



UNIVERSIDADE FEDERAL DA PARAÍBA
CENTRO DE TECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE
ALIMENTOS

BIANCA BEATRIZ TORRES DE ASSIS

FERMENTAÇÃO PROBIÓTICA: UMA ESTRATÉGIA PARA VALORIZAÇÃO DE
FRUTAS NATIVAS SUBEXPLORADAS

JOÃO PESSOA – PB

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos da Universidade Federal da Paraíba - *Campus I*, em cumprimento dos requisitos para obtenção do título de Doutor em Ciência e Tecnologia de Alimentos.

Orientadora: Prof.^a Dr.^a Marciane Magnani

JOÃO PESSOA – PB

2025

Catálogo na publicação
Seção de Catalogação e Classificação

A848f Assis, Bianca Beatriz Torres de.

Fermentação probiótica : uma estratégia para
valorização de frutas nativas subexploradas / Bianca
Beatriz Torres de Assis. - João Pessoa, 2025.
222 f. : il.

Orientação: Marciane Magnani.
Tese (Doutorado) - UFPB/CT.

1. Frutas nativas. 2. Polpas probióticas. 3.
Armazenamento refrigerado. 4. Microbiota colônica. I.
Magnani, Marciane. II. Título.

UFPB/BC

CDU 634.1(043)

Universidade Federal da Paraíba
Centro de Tecnologia
Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos – PPGCTA

ATA DE DEFESA DE TESE
DOUTORADO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

Aos quinze dias do mês de maio do ano de dois mil e vinte e cinco, no Bloco Multimídia (Bolo de Noiva), às 9:30, reuniu-se a Banca Examinadora composta pela Profa. Dra. Marciane Magnani, orientadora do trabalho e presidente da Banca, Profa. Dra. Taliana Kênia Alves Bezerra (Membra Interna/UFPB), Prof. Fábio Anderson Pereira da Silva (Membro Interno/UFPB), Profa. Dra. Lucélia Cabral (Membra Externa à Instituição/UnB) e Prof. Dr. Roger Wagner (Membro Externo à Instituição/UFSM). A reunião teve por objetivo julgar o trabalho da estudante **Bianca Beatriz Torres de Assis**, matrícula nº 20211016570, sob o Título: "FERMENTAÇÃO PROBIÓTICA DE POLPAS DE FRUTAS NATIVAS: ASPECTOS TECNOLÓGICOS, SENSORIAIS E EFEITOS NA MICROBIOTA HUMANA." Os trabalhos foram abertos pela Profa. Dra. Marciane Magnani. A seguir foi dada a palavra à estudante para apresentação do trabalho. Cada Examinador(a) arguiu a doutoranda, com tempos iguais de arguição e resposta. Terminadas as arguições, procedeu-se o julgamento do trabalho, concluindo a Banca Examinadora por sua APROVAÇÃO. Atendidas as exigências da Resolução nº 15/2019/CONSEPE que regulamentam o Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, e nada mais havendo a tratar, foi lavrada a presente Ata, que vai assinada pelos membros da Banca Examinadora e pela doutoranda.

João Pessoa, 15 de maio de 2025.

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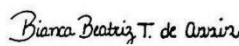
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**A minha mãe, Aldenair da Silva Torres,
Dedico.**

AGRADECIMENTOS

Primeiramente a Deus, que me capacitou durante esta jornada e fez com que as dificuldades fossem minimizadas!

À Universidade Federal da Paraíba, especialmente ao Programa de Pós Graduação em Ciência e Tecnologia de Alimentos (PPGCTA), seu corpo docente e funcionários.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela concessão da bolsa e apoio à pesquisa científica.

À minha querida orientadora, prof.^a Dr.^a Marciane Magnani, por toda dedicação, ensinamentos, ótimos momentos de ajuda, valiosos conselhos tanto na área acadêmica como também na vida pessoal e motivação ao longo de toda jornada. Sou muito grata a senhora por dispor de tempo, paciência, carinho e amizade ao longo destes quatro anos de trabalho juntas. Acredito que essa foi apenas uma primeira etapa e estarei muito feliz em continuar aprendendo com a senhora! Apenas não tenho palavras para agradecer, muito obrigada professora, sou e serei eternamente grata pela sua ajuda! Você foi muito importante para a concretização de um sonho!

À prof.^a Dr.^a Tatiana Colombo Pimentel, pela disponibilidade e grandiosas contribuições para a realização e aprimoramento deste trabalho. Sei o quanto a senhora dispôs de tempo para me ajudar, o seu trabalho foi fundamental na construção da minha formação! Muito obrigada professora!!

À prof.^a Dr.^a Lucélia Cabral, membro da banca examinadora, por todo suporte dado para o desenvolvimento deste trabalho e atenção prestada. Muito obrigada professora!!

A minha mãe, Aldenair da Silva Torres, que com toda garra e persistência me incentivou a continuar mesmo nas horas mais difíceis e me ensinou a nunca desistir.

A minha avó, Severina Neuza Silva Torres, que orou por mim durante todo esse tempo. Eu amo você!

Às minhas amigas da pós-graduação e da vida, Mayara Maria e Ruthchelly Tavares, que com suas amizades me ajudaram em muitos momentos de tensão e dificuldades. Obrigada amigas, vocês são especiais!!

A todos os colegas do Laboratório de Processos Microbianos em Alimentos (LPMA), pelas ajudas sempre que necessário!

Aos técnicos, Whyara Almeida e Gilvandro da Costa pelo acolhimento e ajuda durante o início das minhas análises. Muito Obrigada!

Enfim, agradeço a todos, que de alguma forma contribuíram para a realização deste trabalho e torcem por mim. Muito Obrigada, tenho eterna gratidão.

**“Para tudo há uma ocasião certa,
há um tempo certo para cada
propósito debaixo do céu”.**

Eclesiastes 3:1

RESUMO

O Brasil possui ampla diversidade de frutas nativas que são fonte de compostos bioativos que permanecem subexploradas na região Norte e Nordeste do país. A mangaba (*Hancornia speciosa* Gomes) e bacaba (*Oenocarpus bacaba* Mart.) são frutas brasileiras abundantes na região Nordeste e Norte, respectivamente, que se destacam pelo valor nutricional e apresentam compostos com atividade biológica que podem contribuir para a saúde dos indivíduos. Devido a sazonalidade e elevada perecibilidade estas frutas são majoritariamente consumidas regionalmente na forma de polpas, e permanecem pouco valorizadas. Diante disso, esta tese foi dividida em 5 etapas sendo 2 etapas experimentais. Estas etapas avaliaram os aspectos tecnológicos (pH, sólidos solúveis totais e acidez titulável) e os aspectos funcionais (viabilidade e bioacessibilidade) durante 48 h de fermentação e armazenamento das polpas de mangaba e bacaba adicionadas de *Lactocaseibacillus casei* (LC1) e *Lactobacillus acidophilus* (LA-05). Também foi analisada o conteúdo de açúcares, ácidos orgânicos, compostos fenólicos e composição de voláteis da polpa de mangaba durante 48 h de fermentação e armazenamento, e o conteúdo de açúcares, ácidos orgânicos e compostos fenólicos da polpa de bacaba durante 48 h de fermentação. Na sequência, determinou-se o perfil sensorial da polpa de mangaba fermentada por meio da metodologia Preferred Attribute Elicitation (PAE). Por fim, foi avaliado o potencial prebiótico das polpas de mangaba e bacaba fermentadas em sistemas *in vitro* de fermentação colônica com a microbiota intestinal de adultos veganos e saudáveis, respectivamente, por meio do sequenciamento do 16S rRNA. Os resultados evidenciam que as polpas de mangaba e bacaba são substratos adequados para manter a viabilidade dos probióticos em contagens ≥ 7 log UFC/g e proteger os probióticos durante exposição a condições gastrointestinais simuladas, garantindo sua viabilidade na fração intestinal. As polpas fermentadas probióticas de mangaba e bacaba apresentaram maior concentração de ácidos orgânicos incluindo láctico e propiônico e de fenólicos como de trans-resveratrol, procianidina B1 e B2, epicatequina e outros. A fermentação probiótica aumentou a bioacessibilidade dos compostos epicatequina, procianidina B1, kaempferol 3-glucosídeo e ácido cafeico na polpa de mangaba e da procianidina B1 e B2, trans-resveratrol, cis-resveratrol, catequina, epicatequina, e miricetina na polpa de bacaba em comparação às polpas não fermentadas. A polpa de mangaba fermentada aumentou a abundância relativa dos grupos *Dorea*, *Romboutsia*, *Faecalibacterium* e a polpa de bacaba, por sua vez, aumentou a abundância de *Acidaminococcales*, *Enterobacterales* e *Lactobacillales*, também associados a produção de metabólitos como butirato, acetato e propionato que têm sido associados a efeitos promotores de saúde. As polpas de fruta fermentadas também impactaram positivamente nos índices de diversidade microbiana resultando em uma microbiota equilibrada e funcional. Na avaliação sensorial da polpa fermentada de mangaba foi observada viabilidade probiótica ao longo de 28 dias, estabilidade dos compostos fenólicos bioacessíveis, atividade antioxidante e manutenção dos parâmetros de qualidade. A polpa de mangaba fermentada recebeu maiores escores que a polpa não fermentada para atributos sensoriais como cor, brilho, frescor e aroma até o final do período de armazenamento, suportando o desenvolvimento de um novo produto funcional. Assim, o desenvolvimento das polpas fermentadas a partir de frutas subexploradas é capaz de agregar valor a esses produtos, permitindo o desenvolvimento de novos alimentos com potencial funcional e benefícios à saúde. Além disso, pode contribuir para o desenvolvimento de pequenas cadeias produtivas focadas no aproveitamento sustentável de frutas nativas brasileiras. Adicionalmente, esta tese resultou na escrita de um artigo de revisão de literatura, um capítulo de livro e uma patente com tema relacionado ao objetivo desta tese que compreendem a discussão científica sobre a valorização de frutas pouco exploradas e seu consumo como alimento com benefícios à saúde.

Palavras-chave: frutas nativas, polpas probióticas, viabilidade probiótica, compostos fenólicos, armazenamento refrigerado, microbiota colônica

ABSTRACT

Brazil has a wide diversity of native fruits that are rich sources of bioactive compounds, yet they remain underexplored, particularly in the North and Northeast regions of the country. Mangaba (*Hancornia speciosa* Gomes) and bacaba (*Oenocarpus bacaba* Mart.) are Brazilian fruits abundant in the Northeast and North regions, respectively, and stand out for their nutritional value. They contain bioactive compounds with potential health-promoting properties. Due to their seasonality and high perishability, these fruits are mainly consumed locally in the form of fruit pulps and remain undervalued. In this context, this thesis was divided into five stages, including two experimental phases. These phases evaluated the technological parameters (pH, total soluble solids, and titratable acidity) and functional aspects (probiotic viability and bioaccessibility) during 48 hours of fermentation and refrigerated storage of mangaba and bacaba pulps inoculated with *Lactocaseibacillus casei* (LC1) and *Lactobacillus acidophilus* (LA-05). Additionally, the contents of sugars, organic acids, phenolic compounds, and volatile composition were analyzed in the mangaba pulp over 48 hours of fermentation and storage, and the contents of sugars, organic acids, and phenolic compounds in the bacaba pulp were analyzed during 48 hours of fermentation. Next, the sensory profile of the fermented mangaba pulp was determined using the Preferred Attribute Elicitation (PAE) methodology. Finally, the prebiotic potential of the fermented mangaba and bacaba pulps was evaluated using *in vitro* colonic fermentation systems inoculated with the gut microbiota of vegan and healthy adults, respectively, through 16S rRNA gene sequencing. The results demonstrated that mangaba and bacaba pulps are suitable substrates to maintain probiotic viability at counts ≥ 7 log CFU/g and to protect probiotics during exposure to simulated gastrointestinal conditions, ensuring their viability in the intestinal fraction. The probiotic fermented pulps of mangaba and bacaba showed higher concentrations of organic acids, including lactic and propionic acids, and phenolic compounds such as trans-resveratrol, procyanidin B1 and B2, epicatechin, and others. Probiotic fermentation enhanced the bioaccessibility of epicatechin, procyanidin B1, kaempferol 3-glucoside, and caffeic acid in mangaba pulp, and of procyanidin B1 and B2, trans-resveratrol, cis-resveratrol, catechin, epicatechin, and myricetin in bacaba pulp, when compared to the non-fermented pulps. Fermented mangaba pulp increased the relative abundance of *Dorea*, *Romboutsia*, and *Faecalibacterium*, while fermented bacaba pulp increased the abundance of *Acidaminococcales*, *Enterobacterales*, and *Lactobacillales*—microbial groups associated with the production of health-promoting metabolites such as butyrate, acetate, and propionate. Fermented fruit pulps also positively influenced microbial diversity indices, resulting in a balanced and functional gut microbiota. Sensory evaluation of fermented mangaba pulp revealed probiotic viability over 28 days, stability of bioaccessible phenolic compounds, antioxidant activity, and maintenance of quality parameters. The fermented mangaba pulp received higher scores than the non-fermented version for sensory attributes such as color, brightness, freshness, and aroma throughout the storage period, supporting the development of a new functional product.

Keywords: native fruits, probiotic pulps, probiotic viability, phenolic compounds, refrigerated storage, colonic microbiota

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1 INTRODUÇÃO

A produção de alimentos saudáveis, que contêm nutrientes valiosos que promovem benefícios à saúde e reduzem o risco de doenças, aumentou recentemente (LAOPHONGPHIT *et al.*, 2024). O Brasil tem sido destaque pela grande biodiversidade de frutas comestíveis nativas, porém algumas ainda inexploradas e comercialmente indisponíveis. Estas frutas têm despertado crescente interesse em sua exploração devido ao potencial tecnológico e agroindustrial, alto valor nutricional e características únicas como cor, sabor e aroma (RODRIGUES *et al.*, 2023). Dentre a grande variedade de frutas nativas, destacam-se a mangaba (*Hancornia speciosa* Gomes) e bacaba (*Oenocarpus bacaba* Mart.) (REIS *et al.*, 2022; MORAIS *et al.*, 2022).

A mangaba possui alto teor de compostos fenólicos e boa capacidade antioxidante, sendo ácido clorogênico encontrado na maior concentração, seguido pelo ácido p-cumárico e ácido ferúlico (BARRETO *et al.*, 2024). A bacaba apresenta alto teor de flavonóides totais e fenólicos em sua polpa, em destaque para os compostos procianidina B2, epicatequina e catequina (ASSIS *et al.*, 2025). Essas frutas são sazonais e tem uma vida útil curta devido à sua natureza perecível e suscetibilidade a patógenos pós-colheita (ARRUDA; ARAÚJO; MAROSTICA JUNIOR, 2022; SANTOS *et al.*, 2021). Devido a isto, estas frutas são consumidas na forma de produtos derivados, como as polpas, e sua exploração ocorre por meio da agricultura familiar com vendas no mercado local, pesquisa científica e pequenas indústrias (RODRIGUES *et al.*, 2023).

A crescente demanda do consumidor por produtos probióticos não lácteos impulsionou a pesquisa em veículos alternativos, particularmente matrizes vegetais, que oferecem benefícios adicionais à saúde devido aos seus compostos bioativos. Essa mudança é motivada por fatores como intolerância à lactose, alergias à proteína do leite e a crescente adoção de dietas vegetais (SUZUKI *et al.*, 2024). Estudos publicados têm demonstrado que a fermentação de polpas de frutas utilizando probióticos, pode contribuir com ingredientes funcionais e prolongar a vida de prateleira da fruta (ASSIS *et al.*, 2024).

Além disso, o consumo de frutas está associado a um estilo de vida saudável e reduz a incidência de doenças crônicas e degenerativas (SAUER *et al.*, 2024). Esses benefícios estão relacionados às propriedades antioxidantes, anti-inflamatórias e citoprotetoras dos compostos bioativos encontrados nesses alimentos, incluindo fenólicos, carotenoides, ácido ascórbico e tocoferóis (BORGES *et al.*, 2022). A estratégia tecnológica de fermentação de frutas com bactérias probióticas tem sido sugerida como um método de grande valor para melhorar as propriedades nutritivas e bioativas das matrizes alimentares (ASSIS *et al.*, 2021).

Há estudos que relataram o efeito benéfico da fermentação com diferentes probióticos em matrizes como purês de seriguela, mangaba, manga-rosa e acerola fermentadas com *Lactobacillus casei* 01 e *Lactobacillus acidophilus* 05 (ASSIS *et al.*, 2021), polpa de manga fermentada com *Lactobacillus paracasei* OR1 (MK252696), PP1

(MK253713), *Lacticaseibacillus rhamnosus* MA3 (MK253673) e PP3 (MK253673) (LAOPHPNGPHIT; SIRIPORNADULSIL; SIRIPORNADULSIL, 2023), polpa de goiaba fermentada com *Lactococcus lactis* subsp *lactis* e *Lactobacillus casei* LA-1 (KAUR; GHOSH, 2023), polpa de lichia fermentada por *Lactobacillus plantarum*, *Lactobacillus rhamnosus* e uma cepa mista dos dois (TANG *et al.*, 2023), polpa de kiwi fermentada com *Lactiplantibacillus plantarum* subsp. *Plantarum* (ZHOU *et al.* 2020), e mais atualmente polpa de mangaba fermentada com *Lacticaseibacillus casei* 01 (ASSIS *et al.*, 2024) e polpa de bacaba fermentada com *Lactobacillus acidophilus* 05 (LA-05) and *Lacticaseibacillus casei* 01 (LC1) (ASSIS *et al.*, 2025).

Os processos fermentativos podem ser uma estratégia eficaz para aumentar a bioacessibilidade de polifenóis, os níveis antioxidantes de produtos de frutas e vegetais, e relevantes para a reutilização de componentes da fruta (geralmente desperdiçados após o processamento) com valor biológico suficiente, com indicativo de um histórico seguro de consumo (KAUR; GHOSH, 2023). Por conseguinte, as culturas probióticas utilizadas durante o processo de fermentação podem biotransformar os compostos fenólicos de frutas e derivados, liberando compostos fenólicos conjugados com outros constituintes da matriz alimentar, e gerar compostos com maior bioatividade do que suas estruturas precursoras (YAPA *et al.*, 2023). E isto impõe um impacto positivo na função imunológica, digestão, metabolismo e comunicação cérebro-intestino, embora o efeito das culturas probióticas na biotransformação dos compostos fenólicos pode depender da cepa probiótica e do tipo de fruta (YAPA *et al.*, 2023).

No mais, a microbiota intestinal pode realizar a biotransformação de compostos fenólicos, porém, o consumo de produtos fermentados com compostos fenólicos mais bioacessíveis pode proporcionar efeitos benéficos independente da microbiota do hospedeiro (MACEDO *et al.*, 2023; PAN *et al.*, 2023). Estudos usando modelos colônicos *in vitro*, aplicando a microbiota fecal para metabolizar alimentos e determinar a fração dos compostos biotransformada por esses microrganismos bem como estudos que apresentem o potencial destes produtos como alimentos funcionais em indivíduos submetidos a dieta saudável ou vegana ainda são escassos na literatura. Assim, a importância das frutas nativas, para elaboração de produtos funcionais tanto para alimentar comunidades locais no Brasil quanto para a atual demanda do mercado internacional por novos produtos com sabores e propriedades funcionais, não pode ser subestimada.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar os efeitos do metabolismo dos probióticos *Lactobacillus casei* LC1 e *Lactobacillus acidophilus* LA-05 em inóculo simples no perfil e bioacessibilidade de compostos fenólicos em polpa de mangaba e bacaba, e o potencial prebiótico das polpas de frutas fermentadas em sistemas *in vitro* de fermentação colônica.

2.2 OBJETIVOS ESPECÍFICOS

- I. Avaliar os aspectos tecnológicos (pH, sólidos solúveis totais e acidez titulável) e os aspectos funcionais (viabilidade e bioacessibilidade) durante 48 h de fermentação e armazenamento das polpas de mangaba e bacaba;
- II. Analisar o conteúdo de açúcares, ácidos orgânicos, compostos fenólicos e composição de voláteis da polpa de mangaba durante 48h fermentação e armazenamento, e o conteúdo de açúcares, ácidos orgânicos e compostos fenólicos da polpa de bacaba durante 48h fermentação;
- III. Determinar o perfil sensorial da polpa de mangaba fermentada por meio da metodologia Preferred Attribute Elicitation (PAE);
- IV. Avaliar o potencial prebiótico das polpas de mangaba e bacaba fermentadas em sistemas *in vitro* de fermentação colônica, ou seja, o efeito na modulação da microbiota intestinal de adultos veganos e saudáveis, respectivamente, por meio do sequenciamento do 16S rRNA.

3 REVISÃO DE LITERATURA

3.1 *Hancornia speciosa* Gomes

A *Hancornia speciosa*, popularmente conhecida como mangabeira, é uma planta nativa do Brasil que pertence ao grupo *Eudicotiledoneas*, ordem *Gentianales* e à família das *Apocynaceae* (REIS *et al.*, 2022). Esta planta é encontrada nas regiões Norte, Nordeste, Centro-Oeste e parte do Sudeste do Brasil, abrangendo biomas brasileiros como Cerrado, Caatinga, Pantanal e parte da Mata Atlântica, como também é encontrada em alguns países da América do Sul, como Peru, Bolívia e Paraguai (ASSIS *et al.*, 2021).

A mangabeira possui grande importância devido ao valor nutricional de seus frutos e às suas propriedades medicinais e biotecnológicas, por apresentar em sua composição nutrientes essenciais, que contribuem significativamente com benefícios à saúde (REIS *et al.*, 2022). A fruta fresca é a forma de consumo mais citada na literatura, seguida pelas polpas congeladas instantaneamente ou pelo congelamento convencional e na forma de polpas ou purês fermentados (GONÇALVES *et al.*, 2020; ASSIS *et al.*, 2021; ASSIS *et al.*, 2024).

Uma única árvore de mangaba pode produzir mais de 100 kg de frutos por colheita (REIS *et al.*, 2022), e quando consideramos os sólidos solúveis, é fundamental a aplicação de tecnologias para manter a qualidade nutricional, microbiológica e de segurança desse alimento. Os períodos de safra da mangabeira acontecem entre outubro e março, conhecidos como "frutos de verão", e de junho a agosto, chamados de "frutos de inverno". Contudo, é possível encontrar mangabas esporadicamente em outras épocas do ano (PINHEIRO *et al.*, 2018). A exploração das mangabas ainda é predominantemente extrativista, sem a aplicação de qualquer método de manejo durante a produção (SANTOS *et al.*, 2022).

Figura 1. *Hancornia speciosa* Gomes



Fonte: Autor (2025).

A mangaba possui um formato arredondado, casca lisa e verde, quando madura adquire uma cor amarelo-alaranjado. Seu sabor é uma combinação de doce e ácido, com um aroma característico. O formato da fruta é do tipo baga que apresenta forma elíptica e/ou arredondada com peso, diâmetro longitudinal e diâmetro transversal variando entre 12,55 g e 48,42 g, 3,03 cm e 4,34 cm, e 2,84 cm e 4,34 cm, respectivamente. Cada fruta possui, em geral, de 2 a 15 sementes discóides, achatadas e de cor marrom claro, medindo de 7 a 8 mm de diâmetro (ARRUDA; ARAÚJO; MAROSTICA JUNIOR, 2022).

Estudos sobre a mangaba relataram esta fruta nativa é caracterizada por apresentar baixo pH (3,29 – 3,41) e acidez titulável total (2,75 – 2,78 g ácido cítrico/100 g), e altos valores de sólidos solúveis totais (18,13 – 18,93 °Brix) (ASSIS *et al.*, 2024). A mangaba apresenta baixo teor de lipídios (0,55–1,70%), carboidratos (2,30–10,97%) e energia (29,83–72,30 kcal/100 g), além de relevante teor de fibra alimentar (11,60%), com alto potencial para ser incluída na dieta humana (ARRUDA; ARAÚJO; MAROSTICA JUNIOR, 2022). Devido à sazonalidade e alta perecibilidade da mangaba, seu consumo é baseado principalmente em produtos derivados, como a polpa (SANTOS *et al.*, 2022).

Em relação às fibras alimentares presentes na mangaba, o valor médio obtido (11,60-13%) por Reis *et al.* (2022) e Arruda, Araújo e Marostica Junior (2022), pode levar a exploração da fruta pela indústria alimentícia para o desenvolvimento de alimentos funcionais, uma vez que a ingestão diária de fibras alimentares tem sido associada à saúde e ao bem-estar humano, contribuindo para a prevenção de algumas doenças, como obesidade, diabetes, disfunções cardiovasculares e do trato gastrointestinal.

Outros compostos foram investigados e contribuem significativamente para as particularidades de sabor da mangaba e, conseqüentemente, os seus produtos derivados (TEXEIRA *et al.*, 2020), entre eles estão os compostos voláteis. Na polpa de mangaba foram identificados cerca de oitenta compostos por GC-MS, compreendendo ésteres (30), terpenos (25), aldeídos (11), álcoois (7), cetona (4) e outros (3). A polpa de mangaba apresentou maior concentração de alguns ésteres (acetato de etila, acetato de butila, acetato de 3-metil-3-butenila, acetato de 2-metil-2-butenila, dodecanoato de etila e tetradecanoato de etila), terpenos (p-cimeno e terpinoleno), aldeído (2-nonenal), álcool (3-metil-2-butenol) e cetona (acetoina) (ASSIS *et al.*, 2024).

Além disso, a mangaba tem sido explorada para o desenvolvimento de produtos funcionais com intuito de melhorar a saúde dos consumidores (REIS *et al.*, 2022). Estudos têm demonstrado que a fruta é fonte de diversos nutrientes e compostos bioativos, principalmente os compostos fenólicos ácido clorogênico, rutina, quercetina, kaempferol, ácido p-cumárico, ácido ferúlico, e outros vinte e três identificados por cromatografia líquida (YAMASHITA *et al.*, 2020; DUTRA *et al.*, 2017; BARRETO *et al.*, 2024; PÉREZ-GÁLVEZ; VIERA; ROCA, 2020; CARITÁ *et al.*, 2020; FIERS; LEONARDI; ILIEV, 2020; GONÇALVES *et al.*, 2020, ASSIS *et al.*, 2024). O ácido ascórbico e outros elementos essenciais na dieta como cálcio, zinco, ferro, carotenóides (β -caroteno,

β -criptoxantina, além de α -tocoferol e α -, β - e γ -tocotrienóis) são encontrados na fruta, indicando que o ácido ascórbico e os ácidos fenólicos são relevantes na percepção sensorial dos gostos amargo e ácido, e do sabor adstringente, dependendo de suas concentrações presentes na fruta (ISSAOUI *et al.*, 2020).

Alguns estudos que apresentam o perfil de compostos fenólicos e atividade antioxidante da mangaba estão listados na Tabela 1. Na Tabela 1 estão apresentados os compostos fenólicos identificados na polpa de mangaba com suas respectivas atividades biológicas.

Tabela 1. Compostos fenólicos identificados na polpa de mangaba com suas respectivas atividades biológicas

Compostos identificados	Atividade Biológica	Referências
Quercetina, rutina, quercetina-3- O- hexosídeo, quercetina-3- O- raminogalactosídeo, Quercetina -3- O -galactosídeo, quercetina-pentosídeo e avicularina	Propriedades antioxidantes, antiinflamatórias, antiproliferativas, anticancerígenas, antidiabéticas, antivirais e antiagregante da quercetina, bem como sua utilização no tratamento de doenças neurodegenerativas, câncer, distúrbios cardiovasculares, diabetes, reações alérgicas, inflamação e artrite	SANTOS <i>et al.</i> , 2022
Kaempferol, kaempferol-hexosídeo, kaempferol-3- O-(p - cumaroil)-ramnogalactosídeo e kaempferol-3- O-rutinosídeo	Melhora o sistema de defesa antioxidante e atua na modulação de componentes-chave das vias de transdução celular relacionadas à angiogênese, apoptose, metástase e inflamação, protegendo células normais e inibindo a proliferação de células cancerígenas	SANTOS <i>et al.</i> , 2022
Miricetina	Agente gastroprotetor, antidiabético, antiinflamatório, antiaterosclerótico, antioxidante, hipoglicêmico e citoprotetor eficaz	ZITHA <i>et al.</i> , 2022
Catequina	A alta ingestão de catequina foi associada a um menor risco de desenvolver câncer de pâncreas, câncer de esôfago e diabetes tipo 2, biomarcadores de estresse oxidativo reduzidos, e menor mortalidade por doenças cardiovasculares	ZITHA <i>et al.</i> , 2022
Ácido p -cumárico, ácido m-cumárico, ácido trans-clorogênico, ácido cis-clorogênico, ácido isoclorogênico, ácido 1-O-cafeoilquínico, ácido cafeico, ácido trans-orto-cumárico	O ácido cafeico relacionado a propriedades antioxidantes, incluindo a prevenção de inflamações, cânceres, diabetes e doenças neurodegenerativas.	YAMASHITA <i>et al.</i> , 2020

e éster metílico do ácido 5- O-cafeoilquínico	O ácido <i>p</i> -cumárico tem atividades antimutagênica, antígeno tóxica e antimicrobiana, inibe a melanogênese celular, regula o sistema imunológico e reduz a peroxidação do colesterol LDL e o risco de câncer de estômago	
Ácido ferúlico, ácido <i>trans</i> -3-feruloilquínico, e ácido 5-feruloilquínico	O ácido ferúlico é um fitoquímico que possui atividade antioxidante, antiinflamatória, antimicrobiana, antialérgica, hepatoprotetora, anticancerígena, antitrombótica, antiviral e vasodilatadora, além de ter a capacidade de aumentar a viabilidade espermática e modular a atividade enzimática e expressão gênica	YAMASHITA <i>et al.</i> , 2020
Ácido gálico	Propriedades anti-inflamatória, antibiótica, anticarcinogênica, antioxidante e cardioprotetora	DUTRA <i>et al.</i> , 2017
Ácido elágico	Atividades antioxidante, antimutagênica, antiinflamatória e anticancerígena	DUTRA <i>et al.</i> , 2017
Ácido siríngico	Efeitos antioxidantes, antimicrobianos, anticarcinogênicos, anti-inflamatórios, antidiabéticos, cardioprotetores, hepatoprotetores e neuroprotetores	DUTRA <i>et al.</i> , 2017
Ácido protocatecúico, ácido protocatecúico e ácido gentísico	Anti-inflamatórios, antígeno tóxicos, anticarcinogênicos, antidiabéticos, anti-hiperlipidêmicos, antimicrobianos, antioxidantes, hepatoprotetores e neuroprotetores	ALMEIDA <i>et al.</i> , 2021
Ácido salicílico	Potente agente analgésico e anti-inflamatório e é amplamente utilizado no tratamento de doenças reumáticas e na prevenção de trombose arterial coronária e cerebrovascular	ALMEIDA <i>et al.</i> , 2021

Fonte: Autor (2025)

Com isso, a mangaba tem demonstrado grande potencial para ser utilizada como matéria-prima para produtos inovadores. Portanto, a parte comestível (polpa) da mangaba pode ser um novo ingrediente funcional para o desenvolvimento de alimentos com a presença de compostos bioativos.

3.2 *Oenocarpus bacaba* Mart.

A Amazônia e o Cerrado são biomas brasileiros que abrigam uma notável biodiversidade, com uma variedade de palmeiras (BORGES *et al.*, 2022). O bioma Cerrado é o segundo maior bioma do Brasil e a savana mais biodiversa do mundo, engloba uma grande variedade de frutas que possuem propriedades nutricionais e terapêuticas ainda pouco exploradas (MORAIS *et al.*, 2025). Dentre a ampla diversidade de frutas comestíveis nativas destes biomas, destaca-se o gênero *Oenocarpus*, com existência de duas espécies, conhecidas popularmente como bacabeiras: a bacaba-de-leque (*Oenocarpus distichus* Mart.) e a bacaba ou bacabão (*Oenocarpus bacaba* Mart.), ambas de porte arbóreo e monocaules (MORAIS *et al.*, 2022).

Figura 2. *Oenocarpus bacaba* Mart.



Fonte: Jaqueline Araújo (Arquivo próprio).

No Peru, a bacaba é chamada de ungurauy, na Colômbia de punáma e na Guiana Francesa de camou (MORAIS *et al.*, 2022). É uma palmeira que mede entre 7 e 20 m de comprimento, 15 a 25 cm de diâmetro e possui palmito curto e grosso no ápice. As folhas pinadas, geralmente possuem entre 8 a 17 cm, são regularmente agrupadas e dispostas em diferentes planos (CÓL *et al.*, 2021). Os cachos são robustos, com frutos arredondados de cor púrpura escura e uma semente (NASCIMENTO *et al.*, 2019). Os frutos são comestíveis do tipo baga, redondos na cor púrpura com cerca de 1,4 a 2,0 cm de diâmetro e 1,5 a 3,0 g. O epicarpo maduro tem coloração púrpura escura, enquanto o mesocarpo tem de 1,0 a 1,5 mm de espessura, é mais claro, oleoso e fibroso (CÓL *et al.*, 2021).

A fruta bacaba é sazonal, seu período de colheita se estende de abril a dezembro e apresenta-se como uma opção de mercado prospectivo, sendo a colheita longa e produtiva, podendo chegar a 8 kg por planta/ano (MORAIS *et al.*, 2022). Sua exploração é realizada de forma extrativista e em particular, essa espécie tem despertado interesse devido às suas propriedades e potencial para uso sustentável (CÓL *et al.*, 2021). Os frutos da bacaba são frequentemente consumidos em bebidas consideradas saudáveis. Devido à sua rica composição, frutas nativas brasileiras como a bacaba podem ser incorporadas a probióticos para potencializar suas propriedades funcionais (ASSIS *et al.*, 2025).

Com relação ao processamento da bacaba, essa fruta quando adicionada de água produz uma bebida oleaginosa e mucilagínosa de cor branco-amarelada, conhecida como vinho de bacaba ou leite de bacaba (PUERARI *et al.*, 2015). A polpa tem se destacado por apresentar alto potencial para ser usada como ingrediente funcional na aplicação alimentícia, devido alto teor de lipídios, fibras, ácidos fenólicos, flavonoides principalmente antocianinas, considerável atividade antioxidante e por apresentar teores comparáveis ou superiores a de outras frutas da mesma família, como a do açaí (CÓL *et al.*, 2021; OLIVEIRA *et al.*, 2019). Além da polpa, a fruta é usada na extração de óleo de excelente composição nutricional, rico em fibras, proteínas e carboidratos, fonte de antocianina e antioxidantes, semelhante ao azeite de oliva (FREITAS *et al.*, 2021).

A bacaba contém carotenoides (em torno de 1,4 mg/100 g de óleo), vitamina C (30 mg/100 g de polpa) e outros flavonoides como catequina, epicatequina, quercetina 3-glicosídeo e procianidina B₂ (ASSIS *et al.*, 2025). Estudos recentes sobre a bacaba revelaram que essa oleaginosa é rica em PUFA's ômega-3 e -6, com seus efeitos concentrados no controle de processos inflamatórios, proteção cardiovascular e controle do estresse oxidativo, e por serem ricas em compostos bioativos podem ajudar a prevenir doenças crônicas e o envelhecimento (OLIVEIRA *et al.*, 2019; NASCIMENTO *et al.*, 2019; SANTOS *et al.*, 2021b). Pesquisas relatam que a bacaba pode causar inibição ou redução do processo de carcinogênese, por exercer inibição da proliferação celular por meio da indução do apoptose e além disso, pode ser até classificada como superalimento, por ser fonte promissora de antioxidantes naturais com relevância biológica na adipogênese (COSTA *et al.*, 2017; LAUVAI *et al.*, 2017). Na tabela 2, são apresentados estudos de compostos bioativos e potenciais efeitos biológicos da bacaba na saúde.

Tabela 2. Estudos de compostos bioativos e potenciais efeitos biológicos da bacaba na saúde

Matéria-prima	Compostos identificados	Atividade Biológica	Referências
Fruta	Compostos bioativos	Atividade antioxidante	MORAIS <i>et al.</i> (2022)
Óleo de polpa de fruta	Fenólicos totais e PUFA's	Atividade antioxidante	PINTO <i>et al.</i> (2018)
Pó da fruta	Fenólicos totais e antocianinas	Atividade antioxidante	NASCIMENTO <i>et al.</i> (2019)
Polpa de fruta	Fenólicos flavonoides ^e	Propriedades antioxidantes	CARVALHO <i>et al.</i> (2016)
Polpa de fruta	Fitoquímicos	Atividade enzimática e antioxidante	SANTOS FILHO <i>et al.</i> (2020)
Extrato de polpa de fruta	Compostos fenólicos	Capacidades antiproliferativas; apoptose induzida em células de câncer de mama MCF-7 através da via mitocondrial; caspase-9 de maior ativação	FINCO <i>et al.</i> (2016)

Extrato da polpa	fenólico	Compostos fenólicos	Acúmulo reduzido de lipídios intracelulares e expressão de proteínas de marcadores adipogênicos, incluindo PPAR γ , C/EBP α , FABP4, IR- β e adiponectina; diminuição do acúmulo de lipídeos; inibição da adipogênese.				LAUVAI <i>et al.</i> (2017)
Extratos de polpa aquosa, metanólica e acetônica		Compostos e fenólicos	Propriedades antioxidantes e proteção contra danos ao DNA.				LEBA <i>et al.</i> (2014)

Fonte: Autor (2025)

Considerando a bacaba uma fruta de alto valor nutricional e potencial funcional, existe a necessidade de desenvolver estudos sobre os compostos fenólicos bioacessíveis desta fruta, visto que novas informações sobre o potencial uso da bacaba como polpa funcional pode beneficiar a saúde humana. A importância das frutas nativas com sabores diferenciados e propriedades funcionais, tanto para a alimentação das comunidades locais no Brasil, quanto para a atual demanda do mercado internacional não pode ser subestimada.

3.3 POLPAS DE FRUTAS

O Brasil possui uma grande variedade de frutas, sendo classificado como o terceiro maior produtor mundial com uma produção de 122,7 milhões de toneladas de frutas em 2023. Estes números, no entanto, representam apenas uma fração das frutas vendidas como produtos de exportação (ANUÁRIO BRASILEIRO DE FRUTICULTURA, 2024). A fruticultura é o segmento presente em todos os Estados brasileiros, gerando cerca de 5,6 milhões de empregos, com plantações que englobam mais de 2 milhões de hectares, caracterizada pela geração de renda e desenvolvimento do país (ANUÁRIO BRASILEIRO DE FRUTICULTURA, 2024).

As frutas brasileiras nativas apresentam uma boa aceitação no mercado interno e, principalmente no mercado externo, devido suas características sensoriais únicas e sua elevada concentração de bioativos que podem gerar benefícios à saúde do consumidor (ASSIS *et al.*, 2021). A presença destes compostos favorece a sua valorização e permite atingir um mercado mais específico como o de produtos com propriedade funcional, proporcionando exploração pela agroindústria (ASSIS *et al.*, 2024). Devido às alegações de atividade antioxidante, anti-inflamatória, hipocolesterolêmica e entre outras, seus efeitos têm sido relacionados ao alto teor de vitaminas, carotenóides e polifenóis presentes nestas frutas (COPETTI *et al.*, 2020; BOEING *et al.*, 2020; CASTRO *et al.*, 2020; LAKMAL *et al.*, 2021).

As frutas são comumente consumidas *in natura*, entretanto, a disponibilidade sazonal e a alta atividade de água, causa elevada perecibilidade o que dificultam a sua comercialização

(ALMEIDA *et al.*, 2021). A utilização de métodos tecnológicos de processamento de alimentos visando o prolongamento da vida útil da fruta e de seus produtos alimentícios derivados são necessidades resultantes de sua elevada produção e peculiaridades físico-químicas. Além do congelamento da polpa de fruta, a pasteurização é uma tecnologia amplamente utilizada pela indústria para inativar enzimas e microrganismos deteriorantes dos alimentos, tal processo pode causar alterações sensoriais na fruta, porém, essas alterações não influenciam na aceitabilidade sensorial da polpa da fruta para consumo até um ano de armazenamento congelado (GONÇALVES *et al.*, 2020). Diante disso, o processamento de frutas em polpas de frutas torna-se uma alternativa utilizada para ampliar o consumo destes alimentos e proporcionar a comercialização em distintos mercados (SANTOS *et al.*, 2022).

As polpas de frutas podem ser uma fonte muito rica de compostos, que possuem efeitos anti-inflamatórios, antimutagênicos, anticolesterolêmico, antialérgicos, anticancerígenos, antimicrobianos e antiglicêmicos (GÜZEL *et al.*, 2019), e de fibras alimentares, que são um dos pilares de uma alimentação saudável e equilibrada, pois têm um efeito positivo em doenças como distúrbios gastrointestinais, câncer de cólon, constipação, diabetes, doenças cardiovasculares e elevado colesterol (ERGENE *et al.*, 2019). As polpas também são uma boa fonte de enzimas, vitaminas, óleos e componentes bioativos, como polifenóis e carotenoides (HANOĞLU; KARAOĞLU; BEDİR, 2023).

Com isso, pela definição polpa de fruta é caracterizada como produto não fermentado, não concentrado e não diluído, com teor mínimo de sólidos totais, e obtido a partir da parte comestível do fruto (BRASIL, 2000). A polpa de fruta é uma alternativa prática para o consumo, que se diferencia de outros produtos por possuir sua composição e características sensoriais semelhantes ao do fruto fresco. No Brasil a polpa de fruta destinada a produção e consumo da bebida é regulamentada pela Instrução Normativa Nº 1 de 7 de janeiro de 2000 (BRASIL, 2000), e devido ao seu mercado em expansão, está em andamento uma consulta pública para atualização dessa regulamentação, através da Portaria Nº 58, de 30 de agosto de 2016 (BRASIL, 2016), que inclui frutas nativas e exóticas ainda não contemplados na legislação vigente.

3.3.1 Fermentação de polpas de frutas por probióticos

Devido ao crescente conhecimento público das vantagens dos probióticos para a saúde, preservando o equilíbrio da microbiota intestinal e melhorando as defesas da mucosa contra agentes patogênicos, a procura por alimentos funcionais contendo microrganismos com qualidades probióticas está a aumentar rapidamente (MANOJ *et al.*, 2023). As polpas de frutas, geralmente consumidas e consideradas produtos saudáveis, podem servir como meio alternativo para a incorporação de bactérias probióticas, visto que incluem muitos carboidratos, minerais e vitaminas que os probióticos podem usar como substrato (GOMES *et al.*, 2021).

O consumo de polpas de frutas fermentadas com probióticos poderia melhorar a nutrição humana pela ingestão equilibrada de vitaminas, minerais e carboidratos, além de prevenir doenças dependendo das propriedades probióticas da cultura iniciadora (ASSIS *et al.*, 2021). Além disso, algumas polpas fermentadas contêm pigmentos coloridos, como flavonóides, licopeno, antocianina, β -caroteno e glucosinolatos, que atuam como compostos antioxidantes no corpo, ajudando a eliminar radicais livres nocivos envolvidos em doenças degenerativas (GARCIA *et al.*, 2020).

Assim, para realizar fermentações de polpas de frutas com sucesso, a seleção do probiótico a ser utilizado como cultura iniciadora deve ser baseado principalmente em critérios tecnológicos, sensoriais e/ou nutricionais (DI CAGNO; FILANNINO; GOBBETTI, 2017). Entre as características tecnológicas desejáveis, a cultura probiótica deve ser adaptada à matriz da fruta para crescer rapidamente e acidificar o meio mesmo em baixos valores de pH e temperatura, e devem tolerar e/ou metabolizar compostos fenólicos (RUIZ RODRÍGUEZ *et al.*, 2021). A cultura deve ter a capacidade de fermentar eficazmente diversos substratos de carboidratos, aumentando a densidade e melhorando a biodisponibilidade de nutrientes, garantindo um processo de fermentação completo e bem-sucedido. É também desejável que o probiótico seja capaz de sintetizar compostos antimicrobianos, aumentando a segurança e a extensão do prazo de validade por meio da eliminação de microrganismos deteriorantes e patógenos de origem alimentar (GARCIA *et al.*, 2020; SZUTOWSKA, 2020).

Quanto aos critérios sensoriais, a presença de compostos de aromas em polpas de frutas fermentadas tem um impacto crucial na qualidade sensorial e na aceitação do produto final pelo consumidor. Espera-se que a cultura selecionada sintetize compostos de impacto odorífero ou seus precursores, como açúcares, aminoácidos, lipídeos, pigmentos, permitindo a obtenção de polpas fermentadas com sabores e odores satisfatórios e sabores desagradáveis reduzidos, como aldeídos indesejáveis (SZUTOWSKA, 2020).

Em relação às características metabólicas funcionais é preferível a seleção de cultura probiótica capaz de produzir exopolissacarídeos (EPS). Esses compostos melhoram a reologia das polpas fermentadas principalmente devido à sua alta viscosidade, afetando positivamente a percepção do produto final. Certos EPS apresentam algumas propriedades funcionais, como atividades imunomoduladoras, anticancerígenas, antioxidantes e antibacterianas, e podem aumentar a colonização intestinal por probióticos (AYED; M'HIR; HAMDI, 2020). Além disso, a cultura probiótica pode aumentar a atividade antioxidante da matriz e ser capaz de produzir ou liberar compostos bioativos como peptídeos, vitaminas, aminoácidos e fenólicos (AYED; M'HIR; HAMDI, 2020; GARCIA *et al.*, 2020; SZUTOWSKA, 2020).

Alguns estudos que envolvem a conversão de compostos fenólicos através do processo de fermentação foram relatados na literatura, como por Zhou *et al.* (2020) ao avaliarem a biotransformação de fenólicos em polpa de kiwi induzida pela fermentação por *Lactiplantibacillus plantarum* subsp. *plantarum*; Filannino *et al.* (2020) ao investigarem a capacidade de bactérias

láticas autóctones selecionadas em enriquecer o portfólio de compostos bioativos do abacate (*Persea americana* Mill.); e Yang *et al.* (2022) ao objetivarem obter uma cepa adequada com forte capacidade de transformação de polifenóis na polpa de pêssgo e investigar sua taxa de crescimento e metabolismo no substrato durante a fermentação. Como principais resultados encontrados, observou-se que os teores de fenólicos e flavonóides aumentaram após a fermentação. A fermentação resultou em melhorias na produção de álcoois, aldeídos e no conteúdo de compostos aromáticos, como levou a um aumento acentuado da atividade antioxidante.

Com isso, a etapa de fermentação é uma tecnologia simples e valiosa, de baixo custo e um processo sustentável para manter e/ou melhorar as propriedades nutricionais e sensoriais das matérias-primas e para prolongar a vida útil de frutas e polpas em condições de segurança sanitária (DI CAGNO; FILANNINO; GOBBETTI, 2017). A biotransformação de compostos fenólicos que ocorre durante este processo, auxilia na liberação de fenólicos conjugados, melhorando a bioacessibilidade. E o estudo da bioacessibilidade de fenólicos é importante para estimar o impacto da digestão gastrointestinal das matrizes alimentares nas mensurações subsequentes da biodisponibilidade destes compostos (ASSIS *et al.*, 2021).

Alguns aspectos devem ser considerados quanto ao estudo da bioacessibilidade. Os fatores que causam variações no conteúdo dos compostos fenólicos ao longo da exposição às diferentes etapas da digestão, que podem ser atribuídas a algumas propriedades físicas e bioquímicas do trato gastrointestinal, como alterações de pH, atividade enzimática e temperatura (BARAK *et al.*, 2019). A estrutura química dos compostos, complexidade da matriz alimentar, presença de fibras, conteúdo protéico e uma possível interação molecular com os compostos fenólicos, bem como a presença de inibidores ou potencializadores de absorção, tipo e quantidade dos outros compostos ingeridos, estado fisiológico e tempo de esvaziamento gástrico ou de trânsito intestinal, também influenciam na bioacessibilidade dos compostos fenólicos (SANTIAGO, 2018).

Diversas metodologias têm sido utilizadas para avaliar a bioacessibilidade de fenólicos que incluem modelos *in vivo* em animais e humanos, e modelos *in vitro* como a digestão simulada do trato gastrointestinal (ASSIS *et al.*, 2024). A digestão gastrointestinal *in vitro* simula algumas condições gastrointestinais biológicas (enzimas gástricas e pancreáticas) e químicas (pH, temperatura e sais biliares), como também avalia o quão estáveis e absorvidos são os compostos fenólicos e se há alteração da atividade antioxidante durante a digestão (DUTRA *et al.*, 2017). São modelos de digestão que compreendem as fases oral, gástrica, intestinal e, ocasionalmente, fermentação colônica (GONG *et al.*, 2019). As metodologias *in vitro* têm sido desenvolvidas a fim de possibilitar a investigação do efeito da matriz alimentar sobre a bioacessibilidade de fenólicos, de modo a obter resultados para apoiar reivindicações de relevância biológica desses compostos (PÉREZ-BURILLO *et al.*, 2021).

Estudos sobre a bioacessibilidade de fenólicos foram descritos por Morais *et al.* (2019) ao analisarem o efeito de probióticos no conteúdo de fenólicos em polpa de pitaya vermelha. Mashitoa

et al. (2021a) ao escreverem o impacto da utilização de diferentes cepas de bactérias lácticas (BAL) para a fermentação de purê de mamão e seu efeito nos parâmetros de qualidade e bioacessibilidade de compostos fenólicos durante simulação da digestão gastrointestinal *in vitro*. Mashitoa *et al.* (2021b) ao investigarem as alterações dos principais compostos fenólicos em purê de manga pasteurizado fermentado com diferentes cepas de bactérias lácticas e a bioacessibilidade de diferentes compostos fenólicos durante a digestão gastrointestinal *in vitro*. Assis *et al.* (2021) ao avaliarem o efeito da fermentação de purês de frutas (seriguela, mangaba, manga e acerola) com LA-05 e LC-01, e Kaur e Ghosh (2023) ao analisarem a polpa de goiaba após fermentação com cepas selecionadas de BAL. Os estudos revelaram que a fermentação com culturas probióticas aumentam substancialmente o teor de fenólicos e a bioacessibilidade, e o co-cultivo de ambas as cepas probióticas apresentam resultados promissores para um maior teor e bioacessibilidade de compostos fenólicos e maior atividade antioxidante.

Além do mais, há alguns poucos estudos que utilizam polpas de frutas usando modelos colônicos *in vitro*, aplicando a microbiota fecal para determinar o potencial prebiótico e a fração dos compostos biotransformada por esses microorganismos. Nesta perspectiva, alimentos ricos em compostos fenólicos têm sido investigados quanto aos seus efeitos benéficos na microbiota intestinal humana ligados a propriedades prebióticas (SANDERS *et al.*, 2019).

Alves-Santos *et al.* (2023) demonstraram que a polpa de baru estimulou o crescimento e o metabolismo dos probióticos *Bifidobacterium animalis* subsp. *lactis* BB-12, *Lactobacillus acidophilus* LA-05 e *Lactocaseibacillus casei* L-26. A polpa de baru digerida induziu benefícios significativos à microbiota colônica humana, aumentando a abundância relativa de *Lactobacillus-Enterococcus*, *Bifidobacterium* e *Bacteroides-Provotella*. Os resultados mostram que a polpa de baru apresentou elevado teor de compostos fenólicos (principalmente hesperidina), e possui potenciais propriedades prebióticas a serem exploradas na formulação de novos alimentos promotores a saúde. Martínez *et al.* (2023) avaliaram a polpa de argan, e perceberam que a polpa modulou a microbiota intestinal diminuindo a relação *Firmicutes/Bacteroidetes*, considerando que a polpa de argan pode ser considerada um alimento ou ingrediente funcional com efeitos benéficos em diversos biomarcadores do metabolismo.

Neste contexto, polpas de frutas que podem ser fermentadas por bactérias probióticas constituem uma alternativa promissora para suprir as necessidades mencionadas e promover o consumo de frutas. A fermentação utilizando cepas probióticas poder melhorar o perfil de aroma e sabor junto com o aumento do prazo de validade (NAZHAND *et al.*, 2020; SZUTOWSKA, 2020). Portanto, a presente pesquisa representa uma possibilidade de obtenção de informações preliminares e de conhecimento relevante, indispensáveis à comunidade científica.

3.4 ASPECTOS GERAIS DA MICROBIOTA INTESTINAL HUMANA

O corpo humano é habitado por trilhões de microrganismos cuja atividade metabólica e função coordenada são essenciais para a vida e a saúde. Esses microrganismos podem atingir sua densidade mais alta dentro do compartimento intestinal, aproximadamente $3,8 \times 10^{13}$, excedendo em muito o número de células hospedeiras por um fator de dez, e juntos formam uma grande comunidade microbiana chamada microbioma intestinal (LIU *et al.*, 2023).

A microbiota intestinal é formada principalmente por seis filos: *Firmicutes*, *Bacteroides*, *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia* e *Fusobacteria*. Entre eles, *Firmicutes* e *Bacteroides* são os principais filos, e as proporções desses dois filos bacterianos podem variar sob diferentes condições fisiológicas e patológicas (LIU *et al.*, 2023). As proporções de *Firmicutes* para *Bacteroides* são frequentemente consideradas um marcador preditivo para avaliar o estado de saúde e doença (HOU *et al.*, 2022). Em percentagem, esses dois filos representam 90% da constituição total do filo bacteriano e *Actinobacteria*, *Proteobacteria* e *Verrucomicrobia* são representados em menor grau (KLEMENTE; PAZIENZA, 2019). Manter um equilíbrio em ecossistemas microbianos complexos como o trato gastrointestinal humano envolve uma relação mutualística entre a composição da microbiota, a produção metabólica e a resposta imune do hospedeiro (GOYA-JORGE *et al.*, 2024).

No mais, a composição da microbiota intestinal em organismos normais não é constante e sua composição é alterada por uma variedade de fatores, como ambiente, idade, níveis hormonais, terapia medicamentosa (antibióticos) e hábitos alimentares (LIU *et al.*, 2023). Uma microbiota saudável (ou “normal”) deve ter a chamada capacidade de resistência à colonização para combater e superar a infecção por organismos patogênicos, muitos dos quais são bactérias comensais oportunistas (GOYA-JORGE *et al.*, 2024).

Estudos *in vivo* relataram que as alterações na composição da microbiota intestinal demonstraram exercer um papel importante na manutenção da função da barreira intestinal. De fato, foi documentado que dietas pobres em fibras, ricas em proteínas e ricas em gordura aumentam a inflamação e a permeabilidade intestinal, alterando a translocação de populações bacterianas e metabólitos que modulam a inflamação (SAKKAS *et al.*, 2020; KARL *et al.*, 2017). Por conseguinte, tecnologias de sequenciamento de microbioma de alto rendimento, incluindo sequenciamento do gene 16S rRna, metatranscriptômica, metaproteômica e metabolômica, oferecem os insights mais consideráveis sobre o ecossistema da microbiota intestinal e suas funções metabólicas (SAKKAS *et al.*, 2020).

3.4.1 Efeito de polpas de frutas fermentadas na microbiota

As frutas são consideradas a principal fonte de substâncias ativas, constituídas por polissacarídeos, fibra alimentar, fenólicos, flavonóides e carotenóides que podem alterar e modular a variedade, quantidade, riqueza e diversidade da microbiota intestinal (ALASALVAR *et al.*,

2023). As substâncias ativas nas frutas ou polpas de frutas interagem com as bactérias do ácido láctico, o que facilita a diversidade de grupos bacterianos benéficos, que alteram a proporção de *Firmicutes/Bacteroides* e inibem o crescimento de bactérias nocivas no intestino (BORGONOV *et al.*, 2022). A incorporação de ingredientes bioativos pode regular a estrutura geral e a composição da microbiota intestinal e reduzir a disbiose intestinal. Da mesma forma, estes ingredientes ativos são decompostos e metabolizados no intestino, e estes metabólitos entram na circulação humana através da corrente sanguínea com grandes benefícios à saúde (JIN *et al.*, 2023).

As interações entre os componentes alimentares e as populações intestinais precisam ser consideradas para melhor compreender os efeitos benéficos dos compostos bioativos das frutas ou polpas de frutas no bem-estar geral do hospedeiro e para planejar intervenções dietéticas eficazes (BORGONOV *et al.*, 2022). No entanto, os mecanismos de ação através dos quais os compostos bioativos alteram a composição intestinal apenas começaram a ser explicados e alguns deles permanecem desconhecidos (JIN *et al.*, 2023).

Os polifenóis e outros metabólitos nas frutas ou polpas de frutas podem aumentar a função da barreira intestinal, reduzir o estresse oxidativo, inibir a secreção de citocinas inflamatórias e regular a função imunológica regulando a microbiota intestinal (JIN *et al.*, 2023). As frutas são exemplos de matrizes ricas em polifenóis com propriedades antioxidantes e atividade eliminadora de radicais livres, o que é benéfico para a saúde humana (FRAGA *et al.*, 2019).

Em um estudo, Li *et al.* (2023) descobriram que a intervenção de altas doses de polpa de jujuba fermentada (FJP) regulou o perfil da microbiota, posteriormente restaurou a microbiota, como *Bacteroidetes*, *Bifidobacterium* e *Lactobacillus*. Esta pesquisa forneceu resultados convincentes *in vivo* e evidências de que a suplementação de altas doses de FJP através da dieta diária pode ser uma abordagem promissora para modular a saúde intestinal. Assis *et al.* (2024) estudaram a polpa de mangaba fermentada com o probiótico *Lacticaseibacillus casei* 01 (LC1) e seus efeitos na modulação da microbiota intestinal de adultos veganos saudáveis, assim como também Assis *et al.* (2025) avaliaram a polpa de bacaba fermentada com os probióticos *Lactobacillus acidophilus* 05 (LA-05) e *Lacticaseibacillus casei* 01 (LC1) e seu efeito na modulação da microbiota intestinal de adultos saudáveis. Os resultados mostraram que a fermentação fecal *in vitro* aumentou a abundância de microrganismos benéficos e diminuíram a abundância de microrganismos patogênicos como *Clostridiales*. Esses achados fornecem novas informações sobre o potencial uso da mangaba e da bacaba como polpas funcionais que induzem alterações na microbiota colônica associadas a potenciais benefícios para a saúde humana.

3.4.2 Efeitos na microbiota de veganos

Durante as últimas décadas, padrões alimentares vegetarianos e veganos que comprovadamente estão associados a vários resultados benéficos para a saúde foram adotados por uma proporção crescente de indivíduos nas sociedades ocidentais (MEDAWAR *et al.*, 2019).

A forma mais estritamente regulamentada de vegetarianismo (veganismo) é caracterizada por uma abstinência completa do consumo de carne e alimentos de origem animal, como laticínios, ovos e mel, com uma dieta composta apenas por alimentos vegetais como grãos, vegetais, frutas, legumes, nozes, sementes, gorduras e óleos vegetais (VERONESE; REGINSTER, 2019; LEMALE *et al.*, 2019). Muitas pesquisas têm se concentrado nos efeitos potenciais do veganismo na saúde e no bem-estar. Embora diversos benefícios associados a múltiplas comorbidades médicas tenham sido relatados, como deficiências nutricionais em relação a vitaminas, minerais e proteínas (LEMALE *et al.*, 2019; CHANDRA-HIOE *et al.*, 2019; ALLÈS *et al.*, 2017). O veganismo tem sido amplamente aceito como o protótipo de dieta saudável relacionada à microbiota intestinal (TOMOVA *et al.*, 2019; WONG *et al.*, 2018).

As dietas veganas são fontes de nutrientes para microrganismos e também possuem sua própria microbiota, conferindo efeitos heterogêneos na abundância e na diversidade da microbiota intestinal (MILANOVIC *et al.*, 2019). O consumo de vegetais e frutas a longo prazo se correlaciona com a diversidade da microbiota intestinal e uma maior ingestão de fibras aumenta a prevalência de microrganismos associados a um intestino saudável (KLIMENKO *et al.*, 2018). Em um estudo transversal realizado por Losno *et al.* (2021), *Firmicutes* e *Bacteroidetes* demonstraram representar cerca de 95-97,7% da microbiota intestinal total em veganos, com *Firmicutes* contribuindo com cerca de 56-58,6% e *Bacteroidetes* contribuindo com 39% para o microbiota intestinal total.

Especialmente o maior teor de fibras e polifenóis nas dietas veganas parece desempenhar papel significativo na manutenção de um ecossistema mais diversificado de bactérias benéficas (TOMOVA *et al.*, 2019). Os polifenóis aumentam *Bifidobacterium* spp. e *Lactobacillus* spp., proporcionando proteção cardiovascular, bem como efeitos antibacterianos e anti-inflamatórios (SAKKAS *et al.*, 2020). A maioria desses compostos exibe diversidade estrutural e consiste em flavonóides, ácidos fenólicos, estilbenos, lignanas e secoiridóides. Estes passam para o cólon e são metabolizados por bactérias do cólon que influenciam sua bioatividade, enquanto uma pequena proporção é possivelmente absorvida no intestino delgado (ROWLAND *et al.*, 2018). As frutas ou polpas de frutas são exemplos de boas fontes de polifenóis (RINNINELLA *et al.*, 2019).

Reiss *et al.* (2016) relataram uma maior abundância do filo *Proteobacteria* em veganos e, embora não significativa, uma tendência a uma menor abundância de *Actinobacteria* no grupo vegano. Além disso, várias pesquisas encontraram níveis reduzidos de patobiontes, como *Enterobacteriaceae*, que estão associados à indução de inflamação de baixo grau na microbiota intestinal, e um aumento de bactérias degradadoras de fibras, que incluem *Bifidobacterium*, *Prevotella*, *Bacteroides* e *Clostridium* (TOMOVA *et al.*, 2019; FEDERICI *et al.*, 2017; LOSNO *et al.*, 2021). As dietas veganas e seus principais componentes afetam tanto a composição bacteriana

quanto as vias metabólicas da microbiota intestinal, aumentando os microrganismos benéficos. No entanto, são necessários mais estudos para determinar o impacto destas dietas na microbiota intestinal.

4 DELINEAMENTO EXPERIMENTAL

Etapa I: O Artigo de Revisão sob o título “Microbiota of foods: a comprehensive review of diversity and potential implications”, discute a composição da microbiota de diferentes produtos alimentícios fermentados, como bebidas fermentadas, laticínios, alimentos étnicos e outros, com ênfase nos benefícios e desafios do uso de abordagens ômicas para avaliar a microbiota alimentar. Como abordagem metodológica os artigos em inglês foram pesquisados nas bases de dados PubMed e Scopus entre 2014 e 2024, utilizando combinações de palavras-chave como estratégia de busca. As palavras-chave incluíram “microbiota alimentar”, “microbioma alimentar”, “alimentos fermentados”, “bebidas fermentadas”, “laticínios”, “alimentos asiáticos” e “ômicas”. Um total de 94 artigos foram selecionados a partir dessa busca. Esta revisão limitou-se à busca por artigos originais que avaliaram a microbiota de diferentes produtos alimentícios fermentados utilizando uma abordagem ômica. Diferentes estudos foram incluídos, abordando (i) o perfil das comunidades microbianas de diferentes alimentos, (ii) os fatores que impactam a microbiota alimentar e (iii) as correlações entre comunidades microbianas e propriedades físico-químicas e sensoriais. Artigos originais que utilizaram apenas métodos baseados em cultivo foram excluídos. Os estudos foram divididos em bebidas fermentadas, laticínios, alimentos asiáticos e outros alimentos (alimentos fermentados com poucos estudos na área de microbiota). Foram descritos aspectos metodológicos referentes às técnicas ômicas, plataformas, primers, testes de controle de qualidade e bancos de dados utilizados, bem como as vantagens e os principais obstáculos da abordagem ômica moderna para avaliar o microbioma alimentar. Além disso, foram apresentadas e discutidas as bactérias e fungos dominantes em diversos alimentos, bem como suas relações com as características dos alimentos.

Figura 3. Abordagem metodológica para o artigo de revisão



Fonte: Próprio autor (2025).

Etapa II: O Capítulo de livro sob o título “Bioaccessibility of phenolic compounds in fermented native fruit”, detalha a metodologia para extração de compostos fenólicos de frutas nativas fermentadas e analisa a digestão gastrointestinal simulada para avaliar a bioacessibilidade fenólica. A abordagem metodológica foi baseada para (i) obtenção de frutas nativas *in natura* e processamento da polpa, (ii) obtenção de polpa de fruta fermentada probiótica, (iii) extração de compostos fenólicos de frutas nativas fermentadas, (iv) simulação de digestão gastrointestinal *in vitro* e (v) determinação de compostos fenólicos de frutas nativas fermentadas. Este capítulo foi escrito em virtude da crescente valorização de frutas nativas fermentadas como potenciais alimentos funcionais.

Figura 4. Abordagem metodológica para o capítulo de livro

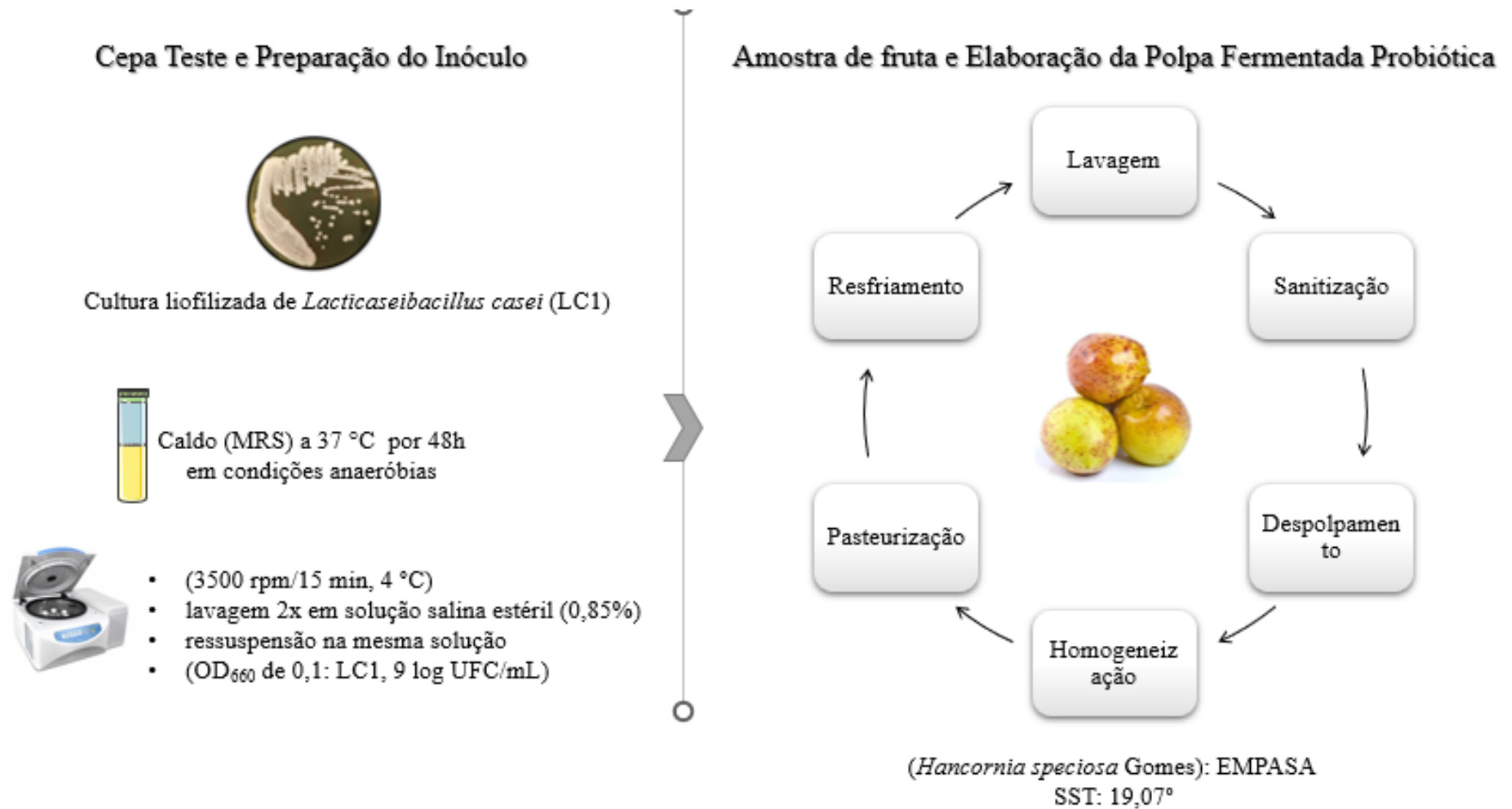


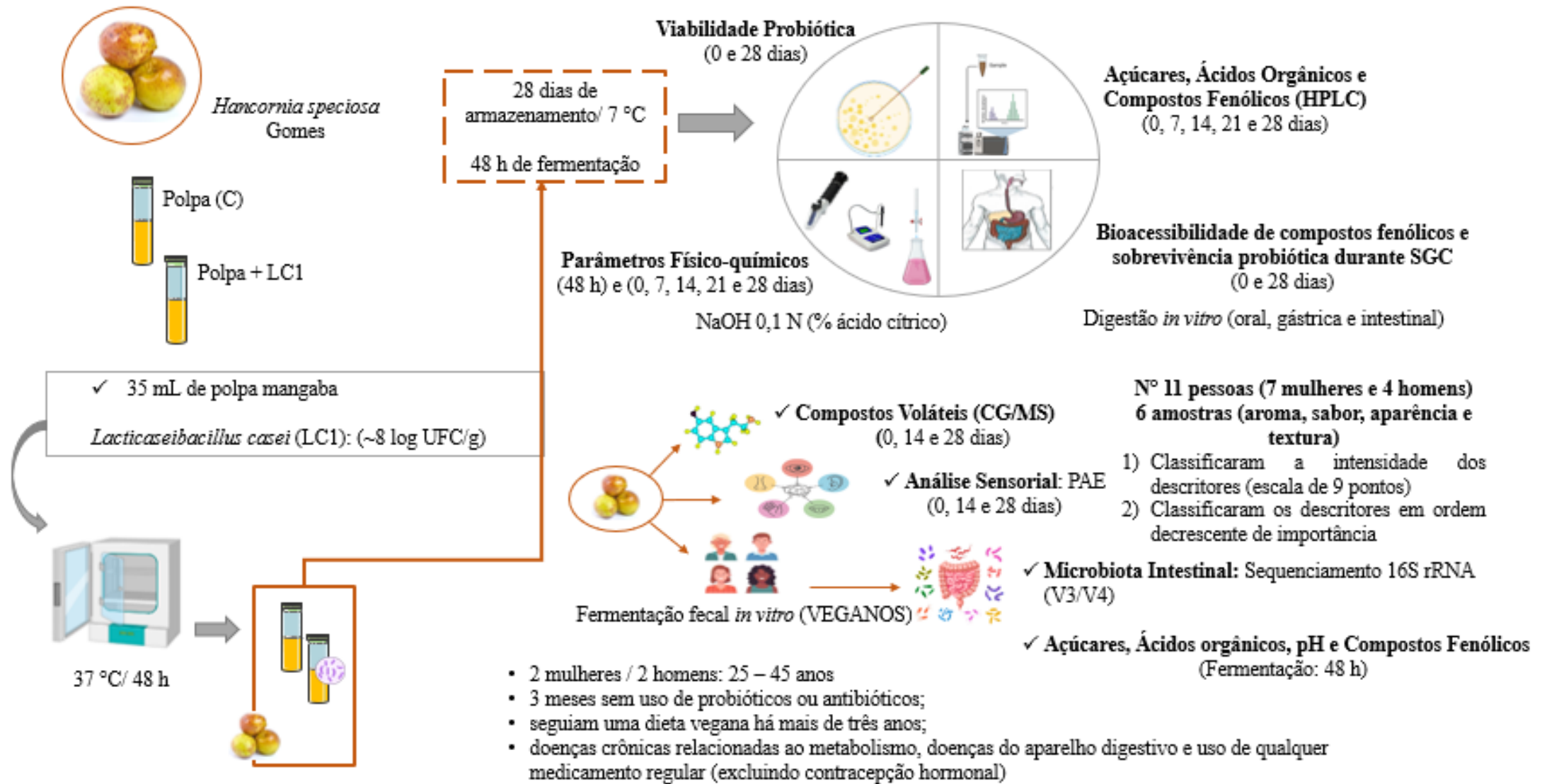
Fonte: Próprio autor (2025).

Etapa III: O Artigo sob o título “Mangaba pulp fermented with *Lactaseibacillus casei* 01 has improved chemical, technological, and sensory properties and positively impacts the colonic microbiota of vegan adults”, avaliou os aspectos funcionais, tecnológicos e sensoriais da polpa de mangaba fermentada com o probiótico *Lactaseibacillus casei* 01 (LC1) durante o armazenamento refrigerado e, determinou o efeito na modulação da microbiota intestinal de adultos veganos

saudáveis por meio do sequenciamento do 16S rRNA. Os experimentos se basearam em 5 etapas principais. Em um primeiro momento foram realizadas a preparação do inóculo da cepa de *Lactocaseibacillus casei* 01 (LC1). Em seguida, realizou-se a elaboração da polpa fermentada probiótica de mangaba (*Hancornia speciosa* Gomes) adicionada de *Lactocaseibacillus casei* (LC1). Posteriormente, foram realizadas as análises ao longo do armazenamento refrigerado para a polpa de mangaba controle e fermentada nos tempos de 1, 7, 14, 21 e 28 dias e as análises durante 48h de fermentação da polpa da mangaba controle e fermentada. A análise sensorial foi escolhida para ser realizada apenas com os tempos 1, 14 e 28 dias de armazenamento da polpa de mangaba. Na última etapa foram desenvolvidos os ensaios de fermentação *in vitro* para as polpas controles e fermentadas da fruta mangaba, utilizando doadores veganos com base no sequenciamento 16S rRNA.

Figura 5. Delineamento experimental da etapa III.





Fonte: Próprio autor (2025).

Etapa IV: O Artigo sob o título “Amazonian native palm pulp (Bacaba) fermented with probiotics influenced physical-chemical technological properties and modulated the bacterial colonic community of healthy adults”, avaliou a composição química e a bioacessibilidade de fenólicos na polpa de bacaba fermentada com *Lactobacillus acidophilus* 05 (LA-05) e *Lacticaseibacillus casei* 01 (LC1), determinou a viabilidade probiótica na polpa fermentada de bacaba e durante a digestão *in vitro* e, determinou o efeito das polpas de bacaba na composição da microbiota intestinal de adultos saudáveis por meio da metabarcodificação do gene 16S rRNA. Os experimentos se basearam em 4 etapas principais. Em um primeiro momento foram realizadas as preparações dos inóculos das cepas de *Lactobacillus acidophilus* 05 (LA-05) e *Lacticaseibacillus casei* 01 (LC1). Em seguida, realizou-se a elaboração das polpas fermentadas probióticas de bacaba (*Oenocarpus bacaba* Mart.) adicionada de *Lactobacillus acidophilus* ou *Lacticaseibacillus casei*, em separado (formulações diferentes). Posteriormente, foram realizadas as análises durante 48h de fermentação da polpa da bacaba controle e fermentada. Na última etapa foram desenvolvidos os ensaios de fermentação *in vitro* para as polpas controles e fermentadas da fruta bacaba, utilizando doadores saudáveis com base no sequenciamento 16S rRNA.

Figura 6. Delineamento experimental da etapa IV.

Cepas Teste e Preparação do Inóculo



Culturas liofilizadas de *Lactobacillus acidophilus* 05 (LA-05) e *Lactocaseibacillus casei* (LC1)



Caldo (MRS) a 37 °C por 48h em condições anaeróbias

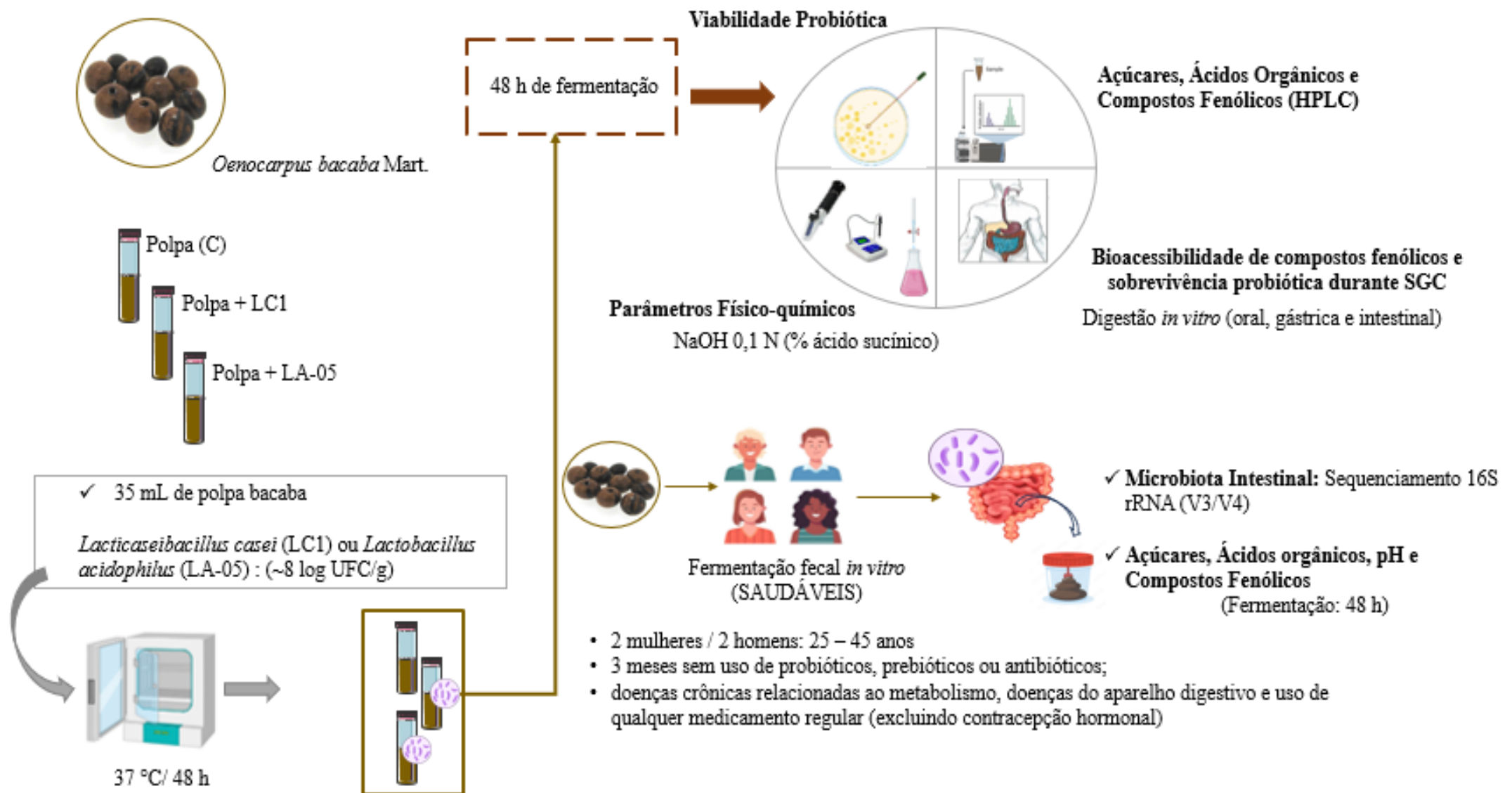


- (3500 rpm/15 min, 4 °C)
- lavagem 2x em solução salina estéril (0,85%)
- ressuspensão na mesma solução
- (OD₆₆₀ de 1,0: LA-05 e 0,1: LC1, 9 log UFC/mL)

Amostras de frutas e Elaboração das Polpas Fermentadas Probióticas



(*Oenocarpus bacaba* Mart.): UFAM
SST: 6,13°



Fonte: Próprio autor (2025).

Etapa V: A Patente sob o título “Processo de fermentação probiótica aplicado a frutas tropicais para produção de alimento funcional”, está submetida ao processo de depósito na Agência UFPB de Inovação Tecnológica - INOVA UFPB. Esta patente tem como objetivo validar o processo fermentativo aplicado a frutas tropicais sob a vantagem de ser utilizada em larga escala pela indústria de alimentos. Além disso, o documento de patente trata-se do resumo da invenção, caracterizado pela descrição do objetivo e as etapas necessárias do processo fermentativo probiótico de frutas tropicais.

Figura 7. Delineamento experimental da etapa V.



Fonte: Próprio autor (2025).

5 RESULTADOS

Os resultados e discussão produzidos durante o desenvolvimento da tese estão expostos em formato de artigo científico, capítulo de livro e patente em atendimento a Norma Complementar nº 01/2024 do PPGCTA.

5.1 ARTIGO DE REVISÃO - Microbiota of foods: a comprehensive review of diversity and potential implications. (Artigo de revisão submetido ao periódico Comprehensive Reviews in Food Science and Food Safety).

ABSTRACT:

Microbial communities play a central role in food ecosystems. Fermented foods, in particular, host complex and dynamic microbiomes that are shaped by raw materials, fermentation substrates, processing environments, and regional production practices. This review provides an in-depth analysis of the microbial diversity in various fermented food products, including beverages, dairy products, Asian foods, and other traditional products, highlighting how microbial composition evolves throughout fermentation and how specific microorganisms contribute to the safety and sensory profiles of the final products. The field has undergone a methodological transformation with the transition from classical culture-based methods to advanced omics technologies. Culture-independent approaches such as metataxonomics, metagenomics, metatranscriptomics, metaproteomics, and metabolomics have enabled more accurate profiling of microbial communities, uncovering both their taxonomic composition and functional roles. Despite the increasing interest in metagenomics and metatranscriptomics, metataxonomic high-throughput sequencing, particularly 16S rRNA and ITS gene analyses, remains the most employed technique due to its lower cost and accessibility. However, it provides limited resolution at the species level and cannot distinguish between live and dead cells. Microbiome characterization using omics has practical implications for the food industry, including the identification of microbial signatures in artisanal foods, the discovery of novel starter cultures or probiotics, and enhanced control over fermentation processes. Findings outline the main methodological approaches, sequencing platforms, primer sets, and bioinformatic tools used in studies, as well as the current limitations and

future directions in the field. Integrative multi-omics strategies is expected to significantly enhance food safety, quality, traceability, and functionality across diverse food systems.

KEYWORDS: microbiome, high-throughput sequencing, dairy products, fermented foods, food quality, culture-independent methods.

1. Introduction

Microbiome studies in food systems have demonstrated the potential to enhance food quality, making it a key research area. This field holds promise for improving safety, sustainability, and production yield while also facilitating the discovery of new strains, probiotics, and mobile genetic elements (Ferrocino et al., 2022; Okoye et al., 2024). The term microbiome was redefined as "the community of microorganisms and their 'theatre of activity' (structural elements, metabolites/signal molecules, and the surrounding environmental conditions) in a defined habitat" (Berg et al. 2020). The food microbiome can originate from multiple sources, including raw materials, the surrounding environment, exposure to human handling, and the geographical area of cultivation or production (Ferrocino et al., 2022). Additionally, factors associated with processing practices, such as the manufacturing stage, moisture levels, and packaging techniques, influence the microbiome profile unique to each type of food (Jarvis et al., 2018; Okoye et al., 2024).

The microbiome also significantly impacts food products, as it influences specific sensory attributes, can contribute to spoilage and safety concerns, and can potentially be transferred from foods to humans (Ferrocino et al., 2023). Therefore, a deeper understanding of microbiomes enables more precise management of food systems, impacting research and industrial practices. Investigating the interactions, functions, and diversity of every microbial species within this complex system is crucial for efficiently monitoring and manipulating food systems, helping to elevate the quality and safety of the produced food (Ferrocino et al. 2022).

Analyzing food microbiomes offers valuable insights into the resident microbial communities and their changes over time, providing significant contributions to various food-related endeavors (Jarvis et al. 2018). For example, the study of microbial communities plays a key

role in monitoring processes and understanding the unique characteristics of fermented foods (Duarte et al., 2024). Microbial diversity plays a crucial role in shaping the chemistry and nutritional properties of food, thereby affecting its yield and quality (Ojeda-Linares et al. 2021). Furthermore, environmental factors significantly influence the composition and functional potential of fermented foods' microbiomes, with studies revealing distinct microbial characteristics in these products based on the fermentation substrate, geographical origin, and processing (Liu et al., 2023; Zhang et al., 2021a).

Therefore, microbiome-scale metabolic reconstruction has become the primary method for uncovering how microorganisms influence the characteristics of food products (Ferrocino et al. 2022). Numerous studies have demonstrated the utility of this approach in elucidating how changes in microbiome composition and function correlate with alterations in factors such as color, pH, and flavor across various food types, like cheeses (D'Incecco et al. 2023), fermented sour meat (Zhong et al. 2021), fermented fish (Sirichoat et al. 2022), fermented soy sauce (Feng et al. 2023), kombucha (Yang et al. 2022), water kefir (Patel et al. 2022), vegetable and cereal fermented beverages (Gutiérrez-Sarmiento et al. 2022; Delgado-Ospina et al. 2022), among others.

Traditional microbiological analyses using culture media have been fundamental in detecting and studying microorganisms in foods. However, these culture-dependent methods have inherent limitations, as they only reveal microorganisms capable of proliferating in synthetic media during analysis. Under stress conditions, some microbial cells may be alive but unable to grow, resulting in a biased and potentially distorted view of the microbial ecology in a sample (Rantsiou et al. 2022). Classical methods also struggle to detect microorganisms in a viable but non-culturable state or those not typically expected in food. These facts lead to the loss of critical information about the entire microbiome. In contrast, -omics approach offers a comprehensive examination of food microbiomes, even identifying small numbers of cells in a sample (Ferrocino et al. 2022).

The shift from classical cultivation-based approaches to modern omics sciences has revolutionized microbiome investigation. In foods containing mixed microbial communities, this approach, known as meta-omics, analyzes macromolecules and metabolites from all present microorganisms, providing a comprehensive overview of microbial activity (Rantsiou et al., 2022).

Omics techniques, such as metataxonomic/metagenomics, metatranscriptomics, metaproteomics, and metabolomics, target DNA, RNA, proteins, and metabolites, respectively, and have been widely used to gain a deeper understanding of the impacts of microorganisms on foods (Okoye et al. 2024; Tilocca et al. 2020).

The choice of the -omics approach largely depends on the type of samples being studied and must align with the research hypothesis and experimental questions. Metataxonomics is efficient for identifying and comparing microbial communities at higher taxonomic levels, such as phylum and genus, but has limited resolution at the species level (Sabater et al. 2021). In contrast, metagenomic approaches, whether amplicon-based or shotgun sequencing, offer a more comprehensive view with higher taxonomic resolution, reaching species or even strain levels. However, the metagenomics limitation is mainly related to the capture of DNA from both living and dead cells, with gene abundance not necessarily correlating with protein or metabolite synthesis. On the other hand, mRNA sequencing does not always predict the translation into functional proteins or metabolites and lacks taxonomic detail. Additionally, metabolites and proteins, which can originate from the host or food ingredients, are highly labile and require specialized methods for collection and preservation (Ferrocino et al., 2022; Okoye et al., 2024).

The selection of -omics tools are typically influenced by a research group's technical and analytical skills, prior experience with these technologies, access to data analysis platforms, and funding availability (Ferrocino et al. 2023). High-throughput sequencing platforms have revolutionized the study of microbiomes in complex food matrices, allowing for a comprehensive understanding of the structure, function, and dynamics of microbial consortia (Sequino et al., 2022; Tilocca et al., 2020). The combination of high-throughput sequencing technologies, which produce extensive datasets, and advanced bioinformatic tools for analyzing and interpreting complex patterns has significantly improved our understanding of the composition and functions of complex microbial populations (Pan and Barrangou 2020).

Considering the microbiome's impact on food product characteristics, the review aimed to present an overview of the microbial groups in different fermented food products, highlighting the advantages and main obstacles of the modern -omics approach to assessing the food microbiome.

2. Methods

PubMed and Scopus databases were searched for English-language articles published between 2014 and 2024, using keyword combinations as the search strategy. Keywords included “food microbiota,” “food microbiome,” “fermented foods,” “fermented beverages,” “dairy products,” “Asian foods,” and “omics.” A total of 94 articles were selected from this search. This review was limited to searching for original articles that evaluated the microbiota of different fermented food products using an -omics approach. Different studies were included, approaching (i) the profile of the microbial communities of different foods, (ii) the factors that impact the food microbiota, and (iii) the correlations between microbial communities with physicochemical and sensorial properties. Original articles that used only cultivation-based methods were excluded.

The studies were divided into fermented beverages, dairy products, Asian foods, and other foods (fermented foods with few studies in the microbiota area). Methodological aspects regarding the omics techniques, platforms, primers, quality control tests, and databases used, as well as the advantages and main obstacles of the modern omics approach to evaluating the food microbiome, were described. Additionally, the dominant bacteria and fungi in various foods, along with their relationships to food characteristics, were presented and discussed.

3. Microbiota of different food products

3.1 *Fermented beverages*

Humans have consumed fermented beverages for millennia. In the past, numerous regions across Asia, South America, Europe, and the Middle East have fermented cereals and other substrates to produce beverages with purported health benefits and preservation (Marsh et al., 2014). There is evidence from archaeological findings of beer and wine fermentation dating back 5,000 years B.C. (Ross et al., 2002). Meanwhile, honey- and fruit-based beverages in China date back to 7,000 B.C., and kombucha production is believed to have originated approximately 220 B.C. (McGovern et al., 2004; Dufresne and Farnworth, 2000).

The presence of microorganisms is crucial in the fermentation process, as it promotes compositional changes in fermented foods and influences their nutritional and sensory profiles (Bao et al., 2019). Microbial community composition varies depending on the process and food matrix. Traditional fermentation often occurs spontaneously under uncontrolled conditions. This spontaneous fermentation process involves a variety of microbial species, including bacteria, fungi, and/or yeasts, which contribute to the sensory characteristics typically associated with the product (Cuvás-Limon et al. 2021).

Several fermented beverages have been traditionally produced in specific geographical regions, acquiring distinct characteristics due to the unique microbiome present in each region. These microbial species are indigenous to the raw materials and play a crucial role in determining the product's distinctive sensory profile (Cuvás-Limon et al., 2021; Smid and Kleerebezen, 2014). Delgado-Ospina et al. (2022) reported that the uniformity of parameters (i.e., environmental and processing conditions and substrates) would lead to a resultant similarity of microbial communities and, thus, products exhibiting comparable properties.

Table 1 presents studies investigating microbial communities using omics techniques for various fermented beverages. A distinct pattern was observed between the bacterial and fungal microbiota. A consistent predominance of phyla Bacillota (formerly Firmicutes) and Pseudomonadota (formerly Proteobacteria) is reported, mainly among beverages fermented with bacteria that produce lactic and acetic acids. These microorganisms are responsible for several physicochemical changes, including the fermentation of carbohydrates, which results in a reduction in pH and the production of antimicrobial substances, thereby creating an environment unfavorable for pathogens (Cuvás-Limon et al., 2021).

Figure 1 illustrates the fermented beverages and their primary microbial composition at the genus level. *Lactobacillus* and amended genera were observed in most beverages, which, according to Cuvás-Limon et al. (2021), are the most frequently isolated lactic acid bacteria (LAB) from naturally fermented foods. This is of special importance, considering that many of the identified strains have been reported as probiotic cultures, such as *Lactobacillus paracasei*, *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, and *Limosilactobacillus reuteri*.

(Patel et al., 2022; Delgado-Ospina et al., 2022). These results underscore the importance of these beverages as potential sources of probiotic strains for functional foods and highlight the need for further studies in this area.

Figure 2 presents the main factors influencing the microbiota composition of food products, which will be discussed throughout this review paper. LAB are also responsible for producing organic acids in palm wine (Djeni et al. 2022). These acids are the principal agents responsible for the wine's sour taste, aroma, color, and consistency. In the context of consortia comprising other genera, *Lactobacillus*, *Leuconostoc*, and *Lactococcus* have been identified as key contributors to the synthesis of volatile compounds, including isoamyl acetate, decanoic acid, and citronellol. These compounds have been shown to play a significant role in imparting the distinctive aroma of Marselan grape wine, thereby enhancing its concentration and complexity (Lu et al., 2020a). As Djeni et al. (2022) also noted, LAB, acetic acid bacteria (AAB), *Acetobacter*, and *Gluconobacter* have been identified as the responsible microorganisms for producing the 'vinegary' flavor in Tody.

In the context of yeast in fermented beverages, the Ascomycota phylum is the most prevalent, followed by the Basidiomycota (Table 1). Yeasts produce organic acids and alcohol, which contribute to the aroma, flavor, and texture of the food products (Maicas 2020).

In the domain of spontaneous fermentation, yeasts have been identified as the indigenous microorganisms that initiate the fermentation process of various beverages, including kombucha and boza (Cuvas-Limon et al. 2021). Moreover, they are employed as starter cultures for the alcoholic fermentation process, with *Saccharomyces cerevisiae* being a prominent strain due to its ability to tolerate ethanol and high fermentation rate (De la Torre-González et al. 2016) (Figure 1). This species contributes to floral and licorice aromas in water kefir (Patel et al., 2022), flavor development in Fangxian huangjiu (Gao et al., 2024), and aroma in palm wine and Tody (Djeni et al., 2022; Das and Tamang, 2023) (Figure 2).

There is growing recognition of the role of non-*Saccharomyces* yeasts in the development of flavor. These organisms have been shown to produce extracellular enzymes aroma precursors, and to increase alcohol concentration. They are also associated with the production of esters that contribute to fruit-like aromas. For example, a correlation has been identified between the genus

Brettanomyces and the presence of citrus attributes in water kefir (Patel et al., 2022). In contrast, *Rhizopus*, *Mucor*, *Wickerhamomyces*, and *Cyberlindnera* have been reported to contribute to the flavor of Fangxian huangjiu (Gao et al., 2024).

Figure 3 illustrates the primary factors that influence the microbiota composition of food products. The dominance of a particular genus and/or species of microorganism can change throughout the beverage fermentation process. In Fangxian huangjiu, *Rhizopus* was prevalent during the pre-fermentation stage, with a subsequent decrease as the process progressed. In contrast, *Saccharomyces*, *Wickerhamomyces*, *Thamnidium*, *Ascoidea*, and *Cyberlindnera*, which initially exhibited lower levels, demonstrated higher levels after fermentation (Gao et al. 2024). A similar phenomenon is observed in the research of Kirchmayr et al. (2024), which discusses the changes in the prevalence of bacteria in Mezcal. Initially, *Rhodospirillales* were more abundant, but at the end of the process, there was a shift towards *Lactobacillales*. These studies show that the fermentation process can facilitate growth and/or inhibit the multiplication of certain microorganisms through various mechanisms, including competition for substrates, the production of metabolites and bacteriocins (e.g., nisin), physicochemical factors, and others (Gao et al. 2024; Gutiérrez-Sarmiento et al. 2022; Cuvas-Limon et al. 2021). They also highlight that changing the fermentation conditions may result in beverages with different microbiota and specific microorganisms can be shaped. These changes may produce beverages with different physicochemical and sensory characteristics, creating opportunities to customize these parameters.

Chinese rice wine, another traditional alcoholic beverage, has a bacterial microbiota consisting of Bacillota, Pseudomonadota, and Actinobacteria phyla. *Bacillus* and *Weissella* dominate in *Fagopyrum tataricum* rice wine (Ren et al. 2019), while *Leuconostoc* and *Pediococcus* dominate in the black glutinous rice wine type (Zhao et al. 2020). As occurred in the fermentation process of Baijiu liquors, *Lactobacillus* was the dominant bacterium at the end of the process (Liu et al. 2019a; Zhao et al. 2020) and contributes to the aroma of Baijiu liquor (Tan et al. 2019) and carbohydrate metabolism (Huang et al. 2020).

As for the fungal microbiota, the two products presented different profiles, with unclassified_O_Saccharomycetales in the *F. tataricum* rice wine (Ren et al. 2019) and

Mucoromycota and Ascomycota in the black glutinous rice wine type (Zhao et al. 2020). The predominant genera (*Rhizopus* and *Saccharomyces*) in black glutinous rice wine (Zhao et al., 2020) appear to be associated with the raw material used in the fermentation process (Chen et al., 2020). Therefore, even when considering the same product type, the raw material has a significant influence on the microbiota of the final products.

A limited number of studies in the present review discuss the presence of potential pathogenic microorganisms within the microbiome. *Escherichia coli* was reported in kombucha samples (Yang et al. 2022), a low occurrence of *Enterobacteriaceae* was reported in Palm wine samples (Djeni et al. 2022), and *Fusarium oxysporus* was detected in Champús sample (Delgado-Ospina et al. 2022). The presence of LAB in beverages is important due to their capacity to inhibit the growth of pathogenic bacteria through the production of fungicidal and bactericidal compounds, as well as acids that reduce the pH level, thereby ensuring food safety (Pérez-Armendáriz et al., 2020). Djeni et al. (2020) discuss the low occurrence of *Enterobacteriaceae* in palm wine samples, proposing that this low prevalence may be attributed to the production of polysaccharides with inhibitory effects on these bacteria, which have pathogenic potential, even in the context of poor hygienic conditions. In addition, Patel et al. (2022) posit that, in certain instances, diversity has been underestimated due to the presence of unidentified yeasts that have not been analyzed. This observation underscores the importance of employing a combination of both culture-dependent and culture-independent techniques to assess the intricate microbial community within specific food products.

3.2 Dairy products

Among fermented foods, dairy products are essential food matrices from both a nutritional and functional perspective and are recommended in the regular diet of consumers of all ages (Ribeiro et al., 2022). Dairy consumption may be an accessible and cost-effective intervention strategy to improve gut health, as several compounds found in dairy foods have shown promise in favorably modulating the gut microbiome and immune function. Fermented dairy foods likely offer

additional benefits for brain health due to the neuroprotective effects of live and active cultures on the gut microbiome and the gut-brain axis (Comerford et al., 2024).

The microbiota of dairy products is highly diverse, and its characterization can provide valuable insights into various aspects of the product, including sensory properties, nutritional composition, shelf life, and safety. Thus, understanding the impact of microbial communities on these aspects is crucial to increasing the quality of the product (Jonnala et al. 2018).

Table 2 presents studies investigating microbial communities using omics techniques for various dairy products. The predominance of the phylum Pseudomonadota was observed in Minas Frescal cheese, followed by the phyla Bacillota and Bacteroidetes (de Paula et al. 2021). Other groups, such as *Pseudomonas*, *Acinetobacter*, and *Escherichia-Shigella*, were also present in most cheeses. They can be associated with contaminated samples once these bacteria are recognized as primary food spoilage and undesirable microorganisms in food processing environments (Arslan et al., 2011; Stellato et al., 2015). The predominance and persistence of *Pseudomonas* and *Acinetobacter* are likely due to their ability to grow at low temperatures, form biofilms, have low growth requirements, and exhibit metabolic versatility (Møretrø and Langsrud 2017; Quintieri et al. 2019).

In addition, the occurrence of *Escherichia-Shigella* in the evaluated cheeses indicates a high association with common post-pasteurization contaminants and has also been linked to raw milk and food handlers in the dairy processing plant (Carvalho et al. 2014; Freitas et al. 2013). Thermal processing in the dairy production chain is recommended for ensuring microbiological safety, as it helps avoid endogenous contamination of food with potentially pathogenic bacteria and reduces bacterial load (de Paula et al., 2021). To ensure the safety of pasteurized milk, on the other hand, adequate pasteurization and prevention of post-pasteurization contamination are crucial.

At the family level, *Enterobacteriaceae* showed greater abundance, and their presence in dairy products generally indicates direct or indirect fecal contamination of the milk or product during the manufacturing process, and therefore, has been associated with poor hygiene conditions (Haddad and Yamani, 2017; Møretrø and Langsrud, 2017). Some *Enterobacteriaceae* also produce lipolytic and proteolytic enzymes that can adversely affect the sensory characteristics of cheese,

with gas-producing strains being linked to texture and flavor defects (de Paula et al. 2021). These bacteria are highly related to the phenomenon of antimicrobial resistance and can become available through food consumption, representing an additional risk factor for consumers (Verraes et al. 2013). The results with Minas Frescal cheese demonstrate the applicability of omics in detecting potential spoilage and pathogenic microorganisms, and these data can be used to improve food quality and safety.

Omics analyses may also contribute to understanding the impact of microbiota on the physicochemical and sensory properties of dairy products. Figure 4 summarizes the main microbial composition, considering the genus level of dairy products. Several microorganisms have been identified in dairy products, mainly *Lactococcus* (Jin et al. 2018; Nero et al. 2021; Dimov et al., 2022, 2023; Gezginc et al. 2022), *Streptococcus* (Nero et al. 2021; Dimov et al. 2023; Golbasi et al. 2023), *Lactobacillus* and amended genera (Dimov et al. 2023; Golbasi et al. 2023), *Staphylococcus* (Dimov et al. 2023), and *Enterococcus* (Dimov et al. 2023).

It is known that raw milk cheese and other dairy products are primarily composed of *Lactococcus* spp. (McSweeney et al. 2000; O'Sullivan et al. 2000; Fernández et al. 2011; Jin et al. 2018), mainly *Lactococcus lactis*, *Lactococcus raffinolactis*, and *Lactococcus garviaea*. They are used in the cheese industry as a starter in fermentation, primarily when pasteurized milk is used to make commercial cheeses. Some advantages of its application during cheese production include the rapid production of acid, the acidification through the output of flavor-developing compounds, the contribution to proteolysis, the conversion of amino acids into flavor compounds (alcohols, ketones, and aldehydes), and citrate utilization and/or fat metabolism (Quigley et al. 2013, Fernández et al. 2010). They may also inhibit pathogens and spoilage agents, and due to the assimilation of melibiose and raffinose, some authors recognize probiotic properties (Gezginc et al. 2022; Dimov et al. 2023). *Lactococcus raffinolactis* ferments lactose and citrate to lactate, formate, acetate, ethanol, and acetoin. Flavor compounds, such as acetoin, have been reported in dairy products and are of interest due to their sensory properties, being responsible for notes of a fermented, buttery flavor (Kimoto-Nira et al., 2012).

Streptococcus, primarily represented by *Streptococcus thermophilus* and *Streptococcus parauberis*, contributes to the final product through acidification, the production of aromatic and volatile compounds, and the synthesis of exopolysaccharides (EPS), which influence rheological properties (Dimov et al., 2023). The EPS produced by *S. thermophilus* exhibits certain probiotic functions and may play a significant role in enhancing the characteristics of fermented dairy products (Xu et al., 2023). *S. parauberis* is widely used as a starter culture due to its ability to rapidly transform lactose to lactate, facilitating a rapid decrease in pH and the production of essential metabolites, including low levels of formate, acetoin, diacetyl, acetaldehyde, and acetate (Santarelli et al. 2008). At the same time, *S. thermophilus* is recognized as the most important microorganism in the dairy industry, improves the ecological environment for lactic acid fermentation, and beneficially reduces cholesterol levels by inhibiting the activity of cholesterol synthase (Mora and Arioli 2014).

In addition, *Lactobacillus* and amended genera contribute to cheese maturation by acidifying and degrading milk proteins. The presence of *Lactobacillus kefiranofaciens* and *Lactiplantibacillus xiangfangensis* makes the product a candidate for functional food, and *L. plantarum* has a beneficial effect on the sensorial characteristics, proteolytic and lipolytic activities, and durability of the product (Gezginc et al., 2022; Dimov et al., 2023).

Furthermore, coagulase-negative *Staphylococcus equorum* plays a crucial role in determining sensory properties due to its proteolytic and lipolytic activities, as well as the production of volatile substances, which distinguishes cheeses containing this microorganism from other similar products (Dimov et al. 2023). Finally, *Enterococcus*, primarily *Enterococcus faecalis* and *Enterococcus italicus*, may contribute to aroma, texture, and flavor or generate bioactive peptides (Dimov et al., 2023).

The literature demonstrates that the production region and cheese type can impact the microbiota of the products (Jin et al., 2018; Kamimura et al., 2019) (Figure 3). In a study of artisanal Brazilian cheeses, differences in the LAB patterns were highlighted, with *Streptococcus*, *Leuconostoc*, *Lactococcus*, and *Lactobacillus* being combined in varying relative abundances according to product type and region of production (Kamimura et al., 2019). At the same time,

marked differences in the structure of the microbiota communities in the cheese dataset from three regions were reported for traditional Buryatian cottage cheese (Jin et al., 2018). These results demonstrate the importance of establishing defined areas for specific products, as the microbiota influences the sensory and physicochemical characteristics of the final products. Furthermore, they may help develop typical microbial signatures for artisanal cheeses, contributing to authentication validation.

3.3 Asian fermented foods

Fermented Asian foods include a large variety of products. Table 3 shows the studies investigating microbial communities using omics techniques for fermented Asian foods. At the same time, Figure 5 summarizes the main microbial composition, considering the genus level of Asian foods.

The studies reported a predominance of *Lactobacillus* (Tan et al. 2019; Huang et al. 2020; Guan et al. 2020a; Zhang et al. 2021c), *Pichia* (Huang et al. 2020), *Saccharomyces* (Huang et al. 2020; Zhang et al. 2021c), *Staphylococcus* (Guan et al. 2020a), *Acetobacter* (Guan et al. 2020a), *Weissella* (Guan et al. 2020a), *Pediococcus* (Guan et al. 2020a), *Bacillus* (Guan et al. 2020a), among others. Changes in the microbiota composition can be observed in response to alterations in cereal composition (Tan et al., 2019), fermentation stage (Tan et al., 2019; Huang et al., 2020; Guan et al., 2020a), and initial temperature (Zhang et al., 2021c) (Figure 3). These results denote the importance of controlling the ingredients used and the fermentation process to produce products with specific microbiota and desirable sensory and physicochemical properties.

Daqu is a saccharifying and fermenting starter derived from the natural fermentation of wheat, barley, and peas through shaping, fermentation, and ripening. It contains various microorganisms, including mold, yeast, and bacteria (Deng et al., 2020; Wang et al., 2021). Different sensory characteristics are obtained by different temperatures in the fermentation process, resulting in white, red, yellow, and black Daqu. The various temperatures do not appear to affect the genera of microorganisms found in the ingredient, with *Kroppenstedtia*, *Bacillus*, and *Saccharopolyspora* being the most frequently reported (Zhang et al. 2021b; Cai et al. 2021a; Wang

et al. 2021; Deng et al. 2020). However, each type of Daqu presents a distinct profile regarding the distribution of these genera, with *Saccharopolyspora* and *Thermomyces* being predominant in black and red Daqu, respectively (Wang et al. 2021; Deng et al. 2020). Daqu appears to also be an essential contributor of the *Aspergillus* genus to the fermentative process of Baiju liquor (Jiao et al. 2022), while fermented grains seem to be an important contributor of *Thermoascus*, *Kazachstania* and *Pichia* (Jiao et al. 2022; Huang et al. 2020; Guan et al. 2020a; Pang et al. 2020; Wang et al. 2021; Zhang et al. 2021b). Therefore, temperature is a crucial parameter to be controlled, resulting in products with distinct microbiota. Furthermore, all ingredients used may contribute to a specific microbiota, and verifying the compatibility and shape of the microbiota is important.

Although *Lactobacillus* was not an abundant genus in Daqu, it was present in pit mud (regardless of the grain types), and its abundance increased throughout the fermentation process (Zhang et al., 2021b; Guan et al., 2020a; Huang et al., 2020; Zhang et al., 2021c). It is important to highlight that this fermentation process is natural, and, as it progresses, new baths are made, a new pit mud is formed as a precipitate from the fermentation, and the impact of the microbiota (mainly consisting of five genera, *Lactobacillus*, *Saccharomyces*, *Pichia*, *Geotrichum*, and *Candida*) of this mixture has been studied for its impact on the sensory characteristics of the liquor. This way, it is possible to modify the sensory attributes of the products by shaping specific microorganisms from specific raw materials (Wang et al. 2019). For example, yeasts, such as *Saccharomyces cerevisiae*, are essential for fermentation, contributing to the development of characteristic aromas and the overall mouthfeel of the product. Variations in the microbial community, driven by environmental factors such as pH, temperature, and flour type, can lead to differences in these sensory properties (Egea et al., 2023; von Gastrow et al., 2023).

Bread is widely consumed worldwide, and those using natural fermentation (such as sourdough as a leaven) have more complex and appreciated sensory characteristics (Egea et al., 2023). Sourdough is a naturally fermented mixture of flour and water in bread-making. The unique characteristics of sourdough bread are attributed to the activities of LAB and yeasts, although the full origins of this microbial community are still not completely understood (Fraberger et al. 2020; von Gastrow et al. 2023). LAB is a group of bacteria crucial in sourdough due to its proteolytic

activity and capacity to form volatile compounds (responsible for the characteristic aroma of the product), as well as antibacterial and antifungal compounds (Aregbe et al. 2019). In sourdough, LAB, such as *Lactobacillus* species, contribute to the production of organic acids and volatile compounds, thereby enhancing the bread's flavor profile and texture (Egea et al., 2023; von Gastrow et al., 2023).

Among the species of LAB, a wide variety is part of sourdough: *Fructilactobacillus sanfranciscensis*, *W. cibaria*, *L. fermentum*, *L. plantarum*, *Limosilactobacillus pontis*, and *Companilactobacillus paralimentarius* (Yan et al. 2019), and *Levilactobacillus brevis* and *Weissella confusum* species (Zhao et al. 2019). *W. cibaria* and *L. plantarum* have good resistance towards drying and can survive the jiaozi process. Furthermore, *W. cibaria* utilizes a higher concentration of monosaccharides as its primary substrate than maltose, the preferred substrate of other *Lactobacillus* (LAB) species (Yan et al. 2019). *L. brevis* and *W. confuse* are species that produce EPS and acid and are, therefore, major contributors to sourdough's texture and flavor/aroma, respectively (Aregbe et al., 2019), which impact the dough's structure and mouthfeel. Additionally, the acidification process, led by LAB (Sun et al. 2023), contributes to the characteristic tangy flavor, a defining feature of sourdough products (Chavan and Chavan 2011).

Unsurprisingly, *Saccharomyces* was the most important genus in sourdough, with *S. cerevisiae* being the most frequently reported species (Yan et al., 2019; Zhao et al., 2019; Sun et al., 2023). Other genera such as *Alternaria* and *Nothophoma* (considered plant pathogens) and *Mucor*, *Aspergillus*, and *Thermoascus* (common indoor and outdoor molds) may be present due to contamination of flour or dough through contact with other parts of the plant or during the manufacturing process (Sun et al. 2023; Yan et al. 2019; Aregbe et al. 2019). Therefore, omics techniques may allow us to identify desired and undesired microorganisms and their potential sources. These unwanted microorganisms, such as molds or plant pathogens, can jeopardize food safety by introducing mycotoxins or pathogens into the product, emphasizing the importance of microbiological control during fermentation to ensure the bread's safety and quality. Variations in the sourdough environment, such as pH, flour composition, or fermentation time, may influence the microbial community structure (Urien et al. 2019). Several studies indicate that the microbiota of

the bakery environment is the primary driver of sourdough microbial diversity (von Gastrow et al., 2023).

Chinese steamed bread (based on wheat or potato flour) and jiaozi (based on mixtures of wheat, corn, and rice flour, among others) are traditional products that are produced from the natural fermentation of a dough starter resulting in a product with characteristic texture, flavor and nutritional value (Yan et al. 2019). Regardless of the raw material or the sourdough manufacturing process, *Lactobacillus* and amended genera and *Weissella* were the present genus in all cases (Sun et al. 2023; Yan et al. 2019; Zhao et al. 2019; Aregbe et al. 2019) while *Pediococcus* genus was only present in millet sourdough (Sun et al. 2023) and potato (Zhao et al. 2019). *Weissella* metabolites, as well as metabolites from other beneficial microorganisms, present in fermentation, not only play a role in flavor development but also help preserve the product by lowering the pH and creating an environment that inhibits the growth of spoilage microorganisms (Lu et al. 2020b; Fessard and Remize 2017).

At the same time, a study on chia sourdough demonstrated increased *Lactobacillus* diversity during fermentation, with *E. faecium*, *E. mundtii*, *W. cibaria*, and *L. rhamnosus* emerging as dominant species in the final stages of propagation (Maidana et al. 2020). These studies demonstrate that processing conditions may also alter the microbiota, increasing the abundance of those with the ability to survive drastic conditions, such as drying.

Chilli peppers follow the same pattern as other products during fermentation, demonstrating genera (*Rosenbergiella*, *Ralstonia*, and *Chromohalobacter* for bacteria and *Alternaria*, and *Stemphylium*, *Cryptococcus* for fungi) and species most related to the contribution of the raw material to spontaneous fermentation at the beginning of fermentation.

At this stage, the initial composition of the microbial community is heavily influenced by the raw material, which contributes to the early development of flavor and aroma precursors through the production of volatile compounds, such as esters and alcohols, by fungi like *Cryptococcus* and *Alternaria* (Xu et al., 2021a). At the end of the fermentation process, it was possible to identify genera (*Pantoea*, *Lactobacillus*, *Curtobacterium*, *Methylobacterium*, and *Sodalis* for bacteria and *Hyphopichia*, *Kodamaea*, and *Meyerozyma* for fungi) and species (*L. brevis*, *Companilactobacillus*

alimentarius, *Companilactobacillus versmoldensis*, *Levilactobacillus namurensis*, and *C. paralimentarius*) more related to tolerance to low pH (due to the production of acids by microorganisms), anaerobiosis, among other conditions (Cai et al. 2021b; Xu et al. 2021a; Ye et al. 2022). As fermentation progresses, the microbial community shifts to include species that contribute to the characteristic sourness and depth of flavor of fermented chili peppers. *Lactobacillus* and related genera are particularly significant, as they are responsible for lactic acid production, which lowers the pH, creating the acidic environment needed for the preservation of the product while also enhancing the flavor profile by producing compounds like lactic acid, acetic acid, and other organic acids (Ye et al. 2022).

In this sense, *Lactobacillus* and other amended genera, for example, occur due to increased oxygen consumption and acid production (Ye et al. 2022), while *Meyerozyma*, *Methylobacterium*, *Alternaria*, and *Debaryomyces* have been associated with the production of important compounds contributing to the aroma of these products (Xu et al. 2021a). These microbial species play a pivotal role in developing the sensory characteristics of chili peppers. For instance, yeasts like *Meyerozyma* and *Methylobacterium* contribute to the production of aromatic compounds, including esters, alcohols, alkenes, a benzene derivative, ketones, piperidine, and phenols, thereby enhancing the overall flavor complexity. At the same time, *Lactobacillus* species help refine the sour and tangy notes (Xu et al. 2021a).

In addition, the *Staphylococcus* genus was the only genus that appeared in both unfermented and fermented chili pepper samples. *Staphylococcus*, while commonly present in the initial stages of fermentation, is a genus that can present safety concerns due to its potential to carry pathogenic strains. The presence of such bacteria in both unfermented and fermented samples emphasizes the importance of carefully controlling fermentation conditions, as the development of harmful microorganisms could jeopardize the safety of the final product. Therefore, regular microbiota monitoring through advanced techniques, such as omics, is crucial for ensuring the microbial quality and safety of fermented chili peppers (Cai et al. 2021b). These studies demonstrate the importance of food characteristics in the selection of some specific microbiota, such as pH and anaerobiosis, influencing the sensory attributes of the final product but also determining its safety by inhibiting

the growth of spoilage or pathogenic microorganisms, ensuring a product that is both flavorful and safe for consumption (Cai et al. 2021b; Xu et al. 2021a; Ye et al. 2022).

Comparing fermented soy products (sauce, paste, and curd), *Bacillus*, *Weissella*, and *Staphylococcus* genera of bacteria were the most reported (Feng et al. 2023; Liu et al. 2021b; Chun et al. 2020; Xie et al. 2019, 2020; Chen et al. 2019a; Kumar et al. 2019), while for fungus *Candida* and *Aspergillus* genera were the most reported (Feng et al. 2023; Chun et al. 2020; Xie et al. 2019; He and Chung 2020). Among the species within the genus *Bacillus* sp. that appear to contribute to the fermentative process, *B. subtilis*, *B. amyloliquefaciens*, *B. licheniformis*, and *B. cereus* species have been reported for Kinema, a traditional fermented soybean consumed in the Sikkim Himalaya (India) (Kumar et al. 2019). These microbial communities play a significant role in shaping the sensory characteristics of fermented soy products. *Bacillus* species, such as *B. subtilis* and *B. amyloliquefaciens*, are involved in the production of amino acids, peptides, and other flavor precursors that contribute to the umami taste and other complex flavors characteristic of fermented soy products (Kumar et al., 2019). The *Weissella* genus produces organic acids that contribute to the flavor of fermented soy products (Feng et al., 2023; Liu et al., 2021b; Chun et al., 2020; Xie et al., 2020; He and Chung, 2020). In addition to organic acids, *Weissella* species also contribute to the formation of other metabolites, such as aldehydes and esters, which enhance the aroma profile and overall sensory experience (Feng et al., 2023; Xie et al., 2020).

While *Bacillus* species contribute significantly to flavor development (previously reported), their presence also raises safety concerns. Some species, such as *B. cereus*, are known to produce toxins that pose a potential health risk if not adequately controlled during fermentation (Kumar et al. 2019). The ability of *Bacillus* species to make these harmful toxins underscores the importance of strict monitoring during the fermentation process to ensure the safety of the final product.

Doubanjiang is a traditional and savory Chinese bean chili paste made from fermented broad beans, chili peppers, soybeans, salt, and flour (Lu et al. 2020b). The fermentation process involves three stages, the first being a natural fermentation of the mixture of beans, wheat flour, salt, and *Aspergillus oryzae* for 2-3 months; the second is the preparation of chili peppers from red peppers; and finally, the two fermented substances are mixed and subjected to open fermentation with stirring

for up to 36 months (Yanget al. 2021a). Doubanjiang's long and complex fermentation process is key to developing its unique flavor profile. The initial stages are dominated by fungal species, such as *Aspergillus oryzae*, which contribute to the breakdown of starches and proteins, producing amino acids, peptides, and other compounds that enhance the savory umami and umami-like flavors typical of the paste. This microbial activity also generates other flavor precursors, such as aldehydes and esters, which are further developed during the later stages of fermentation (Yang et al. 2021a; Lu et al. 2020b).

Some studies have sought to characterize the microbiota responsible for the characteristic flavor of the product, highlighting the predominance of species from the genera *Bacillus* and *Staphylococcus* in the most advanced stages of fermentation (Lu et al., 2020b; Yang et al., 2021b). These bacteria produce lactic acid and other organic acids, which contribute to the characteristic acidity and complexity of the sensory profile. In addition, *Bacillus* participates in the degradation of plant materials, promoting improvements in the texture and consistency of the paste, as well as in the fermentation of proteins and lipids, resulting in compounds that influence aroma and flavor (Liu et al. 2024). *Staphylococcus* species, on the other hand, play a role in the formation of aromatic compounds, such as aldehydes and alcohols, which enrich the sensory complexity of products like Doubanjiang (Zhang et al. 2020).

Pathogenic microorganisms such as some species of *Klebsiella*, *Cronobacter*, and *Acinetobacter* genera also appear in fermented potato (Aregbe et al. 2019), fermented soy sauce and paste (Liu et al. 2021; Xie et al. 2019), and fermented bean paste (Yanget al. 2021b). The presence of these pathogenic bacteria highlights a critical safety concern in the fermentation process. Even though the long fermentation period may inhibit some harmful microbes, these pathogens can survive under certain conditions, potentially compromising the product's safety. This risk highlights the importance of utilizing modern microbiological monitoring methods, including omics techniques, to ensure that pathogenic species are effectively controlled and that the product meets food safety standards (Lu et al., 2020b). Most studies did not report the species of microorganisms. However, *Acinetobacter lwoffii* has been reported in fermented soybean paste (Xie et al., 2020).

Additionally, *Enterococcus faecalis* from the *Enterococcus* genus was found in some studies of fermented products, such as Daqu (Chen et al., 2021) and fermented soybean paste (Xie et al., 2020; Kumar et al., 2019). This microorganism typically lives in the gastrointestinal tract and can also be found in the oral cavity and vaginal tract (Dieterich et al., 2018). Therefore, it is a microorganism typically used in the food industry to indicate hygiene conditions (Boehm and Sassoubre 2014). Although *E. faecalis* is usually not harmful to the human body, commensal bacteria found in healthy individuals can be utilized as a probiotic (Oruc et al., 2021).

Fermented aquatic products are common in Southeast Asia and Northern Europe. They are typically produced from fish, shrimp, and crabs and fermented for a specified period in a suitable environment after adding salt, glutinous rice, spices, or other ingredients (Aarti et al., 2017; Han et al., 2020; Prihanto et al., 2021). The process relies on spontaneous fermentation, driven by microorganisms derived from raw materials and the environment (Ohshima et al., 2019). These microbial communities are essential to the development of sensory characteristics—such as umami, sourness, aroma, and texture—via the production of organic acids, amino acids, and volatile compounds. Therefore, monitoring microbial composition and behavior is crucial for improving both flavor and safety in fermented foods (Wang et al., 2021a).

Chinese fish sauce is produced using a mixture of fish by-products and a large proportion of sea salt as the primary raw materials and is fermented naturally for 15 months. *Shewanella* dominates the early stages, followed by *Halanaerobium* and, finally, *Tetragenococcus* (Du et al. 2019). In Thai fish sauce, *Halomonas* sp. predominates early, succeeded by *Lentibacillus* and *Tetragenococcus*, likely due to its natural occurrence in saline environments (Ohshima et al. 2019). *Shewanella* contributes to umami by breaking down proteins into peptides and amino acids. As fermentation progresses, halotolerant bacteria such as *Halanaerobium* and *Tetragenococcus* thrive, contributing to the complex final flavor and texture while ensuring product safety.

Salinity is a crucial factor in shaping the microbial community, enhancing safety by inhibiting the growth of pathogens. However, some salt-tolerant pathogens may persist, necessitating close monitoring of the microbiota. Phewpan et al. (2019) identified *Tetragenococcus* spp., *Staphylococcus* spp., and *Lactobacillus* spp. as dominant genera in Thai fermented freshwater

fish (Pla-Ra). *Tetragenococcus* spp., halophilic lactic acid bacteria, are key organisms in soy- and seafood-based ferments (Kim et al. 2009; Ohshima et al. 2019). *Staphylococcus* spp. occurs commonly in fermented meats and seafood and is not typically hazardous (Søndergaard and Stahnke 2002). *Lactobacillus* spp. contributes to flavor through glutamate dehydrogenase activity (Tanous et al. 2002). Still, microbial diversity can vary depending on the ingredients, methods, and fermentation duration.

Sirichoat et al. (2022) confirmed the presence of *Tetragenococcus*, *Staphylococcus*, and *Lactobacillus* in Pla-ra but linked different odor intensities to variations in microbiota: *Tetragenococcus* was associated with both weak and strong odor groups, while *Virgibacillus* and *Lentibacillus* were more abundant in moderate and strong odor groups, respectively. Additionally, *Brevibacterium*, *Brachybacterium*, *Chromohalobacter*, and *Terribacillus* were linked to specific aroma profiles, underlining microbiota's role in flavor.

A study on Chinese fermented tangerine fish (*Siniperca chuatsi*) found *Fusobacteria* to be prevalent in the ventral muscle and Pseudomonadota in the dorsal muscle (Shen et al., 2021). Pseudomonadota degrades organic molecules, contributing to nutrient cycling (Zhao and Eun 2020). *Acidaminococcus*, involved in amino acid metabolism, was dominant in the samples but had not been detected in previous studies, possibly due to differences in raw materials (Iglesias-Iglesias et al. 2019; Ji et al. 2017). This reinforces how fish anatomy and environment shape microbial profiles, highlighting the value of -omics tools in detecting novel taxa.

Other traditional fermented foods include Sichuan, Cantonese, and Harbin-style Chinese sausages (Wang et al., 2019; Wang et al., 2021b; Chen et al., 2019b). These products are rich in *Staphylococcus* spp. and *Lactobacillus* spp., which contribute to their fermentation properties (Wang et al. 2018). In Sichuan sausages, Wang et al. (2019) identified *Lactobacillus*, *Weissella*, and *Pediococcus* as core microbiota species. *Weissella* spp. contribute to flavor and inhibit pathogens through acid and bacteriocin production, while *Lactobacillus* and *Pediococcus* assist in proteolysis and the release of flavor compounds (Georgieva et al. 2009; Luckow and Delahunty 2004). Similar microbial dominance was reported in Cantonese sausages, with *Pediococcus* spp. as the primary genus in inoculated fermentation (Wang et al. 2021b).

Fermented vegetables have long been part of Chinese tradition, dating back over 3,000 years (Guan et al. 2020b). Today, they're still made using perishable, seasonal produce through traditional, uncontrolled methods, where the microbial composition determines product quality (Cao et al., 2017; Wang et al., 2017).

There are various types of fermented vegetables in the world, especially in China, such as traditional Chinese pickles (An et al. 2021), Chinese spicy cabbage (Chang et al. 2022), Guangxi Suansun and Guangxi Suancai (Guan et al. 2020b), Suansun (Guan et al. 2020c), Suancai (He et al. 2020; Liang et al. 2020), Idli (Kavitake et al. 2022, Mandhanian et al. 2019), Kimchi (Lee et al. 2019), pickled pepper, pickled *Brassica napobrassica*, tasty wild chive flowers, and pickled cucumber (Liang et al. 2022), Jiang-shui and Suan-cai (Liu et al. 2019b), homemade paocai and Chinese spicy cabbage (Liu et al. 2019c), pickled radishes (Rao et al. 2020a), and Sichuan pickles (Rao et al. 2020b).

An et al. (2021) found that *Lactobacillus* and *Weissella* are dominant in both homemade and industrial pickles, both of which are essential to flavor and safety. The presence of *Weissella* in preserved foods enhances flavor and ensures food safety by producing acid and alcohol, thereby inhibiting the growth of pathogenic bacteria (Chen et al., 2020; Fusco et al., 2015). Chang et al. (2022) observed similar patterns in spicy cabbage, though *Pantoea* and *Pseudomonas* also appeared, possibly due to poor anaerobic conditions in homemade versions (Cao et al. 2017). *Pseudomonas* is an aerobic bacteria that can be increased because homemade spicy cabbage conditions can disturb the fermentation process's anaerobic environment (Cao et al. 2017).

A study on Indian Idli fermentation identified *Bacillota* and *Pseudomonadota* as the dominant bacteria, with *Bacillota* contributing to acid tolerance and EPS production, including glucan and dextran (Kavitake et al., 2022). These traits make them suitable for developing starter cultures. Similarly, *Enterococcus*, *Pediococcus*, and *Weissella* are proposed for enhancing shelf life, flavor, and nutrition (Steinkraus 2002). He et al. (2020) observed similar bacterial profiles in fermented plant foods, such as Suancai. Thus, fermented plant products are promising sources of starter cultures.

3.4 Other products

Other important products with complex microbiomes include cocoa, coffee, and meat and fish-cured products (Table 4). Cocoa fermentation is the initial step in the post-harvest processing of cocoa, a crucial step in producing chocolate. This process occurs naturally and involves a complex microbial ecosystem formed through cross-feeding relationships among yeasts and bacteria. The microbial succession begins with yeasts, followed by LAB, and concludes with acetic acid bacteria (AAB) (Almeida et al., 2020; Verce et al., 2021). Fermentation is essential for developing the desirable taste, aroma, and color characteristics in fermented seeds and chocolate products. Enzymes released during the fermentation process can metabolize substrates in cocoa, producing characteristic aromas. Therefore, understanding the microbial dynamics during fermentation is crucial for improving both yield and quality (Agyirifo et al., 2019; Almeida et al., 2020).

The initial populations of the microbial groups involved in cocoa fermentation vary by location and are influenced by the composition of the fresh pulp and the fermentation practices used (Papalexandratou et al. 2019). Agyirifo et al. (2019) evaluated the microbial communities of Ghanaian fermented cocoa and identified a high percentage of bacteria (90.0%), yeast (9%), and bacteriophages (1%) that originated from the cocoa microbiome. The dominant genera were *Lactobacillus* (20%), *Gluconacetobacter* (9%), *Acetobacter* (7%), and *Gluconobacter* (6%). Microorganisms identified in the study, including *L. plantarum*, *L. fermentum*, *Gluconobacter oxydans*, and *Acetobacter pasteurianus*, produce key aromatic compounds, such as diacetyl, acetoin, butanediol, and phenylacetaldehyde, thus enhancing the aroma and sensory qualities of fermented foods. Despite the specificity of microbial communities according to cocoa origin, other studies have also reported the dominance of *Lactobacillus* and *Acetobacter* among bacteria and *Saccharomyces* among yeasts in fermented cocoa from Nicaragua (Papalexandratou et al., 2019), Brazil (Almeida et al., 2020), and Costa Rica (Verce et al., 2021).

Coffee is one of the most popular and widely consumed beverages worldwide, renowned for its rich and complex flavor, as well as its other sensory characteristics. Coffee fermentation occurs naturally through the breakdown of the mucilage surrounding the coffee beans. During this process,

the naturally occurring epiphytic microorganisms in the coffee metabolize sugars, producing compounds such as organic acids, esters, and alcohols, which directly influence the coffee's characteristics. Thus, understanding the microbial ecology of coffee bean fermentation is crucial for comprehending how microbial growth and metabolism impact coffee quality and for recognizing the distinct attributes of coffee produced in various regions (Elhalis et al., 2020; Martins et al., 2020).

Martins et al. (2020) investigated the influence of different altitudes on the epiphytic microbiota of Brazilian coffee. They found that the greatest diversity of yeasts was present in natural and pulped coffee at altitudes of 1000 m and 800 m, respectively, which contributed to the sensory profiles of aroma and fragrance. Additionally, a study by Elhalis et al. (2020) on Australian coffee found that according to the results of culture-independent methods, *Lactococcus*, *Leuconostoc*, *Gluconobacter*, *Enterobacter*, and *Serratia* dominated the bacterial community during fermentation, while the main yeast was *Hanseniaspora uvarum*. This yeast is associated with the sensorial characteristics of other fermented products, such as wine and dairy products. It is recognized for its high enzymatic activities, which lead to the release of aromatic compounds and an enhanced overall quality of the final product. Many microbial species identified in this study have been previously reported in the wet fermentation of coffee beans in other parts of the world, but some yeast species isolated were region-specific. These results corroborate that the diversity of microorganisms present during coffee processing can vary based on several factors, including fruit composition, processing method, and regional conditions such as temperature, oxygen levels, and altitude (Martins et al., 2020).

Fermented meat and fish products are renowned for their distinctive flavor, texture, and extended shelf life, which is achieved through fermentation aided by microorganisms. Zhong et al. (2021) investigated the relationship between microbial communities and the flavor profile of traditional fermented sour meat. They identified *Lactobacillus*, *Weissella*, *Staphylococcus*, *Kodamaea*, *Hyphopichia*, and *Yarrowia* as the predominant microorganisms, which were closely linked to changes in most flavor compounds. Furthermore, esters, aldehydes, and alcohols were identified as the primary contributors to the distinctive flavor of sour meat.

Regarding the fermented fish, hákarl is produced by curing the Greenland shark (*Somniosus microcephalus*). The dominant microorganisms in this product included *Tissierella*, *Pseudomonas*, *Oceanobacillus*, *Abyssivirga*, and *Lactococcus*. Fresh shark meat is toxic due to high levels of trimethylamine (TMA) or trimethylamine N-oxide (TMAO). Still, the presence of *Pseudomonas* in the samples supports the hypothesis that this microorganism might play a role in detoxifying shark meat by breaking down TMAO or TMA during fermentation (Osimani et al. 2019). Additionally, Zhang et al. (2021a) investigated a dry-cured fish stored for 30 days at two different temperatures. They found that *Staphylococcus*, *Macroccoccus*, and *Acinetobacter* were the leading genera throughout the storage period. *Staphylococcus* is capable of producing enterotoxins, significantly contributing to meat spoilage, and therefore requires greater attention in terms of food quality and safety. The study also revealed that refrigeration at 4°C helps maintain microbial diversity, whereas storage at 25°C leads to the dominance of specific microbiota and a decrease in community diversity. Therefore, storing, transporting, and selling fermented fish at 4°C is recommended to extend its shelf life. This study demonstrates that storage conditions have a significant impact on the microbial composition of food products.

4. Omics techniques in food science

Based on the studies that accessed omics techniques (e.g., metataxonomic, metagenomics, and metatranscriptomics), it was observed that the majority (88% of the total) performed metataxonomic sequencing or high-throughput sequencing on different fermented beverages, dairy products, Asian foods, and other foods. The evaluation of the 16S rRNA gene using the V3-V4 region was prevalent in more than 50 accessed studies (~56% of the total) to evaluate the composition of bacterial communities (Tables 1-4), followed by V4 and V4-V5 regions. The 16S rRNA gene is around 1,500 bp long and has been widely used to identify microbial communities directly from DNA samples (Winand et al. 2019). This gene encodes the small ribosomal subunit (16S rRNA), which is organized into highly conserved regions interspersed with nine variable regions (V-regions), designated V1 to V9 (Abellan-Schneyder et al., 2021; Woese, Kandler, & Wheelis, 1990).

Regarding fungal communities, the internal transcribed spacer (ITS) marker gene, encompassing both ITS1 and ITS2 regions, has been the most preferred in the most accessed studies (~72%) (Tables 1-4). The ITS region has been commonly used as a target in molecular biology techniques, and it has the highest probability of being successful in identifying fungi. The ITS region is 300-1,200 bp long and is located between the small and large subunit (SSU and LSU) rRNA genes, encompassing two spacers, ITS1 and ITS2, separated by the 5.8S rRNA gene (Schoch et al., 2012).

Nowadays, the next-generation sequencing (NGS) methods can sequence a maximum of 2 × 300 bp with high accuracy, employing "universal" primers to access one or more variable regions (Winand et al. 2019). The selection of PCR primers for DNA amplification is a crucial step in 16S rRNA and ITS gene sequencing, enabling direct access to taxonomic profiles from DNA samples without the need for cultivation methods.

The universal primer pairs 338F and 806R were used in more than 28 accessed studies (~30%) to evaluate the 16S rRNA gene, targeting the V3-V4 region (Tables 1-4). Other primer pairs frequently used in the accessed studies were 515F and 806R, followed by 341F and 805R (10 and 6 studies). Nowadays, primer 338F is extensively used in various types of DNA samples. For example, it has been used to compare bacterial taxonomic affiliations in food samples, the human gut, soil, and other samples (Dimitrakopoulou et al., 2021; Kameoka et al., 2021; Zeng and An, 2021). The primer pairs used for ITS, specifically ITS1f and ITS2, were employed in numerous studies (13 studies) (Tables 1-4). The primer ITS1f was designed in pair with another primer (ITS-4B) to identify higher fungi and basidiomycetes (Gardes and Bruns 1993). On the other hand, ITS2 was designed with other primers for amplifying many segments of fungi's nuclear and mitochondrial rDNA genes (nuclear, small, ITS, mitochondrial small, and large) (White et al. 1990). ITS1f and ITS2 have been combined to amplify the ITS1 region of the fungal rRNA operon. However, many studies have utilized the entire ITS region or one of its two subregions, ITS1 and ITS2, for DNA barcoding analyses (Tables 1-4).

There have been discussions about the best fungal markers for sequencing, and the results from many studies have shown that ITS1 is more variable than ITS2. Some studies have indicated

that ITS1 is probably the better choice for studying fungal communities (Mbareche et al. 2020; Wang et al. 2015). On the other hand, ITS2 is shorter than ITS1, thereby reducing amplification bias; there is a lower chance of secondary structures or mismatches during amplification, which consequently results in greater consistency (Yang et al. 2018). Regardless of the chosen region, the taxonomic classification of ITS sequence is notoriously difficult and still more challenging in environmental samples due to their length heterogeneity. When compared to the bacterial 16S rRNA gene, some regions of this gene (for example, V4) are typically around 250 bp; on the other hand, the rRNA internal spacer region 2 (ITS2) can range from ~50 to 800 bp (Kyle and Klassen, 2024). In addition, bioinformatic pipelines and parameters have been extensively tested for the 16S gene; however, with an increase in the length of read sequencing and reduced costs, improvements in ITS taxonomic classification are expected.

Most of the studies used the Illumina platform for sequencing (HiSeq NovaSeq6000 platform, MiSeq, HiSeq 2500, NextSeq 500, and others) (Tables 1-4). Regarding bioinformatics analysis, it was observed that the quality control analysis on the next-generation sequencing data was performed using different software and approaches (Table 1-4). On the other hand, regarding the assessment of taxonomic classification based on sequence, it was observed that most of the accessed studies (~48%) utilized QIIME software in various versions (v1.8.0, v1.9.2, v1.9.1, v2, and others) (Tables 1-4). QIIME (Quantitative Insights into Microbial Ecology) is a software that performs microbial community analysis from bacterial, archaeal, fungal, and viral communities. The wide application of QIIME may be related to its ability to run on a high-performance computer cluster or even a laptop running Windows (Kuczynski et al. 2011). Also, it was observed that the taxonomic classification of 16S reads was usually based on curated databases: Greengenes (McDonald et al. 2012), RDP - Ribosomal Database Project (taxonomy or classifier) (Wang et al. 2007) and SILVA (Yilmaz et al. 2014) in many of accessed studies (Tables 1-4). RDP and Silva databases are based on 16S rRNA sequences from Bacteria, Archaea, and Fungi. On the other hand, the Greengenes database is dedicated to the taxonomy of bacteria and archaea. The RDP database is one of the most popular approaches for taxonomic classification, has been recently updated (RDP Classifier 2.14 (August 2023)) (Wang and Cole 2024), and the SILVA SSU database 138 was

updated in 2019 (<https://www.arb-silva.de/documentation/release-138/>). For instance, Greengenes has not been updated since 2013, but it has recently been updated to a new version of Greengenes2 (McDonald et al., 2024). Regularly updating the database is crucial because it can provide more data for accurate taxonomy and phylogenies classification. Regarding the database for molecular identification of eukaryotes, it was observed that most studies used UNITE (<https://unite.ut.ee>) (Tables 1-4). The UNITE database was launched in 2003 and is considered a web-based database that provides approximately 10 million sequence references related to the nuclear ribosomal ITS region (Abarenkov et al., 2024).

Finally, it was also observed that few studies used metagenomics (whole metagenome shotgun) and metatranscriptomic in the accessed literature (Tables 1-4). Metagenomic and metatranscriptomic sequencing is a culture-independent method that involves sequencing the whole DNA or RNA fragments derived from all organisms present in a single sample, providing taxonomic and functional information (Pérez-Cobas et al., 2020). The widespread use of metataxonomic sequencing compared to metagenomic/metatranscriptomic sequencing is related to the lower cost of sequencing, well-established bioinformatic analytical pipelines, the absence of a requirement for strong computational capacity, and the availability of curated public databases (SILVA, RDP, Greengenes, and others) (Pérez-Losada et al. 2022). On the other hand, metataxonomic analysis, despite its widespread use, presents several disadvantages. This technique cannot discriminate between dead and live microorganisms; this aspect is considered a problem if the metabolisms are the primary concern in the study. In addition, there is a lack of resolution; metataxonomic sequencing achieves the identification of microorganisms at the genus level (not at the species level), and neither the 16S rRNA (for bacteria) nor the ITS genes (for fungi) are used. This scenario is expected to change soon due to the increasing power of new sequencing machines, the decreasing cost of sequencing processes, and the development of user-friendly bioinformatics tools for researchers. Other omics approaches, such as metagenomic (whole-metagenome shotgun sequencing) or metatranscriptomic (RNA sequencing), are helping researchers understand the microbial community structure, function, and interactions within a specific environment with greater resolution. However, it is known that these techniques remain challenging for many research

groups because they require more sophisticated laboratory processes, advanced bioinformatic tools, and computational capacity for fast data processing (Xu et al., 2021b). Despite the challenges, applying omics techniques will substantially improve the understanding of the patterns related to microbial communities in fermented foods, spoilage microbiota, or probiotic products.

5. Final remarks and future trends

The characterization of food microbiota has become an essential focus in food science, offering critical insights into various product aspects, such as sensory properties, nutritional composition, shelf life, and safety. Fermented foods represent a rich and dynamic microbial environment where bacteria, yeasts, and fungi interact to shape product quality, stability, safety, and sensory complexity. Factors such as raw materials, fermentation conditions, geographical origin, and processing stages modulate microbial composition, succession, and dominance throughout fermentation processes.

The evolution from classical culture-dependent methods to modern omics-based, culture-independent techniques, especially high-throughput sequencing, has revolutionized the study of microbial communities. Metataxonomics (16S rRNA and ITS sequencing) has become the most accessible and widely applied approach, allowing researchers to describe the diversity and structure of microbiota with high taxonomic breadth. However, this approach still faces limitations in species-level resolution and viability discrimination. Meanwhile, more comprehensive omics strategies, including metagenomics, metatranscriptomics, metaproteomics, and metabolomics, are gradually being integrated, enabling not only taxonomic identification but also functional characterization and metabolic profiling of microbial communities.

Despite current challenges, such as cost, bioinformatics complexity, and limited access to curated databases, the incorporation of multi-omics approaches, coupled with artificial intelligence and advanced computational modeling, is expected to reshape the future of food microbiome research. These innovations will support the development of predictive models for microbial behavior, help optimize fermentation processes, and enhance the reproducibility and traceability of food products.

In practical terms, understanding the microbiota of fermented foods can inform the design of new starter cultures, enhance the selection of probiotic strains, and facilitate the authentication and valorization of traditional and artisanal products. Moreover, microbiome monitoring has implications for food safety management, particularly in detecting spoilage or pathogenic organisms and controlling their presence throughout the production chain.

In conclusion, the application of omics technologies to food microbiota analysis not only deepens our understanding of microbial ecology in food systems but also opens new avenues for innovation in the development of high-quality, safe, functional, and sustainable food products. As tools and methods continue to evolve, integrating taxonomic and functional data will be key to unlocking the full potential of microbial communities in food science and industry.

Acknowledgments

Thank the “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (Grants # CNPq, (Grants # 402745/2021-3, 303384/2022-0 and 404506/2023-2) and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES-Brazil; Finance code 001 and UFPB/CAPES/PrInt 88881.311776/2018-01).

Conflicts of interests

There is no conflict of interest to be declared.

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Figure Captions

Figure 1 - Main microbial composition of fermented beverages, considering the genus level.

Figure 2 - Impact of microbial composition on the sensory and quality characteristics of food products.

Figure 3 - Main factors influencing the microbiota composition of food products

Figure 4 - Dairy foods and their main microbial composition, considering the genus level.

Figure 5 - Asian foods and their main microbial composition, considering the genus level.

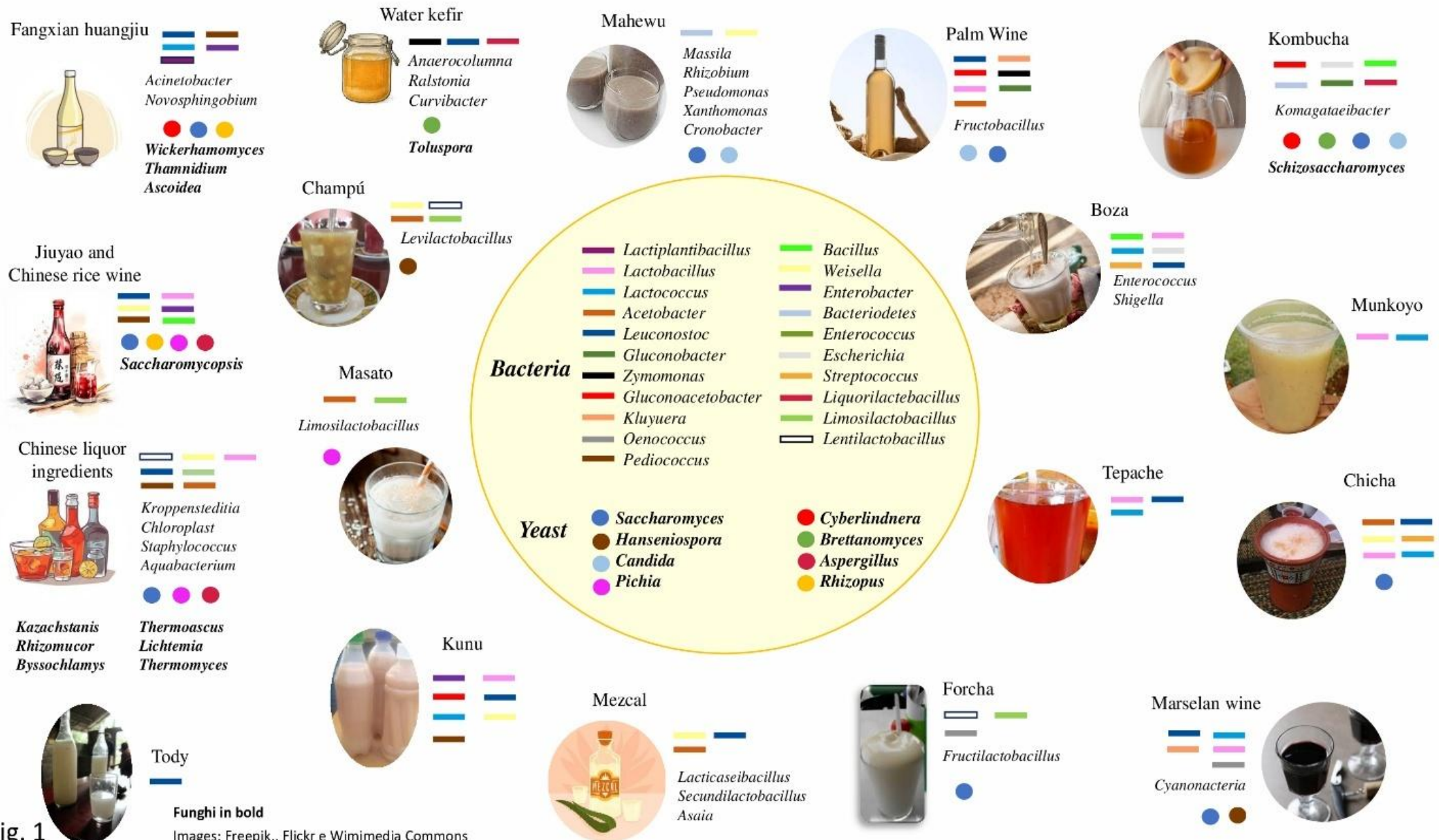
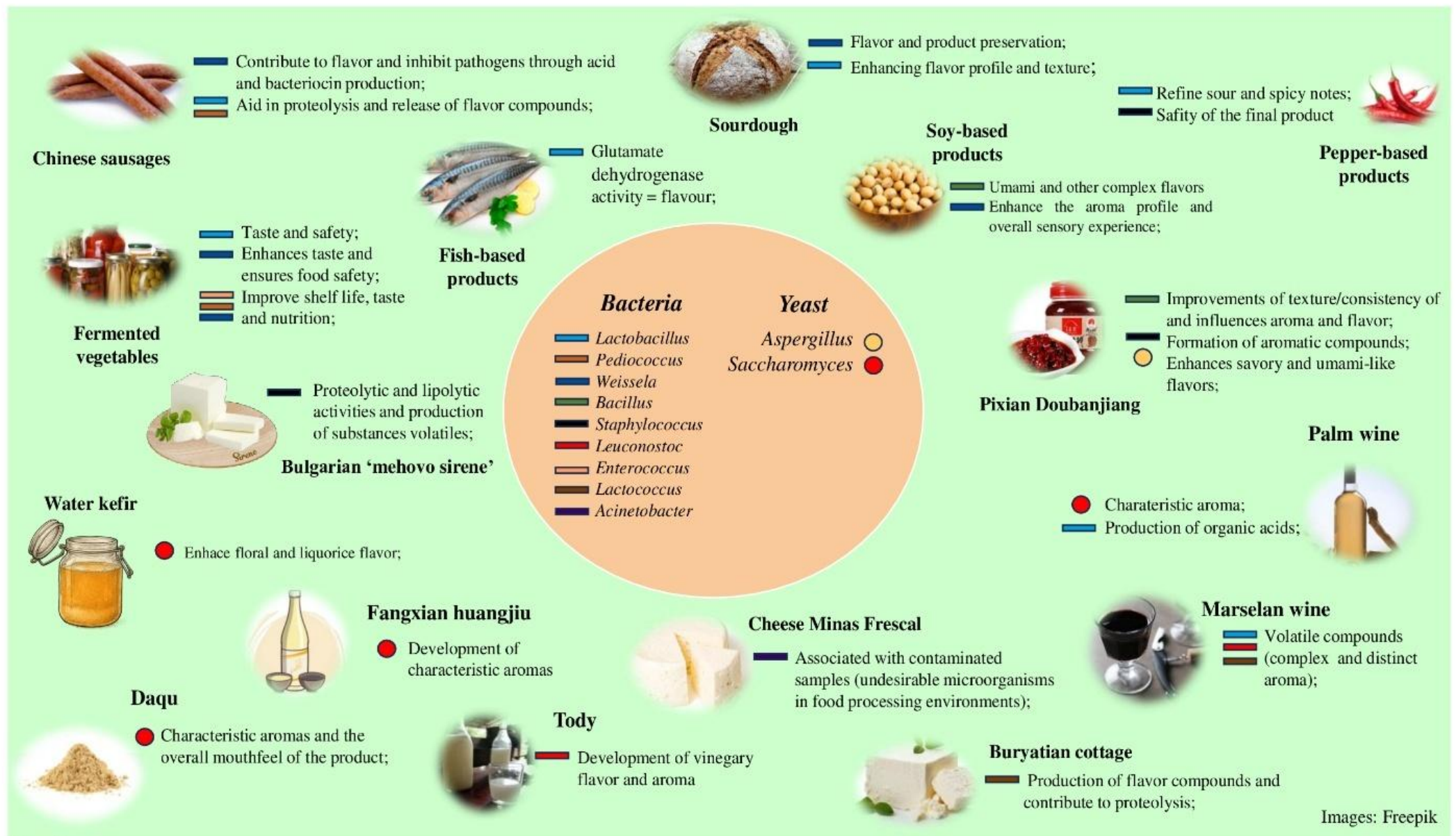
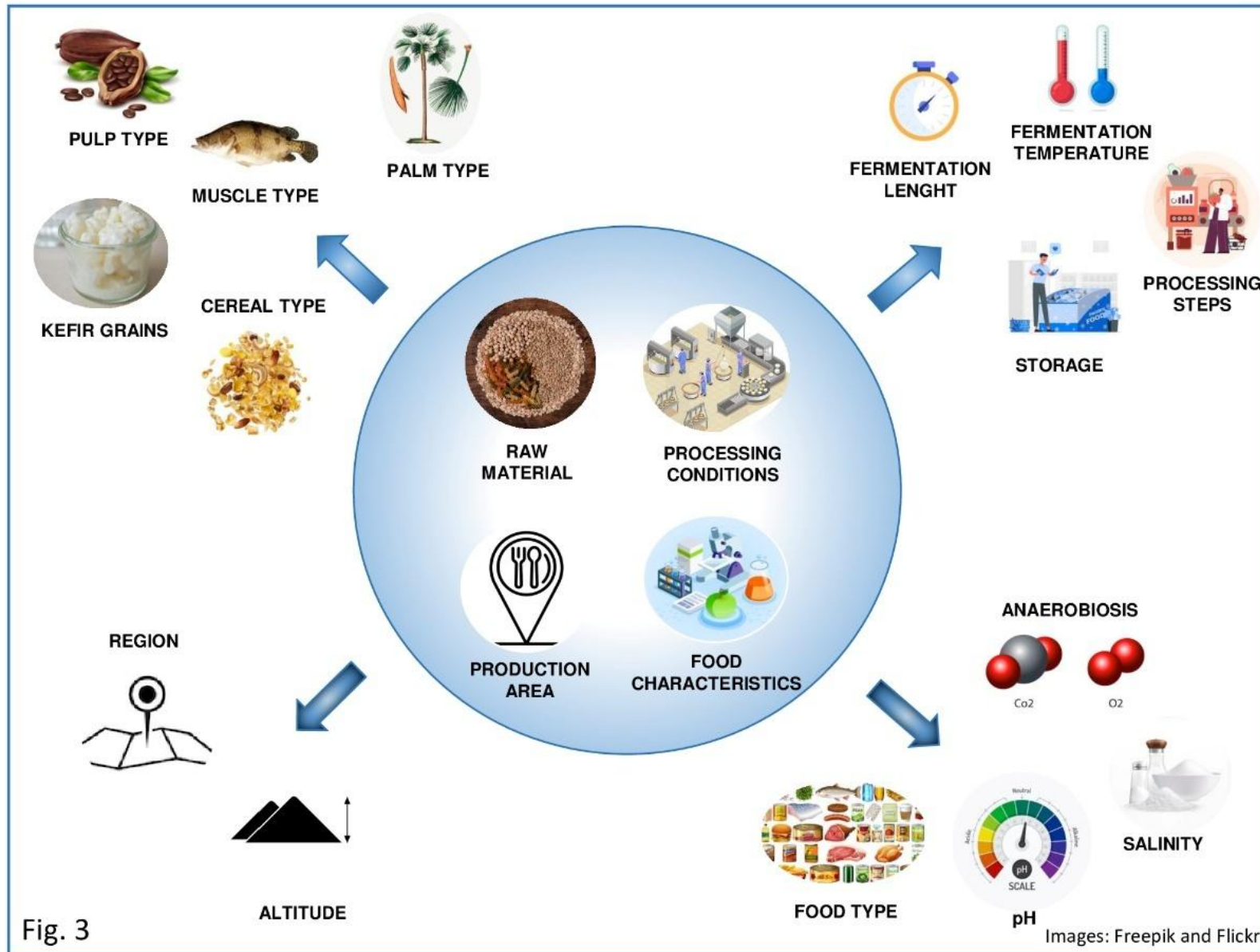


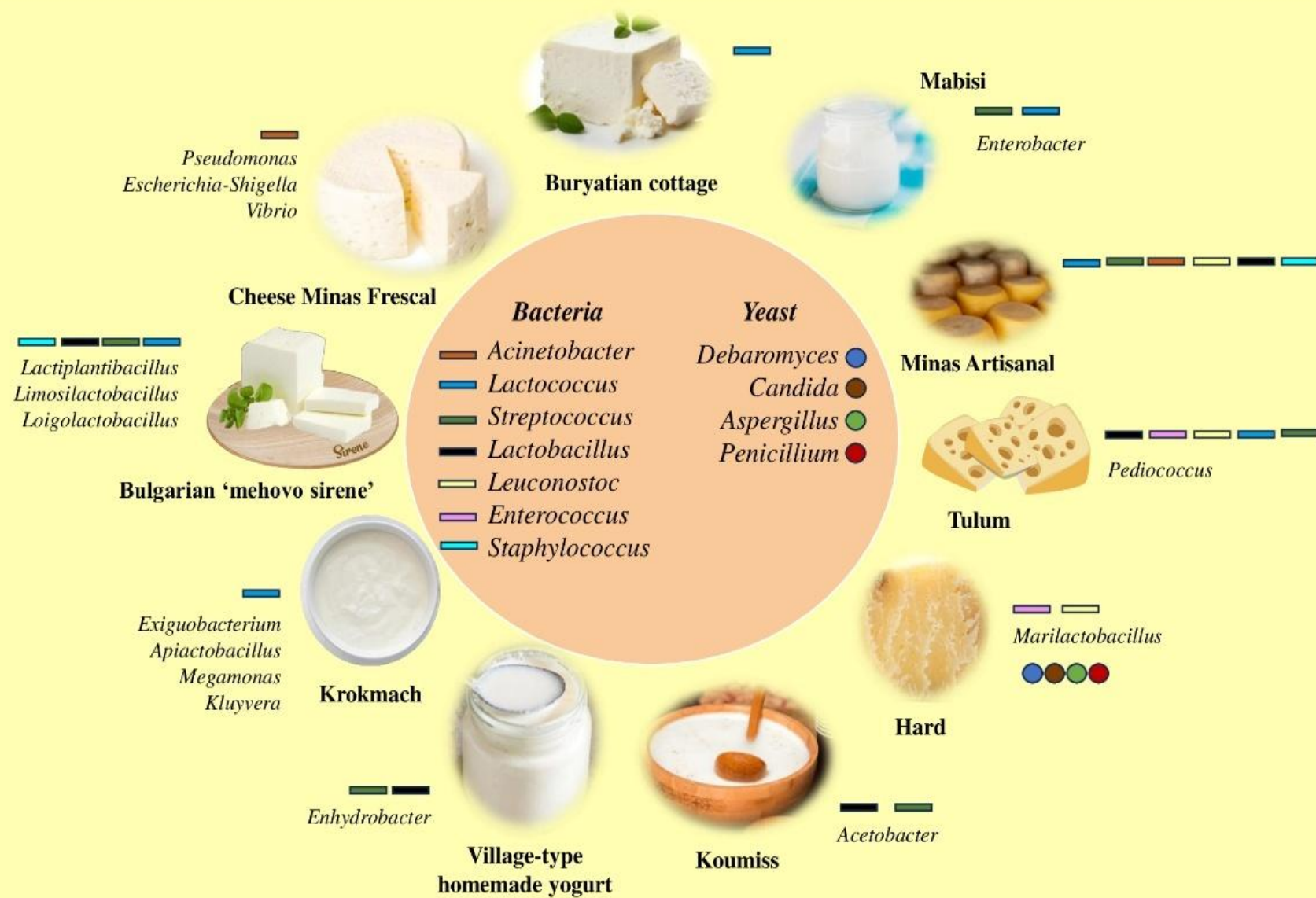
Fig. 1



Images: Freepik

Fig. 2





Images: Freepik

Fig. 4



Fig. 5

Table 1. Microbial communities in fermented beverages using omics techniques.

Sample	Study aim	Methods used	Main findings	Reference
Water kefir	Characterization of the microbial composition of the original water kefir and compare to obtain a microbiological profile.	<u>Metagenomic sequencing:</u> Platform: Illumina MiSeq HTS: V1–V2 region of 16S rRNA. Quality control: not provided Database: SILVA v. 138.1 database.	<u>Bacteria:</u> <i>Anaerocolumna</i> and <i>Ralstonia</i> were the dominant genus of microorganisms in beverages. In grains, the most common genus was <i>Liquorilactobacillus</i> .	Rodríguez et al. (2024)
Water kefir	Study the microbial evolution and the contribution of different microbial groups present in water kefir grains, for further development and optimization of the water kefir manufacturing process.	<u>Metagenomic sequencing:</u> Platform: Illumina HiSeq 2500 Quality control: FastQC v0.11.9 using Trimmomatic v0.33, Bowtie2 and MEGAHIT v1.2.9. Database: NCBI BLAST nonredundant protein.	<u>Bacteria:</u> <i>Zymomonas mobilis</i> was the most abundant species during fermentation, present in kefir liquor and grains. <u>Fungi or Yeast:</u> The dominant species was <i>Brettanomyces bruxellensis</i> . <i>Lentilactobacillus hilgardii</i> , <i>Leuconostoc suionicum</i> , <i>Lactocaseibacillus paracasei</i> , <i>Lactiplantibacillus plantarum</i> , and <i>Liquorilactobacillus satsumensis</i> were dominant in the grains, while <i>Curvibacter sp. PAE-UM</i> , <i>Brettanomyces bruxellensis</i> , <i>Torulaspora delbrueckii</i> , <i>Liquorilactobacillus hordei</i> , <i>Liquorilactobacillus mali</i> , and <i>Leconostoc pseudomesenteroides</i> were predominant in the liquor, especially after 30 h of fermentation.	Patel et al. (2022)

Chicha, Forcha, Champús, and Masato	Study of the fungal and bacterial communities of fermented beverages and elucidate their possible role in fermentation.	<u>Metataxonomic sequencing:</u> Platform: Illumina HiSeq HTS: V3-V4 regions of 16S rDNA and ITS (Internal Transcriber Spacer). Quality control: QIIME 2 v2020.8. Database: Bayesian classifier, Greengenes v13_8, and UNITE databases.	<u>Bacteria:</u> Bacillota was the predominant phylum, and <i>Lactobacillus</i> was the most prevalent genus in all beverages. Forcha samples: <i>Fructilactobacillus sanfranciscensis</i>, <i>Oenococcus oeni</i>, <i>Limosilactobacillus reuteri</i>, <i>Lentilactobacillus buchneri</i>, and <i>Limosilactobacillus fermentum</i> were predominant. Masato samples: the most abundant species were <i>Limosilactobacillus fermentum</i>, <i>Ligilactobacillus ruminis</i>, <i>Limosilactobacillus reuteri</i>, and <i>Acetobacter pasteurianus</i>. Champú samples: <i>Limosilactobacillus fermentum</i>, <i>Weisella koreensis</i>, <i>Lentilactobacillus buchneri</i>, <i>Levilactobacillus brevis</i>, <i>Limosilactobacillus reuteri</i>, and <i>Acetobacter pasteurianus</i> constituted the majority of species Chicha samples: <i>Lactococcus lactis</i> and <i>Weisella koreensis</i> were the dominant organisms. <u>Fungi or Yeast:</u> The analysis of fungal communities revealed the presence of only Ascomycota and Basidiomycota phyla. <i>Saccharomyces</i> was the most prevalent in the Forcha and Chicha samples, while <i>Pichia</i> was the dominant species in the Masato sample and <i>Hanseniaspora</i> in the Champús sample.	Delgado-Ospina et al. (2022)
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Chicha	Investigation of the bacterial diversity associated with Peruvian Chicha de Jora produced in 14 chicherias located in seven different provinces in Peru.	<u>Metataxonomic sequencing:</u> Platform: Illumina Miseq HTS: V3-V4 regions, 16S rRNA sequence (Primers 343F and 802R). Quality control: not provided Database: Greengenes	<u>Bacteria:</u> Bacillota and Pseudomonadota were the dominant phyla in all samples. Bacteroidetes and Actinobacteria were also detected in small amounts in some samples. <i>Lactobacillaceae</i> and <i>Acetobacteraceae</i> were the most representative families, and <i>Leuconostocaceae</i> and <i>Streptococcaceae</i> were also detected in all samples. A small percentage of chicha samples contained other families of bacteria: <i>Clostridiaceae</i>, <i>Bacillaceae</i>, <i>Enterobacteriaceae</i>, <i>Enterococcaceae</i>, <i>Bifidobacteriaceae</i>, <i>Sphingomonadaceae</i>, and <i>Ruminococcaceae</i>.	Bassi et al. (2020)
Tepache	Analyze the physicochemical aspects, the bacterial and fungal community structure, and the predictive metabolic potential of microbial communities during tepache fermentation.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq 2 × 300 HTS: V4-V5 regions 16S rRNA gene (515 F and 926R primers) and ITS2-ITS4 of fungal rRNA operon (ITS86F and ITS4 primers). Quality control: -fastq_filter, fastq_maxlen and fastq_minlen libraries. Database: Ribosomal Database Project (RDP).	<u>Bacteria:</u> Increase of Bacillota, reduction of Pseudomonadota, and complete loss of Bacteroidetes and Acidobacteria phyla during fermentation. Pseudomonadota and Bacteroidetes were only found at the initial time. After 72 hours, <i>Bacilli</i> class members, including the genus <i>Lactobacillus</i>, <i>Leuconostoc</i>, and <i>Lactococcus</i>, were dominant. <u>Fungi or Yeast:</u> The progress on fermentation resulted in an increase of Ascomycota and a reduction of Basidiomycota phylum.	Gutiérrez-Sarmiento et al. (2022)
Boza	Determination of the bacterial microbiota of Boza samples collected from different regions of Turkey and	<u>Metataxonomic sequencing:</u> Platform: Illumina iSeq100 HTS: 16S rRNA gene, V3-V4 region (341F and 806R primer)	<u>Bacteria:</u> <i>Lactococcus</i>, <i>Lactobacillus</i>, <i>Leuconostoc</i>, and <i>Streptococcus</i> were the dominant genera in all samples.	Ucak et al. (2022)

	analysis of the chemical properties.	Quality control: not provided Database: RDP Classifier	Pre-enriched Boza samples were dominated by <i>Lactococcus</i> , <i>Enterococcus</i> , <i>Escherichia/Shigella</i> , <i>Bacillus</i> , and <i>Lactobacillus</i> . Pre-enrichment of Boza samples increased the number of <i>Enterococci</i> and <i>Escherichia/Shigella</i> . All enterococci were identified as <i>Enterococcus faecium</i> .	
Kombucha	Investigate the heterogeneity of nine commercial kombucha samples by employing chemical, metabolomics and metagenomics analyses to gain a deeper understanding of the diversity of kombucha.	<u>Metagenomic sequencing:</u> Platform: Illumina Novaseq 6000 Quality control: KneadData Database: MetaPhlAn 3.0 <u>Metataxonomic sequencing</u> Platform: Illumina Novaseq 6000 HTS: Fungal ITS1 region (ITS1f and ITS2 primers) Quality control: DADA2 Database: UNITE database	<u>Bacteria:</u> The samples were divided according to the dominant bacteria. The first group presented a predominance of <i>Bacillus coagulans</i> and/or <i>Gluconacetobacter liquefaciens</i> . The second group showed a dominance of <i>Liquorilactobacillus nagelii</i> . The last group was dominated by <i>Liquorilactobacillus mali</i> , <i>Gluconobacter</i> , genus <i>Komagataeibacter</i> (mainly <i>K. rhaeticus</i>), <i>Escherichia coli</i> , and <i>Bacteroides thetaiotamicron</i> . All samples contained enteric bacteria <i>Bacteroides thetaiotamicron</i> , <i>Escherichia coli</i> , and <i>Enterococcus faecalis</i> . <u>Fungi or Yeast:</u> <i>Brettanomyces</i> species and <i>Cyberlindnera jadinii</i> dominated the fungal composition, and <i>Saccharomyces cerevisiae</i> was found in all samples.	Yang et al. (2022)
Kombucha	Determination of the microbial composition and functional properties of two Turkish kombuchas during fermentation.	<u>Metagenomic sequencing:</u> Platform: Illumina HiSeq X Ten; Quality control: MGnify v4.1	<u>Bacteria:</u> Pseudomonadota were predominant and attributed to the <i>Acetobacteraceae</i> family during fermentation.	Arikan et al. (2020)

		Database: MapSeq v1.2 and SILVA v128 database	<u>Fungi or Yeast:</u> Ascomycota was the predominant fungal phylum in both samples, and ITS1 sequences were assigned to the genus <i>Zygosaccharomyces</i> .
		<u>Metataxonomic sequencing:</u>	Clustering of the genome bins revealed the presence of three <i>Komagataeibacter</i> genomes and one <i>Z. bailii</i> genome.
		Platform: Illumina MiSeq	
		HTS: V3-V4 region of 16S rRNA gene (primers 341F and 785R) and the fungal ITS region (ITS1F- and ITS2R)	
		Quality control: QIIME2	
		Database: SILVA v128 and UNITE v7.2 databases.	
Kombucha	Study three Kombuchas samples' kinetics, microbial diversity, chemical composition, and functional profile.	<u>Metagenomic shotgun sequencing:</u>	<u>Bacteria:</u> Actinobacteria and Pseudomonadota were identified in all samples. The <i>Acetobacteraceae</i> family was dominant, with the dominant genera being <i>Komagataeibacter</i> , <i>Gluconacetobacter</i> , and <i>Gluconobacter</i> . Additionally, several species from the <i>Acetobacter</i> genus were identified (<i>A. malorum</i> , <i>A. pasteurianus</i> , <i>A. pomorum</i> , and <i>A. tropicalis</i>).
		Platform: NextSeq sequencing platform Illumina	
		Quality control: bwa v. 0.7.15 and CheckM	
		Database: NR database	The dominant bacterial species identified were <i>Komagataeibacter rhaeticus</i> , followed by <i>Gluconacetobacter sp. SXCC1</i> .
			<u>Fungi or Yeast:</u> The <i>Saccharomycetaceae</i> and <i>Schizosaccharomycetaceae</i> families (phylum Ascomycota) were the most prevalent. The four genera of yeast <i>Candida arabinofementans</i> ,

			<i>Brettanomyces bruxellensis</i> , <i>Schizosaccharomyces pombe</i> , and <i>Zygosaccharomyces bailii</i> were identified across the three kombucha liquid phases, with <i>B. bruxellensis</i> being the most prevalent.	
Mabisi and Munkoyo (Zambia)	Profiling the microbial communities of the traditional fermented foods Mabisi and Munkoyo, collected in Zambia, at the end of fermentation and documenting the nutritional composition and their variations from different producers.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq V3 HTS: 16SrRNA Amplicon Sequencing Quality control: cutadapt Database: not provided	<u>Bacteria:</u> The most frequently identified genera in all samples were <i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Streptococcus</i> , <i>Enterobacter</i> , <i>Klebsiella</i> or <i>Acetobacter</i> . In Mabisi samples, <i>Streptococcus</i> , <i>Enterobacter</i> , and <i>Lactococcus</i> were most abundant, whereas in Munkoyo, <i>Lactococcus</i> and <i>Lactobacillus</i> were dominant.	Chileshe et al. (2020)
Munkoyo	Examination of the physiochemical properties of Munkoyo, profiling the bacterial community composition concerning location, variations in processing practices and consumption preferences, and identifying the aroma compounds of the beverage.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq HTS: V3-V4 regions of the 16S rRNA (341F and 785R primers) Quality control: pick_open_reference_otus.py Database: Greengenes (v13.5) rRNA database	The dominant bacterial families identified were <i>Streptococcaceae</i> , <i>Leuconostocaceae</i> , <i>Enterobacteriaceae</i> , <i>Lactabacillales</i> , <i>Bacillaceae</i> , and <i>Aeromonadaceae</i> . The results demonstrated high levels of bacterial diversity, with notable differences observed between samples.	Phiri et al. (2019)
Palm Wine	Identification of the key microbial communities and metabolites associated with the palm wines produced from ron palm (<i>Borassus</i>	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq	<u>Bacteria:</u> Dominance of Bacillota, represented by the <i>Lactobacillaceae</i> and <i>Leuconostocaceae</i> families, and Pseudomonadota phyla, including the	Djeni et al. (2020)

	<i>aethiopum</i>), oil palm (<i>Elaeis guineensis</i>), and rafa palm (<i>Raphia hookeri</i>) in Côte d'Ivoire and attempted to establish the basis of their differences.	HTS: V3-V4 region of 16S rRNA gene (Primers 520 F and 907R) and the fungal ITS2 region (Forward ITS1-F and reverse primer ITS2) Quality control: QIIME v1.8.0 platform Database: UNITE v7.1 (fungal ITS) and M5RNA databases (16S rRNA).	<i>Acetobacteraceae</i>, <i>Sphingomonadaceae</i>, and <i>Enterobacteriaceae</i> families. At the genus level, the genera <i>Lactobacillus</i>, <i>Acetobacter</i>, <i>Leuconostoc</i>, <i>Fructobacillus</i>, <i>Gluconobacter</i>, <i>Zymomonas</i>, <i>Gluconoacetobacter</i>, and <i>Kluyvera</i> were the most abundant in the palm wine samples. <u>Fungi or Yeast:</u> <i>Saccharomyces cerevisiae</i> was the dominant yeast in all three types of palm wine. <i>Hanseniaspora sp.</i> and <i>Hanseniaspora valbyensis</i> were present in raphia palm wine, <i>Hanseniaspora guilliermondii</i> and <i>Saccharomycodes</i> in the oil palm wine.	
Palm Wine (Tuba)	Comparison of the production of Tuba under controlled conditions and those commercialized locally and analyzing the diversity and functionality of the bacterial communities.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq HTS: V3–V4 regions of the 16S rRNA; Primers (341F and 785R 5'primers) Quality control: not provided Database: Greengenes	<u>Bacteria:</u> The predominant genera in all samples were <i>Fructobacillus</i>, <i>Leuconostoc</i>, <i>Gluconacetobacter</i>, <i>Sphingomonas</i>, <i>Vibrio</i>, and some genera of the <i>Enterobacteriaceae</i> family, including potential pathogens such as <i>Erwinia</i>, <i>Klebsiella</i>, <i>Serratia</i>, and <i>Cronobacter</i>.	Astudillo-Melgar et al. (2019)
Marselan wine	Investigate the microorganism diversity and aroma compounds during the spontaneous fermentation (SMF) of 'Marselan' and compare with the inoculated fermentation (IMF).	<u>Metataxonomic sequencing:</u> Platform: Illumina Miseq HTS: 16 S rRNA genes, V3–V4 region (338 F and 806 R primers)	<u>Bacteria:</u> <i>Cyanobacteria</i> and <i>Kluyvera</i> decreased during spontaneous fermentation, while <i>Leuconostoc</i> and <i>Lactococcus</i> increased. The abundance of bacteria in inoculated fermentation was higher than in spontaneous fermentation. In SMF, <i>norank_c_cyanobacteria</i>, <i>Rhodococcus</i>,	Lu et al. (2020)

		ITS region for fungi (ITS1F and ITS2R primers)	<i>Leuconostoc</i> , <i>Oenococcus</i> , and <i>Lactobacillus</i> were the predominant microorganisms.	
		Quality control: QIIME software		
		Database: Silva/Unite Database	<u>Fungi or Yeast</u> : <i>Saccharomyces</i> was the most abundant yeast genera in SMF and IMF, dominating throughout fermentation.	
Wine	Isolation and identification of the yeast populations during spontaneous wine fermentation and analysis of the correlation of the fungal communities with wine aroma profiles.	<u>Metataxonomic sequencing</u> : Platform: Illumina MiSeq HTS: Fungal ITS1 region (ITS1F and ITS2 primers) Quality control: QIIME v1.9.2 and the paired ends were joined using FLASH Database: UNITE fungal ITS database (v7.2)	<u>Fungi or Yeast</u> : Ascomycota was the most abundant phylum in the grape before fermentation, followed by <i>Basidiomycota</i> and <i>Mortierellomycota</i> . Filamentous fungi, especially the genera <i>Cladosporium</i> , <i>Aureobasidium</i> , and <i>Epicoccum</i> , and great populations of <i>Saccharomyces</i> , <i>Hanseniaspora</i> , <i>Basidiomycetous</i> , <i>Vishniacozyma</i> , <i>Rhodotorula</i> , and <i>Sporobolomyces</i> dominated the must/juice. Significant decreases in fungal diversity were observed after the fermentation stages. However, <i>Saccharomyces cerevisiae</i> dominated all samples' fermentations at the middle and end points.	Liu et al. (2021a)
Kunu	Determination of the bacterial community dynamics and diversity during three Kunu formulation processes and evaluation of the effect of processing on the mycotoxin profiles.	<u>Metataxonomic sequencing</u> : Platform: Illumina MiSeq HTS: V3-V4 region of partial 16S rRNA genes (Primers 341F and 805R) Quality control: FastQC software Database: SILVA rRNA reference (Release 128) database	<u>Bacteria</u> : Bacillota dominated most processing stages, while Pseudomonadota dominated the remainder. <i>Lactobacillus</i> was the dominant genus in most of the processing stages. All communities exhibited shared phylotypes dominated by LAB species of <i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Leuconostoc</i> , <i>Pediococcus</i> , and <i>Weissella</i> . Other shared phylotypes included species of <i>Gluconacetobacter</i> , <i>Enterobacter</i> , <i>Lactobacillus</i> ,	Ezekiel et al. (2019)

			<i>Leuconostoc</i> , <i>Lactococcus</i> , <i>Pediococcus</i> , and <i>Weissella</i> .
Mahewu	Determination of the composition and origin of the microbiota involved in the fermentation of Mahewu and establishing the degree of overlap between the microbiota of mahewu and millet malt.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq HTS: V5-V6 regions of the 16S rRNA (784F and 1064R primers) Quality control: LotuS v. 1.565 Database: BLAST against the SILVA v128 database	<u>Bacteria:</u> The most abundant phylum was Pseudomonadota, with <i>Cronobacter</i> as the most abundant genus in all millet malt samples, followed by Bacillota, with <i>Weissella</i> as the most abundant genus. DNA from <i>Rhizobium</i> , <i>Massilia</i> spp., <i>Pseudomonas</i> spp., <i>Xanthomonas</i> , and <i>Bacteroidetes</i> was also identified. <u>Fungi or Yeast:</u> <i>Saccharomyces cerevisiae</i> and <i>Candida glabrata</i> were present in all samples.
Fangxian huangjiu (FH)	Investigate the microbial communities, metabolites, and physicochemical factors of FH samples, correlating the microbial communities, physicochemical characteristics, and volatile compounds and mapping the metabolism, temporal taxonomic taxa, enzymes of starch degradation, and flavor development.	<u>Metagenomic sequencing:</u> Platform: Illumina NovaSeq 6000 Quality control: MEGAHIT Database: KEGG	<u>Bacteria:</u> The main bacterial phyla were Pseudomonadota, Bacillota, Bacteroidetes, and Actinobacteria. <i>Leuconostoc</i> , <i>Lactococcus</i> , <i>Acinetobacter</i> , <i>Enterobacter</i> , and <i>Novosphingobium</i> remained high throughout fermentation. <i>Raoultella</i> and <i>Pseudomonas</i> had high levels at the pre-fermentation stage but low levels at the post-fermentation stage. <i>Lactiplantibacillus</i> and <i>Pediococcus</i> were low in pre-fermentation but remained high in post-fermentation. <u>Fungi or Yeast:</u> Mucoromycota and Ascomycota were the most abundant phyla.

			<i>Rhizopus</i> was found at the pre-fermentation stage, and low levels were detected at the post-fermentation. At the same time, <i>Saccharomyces</i>, <i>Wickerhamomyces</i>, <i>Thamnidium</i>, <i>Ascoidea</i>, and <i>Cyberlindnera</i> remained low at the pre-fermentation stage and showed high levels at the post-fermentation.	
Mezcal	Study of the bacterial composition in mezcal from Guerrero state.	<u>Metataxonomic sequencing:</u> Platform: Ion Torrent PGM™ HTS: V2–V3 region of 16S rRNA (27F and 1495R) Quality control: Trimmomatic Database: Greengenes	<u>Bacteria:</u> <i>Lactobacillales</i>, <i>Rhodospirillales</i>, <i>Enterobacteriales</i>, <i>Flavobacteriales</i>, <i>Actinomycetales</i>, and <i>Bacillales</i> were the most abundant orders. <i>Lactobacillales</i> were more abundant at the end of the fermentation, and <i>Rhodospirillales</i> were more abundant at the beginning. <i>Lactocaseibacillus</i>, <i>Secundilactobacillus</i>, <i>Acetobacter</i>, <i>Asaia</i>, <i>Weissella</i> and <i>Leuconostoc</i> were the most abundant genera in all samples.	Kirchmayr et al. (2024)
Toddy	Study the metabolomics and metagenomics of toddy to obtain the profile of microbial communities and their contribution to the production of metabolites for health benefits.	<u>Metagenomic sequencing:</u> Platform: Nanopore sequencing Quality control: NanoPlot software v1.33.0. Database: NCBI nr + euk database	<u>Bacteria:</u> The phylum Bacillota were the most abundant, followed by Pseudomonadota, Actinomycetota, Bacteroidota, Acidobacteriota, Ascomycota, and Uroviricota. The family <i>Leuconostocaceae</i> was the most prevalent, <i>Leuconostoc</i> was the most abundant genus, and <i>Leuconostoc mesenteroides</i> was the most abundant among other lactic acid bacteria (LAB) species.	Das and Tamang (2023)
Chinese liquor	The effect of hulless barley on the microbiota in Chinese liquor was studied using two	<u>Metataxonomic sequencing:</u>	<u>Bacteria:</u> <i>Pantoea</i>, <i>Acinetobacter</i>, and <i>Staphylococcus</i> ↓with ↑fermentation time	Liu et al. (2019a)

	main cultivars (heilaoya and duli Huang)	Platform: MiSeq benchtop sequencer for 250-bp paired-end sequencing HTS: Bacteria: V3-V4 hypervariable region of the 16S rRNA gene with universal primer sets F338 and barcode-R806. Fungi: internal transcribed spacer (ITS) region with primers ITS1F and ITS2R. Quality control: Qiime pipeline (v1.8.0) Database: Greengenes database (v13.8) and the UNITE fungal ITS database (v6.0).	<i>Weissella</i> and <i>Lactobacillus</i> ↑with ↑fermentation time. <u>Fungi or Yeast:</u> <i>Pichia</i> (↑with ↑fermentation time), <i>Komagataella</i>, <i>Aspergillus</i> (↓with ↑fermentation time), and <i>Saccharomyces</i> (↓with ↑fermentation time) were different in the two types of fermented grain.
Chinese rice wine	Evaluating Bacterial and fungal diversity and their relationship with volatile compounds	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq sequencing HTS: Bacteria: V3-V4 hypervariable regions of the bacterial 16 S rRNA gene were amplified with primers 338 F and 806 R. Fungal: ITS1 was amplified with the ITS1F and ITS2 primers. Quality control: Trimmomatic and merged by FLASH Database: QIIME v1.7.0	<u>Bacteria:</u> Bacillota, Pseudomonadota, and Actinobacteria were the dominant bacterial phyla. Ren et al. (2019) <i>Bacillus</i> was the dominant genus. The 8° day of fermentation showed the abundance of most genera, which decreased until the 12th day. <u>Bacteria:</u> <i>Weissella</i> was the main genus that generated acids and could inhibit the growth of other bacteria. <u>Fungi or Yeast:</u> <i>unclassified_O_Saccharomycetales</i> was the high percent relative abundance.

			The <i>Pichia</i> genus decreased throughout fermentation.	
Black glutinous rice wine	Exploring the correlations between microbial communities with physicochemical properties and flavor components during fermentation	<u>Metataxonomic sequencing:</u> Platform: Illumina Hiseq 2500 platform HTS: Bacteria: V3-V4 region of the bacteria 16S rRNA gene was amplified with primers 338F and 806R Fungi: The ITS1 region of the fungi was amplified with the forward primer ITS1F and the reverse primer ITS1R Quality control: Trimmomatic software v0.33, chimeras removed using UCHIME tool in QIIME software Database: Silva (bacteria) and UNITE (fungi) taxonomic databases.	<u>Bacteria:</u> Bacillota and Pseudomonadota were the predominant communities. <i>Leuconostoc</i> and <i>Pediococcus</i> were the predominant genus. <u>Fungi or Yeast:</u> Mucoromycota and Ascomycota were the predominant communities. <i>Rhizopus</i> and <i>Saccharomyces</i> were the predominant genus. <i>Saccharomyces</i> and <i>Saccharomycopsis</i> were the main yeasts.	Zhao et al. (2020)
Baijiu liquors and fermented grains	Investigating the difference between microbiota dynamics during the fermentation processes with single cereal and mixed cereals	<u>Metataxonomic sequencing:</u> Platform: Miseq Benchtop Sequencer for 250 bp pair-end sequencing HTS: Bacteria: V3-V4 hypervariable region of the 16S rRNA gene was amplified using the	<u>Bacteria:</u> Fermentation using the grain mixture increased the abundance of <i>Clostridiales</i>. <i>Lactobacillus</i> could reflect the microbial succession rate of stage shift in strong-aroma Baijiu fermentation.	Tan et al. (2019)

primer sets F338 and barcode
R806. Fungi, the internal
transcribed spacer (ITS) region was
amplified with primers ITS1F and
ITS2R.

Quality control: removed by
Trimmomatic v0.32 and Uchime
algorithm, clustered using Qiime's
Uparse pipeline

Database: 16S rRNA - Silva v128
and ITS - Unite v7.0.

HTS: High-Throughput Sequencing; ITS: Internal Transcriber Spacer; Ribosomal Database Project: RDP.

Table 2. Microbial communities in dairy products using omics techniques.

Sample	Study aim	Methods used	Insightful results	Reference
Minas Frescal Cheese (MFC)	Understanding microbial community structure in MFC establishes the identity of dominant bacterial populations.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq HTS: V3-V4 hypervariable regions of the 16S rRNA gene were amplified (341F and 806R primers). Quality control: Trimmomatic v0.32 Database: SILVA 132	<u>Bacteria:</u> Pseudomonadota dominated all samples, accounting for an average of 88.7% of all sequences. Groups associated with contamination, such as <i>Pseudomonas</i>, <i>Acinetobacter</i>, and <i>Vibrio</i>, were highly frequent in most brands. The core microbiome shared by 100% of the samples indicated a predominance of the <i>Enterobacteriaceae</i> family.	de Paula et al. (2021)
Minas artisanal cheese (MAC)	Analysis of the microbiota of MAC produced in the Serro regions to identify distinguishable microbial signatures that might be used for authenticity testing.	<u>Metataxonomic sequencing:</u> Platform: MiSeq Sequencing System (Illumina) HTS: V3-V4 region of 16S rRNA; primers 341F and 806R Quality control: not provided Database: Sequence database (NeoRef, Neoprosecta)	<u>Bacteria:</u> <i>Lactococcus lactis</i> was the predominant species in all samples. Core microbiota for all cheese samples included <i>Lactococcus lactis</i>, <i>Streptococcus salivarius</i>, <i>S. thermophilus</i>, and <i>Acinetobacter johnsonii</i>. The <i>Lactobacilli</i> and <i>L. lactis</i> are the dominant lactic groups and species in the Serro MAC, supporting the establishment of a typical microbial signature for this cheese.	Nero et al. (2021)
Artisanal cheeses (Bulgarian ‘mehovo sirene’ cheese)	Characterize the microbiota composition of a Bulgarian artisanal skin bag cheese, point out its distinction in relation to other skin bag cheeses, and prove its safety.	<u>Metataxonomic sequencing:</u> Platform: not provided HTS: V3–V4 of the 16S rRNA gene (341F and 806R primers) Quality control: not provided	<u>Bacteria:</u> The metagenomics study revealed an unusual microbiome comprising beneficial bacteria such as <i>Staphylococcus equorum</i>, <i>Lactococcus lactis</i>, <i>Streptococcus thermophilus</i>, <i>Lactobacillus delbrueckii</i>, <i>Lactobacillus kefiranoferiens</i>, <i>Lactococcus raffinolactis</i>, <i>Lactiplantibacillus plantarum</i>,	Dimov et al. (2023)

		Database: not provided	<i>Limosilactobacillus fermentum</i> and <i>Loigolactobacillus coryniformis</i> .	
Artisanal Brazilian cheeses	Characterization of the microbiota of Brazilian artisanal cheeses produced in different regions, emphasizing the differences between the manufacturing practice and the geographical origin.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq platform HTS: V3-V4 region of the 16S rRNA gene by using primers S-D-Bact-0341 and S-D-Bact-0785-a-A-21 Quality control: PRINSEQ and QIIME 1.9.1 software Database: NCBI nr database	<u>Bacteria:</u> LAB dominated all cheeses, although <i>Enterobacteriaceae</i> and <i>Staphylococcus</i> also occurred in North, Northeast, and Central cheeses. Differences in the LAB patterns were also highlighted: <i>Streptococcus</i> , <i>Leuconostoc</i> , <i>Lactococcus</i> , and <i>Lactobacillus</i> were combined differently in relative abundance according to product type and region of production.	Kaminura et al. (2019)
Tulum cheese	Assessment of the diversity of bacterial populations, which are important as native non-starter flora of the raw milk Turkish artisanal Tulum cheeses using amplicon-based sequencing.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq HTS: V4 region of the 16S rDNA (341F and 805R primers) Quality control: FASTQC v0.11.2 tool and QIIME II (ver.4 2019) analyses platform Database: SILVA reference database (2019).	<u>Bacteria:</u> Bacterial communities of the Tulum cheese samples were found to be a complex microbiota with organisms belonging to the Bacillota and Pseudomonadota. <i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Streptococcus</i> , <i>Leuconostoc</i> , <i>Enterococcus</i> , and <i>Pediococcus</i> genera were the dominant bacterial genera in all samples.	Gezginc et al. (2022)
Hard cheese rind	Assessment of the microbial diversity of wooden shelves used for maturing cheeses with Protected Designation of Origin (PDO) Pecorino di Filiano and Protected Geographical Indication (PGI) Canestrato di	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq system HTS: V3-V4 region of the 16S rRNA gene (341F to 805R primers) and fungal ITS2 region	<u>Bacteria:</u> Mesophilic and thermophilic bacteria dominated the surfaces of all wooden shelves and cheese rinds. The BAL community was represented by <i>Enterococcus</i> spp., <i>Leuconostoc</i> spp., and <i>Marilactibacillus</i> spp.	Busetta et al. (2024)

	Moliterno. The microorganisms associated with the rind of these cheeses were also investigated.	(ITS3/ ITS4 primers) were amplified for bacteria and yeast, respectively Quality control: Qiime2 v2018.2. Database: Greengenes v13_8 (bacteria) and UNITE software v8.0 (fungi).	<u>Fungi or Yeast:</u> Among yeasts, <i>Debaryomyces</i> spp., <i>Candida</i> spp., <i>Aspergillus</i> spp., and <i>Penicillium</i> spp. dominated the filamentous fungal community. Alpha diversity confirmed a strict correlation between the microbiota on wooden shelves and that on cheese rinds in most factories.	
Traditional Buryatian cottage cheese	Characterization of the microbiota communities of cheese Buryatia samples collected based on full-length 16S rRNA profiling.	<u>Metataxonomic sequencing:</u> Platform: PacBio SMRT sequencing and Illumina platform HTS: 16S rRNA gene, the forward 27F, and the reverse 1541R primers. Quality control: QIIME package Database: Ribosomal Database Project (RDP) II	<u>Bacteria:</u> The dominant species was <i>Lactococcus lactis</i> . Principal component analysis and multivariate ANOVA showed marked differences in the structure of the microbiota communities in the cheese data sets from the 3 regions. The results indicate that the bacterial communities of traditional artisan cheeses vary depending on geographic origin.	Jin et al. (2018)
Village-type homemade yogurts	Assessment of the bacterial diversity using next-generation sequencing via metagenomic analysis in village-type homemade yogurts.	<u>Metataxonomic sequencing:</u> Platform: Illumina platform HTS: 16S rRNA gene (V3 e V4 hypervariable regions); (341F and 805R primers) Quality control: QIIME 1.9.1 Database: not provided	<u>Bacteria:</u> <i>Lactobacillus</i> was the most prevalent genera in homemade yogurt samples at the end of all back-slopped yogurt productions, followed by <i>Streptococcus</i> . Besides, <i>Enhydrobacter</i> genera were detected with the third highest relative abundance in almost all yogurts.	Golbasi et al. (2023)

Krokmach (traditional Bulgarian fermented dairy product)	Investigation of microbiota composition by next-generation sequencing.	<u>Metataxonomic sequencing:</u> Platform: Illumina HiSeq HTS: V3–V4 region of the 16S rRNA genes was amplified with the primers 341F and 806R Quality control: not provided Database: SILVA Database	<u>Bacteria:</u> The dominant species was <i>Lactococcus lactis</i> subsp. <i>lactis</i>. The overall composition of <i>Exiguobacterium</i> sp was quite unusual. Presence of <i>Apiactobacillus kunkeei</i>, <i>Megamonas</i> sp., and <i>Kluyvera georgiana</i> among the dominant and subdominant species and genera.	Dimov et al. (2022)
Koumiss	Study of the bacterial community during the fermentation of koumiss, analyzing the dynamics changes in the chemical composition and evaluating the correlation between groups of bacteria and major metabolites.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq HTS: V3-V4 region, 16S rRNA sequence (Primers 341F and 805R) Quality control: not provided Database: GreenGenes 16S rDNA	<u>Bacteria:</u> The four dominant phyla were Bacillota, Pseudomonadota, Actinobacteria, and Bacteroidetes. Bacillota and Pseudomonadota showed a relative abundance during fermentation. Actinobacteria decrease sharply as fermentation continues. Initially, the genera <i>Lactobacillus</i>, <i>Streptococcus</i>, <i>Rothia</i>, <i>Lactococcus</i>, <i>Leuconostoc</i> and <i>Enhydrobacter</i> predominated. After fermentation, <i>Lactobacillus</i>, <i>Streptococcus</i>, <i>Acetobacter</i> prevailed, and <i>Lactobacillus helveticus</i>, <i>Streptococcus parauberis</i>, and <i>A. pasteurianus</i> were the most abundant species. The presence of pathogens (<i>Escherichia</i>, <i>Shigella</i>, and <i>Staphylococcus aureus</i>) was detected at the beginning of the process and gradually decreased as the fermentation progressed.	Bao et al. (2019)

Table 3. Microbial communities in Asian fermented foods using omics techniques.

Sample	Study aim	Methods used	Insightful results	Reference
High-temperature Daqu (material for Chinese Baijiu)	Investigating the differences in microbial communities, physicochemical indices, and potential functions among different high-temperature Daqu with different colors	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq2500 platform HTS: V4 regions of the bacterial 16S rRNA gene and the internal transcribed spacer (ITS1) regions of fungal rRNA genes were amplified using the primers 338f/806r and ITS1f/2043R Quality control: Trimmomatic, merged by FLASH, clustered using UPARSE v7.1, and chimeric sequences were identified and removed using the UCHIME algorithm. Database: RDP Classifier	<u>Bacteria:</u> Bacillota and Actinobacteria were the main bacterial phyla, while <i>Kroppenstedtia</i>, <i>Bacillus</i>, and <i>Saccharopolyspora</i> were the main genera. <u>Fungi or Yeast:</u> Ascomycota was the predominant fungal phylum, while <i>Thermoascus</i>, <i>Thermomyces</i>, and <i>unclassified_o_Eurotiales</i> were the major fungal genera.	Deng et al. (2020)
Low-temperature Daqu	Examining the structure of bacterial and fungal communities in three different types (Hongxin, Houhuo, and Qingcha)	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq sequencer HTS: V3-V4 region of bacterial 16S rRNA was amplified with forward primer 338F and reverse primer 806R. Internal Transcribed Spacer (ITS) regions of fungal rRNA gene were amplified with forward primer ITS1F and reverse primer ITS2R	<u>Bacteria:</u> Bacterial richness and diversity were higher in Hongxin and Qingcha than in Houhuo. Bacillota, Actinobacteria, and Pseudomonadota were the dominant bacterial phyla. <i>Bacillus</i>, <i>Streptomyces</i>, <i>Saccharopolyspora</i>, <i>Lactobacillus</i>, and <i>Thermoactinomyces</i> were the dominant bacterial genera.	Cai et al. (2021a)

		Quality control: in-house Python scripts Database: Greengenes v13.8, RDP (Release 11.5) and SILVA v132.	<u>Fungi or Yeast:</u> Ascomycota was the dominant fungal phylum. <i>Saccharomycopsis</i>, <i>Thermoascus</i>, and <i>Monascus</i> were the dominant fungal genera.	
Daqu	Analyzing the microbial structure of special-flavor Baiju Daqu	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq The bacteria's HTS: 16S rRNA gene was amplified using the forward primer 27F and reverse primer 1492R. The 18S rRNA gene fragments in the fungi were amplified by using the barcoded primer pairs of SSU0817F and SSU1196R Quality Control: quality-filtered by fastp v0.20.0 and merged by FLASH v1.2.7 Database: RDP Classifier v2.2 against the 16S rRNA database (Silva v138)	<u>Bacteria:</u> Lactobacillales (mainly <i>Enterococcus faecalis</i>, <i>Lactiplantibacillus plantarum</i>, and <i>Pediococcus pentosaceus</i> microorganisms), Bacillales, and Rhodospirillales (mainly <i>Acetobacter pasteurianus</i> microorganism) were the phylotype level more found <i>Bacillus licheniformis</i> was the most abundant bacterial community. <u>Fungi or Yeast:</u> Saccharomycetales (<i>Wickerhamomyces anomalus</i> and <i>Pichia anomalus</i> microorganisms mainly), Eurotiales (<i>Aspergillus</i> and <i>Penicillium</i> mainly), and Capnodiales were the phylotype level more found.	Chen et al. (2021)
Yellow water and pit mud (ingredients of Chinese Strong-Flavor Liquor)	Evaluating how the microbes in the yellow water interact with microbes in the mud in the Chinese Strong-Flavor Liquor production	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq HTS: V3–V4 region of the bacterial 16S rRNA gene, and the amplification primers were 338F and 806R Quality control: FLASH and Trimmomatic software	<u>Bacteria:</u> Bacillota increased with fermentation time (30-90 days). Bacteroidetes decreased with fermentation time. The <i>Lactobacillus</i> genus was dominant.	Gao, Wu, Zhang (2020)

Database: Silva (Release 132)			
Pit mud and fermented grains	Evaluating pit mud microbes in the formation of diverse flavor compounds	<u>Metataxonomic sequencing:</u> Platform: Illumina MisSeq platform HTS: Fungi: ITS2 region was amplified with the primers ITS3 and ITS4 Bacteria: V3–V4 hypervariable regions of the 16S rRNA gene were amplified using primers 338F and 806R Quality control: data were filtered by QIIME pipeline (v 1.9.1), chimera sequences removed by UCHIME, and clustered using UPARSE. Database: Silva database (v 138) and the Unite fungal ITS database	<u>Bacteria:</u> <i>Pediococcus</i>, <i>Lactobacillus</i>, and <i>Kroppenstedtia</i> at 0 days and <i>Lactobacillus</i> at the end of fermentation. <i>Caproiciproducens</i>, <i>Caloramator</i>, <i>Sedimentibacter</i> and <i>Caldicoprobacter</i> in general. <u>Fungi or Yeast:</u> <i>Pichia</i> (day 0) and <i>Saccharomyces</i> at the end of fermentation. <i>Pichia</i>, <i>Rhizopus</i>, <i>Aspergillus</i> and <i>Mucor</i> were more abundant in general.
Fermented Grain	Identifying the microbiota in the fermentation process of Chinese strong-flavor liquor by different pit ages and layers of fermentation cellars.	<u>Metataxonomic sequencing:</u> Platform: Illumina platform HTS: Bacteria: primers 338F/806R were used to amplify the V3–V4 regions of the bacterial 16S rRNA gene. Fungi: ITS5/ITS1 was used to amplify ITS1 regions of fungal rRNA Quality control: filtered by QIIME pipeline v1.8.0, aligned and assembled using FLASH v1.2.7.	<u>Bacteria:</u> Bacillota and Pseudomonadota phyla <i>Lactobacillus</i>, <i>Aquabacterium</i>, and <i>Chloroplast</i> genus. <i>Lactobacillus</i> was more abundant in the button layer. <i>Aquabacterium</i> was more abundant in the middle layer. <u>Fungi or Yeast:</u> Ascomycota phyla <i>Aspergillus</i>, <i>Thermoascus</i>,

		Database: Greengenes Database (Release 13.8) and UNITE Database (Release 5.0)	<i>Kazachstania</i> , <i>Pichia</i> , and <i>Rhizomucor</i> genus <i>Thermoascus</i> was more abundant in the middle layer.	
Fermented Grain	Evaluating the microbial and metabolic characteristics of the fermented grain during Baijiu fermentation	<u>Metagenomic shotgun sequencing:</u> Platform: Illumina HiSeq 4000 platform Quality control: Sickle software Database: Taxonomic annotation was performed against the NCBI nr database. The EggNOG v4.5, CAZy v5.0, and KEGG were employed for functional annotation.	The abundance of fungi and bacteria at the beginning of fermentation is practically equal. <u>Bacteria:</u> Bacteria abundance increases with 7 days of fermentation, and there is a decrease in microbial diversity during fermentation. <i>Lactobacillus</i> and <i>Pichia</i> were the most abundant, with <i>Lactobacillus</i> reaching an abundance greater than 90% during fermentation at the end of fermentation. <i>Pichia kudravzerii</i> , <i>Lichtemia ramosa</i> , <i>Leuconostoc citreum</i> , and <i>Bacillus licheniformis</i> were predominant at the beginning of fermentation. <i>Lactobacillus acetotolerans</i> , <i>Lentilactobacillus buchneri</i> , and <i>Lentilactobacillus hilgardii</i> were predominant at the end of fermentation. <u>Fungi or Yeast:</u> <i>Saccharomyces cerevisiae</i> was the largest producer of alcohol.	Huang et al. (2020)
Fermented Grain	Characterizing the microbiota during the fermentation	<u>Metataxonomic sequencing:</u> Platform: Illumina HiSeq 2500 platform	<u>Bacteria:</u>	Guan et al. (2020a)

	processes of Chinese strong-flavor Baijiu differing in cellar ages with consideration for potential relation to physicochemical variables	HTS: Bacteria: V3–V4 hypervariable region of bacterial 16S rRNA genes were amplified with the universal primer pair 338F and 806R. Fungi: primer pair ITS1F and ITS2 targeting the ITS1 regions Quality control: paired via FLASH, de-multiplexed, trimmed, and assessed for chimeras using QIIME software (v. 1.8.0), and Quality-filtered using UCLUST software Database: Silva database (16S rDNA) and Unite database (ITS) in QIIME	Dominant orders: <i>Lactobacillales</i> (↓ with ↑fermentation time), <i>Enterobacteriales</i>, <i>Bacillales</i>, <i>Eurotiales</i>, and <i>Saccharomycetales</i> Dominant genus bacteria: <i>strict aerobes</i> (e.g., <i>Staphylococcus</i> and <i>Acetobacter</i>) or <i>facultative aerobes</i> (e.g., <i>Kosakonia</i>, <i>Lactobacillus</i> (↑with ↑fermentation time), <i>Weissella</i>, <i>Pediococcus</i>, <i>Pantoea</i>, and <i>Bacillus</i>). <u>Fungi or Yeast:</u> <i>Kazachstania</i> with a significant level of <i>Thermoascus</i> (↓ with ↑fermentation time), <i>Aspergillus</i> (↓ with ↑fermentation time), and <i>Saccharomyces</i>.
Fermented grains	Identifying the microbiota present during material pretreatment	<u>Metataxonomic sequencing:</u> Platform: Illumina platform HTS: Bacteria: V3–V4 domains of the 16 S rRNA genes were amplified using primers 338 F and 806 R Fungi: internal transcribed spacer ITS2 regions were amplified with primers ZIT_F and ZITS_R Control quality: QIIME (v.1.17) Database: Silva 123/16 S rRNA database by a QIIME-based wrapper of RDP-classifier (v.2.2). The fungal ITS database was clustered using the USEARCH v7.1.	<u>Bacteria:</u> <i>Cyanobacteria_norank</i> was predominant at the beginning of sorghum soaking. <i>Lactobacillus</i> genus became abundant after sorgho soaking step. <u>Fungi or Yeast:</u> <i>Epicoccum</i> was predominant at the beginning of sorghum soaking, and <i>Cyberlindnera</i> and <i>Pichia</i> at the end. Pang et al. (2020)

Fermented grains	Investigating the diversity and evolution of microbial community in zaopei samples (fermented cereals that are placed inside the pit cellar for alcohol fermentation and formation of flavoring compounds) from individual stacking-fermentation rounds	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq platform HTS: V3–V4 and fungal internal transcribed spacer (ITS) 1 regions of the rRNA were amplified using forward and reverse primers with barcodes. Quality control: QIIME (v1.8.0) Database: Silva 128/16S rRNA and ITS databases were clustered using USEARCH v7.1.	<u>Bacteria:</u> Bacillota and Pseudomonadota were the dominant phylum. <i>Bacillus</i>, <i>Lentibacillus</i>, <i>Kroppenstedtia</i>, <i>Oceanobacillus</i>, and <i>Lactobacillus</i> were abundant in all rounds. <u>Fungi or Yeast:</u> Ascomycota was the dominant fungal phylum. <i>Thermomyces</i>, <i>Byssochlamys</i>, <i>Thermoascus</i>, <i>unclassified_o__Eurotiales</i>, and <i>Aspergillus</i>.	Wang, Huang, Huang (2021)
Fermented grains and environmental microbiota	Evaluating the environmental microbiota in community assembly, the sources of microbiota and the ecological processes of the fermentation were characterized by the sauce-flavor Baijiu	<u>Metataxonomic sequencing:</u> Platform: MiSeq benchtop sequencer HTS: Bacteria: V3-V4 hypervariable region of the 16S rRNA gene was amplified using the universal primer sets 338F and 806R. Fungi: The internal transcribed spacer (ITS 2) region was amplified with primers ITS3 and ITS4 Quality control: QIIME v.1.9.1 Database: Silva 132_16 S rRNA database with a QIIME-based wrapper of RDP-classifier (v.2.2) and BLAST search against the UNITE fungal ITS database	<u>Bacteria:</u> <i>Virgibacillus</i>, <i>Kroppenstedtia</i>, <i>Bacillus</i>, and <i>Oceanobacillus</i> were predominant in the initial fermentation. <i>Lactobacillus</i> was predominant after the initial phase. <i>Pichia</i> was predominant throughout the fermentation process.	Zhang et al. (2021b)

Base liquor, heap fermented grains, and pit fermented grains	Determining the effects of initial temperature on the sauce-flavor Baijiu fermentation, both microbial community and metabolic profiles.	<u>Metataxonomic sequencing:</u> Platform: Illumina Miseq sequencing HTS: Bacteria: V3-V4 hypervariable region of the 16S rRNA gene was amplified using the universal primer sets 338F and 806R with adapter and barcode sequences. Fungi: internal transcribed spacer (ITS 2) region was amplified with primers ITS3 and ITS4 Quality control: processed via QIIME v.1.9.1 Database: Silva 132_16 S rRNA database and UNITE fungal ITS database	<u>Bacteria:</u> High initial temperature increased the abundance of <i>Lactobacillus</i> and <i>Saccharomyces</i>. Zhang et al. (2021c)
JIUYAO (starter used for Huangjiu rice wine)	Evaluating the microbial communities found in JIUYAO from different Chinese province	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq high-throughput sequencing platform HTS: V3–V4 regions of the 16S ribosomal RNA (rRNA) was amplified with the 338F and 806R. Internal Transcribed Spacer (ITS) regions of fungal was amplified with ITS5F and ITS1R primers Quality control: QIIME analysis platform, UCLUST Database: Ribosomal Database Project (RDP)	<u>Bacteria:</u> <i>Pediococcus</i>, <i>Enterobacter</i>, and <i>Weissella</i> were the dominant genus of bacteria. Chen et al. (2020) <u>Fungi or Yeast:</u> <i>Rhizopus</i>, <i>Saccharomycopsis</i>, and <i>Aspergillus</i> were the dominant genus of fungi.

Niandoubao sourdough (fermented proso millet food)	Investigating the composition and drivers of Niandoubao sourdough microbiota	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq platform HTS: V3–V4 region of the bacterial 16S rRNA gene with primers 338F and 806R. Fungal amplification primers were ITS3F and ITS4R Control quality: quality-filtered by fastp v0.20.0, merged by FLASH v1.2.7, clustered using UPARSE v7.1 Database: Silva database for bacteria and the UNITE database for fungi.	<u>Bacteria:</u> <i>Enterobacterales</i> and <i>Lactobacillales</i> order. <i>Kosakonia</i>, <i>Enterobacter</i>, <i>Weissella</i>, <i>Lactococcus</i>, <i>Lactobacillus</i>, <i>Leuconostoc</i>, and <i>Pediococcus</i> genera were dominant. <u>Fungi or Yeast:</u> <i>Saccharomycetales</i>, <i>Pleosporales</i>, <i>Mucorales</i>, <i>Eurotiales</i>, <i>unclassified_c_Sordariomycetes</i>, and <i>unclassified_p_Ascomycota</i> order, <i>Mucor</i>, <i>Saccharomyces</i>, <i>Alternaria</i>, <i>unclassified_p_Ascomycota</i>, and <i>Aspergillus</i> genera were dominant.	Sun et al. (2023)
Traditional type I sourdough and jiaozi	Investigating the microbial diversity of jiaozi and type I sourdoughs	<u>Metataxonomic sequencing:</u> Platform: not provided HTS: The V3-V4 hypervariable regions of all bacterial 16S rRNA genes were amplified with primers 338F and 806R. The fungi rRNA hypervariable regions ITS2 were amplified with primers ITS1F and 2043R. Control quality: clustered with 97% similarity cutoff using UPARSE v7.1, removed chimeric sequences UCHIME.	<u>Bacteria:</u> <i>Lactobacillus</i> and <i>Weissella</i> genera were dominant. LAB: <i>Fructilactobacillus sanfranciscensis</i>, <i>Weissella cibaria</i>, <i>Limosilactobacillus fermentum</i>, <i>Lactiplantibacillus plantarum</i> and <i>Pediococcus pentosaceus</i>. <u>Fungi or Yeast:</u> <i>Saccharomyces</i>, <i>Candida</i>, <i>Alternaria</i>, <i>Aspergillus</i>, <i>Wickerhamomyces</i>, and <i>Penicillium</i> genera.	Yan et al. (2019)

		Database: Silva (SSU128) (16S rRNA) and Unite 7.0 database (ITS).	
Chinese traditional sourdoughs	Evaluating the microbial diversity of 5 different sourdoughs and their effects on potato steamed bread	<u>Metataxonomic sequencing:</u> Platform: Illumina paired-end sequencing HTS: V3-V4 region of the 16S rDNA and ITS region were amplified respectively for the identification of bacteria and fungi Quality control: merged, filtered, and clustered with UCLUST of QIIME software v1.8.0. Database: Silva and Unite	<u>Bacteria:</u> <i>Pediococcus</i> and <i>Lactobacillus</i> genera. <i>Lactobacillus brevis</i> and <i>Weissella confuse</i> species. Zhao, Mu, Sun (2019) <u>Fungi or Yeast:</u> <i>Wickerhamomyces</i> , <i>Saccharomyces</i> , <i>Kazachstania</i> , and <i>Penicillium</i> genera. <i>Wickerhamomyces anomalus</i> , <i>Saccharomyces cerevisiae</i> , <i>Kazachstania humilis</i> species.
Fermented potato	Investigating different pretreatment (high hydrostatic pressure (HFP), boiled (BFP), and steamed (SFP)) before fermentation on the microbial diversity	<u>Metataxonomic sequencing:</u> Platform: Illumina Highseq protocol HTS: V3-V4 region (Bacteria) and ITS region (Fungi) Quality control: UCLUST of QIIME v1.8.0 Database: Silva and UNITE reference gene	<u>Bacteria:</u> Fusobacteria, Deferribacteres, Bacteriodes, Actinobacteria, Pseudomonadota, Bacillota, and Cyanobacteria are the phyla more present. Aregbe, Mu, Sun (2019) <i>Acetobacter</i> , <i>Leuconostoc</i> , <i>Aeromonas</i> , <i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Gluconobacter</i> , <i>Bacillus</i> , <i>Klebsiella</i> , <i>Weissella</i> , and <i>Nicotiana otophora</i> were the genera more present. BFP presented higher bacterial biodiversity. 3SFP: presented less fungal diversity; high abundance of <i>Weissella</i> and <i>Gluconobacter</i> .

Sourdough	Assessment of fungal species diversity in a collection of type I sourdoughs made with organic flour from bakeries with different bread-making practices and from other French regions.	<u>Metataxonomic sequencing</u> Platform: Titanium FLX pyrosequencing (Roche technology) HTS: ITS1 rDNA region (primers pair NSA3/58A2R). Quality control: PRINSEQ tool and QIIME (v.1.8.0). Database: UNITE (v.7.0) completed with the sourdough strain sequences.	<u>Bacteria:</u> <i>Tissierella</i> , followed by <i>Oceanobacillus</i> , <i>Pseudomonas</i> and <i>Abyssivirga</i> was the dominant genus. <u>Fungi or Yeast:</u> <i>Debaryomyces</i> , <i>Candida</i> , and, to a lesser extent, <i>Saccharomyces</i> species were predominant in the fungal community.	Urien et al. (2019)
Sourdough	Investigation of the microbial dispersal along the French sourdough bread-making chain.	<u>Metataxonomic sequencing</u> Platform: Illumina MiSeq. HTS: V3–V4 region of the bacterial 16S rRNA gene (primers pair 343F/784R) and the ITS1 region of the fungi (primer pairs ITS1-F/ITS2); Quality control: dada2 (v.1.16) and FROGS 3.2.2. Database: SILVA (v.138) for bacteria and UNITE (v.8.0) for fungi.	Microbial species present in the flour are mainly not detected in the sourdough. Sourdough yeasts did not come from flour, and probably neither did sourdough LAB.	von Gastrow et al. (2021)
Chia sourdough	Investigating the microbial development during natural chia dough fermentation.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq HTS: V3–V4 region of bacterial 16S rRNA gene (primers pair 343F/802R). Quality control: PANDAseq script	<u>Bacteria:</u> <i>Enterococcus faecium</i> , <i>Enterococcus mundtii</i> , <i>Weissella cibaria</i> , and <i>Lactocaseibacillus rhamnosus</i> were the dominant species in the final fermentation stages, while <i>Bacillus</i> and <i>Clostridium</i> were	Maidana et al. (2020)

		Database: Greengenes.	mainly present during the first fermentation stages.
Zha-Chili	Evaluating the bacterial diversity of zha-chili samples from different regions	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq sequencer HTS: V3-V4 region of 16S rRNA was amplified using forward primer 338F and reverse primer 806R. Quality control: FLASH v1.2.7 and fastp v0.20.0 Database: Greengenes and SILVA databases	<u>Bacteria:</u> Bacillota, Pseudomonadota, and Actinobacteria were the dominant phyla. <i>Lactobacillus</i>, <i>Pseudomonas</i>, <i>Pediococcus</i>, <i>Weissella</i>, and <i>Staphylococcus</i> were the dominant genus. Cai et al. (2021b)
Chili pepper (<i>Capsicum frutescens</i> L.)	Evaluating the bacterial and fungal during the manufacturing process of fermented chili pepper	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq platform HTS: Bacteria: V3 and V4 hypervariable regions of the bacteria 16S rRNA genes were amplified with the primers 338F and 806R. Fungi: ITS region of fungal 18S rDNA was amplified using a broadly conserved primer set (the forward primer ITS1 and the reverse primer ITS4). Quality control: quality-filtered using the Trimmomatic tool, merged using FLASH software, clustered using Usearch v7.1. Database: Silva and Unite v7.0 databases.	<u>Bacteria:</u> <i>Rosenbergiella</i> and <i>Staphylococcus</i> genera. <i>Pantoea</i> was present throughout the fermentation process. <i>Rosenbergiella</i> and <i>Pseudomonas</i> decreased after fermentation. <i>Staphylococcus</i>, <i>Curtobacterium</i>, <i>Methylobacterium</i>, <i>Sodalis</i>, <i>Sphingomonas</i>, and <i>Microbacterium</i> increased after fermentation. <u>Fungi or Yeast:</u> <i>Alternaria</i>, <i>Stemphylium</i>, <i>Cryptococcus</i>, <i>Candida</i>, and <i>Gibberella</i> genera for unfermented sample. Xu et al. (2021a)

			<i>Hyphopichia</i> , <i>Kodamaea</i> , <i>Meyerozyma</i> , <i>Candida</i> , and <i>Debaryomyces</i> genera for fermented sample	
Chinese pickled chili pepper (<i>Capsicum frutescens</i> L.)	Evaluating the dynamics of flavor changes and microbial diversity during the industrial-scale fermentation of pickled chili pepper	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq platform HTS: Bacteria: full-length 16S rRNA (27F and 1492R primers). Fungi: ITS regions of the rRNA were amplified by using ITS9 and ITS4 primers Quality control: Chimera was removed by UCHIME and clustered by the UPARSE algorithm (v7.0.1001) with USEARCH v7.1. Database: SILVA v123 and UNITE database	<u>Bacteria:</u> Beginning of fermentation (<i>Ralstonia</i> and <i>Chromohalobacter</i> genera <i>Ralstonia pickettii</i> and <i>Chromohalobacter beijerinckii</i> species). End of fermentation (<i>Lactobacillus</i> genus and amended: <i>Levilactobacillus brevis</i> , <i>Companilactobacillus alimentarius</i> , <i>Companilactobacillus versmoldensis</i> , <i>Levilactobacillus namurensis</i> , and <i>Companilactobacillus paralimentarius</i> species). <u>Fungi or Yeast:</u> Beginning of fermentation (<i>Zygosaccharomyces</i> , <i>Candida</i> , <i>Wallemia</i> , and <i>Eurotium</i> genera, <i>Zygosaccharomyces bisporus</i> , <i>Candida</i> sp. F15, and <i>Wallemia ichthyophaga</i> species). End of fermentation (<i>Candida</i> , <i>Zygosaccharomyces</i> , and <i>Pichia</i> genera <i>Candida</i> sp. F15, <i>Zygosaccharomyces bisporus</i> , and <i>S. bulderi</i> species).	Ye et al. (2022)
Fermented soy sauce	Evaluating soy sauce produced using koji-	<u>Metataxonomic sequencing:</u>	<u>Bacteria:</u> <i>Staphylococcus</i> and <i>Weissella</i> were the dominant bacterial genera.	Feng et al. (2023)

	making (fermentation made from grains or legumes using <i>Aspergillus oryzae</i>) and moromi fermentation (immersing the resulting koji in a brine solution and left to ferment for several months up to 4 years).	Platform: Illumina MiSeq platform HTS: V4-V5 region of 16S rDNA of bacteria with 27F and 1492R primers ITS rDNA region of fungi with ITS1 and ITS4 primers Quality control: filtered with the QIIME v1.8 Database: EzBioCloud database	<i>Candida</i> , <i>Aspergillus</i> , <i>Zygosaccharomyce</i> , and <i>Wickerhamiella</i> were the dominant fungi genera.
Fermented soy sauce	Evaluating the bacterial communities and the changes in soy sauce during fermentation	<u>Metataxonomic sequencing:</u> Platform: Illumina HiSeq 2500 PE250 HTS: V3-V4 hyper-variable region of 16S rRNA gene: 338F and 806R primers. Quality control: FLASH software (v1.2.7), Trimmomatic software (v 0.33), UCHIME software (v 4.2) Database: Not provided.	<u>Bacteria:</u> <i>Weissella</i>, <i>Bacillus</i>, <i>Lactococcus</i>, <i>Klebsiella</i>, <i>Pediococcus</i>, <i>Escherichia</i>, <i>Acinetobacter</i>, <i>Leuconostoc</i>, <i>Cronobacter</i>, and <i>Kushneria</i> genera. The uncultured bacterium <i>Weissella</i>, uncultured bacterium <i>Acinetobacter</i>, uncultured bacterium <i>Lactococcus</i>, and <i>Leuconostoc pseudomesenteroides</i> species.
Doenjang (traditional Korean fermented soybean paste)	Evaluating microbial abundances and communities of doenjang produced with 9%, 12%, 15%,	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq paired-end sequencing	<u>Bacteria:</u> <i>Bacillus</i>, <i>Staphylococcus</i>, and <i>Clostridium</i> were identified as major bacterial genera.

	and 18% solar salt concentrations	HTS: 341F/ 805R primers targeting bacterial 16S rRNA genes 3271-ITS2F /3271-ITS2R targeting fungal internal transcribed spacer (ITS) regions Quality control: VSEARCH program available in Qiime2 plugin software package ver. 2018.4 Database: Silva (silva-119-99-nb-classifier) and UNITE databases (unite-ver7-99-classifier-01.12.2017).	<u>Fungi or Yeast:</u> <i>Aspergillus</i>, <i>Scopulariopsis</i>, <i>Fusarium</i>, <i>Mucor</i>, and <i>Penicillium</i> were identified as major fungal genera. <i>Weissella</i>, <i>Tetragenococcus</i>, <i>Oceanobacillus</i>, and <i>Debaryomyces</i> were major groups in low-salt doenjang.
Da-jiang (fermented soybean paste)	Understanding the role of bacteria during the fermentation of da-jiang	<u>Metagenomic shotgun sequencing:</u> Platform: Illumina Hiseq2500 Quality control: mapped to Soybean genomes by SOAPaligner (v2.21), predict assembled software Meta-GeneMark (v2.10), clustered using CD-HIT Database: BLAST (v2.2.28+), (KEGG), database	<u>Bacteria:</u> Bacillota and Pseudomonadota Xie et al. (2020) phylum. <i>Leuconostoc</i>, <i>Acinetobacter</i>, <i>Enterobacter</i>, <i>Enterococcus</i>, <i>Pantoea</i>, <i>Lactococcus</i>, <i>Lactobacillus</i>, <i>Pseudomonas</i>, <i>Staphylococcus</i>, <i>Tetragenococcus</i>, and <i>Weissella</i> genera. <i>Leuconostoc mesenteroides</i>, <i>Leuconostoc citreum</i>, <i>Leuconostoc lactis</i>, <i>Lactococcus lactis</i>, <i>Acinetobacter lwoffii</i>, <i>Acinetobacter johnsonii</i>, <i>Enterobacter</i> sp. 638, <i>Enterococcus faecium</i>, and <i>Enterococcus faecalis</i> species.
Shuidouchi fermented soybean	Evaluating the microbial diversity of	<u>Metataxonomic sequencing:</u> Platform: Illumina HiSeq2500 platform	<u>Bacteria:</u> Bacillota, Bacteroidetes, and Pseudomonadota were the predominant phyla Chen et al. (2019a)

	Shuidouchi from different producers in southwest China	HTS: V4 region of 16S rRNA genes were amplified using specific primer (515F and 806R) Quality control: Clusted with QIIME (V 1.7.0) Database: QIIME (V 1.7.0)	<i>Bacillus</i> , <i>Bacteroides</i> , and <i>Lactobacillus</i> were genera more predominant.
Natural fermented plain sufu (Chinese fermented soybean curd)	Exploring core functional microbiota during the production of plain sufu.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq platform HTS: V3–V4 domains of 16S rDNA of bacteria (341F and 806R primers) and the ITS1 rDNA regions of fungi (its1 and its2 primers) Quality control: fitered using QIIME (v1.8.0), merged using FLASH software (ver.1.2.11), clustered USEARCH software (ver.7.0.1090) Database: RDP Classifier (v2.2) trained on the Greengenes and the UNITE database for annotation of bacterial and fungal species, respectively.	<u>Bacteria:</u> Bacillota, Pseudomonadota, Cyanobacteria, and Bacteroidetes phyla <i>Bacillus</i>, <i>Enterobacter</i>, <i>Lactobacillus</i>, <i>Sphingobacterium</i>, <i>Stenotrophomonas</i>, <i>Tetragenococcus</i>, <i>Trabulsiella</i>, <i>Unclassified</i>, and <i>Weissella</i> genera (↓ during the time of fermentation). <u>Fungi or Yeast:</u> <i>Alternaria</i>, <i>Sterigmatomyces</i>, <i>Actinomucor</i>, <i>Fusarium</i>, <i>Debaryomyces</i>, <i>Candida</i> genera.
Kinema (fermented soybean of Sikkim Himalaya)	Evaluating the microbial diversity of Kinema	<u>Metagenomic shotgun sequencing:</u> Platform: Illumina Hiseq 2500 platform Quality control: CLC genomics workbench	<u>Bacteria:</u> Bacillota, Actinobacteria, and Pseudomonadota phylum. Bacillaceae, Staphylococcaceae, and Enterococcaceae family. <i>Bacillus</i>, <i>Staphylococcus</i>, and <i>Enterococcus</i> genera.

		Database: KEGG Orthology (KO) and Clusters of Orthologous Group of Proteins (COGs) database	<i>Bacillus</i> sp.: <i>B. subtilis</i>, <i>B. amyloliquefaciens</i>, <i>B. licheniformis</i>, and <i>B. cereus</i> species. <i>Enterococcus faecalis</i>, <i>Staphylococcus saprophyticus</i>, <i>E. casseliflavus</i>, and <i>E. faecium</i> species.
Chinese horse bean-chili-paste (doubanjiang)	Studying microbial community during the three fermentation phase	<u>Metataxonomic sequencing:</u> Platform: Illumina Miseq sequencer HTS: 16S rDNA of bacteria (338F and 806R primers) and fungal internal transcribed spacer (ITS1F/2043R primers) Control quality: filtered and optimized by software FLASH, chimera sequences were removed QIIME Pipeline Database: RDP classifier Bayesian	<u>Bacteria:</u> <i>Bacillus</i> sp., <i>Staphylococcus</i> sp., <i>Oceanobacillus</i> sp., <i>Candida</i> sp., <i>Zygosaccharomyces</i> sp., and <i>Aspergillus</i> sp. dominated the 1st phase. <i>Bacillus</i> sp., <i>Candida</i> sp., and <i>Zygosaccharomyces</i> sp. were dominant in the 2sd and 3sd phases. Lu et al. (2020b)
Low-salt doubanjiang (broad bean paste) fermentation	Evaluating the microbial dynamic changes during fermentation in different salt concentrations	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq platform HTS: V3–V4 regions of bacterial 16S rRNA genes and ITS2 region of fungi Quality control: FLASH (v1.2.11.), clustered using USEARCH v7.0.1090, chimeras removed using UCHIME v4.2.40	<u>Bacteria:</u> <i>Bacillus</i> and <i>Staphylococcus</i> genera <i>Enterobacter</i>, <i>Prevotella</i>, <i>Erwinia</i>, <i>Trabulsiella</i>, <i>Klebsiella</i>, <i>Cronobacter</i>, and <i>Acinetobacter</i> genera (↑ with ↓ salt concentration). Yang et al. (2021a)

		Database: UNITE and Greengene for genera annotation		
Pixian Doubanjiang	Evaluating the microbial community of Pixian Doubanjiang	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq platform with MiSeq Reagent Kit v3 HTS: bacteria and fungi Quality control: clustered by UCLUST Database: Greengenes and UNITE	<u>Bacteria:</u> <i>Cupriavidus</i> , <i>Burkholderia-Caballeronia-Paraburkholderia</i> , <i>Paucibacter</i> , and <i>Sphingomonas</i> genera. <u>Fungi or Yeast:</u> <i>Saccharomycetales</i> , <i>Issatchenkia</i> , <i>Pichia</i> , <i>Cladosporium</i> , and <i>Sterigmatomyces</i> .	Yang et al. (2021b)
Pixian Doubanjiang	Evaluating the dynamic changes in the bacterial community during the natural fermentation	<u>Metataxonomic sequencing:</u> Platform: Illumina Miseq sequencing HTS: The V4–V5 region of 16S rDNA genes was amplified with universal primers 515F and 909R for bacteria. Internal transcribed spacer (ITS) regions were amplified with the primers ITS4 and ITS3_KYO2 for fungi Quality control: filtered by UCHIME (v4.2.40), clustered utilizing UPARSE software Database: RDP classifier	<u>Bacteria:</u> Bacillota and Pseudomonadota phylum. Bacteroidetes phyla were stable throughout the process. <i>Lactobacillus</i> and <i>Bacillus</i> decreased throughout maturation <i>Escherichia-Shigella</i> increased throughout maturation. <u>Fungi or Yeast:</u> Ascomycota, Basidiomycota, Zygomycota, and Rozellomycota phyla. <i>Penicillium</i> , <i>Wickerhamomyces</i> , <i>Cladosporium</i> , <i>Millerozyma</i> , <i>Alternaria</i> , <i>Mucor</i> , <i>Yamadazyma</i> , and <i>Aureobasidium</i> genera.	Zhang et al. (2020)

Traditional Chinese Fish Sauce	Evaluating the dynamic changes in the bacterial community during the natural fermentation	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq platform HTS: V3-V4 regions of the bacterial 16S rRNA gene with primer 338F and the reverse primer 806R Quality control: filtered with the Trimmomatic software (v0.36), merged with the FLASH software, clustered using UPARSE (v7.1), and chimeric sequences removed using UCHIME Database: Silva (SSU123) 16S rRNA database	<u>Bacteria:</u> Bacillota and Pseudomonadota phyla Pseudomonadota phylum ↓ during fermentation Bacillota phylum ↑ during fermentation. <i>Halanaerobium</i>, <i>Shewanella</i>, <i>Bacillus</i>, <i>Tetragenococcus</i>, <i>Vibrio</i>, <i>Gallicola</i>, <i>Photobacterium</i>, <i>Pseudomonas</i>, and <i>Psychrobacter</i> genera.	Du et al. (2019)
Thai fish sauce	Evaluating the dynamic changes in the bacterial community during the natural fermentation	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq system HTS: The bacterial 16S rRNA gene with primer 515F and the reverse primer 806R Quality control: Trimmomatic Database: RDP classifier and Greengenes ver. 13_8	<u>Bacteria:</u> Pseudomonadota, Actinobacteria, Euryarchaeota, and Fusobacteria phyla were found at the end of fermentation. <i>Halanaerobium</i> sp. was predominant during the fermentation. <i>Lentilactobacillus</i> sp., <i>Halomonas</i> sp., and <i>Tetragenococcus</i> were found during the fermentation.	Ohshima et al. (2019)
Pla-ra (natural Thai fermented freshwater fish product)	Investigating the microbial diversity, glutamyl peptide profiles, and the relationships between them in	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq system HTS: V4 region of 16S rRNA (515F and 806R primers)	<u>Bacteria:</u> 12 genera of bacteria were detected, with dominance of <i>Tetragenococcus</i> spp., <i>Staphylococcus</i> spp., and <i>Lactobacillus</i> spp. Of the 18 glutamyl peptides analyzed, 17 were found, although the amounts detected were below the taste threshold. In the analysis of principle components, the relationship between	Phewpan et al. (2019)

	Thai fermented fish products.	Quality control: FASTQ and Mothur v1.36.1 Database: Greengenes (v. 13_827)	microorganisms and the generation of glutamyl peptides was observed, mainly between <i>Tetragenococcus</i> spp. and <i>Lentibacillus</i> spp. and the generation of γ -Glu-Val-Gly.	
Fermented fish (pla-ra)	Exploring the microbial community composition (bacteria and fungi) of fermented fish (pla-ra) from Northeast Thailand.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq HTS: 16S rRNA gene (all variable regions, V1–V9: Bacteria) and the nuclear ribosomal ITS regions (ITS1 and ITS2: Fungi). Quality control: QIIME software (v. 1.7.0) and FASTQ Toolkit (v. 2.2.0; Illumina) Database: Silva database and ITS1/2 reference sequences UNITE project database (v. 7.2)	<u>Bacteria:</u> The bacterial communities were rich, comprising 402 genera from 28 phyla, including <i>Tetragenococcus</i> , <i>Staphylococcus</i> , <i>Virgibacillus</i> , <i>Lactobacillus</i> , and <i>Lentibacillus</i> . The fungal communities comprised 7 phyla and 60 genera, such as <i>Heterobasidion</i> , <i>Densospora</i> , <i>Exophiala</i> , and <i>Monascus</i> . Odor-association analysis revealed that <i>Brevibacterium</i> , <i>Brachybacterium</i> , and <i>Chromohalobacter</i> were more abundant in the weak-odor group, while <i>Noviherbaspirillum</i> was more abundant in the strong-odor group.	Sirichoat et al. (2022)
Fermented mandarin fish	Evaluating diversity and succession of microbial communities in fermented samples of <i>S. chuatsi</i> .	<u>Metataxonomic sequencing:</u> Platform: Illumina HiSeq 2500 platform HTS: 16S rRNA - V4 region, primers: 515F and 806R Quality control: FLASH (v. 1.2.11) software, UPARSE (v. 7.0.1090) software, and QIIME (v. 1.8.0). Database: RDP Classifier v2.2 and Greengenes database (v. 201305)	Alcohols, nitrogen compounds, aldehydes, and esters were the main flavor components, and three microbial genera (<i>Psychrilyobacter</i> , <i>Fusobacterium</i> , and <i>Acidaminococcus</i>) were closely associated with producing these components. These microorganisms formed characteristic flavor substances such as linalool, trimethylamine, indole, and Geranyl acetate.	Shen et al. (2021)
Fermented mandarin fish	Investigating and comparing three typical fermented	<u>Metataxonomic sequencing:</u> Platform: Illumina HiSeq 2500 platform	<u>Bacteria:</u> <i>Psychrilyobacter</i> , <i>Fusobacterium</i> , and <i>Vibrio</i> were the most dominant in the HF-, MF-, and OF-fermented products, respectively.	Wang et al. (2021a)

<i>(Siniperca chuatsi)</i>	mandarin fish products concerning bacterial profiles and flavor compounds.	HTS: 16S rRNA, V4 region: primers 515F and 806R Quality control: UPARSE (v. 7.0.1090) software. Database: RDP Classifier v2.2 and Greengenes database (v. 201305).	Several bacteria correlated with the production of important flavor compounds, and three genera (<i>Arcobacter</i> , <i>Psychrilyobacter</i> , and <i>Shewanella</i>) were the primary microorganisms contributing to more than six characteristic flavor compounds in fermented mandarin fish products.	
Chouguiyu (traditional Chinese fermented fish)	Investigating the evolving microbiota during Chouguiyu fermentation	<u>Metataxonomic sequencing:</u> Platform: Illumina Hiseq 2500 platform HTS: V4 region of bacterial 16S rRNA: primers 515F and 806R Quality control: Qiime (v. 1.7.0) Database: RDP Classifier (v2.2) and the GreenGene database	Redundancy analysis showed the relationships between evolving microbiota and the formation of Chouguiyu's aroma and taste, and <i>Psychrilyobacter</i> was identified to be the most important bacterial genus contributing to flavor and aroma development, followed by <i>Fusobacterium</i> , <i>Arcobacter</i> , and <i>Oceanisphaera</i> . These genera were positively correlated with Glu, Met, Tyr, Pro, linalool, piperitone, indole, TBARS, TVB-N, and TCA and influenced the flavor and aroma of the Chouguiyu.	Yang et al. (2020)
Suan yu (Chinese traditional fermented fish)	Investigating microbial community diversity and dynamics during Suan yu fermentation and examining the influence of mixed starter culture inoculation on the	<u>Metataxonomic sequencing:</u> Platform: Illumina HiSeq HTS: V3-V4 region of the bacterial 16S rRNA: primers 338F and 806R. ITS1 region of the fungi: forward primer ITS1F and the reverse primer ITS1R Quality control: FASTX- Toolkit Database: Silva databases using the RDP	<u>Bacteria:</u> Bacillota and Ascomycota were the predominant phyla of bacteria and fungi, respectively, in all samples. <i>Lactobacillus</i>, <i>Macroccoccus</i>, and <i>Staphylococcus</i> were the predominating bacteria throughout the fermentation process, regardless of the inclusion of starter cultures. <u>Fungi or Yeast:</u> <i>Saccharomyces</i> was the predominating fungal genus in the early-fermentation stage of samples that inoculated	Zang et al. 2018

	microbial community.		starter cultures (MS), while <i>Candida</i> and <i>Wickerhamomyces</i> dominated the final product. Compared with naturally fermented samples (NS; no starter cultures), <i>Lactococcus</i> , <i>Leuconostoc</i> , <i>Enterococcus</i> , <i>Vibrio</i> , <i>Fusicolla</i> , and <i>Torulaspora</i> were inhibited, and <i>Aureobasidium</i> emerged in samples inoculated with starter cultures.	
Thai traditional fermented shrimp paste	Demonstrating the difference in microbial community structure in shrimp pastes collected from various production regions in Thailand.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq system HTS: V4 region of 16S rRNA (515F and 806R primers) Quality control: USEARCH (v. 8.01623), Mothur (v.1.36.1), and QIIME (v. 9.1.1). Database: Greengenes (ver. 13_8)	<u>Bacteria:</u> <i>Salimicrobium</i> spp. (53.6%) and <i>Salinicoccus</i> spp. (44.0%) were the major population. <i>Alkalibacterium</i> , <i>Macrococcus caseolyticus</i> , <i>Jeotgalicoccus psychophillus</i> , and <i>Staphylococcus</i> spp. became visible. <i>M. caseolyticus</i> and <i>J. psychophilus</i> were detected for the first time in shrimp paste products. Then, in the one-month fermented sample, <i>Lentibacillus</i> spp. was 3.3%. In a two-month fermented sample, <i>Lentibacillus</i> spp. was solely the most abundant microorganism. From aminopeptidase genes analysis, the abundance of <i>Salinococcus</i> spp. during preparation and <i>Lentibacillus</i> spp. while fermentation may relate to the good flavor quality of shrimp paste.	Phewpan et al. 2020
Chinese Sichuan sausages	Evaluating the microbial community diversity and succession of Sichuan Chinese sausages during	<u>Metataxonomic sequencing:</u> Platform: Illumina Miseq HTS: V4 16S rRNA gene regions: primers 515F and 806R. 18S rRNA region of the fungi: universal primers V43NDF and Euk_V4_R.	<u>Bacteria:</u> <i>Lactobacillus</i> spp., <i>Weissella</i> spp., and <i>Pediococcus</i> spp. were the dominant bacterial genus.	Wang, Wang, Zhao (2019)

	spontaneous fermentation.	Quality control: UPARSE software. Database: not provided	
Chinese Cantonese sausages	Evaluating the effects of starter culture on bacterial community diversity and food safety of Cantonese Chinese sausages.	<u>Metataxonomic sequencing:</u> Platform: Illumina Miseq HTS: V3-V4 16S rRNA gene regions: primers 515F and 806R. Quality control: UPARSE software. Database: not provided	<u>Bacteria:</u> <i>Pediococcus</i> spp., <i>Lactobacillus</i> spp., and <i>Staphylococcus</i> spp. were the dominant bacterial genus. Wang et al. (2021b)
Harbin dry sausage	Evaluating the effect of the sodium substitutes on the physical and microbial properties and sensory characteristics in low-sodium Harbin dry sausages during 12 days of fermentation	<u>Metataxonomic sequencing:</u> Platform: Illumina Miseq platform HTS: V3-V4 region of the 16S rRNA gene: 338F and 806R primers. Quality control: QIIME 2.1 analysis platform. Database: Silva database	<u>Bacteria:</u> <i>Weissella</i> , <i>Lactobacillus</i> , and <i>Staphylococcus</i> were more present at day 0 <i>Lactobacillus</i> and <i>Staphylococcus</i> at day 6 <i>Lactobacillus</i> and <i>Staphylococcus</i> genus increased during the fermentation process. Chen et al. (2019b)
Pickles	Evaluating microbial diversity in household and industrial products.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq platform HTS: V3-V4 hypervariable regions of the bacteria 16S rRNA gene: primers 338F and 806R. Fungal 18S sequences with the primers SSU0817F and SSU1196R.	<u>Bacteria:</u> phyla most found in samples in descending order: Bacillota, Pseudomonadota, Cyanobacteria, Bacteroidetes, and Actinobacteria. An et al. (2021) All samples contained LAB. <i>Lactobacillus</i> and <i>Weissella</i> were the microorganisms more abundant.

		Quality control: clustered using UPARSE (v7.1) and chimeric sequences removed using UCHIME (v4.2) Database: Ribosomal Database Program	<u>Fungi or Yeast:</u> The phylum Ascomycota (fungi) was present and predominated in all the samples. <i>Pichia</i>, <i>Hanseniaspora</i>, and unclassified <i>Saccharomycetaceae</i> were yeast that were more present.	
Chinese spicy cabbage	Analyzing the microbial diversity of Chinese spicy cabbage (n=24, homemade or sold in the market)	<u>Metataxonomic sequencing:</u> Platform: Illumina HiSeq platform HTS: V3-V4 region of the 16S rRNA gene: 343F-5 798R primers. Quality control: Trimmomatic software (v0.35), FLASH software (v1.2.11), and QIIME software (v1.8.0). Database: Silva database (v132)	<u>Bacteria:</u> Bacillota, Pseudomonadota, and Bacteroidetes were the dominant bacterial phyla. <i>Lactobacillaceae</i>, <i>Leuconostocaceae</i>, and <i>Enterobacteriaceae</i> were the dominant family. <i>Lactobacillus</i>, <i>Panthenia</i>, and <i>Weisella</i> were the dominant genus.	Chang et al. (2022)
Idli (traditional cereal-legume-based fermented food)	Investigating the complete microbial community profile of traditionally prepared ethnic fermented food (Idli) at various stages of batter fermentation.	<u>Metataxonomic sequencing:</u> Platform: Illumina HiSeq 2500 HTS: V3-V4 region of the 16S rRNA gene: 341F and 518R primers. Quality control: QIIME software (v1.9). Database: RDP Classifier (v2.2) and the GreenGene database (v13.8).	<u>Bacteria:</u> Bacillota and Pseudomonadota were the predominant bacterial phyla. <i>Lactococcus</i>, <i>Weissella</i>, <i>Lactobacillus</i>, <i>Enterococcus</i> were the main genus.	Kavitate et al. (2022)
Idli	Exploring the diversity of the batter by culture-dependent	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq platform.	<u>Bacteria:</u> Bacillota and Pseudomonadota were the dominant phyla.	Mandhanian et al. (2019)

	and independent methods	HTS: V3 region of the 16S rRNA gene: 341F and 518R primers. Quality control: FLASH, Mothur, QIIME (v1.7). Database: Silva database (v132).		
Suancai (Traditional fermented vegetable)	Determining the effect of temperature on the volatile flavor compounds (VFCs) and the bacterial community during the fermentation process.	<u>Metataxonomic sequencing:</u> Platform: Illumina HiSeq 2500 HTS: V3-V4 region 338F and 806R primers. Quality control: not provided Database: not provided	<u>Bacteria:</u> Bacillota and Pseudomonadota were the dominant phyla. <i>Lactobacillus</i>, <i>Serratia</i>, <i>Leuconostoc</i>, <i>Pseudomonas</i>, <i>Pediococcus</i>, and others were the main genus.	He et al. (2020)
Suancai	Investigating the effect of salt concentration on microbial diversity during suancai fermentation.	<u>Metataxonomic sequencing:</u> Platform: Illumina HiSeq 2500 HTS: V3-V4 bacterial region: 338F and 806R primers. ITS1 region of the fungi: ITS1F and ITS2 primers. Quality control: FLASH (v1.2.11), Trimmomatic (v0.33), UCHIME (v8.1) and USEARCH (v10.0). Database: Silva and Unite databases.	<u>Bacteria:</u> Bacillota and Pseudomonadota were detected as the main phylum. <i>Lactobacillus</i> was the dominant genus and was not affected by salt concentration. <u>Fungi or Yeast:</u> Ascomycota was the dominant phyla.	Liang et al. (2020)

Table 4. Microbial communities in other foods using omics techniques.

Sample	Study aim	Methods used	Insightful results	Reference
Cocoa	Assessment of taxonomic and functional aspects of the microbial succession in cocoa fermentation.	<u>Metagenomic shotgun sequencing:</u> Platform: Illumina NextSeq 500 platform. Quality control: Trimmomatic tool. Database: 1) Taxonomic assessment in QIIME: SILVA (v.132) for bacteria and UNITE (v.8.0) for fungi. 2) Taxonomic assignment in MetaPhlan2 3) Annotation of functional repertoire: UniRef90 database	Yeasts and filamentous fungi shaped the bacterial succession. In addition, Quorum Sensing (QS) and Quorum Quenching (QQ) activity may modulate bacterial dominance at different time points during fermentation.	Almeida et al. (2020)
Cocoa	Evaluation of the microbial diversity and behavior during cocoa fermentation on three Costa Rican cocoa fermentation processes.	<u>Metagenomic and Metatranscriptomic sequencing:</u> Platform: Illumina HiSeq 2500. Quality control: FastQC (v.0.11.3) and PRINSEQ (v.0.20.2). Database: BLAST algorithm blastn. DIAMOND was used to compare the metagenomic sequence reads to the NCBI non-redundant protein database (NCBI-nr;	<u>Bacteria:</u> The major players were <i>Limosilactobacillus fermentum</i> and <i>Lactiplantibacillus plantarum</i> among LAB, <i>Acetobacter pasteurianus</i> , and <i>Acetobacter ghanensis</i> among acetic acid bacteria (AAB). <u>Fungi or Yeast:</u> <i>Hanseniaspora opuntiae</i> and <i>Saccharomyces cerevisiae</i> dominated.	Verge et al. (2021)
Cocoa	Investigating microbial succession and sensorial characteristics during Nicaragua fermentations of	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq	<u>Bacteria:</u> <i>Tatumella</i> species (mainly <i>T. terreus</i> and <i>T. punctata</i>) were predominant at the onset of all fermentations, followed by unusually late (generally 2 days into the	Papalexandratou et al. (2019)

	single and blended cocoa types.	HTS: V3 region of bacterial 16S rRNA genes and the ITS2 region of the fungi; Quality control: fastq_mergepairs and fastq_filter scripts using USEARCH (v10). Database: Greengenes (v.13.8) for bacteria and UNITE (v.7.0) for yeasts.	fermentations) appearance of <i>Limosilactobacillus fermentum</i>. <i>Acetobacter</i> spp. was predominant among AAB during all fermentations, but <i>Gluconobacter</i> spp. was involved in some single-variety fermentations.	
Cocoa	Evaluating microbial communities in the cocoa bean fermentation.	<u>Metagenomic shotgun sequencing:</u> Platform: Illumina Hiseq 2000. Quality control: FASTQC tool (v0.11.2) and MG-RAST (v.3.5). Database: M5NR protein database, SEED non-redundant database, KEGG	<u>Bacteria:</u> <i>Lactobacillus</i>, <i>Gluconacetobacter</i>, <i>Acetobacter</i>, and <i>Gluconobacter</i> were the dominant genera identified.	Agyirifo et al. (2019)
Coffee	Evaluating the microbial ecology in the fermentation of Australian coffee beans.	<u>Metataxonomic sequencing:</u> Platform: Illumina MiSeq. HTS: V3–V4 region of bacterial 16S rRNA gene (primers pair 341F/806R) and fungal ITS region (primers pair ITS1F/ITS2); Quality control: PEAR (v.0.9.5), QIIME (v.1.8), USEARCH (v.7.1.1090) and UPARSE software. Database: Greengenes.	<u>Bacteria:</u> The bacterial community was mainly composed of aerobic mesophilic bacteria (AMB), with <i>Citrobacter</i> being the dominant genus. <i>Leuconostoc mesenteroides</i> and <i>Lactococcus lactis</i> were the dominant among the LAB. <u>Fungi or Yeast:</u> <i>Hanseniaspora uvarum</i> and <i>Pichia kudriavzevii</i> were dominant.	Elhalis, Cox and Zhao (2020)
Fermented sour meat	Investigating the microbial composition and volatile	<u>Metataxonomic sequencing:</u>	<u>Bacteria:</u> <i>Lactobacillus</i>, <i>Staphylococcus</i>, <i>Kodamaea</i>, and	Zhong et al. (2021)

	flavor changes during the fermentation of sour meat and exploring the correlation between traditional flavors of this product and its microbial community.	Platform: Illumina MiSeq PE300 /NovaSeq PE250 platform. HTS: V3–V4 region of the bacterial 16S rRNA gene (primers pair 338F/806R) and the ITS1 region of the fungi (primers pair 1737F/2043R). Quality control: fastp (v.0.20.0), FLASH (v.1.2.7), UPARSE (v.7.1). Database: SILVA (v.138).	<i>Yarrowia</i> were the dominant microbial genera correlated with the changes in most flavor substances.
<i>Hákarl</i> (traditional Icelandic fermented fish)	Identifying microbial species present in ready-to-eat <i>hákarl</i> .	<u>PCR-DGGE analysis and Metataxonomic sequencing:</u> Platform: Illumina MiSeq. HTS: V3–V4 region of bacterial 16S rRNA genes and D1/D2 region of fungal 26S rRNA gene. Quality control: QIIME (v.1.9.0) and USEARCH software (v.8.1). Database: GenBank and Greengenes.	<u>Bacteria:</u> <i>Tissierella</i>, <i>Pseudomonas</i>, <i>Oceanobacillus</i>, <i>Abyssivirga</i> and <i>Lactococcus</i> were predominant genera <u>Fungi or Yeast:</u> <i>Candida</i>, <i>Debaryomyces</i>, and <i>Saccharomyces</i> were predominant genera.
Dry-cured fish	Evaluating the diversity and dynamics of the microbial community in vacuum-packed dry-cured fish during storage under two different temperatures and the correlation between	<u>Metataxonomic sequencing:</u> Platform: Illumina HiSeq NovaSeq6000 platform HTS: V4 region of bacterial 16S rRNA genes (primers pair 515F/806R). Quality control: FLASH (v.1.2.7) and QIIME (v.1.9.1).	<u>Bacteria:</u> Bacillota, Pseudomonadota, and Actinobacteria were the dominant phyla, and <i>Staphylococcus</i>, <i>Macroccoccus</i>, and <i>Acinetobacter</i> were the dominant genera throughout the storage process.

lipids oxidation and
microbial profiles.

Database: Greengenes.

5.2 CAPÍTULO DE LIVRO - Bioaccessibility of phenolic compounds in fermented native fruit (Abordagem metodológica aceita como capítulo de livro na segunda edição do livro *Methods and Protocols in Food Science*).

Chapter on " *Basic Methods and Protocols for Assessing the Bioaccessibility of Compounds in Foods and Byproducts*"

Bioaccessibility of phenolic compounds in fermented native fruit

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Thank you for submitting your revised chapter to *Methods and Protocols in Food Science* series as part of the above cited book.

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Abstract

Native fruits are gaining popularity in national and international markets, attributed to their unique flavors and health benefits due to high bioactive concentrations, especially phenolic compounds. The fermentation process of native fruits such as mangaba and bacaba have been highlighted, as they are rich in vitamins and minerals and contribute a considerable quantity of flavonoids, phenolic acids, and stilbenes. More interesting than just evaluating the content of phenolic compounds present in food matrices is determining bioaccessibility through *in vitro* digestion simulations, and it is important to know the fraction of the compounds that are biotransformed by these microorganisms to better understand the potential of these products as functional foods. This chapter details the methodology for extracting phenolic compounds from fermented native fruits and analyzing simulated gastrointestinal digestion to evaluate phenolic bioaccessibility.

Keywords: *In vitro* digestion. Bioaccessibility. Bioactive compounds. Vegetable matrices. Fermentative process.

1. Introduction

Brazil has a large biodiversity of underexplored native fruits that have attracted increasing interest given their technological and agroindustrial potential, high nutritional value, and unique color, flavor, and aroma characteristics [1]. Despite the potential of many native fruits, their perishability limits their wider distribution, and due to seasonality, their consumption is mainly based on derived products, such as pulp [2].

Fruits native to the Amazon region, such as bacaba (*Oenocarpus bacaba* Mart.), and to the Caatinga Biome, such as mangaba (*Hancornia speciosa* Gomes), stand out for their richness in vitamins and minerals and contribute a considerable quantity of phenolic compounds, such as flavonoids, phenolic acids, and stilbenes [1, 3, 4]. Due to their rich composition, native fruits can be combined with probiotics to enhance their technofunctional properties [2, 5]. This growing interest reflects consumer demand for non-dairy probiotic products, driving research into alternative carriers, particularly plant-based matrices, which offer additional health benefits attributed to their phenolic compounds. This shift is driven by factors such as lactose intolerance, milk protein allergies, and the increasing adoption of plant-based diets [6].

Studies have shown that fermenting fruit pulps with probiotics can contribute functional ingredients and extend the shelf life of the fruit [1, 4, 5, 7, 8, 9, 10]. Fermentation processes can be an effective strategy to increase the bioaccessibility of polyphenols and the antioxidant levels of fruit and vegetable products and are relevant for the reuse of fruit components (usually wasted after processing) with sufficient biological value, indicating a safe consumption history [8]. Thus, probiotic cultures employed during the fermentation process are capable of biotransforming the

phenolic compounds present in fruits and their derivatives. This transformation may lead to the release of phenolic compounds conjugated with other components of the food matrix, resulting in metabolites with enhanced bioactivity compared to their original forms [11].

Phenolic compounds contribute health benefits by exerting antioxidant, anti-inflammatory, and anticancer activities. These compounds can act locally or systemically to benefit health. At the local level, they act directly throughout the passage through the gastrointestinal tract, and at the systemic level, the compounds act after absorption [12]. Therefore, more interesting than just evaluating the content of phenolic compounds present in food matrices is determining bioaccessibility [13], as it is crucial to estimate the impact of gastrointestinal digestion on subsequent measurements of phenolic compound bioavailability [14].

Factors that can influence bioaccessibility and should be considered, such as factors that cause variations in the content of phenolic compounds throughout exposure to different stages of digestion, which may be a result of some physical and biochemical properties of the gastrointestinal tract, with emphasis on changes in pH, enzymatic activity and temperature [14]. Other factors such as the chemical structure of the compounds, the complexity of the food matrix, the presence of fibers, protein content and their possible molecular interaction with phenolic compounds, as well as the presence of absorption inhibitors or enhancers, type, and quantity of other compounds ingested, physiological state and time of gastric emptying or intestinal transit also influence the bioaccessibility of phenolic compounds [7].

Studies report that acidic pH, combined with enzymatic activity during gastric digestion, can induce the hydrolysis of some phenolic compounds bound to other food constituents, thereby increasing the phenolic content. Gastric digestion can also reduce the content of phenolic compounds, possibly due to changes in their molecular weight, solubility, and chemical structure [15]. In turn, alkaline conditions can exert a variable influence on the content of phenolic compounds. The alkaline conditions used to simulate the intestinal phase can favor the release of phenolic compounds bound to macromolecules, such as proteins and fibers [14,15]. In contrast, other authors have observed significant decreases in the content of phenolic compounds after intestinal digestion [14].

Methodologies used to assess the bioaccessibility of phenolic compounds include *in vivo* models, which involve studies with animals and humans, as well as *in vitro* models, such as simulated digestion of the gastrointestinal tract. Several *in vitro* methodologies have been developed to simulate the conditions of the gastrointestinal tract, enabling the investigation of the effect of the food matrix on the bioaccessibility of phenolics. This approach aims to obtain results that support the biological relevance of these compounds in the context of nutrition and health [7]. *In vitro* gastrointestinal digestion, which includes the oral, gastric, and intestinal phases, simulates some biological (gastric and pancreatic enzymes) and chemical (pH, temperature, and bile salts)

gastrointestinal conditions, as well as assessing how stable and absorbed the phenolic compounds are and whether there is a change in antioxidant activity during digestion [16].

Considering the growing appreciation of fermented native fruits as potential functional foods, this chapter provides a detailed protocol for *in vitro* digestion of fermented native fruits, as well as the determination of phenolic compound concentrations and their bioaccessibility throughout the digestive process.

2. Materials

2.1 Native fruits

Native fruits (**Figure 1**), *in natura* and subjected to pulp processing.



Figure 1 Examples of native fruits. (A) Mangaba (*Hancornia speciosa* Gomes). (B) Bacaba (*Oenocarpus bacaba* Mart.). (C) Mangaba pulp. (D) Bacaba pulp.

2.2 Obtaining probiotic fermented fruit pulp

Mangaba and bacaba pulps (35 mL) are separately spiked with probiotics (final counts of approximately 8 log CFU/mL) and incubated at 37 °C for 48 h in anaerobic jars. Two formulations are tested: (1) control (no probiotics added) and (2) mangaba or bacaba pulp fermented with probiotics (Figure 2, A and B).

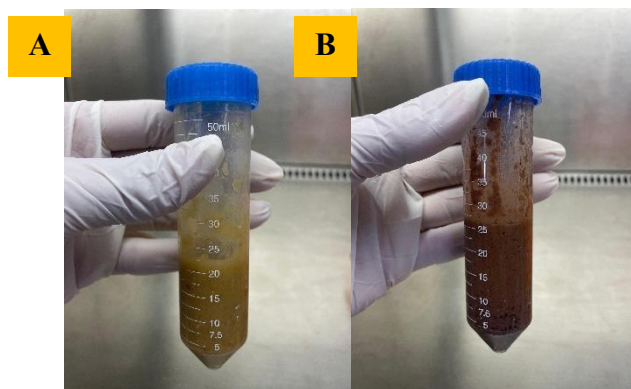


Figure 2 (A) Fermented mangaba pulp. (B) Fermented bacaba pulp.

2.3 Extraction of phenolic compounds from fermented native fruits

- a) Laboratory materials: falcon tubes and syringe filters (0.45 μm).
- b) Equipment: vortex mixer and centrifuge.

2.4 Simulated *in vitro* gastrointestinal digestion

- a) Laboratory materials: Falcon tubes, glass rods, automatic micropipettes, micropipette tips, and Becher and Erlenmeyer flasks.
- b) Reagents: potassium chloride (KCl 0.5M), monopotassium phosphate (KH_2PO_4 0.5M), sodium bicarbonate (NaHCO_3 1M), sodium chloride (NaCl 2M), magnesium chloride hexahydrate ($\text{MgCl}_2(\text{H}_2\text{O})_6$), hydrochloric acid (HCl 1M), sodium hydroxide (NaOH 1M), ammonium carbonate ($\text{CaCl}_2(\text{H}_2\text{O})_2$ 0.3M).
- c) Digestive enzymes: porcine pepsin, porcine pancreatin, and bile salts.
- d) Equipment: analytical balance, pH meter, incubator with orbital shaker, water bath, and horizontal laminar flow hood.

2.4.1 Preparation of solution for *in vitro* digestion

- a) Hydrochloric acid (HCl 6M) solution

In a beaker (100 mL), add 25 mL of ultrapure water, then with a volumetric pipette, slowly add 25 mL of 12 M HCl to the beaker containing ultrapure water. A glass rod will be necessary for careful mixing of the solution and subsequent transfer to a properly labeled bottle (date of preparation and name of the solution). Keep the solution refrigerated between 2 and 5 $^{\circ}\text{C}$.

- b) Sodium hydroxide (NaOH 5M) solution

Weigh 60 g of NaOH on an analytical balance using a spatula and then transfer it to a beaker (250 mL). After this step, add 200 mL of ultrapure water to the beaker containing 60 g of NaOH

and homogenize the solution with a glass rod until the NaOH is completely dissolved. Subsequently, transfer the solution to a 300 mL volumetric flask and complete the volume with ultrapure water. Finally, place the newly prepared solution in a properly labeled bottle (date of preparation and name of the solution) and store it under refrigeration (2 and 5 °C).

2.4.2 Preparation of gastrointestinal fluids and stock solutions

The methodology described by Minekus [17] was used to prepare gastrointestinal fluids and stock solutions. The quantities of each reagent were weighed individually on an analytical balance and transferred to previously calibrated Falcon tubes (Table 1).

Table 1. Concentrations of reagents used in the preparation of simulated digestive fluids and stock solutions

Reagents	Stock concentration s		(SSF) pH 7		(SGF) pH 3		(SIF) pH 7	
			Vol. of stock	Conc. in SSF	Vol. of stock	Conc. in SSF	Vol. of stock	Conc. in SSF
	g/L	mol/L	mL	mmol/L	mL	mmol/L	mL	mmol/L
KCl	37.3	0.5	15.1	15.1	6.9	6.9	6.8	6.8
KH ₂ PO ₄	68	0.5	3.7	3.7	0.9	0.9	0.8	0.8
NaHCO ₃	84	1	6.8	13.6	12.5	25	42.5	85
NaCl	117	2	X	X	11.8	47.2	9.6	38.4
MgCl ₂ (H ₂ O) ₆	30.5	0.15	0.5	0.15	0.4	0.1	1.1	0.33
(NH ₄) ₂ CO ₃	48	0.5	0.06	0.06	0.5	0.5	X	X

SSF: Simulated Salivary Fluid; SGF: Simulated Gastric Fluid; SIF: Simulated Intestinal Fluid.

a) Simulated salivary fluid (SSF)

Add the reagents to a beaker (500 mL) using an automatic micropipette for each solution in the same order and concentrations as in Table 1. Then, homogenize the solution and adjust the pH to 7.0 with sodium hydroxide (NaOH). Once the pH has been adjusted, complete the volume (500 mL) with ultrapure water. Finally, transfer the simulated salivary fluid to a properly labeled amber bottle (name of the solution and date of preparation) and keep it refrigerated (2 and 5 °C).

b) Simulated Gastric Fluid (SGF)

Add the reagents to a beaker (500 mL) using an automatic micropipette for each solution in the same order and concentrations as in Table 1. Then, homogenize the solution and adjust the pH to 3.0 with hydrochloric acid (HCl). After adjusting the pH, makeup to volume (500 mL) with ultrapure water. Finally, transfer the simulated gastric fluid to a properly labeled amber bottle (name of the solution and date of preparation) and keep it refrigerated (2 and 5 °C).

c) Simulated Intestinal Fluid (SIF)

Add the reagents to a beaker (500 mL) using an automatic micropipette for each solution in the same order and concentrations as in Table 1. Then, homogenize the solution and adjust the pH to 7.0 with sodium hydroxide (NaOH). After adjusting the pH, complete the volume (500 mL) with ultrapure water. Finally, transfer the simulated intestinal fluid to a properly labeled amber bottle (name of the solution and date of preparation) and keep it refrigerated (2 and 5 °C).

2.5 Determination of phenolic compounds of fermented native fruits

Phenolic compounds, including their digestive fractions, from fermented native fruits should be identified and quantified by high-performance liquid chromatography (HPLC) coupled with a diode array detector (DAD). The profiles of individual phenolics identified, including their digestive fractions, should be compared with authentic standards. The detection of phenolic compounds by diode array detector (DAD) should be performed as follows:

- a) 220 nm for catechin, epicatechin, epigallocatechin gallate, epicatechin gallate, procyanidin A2, procyanidin B1, and procyanidin B2.
- b) 280 nm for gallic acid, syringic acid, hesperidin, naringenin, and cis-resveratrol
- c) 320 nm for caffeic acid, p-coumaric acid, chlorogenic acid, caftaric acid, and trans-resveratrol.
- d) 360 nm for kaempferol 3-glucoside, quercetin 3-glucoside, rutin, myricetin, and isorhamnetin.
- e) 520 nm for malvidin 3-glucoside, cyanidin 3-glucoside, delphinidin 3-glucoside, pelargonidin 3-glucoside, peonidin 3-glucoside, cyanidin-3,5-diglucoside, malvidin-3,5-diglucoside and pelargonidin-3,5-diglucoside.

3. Methods

3.1 Extraction of phenolic compounds from fermented native fruits

The extraction of phenolic compounds should follow the methodology described by Assis et al. [4]. The samples were centrifuged (3500 x g/15 min) and passed through 0.45 µm syringe filters to obtain the filtrate (Figure 3).



Figure 3 Samples of fermented native fruits subjected to centrifugation

3.2 *In vitro* digestion of fermented native fruit samples

In vitro digestion following the methodology described by Minekus [17] was used to analyze the bioaccessibility of phenolics. Three consecutive steps were followed (Figure 4).



Figure 4 Samples of fermented native fruits subjected to *in vitro* digestion analysis

a) Oral phase

In a Becker, dilute 5 g of the fruit sample with 3.5 mL of SSF (preheated to 37°C). Measure the volume of the final digestion mixture, including the mixture and SSF. Transfer the volume to an Erlenmeyer flask (100 mL). Add 25 μ L of 0.3 M CaCl_2 to reach a total concentration of 1.5 mM in SSF. Add 975 μ L of water. Incubate while mixing for 2 minutes at 37 °C and 90 rpm. Use an automatic micropipette to collect three aliquots (2 mL each), then filter them through a 0.45 μ m syringe filter. Transfer the filtered samples to labeled cryovials and store them in the freezer.

b) Gastric phase

Prewarm the SGF electrolyte stock solution to 37 °C. Add 8 mL of SGF to the above mixture to achieve a final ratio of 1:1 (vol/vol). Adjust the pH to 3.0 by adding hydrochloric acid (HCl). Add 5 µL of $\text{CaCl}_2(\text{H}_2\text{O})_2$ solution. Add 1.6 mL of pepsin solution (2000 U/mL) to the final digestion mixture. Check the pH and adjust it to 3.0 if necessary. Add 0.925 µL of water. Incubate the sample at 37 °C, mixing the digestion mixture sufficiently for 2 h from the point at which pepsin was added. Use an automatic micropipette to collect three aliquots (2 mL each), then filter them through a 0.45 µm syringe filter. Transfer the filtered samples to labeled cryovials and store them in the freezer.

c) Intestinal phase

Preheat the SIF electrolyte stock solution in a water bath at 37 °C. Add 8.5 ml of the SIF to the above mixture to achieve a final ratio of 1:1 (vol/vol). Adjust the pH to 7.0 by adding sodium hydroxide (NaOH). Add 2.5 ml of the bile solution to the SIF/gastric chime solution (place the solution on a rotary wheel mixer at 37 °C for at least 30 min to achieve complete solubilization of the bile). Add 40 µL of the $\text{CaCl}_2(\text{H}_2\text{O})_2$ solution. Add 5 ml of pancreatin to the solution. Check the pH and adjust it to 7.0 if necessary. Add 3.76 ml of water. Incubate the samples at 37 °C in a shaking incubator to thoroughly homogenize the digestive mixture for 2 hours, starting from the point at which the pancreatic enzymes were added. Use an automatic micropipette to collect three aliquots (2 mL each), then filter them through a 0.45 µm syringe filter. Transfer the filtered samples to labeled cryovials and store them in the freezer.

The bioaccessibility for each phenolic compound was determined using Eq. 1.

$$\text{Bioaccessibility (\%)} = (\text{phenolic after digestion} / \text{phenolic in the fruit pulp}) \times 100 \text{ (Eq. 1)}$$

Where: (phenolic after digestion) refers to the phenolic concentration after *in vitro* digestion, and (phenolic in fruit pulp) refers to the phenolic concentration before *in vitro* digestion.

3.3 Determination of phenolic compounds of fermented native fruits

Phenolic compounds are quantified using the filtrates prepared as described in section 3.1. The determination of these compounds follows the methodology described by Lima [18].

Phenols should be determined by high-performance liquid chromatography (HPLC) coupled to a diode array detector (DAD). The Zorbax Eclipse Plus RP-C18 column (100 × 4.6 mm, 3.5 µm) and the Zorbax C18 pre-column (12.6 × 4.6 mm, 5 µm) are used for separation at 35 °C, using acidified water (phase A) and acidified methanol (phase B) at a flow rate of 0.8 mL/min.

Data acquisitions are processed using the software. Calibration curves of external standards are used to quantify phenolics. Compound peaks are identified by comparing their retention times

with external standards. The spectral purity of the peak is checked using the Threshold tool to ensure accuracy of identification compared to the external standard.

4. Notes

- For *in vitro* digestion analysis, it is necessary to prepare enzyme solutions that will be added throughout the digestion phases; however, these solutions should not be stored or used later in other analyses.
- The digestive enzymes should be weighed separately using an analytical balance and dissolved in their respective digestive fluids in the quantities necessary to achieve concentrations of 2000 U/mL of pepsin (porcine gastric mucosa), 100 U/mL of pancreatin (porcine pancreas), and 10 mM of bile salts (bovine bile extract).

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5.3 ARTIGO 1- Mangaba pulp fermented with *Lactocaseibacillus casei* 01 has improved chemical, technological, and sensory properties and positively impacts the colonic microbiota of vegan adults. (Artigo publicado no periódico Food Research International).

Food Research International 186 (2024) 114403



Contents lists available at ScienceDirect

Food Research International

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



Mangaba pulp fermented with *Lactocaseibacillus casei* 01 has improved chemical, technological, and sensory properties and positively impacts the colonic microbiota of vegan adults

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ARTICLE INFO

Keywords:

In vitro fermentation
Bioaccessibility
Probiotic survival
Fermented pulp
Microbial metabolites

ABSTRACT

This study aimed to evaluate the functional, technological, and sensory aspects of mangaba (*Hancornia speciosa* Gomes) fruit pulp fermented with the probiotic *Lactocaseibacillus casei* 01 (LC1) during refrigerated storage (7 °C, 28 days). The effects of the fermented mangaba pulp on the modulation of the intestinal microbiota of healthy vegan adults were also assessed. Mangaba pulp allowed high viability of LC1 during storage and after simulated gastrointestinal conditions (≥ 7 log CFU/g). The fermented mangaba pulp showed lower pH and total soluble solids, and higher titratable acidity, and concentrations of lactic, acetic, citric, and propionic acids during storage compared to non-fermented pulp. Also, it presented a higher concentration of bioaccessible phenolics and volatiles, and improved sensory properties (yellow color, brightness, fresh appearance, and typical aroma and flavor). Fermented mangaba pulp added to *in vitro* cultured colonic microbiota of vegan adults decreased the pH values and concentrations of maltose, glucose, and citric acid while increasing rhamnose and phenolic contents. Fermented mangaba pulp promoted increases in the abundance of *Dorea*, *Romboutsia*, *Faecalibacterium*, *Lachnospira*, and *Lachnospiraceae* ND3007 genera and positively impacted the microbial diversity. Findings indicate that mangaba pulp fermented with LC1 has improved chemical composition and functionality, inducing changes in the colonic microbiota of vegan adults associated with potential benefits for human health.

1. Introduction

Brazil has a great biodiversity of underexplored native fruits that has attracted increasing interest due to their technological and agro-industrial potential, high nutritional value, and unique characteristics of color, flavor, and aroma (Bitencourt et al., 2019). *Hancornia speciosa* Gomes, popularly known as mangaba, is a fruit from the Brazilian Amazon rainforest and Caatinga and Cerrado biomes (Assis et al., 2021). Mangaba has reduced lipids (0.55–1.70 %), carbohydrates (2.30–10.97 %), and energy (29.83–72.30 kcal/100 g) levels, and relevant dietary

fiber content (11.60 %) with high potential to be included in the human diet (Arruda, Araújo & Maróstica Junior, 2022). Due to the seasonality and high perishability of mangaba, its consumption is mainly based on derived products such as pulp (Santos et al., 2022).

The fermentation of fruit pulps using probiotics can improve their functional properties and extend their shelf life (Moraes et al., 2019). Furthermore, probiotic fermentation may improve the products' aromatic profile and sensory characteristics (Macedo et al., 2023; Assis et al., 2021). Finally, probiotic fermented pulps could be an option for vegans, as most probiotic fermented foods are dairy products (Pimentel

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<https://doi.org/10.1016/j.foodres.2024.114403>

Received 30 December 2023; Received in revised form 17 April 2024; Accepted 20 April 2024

Available online 21 April 2024

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et al., 2021).

Previous studies have already fermented fruit pulps or purees with probiotic cultures, such as red pitaya pulp with *Lactobacillus acidophilus* LA-05 or *Bifidobacterium animalis* ssp. *lactis* BB-12 (Morais et al., 2019), kiwi pulp with *Lactiplantibacillus plantarum* subsp. *plantarum* (Zhou et al., 2020), logan pulp with *Lactobacillus fermentum* (Huang et al., 2020) and seriguela, pink mango and acerola purees with *Lactocaseibacillus casei* 01 and *L. acidophilus* La-05 (Assis et al., 2021). However, none of the studies evaluated the potential changes in the physicochemical and sensory characteristics of the products during storage, which is important for commercial purposes.

In a previous study, we observed that *L. casei* 01 could transform the phenolic compounds of mangaba purees and increase the product's antioxidant activity (Assis et al., 2021). Some bioaccessible phenolic compounds have been associated with modulating intestinal microbiota (Macedo et al., 2023). Therefore, we hypothesize that the mangaba pulp fermented with *L. casei* 01 could also modulate the microbiota composition.

Studies with pulps fermented by probiotics using *in vitro* colonic models with the application of human fecal microbiota are still scarce. There are reports of the effects of fruit pulps fermented with probiotics on the microbiota of middle-aged hypertensive adults (Macedo et al., 2023) or healthy omnivores individuals (Guergoletto et al., 2017; Tang et al., 2023). However, as far as the authors know, no study covers the impact of probiotic fruit-derived products on the microbiota of vegan individuals. The difference in intestinal microbiota composition among individuals following vegan and omnivore diets is established (Tomova et al., 2019). It is important to evaluate the impact of pulps fermented with probiotics on the microbiota of this target population.

Therefore, the objectives of this study were i) to evaluate the functional, technological, and sensory aspects of mangaba pulp fermented with the probiotic *Lactocaseibacillus casei* 01 (LC1) during refrigerated storage and ii) to determine the effect on the modulation of the intestinal microbiota of healthy vegan adults through 16S rRNA sequencing.

2. Material and methods

2.1. Probiotic strain and inoculum preparation

A lyophilized culture of *Lactocaseibacillus casei* 01 (LC1) was obtained from Christian Hansen, Ind. With. Ltd. (Valinhos, São Paulo, Brazil). The stock culture was maintained in a cryovial at -20°C in de Man, Rogosa, and Sharpe (MRS) broth (HiMedia, Mumbai, India) containing glycerol (20 mL/100 mL, Sigma Aldrich, St. Louis, USA). To activate the strain, 100 μL of the strain was cultured individually in 9 mL of MRS broth for 24 h at 37°C under anaerobic conditions in an anaerobic flask (Anaerobic System Anaerogen, Oxoid Ltd., Wade Road, UK) with O_2 less than 1 %. Subsequently, 30 μL of the culture was plated on MRS agar and incubated at 37°C for 48 h under the same conditions. Finally, 3 loops were removed and cultured in 9 mL MRS broth for 24 h at 37°C under the same conditions.

To obtain the inoculum, the culture was centrifuged (3500 rpm/15 min, 4°C), washed twice in sterile saline solution (0.85 %, Clavico, Minas Gerais, Brazil) and resuspended in the same solution to obtain suspensions with standard cell counts (OD_{660} of 1.0, 9 log CFU/mL) (Morais et al., 2019).

2.2. Obtaining mangaba and elaborating the probiotic fermented pulp

Hancornia speciosa Gomes was purchased from a regional supplier (EMPASA; João Pessoa, Brazil). The fruits were chosen based on the noticeable absence of infection and mechanical impairment indicators. Furthermore, they were in the commercial maturity stage, with a total soluble solids (TSS) content of 19.07 °Brix.

Fruit processing was carried out on the same day of collection. In preparing the mangaba pulp, the following steps were carried out: a)

washing with sterile distilled water, sanitizing by immersing the fruits in a sodium hypochlorite solution for 5 min (150 ppm, pH 7.2 adjusted with 1 M NaOH); b) pulping with a household utensil (stainless steel blade, 6" Tramontina 23441066, Brazil); c) homogenization with a domestic processor (MixerTurbomix Pro 3 MX31, Arno®, Brazil, 400 W) for 5 min; d) pasteurization in a thermostatic bath at 65°C /30 min and cooling to room temperature ($25 \pm 1^{\circ}\text{C}$) (Zhang et al., 2021).

Fermentation of mangaba pulp was carried out by inoculating LC1 at 8 log CFU/mL in a volume of 35 mL of pulp and incubating at 37°C for 48 h (Assis et al., 2021) under anaerobic conditions in an anaerobic flask (Anaerobic System Anaerogen, Oxoid Ltd., Wade Road, United Kingdom) with $\text{O}_2 < 1\%$. Two formulations were processed: (1) control (without the addition of probiotics) and (2) probiotic fermented mangaba pulp (with the addition of LC1). The formulations for refrigerated storage were packaged in 50 mL flasks and stored at 7°C for 28 days, the common shelf life of pasteurized pulps.

2.3. Viability of probiotics in fermented mangaba pulp

The viable cells of LC1 were enumerated in the probiotic fermented mangaba pulp during the 48 h of fermentation, at times 0 and 28 days of refrigerated storage, and in the *in vitro* digestion stages (according to section 2.7), using the microdrop inoculation technique (Herigstad, Hamilton, & Heersink, 2001). To this end, 100 μL aliquots of the formulation were diluted in sterile saline solution (10^{-1} – 10^{-7}) and vortexed for 30 s. Then, 20 μL aliquots of each dilution were distributed on the MRS agar surface. The plates were incubated at 37°C for 48 h under anaerobic conditions in an anaerobic flask (Anaerobic System Anaerogen, Oxoid Ltd., Wade Road, UK) with $\text{O}_2 < 1\%$. The results were expressed as log CFU/g.

2.4. Physical and chemical parameters of mangaba pulp

The pH, TA, and TSS analyses of the control and probiotic fermented mangaba pulp formulations followed standard procedures (AOAC, 2016). pH values were determined with a digital pH meter (Quimis, São Paulo, Brazil). TA was determined by titrating samples with 0.1 N NaOH, and the results were expressed as a percentage of citric acid. TSS was measured with a digital refractometer (model HI 96801, São Paulo, Brazil) at $25 \pm 1^{\circ}\text{C}$. These analyses were performed during pulp fermentation and storage, and the pH was also evaluated during *in vitro* colonic fermentation.

2.5. Determination of sugars and organic acids in mangaba pulp

For the determination of sugars and organic acids, the control and probiotic fermented mangaba pulp formulations were centrifuged (3500 rpm/15 min), and the supernatant was filtered through a 0.45 μm Millex-HA filter (Millipore Co., Bedford, MA), according to the methodology described by Coelho et al. (2018).

The filtrates were analyzed by High-Performance Liquid Chromatography (HPLC) using an Agilent chromatograph (model 1260 Infinity LC, Agilent Technologies, USA) coupled to a diode array detector (DAD) and a refractive index detector (DIR). The analytical conditions were an Agilent Hi-Plex H column (7.7×300 mm, 8 μm) and H_2SO_4 4 M in ultrapure water as the mobile phase (flow rate 0.5 mL/min).

Peaks of sugars and organic acids were identified by comparing their retention times with those of external standards (Sigma Aldrich, St. Louis, USA). Quantification was based on external standard calibration curves according to a validated method by Coelho et al. (2018). All analyzed compounds presented calibration curves with $R^2 \geq 0.998$. Peak spectral purity was checked using the Threshold tool to ensure identification accuracy compared to the external standard. Sugars and organic acids were determined during storage (0, 7, 14, 21, and 28 of storage) and *in vitro* colonic fermentation.

2.6. Determination of phenolic compounds in mangaba pulp

For the extraction of phenolic compounds, 2 g of the control and probiotic fermented mangaba pulp formulations were macerated and homogenized (1:10) with methanol acid solution (70 % methanol, 29 % H₂O and 1 % hydrochloric acid, v/v/v) by vortexing for 2 min and sonicated at 40 kHz for 30 min at 25 °C (bath USC1800, UNIQUE, Brazil) (Morais et al., 2020).

Then, the extract was centrifuged at 3.500 rpm for 15 min (SL-701 centrifuge; Solab, São Paulo, Brazil), and the supernatant was collected. This procedure was performed twice (Dantas et al., 2019). A 1 mL aliquot of the extract was then filtered through a 0.45 µm Millex-HA filter (Millipore Co., Bedford, MA) and used to quantify phenolic compounds by HPLC.

In quantifying phenolic compounds, 20 µL of the filtrate was injected into the chromatograph as described by Padilha et al. (2017). The chromatographic separation of phenolic compounds was carried out using a Zorbax Eclipse Plus RP-C18 column (100 × 4.6 mm, 3.5 µm) and Zorbax C18 pre-column (12.6 × 4.6 mm, 5 µm). The column temperature was adjusted to 35 °C, and the mobile phases were composed of water (pH 2) acidified with 0.1 mM/L phosphoric acid (phase A) and methanol acidified with 0.5 % phosphoric acid (phase B). The flow rate was maintained at 0.8 mL/min. DAD data acquisitions were processed using OpenLAB CDS ChemStation Edition™ software (Agilent Technologies). The chromatogram of the external standards used to quantify the phenolics is shown in Supplementary Fig. S1.

A comparison of their retention times with external standards (Sigma Aldrich, St. Louis, USA) was used to identify the peaks of phenolic compounds. Quantification was performed using external standard calibration curves according to the validated method described by Padilha et al. (2017). All analyzed compounds presented calibration curves with $R^2 \geq 0.998$. Peak spectral purity was checked using the Threshold tool to ensure identification accuracy compared to the external standard. The phenolic compounds were determined during storage (0, 7, 14, 21, and 28 of storage) and *in vitro* colonic fermentation.

2.7. Determination of bioaccessibility of phenolic compounds and probiotic survival to simulated gastrointestinal conditions

The determination of probiotic survival to SGC and bioaccessibility in control and probiotic fermented mangaba pulp formulations was performed using the *in vitro* digestion method following three sequential phases: oral, gastric, and intestinal, according to the methodology described by Minekus et al. (2014).

In the simulated oral phase, 5 g of control and probiotic fermented mangaba pulp (separately) was mixed with 3.5 mL of simulated salivary fluid (SSF), 0.5 mL of 1500 U/mL of α-amylase in SSF solution, 25 µL of 0.3 M CaCl₂ and 975 µL of water, and the mixture was incubated at 37 ± 2 °C for 2 min with agitation (90 rpm). In the simulation of gastric digestion (SGF), the above mixture was added with 7.5 mL of simulated gastric fluid (SGF), 1.6 mL of 2000 U/mL porcine pepsin in SGF solution, 5 µL of 0.3 M CaCl₂ and 0.695 µL of water, and the pH was adjusted to 3.0 with 1 M HCl. Then, the mixture was incubated at 37 ± 2 °C with agitation (90 rpm) for 2 h. In the intestinal phase, 20 mL of the gastric mixture was added with 11 mL of simulated intestinal fluid (SIF), 5.0 mL of pancreatin solution 800 U/mL in SIF solution, 2.5 mL of fresh bile (160 mM in bile fresh), 40 µL of 0.3 M CaCl₂ and 1.31 mL of water, and the pH adjusted to 7.0 with 1 M NaOH. Then, the mixture was maintained at 37 ± 2 °C with stirring (90 rpm) for 10 min. The bioaccessibility results were determined using Eq. (1) (Dantas et al., 2019).

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

where: intestinal BC = concentration of the phenolic compound in the intestinal fraction; BC sample = concentration of phenolic compound in the fruit pulp.

The probiotic culture was enumerated according to section 2.3 in the oral, gastric, and intestinal phases.

2.8. Determination of volatile compounds in mangaba pulp

In the determination of volatile compounds in control and probiotic fermented mangaba pulp formulations, 3 mL of the sample was used in a 20 mL hermetically sealed glass flask with screw caps containing a Teflon-coated septum. Three subsequent steps were necessary to determine volatile compounds: extraction, separation, and identification. The first step consisted of extracting volatile compounds using the solid phase microextraction (SPME) technique with a Supelco SPME device (Bellafonte, PA, USA) (Fonseca et al., 2021). The fiber used was 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), activated according to the manufacturer's conditions (270 °C/60 min). The internal standard was 1,2-dichlorobenzene (Sigma Aldrich, USA) at a 50 µg/mL concentration.

The second step was the separation of volatile compounds performed on a 7890B gas chromatograph coupled to an Agilent Technologies 5977B mass spectrometer (Little Falls, DE, USA). The column used was the VF-5MS (30 m x 0.25 mm x 0.25 µm). The oven was initially heated to 60 °C for 5 min, followed by an increase to 230 °C at a rate of 10 °C/min, maintaining this temperature for 15 min, totaling 37 min of run time. The system operated in splitless mode. The transfer line between the chromatograph and the mass spectrum was 250 °C. The injector temperature was maintained at 230 °C. The mass spectrometer was operated by electron impact with a source temperature of 230 °C, and with ionization energy of 70 eV, scan range from m/z 35 to m/z 350 to 3.33 scan/s.

The last step was the identification of the compounds carried out by analyzing the fragmentation patterns displayed in the mass spectra, which were confirmed by comparing their mass spectra with those present in the database provided by the NIST equipment (National Institute of Standards & Technology, USA), as well as by their retention rates with those of known compounds, through the injection of homologous n-alkanes (C8-C30). The volatile compounds were determined during storage (0, 7, 14, 21, and 28).

Furthermore, the odor activity value (OAV) was calculated by dividing the concentration of volatile compounds by their odor threshold values and represented the contribution of volatile compounds to the flavor (Olivares et al., 2009). Compounds with a ratio value ≥ 1 were considered responsible for the aroma, and their OAVs were proportional to the aroma profile contributions (Jiang et al., 2022). The OVA was calculated according to the Equation (2).

$$\text{OAV} = (\text{C} / \text{OT}) \quad (2)$$

where, C (µg/g) is the concentration of the compound, OT is the odor threshold value of the compound.

2.9. Sensory evaluation of mangaba pulp by preferred attribute elicitation (PAE)

The Federal University of Paraíba Ethics Committee approved this study under protocol number 65306722.0.0000.5188. Consumers of fruits and derivatives (n = 11, 7 women, 4 men, 15–35 years old) participated in the PAE section, selected based on availability. The PAE methodology was conducted following Grygorczyk et al. (2013). Initially, consumers received the control (CONTR0, CONTR14, and CONTR28) and probiotic fermented pulps (FERM0, FERM14, FERM28). They described the descriptors considered important for the characterization of the products. These descriptors were, by consensus, grouped into categories (aroma, flavor, appearance, and texture). Descriptors with similar meanings or difficult to assess were excluded. Consumers also defined the descriptor terms' anchors considering the attributes' intensity. After a 15 min break, the control and probiotic fermented pulps were randomly and monadically offered, and consumers were

asked to rank each descriptor intensity (9 point scale). Finally, consumers classified the selected descriptors in descending order of importance for the acceptance of the pulps. They were aware that the descriptors could have the same sort of order. The PAE section lasted 120 min.

2.10. *In vitro* fermentation assays with mangaba pulp: Fecal inoculum preparation and fermentation

Four healthy vegan volunteers, consisting of two women and two men aged between 25 and 45 years, who had not used probiotics or antibiotics for at least three months and followed a vegan diet for more than three years, were recruited for this research. Exclusion criteria were chronic diseases related to metabolism, digestive system diseases, and the use of any regular medication (excluding hormonal contraception). Participants were asked to donate a fresh stool sample collected, placed in a suitable plastic container, and transported in an anaerobic jar to the laboratory. They signed the informed consent form. The Federal University of Paraíba Ethics Committee approved the fecal fermentation experiment under protocol number 65306722.0.0000.5188.

To prepare the fecal inoculum according to the methodology of Wang et al. (2020), 5 g of fresh fecal sample (1.25 g of fecal sample from each donor) was mixed with 9 times the volume of autoclaved modified saline solution (NaCl 9.0 g/L, cysteine-HCl 0.5 g/L) to obtain a fecal suspension (10 %) after centrifugation at 4 °C (500 g, 5 min).

The basal nutrient medium was composed of 0.1 g/L NaCl, 0.04 g/L KH_2PO_4 , 2 g/L NaHCO_3 , 0.01 g/L MgSO_4 , 0.04 g/L K_2HPO_4 , 0.01 g/L CaCl_2 , 0.5 g/L L-cysteine, 2.0 mL/L Tween-80, 2.0 g/L yeast extract, 2.0 g/L peptone, 1.0 mL/L resazurin solution (1 %, w/v), 10 $\mu\text{L/L}$ vitamin K, 0.5 g/L bile salts and 0.02 g/L hemin, and the pH of the medium was maintained at 7.0 (Wang et al., 2020).

After autoclaving the basal nutrient medium at 121 °C for 20 min, 9 mL was mixed with 1 mL of fecal suspension (sample CONTROL) and then incubated at 37 °C for 48 h under anaerobic conditions. Two other samples that contained 9 mL of basal nutrient medium mixed with 1 mL of fecal suspension and 0.5 g of digested control pulp (sample MAN-PULP) and 9 mL of basal nutrient medium mixed with 1 mL of fecal suspension and 0.5 g of digested probiotic fermented pulp (sample MAN-PULP_LC1) were also incubated at 37 °C for 48 h under anaerobic conditions. This experiment was performed in a genuine duplicate.

2.11. 16S rRNA sequencing and fecal microbiota analysis

DNA extraction from the samples was performed using a MoBio Power Food DNA isolation kit, Mobio Laboratories Inc., USA, following the manufacturer's procedures. The bacteria were identified via high-throughput sequencing of 16S rRNA V3/V4 region using primers 341F (CCTACGGGGRGCGAGCAG) and 785R (CTACACGGGTATCTAATCC). The 16S rRNA libraries were sequenced on the MiSeq Sequencing System (Illumina Inc., USA) using the MiSeq Reagent V2 kit with paired reads of 250 pb (2x250 pb). The KAPA Library Quantification kit for Illumina was used for multiplexing, clustering, and library sequencing reactions (Illumina, USA) on a MiSeq platform (Canani et al., 2017).

For the analysis and study of microbial diversity, the quality of the raw data from 16S rRNA V3/V4 was checked using FASTQC. After that, the low-quality sequences were filtered and trimmed, and the primers were removed using Cutadapt 3.7 software (Martin, 2011). The remaining sequences were then processed in DADA2 (1.26) for dereplication chimera removal and clustered into amplicon sequence variants (ASVs) (Callahan et al., 2016). Subsequently, ASVs were taxonomically identified based on their similarity to sequences in the bacterial database (16S rRNA SILVA 138) (Quast et al., 2012; Yilmaz et al., 2014). The raw data were submitted to the Sequence Read Archive of the National Center for Biotechnology Information (NCBI) under the Bio-Project PRJNA1044155 (Table S1).

2.12. Statistical analysis

All experiments were performed in triplicate. Results were expressed as mean $\pm$ standard deviation. Data were analyzed using ANOVA, followed by Turkey's test or Student's *t*-test considering the significance of $p < 0.05$ and using Sigma Stat 3.5 software (Jandel Scientific Software, San Jose, California).

PAE data were analyzed using Generalized Procrustes Analysis (GPA) with a matrix of 6 rows (pulp formulations) and 220 columns (descriptors $\times$ number of consumers, 11 consumers, 20 descriptors). The Friedman test analyzed the importance of descriptors (ranking test) using Newell and MacFarlane tables. Statistical analyses were performed using the XLSTAT 2022.2.1 program (Adinsoft®, New York, USA). The microbiota data were submitted to ANOVA, followed by the Tukey test considering the significance of $p < 0.05$ and using XLSTAT 2014 software (Adinsoft®, Paris, France).

3. Results and discussion

3.1. Physicochemical parameters and probiotic viable counts

The cell counts ranged from 8.02 to 8.10 log CFU/g, without significant differences at 24 and 48 h of fermentation ($p > 0.05$; Table S2), indicating that the probiotic culture may adapt to the environment imposed by the fruit pulp as substrate, maintaining the probiotic viability (Kapasob et al., 2017). The fermented mangaba pulp could be considered a probiotic product, as the minimum recommended probiotic count was maintained (6 log CFU/g) (Costa et al., 2019).

Table 1 presents the physicochemical parameters of mangaba pulp during 48 h of fermentation. At the end of fermentation, the probiotic fermented mangaba pulp had the lowest pH value (2.30), the lowest TSS (18.83 °Brix), and the highest TA (2.90 % citric acid) ($p < 0.05$) (Table 1). Probiotics consume sugars from the fruit pulp during fermentation and produce organic acids, such as citric, acetic, or lactic acid, causing decreased pH and TSS values and increased TA (Guergoletto et al., 2017). There were similar results for mangaba puree (Assis et al., 2021) and cashew juice (Kapasob et al., 2017).

3.2. Physical-chemical parameters, sugar profile, and organic acid profile during refrigerated storage of fruit pulp

Table 2 presents the physical-chemical parameters, sugar profile, and organic acid profile of mangaba pulp during 28 days of storage. At the beginning of storage (0 days), the control pulp showed the highest values of pH and TSS (3.41 and 18.93, respectively) and the lowest TA (2.77 % of citric acid, $p < 0.05$). During storage, probiotic fermented mangaba pulp showed similar values ($p < 0.05$) of pH (2.19–2.31).

Table 1
Physicochemical parameters of mangaba pulp fermented by LC1 during 48 h.

Parameters	Time (h)	Control	Fermented
pH	0	3.47 $\pm$ 0.02 ^{Aa}	3.47 $\pm$ 0.02 ^{Aa}
	24	3.45 $\pm$ 0.01 ^{Aa}	2.31 $\pm$ 0.02 ^{Bb}
	48	3.41 $\pm$ 0.04 ^{Aa}	2.30 $\pm$ 0.03 ^{Bb}
Total soluble solids (°Brix)	0	19.07 $\pm$ 0.06 ^{Aa}	19.03 $\pm$ 0.06 ^{Aa}
	24	19.03 $\pm$ 0.06 ^{Aa}	18.93 $\pm$ 0.15 ^{Ba}
	48	18.93 $\pm$ 0.12 ^{Aa}	18.83 $\pm$ 0.12 ^{Bb}
Titratable acidity (% citric acid)	0	2.73 $\pm$ 0.06 ^{Aa}	2.70 $\pm$ 0.05 ^{Aa}
	24	2.77 $\pm$ 0.06 ^{Ab}	2.87 $\pm$ 0.06 ^{Aa}
	48	2.77 $\pm$ 0.03 ^{Ab}	2.90 $\pm$ 0.09 ^{Aa}

Control: non-fermented pulp; Fermented: pulp fermented by LC1. A-B: different capital letters superimposed on the same column denote differences ($p < 0.05$) between the different times for the same treatment, according to the Tukey test or *t* test; a-b: different lowercase letters substituted on the same line denote differences ($p < 0.05$) between the same time for different treatments, according to the Tukey test or *t* test.

Table 2

Content of sugars, organic acids and physicochemical parameters of mangaba pulp fermented by LC1 during 28 days storage.

Sugar (mg/mL)	Control					Fermented				
	Time (Day)									
	0	7	14	21	28	0	7	14	21	28
Glucose	0.03 ± 0.01 ^{Aa}	0.02 ± 0.01 ^{Aa}	0.04 ± 0.03 ^{Aa}	0.06 ± 0.02 ^{Aa}	0.03 ± 0.01 ^{Aa}	0.02 ± 0.01 ^{Aa}	0.06 ± 0.00 ^{Aa}	0.06 ± 0.04 ^{Aa}	0.06 ± 0.03 ^{Aa}	0.06 ± 0.03 ^{Aa}
Fructose	0.03 ± 0.00 ^{Aa}	0.02 ± 0.01 ^{Aa}	0.04 ± 0.02 ^{Aa}	0.06 ± 0.00 ^{Aa}	0.03 ± 0.01 ^{Aa}	0.02 ± 0.01 ^{Aa}	0.06 ± 0.01 ^{Aa}	0.06 ± 0.02 ^{Aa}	0.06 ± 0.00 ^{Aa}	0.06 ± 0.04 ^{Aa}
Organic acids (g/L)										
Citric	6.07 ± 0.02 ^{Ab}	6.02 ± 0.10 ^{Ab}	6.04 ± 0.08 ^{Ab}	6.28 ± 0.15 ^{Ab}	6.26 ± 0.02 ^{Ab}	4.38 ± 0.10 ^{Bc}	12.87 ± 0.01 ^{Aa}	12.60 ± 0.02 ^{Aa}	12.17 ± 0.03 ^{Aa}	12.48 ± 0.03 ^{Aa}
Malic	1.41 ± 0.15 ^{Ba}	1.41 ± 0.01 ^{Ba}	1.47 ± 0.06 ^{Ba}	1.51 ± 0.20 ^{Ba}	1.53 ± 0.03 ^{Ba}	<LOD	<LOD	<LOD	<LOD	<LOD
Succinic	0.59 ± 0.01 ^{Ca}	0.55 ± 0.02 ^{Ca}	0.50 ± 0.05 ^{Ca}	0.50 ± 0.05 ^{Ca}	0.57 ± 0.08 ^{Ca}	0.53 ± 0.02 ^{Ca}	<LOD	<LOD	<LOD	<LOD
Lactic	<LOD	<LOD	<LOD	<LOD	<LOD	6.44 ± 0.09 ^{Ac}	7.79 ± 0.03 ^{Ba}	7.70 ± 0.04 ^{Ba}	7.02 ± 0.07 ^{Bb}	4.54 ± 0.10 ^{Bd}
Formic	0.45 ± 0.06 ^{Ca}	0.44 ± 0.10 ^{Ca}	0.47 ± 0.01 ^{Ca}	0.47 ± 0.01 ^{Ca}	0.47 ± 0.06 ^{Da}	<LOD	0.24 ± 0.04 ^{Cb}	0.23 ± 0.03 ^{Cb}	0.23 ± 0.07 ^{Cb}	0.23 ± 0.02 ^{Db}
Acetic	0.37 ± 0.02 ^{Da}	0.13 ± 0.05 ^{Db}	0.13 ± 0.05 ^{Db}	0.13 ± 0.05 ^{Db}	0.12 ± 0.04 ^{Eb}	0.59 ± 0.04 ^{Ca}	0.37 ± 0.02 ^{Ca}	0.37 ± 0.02 ^{Ca}	0.37 ± 0.03 ^{Ca}	0.40 ± 0.08 ^{Ca}
Propionic	<LOD	<LOD	<LOD	<LOD	<LOD	0.28 ± 0.02 ^{Ca}	0.28 ± 0.03 ^{Ca}	0.28 ± 0.03 ^{Ca}	0.27 ± 0.04 ^{Ca}	0.29 ± 0.02 ^{Da}
pH	3.41 ± 0.04 ^a	3.29 ± 0.03 ^b	3.31 ± 0.03 ^b	3.31 ± 0.02 ^b	3.32 ± 0.02 ^b	2.30 ± 0.03 ^b	2.31 ± 0.02 ^b	2.30 ± 0.03 ^b	2.29 ± 0.03 ^b	2.19 ± 0.01 ^b
TSS (°Brix)	18.93 ± 0.12 ^a	18.13 ± 0.12 ^b	18.13 ± 0.06 ^b	18.17 ± 0.06 ^b	18.18 ± 0.03 ^b	18.83 ± 0.12 ^a	18.03 ± 0.22 ^b	18.03 ± 0.20 ^b	18.05 ± 0.15 ^b	17.87 ± 0.06 ^c
TA (%citric acid)	2.77 ± 0.03 ^b	2.78 ± 0.08 ^b	2.75 ± 0.05 ^b	2.77 ± 0.06 ^b	2.78 ± 0.08 ^b	2.90 ± 0.09 ^a	2.93 ± 0.06 ^a	2.93 ± 0.06 ^a	2.95 ± 0.05 ^a	3.18 ± 0.05 ^a

Control: non-fermented pulp; Fermented: pulp fermented by LC1. < LOD: lower detection level. A-E: different capital letters substituted in the same column denote differences ($p < 0.05$) between different sugars and organic acids for the same treatment during storage, according to Tukey's test or t -test; a-c: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same sugar, organic acid and physical-chemical parameter for different treatments, according to Tukey test or t test.

However, it had lower TSS and higher TA when compared to non-fermented mangaba pulp ($p < 0.05$).

Considering the sugar contents (glucose and fructose), they were kept constant ($p > 0.05$) throughout storage in both formulations. The probiotic fermented mangaba pulp showed higher concentrations of lactic acid, acetic acid, citric acid, and propionic acid and lower concentrations of malic acid and succinic acid during storage ($p < 0.05$). Lactic acid is the main end product of the metabolism of most probiotic cultures, which are essentially homofermentative strains (Costa et al., 2018). Malic acid may sometimes be converted into lactic acid, as the probiotic culture can decarboxylate malate and form lactate and CO₂ using malolactic fermentation. Other routes can also be found, such as the conversion of malate into pyruvate and then into lactate (Giri et al., 2018). Furthermore, LC1 can also produce acetic acid (Miranda et al., 2019). The maintenance of sugar contents may be related to sucrose hydrolyzation by the LC1 and further use of glucose in its metabolism or hydrolyzation of glycosidic bounds of phenolic compounds by the higher acidity. Therefore, increasing the free sugars and counterbalancing the sugar consumed by the probiotic culture (Fernandes et al., 2021). Overall, the probiotic culture kept the metabolic activity during storage, increasing the concentration of organic acids and resulting in higher TA.

3.3. Survival of LC1 during storage in fruit pulp and simulated gastrointestinal conditions

Table 3 presents the survival of the probiotic in the fruit pulp during storage and simulated gastrointestinal conditions (SGC). The probiotic counts were kept constant during refrigerated storage for 28 days (7.95 – 8.02 log CFU/g, $p > 0.05$). During SGC, decreased probiotic counts were observed after the gastric phase for the recently prepared products (day 0) and the oral phase for the stored products (day 28) ($p < 0.05$). These results suggest lower resistance to the SGC after storage, which may be related to the higher acidity of the product (Table 2). However, the

Table 3

Viable cell counts of LC1 in mangaba pulp during the simulated digestion at different storage times.

Phase	Time (Day)	log CFU/g
Before Simulated Digestion (during storage)	0	8.02 ± 0.05 ^{Aa}
	28	7.95 ± 0.04 ^{Aa}
Oral Phase	0	8.00 ± 0.02 ^{Aa}
	28	7.74 ± 0.07 ^{Bb}
Gastric Phase	0	7.90 ± 0.02 ^{Ab}
	28	7.62 ± 0.03 ^{Bc}
Intestinal Phase	0	7.80 ± 0.07 ^{Ab}
	28	7.59 ± 0.03 ^{Bc}

LC1: Fermented pulp by *L. casei*. A-B: different capital letters substituted in the same column denote differences ($p < 0.05$) between the different times for the same phase, according to the Tukey test or t test; a-c: different lowercase letters substituted in the same column denote differences ($p < 0.05$) between the same time for different phases, according to the Tukey test or t test.

probiotic counts were maintained higher than 7 log CFU/g, suggesting that mangaba pulp composition protected the probiotic during the SGC, resulting in adequate probiotic survival.

3.4. Effect of LC1 on phenolic compounds in fruit pulp during storage

Table 4 shows the results of phenolic compounds in mangaba pulp during 28 days of storage. The profile of phenolic compounds in the mangaba pulp is similar to that reported in the corresponding fruit, frozen pulp, or puree (Assis et al., 2021, Dutra et al., 2017). Three distinct groups of phenolics were identified: stilbenes, flavonoids, and phenolic acids. Trans-resveratrol, quercetin 3-glucoside, kaempferol 3-glucoside, and chlorogenic acid were the phenolics found in higher concentrations in mangaba pulp (Table 4).

Probiotic fermentation altered the concentration of some of the

Table 4

Phenolic compounds in mangaba pulp fermented by LC1 during 28 days storage.

Phenolics (mg/100 g)	Treatments	Time (Day)				
		0	7	14	21	28
Stilbenes						
Trans-Resveratrol	Control	0.94 ± 0.10 ^{Bc}	1.03 ± 0.18 ^{Ab}	1.17 ± 0.10 ^{Ab}	1.36 ± 0.15 ^{Aa}	1.18 ± 0.10 ^{Bb}
	Fermented	1.05 ± 0.15 ^{Ab}	1.12 ± 0.05 ^{Ab}	1.11 ± 0.03 ^{Bb}	0.74 ± 0.26 ^{Bc}	1.33 ± 0.08 ^{Aa}
Cis Resveratrol	Control	0.26 ± 0.05 ^{Ab}	0.30 ± 0.08 ^{Ab}	0.35 ± 0.00 ^{Ab}	0.41 ± 0.07 ^{Aa}	0.35 ± 0.10 ^{Aa}
	Fermented	0.37 ± 0.03 ^{Aa}	0.34 ± 0.04 ^{Aa}	0.32 ± 0.06 ^{Aa}	0.21 ± 0.08 ^{Bb}	0.40 ± 0.03 ^{Aa}
Flavonoids						
Catechin	Control	0.15 ± 0.10 ^{Aa}	0.16 ± 0.10 ^{Aa}	0.19 ± 0.02 ^{Aa}	0.21 ± 0.05 ^{Aa}	0.20 ± 0.04 ^{Aa}
	Fermented	0.15 ± 0.08 ^{Aa}	0.17 ± 0.09 ^{Aa}	0.18 ± 0.03 ^{Aa}	0.13 ± 0.10 ^{Aa}	0.21 ± 0.03 ^{Aa}
Epicatechin	Control	0.07 ± 0.01 ^{Bb}	0.10 ± 0.00 ^{Ab}	0.11 ± 0.10 ^{Ab}	0.13 ± 0.10 ^{Ab}	0.08 ± 0.02 ^{Ab}
	Fermented	0.22 ± 0.12 ^{Aa}	0.14 ± 0.05 ^{Ab}	0.13 ± 0.11 ^{Ab}	0.08 ± 0.07 ^{Ab}	0.11 ± 0.10 ^{Ab}
Epicatechin Gallate	Control	0.07 ± 0.05 ^{Ab}	0.09 ± 0.08 ^{Ab}	0.10 ± 0.06 ^{Ab}	0.11 ± 0.05 ^{Ab}	0.10 ± 0.10 ^{Aa}
	Fermented	0.09 ± 0.02 ^{Ab}	0.09 ± 0.00 ^{Ab}	0.08 ± 0.02 ^{Ab}	0.05 ± 0.02 ^{Ab}	0.13 ± 0.08 ^{Ab}
Epigallocatechin Gallate	Control	0.02 ± 0.00 ^{Aa}	0.02 ± 0.00 ^{Aa}	0.04 ± 0.01 ^{Aa}	0.05 ± 0.00 ^{Aa}	0.03 ± 0.02 ^{Aa}
	Fermented	0.04 ± 0.02 ^{Aa}	0.04 ± 0.02 ^{Aa}	0.03 ± 0.03 ^{Aa}	0.02 ± 0.00 ^{Aa}	0.04 ± 0.01 ^{Aa}
Procyanidin B1	Control	0.05 ± 0.03 ^{Ba}	0.06 ± 0.04 ^{Aa}	0.08 ± 0.06 ^{Aa}	0.09 ± 0.03 ^{Aa}	0.09 ± 0.05 ^{Aa}
	Fermented	0.49 ± 0.20 ^{Aa}	0.07 ± 0.04 ^{Ab}	0.08 ± 0.05 ^{Ab}	0.05 ± 0.04 ^{Ab}	0.09 ± 0.07 ^{Ab}
Procyanidin B2	Control	0.14 ± 0.10 ^{Ba}	0.19 ± 0.12 ^{Aa}	0.20 ± 0.06 ^{Aa}	0.22 ± 0.10 ^{Aa}	0.18 ± 0.14 ^{Aa}
	Fermented	0.31 ± 0.20 ^{Aa}	0.22 ± 0.09 ^{Ab}	0.20 ± 0.05 ^{Ab}	0.15 ± 0.07 ^{Bb}	0.21 ± 0.10 ^{Ab}
Hesperidin	Control	0.01 ± 0.00 ^{Bb}	0.14 ± 0.06 ^{Ab}	0.17 ± 0.02 ^{Ab}	0.20 ± 0.08 ^{Ab}	0.16 ± 0.08 ^{Ab}
	Fermented	0.19 ± 0.07 ^{Aa}	0.17 ± 0.04 ^{Aa}	0.15 ± 0.07 ^{Aa}	0.10 ± 0.05 ^{Ba}	0.20 ± 0.00 ^{Ab}
Rutin	Control	0.06 ± 0.05 ^{Aa}	0.07 ± 0.05 ^{Aa}	0.07 ± 0.03 ^{Aa}	0.09 ± 0.05 ^{Aa}	0.08 ± 0.06 ^{Aa}
	Fermented	0.08 ± 0.03 ^{Aa}	0.07 ± 0.04 ^{Aa}	0.07 ± 0.05 ^{Aa}	0.05 ± 0.07 ^{Aa}	0.09 ± 0.02 ^{Aa}
Procyanidin A2	Control	0.09 ± 0.01 ^{Aa}	0.10 ± 0.00 ^{Aa}	0.09 ± 0.01 ^{Aa}	0.08 ± 0.01 ^{Aa}	0.10 ± 0.08 ^{Aa}
	Fermented	0.08 ± 0.00 ^{Ab}	0.07 ± 0.03 ^{Ab}	0.07 ± 0.02 ^{Ab}	0.07 ± 0.00 ^{Ab}	0.11 ± 0.10 ^{Aa}
Quercetin 3-glucoside	Control	5.84 ± 0.25 ^{Bc}	6.44 ± 0.32 ^{Bd}	7.26 ± 0.10 ^{Ac}	8.48 ± 0.22 ^{Ab}	7.39 ± 0.16 ^{Bb}
	Fermented	7.79 ± 0.32 ^{Ab}	6.99 ± 0.18 ^{Ac}	6.93 ± 0.04 ^{Bd}	4.61 ± 0.12 ^{Bc}	8.28 ± 0.09 ^{Ab}
Kaempferol 3-glucoside	Control	0.97 ± 0.19 ^{Bc}	1.08 ± 0.06 ^{Bc}	1.25 ± 0.10 ^{Ab}	1.45 ± 0.09 ^{Ab}	1.26 ± 0.05 ^{Ab}
	Fermented	1.34 ± 0.22 ^{Aa}	1.18 ± 0.08 ^{Ab}	1.18 ± 0.05 ^{Bb}	0.78 ± 0.02 ^{Bc}	1.22 ± 0.10 ^{Ab}
Narigenin	Control	0.26 ± 0.08 ^{Bb}	0.31 ± 0.02 ^{Ab}	0.35 ± 0.03 ^{Ab}	0.41 ± 0.09 ^{Ab}	0.36 ± 0.02 ^{Ab}
	Fermented	0.41 ± 0.16 ^{Aa}	0.34 ± 0.01 ^{Ac}	0.34 ± 0.02 ^{Bc}	0.21 ± 0.03 ^{Bd}	0.41 ± 0.05 ^{Ab}
Myricetin	Control	0.40 ± 0.20 ^{Bb}	0.51 ± 0.03 ^{Ab}	0.57 ± 0.12 ^{Ab}	0.67 ± 0.03 ^{Ab}	0.57 ± 0.01 ^{Ab}
	Fermented	0.64 ± 0.05 ^{Aa}	0.54 ± 0.08 ^{Ab}	0.54 ± 0.10 ^{Ab}	0.36 ± 0.02 ^{Bb}	0.64 ± 0.06 ^{Ab}
Isorhamnetin	Control	0.06 ± 0.03 ^{Ab}	0.07 ± 0.03 ^{Ab}	0.07 ± 0.00 ^{Ab}	0.09 ± 0.00 ^{Ab}	0.08 ± 0.02 ^{Ab}
	Fermented	0.08 ± 0.00 ^{Aa}	0.07 ± 0.02 ^{Aa}	0.07 ± 0.03 ^{Aa}	0.05 ± 0.01 ^{Aa}	0.08 ± 0.02 ^{Aa}
Phenolic Acids						
Syringic Acid	Control	0.17 ± 0.15 ^{Ba}	0.20 ± 0.12 ^{Aa}	0.23 ± 0.09 ^{Aa}	0.28 ± 0.10 ^{Aa}	0.27 ± 0.08 ^{Aa}
	Fermented	0.27 ± 0.10 ^{Ab}	0.22 ± 0.10 ^{Aa}	0.22 ± 0.08 ^{Aa}	0.15 ± 0.00 ^{Bb}	0.29 ± 0.02 ^{Aa}
Gallic Acid	Control	0.71 ± 0.12 ^{Ba}	0.69 ± 0.12 ^{Ab}	<LOD	<LOD	<LOD
	Fermented	0.87 ± 0.08 ^{Ab}	0.67 ± 0.10 ^{Ab}	<LOD	<LOD	<LOD
Chlorogenic Acid	Control	5.41 ± 0.26 ^{Bd}	5.73 ± 0.17 ^{Bc}	6.60 ± 0.08 ^{Ab}	7.70 ± 0.06 ^{Ab}	6.69 ± 0.05 ^{Bb}
	Fermented	7.79 ± 0.34 ^{Ab}	6.30 ± 0.09 ^{Ac}	6.32 ± 0.03 ^{Bc}	4.27 ± 0.02 ^{Bd}	7.45 ± 0.04 ^{Ab}
Caffeic Acid	Control	0.04 ± 0.01 ^{Ba}	0.05 ± 0.02 ^{Ab}	0.05 ± 0.01 ^{Ab}	0.07 ± 0.03 ^{Ab}	0.06 ± 0.00 ^{Ab}
	Fermented	0.71 ± 0.18 ^{Aa}	0.05 ± 0.01 ^{Ab}	0.05 ± 0.04 ^{Ab}	0.04 ± 0.02 ^{Ab}	0.07 ± 0.05 ^{Ab}
Caftaric Acid	Control	0.08 ± 0.07 ^{Aa}	0.10 ± 0.08 ^{Aa}	0.13 ± 0.10 ^{Aa}	0.15 ± 0.01 ^{Aa}	0.14 ± 0.02 ^{Aa}
	Fermented	0.06 ± 0.03 ^{Ab}	0.10 ± 0.05 ^{Ab}	0.12 ± 0.04 ^{Ab}	0.08 ± 0.02 ^{Ab}	0.16 ± 0.01 ^{Ab}
p-Coumaric Acid	Control	<LOD	<LOD	0.01 ± 0.00 ^{Aa}	<LOD	0.01 ± 0.00 ^{Aa}
	Fermented	0.01 ± 0.00 ^{Aa}	0.01 ± 0.00 ^{Aa}	0.01 ± 0.01 ^{Aa}	<LOD	0.02 ± 0.01 ^{Ab}

Control: non-fermented pulp; Fermented: pulp fermented by LC1. Values are expressed as mean ± standard deviation (mg/100 g). < LOD: lower detection level. A-B: different capital letters superscript in the same column for the same compound denote difference ($p < 0.05$) between different treatments for the same time, according to the Tukey test or t test; a-c: Different lowercase letters superscript in the same line for the same compound denote difference ($p < 0.05$) between different times for the same treatment, according to the Tukey test or t test.

phenolic compounds ($p < 0.05$, day 0), as the fermented mangaba pulp showed the highest concentrations ($p < 0.05$) of *trans*-resveratrol, epicatechin, procyanidin B1, procyanidin B2, hesperidin, quercetin 3-glucoside, kaempferol 3-glucoside, narigenin, myricetin, syringic acid, gallic acid, chlorogenic acid, caffeic acid, and p-coumaric acid (Table 4). The probiotic culture metabolism (Table 2) may be associated with the biotransformation capacity (Cheng et al., 2016). Probiotic microorganisms can disintegrate the plant cell wall, synthesize or release different phenolic compounds, convert simple phenolics, or depolymerize high molecular weight phenolics (Oliveira et al., 2020). Furthermore, probiotics can transform phenolic compounds through the action of different glycosyl hydrolases, with the release of aglycone from glycol-conjugated phenolic compounds (Pereira-Caro et al., 2015). Some bacterial species can catalyze O and C deglycosylation, demethylation, dehydroxylation, ester cleavage, carbon-carbon double bond reduction, isomerization, ring fission, and decarboxylation of phenolic compounds

(Braune & Blaut, 2016). Our results demonstrate that the fermentation of mangaba pulp with LC1 resulted in a product with a higher concentration of phenolic compounds from the stilbenes, flavonoids, and phenolic acids groups.

After 7 days of storage, the fermented pulp continued to show the highest concentrations ($p < 0.05$) of quercetin 3-glucoside, kaempferol 3-glucoside, chlorogenic acid, and p-coumaric acid. After 14–21 days of refrigerated storage, the concentration of phenolics in the fermented pulp decreased ($p < 0.05$) for *trans*-resveratrol, *cis*-resveratrol, procyanidin B2, hesperidin, quercetin 3-glucoside, kaempferol 3-glucoside, narigenin, myricetin, syringic acid, and chlorogenic acid. Refrigerated storage can promote the degradation of some phenolic compounds. The decrease in some phenolics may also be associated with their use by probiotic cultures (Assis et al., 2021; Cheng et al., 2016).

On the other hand, an increase in the concentration of some phenolic compounds in the fermented pulp was observed during refrigerated

storage ($p < 0.05$). Trans-resveratrol, epicatechin gallate, procyanidin A2, and quercetin 3-glucoside obtained their highest concentrations in the fermented pulp at the storage end ($p < 0.05$). The increase in lactic acid concentration in mangaba pulp fermented by LC1 (Table 2) may have induced the structural breakdown of the plant cell wall of the fruit pulp, resulting in the release of bound phenolic constituents (Guergetto et al., 2017). Our results demonstrate that fermentation and refrigerated storage may change the phenolic compounds profile. Still, the fermented mangaba pulp showed higher concentrations of these compounds, mainly those typical of mangaba pulp (trans-resveratrol and quercetin 3-glucoside).

The concentration of phenolic compounds is important. However, the biological properties of these compounds depend on their absorption in the gastrointestinal tract (Filannino et al., 2015). These compounds need to be released from the food matrix and absorbed during gastrointestinal digestion to exert their benefits.

3.5. Phenolic compounds after digestion and bioaccessibility during storage

The SGC resulted in reductions in the concentrations of trans-resveratrol, cis-resveratrol, epicatechin gallate, procyanidin B2, hesperidin, rutin, quercetin 3-glucoside, kaempferol 3-glucoside, narigenin, myricetin, syringic acid, chlorogenic acid, caffeic acid, p-coumaric acid and gallic acid in both formulations ($p < 0.05$, Table S3). The reduction in the concentration of phenolic compounds can be explained by the exposure of phenolics to the harsh conditions of the gastrointestinal tract, mainly due to the effects of alkaline and acidic pH conditions on the stability of these phenolic compounds (Oliveira et al., 2021).

However, there was an increase in the catechin compound, and this probably occurred because catechin can be released from polymeric flavonoids due to microbial metabolism and the activity of digestive and intestinal enzymes, which break matrix-bound phenolic compounds (Medeiros et al., 2021). Catechin may exert an antihypertensive effect due to its angiotensin-converting enzyme inhibitory property and induce endothelial recovery and lower blood pressure (Lapi et al., 2020). Finally, reductions in epicatechin, procyanidin B1, and caffeic acid concentrations were observed only for the fermented pulp ($p < 0.05$), which may be associated with their use by probiotic cultures (Assis et al., 2021).

Fermentation with probiotic culture improved the bioaccessibility of phenolic compounds from the fruit pulp during refrigerated storage. Epicatechin, procyanidin B1, kaempferol 3-glucoside, and caffeic acid had increased bioaccessibility during storage ($p < 0.05$, Table S2). However, decreases in the bioaccessibility of trans-resveratrol, catechin, epigallocatechin gallate, rutin, procyanidin A2, myricetin, and syringic acid were observed during the storage of fermented pulp ($p < 0.05$). The increase in bioaccessibility can be explained by the action of probiotic cultures with the enzyme β -glucosidase, which can hydrolyze plant β -glycosides and improve the bioaccessibility of phenolic compounds. Therefore, the probiotic culture can biotransform phenolic compounds into metabolites better absorbed in the gastrointestinal tract (Braga et al., 2018). Consuming fermented products with more bioaccessible phenolic compounds can provide beneficial effects regardless of the host microbiota (Braga et al., 2018). The results suggest that LC1 contributed positively to the profile and bioaccessibility of mangaba pulp phenolics.

3.6. Effect of LC1 on volatile compounds in fruit pulp during storage

Volatile compounds identified in mangaba pulps during refrigerated storage are shown in Table 5. Eighty volatile compounds were identified by GC-MS, comprising esters (30), terpenes (25), aldehydes (11), alcohols (7), ketone (4) and others (3). These compounds contribute markedly to the fruity note of fruit-derived products (Sampaio et al., 2006). A high content of alcohols, ketones, and terpenes may be associated with the floral and fruity notes of fermented fruit products (Chen et al., 2018).

The non-fermented mangaba pulp showed a higher concentration of some esters (ethyl acetate, butyl acetate, 3-methyl-3-butenyl acetate, 2-methyl-2-butenyl acetate, ethyl dodecanoate, and ethyl tetradecanoate), terpenes (p-cymene and terpinolene), aldehyde (2-nonenal), alcohol (3-methyl-2-butenol), and ketone (acetoin). A high content of acetate esters has been linked to improved aroma perception in beverages (Valera et al., 2021). Fruit and green notes are related to the presence of terpinolene and 2-nonenal, while citrus and green aroma may be associated with p-cymene (Gou et al., 2023). Therefore, these volatile compounds may represent part of the volatile profile of mangaba pulps.

The fermentation with probiotic cultures resulted in increases in the concentration of volatile compounds of all groups, such as esters (ethyl butanoate, isoamyl acetate, 3-methyl-2-butenyl acetate, butyl butanoate, hexyl acetate, isoamyl butanoate, 4-pentenyl butyrate, methyl benzoate, benzyl acetate, ethyl benzoate, methyl salicylate, octyl acetate, ethyl phenylacetate, β -phenylethyl acetate, dimethyl salicylate, citronellol acetate, nerol acetate, ethyl 2-methoxy benzoate, isoamyl benzoate, and ethyl hexadecanoate), terpenes (β -myrcene, D-limonene, β -(Z)-ocimene, trans- β -ocimene, isocamphane, linalool oxide, linalool, 1,3,8-p-methatriene, α -terpineol, geraniol, nerolidol, eugenol, β -damascenone, caryophyllene, γ -elemene, aromandendrene, geranyl acetone, humulene, γ -selenene, β -cadinene, and δ -Cadinene), aldehyde (benzaldehyde, benzeneacetaldehyde, (E)-2-octenal, decanal, dimethylbenzaldehyde, (E)-2-decenal, (E-E)-2,4-decadienal, and 2-undecenal), alcohols (3-methyl-3-butenol, benzyl alcohol, octanol, and phenylethyl alcohol), and ketones (6-methyl-5-hepten-2-one, 2-nonanone, and 6-methyl-3,5-heptadien-2-one). The compounds quantified in higher concentrations in the fermented mangaba pulp contribute to sensory characteristics, such as floral notes (linalool, decanal, phenyl ethyl alcohol, ethyl benzoate, β -(Z)-ocimene), sweet aroma (linalool, decanal, benzaldehyde), herbaceous/green aroma (6-methyl-5-hepten-2-one, (E)-2-octenal, α -terpineol), wood aroma (caryophyllene, linalool, humulene, δ -Cadinene), fruity aroma (ethyl butanoate, β -myrcene, octyl acetate, benzyl alcohol, ethyl benzoate), citric notes (D-limonene, undecanal, octanol, 6-methyl-5-hepten-2-one, geraniol), bitter almond note (benzyl alcohol, dimethyl benzaldehyde), and refreshing and mint aroma and flavor (α -terpineol) (Pan et al., 2022, Gou et al., 2023).

Probiotic bacteria can promote bioconversion and produce new aromatic compounds, thus affecting pulp aroma. The butyl hexanoate ester (1.89 $\mu\text{g}/100\text{ g}$) and the isoamyl alcohol (93.10 $\mu\text{g}/100\text{ g}$) were only quantified at the end of storage (28 days) in the fermented pulp ($p < 0.05$). It is considered that alcohols are formed by sugar metabolism, ketone reduction, amino acid metabolism, and fatty acid degradation (Bezerra et al., 2017). In particular, the production of isoamyl alcohol is related to the catabolism of leucine by lactic acid bacteria (Smit et al., 2005).

The volatile flavor compounds in mangaba pulp samples were analyzed by the OAV method, and each compound's contributions to the pulp flavor were evaluated (Table 6). Then, 26 flavoring substances with $\text{OAV} \geq 1$ were found, including 7 esters, 6 terpenes, 7 aldehydes, 2 alcohols, 2 ketones, and 2 others. The compounds isoamyl acetate, D-limonene, geraniol, benzaldehyde, isoamyl alcohol and 6-methyl-5-hepten-2-one indicated to be the main flavor active substances in the fermented mangaba pulp samples at the end of storage. The isoamyl alcohol, 2-nonanone and naphthalene were flavoring substances perceived only after fermentation and storage of mangaba pulp. Furthermore, methyl benzoate, (E)-2-octenal and (E)-2-decenal were the main flavor active substances for all samples compared to the other compounds.

Our results demonstrate that LC1 fermentation contributed to the formation of diverse volatile compounds, which could improve product aroma.

3.7. Sensory evaluation by preferred elicitation attribute (PAE)

The sensory attributes of mangaba pulp considered important by

Table 5
Volatile compounds in mangaba pulp fermented by LC1 during 28 days storage.

Class	Compound (µg/100 g)	LRI ^a	Control			Fermented		
			Time					
			0	14	28	0	14	28
ESTER	Ethyl Acetate	<800	59.19 ± 8.64 ^a	45.08 ± 1.36 ^b	4.56 ± 0.91 ^e	31.35 ± 8.85 ^c	10.79 ± 1.68 ^d	4.52 ± 0.70 ^f
	Ethyl butanoate	800	18.23 ± 3.85 ^b	6.34 ± 0.93 ^c	5.15 ± 1.36 ^d	66.49 ± 9.73 ^a	2.39 ± 0.63 ^f	4.23 ± 0.11 ^e
	Butyl acetate	810	2.62 ± 0.53 ^b	0.50 ± 0.10 ^d	0.57 ± 0.06 ^d	1.36 ± 0.26 ^c	0.55 ± 0.13 ^f	6.62 ± 0.60 ^a
	Isoamyl acetate	871	145.05 ± 11.55 ^d	117.44 ± 28.90 ^e	171.21 ± 3.38 ^c	211.35 ± 7.31 ^b	94.94 ± 7.14 ^f	215.46 ± 21.12 ^a
	3-Methyl-3-butenyl acetate	881	171.60 ± 5.44 ^c	154.90 ± 19.63 ^d	198.40 ± 8.56 ^b	132.38 ± 16.56 ^e	127.63 ± 16.10 ^f	255.95 ± 27.65 ^a
	3-Methyl-2-butenyl acetate	920	64.34 ± 5.38 ^c	54.19 ± 10.04 ^d	67.69 ± 15.13 ^b	97.11 ± 21.82 ^a	54.74 ± 5.60 ^e	53.55 ± 3.41 ^f
	2-Methyl-2-butenyl acetate	926	1.43 ± 0.35 ^b	1.63 ± 0.13 ^b	2.21 ± 0.05 ^a	<LOD	<LOD	<LOD
	Butyl butanoate	994	3.95 ± 0.97 ^b	3.77 ± 1.09 ^b	2.60 ± 0.08 ^e	16.12 ± 0.94 ^a	3.76 ± 0.15 ^c	3.02 ± 0.26 ^d
	Hexyl acetate	1011	5.50 ± 0.04 ^c	4.31 ± 0.35 ^c	4.62 ± 0.49 ^d	6.44 ± 1.49 ^b	5.16 ± 0.36 ^c	12.82 ± 2.09 ^a
	Isoamyl butanoate	1055	4.20 ± 0.83 ^c	2.79 ± 0.21 ^f	3.00 ± 0.75 ^e	8.45 ± 2.02 ^a	4.41 ± 0.07 ^d	7.08 ± 0.91 ^b
	4-Pentenyl butyrate	1065	2.70 ± 0.59 ^b	0.94 ± 0.08 ^d	1.09 ± 0.17 ^c	5.27 ± 1.05 ^a	0.66 ± 0.16 ^c	1.06 ± 0.26 ^c
	Methyl benzoate	1093	25.81 ± 0.29 ^e	24.22 ± 1.91 ^e	27.47 ± 4.73 ^c	56.88 ± 8.48 ^a	28.87 ± 0.58 ^d	29.71 ± 2.98 ^b
	Benzyl acetate	1163	3.30 ± 0.38 ^b	1.69 ± 0.34 ^d	2.81 ± 0.09 ^c	6.34 ± 0.94 ^a	1.01 ± 0.37 ^f	1.44 ± 0.04 ^e
	Benzyl benzoate	1170	39.47 ± 0.01 ^f	38.81 ± 2.07 ^e	52.14 ± 2.38 ^d	101.94 ± 17.83 ^a	60.04 ± 11.48 ^c	75.75 ± 1.57 ^b
	Butyl hexanoate	1186	<LOD	<LOD	<LOD	<LOD	<LOD	1.89 ± 0.13
	Methyl salicylate	1193	17.06 ± 1.04 ^f	17.64 ± 1.84 ^e	24.15 ± 0.86 ^d	40.67 ± 0.78 ^a	23.68 ± 4.48 ^c	34.12 ± 0.22 ^b
	Octyl acetate	1206	0.64 ± 0.04 ^d	0.30 ± 0.00 ^e	0.80 ± 0.08 ^c	0.99 ± 0.13 ^b	0.71 ± 0.16 ^c	1.20 ± 0.06 ^a
	Ethyl phenylacetate	1243	3.81 ± 0.17 ^c	4.86 ± 0.44 ^c	5.25 ± 0.29 ^b	3.99 ± 0.83 ^d	3.83 ± 0.97 ^d	6.65 ± 0.45 ^a
	β-Phenylethyl acetate	1255	3.58 ± 0.25 ^d	2.84 ± 0.56 ^c	4.43 ± 0.36 ^b	5.91 ± 0.35 ^a	2.41 ± 0.09 ^f	4.05 ± 0.08 ^c
	Ethyl salicylate	1265	6.25 ± 1.12 ^c	6.91 ± 0.10 ^d	9.56 ± 0.40 ^b	6.27 ± 0.69 ^c	8.17 ± 2.98 ^a	1.10 ± 0.08 ^e
	Dimethyl salicylate	1337	1.52 ± 0.28 ^f	1.73 ± 0.20 ^e	2.74 ± 0.12 ^d	4.11 ± 0.46 ^b	2.57 ± 0.67 ^c	4.91 ± 0.81 ^a
	Citronellol acetate	1350	6.72 ± 0.95 ^f	8.35 ± 0.22 ^e	10.43 ± 0.07 ^d	15.88 ± 2.10 ^a	10.13 ± 1.35 ^c	15.10 ± 0.19 ^b
	Nerol acetate	1370	2.59 ± 0.47 ^e	2.49 ± 0.47 ^f	3.91 ± 0.38 ^d	4.69 ± 1.49 ^b	4.97 ± 0.89 ^c	8.06 ± 1.27 ^a
	Ethyl decanoate	1392	0.55 ± 0.10 ^c	0.58 ± 0.01 ^c	0.67 ± 0.07 ^c	0.57 ± 0.12 ^c	0.77 ± 0.07 ^b	1.39 ± 0.31 ^a
	Ethyl 2-methoxybenzoate	1403	0.80 ± 0.20 ^e	1.62 ± 0.12 ^c	1.03 ± 0.11 ^d	2.93 ± 0.05 ^a	1.27 ± 0.42 ^c	1.83 ± 0.26 ^b
	Isoamyl benzoate	1437	0.54 ± 0.17 ^e	0.64 ± 0.17 ^d	0.84 ± 0.20 ^c	1.55 ± 0.51 ^a	0.90 ± 0.06 ^c	1.24 ± 0.21 ^b
	Ethyl dodecanoate	1590	2.64 ± 0.84 ^a	0.17 ± 0.05 ^d	0.18 ± 0.04 ^d	0.56 ± 0.14 ^b	0.42 ± 0.03 ^c	0.43 ± 0.06 ^c
	Ethyl tetradecanoate	1790	0.06 ± 0.02 ^a	0.12 ± 0.02 ^a	0.16 ± 0.01 ^a	<LOD	<LOD	<LOD
	Methyl hexadecanoate	1921	0.10 ± 0.03 ^b	0.14 ± 0.01 ^b	0.18 ± 0.05 ^b	0.14 ± 0.01 ^b	0.10 ± 0.10 ^b	0.31 ± 0.06 ^a
	Ethyl hexadecanoate	1991	0.20 ± 0.00 ^d	0.50 ± 0.02 ^c	0.58 ± 0.16 ^b	0.62 ± 0.11 ^b	0.56 ± 0.05 ^c	0.97 ± 0.09 ^a
TERPENE	β-Myrcene	989	4.75 ± 0.21 ^b	3.78 ± 0.72 ^c	2.50 ± 0.68 ^f	11.17 ± 1.25 ^a	2.92 ± 0.41 ^e	3.07 ± 0.86 ^d
	p-Cymene	1023	1.71 ± 0.49 ^a	0.38 ± 0.00 ^e	0.73 ± 0.09 ^c	0.73 ± 0.09 ^c	0.51 ± 0.02 ^d	1.53 ± 0.21 ^b
	D-Limonene	1027	8.34 ± 2.13 ^e	8.75 ± 0.90 ^f	10.21 ± 2.93 ^d	14.94 ± 4.01 ^b	13.24 ± 1.05 ^c	34.15 ± 8.93 ^a
	β (Z) Ocimene	1036	3.93 ± 0.51 ^c	1.51 ± 0.26 ^e	2.75 ± 0.37 ^d	10.13 ± 0.69 ^b	3.45 ± 1.06 ^c	11.79 ± 1.71 ^a
	trans-β Ocimene	1047	4.26 ± 0.75 ^b	2.59 ± 0.43 ^e	3.29 ± 0.53 ^d	4.82 ± 0.94 ^a	3.32 ± 0.56 ^d	4.60 ± 0.18 ^c
	Isocamphane	1052	3.18 ± 0.08 ^d	2.75 ± 0.34 ^e	3.20 ± 0.26 ^c	5.14 ± 0.84 ^b	3.17 ± 0.09 ^d	6.23 ± 1.57 ^a
	Linalool oxide	1072	1.83 ± 0.10 ^c	1.01 ± 0.07 ^f	1.11 ± 0.24 ^e	4.35 ± 0.03 ^b	1.58 ± 0.07 ^d	4.08 ± 0.60 ^a
	Terpinolene	1087	3.77 ± 0.26 ^b	1.56 ± 0.02 ^d	0.65 ± 0.08 ^e	3.28 ± 0.95 ^c	0.18 ± 0.01 ^f	7.05 ± 1.49 ^a
	Linalool	1096	30.41 ± 2.31 ^a	26.10 ± 0.10 ^f	31.64 ± 5.16 ^d	76.50 ± 17.63 ^a	39.27 ± 5.65 ^c	52.63 ± 15.87 ^b
	1,3,8-p-Menthatriene	1122	2.31 ± 0.63 ^b	0.48 ± 0.16 ^f	1.67 ± 0.33 ^c	7.75 ± 0.38 ^a	0.91 ± 0.02 ^e	1.39 ± 0.04 ^d
	α-Terpineol	1190	5.95 ± 0.13 ^d	5.53 ± 0.34 ^e	10.13 ± 1.32 ^c	18.80 ± 0.96 ^a	10.77 ± 0.75 ^c	12.97 ± 0.13 ^b
	Geraniol	1251	2.28 ± 0.12 ^c	1.76 ± 0.44 ^f	3.32 ± 0.21 ^d	5.15 ± 1.41 ^b	3.70 ± 1.23 ^c	6.12 ± 0.93 ^a
	Eugenol	1357	3.37 ± 0.27 ^e	4.40 ± 0.61 ^d	5.64 ± 0.64 ^c	8.75 ± 2.26 ^a	4.51 ± 0.49 ^d	7.33 ± 0.82 ^b
	β-Damascenone	1385	3.44 ± 0.30 ^f	3.91 ± 0.33 ^e	5.65 ± 0.12 ^d	11.19 ± 0.17 ^a	6.45 ± 0.28 ^c	8.84 ± 1.00 ^b
	Caryophyllene	1422	10.60 ± 1.49 ^f	13.22 ± 2.11 ^e	19.71 ± 1.57 ^c	24.94 ± 3.11 ^b	16.20 ± 1.56 ^d	27.19 ± 0.11 ^a
	γ-Elementene	1434	0.24 ± 0.02 ^c	0.32 ± 0.04 ^b	0.42 ± 0.02 ^b	0.30 ± 0.10 ^b	0.24 ± 0.01 ^c	0.58 ± 0.10 ^a
	Aromandendrene	1446	0.23 ± 0.05 ^c	0.48 ± 0.09 ^c	0.43 ± 0.01 ^d	1.23 ± 0.03 ^a	0.42 ± 0.05 ^d	0.63 ± 0.04 ^b
	Geranyl acetone	1449	1.13 ± 0.21 ^d	1.68 ± 0.17 ^b	1.24 ± 0.11 ^d	3.08 ± 0.63 ^a	1.30 ± 0.36 ^c	1.78 ± 0.11 ^b
	Humulene	1460	1.15 ± 0.36 ^f	1.80 ± 0.39 ^d	2.48 ± 0.26 ^b	1.60 ± 0.26 ^c	2.07 ± 0.39 ^c	3.43 ± 0.05 ^a
	γ-Selinene	1481	0.25 ± 0.06 ^c	0.51 ± 0.16 ^c	1.28 ± 0.36 ^a	0.54 ± 0.17 ^c	0.44 ± 0.05 ^d	1.19 ± 0.06 ^b
	Eremophilene	1501	0.68 ± 0.17 ^d	1.21 ± 0.23 ^c	1.52 ± 0.50 ^b	0.73 ± 0.09 ^d	2.01 ± 0.03 ^b	2.25 ± 0.22 ^a
	β-Cadinene	1519	0.20 ± 0.06 ^c	0.23 ± 0.06 ^c	0.20 ± 0.03 ^c	0.47 ± 0.02 ^b	0.33 ± 0.09 ^b	0.60 ± 0.08 ^a
	δ-Cadinene	1527	2.20 ± 0.02 ^e	2.72 ± 0.15 ^d	3.56 ± 0.55 ^c	4.81 ± 0.90 ^b	3.18 ± 0.89 ^c	5.35 ± 0.76 ^a
	Nerolidol	1540	0.26 ± 0.04 ^e	0.30 ± 0.00 ^e	0.35 ± 0.05 ^d	0.56 ± 0.06 ^c	0.58 ± 0.15 ^b	0.82 ± 0.13 ^a
	Cadalene	1680	0.13 ± 0.03 ^b	0.21 ± 0.02 ^b	0.12 ± 0.10 ^b	0.18 ± 0.04 ^b	0.20 ± 0.03 ^b	0.39 ± 0.05 ^a
ALDEHYDE	Benzaldehyde	961	12.84 ± 2.30 ^f	22.09 ± 1.13 ^c	67.35 ± 12.30 ^b	16.47 ± 4.86 ^d	18.46 ± 0.33 ^e	139.74 ± 0.10 ^a
	Benzeneacetaldehyde	1043	3.90 ± 0.94 ^c	2.41 ± 0.22 ^e	2.97 ± 0.58 ^d	5.31 ± 1.81 ^b	1.65 ± 0.45 ^f	7.10 ± 0.53 ^a
	(E)-2-Octenal	1056	6.39 ± 0.94 ^c	4.93 ± 0.12 ^d	7.46 ± 1.66 ^b	16.86 ± 0.86 ^a	6.39 ± 0.96 ^c	9.12 ± 0.03 ^b
	2-Nonenal	1157	1.29 ± 0.19 ^a	0.38 ± 0.10 ^b	<LOD	<LOD	<LOD	<LOD
	Decanal	1201	2.73 ± 0.04 ^f	3.37 ± 0.25 ^d	5.49 ± 0.35 ^a	4.24 ± 0.62 ^b	2.71 ± 0.79 ^e	3.76 ± 0.59 ^c
	2,5-Dimethylbenzaldehyde	1212	0.56 ± 0.06 ^c	0.27 ± 0.05 ^f	0.81 ± 0.08 ^d	9.03 ± 2.28 ^b	20.36 ± 4.08 ^a	1.55 ± 0.03 ^c
	(E)-2-Decenal	1258	5.44 ± 0.82 ^d	6.30 ± 0.66 ^c	8.49 ± 0.94 ^b	10.56 ± 2.70 ^a	5.19 ± 0.05 ^e	8.77 ± 0.75 ^b
	Undecanal	1301	1.73 ± 0.19 ^b	2.06 ± 0.05 ^e	2.73 ± 0.16 ^d	4.68 ± 0.03 ^a	2.38 ± 0.71 ^c	3.26 ± 0.23 ^b
	(E,E)-2,4-Decadienal	1313	1.05 ± 0.02 ^e	2.22 ± 0.11 ^b	1.92 ± 0.09 ^c	2.33 ± 0.11 ^a	0.79 ± 0.01 ^f	1.00 ± 0.21 ^d
	2-Undecenal	1360	1.63 ± 0.38 ^f	2.59 ± 0.86 ^d	4.34 ± 0.03 ^c	4.03 ± 0.95 ^b	3.29 ± 0.05 ^e	5.39 ± 0.90 ^a
ALCOHOL	Tetradecanal	1610	0.04 ± 0.00 ^a	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a	<LOD	<LOD	<LOD
	3-methyl-3-butenol	<800	92.70 ± 2.37 ^e	118.30 ± 3.28 ^c	144.03 ± 0.71 ^b	176.87 ± 17.97 ^a	91.78 ± 10.87 ^f	107.61 ± 8.78 ^d
	Isoamyl alcohol	<800	<LOD	<LOD	<LOD	<LOD	<LOD	93.10 ± 3.30
	3-Methyl-2-butenol	<800	19.97 ± 1.61 ^a	4.10 ± 0.45 ^f	13.00 ± 3.57 ^c	8.23 ± 0.22 ^d	4.37 ± 0.97 ^e	15.99 ± 0.75 ^b
	Hexanol	865	3.92 ± 0.03 ^b	1.27 ± 0.17 ^e	2.16 ± 0.32 ^d	3.48 ± 0.48 ^b	2.23 ± 0.67 ^c	4.95 ± 0.04 ^a
	Benzyl alcohol	1034	2.75 ± 0.59 ^c	1.62 ± 0.23 ^e	0.97 ± 0.05 ^f	4.98 ± 1.46 ^b	2.63 ± 0.23 ^d	6.93 ± 0.54 ^a

(continued on next page)

Table 5 (continued)

Class	Compound (µg/100 g)	LRI ^a	Control			Fermented		
			Time					
			0	14	28	0	14	28
KETONE	Octanol	1069	1.81 ± 0.04 ^d	1.00 ± 0.01 ^e	2.46 ± 0.25 ^c	2.95 ± 0.72 ^a	1.42 ± 0.42 ^d	3.28 ± 0.10 ^b
	Phenylethyl alcohol	1111	27.59 ± 4.41 ^e	24.82 ± 0.15 ^f	39.94 ± 0.55 ^d	69.32 ± 17.53 ^a	39.63 ± 1.20 ^c	47.26 ± 7.95 ^b
	Acetoin	<800	21.81 ± 1.87 ^a	17.08 ± 0.33 ^b	<LOD	<LOD	<LOD	<LOD
	6-Methyl-5-hepten-2-one	986	2.80 ± 0.09 ^e	2.58 ± 0.34 ^e	3.35 ± 0.31 ^b	2.70 ± 0.67 ^d	2.73 ± 0.77 ^c	9.42 ± 1.13 ^a
	2-Nonanone	1089	<LOD	<LOD	<LOD	6.53 ± 0.79 ^a	2.84 ± 0.58 ^c	4.63 ± 0.08 ^b
OTHERS	6-Methyl-3,5-heptadien-2-one	1102	9.92 ± 0.07 ^f	10.59 ± 0.32 ^d	10.10 ± 0.65 ^e	26.91 ± 1.35 ^a	13.11 ± 0.76 ^c	13.83 ± 2.82 ^b
	Benzyl nitrile	1140	3.12 ± 0.58 ^c	1.54 ± 0.30 ^e	1.38 ± 0.02 ^f	10.52 ± 1.30 ^a	2.45 ± 0.40 ^d	3.40 ± 0.59 ^b
	Naphthalene	1183	<LOD	<LOD	0.44 ± 0.11 ^a	<LOD	<LOD	0.38 ± 0.07 ^b
	γ-Decalactone	1467	1.74 ± 0.31 ^f	3.36 ± 0.28 ^e	5.04 ± 1.49 ^c	5.86 ± 1.71 ^b	4.32 ± 1.21 ^d	7.62 ± 0.00 ^a

^a Linear Retention Index. Control: non-fermented pulp; Fermented: pulp fermented by LCI. Values are expressed as mean ± standard deviation (µg/100 g). <LOD: lower detection level; a-f: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same compound for different treatments, according to Tukey's test or *t*-test.

consumers are presented in Table S4. Consumers identified 20 important attributes, being 6 in appearance (fresh appearance, brightness, yellow color, orange color, grainy appearance, brown color), 5 in aroma (typical aroma, fresh aroma, sweet aroma, citric aroma, fermented aroma), 6 in flavor (sweet taste, typical flavor, sour taste, fermented taste, astringent, bitter taste) and 3 in texture (homogeneous texture, pastry texture, and sandy texture). Consumers indicated that fresh appearance, brightness, yellow color, typical and fresh aroma, sweet taste, typical flavor, and homogeneous texture are the most important attributes for product acceptance. On the other hand, the least desired attributes were the grainy appearance, the brown color, the fermented aroma and taste, the astringent and bitter taste, and the sandy texture.

Fig. 1 presents the GPA maps for the control and fermented pulps. A total data variation of 64.12 % was explained in two main components (PC1 = 45.35 % and PC2 = 18.77 %). The total explanation may be significant as the assessors were untrained consumers (Fernandes et al., 2021). The attributes yellow color, orange color, brightness, fresh appearance, typical aroma, sour taste, bitter taste, and typical flavor were positively correlated with F1. In contrast, the grainy appearance, brown color, fermented aroma, sandy texture, and sweet taste were correlated negatively with this axis. In F2, homogeneous texture and fermented taste were negatively correlated. Sweet aroma, citric aroma, pasty texture, and astringent taste showed correlations lower than 0.7, indicating that these attributes were possibly in similar intensities in the products.

The PC1 separated the control formulations (left side of the axis) from the fermented formulations (right side of the axis). The control formulations were characterized by their grainy appearance, brown color, fermented aroma, sandy texture, and sweet taste, which were attributes not desired by consumers (except for sweet taste). At the same time, the fermented formulations were characterized by yellow color, orange color, brightness, fresh appearance, typical aroma, sour taste, bitter taste, and typical flavor, which consumers desired (except for bitter taste). The PC2 separated formulations based on storage time, as the formulations with 0 and 14 days were below the axis, and formulations with 28 days were above the axis. In the formulations with 0 and 14 days, a greater homogeneous texture and fermented taste were observed.

The fermentation of mangaba pulp with LCI improved the typical characteristics of the products (yellow/orange color, brightness, fresh appearance, typical aroma, and flavor). Furthermore, the higher acidity of the probiotic product (Tables 2 and 3) was perceived, as the probiotic mangaba pulps showed a higher sour taste. The impact of refrigerated storage was also observed, mainly by changes in the homogeneous texture and fermented taste.

3.8. Content of sugars, organic acids, pH, and identification of phenolic compounds in fruit pulp during fecal fermentation

The results of variations in sugar and organic acid contents and pH values are presented in Table 7. During colonic fermentation, there was a decrease in pH values in all groups, and the lowest values were registered in the mangaba pulp (MAN: 7.33, $p < 0.05$) and fermented mangaba pulp (MAN_LCI: 7.27) within 48 h ($p < 0.05$). The drop in pH is caused by the production of organic acids resulting from the consumption of sugars present in the fruit pulp, mainly glucose, and fructose, by probiotic cultures and/or other microorganisms present in the fecal samples (Guergoletto et al., 2017).

All groups after colonic fermentation showed an increase in the production of acetic, propionic, and butyric acids and a decrease in the concentration of fructose and succinic, lactic, and formic acids ($p < 0.05$). Reported studies have shown that acetic acid can be used as an energy source for the intestinal microbiota and peripheral tissues and may be related to the inhibition of pathogens and greater abundance of *Lactobacillus* spp. and *Bifidobacterium* spp. (Zhou et al., 2018; Medeiros et al., 2021; Pravasi, 2014). Propionic acid is produced in the human intestine mainly by *Bacteroidetes* and *Firmicutes* (Zhou et al., 2018; Russel et al., 2013). It is an inhibitor of gluconeogenesis and cholesterol synthesis in the liver and has antibacterial and anti-inflammatory effects, protecting the human intestine against pathogens (Havenaar, 2011). Butyric acid exerts the strongest anti-inflammatory effect of all short-chain fatty acids (SCFAs) (Kuczyńska et al., 2011). Furthermore, this acid is the main energy source of intestinal epithelial cells, able to modulate gene expression with beneficial immunoregulatory effect on intestinal epithelial cells and other mucosal cell populations (Markowiak-Kopeć & Śliżewska, 2020).

After 48 h of fermentation, the fermented mangaba pulp (MAN_LCI) showed a higher consumption of maltose, glucose, glycerol, and citric acid ($p < 0.05$). Intestinal bacteria such as *Enterococcus*, *Lactobacillus*, and *Bifidobacterium* spp. can metabolize complex carbohydrates and, mainly, sugars, producing organic acids and decreasing pH values (Medeiros et al., 2021). Lactic acid bacteria species also have the property of using citric acid as a carbon source through the action of citrate lyase in the early stages under favorable acidity, producing acetic acid besides lactic acid (Droux & Bernard, 2017). Thus, citric acid may have been broken down into other compounds by the microbiota, such as acetic acid (Li et al., 2021).

At the same time, rhamnose was found in higher concentrations in the fermented mangaba pulp (MAN_LCI: 0.12 g/L). The presence of rhamnose suggests that in mangaba pulp, probiotic culture possibly degraded rhamnose-containing polysaccharides, such as pectin, into monosaccharides (Kaprashov et al., 2017).

The results of variations in phenolic compound contents are presented in Table 8. Stilbenes, flavonoids, and phenolic acids were detected in the CTL, MAN, and MAN_LCI groups before and after 48 h of

Table 6
Flavour-active compounds in pulp of mangaba identified by OAV.

Class	Compound (µg/100 g)	TV (mg/L)	Control			Fermented		
			OAV-T0	OAV-T14	OAV-T28	OAV-T0	OAV-T14	OAV-T28
ESTER	Ethyl Acetate	20.00	0.03	0.02	0.00	0.02	0.01	0.00
	Ethyl butanoate	0.40	0.33	0.16	0.13	1.66	0.06	0.11
	Butyl acetate	7.50	0.00	0.00	0.00	0.00	0.00	0.01
	Isoamyl acetate	0.50	2.90	2.35	3.42	4.23	1.90	4.31
	3 Methyl 3-butenyl acetate							
	3-Methyl-2-butenyl acetate							
	2-Methyl-2-butenyl acetate							
	Butyl butanoate	0.10	0.40	0.38	0.26	1.61	0.38	0.30
	Hexyl acetate	3.50	0.02	0.01	0.01	0.02	0.01	0.04
	Isoamyl butanoate	0.13	0.32	0.21	0.23	0.65	0.34	0.54
	4-Pentenyl butyrate							
	Methyl benzoate	0.0025	103.24	96.88	109.88	227.52	115.48	118.84
	Benzyl acetate	85.00	0.00	0.00	0.00	0.00	0.00	0.00
	Ethyl benzoate	0.406	6.58	6.47	8.69	16.99	10.01	12.63
	Butyl hexanoate	0.70						0.03
	Methyl salicylate	0.04	4.27	4.41	6.04	10.17	5.92	8.53
	Octyl acetate	0.50	0.01	0.01	0.02	0.02	0.01	0.02
	Ethyl phenylacetate	0.65	0.06	0.07	0.08	0.06	0.06	0.10
	β-Phenylethyl acetate	0.20	0.18	0.14	0.22	0.30	0.12	0.20
	Ethyl salicylate	0.084	0.74	0.82	1.14	0.75	0.97	0.13
	Dimethyl salicylate							
	Citronellol acetate	1.80	0.04	0.05	0.06	0.09	0.06	0.08
	Nerol acetate	1.60	0.02	0.02	0.02	0.03	0.03	0.05
	Ethyl decanoate	0.57	0.01	0.01	0.01	0.01	0.01	0.02
	Ethyl 2-methoxybenzoate							
	Isoamyl benzoate	0.25	0.02	0.03	0.03	0.06	0.04	0.05
	Ethyl dodecanoate	2.00	0.01	0.00	0.00	0.00	0.00	0.00
	Ethyl tetradecanoate	2.50	0.00	0.00	0.00	0.00	0.00	0.00
	Methyl hexadecanoate	87.00	0.00	0.00	0.00	0.00	0.00	0.00
	Ethyl hexadecanoate	2.00	0.00	0.00	0.00	0.00	0.00	0.00
TERPENE	β-Myrcene	0.01	4.75	3.78	2.50	11.17	2.92	3.07
	p-Cymene	7.20	0.00	0.00	0.00	0.00	0.00	0.00
	D-Limonene	0.01	8.34	8.75	10.21	14.94	13.24	34.15
	β-(Z)-Ocimene	34.00	0.00	0.00	0.00	0.00	0.00	0.00
	trans-β-Ocimene	0.034	1.25	0.76	0.97	1.42	0.98	1.35
	Isocamphane							
	Linalool oxide	0.32	0.06	0.03	0.03	0.14	0.05	0.13
	Terpinolene	41.00	0.00	0.00	0.00	0.00	0.00	0.00
	Linalool	15.00	0.02	0.02	0.02	0.05	0.03	0.04
	1,3,8-p-Menthatriene	0.015	1.54	0.32	1.11	5.17	0.61	0.93
	α-Terpineol	1.00	0.06	0.06	0.10	0.19	0.11	0.13
	Geraniol	0.01	2.28	1.76	3.32	5.15	3.70	6.12
	Eugenol	0.002	16.85	22.00	28.20	43.75	22.55	36.65
	β-Damascenone	0.20	0.17	0.20	0.28	0.56	0.32	0.44
	Caryophyllene	0.45	0.24	0.29	0.44	0.55	0.36	0.60
	γ-Elemene							
	Aromandendrene							
	Geranyl acetone	0.06	0.19	0.28	0.21	0.51	0.22	0.30
	Humulene	0.80	0.01	0.02	0.03	0.02	0.03	0.04
	γ-Selinene							
	Eremophilene							
	β-Cadinene							
	δ-Cadinene							
	Nerolidol							
	Cadalene							
ALDEHYDE	Benzaldehyde	0.025	5.14	8.84	26.94	6.59	7.38	55.90
	Benzeneacetaldehyde	2.50	0.02	0.01	0.01	0.02	0.01	0.03
	(E)-2-Octenal	0.00002	3195.00	2465.00	3730.00	8430.00	3195.00	4560.00
	2-Nonenal	0.002	6.45	1.90				
	Decanal	0.0009	30.33	37.44	61.00	47.11	30.11	41.78
	2,5-Dimethylbenzaldehyde	0.20	0.03	0.01	0.04	0.45	1.02	0.08
	(E)-2-Decenal	0.001	54.40	63.00	84.90	105.60	51.90	87.70
	Undecanal	0.001	17.30	20.60	27.30	46.80	23.80	32.60
	(E,E)-2,4-Decadienal	0.00068	15.44	32.65	28.24	34.26	11.62	14.71
ALCOHOL	2-Undecenal							
	Tetradecanal	0.11	0.00	0.00	0.00			
	3-methyl-3-butenol	17.00	0.05	0.07	0.08	0.10	0.05	0.06
	Isoamyl alcohol	0.06						15.52
	3-Methyl-2-butenol							
	Hexanol	4.00	0.01	0.00	0.01	0.01	0.01	0.01
	Benzyl alcohol	900.00	0.00	0.00	0.00	0.00	0.00	0.00
	Octanol	0.90	0.02	0.01	0.03	0.03	0.02	0.04
	Phenylethyl alcohol	0.04	6.90	6.21	9.99	17.33	9.91	11.82

(continued on next page)

Table 6 (continued)

Class	Compound ($\mu\text{g}/100\text{ g}$)	TV (mg/L)	Control			Fermented		
			OAV-T0	OAV-T14	OAV-T28	OAV-T0	OAV-T14	OAV-T28
KETONE	Acetoin	50.00	0.00	0.00				
	6-Methyl-5-hepten-2-one	0.05	0.56	0.52	0.67	0.54	0.55	1.88
	2-Nonanone	0.02				3.27	1.42	2.32
OTHERS	6-Methyl-3,5-heptadien-2-one							
	Benzyl nitrile	0.0672	0.46	0.23	0.21	1.57	0.36	0.51
	Naphthalene	0.00044			10.00			8.64
	γ -Decalactone	0.40	0.04	0.08	0.13	0.15	0.11	0.19

OT: odor threshold value of the compound; OAV: odor activity value.

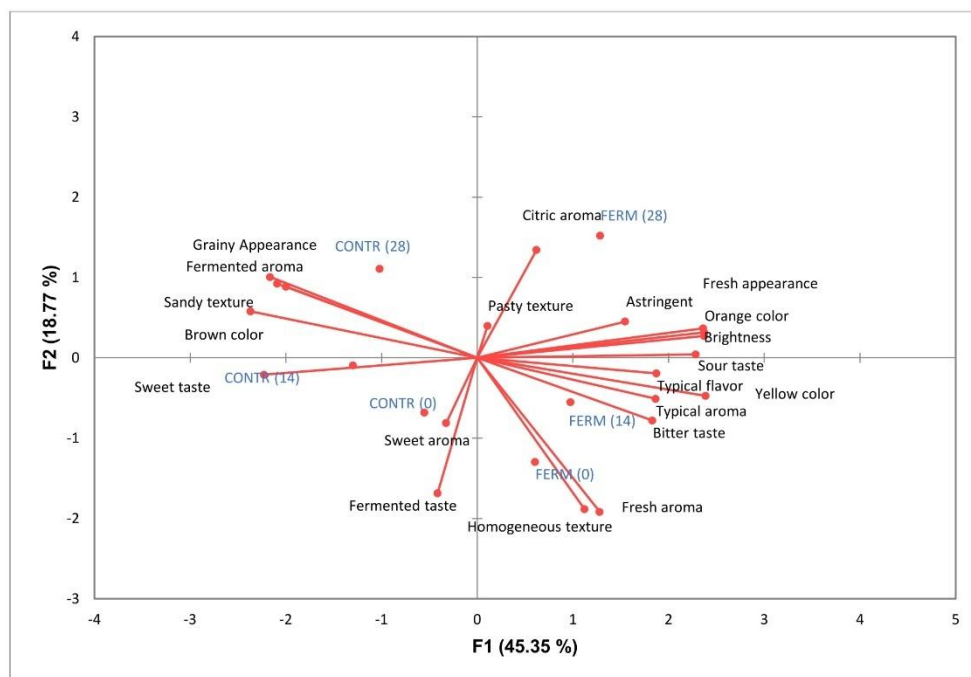


Fig. 1. GPA biplot maps for the control and fermented pulps (F1 x F2).

colonic fermentation. Epicatechin and hesperidin compounds increased in concentration after 48 h of fecal fermentation for all evaluated groups ($p < 0.05$), suggesting a typical change derived from fermentation. Quercetin 3-glucoside, syringic acid, and procyanidin B1 showed the highest concentrations in CTL before fermentation, while epicatechin gallate contents increased in concentration after 48 h ($p < 0.05$). According to previous studies, syringic and gallic acids are mainly degradation products of malvidin-3-glucoside (Hidalgo et al., 2012). At the same time, the addition of fruit pulp (MAN) resulted in an increase in procyanidin A2 and gallic acid after 48 h ($p < 0.05$). Gallic acid is a secondary metabolite of polyphenols well known for its antioxidant characteristics and antihyperlipidemic, cardioprotective, anti-hyperglycemic, and anticancer actions (Ashrafizadeh et al., 2021). Finally, the fermented mangaba pulp (MAN_LC1) showed an increased concentration of procyanidin B1 and gallic acid and a decreased concentration of catechin and procyanidin A2 ($p < 0.05$). Studies indicate that a long fermentation period negatively influences the concentration of some bioactive substances like catechin (Álvarez et al., 2017). Our results demonstrate that the phenolic compounds during fecal

fermentation may vary according to the substrate used (control, mangaba pulp, or fermented mangaba pulp).

3.9. Effect of fruit pulp on the intestinal microbiota

The effect of fermented mangaba pulp on the intestinal microbiota was evaluated using high-throughput sequencing analysis. The richness and α -diversity of the intestinal microbiota, evaluated by the observed indices, Chao1, se.chao1, ACE, se.ACE, Shannon, Simpson, and InvSimpson for all treatments are shown in Table 9.

The se.chao1, Simpson, and InvSimpson indices showed no significant difference between groups ($p > 0.05$). The control feces showed the highest values of the Observed, Chao1, and ACE indices at time 0 (CTL_0h, 771.50, 806.65, and 795.90, respectively) ($p < 0.05$). At the same time, the lowest values of the Observed, ACE, and Chao1 indices were found in the fermented mangaba pulp (MAN_LC1_48h, 539.50, 584.25, and 588.45, respectively). Finally, the fermented mangaba pulp also showed a lower Shannon index value (MAN_LC1_48h, 5.94) compared to CTL_0h, but it did not differ from CTL_48h and MAN_48h

Table 7

Content of sugars, organic acids and pH in colonic fermentation experiments with mangaba pulp fermented by LC1.

Sugar (g/L)	Time (h)					
	0 h			48 h		
	CTL	MAN	MAN_LC1	CTL	MAN	MAN_LC1
Maltose	0.08 ± 0.02 ^{Ba}	0.04 ± 0.00 ^{Ba}	0.03 ± 0.02 ^{Ba}	0.02 ± 0.01 ^{Ba}	0.08 ± 0.02 ^{Aa}	<LOD
Glucose	0.18 ± 0.12 ^{Aa}	0.23 ± 0.10 ^{Aa}	0.14 ± 0.12 ^{Ab}	0.27 ± 0.09 ^{Aa}	0.06 ± 0.01 ^{Ac}	<LOD
Fructose	0.16 ± 0.08 ^{Aa}	0.20 ± 0.04 ^{Aa}	0.13 ± 0.09 ^{Aa}	0.02 ± 0.10 ^{Bb}	0.06 ± 0.08 ^{Ab}	0.10 ± 0.01 ^{Bb}
Glycerol	0.04 ± 0.01 ^{Ba}	0.05 ± 0.03 ^{Ba}	0.05 ± <LOD	0.07 ± 0.01 ^{Ba}	0.06 ± <LOD	<LOD
Rhamnose	<LOD	<LOD	<LOD	<LOD	<LOD	0.12 ± 0.00 ^B
Organic acids (g/L)						
Citric	0.04 ± 0.00 ^{Ca}	0.06 ± 0.01 ^{Ca}	0.04 ± 0.03 ^{Ca}	0.06 ± 0.02 ^{Ca}	0.06 ± 0.03 ^{Ba}	<LOD
Succinic	0.82 ± 0.06 ^{Ac}	0.96 ± 0.02 ^{Ab}	1.01 ± 0.10 ^{Aa}	0.13 ± 0.03 ^{Cc}	0.10 ± 0.10 ^{Cc}	0.10 ± 0.05 ^{Cc}
Lactic	<LOD	<LOD	0.11 ± 0.05 ^C	<LOD	<LOD	<LOD
Formic	0.01 ± 0.01 ^{Cb}	0.02 ± 0.00 ^{Cb}	0.08 ± 0.04 ^{Ca}	<LOD	<LOD	<LOD
Acetic	<LOD	0.11 ± 0.10 ^{Bb}	0.13 ± 0.03 ^{Bb}	0.85 ± 0.10 ^{Aa}	0.81 ± 0.12 ^{Aa}	0.85 ± 0.09 ^{Aa}
Propionic	0.17 ± 0.03 ^{Bc}	0.19 ± 0.00 ^{Bc}	0.23 ± 0.10 ^{Bb}	0.41 ± 0.04 ^{Ba}	0.28 ± 0.20 ^{Ba}	0.39 ± 0.03 ^{Ba}
Butyric	<LOD	<LOD	<LOD	0.38 ± 0.05 ^{Ba}	0.33 ± 0.10 ^{Ba}	0.38 ± 0.08 ^{Ba}
pH						
	8.52 ± 0.02 ^a	8.26 ± 0.09 ^b	8.22 ± 0.05 ^b	7.65 ± 0.02 ^c	7.33 ± 0.04 ^d	7.27 ± 0.04 ^d

CTL: no addition of mangaba pulp, only microbiota in fermentation medium; MAN: non-fermented and digested mangaba pulp; MAN_LC1: mangaba pulp fermented by LC1 and digested. Values are expressed as mean ± standard deviation (g/L). < LOD: lower detection level. A-D: different capital letters substituted in the same column denote differences ($p < 0.05$) between different sugars and organic acids for the same treatment, according to Tukey's test or t -test; a-d: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same sugar, organic acid and pH for different treatments, according to Tukey test or t -test.

($p > 0.05$). The results suggest that probiotic fermentation impacted microbial diversity by reducing the number of species, diversity, and species richness (Kim et al., 2017). It has been postulated that probiotic fermentation may contribute to the survival of these adapted microorganisms in the intestine and the reduction of other species, including pathogenic microorganisms, which may result in reduced microbial diversity (Oliveira et al., 2024).

Regarding the taxonomic affiliations from ASVs, Fig. 2 highlights the main phyla found for all the groups. *Firmicutes* were observed in dominance (from 73.45 % to 98.39 %). Studies show that 60–80 % of the total fecal microbiome belongs to the *Bacteroidetes* and *Firmicutes* phyla, indicating that these phyla dominate the intestinal microbiota in healthy individuals (Retnakumar, et al., 2022). Therefore, the microbiota of the vegan-healthy individuals also shows characteristics observed for non-vegan-healthy individuals.

Similar relative abundance of the phyla *Proteobacteria*, *Bacteroidota*,

Table 8

Phenolic compounds in colonic fermentation experiments with mangaba pulp fermented by LC1.

Phenolics (mg/100 g)	Treatments	Time (h)	
		0	48
Stilbenes			
Cis-Resveratrol	CTL	0.13 ± 0.02 ^{Aa}	0.13 ± 0.00 ^{Aa}
	MAN	0.10 ± 0.03 ^{Aa}	0.11 ± 0.04 ^{Aa}
	MAN_LC1	0.11 ± 0.02 ^{Aa}	0.08 ± 0.03 ^{Aa}
Flavonoids			
Catechin	CTL	0.08 ± 0.04 ^{Aa}	0.05 ± 0.01 ^{Aa}
	MAN	0.05 ± 0.01 ^{Aa}	0.04 ± 0.00 ^{Aa}
	MAN_LC1	0.05 ± 0.02 ^A	<LOD
Epicatechin	CTL	0.02 ± 0.00 ^{Ab}	0.34 ± 0.05 ^{Ba}
	MAN	0.02 ± 0.02 ^{Ab}	0.51 ± 0.15 ^{Aa}
	MAN_LC1	0.02 ± 0.00 ^{Ab}	0.36 ± 0.02 ^{Ba}
Epicatechin Gallate	CTL	<LOD	0.04 ± 0.02
	MAN	<LOD	<LOD
	MAN_LC1	<LOD	<LOD
Procyanidin B1	CTL	0.15 ± 0.00 ^A	<LOD
	MAN	0.01 ± 0.01 ^{Ba}	0.02 ± 0.02 ^{Aa}
	MAN_LC1	<LOD	0.02 ± 0.00 ^A
Procyanidin B2	CTL	0.09 ± 0.06 ^{Aa}	0.08 ± 0.07 ^{Aa}
	MAN	0.08 ± 0.01 ^{Aa}	0.08 ± 0.03 ^{Aa}
	MAN_LC1	0.08 ± 0.03 ^{Aa}	0.07 ± 0.02 ^{Aa}
Hesperidin	CTL	<LOD	0.91 ± 0.12 ^A
	MAN	<LOD	0.99 ± 0.10 ^A
	MAN_LC1	<LOD	0.84 ± 0.20 ^A
Rutin	CTL	<LOD	<LOD
	MAN	<LOD	<LOD
	MAN_LC1	<LOD	<LOD
Procyanidin A2	CTL	0.06 ± 0.01 ^{Ba}	0.09 ± 0.03 ^{Aa}
	MAN	<LOD	0.07 ± 0.03 ^A
	MAN_LC1	0.58 ± 0.25 ^{Aa}	0.06 ± 0.02 ^{Ab}
Quercetin 3 Glucoside	CTL	0.02 ± 0.01	<LOD
	MAN	<LOD	<LOD
	MAN_LC1	<LOD	<LOD
Phenolic Acids			
Syringic Acid	CTL	0.01 ± 0.00	<LOD
	MAN	<LOD	<LOD
	MAN_LC1	<LOD	<LOD
Gallic Acid	CTL	<LOD	<LOD
	MAN	<LOD	0.24 ± 0.10 ^A
	MAN_LC1	<LOD	0.14 ± 0.08 ^B

CTL: no addition of mangaba pulp, only microbiota in fermentation medium; MAN: non-fermented and digested mangaba pulp; MAN_LC1: mangaba pulp fermented by LC1 and digested. Values are expressed as mean ± standard deviation (mg/100 g). < LOD: lower detection level. A-B: different capital letters superscript in the same column for the same compound denote difference ($p < 0.05$) between different treatments for the same time, according to the Tukey test or t -test; a-b: different lowercase letters superscript in the same line for the same compound denote difference ($p < 0.05$) between different times for the same treatment, according to the Tukey test or t -test.

and *Euryarchaeota* were observed for all groups ($p > 0.05$). Adding mangaba pulp without or with LC1 to the feces of healthy vegan adults increased the abundance of *Firmicutes*, and decreased the abundance of *Actinobacteriota* and *Synergistota* ($p < 0.05$), contrary to what was observed for CTL. Only the fermented mangaba pulp could decrease the abundance of *Desulfobacterota* ($p < 0.05$). Consuming food products with high dietary fiber and bioactive compounds, including phenolics, may reduce the *Bacteroidetes* and increase the *Firmicutes* phylum (Medeiros et al., 2021). These results demonstrate that the mangaba pulp and, mainly, fermented mangaba pulp changed the relative abundance of the microbiota phyla during fermentation, contributing to the maintenance of the original phyla and their abundance in the feces.

Clostridia was observed at the class level (Table S5), especially in the CTL_0h group (98.36 %). In fact, the most abundant class in the phylum *Firmicutes* is the *Clostridia* (20.3 %) (Retnakumar, et al., 2022). Similar

Table 9

Alpha diversity indices obtained from 16S rRNA gene amplicon sequencing in colonic fermentation experiments.

	CTL_0h	CTL_48h	MAN_48h	MAN_LC1_48h
Observed	771.50 ± 58.50 ^a	647.00 ± 61.00 ^b	600.50 ± 34.50 ^{bc}	539.50 ± 32.50 ^c
Chao1	806.65 ± 73.35 ^a	686.05 ± 50.05 ^b	655.25 ± 26.85 ^{bc}	588.45 ± 6.55 ^c
se.chao1	11.40 ± 3.90 ^a	11.30 ± 1.90 ^a	14.40 ± 1.30 ^a	13.25 ± 5.45 ^a
ACE	795.90 ± 63.30 ^a	682.65 ± 47.25 ^b	653.70 ± 28.00 ^{bc}	584.25 ± 12.45 ^c
se.ACE	13.50 ± 0.80 ^a	7.55 ± 5.22 ^{ab}	10.30 ± 1.40 ^b	9.30 ± 1.20 ^{ab}
Shannon	6.26 ± 0.07 ^a	6.10 ± 0.20 ^{ab}	6.07 ± 0.05 ^{ab}	5.94 ± 0.14 ^b
Simpson	0.997 ± 0.00 ^a	0.996 ± 0.00 ^a	0.997 ± 0.00 ^a	0.996 ± 0.00 ^a
InvSimpson	403.30 ± 30.50 ^a	327.90 ± 102.80 ^a	326.55 ± 24.55 ^a	287.40 ± 47.90 ^a

CTL_0h: no addition of mangaba pulp, only the microbiota in the fermentation medium, at time 0 h; CTL_48h: no addition of mangaba pulp, only the microbiota in the fermentation medium, after 48 h of fermentation; MAN_48h: addition of unfermented and digested mangaba pulp, after 48 h of fermentation; MAN_LC1_48h: addition of mangaba pulp fermented by *L. casei* and digested after 48 h of fermentation; Values are expressed as mean ± standard deviation (mg/100 g); a-c: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same alpha diversity for different treatments, according to Tukey's test or *t*-test.

relative abundance of *Clostridia*, *Bacilli*, *Gammaproteobacteria*, *Bacteroidia*, and *Methanobacteria* was observed for all groups ($p > 0.05$).

Adding mangaba pulp without or with LC1 to the feces of healthy vegan adults increased the abundance of *Actinobacteria* ($p < 0.05$). Furthermore, it restored the microbiota balance by decreasing the abundance of *Coriobacteriia* and *Synergistota* ($p < 0.05$), contrary to what was observed for CTL. Only the fermented mangaba pulp could decrease the abundance of *Desulfobacterota* ($p < 0.05$). *Actinobacteria* has been reported as an essential player in developing and maintaining intestinal homeostasis (Binda et al., 2018).

At the order level (Table S6), a similar relative abundance of *Oscillospirales*, *Enterobacteriaceae*, *Bacteroidales*, *Erysipelotrichales*, *Methanobacteriales*, and *Monoglobales* was observed for all groups ($p > 0.05$). At the same time, increases in *Peptostreptococcales-Tissierellales* were observed in all groups ($p < 0.05$). The addition of mangaba pulp without or with LC1 to the feces of healthy vegan adults restored the balance of the microbiota by decreasing the abundance of *Coriobacteriales*,

Synergistales, *Desulfobacteriales*, and *Acidaminococcales* ($p < 0.05$), contrary to what was observed for CTL. The *Coriobacteriales* are paradigm pathogens involved in bile acid metabolism, gut barrier, and metabolic dysfunctions, negatively impacting cholesterol homeostasis through increasing cholesterol absorption (Zhao et al., 2017). At the same time, mangaba pulps increased the abundance of *Bifidobacteriales* (0.16 and 0.22 %, respectively) and *Lachnospirales* (77.32 and 76.10 %, respectively) compared to the CTL_48h group ($p < 0.05$).

At the family level (Table S7), a higher abundance of *Lachnospiraceae* was observed in CTL_0h (87.20 %, $p < 0.05$). The *Lachnospiraceae* family keeps the prevalence in the groups added with mangaba pulp without or with LC1 (MAN_48h and MAN_LC1_48h) (77.32 and 76.10 %, respectively). *Lachnospiraceae* produces short-chain fatty acids (SCFAs) from dietary fiber that have anti-inflammatory and modulating effects on the intestinal mucosa, maintaining intestinal health (Hill-Burns et al., 2017).

Similar relative abundance of *Ruminococcaceae*, *Enterobacteriaceae*, *Bacteroidaceae*, *Erysipelatoclostridiaceae*, and *Methanobacteriaceae* were observed for all groups ($p > 0.05$). Furthermore, all groups showed an increase in the abundance of *Peptostreptococcaceae* and *Anaerovoracaceae* and a decrease in the abundance of *Oscillospiraceae* and *Monoglobaceae* during fermentation ($p < 0.05$).

The addition of mangaba pulp without or with LC1 to the feces of healthy vegan adults increased the abundance of *Lachnospiraceae* (77.32 % and 76.10 %, respectively) and *Bifidobacteriaceae* (0.16 and 0.22 %, respectively) and decreased the abundance of *Eggerthellaceae* and *Synergistota* ($p < 0.05$), contrary to what was observed for CTL. Only the fermented mangaba pulp could decrease the abundance of *Desulfobacteriia* ($p < 0.05$). *Bifidobacteriaceae* are responsible for a variety of beneficial health effects, including inhibiting harmful bacterial growth, regulating the gastrointestinal and immune system, regulating intestinal microbial homeostasis, improving the integrity of the intestinal mucosal barrier, and decreasing levels of intestinal lipopolysaccharides (Bonvegna & Cilia, 2023).

At the genus level (Fig. 3), after colonic fermentation, no changes in the abundance of the *[Eubacterium] hallii* group, *Subdoligranulum*, and *Anaerostipes* for all groups compared to the CTL_0h group was observed ($p > 0.05$). Furthermore, a similar decrease of *Blautia*, *[Bacteroides] pectinophilus* group, *Lachnospiraceae* NK4A136 group, *Fusicatenibacter*, and *Agathobacter* was observed for all groups compared to the CTL_0h group ($p < 0.05$). Finally, similar increases in *Cloacibacillus*, *Romboutsia*, and Family XIII AD3011 group were observed ($p < 0.05$). These results suggest that these changes occurred irrespective of the utilization of mangaba pulp.

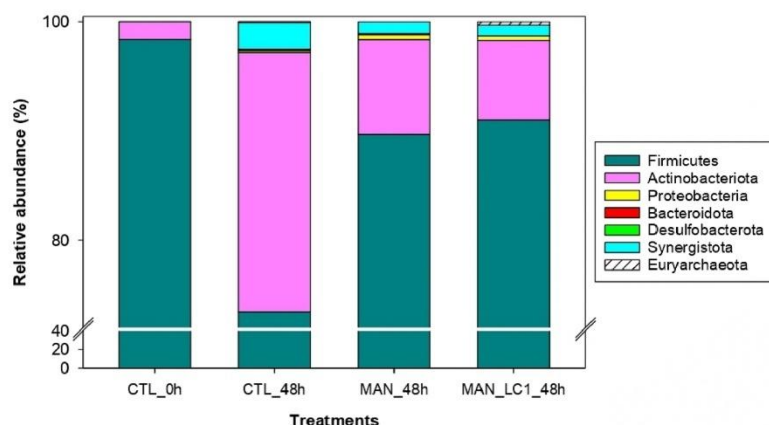


Fig. 2. Relative abundance of bacterial phyla in colonic fermentation experiments with mangaba pulp fermented by LC1.

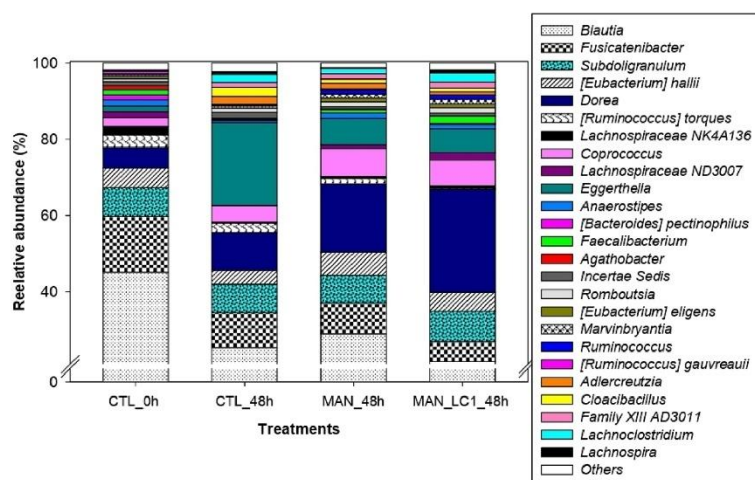


Fig. 3. Relative abundance of bacterial genera in colonic fermentation experiments with mangaba pulp fermented by LC1.

The addition of mangaba pulp without or with LC1 to the feces of healthy vegan adults increased the abundance of *Coprococcus* (7.28 and 6.80 %, respectively), *Marvinbryantia* (1.00 and 1.13 %, respectively), *Ruminococcus* (1.10 and 1.09 %, respectively), and *[Eubacterium] eligens* group (1.02 and 0.97 % respectively) compared to the CTL_48h group ($p < 0.05$) (Fig. 4). *Coprococcus* may produce propionate, known for its impacts on intestinal and general health, to reduce serum cholesterol levels, lipogenesis, and the risk of carcinogenesis. Furthermore, propionate can promote the secretion of important gut hormones like PYY and GLP-1 in human colonic cells (Pingitore et al., 2017; Psichas et al., 2015).

Marvinbryantia has been recently proposed as a probiotic culture and is related to significant levels of isobutyric, isovaleric, and butyric acids and reduction of inflammatory cytokines (Guo et al., 2022). *Ruminococcus* are mucosa-related bacteria; they can improve intestinal barrier integrity and may use polyphenols to improve microbial abundance (Lee

et al., 2018; Cheng et al., 2020). Finally, the *[Eubacterium] eligens* group are lactate-utilizing butyrate producers and can release anti-inflammatory cytokines (Hu et al., 2022). At the same time, adding mangaba pulp to the feces of healthy vegan adults reduced the abundance of *Eggerthella* and *Incertae Sedis* compared to the CTL_48h group ($p < 0.05$). These results demonstrate that mangaba pulp had an important role in microbiota modulation.

When fermented fruit pulp was added to the donors' feces, it increased the abundance of *Dorea* (26.92 %), *Romboutsia* (1.47 %), *Faecalibacterium* (1.90 %), *Lachnospira* (0.77 %), and *Lachnospiraceae* ND3007 (0.10 %) compared to the CTL_48h group ($p < 0.05$). *Dorea* is related to the digestion of carbohydrates and positively affects the host. *Romboutsia* is highly adapted to environments rich in nutrients and has been considered a marker of intestinal stability and negatively correlated with body weight (Wang et al., 2022). It has been reported that the source of 70 % of the energy used by intestinal epithelial cells (IEC) is

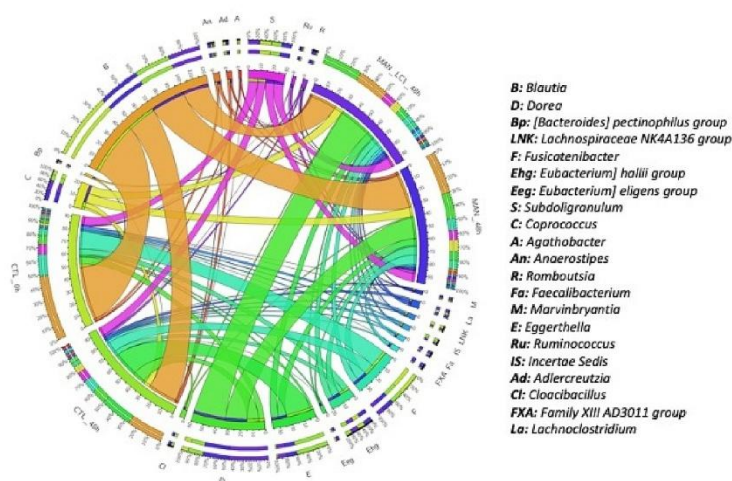


Fig. 4. Circos plot displaying the relative abundance of the main groups inferred by 16S rRNA gene sequencing in each treatment after colonic fermentation experiments.

butyric acid produced by commensal bacteria, especially *Lachnospira* and *Faecalibacterium* (Markowiak-Kopeć & Śliżewska, 2020). *Faecalibacterium prausnitzii* is the only known species among *Faecalibacterium* genera that consists of one of the most abundant and important commensal bacteria in healthy humans. This bacterium produces beneficial short-chain fatty acids through fermentation of dietary fiber and it is currently considered a promising next-generation probiotic to fight gastrointestinal disorders (Markowiak-Kopeć & Śliżewska, 2020). Finally, *Lachnospiraceae* has a butyrogenic effect, utilizing the acetyl-CoA pathway to convert acetate to butyrate (Bester et al., 2023).

At the same time, the fermented fruit pulp resulted in a lower abundance of *Adlercreutzia* and *Lachnospiraceae* compared to the CTL 48h group ($p < 0.05$). *Lachnospiraceae* is a potentially pathogenic bacteria, and consuming polyphenols may reduce its abundance (Pan et al., 2023). Furthermore, *Adlercreutzia* abundance was associated with inflammation and overweight (Niter et al., 2020). These results demonstrate that fermented mangaba pulp can beneficially modulate human microbiota composition.

A growing body of evidence has shown that the gut microbiota significantly impacts our health, protecting against pathogen overgrowth, providing a source of energetic biogenesis, biosynthesizing vitamins, and modulating lipid metabolism (Lynch & Pedersen, 2016). Intestinal microbiotas are gradually considered modulators of the interaction between diet and human health (Makki et al., 2018). Thus, dietary intervention is expected to improve health and prevent disease by modulating the gut microbiota (Han & Xiao, 2020; Chen et al., 2018).

4. Conclusions

This study was the first to evaluate mangaba probiotic fermented pulp during refrigerated storage and its effects on the microbiota of healthy vegan adults during *in vitro* colonic fermentation. Mangaba pulp was a suitable substrate for the growth of LC1, resulting in adequate viability, higher concentration and bioaccessibility of phenolics, adequate volatile composition, and improved sensory aspects. The products kept functional, technological, and sensory properties during refrigerated storage for 28 days at 7 °C. Its colonic fermentation contributed positively to the microbiota modulation, with increased abundances of *Dorea*, *Romboutsia*, *Faecalibacterium*, *Lachnospira*, and *Lachnospiraceae* ND3007 genera. Findings open new possibilities for valorizing an underexploited fruit with health effects.

CRediT authorship contribution statement

Bianca Beatriz Torres de Assis: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Conceptualization. **Tatiana Colombo Pimentel:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Hubert Vidal:** Writing – review & editing, Project administration, Conceptualization. **Marcos dos Santos Lima:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis. **Mércia de Sousa Galvão:** Writing – review & editing, Methodology, Formal analysis. **Marta Suely Madruga:** Writing – review & editing, Funding acquisition, Formal analysis. **Melline Fontes Noronha:** Writing – review & editing, Validation, Software, Formal analysis. **Lucélia Cabral:** Writing – review & editing, Writing – original draft, Software, Formal analysis. **Marciane Magnani:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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.

Acknowledgments

The authors would like to thank the “Coordination for the Improvement of Higher Education Personnel” (CAPES; Financial code 001), CAPES-COFECUB program (88881.711922/2022-01) and “National Council for Scientific and Technological Development” (CNPq; 441287/2020-4; 441280/2020-0; 402745/2021-3, 303384/2022-0 and 404506/2023-2).

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2024.114403>.

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5.4 ARTIGO 2 - Amazonian native palm pulp (Bacaba) fermented with probiotics influenced physical-chemical technological properties and modulated the bacterial colonic community of healthy adults. (Artigo publicado no periódico Food Research International).

Food Research International 209 (2025) 116326



Contents lists available at ScienceDirect

Food Research International

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



Fermentation of Amazonian fruit pulp (bacaba) with distinct probiotics: Impacts on chemical composition, bioaccessibility, and effects on human intestinal microbiota

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ARTICLE INFO

Keywords:

Fermented pulp
Microbiota
Brazilian palm
Oenocarpus bacaba Mart.
Bioaccessibility
Probiotic viability

ABSTRACT

Bacaba (*Oenocarpus bacaba* Mart.) is an underexplored Amazonian fruit rich in polyphenols that can serve as a substrate for probiotic survival and may positively impact on the composition and metabolism of the intestinal microbiota. This study aimed to evaluate the bacaba pulp fermented with probiotics *Lactobacillus acidophilus* 05 (LA-05) and *Lactocaseibacillus casei* 01 (LC1) regarding the chemical composition and probiotics survivability during fermentation (48 h), and the effect on the modulation of the intestinal microbiota of healthy adults through 16S rRNA sequencing. The probiotic-fermented bacaba pulps showed decreased pH and total soluble solids values and sugar content (maltose, glucose, fructose, and rhamnose), and increased titratable acidity values, organic acid content (lactic and tartaric acids), and phenolic compounds concentration compared to the control pulp. Furthermore, it presented adequate probiotic viability after fermentation and simulated gastrointestinal conditions. The bacaba pulp fermented with LC1 showed a higher concentration of butyric acid and phenolic compounds concentration (trans-resveratrol, cis-resveratrol, catechin, procyanidin B₂ and pelargonidin 3-glucoside) and bioaccessibility compared to the control pulp. The bacaba pulp fermented with LA-5 showed a higher concentration of pelargonidin 3-glucoside and procyanidin B₂ compared to the control pulp and the highest bioaccessibility of some phenolic compounds (trans-resveratrol, cis-resveratrol, catechin, epicatechin, procyanidin B₁, procyanidin B₂, myricetin, and isorhamnetin). *In vitro* fecal fermentation reduced the pH and increased the abundance of *Desulfovibrionales*, *Lactobacillales*, and *Peptostreptococcales-Tissierellales* for all treatments. Bacaba pulp with LC1 resulted in the lowest pH values, and increased production of organic acids and concentration of phenolic compounds. Furthermore, both probiotic pulps increased the abundance of *Lactobacillales* and *Acidaminococcales* and decreased the abundance of *Clostridiales*. These findings provide new information about the potential of using bacaba in a functional pulp that may benefit human health through colonic microbiota changes.

1. Introduction

Native Brazilian fruits are gaining popularity in domestic and international markets due to their unique flavors and health benefits from

high bioactive concentrations (Assis et al., 2024). Despite the potential of many lesser-known native fruits, their perishability limits wider distribution (Almeida et al., 2021). Notably, fruits from the Amazonia, like bacaba (*Oenocarpus bacaba* Mart.), are rich in bioactive compounds that

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<https://doi.org/10.1016/j.foodres.2025.116326>

Received 12 February 2025; Received in revised form 25 March 2025; Accepted 30 March 2025

Available online 1 April 2025

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may help prevent chronic diseases and aging, especially their high content of lipids, fibers, antioxidants, and omega-3 and -6 fatty acids, contributing to cardiovascular health and inflammation control (Oliveira et al., 2019; Santos, Biasoto, et al., 2021).

Bacaba fruits are often consumed in beverages considered healthy. Due to the rich composition, native Brazilian fruit such as bacaba can be incorporated with probiotics to enhance their techno-functional properties (Gomes et al., 2021; Santos et al., 2022).

Previous studies have evaluated the fermentation of Brazilian native fruit pulps with probiotic cultures, such as seriguela and mangaba with *Lactocaseibacillus casei* 01 (Assis et al., 2021, 2024), peach pulp with *Lactiplantibacillus plantarum* 21,802, *Lactobacillus brevis* 6239, *Lactiplantibacillus pentosus* 21,798 and 21,832, and *Companilactobacillus alimentarius* 21,852 (Yang et al., 2022), and guava pulp with *Lactococcus lactis* subsp. *lactis* and *L. casei* LA-01 (Kaur & Ghosh, 2023).

The use of the probiotics *Lactobacillus acidophilus* LA-05 and *L. casei* LC1 has demonstrated important results, as during fermentation of fruit pulps (*Spondias purpurea* L. (seriguela), *Hancornia speciosa* (mangaba), *Mangifera indica* L. var. 'Rosa' (pink mango), *Malpighia emarginata* D.C. (acerola), and *Hylocereus undatus* (red pitaya)) probiotics could bio-transform phenolic compounds and release conjugated phenolics, improving bioaccessibility and antioxidant activity with increased production of metabolites (organic acids) (Assis et al., 2021; Morais et al., 2019). Some bioaccessible phenolic compounds have been associated with the modulation of the intestinal microbiota (Macedo et al., 2023) and a positive effect of fruit pulps fermented with *L. casei* LC1 was observed in the microbiota of vegan adults (Assis et al., 2024). Therefore, we hypothesized that bacaba pulp fermented with *L. acidophilus* 05 (LA-05) or *L. casei* 01 (LC1) could have improved chemical composition and phenolics bioaccessibility impacting microbiota composition.

The role of the gut microbiota on human health and disease has attracted widespread attention (Ling et al., 2022; Sarkar et al., 2021). Increasing evidence shows that alterations in the gut microbiota not only cause various gastrointestinal diseases (Pan et al., 2023) but are also associated with other chronic diseases such as metabolic syndrome, Parkinson's disease, and certain types of cancer (Castelli et al., 2021; Li & Chen, 2022; Lim et al., 2017). A growing number of studies indicate that probiotics can influence metabolic pathways, reduce inflammation, and improve intestinal barrier function, potentially leading to weight reduction and improved metabolic health (DiMattia et al., 2024; Guedes et al., 2023; Saadati et al., 2024). Few studies have evaluated fermented fruit pulps to determine the effects of probiotic fruit pulps on intestinal microbiota using *in vitro* colonic models. There are reports of the effects of fruit pulps fermented with probiotics on the microbiota of healthy vegan adults (Assis et al., 2024), middle-aged hypertensive adults (Macedo et al., 2023), and healthy omnivores (Guergoletto et al., 2017; Tang et al., 2023). However, to the authors' knowledge, no prior studies covered the impact of fruit-derived probiotic products on the microbiota of healthy individuals. Furthermore, no studies were reported with bacaba pulp or comparing different probiotic strains.

Therefore, the objectives of this study were i) to evaluate the chemical composition and phenolics bioaccessibility in bacaba pulp fermented with *Lactobacillus acidophilus* 05 (LA-05) and *Lactocaseibacillus casei* 01 (LC1); ii) to determine the probiotic viability in fermented bacaba pulp during *in vitro* digestion; and iii) to determine the effect of bacaba pulps on the composition of the intestinal microbiota of healthy adults through 16S rRNA gene metabarcoding.

2. Material and methods

2.1. Probiotic strains and growth conditions

The probiotic strains were obtained from Christian Hansen, Ind. With. Ltd. (Valinhos, São Paulo, Brazil). The stock cultures were preserved using cryogenic vials at -20°C in Man, Rogosa, and Sharpe (MRS) broth (HiMedia, Mumbai, India) containing glycerol (20 mL/100

mL, Sigma Aldrich, St. Louis, USA). Strain activation was performed using 100 μL of each strain individually grown in 9 mL of MRS broth for 24 h at 37°C under anaerobic conditions in an anaerobic flask (Anaerobic System Anaerogen, Oxoid Ltd., Wade Road, UK) with $\text{O}_2 < 1\%$. Then, 30 μL aliquots of each culture were added to MRS agar, and the plates were incubated at 37°C for 48 h under the same conditions. Subsequently, 3 loops were removed and grown in 9 mL of MRS broth for 24 h at 37°C under the same conditions. The inoculum of each strain was obtained by centrifugation (3500 rpm/15 min, 4°C), two washes with sterile saline solution (0.85 g/100 mL, Ciaviccio, Minas Gerais, Brazil) and resuspension in the same solution to obtain suspensions with standard cells [OD₆₆₀ of 1.0 (LA-05) and 0.1 (LC1), 9 log CFU/mL] (Assis et al., 2021).

2.2. Bacaba fruit and probiotic fermented pulp

Bacaba (*Oenocarpus bacaba* Mart.) fruits were collected at Latitude: -3.10719 and Longitude: -60.02613° 3° $6'$ $26''$ Sul, 60° $1'$ $34''$ in Manaus, Amazonas State. Fruits were selected, sanitized (NaClO 150 ppm; 30 min), immersed in water at 60°C for 15 min, and subjected to mechanical pulping (traditional way of obtaining the pulp). Mechanical pulping was performed in a previously sanitized fruit pulper (stainless steel vertical machine, METVISA® DG.10, Belém, Brazil) in a 2:1 ratio (fruit:water, w/v) and the seeds were discarded resulting in a creamy final product (Cunha et al., 2018).

Bacaba pulp (35 mL) was added with LA-05 or LC1 (final counts approximately 8 log CFU/mL) and incubated at 37°C for 48 h in anaerobic jars using Anaerogen patches, (Oxoid Ltd., United Kingdom). For all the experiments, three formulations were tested: (1) control (no probiotics added), (2) bacaba pulp fermented with probiotic LA-05, and (3) bacaba pulp fermented with probiotic LC1.

The viability of LA-05 and LC1 was verified in bacaba pulps just after inoculation and after 24 and 48 h of fermentation following the proposed by Assis et al. (2024). To determine viable cells for each formulation, 100 μL were serially diluted in a sterile saline solution (10^{-1} – 10^{-6}). Then, 20 μL of each dilution was redistributed onto the surface of the MRS agar, and the plates were incubated for 48 h at 37°C under anaerobic conditions. Cells were enumerated using the microdroplet inoculation technique (Luca & Oroian, 2021). Results for viability were expressed as log CFU/mL.

The physicochemical characteristics of the bacaba pulps (pH, titratable acidity [TA in succinic acid %], and total soluble solids [TSS in °Brix at $25 \pm 1^{\circ}\text{C}$]) followed standard procedures (AOAC, 2016). The analyses were performed before and after pulp fermentation, and pH was also evaluated during *in vitro* colonic fermentation.

2.3. Determination of sugars and organic acids in bacaba pulps

Pulps were centrifuged (3500 $\times$ g/15 min) and passed through a 0.45 μm Millex-HA membrane (Millipore Co., Bedford, MA). The filtrates were analyzed by High-Performance Liquid Chromatography (HPLC) using the 1260 Infinity chromatograph (Agilent Technologies, USA) coupled to a diode array detector (DAD) and a refractive index detector (RID). The column used was the Agilent Hi-Plex H (7.7×300 mm, 8 μm). The analytical conditions were H_2SO_4 (4 M) in ultrapure water as mobile phase, flow rate of 0.7 mL/min, RID temperature of 50°C , column compartment temperature of 70°C , and 10 μL injection volume. The detection was performed at 210 nm in the DAD for the organic acid profile and in RID for the sugar profile (Coelho et al., 2018). Citric, tartaric, malic, succinic, lactic, formic, acetic, propionic, and butyric acids, and maltose, glucose, fructose, and rhamnose (Sigma Aldrich, St. Louis, USA) were evaluated.

Calibration curves of external standards were used in the quantification according to the method validated by Coelho et al. (2018) and the calibration curves had $R^2 \geq 0.998$. Peak identification was performed by comparing the retention times with those of external standards (Sigma

Aldrich, St. Louis, USA). The spectral purity of the peaks was verified using the Threshold tool.

2.4. Determination of phenolic compounds in bacaba pulps and fecal fermentation

To determine the phenolics in bacaba pulp formulations, the extracts were prepared as described for sugars/acids extracts (Section 2.3).

Phenolics were quantified using filtrates as described by Lima et al. (2024). The Zorbax Eclipse Plus RP-C18 column (100 × 4.6 mm, 3.5 μm) and the Zorbax C18 pre-column (12.6 × 4.6 mm, 5 μm) were used for separation at the temperature of 35 °C using acidified water (phase A) and acidified methanol (phase B) at 0.8 mL/min. Data acquisitions were processed using OpenLAB CDS ChemStation Edition™ software (Agilent Technologies).

Standards of malvidin 3-glucoside, cyanidin 3-glucoside, delphinidin 3-glucoside, pelargonidin 3-glucoside, peonidin 3-glucoside, (+)-catechin, (–)-epicatechin, (–) epigallocatechin-gallate, (–)-epicatechin gallate, procyanidin A₂, procyanidin B₁, procyanidin B₂, kaempferol 3-glucoside, quercetin 3-glucoside, rutin, myricetin, and isorhamnetin were from Extrasynthese (Genay, France). Caffeic acid, 3,4 dihydroxybenzoic acid, vanillic acid, gallic acid, 4-hydroxybenzoic acid, vanillin, O-vanillin, (–)-epigallocatechin, *p*-coumaric acid, chlorogenic acid, caftaric acid, quercetin hydrate, syringic acid, ferulic acid, fumaric acid, hesperidin, naringenin, hesperitin, naringin, cyanidin-3,5-diglucoside, malvidin-3,5-diglucoside, and pelargonidin-3,5-diglucoside were from Sigma-Aldrich. *trans*-Resveratrol and *cis*-resveratrol were obtained from Cayman Chemical Company (Michigan, USA).

Calibration curves ($R^2 \geq 0.998$) of external standards were used to quantify phenolics. The peaks of the compounds were identified by comparing their retention times with external standards (Sigma Aldrich, St. Louis, USA). The spectral purity of the peak was checked using the Threshold tool to ensure identification accuracy compared to the external standard.

2.5. Determination of phenolics' bioaccessibility and probiotic survivability to *in vitro* digestion

The *in vitro* digestion following the methodology described by Minekus et al. (2014) was used for analyses of probiotics enumeration and phenolics bioaccessibility. Three consecutive steps were followed. In the oral phase, 5 g of control and probiotic fermented bacaba pulp (separately) were incubated at 37 ± 2 °C for 2 min with shaking (90 rpm) with a mixture of 3.5 mL of simulated salivary fluid (SSF), 0.5 mL of 1500 U/mL α-amylase in SSF solution, 25 μL of 0.3 M CaCl₂ and 975 μL of water. The gastric phase (SGF) was performed using the previous mixture with the addition of 7.5 mL of simulated gastric fluid (SGF), 1.6 mL of 2000 U/mL porcine pepsin in SGF solution, 5 μL of 0.3 M CaCl₂ and 0.695 μL of water, and the pH was adjusted to 3.0 with 1 M HCl under incubation conditions at 37 ± 2 °C with shaking (90 rpm) for 2 h. In the last phase (intestinal phase), the resulting mixture from the gastric phase was added with 11 mL of simulated intestinal fluid (SIF), 5.0 mL of 800 U/mL pancreatin solution in SIF solution, 2.5 mL of fresh bile (160 mM in fresh bile), 40 μL of 0.3 M CaCl₂ and 1.31 mL of water, and the pH adjusted to 7.0 with 1 M NaOH under incubation conditions at 37 ± 2 °C with shaking (90 rpm) for 10 min (Minekus et al., 2014).

Probiotic enumeration and phenolics were determined following the procedures described in sections 2.2 and 2.4. The bioaccessibility for each phenolic compound was determined using Eq. 1.

Bioaccessibility (%) = (phenolic after digestion/ phenolic in the fruit pulp) × 100 (Eq. 1)

2.6. Fecal fermentation assays with bacaba pulps: Fecal inoculum experimental conditions

The participants had at least three months without using probiotics,

prebiotics, or antibiotics, and the absence of chronic diseases related to metabolism, diseases of the digestive system, or use of any regular medication.

Two women and two men (healthy) aged between 25 and 45 were recruited. The number of individuals was based on previous studies and is commonly used for evaluation of probiotic and/or prebiotic foods impact in the microbiota (Assis et al., 2024; Borgonovi, Salgado, et al., 2022; Mafaldo et al., 2024).

The participants were asked to collect a fresh stool sample and place it in a plastic container inside an anaerobic jar for immediate transportation to the laboratory. The donors signed the free and informed consent form approved by the Ethical Committee of the Federal University of Paraíba (65,306,722.0.0000.5188 process).

The fecal inoculum was prepared according to the methodology of Wang et al. (2020) using an equal proportion of each donor's feces (1.25 g fecal sample from each donor: 5 g) diluted in modified saline solution (1:10; NaCl 9.0 g/L, cysteine-HCl 0.5 g/L) to obtain a fecal suspension (10 %) after centrifugation at 4 °C (500 g, 5 min). The composition of the basal nutrient medium (pH 7) used for fecal fermentation was described by Wang et al. (2020). The media was composed by 0.1 g/L NaCl, 0.04 g/L KH₂PO₄, 2 g/L NaHCO₃, 0.01 g/L MgSO₄, 0.04 g/L K₂HPO₄, 0.01 g/L CaCl₂, 0.5 g/L L-cysteine, 2.0 mL/L Tween-80, 2.0 g/L yeast extract, 2.0 g/L peptone, 1.0 mL/L resazurin solution (1 %, w/v), 10 μL/L vitamin K, 0.5 g/L bile salts and 0.02 g/L hemin. Before the experiments, it was autoclaved at 121 °C for 20 min (Wang et al., 2020).

Before the experiments, samples of bacaba pulp were digested using the same procedures described in section 2.5. To run the fecal fermentation, the basal medium was inoculated with the fecal suspension (1:9; v/v; sample CT) and added with non-fermented digested bacaba pulp (sample BT), digested bacaba pulp fermented with LA-05 (sample LAT), and digested bacaba pulp fermented with LC1 (sample LCT). The systems were also incubated at 37 °C for 48 h under anaerobic conditions in a batch system. The fermentation time (48 h) was based on previous studies (Assis et al., 2024; Mafaldo et al., 2024; Wang et al., 2020).

The sugar, organic acids, and phenolic compounds after fecal fermentation were determined according to sections 2.3 and 2.4.

2.7. High-throughput sequencing of the 16S rRNA of intestinal microbiota analysis

The DNA was extracted from control and probiotic bacaba pulp samples using a MoBio Power Food DNA isolation kit, Mobio Laboratories Inc., USA, following the manufacturer's procedures. The 16S rRNA V3/V4 region was amplified using primers 341F (CCTACGGGGRSGCAGCAG) and 785R (CTACCAAGGTATCTAATCC) to identify the bacterial communities by high-throughput sequencing in the DNA samples. The 16S rRNA bacterial libraries were sequenced using the MiSeq Reagent V2 kit with paired reads of 250 pb (2 × 250 pb) in the MiSeq Sequencing System (Illumina Inc., USA). For multiplexing, pooling, and library sequencing reactions (Illumina, USA), the KAPA Library Quantification kit for Illumina was used on a MiSeq platform (Canani et al., 2017).

FASTQC software was used for the initial quality control of the bacterial communities' sequences (16S rRNA V3/V4). After that, the sequences were submitted to Cutadapt 3.7 software (Martin, 2011) to filter and trim low-quality sequences, and remove primers. Subsequently, the DADA2 algorithm (version 1.26) was employed to dereplicate, remove chimeras, and cluster them into amplicon sequence variants (ASVs) (Callahan et al., 2016). The bacterial taxonomic assignment of the ASVs was confronted with sequences within the bacterial database (16S rRNA SILVA 138) (Quast et al., 2012; Yilmaz et al., 2014). Raw data (FASTQ) files have been deposited to the National Center for Biotechnology Information (NCBI), Sequence Read Archive under Bio-Project PRJNA1044155 (Table S1).

2.8. Statistical analysis

Bacaba analyses were performed in triplicate and expressed as mean $\pm$ standard deviation. Physicochemical data, and sugar, organic acid, and phenolic content were analyzed using Analysis of Variance (ANOVA), followed by Turkey's test or Student's *t*-test, Bonferroni e Holm. The latter are multiple comparison tests that identify which of the pairs under analysis are significantly different from each other. The values considered statistically significant when $p < 0.05$ and using Sigma Stat 3.5 software (Jandel Scientific Software, San Jose, California). Microbiota data were submitted to ANOVA, followed by Tukey's test considering the significance of $p < 0.05$ and using XLSTAT 2014 software (Adinsoft®, Paris, France).

3. Results and discussion

3.1. Physicochemical parameters of bacaba pulp

The physicochemical parameters of the bacaba pulp fermented by LC1 or LA-05 during 48 h of fermentation are shown in Table 1. After 48 h of fermentation, the probiotic-fermented bacaba pulps presented lower pH (3.34 and 3.40) and TSS (5.73 and 3.97°Brix) values, and higher TA values (1.93 and 1.20 % succinic acid) ($p < 0.05$) than the control pulp. These results are attributed to the production of organic acids from the consumption of sugars present in the fruit pulp by the probiotic cultures, resulting in higher acidity and lower TSS values (Fonseca et al., 2021). Similar results have already been reported in previous studies (Assis et al., 2021; Assis et al., 2024), demonstrating adequate fermentation process of the fruit pulp by both probiotic strains.

3.2. Survivability of probiotics during fermentation in bacaba pulp and simulated gastrointestinal conditions

Figs. 1 and 2 show the survival of probiotics in the fruit pulp during 48 h of fermentation and *in vitro* digestion, respectively. At the beginning of the fermentation process, LC1 and LA-05 showed similar viable counts (7.58 and 7.56 log CFU/mL, $p > 0.05$, respectively). During fermentation, there was an increase in probiotic counts in the fruit pulp

($p < 0.05$), and both probiotic cultures showed similar viabilities after 48 h (8.64 and 8.30 log CFU/mL, $p > 0.05$, respectively). Therefore, the probiotic cultures could adapt to the environment imposed by the fruit pulp as a substrate, maintaining probiotic viability, regardless of the probiotic strain (Espírito-Santo et al., 2015). One of the leading commercial strains used in fruit and vegetable fermentation is lactic acid bacteria, such as *L. casei* and *L. acidophilus* (Saud et al., 2024; Tang et al., 2022). Fermented bacaba pulp could be considered a probiotic product since, according to Lillo-Pérez et al. (2021), the product's lowest number of probiotic cells to guarantee benefits to the intestinal microbiota is 6 log CFU/mL. Thus, bacaba pulp was a suitable raw material for fermentation with LC1 or LA-5.

During *in vitro* digestion, decreases in probiotic counts were observed for the probiotic-fermented bacaba pulps ($p < 0.05$, Fig. 2). These results suggest some loss of probiotic viability occurred in the *in vitro* digestion, which may be related to the high acidity and presence of bile salts (Assis et al., 2021). However, probiotic counts were maintained above 7 log CFU/mL (LC1: 7.55 and LA-05: 7.67 CFU/mL, respectively), suggesting that the bacaba pulp composition protected the probiotic during *in vitro* digestion, resulting in adequate probiotic survival. Generally, products with a semi-solid structure, such as fruit pulps, present high probiotic survival rates (Mantovani et al., 2020). High survival of *Lactobacillus* and amended genera in fruit-derived products to *in vitro* digestion have already been reported in previous studies (Dunkley & Hekmat, 2020). However, survival may depend on the chemical composition of the fruit pulp (Santos et al., 2023) and no study has reported the utilization of bacaba pulp so far.

3.3. Sugar profile and organic acid profile of bacaba pulp during fermentation

Table 2 presents the sugar and organic acid content of bacaba pulp fermented by LC1 or LA-05. The bacaba pulp was characterized by a higher concentration of rhamnose (13.31 mg/mL), glucose (8.17 mg/mL), and fructose (3.58 mg/mL) as sugars and succinic acid (73.71 g/L), lactic acid (7.18 g/L), and malic acid (4.18 g/L) as organic acids.

After 48 h of fermentation, the probiotic-fermented bacaba pulps showed higher consumption of maltose, glucose, fructose, and rhamnose and increased production of organic acids (lactic and tartaric) than the control pulp ($p < 0.05$). However, fermentation caused decreases in the concentration of other organic acids, such as citric, malic, succinic, formic, acetic, and propionic ($p < 0.05$). The consumption of sugars by probiotic cultures, such as glucose, fructose, and rhamnose, results in the production of organic acids (Li & Chen, 2022; Menezes et al., 2021).

Lactic acid is the primary end product of the metabolism of sugars by most probiotic cultures, which are essentially homofermentative strains (Zhao & Ganzle, 2018). Lactic acid may also be produced using malolactic conversion through decarboxylation of malate and formation of lactate and CO₂, which may be associated with the decrease in malic acid (Ricci et al., 2019). Finally, probiotic cultures may use citric acid and other organic acids as a carbon source to produce lactic acid (Fonseca et al., 2021; Li & Chen, 2022).

In complex media, which feature diverse fermentable sugars and organic acids, the use of compounds by probiotic cultures depends on the substrate and the probiotic strain (Nguyen et al., 2019). Our results demonstrate that, in a general view, both probiotic cultures exhibited similar metabolism during the fermentation of bacaba pulp. However, LC1 formulation presented a higher concentration of lactic and butyric acids and a higher consumption of malic acid ($p < 0.05$), suggesting malolactic conversion in this product. Butyric acid is considered the short-chain fatty acid with the strongest anti-inflammatory effect and is the main energy source for the intestinal epithelial cells (Assis et al., 2024). At the same time, LA-5 formulation showed a lower concentration of rhamnose and glucose, and propionic, butyric, citric, and succinic acids ($p < 0.05$).

L. casei has been described as a probiotic species with improved

Table 1
Physicochemical parameters in bacaba pulp fermented by *L. casei* 01 and *L. acidophilus* 05.

Parameters	Time (h)	Control	LC1	LA 05
pH	0	4.85 $\pm$ 0.14 ^{Ab}	4.64 $\pm$ 0.01 ^{Ab}	4.63 $\pm$ 0.03 ^{Ab}
	24	4.86 $\pm$ 0.02 ^{Ba}	3.43 $\pm$ 0.01 ^{Bc}	3.63 $\pm$ 0.03 ^{Bb}
	48	4.76 $\pm$ 0.04 ^{Ca}	3.34 $\pm$ 0.01 ^{Cb}	3.40 $\pm$ 0.01 ^{Cb}
	0	6.13 $\pm$ 0.12 ^{Ba}	6.05 $\pm$ 0.06 ^{Ab}	6.03 $\pm$ 0.06 ^{Ab}
	24	5.93 $\pm$ 0.06 ^{Bb}	6.00 $\pm$ 0.10 ^{Aa}	4.23 $\pm$ 0.06 ^{Bc}
	48	5.90 $\pm$ 0.10 ^{Ba}	5.73 $\pm$ 0.12 ^{Ba}	3.97 $\pm$ 0.15 ^{Cb}
Total soluble solids (°Brix)	0	0.62 $\pm$ 0.03 ^{Aa}	0.63 $\pm$ 0.06 ^{Ca}	0.57 $\pm$ 0.06 ^{Ca}
	24	0.67 $\pm$ 0.06 ^{Ac}	1.67 $\pm$ 0.06 ^{Ba}	1.03 $\pm$ 0.06 ^{Bb}
	48	0.68 $\pm$ 0.03 ^{Ac}	1.93 $\pm$ 0.15 ^{Aa}	1.20 $\pm$ 0.10 ^{Ab}
Titratable acidity (% succinic acid)	0	0.62 $\pm$ 0.03 ^{Aa}	0.63 $\pm$ 0.06 ^{Ca}	0.57 $\pm$ 0.06 ^{Ca}
	24	0.67 $\pm$ 0.06 ^{Ac}	1.67 $\pm$ 0.06 ^{Ba}	1.03 $\pm$ 0.06 ^{Bb}
	48	0.68 $\pm$ 0.03 ^{Ac}	1.93 $\pm$ 0.15 ^{Aa}	1.20 $\pm$ 0.10 ^{Ab}

Control: non-fermented pulp; LC1: Fermented pulp by *L. casei* 01; LA-05: Fermented pulp by *L. acidophilus* 05. A-C: different capital letters superimposed on the same column denote differences ($p < 0.05$) between the different times for the same treatment, according to the Tukey test or *t*-test; a-c: different lowercase letters substituted on the same line denote differences ($p < 0.05$) between the same time for different treatments, according to Tukey's test or *t*-test.

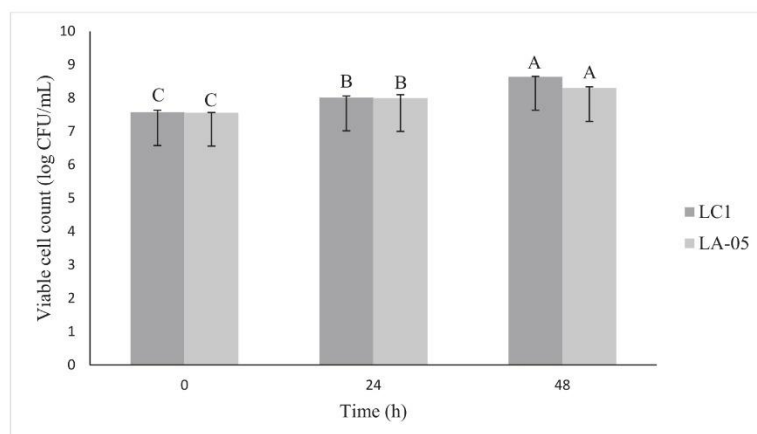


Fig. 1. Probiotic viable cell counts of bacaba pulp fermented by *L. casei* 01 and *L. acidophilus* 05 during 48 h of fermentation.

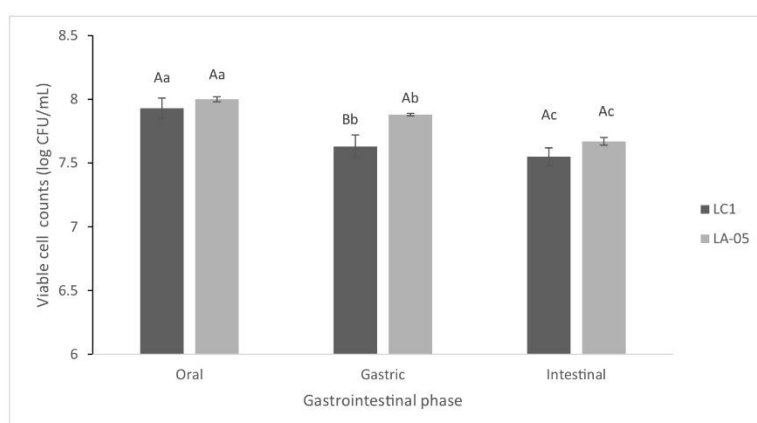


Fig. 2. Probiotic viable cell counts of *L. casei* 01 and *L. acidophilus* 05 in bacaba pulp. Different capital letters denote differences ($p < 0.05$) between the treatments for the same phase, according to the *t*-test. Different lowercase letters denote differences ($p < 0.05$) for the same treatment for different phases according to Tukey's test.

malolactic fermentation capacity than other probiotic cultures, demonstrating a higher consumption of malic acid, resulting in products with higher concentrations of lactic acid (Vargas-Luna et al., 2025). Furthermore, the *Lactocaseibacillus* genus has an improved performance for butyric acid production compared to common starter (*Lactobacillus* and *Streptococcus*) and *Lactiplantibacillus* genus (Luca et al., 2024). Finally, the probiotic cultures have different preferences for carbohydrate utilization, with *L. acidophilus* consuming more glucose and *L. casei* more fructose (Lee et al., 2013), which corroborates the lower glucose content in the product fermented with *L. acidophilus* and the lowest content of fructose in the product fermented with *L. casei*.

3.4. Effect of probiotics on phenolic compounds in bacaba pulp during fermentation

Table 3 presents the results of phenolic compounds in bacaba pulp during 48 h of fermentation. Three distinct groups of phenolics, namely stilbenes, flavonoids, and phenolic acids, were identified in fruit pulp. Procyanidin B₂ (6.85 mg/100 g), epicatechin (3.20 mg/100 g), catechin (1.40 mg/100 g), and myricetin (0.85 mg/100 g) were the phenolics in

higher concentrations in the bacaba pulp (Table 3).

The concentration of catechin, epicatechin, procyanidin B₂, myricetin, p-coumaric acid, and pelargonidin 3-glucoside was increased for all formulations during 48 h of fermentation ($p < 0.05$). Fermentation can lead to the release of bound phenolics and the biotransformation of phenolic compounds by microorganisms (Melini & Melini, 2021; Yang et al., 2023).

Probiotic fermentation changed the concentration of some phenolic compounds after 48 h ($p < 0.05$). Bacaba pulp fermented by LA-05 resulted in higher concentrations of procyanidin B₂ and pelargonidin 3-glucoside ($p < 0.05$). At the same time, bacaba pulp fermented by LC1 resulted in the highest concentrations of cis-resveratrol, trans-resveratrol, catechin, procyanidin B₂, isorhamnetin, and pelargonidin 3-glucoside at the end of fermentation ($p < 0.05$).

Probiotics can act by disintegrating the plant cell wall, synthesizing different phenolic compounds, and modifying simple phenolics (Lizardo et al., 2020; Oliveira et al., 2020). Probiotics may also utilize nutrients from fruit during fermentation and release enzymes, which catalyze the hydrolysis of complex phenolic compounds into smaller molecules that increase phenolic content (Mantzourani et al., 2020; Multari et al., 2020;

Table 2

Content of sugars and organic acids of bacaba pulp fermented by *L. casei* 01 and *L. acidophilus* 05 during 48 h of fermentation.

Organic acids (g/L)	Treatments		
	Control	LC1	LA-05
Citric	3.54 ± 0.02 ^{Da}	1.36 ± 0.00 ^{Cb}	1.11 ± 0.02 ^{De}
Malic	4.18 ± 0.01 ^{Ca}	<LOD	1.51 ± 0.03 ^{Cb}
Succinic	73.71 ± 0.05 ^{Aa}	36.59 ± 0.02 ^{Ab}	31.48 ± 0.00 ^{Ac}
Lactic	7.18 ± 0.04 ^{Bc}	19.37 ± 0.10 ^{Ba}	16.22 ± 0.01 ^{Bb}
Formic	1.56 ± 0.03 ^F	<LOD	<LOD
Acetic	1.10 ± 0.01 ^{Ga}	0.34 ± 0.00 ^{Fb}	0.29 ± 0.02 ^{Fb}
Propionic	2.63 ± 0.10 ^{Ba}	0.73 ± 0.02 ^{Bb}	0.42 ± 0.01 ^{Bc}
Butyric	0.59 ± 0.02 ^{Hb}	1.38 ± 0.02 ^{Ca}	0.33 ± 0.03 ^{Bc}
Tartaric	<LOD	0.19 ± 0.00 ^{Fa}	0.17 ± 0.02 ^{Ga}
Sugar (g/L)			
Maltose	0.65 ± 0.02 ^D	<LOD	<LOD
Glucose	8.17 ± 0.12 ^{Ba}	0.15 ± 0.00 ^{Cb}	0.11 ± 0.01 ^{Cb}
Fructose	3.58 ± 0.05 ^{Ca}	0.21 ± 0.02 ^{Bb}	0.29 ± 0.02 ^{Bb}
Rhamnose	13.31 ± 0.10 ^{Aa}	1.66 ± 0.03 ^{Ab}	1.49 ± 0.00 ^{Bc}

Control: non-fermented pulp; LC1: Fermented pulp by *L. casei* 01; LA-05: Fermented pulp by *L. acidophilus* 05. A-H: different capital letters substituted in the same column denote differences ($p < 0.05$) between the different organic acids or sugars for the same treatment, according to the Tukey test or t-test; a-c: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same organic acid or sugar for different treatment, according to Tukey's test or t-test.

Table 3

Phenolic compounds in bacaba pulp fermented by *L. casei* 01 and *L. acidophilus* 05 during 48 h of fermentation.

Phenolics (ng/100 g)	Treatments	Time (h)	
		0	48
Stilbenes			
Trans-Resveratrol	Control	<LOD	<LOD
	LC1	0.10 ± 0.02 ^b	0.28 ± 0.02 ^A
	LA-05	<LOD	<LOD
Cis-Resveratrol	Control	<LOD	<LOD
	LC1	0.25 ± 0.03 ^b	0.41 ± 0.03 ^A
	LA-05	<LOD	<LOD
Flavonoids			
Catechin	Control	1.40 ± 0.01 ^{Ab}	1.58 ± 0.03 ^{Ba}
	LC1	0.91 ± 0.02 ^{Bb}	1.85 ± 0.02 ^{Aa}
	LA-05	0.89 ± 0.02 ^{Bb}	1.65 ± 0.03 ^{Ba}
Epicatechin	Control	3.20 ± 0.05 ^{Ab}	3.57 ± 0.02 ^{Aa}
	LC1	1.33 ± 0.03 ^{Cb}	1.64 ± 0.00 ^{Ca}
	LA-05	1.62 ± 0.02 ^{Bb}	1.80 ± 0.03 ^{Ba}
Procyanidin B2	Control	6.85 ± 0.10 ^{Bb}	7.12 ± 0.00 ^{Ca}
	LC1	7.02 ± 0.03 ^{Ab}	11.97 ± 0.01 ^{Aa}
	LA-05	6.88 ± 0.02 ^{Bb}	11.54 ± 0.03 ^{Ba}
Pelargonidin 3-Glucoside	Control	<LOD	0.50 ± 0.03 ^C
	LC1	<LOD	0.86 ± 0.01 ^A
	LA-05	<LOD	0.69 ± 0.03 ^B
Quercetin 3-glucoside	Control	0.15 ± 0.01 ^{Ba}	0.14 ± 0.02 ^A
	LC1	0.20 ± 0.02 ^B	<LOD
	LA-05	0.35 ± 0.00 ^A	<LOD
Myricetin	Control	0.85 ± 0.01 ^{Ab}	1.04 ± 0.10 ^{Ba}
	LC1	0.56 ± 0.02 ^{Cb}	1.14 ± 0.05 ^{Aa}
	LA-05	0.65 ± 0.02 ^{Bb}	1.15 ± 0.00 ^{Aa}
Isorhamnetin	Control	<LOD	<LOD
	LC1	<LOD	0.02 ± 0.02
	LA-05	<LOD	<LOD
Phenolic Acids			
p-Coumaric Acid	Control	<LOD	0.17 ± 0.01 ^B
	LC1	<LOD	0.31 ± 0.00 ^A
	LA-05	<LOD	0.12 ± 0.05 ^B

Control: non-fermented pulp; LC1: Fermented pulp by *L. casei* 01; LA-05: Fermented pulp by *L. acidophilus* 05. A-C: different capital letters superscript in the same column for the same compound denote difference ($p < 0.05$) between different treatments for the same phase, according to the Tukey test or t-test; a-b: different lowercase letters superscript in the same line for the same compound denote difference ($p < 0.05$) between different phases for the same treatment, according to the Tukey test or t-test.

Santos, Freitas, et al., 2021). In a narrower view, probiotic cultures have the enzyme β -glucosidase, which can hydrolyze plant β -glucosides, increasing the concentration of phenolic compounds (Braga et al., 2018; Wang et al., 2024). Enzyme activity mainly depends on the carbon source and strain specificity (Cheng et al., 2016). Catechin has stronger antioxidant activity than other flavonoids due to the presence of the catechol moiety (C 3' -OH and C 4' -OH) of the B aromatic ring and its role in eliminating free radicals (Graton et al., 2024). Procyanidin B₂ shows a remarkable inhibitory effect on several types of cancer, including digestive tract cancer, oral squamous cell carcinoma, and breast and prostate cancer (Chen et al., 2023). Therefore, their higher concentration in probiotic-fermented pulps is desired.

It is known that the metabolism of phenolic compounds is specific to each *Lactobacillus* strain (Gaur et al., 2020; Ulbrich et al., 2015). Differences in phenolic content with different probiotic strains may be related to the diversity of hydrolase produced by the strains, leading to the difference in hydrolytic capacity (Zhou et al., 2020). *L. casei* has demonstrated a higher β -glucosidase production compared to other probiotic strains (Jaijoi et al., 2025), which has been attributed to its higher vitality and metabolic efficiency in acid conditions (such as fruit pulps) and strain specificity for enzyme production (Cheng et al., 2016; Wang et al., 2024). Furthermore, the increased lactic acid concentration in bacaba pulp fermented by LC1 (Table 2) may have induced the structural breakdown of the plant cell wall of the fruit pulp, resulting in the release of bound phenolic constituents (Assis et al., 2024). The greater metabolism of LC1 in the fruit pulp (Table 2) may also be associated with the greater biotransformation capacity (Assis et al., 2021), and the increased acidity of LC1 product may increase phenolic compounds content, as phenolic compounds are more stable under acid conditions (Pimentel et al., 2021). On the contrary, *L. acidophilus* demonstrated lower β -glucosidase production and conversion of anthocyanins in mulberry than other strains (*L. plantarum*, *S. thermophilus*, and *B. lactis*) (Cheng et al., 2016).

On the other hand, probiotic-fermented bacaba pulps showed a decrease in the concentration of the quercetin 3-glucoside compound after fermentation ($p < 0.05$). The reduction in some phenolics may be associated with their use by probiotic cultures and the energy metabolism of probiotics, which may also affect the decarboxylation and reduction reactions of phenolics (Li & Chen, 2022). Probiotics may produce glycosidase that acts on the C—O bond at site 3 to remove glycosides. Therefore, probiotics may promote deglycosylation of 3-O-glycosides from quercetin-3-O-glucoside to yield quercetin, which may increase its content in the products or be used as a substrate for new compounds (Gänzle, 2015; Wang et al., 2024). As far as the authors know, this is the first study to evaluate the phenolic profile of bacaba pulp.

3.5. Effect of probiotics on phenolic compounds in bacaba pulp after digestion and bioaccessibility

Table 4 presents the results of phenolic compounds after digestion and bioaccessibility for bacaba pulp fermented by LC1 or LA-05. *In vitro* digestion increased the concentration of catechin, epicatechin, epigallocatechin gallate, procyanidin B₂, and myricetin for all formulations ($p < 0.05$, Table 5). The increase of phenolic compounds in the intestinal fraction may be due to the action of intestinal and digestive enzymes (e. g., pancreatin) that facilitate the release of phenolics bound to the matrix (Dutra et al., 2017; Medeiros et al., 2021). Catechin is a flavonoid with two hydroxyl groups in the B rings, associated with high antioxidant activity (Dutra et al., 2017). In a previous study, Mosele et al. (2015) suggested that the high bioaccessibility of catechin in pomegranate fruit resulted from the partial hydrolysis of proanthocyanidins when exposed to intestinal pH values. Similarly, the increased bioaccessible amounts of some phenolic acids may be related to the chemical rearrangements of some compounds (Dutra et al., 2017).

Procyanidin B1 and isorhamnetin increased their concentrations in

Table 4

Bioaccessibility of phenolic compounds in bacaba pulp fermented by *L. casei* 01 and *L. acidophilus* 05 during 48 h of fermentation.

Phenolics (mg/100 g)	Treatments	Digestion phase		Bioaccessibility (%)
		Before digestion	Intestinal	
Stilbenes				
Trans-Resveratrol	Control	<LOD	<LOD	<LOD
	LA-05	0.25 ± 0.03 ^a	0.19 ± 0.02 ^{ab}	76.00 ^x
		0.15 ± 0.02 ^{ba}	0.05 ± 0.01 ^{bb}	
		0.02 ± 0.01 ^{ba}	0.01 ± 0.01 ^{bb}	
Control	<LOD	<LOD	<LOD	
Cis-Resveratrol	LA 05	0.55 ± 0.01 ^a	0.43 ± 0.02 ^{ab}	78.18 ^x
	LC1	0.41 ± 0.03 ^{ba}	0.29 ± 0.02 ^{bb}	70.73 ^y
		0.03 ^{ba}	0.02 ^{bb}	
Flavonoids				
Catechin	Control	1.58 ± 0.03 ^{ab}	2.00 ± 0.02 ^a	126.58 ^z
	LA 05	1.11 ± 0.02 ^{ab}	2.38 ± 0.00 ^a	214.41 ^x
		1.05 ± 0.03 ^{cb}	1.38 ± 0.02 ^{ba}	
		3.57 ± 0.02 ^{ab}	4.21 ± 0.00 ^a	
Control	3.57 ± 0.02 ^{ab}	4.21 ± 0.00 ^a	117.93 ^z	
Epicatechin	LA 05	1.64 ± 0.00 ^{cb}	2.19 ± 0.01 ^{ba}	133.54 ^x
	LC1	1.80 ± 0.03 ^{bb}	2.25 ± 0.02 ^{ba}	125.00 ^y
		0.03 ^{bb}	0.20 ± 0.03 ^a	
		<LOD	0.17 ± 0.00 ^a	
Epigallocatechin Gallate	LA-05	<LOD	0.16 ± 0.02 ^a	<LOD
Procyanidin B1	Control	<LOD	<LOD	<LOD
	LA 05	2.95 ± 0.04 ^{ab}	3.98 ± 0.03 ^a	134.91 ^x
		2.50 ± 0.02 ^{bb}	3.20 ± 0.02 ^{ba}	
		7.12 ± 0.00 ^{cb}	48.42 ± 0.05 ^{ca}	
Control	7.12 ± 0.00 ^{cb}	48.42 ± 0.05 ^{ca}	680.06 ^z	
Procyanidin B2	LA 05	11.54 ± 0.01 ^{bb}	102.57 ± 0.10 ^a	888.82 ^x
	LC1	11.97 ± 0.03 ^{ab}	100.83 ± 0.04 ^{ba}	842.36 ^y
		0.20 ± 0.05 ^a	<LOD	
		<LOD	<LOD	
Quercetin 3-glucoside	LA-05	0.11 ± 0.01 ^b	<LOD	<LOD
	LC1	0.15 ± 0.02 ^b	<LOD	<LOD
		1.04 ± 0.10 ^{ab}	1.35 ± 0.02 ^{ca}	
		1.14 ± 0.05 ^{ab}	1.72 ± 0.03 ^a	
Myricetin	LA 05	0.05 ^{ab}	0.03 ^a	150.88 ^x
	LC1	1.15 ± 0.00 ^{ab}	1.55 ± 0.02 ^{ba}	134.78 ^y
		<LOD	<LOD	
Isorhamnetin	LA 05	0.45 ± 0.03 ^{ab}	0.90 ± 0.02 ^a	200.00 ^x
	LC1	0.22 ± 0.02 ^{bb}	0.40 ± 0.01 ^{ba}	181.82 ^y
		0.02 ^{bb}	0.01 ^{ba}	
Phenolic Acids				
p-Coumaric Acid	Control	0.17 ± 0.01 ^a	<LOD	<LOD
	LA 05	0.11 ± 0.00 ^a	<LOD	<LOD
		0.12 ± 0.05 ^a	<LOD	
		0.05 ^a	<LOD	
LC1	0.05 ^a	<LOD	<LOD	

Control: non-fermented pulp; LC1: Fermented pulp by *L. casei* 01; LA-05: Fermented pulp by *L. acidophilus* 05. A-C: different capital letters superscript in the same column for the same compound denote difference ($p < 0.05$) between different treatments for the same phase, according to the Tukey test or t-test; a-b: different lowercase letters superscript in the same line for the same compound denote difference ($p < 0.05$) between different phases for the same treatment,

according to the Tukey test or t-test; X-Z: different superscript capital letters in the same column for bioaccessibility values denote difference ($p < 0.05$) between different treatments for the same compound, based on Tukey's test or t-test.

the intestinal fraction only after probiotic fermentation ($p < 0.05$). The increased concentration of lactic acid in probiotic-fermented bacaba pulps (Table 2) may have induced the structural breakdown of the plant cell wall of the fruit pulp, resulting in the release of phenolic constituents bound through enzymatic processes in the *in vitro* digestion (Braga et al., 2018; Guergoletto et al., 2017).

On the other hand, after *in vitro* digestion, the compounds quercetin 3-glucoside, and p-coumaric acid showed a reduction in concentration for all formulations ($p < 0.05$, Table 4). The decrease in the concentration of phenolic compounds can be explained by the exposure of phenolics to adverse conditions in the gastrointestinal tract, mainly due to the effects of alkaline and acidic pH conditions on the stability of these phenolic compounds.

Reductions in trans-resveratrol and cis-resveratrol concentrations were observed when bacaba pulp was fermented by LA-05 or LC1 ($p < 0.05$, Table 4). This decrease in phenolic compounds after fermentation was reported and associated with consuming these compounds through probiotic cultures (Cheng et al., 2016; Oliveira et al., 2021).

The compounds trans-resveratrol, cis-resveratrol, catechin, epicatechin, procyanidin B₁, procyanidin B₂, myricetin, and isorhamnetin showed greater bioaccessibility when bacaba pulp was fermented by LA-05, followed by bacaba pulp fermented by LC1 ($p < 0.05$, Table 4). Catechin is well recognized for its potent antioxidant activity, plays the role of free radical scavenging and protects cells from oxidative damage (Tsega et al., 2025). Furthermore, studies have confirmed that catechin can exhibit many pharmacological effects (Teixeira & Sousa, 2021), including antitumor, anti-inflammatory (Basiricó et al., 2019; Dey et al., 2019), antioxidant (Zhang et al., 2016), antimicrobial activity (Kim, Covington, & Pamer, 2017), cardio-cerebrovascular protection (Morrison et al., 2014), and modulation of neuronal cell apoptosis (Park et al., 2020). The increased bioaccessibility of procyanidin B₂ (PCB₂) may indicate potent antioxidant effects, as it reduces the levels of reactive oxygen species (ROS) in cultured cells, and also has positive effects against ulcerative colitis, cancer, cardiovascular diseases, and Alzheimer's disease (Alsaab et al., 2025). Our results demonstrated that both probiotics improved the bioaccessibility of phenolic compounds, with slightly better results for *L. acidophilus*. Therefore, differences may have occurred between probiotic products during gastrointestinal digestion regarding chemical structure modification, increase or decrease of solubility, or interaction with other compounds, influencing the phenolics' bioaccessibility (Covas-Limon et al., 2022).

In plant foods, phenolic compounds are entrapped in a complex macromolecular matrix. They are commonly released during the different stages of digestion in the gastrointestinal tract so that they can be absorbed in the gut or the small intestine and confer their biological effects. Therefore, higher bioaccessibilities of phenolic compounds are desired to increase intestinal uptake, improve microbial metabolism in the color, and promote antioxidant effects *in vivo* (González-Aguilar et al., 2017). Therefore, fermentation of phenolic compounds increases the bioaccessibility, and the bioaccessible compounds may reach the gut and be used by several members of the microbiota, potentially constituting a (poly)phenol prebiotic pathway leading to the production of SCFA (Rodríguez-Daza et al., 2021). Furthermore, fermented products with more bioaccessible phenolic compounds can provide beneficial effects regardless of the host's microbiota (Assis et al., 2021; Assis et al., 2024). Our results demonstrate that the fermentation of bacaba pulp with probiotics increased the bioaccessibility of the phenolic compounds, which may be related to better health effects, including microbiota modulation.

Table 5

Content of sugars, organic acids and pH in colonic fermentation experiments with bacaba pulp fermented by *L. casei* 01 and *L. acidophilus* 05.

Organic acids (g/L)	Time (hours)							
	0 h				48 h			
	CT	BT	LCT	LAT	CT	BT	LCT	LAT
Citric	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.19 ± 0.05 ^f	<LOD
Succinic	0.10 ± 0.00 ^{Ca}	0.04 ± 0.02 ^{Ca}	<LOD	0.14 ± 0.00 ^{Ba}	0.09 ± 0.00 ^{Da}	0.06 ± 0.03 ^{Ca}	0.05 ± 0.00 ^{Fa}	0.06 ± 0.02 ^{Ca}
Lactic	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.62 ± 0.02 ²	<LOD
Formic	0.07 ± 0.03 ^{Cb}	<LOD	<LOD	0.06 ± 0.02 ^{Db}	<LOD	<LOD	0.59 ± 0.05 ^{2a}	<LOD
Acetic	0.16 ± 0.03 ^{Cd}	0.17 ± 0.00 ^{Bd}	<LOD	0.46 ± 0.10 ^{Bc}	0.90 ± 0.08 ^{Ab}	0.99 ± 0.01 ^{Ab}	1.25 ± 0.10 ^{Ba}	0.95 ± 0.05 ^{Ab}
Propionic	2.67 ± 0.12 ^{Ac}	0.94 ± 0.00 ^{Ad}	0.18 ± 0.01 ^f	3.33 ± 0.02 ^{Ab}	0.46 ± 0.04 ^{Be}	<LOD	7.22 ± 0.03 ^{Aa}	<LOD
Butyric	0.41 ± 0.00 ^{Bb}	<LOD	<LOD	0.29 ± 0.02 ^{Cb}	0.34 ± 0.03 ^{Cb}	0.35 ± 0.00 ^{Bb}	0.68 ± 0.04 ^{Ba}	0.31 ± 0.01 ^{Bb}
Tartaric	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.03 ± 0.02 ^f	<LOD
Malic	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.78 ± 0.10 ^C	<LOD
Sugar (g/L)								
Maltose	0.28 ± 0.03 ^{Aa}	<LOD	<LOD	<LOD	0.05 ± 0.01 ^{Bb}	0.10 ± 0.00 ^{Bb}	0.19 ± 0.03 ^{Bc}	0.10 ± 0.00 ^{Bb}
Glucose	0.36 ± 0.02 ^{Aa}	<LOD	<LOD	<LOD	0.07 ± 0.00 ^{Bb}	0.08 ± 0.02 ^{Bb}	0.15 ± 0.02 ^{Bc}	0.04 ± 0.00 ^{Bb}
Fructose	0.32 ± 0.02 ^{Aa}	<LOD	<LOD	<LOD	0.05 ± 0.00 ^{Bb}	0.08 ± 0.00 ^{Bb}	0.20 ± 0.00 ^{Bc}	0.09 ± 0.00 ^{Bb}
Rhamnose	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.16 ± 0.03 ^B	<LOD
pH	8.73 ± 0.01 ^a	8.46 ± 0.01 ^b	8.40 ± 0.03 ^b	8.43 ± 0.00 ^b	7.39 ± 0.01 ^c	7.22 ± 0.03 ^d	7.08 ± 0.01 ^e	7.17 ± 0.08 ^d

CT: no addition of bacaba pulp, only microbiota in fermentation medium; BT: non-fermented and digested bacaba pulp; LCT: bacaba pulp fermented by LC1 and digested; LAT: bacaba pulp fermented by LA-05 and digested. A-F: different capital letters substituted in the same column denote differences ($p < 0.05$) between different sugars and organic acids for the same treatment, according to Tukey's test or t-test; a-f: different lowercase letters substituted in the same row denote differences ($p < 0.05$) between the same sugars, organic acids and pH for different treatments, according to Tukey's test or t-test.

3.6. Content of sugars, organic acids, and pH in bacaba pulp during fecal fermentation

The results of sugar and organic acid contents and pH values during fecal fermentation are presented in Table 5. After 48 h of fermentation, a decrease in pH was observed for all treatments, emphasizing the treatment added with pulp fermented with LC1, which obtained the lowest value (LCT48h: 7.08, $p < 0.05$). The decrease in the pH value is due to the production of organic acids resulting from the consumption of sugars present in the fruit pulp, mainly glucose and fructose, by probiotic cultures and/or other microorganisms present in the fecal samples, such as *Enterococcus*, *Lactobacillus*, and *Bifidobacterium* spp. (Assis et al., 2024; Medeiros et al., 2021). Furthermore, low colonic pH has been used as a marker of beneficial effects and may influence the production of intestinal metabolites (such as propionic and butyric acids) that can bind to GPR43 proteins that play a role in regulating the human body's energy homeostasis and intestinal immunity (Markowiak-Kopeć & Śliżewska, 2020; Zamora-Gasga et al., 2018).

The treatment added with pulp fermented with LC1 (LCT48h) showed an increase in the production of acids (citric, lactic, formic, acetic, propionic, and butyric acid) and in the concentration of sugars (maltose, glucose, rhamnose, and fructose) when compared to all other treatments ($p < 0.05$). The presence of sugars in cultivation media could indicate a possible ability of the LC1 to degrade polysaccharides in bacaba pulp to monosaccharides. These monosaccharides should be converted into intermediate metabolites and, ultimately, to various organic acids, some of which may benefit the host (Menezes et al., 2021).

Lactic acid is the primary end product of the metabolism of *Lactobacillus* species (homofermentative) (Zhao & Ganzle, 2018), in addition to being one of the leading end products of the metabolism of *Bifidobacterium* species (heterofermentative) (Kaprashov et al., 2017). Acetic acid is produced in the gut during the fermentation of carbohydrates that escape degradation in the upper part of the intestinal tract by lactic acid bacteria and *Bifidobacterium* (Patrignani et al., 2018). It can be used as an energy source for the intestinal microbiota and peripheral tissues and inhibit pathogens (Medeiros et al., 2021; Zhou et al., 2018).

The diverse composition of the gut microbiota is associated with varied metabolic profiles. Gut microbiota-derived metabolites, especially SCFAs, are crucial for gastrointestinal health (Nabavi-Rad et al., 2022). Butyric acid is one of the most studied short-chain fatty acids and is known for its extra- and intrainestinal effects, mainly linked to its

anti-inflammatory and anticancer activities (Aiello et al., 2023). Importantly, butyric acid plays a crucial role in maintaining colonic tissue vitality and has been shown to alleviate the severity of ulcerative colitis, which is a chronic idiopathic inflammatory condition of the colon (Yan et al., 2024). Butyric acid is mainly produced by the fermentation of aldehydes (e.g., glucose, galactose, mannose, and xylose), and it is positively correlated with *Ruminococcaceae*, *Faecalibacterium*, *Roseburia*, and *Coprococcus* (Dietallevi et al., 2021). It stands out as the primary source of energy for intestinal epithelial cells, capable of modulating gene expression with a beneficial immunoregulatory effect on intestinal epithelial cells and other mucosal cell populations (Markowiak-Kopeć & Śliżewska, 2020). Bacteroidetes can produce this acid, regulating the immunity of the intestinal mucosa and acting as a functional marker of healthy anaerobic intestinal microbiota, synthesized from acetic acid or through the condensation of two acetyl CoA molecules (Medrano et al., 2020; Million et al., 2018). Furthermore, *L. casei* can ferment carbohydrates and produce organic acids such as lactic and acetic acid, which serve as substrates for other bacteria that convert these acids into butyric acid (Medrano et al., 2020). Increased butyrate production by probiotic fermentation may be related to the reduction of some bacteria of the order *Clostridiales*, especially pathogenic species such as *Clostridium difficile* (Aiello et al., 2023).

Propionic acid is primarily produced by the fermentation of ketones (e.g., fructose, arabinose, and tagatose) (Rubio-Castillo et al., 2022), could regulate homeostasis and the function of the intestinal barrier (Markowiak-Kopeć & Śliżewska, 2020), and is positively correlated with *Bacteroides* (Dietallevi et al., 2021).

Finally, the detection of formic, citric, and malic acids could be associated with the capability of the cultivated bacterial species/strains to produce these organic acids from plant-derived carbohydrate fermentation (Dessie et al., 2018), as reported in previous investigations with fermentation of fruit or fruit processing by-products (Assis et al., 2024; Kumar et al., 2003).

3.7. Profile of phenolic compounds in bacaba pulp during fecal fermentation

The results of variations in the content of phenolic compounds in bacaba pulp during 48 h of fecal fermentation are presented in Table 6. Two distinct groups of phenolics, namely flavonoids and phenolic acids, were identified in treatments CT, BT, LCT, and LAT before and after 48 h of colonic fermentation. The compounds catechin, epigallocatechin

Table 6

Phenolic compounds in colonic fermentation experiments with bacaba pulp fermented by *L. casei* 01 and *L. acidophilus* 05.

Phenolics (mg/100 g)	Treatments	Time (h)	
		0	48
Flavonoids			
Catechin	CT	0.72 ± 0.02 ^{Ab}	0.90 ± 0.00 ^{Ba}
	BT	0.46 ± 0.00 ^{Bb}	0.79 ± 0.03 ^{Ba}
	LCT	0.65 ± 0.02 ^{Ab}	1.00 ± 0.10 ^{Aa}
	LAT	0.65 ± 0.01 ^{Ab}	0.90 ± 0.00 ^{Ba}
	CT	<LOD	1.30 ± 0.05 ^B
Epigallocatechin Gallate	BT	<LOD	0.30 ± 0.02 ^D
	LCT	<LOD	1.55 ± 0.02 ^A
	LAT	<LOD	0.51 ± 0.00 ^C
Procyanidin B1	CT	0.16 ± 0.03 ^{Ab}	0.30 ± 0.03 ^{Da}
	BT	0.11 ± 0.02 ^{Ab}	0.78 ± 0.03 ^{Ba}
	LCT	0.15 ± 0.02 ^{Ab}	0.95 ± 0.00 ^{Aa}
	LAT	0.18 ± 0.03 ^{Ab}	0.72 ± 0.01 ^{Ba}
	CT	13.87 ± 0.01 ^{Ca}	0.60 ± 0.00 ^B
Procyanidin B2	BT	11.91 ± 0.05 ^D	<LOD
	LCT	18.95 ± 0.10 ^A	<LOD
	LAT	18.61 ± 0.03 ^B	<LOD
	CT	0.28 ± 0.02 ^A	<LOD
Malvidin 3 Glucoside	BT	0.18 ± 0.02 ^B	<LOD
	LCT	0.29 ± 0.03 ^A	<LOD
	LAT	0.29 ± 0.02 ^A	<LOD
	CT	0.58 ± 0.05 ^{Ab}	1.42 ± 0.00 ^{Ca}
Petunidin 3-Glucoside	BT	0.21 ± 0.12 ^{Cb}	1.38 ± 0.02 ^{Ca}
	LCT	0.40 ± 0.03 ^{Bb}	3.14 ± 0.01 ^{Aa}
	LAT	0.40 ± 0.02 ^{Bb}	1.60 ± 0.02 ^{Ba}
Phenolic Acids			
Syringic Acid	CT	<LOD	0.07 ± 0.02 ^B
	BT	<LOD	0.15 ± 0.03 ^B
	LCT	<LOD	0.30 ± 0.02 ^A
	LAT	<LOD	0.09 ± 0.03 ^B

CT: no addition of bacaba pulp, only microbiota in fermentation medium; BT: non-fermented and digested bacaba pulp; LCT: bacaba pulp fermented by LC1 and digested; LAT: bacaba pulp fermented by LA-05 and digested. A-D: different capital letters superscript in the same column for the same compound denote difference ($p < 0.05$) between different treatments for the same time, according to the Tukey test or t-test; a-b: different lowercase letters superscript in the same line for the same compound denote difference ($p < 0.05$) between different times for the same treatment, according to the Tukey test or t-test.

gallate, procyanidin B₁, petunidin 3-glucoside, and syringic acid increased the concentration after 48 h of fecal fermentation for all treatments evaluated ($p < 0.05$). The increase in these compounds brings benefits, as polyphenols protect epithelial cells and prevent inflammation in the intestines, resulting in better intestinal barrier function (Iqbal et al., 2020). This result suggests a typical modification derived from colonic fermentation. According to previous studies, syringic acid is one of the main degradation products of malvidin-3-glucoside (Vázquez-Vázquez et al., 2024).

The addition of bacaba pulp fermented by LA-5 (LAT) after 48 h of fermentation resulted in higher concentrations of procyanidin B₁ and petunidin 3-glucoside, while the pulp with LC1 (LCT) after 48 h of fermentation resulted in higher concentrations for the compounds catechin, epigallocatechin gallate, procyanidin B₁, petunidin 3-glucoside and syringic acid when compared to the control treatment at the same time ($p < 0.05$). Probiotic microorganisms and colonic microbiota can disintegrate the plant cell wall, synthesize or release different phenolic compounds, convert simple phenolics, or depolymerize high molecular weight phenolics (Oliveira et al., 2020). Furthermore, probiotics can transform phenolic compounds through the action of different glycosyl hydrolases, with the release of aglycone from phenolic compounds conjugated with glycol (Pereira-Caro et al., 2015).

Procyanidin B₂ and malvidin 3-glucoside decreased concentration after 48 h of colonic fermentation for all treatments evaluated ($p < 0.05$). The intestinal microbiota's use of phenolic compounds as substrates can occur through enzymatic reactions (deglycosylation,

sulfation, dihydroxylation, and hydrogenation) (Assis et al., 2024). However, not all phenolic compounds found in treatments have been used by the intestinal microbiota and remained stable during colonic fermentation. Furthermore, studies indicate that a long fermentation period negatively influences some bioactive substances' concentration (Li et al., 2024). Overall, flavonoids can regulate and balance the disordered gut microbiota. However, little is known about the impacts of most flavonoids on gut microbiota (Pan et al., 2023). Our findings attest that phenolic compounds during colonic fermentation may vary depending on the substrate used (control, bacaba pulp, or fermented bacaba pulp).

3.8. Effect of bacaba pulp on the modulation of intestinal microbiota

The effect of fermented bacaba pulp by LA-05 and LC1 on the intestinal microbiota of healthy adults was evaluated using 16S rRNA high-throughput sequencing analysis. The richness and α diversity of the intestinal microbiota, assessed by the observed indices, Chao1, se. Chao1, ACE, se. ACE, Shannon, Simpson, and InvSimpson for all treatments are shown in Table 7.

The observed, Chao1, ACE, and Simpson indices, showed no significant difference between treatments ($p > 0.05$). Feces from healthy adults analyzed at the beginning of colonic fermentation showed higher values for the se. Chao1, Shannon, and InvSimpson indices (CT0, 6.55, 6.10, and 337.28, respectively) ($p < 0.05$). At the end of fermentation, lower values for Shannon, and InvSimpson indices were observed in the media with bacaba pulp (BT48, LAT48, LCT48, $p < 0.05$). Shannon and Simpson diversity indices have been traditionally used to measure community diversity (Lemos et al., 2011), suggesting that fermentation time (48 h) and the bacaba pulp addition reduced the community diversity. The se. ACE index presented a higher value when adding bacaba pulp with LA-05 (LAT48, 12.96). The ACE index has been used as an abundance-based coverage estimator of species richness (Zhao & Eun, 2020). Therefore, LA-5 promoted an increase in the species richness.

The taxonomic assignment obtained by sequencing analysis of 16S rRNA genes showed that the ASVs belonged to two main bacterial phyla in bacaba pulps CT0, namely, *Firmicutes* (60.89 %) and *Bacteroidota*

Table 7

Alpha diversity indices obtained from 16S rRNA gene amplicon sequencing in colonic fermentation experiments.

	CT0	CT48	BT48	LAT48	LCT48
Observed	664.00 ± 62.00 ^A	684.00 ± 2.00 ^A	631.50 ± 24.50 ^A	716.50 ± 23.50 ^A	656.50 ± 67.50 ^A
	676.05 ± 67.05 ^A	687.70 ± 2.55 ^A	637.73 ± 21.95 ^A	721.21 ± 26.49 ^A	661.88 ± 68.50 ^A
	6.55 ± 1.49 ^A	2.80 ± 0.25 ^B	3.77 ± 1.04 ^B	3.15 ± 1.42 ^B	3.74 ± 0.45 ^B
se. Chao1	673.02 ± 67.45 ^A	687.61 ± 2.50 ^A	637.27 ± 23.35 ^A	720.93 ± 25.22 ^A	660.77 ± 68.55 ^A
	11.06 ± 11.06 ^A	12.50 ± 12.37 ^A	12.37 ± 12.96 ^A	12.96 ± 12.96 ^A	12.09 ± 12.09 ^A
	0.39 ^D	0.01 ^B	0.20 ^{BC}	0.13 ^A	0.00 ^C
se. ACE	6.10 ± 0.10 ^A	5.91 ± 0.00 ^B	5.73 ± 0.01 ^C	5.83 ± 0.06 ^{BC}	5.82 ± 0.05 ^{BC}
	1.00 ± 1.00 ^A	1.00 ± 1.00 ^A	1.00 ± 1.00 ^A	0.997 ± 0.997 ^A	1.00 ± 1.00 ^A
	0.00 ^A	0.00 ^A	0.00 ^A	0.00 ^A	0.00 ^A
Simpson	337.28 ± 40.05 ^A	260.72 ± 2.06 ^B	200.74 ± 0.10 ^C	213.76 ± 16.99 ^{BC}	219.71 ± 2.20 ^{BC}
InvSimpson					

CT0: no addition of bacaba pulp, only the microbiota in the fermentation medium, at time 0 h; CT48: no addition of bacaba pulp, only the microbiota in the fermentation medium, after 48 h of fermentation; BT48: addition of non-fermented and digested bacaba pulp, after 48 h of fermentation; LAT38: addition of bacaba pulp fermented by LA-05 and digested after 48 h of fermentation; LCT48: addition of bacaba pulp fermented by LC1 and digested after 48 h of fermentation; Values are expressed as mean ± standard deviation (mg/100 g); A-D: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same alpha diversity for different treatments, according to Tukey's test or t-test.

(28.10 %) (Fig. 3). Then, *Actinobacteriota* (6.28 %), and *Proteobacteria* (3.19 %) were found. *Firmicutes* is one of the predominant phyla of the gut microbiota and has been reported to have the ability to increase dietary energy harvest, which includes butyrate-producing genera such as *Faecalibacterium*, *Roseburia*, *Eubacterium*, and *Clostridium* (Zeng et al., 2019). Studies show that 60–80 % of the total fecal microbiome belongs to the *Firmicutes* and *Bacteroidetes* phyla, indicating that these phyla dominate the gut microbiota in healthy individuals (Retnakumar et al., 2022). Among the four main phyla (*Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*), the *Proteobacteria* is the most unstable. The bloom of *Proteobacteria* is a microbial signature of dysbiosis in the gut microbiota (Kumar & Kumar, 2022). Therefore, our results are similar to others concerning the microbiota of healthy adults.

At the phylum level (Fig. 3), after colonic fermentation, adding bacaba pulp without or with LA-05 or LC1 (BAT48, LAT48, and LCT48) to the feces of healthy adults resulted in an increased abundance of *Proteobacteria* and *Bacteroidota* ($p < 0.05$), and decreased abundance of *Firmicutes*, *Desulfobacterota*, and *Actinobacteriota* ($p < 0.05$) (CT48). No differences were observed for *Verrucomicrobiota*, *Synergistota*, and *Euryarchaeota* ($p > 0.05$).

At the class level (Table 8), after colonic fermentation, adding bacaba pulp without or with LA-05 or LC1 (BAT48, LAT48, and LCT48) to the feces of healthy adults resulted in an increased abundance of *Gammaproteobacteria*, *Bacteroidia*, *Bacilli*, and *Negativicutes* ($p < 0.05$, CT48), and decreased abundance of *Clostridia*, *Desulfobacteriota*, *Coriobacteriia*, *Actinobacteria*, and *Verrucomicrobiae* ($p < 0.05$, CT48). The LAT48 showed a higher abundance of *Bacilli* ($p < 0.05$). No differences were observed for *Synergistia* ($p > 0.05$). Notably, the increase in the abundance of *Gammaproteobacteria* is favorable (Jeong et al., 2019; Kim, Le, et al., 2017; Litvak et al., 2017; Rizzatti et al., 2017). *Bacilli* has been shown to improve digestive health by minimizing the human inflammatory response and increasing intestinal barrier function (Cao et al., 2020; Rhyat et al., 2019). It is also involved in altering the microbiota in the human gastrointestinal tract and providing health-promoting

Table 8

Relative abundance of bacterial class in colonic fermentation experiments with bacaba pulp fermented by *L. casei* 01 and *L. acidophilus* 05.

Class	CT0	CT48	BT48	LAT48	LCT48
<i>Gammaproteobacteria</i>	3.19 ± 0.11 ^C	46.52 ± 0.69 ^B	59.10 ± 0.34 ^A	57.40 ± 3.60 ^A	55.61 ± 0.92 ^A
<i>Clostridia</i>	55.62 ± 0.21 ^A	28.89 ± 0.10 ^B	13.37 ± 0.90 ^D	15.03 ± 2.32 ^{CD}	17.25 ± 0.53 ^C
<i>Bacteroidia</i>	28.40 ± 0.73 ^A	8.33 ± 0.41 ^C	10.65 ± 0.39 ^B	11.54 ± 0.15 ^B	11.41 ± 0.51 ^B
<i>Bacilli</i>	3.62 ± 0.31 ^D	3.61 ± 0.18 ^D	4.73 ± 0.28 ^C	6.97 ± 0.53 ^A	6.06 ± 0.23 ^B
<i>Desulfobacteriota</i>	0.64 ± 0.02 ^C	7.96 ± 0.14 ^A	4.52 ± 0.12 ^B	4.98 ± 0.92 ^B	4.79 ± 0.12 ^B
<i>Negativicutes</i>	1.66 ± 0.16 ^{BC}	1.23 ± 0.18 ^C	3.39 ± 0.39 ^A	2.77 ± 0.74 ^A	3.63 ± 0.37 ^A
<i>Coriobacteriia</i>	1.16 ± 0.13 ^A	0.66 ± 0.05 ^B	0.57 ± 0.11 ^B	0.60 ± 0.30 ^B	0.43 ± 0.14 ^B
<i>Actinobacteria</i>	5.62 ± 0.20 ^A	0.77 ± 0.03 ^B	0.40 ± 0.07 ^C	0.41 ± 0.10 ^C	0.38 ± 0.05 ^C
<i>Verrucomicrobiae</i>	0.11 ± 0.01 ^C	0.37 ± 0.15 ^A	0.22 ± 0.03 ^B	0.18 ± 0.09 ^B	0.28 ± 0.01 ^B
<i>Synergistia</i>	1.11 ± 0.12 ^A	0.00 ± 0.00 ^B	0.06 ± 0.02 ^B	0.15 ± 0.02 ^B	0.12 ± 0.12 ^B
<i>Other</i>	0.50 ± 0.02 ^A	0.07 ± 0.02 ^B	0.03 ± 0.03 ^B	0.00 ± 0.00 ^B	0.07 ± 0.07 ^B

CT0: no addition of bacaba pulp, only microbiota in the fermentation medium, at time 0 h; CT48: no addition of bacaba pulp, only the microbiota in the fermentation medium, after 48 h of fermentation; BT48: addition of non-fermented and digested bacaba pulp, after 48 h of fermentation; LAT48: addition of bacaba pulp fermented by LA-05 and digested after 48 h of fermentation; LCT48: addition of bacaba pulp fermented by LC1 and digested after 48 h of fermentation; Values are expressed as mean ± standard deviation (mg/100 g); A-D: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same class for different treatments, according to Tukey's test or t-test.

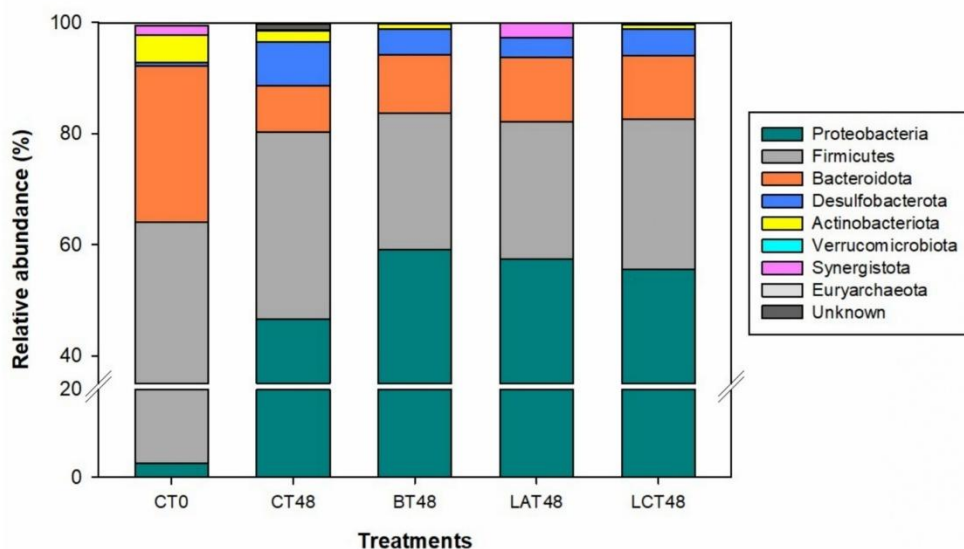


Fig. 3. Relative abundance of bacterial phyla *in vitro* colonic fermentation experiments for each treatment with or without addition of bacaba pulp fermented by *L. casei* 01 and *L. acidophilus* 05.

factors to humans or other hosts, such as GABA and vitamins that stimulate the host's immune system to act against pathogens (Starosila et al., 2017). Specifically, it has been demonstrated that bacteriocins secreted by *Bacilli* play a critical role in suppressing cancer and that they repress and control influenza viral infectivity, which can be achieved by coordinated defense by the altered microbiota in the gut and also by the secretion of several antiviral substances (Kaur & Kaur, 2015; Starosila et al., 2017).

At the order level (Fig. 4), after colonic fermentation, adding bacaba pulp without or with LA-05 or LC1 (BAT48, LAT48, and LCT48) to the feces of healthy adults resulted in an increased abundance of *Enterobacterales*, *Bacteroidales*, *Lactobacillales*, *Desulfovibrionales*, and *Acidaminococcales* ($p < 0.05$, CT48), and decreased abundance of *Peptostreptococcales-Tissierellales*, *Clostridiales*, and *Bifidobacteriales* ($p < 0.05$, CT48). The LAT48 showed a higher abundance of *Lactobacillales* ($p < 0.05$). No differences were observed for *Lachnospirales* and *Oscillospirales* ($p > 0.05$). *Acidaminococcales*, an important order of glutamate-fermenting microorganisms, produces ammonia as the primary end product through glutamate fermentation. Changes in the microbiomes of this order may lead to changes in metabolites related to gamma-glutamyl. Circulating levels of gamma-glutamyl-related bioactive metabolites produced by the gut microbiome permeate the blood-brain barrier, which may play essential roles in the pathogenesis of brain-related diseases (Zhang et al., 2017). An increase in the abundance of *Lactobacillales* has beneficial effects that have been investigated, including reducing the risk of infection, providing resistance to colonization, improving intestinal barrier function, possibly in conjunction with lowering inflammation, modifying bile acid metabolism, and perhaps the cholesterol metabolism. *Lactobacillales* may also act to modulate levels of neurotransmitters involved in mood and behavior, modulate the risk of kidney disease through oxalate degradation, and, most importantly, act as a genetically tractable host for biotherapeutic administration (Walter & O'Toole, 2023).

In addition, information in the literature varies regarding the benefits of *Lactobacillaceae*, such as improving type 2 *Diabetes mellitus*, anti-inflammatory effects and reducing body weight (Wei et al., 2020), producing short-chain fatty acids, which may be useful for energy

metabolism, maintaining intestinal integrity, preventing endotoxemia and intestinal gluconeogenesis (Zhang et al., 2017). A decrease in the abundance of *Clostridiales* is considered a beneficial outcome, as it was addressed by Li et al. (2024) when they identified several genera within the order *Clostridiales* predominantly responsible for amino acid metabolism dysfunction, especially for the metabolism of branched-chain amino acids (BCAAs), which have been shown to affect brain function, mood, and cognition. Therefore, the authors obtained sufficient evidence to conclude that amino acid fermenting *Clostridiales* taxa and their end products SCFAs were important contributors to different psychiatric diseases.

At the family level (Fig. 5), after colonic fermentation, adding bacaba pulp without or with LA-05 or LC1 (BAT48, LAT48, and LCT48) to the feces of healthy adults resulted in an increased abundance of *Enterobacteriaceae*, *Bacteroidaceae*, *Enterococcaceae*, and *Acidaminococcaceae* ($p < 0.05$, CT48), and decreased abundance of *Desulfovibrionaceae*, *Peptostreptococcaceae*, and *Bifidobacteriaceae* ($p < 0.05$, CT48). No differences were observed for *Tannerellaceae*, *Lachnospiraceae*, and *Ruminococcaceae* ($p > 0.05$). Several facultatively anaerobic bacteria, such as those from the families *Enterobacteriaceae* and *Enterococcaceae*, are ubiquitous members of the healthy human gut microbiota but generally at levels considerably (at least 100-fold) lower than those of strictly anaerobic gut commensals (Schaik, 2015).

At the genus level (Table 9), after colonic fermentation, adding bacaba pulp without or with LA-05 or LC1 (BAT48, LAT48, and LCT48) to the feces of healthy adults resulted in an increased abundance of *Acidaminococcus*, *Bacteroides*, *Enterococcus*, and *Escherichia-Shigella* ($p < 0.05$, CT48), and decreased abundance of *Bifidobacterium*, *Bilophila*, *Lachnoclostridium*, *Parabacteroides*, and *Paraclostridium* ($p < 0.05$, CT48). No differences were observed for *Blautia* ($p > 0.05$).

Interactions between dietary components and gut populations need to be considered to understand better the beneficial effects of fruit bioactive compounds on overall host well-being and to plan effective nutritional interventions. However, the mechanisms of action through which bioactive compounds alter intestinal composition have only begun to be explained (Borgonovi, Salgaço, et al., 2022), and some of them remain unclear. Therefore, new studies aiming to investigate these

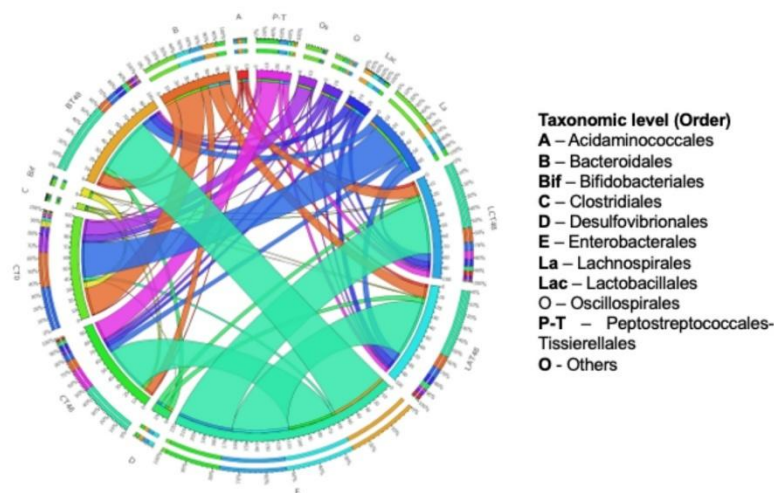


Fig. 4. Circus plot showing the relative abundance of the order inferred by 16S rRNA gene sequencing in each treatment with or without addition of bacaba pulp fermented by *L. casei* 01 and *L. acidophilus* 05 after *in vitro* colonic fermentation experiments. CT0: without addition of bacaba pulp, only microbiota in the fermentation medium, at time 0 h; CT48: without addition of bacaba pulp, only microbiota in the fermentation medium, after 48 h of fermentation; BAT48: addition of unfermented and digested bacaba pulp, after 48 h of fermentation; LAT48: addition of bacaba pulp fermented by LA-05 and digested after 48 h of fermentation; LCT48: addition of bacaba pulp fermented by LC1 and digested after 48 h of fermentation.

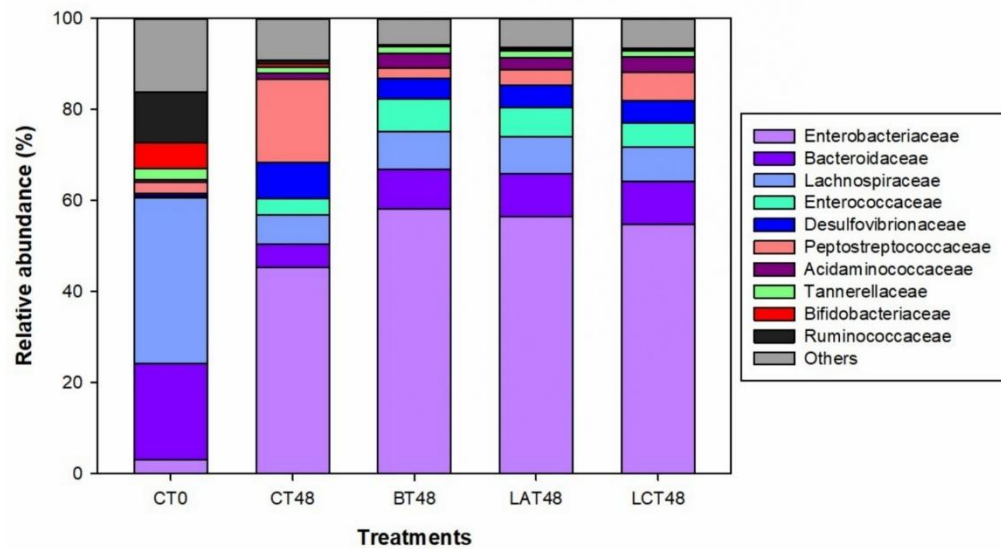


Fig. 5. Relative abundance of bacterial family *in vitro* colonic fermentation experiments for each treatment with or without addition of bacaba pulp fermented by *L. casei* 01 and *L. acidophilus* 05.

Table 9

Relative abundance of bacterial genus in colonic fermentation experiments with bacaba pulp fermented by *L. casei* 01 and *L. acidophilus* 05.

Genus	CT0	CT48	BT48	LAT48	LCT48
<i>Acidaminococcus</i>	0.00 ± 0.00 ^B	0.71 ± 0.14 ^B	2.86 ± 0.52 ^A	2.03 ± 0.79 ^A	2.93 ± 0.48 ^A
<i>Bacteroides</i>	21.19 ± 1.45 ^A	4.94 ± 0.08 ^C	8.63 ± 0.16 ^B	9.37 ± 0.19 ^B	9.37 ± 0.33 ^B
<i>Bifidobacterium</i>	5.62 ± 0.20 ^A	0.77 ± 0.03 ^B	0.40 ± 0.07 ^C	0.41 ± 0.10 ^C	0.38 ± 0.05 ^C
<i>Bilophila</i>	0.32 ± 0.02 ^C	6.56 ± 0.39 ^A	3.45 ± 0.06 ^B	3.48 ± 0.69 ^B	3.38 ± 0.10 ^B
<i>Blautia</i>	17.83 ± 1.13 ^A	0.41 ± 0.19 ^B	1.08 ± 0.06 ^B	0.71 ± 0.01 ^B	0.65 ± 0.05 ^B
<i>Enterococcus</i>	0.25 ± 0.10 ^E	3.53 ± 0.10 ^D	7.19 ± 0.51 ^A	6.33 ± 0.36 ^B	5.38 ± 0.20 ^C
<i>Escherichia-Shigella</i>	2.88 ± 0.09 ^C	44.59 ± 0.72 ^B	57.18 ± 0.40 ^A	55.45 ± 3.23 ^A	53.92 ± 1.04 ^A
<i>Lachnospiraceae</i>	0.02 ± 0.02 ^C	3.34 ± 0.17 ^A	1.73 ± 0.70 ^B	1.75 ± 0.56 ^B	2.01 ± 0.50 ^B
<i>Other</i>	50.63 ± 0.10 ^A	17.72 ± 0.71 ^B	15.68 ± 0.52 ^B	17.61 ± 2.16 ^B	17.53 ± 1.96 ^B
<i>Parabacteroides</i>	1.29 ± 0.04 ^B	2.49 ± 0.20 ^A	1.43 ± 0.16 ^B	1.47 ± 0.02 ^B	1.37 ± 0.22 ^B
<i>Paraclostridium</i>	0.00 ± 0.00 ^D	14.94 ± 0.27 ^A	0.41 ± 0.21 ^D	1.38 ± 0.03 ^C	3.13 ± 0.68 ^B

CT0: no addition of bacaba pulp, only microbiota in the fermentation medium, at time 0 h; CT48: no addition of bacaba pulp, only the microbiota in the fermentation medium, after 48 h of fermentation; BT48: addition of non-fermented and digested bacaba pulp, after 48 h of fermentation; LAT48: addition of bacaba pulp fermented by LA-05 and digested after 48 h of fermentation; LCT48: addition of bacaba pulp fermented by LCI and digested after 48 h of fermentation; Values are expressed as mean ± standard deviation (mg/100 g); a-b: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same class for different treatments, according to Tukey's test or t-test.

mechanisms must be carried out. Furthermore, it is also important to evaluate whether these compounds positively or negatively affect beneficial microorganisms, such as lactic acid bacteria, as they are generally used to modulate the intestinal microbiota.

Our study provides important information about the functional benefits of bacaba pulp fermentation and its impact on the microbiota of healthy individuals. Previous studies have also reported increased bioaccessibility of phenolic compounds and antioxidant activity after probiotic fermentation of fruit pulps, such as for red pitaya fermented with *L. acidophilus* LA-05 or *B. lactis* BB-12 (Morais et al., 2019), seriguela, mangaba, mango, and acerola fermented with *L. acidophilus* LA-05 and *L. casei* LCI (Assis et al., 2021) and lychee pulp fermented with *L. plantarum* and *Limosilactobacillus fermentum*. At the same time, improvements on the microbiota composition were reported for lychee pulp fermented by *L. plantarum* and *L. rhamnosus* (Tang et al., 2023) or mangaba pulp fermented with *L. casei* 01 (Assis et al., 2024). However, the effect depended on the probiotic strain, fruit pulp (Assis et al., 2021), and group (healthy individuals, vegans, etc) suggesting the need to evaluate the compatibility of fruit pulps and the selected probiotics.

Our study also highlights the nutritional and microbiota-modulating benefits of fermented bacaba pulp. However, future studies are needed to evaluate its potential for commercialization purposes, such as product stability during cold storage with the establishment of shelf life, the impact of probiotic fermentation on the sensory attributes and consumer acceptance of the products, and the verification of regulatory aspects. The bacaba pulp could be commercialized as a functional food or evaluated as dietary supplementation. As the global population is increasingly adopting a healthy lifestyle, focusing on better eating habits, this issue drives the integration of health-promoting ingredients into foods and beverages, leading to the development of functional products that meet these demands (Peruzzolo et al., 2025). Furthermore, clinical or animal trials are necessary to substantiate the results obtained, since *in vitro* fecal fermentation models cannot fully simulate the complex *in vivo* environment, and this limitation needs to be acknowledged.

4. Conclusions

This study was the first to evaluate fermented bacaba pulp for probiotic metabolism and survival and its effect on modulating the microbiota of healthy adults. Bacaba pulp was a suitable substrate for probiotic growth, presenting adequate viability, and higher concentrations and bioaccessibility of phenolics. Sequencing of 16S rRNA indicated positive results in modulating the microbiota of healthy adults, with an increase in the abundance of the order *Acidaminococcales*, *Enterobacterales*, and *Lactobacillales*. These findings provide theoretical evidence for using bacaba pulp as a functional food.

CRediT authorship contribution statement

Bianca Beatriz Torres de Assis: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lucélia Cabral:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Francieli Araújo Silva:** Writing – review & editing, Writing – original draft, Formal analysis. **Jaqueline de Araújo Bezerra:** Writing – review & editing, Writing – original draft, Conceptualization. **Melline Fontes Noronha:** Software, Methodology, Investigation, Formal analysis, Data curation. **Hubert Vidal:** Writing – review & editing, Writing – original draft, Project administration, Conceptualization. **Marcos dos Santos Lima:** Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Data curation. **Tatiana Colombo Pimentel:** Writing – review & editing, Writing – original draft, Visualization, Validation, Conceptualization. **Marciane Magnani:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Data curation, Conceptualization.

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.

Acknowledgments

The authors would like to thank the “Coordination for the Improvement of Higher Education Personnel” (CAPES; Financial code 001), CAPES-COFECUB program (88881.711922/2022-01) and “National Council for Scientific and Technological Development” (CNPq; 441287/2020-4; 441280/2020-0; 402745/2021-3; 303384/2022-0 and 404506/2023-2).

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2025.116326>.

Data availability

Data will be made available on request.

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5.5 PATENTE - Processo de fermentação probiótica aplicado a frutas tropicais para produção de alimento funcional. (Patente submetida ao processo de depósito na Agência UFPB de Inovação Tecnológica - INOVA UFPB).

“PROCESSO DE FERMENTAÇÃO PROBIÓTICA APLICADO A FRUTAS TROPICAIS PARA PRODUÇÃO DE ALIMENTO FUNCIONAL”

As culturas probióticas utilizadas durante o processo de fermentação podem biotransformar os compostos fenólicos de frutas e derivados, liberando compostos fenólicos conjugados com outros constituintes da matriz alimentar, e gerando compostos com maior bioatividade do que suas estruturas precursoras, embora o efeito das culturas probióticas na biotransformação dos compostos fenólicos pode depender da cepa probiótica e do tipo de fruta. Os processos fermentativos podem ser uma estratégia eficaz para aumentar a bioacessibilidade de polifenóis, os níveis antioxidantes de produtos de frutas e vegetais, e relevantes para a reutilização de componentes da fruta (geralmente desperdiçados após o processamento) com valor biológico suficiente, com indicativo de um histórico seguro de consumo. Há estudos que relataram o efeito benéfico da fermentação com diferentes probióticos em matrizes como purês de seriguela, mangaba, manga-rosa e acerola fermentadas com *Lactocaseibacillus casei* 01 e *Lactobacillus acidophilus* 05, polpa de mangaba fermentada com *Lactocaseibacillus casei* 01 e polpa de bacaba fermentada com *Lactobacillus acidophilus* 05 (LA-05) and *Lactocaseibacillus casei* 01 (LC1), no entanto os processos fermentativos são variáveis e complexos e precisam de etapas validadas para o processo ocorrer eficazmente. Por esta razão, a presente invenção busca solucionar esse entrave, através do direcionamento das etapas que ocorrem antes e durante a fermentação de frutas tropicais com culturas probióticas, para a obtenção de um alimento funcional com histórico seguro de consumo. Esta invenção possui ampla aplicação na área de biotecnologia, sobretudo, na produção de alimentos funcionais a partir da fermentação com culturas probióticas com aplicabilidade principalmente a indústria de alimentos e pequenas cadeias produtivas que estão focadas no aproveitamento e valorização de frutas tropicais. Até o presente momento não foram encontrados relatos sobre a utilização de processos fermentativos com culturas probióticas em frutas tropicais, portanto, as invenções conhecidas no estado da técnica são completamente distintas do revelado na presente invenção. O presente documento trata-se do resumo da invenção, caracterizado pela descrição do objetivo e as etapas necessárias do processo fermentativo probiótico de frutas tropicais.

6 CONSIDERAÇÕES FINAIS

Os achados desta pesquisa contribuem com informações para a valorização de frutas pouco exploradas e seu consumo como alimento funcional com efeitos benéficos à saúde. A pesquisa demonstrou que as polpas de mangaba e bacaba foram um substrato adequado para o crescimento de probióticos, resultando em viabilidade adequada após a fermentação e condições gastrointestinais simuladas e maior concentração e bioacessibilidade de fenólicos em comparação à polpa controle. As polpas fermentadas probióticas de mangaba e bacaba apresentaram maior concentração de ácidos orgânicos incluindo láctico e propiônico. Além disso, as polpas de mangaba e bacaba fermentadas promoveram aumento na abundância de grupos bacterianos associados a efeitos positivos na microbiota humana contribuindo positivamente para a modulação da microbiota. A polpa de mangaba fermentada aumentou a abundância relativa dos grupos *Dorea*, *Romboutsia*, *Faecalibacterium* e a polpa de bacaba, por sua vez, aumentou a abundância de *Acidaminococcales*, *Enterobacterales* e *Lactobacillales*. As polpas de fruta fermentadas também impactaram positivamente nos índices de diversidade microbiana resultando em uma microbiota equilibrada e funcional. Na avaliação sensorial da polpa fermentada de mangaba como um novo produto funcional durante o armazenamento foi observada viabilidade probiótica ao longo de 28 dias, estabilidade dos compostos fenólicos bioacessíveis, atividade antioxidante e manutenção dos parâmetros de qualidade. A polpa de mangaba fermentada recebeu maiores escores que a polpa não fermentada para atributos sensoriais como cor, brilho, frescor e aroma até o final do período de armazenamento, suportando o desenvolvimento de um novo produto funcional.

Esta pesquisa foi a primeira a avaliar a polpa de mangaba e bacaba fermentadas quanto ao metabolismo e sobrevivência de probióticos e seu efeito na modulação da microbiota intestinal de adultos saudáveis e veganos, respectivamente. Os resultados fornecem novas informações sobre o potencial do uso da mangaba e da bacaba como polpas funcionais que podem beneficiar a saúde humana por meio de alterações na microbiota colônica. Além disso, o conhecimento gerado atende um eixo prioritário de direcionamento da ciência para o reconhecimento das riquezas da biodiversidade brasileira e sua exploração sustentável e uso para promoção da saúde humana. Os produtos gerados reforçam a potencialidade da bioeconomia nas regiões Norte e Nordeste a projeção do país. Para pesquisas futuras recomenda-se estudos clínicos para confirmar os efeitos moduladores destas polpas fermentadas probióticas na microbiota intestinal humana e seus impactos na saúde.

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8 PRINCIPAIS CONTRIBUIÇÕES ACADEMICOS E SOCIAIS

O estudo desenvolvido na presente tese sobre fermentação probiótica visando uma estratégia para valorização de frutas nativas subexploradas está alinhado a alguns dos Objetivos de Desenvolvimento Sustentável (ODS) da Agenda 2030 das Nações Unidas (ONU). A tese desenvolvida está diretamente relacionada a ODS 1 Erradicação da pobreza, ODS 2 (Fome zero e agricultura sustentável), ODS 3 (Saúde e bem-estar), ODS 8 (Trabalho decente e crescimento econômico), ODS 10 (Redução das desigualdades), ODS 11 (Cidades e comunidades sustentáveis), ODS 12 (Consumo e produção responsáveis), ODS 13 (Ação contra mudança global do clima), e ODS 15 (Vida terrestre), refletindo a importância dos resultados gerados e seu potencial de aplicação. O desenvolvimento das polpas fermentadas probióticas a partir de frutas subexploradas como a mangaba e a bacaba em paralelo a comprovação científica dos efeitos benéficos a saúde dos indivíduos, permitiu o desenvolvimento de novos alimentos com potencial funcional.

As polpas fermentadas de mangaba e bacaba atendem as atuais demandas do mercado consumidor por produtos nutritivos, que contribuam para além da nutrição básica e resultantes de uma exploração sustentável do meio ambiente. Estes produtos atendem o crescente nicho mercadológico de produtos de origem vegetal e sem lactose. Há impactos positivos na bioeconomia que surge como opção para as populações locais e habitantes das regiões Norte e Nordeste, onde existe uma diversidade de frutas ainda obtidas de forma extrativista. Os resultados suportam o desenvolvimento de novos produtos funcionais que podem ser desenvolvidos por associações locais para geração de renda contribuindo para a fixação nestas regiões e para a economia circular. A exploração sustentável de frutas nativas contribui para a preservação da biodiversidade brasileira, alinhadas ao combate dos desafios impostos pelas mudanças climáticas e desmatamento.

Desta forma, os produtos propostos podem contribuir para a promoção da Segurança Alimentar e Nutricional pela utilização de frutas subexploradas, que são fonte de nutrientes e de compostos bioativos, contribuindo indiretamente, pela geração de renda, para a mitigação da fome. Além disso, destaca-se a oportunidade de fomentar o desenvolvimento econômico e social nas regiões menos desenvolvidas do Brasil. O conhecimento gerado atende um eixo prioritário de direcionamento da ciência para o reconhecimento das riquezas da biodiversidade brasileira e sua exploração sustentável e uso para promoção da saúde humana. Os produtos gerados reforçam a potencialidade da bioeconomia nas regiões Norte e Nordeste a projeção do país.

Além do artigo de revisão, capítulo de livro, patente e artigos originais apresentados nos tópicos anteriores, esta tese originou outros impactos acadêmicos:

- I. Publicação de um artigo como co-autora: “Prebiotics in non-dairy products: Technological and physiological functionality, challenges, and perspectives” no ano de 2022, na revista Food Bioscience.

- II. Publicação de um artigo como co-autora, sob título “Evaluation of potentially probiotic yeasts and *Lactiplantibacillus plantarum* in co-culture for the elaboration of a functional plant-based fermented Beverage” no ano 2022, na revista Food Research International.
- III. Publicação de um artigo como co-autora, sob título “Organic arabic coffee husk: Antioxidant and cytoprotective properties and potential impacts on selected human intestinal bacterial populations of individuals with diabetes” no ano 2024, na revista Food Research International.
- IV. Participação em Congresso Brasileiro com resumo submetido e aceito na modalidade pôster e apresentação, sob título “Impacts of bacaba fruit fermented with probiotics on gut microbiota” no ano 2024, no 29ª CBCTA – Congresso Brasileiro de Ciência e Tecnologia de Alimentos, em Florianópolis, com o tema “O futuro dos alimentos: inovação, saúde e sustentabilidade na cadeia produtiva de alimentos”.
- V. Participação em Congresso Internacional com resumo submetido e aceito na modalidade pôster, sob título “Can probiotics be used as biotechnological tools to increase bioaccessible phenolics in soursop pulp?” no ano 2021, no IAFP – Internacional Association for Food Protection.
- VI. Participação e Premiação em 1º lugar no 23ª Prêmio Péter Murányi da Fundação Péter Murányi, na categoria alimentação, juntamente com outros colaboradores com o projeto intitulado “Biotransformação para valorização de frutas nativas subexploradas no Norte e Nordeste do Brasil”.

APÊNDICE

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

O Sr.(a) está sendo convidado(a) a participar do projeto de pesquisa “Fermentação probiótica de polpas de frutas nativas: Aspectos tecnológicos, sensoriais e efeito na microbiota humana”, de responsabilidade do pesquisador Bianca Beatriz Torres de Assis.

O objetivo deste estudo é avaliar as polpas de mangaba e bacaba fermentadas probióticas na modulação da microbiota intestinal de adultos saudáveis e/ou veganos. Os resultados desta pesquisa contribuirão com a sociedade, por meio da geração de dados sobre como composição da população bacteriana intestinal de adultos saudáveis e/ou veganos, e como a modulação pode ser feita via dieta utilizando polpa de mangaba ou bacaba, oferecendo assim melhor qualidade de vida para estes indivíduos. Além disto, a presente pesquisa representa uma possibilidade de obtenção de informações que podem contribuir e ampliar a disponibilidade de alimentos funcionais, além de agregar valor a um produto agrícola importante para o Brasil.

A participação nesta pesquisa consistirá na doação de fezes para a realização das análises que possibilitem identificar e quantificar a população bacteriana intestinal dos participantes, bem como os metabólitos produzidos por estas bactérias.

São esperados os seguintes benefícios desta participação: contribuição na geração de dados sobre a modulação da microbiota intestinal para atenuação dos efeitos de disbiose, em adultos saudáveis e/ou adultos veganos. Considerando que toda pesquisa oferece algum tipo de risco, nesta pesquisa podem haver riscos mínimos associados ao desenvolvimento do estudo, tais como desconforto na coleta do material fecal e doação do material fecal.

As informações fornecidas por você serão consideradas confidenciais e de conhecimento apenas dos pesquisadores responsáveis. Os participantes da pesquisa não serão identificados em nenhum momento, mesmo quando os resultados desta pesquisa forem divulgados em qualquer forma, garantindo o anonimato.

Não será cobrado nada, não haverá gastos decorrentes de sua participação, se houver algum dano decorrente da pesquisa, o participante será indenizado nos termos da Lei.

Gostaríamos de deixar claro que a participação é voluntária e você tem o direito de desistir da participação nesta pesquisa a qualquer momento, sem nenhuma penalidade ou prejuízo de qualquer natureza.

Desde já, agradecemos a atenção e a participação e colocamo-nos à disposição para maiores informações. (Bianca Beatriz Torres de Assis, (83) 998077193, biancabtassis@gmail.com)

Esse termo terá suas páginas rubricadas pelo pesquisador que aplicará o termo e pelo(s) participantes e será assinado em duas vias, das quais uma ficará com o participante e a outra com pesquisador.

Ao fornecer o meu consentimento assinando este Termo, eu declaro que recebi todas as informações sobre o projeto de pesquisa de forma clara e completa e que minhas perguntas foram respondidas satisfatoriamente. Tenho conhecimento de que posso

desistir da participação a qualquer momento e que receberei uma via deste documento assinado por mim.

Participante

Nome Completo do Participante da Pesquisa

Pesquisador

Eu confirmo que expliquei a natureza e o objetivo desta pesquisa, e os possíveis riscos e benefícios ao participante da pesquisa.

Nome do Pesquisador

Assinatura do Pesquisador

Data (dd-MM-aaaa)

ANEXO

PARECE CONSUBSTANCIADO DO CEP

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



PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: POLPAS DE FRUTAS DOS BIOMAS BRASILEIROS FERMENTADAS COM PROBIÓTICOS: ASPECTOS FUNCIONAIS, SENSORIAIS E TECNOLÓGICOS

Pesquisador: BIANCA BEATRIZ TORRES DE ASSIS

Área Temática:

Versão: 2

CAAE: 65306722.0.0000.5188

Instituição Proponente: Universidade Federal da Paraíba/Centro de Tecnologia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 6.785.059

Apresentação do Projeto:

O projeto intitulado: POLPAS DE FRUTAS DOS BIOMAS BRASILEIROS FERMENTADAS COM PROBIÓTICOS: ASPECTOS FUNCIONAIS, SENSORIAIS E TECNOLÓGICOS, pertence ao PPGCTA/CT/UFPB.

Introdução:

O Brasil possui uma grande biodiversidade de frutas nativas, porém algumas ainda inexploradas e comercialmente indisponíveis. No entanto, as frutas nativas do Brasil têm despertado crescente interesse na exploração devido ao seu potencial tecnológico e agroindustrial, alto valor nutricional e características únicas como cor, sabor e aroma (BITENCOURT et al., 2019). Dentre a grande variedade de frutas nativas, destacam-se a mangaba (*Hancornia speciosa* Gomes) e bacaba (*Oenocarpus bacaba* Mart.) (REIS et al., 2022; MORAIS et al., 2022). A mangaba possui alto teor de compostos fenólicos e boa capacidade antioxidante, sendo ácido clorogênico (150 ± 5 mg/g) e rutina (120 ± 8 mg/g) os principais (SANTOS et al., 2022). A bacaba apresenta alto teor de flavonóides totais e fenólicos em sua polpa, em torno de 16,41 mg de quercetina/100g e 98 mg de ácido gálico/100g (SANTOS FILHO et al., 2020). Essas frutas são sazonais e tem uma vida útil curta devido à sua natureza

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Continuação do Parecer: 6.785.059

perecível e suscetibilidade a patógenos pós-colheita (ARRUDA; ARAÚJO; MAROSTICA JUNIOR, 2022; SANTOS et al., 2021). Devido a isto, estas frutas são consumidas na forma de produtos derivados, como as polpas. Estudos têm demonstrado que a fermentação de polpas de frutas utilizando probióticos, pode contribuir com ingredientes funcionais e prolongar a vida útil da fruta (MORAIS et al., 2019). Há estudos que relataram o efeito benéfico da fermentação com diferentes probióticos em matrizes como polpa de manga fermentada com *Lactocaseibacillus paracasei* OR1 (MK252696), PP1 (MK253713), *Lactocaseibacillus rhamnosus* MA3 (MK253673) e PP3 (MK253673) (LAOPHPNGPHIT; SIRIPORNADULSIL, 2023), polpa de goiaba fermentada com *Lactococcus lactis* subsp *lactis* e *Lactobacillus casei* LA-1 (KAUR; GHOSH, 2023), polpa de lichia fermentada por *Lactobacillus plantarum*, *Lactobacillus rhamnosus* e uma cepa mista dos dois (TANG et al., 2023), polpa de kiwi fermentada com *Lactiplantibacillus plantarum* subsp. *Plantarum* (ZHOU et al. 2020), polpa de logan fermentada com *Lactobacillus fermentum* (HUANG et al., 2019), polpa de pitaya vermelha fermentada com *Lactobacillus acidophilus* LA-05 ou *Bifidobacterium animalis* ssp. *lactis* BB-12 (MORAIS et al., 2019) e purês de seriguela, mangaba, mangarosa e acerola fermentadas com *Lactocaseibacillus casei* 01 e *Lactobacillus acidophilus* 05 (ASSIS et al., 2021). As matérias-primas, como as frutas ou polpas de frutas, podem ser transformadas através da fermentação em novos produtos com melhor qualidade nutricional, propriedades melhoradas para a saúde intestinal, funcionalidades biológicas específicas e substâncias bioativas (FILANNINO et al., 2019; GARCIA et al., 2016). Em vista disso, os processos fermentativos podem ser uma estratégia eficaz para aumentar a bioacessibilidade de polifenóis, os níveis antioxidantes de produtos de frutas e vegetais, e relevantes para a reutilização de componentes da fruta (geralmente desperdiçados após o processamento) com valor biológico suficiente. E no mais, os produtos fermentados geralmente possuem um histórico seguro de consumo (KAUR; GHOSH, 2023). Por conseguinte, as culturas probióticas utilizadas durante o processo de fermentação podem biotransformar os compostos fenólicos de frutas e derivados, liberando compostos fenólicos conjugados com outros constituintes da matriz alimentar, e gerando compostos com maior bioatividade do que suas estruturas precursoras (VALERO-CASES et al., 2017; DELGADO et al., 2019; KAPRASOB et al., 2017). Assim, fermentações de frutas com bactérias probióticas tem sido sugerida como um método de grande valor para melhorar suas propriedades nutricionais e bioativas (MARCO et al., 2017). O efeito das culturas probióticas na biotransformação dos compostos fenólicos pode depender da cepa probiótica e do tipo de fruta (MORAIS et al., 2019). No mais, a microbiota intestinal pode realizar a biotransformação de compostos fenólicos, porém, o consumo de

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produtos fermentados com compostos fenólicos mais bioacessíveis podem proporcionar efeitos benéficos independente da microbiota do hospedeiro (BRAGA et al., 2018). A microbiota é comumente analisada por sequenciamento de 16S rRNA (SAWADA et al., 2021). Estudos usando modelos colônicos in vitro, aplicando a microbiota fecal para metabolizar alimentos e determinar a fração dos compostos biotransformada por esses microrganismos bem como estudos que apresentem o potencial destes produtos como alimentos funcionais ainda são escassos na literatura. Diante do exposto o presente estudo foi realizado para avaliar os aspectos tecnológicos (pH, sólidos solúveis totais e acidez titulável) durante fermentação de 48h das polpas de frutas fermentadas (mangaba e bacaba), e os aspectos funcionais (viabilidade e bioacessibilidade) durante armazenamento; analisar o conteúdo de açúcares, ácidos orgânicos, compostos fenólicos e composição de voláteis das polpas de frutas fermentadas durante fermentação e armazenamento; determinar o perfil sensorial da polpa de mangaba fermentada por meio da metodologia Preferred Attribute Elicitation (PAE), e por fim, avaliar o potencial prebiótico das polpas de frutas fermentadas em sistemas in vitro de fermentação colônica.

Hipótese:

O metabolismo de probióticos (*L. acidophilus* LA-05 e *L. casei* LC1) influência nos aspectos funcionais, sensoriais, tecnológicos em polpas de acerola e bacaba dos biomas brasileiros ao longo do armazenamento de 28 dias, e estas podem servir de substrato para modulação da microbiota intestinal, estimulando as bactérias benéficas e promovendo a redução de bactérias patogênicas

Metodologia:

Culturas liofilizadas de *Lactobacillus acidophilus* LA-05 e *Lactobacillus casei* LC-01 foram obtidas da Christian Hansen Ind. Com. Ltda. (Valinhos, Brasil). Para obtenção do inóculo das cepas, as culturas foram centrifugadas (3500 rpm /15 min, 4 °C), lavadas em solução salina estéril e ressuspensas no mesmo meio para obtenção de suspensões celulares padrão. As densidades ópticas (OD) de 1,0 e 0,1 a 660 nm foram usadas para obtenção 9 log UFC/mL para *L. acidophilus* LA-05 e *L. casei* LC1, respectivamente. A fruta mangaba foi adquirida de um

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distribuidor local(EMPASA; João Pessoa, Brasil) e a bacaba através de parceria com a Universidade Federal do Amazonas (UFAM). A fermentação das polpas de mangaba e bacaba foram realizadas inoculando LA-05 e LC1 a 8 log UFC/g em um volume de 35 mL de cada polpa e incubando a 37 °C por 24 h (Assis et al., 2021) em condições anaeróbias em frasco anaeróbio (Anaerobic System Anaerogen, Oxoid Ltd., Wade Road, Reino Unido) com O₂ tica (com adição de LC1), e três formulações para a polpa de bacaba: (1) controle (sem adição de probióticos), (2) polpa de bacaba fermentada probiótica (com adição de LA-05) e (3) polpa de bacaba fermentada probiótica (com adição de LC1). As formulações de mangaba e bacaba foram analisadas antes e durante a fermentação quanto à viabilidade probiótica, valores de pH, acidez titulável e valores de SST. Além disso, apenas as formulações de mangaba foram avaliadas durante o armazenamento refrigerado (7 °C/28 dias) quanto à viabilidade probiótica, parâmetros físico-químicos, teor de açúcares, ácidos orgânicos, perfil de compostos fenólicos, bioacessibilidade, compostos voláteis e sensorial. E por fim, o efeito das formulações de mangaba e bacaba na microbiota colônica com consequente avaliação dos valores de pH, teor de açúcares, ácidos orgânicos e perfil fenólico. As células viáveis de *L. acidophilus* e *L. casei* foram enumeradas em polpas de frutas ao longo do armazenamento (1, 14 e 28 dias) e nas etapas da digestão in vitro, usando uma técnica de inoculação em microgotas (HERIGSTAD; HAMILTON; HEERSINK, 2001). As análises de pH, acidez titulável (AT) e sólidos solúveis totais (SST) das amostras de polpas de frutas fermentadas serão realizadas seguindo procedimentos padrão (AOAC, 2016). A avaliação da bioacessibilidade em polpas de frutas pelo método de digestão in vitro foi realizada seguindo três fases sequenciais: oral, gástrica e intestinal, de acordo com a metodologia descrita por Minekus et al. (2014). O conteúdo de açúcares e ácidos orgânicos das amostras foram analisados por HPLC segundo COELHO et al. (2018). Para os compostos fenólicos foi utilizado a metodologia de PADILHA et al. (2017) e para os compostos voláteis a metodologia de a MONTEIRO et al. (2018). A análise sensorial foi realizada pelo método Preferred Elicitation Attribute (PAE) segundo Grygorczyk et al. (2013). Para o estudo dos ensaios de fermentação in vitro foram necessários o recrutamento de 4 voluntários (veganos) para análise com polpa de mangaba fermentada e 4 voluntários (saúdáveis) para análise com polpa de bacaba fermentada, constituídos por duas mulheres e dois homens com idades entre 25 e 45 anos, que não usavam probióticos ou antibióticos há pelo menos três meses e seguiam uma dieta vegana há mais de três anos (para os voluntários veganos). A preparação do inóculo fecal seguiu metodologia descrita por Wang et al. (2020). A extração de DNA das amostras foi realizada utilizando um kit de isolamento de DNA MoBio Power Food, Mobio Laboratories Inc.,

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EUA, seguindo os procedimentos do fabricante. As bactérias foram identificadas através de sequenciamento de alto rendimento da região 16S rRNA V3/V4 usando os primers 341F (CCTACGGGGRSGCAGCAG) (Wang & Qian, 2009) e 785R (CTACCAGGGTATCTAATCC) (Amann et al., 1995).

Critério de Inclusão:

Voluntários Veganos saudáveis sem precedentes de doenças do intestino grosso, que não fizeram utilização de antibióticos por pelo menos seis meses e seguiram dietas normais (sem o uso de alimentos probióticos ou prebióticos concentrados).

Critério de Exclusão:

Os voluntários que não respeitarem aos critérios de seleção.

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar as polpas de frutas mangaba e bacaba fermentadas com probióticos (*L. acidophilus* LA-05 e *L. casei* LC1) quanto aos aspectos funcionais, sensoriais e tecnológicos, e o efeito de modulação da microbiota intestinal.

Objetivo Secundário:

Avaliar os aspectos tecnológicos (pH, sólidos solúveis totais e acidez titulável) durante fermentação de 48h das polpas de frutas fermentadas (mangaba e bacaba), e os aspectos funcionais (viabilidade e bioacessibilidade) durante armazenamento; analisar o conteúdo de açúcares, ácidos orgânicos, compostos fenólicos e composição de voláteis das polpas de frutas fermentadas durante fermentação e armazenamento; determinar o perfil sensorial da polpa de mangaba fermentada por meio da metodologia Preferred Attribute Elicitation (PAE), e por fim, avaliar o potencial prebiótico das polpas de frutas fermentadas em sistemas in vitro de fermentação colônica

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Avaliação dos Riscos e Benefícios:

Os riscos são mínimos, pois todas as amostras em estudo serão verificadas seguindo a RDC n° 12 de 2 de janeiro de 2001 da Anvisa antes da realização da análise sensorial. A análise sensorial das polpas de frutas fermentadas e ensaios de fermentação in vitro se iniciará após aprovação pelo Comitê de Ética em Pesquisa em Seres Humanos do Centro de Ciências da Saúde/UFPB.

Comentários e Considerações sobre a Pesquisa:

Desenho Experimental realizado em 4 fases sequenciais: fase 1 - preparação do inóculo, fase 2 - elaboração das polpas de frutas fermentadas probióticas, fase 3 - análises ao longo do armazenamento (1, 14 e 28 dias) e fase 4 - ensaios de fermentação in vitro.

Considerações sobre os Termos de apresentação obrigatória:

O projeto em tela encontra-se dentro da Resolução 466/12, e das Normas Operacionais 001/2013.

Recomendações:

Recomenda-se aprovar

Conclusões ou Pendências e Lista de Inadequações:

Aprovado

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BASICAS_231126_2_E1.pdf	26/03/2024 15:33:41		Aceito
Folha de Rosto	folha_rosto_bianca_ppgcta.pdf	13/11/2022 22:50:36	BIANCA BEATRIZ TORRES DE ASSIS	Aceito
Outros	Certidao_PPGCTA.pdf	27/07/2022 15:16:33	BIANCA BEATRIZ TORRES DE ASSIS	Aceito
Outros	Carta_de_Anuencia.docx	27/07/2022 15:15:56	BIANCA BEATRIZ TORRES DE ASSIS	Aceito
Outros	Instrumento_De_Coleta.docx	27/07/2022 15:11:16	BIANCA BEATRIZ TORRES DE ASSIS	Aceito

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Orçamento	Orcamento_DOC.docx	27/07/2022 15:08:20	BIANCA BEATRIZ TORRES DE ASSIS	Aceito
Cronograma	Cronograma.docx	27/07/2022 15:05:16	BIANCA BEATRIZ TORRES DE ASSIS	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.doc	27/07/2022 15:04:13	BIANCA BEATRIZ TORRES DE ASSIS	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_DOC_BIANCA_BEATRIZ_d ocx	27/07/2022 14:57:47	BIANCA BEATRIZ TORRES DE ASSIS	Aceito

Situação do Parecer:

Aprovado

Necessita apreciação da CONEP:

Não

JOAO PESSOA, 24 de Abril de 2024

Assinado por:

Eliane Marques Duarte de Sousa
(Coordenador(a))

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DOCUMENTO DE CONFIRMAÇÃO DE ACEITE DO CAPÍTULO DE LIVRO

Publishing Agreement

SPRINGER NATURE

for Contributions in Collected Works

This Publishing Agreement (this "Agreement") has been approved by and entered into between:

Bianca Beatriz Torres de Assis¹, Marcos dos Santos Lima², Jaqueline de Araújo Bezerra³, Tatiana Colombo Pimentel⁴, Marciane Magnani^{1*}

1Laboratory of Microbial Processes in Foods, Department of Food Engineering, Center of Technology, Federal University of Paraíba, Campus I, 58051-900 João Pessoa, Paraíba, Brazil

2Department of Food Technology, Federal Institute of Sertão Pernambucano, Petrolina, Pernambuco, Brazil

3Federal Institute of Education, Science and Technology of Amazonas (IFAM), Manaus, Amazonas, Brazil

4Federal Institute of Education Science and Technology of Parana (IFPR), Paranavaí, Paraná, Brazil (the "Author")

whereas, in the event that the Author is more than one person, **Marciane Magnani** serves as corresponding author

(the "Corresponding Author")

on the one part and

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1 New York Plaza, New York, NY 10004, U.S.A.

(the "Publisher")

on the other part;

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The Publisher intends to publish the Author's contribution in a collected work provisionally entitled:

Bioaccessibility of Compounds in Foods and Byproducts

(the "Work")

edited by: **Marciane Magnani and Tatiana Colombo Pimentel**

(the "Editor")

The Publisher intends to publish the Work under the imprint Springer.

The Work may be published in the book series **Methods and Protocols in Food Science**.

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When the Author is more than one person then, unless otherwise indicated in this Agreement or agreed in writing by the Publisher:

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(b) the Corresponding Author hereby warrants and represents that all co-authors of the contribution have expressly agreed that the Corresponding Author has full right, power and authority to sign this

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- (a) all applicable anti-bribery and corruption laws; and
- (b) all applicable data protection and electronic privacy and marketing laws and regulations; and
- (c) the Publisher's ethic rules (available at <https://www.springernature.com/gp/authors/book-authors-code-of-conduct>), as may be updated by the Publisher at any time in its sole discretion. The Publisher shall notify the Author in the event of material changes by email or other written means (the "**Applicable Laws**").

If the Author is in material breach of any of the Applicable Laws or otherwise in material breach of accepted ethical standards in research and scholarship, or becomes the subject of any comprehensive or selective sanctions issued in any applicable jurisdiction (e.g. being subject to the OFAC sanctions list) or if, in the opinion of the Publisher, at any time any act, allegation or conduct of or about the Author prejudices the production or successful exploitation of the Contribution and the Work or brings the name and/or reputation of the Publisher or the Work into disrepute, or is likely to do so, then the Publisher may terminate this Agreement in accordance with the Clause "**Termination**".

- 7.3 The Publisher reserves the right to amend and/or require the Author to amend the Contribution at any time to remove any actual or potential breach of the above warranties and representations or otherwise unlawful part(s) which the Publisher or its internal or external legal advisers identify at any time. Any such amendment or removal shall not affect the warranties and representations given by the Author in this Agreement.

8. Author's Discount and Electronic Access

- 8.1 The Author, or each co-author, is entitled to purchase for their personal use the Work and other books published by the Publisher at a discount of 40% off the list price, for as long as there is a contractual arrangement between the Author and the Publisher and subject to any applicable book price law or regulation. The copies must be ordered from the affiliated entity of the Publisher (Springer Nature Customer Service Center GmbH or Springer Nature Customer Service Center LLC, respectively). Resale of such copies is not permitted.
- 8.2 The Publisher shall provide the electronic final published version of the Work to the Author, provided that the Author has included their e-mail address in the manuscript of the Contribution.

9. Consideration

- 9.1 The Parties agree that the Publisher's agreement to its contractual obligations in this Agreement in respect of its efforts in considering publishing and promoting the Contribution and the Work is good and valuable consideration for the rights granted and obligations undertaken by the Author under this Agreement, the receipt, validity and sufficiency of which is hereby acknowledged by the Author. The Parties expressly agree that no royalty, remuneration, licence fee, costs or other moneys whatsoever shall be payable to the Author.
- 9.2 The Publisher and the Author each have the right to authorise collective management organisations ("CMOs") of their choice to manage some of their rights. Reprographic and other collectively managed rights in the Contribution ("**Collective Rights**") have been or may be licensed on a non-exclusive basis by each of the Publisher and the Author to their respective CMOs to administer the Collective Rights under their reprographic and other collective licensing schemes ("**Collective Licences**"). Notwithstanding the other provisions of this Clause, the Publisher and the Author shall each receive and retain their share of revenue from use of the Contribution under Collective Licences from, and in accordance with, the distribution terms of their respective CMOs. To the fullest extent permitted by law, any such revenue is the sole property of the Publisher and the Author respectively and, if applicable, the registration and taxation of that revenue is the sole responsibility of the respective recipient party. The Publisher and the Author shall cooperate as necessary in the event of any change to the licensing arrangements set out in this Clause.

10. New Editions

- 10.1 The Publisher has the sole right to determine whether to publish any subsequent edition of the Work containing an updated version of the Contribution, but only after reasonable consultation with the Author. Once notified by the Publisher that an update of the Contribution is deemed necessary, the Author agrees to deliver an updated manuscript in accordance with the terms of the Clause "**The Author's Responsibilities**" and the other relevant provisions of this Agreement, together with the material for any new illustrations and any other supporting content including media enhancements, within a reasonable period of time (as determined by the Publisher) after such notification. Substantial changes in the nature or size of the Contribution require the written approval of the Publisher at its sole discretion. The terms of this Agreement shall apply to any new edition of the Work that is published under this "**New Editions**" Clause.
- 10.2 If the Author, for whatever reason, is unwilling, unable or fails (including as a result of death or incapacity) to submit an updated manuscript that meets the terms of this Agreement within the above stated period, then the Publisher is entitled to revise, update and publish the content of the existing edition or to designate one or more individuals (which, where co-authors have entered into this Agreement, may be one or more of the co-authors) to prepare this and any future editions provided that the new editions shall not contain anything that is a derogatory use of the Author's work that demonstrably damages the Author's academic reputation. In such case, the Author shall not participate in preparing any subsequent editions. The Author agrees that the Publisher shall be entitled but not

obliged to continue to use the name of the Author on any new editions of the Work together with the names of the person or persons who contributed to the new editions. Should the Author or the Author's successors object to such continuing use then they must notify the Publisher in writing when first contacted by the Publisher in connection with any new edition.

11. Termination

- 11.1 In addition to the specific rights of termination set out in the Clause "**The Publisher's Responsibilities**" and the Clause "**The Author's Responsibilities**", either Party shall be entitled to terminate this Agreement forthwith by notice in writing to the other Party if the other Party commits a material breach of the terms of the Agreement which cannot be remedied or, if such breach can be remedied, fails to remedy such breach within 45 days of being given written notice to do so.
- 11.2 Termination of this Agreement, howsoever caused, shall not affect:
- (a) any subsisting rights of any third party under any licence or sub-licence validly granted by the Publisher prior to termination and the Publisher shall be entitled to retain its share of any sum payable by any third party under any such licence or sub-licence;
 - (b) except where stated otherwise in this Agreement, any claim which either Party may have against the other for damages or otherwise in respect of any rights or liabilities arising prior to the date of termination;
 - (c) the Publisher's right to continue to sell any copies of the Work which are in its power, possession or control as at the date of expiry or termination of this Agreement for a period of six months on a non-exclusive basis.

12. General Provisions

- 12.1 This Agreement, and the documents referred to within it, constitute the entire agreement between the Parties with respect to the subject matter hereof and supersede any previous agreements, warranties, representations, undertakings or understandings. Each Party acknowledges that it is not relying on, and shall have no remedies in respect of, any undertakings, representations, warranties, promises or assurances that are not set forth in this Agreement. Nothing in this Agreement shall exclude any liability for or remedy in respect of fraud, including fraudulent misrepresentation. This Agreement may be modified or amended only by agreement of the Parties in writing. For the purposes of modifying or amending this Agreement, "in writing" requires either a written document signed by both the Parties or an electronic confirmation by both the Parties with DocuSign or a similar e-signature solution. Any notice of termination and/or reversion and, where applicable, any preceding notices (including any requesting remediable action under the Clause "**Termination**") must be provided in writing and delivered by post, courier or personal delivery addressed to the physical address of the relevant Party as set out at the beginning of this Agreement or any replacement address notified to the other Party for this purpose. All such notices shall become effective upon receipt by the other Party. Receipt is deemed to have taken place five working days after the respective notice was sent by post or left at the address by courier or personal delivery. If the Publisher is the terminating Party the notice need only be provided to the address of the Corresponding Author. If the Author is the terminating Party a copy of the notice must also be sent to the Publisher's Legal Department located at Heidelberger Platz 3, 14197 Berlin, Germany.
- 12.2 Nothing contained in this Agreement shall constitute or shall be construed as constituting a partnership, joint venture or contract of employment between the Publisher and the Author. No Party may assign this Agreement to third parties but the Publisher may assign this Agreement or the rights received hereunder to its affiliated companies. In this Agreement, any words following the terms "include", "including", "in particular", "for example", "e.g." or any similar expression shall be construed as illustrative and shall not limit the sense of the words preceding those terms.
- 12.3 If any difference shall arise between the Author and the Publisher concerning the meaning of this Agreement or the rights and liabilities of the Parties, the Parties shall engage in good faith discussions to attempt to seek a mutually satisfactory resolution of the dispute. This Agreement shall be governed by, and shall be construed in accordance with, the laws of New York State. The courts of competent jurisdiction in New York, N.Y. shall have the exclusive jurisdiction.
- 12.4 A person who is not a party to this Agreement (other than an affiliate of the Publisher) has no right to enforce any terms or conditions of this Agreement. This Agreement shall be binding upon and inure to

the benefit of the successors and assigns of the Publisher. If one or more provisions of this Agreement are held to be unenforceable (in whole or in part) under applicable law, each such provision shall be deemed excluded from this Agreement and the balance of the Agreement shall remain valid and enforceable but shall be interpreted as if that provision were so excluded. If one or more provisions are so excluded under this Clause then the Parties shall negotiate in good faith to agree an enforceable replacement provision that, to the greatest extent possible under applicable law, achieves the Parties' original commercial intention.

The Corresponding Author signs this Agreement on behalf of any and all co-authors.

Signature of Corresponding Author:

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Appendix “Author’s Self-Archiving Rights”

The Publisher acknowledges that the Author retains rights to archive the Contribution but only subject to and in accordance with the following provisions:

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A “Preprint” is defined as the Author’s version of the Contribution submitted to the Publisher but before any peer review or any other editorial work by or on behalf of the Publisher has taken place. The Author may make available the Preprint of the Contribution for personal and private reading purposes only on any of:
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(b) a legally compliant, non-commercial preprint server, such as but not limited to arXiv, bioRxiv and RePEc; provided always that once the “Version of Record” (as defined below) of the Contribution has been published by or on behalf of the Publisher, the Author shall immediately ensure that any Preprint made available above shall contain a link to the Version of Record and the following acknowledgement: *“This is a preprint of the following chapter: [author of the chapter], [chapter title], published in [book title], edited by [editor of the book], [year of publication], [publisher (as it appears on the cover of the book)] reproduced with permission of [publisher (as it appears on the copyright page of the book)]. The final authenticated version is available online at: [http://dx.doi.org/\[insert DOI\]](http://dx.doi.org/[insert DOI])”.*
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Appendix "Author's Reuse Rights"

1. The Publisher acknowledges that the Author retains the ability to copy, distribute or otherwise reuse the Contribution, without the requirement to seek specific prior written permission from the Publisher, ("Reuse") subject to and in accordance with the following provisions:
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