± 0.12, pH values of 2.60 ± 0.03, TA of 1.49 ± 0.02 %citric acid, and counts of 5.83 log CFU/mL for yeast and 3.82 log CFU/mL for LAB. Soursop pulp kept higher yeast counts at the end of fermentation time (6.11 log CFU/mL, $p < 0.05$), while no differences in LAB counts among fruit juices were observed (6.93–6.97 log CFU/mL, $p \geq 0.05$). The low acidity and higher TSS values of the soursop juice may have been the determining factor for the survival of yeasts in this food matrix.

Yeasts were selected according to the square of the number of morphotypes observed on the plates. After simple staining using methylene blue and fermentation tests (data not shown), 27 isolates presumably classified as yeast were submitted for identification by MALDI-TOF. Yeasts belonging to the genus *Hanseniaspora* were found in more significant numbers among the identified isolates (n = 15). The second most frequent genus of isolated yeast was *Issatchenkia* (n = 4) and *Candida* (n = 4). In their study, Okigbo and Obire (2009) also reported *Candida* sp. strains in fresh and rotten soursop. Yeasts belonging to the genera *Kodamea* (n = 1) and *Saccharomyces* (n = 1) were identified in a smaller number. At this stage, only two of the isolates could not be determined (score < 1.699). One of the isolates had a low identification score (*Candida carpophila* – 1.732), and all the other isolates had scores greater than 2.000.

The strains *H. opuntiae* 125 and *I. terricola* 129 isolated from umbu-cajá, and *H. opuntiae* 148 from soursop, with scores of 2.313, 2.147, and 2.288, respectively, were selected for technological application in soursop and umbu-cajá pulps. These strains showed the best identification scores among the non-pathogenic species, had a history of use in food, whether to produce compounds, aroma improvement, as biocontrol agents, among others (Pimentel, Oliveira, et al., 2021), and did not produce excessive alcohol. Furthermore, none of the selected strains showed gelatinase, hemolytic, or DNAse activity compared to *S. aureus* ATCC 25,923 (positive control strain), which are important safety characteristics required for strains used in the food industry.

3.2. Fermentation of soursop and umbu-cajá pulps with selected yeasts

Soursop pulp showed pH values of 3.74 ± 0.01, TSS of 12.33 ± 0.12 and TA of 0.81 ± 0.03 %citric acid, while umbu-cajá pulp had pH values of 2.50 ± 0.02, TSS of 12.33 ± 0.12 and TA of 1.70 ± 0.01 %citric acid. The fermentation process did not cause changes in the pH values of the pulps ($p \geq 0.05$). The pH value maintenance may be associated with the organic acid balance after fermentation. However, the TSS values differed from the initial values ($p < 0.05$), with the highest reduction occurring in pulps fermented by *H. opuntiae* 148 and the lowest in pulps fermented by *I. terricola* 129 (data not shown). Differences in TSS reductions suggest different metabolic activities of the yeasts in the fruit pulps.

The initial counts (time 0) of *H. opuntiae* 125, *I. terricola* 129, and *H. opuntiae* 148 ranged from 6.31 ± 0.03 to 6.44 ± 0.05 log CFU/mL for soursop pulps and from 6.32 ± 0.07 to 6.47 ± 0.03 log CFU/mL for umbu-cajá pulps (Table S1; $p \geq 0.05$). At the end of the fermentation, it was possible to observe an increase in yeast counts in all fruit pulps ($p < 0.05$). *H. opuntiae* 125 was isolated from umbu-cajá, and it showed the highest viability in umbu-cajá pulp compared to the other yeast strains ($p < 0.05$). At the same time, *I. terricola* 129, also isolated from umbu-cajá, showed the lowest counts in soursop pulp, and *H. opuntiae* 148 isolated from soursop showed the lowest counts in umbu-cajá pulp ($p < 0.05$) (Table S1). In this way, autochthonous microorganisms may be more adapted to the matrix and perform better fermentation processes (Pimentel, Oliveira, et al., 2021). *H. opuntiae* 125 also showed the highest viability in soursop pulp ($p < 0.05$). This yeast strain showed a higher ability to increase its viability during fermentation regardless of the fruit pulp used.

3.3. Fermented fruit pulps profile of sugars, organic acids, and alcohol

Table 1 shows the values of sugar and alcohol concentrations before and after the fermentation of each pulp, and Table 2 shows the values for organic acids. The pulp fermentation with yeast strains did not change the fructose, citric acid, malic acid, and lactic acid contents in both fermented pulps ($p \geq 0.05$). At the same time, higher concentrations of propionic acid were observed in the fermented umbu-cajá pulps

Table 1
Concentrations of sugar and alcohol in fermented and non-fermented pulp fruits pulps (72 h) by *H. opuntiae* 125, *I. terricola* 129 or *H. opuntiae* 148.

Sample	Treatments	Sugars (g/L)			Alcohols (g/L)
		Glucose	Fructose	Maltose	Ethanol
Soursop	Control	42.00 ± 3.54^{A}	44.93 ± 3.77^{A}	0.46 ± 0.05^{A}	0.37 ± 0.02^{D}
	H. opuntiae 125	37.15 ± 3.31^{A}	40.36 ± 1.37^{A}	0.53 ± 0.02^{A}	25.51 ± 0.00^{B}
	I. terricola 129	44.90 ± 2.34^{A}	49.68 ± 2.85^{A}	0.58 ± 0.03^{A}	5.27 ± 0.00^{C}
	H. opuntiae 148	26.64 ± 2.38^{B}	35.63 ± 1.32^{A}	0.58 ± 0.08^{A}	30.32 ± 0.00^{A}
Umbu-cajá	Control	30.98 ± 1.24^{A}	29.92 ± 1.17^{A}	19.97 ± 1.23^{B}	0.20 ± 0.01^{C}
	H. opuntiae 125	29.45 ± 0.38^{A}	27.46 ± 2.36^{A}	23.96 ± 2.94^{AB}	23.68 ± 0.55^{A}
	I. terricola 129	30.10 ± 2.80^{A}	27.06 ± 0.13^{A}	29.92 ± 0.56^{A}	4.39 ± 0.13^{B}
	H. opuntiae 148	26.20 ± 1.22^{A}	26.22 ± 1.01^{A}	27.39 ± 0.48^{AB}	23.63 ± 1.04^{A}

Values are expressed as the mean ± standard deviation; n = 9. Control: non-fermented pulp; Fermented pulps: *H. opuntiae* 125, *I. terricola* 129 or *H. opuntiae* 148; A-D: different capital letters in the same column denote differences ($p < 0.05$) among treatments for the same fruit pulp, according to the Tukey test.

Table 2
Concentrations of organics acids in fermented and non-fermented pulp fruits pulps (72 h) by *H. opuntiae* 125, *I. terricola* 129 or *H. opuntiae* 148.

Sample	Treatments	Organic acids (g/L)						
		Citric	Malic	Tartaric	Acetic	Propionic	Succinic	Lactic
Soursop	Control	2.07 ± 0.18^{A}	5.90 ± 0.53^{A}	0.03 ± 0.00^{A}	0.08 ± 0.00^{B}	<LOD	3.56 ± 0.32^{A}	<LOD
	H. opuntiae 125	2.29 ± 0.09^{A}	6.38 ± 0.21^{A}	0.03 ± 0.00^{AB}	0.18 ± 0.01^{A}	<LOD	1.85 ± 0.00^{B}	<LOD
	I. terricola 129	2.41 ± 0.15^{A}	6.69 ± 0.41^{A}	0.03 ± 0.00^{AB}	0.16 ± 0.01^{A}	<LOD	1.82 ± 0.00^{B}	<LOD
	H. opuntiae 148	2.27 ± 0.17^{A}	6.20 ± 0.17^{A}	0.02 ± 0.00^{B}	0.23 ± 0.04^{A}	<LOD	2.61 ± 0.00^{B}	<LOD
Umbu-cajá	Control	0.14 ± 0.02^{AB}	1.49 ± 0.06^{A}	0.02 ± 0.00^{B}	<LOD	0.18 ± 0.00^{B}	0.37 ± 0.01^{A}	0.47 ± 0.02^{A}
	H. opuntiae 125	0.07 ± 0.01^{B}	1.33 ± 0.16^{A}	0.05 ± 0.00^{A}	0.28 ± 0.01^{A}	0.23 ± 0.01^{A}	0.09 ± 0.01^{C}	0.65 ± 0.04^{A}
	I. terricola 129	0.17 ± 0.02^{A}	0.82 ± 0.06^{A}	0.02 ± 0.00^{B}	0.06 ± 0.01^{C}	0.27 ± 0.01^{A}	0.07 ± 0.00^{C}	0.37 ± 0.08^{A}
	H. opuntiae 148	0.11 ± 0.00^{AB}	1.29 ± 0.20^{A}	0.06 ± 0.00^{A}	0.23 ± 0.01^{B}	0.24 ± 0.01^{A}	0.19 ± 0.00^{B}	0.61 ± 0.00^{A}

Values are expressed as the mean ± standard deviation; n = 9. < LOD: below detection limit; Control: non-fermented pulp; Fermented pulps: *H. opuntiae* 125, *I. terricola* 129 or *H. opuntiae* 148; A-C: different capital letters in the same column denote differences ($p < 0.05$) among treatment for the same fruit pulp, according to the Tukey test.

Table 3
Concentration of volatile compounds (ng/mL) detected by GC–MS in fruits pulps before and after fermentation (72 h) by yeast cultures *H. opuntiae* 125, *I. terricola* 129 or *H. opuntiae* 148 and their sensory attributes.

Volatile compounds	Sensory attributes*	LRI	Soursop pulp				Umbu-cajá pulp			
			Control	*H. opuntiae* 125	*I. terricola* 129	*H. opuntiae* 148	Control	*H. opuntiae* 125	*I. terricola* 129	*H. opuntiae* 148
Higher alcohols										
1,6-octadien-3-ol,3,7-dimethyl	Spicy, citrus, taste, floral, woody, sweet, with a green, spicy tropical nuance	1082	29.81^{D}	36.97^{C}	59.66^{A}	52.58^{B}	200.07^{B}	176.74^{C}	249.37^{A}	136.01^{D}
3-methyl-1-butanol	Malty	697	–	152.08^{C}	220.36^{A}	174.57^{B}	–	98.11^{B}	183.66^{A}	65.44^{C}
cis-3,7-dimethyl-2,6-octadien-1-ol	Floral, Fruit flavor	1228	–	–	–	–	72.72^{A}	52.60^{B}	59.98^{B}	53.83^{B}
1-phenylethanol		1136	–	45.59^{C}	107.32^{A}	83.65^{B}	–	31.33^{C}	71.21^{A}	42.85^{B}
2-methyl-1-propanol	Malty	597	–	–	93.80^{A}	95.75^{A}	–	–	12.55^{A}	11.11^{B}
α-terpineol	Lime, peach, floral flavor, sweet, lime taste	1137	1.60^{A}	1.35^{B}	1.65^{A}	1.27^{B}	109.71^{B}	104.60^{B}	131.62^{A}	85.00^{C}
Geraniol	–	1228	–	–	–	–	72.72^{A}	52.60^{B}	59.98^{B}	53.83^{B}
Phenol, 2,4-bis (1,1-dimethylethyl)	–	1555	1.97^{C}	5.05^{A}	5.30^{A}	3.17^{B}	1.63^{C}	4.49^{B}	4.64^{B}	5.89^{A}
Total higher alcohols			**33.37^{D}**	**241.05^{C}**	**488.11^{A}**	**411.00^{B}**	**456.86^{C}**	**520.49^{B}**	**773.00^{A}**	**453.97^{C}**
Aldehyde										
Benzaldehyde	Almond odor	1208	37.18^{A}	–	1.45^{B}	–	104.33^{A}	–	4.27^{B}	–
Total aldehyde			**37.18^{A}**	**–**	**1.45^{B}**	**–**	**104.33^{A}**	**–**	**4.27^{B}**	**–**
Acids										
Hexanoic acid	Fatty-rancid odor, acrid-acid	974	99.82^{A}	67.44^{D}	85.18^{B}	74.20^{C}	–	–	–	–
2-propenoic	–	1267	85.31^{A}	60.11^{B}	64.26^{B}	56.94^{C}	14.41^{B}	15.75^{A}	10.47^{C}	7.86^{D}
Acetic acid	Strong odor of vinegar	576	9.32^{D}	88.09^{A}	30.24^{C}	82.95^{B}	4.77^{D}	60.40^{B}	22.69^{C}	114.86^{A}
Benzoic acid	–	1160	–	–	–	–	21.69^{A}	13.49^{B}	20.25^{A}	13.23^{B}
Octanoic acid	Slightly sour taste	1173	3.50^{C}	6.03^{A}	3.33^{C}	4.80^{B}	2.72^{C}	6.88^{A}	3.15^{B}	6.52^{A}
n-hexadecanoic acid	Faint oily aroma	1978	–	1.54^{A}	–	1.18^{B}	1.07^{B}	2.12^{A}	–	1.11^{B}
Isobutyric acid	Sweat, bitter; cheese, rancid	711	–	–	15.12	–	–	–	22.52	–
Total acids			**197.95^{B}**	**223.21^{A}**	**198.13^{B}**	**220.07^{A}**	**44.66^{D}**	**98.63^{B}**	**79.08^{C}**	**143.57^{A}**
Ester										
Methyl salicylate	Odor and taste of wintergreen	1281	2.22^{A}	0.86^{C}	1.45^{B}	–	9.33^{A}	6.93^{C}	7.72^{B}	5.89^{D}
2-hexenoic acid, methyl ester	–	892	114.50^{C}	258.06^{A}	195.67^{B}	268.76^{A}	–	–	–	–
Total ester			**116.71^{C}**	**258.93^{A}**	**197.12^{B}**	**268.76^{A}**	**9.33^{A}**	**6.93^{C}**	**7.72^{B}**	**5.89^{D}**
Terpene										
β-caryophyllene		1494	6.04^{B}	5.21^{C}	3.75^{D}	11.21^{A}	138.64^{A}	74.78^{C}	84.62^{B}	74.87^{C}
Total terpene			**6.04^{B}**	**5.21^{C}**	**3.75^{D}**	**11.21^{A}**	**138.64^{A}**	**74.78^{C}**	**84.62^{B}**	**74.87**
Acetate										
2-phenylethyl acetate	Apple, honey, roses, sweet; flowery	1259	–	465.96^{A}	17.79^{B}	463.52^{A}	–	396.95^{A}	4.54^{B}	362.14^{A}
Total acetate			**–**	**465.96^{A}**	**17.79^{B}**	**463.52^{A}**	**–**	**396.95^{A}**	**4.54^{B}**	**362.14^{A}**

Values are expressed as the mean of concentration (ng/mL). Control: non-fermented pulp; Fermented pulps: *H. opuntiae* 125, *I. terricola* 129 or *H. opuntiae* 148; A-D: different capital letters in the same row for the same volatile compound (or sum of the volatile group) denote difference ($p < 0.05$) among treatments for the same fruit, based on the Tukey test. *Sensory attributes are taken from https://pubchem.ncbi.nlm.nih.gov/; https://www.flavornet.org; https://www.femaflavor.org/.

compared to control, regardless of the yeast strain used ($p < 0.05$). Propionic acid is an energy source for colon cells, and it is associated with the inhibition of cholesterol and fat synthesis and gluconeogenesis (Fonseca et al., 2021). In this way, higher propionic acid concentrations may represent a positive result in the fermented umbu-cajá pulps.

The fermentation of umbu-cajá pulp with *H. opuntiae* 125, isolated from umbu-cajá, resulted in the highest consumption of succinic acid and production of ethanol, acetic acid, and tartaric acid. *I. terricola* 129 was the strain that produced the least ethanol in both pulps ($p < 0.05$). At the same time, it produced maltose in the pulp of umbu-cajá, the fruit from which it was isolated. Some yeasts can produce β-amylase enzymes that metabolize starch to produce maltose. Consumption of succinic acid and ethanol and acetic acid production was also observed ($p < 0.05$). The *H. opuntiae* 148 strain consumed the glucose and produced the most ethanol in soursop pulp, the isolation source fruit. At the same time, decreases in tartaric acid and succinic acid, and acetic acid production were observed ($p < 0.05$). Yeasts may use sugars (mainly glucose) during fermentation with the production of ethanol and acetic acid (Rêgo et al., 2020). Some organic acids may also be consumed during fermentation, such as succinic acid (Qiu et al., 2021). Decreased succinic acid content may be important from a sensory point of view. Its salty and bitter taste is considered unpleasant (Vilela, 2019). Overall, the results suggest higher metabolic activity of yeast strains in the isolated fruit pulps.

3.4. Volatile organic compounds profile of fermented fruit pulps

The volatile compounds identified in the fermented soursop and umbu-cajá pulps are shown in Table 3. Twenty volatile compounds were identified by GC–MS, comprising alcohols, aldehydes, acids, esters, terpenes, and acetates. Specific compounds were identified in all formulations (1,6-octadien-3-ol,3,7-dimethyl, α-terpineol, phenol, 2,4-bis (1,1-dimethylethyl), 2-propenoic, acetic acid, octanoic acid, and β-caryophyllene), which are described as responsible for sweet, woody, fermented fruit, and sour aromas (Freitas, Magalhães, Alves Filho, & Garruti, 2021, Buljeta, Pichler, Ivić, Šimunović, & Kopjar, 2021).

Umbu-cajá pulp showed three exclusively compounds (*cis*-3,7-dimethyl-2,6-octadien-1-ol, geraniol, and benzoic acid), which are associated with floral and fruit aromas (Giuffrida et al., 2020). At the same time, soursop pulp showed two exclusively compounds (hexanoic acid, and 2-hexenoic acid, methyl ester), associated with sweet, sour, and grassy aromas (Freitas et al., 2021, Buljeta et al., 2021).

Higher alcohols corresponded to major volatiles ($p < 0.05$) in both fermented fruit pulps (Fig. S1), especially in umbu-cajá pulps fermented by *I. terricola* (Table 3). These compounds are formed predominantly from the degradation of amino acids by the Ehrlich pathway, which is the most studied and discussed pathway. However, higher alcohols may also be formed by *de novo* biosynthesis of amino acids from sugars, mainly glucose (Yan, Xiangsong, & Xiang, 2019, Benucci et al., 2021). Monoterpene alcohols such as α-terpineol and geraniol have also been found in fruit wines. Specifically, α-terpineol was the major compound in wine made from umbu (Duarte et al., 2010), a fruit belonging to the same family of umbu-cajá. In our study, the major higher alcohol in umbu-cajá samples was 1,6-octadien-3-ol,3,7-dimethyl, mainly in the umbu-cajá pulp fermented by *I. terricola* 129 (Table 3). On the other hand, the two *Hanseniaspora* strains had reduced amounts of higher alcohols (Table 3). These results can be explained by *Hanseniaspora* yeast's ability to convert higher alcohols into esters (Giorello et al., 2018).

Following higher alcohols, acetate was abundant in soursop samples fermented by *H. opuntiae* 148 or *H. opuntiae* 125 ($p < 0.05$), which had similar effects on most volatiles, regardless of the fermented fruit pulp (Table 3). On the other hand, acetate was among the minor volatiles in soursop fermented with *I. terricola* 129 and almost absent in fermented umbu-cajá fermented with this yeast (Table 3). There was no acetate in the control samples, either soursop or umbu-cajá, demonstrating that this compound was derived from yeast metabolism. High acetate esters contents have been related to enhanced aroma perception in fermented beverages (Valera, Olivera, Boido, Dellacassa, & Carrau, 2021). Therefore, the observed results may be considered for further sensory evaluation.

Another volatile found mainly in pulps fermented by *H. opuntiae* 148 and *H. opuntiae* 125 was the 2-phenylethyl acetate (Table 3). It is a metabolite of phenylalanine's amino acid and is responsible for the desirable floral aroma, especially roses, in wines (Cordente et al., 2018). The 2-phenylethyl acetate has been associated with fermentation by non-*Saccharomyces* yeasts, including species of the genus *Hanseniaspora* that produce large quantities given fruity aroma in wines (Mota-Gutierrez, Barbosa-Pereira, Ferrocino, & Cocolin, 2019). Otherwise, 3-methyl-1-butanol and 2-methyl-1-propanol, associated with malty aroma were mainly produced during fermentation with *I. terricola* 129 ($p < 0.05$) (Table 3).

Benzaldehyde and β-caryophyllene, the aldehyde and terpene identified in fermented pulps, respectively, decreased during fermentation ($p < 0.05$, except for soursop pulp fermented with *H. opuntiae* 148) (Table 3), while volatile compounds (e.g. 1-phenylethanol) already described in fermented cocoa (Mota-Gutierrez et al., 2019) and cocoa and cupuaçu wines (Duarte et al., 2010) were formed in the fermented fruit pulps.

The PCA was able to explain 83.22 % of the volatiles data variability in fermented soursop pulp by the yeast strains (D1: 55.26 % and D2: 27.96 %; Fig. S2) and 99.26 % in fermented umbu-cajá pulp (D1: 82.22 % and D2: 17.04 %; Fig. S2). Considering the PCA, it is possible to observe the opposite behavior of *H. opuntiae* 148 or *H. opuntiae* 125 to *I. terricola* 129 concerning the values for acetate and higher alcohols.

The results indicate that fermentation of fruit pulps by the tested yeasts, mainly from its fruit source, defines the volatile composition contributing to unique characteristics in the derived fermented pulp.

3.5. Phenolic compounds in fermented and unfermented fruit pulps

The flavonoids and phenolic acids identified in the fermented soursop and umbu-cajá pulps are shown in Tables 4 and 5, respectively. Better visualization of data may be observed in the PCA map. PCA was able to explain the data with variability of 74.99 % (D1: 51.71 % and D2: 23.28 %, Fig. S3). The parameters with correlation coefficients higher than 0.7 (absolute values) with each component were considered important (Assis et al., 2021). Umbu-cajá pulp, located on the right side of the axis, was characterized by higher concentrations of *cis*-resveratrol, procyanidin A2, quercetin 3-glucoside, kaempferol 3-glucoside, myricetin, syringic acid, rutin, caftaric acid, and chlorogenic acid. Furthermore, it showed higher bioaccessibility of procyanidin A2, caffeic acid, caftaric acid, and chlorogenic acid. On the other hand, soursop pulp, located on the left side, was characterized by higher concentrations of catechin, epicatechin, epigallocatechin gallate, procyanidin B1, caffeic acid, and p-coumaric acid. The results suggest that umbu-cajá pulp is a matrix with higher concentration and types of phenolic compounds than soursop pulp.

The fermentation of soursop pulp resulted in increased procyanidin B1 content and decreased kaempferol 3-glucoside contents, regardless of the yeast strain used ($p < 0.05$). The fermentation of soursop pulp using *H. opuntiae* 125 resulted in products with lower gallic acid content ($p < 0.05$). On the contrary, the fermentation with *I. terricola* 129 increased the epicatechin and procyanidin B2 contents, and the fermentation with *H. opuntiae* 148 increased the *cis*-resveratrol and procyanidin A2 contents ($p < 0.05$). cis-resveratrol is known for its antioxidant, anti-cancer, anti-inflammatory, anti-aging, cardioprotective, and neuroprotective effects (Ibrahim, Yan, Xu, Yang, & Yan, 2021), and procyanidin A2 has an antidiabetic effect, especially in s type 2 diabetes, being able to increase glucose uptake in hepatocytes and myoblasts (Shay et al., 2015). The fermentation of umbu-cajá pulp increased procyanidin A2 and B1, caftaric acid and chlorogenic acid, regardless of the yeast strain used ($p < 0.05$). The fermentation of umbu-cajá pulp using *H. opuntiae* 125

Table 4
Bioaccessibility of phenolic compounds in unfermented soursop pulp (control) and fermented by yeasts *H. opuntiae* 125, *I. terricola* 129 or *H. opuntiae* 148.

Sample	Soursop pulp			
Phenolics (mg/L)	Treatments	Concentration before digestion	Concentration after digestion	Bioaccessibility (%)
Estilbenos				
cis-resveratrol	Control	0.27 ± 0.04^{ABb}	2.03 ± 0.12^{Aa}	771.62 ± 88.87^{A}
	H. opuntiae 125	0.18 ± 0.00^{Cb}	0.64 ± 0.08^{Ba}	350.05 ± 48.58^{B}
	I. terricola 129	0.19 ± 0.00^{BCb}	0.58 ± 0.05^{Ba}	313.52 ± 21.26^{B}
	H. opuntiae 148	0.33 ± 0.03^{Ab}	0.71 ± 0.02^{Ba}	218.12 ± 15.86^{B}
Flavonoids				
Catechin	Control	2.48 ± 0.20^{Aa}	0.94 ± 0.03^{Ab}	38.35 ± 4.41^{A}
	H. opuntiae 125	2.67 ± 0.10^{Aa}	1.25 ± 0.13^{Ab}	46.93 ± 5.62^{A}
	I. terricola 129	2.68 ± 0.07^{Aa}	1.33 ± 0.22^{Ab}	49.67 ± 7.93^{A}
	H. opuntiae 148	3.18 ± 0.48^{Aa}	1.30 ± 0.06^{Ab}	41.56 ± 4.52^{A}
Epicatechin	Control	1.22 ± 0.15^{Ba}	0.13 ± 0.01^{Bb}	10.44 ± 0.94^{B}
	H. opuntiae 125	0.95 ± 0.08^{Ba}	0.23 ± 0.03^{Bb}	24.51 ± 4.48^{A}
	I. terricola 129	1.82 ± 0.17^{Aa}	0.58 ± 0.12^{Ab}	31.70 ± 5.49^{A}
	H. opuntiae 148	1.18 ± 0.16^{Ba}	0.31 ± 0.04^{Bb}	25.76 ± 0.20^{A}
Epicatechin gallate	Control	<LOD	0.20 ± 0.02^{Aa}	ND
	H. opuntiae 125	<LOD	<LOD	ND
	I. terricola 129	<LOD	<LOD	ND
	H. opuntiae 148	<LOD	<LOD	ND
Epigallocatechin gallate	Control	1.10 ± 0.05^{Aa}	<LOD	ND
	H. opuntiae 125	1.16 ± 0.02^{Aa}	0.32 ± 0.05^{Ab}	27.88 ± 4.64^{A}
	I. terricola 129	1.07 ± 0.04^{Aa}	0.34 ± 0.04^{Ab}	31.51 ± 4.35^{A}
	H. opuntiae 148	1.45 ± 0.25^{Aa}	0.34 ± 0.04^{Ab}	23.73 ± 2.12^{A}
Procyanidin A2	Control	2.23 ± 0.22^{Ba}	1.42 ± 0.04^{Ab}	64.32 ± 7.66^{A}
	H. opuntiae 125	<LOD	0.59 ± 0.00^{Ca}	ND
	I. terricola 129	<LOD	1.05 ± 0.14^{Ba}	ND
	H. opuntiae 148	3.18 ± 0.14^{Aa}	<LOD	ND
Procyanidin B1	Control	0.82 ± 0.14^{Ca}	<LOD	ND
	H. opuntiae 125	1.06 ± 0.02^{ABa}	0.32 ± 0.03^{Ab}	30.22 ± 2.52^{A}
	I. terricola 129	1.33 ± 0.03^{Aa}	0.38 ± 0.07^{Ab}	28.84 ± 4.87^{A}
	H. opuntiae 148	1.25 ± 0.17^{Aa}	0.33 ± 0.05^{Ab}	27.16 ± 5.98^{A}
Procyanidin B2	Control	3.65 ± 0.21^{Bb}	21.63 ± 2.08^{ABa}	591.23 ± 26.89^{B}
	H. opuntiae 125	2.39 ± 0.03^{Db}	26.89 ± 3.60^{Aa}	1,126.67 ± 162.90^{A}
	I. terricola 129	5.72 ± 0.21^{Ab}	19.31 ± 1.59^{Ba}	337.92 ± 26.61^{B}
	H. opuntiae 148	2.93 ± 0.07^{Cb}	27.92 ± 1.44^{Aa}	953.84 ± 45.18^{A}
Rutin	Control	<LOD	0.02 ± 0.00^{Aa}	ND
	H. opuntiae 125	<LOD	<LOD	ND
	I. terricola 129	<LOD	<LOD	ND
	H. opuntiae 148	<LOD	<LOD	ND
Kaempferol 3-glucoside	Control	0.02 ± 0.00^{Ab}	0.10 ± 0.00^{Aa}	489.95 ± 111.10^{A}
	H. opuntiae 125	<LOD	<LOD	ND
	I. terricola 129	<LOD	<LOD	ND
	H. opuntiae 148	<LOD	<LOD	ND
Quercetin 3-glucoside	Control	0.17 ± 0.03^{Ab}	4.28 ± 0.09^{Aa}	2,540.14 ± 414.31^{A}
	H. opuntiae 125	0.16 ± 0.00^{Aa}	0.15 ± 0.01^{Ba}	93.20 ± 8.09^{B}
	I. terricola 129	0.17 ± 0.00^{Aa}	0.13 ± 0.01^{Bb}	76.36 ± 3.66^{B}
	H. opuntiae 148	0.18 ± 0.01^{Aa}	0.13 ± 0.01^{Bb}	71.26 ± 4.49^{B}
Narigenin	Control	<LOD	0.32 ± 0.02^{Aa}	ND
	H. opuntiae 125	<LOD	<LOD	ND
	I. terricola 129	<LOD	0.36 ± 0.03^{Aa}	ND
	H. opuntiae 148	<LOD	<LOD	ND
Myricetin	Control	0.22 ± 0.02^{Aa}	0.26 ± 0.02^{Aa}	120.72 ± 21.02^{A}
	H. opuntiae 125	0.24 ± 0.02^{Aa}	0.11 ± 0.02^{Bb}	44.22 ± 4.45^{B}
	I. terricola 129	0.22 ± 0.01^{Aa}	0.13 ± 0.01^{Bb}	59.70 ± 8.24^{B}
	H. opuntiae 148	0.24 ± 0.01^{Aa}	<LOD	ND
Phenolic acids				
Caftaric acid	Control	<LOD	0.81 ± 0.01^{Aa}	ND
	H. opuntiae 125	<LOD	<LOD	ND
	I. terricola 129	<LOD	<LOD	ND
	H. opuntiae 148	<LOD	<LOD	ND
Caffeic acid	Control	0.72 ± 0.12^{Aa}	<LOD	ND
	H. opuntiae 125	0.71 ± 0.03^{Aa}	0.33 ± 0.05^{Ab}	47.09 ± 8.89^{A}
	I. terricola 129	0.86 ± 0.02^{Aa}	0.42 ± 0.06^{Ab}	48.99 ± 5.74^{A}
	H. opuntiae 148	0.91 ± 0.14^{Aa}	0.36 ± 0.03^{Ab}	40.28 ± 3.10^{A}
Chlorogenic acid	Control	<LOD	0.21 ± 0.01^{Aa}	ND
	H. opuntiae 125	<LOD	<LOD	ND
	I. terricola 129	<LOD	<LOD	ND
	H. opuntiae 148	<LOD	<LOD	ND
Gallic acid	Control	3.13 ± 0.13^{Ab}	15.37 ± 0.24^{Aa}	492.67 ± 19.78^{B}
	H. opuntiae 125	2.13 ± 0.25^{Bb}	14.68 ± 0.57^{Aa}	703.46 ± 115.28^{A}
	I. terricola 129	2.83 ± 0.02^{Ab}	11.84 ± 0.97^{Ba}	418.72 ± 33.55^{B}

(continued on next page)

Table 4 (*continued*)

Sample	Soursop pulp			
Phenolics (mg/L)	Treatments	Concentration before digestion	Concentration after digestion	Bioaccessibility (%)
	H. opuntiae 148	2.86 ± 0.33^{Ab}	16.74 ± 0.68^{Aa}	591.06 ± 41.14^{AB}
Syringic acid	Control	0.29 ± 0.01^{Aa}	0.20 ± 0.01^{Ab}	68.00 ± 3.24^{A}
	H. opuntiae 125	0.29 ± 0.01^{Aa}	0.07 ± 0.01^{Bb}	23.40 ± 4.10^{B}
	I. terricola 129	0.29 ± 0.01^{Aa}	0.05 ± 0.01^{Bb}	16.26 ± 3.36^{B}
	H. opuntiae 148	0.35 ± 0.06^{Aa}	0.07 ± 0.01^{Bb}	21.20 ± 1.48^{B}
ρ_Coumaric acid	Control	6.85 ± 0.59^{ABa}	0.26 ± 0.02^{Bb}	3.80 ± 0.49^{B}
	H. opuntiae 125	6.48 ± 0.20^{Ba}	5.61 ± 0.63^{Aa}	86.86 ± 11.32^{A}
	I. terricola 129	8.52 ± 0.20^{Aa}	5.81 ± 0.66^{Ab}	67.96 ± 6.09^{A}
	H. opuntiae 148	8.07 ± 1.01^{ABa}	6.62 ± 0.23^{Aa}	83.02 ± 8.30^{A}

Values are expressed as the mean ± standard deviation; n = 9. < LOD: below the detection limit; Control: non-fermented pulp; Fermented pulps: *H. opuntiae* 125, *I. terricola* 129 or *H. opuntiae* 148; ND: not detected; A-D: different capital letters in the same column for the same compound denote difference ($p < 0.05$) among treatments, based on the Tukey test. a-b: different lowercase letters superscript on the same row denotes difference ($p < 0.05$) between before digestion and intestinal phase for the same treatments, based on the *t*-test.

resulted in products with higher contents of *cis*-resveratrol, epicatechin, quercetin 3-glucoside, myricetin, caffeic acid, syringic acid, and p-coumaric acid, and lower concentrations of procyanidin B2, rutin, and gallic acid ($p < 0.05$). The fermentation with *I. terricola* 129 increased the contents of epicatechin gallate, procyanidin B2, epigallocatechin gallate, and caffeic acid and decreased catechin and quercetin contents 3-glucoside, and rutin ($p < 0.05$). Finally, the fermentation with *H. opuntiae* 148 increased the contents of quercetin 3-glucoside, epicatechin, myricetin, syringic acid, and p-coumaric acid, and decreased the contents of procyanidin B2 and gallic acid.

The results suggest a behavior of increase in the concentration of many phenolic compounds in the fruit pulps after fermentation with yeasts, and the effect depends on the pulp type and yeast strain. Microorganisms may break down the fruit cell wall and release, synthesize, convert, or depolymerize phenolic compounds (Assis et al., 2021). Yeasts may produce β-glucosidase, transforming bound phenolic compounds (glycosides) to free forms (aglycones), which is associated with increased bioactivity (Rai et al., 2019). Differences in the yeast degradation ability and metabolic pathways may explain differences in the phenolic compounds impacted by fermentation. The decrease in the concentration of some phenolic compounds may be related to their consumption by yeasts during fermentation (Cheng, Liu, Chen, Zhang, & Zhang, 2016).

The digestive process resulted in reduced content of most of the phenolic compounds, such as epicatechin, catechin, procyanidin B1, epigallocatechin gallate, caffeic acid, and syringic acid for soursop pulp and catechin, *cis*-resveratrol, epigallocatechin gallate, epicatechin, procyanidin A2 and B1, rutin, kaempferol 3-glucoside, quercetin 3-glucoside, myricetin, caftaric acid, chlorogenic acid, and syringic acid for umbu-cajá pulps ($p < 0.05$). On the other hand, increased concentrations of procyanidin B2, *cis*-resveratrol, and gallic acid were observed in soursop pulp, and increased concentrations of procyanidin B2, caffeic acid, gallic acid, and p-coumaric acid in umbu-cajá pulps ($p < 0.05$). The high phenolic compound concentration in the intestinal fraction may occur due to enzymes in the gastrointestinal tract, such as pancreatin, which can release phenolic compounds in the food matrix (Bouayed, Hoffmann, & Bohn, 2011). On the other hand, decreases may be related to the exposure of phenolic compounds to the adverse conditions of the simulated gastrointestinal tract, decreasing their stability (Assis et al., 2021).

3.6. *Bioaccessibility of phenolic compounds in fermented fruit pulp and viability of selected strains under gastrointestinal tract simulated conditions*

The bioaccessibility of phenolic compounds identified in fermented and unfermented soursop and umbu-cajá pulps are presented in Tables 4 and 5. The fermentation of soursop pulps resulted in increases in the bioaccessibility of epicatechin, procyanidin B1, epigallocatechin gallate, caffeic acid, and p-coumaric acid, regardless of the yeast used in the fermentation ($p < 0.05$). Epicatechin is a compound that can neutralize reactive oxygen species, modulating cell signaling, including pathways involved in cell proliferation. Its action has been alleged to be promising in improving results in patients undergoing chemotherapy and radiotherapy (Shay et al., 2015). However, lower concentrations were observed for *cis*-resveratrol, procyanidin A2, quercetin 3-glucoside, kaempferol 3-glucoside, syringic acid, and myricetin in the fermented pulps ($p < 0.05$). Although the concentrations of quercetin 3-glucoside, kaempferol 3-glucoside, and myricetin were low compared to the other components, there was an increase in their bioaccessibility, indicating that there was the release of sugars through hydrolysis. The hydrolysis of these glycosylated components and of other plant components such as cellulose present in cell walls, probably due to the action of β-glycosidases enzymes, releases sugars and may explain the lack of changes in sugar levels in some formulations. The β-glycosidases produced by several non-*Saccharomyces* yeasts tolerate alcohol and are more active than those produced by *Saccharomyces* strains (Xu et al., 2022).

The fermentation of soursop pulp using *H. opuntiae* 125 increased the bioaccessibility of procyanidin B2 and gallic acid, while the fermentation using *H. opuntiae* 148 increased the procyanidin B2 bioaccessibility ($p < 0.05$). Procyanidin B2 is a compound recognized for its antioxidant effect and benefits concerning the prevention of cognitive impairment and the impact on the intestinal microbiota (Xiao et al., 2018).

The fermentation of umbu-cajá pulps decreased the bioaccessibility of procyanidin A2, caftaric acid, chlorogenic acid, and syringic acid, regardless of the yeast used in the fermentation ($p < 0.05$). The fermentation of umbu-cajá pulp using *H. opuntiae* 125 increased the bioaccessibility of procyanidin B2, gallic acid, and p-coumaric acid and decreased the bioaccessibility of quercetin 3-glucoside ($p < 0.05$). The fermentation using *I. terricola* 129 increased the bioaccessibility of epicatechin, catechin, epigallocatechin gallate, and epicatechin gallate ($p < 0.05$). Finally, the fermentation using *H. opuntiae* 148 increased the bioaccessibility of *cis*-resveratrol, procyanidin B2, gallic acid, and p-coumaric acid ($p < 0.05$).

Fig. 1 shows the viability of the strains during and at the end of the *in vitro* digestion. Before the simulation, all yeasts had counts above 7 log CFU/mL in all fermented pulps. In this way, both fruit pulps were suitable substrates for the growth of yeasts, originating fermented products with high counts (Assis et al., 2021). *H. opuntiae* 125 showed the highest initial counts in fermented pulps. In contrast, *I. terricola* 129 showed the lowest counts in soursop pulp and *H. opuntiae* 148 the lowest in umbu-cajá pulp ($p < 0.05$). During simulated gastric conditions, the yeast counts decreased in all formulations ($p < 0.05$), except for *I. terricola* 129 in soursop pulp ($p \geq 0.05$). Yeast viability was maintained above 6 log CFU/mL for *H. opuntiae* 125 in both pulps and *I. terricola* 129 in soursop pulp ($p < 0.05$), which is the recommended probiotic survival in the gastrointestinal tract (Costa et al., 2019). On the other formulations, yeast survival higher than 4.5 log CFU/mL was observed,

Table 5
Bioaccessibility of phenolic compounds in unfermented umbu-cajá pulp (control) and fermented by yeasts *H. opuntiae* 125, *I. terricola* 129 or *H. opuntiae* 148.

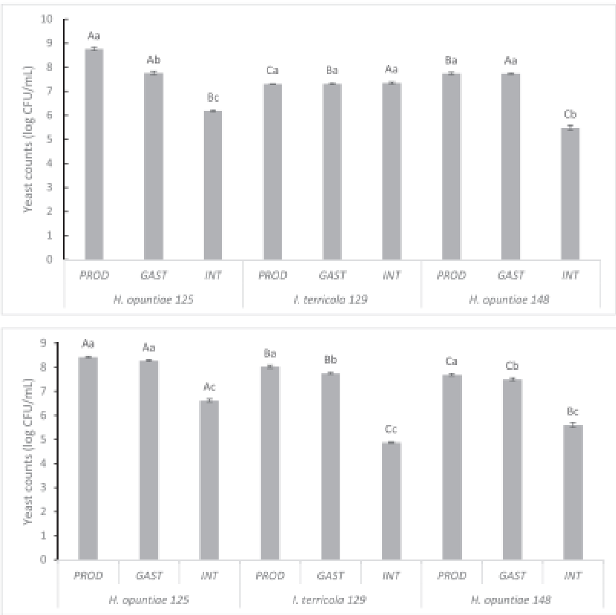
Sample	Umbu-cajá pulp			
Phenolics (mg/L)	Treatments	Concentration before digestion	Concentration after digestion	Bioaccessibility (%)
Estilbenos				
cis-resveratrol	Control	4.75 ± 0.35^{Ba}	2.24 ± 0.05^{Ab}	47.51 ± 4.76^{B}
	H. opuntiae 125	6.53 ± 0.53^{Aa}	1.64 ± 0.23^{BCb}	24.99 ± 2.52^{B}
	I. terricola 129	4.26 ± 0.76^{Ba}	1.38 ± 0.09^{Cb}	33.24 ± 3.86^{B}
	H. opuntiae 148	0.24 ± 0.01^{Cb}	1.91 ± 0.17^{ABa}	799.12 ± 84.49^{A}
Flavonoids				
Catechin	Control	1.99 ± 0.31^{Aa}	0.98 ± 0.01^{Ab}	50.21 ± 7.20^{B}
	H. opuntiae 125	1.78 ± 0.08^{Aa}	0.79 ± 0.11^{Ab}	44.07 ± 4.78^{B}
	I. terricola 129	1.21 ± 0.06^{Ba}	0.88 ± 0.07^{Ab}	72.83 ± 3.83^{A}
	H. opuntiae 148	1.89 ± 0.01^{Aa}	0.83 ± 0.03^{Ab}	43.72 ± 1.52^{B}
Epicatechin	Control	0.34 ± 0.03^{Ba}	0.13 ± 0.01^{Bb}	39.55 ± 3.89^{B}
	H. opuntiae 125	0.41 ± 0.02^{Aa}	0.14 ± 0.01^{Bb}	33.80 ± 3.83^{B}
	I. terricola 129	0.21 ± 0.01^{Ca}	0.26 ± 0.03^{Aa}	122.48 ± 10.94^{A}
	H. opuntiae 148	0.44 ± 0.02^{Aa}	0.10 ± 0.01^{Bb}	23.85 ± 2.61^{B}
Epicatechin gallate	Control	<LOD	0.21 ± 0.01^{Aa}	ND
	H. opuntiae 125	<LOD	<LOD	ND
	I. terricola 129	0.29 ± 0.01^{Aa}	$<0.20 \pm 0.02^{Ab}$	70.24 ± 10.05^{A}
	H. opuntiae 148	<LOD	<LOD	ND
Epigallocatechin gallate	Control	0.03 ± 0.00^{Ca}	<LOD	ND
	H. opuntiae 125	0.06 ± 0.01^{Ba}	<LOD	ND
	I. terricola 129	0.11 ± 0.00^{Ab}	0.40 ± 0.01^{Aa}	364.01 ± 18.20^{A}
	H. opuntiae 148	0.07 ± 0.00^{Ba}	<LOD	ND
Procyanidin A2	Control	5.30 ± 0.20^{Ba}	1.94 ± 0.04^{Ab}	36.55 ± 1.34^{A}
	H. opuntiae 125	6.53 ± 0.20^{Aa}	1.37 ± 0.13^{Bb}	20.95 ± 1.27^{B}
	I. terricola 129	6.42 ± 0.11^{Aa}	1.12 ± 0.07^{Bb}	17.41 ± 1.37^{B}
	H. opuntiae 148	6.72 ± 0.16^{Aa}	1.43 ± 0.28^{ABb}	21.42 ± 4.78^{B}
Procyanidin B1	Control	0.21 ± 0.01^{Ca}	0.11 ± 0.01^{Bb}	54.55 ± 8.57^{AB}
	H. opuntiae 125	0.42 ± 0.05^{ABa}	0.12 ± 0.01^{Bb}	30.46 ± 5.23^{B}
	I. terricola 129	0.37 ± 0.01^{Ba}	0.27 ± 0.02^{Ab}	74.03 ± 5.50^{A}
	H. opuntiae 148	0.49 ± 0.02^{Aa}	<LOD	ND
Procyanidin B2	Control	3.89 ± 0.20^{Bb}	16.70 ± 0.75^{Aa}	430.55 ± 37.46^{B}
	H. opuntiae 125	0.37 ± 0.03^{Cb}	17.59 ± 0.75^{Aa}	$4{,}800.89 \pm 140.51^{A}$
	I. terricola 129	6.36 ± 0.07^{Ab}	15.91 ± 0.89^{Aa}	249.93 ± 14.11^{B}
	H. opuntiae 148	0.42 ± 0.02^{Cb}	18.76 ± 1.78^{Aa}	$4{,}443.77 \pm 496.27^{A}$
Rutin	Control	0.03 ± 0.00^{Aa}	<LOD	ND
	H. opuntiae 125	0.03 ± 0.00^{Ba}	<LOD	ND
	I. terricola 129	<LOD	<LOD	ND
	H. opuntiae 148	0.03 ± 0.00^{Aa}	<LOD	ND
Kaempferol 3-glucoside	Control	0.24 ± 0.01^{ABa}	0.09 ± 0.01^{Ab}	36.24 ± 3.06^{A}
	H. opuntiae 125	0.27 ± 0.01^{Aa}	0.07 ± 0.01^{Ab}	24.41 ± 3.62^{A}
	I. terricola 129	0.21 ± 0.02^{Ba}	0.08 ± 0.01^{Ab}	37.12 ± 6.55^{A}
	H. opuntiae 148	0.28 ± 0.02^{Aa}	0.08 ± 0.00^{Ab}	29.66 ± 2.97^{A}
Quercetin 3-glucoside	Control	10.16 ± 0.08^{Ba}	4.31 ± 0.05^{Ab}	42.47 ± 0.82^{A}
	H. opuntiae 125	12.09 ± 0.47^{Aa}	3.22 ± 0.35^{Bb}	26.58 ± 1.94^{B}
	I. terricola 129	9.38 ± 1.10^{Ca}	3.39 ± 0.30^{Bb}	37.05 ± 7.81^{AB}
	H. opuntiae 148	12.55 ± 0.13^{Aa}	3.94 ± 0.30^{ABb}	31.41 ± 2.57^{AB}
Narigenin	Control	<LOD	<LOD	ND
	H. opuntiae 125	<LOD	<LOD	ND
	I. terricola 129	<LOD	<LOD	ND
	H. opuntiae 148	<LOD	0.34 ± 0.00^{Aa}	ND
Myricetin	Control	1.01 ± 0.00^{Ba}	0.21 ± 0.01^{Bb}	20.39 ± 0.75^{A}
	H. opuntiae 125	1.34 ± 0.05^{Aa}	0.29 ± 0.06^{ABb}	21.43 ± 3.67^{A}
	I. terricola 129	0.95 ± 0.13^{Ba}	0.23 ± 0.04^{ABb}	25.77 ± 8.03^{A}
	H. opuntiae 148	1.35 ± 0.03^{Aa}	0.38 ± 0.06^{Ab}	27.88 ± 4.73^{A}
Phenolic acids				
Caftaric acid	Control	1.76 ± 0.02^{Ba}	0.88 ± 0.02^{Ab}	49.72 ± 1.19^{A}
	H. opuntiae 125	2.20 ± 0.07^{Aa}	0.62 ± 0.07^{Bb}	28.18 ± 2.64^{B}
	I. terricola 129	2.15 ± 0.07^{Aa}	0.68 ± 0.01^{Bb}	31.51 ± 0.71^{B}
	H. opuntiae 148	2.30 ± 0.06^{Aa}	0.71 ± 0.05^{Bb}	30.89 ± 2.80^{B}
Caffeic acid	Control	0.04 ± 0.01^{Bb}	0.18 ± 0.01^{Ba}	463.71 ± 57.57^{A}
	H. opuntiae 125	0.08 ± 0.01^{Ab}	0.13 ± 0.02^{Ca}	175.57 ± 22.20^{C}
	I. terricola 129	0.08 ± 0.01^{Ab}	0.25 ± 0.01^{Aa}	$324.73 \pm 33{,}09^{B}$
	H. opuntiae 148	0.06 ± 0.00^{ABb}	0.14 ± 0.01^{BCa}	263.26 ± 25.49^{BC}
Chlorogenic acid	Control	0.56 ± 0.03^{Ba}	0.22 ± 0.01^{Ab}	39.48 ± 3.48^{A}
	H. opuntiae 125	0.82 ± 0.04^{Aa}	0.15 ± 0.02^{Bb}	18.68 ± 1.79^{B}
	I. terricola 129	0.71 ± 0.07^{Aa}	0.16 ± 0.01^{Bb}	23.04 ± 0.80^{B}
	H. opuntiae 148	0.84 ± 0.02^{Aa}	0.19 ± 0.02^{ABb}	22.34 ± 2.53^{B}
Gallic acid	Control	2.89 ± 0.12^{Ab}	10.63 ± 0.14^{Ba}	368.29 ± 12.53^{B}
	H. opuntiae 125	2.28 ± 0.07^{BCb}	13.62 ± 0.70^{Aa}	598.67 ± 31.55^{A}
	I. terricola 129	2.53 ± 0.17^{ABb}	10.72 ± 0.63^{Ba}	424.82 ± 4.48^{B}

(continued on next page)

Table 5 (*continued*)

Sample	Umbu-cajá pulp			
Phenolics (mg/L)	Treatments	Concentration before digestion	Concentration after digestion	Bioaccessibility (%)
	H. opuntiae 148	2.08 ± 0.09[Cb]	12.02 ± 1.20[ABa]	577.29 ± 32.02[A]
Syringic acid	Control	0.55 ± 0.03[Ca]	0.17 ± 0.01[Ab]	31.37 ± 0.60[A]
	H. opuntiae 125	0.85 ± 0.05[Aa]	0.18 ± 0.03[Ab]	20.51 ± 2.93[B]
	I. terricola 129	0.58 ± 0.04[Ca]	0.11 ± 0.01[Bb]	18.45 ± 2.10[B]
	H. opuntiae 148	0.71 ± 0.04[Ba]	0.15 ± 0.01[ABb]	21.58 ± 0.80[B]
ρ_Coumaric acid	Control	<LOD	0.31 ± 0.02[Aa]	ND
	H. opuntiae 125	0.04 ± 0.00[Ab]	0.18 ± 0.03[Ba]	419.02 ± 48.62[A]
	I. terricola 129	<LOD	0.21 ± 0.02[Ba]	ND
	H. opuntiae 148	0.04 ± 0.00[Bb]	0.20 ± 0.03[Ba]	563.65 ± 83.08[A]

Values are expressed as the mean ± standard deviation; n = 9. < LOD: below the detection limit; Control: non-fermented pulp; Fermented pulps: *H. opuntiae* 125, *I. terricola* 129 or *H. opuntiae* 148; ND: not detected; A-D: different capital letters in the same column for the same compound denote difference ($p < 0.05$) among treatments, based on the Tukey test. a-b: different lowercase letters superscript on the same row denotes difference ($p < 0.05$) between before digestion and intestinal phase for the same treatments, based on the *t*-test.



(A)

(B)

Fig 1. Viable cell counts of *H. opuntiae* 125, *I. terricola* 129 or *H. opuntiae* 148 before simulated digestion (a), gastric phase (b) and intestinal phase (c). (A) soursop pulp, (B) umbu-cajá pulp.

demonstrating that yeast had a limited gastrointestinal resistance (Costa et al., 2019). The results indicate that the soursop pulp composition (higher sugar content and lower acidity) protected the cultures during the *in vitro* digestion, resulting in more remarkable survival of the microorganisms. Further studies focusing on the probiotic potential of these strains should clarify their possible effects on gut microbiota.

Better visualization of the data may be observed in a PCA map. For soursop, D1 explained 52.34 % of the variability contained in the original variables, and principal D2 explained 26.45 %, totaling 78.79 % of the total variability (Figure S3B). The fermented pulps, located on the right side, were characterized by the higher concentration of procyanidin B1 and bioaccessibility of catechin, epicatechin, procyanidin B1, epigallocatechin gallate, p-coumaric, which was associated with the presence of yeasts in the product. The control product, located at the left side of the axis, was characterized by the highest kaempferol 3-glucoside and bioaccessibility of *cis*-resveratrol, quercetin 3-glucoside, kaempferol 3-glucoside, myricetin, and syringic acid. The soursop pulp fermented with *H. opuntiae* 148, located above the D2 axis, showed higher concentrations of *cis*-resveratrol, epigallocatechin gallate, catechin, procyanidin A2, quercetin 3-glucoside, and syringic acid. For soursop pulp, higher biotransformation of phenolic compounds was observed for *H. opuntiae* 148, isolated from soursop juice. These results corroborate the higher metabolic activity considering organic acids, ethanol production, and volatile organic compounds formation.

For umbu-cajá pulp, D1 explained 47.36 % of the variability contained in the original variables, and D2 explained 43.96 %, totaling 91.32 % of the total variability (Figure S3C). The fermented pulps, located on the right side, were characterized by a higher concentration of epigallocatechin gallate, procyanidin A2 and B1, caffeic acid, caftaric acid, and chlorogenic acid, and bioaccessibility of gallic acid, which was associated with the presence of yeasts in the product. The control product, located at the left side of the axis, was characterized by the highest concentration of gallic acid and bioaccessibility of procyanidin B1, quercetin 3-glucoside, caffeic acid, syringic acid, caftaric acid, and chlorogenic acid. The umbu-cajá pulp fermented with *I. terricola* 129, located below the D2 axis, showed higher concentrations of procyanidin B1 and bioaccessibility of epicatechin gallate, catechin, epicatechin, procyanidin A2, epigallocatechin gallate, epicatechin gallate, and kaempferol 3-glucoside. For umbu-cajá pulp, the higher biotransformation of phenolic compounds was observed for *I. terricola* 129, isolated from umbu-cajá juice. These results corroborate the higher metabolic activity considering organic acids, ethanol production, and volatile organic compounds formation. *H. opuntiae* 125 demonstrated great adaptability in both fruit pulps and the ability to biotransform phenolic compounds.

4. Conclusions

For the first time, soursop and umbu-cajá pulps from the Brazilian Caatinga were fermented using yeasts isolated from the natural fermentation of these same fruit juices. Fruit pulps were suitable substrates for yeasts, resulting in good metabolic activity, high viability, and increased bioaccessibility of phenolic compounds, in parallel to changes in the volatile contents. These results provide strong evidence that fermentation can be a potential technology to develop fruit products with more significant amounts of bioaccessible phenolics, opening opportunities for different applications in the food industry. We could observe that the use of selected autochthonous yeasts in the elaboration of fermented pulps enhanced the added value of the products.

CRediT authorship contribution statement

Elvira de Lourdes Chaves Macêdo: Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Tatiana Colombo Pimentel:** Visualization, Writing – original draft, Writing – review & editing. **Dirceu de Sousa Melo:** Conceptualization, Investigation, Visualization, Writing – review & editing. **Angélica Cristina de Souza:** Conceptualization, Investigation, Visualization, Writing – review & editing. **Janne Santos de Morais:** Visualization, Writing – review & editing. **Marcos dos Santos Lima:** Investigation, Visualization, Writing – review & editing. **Disney Ribeiro Dias:** Conceptualization, Supervision, Visualization, Writing – review & editing. **Rosane Freitas Schwan:** Conceptualization, Supervision, Visualization, Writing – review & editing. **Marciane Magnani:** Conceptualization, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134200.

References

Amorim, J. C., Piccoli, R. H., & Duarte, W. F. (2018). Probiotic potential of yeasts isolated from pineapple and their use in the elaboration of potentially functional fermented beverages. *Food Research International, 107*, 518–527. https://doi.org/10.1016/j.foodres.2018.02.054

AOAC (2016). Official Methods of Analysis. 20th ed. AOAC Intl., Gaithersburg, MD (20th ed.). Gaithersburg, MD: AOAC Intl.

Arroyo-López, F. N., Romero-Gil, V., Bautista-Gallego, J., Rodríguez-Gómez, F., Jiménez-Díaz, R., … Garrido-Fernández, A. (2012). Yeasts in table olive processing: Desirable or spoilage microorganisms? *International Journal of Food Microbiology, 160*(1), 42–49. https://doi.org/10.1016/j.ijfoodmicro.2012.08.003

Assis, B. B. T., Pimentel, T. C., Dantas, A. M., Lima, M. S., Borges, G. D. S. C., & Magnani, M. (2021). Biotransformation of the Brazilian Caatinga fruit-derived phenolics by *Lactobacillus acidophilus* La-5 and *Lacticaseibacillus casei* 01 impacts bioaccessibility and antioxidant activity. *Food Research International, 146*, Article 110435. https://doi.org/10.1016/j.foodres.2021.110435

Benucci, I., Cecchi, T., Lombardelli, C., Maresca, D., Mauriello, G., & Esti, M. (2021). Novel microencapsulated yeast for the primary fermentation of green beer: Kinetic behavior, volatiles and sensory profile. *Food Chemistry, 340*, Article 127900. https://doi.org/10.1016/j.foodchem.2020.127900

Bouayed, J., Hoffmann, L., & Bohn, T. (2011). Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastrointestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. *Food Chemistry, 128*(1), 14–21. https://doi.org/10.1016/j.foodchem.2011.02.052

Buljeta, I., Pichler, A., Ivić, I., Šimunović, J., & Kopjar, M. (2021). Encapsulation of fruit flavor compounds through interaction with polysaccharides. *Molecules, 26*(14), 4207. https://doi.org/10.3390/molecules26144207

Carvalho, B. F., Ávila, C. L. S., Bernardes, T. F., Pereira, M. N., Santos, C., & Schwan, R. F. (2017). Fermentation profile and identification of lactic acid bacteria and yeasts of rehydrated corn kernel silage. *Journal of Applied Microbiology, 122*(3), 589–600. https://doi.org/10.1111/jam.13371

Cheng, J. R., Liu, X. M., Chen, Z. Y., Zhang, Y. S., & Zhang, Y. H. (2016). Mulberry anthocyanin biotransformation by intestinal probiotics. *Food Chemistry, 213*, 721–727. https://doi.org/10.1016/j.foodchem.2016.07.032

Coelho, E. M., Padilha, C. V. S., Miskinis, G. A., Sá, A. G. B., Pereira, G. E., Azevêdo, L. C., & Lima, M. S. (2018). Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: Method validation and characterization of products from northeast Brazil. *Journal of Food Composition and Analysis, 66*, 160–167. https://doi.org/10.1016/j.jfca.2017.12.017

Cordente, A. G., Solomon, M., Schulkin, A., Francis, I. L., Barker, A., Borneman, A. R., & Curtin, C. D. (2018). Novel wine yeast with ARO4 and TYR1 mutations that overproduce 'floral'aroma compounds 2-phenylethanol and 2-phenylethyl acetate. *Applied Microbiology and Biotechnology, 102*(14), 5977–5988. https://doi.org/10.1007/s00253-018-9054-x

Costa, G. M., Paula, M. M., Barão, C. E., Klososki, S. J., Bonafé, E. G., Visentainer, J. V., … Pimentel, T. C. (2019). Yoghurt added with *Lactobacillus casei* and sweetened with natural sweeteners and/or prebiotics: Implications on quality parameters and probiotic survival. *International Dairy Journal, 97*, 139–148. https://doi.org/10.1016/j.idairyj.2019.05.007

Duarte, W. F., Dias, D. R., Oliveira, J. M., Teixeira, J. A., Silva, J. B. D. A., & Schwan, R. F. (2010). Characterization of different fruit wines made from cacao, cupuassu, gabiroba, jaboticaba and umbu. *LWT-Food Science and Technology, 43*(10), 1564–1572. https://doi.org/10.1016/j.lwt.2010.03.010

Farinazzo, F. S., Madeira, T. B., Fernandes, M. T. C., Mauro, C. S. I., Tomal, A. A. B., … Garcia, S. (2020). Organic and conventional apple fermented by *Saccharomyces boulardii* – The effect of the antioxidant quercetin on cellular oxidative stress. *British Food Journal, 123*(2), 520–534. https://doi.org/10.1108/BFJ-07-2019-0564

Fonseca, H. C., Melo, D. S., Ramos, C. L., Dias, D. R., & Schwan, R. F. (2021). Probiotic properties of lactobacilli and their ability to inhibit the adhesion of enteropathogenic bacteria to Caco-2 and HT-29 cells. *Probiotics and Antimicrobial Proteins, 13*(1), 102–112. https://doi.org/10.1007/s12602-020-09659-2

Freire, A. L., Ramos, C. L., Souza, P. N. C., Cardoso, M. G. B., & Schwan, R. F. (2017). Nondairy beverage produced by controlled fermentation with potential probiotic starter cultures of lactic acid bacteria and yeast. *International Journal of Food Microbiology, 248*, 39–46. https://doi.org/10.1016/j.ijfoodmicro.2017.02.011

Freitas, A. S., Magalhães, H. C. R., Alves Filho, E. G., & Garruti, D. D. S. (2021). Chemometric analysis of the volatile profile in peduncles of cashew clones and its correlation with sensory attributes. *Journal of Food Science, 86*, 5120–5136. https://doi.org/10.1111/1750-3841.15957

Giorello, F., Valera, M. J., Martin, V., Parada, A., Salzman, V., … Carrau, F. (2018). Genomic and transcriptomic basis of *Hanseniaspora vineae*'s impact on flavor diversity and wine quality. *Applied and Environmental Microbiology, 85*(1), e01959–e02018. https://doi.org/10.1128/AEM.01959-18

Giuffrida, D., Martínez, N., Arrieta-Garay, Y., Fariña, L., Boido, E., & Dellacassa, E. (2020). Valorisation of *Schinus molle* fruit as a source of volatile compounds in foods as flavours and fragrances. *Food Research International, 133*, Article 109103. https://doi.org/10.1016/j.foodres.2020.109103

Guergoletto, K. B., Bonifacio, K. L., Barbosa, D. S., Valezi, D. F., Salviato, A., … Garcia, S. (2020). Influence of spray-drying and room temperature storage on the anti-and prooxidant properties of fermented juçara pulp. *Food Technology and Biotechnology, 58*(1), 29. https://doi.org/10.17113/ftb.58.01.20.6335.

Ho, C. W., Chang, L. S., Muzni, S. K. S., Fazry, S., Lazim, A., … Lim, S. J. (2021). Functional beverage production using acetous fermentation of soursop: Physicochemical, toxicity and organoleptic properties. *Food Bioscience, 39*, Article 100812. https://doi.org/10.1016/j.fbio.2020.100812

Ibrahim, G. G., Yan, J., Xu, L., Yang, M., & Yan, Y. (2021). Resveratrol production in yeast hosts: Current status and perspectives. *Biomolecules, 11*(6), 830. https://doi.org/10.3390/biom11060830

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, B., … Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food–an international consensus. *Food & Function, 5*(6), 1113–1124. https://doi.org/10.1039/c3fo60702j

Morais, S. G. G., Borges, G. D. S. C., Lima, M. S., Martín-Belloso, O., & Magnani, M. (2019). Effects of probiotics on the content and bioaccessibility of phenolic compounds in red pitaya pulp. *Food Research International, 126*, Article 108681. https://doi.org/10.1016/j.foodres.2019.108681

Mota-Gutierrez, J., Barbosa-Pereira, L., Ferrocino, I., & Cocolin, L. (2019). Traceability of functional volatile compounds generated on inoculated cocoa fermentation and its potential health benefits. *Nutrients, 11*(4), 884. https://doi.org/10.3390/nu11040884

Nyanga, L. K., Nout, M. J. R., Smid, E. J., Boekhout, T., & Zwietering, M. H. (2013). Fermentation characteristics of yeasts isolated from traditionally fermented masau (*Ziziphus mauritiana*) fruits. *International Journal of Food Microbiology, 166*(3), 426–432. https://doi.org/10.1016/j.ijfoodmicro.2013.08.003

Okigbo, R. N., & Obire, O. (2009). Mycoflora and production of wine from fruits of soursop (*Annona Muricata* L.). *International Journal of Wine Research, 1*(1), 1–9. https://doi.org/10.2147/IJWR.S4667

Padilha, C. V. S., Miskinis, G. A., Souza, M. E. A. O., Pereira, G. E., Oliveira, D., … Lima, M. S. (2017). Rapid determination of flavonoids and phenolic acids in grape juices and wines by RP-HPLC/DAD: Method validation and characterization of commercial products of the new Brazilian varieties of grape. *Food Chemistry, 228*, 106–115. https://doi.org/10.1016/j.foodchem.2017.01.137

Palachum, W., Choorit, W., Manurakchinakorn, S., & Chisti, Y. (2020). Guava pulp fermentation and processing to a vitamin B12-enriched product. *Journal of Food Processing and Preservation, 44*(8), Article e14566. https://doi.org/10.1111/jfpp.14566

Pereira, F. R. A., Pereira, W. E., Pessoa, A. M. S., & Vasconcelos, E. S. A. G. (2021). Biometry in Umbu fruits from the semi-arid region of Paraiba. *Revista Brasileira de Fruticultura, 43*(6). https://doi.org/10.1590/0100-29452021808

Pimentel, T. C., Costa, W. K. A., Barão, C. E., Rosset, M., & Magnani, M. (2021). Vegan probiotic products: A modern tendency or the newest challenge in functional foods. *Food Research International, 140*, Article 110033. https://doi.org/10.1016/j.foodres.2020.110033

Pimentel, T. C., Oliveira, L. I. G., Macedo, E. D. L. C., Costa, G. N., Dias, D. R., … Magnani, M. (2021). Understanding the potential of fruits, flowers, and ethnic beverages as valuable sources of techno-functional and probiotics strains: Current scenario and main challenges. *Trends in Food Science & Technology, 114*, 25–59. https://doi.org/10.1016/j.tifs.2021.05.024

Qiu, X., Yu, L., Wang, W., Yan, R., Zhang, Z., Yang, H., … Zhu, B. (2021). Comparative evaluation of microbiota dynamics and metabolites correlation between spontaneous and inoculated fermentations of Nanfeng tangerine wine. *Frontiers in Microbiology, 12*. https://doi.org/10.3389/fmicb.2021.649978

Rai, A. K., Pandey, A., & Sahoo, D. (2019). Biotechnological potential of yeasts in functional food industry. *Trends in Food Science & Technology, 83*, 129–137. https://doi.org/10.1016/j.tifs.2018.11.016

Rêgo, E. S. B., Rosa, C. A., Freire, A. L., Machado, A. M. R., Gomes, F. C. O., … Padilha, F. F. (2020). Cashew wine and volatile compounds produced during fermentation by *non-Saccharomyces* and *Saccharomyces* yeast. *LWT-Food Science and Technology, 126*, Article 109291. https://doi.org/10.1016/j.lwt.2020.109291

Rodríguez-Roque, M. J., Rojas-Graü, M. A., Elez-Martínez, P., & Martín-Belloso, O. (2013). Changes in vitamin C, phenolic, and carotenoid profiles throughout in vitro gastrointestinal digestion of a blended fruit juice. *Journal of Agricultural and Food Chemistry, 61*(8), 1859–1867. https://doi.org/10.1021/jf3044204

Sabidi, S., Koh, S. P., Shukor, S. A., Sharifudin, S. A., & Sew, Y. S. (2020). Safety assessment of fermented jackfruit (*Artocarpus heterophyllus*) pulp and leaves in Sprague-Dawley rats. *Food Science & Nutrition, 8*(8), 4370–4378. https://doi.org/10.1002/fsn3.1734

Sengun, I. Y., Nielsen, D. S., Karapinar, M., & Jakobsen, M. (2009). Identification of lactic acid bacteria isolated from Tarhana, a traditional Turkish fermented food. *International Journal of Food Microbiology, 135*(2), 105–111. https://doi.org/10.1016/j.ijfoodmicro.2009.07.033

Shay, J., Elbaz, H. A., Lee, I., Zielske, S. P., Malek, M. H., & Hüttemann, M. (2015). Molecular mechanisms and therapeutic effects of (−)-epicatechin and other polyphenols in cancer, inflammation, diabetes, and neurodegeneration. *Oxidative Medicine and Cellular Longevity, 2015*, Article 181260. https://doi.org/10.1155/2015/181260

Singh, T. P., Kaur, G., Malik, R. K., Schillinger, U., Guigas, C., & Kapila, S. (2012). Characterization of intestinal *Lactobacillus reuteri* strains as potential probiotics. *Probiotics Antimicrob Proteins, 4*(1), 47–58. https://doi.org/10.1007/s12602-012-9090-2

Valera, M. J., Olivera, V., Boido, E., Dellacassa, E., & Carrau, F. (2021). Wine aroma characterization of the two main fermentation yeast species of the apiculate genus *Hanseniaspora*. *Fermentation, 7*(3), 162. https://doi.org/10.3390/fermentation7030162

Vilela, A. (2019). Use of nonconventional yeasts for modulating wine acidity. *Fermentation, 5*(1), 27. https://doi.org/10.3390/fermentation5010027

Xiao, Y., Dong, J., Yin, Z., Wu, Q., Zhou, Y., & Zhou, X. (2018). Procyanidin B2 protects against d-galactose-induced mimetic aging in mice: Metabolites and microbiome analysis. *Food and Chemical Toxicology, 119*, 141–149. https://doi.org/10.1016/j.fct.2018.05

Xu, A., Xiao, Y., He, Z., Liu, J., Wang, Y., … Zhu, D. (2022). Use of non-*Saccharomyces* yeast co-fermentation with *Saccharomyces cerevisiae* to improve the polyphenol and volatile aroma compound contents in nanfeng tangerine wines. *Journal of Fungi, 8*(2), 128. https://doi.org/10.3390/jof8020128

Yan, S., Xiangsong, C., & Xiang, X. (2019). Improvement of the aroma of lily rice wine by using aroma-producing yeast strain *Wickerhamomyces anomalus* HN006. *AMB Express, 9*(1), 1–14. https://doi.org/10.1186/s13568-019-0811-8

Supplementary table

Table S1. Viable cell counts of *H. opuntiae* 125, *I. terricola* 129 or *H. opuntiae* 148 in soursop and umbu-cajá fruit pulps before and after fermentation.

Sample	**Treatments (log CFU/g)**	**Time (h)**	
		0	**72**
Soursop	*H. opuntiae* 125	6.42 ± 0.09^{Ab}	8.76 ± 0.03^{Aa}
	I. terricola 129	6.31 ± 0.03^{Ab}	7.31 ± 0.05^{Ca}
	H. opuntiae 148	6.44 ± 0.05^{Ab}	7.74 ± 0.04^{Ba}
Umbu-cajá	*H. opuntiae* 125	6.40 ± 0.05^{Ab}	8.43 ± 0.05^{Aa}
	I. terricola 129	6.47 ± 0.03^{Ab}	8.08 ± 0.01^{Ba}
	H. opuntiae 148	6.32 ± 0.07^{Ab}	7.70 ± 0.04^{Ca}

Values are expressed as the mean ± standard deviation in Log CFU/g; Fermented pulps (*H. opuntiae* 125, *I. terricola* 129 or *H. opuntiae* 148); n=9. A-C: different capital letters in the same column denote differences ($p < 0.05$) among treatments for the same fruit pulp, according to the Tukey test; a-b: different lowercase letters on the same row denote differences ($p < 0.05$) in the same treatment for fermentation time, according to the t-test.

Supplementary figures

Fig. S1. Area percentage of volatiles group identified by GC-MS in fermented pulps and respective controls.

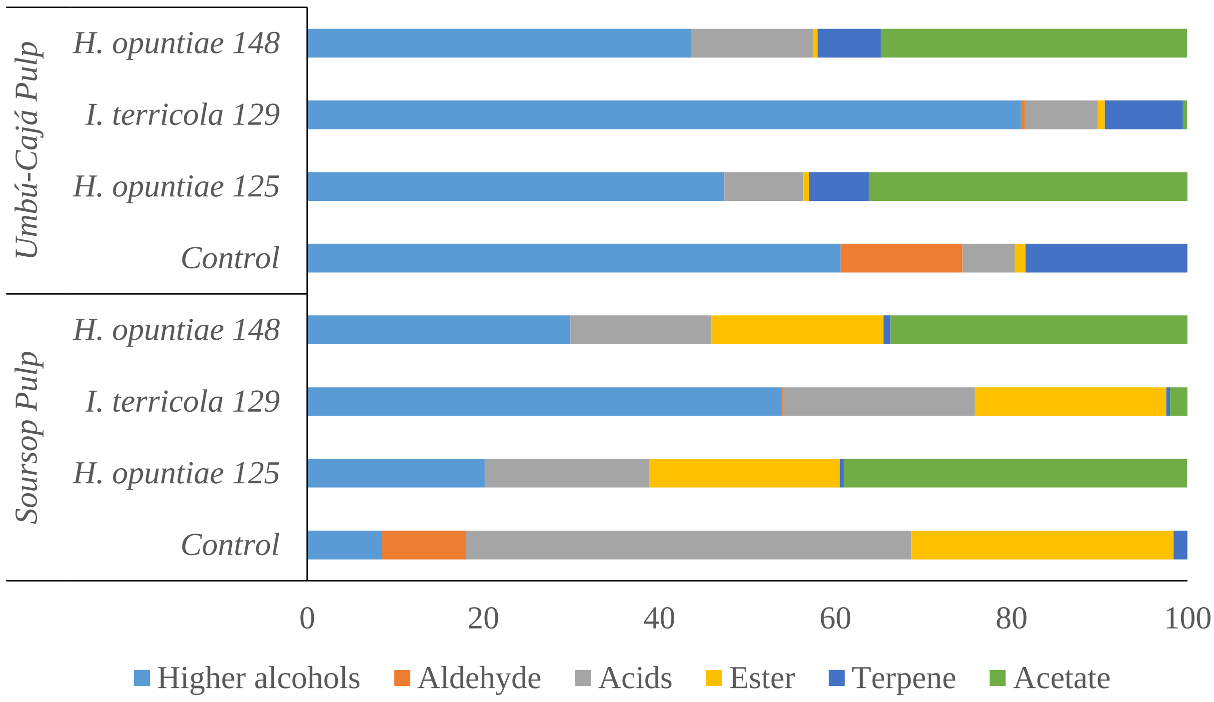
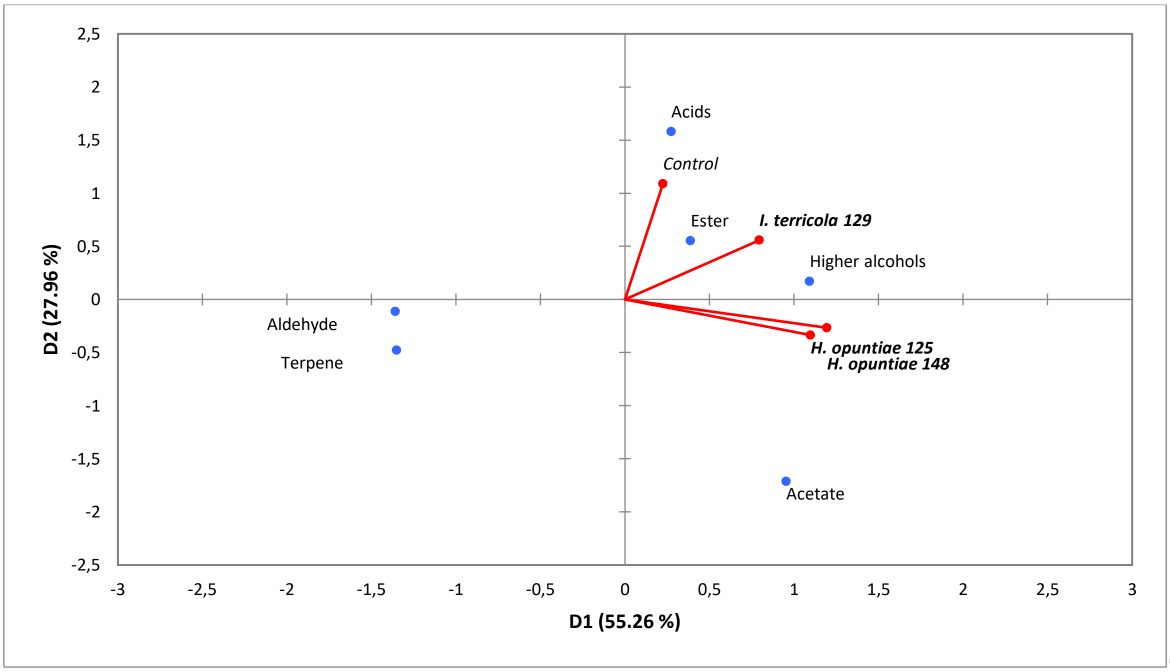


Fig. S2. Principal component analysis (PCA) of groups of volatile compounds in fruit pulps: volatile compound (circles) and formulations (vectors). (A) soursop pulp, (B) umbu-cajá pulp.



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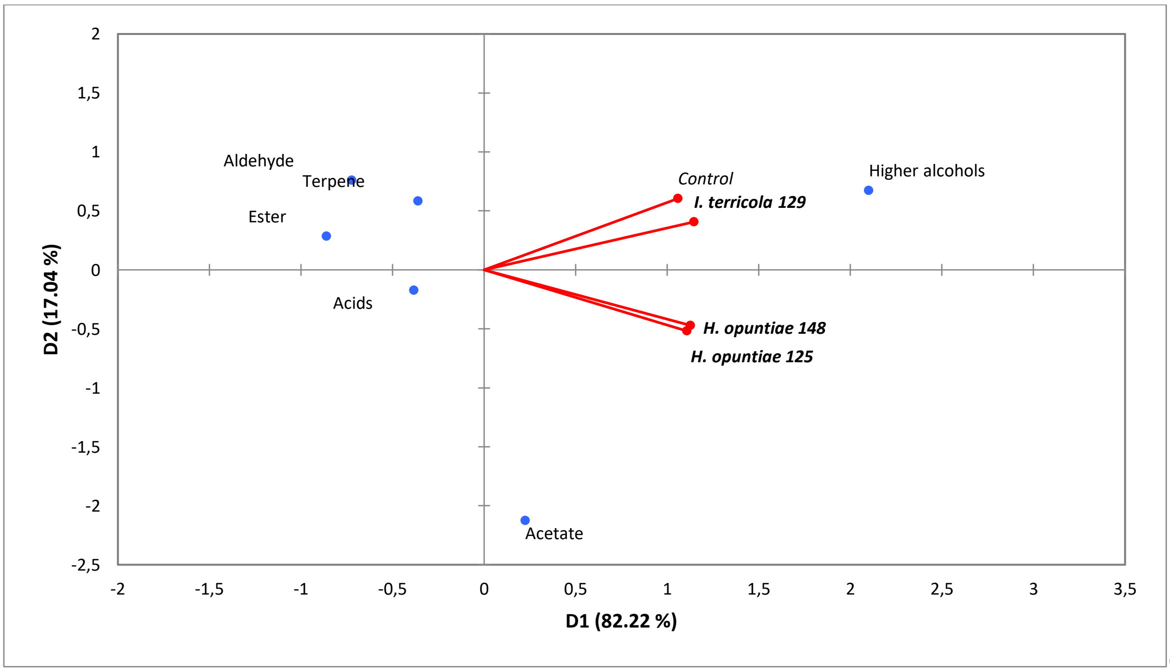
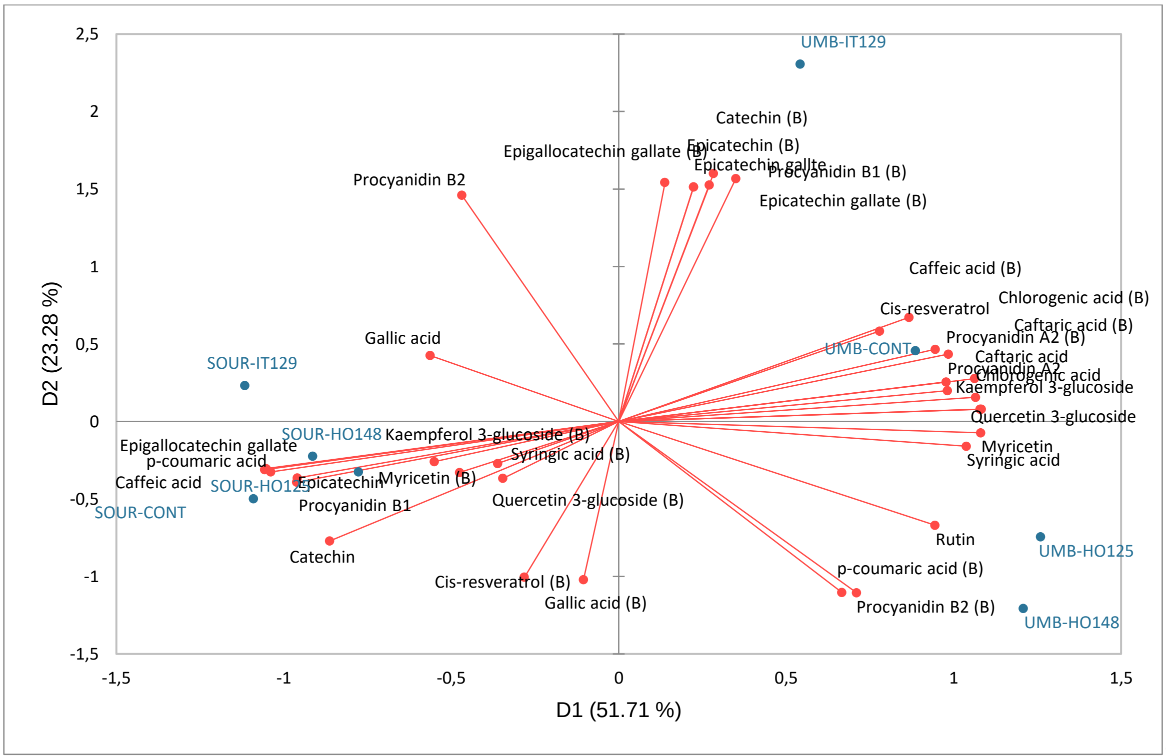
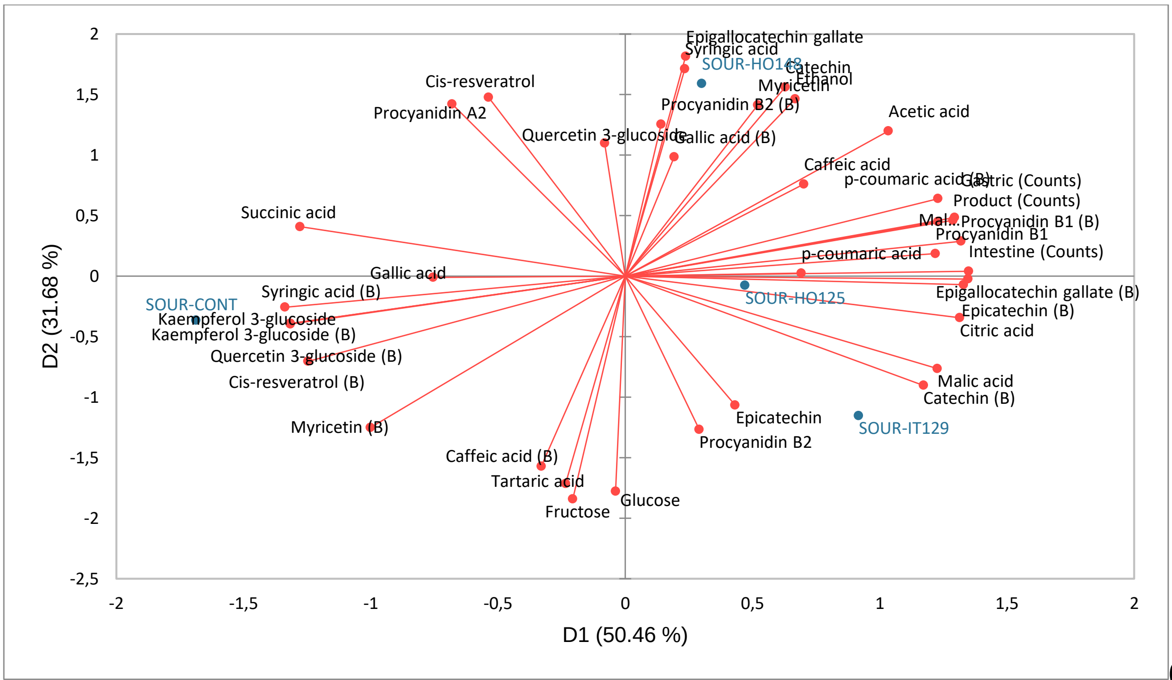


(B)

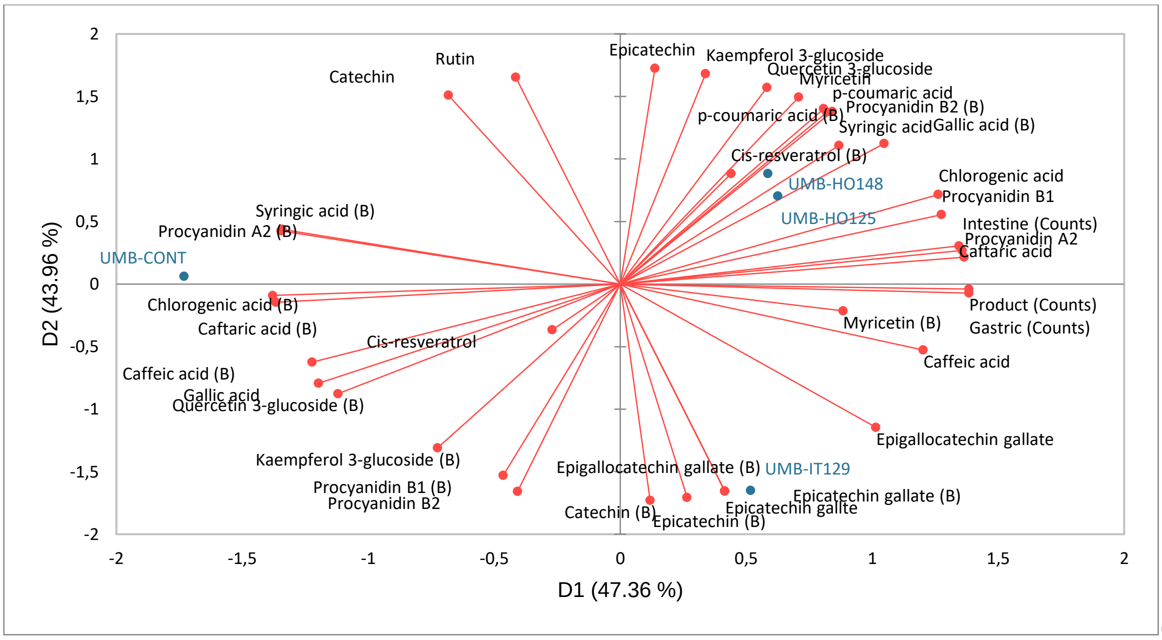
Fig. S3. Principal component analysis (PCA) of the phenolic compounds in fruit pulps after Varimax transformation (D): phenolics compounds and bioaccessible phenolic compounds (B) (vectors) and formulations (circles). (A) both fruits, (B) soursop pulp, (C) umbu-cajá pulp.



(A)



(B)



(C)

UMB-CONT (umbu pulp, no yeast), UMB-IT129 (umbu pulp, *I. terricola* 129), UMB-HO148 (umbu pulp, *H. opuntiae* 148), UMB-HO125 (umbu pulp, *H. opuntiae* 125), SOUR-CONT (soursop pulp, no yeast), SOUR-IT129 (soursop pulp, *I. terricola* 129), SOUR-HO148 (soursop pulp, *H. opuntiae* 148), SOUR-HO125 (sousrop pulp, *H. opuntiae* 125).

APÊNDICE B – ARTIGO II

"Effects of fermented soursop and umbu-cajá pulps on the colonic microbiota of middle-aged hypertensive adults"

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Effects of yeast fermented soursop and umbu-cajá pulps on the colonic microbiota of middle-aged hypertensive adults

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Annona muricata L.

ABSTRACT

The effects of umbu-cajá and soursop pulps fermented by the yeast *Issatchenkia terricola* 129 on the colonic microbiota of middle-aged hypertensive adults were evaluated *in vitro* fermentation during 48 h. The relative abundance of distinct bacterial groups, pH values, and contents of organic acids, sugars, and phenolic compounds were determined at 0, 24, and 48 h of fermentation. *Saccharomyces boulardii* CNCM I-745, a recognized probiotic yeast, was used for comparison purposes. During colonic fermentation, maltose, glucose, and fructose were metabolized, resulting in reduced pH values and decreased relative abundance of *Bacteroides* spp./*Prevotella* spp. Fermented soursop pulp with *I. terricola* 129 improved the colonic microbiota by increasing the relative abundance of *Bifidobacterium* spp. and *Lactobacillus* spp. and reducing the relative abundance of *Eubacterium rectale*/*Clostridium coccoides* and *Clostridium histolyticum*, resulting in the highest prebiotic index. At the same time, the consumption of rhamnose and gallic acid and higher contents of propionic and acetic acids and procyanidin B2 were observed. The effects on colonic microbiota were less pronounced for *S. boulardii* followed by umbu-cajá pulp with *I. terricola* 129. The results indicate that fermented soursop pulp with *I. terricola* 129 may modulate the colonic microbiota of middle-aged hypertensive adults.

1. Introduction

Exotic and tropical fruits have attractive sensory properties and important nutritional and therapeutic value, increasing their demand in domestic and international markets (Albuquerque et al., 2016; Maia et al., 2019; Sarkar et al., 2022). The Northeast region of Brazil, mainly the Caatinga Biome, has a diversity of underexplored fruits. Soursop (*Annona muricata* L.) is a fruit from the Annonaceae family and native to the Caribbean and American tropics. It is a source of carbohydrates, micronutrients, and several bioactive compounds, such as acetogenins, alkaloids, and phenolic compounds, which may show health beneficial effects (Agu & Okolie, 2017; Chang et al., 2018). Umbu-cajá belongs to the Anacardiaceae family and originates from the natural cross between umbu (*Spondias tuberosa*) and cajá (*Spondias mombin*). It is explored primarily in an extractive way and has attracted attention due to its contents of bioactive compounds, primarily polyphenols (Pereira et al., 2021). However, research on these fruits is lacking, especially in exploring them as raw materials for biotechnological applications and developing new products.

Fermented pulps may add value to these fruits, generating attractive beverages for a public demanding new functional fruit-based products with beneficial effects on health (Amorim et al., 2018). Fermentation may contribute to more desirable sensory properties and aromatic profiles and the starter culture may transform the phenolic compounds, improving their bioactivity (Sabidi et al., 2020). The utilization of yeasts as starter cultures may result in products of higher quality and health

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effects, mainly if they are endogenous to the fruits (Emer et al., 2021).

Saccharomyces boulardii CNCM I-745 was first isolated from fruits and used as a probiotic culture (Wang et al., 2022). The ingestion of this yeast showed numerous health effects, including regeneration and/or modulation of the intestinal microbiota after diarrhea (Moré & Swidsinski, 2015) and antibiotic administration (Kabbani et al., 2017), as well as improving the lipidemic profile in hypercholesterolemic hamsters (Briand et al., 2019). However, there is a need for the isolation and utilization of other yeasts with biotechnological and health effects (Farinazzo et al., 2020).

In our previous study, we isolated yeasts from naturally fermented soursop and umbu-cajá fruit juices (Issatchenkia terricola 129, Hanseniaspora opuntiae 125, and Hanseniaspora opuntiae *148*). We used them in the fermentation of their pulps. I. terricola 129 presented higher metabolic activity and production of volatiles capable of providing desirable aromas in the fermented product and low ethanol production. Furthermore, it increased the bioaccessibility and concentration of many phenolic compounds (Macêdo et al., 2022). Some of these phenolic compounds may modulate the gut microbiota composition (Calderón-Pérez et al., 2021) and show hypotensive effects due to their capacity to inhibit the angiotensin-converting enzyme I (ACE) (Alu'datt et al., 2017).

We hypothesize that soursoup and umbu-cajá pulps fermented with I. terricola 129 could modulate the intestinal microbiota of hypertensive individuals. To test this hypothesis, we used an *in vitro* colonic fermentation experiment to evaluate the effects of umbu-cajá and soursop pulps fermented with I. terricola 129 on specific intestinal microbial groups of middle-aged hypertensive adults. The experiments were carried out for 48 h and the microbial metabolic activity was evaluated by quantifying the contents of organic acids, sugars, and phenolic compounds. At the same time, the probiotic yeast strain *Saccharomyces boulardii* CNCM I-745 was used for comparison reasons.

2. Material and methods

2.1. Genetic sequencing of the strain selected for the study

Issatckenkia terricola 129 was the yeast used in the fermentation of umbu-cajá and soursop pulps. This strain was isolated from naturally fermented fruit juices and preliminarily identified via MALDI-TOF MS. The safety was assessed by hemolytic, gelatinase, and DNAse activities (Macêdo et al., 2022). In this study, QIAamp DNA Mini Kit was used to extract the DNA from the isolate following the "DNA Purification from Tissues" protocol (Qiagen, Hilden, Germany). The ITS region was amplified using ITS1 and ITS4 primers (Manter & Vivanco, 2007). The PCR products were sent for sequencing at the ACTgene company. The sequences were compared with the GenBank database using the Basic Local Alignment Tool (BLAST) program (National Center for Biotechnology Information, Bethesda, Maryland, USA) to identify the isolate.

2.2. Fermentation of fruit pulps

Fruit pulps (without preservatives) processed in the Pé de Fruta (umbu-cajá) (PB, Brazil) and Fazenda Mangai (soursop) industrial plants were obtained for the preparation of the fermented pulp. The yeast *I. terricola* 129 was inoculated into Yeast Extract Peptone Dextrose (YEPD) broth (5 mL, containing 1% yeast extract (w/v), 2% peptone (w/v), 1.5% agar (w/v)) (Acumedia, Lansing, Michigan, USA), and 2% D-glucose (w/v) (Dinâmica Química Contemporânea, Indaiatuba, SP, Brazil), at pH 3.5, and incubated at 30 ± 1 °C for 24 h. The culture was centrifuged (3500 g, 4 °C, 15 min), washed twice with sterile peptone water (0.1%, HiMedia, Mumbai, India), and resuspended in the same solution (5 mL, Freire et al., 2017). Next, 0.4 μL of the resuspended culture (6 log CFU/mL) was inoculated in 40 mL of soursop or umbu-cajá pulp previously pasteurized (80 °C for 5 min) and cooled using an ice bath. The inoculated tubes and their respective non-inoculated controls were incubated in BOD (Caltech Ind. Com. Ltda., Franca, SP, Brazil) for 48 h at 14 °C using a static fermentation. The physicochemical characteristics of the fruit pulps are presented in Table S1. The fermented products have yeast counts higher than 7 log CFU/mL (Macêdo et al., 2022). Finally, the fermented and non-fermented pulps were submitted to pH measurements at 0, 24, and 48 h of fermentation (Freire et al., 2017).

2.3. *In vitro* digestion of fermented fruit pulps

The *in vitro* digestion of fermented umbu-cajá and soursop pulps was carried out according to Minekus et al. (2014). The oral phase was simulated using simulated salivary fluid (SSF) (3.5 mL), which was homogenized with the fermented pulps (5 g), and the mixture was incubated under stirring (90 rpm) at 37 ± 1 °C for 2 min in an incubator of orbital agitation (Thoth Equipamentos, model 6420, Piracicaba, SP, Brazil). The SSF consisted of a solution of 25 μL of 0.3 M $CaCl_2$ and 975 μL of water containing 0.5 mL of 1500 U/mL α-amylase (Sigma-Aldrich, St. Louis, Missouri, USA). Then, simulated gastric fluid (SGF) (7.5 mL), consisting of a mixture of 5 μL of 0.3 M $CaCl_2$, 0.695 μL of water, and 1.6 mL of 2000 U/mL porcine pepsin (Sigma-Aldrich), was included to simulate gastric digestion. In this step, the pH was adjusted to 3.0 using HCl (1 M) and the mixture was incubated with shaking at 37 ± 1 °C for 2 h. At the end of this time, the gastric mixture (20 mL) was added with 2.5 mL of fresh bile (Sigma-Aldrich, 160 mM of fresh bile), simulated intestinal fluid (SIF) (11 mL) and 5.0 mL of 800 U/mL pancreatin solution (Sigma-Aldrich) in SIF solution, 40 μL of 0.3 M $CaCl_2$, and 1.31 mL of water, aiming to simulate the intestinal phase. In this step, the pH was adjusted to 7.0 with NaOH (1 M) and the mixture was incubated with shaking (90 rpm) at 37 ± 2 °C for 2 h. As needed, the pH values were controlled and adjusted at each stage of the *in vitro* digestion. The final contents of the digestions were dialyzed in regenerated cellulose dialysis tubes (14 KDa cut-off, Sigma-Aldrich) for 18 h against 0.01 M NaCl at 5 ± 0.5 °C (Guergoletto et al., 2016). Afterward, the NaCl solution was discarded and replaced by a new solution for another 2 h of dialysis. The dialyzed samples were used in the colonic fermentation experiment.

2.4. Human fecal inoculum of middle-aged hypertensive adults

The inoculum preparation was performed using fresh fecal samples donated by four hypertensive adult volunteers (two women and two men, aged between 45 and 59 years) after approval by the Institutional Committee for Ethics in Research with Human Beings (Federal University of Paraíba, João Pessoa, PB, Brazil, Opinion number 5.315.511). The inclusion criteria established were people without gastrointestinal or colon disease, who followed a regular omnivorous diet, did not use probiotic foods or concentrated prebiotics, and had not used antibiotics during the six months before the study. Furthermore, they were diagnosed with hypertension, a non-communicable chronic disease characterized by systolic and diastolic blood pressures equal to or greater than 140 mm Hg and 90 mm Hg, respectively, without using antihypertensive medication and assessed by a trained professional (Barroso et al., 2021). Hypertensive adult feces were selected because the fermented fruit pulps showed anti-hypertensive activity evaluated in preliminary *in vitro* tests (data not shown).

Volunteers received specific instructions for sample collection and a hygienic collection/storage kit containing gloves, a mask, and a sterile bottle. After collection, the flasks with the feces were placed in packaging with an anaerobic generator system (AnaeroGen, Oxoid, Basingstoke England) and sent to the laboratory. Then, the fecal samples were mixed in an equal proportion for the donors (1:1:1:1) and diluted (1:10) with autoclaved modified physiological saline solution (NaCl 8.5 g/L, cysteine-HCl 0.5 g/L, Sigma-Aldrich) to obtain a fecal suspension. The suspension was homogenized under agitation (200 rpm, 2 min) and filtered using triple layer gauze to remove larger particles and stored in sterile flasks under anaerobic conditions at 37 ± 1 °C (AnaeroGen)

(Massa et al., 2022).

2.5. Culture media and colonic fermentation

The basal nutrient medium for colonic fermentation was composed of 4.5 g/L of KCl, 4.5 g/L of NaCl, 10.69 g/L of $MgSO_4$, 0.5 g/L of $NaHCO_3$, 0.5 g/L KH_2PO_4, 0.8 g/L of L-cysteine, 0.4 g/L bile salt, 0.5 g/L K_2HPO_4, 0.005 g/L $FeSO_4$, 0.08 g/L $CaCl_2$, and 1 mL/L Tween 80. As an anaerobic indicator, 4 mL/L of resazurin solution (0.025%, v/v) was added to the medium, and distilled water was used to dilute the components. The pH of the basal medium was adjusted to 6.8 with the addition of HCl (1 M) (Andrade et al., 2020). The colonic fermentation was performed using 40% of basal nutrient medium (v/v), 40% of human fecal inoculum (v/v), and 20% of the soursop or umbu-cajá pulp (v/v) in a final volume of 40 mL. Colonic fermentation batches using *Saccharomyces boulardii* CNCM I-745 (7 log CFU/mL) and without adding fruit pulps (control) were performed for comparison purposes. The treatments mentioned were denoted as SOUR-IT129 (soursop pulp fermented with *I. terricola* 129), UMB-IT129 (umbu-cajá pulp fermented with *I. terricola* 129), SB (CNCM I-745) (*S. boulardii* CNCM I-745), and control. After preparation, the cultures were incubated under anaerobiosis (AnaeroGen) for 48 h at 37 ± 1 °C (Massa et al., 2022).

2.6. *In situ* hybridization fluorescence coupled with multiparametric flow cytometry

The abundance of selected microbial groups was analyzed using the fluorescence in situ hybridization (FISH) technique with selected oligonucleotide probes designed to target specific regions of the 16 S rRNA gene of these microorganisms combined with the multiparametric flow cytometry (MFC) to evaluate the ability of fermented pulps to modulate the human intestinal microbiota during *in vitro* colonic fermentation (Conterno et al., 2019; Menezes et al., 2021). The probes used in the experiment were Bif 164, specific for *Bifidobacterium*; Lab 158, specific for *Lactobacillus* spp./*Enterococcus* spp.; Bac 303, specific for *Bacteroides* spp./*Prevotella* spp.; Erec 482, specific for *Eubacterium rectale*/*Clostridium coccoides*; and Chis 150, specific for *Clostridium histolyticum*. The selection of the bacterial groups was based on their representativeness in the fecal microbiota and their association with positive or negative metabolic responses (Medeiros et al., 2021). Furthermore, these groups have been used as markers in previous studies that evaluate the impact of functional ingredients administration on the gut microbiota (Albuquerque et al., 2021; Massa et al., 2022; Medeiros et al., 2021; Menezes et al., 2021).

The probes were labeled with the fluorescent dye Cy3 (Sigma-Aldrich) (Menezes et al., 2021; Rodrigues et al., 2016) and the SYBR Green marker (Molecular Probes, Invitrogen, Carlsbad, California, USA) was used for double-stranded DNA labeling to enumerate the total bacterial population in each evaluated group (Conterno et al., 2019). Stabilization of the cell structure of the cultures was performed at 0, 24, and 48 h using 375 μL aliquots that were fixed at 4 °C (overnight) with 1125 μL of filtered paraformaldehyde solution (4%, w/v). The aliquots were centrifuged (10,000×*g*, 5 min, 4 °C), washed twice with 1 M PBS (10,000×*g*, 5 min, 4 °C), resuspended in 300 μL of PBS:99% ethanol (1:1 v/v), filtered with a membrane filter with a pore size of 0.45 μm (Whatman®) and stored at −20 °C.

In situ hybridization was performed by diluting 10 μL of the fixed cell suspension in 190 μL of 1X PBS (Gibco, Gaithersburg, Maryland, USA; pH 7.2), followed by centrifugation at 4000×*g* for 15 min at 4 °C and discarding of the supernatant. The cells were then resuspended in 200 μL of Tris-EDTA buffer (100 mM Tris-HCl and 50 mM EDTA; pH 8) and centrifuged under the same conditions as above. The samples were treated with lysozyme (1 mg/mL) diluted in 200 μL of Tris-EDTA and incubated for 10 min at room temperature (25 ± 0.5 °C) in a dark place to promote permeabilization of the cells that then received the Lab 158 and Bif 164 probes, and centrifuged (4000×*g*, 15 min, 4 °C). The samples resuspended in 45 μL of hybridization buffer composed of 0.9 M NaCl, 20 mM Tris-HCl (pH 7.5), and 0.1% sodium dodecyl sulfate (SDS) (w/v) and added 5 μL of fluorescent oligonucleotide probe (50 ng/μL) being kept in the dark and at the appropriate hybridization temperature for each probe (45 °C for Bac 303 or 50 °C for the other probes). After 4 h, the samples were centrifuged (4000×*g*, 15 min, 25 °C), resuspended in 200 μL of hybridization buffer without the addition of DSS, and kept in the dark for 30 min under the appropriate washing temperature for each probe (45 or 50 °C, as mentioned above) to remove unconnected probes. The samples were again centrifuged (4000×*g*, 15 min, 25 °C), resuspended in 200 μL of 1X PBS and 20 μL of SYBR Green (1:1000 stock solution diluted in dimethyl sulfoxide ≥99.9%, Sigma-Aldrich), incubated for 10 min in the dark at room temperature (25 ± 0.5 °C), centrifuged (4000×*g*, 15 min, 25 °C) and resuspended with 200 μL of 1X PBS. For each prepared sample, a blank sample was also prepared (without the oligonucleotide probe and SYBR Green). A sample labeled only with SYBR Green, using the same method as the hybridized samples, defined the threshold of the gates of the flow cytometer (BD Accuri C6, New York, New Jersey, USA), revealing the potential autofluorescence of the samples and excluding false positives.

The MFC principle consists of passing fluorescent signals from individual cells through a laser zone, being collected as logarithmic signals (pulse area measurements) by channels FL1 (SYBR Green) and FL2 (Lab 158, Bif 164, Bac 303, Chis 150, and Erec 482). The configuration was performed, so the samples were in low flow, with the threshold level for direct dispersion (FSC) set to 30,000 and 10,000 events collected for each sample. The BD Accuri™ C6 Plus Software (Becton Dickinson, Biosciences) was used to record the fluorescence emission cytograms. Results were expressed as the relative abundance (percentage) of bacterial group cells hybridized by each specific Cy3 probe (recorded as fluorescent events) compared to the total number of bacteria enumerated with the SYBR Green stain (Conterno et al., 2019).

2.7. Determination of the prebiotic index

The determination of the prebiotic index was performed after calculating the relative abundance (percentage) of each bacterial group measured based on the results of the FISH-FC (Albuquerque et al., 2021), for which the following equation was used:

Prebiotic index = %Lab + %Bif − %Bac − %Chis − %Erec *Eq. (1)*.

Where %Lab = (*Lactobacillus* spp./*Enterococcus* spp. relative abundance at 24 or 48 h) − (relative abundance of this bacterial group at time zero); %Bif = (relative abundance of *Bifidobacterium* spp. at 24 or 48 h) − (relative abundance of this bacterial group at time zero); %Bac = (relative abundance of *Bacteroides* spp./*Prevotella* spp. at 24 or 48 h) − (relative abundance of this bacterial group at time zero); %Chis = (relative abundance of *C. histolyticum* at 24 or 48 h) − (relative abundance of this bacterial group at time zero); and %Erec = (relative abundance of *E. rectale*/*C. coccoides* in 24 or 48 h) − (relative abundance of this bacterial group at time zero). Positive results for the prebiotic index indicate an overall beneficial alteration of the gut microbiota, while negative results indicate an undesirable overall alteration (Albuquerque et al., 2021; Gunathilakea et al., 2018).

2.8. Determination of pH and metabolites during colonic fermentation

Aliquots of batch cultures corresponding to treatments SOUR-IT129, UMB-IT129, SB (CNCM I-745), and control at 0, 24, and 48 h were centrifuged (3500 g, 15 min) (Centrifuge SL-701; Solab, São Paulo, SP, Brazil). Then, the supernatant was filtered (Millex-HA membrane, 0.45 μm, Millipore, Bedford, Massachusetts, USA). The determination of sugars and organic acids was performed by High-Performance Liquid Chromatography (HPLC). An Agilent chromatograph (model 1260 Infinity LC, Agilent Technologies, Santa Clara, California, USA) coupled to a diode array (DAD) and a refractive index detector (RID) was used. Ultrapure water was used for the 4 M dilution of H2SO4 as the mobile phase at a flow rate of 0.5 mL/min on an Agilent Hi-Plex H column (7.7 × 300 mm, 8 μm). Phenolic compounds were also determined by HPLC by using a Zorbax pre-column (C18; 12.6 × 4.6 mm, 5 μm) and a Zorbax column (Eclipse Plus RP-C18; 100 × 4.6 mm, 3.5 μm). Acidified water (pH 2; 0.1 mol/L phosphoric acid; phase A) and acidified methanol (99.5:05; phosphoric acid; phase B) were used as mobile phases. The column temperature was kept at 35 °C, while the flow rate was kept at 0.8 mL/min. The OpenLAB CDS ChemStation Edition™ (Agilent Technologies) software was used to process the data acquisitions. The peaks of sugars, organic acids, and phenolic compounds were identified by comparing their retention times to external standards (Table S2, Supplementary Table). External standard calibration curves following validated methods were used to quantify sugars and organic acids (Coelho et al., 2018) and phenolic acids (Padilha et al., 2017). The threshold tool was used to verify the peaks spectral purity to ensure identification accuracy compared to the external standards. All compounds showed calibration curves with $R^2 \geq 0.998$.

2.9. *Statistical analysis*

Analyzes were performed in triplicate on three independent occasions. Data were analyzed using analysis of variance (ANOVA) and Student's t-test or Tukey's test ($p < 0.05$). Principal component analysis (PCA) and Pearson's correlation were performed using the data of the abundance of bacterial groups, short-chain fatty acids (SCFA), and phenolic compounds to observe the influence of each compound on the percentages recorded for each group, or intragroup influences. The matrix consisted of 5 columns and 11 rows. Statistical analyzes were performed using XLSTAT® 2021.4.1 software (Addinsoft™, Paris, France).

3. Results and discussion

3.1. *DNA sequencing of selected strain*

The strain was identified as *Issatchenkia terricola* (*Issatchenkia terricola* 129) by DNA sequencing, showing 100% congruence with the preliminary identification with MALDI-TOF MS technique (Macêdo et al., 2022). The sequence was deposited in the National Center for Biotechnology Information (Access Number KP132531.1; Table S3) and the strain was integrated into the Microorganisms Culture Collection of Agricultural Microbiology at the Federal University of Lavras (MG, Brazil) under the code CCMA 2040.

3.2. *Changes in the relative abundance of bacterial populations during colonic fermentation*

The changes that occurred in the relative abundance of the measured bacterial groups during the colonic fermentation are shown in Fig. S1, and Table 1 and S4. *Bacteroides* spp./*Prevotella* spp. (Bac) had the highest relative abundance at time zero, followed by *C. histolyticum* (Chis), *Lactobacillus* spp./*Enterococcus* spp. (Lab), *E. rectale/C. coccoides* (Erec) and *Bifidobacterium* spp. (Bif). Bacteria belonging to the genus *Bacteroides* have been found in high abundance in gut microbiota of hypertensive individuals (Calderón-Perez et al., 2020, 2021). Both fruit pulps and SB (CNCM I-745) caused a higher abundance of Bac than control during fermentation ($p < 0.05$).

SOUR-IT129 significantly increased the relative abundances of *Lactobacillus* spp. (+199.93%) and *Bifobacterium* spp. (+708.46%) and decreased those of *C. histolyticum* (−60.48%), and *E. rectale* (−74.89%) during colonic fermentation for 48 h ($p < 0.05$). The fungal and bacterial populations of the gut are closely linked, influencing each other and, consequently, the host metabolism (Shuai et al., 2022). The modulation of lactobacilli and *Bifidobacterium* populations by yeasts can benefit the

Table 1
Hybridized bacterial groups in the treatments SOUR-IT129, UMB-IT129, SB (CNCM I-745), and Control at time zero, 24, and 48 h of *in vitro* colonic fermentation.

Bacterial group	Samples	Fermentation (time)		
		0 h*	24 h*	48 h*
Lactobacillus spp./ *Enterococcus* spp. (Lab 158)	SOUR-IT129	9.12 (0.37)^{Ac}	9.67 (0.40)^{Bb}	27.34 (0.54)^{Aa}
	UMB-IT129	9.12 (0.54)^{Ac}	15.69 (0.61)^{Ab}	16.67 (0.69)^{Ba}
	SB (CNCM I-745)	9.12 (0.31)^{Aa}	3.06 (0.15)^{Dc}	7.08 (0.31)^{Cb}
	Control	9.12 (0.03)^{Aa}	5.71 (0.26)^{Cb}	3.98 (0.16)^{Dc}
Bifidobacterium spp. (Lab 164)	SOUR-IT129	2.89 (0.10)^{Ac}	27.73 (0.65)^{Aa}	23.37 (0.56)^{Ab}
	UMB-IT129	2.89 (0.10)^{Ac}	8.55 (0.66)^{Bb}	11.22 (0.38)^{Ba}
	SB (CNCM I-745)	2.89 (0.11)^{Ac}	6.88 (0.29)^{Cb}	7.40 (0.30)^{Da}
	Control	2.89 (0.17)^{Ac}	3.66 (0.16)^{Db}	9.11 (0.39)^{Ca}
Bacteroides spp./*Prevotella* spp. (Bac 303)	SOUR-IT129	25.05 (0.43)^{Aa}	17.36 (0.73)^{Bc}	19.98 (0.49)^{Bb}
	UMB-IT129	25.05 (0.60)^{Ac}	50.71 (0.84)^{Aa}	32.41 (0.69)^{Ab}
	SB(CNCM I-745)	25.05 (0.71)^{Aa}	18.97 (0.53)^{Cb}	6.11 (0.27)^{Cc}
	Control	25.05 (0.50)^{Aa}	7.00 (0.20)^{Db}	3.26 (0.10)^{Dc}
C. histolyticum (Chis 150)	SOUR-IT129	12.55 (0.35)^{Aa}	6.91 (0.31)^{Db}	4.80 (0.13)^{Cc}
	UMB-IT129	12.55 (0.56)^{Ac}	51.68 (0.83)^{Aa}	34.62 (0.65)^{Ab}
	SB (CNCM I-745)	12.55 (0.36)^{Ab}	30.55 (0.83)^{Ba}	10.58 (0.52)^{Bc}
	Control	12.55 (0.52)^{Aa}	8.35 (0.64)^{Cb}	4.17 (0.17)^{Dc}
E. rectale/C. coccoides (Erec 482)>	SOUR-IT129	8.32 (0.21)^{Aa}	4.16 (0.17)^{Db}	2.09 (0.09)^{Dc}
	UMB-IT129	8.32 (0.17)^{Ac}	28.20 (0.53)^{Aa}	21.23 (0.81)^{Ab}
	SB (CNCM I-745)	8.32 (0.26)^{Aa}	7.51 (0.35)^{Bb}	5.99 (0.30)^{Bc}
	Control	8.32 (0.24)^{Aa}	6.10 (0.18)^{Cb}	2.72 (0.09)^{Cc}

Values are expressed as average of three independent experiments performed in triplicate. Standard deviation expressed between bracts. *Results are expressed as percentages. A-D: different superscript capital letters in the same fermentation time denote difference ($p < 0.05$) among treatments, based on Tukey's test. a-c: different superscript lowercase letters for the same treatment at different times denote difference ($p < 0.05$), based on Tukey's test. SOUR-IT129 (soursop pulp fermented with *I. terricola* 129), UMB-IT129 (umbu-cajá pulp fermented with *I. terricola* 129), SB(CNCM I-745) (*S. boulardii* CNCM I-745), and control.

organism. Studies have shown that *Lactobacillus* species can prevent dysbiosis and oxidative stress by treating the endothelial dysfunction that occurs in arterial hypertension (Palmu et al., 2021; Robles-Vera et al., 2018). Similar effects were reported concerning *Bifidobacterium* populations, which caused improvements in the intestinal microbiota composition and the reduction of oxidative stress, preventing aortic injuries and treating endothelial dysfunction mainly due to the increase in acetic acid production (Lu et al., 2022; Robles-Vera et al., 2020). On the contrary, the increased relative abundance of *E. rectale* was related to increased risks for cardio and cerebrovascular events in patients with refractory hypertension (Jiao et al., 2022). Finally, *C. histolyticum* is recognized as an enteric pathogen and its lower abundance in SOUR-IT129 treatment may be associated with the decreased pH values in the medium, being a limiting factor for this microorganism (Albuquerque et al., 2021).

In the SB (CNCM I-745) and control treatments, there was an increase in the relative abundance of *Bifidobacterium* spp. (155.8% and

220.19%, respectively, $p < 0.05$) and decrease in *C. histolyticum* and *E. rectale/C. coccoides* abundance during fermentation ($p < 0.05$), which are positive results. However, *Lactobacillus* spp. relative abundance was also reduced ($p < 0.05$). In the UMB-IT129 treatment, there was a significant increase in all measured bacterial populations ($p < 0.05$). Considering these results, the effects caused mainly by the SOUR-IT129 treatment can benefit the health of hypertensive individuals due to the increase in *Lactobacillus* spp. and *Bifidobacterium* spp. populations and the reduction of *C. histolyticum* and *E. rectale/C. coccoides* populations.

3.3. Determination of prebiotic index

The prebiotic indexes calculated for the evaluated treatments are shown in Table 2. The results recorded for the SOUR-IT129 treatment were positive and increased over time (from 42.89 ± 0.52 to 57.76 ± 0.94) ($p < 0.05$), demonstrating a desirable modulation of the colonic microbiota. This result may be associated with the highest impacts of SOUR-IT129 causing increases in the populations of beneficial microorganisms (*Lactobacillus* spp. and *Bifidobacterium* spp.), besides decreasing undesirable microorganisms (*C. histolyticum* and *E. rectale/C. coccoides*). Increases in the prebiotic index during fermentation were also observed for the other treatments, such as control (from 21.85 ± 0.17 to 38.86 ± 0.96), SB (CNCM I-745) (from -13.17 ± 0.43 to 25.70 ± 0.63), and UMB-IT129 (from -72.44 ± 0.27 to -26.46 ± 0.37). However, the prebiotic indexes of control and SB (CNCM I-745) were lower than the SOUR-IT129 ($p < 0.05$), primarily due to the decrease in *Lactobacillus* spp. abundance in the former treatments. Finally, the prebiotic index of UMB-IT129 was negative, which may be associated with its impacts on the modulation of all measured microbial groups, including undesirable microorganisms.

3.4. Evolution of pH and metabolism of sugars and organic acids during colonic fermentation

Results of the variation in pH values and contents of sugars and organic acids are shown in Table 3. During colonic fermentation, there was a decrease in pH values in all treatments and the lowest value was recorded in the SB treatment (CNCM I- 745) (5.48 ± 0.07) ($p < 0.05$), followed by the SOUR-IT129 treatment (5.93 ± 0.03). There was a decrease in glucose, fructose, maltose, citric acid, and tartaric acid contents at the end of 48 h of fermentation ($p < 0.05$). Rhamnose was only found in SOUR-IT129 treatment and it was transformed during fermentation ($p < 0.05$). Gut bacteria, such as *Enterococcus*, *Lactobacillus*, and *Bifidobacterium* spp. may metabolize complex carbohydrates and, mainly, sugars, resulting in organic acid production and decreased pH values (Medeiros et al., 2021).The highest prebiotic index of SOUR-IT129 treatment may be partially related to the presence of rhamnose in the medium, which was used by the beneficial microbiota

Table 2
Prebiotic indexes of the treatments SOUR-IT129, UMB-IT129, SB (CNCM I-745), and control at time 24 and 48 h of *in vitro* colonic fermentation.

Samples	Fermentation (time)	
	24 h	48 h
SOUR-IT129	42.89 ± 0.52^{Ab}	57.76 ± 0.94^{Aa}
UMB-IT129	-72.44 ± 0.27^{Db}	-26.46 ± 0.37^{Da}
SB(CNCM I-745)	-13.17 ± 0.43^{Cb}	25.70 ± 0.63^{Ca}
Control	21.85 ± 0.17^{Bb}	36.86 ± 0.96^{Ba}

Values are expressed as average ± standard deviation, n = 3. A-D: different superscript capital letters in the same fermentation time denote difference ($p < 0.05$) among treatments, based on Tukey's test. a-b: different superscript lowercase letters for the same treatment at different times denote difference ($p < 0.05$), based on Student's t-test. SOUR-IT129 (soursop pulp fermented with *L. terricola* 129), UMB-IT129 (umbu-cajá pulp fermented with *L. terricola* 129), SB (CNCM I-745) (*S. boulardii* CNCM I-745), and control.

Table 3
PH values, sugar, and organic acid contents in the treatments SOUR-IT129, UMB-IT129, SB (CNCM I-745), and Control at time zero, 24 and 48 h of *in vitro* colonic fermentation.

Parameters*	Samples	Fermentation (time)		
		0 h	24 h	48 h
pH	SOUR-IT129	7.65 ± 0.03^{Aa}	7.41 ± 0.06^{ABb}	5.93 ± 0.03^{Cc}
	UMB-IT129	7.65 ± 0.03^{Aa}	7.29 ± 0.05^{Bb}	6.23 ± 0.04^{Bc}
	SB (CNCM I-745)	7.65 ± 0.04^{Aa}	7.53 ± 0.04^{Aa}	5.48 ± 0.07^{Db}
	Control	7.65 ± 0.06^{Aa}	7.53 ± 0.05^{Aa}	6.99 ± 0.10^{Ab}
Maltose	SOUR-IT129	<LOD	<LOD	<LOD
	UMB-IT129	0.01 ± 0.00^{Ab}	0.12 ± 0.01^{Aa}	<LOD
	SB (CNCM I-745)	<LOD	0.07 ± 0.01^{Ba}	<LOD
	Control	<LOD	<LOD	0.05 ± 0.00^{Aa}
Glucose	SOUR-IT129	0.40 ± 0.02^{Aa}	0.05 ± 0.00^{Ab}	<LOD
	UMB-IT129	0.28 ± 0.03^{Ba}	<LOD	<LOD
	SB (CNCM I-745)	0.01 ± 0.00^{Ca}	<LOD	<LOD
	Control	<LOD	<LOD	<LOD
Fructose	SOUR-IT129	0.31 ± 0.03^{Aa}	<LOD	<LOD
	UMB-IT129	0.18 ± 0.01^{Ba}	<LOD	<LOD
	SB (CNCM I-745)	<LOD	<LOD	<LOD
	Control	<LOD	<LOD	<LOD
Rhamnose	SOUR-IT129	0.08 ± 0.01^{Aa}	<LOD	<LOD
	UMB-IT129	<LOD	<LOD	<LOD
	SB (CNCM I-745)	<LOD	<LOD	<LOD
	Control	<LOD	<LOD	<LOD
Citric	SOUR-IT129	0.03 ± 0.00^{Aa}	<LOD	<LOD
	UMB-IT129	0.01 ± 0.00^{Ba}	<LOD	<LOD
	SB (CNCM I-745)	<LOD	<LOD	<LOD
	Control	<LOD	<LOD	<LOD
Tartaric	SOUR-IT129	0.01 ± 0.00^{Aa}	<LOD	<LOD
	UMB-IT129	0.01 ± 0.00^{Aba}	<LOD	<LOD
	SB (CNCM I-745)	0.01 ± 0.00^{Ba}	<LOD	<LOD
	Control	<LOD	0.01 ± 0.00^{Aa}	<LOD
Succinic	SOUR-IT129	0.08 ± 0.00^{Ba}	0.05 ± 0.00^{Cb}	0.07 ± 0.00^{Ba}
	UMB-IT129	0.06 ± 0.01^{Ba}	0.03 ± 0.00^{Cb}	0.06 ± 0.00^{Ba}
	SB (CNCM I-745)	0.06 ± 0.00^{Bb}	0.09 ± 0.00^{Ba}	0.07 ± 0.00^{Bb}
	Control	0.13 ± 0.01^{Ab}	0.15 ± 0.01^{Ab}	0.24 ± 0.02^{Aa}
Formic	SOUR-IT129	0.05 ± 0.00^{Aa}	0.01 ± 0.00^{Bb}	<LOD
	UMB-IT129	0.03 ± 0.00^{Bb}	0.05 ± 0.00^{Aa}	0.02 ± 0.00^{Ac}
	SB (CNCM I-745)	0.02 ± 0.00^{Ca}	<LOD	<LOD
	Control	<LOD	<LOD	0.02 ± 0.00^{Aa}
Acetic	SOUR-IT129	0.27 ± 0.02^{Bb}	0.75 ± 0.03^{Aa}	0.71 ± 0.03^{Aa}
	UMB-IT129	0.22 ± 0.02^{Bb}	0.78 ± 0.03^{Aa}	0.79 ± 0.03^{Aa}
	SB (CNCM I-745)	0.21 ± 0.02^{Bb}	0.44 ± 0.02^{Ba}	0.50 ± 0.02^{Ba}
	Control	0.54 ± 0.03^{Aa}	0.49 ± 0.02^{Ba}	0.20 ± 0.02^{Cb}
Propionic	SOUR-IT129		1.22 ± 0.06^{Aa}	

(continued on next page)

Table 3 (*continued*)

Parameters*	Samples	Fermentation (time)		
		0 h	24 h	48 h
		0.90 ± 0.08^{Bb}		0.84 ± 0.04^{Ab}
	UMB-IT129	0.93 ± 0.07^{Bb}	1.17 ± 0.05^{Aa}	0.87 ± 0.03^{Ab}
	SB (CNCM I-745)	0.82 ± 0.04^{Ba}	0.29 ± 0.02^{Bb}	0.17 ± 0.01^{Cb}
	Control	0.17 ± 0.01^{Ac}	0.32 ± 0.02^{Bb}	0.66 ± 0.04^{Ba}

Values are expressed as average ± standard deviation, n = 3. *Results of sugars and organic acids are expressed as g/L. <LOD: lower than the detection limit. A-D: different superscript capital letters in the same fermentation time denote difference ($p < 0.05$) among treatments, based on Tukey's test. a-c: different superscript lowercase letters for the same treatment at different times denote difference ($p < 0.05$), based on Tukey's test. SOUR-IT129 (soursop pulp fermented with *L. terricola* 129), UMB-IT129 (umbu-cajá pulp fermented with *L. terricola* 129), SB (CNCM I-745) (*S. boulardii* CNCM I-745), and control.

during fermentation.

Control treatment showed a different behavior for short-chain fatty acids (SCFA) during colonic fermentation, with increases in succinic, formic, and propionic acids and decreases in acetic acid content ($p < 0.05$). On the other hand, fermented pulps and SB (CNCM I-745) showed stability of succinic acid ($p \geq 0.05$), decreases in formic acid, and increases in acetic acid ($p < 0.05$). At the end of fermentation, control treatment showed higher succinic acid content, while SOUR-IT129 and UMB-IT129 treatments presented higher propionic and acetic acid contents ($p < 0.05$). Increased succinic acid contents in the fecal samples have been linked to adverse effects, such as stimulating increased production of free radicals and interleukins, aggravating the inflammatory response (Liu et al., 2022). It has also been found elevated in fecal samples from overweight individuals (Wan et al., 2020) and associated with metabolism-related cardiovascular disorders (Serena et al., 2018). This SCFA is a metabolism of the microorganisms of the phylum *Bacteroidetes*, which include the genera *Bacteroides* spp. and *Prevotella* spp. and the succinate pathway is the primary means for propionate formation. In this pathway, deoxy sugars, such as fucose and rhamnose, are used (Sharon et al., 2014). The higher abundance of *Bacterioidetes* spp. in the fermented pulps may have resulted in a higher propionic acid formation, while succinic acid was maintained in control. Propionic acid may regulate intestinal barrier function and homeostasis (Massa et al., 2022). At the same time, acetic acid production may be related to enteropathogens inhibition and a higher abundance of *Lactobacillus* spp. and *Bifidobacterium* spp. (Medeiros et al., 2021). Acetic acid is a source of energy for the liver and peripheral tissues (Pravasi, 2014).These results demonstrate that fermented pulps and SB (CNCM I- 745) induced a more desired metabolic profile during colonic fermentation than control.

3.5. *Changes in the quantities of phenolic compounds during colonic fermentation*

Stilbenes, phenolic acids, and flavonoids were detected in SOUR-IT129 and/or UMB-IT129 treatments at time zero (Table 4). However, only three phenolic compounds were identified after 24 and 48 h of colonic fermentation. Phenolic compounds show poor absorption in the small intestine and may be metabolized by the gut microbiota, modulating the abundance of beneficial microorganisms (Massa et al., 2022). They may have been used as a substrate by *Lactobacillus* spp. and *Bifidobacterium* spp. in SOUR-IT129 and UMB-IT129 treatment (Medeiros et al., 2021), demonstrating the potential prebiotic effect of the phenolic compounds.

Catechin, procyanidin B2, and gallic acid decreased in SOUR-IT129 and UMB-IT129 treatment ($p < 0.05$) but were detected up to the end

Table 4
Phenolic compounds contents (mg/L) in the treatments SOUR-IT129 and UMB-IT129 at 24 and 48 h of *in vitro* colonic fermentation.

Parameters	Samples*	Fermentation (time)		
		0 h	24 h	48 h
Cis-resveratrol	SOUR-IT129	0.66 ± 0.04^{Ba}	<LOD	<LOD
	UMB-IT129	1.48 ± 0.09^{Aa}	<LOD	<LOD
Catechin	SOUR-IT129	1.40 ± 0.11^{Aa}	0.43 ± 0.02^{Ab}	0.42 ± 0.03^{Ab}
	UMB-IT129	0.97 ± 0.08^{Ba}	0.33 ± 0.02^{Bb}	0.37 ± 0.02^{Ab}
Epicatechin	SOUR-IT129	0.65 ± 0.06^{Aa}	<LOD	<LOD
	UMB-IT129	0.24 ± 0.02^{Ba}	<LOD	<LOD
Epigallocatechin gallate	SOUR-IT129	0.37 ± 0.06^{Aa}	<LOD	<LOD
	UMB-IT129	0.44 ± 0.03^{Aa}	<LOD	<LOD
Procyanidin A2	SOUR-IT129	0.99 ± 0.07^{Ba}	<LOD	<LOD
	UMB-IT129	1.23 ± 0.11^{Aa}	<LOD	<LOD
Procyanidin B1	SOUR-IT129	0.34 ± 0.02^{Aa}	<LOD	<LOD
	UMB-IT129	0.33 ± 0.03^{Aa}	<LOD	<LOD
Procyanidin B2	SOUR-IT129	19.70 ± 0.89^{Aa}	0.88 ± 0.05^{Ac}	1.01 ± 0.05^{Ab}
	UMB-IT129	16.14 ± 1.40^{Ba}	0.58 ± 0.04^{Bc}	0.74 ± 0.05^{Bb}
Kaempferol 3-glucoside	SOUR-IT129	<LOD	<LOD	<LOD
	UMB-IT129	0.10 ± 0.01^{Aa}	<LOD	<LOD
Quercetin 3-glucoside	SOUR-IT129	0.14 ± 0.01^{Ba}	<LOD	<LOD
	UMB-IT129	3.79 ± 0.31^{Aa}	<LOD	<LOD
Narigenin	SOUR-IT129	0.42 ± 0.03^{Aa}	<LOD	<LOD
	UMB-IT129	<LOD	<LOD	<LOD
Myricetin	SOUR-IT129	0.16 ± 0.01^{Ba}	<LOD	<LOD
	UMB-IT129	0.19 ± 0.02^{Aa}	<LOD	<LOD
Caftaric acid	SOUR-IT129	<LOD	<LOD	<LOD
	UMB-IT129	0.76 ± 0.05^{Aa}	<LOD	<LOD
Caffeic acid	SOUR-IT129	0.44 ± 0.02^{Aa}	<LOD	<LOD
	UMB-IT129	0.26 ± 0.01^{Ba}	<LOD	<LOD
Chlorogenic acid	SOUR-IT129	<LOD	<LOD	<LOD
	UMB-IT129	0.19 ± 0.02^{Aa}	<LOD	<LOD
Gallic acid	SOUR-IT129	10.65 ± 0.80^{Aa}	1.35 ± 0.05^{Bb}	0.71 ± 0.05^{Bc}
	UMB-IT129	9.57 ± 0.83^{Ba}	1.72 ± 0.09^{Ab}	1.08 ± 0.10^{Ac}
Syringic acid	SOUR-IT129	0.06 ± 0.00^{Ba}	<LOD	<LOD
	UMB-IT129	0.14 ± 0.01^{Aa}	<LOD	<LOD
ρ_Coumaric acid	SOUR-IT129	6.14 ± 0.27^{Aa}	<LOD	<LOD
	UMB-IT129	<LOD	<LOD	<LOD

Values are expressed as average ± standard deviation, n = 3. <LOD: lower than the detection limit. Treatment SB (CNCM I-745) had no phenolic compounds detected over the fermentation. A-C: different superscript capital letters in the same fermentation time denote difference ($p < 0.05$) among treatments, based on Tukey's test. a-c: different superscript lowercase letters for the same treatment at different times denote difference ($p < 0.05$), based on Tukey's test.

of the fermentation. It probably occurred because catechin and gallic acid can be released from polymeric flavonoids due to microbial metabolization, while procyanidin B2 can be formed by the polymerization of catechin and epicatechin (Medeiros et al., 2022).

Procyanidin B2 has been reported for its inhibitory effects on oxidative stress, lowering blood pressure and improving renal function in rats (Yao et al., 2021), in addition to reducing vascular calcification due to its potent antioxidant action (Liang et al., 2021). Gallic acid is a secondary metabolite of polyphenols and is well known for its antioxidant characteristic and *anti*-hyperlipidemic, cardioprotective, anti-hyperglycemic, and anticancer actions (Ashrafizadeh et al., 2021; Zanwar et al., 2014). Catechin can exert an antihypertensive effect due to its angiotensin-converting enzyme inhibitory property, as well to induce endothelial recovery and reduce blood pressure (Lapi et al., 2020). Considering that phenolic compounds are mainly absorbed in the colon, their during colonic fermentation may be beneficial for hypertensive consumers. Further *in vivo* studies with hypertensive individuals can focus on the benefit of the phenolic compounds in fermented soursop and umbu-cajá pulps through the intestine axis.

3.6. Colonic fermentation and the correlation of the relative abundance of the evaluated microbial groups with the SCFA contents and phenolic compounds

A PCA map was used to assess the relationship between the relative

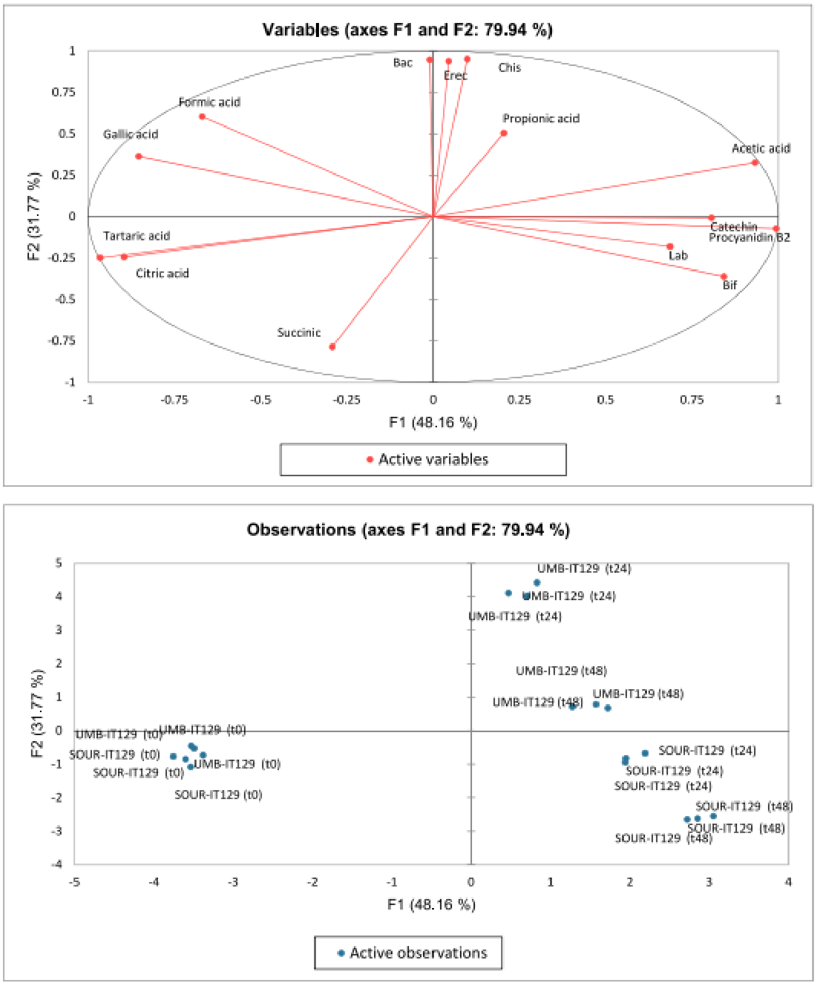

Fig. 1. Variables of the principal component analysis (PCA) with relative abundance of hybridized bacterial groups, short chain fatty acids, and phenolic compounds analyzed during *in vitro* colonic fermentation in the treatments SOUR-IT129, UMB-IT129 at time zero, 24, and 48 h. SOUR-IT129: soursop pulp fermented with *I. terricola* 129; UMB-IT129: umbu-cajá pulp fermented with *I. terricola* 129; Control: only with the addition of fecal inoculum. Lab: *Lactobacillus* spp./*Enterococcus* spp., Bif: *Bifidobacterium* spp., Bac: group of *Bacteroides* spp./*Prevotella* spp., Chis: *Clostridium histolyticum*, and Erec: *Eubacterium rectall/Clostridium coccoides*.

abundance of each bacterial group with the values recorded for SCFA and phenolic compounds (Fig. 1). The analysis explained the data with a total variance of 79.94% (F1: 48.16% and F2: 31.77%). The SOUR-IT129 treatment showed higher concentrations of procyanidin B2, catechin, and acetic acid and higher abundances of *Lactobacillus* spp. and *Bifidobacterium* spp. In contrast, propionic acid stood out in the UMB-IT129 treatment along with the abundance of *Bacterioides, C. histolyticum*, and *E. rectale/C. coccoides* at both 24 and 48 h of fermentation. PCA map evidenced the positive effect of SOUR-IT129 on the modulation of beneficial gut microbial groups (*Lactobacillus* spp. and *Bifidobacterium* spp.).

The increase in acetic acid was positively correlated with the contents of catechin (0.82), procyanidin B2 (0.90), and the abundance of *Lactobacillus* spp. (0.54) and *Bifidobacterium* spp. (0.67) (Table S5). The results indicate that a higher metabolic activity due to the higher abundance of microbiota resulted in the production of SCFA and higher contents of phenolic compounds.

4. Conclusions

This study was the first to evaluate the effects of soursop and umbu-cajá pulps fermented by yeast on the microbiota of middle-aged hypertensive adults during *in vitro* colonic fermentation. Fermented soursop pulps with *I. terricola* 129 improved the microbiota by increasing the populations of *Lactobacillus* spp. and *Bifidobacterium* spp. and reducing the populations of *E. rectale/C. coccoides* and *C. histolyticum*, resulting in the highest prebiotic index among the tested products. Fermented soursop pulp increased the metabolic activity of the colonic microbiota, with the consumption of rhamnose and gallic acid and increasing the concentrations of acetic and propionic acids and procyanidin B2. The results indicate that fermented soursop pulp with yeast *I. terricola* 129 may modulate the colonic microbiota of middle-aged hypertensive adults. Clinical studies are needed to confirm the modulatory effects of these fermented pulps on human gut microbiota and their health impacts.

Credit author statement

ELCM, ELS, TCP and MM participated in the conceptualization of the study; ELCM, JSM, DSM, ACS, MSL, KBS and NPAR performed the investigation; ELCM, KBS, NPAR, TCP and MM worked in the formal analysis of the data; ELCM, TCP and MM wrote the original draft of the manuscript. All authors were involved in review, editing and visualization. ELS and MM provided the resources for development of the study and worked in the supervision; MM was responsable by the project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2022.102309.

References

Agu, K. C., & Okolie, P. N. (2017). Proximate composition, phytochemical analysis, and in vitro antioxidant potentials of extracts of *Annona muricata* (Soursop). *Food Sciences and Nutrition, 5*(5), 1029–1036. https://doi.org/10.1002/fsn3.498

Albuquerque, T. M. R., Magnani, M., Lima, M. S., Castellano, L. R. C., & de Souza, E. L. (2021). Effects of digested flours from four different sweet potato (*Ipomoea batatas* L.) root varieties on the composition and metabolic activity of human colonic microbiota in vitro. *Journal of Food Science, 86*, 3707–3719. https://doi.org/10.1111/1750-3841.15852

Albuquerque, T. G., Santos, F., Sanches-Silva, A., Oliveira, M. B., Bento, A. C., & Costa, H. S. (2016). Nutritional and phytochemical composition of *Annona cherimola* Mill. fruits and by-products: Potential health benefits. *Food Chemistry, 193*, 187–195. https://doi.org/10.1016/j.foodchem.2014.06.044

Alu'datt, M. H., Rababah, T., Alhamad, M. N., Al-Mahasneh, M. A., Ereifej, K., Al-Karaki, G., … Ghozlan, K. A. (2017). Profiles of free and bound phenolics extracted from Citrus fruits and their roles in biological systems: Content, and antioxidant, anti-diabetic and anti-hypertensive properties. *Food & Function, 8*(9), 3187–3197. https://doi.org/10.1039/c7fo00212b

Amorim, J. C., Piccoli, R. H., & Duarte, W. F. (2018). Probiotic potential of yeasts isolated from pineapple and their use in the elaboration of potentially functional fermented beverages. *Food Research International, 107*, 518–527. https://doi.org/10.1016/j.foodres.2018.02.054

Andrade, R. M. S. de, Silva, S., Costa, C. M. D. S. F., Veiga, M., Costa, E., Ferreira, M. S. L., … Pintado, M. E. (2020). Potential prebiotic effect of fruit and vegetable byproducts flour using in vitro gastrointestinal digestion. *Food Research International, 137*, Article 109354. https://doi.org/10.1016/j.foodres.2020.109354

Ashrafizadeh, M., Zarrabi, A., Mirzaei, S., Hashemi, F., Samarghandian, S., Zabolian, A., … Varma, R. S. (2021). Gallic acid for cancer therapy: Molecular mechanisms and boosting efficacy by nanoscopical delivery. *Food and Chemical Toxicology, 157*, Article 112576. https://doi.org/10.1016/j.fct.2021.112576

Barroso, W. K. S., Rodrigues, C. I. S., Bortolotto, L. A., Mota-Gomes, M. A., Brandão, A. A., Feitosa, A. D. D. M., … Nadruz, W. (2021). Brazilian guidelines for arterial hypertension – 2020. *Brazilian Archives of Cardiology, 116*, 516–658. https://doi.org/10.36660/abc.20201238 ([In Portuguese]).

Briand, F., Sulpice, T., Giammarinaro, P., & Roux, X. (2019). *Saccharomyces boulardii* CNCM I-745 changes lipidemic profile and gut microbiota in a hamster hypercholesterolemic model. *Beneficial Microbes, 10*(5), 555–567. https://doi.org/10.3920/BM2018.0134

Calderón-Pérez, L., Gosalbes, M. J., Yuste, S., Valls, R. M., Pedret, A., Llauradó, E., … Solà, R. (2020). Gut metagenomic and short chain fatty acids signature in hypertension: A cross-sectional study. *Scientific Reports, 10*(1), 1–16. https://doi.org/10.1038/s41598-020-63475-w

Calderón-Pérez, L., Llauradó, E., Companys, J., Pla-Pagà, L., Pedret, A., Rubió, L., … Valls, R. M. (2021). Interplay between dietary phenolic compound intake and the human gut microbiome in hypertension: A cross-sectional study. *Food Chemistry, 344*, Article 128567. https://doi.org/10.1016/j.foodchem.2020.128567

Chang, L. S., Karim, R., Abdulkarim, S. M., Yusof, Y. A., & Ghazali, H. M. (2018). Storage stability, color kinetics and morphology of spray-dried soursop (*Annona muricata* L.) powder: Effect of anticaking agents. *International Journal of Food Properties, 21*(1), 1937–1954. https://doi.org/10.1080/10942912.2018.1510836

Coelho, E. M., Padilha, C. V. S., Miskinis, G. A., Sá, A. G. B., Pereira, G. E., Azevêdo, L. C., & Lima, M. S. (2018). Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: Method validation and characterization of products from northeast Brazil. *Journal of Food Composition and Analysis, 66*, 160–167. https://doi.org/10.1016/j.jfca.2017.12.017

Conterno, L., Martinelli, F., Tamburini, M., Fava, F., Mancini, A., Sordo, M., … Tuohy, K. (2019). Measuring the impact of olive pomace enriched biscuits on the gut microbiota and its metabolic activity in mildly hypercholesterolaemic subjects. *European Journal of Nutrition, 58*(1), 63–81. https://doi.org/10.1007/s00394-017-1572-2

Emer, C. D., Marques, S., Colla, L. M., & Reinehr, C. O. (2021). Biogenic amines and the importance of starter cultures for malolactic fermentation. *Australian Journal of Grape and Wine Research, 27*(1), 26–33. https://doi.org/10.1111/ajgw.12462

Farinazzo, F. S., Madeira, T. B., Fernandes, M. T. C., Mauro, C. S. I., Tomal, A. A. B., Nixdorf, S. L., & Garcia, S. (2020). Organic and conventional apple fermented by *Saccharomyces boulardii*–The effect of the antioxidant quercetin on cellular oxidative stress. *British Food Journal.* https://doi.org/10.1108/BFJ-07-2019-0564

Freire, A. L., Ramos, C. L., da Costa Souza, P. N., Cardoso, M. G. B., & Schwan, R. F. (2017). Nondairy beverage produced by controlled fermentation with potential probiotic starter cultures of lactic acid bacteria and yeast. *International Journal of Food Microbiology, 248*, 39–46. https://doi.org/10.1016/j.ijfoodmicro.2017.02.011

Guergoletto, K. B., Costabile, A., Flores, G., Garcia, S., & Gibson, G. R. (2016). In vitro fermentation of juçara pulp (*Euterpe edulis*) by human colonic microbiota. *Food Chemistry, 196*, 251–258. https://doi.org/10.1016/j.foodchem.2015.09.048

Gunathilakea, K. D. P. P., Ranaweera, K. K. D. S., & Rupasinghe, H. P. V. (2018). Change of phenolics, carotenoids, and antioxidant capacity following simulated gastrointestinal digestion and dialysis of selected edible green leaves. *Food Chemistry, 245*, 371–379. https://doi.org/10.1016/j.foodchem.2017.10.096

Jiao, J., Zhang, Y., Han, P., & Zhai, S. (2022). A preliminary study on the value of intestinal flora in predicting major adverse cardiovascular and cerebrovascular events in patients with refractory hypertension. *Computational and Mathematical Methods in Medicine*. https://doi.org/10.1155/2022/7723105, 2022.

Kabbani, T. A., Pallav, K., Dowd, S. E., Villafuerte-Galvez, J., Vanga, R. R., Castillo, N. E., ... Kelly, C. P. (2017). Prospective randomized controlled study on the effects of *Saccharomyces boulardii* CNCM I-745 and amoxicillin-clavulanate or the combination on the gut microbiota of healthy volunteers. *Gut Microbes, 8*(1), 17–32. https://doi.org/10.1080/19490976.2016.1267890

Lapi, D., Stornaiuolo, M., Sabatino, L., Sommella, E., Tenore, G., Daglia, M., ... Novellino, E. (2020). The pomace extract taurisolo protects rat brain from ischemia-reperfusion injury. *Frontiers in Cellular Neuroscience, 14*, 3. https://doi.org/10.3389/fncel.2020.00003

Liang, Y., Chen, G., Zhang, F., Yang, X., Chen, Y., Duan, Y., ... Han, J. (2021). Procyanidin B2 reduces vascular calcification through inactivation of ERK1/2-RUNX2 pathway. *Antioxidants, 10*(6), 916. https://doi.org/10.3390/antiox10060916

Liu, H., Zhang, H., Zhang, X., Chen, Q., & Xia, L. (2022). Role of succinic acid in the regulation of sepsis. *International Immunopharmacology, 110*, Article 109065. https://doi.org/10.1016/j.intimp.2022.109065

Lu, W., Wang, Y., Fang, Z., Wang, H., Zhu, J., Zhai, Q., ... Chen, W. (2022). *Bifidobacterium longum* CCFM752 prevented hypertension and aortic lesion, improved antioxidative ability, and regulated gut microbiome in spontaneously hypertensive rats. *Food & Function*. https://doi.org/10.1039/D1FO04446J

Macêdo, E. L. C., Pimentel, T. C., Melo, D. S., Souza, A. C., Morais, J. S., Lima, M. S., Dias, D. R., Schwan, R. F., & Magnani, M. (2022). Yeasts from fermented Brazilian fruits as biotechnological tools for increasing phenolics bioaccessibility and improving the volatile profile in derived pulps. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2022.134200

Maia, G. A., da Silva, L. M. R., do Prado, G. M., Fonseca, A. V. V., de Sousa, P. H. M., & de Figueiredo, R. W. (2019). Development of mixed beverages based on tropical fruits. In *Non-alcoholic beverages* (pp. 129–162). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-815270-6.00005-0.

Manter, D. K., & Vivanco, J. M. (2007). Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. *Journal of Microbiological Methods, 71*(1), 7–14. https://doi.org/10.1016/j.mimet.2007.06.016

Massa, N. M. L., de Oliveira, S. P. A., Rodrigues, N. P. A., Menezes, F. N. D. D., Lima, M. S., Magnani, M., & de Souza, E. L. (2022). In vitro colonic fermentation and potential prebiotic properties of pre-digested jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg) by-products. *Food Chemistry, 388*, Article 133003. https://doi.org/10.1016/j.foodchem.2022.133003

Medeiros, V. P. B., de Souza, E. L., de Albuquerque, T. M. R., da Costa Sassi, C. F., Lima, M. S., Sivieri, K., ... Magnani, M. (2021). Freshwater microalgae biomasses exert a prebiotic effect on human colonic microbiota. *Algal Research, 60*, Article 102547. https://doi.org/10.1016/j.algal.2021.102547

Menezes, F. N. D. D., Melo, F. H. C., Vieira, A. R. S., Almeida, É. T., Lima, M. S., Aquino, J. S., ... Souza, E. L. (2021). Acerola (*Malpighia glabra* L.) and guava (*Psidium guayaba* L.) industrial processing by-products stimulate probiotic *Lactobacillus* and *Bifidobacterium* growth and induce beneficial changes in colonic microbiota. *Journal of Applied Microbiology, 130*(4), 1323–1336. https://doi.org/10.1111/jam.14824

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, Bourlieu, C., ... Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food–an international consensus. *Food & Function, 5*(6), 1113–1124. https://doi.org/10.1039/c3fo60702j

Moré, M. I., & Swidsinski, A. (2015). Saccharomyces boulardii CNCM I-745 supports regeneration of the intestinal microbiota after diarrheic dysbiosis–a review. *Clinical and Experimental Gastroenterology, 8*, 237. https://10.2147/CEG.S85574.

Padilha, C. V. S., Miskinis, G. A., Souza, M. E. A. O., Pereira, G. E., Oliveira, D., ... Lima, M. S. (2017). Rapid determination of flavonoids and phenolic acids in grape juices and wines by RP-HPLC/DAD: Method validation and characterization of commercial products of the new Brazilian varieties of grape. *Food Chemistry, 228*, 106–115. https://doi.org/10.1016/j.foodchem.2017.01.137

Palmu, J., Lahti, L., & Niiranen, T. (2021). Targeting gut microbiota to treat hypertension: A systematic review. *International Journal of Environmental Research and Public Health, 18*(3), 1248. https://doi.org/10.3390/ijerph18031248

Pereira, F. R. A., Pereira, W. E., Pessoa, A. M. D. S., & Vasconcelos, E. S. A. G. D. (2021). Biometry in umbuzeiro fruit from Paraibano semiarid. *Revista Brasileira de Fruticultura, 43*. https://doi.org/10.1590/0100-29452021808 ([In Portuguese]).

Pravasi, S. D. (2014). Acetic acid. In Philip Wexler (Ed.), *Encyclopedia of toxicology* (Third Edition, pp. 33–35). Academic Press. https://doi.org/10.1016/B978-0-12-386454-3.00216-5.

Robles-Vera, I., de la Visitación, N., Toral, M., Sánchez, M., Romero, M., Gómez-Guzmán, M., ... Duarte, J. (2020). Probiotic *Bifidobacterium breve* prevents DOCA-salt hypertension. *The FASEB Journal, 34*(10), 13626–13640. https://doi.org/10.1096/fj.202001532R

Robles-Vera, I., Toral, M., de la Visitación, N., Sánchez, M., Romero, M., Olivares, M., ... Duarte, J. (2018). The probiotic *Lactobacillus fermentum* prevents dysbiosis and vascular oxidative stress in rats with hypertension induced by chronic nitric oxide blockade. *Molecular Nutrition & Food Research, 62*(19), Article 1800298. https://doi.org/10.1002/mnfr.201800298

Rodrigues, D., Walton, G., Sousa, S., Rocha-Santos, T. A., Duarte, A. C., Freitas, A. C., & Gomes, A. M. (2016). In vitro fermentation and prebiotic potential of selected extracts from seaweeds and mushrooms. *LWT - Food Science and Technology, 73*, 131–139. https://doi.org/10.1016/j.lwt.2016.06.004

Sabidi, S., Koh, S. P., Abd Shukor, S., Adzni Sharifudin, S., & Sew, Y. S. (2020). Safety assessment of fermented jackfruit (*Artocarpus heterophyllus*) pulp and leaves in Sprague-Dawley rats. *Food Sciences and Nutrition, 8*(8), 4370–4378. https://doi.org/10.1002/fsn3.1734

Sarkar, T., Salauddin, M., Roy, A., Sharma, N., Sharma, A., Yadav, S., ... Simal-Gandara, J. (2022). Minor tropical fruits as a potential source of bioactive and functional foods. *Critical Reviews in Food Science and Nutrition*, 1–45. https://doi.org/10.1080/10408398.2022.2033953

Serena, C., Ceperuelo-Mallafré, V., Keiran, N., Queipo-Ortuño, M. I., Bernal, R., Gomez-Huelgas, R., ... Fernández-Veledo, S. (2018). Elevated circulating levels of succinate in human obesity are linked to specific gut microbiota. *The ISME Journal, 12*(7), 1642–1657. https://doi.org/10.1038/s41396-018-0068-2

Sharon, G., Garg, N., Debelius, J., Knight, R., Dorrestein, P. C., & Mazmanian, S. K. (2014). Specialized metabolites from the microbiome in health and disease. *Cell Metabolism, 20*(5), 719–730. https://doi.org/10.1016/j.cmet.2014.10.016

Shuai, M., Fu, Y., Zhong, H. L., Gou, W., Jiang, Z., Liang, Y., ... Zheng, J. S. (2022). Mapping the human gut mycobiome in middle-aged and elderly adults: Multiomics insights and implications for host metabolic health. *Gut, 71*(9), 1812–1820. https://doi.org/10.1136/gutjnl-2021-326298

Wang, R., Sun, J., Lassabliere, B., Yu, B., & Liu, S. Q. (2022). Green tea fermentation with *Saccharomyces boulardii* CNCM I-745 and *Lactiplantibacillus plantarum* 299V. *Lebensmittel-Wissenschaft und -Technologie, 157*, Article 113081. https://doi.org/10.1016/j.lwt.2022.113081

Wan, Y., Yuan, J., Li, J., Li, H., Yin, K., Wang, F., & Li, D. (2020). Overweight and underweight status are linked to specific gut microbiota and intestinal tricarboxylic acid cycle intermediates. *Clinical Nutrition, 39*(10), 3189–3198. https://doi.org/10.1016/j.clnu.2020.02.014

Yao, Y., Liu, T., Yin, L., Man, S., Ye, S., & Ma, L. (2021). Polyphenol-rich extract from litchi chinensis seeds alleviates hypertension-induced renal damage in rats. *Journal of Agricultural and Food Chemistry, 69*(7), 2138–2148. https://doi.org/10.1021/acs.jafc.0c07046

Zanwar, A. A., Badole, S. L., Shende, P. S., Hegde, M. V., & Bodhankar, S. L. (2014). Role of gallic acid in cardiovascular disorders. In *Polyphenols in human health and disease* (pp. 1045–1047). Academic Press. https://doi.org/10.1016/B978-0-12-398456-2.00080-3.

Supplementary tables

Table S1. Physicochemical parameters of fruit pulps fermented by *I. terricola* 129.

Sample	Treatment	pH	Total soluble solids (°Brix)	Titratable acidity (% citric acid)
Soursop	Control	3.74 ± 0.01^{A}	12.33 ± 0.12^{A}	0.81 ± 0.03^{A}
	I. terricola 129	3.69 ± 0.02^{A}	12.33 ± 0.12^{A}	0.81 ± 0.02^{A}
Umbu-cajá	Control	2.50 ± 0.02^{A}	12.33 ± 0.12^{A}	1.70 ± 0.01^{A}
	I. terricola 129	2.53 ± 0.02^{A}	12.40 ± 0.00^{A}	1.69 ± 0.03^{A}

Control - non-fermented pulp; Values are expressed as the mean ± standard deviation; n=9.
A: different capital letters in the same column denote differences ($p < 0.05$) between different treatments for the same fruit pulp, based on Tukey's test.

Table S2 External standard used to quantify phenolic compounds, sugars, and organic acids.

Compound	**Brand**
Phenolic compounds	
cis-resveratrol and trans-resveratrol	Cayman Chemical Company (Ann Arbor, MI, USA)
chlorogenic, gallic, syringic, p-coumaric, caffeic and caftaric acids, catechin, epicatechin, hesperidin, naringenin, procyanidin B1 and B2, delphinidin 3-glucoside, cyanidin 3,5-diglucoside, cyanidin 3-glucoside, malvidin 3,5-diglucoside, malvidin 3-glucoside, and pelargonidin 3,5-diglucoside	Sigma-Aldrich (St. Louis, MA, USA)
procyanidin A2, kaempferol 3-glycoside, quercetin 3-glycoside, quercetin 3-rutinoside (rutin), myricetin, epicatechin gallate, epigallocatechin gallate, pelargonidin 3-glycoside, petunidin 3-glycoside, and peonidine 3-O-glycosides	Extrasynthesis (Genay, France)
Sugars	
glucose and fructose	Sigma-Aldrich (St. Louis, MA, USA)
maltose and rhamnose	Chem Service (West Chester, PA, USA)
Organic acids	
citric, tartaric, malic, succinic, lactic, formic, acetic, propionic, and butyric acids	Química Vetec (Rio de Janeiro, RJ, Brazil)

All standards had a purity level of ≥ 98%.

Table S3. Identification code of yeast isolate, partial sequence, species identified, and accession number in the corresponding GenBank.

Code	Sequence	Species identified	Access number*
129	AAACTTTCAACAACGGATCTCTTG GTTCTCGCATCGATGAAGAGCGC AGCGAAATGCGATACCTAGTGTG AATTGCAGCCATCGTGAATCATC GAGTTCTTGAACGCACATTGCGC CCCCTGGTATTCCGGGGGGCATG CCTGTTTGAGCGTCGTTTCTATCT CACGCAAGTGGAGCTGGCCCGGC CTTGGCCCCGCCGAAAAGAAACG AGGGCGAAGCGAACTATGTTGTG CGCCGACCCCAGCTATCAAGCTC GACCTCAAATCAGGTAGGAATAC CCGCTGAACTTAAGCATATCAAT AAGCGGAGGA	*Issatchenkia terricola*	KP132531.1

*Database access number of Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

Table S4. Alterations (increase or decrease) in the relative abundance of distinct bacterial groups in the treatments SOUR-IT129, UMB-IT129, SB (CNCM I-745), and control at time 24 and 48 h of *in vitro* colonic fermentation.

Bacterial group	**Samples**	**Fermentation (time)**	
		24 h*	**48 h***
Lactobacillus spp./ *Enterococcus* spp. (Lab 158)	SOUR-IT129	6.07 (1.24)	199.93 (1.53)
	UMB-IT129	72.09 (1.39)	82.86 (1.53)
	SB(CNCM I-745)	-66.40 (0.61)	-22.39 (0.62)
	Control	-37.33 (0.78)	-56.38 (0.61)
Bifidobacterium spp. (Lab 164)	SOUR-IT129	859.58 (9.45)	708.46 (7.65)
	UMB-IT129	195.82 (4.15)	288.15 (4.61)
	SB(CNCM I-745)	138.28 (3.04)	155.88 (5.84)
	Control	26.72 (2.45)	215.31 (4.83)
Bacteroides spp./ *Prevotella* spp. (Bac 303)	SOUR-IT129	-30.69 (0.06)	-20.25 (0.26)
	UMB-IT129	102.47 (0.10)	29.41 (0.08)
	SB(CNCM I-745)	-24.25 (0.03)	-75.60 (0.28)
	Control	-71.67 (0.21)	-87.35 (0.05)
C. histolyticum (Chis 150)	SOUR-IT129	-44.98 (±0.49)	-60.48 (0.74)
	UMB-IT129	311.72 (1.35)	175.81 (0.55)
	SB(CNCM I-745)	143.40 (1.11)	-15.73 (0.08)
	Control	-33.47 (±0.59)	-66.77 (±0.04)
E. rectale/ C. coccoides (Erec 482)	SOUR-IT129	-50.03 (0.37)	-74.89 (0.86)
	UMB-IT129	238.78 (1.02)	155.05 (0.64)
	SB(CNCM I-745)	-9.80 (1.18)	-28.01 (0.18)
	Control	-26.76 (0.12)	-67.37 (0.34)

Values are expressed as average of three independent experiments performed in triplicate. Standard deviation expressed between bracts. *Results are expressed in percentage and are relative to the comparison between the abundances at time 24 or 48 with time zero. SOUR-IT129 (soursop pulp fermented with *I. terricola* 129), UMB-IT129 (umbu-cajá pulp fermented with *I. terricola* 129), SB (CNCM I-745) (*S. boulardii* CNCM I-745), and control.

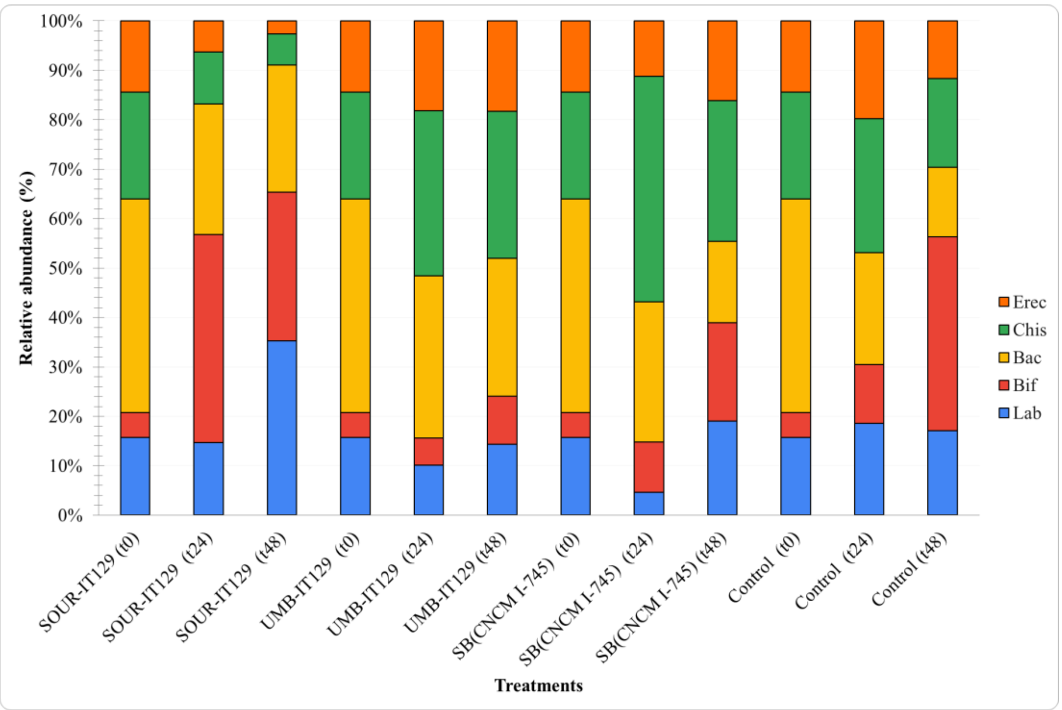
Table S5. Correlation matrix (Pearson) between different SCFA values, phenolic compounds, and relative abundances of bacterial groups during *in vitro* colonic fermentation.

Variables	Citric acid	Tartaric acd	Succinic acd	Formic acid	Acetic acid	Propionic acid	Catechin	Procyanidin B2	Gallic acid	Lab	Bif	Bac	Chis	Erec
Citric acid	**1.00**	**0.94**	**0.58**	**0.60**	**-0.88**	-0.29	**-0.51**	**-0.87**	**0.63**	**-0.55**	**-0.67**	-0.20	-0.30	-0.26
Tartaric acid	**0.94**	**1.00**	**0.49**	**0.51**	**-0.98**	-0.31	**-0.77**	**-0.94**	**0.73**	**-0.59**	**-0.73**	-0.21	-0.33	-0.28
Succinic acid	**0.58**	**0.49**	**1.00**	-0.18	**-0.48**	**-0.69**	-0.08	-0.23	-0.18	0.15	-0.11	**-0.63**	**-0.65**	**-0.61**
Formic acid	**0.60**	**0.51**	-0.18	**1.00**	-0.40	0.25	-0.32	**-0.70**	**0.76**	**-0.55**	**-0.75**	**0.60**	**0.50**	**0.51**
Acetic acid	**-0.88**	**-0.98**	**-0.48**	-0.40	**1.00**	0.37	**0.82**	**0.90**	**-0.69**	**0.54**	**0.67**	0.29	0.41	0.36
Propionic acid	-0.29	-0.31	**-0.69**	0.25	0.37	**1.00**	0.24	0.20	0.26	-0.34	0.31	0.28	0.28	0.24
Catechin	**-0.51**	**-0.77**	-0.08	-0.32	**0.82**	0.24	**1.00**	**0.81**	**-0.74**	**0.49**	**0.72**	-0.02	0.07	0.03
Procyanidin B2	**-0.87**	**-0.94**	-0.23	**-0.70**	**0.90**	0.20	**0.81**	**1.00**	**-0.86**	**0.69**	**0.88**	-0.08	0.02	-0.03
Gallic acid	**0.63**	**0.73**	-0.18	**0.76**	**-0.69**	0.26	**-0.74**	**-0.86**	**1.00**	**-0.79**	**-0.73**	0.27	0.17	0.20
Lab	**-0.55**	**-0.59**	0.15	**-0.55**	**0.54**	-0.34	**0.49**	**0.69**	**-0.79**	**1.00**	0.44	0.04	0.03	-0.03
Bif	**-0.67**	**-0.73**	-0.11	**-0.75**	**0.67**	0.31	**0.72**	**0.88**	**-0.73**	0.44	**1.00**	-0.45	-0.37	-0.43
Bac	-0.20	-0.21	**-0.63**	**0.60**	0.29	0.28	-0.02	-0.08	0.27	0.04	-0.45	**1.00**	**0.97**	**0.95**
Chis	-0.30	-0.33	**-0.65**	**0.50**	0.41	0.28	0.07	0.02	0.17	0.03	-0.37	**0.97**	**1.00**	**0.99**

Values in bold are different from 0 with a significance level α=0.05. SCFA = short-chain fatty acid. Bif: *Bifidobacterium* spp.; Lab: *Lactobacillus* spp./*Enterococcus* spp.; Bac: *Bacteroides* spp./*Prevotella* spp.; Erec: *Eubacterium rectale/Clostridium coccoides*; and Chis: *Clostridium histolyticum*.

Supplementary figure

Fig S1. Relative abundance within the measured bacterial groups during *in vitro* human colonic fermentation in in the treatments SOUR-IT129, UMB-IT129, SB (CNCM I-745), and Control at time zero, 24 and 48 h.



SOUR-IT129: soursop pulp fermented with *I. terricola* 129; UMB-IT129: umbu-cajá pulp fermented with *I. terricola* 129; SB (CNCM I-745): *S. boulardii* CNCM I-745; Control: only with the addition of fecal inoculum. Lab: group of *Lactobacillus* spp./*Enterococcus* spp., Bif: *Bifidobacterium* spp., Bac: *Bacteroides* spp./*Prevotella* spp., Chis: *Clostridium histolyticum*, and Erec: *Eubacterium rectall*/*Clostridium coccoides*.

ANEXO A – PARECER CONSUBSTANCIADO DO COMITÊ DE ÉTICA

CENTRO DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE FEDERAL DA PARAÍBA - CCS/UFPB

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: POTENCIAL BIOTECNOLÓGICO DE MICRORGANISMOS ISOLADOS DE FRUTAS DA CAATINGA FERMENTADAS

Pesquisador: ELVIRA DE LOURDES CHAVES MACEDO

Área Temática:

Versão: 1

CAAE: 55854822.5.0000.5188

Instituição Proponente: Centro De Ciências da Saúde

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 5.315.511

Apresentação do Projeto:

Trata-se de analisar o projeto de pesquisa da doutoranda Elvira de Lourdes Chaves Macedo, do Programa de Pós-graduação em Ciências da Nutrição do Centro de Ciências da Saúde da Universidade Federal da Paraíba, sob a orientação da Professora Dra. Marciane Magnani.

Objetivo da Pesquisa:

Os objetivos serão: i) o isolamento e identificação da microbiota dominante em fermentação espontânea de graviola e umbu-cajá; ii) a avaliação do potencial biotecnológico de leveduras isoladas selecionadas para produção de polpas fermentadas; iii) caracterização das polpas fermentadas obtidas quanto aos aspectos físico-químicos (ph, sólidos solúveis e acidez titulável), perfil de açúcares, ácidos orgânicos, compostos voláteis e fenólicos (perfil e bioacessibilidade); iv) avaliar atividade antidiabética e anti-hipertensiva in vitro e v) avaliar os efeitos das polpas de frutas fermentadas na modulação na microbiota intestinal humana de hipertensos e diabéticos in vitro.

Avaliação dos Riscos e Benefícios:

Riscos: os voluntários podem sentir-se constrangidos durante o momento da coleta do material fecal.

Benefícios: A pesquisa apresenta abordagem inovadora considerando os aspectos tecnológicos e econômicos, por utilizar frutas do bioma Caatinga ainda pouco exploradas. Ela poderá definir benefícios da fermentação dessas frutas com leveduras autóctones como meio para melhoria do perfil nutricional, principalmente no que diz respeito à bioacessibilidade de compostos fenólicos. Além disso, avaliará os efeitos desses produtos fermentados na modulação na microbiota intestinal humana in vitro tendo potencial de gerar produtos derivados com maior valor agregado. A execução deste projeto de tese apresenta a possibilidade de gerar informações que subsidiem a elaboração de produtos com características funcionais.

Comentários e Considerações sobre a Pesquisa:

Trata-se de uma pesquisa experimental em laboratório (in vitro) realizada no Laboratório de Processos Microbianos da Universidade Federal da Paraíba (UFPB) em parceria com pesquisadores e laboratórios da Universidade Federal de Lavras (UFLA) e do Instituto Federal do Sertão Pernambucano (IFSertãoPE). Os frutos de graviola (Annona muricata L.), umbu-cajá (Spondias spp.) em estádio de maturação horticultural foram adquiridos em comércio local (João Pessoa – Paraíba, Brasil. As analises laboratoriais já foram realizadas e o encaminhamento dessa pesquisa ao comitê de ética em pesquisa se enquadra no envolvimento de algumas analises serem realizadas com a doação de material fecal, o qual será colhido no referido dia e armazenado em frasco anaeróbio, em pacientes diabéticos e hipertensos, adultos, com idades entre 20 e 59 anos, de ambos os sexos, em número de quatro voluntários adultos hipertensos (dois homens e duas mulheres) e quatro voluntários adultos diabéticos (dois homens e duas mulheres). Os critérios de inclusão estabelecidos serão pessoas que não sofram de nenhuma doença do gastrointestinal ou do cólon, sigam dieta onívora regular sem o uso de alimentos probióticos ou prebióticos concentrados e que não tenham utilizado antibióticos durante os seis meses anteriores ao estudo. As fezes serão coletadas em tubos estéreis dispostos em frasco com sistema gerador de anaerobiose (AnaeroGen, Oxoid, Basingstoke Inglaterra). A polpa não fermentada de cada fruta será utilizada como controle. A suspensão fecal será considerada como grupo de origem. O grupo de origem, controle e polpa fermentada serão registrados como ORI, CTRL e PF.

Considerações sobre os Termos de apresentação obrigatória:

O projeto em tela se encontra bem instruído de acordo com a Resolução 466/12 que rege as pesquisas envolvendo seres humanos.

Recomendações:

Recomenda-se manter a metodologia proposta e acrescentar a página da plataforma Brasil os benefícios que constam no Termo de Consentimento Livre e Esclarecido.

Conclusões ou Pendências e Lista de Inadequações:

Sem pendências.

Considerações Finais a critério do CEP:

Certifico que o Comitê de Ética em Pesquisa do Centro de Ciências da Saúde da Universidade Federal da Paraíba – CEP/CCS aprovou a execução do referido projeto de pesquisa. Outrossim, informo que a autorização para posterior publicação fica condicionada à submissão do Relatório Final na Plataforma Brasil, via Notificação, para fins de apreciação e aprovação por este egrégio Comitê.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1896295.pdf	15/02/2022 16:00:55		Aceito
Folha de Rosto	folha_rosto_elvira.pdf	15/02/2022 16:00:12	ELVIRA DE LOURDES CHAVES MACEDO	Aceito
Declaração de Instituição e Infraestrutura	certidao.pdf	10/02/2022 14:45:47	ELVIRA DE LOURDES CHAVES MACEDO	Aceito
Declaração de Instituição e Infraestrutura	anuencia_marciane.pdf	10/02/2022 14:44:44	ELVIRA DE LOURDES CHAVES MACEDO	Aceito
Projeto Detalhado / Brochura Investigador	tese_elvira.docx	10/02/2022 14:37:45	ELVIRA DE LOURDES CHAVES MACEDO	Aceito
Orçamento	orcamento_elvira.docx	10/02/2022 14:35:56	ELVIRA DE LOURDES CHAVES MACEDO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_elvira.doc	10/02/2022 14:35:36	ELVIRA DE LOURDES CHAVES MACEDO	Aceito
Cronograma	cronograma_elvira.docx	10/02/2022 14:34:42	ELVIRA DE LOURDES CHAVES MACEDO	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:
Não

JOAO PESSOA, 28 de Março de 2022

Assinado por:
Eliane Marques Duarte de Sousa
(Coordenador(a))

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ANEXO B – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Prezado (a) Senhor (a)

Esta pesquisa é sobre a avaliação do potencial biotecnológico de leveduras isoladas a partir graviola e umbu-cajá naturalmente fermentadas e está sendo desenvolvida pela pesquisadora Elvira de Lourdes Chaves Macêdo, aluna do Programa de Pós-Graduação em Ciências da Nutrição, da Universidade Federal da Paraíba (UFPB), sob a orientação da Profa. Dra. Marciane Magnani, professora do Programa de Pós-Graduação em Ciências da Nutrição do Departamento de Nutrição (UFPB).

Os objetivos do estudo são avaliar o potencial biotecnológico de leveduras isoladas a partir graviola e umbu-cajá naturalmente fermentadas. A realização desta pesquisa trará informações científicas atualmente escassas no campo de estudo acerca do potencial biotecnológico de leveduras isoladas a partir de fontes autóctones. A pesquisa apresenta abordagem inovadora considerando os aspectos tecnológicos e econômicos, por utilizar frutas do bioma Caatinga ainda pouco exploradas. Ela poderá definir benefícios da fermentação dessas frutas com leveduras autóctones como meio para melhoria do perfil nutricional, principalmente no que diz respeito à bioacessibilidade de compostos fenólicos. Além disso, avaliará os efeitos desses produtos fermentados na modulação na microbiota intestinal humana *in vitro* tendo potencial de gerar produtos derivados com maior valor agregado. A execução deste projeto de tese apresenta a possibilidade de gerar informações que subsidiem a elaboração de produtos com características funcionais.

Os conhecimentos obtidos neste estudo fornecerão conceitos fundamentais sobre uso de leveduras para a fermentação aumentando a bioacessibilidade de compostos específicos. Além disso, a realização da pesquisa possibilitará identificar efeitos benéficos promotores de saúde a partir do consumo dos fermentados de fruta, gerando informações de interesse para elaboração de novos produtos funcionais, aspecto de corrente interesse à indústria alimentícia e a comunidade que busca por consumo de uma alimentação com efeitos benéficos para a saúde.

Em virtude que este TCLE se encontra em mais de uma página, as demais serão rubricadas pelo pesquisador e sujeito da pesquisa.

Solicitamos a sua colaboração para realizar a doação de material fecal, o qual será colhido no referido dia e armazenado em frasco anaeróbio cedido pelos envolvidos na pesquisa, como também sua autorização para apresentar os resultados deste estudo em eventos da área de saúde e, eventualmente, publicação em revista científica. Por ocasião da publicação dos resultados, seu nome será mantido em sigilo. Informamos que essa pesquisa não oferece riscos, previsíveis, para a sua saúde, uma vez que os resultados obtidos servirão de base para definir os efeitos da interação dos fermentados de graviola e umbu-cajá com a microbiota colônica.

Esclarecemos que sua participação no estudo é voluntária e, portanto, o(a) senhor(a) não é obrigado(a) a fornecer as informações e/ou colaborar com as atividades solicitadas pela Pesquisadora. Caso decida não participar do estudo, ou resolver a qualquer momento desistir do mesmo, não sofrerá nenhum dano.

Os pesquisadores estarão a sua disposição para qualquer esclarecimento que considere necessário em qualquer etapa da pesquisa.

Diante do exposto, declaro que fui devidamente esclarecido(a) e dou o meu consentimento para participar da pesquisa e para publicação dos resultados. Estou ciente que receberei uma cópia desse documento.

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Assinatura do Participante da Pesquisa

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Assinatura da Testemunha

Contato do Pesquisador (a) Responsável:

Caso necessite de maiores informações sobre o presente estudo, favor ligar para a pesquisadora: Elvira de Lourdes Chaves Macêdo.

Endereço (Setor de Pesquisa): Laboratório de Processos Microbianos em Alimentos, Departamento de Engenharia de Alimentos da Universidade Federal da Paraíba (UFPB), Campus I, - Cidade Universitária – CEP 58051-900 – João Pessoa – PB Telefone: (83) 3216-7576

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Atenciosamente,

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Assinatura do Pesquisador Responsável