UNIVERSIDADE FEDERAL DA PARAÍBA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA NUTRIÇÃO

SÔNIA PAULA ALEXANDRINO DE OLIVEIRA

IMPACTOS DE DIFERENTES PARTES DA BETERRABA VERMELHA (*Beta vulgaris* L.) SOBRE O CRESCIMENTO E ATIVIDADE METABÓLICA DE PROBIÓTICOS E DA MICROBIOTA COLÔNICA HUMANA

João Pessoa

2023

SÔNIA PAULA ALEXANDRINO DE OLIVEIRA

IMPACTOS DE DIFERENTES PARTES DA BETERRABA VERMELHA (*Beta vulgaris* L.) SOBRE O CRESCIMENTO E ATIVIDADE METABÓLICA DE PROBIÓTICOS E DA MICROBIOTA COLÔNICA HUMANA

João Pessoa

2023

SÔNIA PAULA ALEXANDRINO DE OLIVEIRA

IMPACTOS DE DIFERENTES PARTES DA BETERRABA VERMELHA (*Beta vulgaris* L.) SOBRE O CRESCIMENTO E ATIVIDADE METABÓLICA DE PROBIÓTICOS E DA MICROBIOTA COLÔNICA HUMANA

Tese apresentada ao Programa de Pós-graduação em Ciência da Nutrição, Centro de Ciência da Saúde, Universidade Federal da Paraíba, em cumprimento aos requisitos para obtenção do título de Doutora em Ciências da Nutrição

Orientador: Prof. Dr. Evandro Leite de Souza

João Pessoa

2023

Catalogação na publicação
Seção de Catalogação e Classificação

O48i Oliveira, Sônia Paula Alexandrino de.
Impactos de diferentes partes da beterraba vermelha (Beta vulgaris L.) sobre o crescimento e atividade metabólica de probióticos e da microbiota colônica humana / Sônia Paula Alexandrino de Oliveira. - João Pessoa, 2023.
115 f. : il.

Orientação: Evandro Leite de Souza.
Tese (Doutorado) - UFPB/CCS.

1. Nutrição - Ciências. 2. Beta vulgaris L. 3. Microbiota intestinal. 4. Ingrediente funcional. 5. Propriedades prebióticas. I. Souza, Evandro Leite de. II. Título.

UFPB/BC CDU 612.39(043)

Elaborado por GRACILENE BARBOSA FIGUEIREDO - CRB-15/794

SÔNIA PAULA ALEXANDRINO DE OLIVEIRA

IMPACTOS DE DIFERENTES PARTES DA BETERRABA VERMELHA (*Beta vulgaris* L.) SOBRE O CRESCIMENTO E ATIVIDADE METABÓLICA DE PROBIÓTICOS E DA MICROBIOTA COLÔNICA HUMANA

Tese defendida em 31/08/2023.

BANCA EXAMINADORA

Profº. Dr. Evandro Leite de Souza
PPGCN/DN/CCS/UFPB
Presidente da Banca Examinadora
Orientador

Prof. Dr. Fillipe de Oliveira Pereira
PPGCN/DN/CCS/UFPB
Examinador Interno

Kataryne Árabe Rimá de Oliveira

Profa. Dra. Kataryne Árabe Rimá de Oliveira
PPGCN/DN/CCS/UFPB
Examinadora Interna

Profa. Dra. Noadia Priscila Araujo Rodrigues
DG/CTDR/UFPB
Examinadora Externa

Profa. Dra. Ana Elizabeth Cavalcante Fai
DNBE/Instituto de Nutrição/UERJ
Examinadora Externa

AGRADECIMENTOS

Em primeiro lugar, agradeço a Deus pelo dom da vida, pelo seu amor infinito, sem ele nada sou;

Aos meus pais Luciene Rodrigues da Silva Alexandrino e João Batista Alexandrino pelos ensinamentos de vida, amor, compreensão, confiança, incentivo e dedicação. Obrigada por se fazerem presente em todos os momentos sejam eles bons ou ruins, e por acreditarem em mim. Vocês terão meu amor, admiração e agradecimento eterno;

Ao meu esposo Dorivaldo, por toda compreensão sobre as horas dedicadas à pesquisa, períodos intensos nos quais se mostrou paciente e da sua forma, me incentivou a prosseguir e me ajudou na logística de muita coisa;

Às minhas preciosidades, minhas filhas Laura e Sarah, meu amor mais puro e verdadeiro. Meu motivo para nunca desistir são vocês;

Às minhas irmãs Sandra e Silvia, pelo apoio e pela ajuda em todos os momentos em que precisei;

Agradeço ao Prof. Dr. Evandro leite de Souza, por ter-me deixado fazer parte do seu grupo de trabalho e ter acreditado na minha capacidade. Pela competência na orientação, pelo tempo dedicado, paciência e disposição para a melhor condução desse trabalho;

A professora Dra. Maria Lúcia da Conceição, a quem tenho grande carinho, que me acolheu no laboratório e me ensinou não apenas microbiologia, mas a ser servidora pública. Agradeço por todo seu tempo dedicado e por servir de exemplo de profissional e ser humano;

Agradeço a minha colega e amiga Heloísa, sempre disponível a me ajudar com a pesquisa e qualquer situação pessoal. Muito obrigada por todo companheirismo e disponibilidade;

A Nayara Massa e Jaielison Yandro, pelo apoio e disponibilidade nas mais diversas situações durante o doutoramento;

Aos colegas do LMBA, que partilharam comigo os sabores e dissabores da pesquisa, mantenham o foco e perseverança de que tudo terminará bem;

Agradeço à Profa. Dra. Thatyane M. R. de Albuquerque e Profa. Dra. Noádia Priscila A. Rodrigues pelo apoio e contribuições para que esse trabalho fosse realizado;

Ao professor Dr. Marcos dos Santos Lima pelos esclarecimentos e suporte necessário para a execução de algumas análises;

A banca examinadora desta tese por todas as correções e sugestões para enriquecimento do trabalho;

Ao Departamento de Nutrição/CCS/UFPB e ao Programa de Pós-Graduação em Ciências da Nutrição/CCS/UFPB, pela oportunidade;

A PROPESQ/UFPB pelo suporte financeiro para a realização do trabalho por meio da Chamada Interna Produtividade em Pesquisa PROPESQ/PRPG/UFPB Nº 01/2021 - Programa de Apoio à Pesquisa da UFPB (Código do Projeto: PVG14839-2021);

Ao corpo editorial dos periódicos “Critical Reviews in Food Science and Nutrition” (Doi: https://doi.org/10.1080/10408398.2020.1768510), “Food Bioscence” (Doi: https://doi.org/10.1016/j.fbio.2023.102439) e “Food Research International” (Doi: https://doi.org/10.1016/j.foodres.2023.112998) pelo reconhecimento do mérito e publicação dos artigos científicos derivados da tese.

Muito obrigada!

RESUMO

Padrões dietéticos que incluem o consumo regular de compostos prebióticos são capazes de modular a composição e metabolismo da microbiota intestinal humana, exercendo papel importante na saúde do hospedeiro. A beterraba vermelha (*Beta vulgaris* L.) é uma importante espécie olerícola com consideravel aporte de nutrientes e de compostos bioativos, com destaque para as fibras dietéticas, compostos fenólicos e betalaínas. Esse trabalho teve como objetivo avaliar o potencial prebiótico de liofilizados obtidos de diferentes partes da beterraba vermelha por meio da verificação de seus efeitos sobre o crescimento e metabolismo de cepas probióticas (*Lactobacillus acidophilus* LA-05, *Lacticaseibacillus casei* L-26 e *Limosilactobacillus fermentum* 296) e da microbiota colônica humana em condições *in vitro*. Os ensaios foram realizados com liofilizados das partes da raiz (FDBR) e dos talos/folhas (FDBSL) da beterraba vermelha cultivar Early wonder. FDBR e FDBSL foram submetidos às análises de determinação da composição nutricional e parâmetros físico-químicos. Em seguida, cepas de *Lactobacillus* foram expostas a condições simuladas do trato gastrointestinal na presença de FDBR e FDBSL, quando foram avaliados aspectos relacionados a viabilidade celular e funcionalidade fisiológica, capacidade antioxidante total e compostos fenólicos totais após as três fases principais da digestão (boca, estômago e intestino). Em uma segunda etapa, novas amostras de FDBR e FDBSL foram submetidas a digestão gastrointestinal simulada e o produto da digestão foi avaliado em ensaios de fermentação usando-os como única fonte de carbono para o cultivo das cepas probióticas e para avaliação da modulação da microbiota colônica utilizando inóculo fecal. Foi calculado o escore de atividade prebiótica e a atividade metabólica microbiana foi monitorada por meio da determinaão de pH e dos conteúdos de açúcares e ácidos orgânicos nos diferentes sistemas de cultivo. Também foram monitorados o perfil de fenólicos e capacidade antioxidante total durante as fases da digestão simulada e fermentação com inóculo fecal. FDBR e FDBSL apresentaram altos teores de fibras solúveis (5,25 e 11,10 g/100 g) e insolúveis (21,43 e 37,03 g/100 g), pectinas (8,45 e 9,51 g/100 g), betalaínas (3,36–28,99 g/100 g) e uma variedade de compostos fenólicos. FDBR e FDBSL estimularam o crescimento das cepas probióticas testadas com altas contagens de células viáveis (>9 log UFC/mL), aumento da produção de ácidos acético, butírico, lático e propiônico causando alterações nos teores de compostos fenólicos durante 72 h de cultivo. Ainda, apresentaram valores positivos de índices prebióticos (≥0,17) para as diferentes ceas testadas. Durante a exposição a digestão simulada, FDBR e FDBSL aumentaram a sobrevivência e manutenção das funções fisiológicas ativas nas cepas probióticas, indicado pela alta contagem de células viáveis (≥3,5 log UFC/mL) e percentual de células fisiologicamente ativas (>25,2 % para FDBR e 23,1> % para FDBSL). FDBR e FDBSL aumentaram a abundância de *Lactobacillus* spp./*Enterococcus* spp. (3,64-7,60%) e *Bifidobacterium* spp. (2,76-5,78%) e diminuíram a abundância de *Bacteroides* spp./*Prevotella* spp. (9,56-4,18%), *Clostridium histolyticum* (1,62-1,15%) e *Eubacterium rectale/Clostridium coccoides* (2,33-1,49%) durante 48 h de fermentação colônica, resultando em índices prebióticos positivos (> 3,61). FDBR e FDBSL promoveram intensa atividade metabólica, evidenciada pela diminuição do pH, aumento da produção de ácido lático, acético, butírico e propiônico, e consumo de glicose e frutose ao longo do tempo no cultivo dos probióticos e nos ensaios de fermentação colônica *in vitro*. Alterações no conteúdo de compostos fenólicos e alta capacidade antioxidante durante a fermentação colônica também foram observados. FBRD e FDBSL mostraram potenciais propriedades prebióticas, contribuindo na modulação benéfica da composição e atividade metabólica da microbiota intestinal humana, podendo ser explorados na formulação de produtos alimentícios e suplementos dietéticos funcionais.

Palavras-chave: *Beta vulgaris* L.; propriedades prebióticas; microbiota intestinal; ingrediente funcional.

ABSTRACT

Dietary patterns that include regular consumption of prebiotic compounds are capable of modulating the composition and metabolism of the human intestinal microbiota, playing an important role in the health of the host. Red beet (*Beta vulgaris* L.) is an important vegetable species with a considerable supply of nutrients and bioactive compounds, especially dietary fiber, phenolic compounds and betalains. The aim of this study was to evaluate the prebiotic potential of freeze-dried products obtained from different parts of red beet by checking their effects on the growth and metabolism of probiotic strains (*Lactobacillus acidophilus* LA-05, *Lacticaseibacillus casei* L-26 and *Limosilactobacillus fermentum* 296) and the human colonic microbiota under *in vitro* conditions. The tests were carried out with freeze-dried parts of the root (FDBR) and stalk/leaf (FDBSL) of the red beet cultivar Early wonder. FDBR and FDBSL were analyzed to determine their nutritional composition and physicochemical parameters. Next, *Lactobacillus* strains were exposed to simulated conditions of the gastrointestinal tract in the presence of FDBR and FDBSL, when aspects related to cell viability and physiological functionality, total antioxidant capacity and total phenolic compounds were evaluated after the three main phases of digestion (mouth, stomach and intestine). In a second stage, new samples of FDBR and FDBSL were subjected to simulated gastrointestinal digestion and the digestion product was evaluated in fermentation trials using them as the sole carbon source for the cultivation of probiotic strains and to assess the modulation of the colonic microbiota using fecal inoculum. The prebiotic activity score was calculated and microbial metabolic activity was monitored by determining pH and the content of sugars and organic acids in the different cultivation systems. The phenolic profile and total antioxidant capacity were also monitored during the simulated digestion and fermentation phases with fecal inoculum. FDBR and FDBSL showed high levels of soluble (5.25 and 11.10 g/100 g) and insoluble fiber (21.43 and 37.03 g/100 g), pectins (8.45 and 9.51 g/100 g), betalains (3.36-28.99 g/100 g) and a variety of phenolic compounds. FDBR and FDBSL stimulated the growth of the probiotic strains tested with high viable cell counts (>9 log CFU/mL), increased production of acetic, butyric, lactic and propionic acids and caused changes in the levels of phenolic compounds during 72 h of cultivation. They also showed positive prebiotic index values (≥0.17) for the different ceas tested. During exposure to simulated digestion, FDBR and FDBSL increased the survival and maintenance of active physiological functions in the probiotic strains, as indicated by the high viable cell count (≥3.5 logs CFU/mL) and percentage of physiologically active cells (>25.2% for FDBR and 23.1% for FDBSL). FDBR and FDBSL increased the abundance of *Lactobacillus* spp./*Enterococcus* spp. (3.64-7.60%) and *Bifidobacterium* spp. (2.76-5.78%) and decreased the abundance of Bacteroides spp./Prevotella spp. (9.56-4.18%), *Clostridium histolyticum* (1.62-1.15%) and *Eubacterium rectale/Clostridium coccoides* (2.33-1.49%) during 48 h of colonic fermentation, resulting in positive prebiotic indices (> 3.61). FDBR and FDBSL promoted intense metabolic activity, evidenced by a decrease in pH, an increase in the production of lactic, acetic, butyric and propionic acid, and the consumption of glucose and fructose over time in the probiotic culture and in the *in vitro* colonic fermentation assays. Changes in the content of phenolic compounds and high antioxidant capacity during colonic fermentation were also observed. FBRD and FDBSL showed potential prebiotic properties, contributing to the beneficial modulation of the composition and metabolic activity of the human intestinal microbiota, and could be exploited in the formulation of functional food products and dietary supplements.

Keywords: *Beta vulgaris* L.; prebiotic properties; intestinal microbiota; functional ingredient.

LISTA DE TABELAS

TABELAS DA TESE

TABELAS DO APÊNDICE B – ARTIGO DE REVISÃO

TABELAS DO APÊNDICE C – ARTIGO ORIGINAL I

TABELAS DO APÊNDICE D - ARTIGO ORIGINAL 2

LISTA DE FIGURAS

FIGURAS DA TESE

FIGURAS DO APÊNDICE C - ARTIGO ORIGINAL 1

FIGURAS DO APÊNDICE D – ARTIGO ORIGINAL 2

SUMÁRIO

1 INTRODUÇÃO

A beterraba (*Beta vulgaris* L.) é uma importante espécie olerícola amplamente difundida na América, Europa e Ásia, que apresenta a raiz como o produto comercial mais importante. Entretanto, talos e folhas da beterraba também podem ser utilizados na alimentação humana, apresentando características funcionais semelhantes a raiz devido ao seu elevado teor de fibras solúveis e insolúveis e pigmentos antioxidantes, a exemplo de betalaínas, que podem conferir propriedades bioativas com efeitos positivos sobre a saúde, possibilitando a exploração integral dessa espécie vegetal para fins alimentares (COSTA *et al*., 2017; DE OLIVEIRA *et al*., 2022). No Brasil, o cultivo de beterraba com finalidade comercial é realizado, principalmente, com cultivares de mesa, conhecidos como beterraba hortícola ou vermelha (CHHIKARA *et al*., 2019; TIVELLI *et al*., 2011).

Diversos benefícios à saúde decorrentes do consumo da beterraba vermelha têm sido relacionados com suas propriedades antioxidantes, antimicrobianas, anti-inflamatórias, diuréticas e, mais recentemente, moduladoras da microbiota intestinal (BABARYKIN *et al*., 2019; CHHIKARA *et al*., 2019; DE OLIVEIRA *et al*., 2021). A beterraba vermelha possui fibras dietéticas solúveis e insolúveis, compreendendo hemicelulose, celulose e pectina, das quais podem derivar oligossacarídeos com diferentes estruturas e, consequentemente, com diversas funções biológicas (HOLCK *et al*., 2011). Estudos com oligossacarídeos derivados da pectina extraída da beterraba indicam que esses componentes possuem propriedades prebióticas, com efeitos fisiológicos benéficos sobre a saúde intestinal em decorrência da modulação da microbiota intestinal e estímulo da produção de ácidos graxos de cadeia curta (GÓMEZ *et al*., 2019; LARSEN *et al*., 2018; WANG *et al*., 2023).

Além das fibras, a beterraba vermelha possui conteúdo elevado de compostos fenólicos e pigmentos, destacando-se as betalaínas, que são amplamente investigados em relação aos seus efeitos antioxidantes (PUNIA BANGAR *et al*., 2022; RAMÍREZ-MELO *et al*., 2022; SADOWSKA-BARTOSZ; BARTOSZ, 2021). Os compostos fenólicos mais encontrados na beterraba são das classes dos ácidos fenólicos, a exemplo dos ácidos ferúlico, caféico, cumárico e siríngico, e dos flavonoides, a exemplo da catequina, epicatequina, kaempferol e rutina (PUNIA BANGAR *et al*., 2022), que já foram apontados como coadjuvantes na estimulação do crescimento de probióticos e modulação da microbiota intestinal (DE OLIVEIRA *et al*., 2023a, 2023b; RODRÍGUEZ-DAZA *et al*., 2021). Estudos com foco nos efeitos das betalaínas sobre probióticos e microbiota intestinal são ainda escassos. No entanto, são apontados efeitos positivos de betalaínas e betacianinas sobre a modulação de populações microbianas do intestino humano (CAPPER *et al*., 2020; WANG *et al*., 2022; WANG *et al*., 2018).

Nos últimos anos, tem ocorrido crescente busca pela descoberta de alimentos com funcionalidades que promovam benefícios a saúde dos consumidores, com destaque para os alimentos capazes de afetar positivamente a saúde intestinal humana (DAMIÁN *et al*., 2022; GALANAKIS, 2021). Padrões alimentares que incluem a ingestão regular de componentes prebióticos exercem influência direta na diversidade da microbiota intestinal, bem como na sua atividade metabólica, com a produção de compostos derivados, a exemplo de ácidos graxos de cadeia curta, que influenciam diferentes aspectos relacionado à saúde humana, como a modulação do sistema imunológico e gastrointestinal, melhora da motilidade gastrointestinal e redução dos níveis de colesterol sérico (DE SOUZA *et al*., 2019; SANDERS *et al*., 2019; SHAH *et al*., 2020).

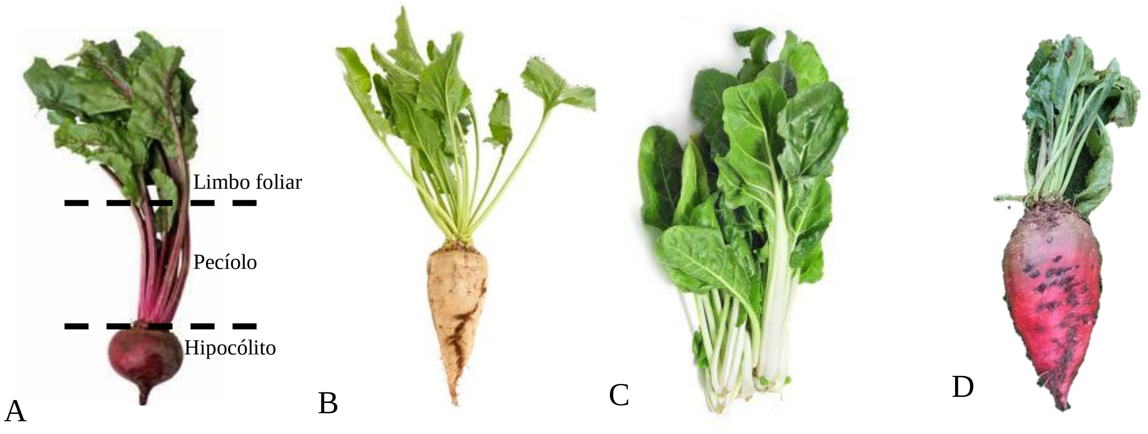
Considerando a necessidade de busca de novas matérias primas naturais e melhor aproveitamento e valorização integral de recursos alimentares ricos em nutrientes e compostos bioativos, em soma à crescente demanda dos consumidores por alimentos com propriedades funcionais, este estudo teve como objetivo investigar as propriedades prebióticas de diferentes partes da beterraba vermelha por meio da determinação *in vitro* dos seus impactos sobre o crescimento e aspectos relacionados à atividade metabólica de cepas probióticas e da microbiota colônica de humanos saudáveis.

2 REFERENCIAL TEÓRICO

2.1 BETERRABA VERMELHA (*Beta vulgaris* L. subsp. *vulgaris*)

A beterraba (*Beta vulgaris* L. subsp. *vulgaris*) é uma importante hortaliça da família Chenopodiaceae, originária do sul e do leste da Europa e norte da África. A subespécie *vulgaris* possui quatro principais biótipos catalogados de acordo com características morfológicas e uso final (Figura 1), a citar: beterraba foliar (Leaf Beet Group, *Beta vulgaris* cicla), açucareira (Sugar Beet Group, *Beta vulgaris* saccharifera), forrageira (Fodder Beet Group, *Beta vulgaris* crassa) e hortícola (Gardem Beet Group, *Beta vulgaris* rubra) (LEWELLEN; PANELLA; HARVESON, 2009). A beterraba hortícola, também conhecida como beterraba vermelha ou beterraba de mesa, é o biótipo cultivado no Brasil. A divisão anatômica da beterraba hortícola (Figura 1/A) consiste em raiz (hipocótilo) e parte aérea composta por hastes (pecíolo) semi-rígidas, de coloração vermelha a roxa, e folhas (limbo foliar) tenras, com coloração e formatos variados (TIVELLI *et al*., 2011; TRANI; CANTARELLA; TIVELLI, 2005).

Figura 1. Grupos principais de *Beta vulgaris* L. subsp. *vulgaris*



Legenda: A: Beterraba hortícola; B: Beterraba açucareira; C: Beterraba folhosa; D: Beterraba forrageira.
Fontes: A: Autor, 2023; B: https://www.scientificamerican.com/article/sugar-beets-make-hemoglobin/; C: https://www.isla.com.br/produto/acelga-verde-escura/18; D: https://lgseeds.co.uk/product/blaze/.

As cultivares de beterraba hortícola cultivadas no Brasil costumam ser de origem norte-americana ou europeia, com raiz tuberosa de formato globular e constituem o grupo denominado Wonder. As principais regiões produtoras são a Sudeste e Sul, por apresentarem temperaturas amenas a frias, que são propícias para o cultivo desse tubérculo. Dados do mapeamento realizado pela Confederação da Agricultura e Pecuária do Brasil (CNA) em 2017 contabilizaram a comercialização de mais de 218 mil toneladas de beterraba vermelha no Brasil, produzidas em uma área com mais de 10 mil hectares, sendo o Paraná o principal estado produtor, seguido por Minas Gerais e São Paulo. Apesar de ser um tubérculo resistente, as perdas da produção de beterraba podem alcançar 20% da sua produção, sendo deixadas no campo ou descartadas no momento da classificação por não apresentarem padrão desejado pelo consumidor (TIVELLI *et al*., 2011; CNA, 2017).

Todas as partes da beterraba (raiz, talos e folhas) podem ser utilizados na alimentação humana, porém a raiz é a parte do vegetal com maior importância comercial. A raiz da beterraba costuma ser consumida crua, cozida ou em preparações alimentícias (saladas, sopas, assados e conservas) ou de bebidas, enquanto talos e folhas podem ser utilizados na preparação de receitas, como saladas e refogados (TIVELLI *et al*., 2011). Recentemente, com a demanda atual por alimentos nutritivos, com funcionalidades tecnológicas e de promoção à saúde, a indústria de alimentos e farmacêutica tem buscado na beterraba o desenvolvimento de produtos inovadores levando em consideração a presença de corantes naturais (betalaínas) com propriedades antioxidantes e outros compostos de interesse presentes em elevas quantidades (PUNIA BANGAR *et al*., 2022; THIRUVENGADAM *et al*., 2022).

A indústria alimentícia utiliza a beterraba na forma de suco, polpa, bagaço, pó e extratos, que podem ainda ser utilizados no desenvolvimento de produtos alimentícios funcionais enriquecidos, a exemplo de massas, produtos de panificação (pães, bolos e salgados), derivados lácteos (sorvetes, iogurtes, requeijão, manteiga e bebidas lácteas), salgadinhos extrusados, bebidas fermentadas, embutidos cárneos (salsichas, linguiças e hambúrgueres), películas e coberturas (PUNIA BANGAR *et al*., 2022).

A parte aérea da beterraba (talos e folhas) costuma servir apenas para a alimentação animal, fertilizante orgânico ou são descartados no ambiente, aumentando a geração de resíduos agroindustriais, embora estudos tenham apontado que alguns compostos fenólicos e minerais podem ser predominantes nessas porções do que na própria raiz da beterraba, indicando a necessidade de investigação sobre seu aproveitamento (COSTA *et al*., 2017; LORIZOLA *et al*., 2018; KALE *et al*., 2018; SLATNAR *et al*., 2015).

A beterraba possui em sua composição aporte de macro e micronutrientes importantes do ponto de vista nutricional, apresentando fibras dietéticas, minerais (potássio, sódio, ferro, cobre, magnésio, cálcio, fósforo e zinco), vitaminas (vitamina A, tiamina, riboflavina, niacina, ácido ascórbico e vitaminas do complexo B), aminoácidos essenciais e não essenciais, fitoesteróis, pigmentos antioxidantes, como as betalaínas, ácidos fenólicos, principalmente, ácido ferúlico, ácido siríngico, epicatequina e kaempferol. Também há um grande interesse sobre sua abundância de nitrato (NO_3^-), devido ao potencial benéfico para a saúde cardiovascular, com a produção endógena do óxido nítrico (NO) (BAIÃO; DA SILVA; PASCHOALIN, 2020; PANGHAL *et al*., 2017; SHALABY; HASSENIN, 2020).

A composição nutricional da beterraba fresca apresenta variabilidade com base na parte anatômica da planta (raiz, caule, folha e casca), das variedades genéticas, condições edafoclimáticas, condições de colheita e pós-colheita (CHHIKARA *et al*., 2019; SAWICKI, BĄCZEK E WICZKOWSKI, 2016; TAKACS-HAJOS; VARGAS-RUBOCZKI, 2022). Dados apresentados pela Tabela Brasileira de Composição de Alimentos (TBCA) e de estudos científicos publicados sobre a composição de nutrientes presentes na raiz, folhas e talos de beterraba são apresentados na Tabela 1.

Tabela 1. Composição nutricional aproximada da raiz, talos e folhas da beterraba vermelha.

Componentes	Quantidade por 100g		
	Raiz (TBCA, 2023)	Talos (Storck et al., 2013)	Folhas (Storck et al., 2013)
Umidade (%)	86,0	94,5	93,2
Valor energético (cal)	46	13,7	18,3
Proteína (g)	1,95	1,13	2,20
Lipídeo (g)	0,10	0,2	0,13
Carboidrato (g)	11,1	13,7	2,39
Fibra Alimentar (g)	3,37	0,73	0,74
Cinzas (g)	0,87	1,7	1,37
Cálcio (mg)	14,4	-	-
Ferro (mg)	0,32	-	-
Fósforo (mg)	19,4	-	-
Potássio (mg)	375,0	-	-
Vitamina C (mg)	3,2	-	-

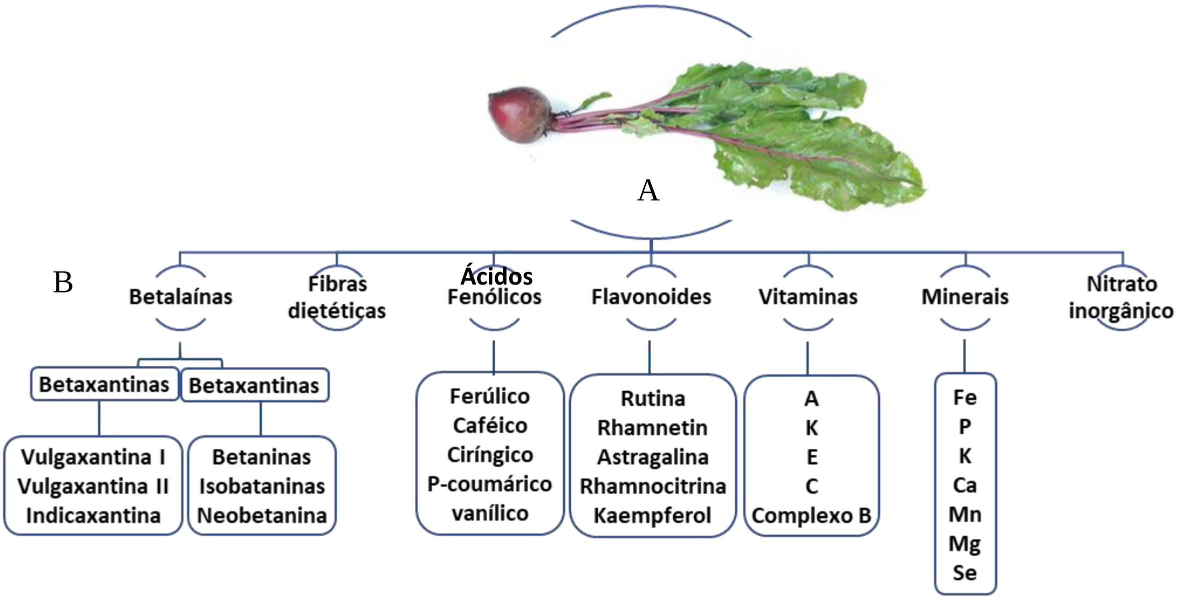
Fonte: Storck *et al*. (2013); TBCA, 2023.

A beterraba vermelha possui um aporte de fibras dietéticas solúveis, como a pectina, e insolúveis, como a celulose e algumas hemiceluloses, as quais podem derivar oligossacarídeos com diferentes estruturas e, consequentemente, com diferentes propriedades biológicas (HOLCK *et al*., 2011). A exploração integral de materiais derivados da beterraba tem se mostrado eficiente na produção enzimática de pectino-oligossacarídeos a partir da polpa ou de subprodutos fibrosos, produzindo carboidratos com propriedades físico-químicas e fisiológicas benéficas para humanos, tais como o estímulo seletivo de bactérias no cólon (CONCHA OLMOS; ZÚÑIGA HANSEN, 2012; DE OLIVEIRA *et al*., 2021; MARTINEZ *et al*., 2015).

A capacidade das pectinas e oligossacarídeos pécticos de estimular o crescimento de populações bacterianas específicas tem sido também descrita (DE OLIVEIRA *et al*., 2023a; GÓMEZ *et al*., 2019; MALIK, BORA; SHARMA, 2019).

Com base nos estudos disponíveis, a beterraba aparece como fonte de agentes promotores de saúde, com potencial de serem utilizados para a prevenção e tratamento terapêutico em diferentes patologias, podendo atuar na prevenão de de alguns tipos de câncer, doenças cardiovasculares, cerebrovasculares, hepáticas e renais (CHEN *et al*., 2021; CLIFFORD *et al*., 2015). A beterraba vermelha contém compostos bioativos que podem apresentar efeitos na promoção da saúde, incluindo as betalaínas, ácido ascórbico, flavonóides, carotenóides, polifenóis, saponinas e elevadas quantidades de nitrato, como mostra a Figura 2 (CLIFFORD *et al*., 2015; SHALABY; HASSENIN, 2020).

Figura 2. Principais nutrientes e compostos bioativos da beterraba vermelha.



Fonte: A: Autor, (2023); B: adaptado de Domínguez *et al*. (2020).

A beterraba e seus subprodutos são fonte de compostos fenólicos, como ácido gálico, vanilina, ácido hidroxibenzóico, ácido siríngico, cianidina-3-O-glicosídeo, catequina, ácido ferúlico. A beterraba também é um dos poucos vegetais que contêm um grupo de pigmentos denominado betalaínas com destacáveis propriedades bioativas (CLIFFORD *et al*., 2015; FU *et al*., 2020; TOSSI *et al*., 2021). As betalaínas são os pigmentos majoritários na beterraba, sendo caracterizadas como pigmentos nitrogenados hidrossolúveis derivados do ácido betalâmico e os principais responsáveis pelas propriedades antioxidantes do vegetal. As betalaínas podem ser classificadas em betacianinas (cor vermelho-violeta) e betaxantinas (amarelo-laranja), estando associadas com a coloração típica das raízes da beterraba (FU *et al*., 2020; TOSSI *et al*., 2021).

Diversas pesquisas com betalaínas provenientes da beterraba enfatizam seu potencial antioxidante e consequente efeito sobre a saúde (ASRA *et al*., 2020; BACKES; GENENA, 2020; KAPOOR; SAWICKI; WICZKOWSKI, 2018; FENG, 2022), bem como de betalaínas e outros bioativos de extratos da beterraba (EDZIRI *et al*., 2019; TUTUNCHI *et al*., 2019), de resíduos da produção de açúcar, sucos e outras formas alimentícias (GÓMEZ *et al*., 2019; MROCZEK *et al*., 2019), além de partes comestíveis não usuais, como talos e folhas (MROCZEK *et al*., 2019; NINFALI; ANGELINO, 2013). No entanto, diversas outras classes de compostos fenólicos também encontrados em beterrabas tem chamado atenção não somente por seu potencial antioxidante, mas por seus mecanismos de biotransformação e interação com a microbiota intestinal (RODRÍGUEZ-DAZA *et al*., 2021).

A pectina da beterraba sacarina estimulou o crescimento e atividade metabólica de espécies de *Bifidobacterium* e *Lactobacillus* em sistemas de fermentação inoculados com microbiota fecal (ONUMPAI *et al*., 2011), enquanto os oligossacarídeos pécticos extraídos da polpa também têm demonstrado destacável propriedade bifidogênica (GÓMEZ *et al*., 2016; GÓMEZ *et al*., 2019). A pectina da beterraba açucareira, constituída, principalmente, de ácido urônico (63 mol%), galactose (18 mol%) e arabinose (13 mol%), mostrou capacidade de aumentar a população de *Lactobacillus* e *Lachnospiraceae* no ceco de ratos *Wistar* (TIAN *et al*., 2016).

A administração de suco concentrado de beterraba por 14 dias afetou a abundância de alguns táxons do microbioma intestinal de adultos saudáveis, com aumento da população de *Akkermansia muciniphila* e diminuição da população de *Bacteroides fragilis* após três dias de consumo (WANG *et al*., 2023). Betacianinas nativas e catabólitos detectados nas fezes após o consumo do suco correlacionaram-se positivamente com a abundância de populações de *Bifidobacterium* e *Coprococcus* e inversamente com a abundância da população de *Ruminococcus*, causando aumento no teor de ácido (iso)butírico, sendo indicadores de efeito benefício da beterraba vermelha sobre a saúde intestinal e sistêmica (WANG *et al*., 2023).

Estudo com suco misto preparado com beterraba, maçã, limão e gengibre administrado a adultos saudáveis resultou em diminuição da população fecal de *Firmicutes* e *Proteobacteria* e aumento da população de *Bacteroidetes* e *Cyanobacteria* nas fezes após 15 dias de consumo. Ainda, foram observados efeitos adicionais, como perda de peso, aumento da produção de vasodilatador NO- e diminuição da oxidação lipídica, que foram associados com o elevado conteúdo de nitratos no suco em decorrência do uso de beterraba como ingrediente (HENNING *et al*., 2017).

Estudos com sistemas de fermentações *in vitro* com beterraba ou oligossacarídeos derivados da beterraba têm indicado que esses componentes podem agir como substrato, estimulando o crescimento de diferentes cepas probióticas (DE OLIVEIRA *et al*., 2023a; GÓMEZ *et al*., 2019; MALIK, BORA, SHARMA, 2019).

Substâncias pécticas extraídas da beterraba têm demonstrado capacidade de melhorar a integridade intestinal e proliferação da mucosa em estudos *in vivo*, além de favorecer a adesão de cepas de *Lactobacillus* às células epiteliais (LARSEN *et al*., 2018; PARKAR; TROWER; STEVENSON, 2013). Similarmente, pectinoligossacarídeos extraídos de subprodutos de beterraba, quando adicionados em caldo de fermentação, têm demonstrado efeitos estimuladores do crescimento de cepas de *Lactobacillus* e *Bifidobacterium*, em particular, *L. reuteri*, *L. rhamnosus* e *L. plantarum*, demostrando também produção acentuada de ácido lático, acético e fórmico (GÓMEZ *et al*., 2019).

A inserção de matrizes contendo beterraba também pode melhorar a sobrevivência de bactérias probióticas às condições adversas da digestão intestinal. *Lacticaseibacillus paracasei* LS14 foram encapsulados em hidrogéis produzidos com proteína de soja e pectina de beterraba, o que resultou em maior capacidade de sobrevivência da cepa após simulação da digestão gastrointestinal. A estrutura mais densa do gel formado quando da exposição as condições gastrointestinais foi relacionana com a menor degradação mecânica e sensibilidade de *L. paracasei* a ação das enzimas e variação de pH utilizado no ensaio (YAN *et al*., 2021).

Ainda, foi relatado que pectinas de beterraba sacarina com alto teor de metoxilação têm potencial para melhorar a sobrevivência de espécies probióticas de *Lactobacillus* expostas a estresses gastrointestinais, como baixo pH e enzimas digestivas, indicando que características estruturais como grau de metoxilação e ramificação e interações eletrostáticas entre o substrato e célula bacteriana estão relacionados com o efeito de proteção (LARSEN *et al*., 2018).

Baseado nessas evidências, a beterraba vermelha, incluindo raiz, talos e folhas, além de possuir um aporte de nutrientes de elevado interesse para os consumidores e indústria de alimentos, apresenta propriedades bioativas resultantes, principalmente, da presença de fibras, betalaínas e compostos fenólicos, que podem conferir uma gama de benefícios à saúde, sejam provenientes do consumo *in natura* ou processado na forma de sucos e pós dessa espécie vegetal, como também por meio de substâncias isoladas da raiz ou de seus subprodutos. A crescente pressão do mercado de alimentos devido ao contínuo crescimento populacional e ao aumento das preocupações com a alimentação saudável e sustentável tem incentivado a busca por fontes alternativas de alimentação para atender às necessidades nutricionais da população (MILIÃO *et al*., 2022). Apesar das evidências dos benefícios nutricionais e funcionais da beterraba, ainda são escassos os estudos que investigam o potencial das partes menos consumidas dessa planta, como os talos e folhas. O aproveitamento dessas partes da beterraba pode reduzir o desperdício de alimentos e enriquecer o valor nutricional das refeições, tornando-as uma opção relevante como Planta Alimentícia Não Convencional (PANC). Portanto, explorar o uso dos talos e folhas de beterraba pode representar uma oportunidade para diversificar a oferta alimentar e promover a sustentabilidade, com uma abordagem alinhada as práticas da economia circular que prioriza o uso dos recursos naturais em processos integrados de inserção em novos ciclos, otimizando os processos produtivos (LORIZOLA *et al*., 2018).

2.2 COMPONENTES PREBIÓTICOS

A comunidade científica e o perfil dos consumidores atuais estão interessados em alimentos e hábitos alimentares mais saudáveis, refletindo, assim, no crescente mercado de alimentos que contêm ingredientes e/ou microrganismos promotores de saúde (DAMIÁN *et al*., 2022; VICENTINI; LIBERATORE; MASTROCOLA, 2016). Não existe consenso científico sobre a definição de alimento funcional, sendo essencialmente um termo de mercado com diferentes definições e regulamentos, dependendo do país, mas que sempre inclui alimentos que trazem, além da sua função nutricional, algum benefício para a saúde do consumidor (BUCALOSSI *et al*., 2020; SZAKÁLY *et al*., 2019).

A definição de prebiótico passou por atualizações ao longo do tempo. A definição aceita mais amplamente ocorreu em 1995, por Gibson e Roberfroid, que ditaram como "um ingrediente alimentar não digerível que afeta beneficamente o hospedeiro ao estimular seletivamente o crescimento e/ou atividade de uma ou de um número limitado de bactérias no cólon e, assim, melhora a saúde do hospedeiro" (GIBSON *et al*., 1995). Recentemente, a Associação Científica Internacional de Probióticos e Prebióticos (ISAPP) convocou um painel de especialistas para revisar a definição de prebióticos, atualizando o termo para a seguinte definição: “um substrato fermentável que alcance o cólon humano e seja seletivamente fermentado por microrganismos hospedeiros, conferindo, assim, efeitos benéficos à saúde” (GIBSON *et al*., 2017).

Anteriormente, os estudos para a identificação de substratos prebióticos se concentravam na avaliação de carboidratos, particularmente de oligossacarídeos. No entanto, uma variedade de novos componentes, a exemplo de compostos fenólicos, peptídeos e ácidos graxos provenientes de diversas fontes alimentares, têm despertado interesse em relação às suas propriedades prebióticas (CUNNINGHAM *et al*., 2021; GÓMEZ *et al*., 2016; LI *et al*., 2016; ZHANG; JU; ZUO, 2018).

Para considerar um ingrediente alimentar como prebiótico, tal ingrediente deve cumprir alguns requisitos: (a) Apresentar resistência à acidez gástrica; (b) não deve ser hidrolisado por enzimas de mamíferos e nem absorvido na parte superior do trato gastrointestinal; (c) deve ser fermentado seletivamente por bactérias intestinais, estimulando seu crescimento; e (d) tornar-se metabolicamente ativo alterando a microbiota colônica em direção a uma composição associada à saúde e bem-estar (SENÉS-GUERRERO *et al*., 2020).

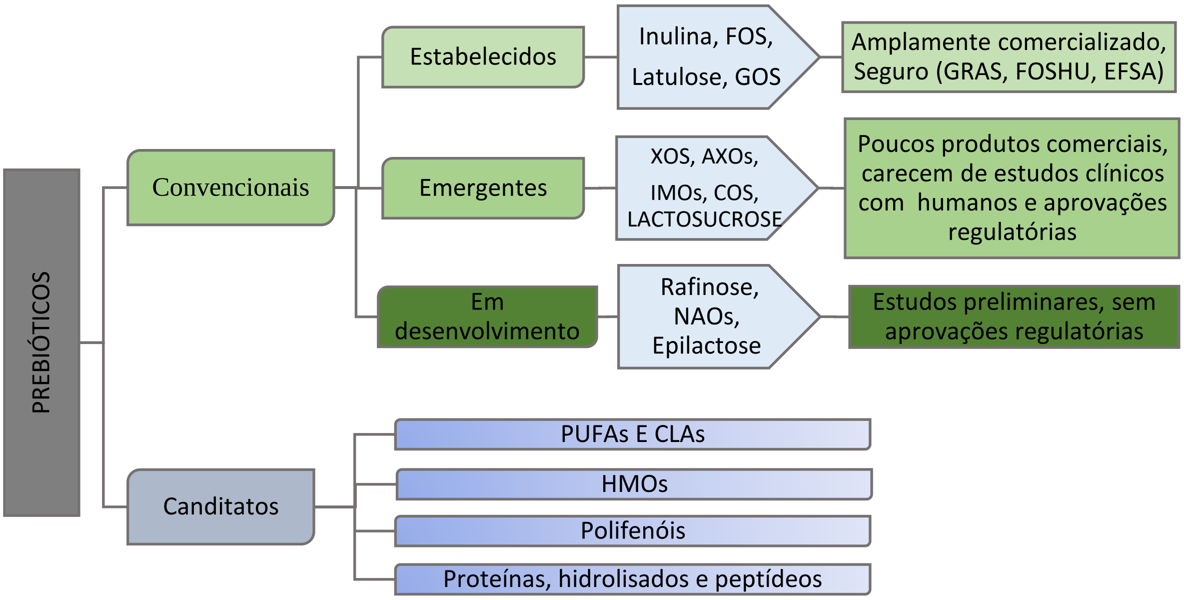
Os benéficos causados pelo consumo de prebióticos não se limitam ao estímulo da multiplicação da microbiota intestinal benéfica, estando também associado com diferentes benefícios à saúde humana, incluindo a modulação do sistema imunológico, melhoria da função gastrointestinal, aumento da absorção de minerais, modulação do metabolismo energético, redução do risco de infecções intestinais (SANDERS *et al*., 2019; WANG *et al*., 2012), melhora da motilidade gastrointestinal e redução dos níveis de colesterol sérico (ASHAOLU, 2020; ROBERFROID *et al*., 2010; SANDERS *et al*., 2019).

Nessa perspectiva, mercado global de prebióticos tem crescido nos últimos anos, e, por isso, o aumento no interesse no desenvolvendo de novas alternativas para obter processos sustentáveis e eficientes para aplicação em larga escala (DONG *et al*., 2023; MANO *et al*., 2018). Os prebióticos mais documentados até o momento são oligossacarídeos resistentes, como frutanos (frutoligosacarídeos, oligofrutose e inulina) e galactanos. No entanto, a busca por novas substâncias alternativas que cumpram a função prebiótica tem aumentado, expandido também às suas possíveis fontes. Estudos sobre novas fontes de substâncias prebióticas, bem como abordagens variadas sobre o impacto do consumo na forma nutracêutica ou como constituintes de uma matriz alimentar, têm sido amplamente abordados, relacionados, principalmente, à presença de uma mistura eficiente de compostos bioativos, antioxidantes e fibras alimentares nesses novos alimentos fontes (FERREIRA *et al*., 2020; KHANGWAL; SHUKLA, 2019).

Além das substâncias à base de carboidratos derivadas de plantas, substâncias à base de leveduras e não carboidratos**,** incluindo compostos fenólicos, ácidos graxos polinsaturados (PUFAs) e outros substâncias, têm sido reportados como candidatos a uso como prebióticos (Figura 3). Porém, mais estudos são necessários para comprovar esses efeitos, especialmente em humanos (CARDOSO *et al*., 2021; CUNNINGHAM *et al*., 2021; REZENDE; LIMA;

NAVES, 2021). Tem sido discutido não apenas sobre novas fontes de prebióticos emergentes, mas também sobre suas características físico-química, mercado, legislação, atividade biológica e aplicação tecnológica (CARDOSO *et al*., 2021 PLAMADA, VODNAR, 2022).

Figura 3. Classificação dos prebióticos de acordo com seu desenvolvimento e "status" regulatório.



Fonte: Adaptado de Cardoso *et al*., (2020).
Legenda: FOS (frutooligossacarídeos), GOS (galactooligossacarídeos), XOS (xilooligossacarídeos), AXOS (arabinoxilooligossacarídeos), IMOs (isomaltooligossacarídeos), HMOs (Oligossacarideos do leite humano), COS (quitooligossacarídeos), NAOs, neogaro-oligossacarídeos, PUFAs (ácidos graxos polinsaturados) e CLAs (ácido linoleico conjugado), GRAS (Generally Recognized as Safe), FOSHU (Food for Specified Health Uses), EFSA (European Food Safety Authority).

Dados científicos apotam diferentes candidatos como potenciais prebióticos, os quais apresentam em comum alta resistência à digestão, habilidade de estimular o crescimento de bactérias benéficas e de produzir ácidos graxos de cadeia curta (AGCC). Dentre esses tem-se: xilooligosacarídeos (XOS) (compostos por cadeias de resíduos de xilose) (AMORIM *et al*., 2020a), isomalto-oligossacarídeos (IMOs) (mistura de oligossacarídeos compostos por uma unidade de maltose e até oito unidades de glicose unidas por ligações glicosídicas) (WU; JOHNSON-HENRY; SHERMAN, 2020), quito-oligossacarídeos solúveis em água (COS, quitosana ou quitina despolimerizada, proveniente de exoesqueletos de artrópodes) (LIU *et al*., 2020), lactosacarose (trisacarídeo composto por glicose, galactose e frutose) (LI *et al*., 2016), rafinose (resíduos de galactose, glicose e frutose) (AMORIM *et al*., 2020b; MAO *et al*., 2018), oligossacarídeos do leite humano (HMOs) (RAY *et al*., 2019; CHENG; YEUNG, 2021), ácidos graxo polinsaturados (PUFFAs, compostos por um ácido carboxílico e uma cauda alifática que contém mais de uma ligação dupla carbono-carbono) (LI *et al*., 2019) e proteínas (proteínas, hidrolisados de proteínas e peptídeos) (ZHANG *et al*., 2020).

A pectina é um heteropolissacarídeo abundante na parede celular primária e lamela média das plantas, formada por homogalacturonana (HG), ramnogalacturonana I (RGI), ramnogalacturonana II (RGII) e xilogalacturonana (XG), que podem se organizar em esruturas diversas de acordo com a fonte e o método de extração (ZDUNEK; PIECZYWEK; CYBULSKA, 2021). Pectinas tem se mostrado como candidato a prebiótico, uma vez que não é digerida no trato gastrointestinal, sendo fermentada pela microbiota colônica humana (SCOTT *et al.,* 2020). Estudos têm demonstrado que a pectina de diferentes fontes, como algas marinhas, soja ou cítricos, pode ser fermentada por membros dos filos *Bacteroidetes* e *Firmicutes* e produzir metabólitos relacionados a benefícios sobre a saúde intestinal (ELSHAHED *et al*., 2021; HURTADO-ROMERO *et al*., 2020).

As propriedades estruturais da pectina, incluindo o grau de esterificação e as cadeias ramificadas, desempenharam papéis críticos na diversidade, enterótipos ou composição distinta da microbiota intestinal (TAN; NIE, 2020). Pesquisa recente realizou a administração de dieta rica em gordura contendo diferentes concentrações (0%, 1%, 3%, 5%, 8% ou 12%) de pectina de maçã por oito semanas em camundongos C57BL/6J, proporcionando aumento das populações de bactérias que degradam a pectina, a exemplo de *Faecalibaculum* e *Ruminococcus,* redução de lesões colônicas, aumento da produção de AGCC (dose dependente) e modulação da microbiota intestinal, onde as populações de *Lactobacillus, Faecalibaculum* e *Coriobacteriaceae* apresentaram correlações positivas com os níveis de acetato, propionato e butirato (ZHAO *et al*., 2022).

A despolimerização da pectina resulta em pectinoligosacarídeos (POS) que podem ser usados como novos ingredientes funcionais com efeitos prebióticos. Esses oligossacarídeos estruturalmente complexos têm se mostrado melhores candidatos prebióticos do que as pectinas nativas em ensaios *in vitro* e *in vivo* (GÓMEZ *et al*., 2016; MIGUEZ *et al*., 2020; YEUNG *et al*., 2021).

Efeitos da pectina ou POS derivados da beterraba sobre a composição da microbiota intestinal foram recentemente revisados, mostrando que POS da beterraba podem modular positivamente a composição e atividade da microbiota intestinal, destacando-se os efeitos bifidogênicos, além de estimular o crescimento e metabolismo de probióticos, o que pode induzir efeito semelhante ao prebiótico (DE OLIVEIRA *et al*., 2021).

Além das fibras, compostos fenólicos também apresentam potencial atividade prebiótica, uma vez que, quando atingem o cólon, entram em contato direto com os microrganismos intestinais, proporcionando interação complexa e multidirecional (MASSA *et al.*, 2020; SUN *et al.*, 2018). Compostos fenólicos são metabólitos secundários sintetizados pelas plantas e alguns outros organismos em situações adversas, como na presença de patógenos ou condições climáticas não favoráveis.

Os compostos fenólicos são categorizados como flavonoides (flavonas, flavanonas, flavonóis, flavanóis, isoflavonas e antocianinas) e moléculas não flavonoides (ácidos fenólicos, ácidos hidroxicinâmicos, lignanas, estilbenos e taninos) (DI LORENZO *et al.*, 2021). Em sua maioria, os compostos fenólicos são encontrados na forma conjugada (glicosilada) ou ligados com macromoléculas de alta complexidade, como fibras dietética, apresentando baixa absorção no intestino delgado (ALVES-SANTOS *et al.*, 2020).

A biotransformação de compostos fenólicos derivados da dieta pode estimular o crescimento de bactérias probióticas específicas, bem como de bactérias comensais que compõem a microbiota intestinal (principalmente *Lactobacillus* spp., *Bifidobacterium* spp., mas também *Akkermansia* spp., *Roseburia* spp. e *Faecalibacterium* spp.), influenciando aspectos como adesão, crescimento, sobrevivência e funcionalidade, além de modular a composição da microbiota intestinal por meio da inibição de bactérias patogênicas (MOORTHY *et al*., 2020; OZDAL *et al*., 2016; SANDERS *et al*., 2019 SINGH *et al*., 2019). Outras interações mutuamente benéficas entre compostos fenólicos, microbiota intestinal e hospedeiro têm sido relatadas, como aumento da bioacessibilidade e biodisponibilidade desses compostos durante a fermentação colônica (ALVES-SANTOS *et al*., 2020; MARTINOV *et al*., 2017; MA; CHEN, 2020).

Há exemplos na literatura sobre compostos fenólicos dietéticos com promissora atividade prebiótica, sendo provenientes de diferentes fontes alimentares, a exemplo de vinho tinto (NAUMOVSKI; PANAGIOTAKOS; D'CUNHA, 2020), subprodutos do processamento da jabuticaba (MASSA *et al*., 2020; SUN *et al*., 2018), extratos de frutas (ALVES-SANTOS *et al*., 2020; COMAN *et al*., 2018; PENG *et al*., 2021) e chás (SUN *et al*., 2018).

Ensaios *in vitro* mostraram que compostos fenólicos, como catequina, ácido gálico, ácido protocatecuico e vanílico, foram capazes de estimular o crescimento de *L. rhamnosus* GG ATCC 53103 e *L. acidophilus* NRRLB 4495 em meio laboratorial, além de inibir o crescimento de *Escherichia coli* e *Salmonella* Typhimurium (PACHECO-ORDAZ *et al*., 2018). Antocianinas dos frutos de *Lycium ruthenicum* Murray ("goji berry") promoveram aumento da abundância relativa de *Lactobacillus* spp. e *Bifidobacterium* spp. e redução da abundância relativa de *Escherichia* spp./*Shigella* spp. na microbiota intestinal de indivíduos com síndrome inflamatória intestinal (PENG *et al*., 2021).

À medida que o foco na sustentabilidade, custos e escala de produção emergem, novas fontes naturais são buscadas, com premissa tanto na valorização de resíduos e subprodutos vegetais por meio da produção de novos substratos direcionados à modulação da microbiota intestinal, quanto como estratégia de aproveitamento integral dos recursos naturais, redução do desperdício e menor geração de resíduos (CUI *et al*., 2019; SABATER *et al*., 2021). Subprodutos agroindustriais, como resíduos de processamento, cascas, sementes, talos e folhas, também têm sido apontados como fontes promissoras de ingrediente de valor agregado, como fibras alimentares e compostos fenólicos com potencial efeito prebiótico (MENEZES *et al*., 2021; SERRANO-CASAS *et al*., 2017).

Pesquisas com subprodutos vegetais, a exemplo de subprodutos da beterraba açucareira (LARSEN et al., 2019; GÒMEZ et al., 2019, YU et al., 2022), caju (fibra) (DUARTE *et al*., 2017; MENEZES *et al*., 2021b) e abacaxi (casca e caule) (CAMPOS *et al*., 2020), grãos de café verde (DESAI *et al*., 2016), agave e farinha de banana verde (ALVARADO-JASSO *et al*., 2020), vagens de *Prosopis nigra* como substrato para produção enzimática de FOS (ROMANO *et al*., 2019) e macroalgas marinhas (PRAVEEN *et al*., 2019), indicam o potencial prebiótico desses materiais, uma vez que atuaram diretamente sobre a sobrevivência e estimulação de bactérias benéficas e produção de metabólitos importantes, como os AGCC.

2.3 EFEITO DE DIFERENTES SUBSTRATOS ALIMENTARES SOBRE A MICROBIOTA BACTERIANA INTESTINAL

O intestino humano é colonizado por uma grande variedade de microrganismos vivos, que formam um ecossistema dinâmico essencial para a manutenção da saúde humana. Estima-se, que o número de microrganismos que colonizam o intestino, denominado 'microbiota intestinal, seja dez vezes maior do que o de células do corpo humano e carregue 150 vezes mais genes do que o genoma humano inteiro (TSIGALOU *et al*., 2021).

Nos últimos anos, o interesse por maiores esclarecimentos sobre a relação entre a composição e função da microbiota colônica humana tem aumentado exponencialmente. São apontados como dominantes na microbiota intestinal os filos bacterianos *Firmicutes*, *Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria* e *Verrucomicrobia*, com os dois filos *Firmicutes* e *Bacteroidetes* representando 90 % da microbiota intestinal. O filo *Firmicutes* é composto por mais de 200 gêneros diferentes, como *Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus* e *Ruminococcus*. *Bacteroides* e *Prevotella* consistem nos gêneros predominantes no filo *Bacteroidetes*. O filo *Actinobacteria* é proporcionalmente menos abundante, sendo representado principalmente pelo gênero *Bifidobacterium* (RINNINELLA *et al*., 2019a; 2019b; RUAN *et al*., 2020; VAN TREUREN; DODD, 2020).

Cada indivíduo alberga um padrão único de composição bacteriana, determinado em parte pelo genótipo do hospedeiro e pela colonização inicial que ocorre com a transmissão materno-fetal durante a gravidez, onde o histórico genético do recém-nascido, a microbiota intestinal materna, o ambiente intrauterino, o tipo do parto, a alimentação e o ambiente externo ao qual é exposto podem influenciar a colonização microbiana do seu trato gastrointestinal (VANDENPLAS *et al*., 2020; YANG *et al*., 2021). Na fase adulta, a microbiota intestinal de um indivíduo pode sofrer variações na sua diversidade devido a influências externas, como padrões dietéticos, uso de antibióticos, estilo de vida, estresse ambiental, status socioeconômico e geografia (CHENG; NING, 2019; DAVENPORT *et al*., 2014; RUAN *et al*., 2020). A composição genética microbiana também pode mudar com o tempo, por exemplo, devido à ocorrência de mutagênese (SUN *et al*., 2020).

Diferentes estudos definem que a microbiota intestinal tem um papel fundamental na mediação do metabolismo humano, atuando na produção de vitaminas, aminoácidos, AGCC, manutenção da barreira epitelial, inibição da adesão de patógenos nas superfícies intestinais, degradação de fontes de carbono originalmente não digeríveis e modulação do sistema imunológico (CHEN *et al*., 2021; SÁNCHEZ *et al*., 2017).

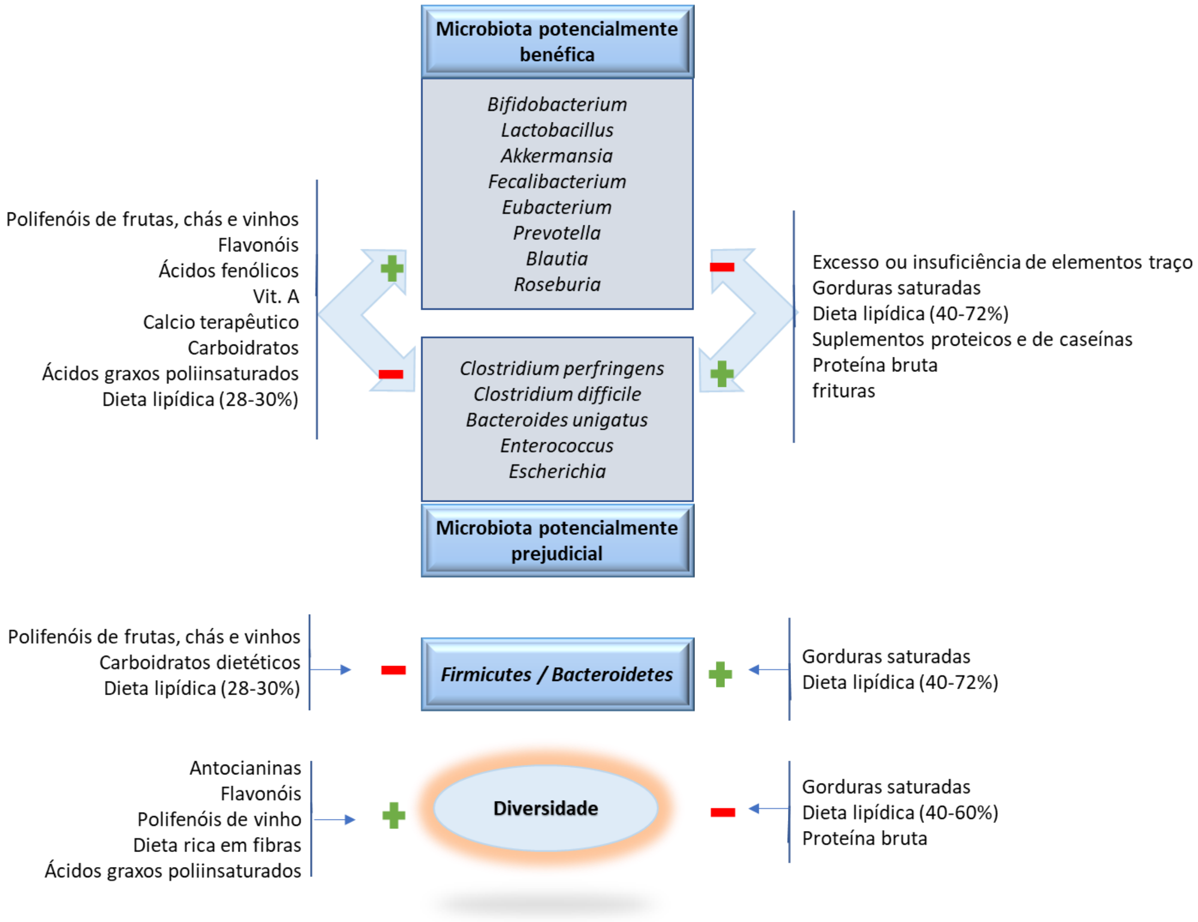
A microbiota pode vir a ser considerada saudável quando se mostra diversificada, equilibrada e resiliente, com capacidade de retornar a um estado de equilíbrio após perturbações. Esse equilíbrio pode ser chamado de homeostase, enquanto as alterações na composição e função da microbiota levam ao conceito de disbiose, que podem estar associadas com a presença de várias doenças crônicas, como disturbuios intestinais (doença celíaca, síndrome do intestino irritável, doença inflamatória intestinal) e extraintestinais (doenças cardiovasculares, hepáticas e renais, síndrome metabólica, alergias, obesidade) (ANDRADE *et al*., 2020; CARDING *et al*., 2015; MENEZES *et al*., 2021a; RUAN *et al*., 2020).

Evidências científicas demonstram que remodelar as interações hospedeiro-microbiota por meio da nutrição é uma via terapêutica para promoção da saúde e prevenção de doenças. A dieta é um importante determinante da configuração da microbiota, sendo o alimento a principal fonte de energia para os microrganismos intestinais. Assim, mudanças nos padrões de macronutrientes dietéticos, principalmente carboidratos, podem resultar em variações rápidas na estrutura populacional da microbiota intestinal por meio da modulação da abundância de determinadas espécies e das suas funções individuais ou coletivas (Figura 4) (KOLODZIEJCZYK; ZHENG; ELINAV, 2019; RAMOS; MARTÍN, 2021; YANG *et al*., 2020; ZHANG; JU; ZUO, 2018).

Padrões dietéticos podem influenciar fortemente a composição da microbiota intestinal, podendo, em alguns casos, como nas dietas pobres em fibras, reduzir a diversidade microbiana intestinal em relação aos filos e gêneros, levando à disbiose, com alteração da função de barreira e permeabilidade e ativação anormal de células imunes, repercutindo em alta incidência de doenças crônicas (RINNINELLA *et al*., 2019b; YANG *et al*., 2020).

Figura 4. Efeito dos nutrientes na microbiota intestinal

.



Fonte**:** adaptada de Yang *et al*. (2020).

Padrões alimentares, como vegetariano e vegano, tendem a promover maior riqueza de *Bacteroidetes* na microbiota intestinal quando comparada a dietas onívoras (LOSASSO *et al*., 2018). Dietas cetogênicas resultam em aumento da ingestão de gorduras e redução da ingestão de carboidratos, causando diminuição do conteúdo de polissacarídeos associado com diminuição das populações de microrganismos intestinais benéficos, a exemplo de bifidobactérias (RINNINELLA *et al.*, 2019b). O uso de dieta cetogênicas em crianças promovem diferenças na composição taxonômica e funcional da microbiota, apresentando diminuição na abundância de bifidobactérias, *Eubacterium rectale* e *Dialister* e aumento de *Escherichia coli* (LINDEFELDT *et al.*, 2019).

As mudanças cíclicas na microbiota intestinal humana devido à variação sazonal na dieta, especialmente para pessoas que vivem em sociedades tradicionais, também é um exemplo relevante de como a dieta tem grande influência na formação da microbiota (KOLODZIEJCZYK; ZHENG; ELINAV, 2019). A dieta mediterrânea, que é amplamente aceita como padrão de dieta saudável, por ser baseada no consumo de uma variedade de alimentos vegetais, com alto percentual de fibras, acarreta em um perfil bacteriano com maior abundância de *Bacteroidetes* e menor proporção de *Firmicutes* em relação aos *Bacteroidetes* (TSIGALOU *et al.*, 2021).

Padrão alimentar caracterizado pela baixa ingestão de fibras dietéticas e maior consumo de gordura e açúcar, pode reduzir a diversidade microbiana. O baixo consumo de fibra alimentar prejudica a microbiota intestinal, resultando em disfunções que contribuem para o aumento de doenças, podendo levar também ao desaparecimento de cepas bacterianas benéficas (SONNENBURG *et al.*, 2016; ZHANG; JU; ZUO, 2018).

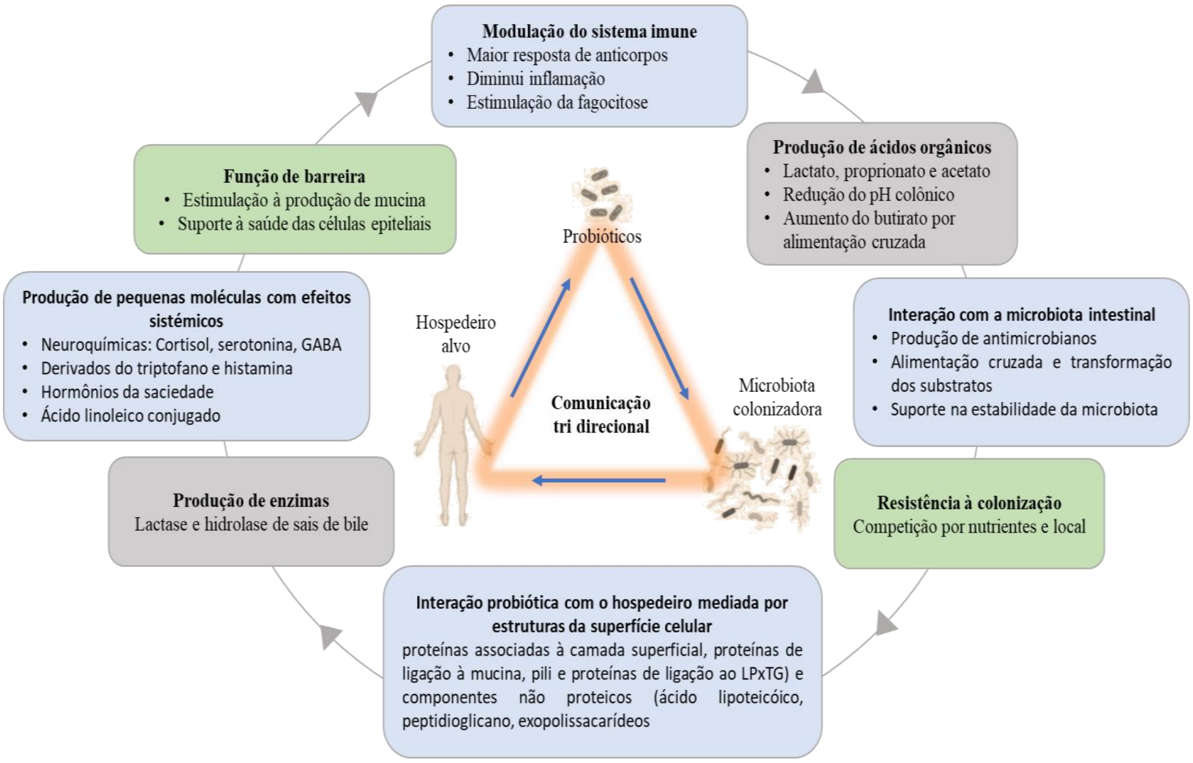
Carboidratos podem ser absorvidos parcialmente no intestino delgado, a exemplo de monossacarídeos e dissacarídeos, enquanto outros mais complexos, como as fibras, que não sofrem digestão, são fermentados no cólon por microrganismos presentes neste ambiente. Quando os carboidratos não digeridos chegam ao cólon, estruturas complexas são hidrolisadas por enzimas extracelulares produzidas pela microbiota, que clivam esse substrato em uma estrutura mais simples que pode ser importada para a célula para ser fermentada, gerando ATP e metabólitos, como ácidos orgânicos (GONG *et al.*, 2020; PAYLING *et al.*, 2020).

Estudos revelam que os prebióticos geralmente causam aumento da prevalência de *Bifidobacterium*, seguido por aumento de *Lactobacillus*, os quais costumam ser probióticos marcadores de prebiose por excelência (CATARINO *et al.*, 2021; WANG *et al.*, 2020), embora a definição atual de prebiótico permita a ampliação dos microrganismos alvo, englobando algumas espécies de gêneros antes reconhecidas como patogênicas. Algumas fibras dietéticas, incluindo arabinoxilanos, galacto-oligossacarídeos, inulina e oligofrutose, podem modular positivamente a microbiota, aumentando a população de bactérias benéficas, como

Bifidobacterium, *Lactobacillus*, *Akkermansia*, *Fecalibacterium*, *Roseburia*, *Bacteroides*, *Prevotella*, *Clostridium* e *Ruminococcus* (YANG *et al*., 2020).

Nesse contexto, ação prebiótica pode ocorrer sobre microrganismos conhecidos como probióticos. Os probióticos podem ser definidos como microrganismos vivos que, quando administrados em quantidades adequadas, conferem benefícios à saúde do hospedeiro (HILL *et al*., 2014), com mecanismos de ação relacionados a proteção contra patógenos, modulação da microbiota intestinal, produção de enzimas e AGCC, restauração da homeostase intestinal com melhora na função de barreira e modulação do sistema imune, como apresentado na Figura 5 (SANDERS et al., 2019; YANG et al., 2020).

Figura 5. Mecanismo de ação dos probióticos



Fonte: Adaptado de Sanders *et al*., 2019.

Bifidobacterium e *Lactobacillus* são considerados os principais alvos microbianos para a açao prebiótica devido aos seus resultados benéficos na saúde intestinal com resultados sistêmicos (HILL *et al*., 2014; SANDERS *et al*., 2019). O gênero *Lactobacillus* é amplamente distribuído na natureza, sendo isolado de várias matrizes alimentares, incluindo materiais vegetais, produtos lácteos, produtos fermentados e leite materno. As cepas desse gênero geralmente receberam uma presunção qualificada de segurança (QPS) da “European Food Safety Authority” (EFSA) e “status” de geralmente reconhecido como seguro (GRAS) da “United States Food and Drug Administration” (US FDA), sendo algumas amplamente comercializadas, a exemplo de *L. acidophillus* La-05 e *L. paracasei* L-26 (CUI & CU, 2021; EFSA, 2017; ZHANG *et al*., 2018). Outras cepas, a exemplo de *Limosilactobacillus fermentum* 296, tem sido citada como segura e capaz de exercer diversos efeitos positivos sobre a saúde do hospedeiro em diversos ensaios *in vitro* e *in vivo* (CRUZ NETO *et al*., 2023; DE ARAÚJO et al., 2022; DE ALBUQUERQUE *et al*., 2018).

As populações de algumas bactérias intestinais (por exemplo, *Bacteroides* e *Clostridium*) tendem a diminuir após a ingestão de prebióticos, possivelmente devido à inibição competitiva de outras espécies colonizadoras que fermentam preferencialmente os prebióticos no intestino, bem como pela produção de ácidos orgânicos, especialmente os AGCC. Os AGCC induzem alterações no microambiente intestinal, promovendo a redução do pH colônico, impedindo que bactérias patogênicas se adaptem facilmente a esse ambiente (RAMOS; MARTÍN, 2021; WANG *et al*., 2020). Um estudo de revisão sobre o uso de prebióticos para alterações na microbiota intestinal apontou que, em ensaios com amostras fecais humanas, há evidência de aumentos significativos para o gênero *Bifidobacterium,* após a intervenção com prebióticos (WANG *et al*., 2020).

Além dos carboidratos, estudos recentes têm apontado que a interação entre compostos fenólicos e microrganismos benéficos da microbiota intestinal trazem benefícios à saúde por meio da estimulação do crescimento, sobrevivência e funcionalidade de microrganismos probióticos, bem como pela atividade dos metabólitos resultantes da sua biotransformação, sugerindo-os como compostos prebióticos (DE SOUZA *et al*., 2019; WANG; QI; ZHENG, 2022). Compostos fenólicos dietéticos são apontados como fortes candidatos a prebióticos, com potencial de modular a microbiota intestinal, em especial a diversidade bacteriana e proporção entre *Firmicutes/Bacteroidetes,* com ênfase em espécies probióticas pertencentes aos gêneros *Lactobacillus* (PARKAR; TROWER; STEVENSON, 2013; WANG *et al*., 2018 WANG, QI, ZHENG, 2022).

Sendo a beterraba uma hortaliça com aporte considerável de nutrientes e bioativos, com todas as suas porções anatômicas ricas em betalaínas, compostos fenólicos e fibras dietéticas, se faz necessário a busca por possibilidades de seu aproveitamento integral como ingrediente funcional. A identificação de possíveis propriedades benéficas de diferentes partes da beterraba, como estimulação do crescimento, proteção frente aos efeitos adversos da digestão gastrointestinal por meio da manutenção da funcionalidade e da capacidade de sobrevivência de probióticos, bem como dos seus impactos sobre a microbiota intestinal humana, torna-se importante para valorização tanto da raiz quanto dos subprodutos da beterraba, estando alinhados com a perspectiva agroalimentar sustentável e de economia circular.

No contexto atual da produção de alimentos, os processos de fabricação são otimizados buscando menor dependência de matérias primas virgens, priorizando insumos renováveis, aliando, assim, o desenvolvimento econômico ao melhor uso dos recursos naturais (JIMENEZ-LOPEZ *et al*., 2020). Toma-se, como exemplo, a cultura da beterraba, onde partes comestíveis não usuais, como talos e folhas, bem como beterrabas fora do padrão de comercialização podem ser utilizadas para a produção de ingredientes de valor agregado para a indústria alimentícia e farmacêutica.

Uma abordagem relevante sobre a temática da tese é apresentada no artigo de revisão intutulado como “A review on bioactive compounds of beet (*Beta vulgaris* L. subsp. vulgaris) with special emphasis on their beneficial effects on gut microbiota and gastrointestinal health”, publicado no periódico “Critical Reviews in Food Science and Nutrition” no ano de 2020 (APÊNDICE B), o qual foi um dos produtos dessa tese. A revisão fornece uma atualização e discute a literatura disponível sobre os nutrientes e compostos bioativos presentes na beterraba e sua capacidade de causar efeitos benéficos sobre a microbiota intestinal, com repercussões em diversos parâmetros indicadores da saúde intestinal. A publicação do artigo de revisão na tese está em consonância com a política de permissões para uso acadêmico descrito no “Taylor & Francis Journal” e encontra-se disponível para acesso em https://doi.org/10.1080/10408398.2020.1768510

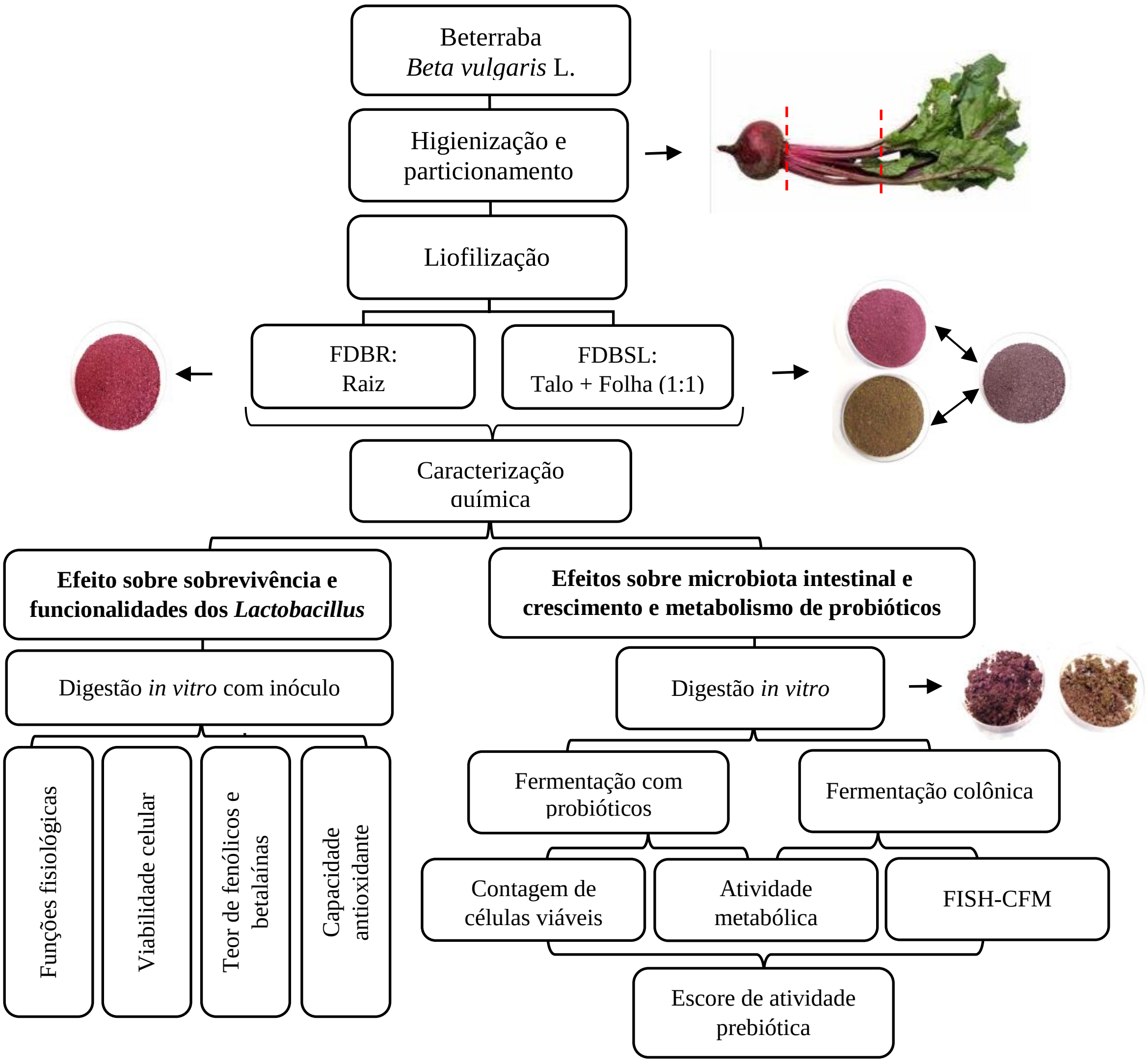
3 MATERIAIS E MÉTODOS

3.1 LOCAL DA PESQUISA E DELINEAMENTO EXPERIMENTAL

Os experimentos foram realizados, em sua maioria, no Laboratório de Microbiologia e Bioquímica de Alimentos (LMBA) do Departamento de Nutrição - Universidade Federal da Paraíba (UFPB) - Campus I, João Pessoa – PB. As análises cromatrográficas ocorreram no Laboratório Experimental de Alimentos (LEA) - Departamento de Tecnologia de Alimentos - Instituto Federal do Sertão Pernambucano (IF Sertão-PE), Petrolina – PE.

Inicialmente, os liofilizados da beterraba foram obtidos após higienização e porcionamento do vegetal. A parte raiz foi denominada FDBR e a mistura de talo e folhas de FDBSL. FDBR e FDBSL foram submetidos às análises de determinação da composição nutricional e parâmetros físico-químicos, seguido por digestão gastrointestinal *in vitro*, onde as cepas de *Lactobacillus* testadas foram expostas aos liofilizados de beterraba, sendo avaliados parâmetros relacionados a fisiologia e viabilidade celular bacteriana, capacidade antioxidante total e compostos fenólicos totais durante as três fases principais da digestão (boca, estômago e intestino). Em segundo momento do estudo, FDBR e FDBSL passaram por processo de digestão simulada e o produto da digestão foi avaliado em dois ensaios de fermentação distintos com três cepas probióticas, onde foi realizada a contagem das células viáveis em placas, seguindo com ensaios de fermentação com inóculo fecal humano, quantificando grupos bacterianos utilizando a técnica de fluorescência de hibridização *in situ* (FISH), seguido por análise de citometria de fluxo multiparamétrica (CFM). Nas duas fermentações foi realizado o monitoramento de indicadores de atividade metabólica (pH, perfil de açúcares, ácidos orgânicos e perfil de compostos fenólicos) presentes no meio fermentado ao longo do tempo. Em seguida, foi realizada a avaliação do escore prebiótico para cada fermentação *in vitro*. O desenho do estudo encontra-se apresentado na Figura 6.

Figura 6. Delineamento experimental da pesquisa.



Fonte: Autor, 2023.
Legenda: FDBR, liofilizado de raiz de beterraba; FDBSL, liofilizado de talo e folha de beterraba; FISH, hibridização por fluorescência *in situ*; CFM, citometria de fluxo multiparamétrica.

3.1.1 Aquisição das amostras de beterraba e obtenção dos liofilizados

Para a realização desse estudo, foram coletadas amostras de beterraba vermelha (*Beta vulgaris* L., cultivar Maravilha ou “early wonder” de três diferentes produtores da agricultura familiar (10 amostras de cada) em seu estado integral (raiz, talos e folhas) (Figura 7), obtidas em feiras agroecológicas na cidade de João Pessoa. As amostras passaram pelas etapas de lavagem, sanitização com solução de hipoclorito de sódio (150 ppm, 15 minutos) e enxague.

As beterrabas higienizadas foram fracionadas em raiz, talos e folhas e cada parte foi prontamente processada em processador elétrico doméstico usando disco ralador de 1,2 mm (modelo RI0702, Philips Walita, São Paulo). Todo o material foi acondicionado em pratos plásticos e congelados a -18 ºC por 24 h, e, subsequentemente, submetidos ao processo de liofilização por 24 horas (temperatura -55 ± 2 ºC, pressão do vácuo <138µHg, velocidade 1 mm/horas) usando liofilizador de bancada (modelo L-101, LIOTOP, São Carlos, Brasil). O material seco foi novamente processado e tamisado através de peneira fina para obtenção de um pó com tamanho de partícula <1,0 mm. O pó obtido foi acondicionado em sacos de polietileno laminado e armazenados sob refrigeração (4 ± 0,5 ºC) até uso nos experimentos. Dos liofilizados obtidos, talos e folhas foram misturados em igual proporção, obtendo-se, assim, duas amostras de liofilizados: raiz (FDBR) e talo + folha (FDBSL).

Figura 7. Beterraba vermelha (*Beta vulgaris* L.) cv. Early wonder.

Fonte: Autor, 2023

3.1.2 Caracterização físico-química dos liofilizados de beterraba

3.1.2.1 Composição centesimal

A análise dos parâmetros físico-químicos dos liofilizados FDBR e FDBSL foram realizadas utilizando os procedimentos descritos pela AOAC (2016) para obtenção dos teores de umidade (AOAC 940.26), cinzas (AOAC 940.26), carboidratos, proteínas (AOAC 935.58) e lipídios (AOAC 920.39). Os conteúdos de fibras insolúveis e solúveis foram determinados utilizando método enzimático-gravimétrico (TOBARUELA *et al*., 2018) e teor de pectina como pectato de cálcio (RANGANNA, 1979).

3.1.2.2 Quantificação de açúcares e ácidos orgânicos

Para determinação do conteúdo de açúcares (glicose e frutose), foi preparado um extrato aquoso do FDBR e FDBSL. Inicialmente, 1 g de cada amostra foi homogeneizada com 5 mL de água ultra purificada (Sistema de Purificação de Água Integral Milli-Q®, EMD Millipore, Billerica, MA, EUA) por 10 minutos com aparelho Turratec (Tecnal, Piracicaba, São Paulo, Brasil). A suspensão foi centrifugada (4000 x *g*, 15 minutos, 4 ºC) e o sobrenadante foi filtrado com filtro de 0,45 µm (Whatman®, GE Healthcare, Chicago, IL, EUA).

Para determinação dos conteúdos de ácido lático, cítrico, succínico, fórmico e málico e/ou AGCC (acético, butírico e propiônico), foi realizada uma extração ácida, onde 1 g da amostra foi diluída em 5 mL de ácido sulfúrico 4 mM/L, solubilizada utilizando agitador tipo Vortex (Tecnal), e, em seguida, centrifugado (58136 x *g*, 10 minutos, 4 °C). O sobrenadante foi coletado utilizando agulha acoplada a uma seringa e, em seguida, filtrado com filtro de seringa de 0,45 µm (Whatman®, GE Healthcare, Chicago, IL, EUA).

Para cada amostra, foi realizada uma injeção em sistema de cromatografia líquida de alta eficiência LC 1260 Infinity (Agilent Technologies, Santa Clara, Califórnia, EUA), equipado com bomba de solvente quaternário (modelo G1311C), desgaseificador, compartimento de coluna termostato (modelo G1316A) e amostrador automático (modelo G1329B) acoplado a detector de matriz de diodo (DAD) (modelo G1315D) e detector de índice de refração (RID) (modelo G1362A). Durante a análise, a coluna Agilent Hi-Plex H (300 x 7,7 mm) com tamanho de partícula de 8,0 µm e coluna de guarda PL Hi-Plex H (5 x 3 mm) (Agilent Technologies) foram mantidas a 50 °C, o volume de injeção foi de 10 µL, vazão de 0,5 mL/minutos, fase móvel 4 mM H_2SO_4 em água ultrapura e corrida de 20 minutos. Os dados obtidos foram processados com o software OpenLAB CDS ChemStation EditionTM (Agilent Technologies). Os picos e as áreas médias de pico foram utilizados para quantificação dos compostos por meio da comparação dos seus tempos de retenção com os padrões de ácidos orgânicos e açúcares, expressando os resultados em g/L (COELHO *et al.*, 2018; LIMA *et al.*, 2019).

Para realização dessas análises, os padrões de glicose e frutose foram obtidos da Sigma-Aldrich (St. Louis, MA, EUA); os padrões dos ácidos acético, butírico, cítrico, fórmico, lático, propiônico, succínico e málico foram obtidos da Vetec Química Fina (Rio de Janeiro, Brasil); todos o com uma pureza de ≥ 99 %. A água ultrapura foi obtida de sistema de ultra purificação de água (Milli-Q®) e o ácido sulfúrico foi obtido da Merck (Darmstadt, Alemanha).

3.1.2.3 Quantificação de compostos fenólicos

A execução dessa análise se deu por meio de hidrólise ácida, onde 2 g de cada amostra (FDBR e FDBSL) foram acidificadas com HCl 0,1 M (Neon, Suzano, São Paulo, Brasil) para atingir pH 2, seguindo-se por mistura com 10 mL de metanol (Neon): água destilada (70:30, v/v) e tratamento com ultrassom (60 minutos, 37 kHz, 30 ºC). A solução foi centrifugada (4000 x *g*, 15 minutos, 4 °C) e o sobrenadante foi filtrado com filtro de tamanho de poro de 0,45 μm (Whatman®).

Para separação e quantificação dos compostos fenólicos foi utilizado cromatógrafo Agilent (modelo 1260 Infinity LC, Agilent Technologies) equipado com bomba quaternária de solventes (modelo G1311C), desgaseificador, compartimento termostático da coluna (modelo G1316A) e amostrador automático (modelo G1329B), juntamente com detector de matriz de diodos (DAD) (modelo G1315D) e detector de índice de refração (DIR) (modelo G1362A). As condições analíticas foram: pré-coluna Zorbax C18 (12,6 × 4,6 mm, 5 μm) (Zorbax, EUA) e coluna Zorbax Eclipse Plus RP-C18 (100 × 4,6 mm, 3,5 μm) utilizadas para a separação cromatográfica dos compostos fenólicos. A fase móvel foi uma solução de água acidificada com ácido fosfórico 0,1 M (pH 2,0, fase A) e metanol acidificado com ácido fosfórico a 0,5 % (fase B) com taxa de fluxo de 0,8 mL/minutos. Os dados foram processados com o software OpenLAB CDS ChemStation EditionTM (Agillent Technologies). Os picos das amostras de CLAE foram identificados comparando seus tempos de retenção com aqueles encontrados para os padrões de compostos fenólicos (Sigma Aldrich). Foram realizadas injeções duplicadas e as áreas médias dos picos foram usadas para quantificação dos compostos fenólicos identificados, expressando os resultados em mg/g (PADILHA *et al.*, 2017).

3.1.2.4 Quantificação de betalaínas

Para determinação do teor de betalaínas, um extrato aquoso (1%, p/v) foi preparado e macerado por congelamento por aproximadamente 12 horas. Depois de descongelar, o extrato aquoso foi centrifugado duas vezes (8536×g, 20 minutos, 4 ºC), o sobrenadante (1 mL) foi diluído com água destilada (24 mL) e as leituras de absorbância (538 nm e 476 nm) foram realizadas em triplicata em espectrofotômetro (Biotek Multi-Detecção Synergy HTX, Winooski, VT, EUA). A absorções de luz em 538 nm e 476 nm foram usadas para calcular o conteúdo de betacianinas e betaxantinas (mg/100 g), respectivamente. A absorção a 600 nm foi medido para corrigir a ocorrência de pequenas impurezas. O conteúdo total de betalaínas foi expresso como a soma de betacianinas e betaxantinas (NILSSON, 1970; VON ELBE, 2001).

3.2 AVALIAÇÃO DO EFEITO DE FDBR E FDBSL SOBRE A SOBREVIÊNCIA DE CEPAS DE *Lactobacillus* QUANDO EXPOSTAS A DIGESTÃO GASTROINTESTINAL SIMULADA

3.2.1 Preparo dos inóculos bacterianos

Para a realização destes experimentos foram utilizadas três cepas de microrganismos reconhecidos como probióticos, a saber: *Lactobacillus acidophilus* (LA-05), *Lacticaseibacillus casei* (L-26) (anteriormente denominado *Lactobacillus casei* L-26) (ZHENG *et al*., 2020) e *Limosilactobacillus fermentum* (L-296) (DE ALBUQUERQUE *et al*., 2018; GARCIA *et al*., 2016). As cepas La-05 e L-26 foram fornecidas pela Coleção de Microrganismos da Faculdade de Biotecnologia da Universidade Católica Portuguesa (Porto, Portugal). A cepa *L. fermentum* 296 foi isolada de subprodutos de frutas e previamente caracterizada em estudos *in vitro* e *in vivo* como tendo aptidões para uso como probióticos (CAVALCANTE *et al.,* 2019; DE ALBUQUERQUE *et al*., 2018; DE LUNA FREIRE *et al*., 2021; DE OLIVEIRA *et al*., 2020; GARCIA *et al*., 2016). Para utilização nos ensaios, cada cepa foi cultivada em caldo de Man, Rogosa e Sharpe (MRS) (HiMedia, Mumbai, Índia) a 37 ± 1 °C por 24 horas sob anaerobiose (AnaeroGen, Oxoid, Basingstoke, Inglaterra). Em seguida, as culturas foram centrifugadas (4500 x *g,* 15 minutos, 4° C), lavadas duas vezes e ressuspensas em solução salina tamponada com fosfato esterilizada (8 g/L de NaCl, 0,2 g/L de KCl, 1,44 g/L de Na_2HPO4, 0,24 g/L de KH_2PO_4) para obter suspensões celulares com densidade óptica a 655 nm (DO655) de 0,3 - 0,5. Estas suspensões apresentaram contagens de células viáveis de aproximadamente 6 log de unidades formadoras de colônias (UFC) por mL (UFC/mL) quando inoculadas em ágar MRS (HiMedia) (DE ALBUQUERQUE *et al*., 2018; DE SOUSA *et al*., 2015).

3.2.2 Simulação da digestão gastrointestinal

O processo de simulação ocorreu conforme os estágios referentes às fases da digestão oral, estomacal e intestinal. A agitação mecânica foi utilizada para simular os movimentos peristálticos e todas as etapas foram realizadas sob temperatura de 37 °C ± 1 °C em estufa incubadora (TE-424, Tecnal) (DE ALBUQUERQUE *et al.*, 2020).

Foram testados os liofilizados FDBR, FDBSL, FOS (controle positivo) e um sistema com adição de tampão de fosfato (HiMedia) usado como controle negativo. Inicialmente, 2,5 g da amostra liofilizada foi misturada com 25 mL de água destilada esterilizada e homogeneizados por 2 minutos. A seguir, cada amostra foi inoculada assepticamente com 5 mL da suspensão de inóculo de cada cepa (La-05, L-26 e L-296 com contagem final de células viáveis entre 6 e 7 log de UFC/mL) e homogeneizados por 2 minutos antes de prosseguir para as etapas seguintes.

A etapa boca foi simulada por meio da adição de solução de α-amylase (Sigma Aldrich) na concentração de 100 U/mL de CaCl2 a 1 mM e pH ajustado para 6,9 com solução de NaHCO3 1M sob agitação de 200 rpm com tempo de exposição de 2 minutos a 37 ± 1 ^{o}C. Na etapa que simula as condições estomacais, foi adicionado solução de pepsina (Sigma Aldrich) na concentração de 25 mg/mL de HCl a 0,1 N, adicionada a uma razão de 0,05 mL/mL e com redução gradual do pH da mistura até 2,0, usando solução de HCl a 1N e agitação de 130 rpm a 37 ± 0,5 ^{o}C durante 120 minutos. A etapa da fase intestinal foi simulada com com adição de solução contendo pancreatina (Sigma Aldrich) na concentração de 2 g de pancreatina/L de $NaHCO_3$ a 0,1M e solução de sais de bile (Sigma Aldrich) na concentração de 12 g de sais de bile bovino/L de $NaHCO_3$ a 0,1M com ajuste de pH para 6,0 com $NaHCO_3$ a 0,1M, sendo expostos durante 120 minutos sob agitação de 45 rpm a 37 ± 1 ^{o}C.

3.2.3 Enumeração de células viáveis das cepas de *Lactobacillus* durante a digestão gastrointestinal simulada

Para avaliar a viabilidade das cepas expostas aos liofilizados de beterraba (FDBR e FDBSL) durante o processo de digestão simulada, alíquotas de 100 µL foram retiradas de cada fase da digestão simulada (boca, estômago e intestino), diluídas seriadamente (10^{-1}-10^{-5}) em solução salina tamponada estéril e alíquotas de 10 µL foram inoculadas em ágar MRS (HiMedia), por meio da técnica de microgotas, e incubadas sob anaerobiose (AnaeroGen, Oxoid, Basingstoke, Inglaterra) a 37 ± 1 ^{o}C por 48 horas. Ao final do período de incubação, as colônias visíveis foram enumeradas e os resultados expressos em log UFC/mL com limite de detecção de 2 log UFC/mL (DUARTE *et al*., 2017).

3.2.4 Avaliação do estado fisiológico das cepas de *Lactobacillus* durante a digestão gastrointestinal simulada

3.2.4.1 Procedimento de coloração e análise de citometria de fluxo multiparamétrica

Após a exposição a cada uma das etapas da digestão gastrointestinal simulada, alíquota de 1,5 mL foi retirada e filtrada em membrana de celulose regenerada (0,45 µm, Whatman®), sendo o filtrado obtido lavado duas vezes e ressuspenso em solução salina tamponada com fosfato e submetidas ao procedimento de coloração com iodeto de propídio (PI, Sigma-Aldrich) na concentração de 10 µg/mL e diacetato de carboxifluoresceína (cFDA, Sigma-Aldrich) na concentração de 2,5 µg/mL. As amostras submetidas à dupla coloração foram incubadas por 15 minutos em ambiente protegido da luz. Em seguida, estas suspensões foram centrifugadas (4500 x g, 10 minutos, 4 °C) e lavadas em igual volume de tampão fosfato salino esterilizado a fim de remover o excesso dos fluorocromos. Finalmente, foram ressuspendidas em solução salina tamponada com fosfato estéril. Assim, as suspensões de células estavam adequadas para análise em citômetro de fluxo multiparamétrico (RODRIGUES *et al*., 2022).

A coloração com estes fluorocromos foi realizada para avaliar a integridade da membrana citoplasmática (corante PI) e presença de atividade respiratória/metabólica (corante cFDA), respectivamente, nas células dos isolados inoculados nas diferentes amostras (liofilizados e controles positivo e negativo) (DE SOUZA PEDROSA *et al*., 2020; PAPARELLA *et al*., 2008).

3.2.4.2 Análise de dados e interpretação dos resultados

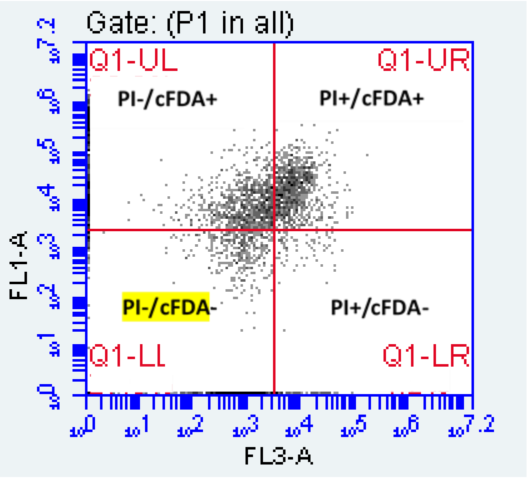
As análises foram realizadas com uso de citômetro de fluxo com emissão de laser de argônio a 488 nm e 640 nm (BD Accuri C6, Becton Dickinson, Franklin Lakes, NJ, EUA). O detector de fluorescência denominado de canal FL1 detecta luz com comprimento de onda de 533 nm ± 30 nm e foi utilizado para coletar fluorescência na cor verde, a fim de verificar marcações com cFDA. O detector de fluorescência denominado de canal FL3 detecta luz com comprimentos de onda > 670 nm foi utilizado para coletar fluorescência na cor vermelha, a fim de verificar marcações com PI.

Os níveis do limiar para aquisição de dados foram definidos com os detectores de dispersão de luz. Na dispersão direta (foward scatter, FSC) utilizou-se o valor de 30.000 e na dispersão lateral (side scatter, SSC) foi utilizado o valor de 12.000 com o intuito de eliminar partículas muito menores do que as células intactas e delimitar as células bacterianas, respectivamente.

Cada aquisição de amostra foi feita com uma configuração de baixa taxa de fluxo (12 µL/minuto) e um total de 10000 eventos foram analisados por amostra. Foi utilizada uma compensação automática para os dados obtidos. A análise dos dados foi realizada com o software BD Accuri C6 (Becton Dickinson and Company) (PAPARELLA *et al*., 2008; DE SOUZA PEDROSA *et al*., 2020).

Para analisar os dados de dupla marcação foram projetados gráficos de densidade FL1 no eixo Y versus FL3 no eixo X. Os gráficos FL1 versus FL3 foram demarcados com quatro quadrantes, sendo um para cada subpopulação celular de acordo com a fluorescência detectada nas leituras como mostra a Figura 8 (PAPARELLA *et al*., 2008)

Figura 8. Interpretação do citograma da dispersão das subpopulações marcadas com iodeto de propidium (PI) e carboxifluoresceína diacetato (cFDA).



Fonte: Autor, 2023.

Legenda: PI+ (membrana permeabilizada), PI- (membrana intacta), cFDA + (com atividade enzimática) e cFDA – (sem atividade enzimática); PI-/cFDA+ (células vivas), PI+/cFDA+ (células injuriadas), PI+/cFDA- (células mortas); PI-/cFDA- (células não marcadas).

3.2.5 Avaliação da capacidade antioxidante dos liofilizados durante a digestão simulada

A capacidade antioxidante de FDBR e FDBSL foi avaliada através do uso de dois métodos: eliminação do radical DPPH (2,2-difenil-1-picrilhidrazil) e capacidade sequestradora do radical ABTS●+ (ácido 2,2'-azino-bis(ácido 3-etilbenzotiazolina-6-sulfônico) (LEE *et al*., 2020; SHEHATA *et al*., 2021). Para análise de DPPH, após exposição a cada etapa da digestão simulada, alíquotas (0,05 mL) das amostras foram reagidas com solução de DPPH 0,2 mM (Sigma-Aldrich) em metanol (0,1 mL), agitadas vigorosamente e mantidas por 30 minutos no escuro para logo em seguida ter a absorbância lida a 517 nm com um espectrofotômetro (Biotek Multi-Detection Synergy HTX). O controle foi preparado com água substituindo a amostra.

O cátion radical ABTS (ABTS●+) foi gerado com a reação de 5 mL de solução aquosa de ABTS (7 mM) + 88 μL de solução de persulfato de potássio (140 mM). A mistura foi mantida no escuro (14 h, 28 ± 1 ºC) antes do uso e diluída com etanol para atingir uma absorbância de 0,7 ± 0,02 unidades a 734 nm usando um espectrofotômetro. Alíquotas (0,05 mL) das amostras foram reagidas com 0,1 mL da solução de radical ABTS azul-verde, agitadas e mantidas no escuro à temperatura ambiente (25 ± 1 °C) por 6 minutos. Em seguida foram observadas as diminuições da absorbância a 734 nm usando espectrofotômetro. Todos os testes foram realizados em triplicata e o resultado expresso em percentual de atividade de eliminação do radical.

3.3 ENSAIOS PARA AVALIAÇÃO DAS PROPRIEDADES PREBIÓTICAS

Ensaios *in vitro* utilizando sistemas de fermentação foram realizados para avaliar os efeitos de FDBR e FDBSL sobre a sobrevivência e proteção de cepas de *Lactobacillus* com alegação probiótica durante a passagem gastrointestinal. Fermentação *in vitro* utilizando inóculo fecal humano também foi usado para avaliar o impacto dos liofilizados de beterraba sobre a modulação de grupos bacterianos distintos da microbiota intestinal, bem como sobre seu metabolismo. Um índice matemático foi utilizado para obter a média quantitativa da influência dos substratos testados sobre a seletividade no crescimento de bactérias benéficas.

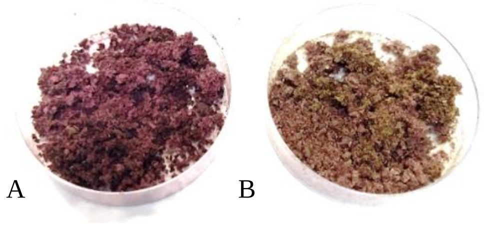
3.3.1 Digestão gastrointestinal *in vitro* dos liofilizados

Os liofilizados FDBR e FDBSL foram submetidos a uma digestão gastrointestinal *in vitro* para ser transformado em material com características similares àquelas quando atingem o cólon humano. Para isso, 5g de cada amostra foi solubilizada em 50 mL de água destilada estéril e prosseguindo para as etapas de digestão oral, estomacal e intestinal conforme descrito no tópico 3.2.2.

Todo o material proveniente da digestão simulada, foi transferido para um tubo de diálise de celulose regenerada de 1 kDa de peso molecular (Spectra/Por_ 6, Spectrum Europe, Holanda) e dialisada em contrafluxo de NaCl 0,01M a 5 ± 1 ºC para remover monossacarídeos livres presentes no subproduto pré-digerido. Após 18 horas, os fluidos de diálise foram substituídos por nova solução de NaCl 0,01M a 5 ± 1 ºC e o processo continuou por um período adicional de 2 horas. As amostras digeridas dialisadas foram liofilizadas novamente (Figura 9

A – B) e armazenadas seguindo os mesmos procedimentos descritos no tópico 3.1.1 (GUERGOLETTO *et al.*, 2016; MASSA *et al.*, 2020).

Figura 9. FDBR (“freeze-dried red beet root”) E FDBSL (“freeze-dried red beet stem and leaves”) após digestão simulada



Fonte: Autor, 2023.
Legenda: A, liofilizado da raiz de beterraba após digestão e diálise; B, liofilizado do talo e folha de beterraba após digestão e diálise.

3.3.2 Avaliação do potencial prebiótico sobre cepas de probióticos

3.3.2.1 Preparo dos inóculos

Para a realização destes experimentos foram utilizadas três cepas de microrganismos apontados como probióticos, a saber: *Lactobacillus acidophilus* (LA-05), *Lacticaseibacillus casei* (L-26) e *Limosilactobacillus fermentum* (L-296) e preparados inóculos em forma de suspensão de acordo com o tópico 3.2.1.

As cepas *Escherichia coli* ATCC 8739 e *E. coli* ATCC 11775 foram utilizadas para preparar um inóculo entérico misto para uso nos ensaios de determinação do escore prebiótico. As cepas foram cultivadas separadamente em caldo de infusão de cérebro-coração (BHI) (HiMedia, Mumbai, Índia) a 37 ºC por 18 – 20 h, as culturas foram centrifugadas (4500 x *g*, 15 minutos, 4 ° C), lavadas e ressuspensas em solução salina esterilizada (NaCl 8,5 g/L) para obter uma suspensão de células com contagens de aproximadamente 6 log CFU/mL quando cultivada em ágar BHI (HiMedia). O inóculo da mistura entérica foi obtido pela mistura (1:1) das suspensões das duas cepas de *E. coli* (DUARTE *et al.*, 2017). Estoques de todas as cepas testadas foram mantidos em ágar MRS ou BHI contendo glicerol (150 g/L; Sigma-Aldrich) a -20 ºC.

3.3.2.2 Meios de cultivo

O caldo MRS com fonte de carbono modificada foi utilizado como meio basal para avaliar os potenciais efeitos prebióticos do FDBR e FDBSL liofilizados submetidos à digestão gastrointestinal *in vitro* (SÁNCHEZ-ZAPATA *et al*., 2013; SOUSA *et al*., 2015; DUARTE *et al*., 2017). A composição dos caldos MRS modificados utilizados nestes ensaios foram: triptona 10 g/L, extrato de carne 8 g/L, extrato de levedura 4 g/L, hidrogenofosfato dipotássico 2 g/L, tween 80 1 g/L, acetato de sódio 5 g/L, citrato de amônio tribásico 2 g/L, sulfato de magnésio 0,2 g/L, sulfato de manganês 0,04 g/L e 20 g/L da respectiva fonte de carbono. Para monitorar o crescimento das cepas probióticas testadas, quatro diferentes caldos de cultivo foram preparados com base na única fonte de carbono utilizada na sua composição: 1: caldo contendo glicose 20 g/L (ingrediente não prebiótico, padrão MRS; HUEBNER; WEHLING; HUTKINS, 2007); 2: caldo contendo fruto-oligossacarídeos 20 g/L (FOS, ingrediente prebiótico; GIBSON *et al*., 2017); 3 e 4: caldo contendo o FDBR e FDBSL liofilizado submetidos à digestão *in vitro* (respectivamente: 20 g/L; e 5: caldo sem adição da fonte de carbono (controle negativo).

Todos os ingredientes utilizados para preparar os caldos de cultivo foram obtidos da Sigma-Aldrich, com exceção do FOS que foi obtido da Galena Ltd. (Campinas, São Paulo, Brasil).

3.3.2.3 Enumeração de células viáveis das cepas probióticas

A análise do crescimento das cepas probióticas testadas quando cultivadas nos diferentes caldos ocorreu por meio da contagem de células viáveis. Inicialmente, o inóculo da respectiva cepa testada (200 µL) foi dispensado em frascos esterilizados contendo 10 mL do caldo de cultivo específico (contagem final de células viáveis de aproximadamente 6 log UFC/mL). Em seguida, as misturas foram homogeneizadas utilizando Vortex durante 30 segundos e incubadas a 37 ± 1 °C. Em diferentes tempos de incubação (zero – logo após a homogeneização e após, 12, 24, 48 e 72 horas), alíquotas de 100 µL de cada mistura foram diluídas em série em solução salina esterilizada (1:9) e, posteriormente, alíquotas de 10 µL de cada diluição foram plaqueadas em ágar MRS usando a técnica de inoculação por microgota. As placas foram incubadas a 37 ± 1°C durante 24-48 horas em condições de aerobiose (AnaeroGen, Oxoid, Basingstoke, Inglaterra). Os resultados das contagens de células viáveis foram expressos como log UFC/mL (DE ALBUQUERQUE *et al*., 2020).

3.3.2.3 Determinação do índice de atividade prebiótica

Para a determinação do índice da atividade prebiótica dos liofilizados FDBR e FDBSL digeridos, um inóculo de cada uma das cepas teste de probióticos foi distribuído (2 %, v/v) em frascos esterilizados contendo 10 mL de caldo MRS com glicose (20 g/L), FDBR (20 g/L), FDBSL (20 g/L) ou FOS (20 g/L). Paralelamente, suspensões da mistura de patógenos entéricos foram inoculadas (2 %, v/v) em caldo M9 contendo glicose (20 g/L), FDBR (20 g/L), FDBSL (20 g/L) ou FOS (20 g/L). Os caldos contendo os patógenos entéricos foram incubados a 37 °C sob aerobiose. Em dois diferentes intervalos de tempo (zero – logo após homogeneização e 48 horas após incubação), alíquotas de cada sistema foram diluídas seriadamente e inoculadas em meios seletivos (ágar MRS para *Lactobacillus* e ágar eosina azul de metileno para *E. coli*, HiMedia). As placas foram incubadas a 37 ± 1 °C durante 24-48 horas (DUARTE *et al*., 2017) e os resultados de log UFC/mL obtidos, usados na **Equação 1** para determinação do índice prebiótico.

Equação 1: A determinação do índice prebiótico

$$\textbf{Índice prebiótico} = \left(\frac{\underset{(48\ \text{h})}{\text{PRO em PRE}} - \underset{(0\ \text{h})}{\text{PRO em PRE}}}{\underset{(48\ \text{h})}{\text{PRO em GLI}} - \underset{(0\ \text{h})}{\text{PRO em GLI}}}\right) - \left(\frac{\underset{(48\ \text{h})}{\text{ME em PRE}} - \underset{(0\ \text{h})}{\text{ME em PRE}}}{\underset{(48\ \text{h})}{\text{ME em GLI}} - \underset{(0\ \text{h})}{\text{ME em GLI}}}\right) \qquad \textit{Eq. 1}$$

Legenda: Resultados em log de UFC/mL para PRO (probióticos), PRE (prebióticos), GLI (glicose), ME (mistura entérica)

A obtenção de valor de escore de atividade prebiótica positivo indica efeitos estimuladores seletivos do componente examinado sobre o crescimento da cepa probiótica em detrimento da mistura entérica e, consequentemente, potencial atividade prebiótica (DE ALBUQUERQUE *et al*., 2020; ZHANG *et al*., 2018).

3.3.2.4 Monitoramento de indicadores de atividade metabólica

Os efeitos de FDBR e FDBSL no metabolismo probiótico foram avaliados com as medidas de pH, açúcares e ácidos orgânicos nos meios de cultivo com FDBR, FDBSL, FOS e glicose nos tempos 0, 24, 48 e 72 horas. O conteúdo de diferentes compostos fenólicos também foi mensurado, mas apenas nos tempos 0, 24 e 72 horas. O pH foi mensurado usando potenciômetro digital (Q400AS, Quimis, Sao Paulo, SP, Brazil). Nos períodos citados, uma alíquota de 2 mL foi coletada, centrifugada (6402×g, 10 minutos, 4 ◦C) e o sobrenadante filtrado em filtro de seringa com poro de 0,45-µm (Whatman). Do filtrado resultante, uma alíquota de 0,5 mL foi retira e acrescida em igual volume de metanol:água (70:30 v/v) para análise cromatográfica de fenólicos conforme item 3.2.2.2. O volume restante do filtrado seguiu para análise de açúcares e ácidos orgânicos como descrito no item 3.2.2.3.

3.4 ENSAIOS COM SISTEMAS DE FERMENTAÇÃO COLÔNICA

3.4.1 Procedimentos éticos

Considerando as exigências do Conselho Nacional de Saúde, este estudo foi submetido à apreciação pelo Comitê de Ética e Pesquisa em Seres Humanos do Centro de Ciências da Saúde da Universidade Federal da Paraíba – CEP/CCS, o qual aprovou sua execução (parecer nº 4.251.958) em 01 de setembro de 2020 (Anexo A). Este procedimento está baseado na Resolução 466/12 (CNS-MS, 2012), que aprova as diretrizes e normas regulamentadoras de pesquisas envolvendo seres humanos e estabelece que "toda pesquisa envolvendo seres humanos deverá ser submetida à apreciação de um Comitê de Ética em Pesquisa". Os indivíduos participantes da pesquisa foram esclarecidos acerca da temática da pesquisa e, em seguida, fizeram assinatura do Termo de Consentimento Livre e Esclarecido (Apêndice A).

3.4.2 Coleta das amostras fecais

As amostras fecais frescas foram doadas por quatro voluntários adultos saudáveis, sendo dois homens e duas mulheres, com idades entre 18-40 anos. Como critérios de inclusão, os doadores deveriam seguir dieta onívora regular, sem precedentes de doença gastrointestinal, sem uso frequente de alimentos probióticos ou prebióticos, além de não ter feito uso de antibióticos ou qualquer outro medicamento de uso controlado por, pelo menos, seis meses antes da coleta (GUERGOLETTO *et al.*, 2016; ANDRADE *et al.*, 2020). Os doadores receberam instruções específicas para a coleta/ armazenamento adequado das amostras. Um kit para coleta foi disponibilizado para cada doador, contendo frascos coletores esterilizados, luvas e espátula descartável, além de álcool 70%.

As amostras foram coletadas em até 30 minutos após a excreção, sendo transportadas para o LMBA/UFPB em jarra de anaerobiose (Anaerojar, Oxoid, Basingstoke, Inglaterra) contendo gerador de gás anaeróbico (AnaeroGen, Oxoid, Basingstoke, Inglaterra) e utilizadas imediatamente (RODRIGUES *et al*., 2016).

3.4.3 Preparação do inóculo fecal humano

As amostras fecais frescas coletadas de cada doador foram misturadas em igual quantidade (1:1:1:1), para formar um único inóculo (120 g) e diluídas (1:10) em solução constituída de 0,5 g/L de cisteína-HCl e 8,5 g/L de NaCl (Sigma-Aldrich), e, posteriormente, homogeneizadas por 2 minutos sob agitação (200 rpm). O inóculo fecal diluído e homogeneizado foi filtrado com o uso de uma camada tripla de gazes esterilizadas para remoção de partículas grandes e armazenadas (37 ± 1 °C) em frascos esterilizados sob condições anaeróbicas (AnaeroGen, Oxoid, Basingstoke, Inglaterra) (ANDRADE *et al*., 2020; MENEZES *et al*., 2021b). Os ingredientes usados para preparar o meio de pré-cultura foram obtidos da Sigma-Aldrich.

3.4.4 Preparação do meio de fermentação colônica *in vitro*

Para o processo de fermentação, foi formulado 1 L de meio de crescimento, com a seguinte composição: 4,5 g de NaCl, 4,5 g de KCl, 1,5 g de $NaHCO_3$, 0,69 g de $MgSO_4$, 0,8 g de L-cisteína, 0,5 g de KH_2PO_4, 0,5 g de K_2HPO_4, 0,4 g de sal biliar, 0,08 g $CaCl_2$, 0,005 g de $FeSO_4$, 1 mL de Tween 80 e 4 mL de solução de resazurina (0,025 %, v/v) como indicador anaeróbio, diluído em água destilada e esterilizado por autoclavagem (121 °C, 1 atm, 15 minutos) (BIANCHI *et al*., 2011). Culturas descontínuas de fermentação (40 mL) foram formuladas com 40% de meio de crescimento (v/v), 40% da pré-cultura do inóculo fecal humano (v/v) e 20% do FDBR ou FDBSL (p/v) digerido segundo o tópico 3.3.1, seguido por incubação sob anaerobiose (AnaeroGen, Oxoid, Basingstoke, Inglaterra) por 48 horas a 37 ± 1 °C (MENEZES *et al*., 2020). Para compor o experimento também foi preparado um meio fermentativo com FOS (20% p/v) e um meio sem adição de substrato fermentável, para serem testados como controles positivos e negativos, respectivamente (GUERGOLETTO *et al*., 2016; MENEZES *et al*., 2021b). Os ingredientes utilizados para formular os meios de fermentação foram obtidos da Sigma Aldrich.

3.4.5 Quantificação das populações bacterianas com hibridização por fluorescência *in situ* (FISH) e citometria de fluxo (CF)

A técnica de hibridização por fluorescência *in situ* (FISH) com sondas oligonucleotídicas selecionadas e projetadas para atingir regiões específicas do gene 16S rRNA de diferentes grupos microbianos combinada com citometria de fluxo multiparamétrica (CFM) foi utilizada para avaliar a capacidade do FDBR e FDBSL digerido em modular a microbiota intestinal humana em sistemas de fermentação colônica *in vitro* (CONTERNO *et al*., 2019; MENEZES *et al*., 2021). Foram utilizadas cinco diferentes sondas (Tabela 2). As sondas foram sintetizadas comercialmente e marcadas com o corante fluorenscente Cy3 (Sigma Aldrich) capaz de hibridizar a região específica do gene 16S rRNA de grupos bacterianos selecionados (RODRIGUES *et al*., 2016; MENEZES *et al*., 2020). O marcador SYBR Green (Molecular Probes, Invitrogen, Carlsbad, CA, EUA) foi usado para enumerar a população total de bactérias por meio da marcação da fita dupla de DNA (CONTERNO *et al*., 2019).

Tabela 2. Sondas de oligonucleotídeos de rRNA 16S e condições de hibridização utilizadas nas análises com hibridização fluorescente *in situ* acoplada a citometria de fluxo multiparamétrica (FISH-CFM).

Sonda	Especificidade	Sequência	Temperatura (ºC)
Bac 303	*Bacteroides* spp./*Prevotella* spp.	CCAATGTGGGGGACCTT	45 ºC
Bif 164	*Bifidobacterium* spp.	CATCCGGCATTACCACCC	50 ºC
Chis 150	*Clostridium histolyticum*	TTATGCGGTATTAATCTYCCTTT	50 ºC
Erec 482	*Clostridium coccoides/Eubacterium rectale*	GCTTCTTAAGTCARGTACCG	50 ºC
Lab 158	*Lactobacillus* spp. /*Enterococcus* spp;	GGTATTAGCAYCTGTTTCCA	50 ºC

No tempo zero (logo após a homogeneização) e após 24 e 48 horas de fermentação, alíquotas de 375 μL das culturas foram fixadas com 1125 μL de solução de paraformaldeído filtrada (4 %, p/v) para estabilizar a estrutura celular, ficando em *overnight* a 4 ºC. Após esse período, as alíquotas foram centrifugadas (10000 × *g*, 5 minutos, 4 °C), lavadas duas vezes com PBS 1 M, ressuspensas em 300 µL de PBS:etanol 99 % (1:1 v/v), filtradas com filtro de membrana tamanho de poro 0,45 µm (Whatman®) e armazenadas a -20 ºC.

Para a realização da hibridização *in situ*, 10 µL da suspensão contendo as células fixadas foram ressuspensas em 190 µL de PBS 1X (Gibco, Gaithersburg, EUA; pH 7.2), centrifugadas (4000 × *g*, 15 minutos, 4 °C), descartado o sobrenadante, ressuspensas em 200 µL de tampão Tris-EDTA (100 mM Tris-HCl and 50 mM EDTA; pH 8) e centrifugadas (4000 × *g*, 15 minutos, 4 °C). As amostras foram tratadas com 200 µL de Tris-EDTA contendo lisozima (1 mg/mL) e incubadas por 10 minutos no escuro a temperatura ambiente (25 ± 0,5 °C) para permeabilizar as células que receberam as sondas Lab 158 e Bif 164, seguindo por centrifugação (4000 × *g*, 15 minutos, 4 °C). As amostras foram ressuspensas em 45 µL de tampão de hibridização [0,9 M NaCl, 20 mM Tris-HCl (pH 7,5), 0,1 % (p/v) de dodecil sulfato de sódio (DSS)] adicionado de 5 µL de sonda oligonucleotídica fluorescente (50 ng/µL) e mantidas sob temperatura de hibridização apropriada para cada sonda (45 ou 50 °C) no escuro por 4 horas.

Após essa etapa, as amostras foram centrifugadas (4000 × g, 15 minutos, 25 °C), ressuspensas em 200 µL de tampão de hibridização sem DSS e mantidas sob temperatura de lavagem apropriada para cada sonda (45 ou 50 °C) no escuro por 30 minutos para retirar sondas não ligadas. As amostras foram centrifugadas (4000 × *g*, 15 minutos, 25 °C), ressuspensas em 200 µL de PBS 1X e 20 µL de SYBR Green (1:1000 solução estoque diluída em dimetil sulfóxido ≥99.9 %, Sigma-Aldrich), incubadas por 10 minutos no escuro sob temperatura ambiente (25 ± 0,5 °C), centrifugadas (4000 × *g*, 15 minutos, 25 °C) e ressuspensas com 200 µL de PBS 1X.

Uma amostra em branco (sem a sonda oligonucleotídica e sem SYBR Green), e uma amostra marcada somente com SYBR Green, foram preparadas para todas as amostras, seguindo os mesmos passos das amostras hibridizadas, como um controle para definir o limiar das comportas do citômetro de fluxo (BD Accuri C6, New Jersey, EUA), o que permite revelar o potencial de autofluorescência das amostras, excluindo os falsos positivos. Na análise de CFM, os sinais fluorescentes das células individuais passam através de uma zona de laser, sendo coletados como sinais logarítmos. Os sinais fluorescentes (medidas da área de pulso) foram coletados pelos canais FL1 (SYBR Green) e FL2 (Lab 158, Bif 164, Bac 303, Chis 150 e Erec 482). A aquisição das amostras foi configurada para passar em um baixo fluxo, com nível limite para dispersão direta (FSC) ajustado para 30.000 e com total de 10.000 eventos coletados para cada amostra. Os citogramas de emissão de fluorescência foram registrados com o software BD Accuri C6 (Becton Dickinson and Company). Os resultados foram expressos como abundância (porcentagem relativa) de células hibridizadas com cada sonda Cy3 específica de grupo bacteriano (registrados como eventos fluorescentes) em relação ao total de bactérias enumeradas com coloração SYBR Green (DE ALBUQUERQUE *et al.*, 2021).

3.4.6 Determinação do índice prebiótico

Para obter uma medida comparativa quantitativa geral do equilíbrio entre diferentes populações bacterianas nas amostras de fermentação colônica, bem como para comparar a influência do FDBR e FDBSL na modulação microbiana seletiva durante a fermentação colônica, um índice prebiótico foi calculado com dados obtidos na análise de FISH-CFM. A equação usada foi descrita por Albuquerque *et al.* (2021). Após o cálculo da abundância relativa (porcentagem relativa), foi feita uma correlação com o número total (abundância, %) de bactérias enumeradas, sendo utilizada na **equação 2**:

Equação 2. Índice prebiótico de modulação microbiana

$$\text{Índice prebiótico} = \%\ \text{Lab} + \%\ \text{Bif} - \%\ \text{Bac} - \%\ \text{Chis} - \%\ \text{Erec}\quad (\textit{Eq. 2})$$

Onde: % Lab = abundância encontrada para Lab após 24 ou 48 horas - abundância encontrada para Lab no tempo zero; % Bif = abundância encontrada para Bif após 24 ou 48 horas - abundância encontrada para Bif no tempo zero; % Bac = abundância encontrada para Bac após 24 ou 48 horas - abundância encontrada para Bac no tempo zero; % Chis = abundância encontrada para Chis após 24 ou 48 horas - abundância encontrada para Chis no tempo zero; e % Erec = abundância encontrada para Erec após 24 ou 48 horas - abundância encontrada para Erec no tempo zero.

A equação assume que um aumento na população de *Lactobacillus* spp./*Enterococcus* spp. (hibridizado pela sonda Lab 158) e/ou *Bifidobacterium* spp. (hibridizado pela sonda Bif 164) é indicador de efeito positivo sobre a modulação da microbiota intestinal, enquanto o aumento na população de *Bacteroides* spp./*Prevotella* spp. (hibridizado pela sonda Bac 303), *C. histolyticum* (hibridizado pela sonda Chis 150) e *E. rectale/C. coccoides* (hibridizado pela sonda Erec 482) é indicador de efeito negativo. Mudanças na abundância destes grupos são inseridas na equação em relação aos seus níveis iniciais (abundância). Se um grupo bacteriano mostra diferenças negativas entre o tempo de fermentação e o tempo zero, o sinal (positivo ou negativo) imediatamente anterior a essa sonda é alterado na equação. Isso permite normalizar o uso da equação para um número variado de sondas. A obtenção de um valor de índice prebiótico positivo indica um equilíbrio benéfico dos grupos bacterianos identificados na fermentação colônica induzida pelo substrato examinado e, consequentemente, potencial atividade prebiótica. Por sua vez, a obtenção de um valor de índice prebiótico negativo indica uma modulação indesejável da microbiota pelo substrato examinado (DE ALBUQUERQUE *et al*., 2021).

3.4.7 Monitoramento de indicadores de atividade metabólica durante a fermentação colônica

Alíquotas de 1 mL foram coletadas no tempo zero e após 24 e 48 horas de fermentação para determinação do pH e dos conteúdos de açúcares, AGCC (acético, butírico e propiônico) e compostos fenólicos, seguindo os mesmos procedimentos descritos no tópico 3.3.2.4. Os valores de pH foram medidos com um potenciômetro digital (método 981.12, AOAC, 2016).

3.4.8 Avaliação da capacidade antioxidante dos liofilizados durante a fermentação colônica

A capacidade antioxidante das diferentes formulações nos sistemas de fermentação colônica foi avaliada no tempo zero (logo após a homogeneização dos componentes do meio de fermentação) e após 24 e 48 horas de fermentação. Foram utilizados métodos já descritos no tópico 3.2.5.

3.5 ANÁLISES ESTATÍSTICAS

Todos os ensaios foram conduzidos em triplicata e em três experimentos distintos, sendo os resultados expressos como média ± desvio padrão. Para avaliar os dados quanto a normalidade, a homogeneidade e/ou a homoscedasticidade foram utilizados os testes de Kolmogorov-Smirnov e o teste de Levene ($p<0,05$). Os dados parâmetros físico-químicos de FDBR e FDBSL foram submetidos ao teste *t* Student ($p \leq 0,05$), enquanto as contagens de células viáveis de *Lactobacillus* nestes substratos e seus respectivos scores de atividade prebiótica foram submetidos a análise de variância (ANOVA) seguida do teste post hoc de Tukey, utilizando $p \leq 0,05$ para significância estatística. O software GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, EUA) foi usado para executar a análise estatística. Teste de correlação de Pearson foi aplicado para avaliar a correlação entre variáveis analisadas durante 72 horas de cultivo dos probióticos nos meios FDBR e FDBSL (contagens de células viáveis, pH, consumo de acúcares e perfil de fenólicos), durante a exposição a digestão gastrointestinal simulada (capacidade antioxidante e perfil de compostos fenólico, contagem de células probióticas viáveis e tamanho de subpopulações de células vivas e injuriadas) e durante a fermentação colônica (abundancia relativa dos grupos bacterianos monitorados, valores de pH e ácidos graxos de cadeia curta). Análise de componentes principais (PCA) foi executada com dados sobre a abundância relativa de grupos bacterianos medidos, valores de pH, AGCC e índice prebiótico durante a fermentação colônica. Foi usando o software R, versão 2.15.3, (Ross Ihaka e Robert Gentleman, University of Auckland, Nova Zelândia) para executar a análise estatística.

4 RESULTADOS

Os resultados da tese são apresentados na forma de dois artigos originais, dispostos nos apêndices C e D. O artigo apresentado no apêndice C é intitulado "*Different parts from the whole red beet (Beta vulgaris* L.) *valorization with stimulatory effects on probiotic lactobacilli and protection against gastrointestinal conditions***"**, publicado no periódico Food Bioscience (https://doi.org/10.1016/j.fbio.2023.102439), o qual avaliou os efeitos *in vitro* de liofilizados de diferentes partes [raiz (FDBR) e mistura de talos e folhas (FDBSL)] de beterraba vermelha sobre o crescimento e atividade metabólica de cepas probióticas de *Lactobacillus*, bem como sobre a sua sobrevivência e estado fisiológico quando expostas à condições gastrointestinais simuladas (SGIC). Também foi monitorado e discutido alterações na capacidade antioxidante dos liofilizados durante a digestão gastrointestinal.

Os resultados demonstraram que FDBR e FDBSL apresentam altos teores de fibras solúveis (5,25 e 11,10 g/100 g) e insolúveis (21,43 e 37,03 g/100 g), pectinas (8,45 e 9,51 g/100 g), betalaínas (3,36 – 28,99 g/100 g) e uma variedade de compostos fenólicos. FDBR e FDBSL estimularam o crescimento das cepas probióticas com altas contagens de células viáveis (>9 log UFC/mL), consumo de açúcares e produção de ácido lático e AGCC (ácido acético, butírico e propiônico) durante 72 horas de cultivo. Na análise do escore prebiótico, FDBR e FDBSL tiveram escores de atividade prebiótica positivos para os três probioticos testados (⩾0,17), indicando efeitos de estimulação seletiva sobre esses microrganismos, com provável associação ao teor de fibras alimentares e compostos fenólicos dos substratos testados. FDBR e FDBSL aumentaram a sobrevivência e manutenção de funções fisiológicas ativas nos probióticos testados durante a exposição ao SGIC. O conteúdo de compostos fenólicos e a capacidade antioxidante de FDBR e FDBSL aumentou após a exposição às condições do estômago, enquanto a capacidade antioxidante diminuiu após exposição a condições intestinais. Assim, os efeitos estimuladores e protetores de FDBR e FDBSL em cepas probióticas os tornam candidatos a serem explorados na formulação de alimentos funcionais ou suplementos dietéticos com efeitos positivos sobre a microbiota intestinal associados a potenciais efeitos prebióticos.

O artigo apresentado no apêndice D*, "Investigating the effects of conventional and unconventional edible parts of red beet (Beta vulgaris L.) on target bacterial groups and metabolic activity of human colonic microbiota to produce novel and sustainable prebiotic ingredientes",* publicado no periódico Food Research International (https://doi.org/10.1016/j.foodres.2023.112998), investigou os efeitos de FDBR e FDBSL sobre a abundância e atividade metabólica de grupos bacterianos considerados como os principais fermentadores e representativos da microbiota intestinal humana. Também foram avaliados valores de pH, açúcar, AGCC, compostos fenólicos e capacidade antioxidante durante 48 horas de fermentação colônica.

Os resultados mostraram que FDBR e FDBSL, de forma geral, aumentaram a abundância relativa de *Lactobacillus* spp./*Enterococcus* spp. (3,64 - 7,60 %) e *Bifidobacterium* spp. (2,76 - 5,78 %) e diminuíram a abundância relativa de *Bacteroides* spp./*Prevotella* spp. (9,56 - 4,18 %), *C. histolyticum* (1,62 - 1,15%) e *E. rectale*/*C. coccoides* (2,33 - 1,49 %) durante 48 horas de fermentação colônica. FDBR e FDBSL apresentaram índices prebióticos positivos (> 3,61) durante a fermentação colônica, indicando efeitos estimulatórios seletivos sobre grupos bacterianos intestinais benéficos. FDBR e FDBSL também aumentaram a atividade metabólica da microbiota colônica humana, evidenciada pela diminuição do pH, consumo de açúcar, produção de AGCC, alterações no conteúdo de compostos fenólicos e manutenção da capacidade antioxidante durante a fermentação colônica.

O desenvolvimento dessa tese forneceu informações relevantes sobre os aspectos nutricionais e funcionais de diferentes partes da beterraba vermelha. Componentes com alegação de saúde, como fibras solúveis e insolúveis, betalaínas e compostos fenólicos, foram encontrados em concentrações relevantes tanto na raiz como nos talos e folhas da beterraba vermelha. Esses mesmos compostos bioativos podem ser responsáveis pela eficácia desses materiais em proteger as cepas probióticas das condições adversas durante a passagem no trato gastrointestinal, ajudando a manter sua viabilidade e funcionalidade ao atingir o intestino para exercer seus efeitos benéficos sobre o hospedeiro. Foi observada a estimulação do crescimento seletivo das cepas probióticas e alterações na composição e atividade metabólica da microbiota intestinal humana, evidenciados pelo aumento do número de células viáveis das cepas probióticas, alterações no pH, produção de metabólitos e alterações no conteúdo de açúcares e compostos fenólicos ao longo do tempo.

Índices prebiótico positivos para fermentações com bactérias probióticas e microrganismos de origem colônica indicam a capacidade dos liofilizados de beterraba de serem usados como novos ingredientes prebióticos em potencial, encorajando o consumo da raíz, caules e folhas de beterraba vermelha como alimentos funcionais e como fontes de ingredientes prebióticos sustentáveis e de valor agregado, sendo uma estratégia para a valorização da cultura da beterraba vermelha.

Entretanto, algumas limitações podem ser compreendidas no desenvolvimento do trabalho devido a dificuldade dos ensaios de natureza *in vitro* em mimetizar os complexos eventos físico-químicos e fisiológicos que ocorrem no corpo humano, mais especificamente no trato digestório. Pesquisas futuras sobre o potencial prebiótico da beterraba vermelha poderiam usar recursos metodológicos já estudados para extrair compostos da raíz, talos e folhas da beterraba vermelha, a exemplo de poli e oligossacarídeos, compostos fenólicos e pigmentos, para avaliar os efeitos dessas substancias de forma isolada sobre probióticos reconhecidos e na modulação da microbiota instestinal humana, aliando testes *in vitro* de alto desempenho com avaliações *in vivo* com o intuito de elucidar melhor os seus impactos sobre a saúde humana e mecanismos subjacentes.

REFERÊNCIAS

ALVARADO-JASSO, G. M. *et al*. Prebiotic effects of a mixture of agavins and green banana flour in a mouse model of obesity. **Journal of Functional Foods**, v. 64, 2020. https://doi.org/10.1016/j.jff.2019.103685

ALVES-SANTOS, A. M. *et al*. Prebiotic effect of dietary polyphenols: A systematic review. **Journal of Functional Foods**, v. 74, n. 104169, 2020. https://doi.org/10.1016/j.jff.2020.104169

AMORIM, C. *et al*. *In vitro* assessment of prebiotic properties of xylooligosaccharides produced by *Bacillus subtilis* 3610. **Carbohydrate Polymers**, v. 229, 2020a. https://doi.org/10.1016/j.carbpol.2019.115460

AMORIM, C. *et al*. *In vitro* fermentation of raffinose to unravel its potential as prebiotic ingredient. **LWT**, v. 126, 2020b. https://doi.org/10.1016/j.lwt.2020.109322

ANDRADE, R. M. S. DE *et al*. Potential prebiotic effect of fruit and vegetable byproducts flour using *in vitro* gastrointestinal digestion. **Food Research International**, v. 137, 2020. https://doi.org/10.1016/j.foodres.2020.109354

AOAC. Official Methods of Analysis of AOAC International - 20th Edition, 2016. **20th ed. Gaithersburg: AOAC**., 2016.

ASHAOLU, T. J. Immune boosting functional foods and their mechanisms: A critical evaluation of probiotics and prebiotics. **Biomedicine and Pharmacotherapy**, v. 130, n. 110625, 2020. https://doi.org/10.1016/j.biopha.2020.110625

ASRA, R. *et al*. Physicochemical study of betasianin and antioxidant activities of red beet tubers (*Beta vulgaris* L.). **Journal of Pharmaceutical and Sciences**, v. 3, n. 1, 2020. https://doi.org/10.36490/journal-jps.com.v3i1.35

BABARYKIN, D. *et al*. Red beet (*Beta vulgaris*) impact on human health. **Journal of Biosciences and Medicines**, v. 07, n. 03, 2019. https://doi.org/10.4236/jbm.2019.73007.

BACKES, E.; GENENA, A. K. Optimisation of the extraction process of antioxidant capacity compounds from beet (*Beta vulgaris* L.) stalk. **Semina:Ciencias Agrarias**, v. 41, n. 6, 2020. https://doi.org/10.5433/1679-0359.2020v41n6p2621

BAIÃO, D. DOS S.; DA SILVA, D. V. T.; PASCHOALIN, V. M. F. Beetroot, a remarkable vegetable: Its nitrate and phytochemical contents can be adjusted in novel formulations to benefit health and support cardiovascular disease therapies. **Antioxidants**, v. 9, n. 10, p. 960, 2020. https://doi.org/10.3390/antiox9100960

BIANCHI, F. *et al*. Development of a headspace solid-phase microextraction gas chromatography-mass spectrometric method for the determination of short-chain fatty acids from intestinal fermentation. **Food Chemistry**, v. 129, n. 1, p. 200-205, 2011. https://doi.org/10.1016/j.foodchem.2011.04.022

BUCALOSSI, G. *et al*. Functional and sensory properties of phenolic compounds from unripe grapes in vegetable food prototypes. **Food Chemistry**, v. 315, 2020. https://doi.org/10.1016/j.foodchem.2020.126291

CAMPOS, D. A. *et al*. Impact of functional flours from pineapple by-products on human intestinal microbiota. **Journal of Functional Foods**, v. 67, 2020. https://doi.org/10.1016/j.jff.2020.103830

CARDING, S., et al. Dysbiosis of the gut microbiota in disease. **Microbial ecology in health and disease**, v. 26, p. 26191, 2015. http://dx.doi.org/10.3402/mehd.v26.26191

CARDOSO, B. B. *et al*. Novel and emerging prebiotics: Advances and opportunities. **Advances in Food and Nutrition Research**. v. 95. P. 41-95, 2021. https://doi.org/10.1016/bs.afnr.2020.08.001

CATARINO, M. D. *et al*. Impact of phlorotannin extracts from *Fucus vesiculosus* on human gut microbiota. **Marine Drugs**, v. 19, n. 7, p. 375 2021. https://doi.org/10.3390/md19070375

CAVALCANTE, R. G. S. *et al*. The probiotic *Lactobacillus fermentum* 296 attenuates cardiometabolic disorders in high fat diet-treated rats. **Nutrition, Metabolism and Cardiovascular Diseases**, v. 29, n. 12, p.1408-4117, 2019. https://doi.org/10.1016/j.numecd.2019.08.003

CHENG, M.; NING, K. Stereotypes about enterotype: The old and new ideas. Genomics, proteomics and bioinformatics. **Beijing Genomics Institute**, v. 17, n. 1, p. 4-12, 2019. https://doi.org/10.1016/j.gpb.2018.02.004

CHENG, Y. J.; YEUNG, C. Y. Recent advance in infant nutrition: Human milk oligosaccharides. **Pediatrics and Neonatology**, v. 62, n. 4, p. 347-353, 2021. https://doi.org/10.1016/j.pedneo.2020.12.013

CHEN, L. *et al*. Beetroot as a functional food with huge health benefits: Antioxidant, antitumor, physical function, and chronic metabolomics activity. **Food Science and Nutrition**, v. 9, p. 6406–6420, 2021a. https://doi.org/10.1002/fsn3.2577

CHEN, L. *et al*. The long-term genetic stability and individual specificity of the human gut microbiome. **Cell**, v. 184, n. 9, p. 2302-2320, 2021b. https://doi.org/10.1016/j.cell.2021.03.024

CHHIKARA, N. *et al*. Bioactive compounds of beetroot and utilization in food processing industry: A critical review. **Food Chemistry**, v. 272, p. 192-200, 2019. https://doi.org/10.1016/j.foodchem.2018.08.022

CLIFFORD, T. *et al*. The potential benefits of red beetroot supplementation in health and disease. **Nutrients**, v. 7, p. 2801-2822, 2015. https://doi.org/10.3390/nu7042801

CNA- Confederação da Agricultura e Pecuária do Brasil. Mapeamento e quantificação da cadeia produtiva de hortaliças. Brasília, DF: **CNA**, p. 79, 2017.

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

COMAN, M. M. *et al*. Polyphenol content and *in vitro* evaluation of antioxidant, antimicrobial and prebiotic properties of red fruit extracts. **European Food Research and Technology**, v. 244, n. 4, p. 735-745, 2018. https://doi.org/10.1007/s00217-017-2997-9

CONCHA OLMOS, J.; ZÚÑIGA HANSEN, M. E. Enzymatic depolymerization of sugar beet pulp: Production and characterization of pectin and pectic-oligosaccharides as a potential source for functional carbohydrates. **Chemical Engineering Journal**, v. 192, p. 29-36, 2012. https://doi.org/10.1016/j.cej.2012.03.085

CONTERNO, L. *et al*. Measuring the impact of olive pomace enriched biscuits on the gut microbiota and its metabolic activity in mildly hypercholesterolaemic subjects. **European Journal of Nutrition**, v. 58, n. 1, p. 63-81, 2019. https://doi.org/10.1007/s00394-017-1572-2

COSTA, A. P. D. *et al*. Minimally processed beetroot waste as an alternative source to obtain functional ingredients. **Journal of Food Science and Technology**, v. 54, n. 7, p. 2050-2058, 2017. https://doi.org/10.1007/s13197-017-2642-4

CUI, J. *et al*. Dietary fibers from fruits and vegetables and their health benefits via modulation of gut microbiota. **Comprehensive Reviews in Food Science and Food Safety**, v.18, p. 1514-1532, 2019. https://doi.org/10.1111/1541-4337.12489

CUI, Y.; QU, X. Genetic mechanisms of prebiotic carbohydrate metabolism in lactic acid bacteria: Emphasis on *Lacticaseibacillus casei* and *Lacticaseibacillus paracasei* as flexible, diverse and outstanding prebiotic carbohydrate starters. **Trends in Food Science & Technology**, v. 115, p. 486–499, 2021. https://doi.org/10.1016/j.tifs.2021.06.058

CUNNINGHAM, M. *et al*. Shaping the future of probiotics and prebiotics. **Trends in Microbiology**, v. 29, n. 8, p. 667-685, 2021. https://doi.org/10.1016/j.tim.2021.01.003

CRUZ NETO, J.P.R., *et al*. Safety evaluation of a novel potentially probiotic *Limosilactobacillus fermentum* in Rats. **Probiotics & Antimicrobial Proteins**, p. 1-11, 2023. https://doi.org/10.1007/s12602-023-10077-3

DAMIÁN, M. R. *et al*. Functional foods, nutraceuticals and probiotics: A focus on human health. **Microorganisms**, v. 10, n. 5, p. 1065, 2022. https://doi.org/10.3390/microorganisms10051065

DAVENPORT, E. R. *et al*. Seasonal variation in human gut microbiome composition. **PLoS ONE**, v. 9, n. 3, 2014. https://doi.org/10.1371/journal.pone.0090731

DE ALBUQUERQUE, T. M. R. *et al*. *In vitro* characterization of *Lactobacillus* strains isolated from fruit processing by-products as potential probiotics. **Probiotics and Antimicrobial Proteins**, v. 10, n. 4, p. 704-716, 2018. https://doi.org/10.1007/s12602-017-9318-2

DE ALBUQUERQUE, T. M. R. *et al*. Potential prebiotic properties of flours from different varieties of sweet potato (*Ipomoea batatas* L.) roots cultivated in Northeastern Brazil. **Food Bioscience**, v. 36, 2020. https://doi.org/10.1016/j.fbio.2020.100614

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

DE ARAÚJO, H. F. G., *et al.* Potentially probiotic *Limosilactobacillus fermentum* fruit-derived strains alleviate cardiometabolic disorders and gut microbiota impairment in male rats fed a high-fat diet. **Probiotics and Antimicrobial Proteins**, v. 14, p. 349-359, 2022. https://doi.org/10.1007/s12602-021-09889-y

DE LUNA FREIRE, M. O. *et al*. Effects of a mixed *Limosilactobacillus fermentum* formulation with claimed probiotic properties on cardiometabolic variables, biomarkers of inflammation and oxidative stress in male rats fed a high-fat diet. **Foods**, v. 10, n. 9, 2021. https://doi.org/10.3390/foods10092202

DE OLIVEIRA, S. P. A. *et al*. A review on bioactive compounds of beet (*Beta vulgaris* L. subsp. *vulgaris)* with special emphasis on their beneficial effects on gut microbiota and gastrointestinal health. **Critical Reviews in Food Science and Nutrition**, v. 61, n. 12, p. 2022-2033, 2021. https://doi.org/10.1080/10408398.2020.1768510

DE OLIVEIRA, S. P. A. *et al*. Different parts from the whole red beet (*Beta vulgaris* L.) valorization with stimulatory effects on probiotic *Lactobacill*i and protection against gastrointestinal conditions. **Food Bioscience**, v. 52, n. 102439, 2023a. https://doi.org/10.1016/j.fbio.2023.102439

DE OLIVEIRA, S. P. A. *et al*. Investigating the effects of conventional and unconventional edible parts of red beet (*Beta vulgaris* L.) on target bacterial groups and metabolic activity of human colonic microbiota to produce novel and sustainable prebiotic ingredients. **Food Research International**, v. 171, n. 112998, 2023b. https://doi.org/10.1016/j.foodres.2023.112998

DE OLIVEIRA, Y. *et al*. Oral administration of *Lactobacillus fermentum* post-weaning improves the lipid profile and autonomic dysfunction in rat offspring exposed to maternal dyslipidemia. **Food and Function**, v. 11, n. 6, p. 558-5594, 2020. https://doi.org/10.1039/D0FO00514B

DESAI, M. S. *et al*. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. **Cell**, v. 167, n. 5, p. 1339-1353, 2016. https://doi.org/10.1016/j.cell.2016.10.043

DE SOUZA, E. L. *et al*. Potential interactions among phenolic compounds and probiotics for mutual boosting of their health-promoting properties and food functionalities–A review. **Critical Reviews in Food Science and Nutrition**, v. 59, n. 10, p. 1645-1659, 2019. https://doi.org/10.1080/10408398.2018.1425285

DE SOUZA PEDROSA, G. T. *et al*. Physiological alterations involved in inactivation of autochthonous spoilage bacteria in orange juice caused by *citrus* essential oils and mild heat. **International Journal of Food Microbiology**, v. 334, n. 108837, 2020. https://doi.org/10.1016/j.ijfoodmicro.2020.108837

DI LORENZO, C. *et al*. Polyphenols and human health: The role of bioavailability. **Nutrients**, v. 13, n. 273, 2021. https://doi.org/10.3390/nu13010273

DOMÍNGUEZ, R. *et al*. Red beetroot: A potential source of natural additives for the meat industry. **Applied Sciences (Switzerland)**, v. 10, n. 23, p. 8340, 2020. https://doi.org/10.3390/app10238340

DONG, C. D., et al. Bioprocess development for the production of xylooligosaccharide prebiotics from agro-industrial lignocellulosic waste. **Heliyon**, v. 9, 2023. https://doi.org/10.1016/j.heliyon.2023.e18316

DOS SANTOS LIMA, M. *et al*. Determining 1-kestose, nystose and raffinose oligosaccharides in grape juices and wines using HPLC: method validation and characterization of products from Northeast Brazil. **Journal of Food Science and Technology**, v. 56, n. 10, p. 4575-4584, 2019. https://doi.org/10.1007/s13197-019-03936-x

DUARTE, F. N. D. *et al*. Potential prebiotic properties of cashew apple (*Anacardium occidentale* L.) agro-industrial byproduct on *Lactobacillus* species. **Journal of the Science of Food and Agriculture**, v. 97, n. 11, p. 3712-3719, 2017. https://doi.org/10.1002/jsfa.8232

EDZIRI, H. *et al*. Phytochemical analysis, antioxidant, anticoagulant and *in vitro* toxicity and genotoxicity testing of methanolic and juice extracts of *Beta vulgaris* L. **South African Journal of Botany**, v. 126, p. 170-175, 2019. https://doi.org/10.1016/j.sajb.2019.01.017

EFSA BIOHAZ. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 7: Suitability of taxonomic units notified to EFSA**. EFSA Journal, v.** 16, n. 1, p. 43, 2018. https://doi.org/10.2903/j.efsa.2018.5131

ELBE, J. H. Betalains. **Current Protocols in Food Analytical Chemistry**, v. 00, n. 1, p. f3.1, 2001. https://doi.org/10.1002/0471142913.faf0301s00

ELSHAHED, M. S. *et al*. Pectin in diet: Interactions with the human microbiome, role in gut homeostasis, and nutrient-drug interactions. **Carbohydrate Polymers**, v. 255, n. 117388, 2021. https://doi.org/10.1016/j.carbpol.2020.117388

FERREIRA, R. DOS S. *et al*. Relationship between intestinal microbiota, diet and biological systems: an integrated view. **Critical Reviews in Food Science and Nutrition**, v.62, n. 5, p. 1166-1186, 2022. https://doi.org/10.1080/10408398.2020.1836605

FU, Y. *et al*. Red beetroot betalains: Perspectives on extraction, processing, and potential health benefits. **Journal of Agricultural and Food Chemistry**, v. 68, n. 42, p. 11595–11611, 2020. https://doi.org/10.1021/acs.jafc.0c04241

GALANAKIS, C. M. Functionality of food components and emerging technologies. **Foods**, v. 10, p. 128, 2021. https://doi.org/10.3390/foods10010128

GARCIA, E. F. *et al*. Identification of lactic acid bacteria in fruit pulp processing by-products and potential probiotic properties of selected *Lactobacillus* strains. **Frontiers in Microbiology**, v. 7, 2016. https://doi.org/10.3389/fmicb.2016.01371

GIBSON, G. R. *et al*. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. **Gastroenterology**, v. 108, n. 4, p. 975-982, 1995. https://doi.org/10.1016/0016-5085(95)90192-2

GIBSON, G. R. *et al*. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. **Nature Reviews Gastroenterology and Hepatology**, v. 14, p. 491-502, 2017. https://doi.org/10.1038/nrgastro.2017.75

GÓMEZ, B. *et al*. Prebiotic potential of pectins and pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp: A comparative evaluation. **Journal of Functional Foods**, v. 20, p. 108–121, 2016. https://doi.org/10.1016/j.jff.2015.10.029

GÓMEZ, B. *et al*. Emerging prebiotics obtained from lemon and sugar beet byproducts: Evaluation of their *in vitro* fermentability by probiotic bacteria. **LWT – Food Science and Technology**, v. 109, p. 17-25, 2019. https://doi.org/10.1016/j.lwt.2019.04.008

GONG, X. *et al*. The interactions between gut microbiota and bioactive ingredients of traditional Chinese medicines: A review. **Pharmacological Research**, v. 157, n. 104824, 2020. https://doi.org/10.1016/j.phrs.2020.104824

GUERGOLETTO, K. B. *et al*. *In vitro* fermentation of juçara pulp (*Euterpe edulis*) by human colonic microbiota. **Food Chemistry**, v. 196, p. 251-258, 2016. https://doi.org/10.1016/j.foodchem.2015.09.048

HENNING, S. M. *et al*. Health benefit of vegetable/fruit juice-based diet: Role of microbiome. **Scientific Reports**, v. 7, n. 1, 2017. https://doi.org/10.1038/s41598-017-02200-6

HILL, C. *et al*. Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. **Nature Reviews Gastroenterology and Hepatology**, v. 11, n. 8, p. 506-514, 2014. https://doi.org/10.1038/nrgastro.2014.66

HOLCK, J. *et al*. Tailored enzymatic production of oligosaccharides from sugar beet pectin and evidence of differential effects of a single DP chain length difference on human faecal microbiota composition after *in vitro* fermentation. **Process Biochemistry**, v. 46, n. 5, pp. 1039-1049, 2011. https://doi.org/10.1016/j.procbio.2011.01.013

HUEBNER, J.; WEHLING, R. L.; HUTKINS, R. W. Functional activity of commercial prebiotics. **International Dairy Journal**, v. 17, n. 7, pp. 770-775, 2007. https://doi.org/10.1016/j.idairyj.2006.10.006

HURTADO-ROMERO, A. *et al*. Innovative technologies for the production of food ingredients with prebiotic potential: Modifications, applications, and validation methods. **Trends in Food Science and Technology**, v. 104, p. 117-131, 2020. https://doi.org/10.1016/j.tifs.2020.08.007

JIMENEZ-LOPEZ, C., et al. Agriculture waste valorisation as a source of antioxidant phenolic compounds within a circular and sustainable bioeconomy. **Food & function**, v. 11, n. 6, p. 4853-4877, 2020. https://doi.org/10.1039/D0FO00937G

KAPOOR, R.; FENG, H. Characterization of physicochemical, packing and microstructural properties of beet, blueberry, carrot and cranberry powders: The effect of drying methods. **Powder Technology**, v. 395, p. 290-300, 2022. https://doi.org/10.1016/j.powtec.2021.09.058

KHANGWAL, I.; SHUKLA, P. Potential prebiotics and their transmission mechanisms: Recent approaches. **Journal of Food and Drug Analysis**, v. 27, n. 3, p. 649-656, 2019. https://doi.org/10.1016/j.jfda.2019.02.003

KOLODZIEJCZYK, A. A.; ZHENG, D.; ELINAV, E. Diet–microbiota interactions and personalized nutrition. **Nature Reviews Microbiology**, v. 17, p. 742-753, 2019. https://doi.org/10.1038/s41579-019-0256-8

MASSA, N. M. L. *et al*. Effects of digested jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg) by-product on growth and metabolism of *Lactobacillus* and *Bifidobacterium* indicate prebiotic properties. **LWT – Food Science and Technology**, v. 131, 2020. https://doi.org/10.1016/j.lwt.2020.109766

LARSEN, N. *et al*. The effect of pectins on survival of probiotic *Lactobacillus* spp. in gastrointestinal juices is related to their structure and physical properties. **Food Microbiology**, v. 74, p. 11-20, 2018. https://doi.org/10.1016/j.fm.2018.02.015

LEE, S. Y. *et al*. Changes in the content and bioavailability of onion quercetin and grape resveratrol during *in vitro* human digestion. **Foods**, v. 9, n. 6, 2020. https://doi.org/10.3390/foods9060694

LINDEFELDT, M. *et al*. The ketogenic diet influences taxonomic and functional composition of the gut microbiota in children with severe epilepsy. **npj Biofilms and Microbiomes**, v. 5, n. 1, 2019. https://doi.org/10.1038/s41522-018-0073-2

LI, P. JUN *et al*. Pectic oligosaccharides hydrolyzed from orange peel by fungal multi-enzyme complexes and their prebiotic and antibacterial potentials. **LWT - Food Science and Technology**, v. 69, p. 203-210, 2016. https://doi.org/10.1016/j.lwt.2016.01.042

LIU, W. *et al*. Effect of chitooligosaccharides on human gut microbiota and antiglycation. **Carbohydrate Polymers**, v. 242, 2020. https://doi.org/10.1016/j.carbpol.2020.116413

LI, Z. *et al*. Enhanced antioxidant activity for apple juice fermented with *Lactobacillus plantarum* ATCC14917. **Molecules**, v. 24, n. 1, 2019. https://doi.org/10.3390/molecules24010051

LORIZOLA, I. M. *et al*. Beet stalks and leaves (*Beta vulgaris* L.) protect against high-fat diet-induced oxidative damage in the liver in mice. **Nutrients**, v. 10, n. 7, 2018. https://doi.org/10.3390/nu10070872

LOSASSO, C. *et al*. Assessing the influence of vegan, vegetarian and omnivore oriented westernized dietary styles on human gut microbiota: A cross sectional study. **Frontiers in Microbiology**, v. 9, n. 03, 2018. https://doi.org/10.3389/fmicb.2018.00317

MA, G.; CHEN, Y. Polyphenol supplementation benefits human health via gut microbiota: A systematic review via meta-analysis. **Journal of Functional Foods**, v. 66, n. 103829, 2020. https://doi.org/10.1016/j.jff.2020.103829

MALIK, M.; BORA, J.; SHARMA, V. Growth studies of potentially probiotic lactic acid bacteria (*Lactobacillus plantarum, Lactobacillus acidophilus*, and *Lactobacillus casei*) in carrot and beetroot juice substrates. **Journal of Food Processing and Preservation**, v. 43, n. 11, 2019. https://doi.org/10.1111/jfpp.14214

MANO, M. C. R. *et al*. Oligosaccharide biotechnology: an approach of prebiotic revolution on the industry. **Applied Microbiology and Biotechnology**, v. 102, p. 17-37, 2018. https://doi.org/10.1007/s00253-017-8564-2

MAO, B. *et al*. *In vitro* fermentation of raffinose by the human gut bacteria. **Food and Function**, v. 9, n. 11, p. 5824-5831, 2018. https://doi.org/10.1039/C8FO01687A

MARTINEZ, R. M. *et al*. Anti-inflammatory activity of betalain-rich dye of *Beta vulgaris*: Effect on edema, leukocyte recruitment, superoxide anion and cytokine production. **Archives of Pharmacal Research**, v. 38, n. 4, p. 494-504, 2015. https://doi.org/10.1007/s12272-014-0473-7

MARTINOV, J. *et al*. Apple pectin-derived oligosaccharides produce carbon dioxide radical anion in Fenton reaction and prevent growth of *Escherichia coli* and *Staphylococcus aureus*. **Food Research International**, v. 100, p. 132-136, 2017. https://doi.org/10.1016/j.foodres.2017.08.040

MENEZES, F. N. D. D. *et al*. Acerola (*Malpighia glabra* L.) and guava (*Psidium guayaba* L.) industrial processing by-products stimulate probiotic *Lactobacillus* and *Bifidobacterium* growth and induce beneficial changes in colonic microbiota. **Journal of Applied Microbiology**, v. 130, n. 4, p. 1326-1336, 2021a. https://doi.org/10.1111/jam.14824

MENEZES, F. N. D. D. *et al*. Impact of cashew (*Anacardium occidentale* L.) by-product on composition and metabolic activity of human colonic microbiota *in vitro* indicates prebiotic properties. **Current Microbiology**, v. 78, n. 6, p. 2264-2274, 2021b. https://doi.org/10.1007/s00284-021-02502-z

MIGUEZ, B. *et al*. Prebiotic effects of pectooligosaccharides obtained from lemon peel on the microbiota from elderly donors using na: *In vitro* continuous colon model (TIM-2). **Food & Function**, v. 11, n. 11, p. 9984-9999, 2020. https://doi.org/10.1039/D0FO01848A

MILIÃO, G. L *et al*. Unconventional food plants: Nutritional aspects and perspectives for industrial applications. Future Foods, v. 5, n. 100124, 2022. https://doi.org/10.1016/j.fufo.2022.100124

MOORTHY, M. *et al*. Prebiotic potential of polyphenols, its effect on gut microbiota and anthropometric/clinical markers: A systematic review of randomised controlled trials. **Trends in Food Science and Technology**, v. 99, p. 634-649, 2020. https://doi.org/10.1016/j.tifs.2020.03.036

MROCZEK, A. *et al*. MS/MS and UPLC-MS profiling of triterpenoid saponins from leaves and roots of four red beet (*Beta vulgaris* L.) cultivars. **Phytochemistry Letters**, v. 30, p. 333-337, 2019. https://doi.org/10.1016/j.phytol.2019.02.015

NAUMOVSKI, N.; PANAGIOTAKOS, D. B.; D'CUNHA, N. M. Untangling the 2-way relationship between red wine polyphenols and gut microbiota. **Gastroenterology**, v. 158, n. 1, p. 48-51, 2020. https://doi.org/10.1053/j.gastro.2019.10.015

NILSSON, T. Studies into the pigments in beetroot (*Beta vulgaris* L. ssp. *vulgaris* var. rubra L.). **Lantbrukshogskolans Annaler**, v. 36, p. 179-219, 1970.

NINFALI, P.; ANGELINO, D. Nutritional and functional potential of *Beta vulgaris* cicla and rubra. **Fitoterapia**, v. 89, p. 188-199, 2013. https://doi.org/10.1016/j.fitote.2013.06.004

ONUMPAI, C. *et al*. Microbial utilization and selectivity of pectin fractions with various structures. **Applied and Environmental Microbiology**, v. 77, n. 16, 2011. https://doi.org/10.1128/AEM.00179-11

OZDAL, T. *et al*. The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. **Nutrients**, v. 8, n. 2, p. 78, 2016. https://doi.org/10.3390/nu8020078

PACHECO-ORDAZ, R. *et al*. Effect of phenolic compounds on the growth of selected probiotic and pathogenic bacteria. **Letters in Applied Microbiology**, v. 66, n. 1, p. 25-31, 2018. https://doi.org/10.1111/lam.12814

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

PANGHAL, A. *et al*. Development of probiotic beetroot drink. **Current Research in Nutrition and Food Science**, v. 5, n. 3, 2017. https://dx.doi.org/10.12944/CRNFSJ.5.3.10

PAPARELLA, A. *et al*. Flow cytometric assessment of the antimicrobial activity of essential oils against *Listeria monocytogenes*. **Food Control**, v. 19, n. 12, p. 1174-1182, 2008. https://doi.org/10.1016/j.foodcont.2008.01.002

PARKAR, S. G.; TROWER, T. M.; STEVENSON, D. E. Fecal microbial metabolism of polyphenols and its effects on human gut microbiota. **Anaerobe**, v. 23, p. 12-19, 2013. https://doi.org/10.1016/j.anaerobe.2013.07.009

PAYLING, L. *et al*. The effects of carbohydrate structure on the composition and functionality of the human gut microbiota. **Trends in Food Science and Technology**, v. 97, p. 233-148, 2020. https://doi.org/10.1016/j.tifs.2020.01.009

PENG, Y. *et al*. Prebiotic effects *in vitro* of anthocyanins from the fruits of *Lycium ruthenicum* Murray on gut microbiota compositions of feces from healthy human and patients with inflammatory bowel disease. **LWT – Food Science and Technology**, v. 149, 2021. https://doi.org/10.1016/j.lwt.2021.111829

PLAMADA, D.; VODNAR, D. C. Polyphenols—gut microbiota interrelationship: A transition to a new generation of prebiotics. **Nutrients**, v. 14, p. 137, 2022. https://doi.org/10.3390/nu14010137

PRAVEEN, M. A. *et al*. An overview of extraction and purification techniques of seaweed dietary fibers for immunomodulation on gut microbiota. **Trends in Food Science and Technology**, v. 92, p. 46-64, 2019. https://doi.org/10.1016/j.tifs.2019.08.011

PUNIA BANGAR, S. *et al*. Bioactive potential of beetroot (*Beta vulgaris*). **Food Research International**, n. 111556, 2022. https://doi.org/10.1016/j.foodres.2022.111556

RAMÍREZ-MELO, L. M. *et al*. Optimization of antioxidant activity properties of a thermosonicated beetroot (*Beta vulgaris* L.) juice and further *in vitro* bioaccessibility comparison with thermal treatments. **LWT – Food Science and Technology**, v. 154, 2022. https://doi.org/10.1016/j.lwt.2021.112780

RAMOS, S.; MARTÍN, M. Á. Impact of diet on gut microbiota. **Current Opinion in Food Science**, v. 37, p. 83-90, 2021. https://doi.org/10.1016/j.cofs.2020.09.006

RANGANNA, S. **Manual Analysis of Fruits and Vegetables Product**. Second edition. New Delhi: Tata McGraw Hill Co. Ltd., 1979.

RAY, C. *et al*. Human Milk Oligosaccharides: The Journey Ahead. **International Journal of Pediatrics (United Kingdom)**, v. 2019, 2019. https://doi.org/10.1155/2019/2390240

REZENDE, E. S. V.; LIMA, G. C.; NAVES, M. M. V. Dietary fibers as beneficial microbiota modulators: A proposal classification by prebiotic categories. **Nutrition,** v. 89, 2021. https://doi.org/10.1016/j.nut.2021.111217

KALE. *et al*. Studies on evaluation of physical and chemical composition of beetroot (*Beta vulgaris* L.). **International Journal of Chemical Studies**, v. 6, n. 2, p. 2977-2999, 2018.

RINNINELLA, E. *et al*. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. **Microorganisms**, v. 7, n. 1, 2019a. https://doi.org/10.3390/microorganisms7010014

RINNINELLA, E. *et al*. Food components and dietary habits: Keys for a healthy gut microbiota composition. **Nutrients**, v. 11, n. 10, p. 2393, 2019b. https://doi.org/10.3390/nu11102393

ROBERFROID, M. *et al*. Prebiotic effects: Metabolic and health benefits. **British Journal of Nutrition**, v. 104, p. s1-s63, 2010. https://doi.org/10.1017/S0007114510003363

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

RODRIGUES, N. P. A. *et al*. Dynamics of physiological responses of potentially probiotic fruit-derived *Limosilactobacillus fermentum* in apple and orange juices during refrigeration storage and exposure to simulated gastrointestinal conditions. **Archives of Microbiology**, v. 204, n. 38, 2022. https://doi.org/10.1007/s00203-021-02672-1

RODRÍGUEZ-DAZA, M. C. *et al*. Polyphenol-mediated gut microbiota modulation: toward prebiotics and further. **Frontiers in Nutrition**, v. 8, 2021. https://doi.org/10.3389/fnut.2021.689456

ROMANO, N. *et al*. Flour from mature *Prosopis nigra* pods as suitable substrate for the synthesis of prebiotic fructo-oligosaccharides and stabilization of dehydrated *Lactobacillus delbrueckii* subsp. *bulgaricus*. **Food Research International**, v. 121, 2019. https://doi.org/10.1016/j.foodres.2018.12.016

RUAN, W. *et al*. Healthy human gastrointestinal microbiome: Composition and function after a decade of exploration. **Digestive Diseases and Sciences**, v. 65, p. 695-705, 2020. https://doi.org/10.1007/s10620-020-06118-4

SABATER, C. *et al*. Vegetable waste and by-products to feed a healthy gut microbiota: Current evidence, machine learning and computational tools to design novel microbiome-targeted foods. **Trends in Food Science & Technology**, v. 118, p. 399–417, 2021. https://doi.org/10.1016/j.tifs.2021.10.002

SADOWSKA-BARTOSZ, I.; BARTOSZ, G. Biological properties and applications of betalains. **Molecules**, v. 26, n. 9, p. 2520, 2021. https://doi.org/10.3390/molecules26092520

SÁNCHEZ, B. *et al*. Probiotics, gut microbiota, and their influence on host health and disease. **Molecular Nutrition and Food Research**, v. 61, 2017. https://doi.org/10.1002/mnfr.201600240

SANDERS, M. E. *et al*. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. **Nature Reviews Gastroenterology and Hepatology**, v. 16, n.10, p. 605-616, 2019. https://doi.org/10.1038/s41575-019-0173-3

SAWICKI, T.; WICZKOWSKI, W. The effects of boiling and fermentation on betalain profiles and antioxidant capacities of red beetroot products. **Food Chemistry**, v. 259, 2018. https://doi.org/10.1016/j.foodchem.2018.03.143

SCOTT, K. P. *et al*. Developments in understanding and applying prebiotics in research and practice - An ISAPP conference paper. **Journal of Applied Microbiology**, v. 128, n. 4, p. 934-949, 2020. https://doi.org/10.1111/jam.14424

SENÉS-GUERRERO, C. *et al*. Dietary fiber and gut microbiota. Em: **Food Engineering Series**. [s.l.], p. 277–298, 2020. https://doi.org/10.1007/978-3-030-38654-2_12

SERRANO-CASAS, V. *et al*. Improvement of lactic acid bacteria viability in acid conditions employing agroindustrial co-products as prebiotic on alginate ionotropic gel matrix co-encapsulation. **Journal of Functional Foods**, v. 38, p. 293-297, 2017. https://doi.org/10.1016/j.jff.2017.09.048

SHAH, B. R. *et al*. Effects of prebiotic dietary fibers and probiotics on human health: With special focus on recent advancement in their encapsulated formulations. **Trends in Food Science and Technology**, v. 102, p.178-192, 2020. https://doi.org/10.1016/j.tifs.2020.06.010

SHALABY, H. S.; HASSENIN, A. S. H. Effects of fortification stirred yoghurt with red beet powder (RBP) on hypercholesterolemia rats. **European Journal of Agriculture and Food Sciences**, v. 2, n. 5, 2020. https://doi.org/10.24018/ejfood.2020.2.5.61

SHEHATA, M. G. *et al*. Antioxidant and antimicrobial activities and UPLC-ESI-MS/MS polyphenolic profile of sweet orange peel extracts. **Current Research in Food Science**, v. 4, p. 326-335, 2021. https://doi.org/10.1016/j.crfs.2021.05.001

SINGH, A. K. *et al*. Beneficial effects of dietary polyphenols on gut microbiota and strategies to improve delivery efficiency. **Nutrients**, v. 11, n. 9, 2019. https://doi.org/10.3390/nu11092216

SLATNAR, A. *et al*. HPLC-MSn identification of betalain profile of different beetroot (*Beta vulgaris* L. ssp. vulgaris) parts and cultivars. **Journal of Food Science**, v. 80, n. 9, p. 1-2, 2015. https://doi.org/10.1111/1750-3841.12977

SONNENBURG, E. D. *et al*. Diet-induced extinctions in the gut microbiota compound over generations. **Nature**, v. 529, n. 7585, p. 212-215, 2016. https://doi.org/10.1038/nature16504

SOUSA, S. *et al*. *In vitro* evaluation of yacon (*Smallanthus sonchifolius*) tuber flour prebiotic potential. **Food and Bioproducts Processing**, v. 95, p. 96-105, 2015. https://doi.org/10.1016/j.fbp.2015.04.003

STORCK, C. R. *et al*. Folhas, talos, cascas e sementes de vegetais: Composição nutricional, aproveitamento na alimentação e análise sensorial de preparações. **Ciência Rural**, v. 43, n. 3, 2013. https://doi.org/10.1590/S0103-84782013000300027

SUN, H. *et al*. The modulatory effect of polyphenols from green tea, oolong tea and black tea on human intestinal microbiota *in vitro*. **Journal of Food Science and Technology**, v. 55, n. 1, 2018. https://doi.org/10.1007/s13197-017-2951-7

SZAKÁLY, Z. *et al*. A modified model of the willingness to pay for functional foods. **Appetite**, v. 138, p. 94-101, 2019. https://doi.org/10.1016/j.appet.2019.03.020

Tabela Brasileira de Composição de Alimentos (TBCA). Universidade de São Paulo (USP). Food Research Center (FoRC). Versão 7.2. São Paulo, 2023. [Acesso em: 07.2023]. Disponível em: http://www.fcf.usp.br/tbca.

TAKÁCS-HÁJOS, M.; VARGAS-RUBÓCZKI, T. Evaluation of bioactive compounds in leaf and root of five beetroot varieties. **Journal of Agriculture and Food Research**, v. 7, 2022. https://doi.org/10.1016/j.jafr.2022.100280

TAN, H.; NIE, S. Deciphering diet-gut microbiota-host interplay: Investigations of pectin. **Trends in Food Science & Technology**, v. 106, p. 171–181, 2020. https://doi.org/10.1016/j.tifs.2020.10.010

THIRUVENGADAM, M. *et al*. A comprehensive review of beetroot (*Beta vulgaris* L.) bioactive components in the food and pharmaceutical industries. **Critical Reviews in Food Science and Nutrition**, p. 1-33, 2022. https://doi.org/10.1080/10408398.2022.2108367

TIAN, L. *et al*. Effects of pectin supplementation on the fermentation patterns of different structural carbohydrates in rats. **Molecular nutrition & food research**, v. 60, n. 10, p. 2256-2266, 2016. https://doi.org/10.1002/mnfr.201600149

TIVELLI, S. W. *et al*. Beterraba: Do plantio à comercialização. **Boletim Técnico IAC, Campinas, n. 210, 2011**, v. 32, n. 4, 2011.

TOBARUELA, E. DE C. *et al*. Application of dietary fiber method AOAC 2011.25 in fruit and comparison with AOAC 991.43 method. **Food Chemistry**, v. 238, p. 87-93, 2018. https://doi.org/10.1016/j.foodchem.2016.12.068

TOSSI, V. E. *et al*. Casting light on the pathway to betalain biosynthesis: A review. **Environmental and Experimental Botany**, v. 186, 2021. https://doi.org/10.1016/j.envexpbot.2021.104464

TRANI, P. E.; CANTARELLA, H.; TIVELLI, S. W. Produtividade de beterraba em função de doses de sulfato de amônio em cobertura. **Horticultura Brasileira**, v. 23, n. 3, 2005. https://doi.org/10.1590/S0102-05362005000300007

TSIGALOU, C. *et al*. Gut microbiome and Mediterranean diet in the context of obesity. Current knowledge, perspectives and potential therapeutic targets. **Metabolism Open**, v. 9, n. 100081, 2021. https://doi.org/10.1016/j.metop.2021.100081

TUTUNCHI, P. *et al*. Extraction of red beet extract with β-cyclodextrin-enhanced ultrasound assisted extraction: A strategy for enhancing the extraction efficacy of bioactive compounds and their stability in food models. **Food Chemistry**, v. 297, 2019. https://doi.org/10.1016/j.foodchem.2019.124994

VANDENPLAS, Y. *et al*. Factors affecting early-life intestinal microbiota development. **Nutrition,** v. 78, n. 110812, 2020. https://doi.org/10.1016/j.nut.2020.110812

VAN TREUREN, W.; DODD, D. Microbial contribution to the human metabolome: Implications for health and disease. **Annual Review of Pathology: Mechanisms of Disease**, v. 15, p. 345-369, 2020. https://doi.org/10.1146/annurev-pathol-020117-043559

VICENTINI, A.; LIBERATORE, L.; MASTROCOLA, D. Functional foods: Trends and development of the global market. **Italian Journal of Food Science**, v. 28, n. 2, 2016.

WANG, L. *et al*. Green tea polyphenols modulate colonic microbiota diversity and lipid metabolism in high-fat diet treated HFA mice. **Journal of Food Science**, v. 83, n. 3, p. 864-873, 2018. https://doi.org/10.1111/1750-3841.14058

WANG, S. *et al*. Fermented milk supplemented with probiotics and prebiotics can effectively alter the intestinal microbiota and immunity of host animals. **Journal of Dairy Science**, v. 95, n. 9, p. 4813-4822, 2012. https://doi.org/10.3168/jds.2012-5426

WANG, S. *et al*. Rational use of prebiotics for gut microbiota alterations: Specific bacterial phylotypes and related mechanisms. **Journal of Functional Foods**, v. 66, p. 103838, 2020. https://doi.org/10.1016/j.jff.2020.103838

WANG, X.; QI, Y.; ZHENG, H. Dietary polyphenol, gut microbiota, and health benefits. **Antioxidants**, v. 11, n.6, 2022. https://doi.org/10.3390/antiox11061212

WANG, Y. *et al*. Effect of two-week red beetroot juice consumption on modulation of gut microbiota in healthy human volunteers – A pilot study. **Food Chemistry**, v. 406, p. 134989, 2023. https://doi.org/10.1016/j.foodchem.2022.134989

WU, R. Y.; JOHNSON-HENRY, K. C.; SHERMAN, P. M. Prebiotics and human milk oligosaccharides. **Encyclopedia of Gastroenterology**, v. 2, p. 278–286, 2020. https://doi.org/10.1016/B978-0-12-801238-3.66049-X

YANG, L. *et al*. Recent advances of intestinal microbiota transmission from mother to infant. **Journal of Functional Foods**, v. 87, 2021. https://doi.org/10.1016/j.jff.2021.104719

YANG, Q. *et al*. Role of dietary nutrients in the modulation of gut microbiota: A narrative review. **Nutrients,** v. 12, n. 2, p. 381, 2020. https://doi.org/10.3390/nu12020381

YAN, W. *et al*. Interpenetrating polymer network hydrogels of soy protein isolate and sugar beet pectin as a potential carrier for probiotics. **Food Hydrocolloids**, v. 113, p. 106453, 2021. https://doi.org/10.1016/j.foodhyd.2020.106453

YEUNG, Y. K. *et al*. Structural, antioxidant, prebiotic and anti-inflammatory properties of pectic oligosaccharides hydrolyzed from okra pectin by Fenton reaction. **Food Hydrocolloids**, v. 118, 2021. https://doi.org/10.1016/j.foodhyd.2021.106779

YU, C., *et al*. Structure and fermentation characteristics of five polysaccharides sequentially extracted from sugar beet pulp by different methods. **Food Hydrocolloids**, v. 126, p. 107462, 2022. https://doi.org/10.1016/j.foodhyd.2021.107462

ZDUNEK, A., PIECZYWEK, P. M., & CYBULSKA, J. The primary, secondary, and structures of higher levels of pectin polysaccharides. **Comprehensive Reviews in Food Science and Food Safety**, v. 20, p. 1101-1117, 2021.

ZHANG, C. *et al*. The potential of proteins, hydrolysates and peptides as growth factors for: Lactobacillus and Bifidobacterium: Current research and future perspectives. **Food and Function**, v. 11, n. 3, p. 1946-1957, 2020. https://doi.org/10.1039/C9FO02961C

ZHANG, N.; JU, Z.; ZUO, T. Time for food: The impact of diet on gut microbiota and human health. **Nutrition**, v. 51, p. 80-85, 2018. https://doi.org/10.1016/j.nut.2017.12.005

ZHANG, S. *et al*. Preparation and prebiotic potential of pectin oligosaccharides obtained from citrus peel pectin. **Food Chemistry**, v. 244, p. 232-237, 2018. https://doi.org/10.1016/j.foodchem.2017.10.071

ZHAO, Y. *et al*. Dose-dependent effects of apple pectin on alleviating high fat-induced obesity modulated by gut microbiota and SCFAs. **Food Science and Human Wellness**, v. 11, p.143-154, 2022. https://doi.org/10.1016/j.fshw.2021.07.015

ZHENG, J. *et al*. A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus *Lactobacillus beijerinck* 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. **International Journal of Systematic and Evolutionary Microbiology**, v. 70, n. 4, 2020. https://doi.org/10.1099/ijsem.0.004107

APÊNDICES

APÊNDICE A – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

UNIVERSIDADE FEDERAL DA PARAÍBA CENTRO DE CIÊNCIAS DA SAÚDE PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA NUTRIÇÃO TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Prezado (a) Senhor (a)

Esta pesquisa é sobre avaliação do efeito prebiótico de farinhas obtidas de diferentes partes de beterraba utilizando sistema de fermentação *in vitro* com inóculo fecal humano e está sendo desenvolvida pela pesquisadora Sônia Paula Alexandrino de Oliveira, aluna do Programa de PósGraduação em Ciências da Nutrição, da Universidade Federal da Paraíba (UFPB), sob a orientação do Prof. Dr. Evandro Leite de Souza, professor do Programa de Pós-Graduação em Ciências da Nutrição do Departamento de Nutrição (UFPB).

Os objetivos do estudo são obter farinhas da raiz, talos e folhas de beterraba vermelha e avaliar o potencial prebiótico das mesmas utilizando sistemas de fermentação *in vitro*, com o inóculo fecal humano. A realização desta pesquisa trará informações científicas atualmente escassas acerca da relação do potencial prebiótico da beterraba hortícola. A pesquisa apresenta abordagem inovadora considerando os aspectos tecnológicos, econômicos e de sustentabilidade, por utilizar além da parte comercial da beterraba, a raiz, co-produtos da cultura da beterraba (talos e folhas), que possui também elevada concentração de polifenóis e carboidratos não digeríveis, definindo benefícios do uso na alimentação e possíveis interações benéficas com probióticos.

A execução deste projeto de tese apresenta o potencial de gerar informações que subsidiem a elaboração de ingredientes funcionais, aspecto de corrente interesse à indústria alimentícia e a comunidade que busca por consumo de uma alimentação mais saudável. Em virtude que este TCLE se encontra em mais de uma página, as demais serão rubricadas pelo pesquisador e sujeito da pesquisa.

Solicitamos a sua colaboração para realizar a doação de material fecal, o qual será colhido no referido dia e armazenado em frasco de coleta cedido pelos envolvidos na pesquisa, como também sua autorização para apresentar os resultados deste estudo em eventos da área de saúde e, eventualmente, publicação em revista científica. Por ocasião da publicação dos resultados, seu nome será mantido em sigilo. Informamos que essa pesquisa não oferece riscos, previsíveis, para a sua saúde e que os resultados obtidos servirão de base para definir os efeitos prebióticos de farinhas obtidas de raiz, talos e folhas de beterraba. Esclarecemos que sua participação no estudo é voluntária e, portanto, o(a) senhor(a) não é obrigado(a) a fornecer as informações e/ou colaborar com as atividades solicitadas pela pesquisadora.

Caso decida não participar do estudo, ou resolver a qualquer momento desistir do mesmo, não sofrerá nenhum dano moral ou financeiro. Os pesquisadores estarão a sua disposição para qualquer esclarecimento que considere necessário em qualquer etapa da pesquisa. Diante do exposto, declaro que fui devidamente esclarecido(a) e dou o meu consentimento para participar da pesquisa e para publicação dos resultados. Estou ciente que receberei uma cópia desse documento.

Assinatura do Participante da Pesquisa

Assinatura da Testemunha

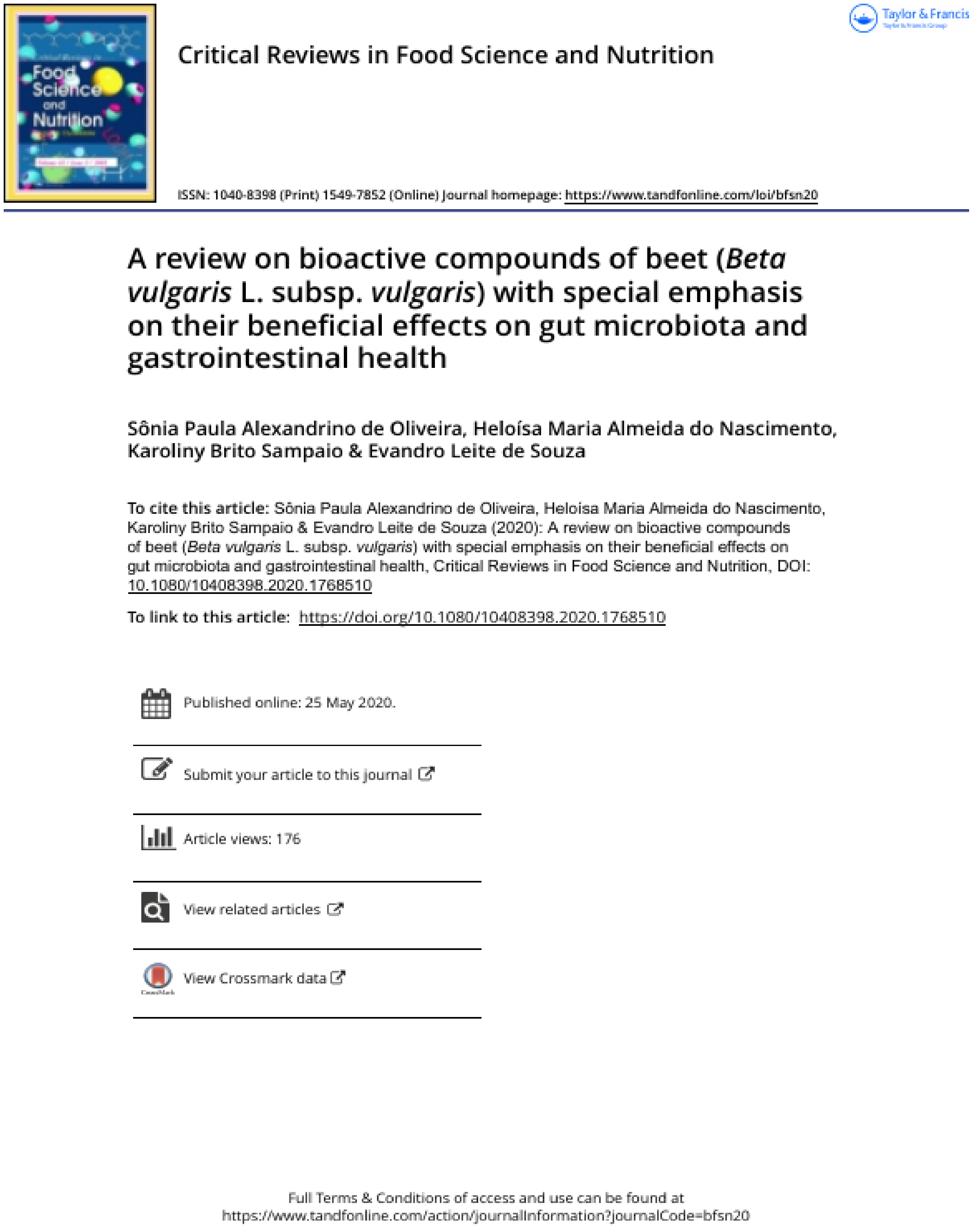
Contato do Pesquisador (a) Responsável:
Caso necessite de maiores informações sobre o presente estudo, favor ligar para a pesquisadora: Sônia Paula Alexandrino de Oiveira.
Endereço (Setor de Pesquisa): Laboratório de Microbiologia de Alimentos, Departamento de Nutrição do Centro de Ciências da Saúde da Universidade Federal da Paraíba (UFPB), Campus I - Cidade Universitária – CEP 58051-900 – João Pessoa – PB Telefone: (83) 3216-7807/ (83) 98841-1246
Comitê de Ética em Pesquisa do Centro de Ciências da Saúde da Universidade Federal da Paraíba Campus I - Cidade Universitária - 1º Andar – CEP 58051-900 – João Pessoa/PB
☎ (83) 3216-7791 – E-mail: **comitedeetica@ccs.ufpb.br**

Atenciosamente,

Assinatura do Pesquisador Responsável

Assinatura do Pesquisador Participante

APÊNDICE B – Artigo de revisão (https://doi.org/10.1080/10408398.2020.1768510).



Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

A review on bioactive compounds of beet (*Beta vulgaris* L. subsp. *vulgaris*) with special emphasis on their beneficial effects on gut microbiota and gastrointestinal health

Sônia Paula Alexandrino de Oliveira, Heloísa Maria Almeida do Nascimento, Karoliny Brito Sampaio & Evandro Leite de Souza

To cite this article: Sônia Paula Alexandrino de Oliveira, Heloisa Maria Almeida do Nascimento, Karoliny Brito Sampaio & Evandro Leite de Souza (2020): A review on bioactive compounds of beet (*Beta vulgaris* L. subsp. *vulgaris*) with special emphasis on their beneficial effects on gut microbiota and gastrointestinal health, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2020.1768510

To link to this article: https://doi.org/10.1080/10408398.2020.1768510

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2020.1768510

Taylor & Francis
Taylor & Francis Group

REVIEW

A review on bioactive compounds of beet (*Beta vulgaris* L. subsp. *vulgaris*) with special emphasis on their beneficial effects on gut microbiota and gastrointestinal health

Sônia Paula Alexandrino de Oliveira, Heloísa Maria Almeida do Nascimento, Karoliny Brito Sampaio, and Evandro Leite de Souza

Laboratory of Food Microbiology, Department of Nutrition, Health Sciences Center, Federal University of Paraíba, João Pessoa, Brazil

ABSTRACT
This review discusses the available literature concerning the bioactive compounds of beet (*Beta vulgaris* L.) and their ability to modulate the gut microbiota and parameters indicative of gastrointestinal health. Data of published literature characterize beet as a source of a variety of bioactive compounds (e.g. diet fiber, pectic-oligosaccharides, betalains and phenolics) with proven beneficial effects on human health. Beet extracts and pectin and pectic-oligosaccharides from beet have shown able to modulate positively gut microbiota composition and activity, with noticeable bifidogenic effects, in addition to stimulate the growth and metabolism of probiotics. Beet betalains and phenolics seem to increase the production of metabolites (e.g. short chain fatty acids) by gut microbiota and probiotics, which are linked with different beneficial effects on host health. The outstanding contents of betalains and phenolics with antioxidant, anti-inflammatory and anti-carcinogenic properties have been linked to the positive effects of beet on gastrointestinal health. Beet should be a healthy choice for use in domestic meal preparations and a source of ingredients to formulate added-value functionalized food products.

KEYWORDS
Beta vulgaris L; bioactive compounds; gut health; modulation

Introduction

Beet (*Beta vulgaris* L. subsp. *vulgaris*), Chenopodiaceae family, is a plant originated from southern and eastern Europe and northern Africa. The world beet root production in 2017 reached 301 million tons with a harvested area of 4.89 million hectares. Europe ranks the first in beet root production (207.9 tons), representing 69% of world beet production, followed by Asia (42.7 tons), America (34.3 tons) and Africa (15.9 tons) (FAOSTAT, 2019).

Beet subspecies are formed by four main biotypes cataloged considering their specific morphological characteristics and end-use, to cite: leaf (leaf beet group), sugar (sugar beet group), forage (fodder beet group) and vegetable beet (garden beet group). In leaf beet, popularly known as Mangold, spinach beet and Swiss chard, the leaves and petioles are the most valued parts because of the typical small dimeter of the roots. In sugar beet, the roots have high sucrose contents, being usually used for sugar extraction and ethanol production, while the leaves are destined commonly for animal feed. Roots, stems and leaves of horticultural beet, popularly known as red beet or table beet, are used for human consumption and roots are the most commercially valued part of the plant because of the outstanding nutritional composition (Lange, Brandenburg, and Bock 1999; Batista et al. 2016).

Beet anatomical division consists of roots (hypocotyl), sprout (composed by semi-rigid red to purple stems, also named petiole) and leaves (leafy limb), which are tender and have varied color and shape depending on beet cultivar (Kumar 2015; Ninfali and Angelino 2013). The most valued and considered usually edible part of beet is the root, which has received increasing interest for use in food formulation because of the attractive color and presence of different nutrients and bioactive compounds, being recognized as a super food because of their unusually high contents of antioxidants, vitamins and other constituents able to exert health promoting properties (Chawla et al. 2016; Kumar 2015; Maheshwari, Parmar, and Joseph 2013). There has been a variety of beet-based preparations available for human consumption, to cite: in raw or cooked form as ingredient to salads, cooked as stews, minimally processed, crunch slices and pickles. Beet have been also used to formulate functionalized products, such as juices, gels, fermented beverages, dried powders (tablets and capsules) and crunchy beet root slices. Recently, beet products have attracted increased attention because of their proven blood pressure-lowering properties and ability to induce improvements in endothelial function (Chhikara et al. 2019; Wiczkowski et al. 2018).

Extracts and beet powders have been also considered potential alternatives to replace synthetic colorants in food

CONTACT Evandro Leite de Souza evandroleitesouza@ccs.ufpb.br Universidade Federal da Paraíba, Centro de Ciências da Saúde, Departamento de Nutrição, Campus I, 58051-900, Cidade Universitária, João Pessoa, Paraíba, Brazil.

industry. Betalains are well-known natural pigments with red-violet and yellow-orange tonalities and stability over a wide pH range, besides to be considered safe for human consumption. However, the practical application of betalains as food pigments has been limited due to their sensitivity to degradation when exposed to light and high temperatures during food processing and storage (Esatbeyoglu et al. 2015; Halwani, Sindi, and Jambi 2018; Polturak and Aharoni 2018).

In recent years, there has been a growing interest on the biological activities of beet, notably of red beet, including their positive effects on gastrointestinal health (Babbar et al. 2017; Baião et al. 2017; Clifford et al. 2015; Chhikara et al. 2019; Ninfali et al. 2017). Additionally, beet consumption has been considered an adjuvant therapeutic option in a range of pathologies associated with oxidative stress and inflammation. These possible properties and applications have been primarily associated with the presence of betalains in beet, which have displayed antioxidant, anti-inflammatory and chemo-preventive activities in in vitro and in vivo experiments (Clifford et al. 2015; Lechner and Stoner 2019; Rahimi et al. 2019).

Some investigations have shown that carbohydrates present in beet, primarily pectin, could influence the gastric emptying, nutrients absorption in small intestine and fermentation in large intestine (Prandi et al. 2018; Tian et al. 2016). Metabolization of oligo and polysaccharides from beet by bacteria forming the gut microbiota has indicated the ability of these components to modulate positively gut microbial communities and stimulate the production of specific metabolites, which are indicative of potential prebiotic properties (Gómez et al. 2016; Holck et al. 2011; Leijdekkers et al. 2014; Vigsnaes et al. 2011). Prebiotic effects of oligo and polysaccharides and phenolics have received increasing attention because of the evidence that gut microbial communities play an important role in human health, with impacts on host metabolism, physiology, nutrition and immune functions (Danneskiold-Samsøe et al. 2019; Guinane and Cotter 2013). Compositional and functional disruption of gut microbiota has been linked to different pathological intestinal conditions (e.g. obesity and malnutrition), systematic diseases (e.g. diabetes and hypertension) and chronic inflammatory diseases (e.g. inflammatory bowel diseases) (Cavalcanti Neto et al. 2018; Cui et al. 2019; Shamoon, Martin, and O'Brien 2019).

This review discusses the available literature concerning the bioactive compounds in beet and their ability to modulate the composition and function of gut microbiota and different parameters indicative of gastrointestinal health.

Nutrients and bioactive compounds in beet

Results of investigations with focus on beet composition have shown the presence of a variety of nutrients, including high amounts of dietary fiber, minerals (e.g. potassium, sodium, iron, copper, magnesium, calcium, phosphorus and zinc), vitamins (e.g. vitamin A, thiamin, riboflavin, niacin, ascorbic acid and B complex vitamins), essential amino acids, phytosterols, phenolics and betalains (Kumar 2015; Kushwaha et al. 2018; Nemzer et al. 2011; Wang, Beltranena, and Zijlstra 2016). Data of nutritional composition of different fresh beet samples have been variable according to beet cultivar, edaphoclimatic characteristics and harvest and postharvest conditions. Red beet grown under organic agricultural practices have shown typically improved nutritional characteristics, higher contents of total polyphenols and betalains, as well as higher antioxidant capacity when compared to conventionally grown red beet (Carrillo et al. 2017; Chhikara et al. 2019).

A study assessing the nutritional and betalainic profile of red beet dried extracts found that contents of nutrients and betalains depended on the type of drying technique (e.g. air-dried, freeze-dried and spray-dried). Contents of nutrients in beet dried extracts were in the range of 2.17–6.14% for ashes, <0.007–1.52% for lipids, 3.81–12.4% for proteins, <1–557% for vitamin C and 75.4–89% for carbohydrates, including fibers (>1–21.8%) and sugars (30.8–62.5%). Contents of fructose, glucose and sucrose in dried red beet extracts were in the range of 0.8–1.9, 1.1–2 and 30.8–62.5%, respectively. Potassium, calcium and magnesium were the minerals found in highest contents in beet extracts and glutamic acid was the most prevalent amino acid (Nemzer et al. 2011).

High contents of bioactive compounds have been purified from beet samples, including carotenoids (mainly *β*-carotene and lutein), saponins (e.g. oleanolic acid and several betavulgarosides), flavonoids (rutin, kaempferol, rhamnetin, rhamnocitrin and astragalin), phenolic acids (e.g. ferulic acid, caffeic acid, p-coumaric acid, syringic acid and vanillic acid) and nitrate (644–1800 mg/kg) (Lechner and Stoner 2019; Lidder and Webb 2013).

Betalains have been the most studied bioactive compounds in beet. Betalains are water soluble indole-derived pigments found in plants belonging to Caryophyllales family. Predominant forms of betalains are betacyanin (red-violet) and betaxanthin (yellow-orange) (Ninfali and Angelino 2013). Red beet root is the primary source of betalains in western diets (Esatbeyoglu et al. 2015). Natural betalain can serve not only as safe additives to add natural color or antioxidant properties to food, pharmaceutical and cosmetic formulations, but also with the end of promoting beneficial effects on health, preventing the occurrence of chronic-degenerative diseases, such as hypertension, dyslipidemia, cancer and cardiovascular diseases (Rahimi et al. 2019). Beet is an allowed source of betalains approved for use as food additives in USA (Title 1 of Code of Federal Regulations, 21 DFR 73, 40) and European Union (E-162) (Martins et al. 2017).

Total betalain contents in a range of 0.8 and 1.3 g/L were found in fresh juice prepared with seven different beet varieties, being approximately 60 and 40% of betacyanins and betaxanthins, respectively. Hydroxycinnamic acids were also detected in beet fresh juices (Wruss et al. 2015). Phenolic compounds identified in extracts from *B. vulgaris* cv. Detroit Dark Red were 4-hydroxybenzoic acid (0.012 mg/g), chlorogenic acid (0.018 mg/g), caffeic acid (0.037 mg/g), catechin

(0.047 mg/g) and epicatechin (0.032 mg/g) (Georgiev et al. 2010).

Consumption of beet juice and beet booster shots has been an attracting niche market, being recognized as adjuvants for health and physical performance (Domínguez et al. 2018). Red beet juices were previously evaluated, including sixteen commercial beet root juices and five commercial beet powders. Contents of different compounds (e.g. minerals, betalains, oxalic acid, phenolic acids, nitrates and sugars) in juices prepared with seven red beet varieties, sixteen commercial beet root juices and five commercial beet powders were measured. Highest variations were found in contents of nitrates (565–4626 mg/L) in commercial beet juice samples, which were mostly associated with typical variation in levels of this ion in beet samples. High nitrate contents have been the main reason for use of beet juice as a high-sport performance booster (Wruss et al. 2015).

Beet leaves have been considered good sources of some nutrients. Dehydrated red beet leaves have shown presence of important polyunsaturated fatty acids, such as ω-6 and ω-3 (ΣPUFAs 48.3 mg/g) and minerals, such as calcium (1864.85 mg/kg), iron (256.30 mg/kg), magnesium (1.79 mg/kg), potassium (20,784.90 mg/kg) and sodium (256.30 mg/kg) (Biondo et al. 2014). Beet fiber has been described as a material derived from all plants belonging to the species *B. vulgaris* and comprises typically cellulose (22–24%), hemicelluloses (30%) and pectin (25%), with a ratio of insoluble to soluble fiber of approximately 2:1. Sugar beet fiber has a fiber content varying from 70 to 80 g/100 g and a limited content of protein (5%), ash (3%) and moisture (7%) (Harland 2018; Sivapragasam et al. 2014). Sugar beet pulp has higher contents of pectin than other lignocellulosic biomass, in addition to have an average 3–4% of sucrose, being considered a good sugar source (Panagiotopoulos et al. 2010).

Although not used commonly for human consumption, sugar beet pulp is an important by-product from beet sugar industry. Due to the high pectin content, sugar beet pulp has become an important source of pectins with good emulsifying properties (Ai et al. 2019). Beet pectin is mainly composed of homogalacturonan, rhamnogalacturonan-I and rhamnogalacturonan-II regions and nanostructures, such as galacturonic acid, rhamnose, arabinose, galactose, glucose, xylose, neutral sugars and ferulic acid (Larsen et al. 2019; Liu et al. 2019). Sugar beet pectin has usually a high content of rhamnogalacturonan-I (RG-I) region. RG-I-rich pectins with a high number of side chains have been indicated as potential sources of a new class of prebiotics named pectin-derived oligosaccharides (Babbar et al. 2017; Gullón et al. 2013; Mao et al. 2019).

Researchers have investigated methods for extraction and characterization of oligosaccharides from beet pectin, involving chemical (Mao et al. 2019; Martínez et al. 2009), enzymatic (Babbar et al. 2017; Concha and Zúñiga 2012; Leijdekkers et al. 2013) and combined methods (Chen, Meng, et al. 2015; Guo et al. 2017). Oligosaccharides obtained with beet pectin hydrolysis have been indicated for use as prebiotics, which should add value to an important sugar beet agro-industrial by-product (Babbar et al. 2017).

Biotransformation of beet betalains

Biotransformation of bioactive compounds naturally found in foods is influenced by their physicochemical properties, food matrix constitution and food processing conditions (Oliveira and Bastos 2011). Studies on the kinetics and absorption of bioactive compounds with measurements of plasma levels and/or urinary excretion have shown that metabolites found in blood resulting from digestive and hepatic activity differ generally from native dietary compounds, indicating that the most abundant compounds in a food are not necessarily those leading to the highest concentrations of active metabolites in target tissues (Manach et al. 2005). Effects of these active metabolites on living tissues could be also affected by their specific chemical structure, metabolism and composition and function of host gut microbiota (Chhikara et al. 2019; Sawicki et al. 2018).

Betalains are hydrophilic pigments formed by betalamic acid and subdivided into two groups based on their chemical composition and structure, to cite: betacyanins (red-violet), derived from condensation of betalamic acid with cyclo-3,4-dihydroxyphenylalanine (cyclo-DOPA); and betaxanthins (yellow-orange), derived from condensation of betalamic acid immonium with amines and distinct amino acids. Betacyanins are classified into four groups: betanin-type, amaranth-type, gomfrenine-type and type-2-decarboxybetanine (Esatbeyoglu et al. 2015).

Tyrosine is the precursor of betalains and its biosynthesis occurs in plant cytoplasm, involving three main enzymes: tyrosinase, 4,5-DOPA-extradiol dioxygenase and betanidin-glucosyltransferase (Esatbeyoglu et al. 2015). Betalains biosynthesis begins with the hydroxylation of tyrosine with molecular oxygen through the action of tyrosinase (Gandía-Herrero and García-Carmona 2012). Formed betalamic acid is the intermediate point in synthesis of all betalains, being responsible for bioactive properties of plant pigments (Esatbeyoglu et al. 2015).

Orally administered betanin, the major pigment in red beet, is poorly absorbed in small intestine, being mostly metabolized in large intestine (Khan 2016). Results of a clinical trial with healthy individuals showed that, after supplementation with a single dose of a commercial red beet juice, the amount of intact betalains (betanin and isobetanin) recovered in urine was 1001 ± 273 mg, which corresponded to 0.28 ± 0.08% of total orally administered dose. Maximum excretion rate (91.7 ± 30.1 mg/h) of betalains was found after 3 h of oral administration. Terminal elimination rate constant (kz), half-life and expected total betalain amount excreted in urine were 0.097 ± 0.021/h, 7.43 ± 1.47/h and 1228 ± 291 mg, respectively. Urinary excretion of non-metabolized betalains seemed to be fast and mono-exponential, indicating a one-compartment model, as well as that bioavailability of betalains was low because the minor portion of systemic elimination occurs via renal clearance. These results indicated that measurements of non-metabolized compounds and their metabolites in plasma, urine and bile should be investigated to a better comprehension of betalain bioavailability (Frank et al. 2005).

Effects of supplementation with fermented beet juice for six weeks were studied in 24 healthy volunteers. The experiment was done in two periods: during the first period

(1 week), volunteers were deprived of products with betalain pigments; and during the second period (7 weeks), volunteers had their diet enriched with industrialized fermented beet juice (dose of 200 mL/60 kg of body weight). Twelve betalain derivatives were found in blood plasma and urine of healthy volunteers after beet juice consumption. Highest betalain levels in blood plasma (87.65 ± 15.71 nmol/L) and urine (1.14 ± 0.12 μmol) were found after the first and second week of beet root juice consumption, respectively. Long-term and regular consumption of beet juice caused stabilization of betalain profile in physiological fluids, which included native betalains and their decarboxylated and dehydrogenated metabolites (Sawicki et al. 2018).

In addition, phenolic compounds found in red beet, such as epicatechin, catechin hydrate, 4-hydroxybenzoic acid and caffeic acid, could exert synergistic effects with betalains to increase mutually their antioxidant effects. Hairy root cultures of red beet (*B. vulgaris* cv. Detroit Dark Red) were collected and used for betalain extraction in order to compare the antioxidant activities of betalain extracts from hairy roots and intact red beet. Hairy root extract had higher antioxidant activity than intact beet extract, which was mostly associated with the 20-fold higher total phenolic content found in hairy root extract (944 ± 22 mg FAE/g DE) when compared to intact beet extract (47 ± 09 mg FAE/g DE). Although to a lesser extent, betalain content in hairy root extract (47.11 ± 1.27 mg/g) were also increased when compared to intact beet extract (39.76 ± 0.98 mg/g), which could have also contributed to the higher antioxidant activity found in hairy root extract. These results suggested that phenolic compounds could exert synergistic effects with betalains to enhance the antioxidant properties (Georgiev et al. 2010). Antioxidant activity of betalains depends on their chemical structure and electron donor capacity. Results of investigations on structure-activity relationship in betalains have indicated that the antiradical activity for the simplest pigments is enhanced by the connection of betalain characteristic electron resonance system with an aromatic ring. Betalamic acid, the central structure of betalains, has strong antiradical and antioxidant activities (Khan 2016; Gandía-Herrero, Escribano, and García-Carmona 2016; Slimen, Najar, and Abderrabba 2017).

Although the bioavailability of beet betalains has not been individually and clearly determined, the achievement of these data could be valuable to give a better understanding of their potential for use in health-promoting dietary interventions (Clifford et al. 2015).

Modulatory effects of beet on gut microbiota

Retrieved studies assessing the in vitro effects of beet and beet bioactive compounds on intestinal microbiota and probiotics with information on tested beet part, bioactive compounds, examined doses, experimental models, measured parameters and main results are shown in Table 1. Non-sucrose polysaccharides from sugar beet pulp, composed of cellulose, hemicelluloses and pectin, have shown resistant to simulated human gastrointestinal conditions and able to reach the colon where could be fermented by gut microbiota (Sivapragasam et al. 2014). Pectin-derived oligosaccharides have shown also resistant to enzymes present in foregut (stomach and small intestine), but they can be fermented by microorganisms found in large intestine, resulting in production of short-chain fatty acids (SCFA) and stimulation of growth and/or activity of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus* species (Gullón et al. 2011; Prandi et al. 2018).

Some studies have investigated the effects of beet consumption on gut microbiota composition, which may induce a prebiotic-like effect. Prebiotics are substrates utilized selectively by microorganisms forming the gut microbiota, conferring a variety of health benefits to the host (Gibson et al. 2017). Pectin and pectin-derived oligosaccharides were cited as prebiotics due to their capacity of modulating the gut microbiota composition, particularly increasing the population of the bacterial species *Faecalibacterium prausnitzii* and *Roseburia intestinalis*. These positive effects on gut microbiota reported to pectin-derived oligosaccharides were similar or even higher than those caused by the commercial prebiotics fructooligosaccharides and galactooligosaccharides (Gullón et al. 2013).

Sugar beet pectin has high methoxyl pectins with relatively higher fractions of rhamnogalacturonan and neutral sugars (galactose, arabinose and rhamnose). After an in vitro fermentation using a TIM-2 colon model, the production of propionic acid was relatively high (43.3 mmol) in media with sugar beet pectin, which correlated positively with a relative high abundance of *Prevotella copri* and *Ruminococcus* spp., indicating that these bacterial species were able to produce propionic acid from sugar beet pectin fermentation. Additionally, abundance of *Oscillospira, Blautia, Dorea, Ruminococcus, Coprococcus, R. torques, Lachnospiraceae* and *Clostridiales*, within the phylum Firmicutes, and *Paraprevotella, B. uniformis, B. ovatus, P. distasonis* and *Prevotella*, within the phylum Bacteroidetes, were increased during sugar beet pectin fermentation. These results indicated that microbial gut communities could be specifically modulated by sugar beet pectin (Larsen et al. 2019).

Administration of a standard diet (RMH-B) with 3% (w/w) of sugar beet pectin, mainly composed of uronic acid (63 mol%), galactose (18 mol%) and arabinose (13 mol%), for seven weeks increased the cecal population of *Lactobacillus* spp. and *Lachnospiraceae* spp. in Wistar rats. Stimulatory effects of sugar beet pectin on these two microbial groups were higher than those caused by a soy pectin enriched diet. These results indicated that dietary supplementation with sugar beet pectin could modulate positively microbiota composition and stimulate SCFA production in large intestine (Tian et al. 2016).

Some studies have focused on production of sugar beet pectic oligosaccharides with application of different methods, including enzymatic and acid hydrolysis, being also suggested the ability of these compounds to stimulate the growth of specific gut bacterial populations (Concha and Zúñiga 2012; Goméz et al. 2016; Gómez et al. 2019). Pectic oligosaccharides obtained with sugar beet pectin autohydrolysis and purified with membrane filtration induced a shift in

Table 1. Retrieved studies assessing the in vitro effects of beet and beet bioactive compounds on intestinal microbiota and probiotics.

References	Tested beet part or product	Bioactive compounds	Experimental models/ measured parameters	Main results
Holck et al. 2011	Sugar beet pectin (3%, w/w)	Homogalacturonan (HG) and rhamnogalacturonan I (RGI) (1.4%, w/w) by enzymatic cleavage.	Fermentation with fecal samples from patients with ulcerative colitis (UC)	Pectic-oligosaccharides with slightly different structures had different effects on Bacteroidetes and Firmicutes
Holck et al. 2010	Sugar beet pulp	Feruloylated and Nonferuloylated Arabino-oligosaccharides from sugar beet pectin (5 g/L)	Fermentation with health human fecal samples	Selective stimulation of bifidobacteria by feruloylated and nonferuloylated long-chain arabino-oligosaccharides
Klewicka and Czyżowska 2011	Beet juice (0.7 L/kg)	Not informed	Fermentation of beet juice with *Lactobacillus brevis* and *L. paracasei*	*L. brevis* and *L. paracasei* had high counts in fermented beet juice up to 30 days of refrigerated storage. Fermented beet juice had anti-mutagenic activity
Onumpai et al. 2011	Sugar beet pectin	Beet arabinan (1%, w/v)	Fermentation with human fecal inoculum	Stimulation of *Bifidobacterium* and *Lactobacillus* growth species and increased abundance of Bacteroides-Prevotella group
Gómez et al. 2016	Sugar beet pulp	Pectic oligosaccharides (5 and 10 g/L)	Fermentation with human fecal cultures	Increased populations of different beneficial bacterial species, with remarkable bifidogenic effects
Tian et al. 2016	Sugar beet	Pectin (3%, w/w)	Wistar rats fed a standard diet chow (RMH-B) with 3% (w/w) sugar beet pectin for 7 weeks	Increased cecal population of *Lactobacillus* spp. and *Lachnospiraceae* spp.
Zhang, Lin, and Zhong 2016	Sugar beet	Pectin (1- 4%, w/v)	Used to prepare solid/oil/ water emulsions to encapsulate *L. salivarius*	Improvement in survival rates of *L. salivarius* under different environmental stresses, including a simulated gastrointestinal digestion
Chung et al. 2017	Sugar beet pectin (3%, w/w)	Homogalacturonan oligomer (degrees of polymerization (DP) DP4 (45 mg/g) and DP5 (37 mg/g)	Utilization as carbon source by *Faecalibacterium prausnitzii, Eubacterium eligens* and *Bacteroides thetaiotaomicron*	*E. eligens* was the most effective to degrade diet-derived pectins, whit ability to grow on purified pectic-oligosaccharides. Purified pectic-oligosaccharides promoted the growth of beneficial Firmicutes species
Henning et al. 2017	Beet root	Not informed	Determination of fecal microbiota composition of healthy adults at phylum level after four days of beet juice consumption	Consumption of mixed juice caused weight loss, increased plasma levels of nitric oxide and decreased lipid oxidation. These effects were primarily associated with the high contents of fiber and nitrate in beet root pulp
Panghal et al. 2017	Fresh beet juice (700 mL)	Not informed	Fermentation of pasteurized beet juice	*L. plantarum, L. rhamnosus* and *L. delbrueckii* increased antioxidant activity and contents of total phenols and flavonoid in fermented juice
Mohsen, Alsaman, and Mahrous 2018	Fresh red beet (200 g/L)	Not informed	Fermentation with *L. plantarum* P108, *L. acidophilus* P110	Fermented beet root had increased total phenolic content and antioxidant activity. Beet root fermented with *L. acidophilus* P110 had highest total phenolic content and antioxidant activity
Münnich et al. 2017	Molasse sugar beet pulp (0–400 g/kg)	Not informed	Determination of ruminal microbial communities and fermentation profile of molasse sugar beet pulp added of ruminal fluid and solid cow digesta	Beet pulp stimulated ruminal acetate production and propionate fermentation. High replacement rates of beet pulp caused decreased utilization of ammonia and higher ruminal methane production
Prandi et al. 2018	Sugar beet pulp	Pectin and Pectin-oligo saccharide mixtures (1%, w/w)	Fermentation with *Lactococcus lactis, L. amylovorus, L. casei, L. plantarum, L. rhamnosus, L. curvatus, L. fermentum, L. acidophilus* and *L. delbrueckii*	Different fractions stimulated the growth of *Lactobacillus* species, with the exception of *L. lactis*. Pectin oligosaccharides with low polymerization degree arabinans and little or no free galacturonic acid were the most effective to stimulate *Lactobacillus* species
Gomez et al. 2019	Sugar beet pulp by-product	Pectic oligosaccharides (10 g/L)	Substitute of glucose (10 g/L) as a carbon source in laboratory medium	Stimulation of the growth of probiotic *Lactobacillus* isolates, especially of *L. reuteri, L. plantarum* and *L. rhamnosus*

(continued)

Table 1. Continued.

References	Tested beet part or product	Bioactive compounds	Experimental models/ measured parameters	Main results
Larsen et al. 2019	Sugar beet	Pectin with higher fractions of rhamnogalacturonan and neutral sugars (7.5 g)	Fermentation using a TIM-2 colon model	Increases in population of *Oscillospira, Blautia, Dorea, Ruminococcus, Coprococcus, R. torques, Lachnospiraceae* and *Clostridiales*, within phylum Firmicutes, as well as of *Paraprevotella, B. uniformis, B. ovatus, P. distasonis and Prevotella*, within phylum Bacteroidetes
Mladenović et al. 2019	Sugar beet molasses (5 - 25%, w/v)	Not informed	Adaptation of *L. paracasei* NRRL B-4564 to sugar beet molasses to ensure substrate utilization and enhanced lactic acid production on molasses-enriched distillery stillage	Adapted *L. paracasei* NRRL B-4564 enhanced the lactic acid and biomass production. Sugar beet molasses was shown a source of sugars molasses, nitrogen and minerals required for growth of lactic acid bacteria and increased lactic acid production

abundance of populations of different beneficial bacterial species, with a remarkable bifidogenic effect revealed by increases in *Bifidobacterium* spp. counts from 11.8% to 23.4% during fermentation. Additionally, the results of this study indicated that sugar beet pectic oligosaccharides induced a more remarkable prebiotic effect than pectin, besides to act similarly to or better than fructooligosaccharides (Goméz et al. 2016). Structure of oligosaccharides fractionated from beet pectins (beet arabinan) were reported to increase the populations of *Bifidobacterium* spp. and *Lactobacillus* spp. during fermentation with a human fecal inoculum, with an outstanding bifidogenic effect (Onumpai et al. 2011).

Pectic oligosaccharides obtained through enzymatic hydrolysis of sugar beet pulp derived from industrial beet by-products were also capable of stimulating the growth of probiotic *Lactobacillus* isolates, especially of *L. reuteri*, *L. plantarum* and *L. rhamnosus*, in laboratory media. These results indicated that sugar beet-derived pectic oligosaccharides could confer beneficial effects on gut microbiota by modulating the growth of probiotic bacteria (Gómez et al. 2019).

Administration of a mixed juice prepared with beet, apple, lemon and ginger to healthy adults that consumed only the tested mixed juice for three days caused a decrease in relative fecal abundance of Firmicutes and Proteobacteria, besides to an increase in relative fecal abundance of Bacteroidetes and Cyanobacteria. Relative fecal abundance of some bacterial genera was also increased, to cite: *Halospirulina* (1467%), *Paraprevotella* (348%), *Barnesiella* (200%), *Odoribacter* (200%) and *Bacteroides* (144%). Otherwise, relative fecal abundance of other bacterial genera was decreased, to cite: *Streptococcus* (8%), *Subdoligranulum* (30%), *Eisenbergiella* (40%), *Ruminiclostridium* (50%) and *Dialister* (67%). These data indicated that administration of mixed juice with beet root was capable of inducing alterations in human gut microbiota composition. Consumption of mixed juice also caused weight loss, increased plasma levels of nitric oxide (vasodilator) and decreased lipid oxidation, which were associated primarily with high contents of fiber and nitrate in beet pulp used to prepare the juice (Henning et al. 2017).

Capability of probiotic bacteria of boosting the health promoting properties of beet juice have also been reported. Fermentation of pasteurized beet juice by probiotic *Lactobacillus plantarum*, *L. rhamnnosus* and *Lactobacillus delbrueckii* resulted in increased contents of total phenolics and flavonoids and antioxidant activity in fermented juice. Fermentation of beet juice by probiotics caused probably structural disintegration of cell wall in beet root cells with the release or synthesis of different antioxidant compounds (Panghal et al. 2017).

Fermentation of beet root juice by probiotic *Lactobacillus brevis* and *L. paracasei* resulted in increased contents of betanin, isobetanin and neobetanin, as well as of betanidine and isobetanidine, which were not found in unfermented beet root juices. Betanidine and isobetanidine are aglycones with ability to neutralize free radicals. Betanidin and its isomer isobetanidin were formed in fermented beet root juice possibly as a result of the activity of *ß*-glucosidase produced by tested probiotics, which catalyzes the transformation of betanin into betanidine. *L. brevis* and *L. paracasei* had high counts (approximately 8.15 log CFU/mL) in fermented beet juice during 30 days of refrigerated storage (Klewicka and Czyżowska 2011).

Beet juice fermented by probiotic *Lactobacillus casei* and *L. brevis* for 48 h at 30 °C was administered in three different volumes (1.5, 3 and 6 mL daily) for Wistar rats fed a basic casein diet for four weeks. Consumption of fermented beet root juice (6 mL daily) modulated positively cecal microflora and metabolic parameters of rats. Fecal counts of *Lactobacillus* spp., *Bifidobacterium* spp., *Bacteriodes* spp. and *Enterococcus* spp. were in the range of 8.2–8.6, 6.2–7.5, 8.0–8.3 and 7.3–7.7 log CFU/g, respectively, during fermentation. Fecal counts of *Clostridium* spp. increased by 1.1–1.6 log CFU/g, while fecal counts of Enterobacteriaceae decreased by 0.8–2.1 log CFU/g in rats. Furthermore, administration of beet root fermented juice increased the fecal contents of SCFA in rats, besides to decrease the activity of α-glucosidase and β-glucuronidase, which were indicative of reduced post-prandial hyperglycemia and enterohepatic circulation of toxic compounds, respectively. These results indicated that consumption of beet juice fermented by probiotic *L. casei* and *L. brevis* induced positive effects on cecal microbiota and metabolic parameters in rats (Klewicka, Zduńczyk, and Juśkiewicz 2009).

Effects of beet on gastrointestinal diseases

Retrieved studies assessing the effects of beet and beet bioactive compounds on parameters indicative of gastrointestinal health with information on tested beet part or product, bioactive compounds, examined doses, experimental models, measured parameters and main results are shown in Table 2. Beet exert a variety of beneficial effects to human health, which are mediated by different naturally occurring bioactive compounds, most notably betalains, phenolics, soluble fiber and pectin (Chhikara et al. 2019; Clifford et al. 2015; Martinez et al. 2015). Inflammation is a biological response of immune system triggered by different factors, such as pathogens, damaged cells and toxic compounds, which may induce acute and/or chronic inflammatory responses in gastrointestinal tract, leading to tissue damage and disease (Chen et al. 2018). There has been evidence that betalains and betaine can exert anti-inflammatory effects by interfering with pro-inflammatory signaling cascades. Nuclear Factor-Kappa B (NF-κB) cascade is the most important cascade in inflammatory processes, being involved in activation and transcription of most target genes that regulate and amplify inflammatory response (i.e. cytokines, chemokines, apoptotic and phagocytic cells). Betalains and betaine can reduce the levels of pro-inflammatory cytokines (e.g. TNF-α, IL-6, IL-8 and IL-1β), reactive oxygen and nitrogen species, as well as the activity of cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) (Clifford et al. 2015; Lechner and Stoner 2019).

A study evaluated the free-radical scavenging activity of betanin in human hepatoma cell lines (HT-29) and confirmed protective effects of betanin to avoid DNA-damage. Beet betalains (15 μmol/L) decreased DNA-damage caused by H_2O_2 in HT-29 enterocytes (measured by the so-called Comet-assay), as well as increased the transcription of nuclear factor erythroid 2-related factor 2 (Nrf2), which induces endogenous cellular antioxidant defense mechanisms. These data indicate that betanin can act as both a free radical scavenger and inducer of antioxidant defense mechanisms in cells (Esatbeyoglu et al. 2014).

Different mechanisms could be involved in hepatoprotective and anticarcinogenic effects of betanin. Hepatoprotective and anticarcinogenic mechanisms of betanin were evaluated considering its influence on activation of Nrf2 and expression of Glutathione S-transferase and NAD(P)H:quinone oxidoreductase in two cell lines (non-tumor human hepatocytes THLE-2 and hepatocellular carcinoma cells HepG2). Betanin (2, 10 and 20 μM) activated the Nrf2-ARE binding sequence in non-tumor human hepatic cell lines (translocation of Nrf2 from the cytosol to the nucleus) and increased the expression of mRNA of phase II detoxifying enzymes, including NAD(P)H:quinone oxidoreductase 1 and Glutathione S-transferase activity (Krajka-Kuźniak et al. 2013).

Effects of phenolics of beet stalks and leaves on liver oxidative damage in mice fed a high-fat (HF) diet were investigated. HF diet mice groups were supplemented during eight weeks with dehydrated beet stalks and leaves or beet stalks and leaf ethanol extract. Dehydrated beet stalks and leaves attenuated the deleterious effects of a HF diet on lipid metabolism, reduced fasting blood glucose levels, ameliorated cholesterol levels and reduced Glutathione-peroxidase and Glutathione-reductase activities in mice. However, ethanolic extract from beet stalks and leaves did not prevent liver damage caused by HF diet. Presence of flavonoids, such as vitexin derivatives, in beet stalks and leaves were reported to protect the liver from damage induced by HF diet. These results indicated that dehydrated beet leaves were more biologically active than ethanolic beet leaves extract, probably because of the interactions of phenolics with other components in dehydrated leaves, especially proteins. Phenolics can interact with proteins leading to formation of soluble and insoluble complexes, which could affect their absorption and biological activities (Lorizola et al. 2018).

Protective effects of long-term feeding (28 days) with beet juice (8 mL/kg body weight) on phase I and phase II enzymes, DNA damage and liver injury induced by hepatocarcinogenic *N*-nitrosodiethylamine (NDEA) was evaluated in rats. Beet juice consumption conferred hepatic protection against a range of inflammatory markers induced by NDEA administration, such as alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase, lactate dehydrogenase, gamma glutamyl transferase, albumin, bilirubin, creatinine and blood urea nitrogen. Long term feeding with beet juice had protective effects against oxidative liver damage induced by *N*-nitrosodiethylamine in rats (Krajka-Kuźniak et al. 2012).

Chemoprevention of cancer with bioactive foods or their extracted/purified components has been associated with normalization of expression of different genes (Lechner and Stoner 2019). Identification of plant compounds (e.g. polyphenols) capable of inducing the killing of tumors through apoptosis has been recognized as a promising strategy to avoid proliferation of cancer cells (Chen, Zhao, and Yu 2016). The relationship between chemical structure and anti-cancer activity for a range of sugar beet pectins extracted and modified with different methods was evaluated by measuring cell viability and apoptosis detection in HT29 and DLD1 colon cancer cells. Pectin extracted from sugar beet pulp differed considerably from pectin extracted from citrus peel. Obtained sugar beet pectin had a higher neutral sugar and lower GalA content, as well as a lower degree of esterification (DE) and higher degree of acetylation (DAc) when compared to citrus peel pectin. Alkali treatment increased the ratio of rhamnogalacturonan I (RGI) to homogalacturonan and increased the anti-cancer effects of pectin, indicating an important role of neutral sugar side chains, such as galatan and arabinan, for pectin bioactivity (Maxwell et al. 2016).

Phenolics, such as gallic acid (GA), cyanidin-3-O-glucoside chloride (CGC) and epicatechin (EP), were extracted from sugar beet molasses and screened for antioxidant and cytotoxicity effects on human colon (CaCO-2) and hepatocellular (HepG2) cancer cell lines. GA had the strongest antioxidant activities and its antitumor activities increased in a dose-dependent manner. In particular, CGC (400 μg/

Table 2. Retrieved studies assessing the effects of beet and beet bioactive compounds on parameters indicative of gastrointestinal health.

References	Tested beet part or product	Bioactive compounds	Experimental models/ measured parameters	Main results
Klewicka et al. 2012	Beet root	Beet root juice fermented by *Lactobacillus brevis* 0944 and *Lactobacillus paracasei* 0920 (0.8 L/Kg)	Effects against aberrant crypt foci formation and genotoxicity of fecal water in rats	Beet root reduced the number of aberrant crypt foci in rats treated with N-Nitroso-N-methylurea, in addition to increase the counts of *Lactobacillus/ Enterococcus* adhered to colonic epithelium
Krajka-Kuźniak et al. 2012	Beet root juice (8 mL/Kg)	Not informed	Protective action of beet root juice on hepatocarcinogenic N-nitrosodiethylamine-induced liver injury in rats	Administration of beet juice for 28 days had a protective effect against oxidative damage to liver
Kapadia et al. 2013	Red beet root extracts	Not informed	Potential antiproliferative synergistic activity of red beet extract with the drug doxorubicin against pancreatic tumor cells	Extracts from red beet root in combined concentrations attenuated the efficacy of the chemotherapeutic drug doxorubicin
Krajka-Kuźniak et al. 2013	Beet root	Betanin (2, 10 and 20 µM)	Evaluation of the hepatoprotective and anticarcinogenic effect of betanin in non-tumor hepatocytes and hepatocellular carcinoma cells	Betanin induced the expression of detoxifying enzymes, indicating that it is partly responsible for the beet hepatoprotective activity
Esatbeyoglu et al, 2014	Red beet extract diluted with dextrin	Betanin (10 µL)	Evaluation the free-radical scavenging activity of betanin on counteract hydrogen peroxide induced DNA damage in human liver hepatoma cells	Betanin acted as a free radical scavenger and n inducer of endogenous cellular enzymatic antioxidant defense mechanisms
Chen, Meng, et al. 2015	Sugar beet molasses	Gallic acid, cyanidin-3-O-glucoside chloride and epicatechin extracted from sugar beet molasses (400 µg/mL)	The phenolics were screened for antioxidant activity and cytotoxicity effects measured by methyl thiazolyl tetrazolium assay against human colon (CaCO-2) and hepatocellular cancer cell lines	Gallic acid had the strongest antioxidant activities and its antitumor activities increased in a dose-dependent manner. Cyanidin-3-O-glucoside chloride caused inhibition of the proliferation of human colon and hepatocellular carcinoma cell lines
Nowacki et al. 2015	Fresh red beet root extracts, with a mix of betanin (64%, w/v) and isobetanin (36%, w/v)	Betanin and isobetanin	Human colorectal cell lines (HT-29 ATCC® HTB-38). All cells were cultured as monolayer (2D) and as aggregates (3D) and treated with betanin-enriched beet root extract for 48 h	Betanin concentrate inhibited the proliferation of cancer cells. HT-29 cells were not sensitive to betanin concentrate at 40 µM and cell proliferation was not decreased
Maxwell et al. 2016	Not informed	Sugar beet pectin (1 mg/mL)	Relationship between pectin structure and anti-cancer activity for a range of sugar beet pectins, extracted and modified in a variety of ways	Modified sugar beet pectin (by alkali treatment) increased the ratio of rhamnogalacturonan I to homogalacturonan and apoptosis induction
Farabegoli et al. 2017	Not informed	Betaxanthins (0.35 ng/mL) and betacyanins (0.25 ng/ mL) from red beet root	Investigated in CaCo-2 colon cancer cell lines	Betaxanthins and betacyanins from red beet root reduced the expression of pro-inflammatory markers and caused down regulation of anti-apoptotic protein Bcl-2
Lorizola et al. 2018	Beet stalks and leaves (0.5%, w/w)	Not informed	Liver oxidative damage in mice fed a high-fat diet and supplemented during 8 weeks with dehydrated beet stalks and leaves	Beet stalks and leaves attenuated the deleterious effects of a HF diet on lipid metabolism, reduced fasting blood glucose levels, ameliorated cholesterol levels and reduced Glutathione-peroxidase and Glutathione-reductase activities

mL) caused inhibition of 94.9 and 87.3% in proliferation of human colon and hepatocellular carcinoma cell lines, respectively (Chen, Meng, et al. 2015).

Cytotoxic activity of betaxanthins (R1) and betacyanins (R2) from red beet (cv. *Detroit*) were investigated in CaCo-2 colon cancer cell lines when these compounds were tested individually or in combination with vitexin-2-O-xyloside (the main cytotoxic flavonoid from beet seeds). Betalains tested individually even in low concentrations (0.25–0.35 µg/mL) had toxic effects on CaCo-2 cell lines. Combinations of vitexin-2-O-xyloside + betaxanthins, vitexin-2-O-xyloside + betacyanin and vitexin-2-O-xyloside + betaxanthins + betacyanins had synergistic toxic effects on CaCo-2 cell lines, being the highest toxic effects found after a 72 h-exposure. These data indicated the use of a cocktail of betaxanthins, betacyanins and vitexin-2-O-xyloside as a chemopreventive alternative against colon cancer due to the capability of betalains of reducing the expression of pro-inflammatory markers and causing a down regulation of anti-apoptotic protein Bcl-2 (Farabegoli et al. 2017).

A study evaluated the effects of extracts from fresh red beet containing a mix of betanin and its stereoisomer isobetanin (64% and 34%, respectively) on human colorectal cell lines (HT-29 ATCC® HTB-38). Cells were cultured as monolayer (2D) and aggregates (3D) and treated with betanin-enriched beet extract for 48 h. Betanin concentrate inhibited the proliferation of cancer cells, which was associated with induction of apoptotic cell death and autophagic activity for cancer cells. However, HT-29 cells were not sensitive to betanin concentrate at 40 µM and cancer cell proliferation was not decreased (Nowacki et al. 2015).

Potential antiproliferative synergistic activity of red beet extract with the drug doxorubicin was evaluated against pancreatic tumor cells (PaCa) in exponential growth phase. Different concentrations of beet extract and doxorubicin (0.29–290 µg/mL) in different combinations were tested for cytotoxic effects with measurements of viability of PaCa after 72 h of incubation. Results indicated a reduction in effective dose of doxorubicin (a chemotherapeutic drug) when combined (1:5 ratio) with beet extract (IC_{50}, IC_{75} and IC_{90}) to decrease the viability of PaCa. The authors stated that red beet extract in selected combined concentrations could enhance the therapeutic efficacy of chemotherapeutic drugs to reduce toxic side-effects as a consequence of the reduction of drug effective dose, in addition to prevent development of decreased cancer cell sensitivity to drug treatment (Kapadia et al. 2013).

Protective effects of beet juice fermented by probiotic *L. brevis* and *L. paracasei* against aberrant crypt foci formation and genotoxicity of fecal water in rats was reported. Beet juice reduced the number of aberrant crypt foci and extensive aberrations in rats treated with *N*-Nitroso-*N*-methylurea (used as carcinogen). Fecal water obtained from rats fed with N-Nitroso-N-methylurea-containing diet induced pronounced cytotoxic and genotoxic effects in Caco-2 cell lines, but administration of fermented beet juice abolished these effects. Betalains in fermented beet root juice was reported as a potential biologically active agent inhibiting the development of cancer cells. Additionally, the beet juice administrations increased the number of bacterial cells, including *Lactobacillus*/*Enterococcus*, adhered to colonic epithelium. These results indicated that fermented beet juice could be a functional food with capability of preventing precancerous alteration induced by carcinogens, in addition to decrease the cyto- and genotoxicity of fecal water (Klewicka et al. 2012).

Conclusion and future perspectives

Available literature has demonstrated conversely that beet has outstanding nutritional value and presence of a variety of bioactive compounds. There has been consistent evidence that non-sucrose polysaccharides, namely pectin and pectic oligosaccharides, from beet can modulate positively the gut microbiota composition and function, in addition to exert stimulatory effects on growth and metabolism of probiotic bacteria, indicating that beet could exert prebiotic properties. Positive effects of beet betalains and phenolics on gut microbiota and probiotics have also resulted in the production of bacterial metabolites, namely SCFA, which have been linked to an array of beneficial effects induced by prebiotics on host health. High contents of betalains and some phenolics with anti-inflammatory, antioxidant and anti-carcinogenic properties have been associated with positive effects induced by beet on parameters indicative of gastrointestinal health. Fermentation of beet products by probiotics seems to impact positively on the beneficial biological effects of these products on gastrointestinal health. Beet should be considered a healthy food option for use by consumers in different domestic meal preparations as well as by food industry as an ingredient to formulated added-value functionalized food products.

Disclosure statement

Authors declare no conflict of interest related to the content of this manuscript.

Funding

Authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) for partial funding of this research (Finance code 001).

ORCID

Evandro Leite de Souza http://orcid.org/0000-0003-4927-9383

References

Ai, C., X. Guo, J. Lin, T. Zhang, and H. Meng. 2019. Characterization of the properties of amphiphilic, alkaline soluble polysaccharides from sugar beet pulp. *Food Hydrocolloids* 94:199–209. doi: 10.1016/j.foodhyd.2019.03.022.

Babarykin, D., G. Smirnova, J. Markovs, S. Vasiljeva, N. Basova, R. Simanis, and L. Viksna. 2019. Therapeutic effect of fractionated by ultrafiltration red beetroot (*Beta vulgaris* L.) juice in rats with food-induced fatty liver. *European Journal of Biological Research* 9:1–9. 10.5281/zenodo.2541075.

Babbar, N., W. Dejonghe, S. Sforza, and K. Elst. 2017. Enzymatic pectic oligosaccharides (POS) production from sugar beet pulp using response surface methodology. *Journal of Food Science and Technology* 54 (11):3707–15. doi: 10.1007/s13197-017-2835-x.

Baião, D., C. de Freitas, L. Gomes, D. da Silva, A. Correa, P. Pereira, E. Aguila, and V. Paschoalin. 2017. Polyphenols from root, tubercles and grains cropped in Brazil: Chemical and nutritional characterization and their effects on human health and diseases. *Nutrients* 9 (9): 1044. doi: 10.3390/nu9091044.

Batista, M. A. V., F. S. Bezerra Neto, M. L. Silva, M. M. Q. Ambrósio, and J. L. X. L. Cunha. 2016. Soil-plant attributes and beet production influenced by fertilization with species of Brazilian Caatinga. *Horticultura Brasileira* 34:31–38. doi: 10.1590/S0102-053620160000100005.

Biondo, P. B. F., J. S. Boeing, E. O. Barizão, N. E. Souza, M. Matsushita, C. C. Oliveira, M. Boroski, and J. V. Visentainer. 2014. Evaluation of beetroot (*Beta vulgaris* L.) leaves during its developmental stages: A chemical composition study. *Food Science and Technology* 34 (1):94–101. doi: 10.1590/S0101-20612014005000007.

Carrillo, C., R. Rey, M. Hendrickx, M. Del Mar Cavia, and S. Alonso-Torre. 2017. Antioxidant capacity of beetroot: Traditional vs novel approaches. *Plant Foods for Human Nutrition (Dordrecht, Netherlands)* 72 (3):266–73. doi: 10.1007/s11130-017-0617-2.

Cavalcanti Neto, M. P., J. S. Aquino, L. F. R. da Silva, R. O. Silva, K. S. L. Guimarães, Y. de Oliveira, E. L. de Souza, M. Magnani, H. Vidal, and J. L. de Brito Alves. 2018. Gut microbiota and probiotics intervention: A potential therapeutic target for management of cardiometabolic disorders and chronic kidney disease? *Pharmacological Research* 130:152–63. doi: 10.1016/j.phrs.2018.01.020.

Chawla, H., M. Parle, K. Sharma, and M. Yadav. 2016. Beetroot: A health promoting functional food. *Nutraceuticals* 1:8–12.

Chen, M., H. Meng, Y. Zhao, F. Chen, and S. Yu. 2015. Antioxidant and in vitro anticancer activities of phenolics isolated from sugar beet molasses. *BMC Complementary and Alternative Medicine* 15 (313):313–8. doi: 10.1186/s12906-015-0847-5.

Chen, M., Y. Zhao, and S. Yu. 2015. Optimisation of ultrasonic-assisted extraction of phenolic compounds, antioxidants, and anthocyanins from sugar beet molasses . *Food Chemistry* 172:543–5501. doi: 10.1016/j.foodchem.2014.09.110.

Chen, M., Z. Zhao, and S. Yu. 2016. Cytotoxicity and apoptotic effects of polyphenols from sugar beet molasses on colon carcinoma cells in vitro. *International Journal of Molecular Sciences* 17 (7):993–10. doi: 10.3390/ijms17070993.

Chen, L., H. Deng, H. Cui, J. Fang, Z. Zuo, J. Deng, Y. Li, X. Wang, and L. Zhao. 2018. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* 9 (6):7204–18. doi: 10.18632/oncotarget.23208.

Chhikara, N., K. Kushwaha, P. Sharma, Y. Gat, and A. Panghal. 2019. Bioactive compounds of beetroot and utilization in food processing industry: A critical review. *Food Chemistry* 272:192–200. doi: 10.1016/j.foodchem.2018.08.022.

Chung, W. S. F., M. Meijerink, B. Zeuner, J. Holck, P. Louis, A. S. Meyer, J. M. Wells, H. J. Flint, and S. H. Duncan. 2017. Prebiotic potential of pectin and pectic oligosaccharides to promote anti-inflammatory commensal bacteria in the human colon. *FEMS Microbiology Ecology* 93 (11):1–9. doi: 10.1093/femsec/fix127.

Clifford, T., G. Howatson, D. J. West, and E. J. Stevenson. 2015. The potential benefits of red beetroot supplementation in health and disease. *Nutrients* 7 (4):2801–22. doi: 10.3390/nu7042801.

Concha, J., and M. E. Zúñiga. 2012. Enzymatic depolymerization of sugar beet pulp: Production and characterization of pectin and pectic-oligosaccharides as a potential source for functional carbohydrates. *Chemical Engineering Journal* 192:29–36. 10.1016/j.cej.2012.03.085.

Cui, J., Y. Lian, C. Zhao, H. Du, Y. Han, W. Gao, H. Xiao, and J. Zheng. 2019. Dietary fibers from fruits and vegetables and their health benefits via modulation of gut microbiota. *Comprehensive Reviews in Food Science and Food Safety* 18 (5):1514–32. doi: 10.1111/1541-4337.12489.

Danneskiold-Samsøe, N. B., H. D. F. Q. Barros, R. Santos, J. L. Bicas, C. B. B. Cazarin, L. Madsen, K. Kristiansen, G. M. Pastore, S. Brix, and M. R. Maróstica Júnior. 2019. Interplay between food and gut microbiota in health and disease. *Food Research International (Ottawa, Ont.)* 115:23–31. doi: 10.1016/j.foodres.2018.07.043.

Domínguez, R., J. L. Maté-Muñoz, E. Cuenca, P. García-Fernandez, F. Mata-Ordoñez, M. C. Lozana-Estevan, P. Veiga-Herreros, S. F. Silva, and M. V. Garnacho-Castaño. 2018. Effects of beetroot juice supplementation on intermittent high-intensity exercise efforts. *Journal of the International Society of Sports Nutrition* 15 (15):2–12. doi: 10.1186/s12970-017-0204-9.

Esatbeyoglu, T., A. E. Wagner, R. Motafakkerazad, Y. Nakajima, S. Matsugo, and G. Rimbach. 2014. Free radical scavenging and antioxidant activity of betanin: Electron spin resonance spectroscopy studies and studies in cultured cells. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association* 73:119–26. doi: 10.1016/j.fct.2014.08.007.

Esatbeyoglu, T., A. E. Wagner, V. B. Schini-Kerth, and G. Rimbach. 2015. Betanin-A food colorant with biological activity. *Molecular Nutrition & Food Research* 59 (1):36–47. doi: 10.1002/mnfr.201400484.

Food and Agriculture Organization of the United Nations (FAOSTAT). 2019. *FAOSTAT statistical database. [Rome]*. Accessed October 17, 2019. http://www.fao.org/faostat/en/?#data/QC.

Farabegoli, F.,. E. S. Scarpa, A. Frati, G. Serafini, A. Papi, E. Spisni, E. Antonini, E. Benedetti, and P. Ninfali. 2017. Betalains increase vitexin-2-O-xyloside cytotoxicity in CaCo-2 cancer cells . *Food Chemistry* 218:356–64. doi: 10.1016/j.foodchem.2016.09.112.

Frank, T., F. C. Stintzing, R. Carle, I. Bitsch, D. Quaas, G. Strass, R. Bitsch, and M. Netzel. 2005. Urinary pharmacokinetics of betalains following consumption of red beet juice in healthy humans. *Pharmacological Research* 52 (4):290–7. doi: 10.1016/j.phrs.2005.04.005.

Gandía-Herrero, F., and F. García-Carmona. 2012. Characterization of recombinant *Beta vulgaris* 4,5-DOPA-extradiol-dioxygenase active in the biosynthesis of betalains. *Planta* 236 (1):91–100. doi: 10.1007/s00425-012-1593-2.

Gandía-Herrero, F., J. Escribano, and F. García-Carmona. 2016. Biological Activities of Plant Pigments Betalains. *Critical Reviews in Food Science and Nutrition* 56 (6):937–45. 10.1080/10408398.2012.740103.

Georgiev, V. G., J. Weber, E. Kneschke, P. N. Denev, T. Bley, and A. I. Pavlov. 2010. Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot *Beta vulgaris* cv. Detroit Dark Red. *Plant Foods for Human Nutrition (Dordrecht, Netherlands)* 65 (2):105–11. doi: 10.1007/s11130-010-0156-6.

Gibson, G. R., R. Hutkins, M. E. Sanders, S. L. Prescott, R. A. Reimer, S. J. Salminen, K. Scott, C. Stanton, K. S. Swanson, P. D. Cani, et al. 2017. Expert consensus document: The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews. Gastroenterology & Hepatology* 14 (8):491–502. doi: 10.1038/nrgastro.2017.75.

Gómez, B., B. Gullón, R. Yáñez, H. Schols, and J. L. Alonso. 2016. Prebiotic potential of pectins and pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp: A comparative evaluation. *Journal of Functional Foods* 20:108–21. doi: 10.1016/j.jff.2015.10.029.

Gómez, B., C. Peláez, M. C. Martínez-Cuesta, J. C. Parajó, J. L. Alonso, and T. Requena. 2019. Emerging prebiotics obtained from lemon and sugar beet byproducts: Evaluation of their *in vitro* fermentability by probiotic bacteria. *LWT* 109:17–25. doi: 10.1016/j.lwt.2019.04.008.

Gorbunova, N., A. Bannikova, A. Evteev, I. Evdokimov, and S. Kasapis. 2018. Alginate-based encapsulation of extracts from *Beta vulgaris* cv. beet greens: Stability and controlled release under simulated gastrointestinal conditions. *LWT* 93:442–9. doi: 10.1016/j.lwt.2018.03.075.

Guinane, C. M., and P. D. Cotter. 2013. Role of the gut microbiota in health and chronic gastrointestinal disease: Understanding a hidden metabolic organ. *Therapeutic Advances in Gastroenterology* 6 (4): 295–308. doi: 10.1177/1756283X13482996.

Gullón, B., P. Gullón, Y. Sanz, J. L. Alonso, and J. C. Parajó. 2011. Prebiotic potential of a refined product containing pectic oligosaccharides. *LWT - Food Science and Technology* 44 (8):1687–96. doi: 10.1016/j.lwt.2011.03.006.

Gullón, B., B. Gómez, M. Martínez-Sabajanes, R. Yáñez, J. C. Parajó, and J. L. Alonso. 2013. Pectic oligosaccharides: Manufacture and functional properties. *Trends in Food Science & Technology* 30 (2): 153–61. doi: 10.1016/j.tifs.2013.01.006.

Guo, X., X. Guo, H. Meng, B. Zhang, and S. Yu. 2017. Using the high temperature resistant pH electrode to auxiliary study the sugar beet pectin extraction under different extraction conditions. *Food Hydrocolloids*. 70:105–13. doi: 10.1016/j.foodhyd.2017.03.032.

Halwani, A. F., H. A. Sindi, and H. A. Jambi. 2018. Characterization of physical properties of red beet pigments. *Journal of Biochemical Technology* 9 (2):16–20.

Harland, J. I. 2018. Authorised EU health claim for sugar beet fibre. In *Series in food science, technology and nutrition*, ed. M. J. Sadler, vol. 3, 113–28. Woodhead Publishing. doi: 10.1016/B978-0-08-100922-2.00008-5.

Henning, S. M., J. Yang, P. Shao, R. Lee, J. Huang, A. Ly, M. Hsu, Q. Lu, G. Thames, D. Heber, et al. 2017. Health benefit of vegetable/fruit juice-based diet: Role of microbiome. *Nature* 7:1–9. 10.1038/s41598-017-02200-6.

Holck, J., K. Hjernø, A. Lorentzen, L. K. Vigsnaes, L. Hemmingsen, T. R. Licht, J. D. Mikkelsen, and A. S. Meyer. 2011. Tailored enzymatic production of oligosaccharides from sugar beet pectin and evidence of differential effects of a single DP chain length difference on human faecal microbiota composition after in vitro fermentation. *Process Biochemistry* 46 (5):1039–49. doi: 10.1016/j.procbio.2011.01.013.

Holck, J., A. Lorentzen, L. K. Vigsnaes, T. R. Licht, J. D. Mikkelsen, and A. S. Meyer. 2010. Feruloylated and nonferuloylated arabino-oligosaccharides from sugar beet pectin selectively stimulate the growth of *Bifidobacterium* spp. in human fecal *in vitro* fermentations. *Journal of Agricultural and Food Chemistry* 59 (12):6511–9. 10.1021/jf200996h.

Kapadia, G. J., G. S. Rao, C. Ramachandran, A. Lida, N. Suzuki, and H. Tokuda. 2013. Synergistic cytotoxicity of red beetroot (*Beta vulgaris* L.) extract with doxorubicin in human pancreatic, breast and prostate cancer cell lines. *Journal of Complementary & Integrative Medicine* 10 (1):1–10. 10.1515/jcim-2013-0007.

Khan, M. I. 2016. Plant betalains: Safety, antioxidant activity, clinical efficacy, and bioavailability. *Comprehensive Reviews in Food Science and Food Safety* 15 (2):316–30. doi: 10.1111/1541-4337.12185.

Klewicka, E., and A. Czyżowska. 2011. Biological stability of lactofermented beetroot juice during refrigerated storage. *Polish Journal of Food and Nutrition Sciences* 61 (4):251–6. doi: 10.2478/v10222-011-0028-2.

Klewicka, E., A. Nowak, Z. Zduńczyk, B. Cukrowska, and J. Błasiak. 2012. Protective effect of lactofermented beetroot juice against aberrant crypt foci formation and genotoxicity of fecal water in rats. *Experimental and Toxicologic Pathology: Official Journal of the Gesellschaft Fur Toxikologische Pathologie* 64 (6):599–604. doi: 10.1016/j.etp.2010.12.001.

Klewicka, E., Z. Zduńczyk, and J. Juśkiewicz. 2009. Effect of lactobacillus fermented beetroot juice on composition and activity of cecal microflora of rats. *European Food Research and Technology* 229 (1): 153–7. doi: 10.1007/s00217-009-1036-x.

Krajka-Kuźniak, V., H. Szaefer, E. Ignatowicz, T. Adamska, and W. Baer-Dubowska. 2012. Beetroot juice protects against N-Nitrosodiethylamine-induced liver injury in rats. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association* 50 (6):2027–33. doi: 10.1016/j.fct.2012.03.062.

Krajka-Kuźniak, V., J. Paluszczak, H. Szaefer, and W. Baer-Dubowska. 2013. Betanin, a beetroot component, induces nuclear factor erythroid-2-related factor 2-mediated expression of detoxifying/antioxidant enzymes in human liver cell lines. *The British Journal of Nutrition* 110 (12):2138–49. doi: 10.1017/S0007114513001645.

Kumar, Y. 2015. Beetroot: A super food. *International Journal of Engineering Studies and Technical Approach* 1:20–6.

Kushwaha, R., V. Kumar, G. Vyas, and J. Kaur. 2018. Optimization of different variable for eco-friendly extraction of betalains and phytochemicals from beetroot pomace. *Waste and Biomass Valorization* 9 (9):1485–94. doi: 10.1007/s12649-017-9953-6.

Lange, W., W. A. Brandenburg, and T. S. M. Bock. 1999. Taxonomy and cultonomy of beet (*Beta vulgaris* L.). *Botanical Journal of the Linnean Society* 130 (1):81–96. doi: 10.1111/j.1095-8339.1999.tb00785.x.

Larsen, N., T. B. Cahú, S. Marta, I. Saad, A. Blennow, and L. Jespersen. 2018. The effect of pectins on survival of probiotic *Lactobacillus* spp. in gastrointestinal juices is related to their structure and physical properties. *Food Microbiology* 74:11–20. doi: 10.1016/j.fm.2018.02.015.

Larsen, N., C. B. Souza, L. Krych, T. B. Cahú, M. Wiese, W. Kot, K. M. Hansen, A. Blennow, K. Venema, and L. Jespersen. 2019. Potential of pectins to beneficially modulate the gut microbiota depends on their structural properties. *Frontiers in Microbiology* 10: 223–13. doi: 10.3389/fmicb.2019.00223.

Lechner, J. F., and G. D. Stoner. 2019. Red beetroot and betalains as cancer chemopreventative agents. *Molecules* 24 (8):1602–12. doi: 10.3390/molecules24081602.

Leijdekkers, A. G. M., M. Aguirre, K. Venema, G. Bosch, H. Gruppen, and H. A. Schols. 2014. In vitro fermentability of sugar beet pulp derived oligosaccharides using human and pig fecal inocula. *Journal of Agricultural and Food Chemistry* 62 (5):1079–87. doi: 10.1021/jf4049676.

Leijdekkers, A. G. M., J. P. M. Bink, S. Geutjes, H. A. Schols, and H. Gruppen. 2013. Enzymatic saccharification of sugar beet pulp for the production of galacturonic acid and arabinose; a study on the impact of the formation of recalcitrant oligosaccharides. *Bioresource Technology* 128:518–25. doi: 10.1016/j.biortech.2012.10.126.

Lidder, S., and A. J. Webb. 2013. Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway. *British Journal of Clinical Pharmacology* 75 (3): 677–96. doi: 10.1111/j.1365-2125.2012.04420.x.

Liu, Z., F. Pi, X. Guo, X. Guo, and S. Yu. 2019. Characterization of the structural and emulsifying properties of sugar beet pectins obtained by sequential extraction. *Food Hydrocolloids* 88:31–42. doi: 10.1016/j.foodhyd.2018.09.036.

Lorizola, I. M., C. P. B. Furlan, M. Portovedo, M. Milanski, P. B. Botelho, R. M. N. Bezerra, B. R. Sumere, M. A. Rostagno, and C. D. Capitani. 2018. Beet stalks and leaves (*Beta vulgaris* L.) protect against high-fat diet-induced oxidative damage in the liver in mice. *Nutrients* 10 (7):872–16. doi: 10.3390/nu10070872.

Maheshwari, R. K., V. Parmar, and L. Joseph. 2013. Latent therapeutic gains of beetroot juice. *World Journal of Pharmaceutical Research* 2: 804–20.

Manach, C., G. Williamson, C. Morand, A. Scalbert, and C. Rémésy. 2005. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American Journal of Clinical Nutrition* 81 (1 Suppl):230S–242. doi: 10.1093/ajcn/81.1.230S.

Mao, Y., R. Lei, J. Ryan, F. A. Rodriguez, B. Rastall, A. Chatzifragkou, C. Winkworth-Smith, S. E. Harding, R. Ibbett, and E. Binner. 2019. Understanding the influence of processing conditions on the extraction of rhamnogalacturonan-I "hairy" pectin from sugar beet pulp. *Food Chemistry: X* 2 (2):100026 doi: 10.1016/j.fochx.2019.100026.

Martínez, M., B. Gullón, H. A. Schols, J. L. Alonso, and J. C. Parajó, 2009. Assessment of the production of oligomeric compounds from sugar beet pulp. *Industrial & Engineering Chemistry Research* 48 (10):4681–7. doi: 10.1021/ie8017753.

Martinez, R. M., D. T. Longhi-Balbinot, A. C. Zarpelon, L. Staurengo-Ferrari, M. M. Baracat, S. R. Georgetti, R. C. Sassonia, W. A. Verri, and R. Casagrande. 2015. Anti-inflammatory activity of betalain-rich dye of *Beta vulgaris: effect on edema, leukocyte recruitment, superoxide anion and cytokine production. Arch. Pharm. Res* 38 (4): 494–504. doi: 10.1007/s12272-014-0473-7.

Martins, N., C. L. Roriz, P. Morales, L. Barros, and I. C. Ferreira. 2017. Coloring attributes of betalains: A key emphasis on stability and future applications. *Food & Function* 8 (4):1357–72. doi: 10.1039/c7fo00144d.

Maxwell, E. G., I. J. Colquhoun, H. K. Chau, A. T. Hotchkiss, K. W. Waldron, V. J. Morris, and N. J. Belshaw. 2016. Modified sugar beet pectin induces apoptosis of colon cancer cells via an interaction with the neutral sugar side-chains. *Carbohydrate Polymers* 136: 923–9. doi: 10.1016/j.carbpol.2015.09.063.

Mladenović, D., J. Pejin, S. Kocić-Tanackov, A. Djukić-Vuković, and L. Mojović. 2019. Enhanced lactic acid production by adaptive evolution of *Lactobacillus paracasei* on agro-industrial substrate. *Applied Biochemistry and Biotechnology* 187 (3):753–69. doi: 10.1007/s12010-018-2852-x.

Mohammed, A. A., S. F. H. Al-Mugdadi1, B. T. Al-Sudani1, and J. K. Kamel. 2018. Beetroot juice as an alternative growth and maintenance medium fox six isolates of *Mycobacterium* spp. *Journal of Pharmaceutical Sciences and Research* 10:1881–4.

Mohsen, S., M. Alsaman, and M. Mahrous. 2018. A Study of the effect of lactic acid bacteria on antioxidant content of beetroot (*Beta vulgaris*). *Biotechnology Research* 4:103–8.

Münnich, M., R. Khiaosa-Ard, F. Klevenhusen, A. Hilpold, A. Khol-Parisini, and Q. Zebeli. 2017. A meta-analysis of feeding sugar beet pulp in dairy cows: Effects on feed intake, ruminal fermentation, performance, and net food production. *Animal Feed Science and Technology* 224:78–89. doi: 10.1016/j.anifeedsci.2016.12.015.

Nemzer, B., Z. Pietrzkowski, A. Spórna, P. Stalica, W. Thresher, T. Michałowski, and S. Wybraniec. 2011. Betalainic and nutritional profiles of pigment–enriched red beet root (*Beta vulgaris* L.) dried extracts. *Food Chemistry* 127 (1):42–53. doi: 10.1016/j.foodchem.2010.12.081.

Ninfali, P., and D. Angelino. 2013. Nutritional and functional potential of *Beta vulgaris cicla* and *rubra*. *Fitoterapia* 89:188–99. doi: 10.1016/j.fitote.2013.06.004.

Ninfali, P., E. Antonini, A. Frati, and E. S. Scarpa. 2017. C-Glycosyl flavonoids from *Beta vulgaris* cicla and betalains from *Beta vulgaris* rubra: Antioxidant, anticancer and antiinflammatory activities-a review. *Phytotherapy Research: PTR* 31 (6):871–84. doi: 10.1002/ptr.5819.

Nowacki, L., P. Vigneron, L. Rotellini, H. Cazzola, F. Merlier, E. Prost, R. Ralanairina, J. Gadonna, C. Rossi, and M. Vayssade. 2015. Betanin-enriched red beetroot (*Beta vulgaris* L.) extract induces apoptosis and autophagic cell death in MCF-7 cells. *Phytotherapy Research: PTR* 29 (12):1964–73. doi: 10.1002/ptr.5491.

Oliveira, D. M., and D. H. M. Bastos. 2011. Biodisponibilidade de ácidos fenólicos. *Química Nova* 34 (6):1051–6. doi: 10.1590/S0100-40422011000600023.

Onumpai, C., S. Kolida, E. Bonnin, and R. A. Rastall. 2011. Microbial utilization and selectivity of pectin fractions with various structures. *Applied and Environmental Microbiology* 77 (16):5747–54. doi: 10.1128/AEM.00179-11.

Panagiotopoulos, I. A., R. R. Bakker, G. J. Vrije, K. Urbaniec, E. G. Koukios, and P. A. M. Claassen. 2010. Prospects of utilization of sugar beet carbohydrates for biological hydrogen production in the EU. *Journal of Cleaner Production* 18:S9–S14. doi: 10.1016/j.jclepro.2010.02.025.

Panghal, A., K. Virkar, V. Kumar, S. B. Dhull, Y. Gat, and N. Chhikara. 2017. Development of probiotic beetroot drink. *Current Research in Nutrition and Food Science Journal* 5 (3):257–62. doi: 10.12944/CRNFSJ.5.3.10.

Parkar, S. G., E. L. Redgate, R. Wibisono, X. Luo, E. T. H. Koh, and R. Schröder. 2010. Gut health benefits of kiwifruit pectins: Comparison with commercial functional polysaccharides. *Journal of Functional Foods* 2 (3):210–8. doi: 10.1016/j.jff.2010.04.009.

Polturak, G., and A. Aharoni. 2018. "La Vie en Rose": Biosynthesis, Sources, and Applications of Betalain Pigments. *Molecular Plant* 11 (1):7–22. doi: 10.1016/j.molp.2017.10.008.

Prandi, B., S. Baldassarre, N. Babbar, E. Bancalari, P. Vandezande, D. Hermans, G. Bruggeman, M. Gatti, K. Elst, and S. Sforza. 2018. Pectin oligosaccharides from sugar beet pulp: Molecular characterization and potential prebiotic activity. *Food & Function* 9 (3): 1557–69. doi: 10.1039/c7fo01182b.

Rahimi, P., S. Abedimanesh, S. A. Mesbah-Namin, and A. Ostadrahimi. 2019. Betalains, the nature-inspired pigments, in health and diseases. *Critical Reviews in Food Science and Nutrition* 59 (18):2949–78. doi: 10.1080/10408398.2018.1479830.

Sánchez-Zapata, E., J. Fernández-López, J. A. Pérez-Alvarez, J. Soares, S. Sousa, A. M. P. Gomes, and M. M. E. Pintado. 2013. In vitro evaluation of "horchata" co-products as carbon source for probiotic bacteria growth. *Food and Bioproducts Processing* 91 (3):279–86. doi: 10.1016/j.fbp.2012.11.003.

Sawicki, T., J. Topolska, E. Romaszko, and W. Wiczkowski. 2018. Profile and content of betalains in plasma and urine of volunteers after long-term exposure to fermented red beet juice. *Journal of Agricultural and Food Chemistry* 66 (16):4155–63. doi: 10.1021/acs.jafc.8b00925.

Shamoon, M., N. M. Martin, and C. L. O'Brien. 2019. Recent advances in gut microbiota mediated therapeutic targets in inflammatory bowel diseases: Emerging modalities for future pharmacological implications. *Pharmacological Research* 148:104344 doi: 10.1016/j.phrs.2019.104344.

Sivapragasam, N., P. Thavarajah, J.-B. Ohm, and D. Thavarajah. 2014. Enzyme resistant carbohydrate based micro-scale materials from sugar beet (*Beta vulgaris* L.) pulp for food and pharmaceutical applications. *Bioactive Carbohydrates and Dietary Fibre* 3 (2):115–21. doi: 10.1016/j.bcdf.2014.03.004.

Slimen, I. B., T. Najar, and M. Abderrabba. 2017. Correction to chemical and antioxidant properties of betalains. *J. Agric. Food Chem* 65 (7):1466 doi: 10.1021/acs.jafc.7b00512.

Tian, L., J. Scholte, K. Borewicz, B. Bogert, H. Smidt, A. J. W. Scheurink, H. Gruppen, and H. A. Schols. 2016. Effects of pectin supplementation on the fermentation patterns of different structural carbohydrates in rats. *Molecular Nutrition & Food Research* 60 (10): 2256–2266. doi: 10.1002/mnfr.201600149.

Vigsnaes, L. K., A. S. Meyer, J. Holck, and T. R. Licht. 2011. In vitro fermentation of sugar beet arabino-oligosaccharides by fecal microbiota obtained from patients with ulcerative colitis to selectively stimulate the growth of *Bifidobacterium* spp. and *Lactobacillus* spp. *Applied and Environmental Microbiology* 77 (23):8336–8344. doi: 10.1128/AEM.05895-11.

Wang, L. F., E. Beltranena, and R. T. Zijlstra. 2016. Diet nutrient digestibility and growth performance of weaned pigs fed wheat dried distillers grains with solubles (DDGS). *Animal Feed Science and Technology* 218:26–32. doi: 10.1016/j.anifeedsci.2016.05.005.

Wiczkowski, W.,. E. Romaszko, D. Szawara-Nowak, and M. K. Piskula. 2018. The impact of the matrix of red beet products and interindividual variability on betacyanins bioavailability in humans. *Food Research International (Ottawa, Ont.)* 108:530–538. doi: 10.1016/j.foodres.2018.04.004.

Wruss, J., G. Waldenberger, S. Huemer, P. Uygun, P. Lanzerstorfer, U. Müller, O. Höglinger, and J. Weghuber. 2015. Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. *Journal of Food Composition and Analysis* 42:46–55. doi: 10.1016/j.jfca.2015.03.005.

Zhang, Y., J. Lin, and Q. Zhong. 2016. S/O/W emulsions prepared with sugar beet pectin to enhance the viability of probiotic *Lactobacillus salivarius* NRRL B-30514. *Food Hydrocolloids*. 52:804–810. doi: 10.1016/j.foodhyd.2015.08.020.

APÊNDICE C – ARTIGO ORIGINAL 1 (https://doi.org/10.1016/j.fbio.2023.102439).

Food Bioscience 52 (2023) 102439

Contents lists available at ScienceDirect

Food Bioscience

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

Different parts from the whole red beet (*Beta vulgaris* L.) valorization with stimulatory effects on probiotic lactobacilli and protection against gastrointestinal conditions

Sônia Paula Alexandrino de Oliveira[a], Heloísa Maria Almeida do Nascimento[a], Noádia Priscilla Araújo Rodrigues[b], Karoliny Brito Sampaio[a], Marcos dos Santos Lima[c], Maria Lúcia da Conceição[a], Evandro leite de Souza[a,*]

[a] *Laboratory of Food Microbiology, Department of Nutrition, Health Science Center Federal University of Paraíba, João Pessoa, PB, Brazil*
[b] *Department of Gastronomy, Center of Technology and Regional Development, Federal University of Paraíba, João Pessoa, PB, Brazil*
[c] *Department of Food Technology, Federal Institute of Sertão de Pernambuco, Petrolina, PE, Brazil*

ARTICLE INFO

Keywords:
Beet
Lactobacillus
Growth promotion
Protection
Functional ingredient

ABSTRACT

This study evaluated the effects of freeze-dried red beet (*Beta vulgaris* L.) root (FDBR) and stem/leaves (FDBSL) on the growth and metabolic activities of the probiotics *Lactobacillus acidophilus* La-05, *Lacticaseibacillus casei* 26, and *Lismosilactobacillus fermentum* 296, as well as on their survival and physiological states when exposed to simulated gastrointestinal conditions (SGIC). The changes in the contents of phenolic compounds and antioxidant activity of FDBR and FDBSL when exposed to SGIC were also evaluated. FDBR and FDBSL had high contents of soluble (5.25 and 11.10 g/100 g) and insoluble fiber (21.43 and 37.03 g/100 g), pectins (8.45 and 9.51 g/100 g), betalains (3.36–28.99 g/100 g), and a variety of phenolic compounds. FDBR and FDBSL stimulated the growth of the probiotic strains with high viable counts (>9 log CFU/mL), the production of acetic, butyric, lactic, and propionic acids, and alterations in the contents of phenolic compounds during 72 h of cultivation. FDBR and FDBSL had positive prebiotic activity scores on the tested probiotics (≥0.17) linked to selective stimulatory effects. FDBR and FDBSL increased the survival and maintenance of active physiological functions in the probiotic during exposure to SGIC. The contents of phenolic compounds and the antioxidant activity of FDBR and FDBSL increased after exposure to the stomach conditions, while the antioxidant activity decreased after exposure to intestine conditions. The stimulatory and protective effects of FDBR and FDBSL on probiotic strains could be associated with their dietary fiber and phenolic compound contents, which could be exploited in the formulation of functional foods or dietary supplements.

1. Introduction

Red beet (*Beta vulgaris* L.), Chenopodiaceae family, is an important vegetable native to the Mediterranean region, with the highest production in Europe, North America, and Asia and consumed worldwide (Chhikara et al., 2019a, 2019b). The root is the most popular and commercialized edible part of red beet, while the leaves and stems are generally discarded with the generation of large amounts of domestic and industrial by-products (Abdo et al., 2022; Fernandez et al., 2020). Red beet has a variety of nutrients and bioactive compounds effective to treat and or prevent various diseases. Betalains, phenolic acids, and flavonoids have been linked to the bioactive properties of red beet, including antihypertensive, antioxidant, anti-inflammatory, antitumor, and hepatoprotective effects (Babarykin et al., 2019; Chen et al., 2021; Chhikara et al., 2019a, 2019b).

The beneficial effects of beet root on the gastrointestinal tract have been typically associated with its dietary fiber contents, causing improvements in the intestinal peristalsis, reducing colon lesion incidence, and modulating the intestinal microbiota (Babarykin et al., 2019; de Oliveira et al., 2020; Panghal et al., 2017). Red beet is rich in soluble and insoluble dietary fiber, including pectin, from which derives oligosaccharides with different structures and variable biological properties (Gómez et al., 2019; Holck et al., 2011). The ability of pectins and pectic oligosaccharides extracted from beet root to stimulate the growth of

---

* Corresponding author.
E-mail address: els@academico.ufpb.br (E. de Souza).

https://doi.org/10.1016/j.fbio.2023.102439
Received 16 June 2022; Received in revised form 25 January 2023; Accepted 26 January 2023

specific beneficial bacterial populations has been reported (Gómez et al., 2016; Ozcan et al., 2021). However, integrative investigations on the potential functional properties and effects of conventional edible beet part (root) and beet by-products (stem and leaves) on probiotics are still scarce.

Previous investigations have shown the stimulatory effects of dietary phenolic compounds on the growth of probiotics belonging to species found as part of the human intestinal microbiota. These studies have associated the degradation of phenolic compounds and the production of organic acids with the growth and metabolism of probiotics (Alves-Santos et al., 2020; Malik et al., 2019; Sampaio et al., 2021). Probiotic bacteria are live microorganisms capable of conferring a health benefit to the host whether administered in adequate amounts (Hill et al., 2014). *Lactobacillus* species are the most traditionally studied as probiotics with beneficial effects on intestinal health (Huang et al., 2022; Minj et al., 2021). As the primary target of probiotics is the intestine, these microorganisms must survive the hostile gastric environment and remain metabolically active in the lower gastrointestinal tract to confer the claimed health properties (Sampaio et al., 2021). Plant matrices rich in dietary fiber and phenolic compounds could be substrates to enhance the growth of probiotics, as well as to impact positively on their tolerance to gastrointestinal stressing conditions, increasing their survival and colonization in the intestine (de Bellis et al., 2021; Rasika et al., 2021).

Considering the search for novel functional ingredients and the need for the sustainable integral valorization of food resources in line with the circular economy in the agroindustry sector, this study hypothesized that ingredients from the beet whole valorization could have stimulatory effects on probiotics and protect these microorganisms from cell damage during the gastrointestinal passage, helping to keep their survival and functionality. To test this hypothesis, this study evaluated the physicochemical characteristics of powdered beet parts (root and stem/leaves) and their effects on the growth and metabolic activity of different probiotic lactobacilli, as well as on their survival and physiological states when exposed to simulated gastrointestinal conditions. The changes in the phenolic profile and antioxidant activities of the powdered beet parts when exposed to simulated gastrointestinal conditions were evaluated.

2. Materials and methods

2.1. Acquisition and freeze-drying of beet samples

Red beet (*Beta vulgaris* L., early wonder cultivar) samples (root, stalks, and leaves; n: 30, approximately 3.5 kg) in the commercial maturation stage were obtained from three different suppliers (10 samples from each supplier) in local agroecological markets (João Pessoa, PB, Brazil). The red beet samples were washed with running potable water, sanitized with a sodium hypochlorite solution (150 ppm, 15 min), and rinsed with potable water. Beet samples were fractionated into roots, stalks, and leaves, and processed with a domestic electronic processor (1.2 mm grater disc). The processed materials were frozen (-18 ± 2 °C, 24 h), dehydrated (-55 ± 2 °C, <138μHg, 1 mm/h, 24 h) with a freeze-dryer (L-101, Liotop, São Carlos, SP, Brazil), sieved to obtain a powder with a particle size of <1 mm, packed in laminated metalized bi-axially oriented polypropylene (BOPP) bags, and stored under refrigeration (4 ± 0.5 °C, maximum period of one month). The powders from stalks and leaves were mixed (1:1, w/w). Two different powdered materials were tested separately: freeze-dried red beet root (FDBR) and freeze-dried red beet stalks + freeze-dried red beet leaves (FDBSL).

2.2. Physicochemical characterization of FDBR and FDBSL

The moisture (AOAC 940.26), ash (AOAC 940.26), protein (AOAC 935.58), and lipid contents (AOAC 920.39) of FDBR and FDBSL were determined with standard procedures (AOAC, 2016). Insoluble and soluble fiber contents were determined with an enzymatic-gravimetric method (Tobaruela et al., 2018), and pectin content (calcium pectate) was determined with a standard method (Ranganna, 1979, pp. 31–32). For determination of the contents of betalains, an aqueous extract (1%, w/v) was prepared and macerated by freezing overnight. After thawing, the aqueous extract was centrifuged two times (8536×*g*, 20 min, 4 °C), the supernatant (1 mL) was diluted with distilled water (24 mL), and absorbance readings (538 nm and 476 nm) were performed in triplicate. The absorbance was read at 517 nm with a spectrophotometer (Biotek Multi-Detection Synergy HTX, Winooski, VT, USA). The light absorptions at 538 nm and 476 nm were used to calculate the contents of betacyanins and betaxanthins (mg/100 g), respectively. The absorption at 600 nm was measured to correct the occurrence of small impurities. The total contents of betalains were expressed as the sum of betacyanins and betaxanthins (Nilsson, 1970; von Elbe, 2001).

FDBR and FDBSL aqueous extracts were prepared to determine the contents of sugars (fructose and glucose). An aliquot (1 g) of the extract was homogenized (10 min) with ultra-purified water (5 mL, Milli Q Advantage A10 Water Purification System, Merck Millipore, Burlington, VM, USA), centrifuged (4268×*g*, 15 min, 24 °C), and the supernatant was filtered (0.45-μm pore size; Wattman, Chicago, IL, USA). FDBR and FDBSL methanol extracts were prepared to determine the contents of phenolic compounds. An aliquot (1 g) of FDBR or FDBSL was mixed with methanol (10 mL, Neon, São Paulo, SP, Brazil): distilled water (70:30, v/v), treated with ultrasound (60 min, 37 kHz, 28 °C, Ultrasonic, Tecnal, Piracicaba, SP, Brazil), and centrifuged (4268×*g*, 15 min, 4 °C). These procedures were repeated two times and the collected supernatants were mixed and filtered (0.45 μm pore size, Whatman).

Separation and quantification of sugars and phenolic compounds were done with high-performance liquid chromatography (HPLC) using an Agilent chromatograph (model 1260 Infinity LC, Agilent Technologies, St. Clara, CA, USA) and analytical conditions previously described (Coelho et al., 2018). An Agilent Hi-Plex H ion exchange column (7.7 × 300 mm, 8 μm, Agilent Technologies) was used for sugar analysis, while a Zorbax C18 (12.6 × 4.6 mm, 5 μm, Agilent Technologies) pre-column and a Zorbax Eclipse Plus RP-C18 (100 × 4.6 mm, 3.5 μm; Agilent Technologies) were used for phenolic compound analysis (Padilha et al., 2017). The limit of detection and the limit of quantification of sugars, organic acids, and phenolic compounds are shown in supplementary material data (S1).

2.3. Microorganisms and preparation of cultivation media

Three probiotic strains, namely *Lactobacillus acidophilus* LA-05 (Chr. Hansen, Hørsholm, Denmark), *Lacticaseibacillus casei* LAFTI L-26 (DSM Food Specialties, Sydney, Australia), and *Limosilactobacillus fermentum* 296 (Duarte et al., 2017; Garcia et al., 2016; Massa et al., 2020; Sampaio et al., 2021), were tested separately in the experiments. These strains were cultivated (20–24 h, 37 °C) in de Man, Rogosa, and Sharpe (MRS) broth (HiMedia, Mumbai, India), centrifuged (4500×*g*, 15 min, 4 °C), washed, and re-suspended in sterile saline solution (NaCl 8.5 g/L; FMaia, Belo Horizonte, MG, Brazil) to obtain a suspension with viable cell counts of approximately 7 log CFU/mL. The experiments with the probiotic strains were performed with anaerobic incubation (AnaeroGen, Basingstoke, New England).

Escherichia coli ATCC 8739 and *E. coli* ATCC 11775 were used to prepare a mixed enteric inoculum to determine the prebiotic activity scores. The strains were cultivated (18–20 h, 37 °C) separately in brain-heart infusion (BHI) broth (HiMedia), centrifuged (4268×*g*, 15 min, 4 °C), washed, and re-suspended in sterile saline solution to obtain a cell suspension with viable cell counts of approximately 7 log CFU/mL. The enteric mixture inoculum was obtained by mixing (1:1 rate) the suspensions of the two *E. coli* strains (Duarte et al., 2017).

Modified MRS broths were prepared by replacing their main carbon source: i) MRS broth with FDBR (20 g/L); ii) MRS broth with FDBSL (20 g/L); iii) MRS broth with commercial fructooligosaccharides (FOS) from chicory (20 g/L, Sigma-Aldrich), a well-known prebiotic (de Albuquerque et al., 2020; Massa et al., 2020); and iv) MRS broth with glucose (20 g/L, Sigma-Aldrich), as a standard medium. The composition of the modified MRS broths used in these assays was: tryptone 10 g/L, meat extract 8 g/L, yeast extract 4 g/L, di-potassium hydrogen phosphate 2 g/L, tween 80 1 g/L, sodium acetate 5 g/L, tribasic ammonium citrate 2 g/L, magnesium sulfate 0.2 g/L, manganese sulfate 0.04 g/L, and the respective examined carbon source (20 g/L) (de Albuquerque et al., 2020; Massa et al., 2020).

2.4. *Determination of the prebiotic activity scores*

An aliquot of the probiotic strain suspension (0.2 mL) was homogenized with 10 mL of MRS broth with FDBR (20 g/L), FDBSL (20 g/L), FOS (20 g/L), and glucose (20 g/L). In parallel, an aliquot (0.2 mL) of the enteric mixture inoculum was homogenized with M9 broth (10 mL, Sigma-Aldrich) with FDBR (20 g/L), FDBSL (20 g/L), FOS (20 g/L), and glucose (20 g/L). At two different incubation time intervals (zero – just after homogenization and 48 h), an aliquot (1 mL) of each cultivation medium was serially diluted (1:9, v/v) in sterile saline solution (10^{-1} – 10^{-8}), and the dilutions (10 μL) were inoculated on MRS agar or eosin methylene blue agar (HiMedia) for probiotic and enteric mixture enumeration, respectively. After an incubation of 48 h at 37 °C, the visible colonies on agar were enumerated (CFU/mL) and the prebiotic activity score was calculated with the formula:

Prebiotic activity score = [(probiotic log CFU/mL on prebiotic at 48 h – probiotic log CFU/mL on prebiotic at 0 h) / (probiotic log CFU/mL on glucose at 48 h – probiotic log CFU/mL on glucose at 0 h)] – [(enteric log CFU/mL on prebiotic at 48 h – enteric log CFU/mL on prebiotic at 0 h) / (enteric log CFU/mL on glucose at 48 h – enteric log CFU/mL on glucose at 0 h)] (Eq. 1)

A positive prebiotic activity score indicates selective stimulatory effects of the examined component on probiotic growth in detriment to the enteric mixture and, consequently, a potential prebiotic activity (de Albuquerque et al., 2020; Huebner et al., 2007).

2.5. *Measurements of the effects of FDBR and FDBSL on probiotics*

2.5.1. *Measurements of the effects on the probiotic growth*

The effects of FDBR and FDBSL on the growth of the probiotic strains were evaluated by enumerating the viable cell counts over time. An aliquot of the probiotic suspension (0.2 mL) was homogenized with MRS broth (10 mL) with FDBR, FDBSL, FOS, and glucose (20 g/L) (final viable cell count of approximately 6 log CFU/mL). At different incubation time intervals (time zero and 12, 24, 48, and 72 h, 37 °C), an aliquot of the cultivation medium (100 μL) was serially diluted (1:9, v/v) in sterile saline solution (10^{-1} – 10^{-8}), and the dilutions (10 μL) were inoculated on MRS agar. At the end of the incubation period (37 °C, 48 h), the visible colonies on agar were enumerated (CFU/g) and the results were expressed as log CFU/mL (de Albuquerque et al., 2020).

2.5.2. *Measurements of the effects on the probiotic metabolism*

The effects of FDBR and FDBSL on the probiotic metabolism were evaluated with the measurements of pH, sugars, organic acids, and phenolic compounds in the cultivation media (20 g/L) with FDBR, FDBSL, FOS, and glucose over time. The pH of the cultivation media was measured with a digital potentiometer (Q400AS, Quimis, São Paulo, SP, Brazil) (method 981.12, AOAC, 2016) at time zero, 12, 24, 48, and 72 h. Contents of sugars (glucose and fructose), lactic acid, short-chain fatty acids (SCFA, *i.e.*, acetic, propionic, and butyric acids), and phenolic compounds were measured at time zero, 24, and 72 h. At each cultivation period, an aliquot (2 mL) of the medium was collected, centrifuged (6402×*g*, 10 min, 4 °C), and the supernatant was filtered (0.45-μm pore size, Whatman). The contents of sugars, lactic acid, SCFA, and phenolic compounds were determined with HPLC using an Agilent chromatograph (model 1260 Infinity LC, Agilent Technologies, St. Clara, USA) and analytical conditions previously described (Coelho et al., 2018; Massa et al., 2020; Padilha et al., 2017).

2.6. *Measurements of the protective effects of FDBR and FDBSL on the probiotics during exposure to simulated gastrointestinal conditions*

The protective effects of FDBR and FDBSL on the probiotic strains were evaluated with the enumeration of the viable cell counts (CFU/mL) and the sizes of the cell subpopulations (percent) with distinct physiological states during exposure to simulated gastrointestinal conditions (SGIC). The conditions mimicking the mouth, stomach, and intestine were reached with continuous adjustments in pH values, use of digestive enzymes (α-amylase, pepsin, and pancreatin), bile salts, and orbital mechanical agitation specific to each step (de Albuquerque et al., 2020; Rodrigues et al., 2022) (Supplementary material data, S2). Initially, an aliquot (0.1 g) of FDBR, FDBSL, and FOS was mixed with sterile distilled water (25 mL) and homogenized (2 min). Each diluted sample was inoculated aseptically with a fresh suspension (5 mL) of the tested probiotic strain (final viable cell count 6–7 log CFU/mL), homogenized (2 min), and submitted to the conditions simulating the mouth, stomach, and intestine.

2.6.1. *Enumeration of probiotic viable cells during exposure to SGIC*

An aliquot (100 μL) was taken after exposure to each simulated gastrointestinal step, serially diluted (1:9, 10^{-1} – 10^{-5}) in a sterile saline solution, and each dilution (10 μL) was inoculated on MRS agar. At the end of the incubation period (37 °C, 48 h), the visible colonies on agar were enumerated and the results were expressed as log CFU/mL.

2.6.2. *Assessment of the physiological state of probiotics during exposure to SGIC*

An aliquot (1.5 mL) was taken after the exposure to each simulated gastrointestinal step, filtered with a regenerated cellulose membrane (0.45 μm, Whatman), the filtrate was washed two times and resuspended in phosphate buffer saline (PBS, 8 g/L NaCl, 0.2 g/L KCl, 1.44 g/L Na_2HPO_4, 0.24 g/L KH_2PO_4, pH 7.4), and submitted to the staining (15 min, room temperature, 22 °C, under light protection) with propidium iodide (PI, 10 μg/mL, Sigma-Aldrich) and carboxyfluorescein diacetate (cFDA, 2.5 μg/mL, Sigma-Aldrich) to evaluate cytoplasmic membrane integrity and enzymatic activity, respectively. The suspensions were centrifuged (4268×*g*, 10 min, 4 °C), washed (1:1, v/v) with PBS to remove excess fluorochromes, and the precipitate was resuspended in PBS. The cytometric analyzes were performed with a flow cytometer with argon laser emission at 488 nm and 640 nm (BD Accuri C6, Becton Dickinson, Franklin Lakes, NJ, USA) using previously described analytical conditions (Rodrigues et al., 2022). Cell subpopulations characterized as PI-cFDA+ were considered non-permeabilized cells with enzymatic activity (living cells), PI + cFDA-were considered permeabilized cells without enzymatic activity (dead cells), and PI + cFDA+ were considered permeabilized cells with enzymatic activity (injured cells) (Rodrigues et al., 2022).

2.7. *Measurements of the antioxidant activity and phenolic compound contents of FDBR and FDBSL during exposure to SGIC*

The antioxidant activity of FDBR and FDBSL was evaluated with DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) methods (Lee et al., 2020; Shehata et al., 2021) after exposure to each step of the SGIC. Aliquots (0.05 mL) of the samples were reacted with DPPH solution in methanol (0.15 mL), shaken vigorously, and kept (30 min) in the dark. The absorbance was read at 517 nm with a spectrophotometer (Biotek Multi-Detection Synergy HTX). DPPH scavenging activity was measured at a wavelength of 517 nm. Controls were prepared with water replacing the sample. DPPH radical-scavenging activity (%) was calculated with the equation:

DPPH radical scavenging activity (%) = [(ABScontrol - ABSsample)]/(ABScontrol)] x 100 (Eq. 2)

Where ABScontrol is the absorbance of the DPPH radical + water and Abs sample is the absorbance of DPPH radical + tested sample.

The ABTS radical cation (ABTS•+) was generated with the reaction of 5 mL of aqueous ABTS solution (7 mM) + 88 μL of potassium persulfate solution (140 mM). The mixture was kept in the dark (14 h, 28 ± 0.5 °C) before use and diluted with ethanol to obtain an absorbance of 0.7 ± 0.02 units at 734 nm. Aliquots (0.05 mL) of the samples reacted with ABTS radical solution (0.15 mL) in the dark. The decrease of absorbance at 734 nm was measured after 6 min.

The percentage inhibition was calculated with the equation: ABTS radical scavenging activity (%) = [(ABScontrol - ABSsample)]/(ABScontrol)] x 100 (Eq. 3)

Where ABScontrol is the absorbance of ABTS radical + water and ABSsample is the absorbance of ABTS radical + tested sample.

Extracts (1:1, v/v) with methanol 70% (70:30, v/v) were prepared with samples collected after exposure to each step of the SGID to measure the phenolic contents. The samples were centrifuged (4000×*g*, 15 min, 4 °C) and the supernatants were filtered (0.45 μm pore size; Whatman). Separation and quantification of phenolic compounds were performed with high-performance liquid chromatography (HPLC) using an Agilent chromatograph (model 1260 Infinity LC, Agilent Technologies) and analytical conditions previously described (Massa et al., 2020; Padilha et al., 2017).

2.8. *Statistical analysis*

The experiments were performed in triplicate on three different occasions. Results were expressed as average ± standard deviation. Kolmogorov-Smirnov normality test determined data normal distribution. Data were submitted to a Student's *t*-test or analysis of variance (ANOVA) followed by Tukey's test using GraphPad Prism 8.0 (GraphPad Software, La Jolla, USA). A p-value of <0.05 was considered for statistical significance. Pearson's correlation test assessed the relationship between the variables using R software (version 2.15.3, Ross Ihaka and Robert Gentleman, University of Auckland, New Zealand).

3. Results

3.1. *Physicochemical characteristics of FDBR and FDBSL*

FDBR and FDBSL had distinct physicochemical characteristics. The contents of lipids, proteins, ash, sodium, pectin, and total dietary fiber were higher in FDBSL compared to FDBR ($p > 0.05$), while the contents of sugars (glucose and fructose) were higher in FDBR ($p > 0.05$). FDBR and FDBSL had high contents of total dietary fiber, particularly soluble fiber. FDBR had higher contents of betalains compared to FDBSL ($p > 0.05$). The contents of betacyanins and betaxanthins were five- and three-fold higher in FDBR than in FDBSL (Table 1).

Different classes of phenolic compounds were found in FDBR and FDBSL, with large variations in their contents, including phenolic acids, flavanols, flavanones, and stilbenes. Procyanidin B1, procyanidin B2, gallic acid, epicatechin, kaempferol 3-glucoside, caftaric acid, and epicatechin gallate were the prevalent phenolic compounds in FDBR. Kaempferol 3-glucoside, procyanidin B2, gallic acid, caftaric acid, hesperidin, catechin, and naringenin were the prevalent phenolic compounds in FDBSL (Table 1).

3.2. *Effects of FDBR and FDBSL on probiotic lactobacilli*

Table 1

Physicochemical characteristics (average ± standard deviation, n = 3) of freeze-dried red beet root (FDBR) and freeze-dried red beet stem and leaves (FDBSL).

Parameters	Contents	
	FDBR	FDBSL
pH	6.31 ± 0.0^{A}	6.10 ± 0.1^{B}
Moisture (g/100 g)	14.3 ± 0.8^{A}	7.96 ± 1.5^{B}
Lipids (g/100 g)	1.47 ± 0.9^{A}	3.33 ± 1.2 ^{A}
Ashes (g/100 g)	13.50 ± 0.2^{B}	19.65 ± 1.5 ^{A}
Protein (g/100 g)	13.10 ± 0.1^{B}	16.10 ± 0.1^{A}
Pectin (g/100 g)	8.45 ± 0.5^{A}	9.51 ± 0.3^{A}
Sodium (mg/100 g)	1.85 ± 0.3^{A}	3.11 ± 0.9^{A}
Soluble fiber (g/100 g)	5.28 ± 0.9^{B}	11.10 ± 1.2^{A}
Insoluble fiber (g/100 g)	21.43 ± 1.5^{B}	37.03 ± 1.3^{A}
Total dietary fiber (g/100 g)	26.71 ± 1.8^{B}	48.14 ± 1.3^{A}
Sugars		
Fructose (g/L)	2.76 ± 0.02^{A}	0.42 ± 0.01^{B}
Glucose (g/L)	3.54 ± 0.03^{A}	0.68 ± 0.08^{B}
Betalains (mg/100 g)		
Betacyanins	28.99 ± 0.9^{A}	5.64 ± 1.3^{B}
Betaxanthins	11.88 ± 1.2^{A}	3.36 ± 0.6^{B}
Phenolic compounds (mg/g)		
Phenolic acids		
Caffeic acid	0.23 ± 0.02^{A}	0.22 ± 0.06^{A}
Caftaric acid	1.96 ± 0.09^{B}	4.86 ± 0.04^{A}
Chlorogenic acid	1.07 ± 0.03^{A}	0.11 ± 0.02^{B}
Gallic acid	3.65 ± 0.02^{B}	14.72 ± 0.02^{A}
Syringic acid	0.51 ± 0.03^{A}	0.52 ± 0.02^{A}
Flavanols		
Catechin	0.54 ± 0.02^{B}	3.29 ± 0.04^{A}
Epigallocatechin gallate	0.06 ± 0.00^{B}	0.11 ± 0.02^{A}
Epicatechin	2.64 ± 0.03^{A}	1.02 ± 0.05^{B}
Epicatechin gallate	1.96 ± 0.01^{A}	0.71 ± 0.08^{B}
Procyanidin A2	<LOD	0.88 ± 0.02
Procyanidin B1	4.05 ± 0.04 ^{A}	0.16 ± 0.02^{B}
Procyanidin B2	41.97 ± 0.01^{A}	21.26 ± 0.01^{B}
Flavanones		
Hesperidin	0.58 ± 0.01^{B}	3.62 ± 0.05^{A}
Naringenin	1.09 ± 0.02^{B}	2.36 ± 0.01^{A}
Flavonols		
Kaempferol 3-glucoside	2.19 ± 0.03^{B}	29.56 ± 0.04^{A}
Myricetin	0.28 ± 0.06^{A}	0.39 ± 0.05^{A}
Quercitin 3-glucoside	0.16 ± 0.08^{B}	0.50 ± 0.01^{A}
Stilbenes		
cis-Resveratrol	1.39 ± 0.02^{A}	1.16 ± 0.02^{B}
trans-Resveratrol	<LOD	0.1 ± 0.02

<LOD: below the limit of detection. LOD values are shown in supplementary material data (S2). Different superscript capital letters in the same row indicate a significant difference ($p < 0.05$) among samples of freeze-dried beet parts (FDBR and FDBSL), based on Tukey's test.

The probiotics L. *acidophilus* LA-05, *L. casei* L26, and *L. fermentum* 296 had high viable cell counts (>9 log CFU/mL) at 72 h of cultivation in media with FDBR, FDBSL, and FOS. The viable cell counts of these strains increased by more than 3 logs in media with FDBR and FDBSL at 72 h compared to time zero (baseline). The viable cell counts of *L. fermentum* 296 at 72 h of cultivation were higher ($p < 0.05$) in media with FDBR and FDBSL compared to media with FOS and glucose. The viable cell counts of *L. acidophilus* LA-05 and *L. casei* L-26 were similar in media with FDBR, FDBSL, and FOS ($p > 0.05$), which were higher compared to media with glucose ($p < 0.05$) (Fig. 1a -1c).

The cultivation of the probiotic strains caused a decrease in the pH values of the media over time regardless of the added carbon source. The lowest pH values in the cultivation media were achieved up to 12 or 24 h of cultivation ($p < 0.05$), except for the medium with FDBSL where the pH value did not change from 12 to 72 h ($p > 0.05$) (Fig. 1a–c). The largest decrease in pH values (−3.8) occurred overall in the medium with FOS. The variations in the pH values of the media with FDBSL were similar over the 72 h of cultivation regardless of the inoculated probiotic strain ($p > 0.05$) (Fig. 1a–c).

FDBR, FDBSL, and FOS had positive prebiotic activity scores on *L. acidophilus* LA-05, *L. casei* L-26, and *L. fermentum* 296. FDBR, FDBSL, and FOS had similar prebiotic activity scores on *L. fermentum* 296 ($p > 0.05$). FDBSL had similar prebiotic activity scores on *L. casei* L-26 and *L. acidophilus* LA-05 ($p > 0.05$), while FDBR and FOS had similar

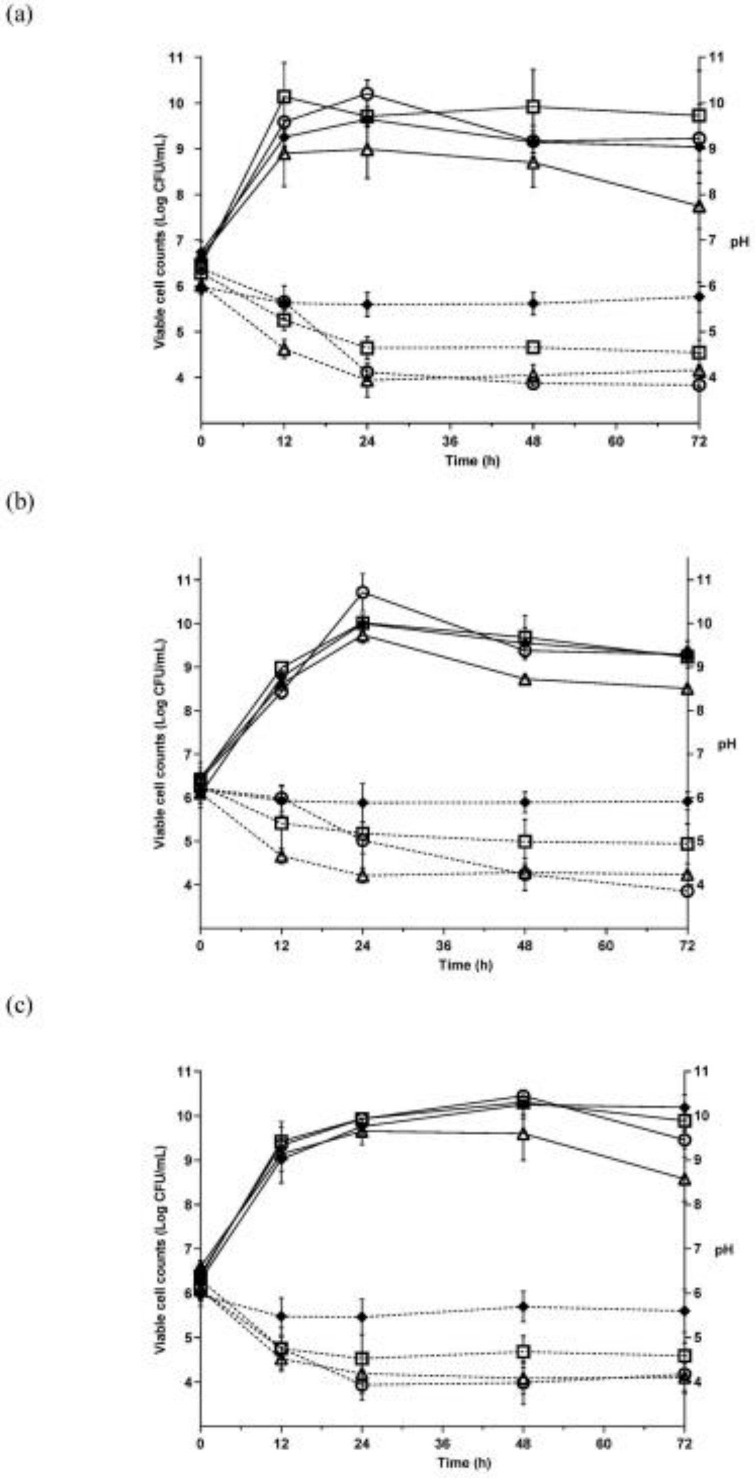


Fig. 1. Viable cell counts (—) of *L. acidophilus* LA-5 (a), *L. casei* L-26 (b), and *L. fermentum* L-296 (c) and pH values (—) in cultivation media with freeze-dried red beet root (FDBR, 20 g/L), freeze-dried red beet stem and leaves (FDBSL, 20 g/L, □), glucose (20 g/L Δ) (average ± standard deviation, n = 3).

prebiotic activity scores on *L. acidophilus* LA-05 and *L. fermentum* 296 ($p > 0.05$). FDBR had higher prebiotic activity scores on *L. acidophilus* LA-05 and *L. casei* L-26 compared to FDBSL ($p < 0.05$) (Table 2).

Fructose and glucose contents decreased in the media during the 72 h of cultivation regardless of the added carbon source and inoculated probiotic strain ($p < 0.05$) (Table 3). Lactic acid contents were increased in the different media at 72 h of cultivation regardless of the inoculated probiotic strain ($p < 0.05$), although the media with FDBSL and glucose inoculated with *L. acidophilus* LA-05 and *L. fermentum* 296 had a decrease in lactic acid content at 24 h of cultivation ($p < 0.05$). Media with FDBR, FOS, and glucose had higher contents of lactic acid at 72 h of cultivation regardless of the inoculated probiotic strain ($p < 0.05$). The acetic acid contents increased over time in media with FDBR, FDBSL, and FOS inoculated with *L. acidophilus* LA-05 and *L. casei* L-26 ($p < 0.05$). The acetic acid contents increased during the 72 h of cultivation in media with FDBR, FDBSL, FOS, and glucose inoculated with *L. fermentum* 296 ($p < 0.05$), and the highest acetic acid contents were detected at 24 h of cultivation. The butyric acid contents were increased at 72 h of cultivation regardless of the media and inoculated probiotic strain ($p < 0.05$). Propionic acid contents increased in media with FDBR, FDBSL, and FOS inoculated with *L. acidophilus* LA-05 and *L. casei* L-26 during the 72 h of cultivation and decreased in media with glucose ($p < 0.05$). Propionic acid contents were decreased at 72 h of cultivation regardless of the cultivation media when inoculated with *L. fermentum* 296 (Table 3).

The contents of phenolic compounds in media with FDBR and FDBSL inoculated with *L. acidophilus* LA-05, *L. casei* L-26, and *L. fermentum* 296 were altered distinctly during the 72 h of cultivation (Table 4). The contents of gallic acid increased and decreased in media with FDBR and FDBSL, respectively, regardless of the inoculated probiotic strain ($p < 0.05$). The contents of caftaric acid decreased during the 72 h of cultivation in media with FDBR and FDBSL inoculated with *L. acidophilus* LA-05, *L. casei* L-26, and *L. fermentum* 296 ($p < 0.05$). The contents of syringic acid did not change over time regardless of the cultivation media and inoculated probiotic strain ($p > 0.05$).

The contents of catechin, epicatechin gallate, and procyanidin B2 increased, and the contents of epicatechin and procyanidin B1 decreased during the 72 h of cultivation in media with FDBR and FDBSL inoculated with *L. acidophilus* LA-05 and *L. fermentum* 296 ($p < 0.05$). The contents of procyanidin A2 decreased during the 72 h of cultivation in media with FDBR and FDBSL inoculated with *L. acidophilus* LA-05, *L. casei* L-26, and *L. fermentum* 296 ($p < 0.05$). The contents of kaempferol 3-glucoside, myricetin, hesperidin, and *cis*-resveratrol increased during the 72 h of cultivation in media with FDBSL regardless of the inoculated probiotic strain ($p < 0.05$) (Table 4).

The probiotic viable cell counts in media with FDBR correlated negatively with the pH values ($r = -0.68$) and contents of glucose ($r = -0.78$), epicatechin ($r = -0.73$), and procyanidin B1 ($r = -0.82$), while correlated positively with the contents of gallic acid ($r = 0.83$), syringic acid ($r = 0.64$), and procyanidin B2 ($r = 0.53$). The pH values in media with FDBR correlated negatively with the contents of lactic acid ($r = -0.63$), gallic acid ($r = -0.65$), procyanidin B2 ($r = -0.63$), and *cis*-resveratrol ($r = -0.73$), and correlated positively with the contents of fructose ($r = 0.89$), glucose ($r = 0.84$), quercetin 3-glucoside ($r = 0.88$), and procyanidin B1 ($r = 0.65$) (Fig. 2a).

Table 2

Prebiotic activity scores (average ± standard deviation, n = 3) of freeze-dried red beet root (FDBR), freeze-dried red beet stems and leaves (FDBSL), and fructooligosaccharides (FOS) on *L. acidophilus* LA-05, *L. casei* L-26, and *L. fermentum* 296.

Strains	FDBR	FDBSL	FOS
L. acidophilus LA-05	0.43 ± 0.02^{Ba}	0.17 ± 0.04^{Cb}	0.48 ± 0.01^{Aa}
L. casei L-26	0.28 ± 0.03^{Bb}	0.17 ± 0.08^{Bb}	0.40 ± 0.02^{Ab}
L. fermentum L-296	0.44 ± 0.02^{ABa}	0.43 ± 0.01^{Aa}	0.45 ± 0.03^{Aab}

A – B: Different superscript capital letters in the same row indicate a significant difference ($p < 0.05$), based on Tukey's test; a - b: Different superscript lowercase letters in the same column denote a significant difference ($p < 0.05$), based on Tukey's test.

The probiotic viable cell counts in media with FDBSL correlated negatively with the pH values ($r = -0.77$) and contents of glucose ($r = -0.75$) and fructose ($r = -0.78$). The probiotic viable cell counts in media with FDBSL correlated positively with the contents of acetic acid ($r = 0.52$) and lactic acid ($r = 0.41$), as well as with the contents of kaempferol 3-glucoside ($r = 0{,}57$) and myricetin ($r = 0{,}58$). The pH values in media with FDBSL correlated negatively with the contents of lactic acid ($r = -0.64$) and myricetin ($r = -0.63$) and correlated positively with the contents of fructose ($r = 0.76$), glucose ($r = 0.62$), epicatechin ($r = 0.53$), and procyanidin B1 ($r = 0.52$) (Fig. 2a).

3.3. *Effects of FDBR and FDBSL on probiotic lactobacilli during exposure to SGIC*

The viable cell counts of *L. acidophilus* LA-05, *L. casei* L-26, and *L. fermentum* 296 during the continuous exposure to the SGIC (mouth, stomach, and intestine steps) in the presence of FDBR and FDBSL were ≥3.6 and ≥ 3.5 log CFU/mL, respectively, and these counts were overall similar to those found in the presence of FOS ($p > 0.05$). The viable cell counts of the same tested probiotic strain were similar when exposed to the different steps of the SGIC in the presence of FDRB and FDBSL ($p > 0.05$), but were lower than those found after the exposure to the stomach and intestine steps in the presence of glucose ($p < 0.05$). The viable cell counts of the probiotic strains were below the limit of detection when exposed to the stomach and or intestine steps in the presence of glucose (Table 5).

The cell subpopulations of *L. acidophilus* LA-05, *L. casei* L-26, and *L. fermentum* 296 with intact cellular membrane and enzymatic activity (live active cells, PI-cFDA+) were of ≥23% during the exposure to the stomach and intestine steps in the presence of FDBR and FDBSL, which were higher than those found in the presence of glucose ($p < 0.05$) (Table 5). The tested probiotic strains exposed to the stomach and intestine conditions in the presence of FDBR and FDBSL had larger subpopulations of live active cells compared to permeabilized cells with enzymatic activity (PI + cFDA+, injured cells, ≤8.7%) and cells with permeabilized membranes and without enzymatic activity (PI + cFDA-, dead cells, ≤5.5%) ($p < 0.05$). The probiotic strains exposed to the stomach and intestine conditions in the presence of FOS and glucose had higher sizes of subpopulations of dead cells (≥9.6%) than when exposed to the same conditions in the presence of FDBR and FDBSL ($p < 0.05$) (Table 5).

The probiotic viable cell counts correlated positively with the sizes of subpopulations of live cells (PI-cFDA+) when exposed to the mouth ($r = 0.56$, $r = 0.81$) and stomach step ($r = 0.99$, $r = 0.84$) in the presence of FDBR and FDBSL. The probiotic viable cell counts correlated positively with the sizes of subpopulations of permeabilized cells with enzymatic activity (PI + cFDA+) when exposed to the mouth ($r = 0.55$, $r = 0.72$) and intestine steps ($r = 0.88$, $r = 0.86$) in the presence of FDBR and FDBSL (Fig. 2b).

3.4. *Changes in phenolic compound contents and antioxidant activities of FDBR and FDBSL during exposure to SGIC*

The contents of the phenolic compounds and the antioxidant activity of FDBR and FDBSL, as measured by DPPH and ABTS method, were determined during the SGIC (Table 6). The highest contents of caftaric acid, chlorogenic acid, catechin, and *cis*-resveratrol in FDBR and FDBSL were found after exposure to the stomach step. The contents of chlorogenic acid and procyanidin B2 increased in FDBR and FDBSL after exposure to the intestine step, while the contents of gallic acid, syringic acid, epicatechin, epicatechin gallate, procyanidin B1, and hesperidin decreased. The highest antioxidant activity in FDBR and FDBSL

Table 3

Contents (average ± standard deviation, n = 3) of lactic acid, short-chain fatty acids (acetic, butyric, and propionic acids), and sugars in media with freeze-dried red beet root (FDBR, 20 g/L), freeze-dried red beet stem and leaves (FDBSL, 20 g/L), glucose (20 g/L), or fructooligosaccharides (FOS, 20 g/L) inoculated with *L. acidophilus* LA-05, *L. casei* L-26, or *L. fermentum* L-296 during 72 h of cultivation.

Media		Probiotic strain/Time of cultivation											
		L. acidophilus LA-05				*L. casei* L-26				*L. fermentum* 296			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
Organic acid (g/L)rowheadrowhead													
Latic acid	FDBR	0.27 ± 0.02^{Da}	6.85 ± 0.04^{Cc}	7.63 ± 0.06^{Bc}	10.54 ± 0.06^{Ac}	0.22 ± 0.05^{Da}	6.67 ± 0.05^{Cb}	8.07 ± 0.04^{Bc}	8.65 ± 0.05^{Ac}	0.73 ± 0.03^{Db}	4.48 ± 0.07^{Bb}	3.58 ± 0.04^{Cb}	5.29 ± 0.06^{Ac}
	FDBSL	0.27 ± 0.04^{Da}	2.73 ± 0.05^{Ad}	2.56 ± 0.07^{Bd}	1.84 ± 0.08^{Cd}	0.17 ± 0.05^{Ba}	1.61 ± 0.03^{Ad}	1.52 ± 0.02^{Ad}	1.71 ± 0.03^{Ad}	0.99 ± 0.05^{Ca}	1.55 ± 0.06^{Ad}	1.06 ± 0.01^{Cd}	1.16 ± 0.05^{Bd}
	FOS	0.26 ± 0.01^{Da}	7.83 ± 0.06^{Cb}	16.91 ± 0.03^{Ba}	17.64 ± 0.04^{Aa}	0.09 ± 0.03^{Db}	3.11 ± 0.04^{Cc}	17.13 ± .05^{Aa}	16.79 ± 0.04^{Ba}	0.23 ± 0.01^{Dc}	1.74 ± 0.02^{Cc}	2.32 ± 0.02^{Bc}	8.38 ± 0.04^{Aa}
	Glucose	0.28 ± 0.03^{Da}	16.78 ± 0.01^{Aa}	15.35 ± 0.01^{Bb}	12.86 ± 0.07^{Cb}	0.10 ± 0.01^{Cb}	9.87 ± 0.06^{Ba}	9.83 ± 0.04^{Bb}	11.86 ± 0.03^{Ab}	0.07 ± 0.02^{Dd}	12.02 ± 0.08^{Aa}	9.58 ± 0.07^{Ba}	7.65 ± 0.05^{Cb}
Acetic acid	FDBR	3.14 ± 0.02^{Db}	3.27 ± 0.04^{Cb}	3.74 ± 0.05^{Ba}	3.88 ± 0.06^{Aab}	3.00 ± 0.04^{Cb}	3.91 ± 0.03^{Ba}	4.03 ± 0.02^{Ba}	5.01 ± 0.03^{Aa}	1.75 ± 0.03^{Dc}	3.00 ± 0.06^{Ab}	2.40 ± 0.01^{Cb}	2.83 ± 0.02^{Ba}
	FDBSL	3.10 ± 0.01^{Cb}	3.00 ± 0.04^{Bc}	3.43 ± 0.03^{Ac}	3.49 ± 0.04^{Ac}	2.54 ± 0.06^{Cc}	3.77 ± 0.01^{Bb}	3.69 ± 0.02^{Ab}	4.10 ± 0.02^{Ab}	2.78 ± 0.05^{Ca}	3.15 ± 0.03^{Ac}	2.20 ± 0.01^{Db}	2.79 ± 0.03^{Ba}
	FOS	2.98 ± 0.04^{Cb}	3.71 ± 0.05^{Aa}	3.65 ± 0.04^{ABb}	3.80 ± 0.06^{Bb}	2.25 ± 0.04^{Dd}	3.13 ± 0.03^{Cd}	3.74 ± 0.04^{Ab}	3.64 ± 0.02^{Ac}	1.68 ± 0.03^{Cd}	2.78 ± 0.04^{Ad}	2.08 ± 0.03^{Bb}	1.98 ± 0.01^{Bb}
	Glucose	3.40 ± 0.02^{Aa}	2.51 ± 0.04^{Bd}	2.49 ± 0.03^{Bd}	2.16 ± 0.01^{Cd}	3.20 ± 0.02^{Da}	3.40 ± 0.02^{Cc}	3.68 ± 0.03^{Bb}	4.01 ± 0.05^{Ab}	1.99 ± 0.01^{Db}	3.92 ± 0.06^{Aa}	3.09 ± 0.02^{Ba}	2.57 ± 0.04^{Ca}
Butyric acid	FDBR	0.64 ± 0.01^{Aa}	0.69 ± 0.01^{Ab}	0.71 ± 0.01^{Aa}	0.71 ± 0.01^{Aa}	0.32 ± 0.04^{Bb}	0.78 ± 0.06^{Aa}	0.80 ± 0.02^{Aa}	0.79 ± 0.03^{Aa}	0.34 ± 0.06^{Cb}	0.51 ± 0.03^{Ab}	0.41 ± 0.02^{Bb}	0.40 ± 0.02^{Ba}
	FDBSL	0.66 ± 0.04^{Aa}	0.60 ± 0.02^{Ad}	0.66 ± 0.04^{Ab}	0.66 ± 0.02^{Ab}	0.39 ± 0.02^{Ca}	0.56 ± 0.01^{Bc}	0.68 ± 0.03^{Ab}	0.67 ± 0.01^{Ab}	0.44 ± 0.05^{Aa}	0.38 ± 0.01^{Ac}	0.48 ± 0.03^{Ab}	0.47 ± 0.03^{Aa}
	FOS	0.63 ± 0.02^{Aa}	0.63 ± 0.02^{Ac}	0.63 ± 0.04^{Ac}	0.60 ± 0.05^{Ad}	0.42 ± 0.05^{Da}	0.64 ± 0.01^{Cb}	0.82 ± 0.01^{Aa}	0.84 ± 0.02^{Aa}	0.34 ± 0.03^{Bb}	0.61 ± 0.04^{Aa}	0.65 ± 0.05^{Aa}	0.40 ± 0.02^{Ba}
	Glucose	0.59 ± 0.01^{Ba}	0.79 ± 0.03^{Aa}	0.65 ± 0.02^{Bb}	0.63 ± 0.01^{ABc}	0.48 ± 0.01^{Ca}	0.61 ± 0.02^{Bb}	0.78 ± 0.05^{Aa}	0.77 ± 0.08^{Aa}	0.35 ± 0.01^{Cb}	0.53 ± 0.02^{Ab}	0.53 ± 0.03^{Ab}	0.41 ± 0.05^{Ba}
Propionic acid	FDBR	1.63 ± 0.05^{Db}	2.53 ± 0.03^{Cc}	3.07 ± 0.02^{Bb}	4.32 ± 0.05^{Ab}	1.63 ± 0.02^{Db}	1.91 ± 0.03^{Bc}	1.83 ± 0.03^{Cb}	2.16 ± 0.03^{Ab}	1.28 ± 0.09^{Ac}	0.59 ± 0.05^{Cd}	0.49 ± 0.04^{Cd}	0.73 ± 0.01^{Bb}
	FDBSL	1.55 ± 0.03^{Dc}	4.97 ± 0.06^{Ca}	5.46 ± 0.07^{Ba}	5.59 ± 0.03^{Aa}	1.28 ± 0.02^{Cd}	4.91 ± 0.05^{Ba}	4.90 ± 0.06^{Ba}	6.16 ± 0.05^{Aa}	6.51 ± 0.07^{Aa}	4.37 ± 0.08^{Ba}	3.30 ± 0.05^{Da}	3.83 ± 0.05^{Ca}
	FOS	1.50 ± 0.02^{Dc}	2.90 ± 0.05^{Cb}	3.01 ± 0.02^{Bc}	3.35 ± 0.04^{Ac}	1.17 ± 0.04^{Dc}	2.72 ± 0.01^{Ab}	1.82 ± 0.04^{Cb}	2.04 ± 0.03^{Bc}	1.13 ± 0.04^{Cd}	1.98 ± 0.06^{Ab}	1.58 ± 0.03^{Bb}	0.54 ± 0.03^{Dc}
	Glucose	1.72 ± 0.03^{Aa}	0.64 ± 0.01^{Dd}	0.95 ± 0.01^{Bd}	0.77 ± 0.01^{Cd}	1.68 ± 0.03^{Aa}	0.82 ± 0.01^{Dd}	0.91 ± 0.01^{Cc}	1.09 ± 0.01^{Bd}	1.41 ± 0.03^{Ab}	1.04 ± 0.01^{Bc}	0.74 ± 0.01^{Cc}	0.57 ± 0.01^{Dc}
Sugars (g/L)rowheadrowhead													
Glucose	FDBR	2.30 ± 0.02^{b}	<LOD	<LOD	<LOD	2.68 ± 0.04^{b}	<LOD	<LOD	<LOD	2.15 ± 0.04^{b}	<LOD	<LOD	<LOD
	FDBSL	0.60 ± 0.01^{d}	<LOD	<LOD	<LOD	0.47 ± 0.03^{d}	<LOD	<LOD	<LOD	1.18 ± 0.03^{d}	<LOD	<LOD	<LOD
	FOS	1.72 ± 0.02^{c}	<LOD	<LOD	<LOD	1.21 ± 0.03^{c}	<LOD	<LOD	<LOD	1.32 ± 0.03^{c}	<LOD	<LOD	<LOD
	Glucose	13.98 ± 0.05^{Aa}	0.04 ± 0.01^{B}	<LOD	<LOD	12.78 ± 0.06^{a}	<LOD	<LOD	<LOD	10.68 ± .04^{Aa}	0.32 ± 0.01^{B}	<LOD	<LOD
Fructose	FDBR	2.69 ± 0.03^{b}	<LOD	<LOD	<LOD	2.13 ± 0.03^{Ab}	2.14 ± 0.01^{Ab}	<LOD	<LOD	1.64 ± 0.02^{b}	<LOD	<LOD	<LOD
	FDBSL	0.24 ± 0.05^{d}	<LOD	<LOD	<LOD	0.18 ± 0.01^{d}	<LOD	<LOD	<LOD	0.36 ± 0.01^{Ad}	0.01 ± 0.00^{Bb}	0.01 ± 0.01^{Bb}	<LOD
	FOS	7.96 ± 0.06^{Aa}	4.45 ± 0.07^{Ba}	0.47 ± 0.02^{C}	0.07 ± 0.02^{D}	7.12 ± 0.05^{Aa}	3.17 ± 0.07^{Ba}	0.19 ± 0.01^{Da}	0.52 ± 0.01^{Ca}	5.41 ± 0.05^{Aa}	3.84 ± 0.04^{Ba}	3.36 ± 0.03^{Ca}	1.19 ± 0.05^{D}
	Glucose	0.96 ± 0.02^{Ac}	0.02 ± 0.01^{Bb}	<LOD	<LOD	0.87 ± 0.01^{Ac}	0.20 ± 0.02^{Bc}	0.18 ± 0.02^{Ca}	0.09 ± 0.03^{Db}	0.91 ± 0.01^{c}	<LOD	<LOD	<LOD

A – D: different superscript capital letters in the same row for the same cultivation medium and probiotic strain denote differences ($p < 0.05$), based on Tukey's test; a-d: different superscript small letters in the same column at the same time interval and measured parameter denote difference ($p < 0.05$), based on Tukey's test.

measured by either DPPH or ABTS was detected after the exposure to the stomach step, with a decrease after the exposure to the intestine step (Table 6).

The results of DPPH and ABTS tests for FDBR (r = 0.78) and FDBSL (r = 0.53) correlated positively during the exposure to the SGIC (Fig. 2c). The results of DPPH and ABTS tests during exposure to the SGIC correlated positively ($r > 0.54$) with the contents of epigallocatechin, procyanidin A2, and kaempferol 3-glucoside. The results of DPPH and ABTS tests of FDBR correlated negatively with the other measured phenolic compounds ($r \leq -0.58$). The highest positive correlations of the results of ABTS test ($r \geq 0.68$) were with the contents of epigallocatechin gallate, procyanidin A2, kaempferol 3-glucoside, myricetin, and *cis*-resveratrol. The highest positive correlations of the results of DPPH test ($r \geq 0.83$) were with the contents of kaempferol 3-glucoside, procyanidin A2, epigallocatechin gallate, catechin, and quercitin 3-glucoside.

The results of DPPH and ABTS tests for FDBSL during the exposure to the SGIC correlated positively with the contents of *cis*-resveratrol, caftaric acid, syringic acid, caffeic acid, and gallic acid, and correlated negatively with the contents of procyanidin A2. The highest positive correlations of DPPH results ($r > 0.66$) for FDBSL were with the contents of gallic acid, syringic acid, caffeic acid, caftaric acid, epicatechin,

Table 4
Contents (average ± standard deviation, n = 3) of phenolic compounds in media with freeze-dried red beet root (FDBR, 20 g/L), freeze-dried red beet stem and leaves (FDBSL, 20 g/L), glucose (20 g/L), or fructooligosaccharides (FOS, 20 g/L) inoculated with *L. acidophilus* LA-05, *L. casei* L-26, or *L. fermentum* L-296 during 72 h of cultivation.

Phenolics (mg/L)	Strain/media/Time of incubation																	
	L. acidophilus LA-05						*L. casei* L-26						*L. fermentum* L-296					
	FDBR			FDBSL			FDBR			FDBSL			FDBR			FDBSL		
	0 h	24 h	72 h	0 h	24 h	72 h	0 h	24 h	72 h	0 h	24 h	72 h	0 h	24 h	72 h	0 h	24 h	72 h
Phenolic acids																		
Syringic acid	0.23 ± 0.03^{A}	0.28 ± 0.02^{A}	0.25 ± 0.01^{A}	0.32 ± 0.03^{A}	0.30 ± 0.03^{A}	0.31 ± 0.01^{A}	0.23 ± 0.03^{A}	0.24 ± 0.05^{A}	0.29 ± 0.01^{A}	0.22 ± 0.01 ^{A}	0.23 ± 0.01 ^{A}	0.25 ± 0.07 ^{A}	0.20 ± 0.02^{A}	0.30 ± 0.01^{A}	0.26 ± 0.02^{A}	0.26 ± 0.01^{A}	0.28 ± 0.03^{A}	0.28 ± 0.01^{A}
Caftaric acid	0.34 ± 0.02^{A}	0.19 ± 0.02^{B}	0.17 ± 0.02^{B}	1.21 ± 0.06^{A}	0.29 ± 0.03^{C}	0.40 ± 0.05^{B}	0.29 ± 0.01^{A}	0.23 ± 0.01^{A}	0.17 ± 0.03^{B}	1.17 ± 0.03^{A}	0.32 ± 0.04^{B}	0.31 ± 0.01^{B}	0.26 ± 0.03^{B}	0.45 ± 0.01^{A}	0.37 ± 0.05^{A}	1.45 ± 0.05^{A}	0.27 ± 0.01^{C}	0.48 ± 0.02^{B}
Gallic acid	1.25 ± 0.03^{C}	2.11 ± 0.05^{B}	4.14 ± 0.04^{A}	3.81 ± 0.05^{A}	3.43 ± 0.03^{B}	3.16 ± 0.07^{C}	1.61 ± 0.04^{B}	1.34 ± 0.09^{B}	3.11 ± 0.07^{A}	3.00 ± 0.05^{A}	2.57 ± 0.07^{B}	2.64 ± 0.02^{B}	0.45 ± 0.01^{C}	2.17 ± 0.07^{B}	2.89 ± 0.09^{A}	3.38 ± 0.07^{A}	1.10 ± 0.05^{C}	2.73 ± 0.10^{B}
Flavanols																		
Catechin	2.59 ± 0.04^{B}	2.50 ± 0.05^{B}	2.86 ± 0.02^{A}	2.34 ± 0.04^{B}	2.40 ± 0.03^{B}	2.79 ± 0.05^{A}	2.58 ± 0.02^{A}	2.53 ± 0.04^{A}	2.52 ± 0.07^{A}	2.69 ± 0.03^{A}	2.48 ± 0.05^{B}	2.46 ± 0.05^{B}	2.40 ± 0.09^{B}	2.38 ± 0.05^{B}	2.67 ± 0.11^{A}	2.39 ± 0.07^{C}	2.63 ± 0.03^{A}	2.70 ± 0.01^{A}
Epigallocatechin gallate	0.20 ± 0.03^{A}	0.19 ± 0.02^{A}	0.20 ± 0.05^{A}	0.27 ± 0.02^{A}	0.25 ± 0.03^{A}	0.26 ± 0.03^{A}	0.21 ± 0.01^{A}	0.20 ± 0.01^{A}	0.22 ± 0.03^{A}	0.19 ± 0.05^{A}	0.27 ± 0.03^{A}	0.26 ± 0.01^{A}	0.19 ± 0.03^{A}	0.27 ± 0.01^{A}	0.27 ± 0.04^{A}	0.25 ± 0.01^{A}	0.19 ± 0.02^{A}	0.27 ± 0.02^{A}
Epicatechin	3.52 ± 0.04^{A}	2.72 ± 0.06^{B}	2.50 ± 0.07^{B}	2.96 ± 0.04^{A}	2.19 ± 0.05^{B}	1.72 ± 0.01^{C}	3.46 ± 0.07^{A}	2.33 ± 0.04^{B}	2.48 ± 0.07^{B}	2.07 ± 0.04^{C}	2.84 ± 0.07^{A}	2.45 ± 0.09^{B}	3.21 ± 0.07^{A}	2.63 ± 0.09^{B}	2.29 ± 0.10^{C}	3.08 ± 0.13^{A}	1.99 ± 0.07^{B}	1.80 ± 0.09^{B}
Epicatechin gallate	0.49 ± 0.02^{C}	1.07 ± 0.05^{A}	0.90 ± 0.03^{B}	0.52 ± 0.03^{C}	0.73 ± 0.03^{B}	1.12 ± 0.07^{A}	0.49 ± 0.03^{A}	0.33 ± 0.01^{B}	0.40 ± 0.03^{A}	1.64 ± 0.03^{A}	0.38 ± 0.01^{B}	0.36 ± 0.02^{B}	0.45 ± 0.01^{A}	0.33 ± 0.02^{B}	0.46 ± 0.02^{A}	0.29 ± 0.01^{C}	2.25 ± 0.03^{A}	0.44 ± 0.01^{B}
Procyanidin A2	2.79 ± 0.03^{AB}	2.65 ± 0.02^{B}	2.81 ± 0.05^{A}	2.10 ± 0.05^{A}	1.93 ± 0.04^{B}	1.67 ± 0.01^{C}	2.73 ± 0.06^{A}	2.52 ± 0.05^{B}	2.41 ± 0.02^{B}	2.30 ± 0.06^{A}	2.11 ± 0.05^{B}	2.00 ± 0.04^{B}	2.54 ± 0.09^{A}	1.75 ± 0.06^{B}	1.81 ± 0.02^{B}	2.03 ± 0.07^{A}	2.05 ± 0.05^{A}	1.66 ± 0.02^{B}
Procyanidin B1	1.9 5 ± 0.03^{A}	1.65 ± 0.02^{B}	1.74 ± 0.01^{B}	1.58 ± 0.04^{A}	1.56 ± 0.02^{A}	1.5 1 ± 0.02^{A}	1.94 ± 0.01^{A}	1.57 ± 0.03^{B}	1.64 ± 0.09^{B}	1.49 ± 0.08^{A}	1.57 ± 0.07^{A}	1.59 ± 0.07^{A}	1.76 ± 0.07^{A}	1.58 ± 0.09^{AB}	1.47 ± 0.07^{B}	1.62 ± 0.05^{A}	1.43 ± 0.07^{B}	1.43 ± 0.09^{B}
Procyanidin B2	1.29 ± 0.01^{A}	1.31 ± 0.02^{A}	1.35 ± 0.01^{A}	1.44 ± 0.02^{A}	1.28 ± 0.10^{B}	1.50 ± 0.03^{A}	1.28 ± 0.02^{A}	1.28 ± 0.01^{A}	1.20 ± 0.03^{A}	1.22 ± 0.03^{A}	1.29 ± 0.03^{A}	1.29 ± 0.02^{A}	1.19 ± 0.01^{B}	1.30 ± 0.05^{B}	1.46 ± 0.03^{A}	1.24 ± 0.03^{B}	1.25 ± 0.05^{B}	1.45 ± 0.03^{A}
Kaempferol 3-glucoside	0.27 ± 0.01^{A}	0.04 ± 0.03^{B}	0.03 ± 0.01^{B}	3.41 ± 0.05^{C}	5.18 ± 0.01^{B}	5.49 ± 0.03^{A}	0.11 ± 0.01^{A}	0.08 ± 0.02^{A}	0.07 ± 0.03^{A}	3.02 ± 0.03^{C}	5.32 ± 0.09^{B}	5.59 ± 0.10^{A}	0.07 ± 0.01^{C}	3.53 ± 0.06^{B}	5.64 ± 0.04^{A}	4.54 ± 0.09^{C}	5.15 ± 0.07^{B}	5.42 ± 0.02^{A}
Myricetin	0.09 ± 0.01	<LOD	<LOD	0.21 ± 0.01^{C}	0.35 ± 0.01^{B}	1.06 ± 0.01^{A}	0.07 ± 0.02^{A}	0.09 ± 0.02^{A}	<LOD	<LOD	0.52 ± 0.01^{B}	0.62 ± 0.03^{A}	0.28 ± 0.01^{C}	0.41 ± 0.01^{B}	0.90 ± 0.03^{A}	0.51 ± 0.02^{C}	0.67 ± 0.01^{B}	0.76 ± 0.05^{A}
Flavanones																		
Hesperidin	0.75 ± 0.03	<LOD	<LOD	0.65 ± 0.01^{C}	0.83 ± 0.03^{B}	0.99 ± 0.01^{A}	0.58 ± 0.07	<LOD	<LOD	1.03 ± 0.02^{B}	0.90 ± 0.01^{C}	1.36 ± 0.04^{A}	0.52 ± 0.01^{C}	0.67 ± 0.03^{B}	1.03 ± 0.05^{A}	0.81 ± 0.02^{B}	0.89 ± 0.02^{B}	0.99 ± 0.01^{A}
Stilbenes																		
cis-Resveratrol	<LOD	<LOD	<LOD	1.15 ± 0.07^{A}	0.54 ± 0.05^{C}	1.09 ± 0.03^{B}	<LOD	<LOD	<LOD	<LOD	0.56 ± 0.01^{B}	1.02 ± 0.03^{A}	0.18 ± 0.02^{C}	1.19 ± 0.02^{A}	1.27 ± 0.05^{B}	0.55 ± 0.01^{C}	1.02 ± 0.07^{B}	1.27 ± 0.09^{A}

<LOD: below the limit of detection. LOD values are shown in supplementary material data (S2). A – C: different superscript capital letters in the same row for the same cultivation media and tested strain denote differences ($p < 0.05$), based on Tukey's test.

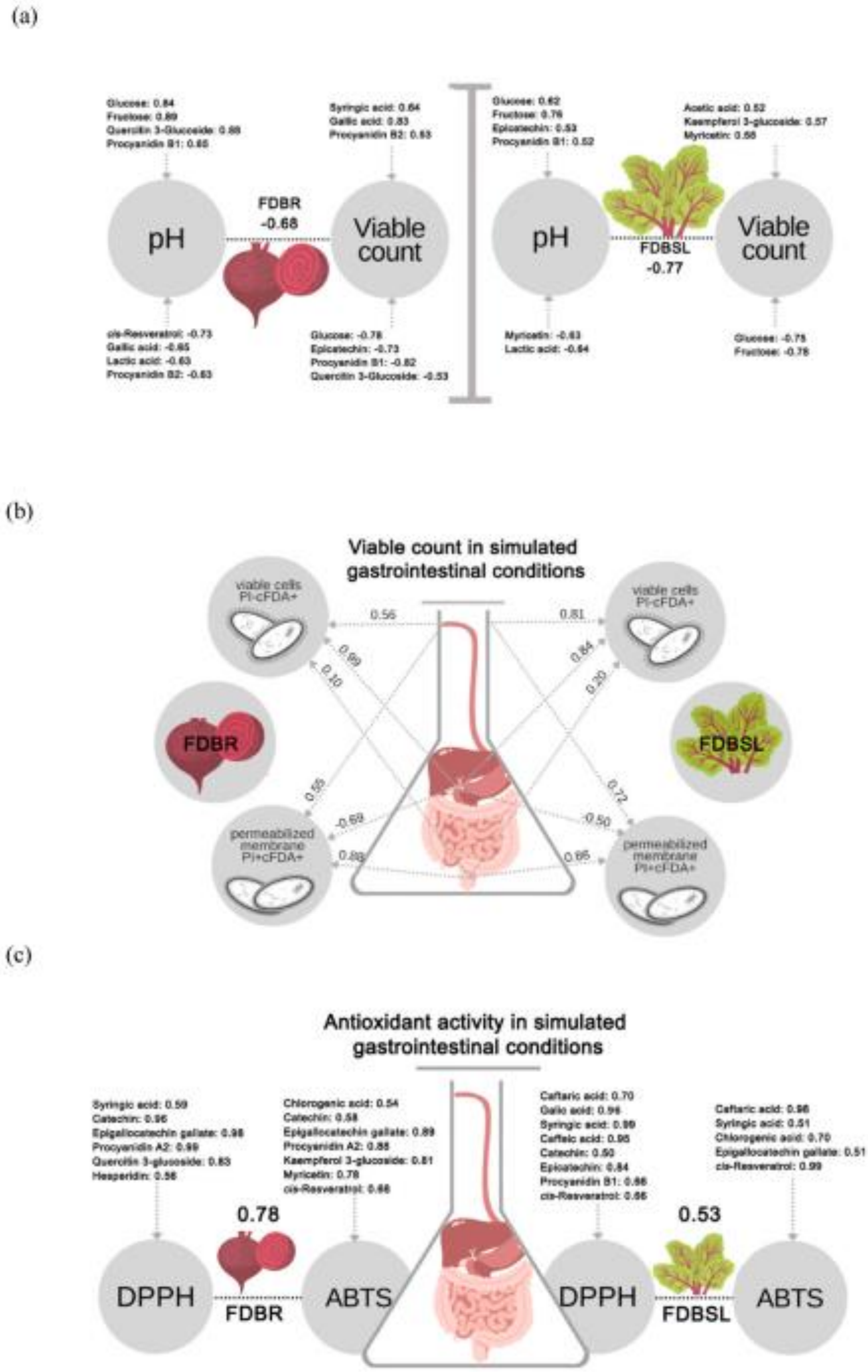


Fig. 2. Schematic results of the correlation between variables at 72 h of cultivation of the lactobacilli probiotic strains in media with FDBR and FDBSL (a), the viable counts of probiotic strains and the sizes of cell subpopulations with different physiological states (PI-cFDA+; PI + cFDA+) during the exposure to the simulated gastrointestinal conditions (SGIC) in the presence of FDBR and FDBSL (b), and the antioxidant activity (DPPH and ABTS) of FDBR and FDBSL during the exposure to SGIC (c).

Table 5

Viable cell counts (Log CFU/mL, average ± standard deviation, n = 3) and size (%, average ± standard deviation, n = 3) of cell subpopulations with different physiological states of *L. acidophilus* LA-05, *L. casei* L-26, and *L. fermentum* 296 during exposure to different steps of an *in vitro* gastrointestinal digestion in the presence of freeze-dried red beet root (FDBR), freeze-dried red beet stem and leaves (FDBSL, 20 g/L), fructooligosaccharides (FOS, 20 g/L), or glucose (20 g/L).

Substrate		*L. acidophilus* LA-05				*L. casei* L-26				*L. fermentum* L-296			
	Digestion step	Viable count (Log CFU/mL)	Size of cell subpopulation (%)			Viable count (Log CFU/mL)	Size of cell subpopulation (%)			Viable count (Log CFU/mL)	Size of cell subpopulation (%)		
			PI-cFDA+	PI + cFDA +	PI + cFDA-		PI-cFDA+	PI + cFDA +	PI + cFDA-		PI-cFDA+	PI + cFDA+	PI + cFDA-
FDBR	Mouth	6.5 ± 0.9^{a}	49.6 ± 0.2^{Aa}	28.7 ± 0.1^{Ba}	2.1 ± 0.4^{Cc}	6.2 ± 1.4^{a}	54.6 ± 0.4^{Aa}	11.8 ± 0.1^{Ba}	2.0 ± 0.2^{Cc}	6.4 ± 0.1^{a}	48.8 ± 0.1^{Aa}	13.4 ± 0.1^{Ba}	1.3 ± 0.1^{Cb}
	Stomach	5.9 ± 0.5^{a}	39.4 ± 0.6^{Ab}	8.7 ± 0.1^{Bb}	2.6 ± 0.3^{Cb}	3.8 ± 0.7^{a}	35.6 ± 0.3^{Ab}	5.7 ± 0.3^{Bb}	2.8 ± 0.2^{Cb}	6.0 ± 0.3^{a}	32.6 ± 0.2^{Ab}	6.6 ± 0.1^{Bb}	2.9 ± 0.3^{Ca}
	Intestine	4.1 ± 0.2^{b}	29.1 ± 0.4^{Ac}	4.2 ± 0.2^{Bc}	4.2 ± 0.06^{Ca}	4.0 ± 0.6^{a}	28.8 ± 0.2^{Ac}	3.1 ± 0.2^{Bc}	2.9 ± 0.1^{Ba}	3.6 ± 0.5^{b}	25.2 ± 0.5^{Ac}	4.3 ± 0.7^{Bc}	2.5 ± 0.2^{Ca}
FDBSL	Mouth	6.2 ± 1.1^{a}	49.7 ± 0.1^{Aa}	10.1 ± 0.2^{Ba}	3.2 ± 0.1^{Cb}	6.1 ± 0.7^{a}	61.0 ± 0.4^{Aa}	12.5 ± 0.5^{Ba}	2.3 ± 0.6^{Cc}	6.5 ± 1.2^{a}	53.7 ± 0.2^{Aa}	10.0 ± 0.6^{Ba}	2.7 ± 0.3^{Ca}
	Stomach	5.6 ± 0.7^{a}	48.7 ± 0.1^{Ab}	4.5 ± 0.1^{Bc}	3.2 ± 0.2^{Cb}	4.5 ± 0.3^{ab}	39.2 ± 0.8^{Ab}	5.2 ± 0.3^{Bc}	4.5 ± 0.5^{Cb}	5.2 ± 0.5^{a}	40.4 ± 0.2^{Ab}	7.5 ± 0.3^{Bb}	2.8 ± 0.2^{Ca}
	Intestine	3.6 ± 0.8^{b}	34.5 ± 0.1^{Ac}	6.0 ± 0.2^{Bb}	5.4 ± 0.1^{Ca}	3.9 ± 0.3^{b}	27.3 ± 0.6^{Ac}	7.5 ± 0.3^{Bb}	5.5 ± 0.1^{Ca}	3.5 ± 0.3^{b}	23.1 ± 0.3^{Ac}	6.3 ± 0.1^{Bb}	2.6 ± 0.3^{Ca}
FOS	Mouth	6.6 ± 0.5^{a}	51.6 ± 0.6^{Aa}	13.1 ± 0.2^{Ba}	8.3 ± 0.6^{Cb}	6.5 ± 0.8^{a}	42.8 ± 1.0^{Ab}	30.7 ± 0.7^{Ba}	28.2 ± 0.5^{Ba}	6.4 ± 0.2^{a}	54.0 ± 0.1^{Aa}	19.7 ± 0.2^{Cb}	26.0 ± 0.5^{Bb}
	Stomach	5.7 ± 0.3^{a}	11.1 ± 0.1^{Cb}	29.7 ± 0.1^{Ab}	21.5 ± 0.2^{Ba}	5.1 ± 0.3^{b}	26.8 ± 0.2^{Bc}	21.1 ± 0.3^{Cb}	28.6 ± 0.3^{Aa}	5.3 ± 0.7^{ab}	27.4 ± 0.3^{Cb}	38.0 ± 0.1^{Ba}	42.2 ± 0.4^{Aa}
	Intestine	3.9 ± 0.6^{b}	8.5 ± 0.5^{Bc}	8.2 ± 0.4^{Bc}	9.6 ± 0.1^{Ab}	3.9 ± 0.2^{c}	24.6 ± 0.4^{Aa}	15.1 ± 0.5^{Bc}	11.4 ± 0.2^{Cb}	3.3 ± 0.4^{b}	22.2 ± 0.1^{Ac}	7.3 ± 0.5^{Cc}	9.8 ± 0.2^{Bc}
Glucoseve control	Mouth	6.0 ± 1.2^{a}	42.4 ± 0.1^{Aa}	4.1 ± 0.6^{Bb}	3.1 ± 0.3^{Cb}	6.1 ± 0.6	48.5 ± 0.2^{Aa}	7.4 ± 0.6^{Bb}	2.2 ± 0.4^{Cc}	5.8 ± 0.9^{a}	40.3 ± 0.6^{Aa}	10.6 ± 0.1^{Ba}	3.8 ± 0.2^{Ca}
	Stomach	3.0 ± 1.1^{b}	16.3 ± 0.2^{Bb}	8.2 ± 0.3^{Ca}	17.6 ± 0.4^{Aa}	<LOD	20.9 ± 0.1^{Ab}	9.9 ± 0.3^{Ba}	10.9 ± 0.1^{Ba}	2.5 ± 0.2^{b}	19.7 ± 0.3^{Ab}	9.7 ± 0.3^{Ba}	9.1 ± 0.4^{Cb}
	Intestine	<LOD	4.1 ± 1.0^{Bc}	4.5 ± 0.8^{Bc}	10.8 ± 0.7^{Ac}	<LOD	9.6 ± 0.4^{Ac}	5.5 ± 0.2^{Bc}	15.4 ± 0.6^{Bb}	<LOD	6.2 ± 0.1^{Bc}	3.8 ± 0.2^{Cb}	13.2 ± 0.2^{Ab}

<LOD: below the limit of detection (2 Log CFU/mL). PI-cFDA+: non-permeabilized cells with enzymatic activity; PI + cFDA+: permeabilized cells with enzymatic activity; PI + cFDA-: permeabilized cells without enzymatic activity. A – C: Different superscript capital letters in the same row indicate a significant difference ($p < 0.05$) between sizes of cell subpopulation for the same step of the digestion and strain), based on Tukey's test; a - c: Different superscript lowercase letters in the column denote a significant difference ($p < 0.05$) between the viable cell counts or sizes of cell subpopulation at distinct digestion steps for the same sample, based on Tukey's test.

procyanidin B1, and *cis*-resveratrol, while for ABTS results ($r > 0.70$) were with the contents of *cis*-resveratrol, caftaric acid, and chlorogenic acid (Fig. 2c).

4. Discussion

The results showed that FDBR and FDBSL are distinct regarding the contents of some nutrients and bioactive compounds probably because these materials were produced from different red beet anatomical parts. Red beet root typically has higher contents of sugars and betalains, while the beet aerial parts (stem and leaves) have higher contents of phenolic compounds (Sawicki et al., 2016; Takács-Hájos & Vargas-Rubóczki, 2022), which agreed with the results of this study. The presence of pectin and soluble and insoluble fibers resistant to digestion and absorption in the human small intestine, as well as phenolic compounds, characterizes FDBR and FDBSL as energy source substrates for intestinal microorganisms (de Oliveira et al., 2020; Zhang et al., 2022). High contents of total dietary fiber, particularly insoluble fiber (>75% of the total dietary fiber), were reported for red beet bagasse (Hotchkiss et al., 2022) and red beet husk (Seremet et al., 2020). Red beet pectin, consisting mostly of arabinose and galactose-rich pectic oligosaccharides, is a predominant polysaccharide in the beet fiber composition (Hotchkiss et al., 2022).

The probiotics L. *acidophilus* LA-05, *L. casei* L26, and *L. fermentum* 296 kept high viable cell counts during 72 h in media with FDBR, FDBSL, and FOS, indicating the use of these substances as a sole carbon source to maintain the probiotic growth over time. Although the media with FDBR and FOS had a decrease in pH values during the measured cultivation period, the medium with FDBSL had little pH variation over time. These results could indicate the capability of FDBSL to buffering the pH of the cultivation media. Early studies reported high viable cell counts of *L. acidophilus* and *L. casei* (>8 log CFU/mL) and a small reduction in pH values in beet juices during 72 h of fermentation (Malik et al., 2019). The incorporation of sugar beet pectic polysaccharides into a laboratory medium also increased the viable cell counts of *Lactobacillus* and *Bifidobacterium* over time (Gómez et al., 2019)

The cultivation media with FDBR and FDBSL showed positive prebiotic activity scores on *L. acidophilus* LA-05, *L. casei* L26, and *L. fermentum* 296, indicating their ability to stimulate the growth of probiotics rather than enteric pathogens (Massa et al., 2020; Yu et al., 2022). However, the media with FDBR and FDBSL had different prebiotic activity scores on the examined probiotics. It could be due to the differences in nutrients available in the cultivation media for use by the inoculated probiotics, as well as to a strain-dependent effect. The presence or absence of specific genes in different *Lactobacillus* strains directly affects their metabolic diversity and, consequently, their ability to use certain nutrients, generate metabolites, and growth behavior in a cultivation medium (Moumita & Das, 2022; Okoye et al., 2022).

The decrease in glucose and fructose contents in parallel to the increase in the viable cell counts of the examined probiotics in the media with FDBR and FDBSL during the measured cultivation period indicates that the consumption of these intrinsic sugar and fiber sources promotes

Table 6

Contents of phenolic compounds (mg/L, average ± standard deviation, n = 3) and antioxidant activity of freeze-dried red beet root (FDBR) and freeze-dried red beet stem and leaves (FDBSL) when exposed to simulated gastrointestinal conditions.

Phenolic compounds (mg/L)	Substrate	Digestion step		
		Mouth	Stomach	Intestine
Phenolic acids				
Caftaric acid	FDBR	0.96 ± 0.03^{Bb}	1.73 ± 0.05^{Ab}	0.92 ± 0.02^{Bb}
	FDBSL	3.80 ± 0.05^{Ba}	6.31 ± 0.02^{Aa}	3.67 ± 0.03^{Ca}
Gallic acid	FDBR	3.65 ± 0.06^{Ab}	0.91 ± 0.03^{Bb}	<LOD
	FDBSL	16.11 ± 0.09^{Aa}	10.58 ± 0.05^{Ba}	<LOD
Syringic acid	FDBR	0.52 ± 0.01^{Aa}	0.42 ± 0.03^{Bb}	0.30 ± 0.01^{Ca}
	FDBSL	0.52 ± 0.05^{Aa}	0.55 ± 0.02^{Aa}	0.39 ± 0.01^{Ba}
Caffeic acid	FDBR	0.16 ± 0.03^{Ca}	0.25 ± 0.03^{Ba}	0.36 ± 0.02^{Aa}
	FDBSL	0.16 ± 0.04^{Aa}	0.15 ± 0.08^{Ab}	0.08 ± 0.01^{Ab}
Chlorogenic acid	FDBR	0.22 ± 0.06^{Ca}	0.39 ± 0.02^{Aa}	0.40 ± 0.02^{Aa}
	FDBSL	0.14 ± 0.04^{Bb}	0.24 ± 0.09^{Ab}	0.23 ± 0.08^{Ab}
Flavonols				
Catechin	FDBR	0.95 ± 0.02^{Bb}	1.05 ± 0.02^{Aa}	0.55 ± 0.02^{Cb}
	FDBSL	1.29 ± 0.03^{Aa}	1.25 ± 0.04^{Aa}	1.24 ± 0.04^{Aa}
Epigallocatechin gallate	FDBR	0.16 ± 0.02^{Ba}	0.21 ± 0.01^{Aa}	0.13 ± 0.03^{Bb}
	FDBSL	0.08 ± 0.01^{Cb}	0.28 ± 0.01^{Ba}	0.32 ± 0.01^{Aa}
Epicatechin	FDBR	1.96 ± 0.09^{Aa}	0.47 ± 0.02^{Bb}	0.28 ± 0.02^{Ca}
	FDBSL	0.94 ± 0.05^{Ab}	0.72 ± 0.01^{Ba}	0.35 ± 0.02^{Ca}
Epicatechin gallate	FDBR	0.52 ± 0.02	<LOD	<LOD
	FDBSL	1.05 ± 0.01^{A}	0.51 ± 0.01^{B}	0.37 ± 0.03^{C}
Procyanidin A2	FDBR	0.40 ± 0.1^{ABb}	0.43 ± 0.03^{Ba}	0.38 ± 0.01^{Ab}
	FDBSL	1.03 ± 0.06^{Ba}	0.44 ± 0.05^{Ca}	1.41 ± 0.06^{Aa}
Procyanidin B1	FDBR	8.55 ± 0.07^{Aa}	3.92 ± 0.09^{Ba}	0.44 ± 0.02^{Ca}
	FDBSL	0.58 ± 0.05^{Ab}	0.38 ± 0.01^{Bb}	0.25 ± 0.02^{Cb}
Procyanidin B2	FDBR	15.89 ± 0.1^{Ca}	18.23 ± 0.05^{Ba}	24.83 ± 0.03^{Aa}
	FDBSL	16.56 ± 0.01^{Ba}	16.27 ± 0.01^{Cb}	38.36 ± 0.06^{Ab}
Kaempferol 3-glucoside	FDBR	<LOD	<LOD	<LOD
	FDBSL	15.39 ± 0.03^{Aa}	14.57 ± 0.06^{Ba}	14.47 ± 0.07^{B}
Quercitin 3-glucoside	FDBR	0.15 ± 0.02^{Ab}	0.13 ± 0.01^{Ab}	<LOD
	FDBSL	0.20 ± 0.07^{Ca}	0.33 ± 0.02^{Ba}	0.52 ± 0.04^{A}
Rutin	FDBR	<LOD	<LOD	<LOD
	FDBSL	0.23 ± 0.03^{C}	1.46 ± 0.06^{A}	0.52 ± 0.02^{B}
Myricetin	FDBR	<LOD	0.14 ± 0.01^{Ab}	0.10 ± 0.01^{Bb}
	FDBSL	0.18 ± 0.03^{B}	0.24 ± 0.01^{Ba}	0.53 ± 0.03^{Aa}
Flavonones				
Hesperidin	FDBR	3.01 ± 0.02^{Ab}	2.08 ± 0.05^{Bb}	1.08 ± 0.02^{Cb}
	FDBSL	19.93 ± 0.02^{Aa}	2.72 ± 0.05^{Ba}	2.79 ± 0.03^{Ba}
Stilbenes				
cis-Resveratrol	FDBR	<LOD	0.15 ± 0.02^{Ab}	0.13 ± 0.05^{Ab}
	FDBSL	5.40 ± 0.03^{A}	5.49 ± 0.05^{Aa}	5.40 ± 0.02^{Aa}
Antioxidant activity (%)				
DPPH	FDBR	64.6 ± 0.1^{Bb}	96.6 ± 0.3^{Aa}	26.0 ± 0.4^{Cb}
	FDBSL	76.7 ± 0.3^{Ba}	88.0 ± 0.2^{Ab}	34.8 ± 0.2^{Ca}
ABTS	FDBR	52.8 ± 0.2^{Ca}	69.1 ± 0.4^{Ab}	54.5 ± 0.2^{Bb}
	FDBSL	61.4 ± 0.3^{Ca}	73.4 ± 0.3^{Aa}	65.3 ± 0.3^{Ba}

<LOD: below the limit of detection. A – C: Different superscript capital letters in the same row indicate a significant difference ($p < 0.05$) between the contents of the same phenolic compound at different steps of the simulated gastrointestinal digestion, based on Tukey's test; a - b: Different superscript lowercase letters in the column denote a significant difference ($p < 0.05$) for the same phenolic compounds in different samples, based on students' *t*-test or Tukey's test.

a decrease in the pH and the production of organic acids, including SCFA (Massa et al., 2020). Particularly, the production of SCFA (namely acetic, propionic, and butyric acids) from fiber degradation and metabolism by beneficial intestinal microorganisms (including lactobacilli) is the main energy source for colonic epithelial cells, besides affecting directly the proliferation of enteric pathogens since the acid accumulation inhibit acid-sensitive pathogens (de Bellis et al., 2021; Suissa et al., 2022).

In addition to producing energy, the oxidation of carbohydrates during fermentation generates lactic and acetic acids as the main end-products, which can be transformed into other organic acids by intestinal bacteria (Suissa et al., 2022). The higher lactic acid contents in media with FDBR inoculated with the examined probiotics at 24 h of cultivation compared to medium with FDBSL probably occurred because of the higher contents of glucose and fructose available in the former. An early study reported increased production of SCFA, especially propionic and butyric acids, during a fecal fermentation of beet leaf extract capsules (Aguirre-Calvo et al., 2020).

The viable cell counts of the probiotic strains correlated positively with the contents of lactic acid, SCFA, and phenolic compounds (namely gallic acid, kaempferol 3-glucoside, and myricetin), while correlated negatively with pH values and glucose contents in media with FDBR and FDBSL. These results could be linked to the ability of the inoculated probiotics to metabolize carbohydrates with the production of organic acids and the reduction of the pH in the cultivation media. Some probiotic lactobacilli can cause the interconversion of phenolic compounds, which could be associated with the alterations in the contents of some phenolic compounds in media with FDBR and FDBSL (Cui & Qu, 2021).

FDBR and FDBSL have phenolic acids (e.g., gallic acid, caftaric acid, and chlorogenic acid) and flavonoids (e.g., kaempferol 3-glucoside, procyanidin B1, procyanidin B2, catechin, epicatechin, and epicatechin gallate) stimulatory to probiotic growth (de Souza et al., 2019; Gong et al., 2020). Some lactobacilli can de-carboxylate, de-esterify, de-methylate, and de-glycosylate phenolic compounds with the production of bioactive derivatives (Li et al., 2019). The decrease in the contents of some phenolic compounds in the cultivation media with FDBR and FDBSL could result from their degradation by the inoculated probiotic strains.

The increase or decrease in the contents of specific phenolic compounds varied with the examined probiotic strain and fermented red beet substrate. Phenolic compounds are converted into specific bioactive metabolites depending on the metabolic pathways used by specific bacterial strains. This conversion is influenced by the substrate composition, which affects the metabolic pathways and biochemical transformations during fermentation (Morais et al., 2019). An early investigation reported alterations in red beet phenolic profile during a spontaneous fermentation, with an overall reduction in the contents of phenolic acids and free flavonoids, and an increase in the contents of free phenolic acids and conjugated flavonoids (Piatosz et al., 2020).

The efficacy of probiotics could be improved when these microorganisms are incorporated into some food matrices since the interactions with food components protect microbial cells as they pass through the gastrointestinal tract (Fiocco et al., 2020; Lillo-Pérez et al., 2021). The maintenance of the viability and the proper release of the probiotic cells through the gastrointestinal tract are important to reach their beneficial effects on the host (Panghal et al., 2019; Sampaio et al., 2021). FDBR and FDBSL provided protection to *L. acidophilus* LA-05, *L. casei* 26, and *L. fermentum* 296 during exposure to the SGIC, with viable cell counts of $\geq$3.5 log CFU/mL after exposure to the intestinal step, which were higher compared to the negative control. Similar results were reported for *L. paracasei* LS14 in soy protein isolate hydrogels and sugar beet pectin (Yan et al., 2021), *L. casei* in coffee kombucha (Bueno et al., 2021), *L. fermentum* 296 in nutraceutical formulations (Sampaio et al., 2021), and *L. fermentum* 139 and *L. fermentum* 263 in apple and orange juices (Rodrigues et al., 2022) exposed to SGIC.

The high acidity in the gastric environment and the high concentration of bile salts in the proximal intestine are the main factors affecting the survival and functionality of probiotics after ingestion (Sampaio et al., 2021). *L. acidophilus* La-05, *L. casei* 26, and *L. fermentum* 296 kept high sizes of cell subpopulations with physiological functionalities during exposure to the SGIC in the presence of FDBR and FDBSL. These cell subpopulations kept their viability with cytoplasmic membrane integrity and presence of functioning enzymatic/metabolic activities up to reaching the intestinal step. It indicates the efficacy of FDBR and FDBSL to protect the tested probiotic strains from cell damage and functionality loss caused by the harsh conditions found in the gastrointestinal tract. The high contents of dietary fibers and phenolic compounds in FDBR and FDBSL could protect probiotic cells from acidic conditions, hydrolytic action of digestive enzymes, antimicrobial and detergent-like properties of bile salts, and osmotic restriction during the gastrointestinal tract passage (Fiocco et al., 2020; Lillo-Pérez et al., 2021).

Relevant sizes of cell subpopulations of the tested probiotics were characterized as having permeabilized cells with enzymatic activity (i.e., injured cells) during the exposure to the SGIC in the presence of FDBR and FDBSL. Cells with these characteristics are called VBNC (viable but non-culturable) (Rodrigues et al., 2022). The damage occurring in these cells makes it impossible to enumerate their colonies in a solid medium, although they still preserve active metabolic characteristics associated with viable cell functioning (Rodrigues et al., 2022; Zhang et al., 2021). It could reinforce the protective effects of FDBR and FDBSL avoiding the inactivation of the probiotic cells since probiotic nonculturable cells could keep several characteristics of living cells and provide health benefits to the host (Howarth & Wang, 2013; Rodrigues et al., 2022).

The antioxidant activity of a food matrix is a consequence of the combined contribution of several antioxidants. Some of these antioxidants, such as phenolic compounds and pigments, are released from the food matrix during the gastrointestinal tract passage. This release occurs due to direct solubilization in the intestinal fluids and the action of digestive enzymes to produce compounds more accessible and available for absorption (Ketnawa et al., 2021). The contents of the phenolic compounds and the antioxidant activity of FDBR and FDBSL were altered during exposure to SGIC. Still, the contents of some phenolic compounds and the antioxidant activity of FDBR and FDBSL correlated positively during the exposure to SGIC.

FDBR and FDBSL had the highest antioxidant activity in the stomach step. The contact of FDBR and FDBSL with an acidic condition and enzymes (e.g., pepsin) in the stomach could degrade the matrix and release some antioxidants, such as phenolic compounds, flavonoids, and betalains, and, consequently, increase the antioxidant activity (Gómez-García et al., 2022; Wang et al., 2020). In turn, the higher pH could be linked to the reduced antioxidant activity of FDBR and FDBSL in the intestine step since phenolic compounds are sensitive to a slightly alkaline environment (Wang et al., 2020). Additionally, the co-digestion of dietary fibers and phenolic compounds causes interaction and association between them in the digestive tract, affecting their bioaccessibility and bioactivities (Jakobek & Matić, 2019, 2019e; Čepo et al., 2020). Early investigations have reported similar results for home-processed red beetroot (Guldiken et al., 2016), beet jam (Wang et al., 2020), encapsulated leaf extract (Aguirre-Calvo et al., 2020), and red beet juice (Desseva et al., 2020).

The maintenance of the antioxidant activity of FDBR and FDBSL during exposure to gastrointestinal conditions could be a result of the interactions between phenolic compounds and betalains, which could protect the gastrointestinal tract from oxidative damage (Wang et al., 2020), as well as decrease the damage caused by the oxidative stress on the probiotic cells when exposed to this environment, helping to keep their viability and functionality (Malik et al., 2019).

5. Conclusion

FDBR and FDBSL have varying contents of insoluble and soluble fibers, pectin, betalains, and phenolic compounds, which could stimulate selectively probiotic lactobacilli. These stimulatory effects resulted in increased probiotic viable cell counts, production of health-related metabolites, and alterations in the contents of phenolic compounds over time. The high viable cell counts and abundance of metabolically active cells of the examined probiotics when exposed to SGIC in the presence of FDBR and FDBSL indicate the efficacy of these materials to protect probiotic microorganisms from the harsh conditions during the gastrointestinal tract passage, helping to keep their viability and functionality upon reaching the intestine to exert their beneficial effects on the host. Changes in the phenolic profile and antioxidant activity of FDBR and FDBSL during the exposure to SGID provide evidence of the interactions between probiotics and bioactive rich matrices, affecting their health-related properties. Both the conventional edible part and the by-products of red beet are sources of ingredients with stimulatory effects on probiotics and possibilities to be exploited in the formulation of functional foods or dietary supplements in the view of a whole valorization of a vegetable with high production and consumption worldwide.

CRediT authorship contribution statement

Conceptualization: SPAO, ELS. Data curation: SPAO, ELS. Formal analysis: SPAO, HMAN, ELS. Funding acquisition: ELS. Investigation: SPAO, HMAN, KBS, MSL, MLC; Methodology: SPAO, NPAR, ELS. Project administration: ELS. Supervision: ELS. Validation: SPAO, ELS. Visualization; Writing – original draft: SPAO, ELS. Writing – review & editing: SPAO, ELS.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors of the paper "**Different parts from the whole red beet (*Beta vulgaris* L.) valorization with stimulatory effects on probiotic lactobacilli and protection against gastrointestinal conditions**" submitted to Food Bioscience 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 thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) for partial funding of this research (Finance code 001), as well as the Universidade Federal da Paraíba (Brazil) for the funding support for this research (Grant number PVG14839-2021).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2023.102439.

References

Abdo, E. M., Shaltout, O. E.-S., Ali, S., & Mansour, H. M. M. (2022). A functional orange juice fortified with beetroot by-products attenuates hyperlipidemia and obesity induced by a high-fat diet. *Antioxidants, 11*(3), 457. https://doi.org/10.3390/antiox11030457

Aguirre-Calvo, T. R., Molino, S., Perullini, M., Rufián-Henares, J.Á., & Santagapita, P. R. (2020). Effects of: *In vitro* digestion-fermentation over global antioxidant response and short chain fatty acid production of beet waste extracts in Ca(ii)-alginate beads. *Food & Function, 11*(12). https://doi.org/10.1039/d0fo02347g

de Albuquerque, T. M. R., Borges, C. W. P., Cavalcanti, M. T., Lima, M. dos S., Magnani, M., & de Souza, E. L. (2020). Potential prebiotic properties of flours from different varieties of sweet potato (*Ipomoea batatas* L.) roots cultivated in Northeastern Brazil. *Food Bioscience, 36*. https://doi.org/10.1016/j.fbio.2020.100614

Alves-Santos, A. M., Sugizaki, C. S. A., Lima, G. C., & Naves, M. M. V. (2020). Prebiotic effect of dietary polyphenols: A systematic review. *Journal of Functional Foods, 74*. https://doi.org/10.1016/j.jff.2020.104169

AOAC. (2016). *Official methods of analysis of AOAC international* (20th ed.). Gaithersburg: AOAC, 2016.

Babarykin, D., Smirnova1, G., Pundinsh, I., Vasiljeva, S., Krumina, G., & Agejchenko, V. (2019). Red beet (*Beta vulgaris*) impact on human health. *Journal of Biosciences and Medicines, 7*, 61–79. https://doi.org/10.4236/jbm.2019.73007

de Bellis, P., Sisto, A., & Lavermicocca, P. (2021). Probiotic bacteria and plant-based matrices: An association with improved health-promoting features. *Journal of Functional Foods, 87*, Article 104821. https://doi.org/10.1016/J.JFF.2021.104821

Bueno, F., Chouljenko, A., & Sathivel, S. (2021). Development of coffee kombucha containing *Lactobacillus rhamnosus* and *Lactobacillus casei*: Gastrointestinal simulations and DNA microbial analysis. *LWT–Food Science and Technology, 142*. https://doi.org/10.1016/j.lwt.2021.110980

Čepo, D. V., Radić, K., Šalov, M., Turčić, P., Anić, D., & Komar, B. (2020). Food (matrix) effects on bioaccessibility and intestinal permeability of major olive antioxidants. *Foods, 9*(12). https://doi.org/10.3390/foods9121831

Chen, L., Zhu, Y., Hu, Z., Wu, S., & Jin, C. (2021). Beetroot as a functional food with huge health benefits: Antioxidant, antitumor, physical function, and chronic metabolomics activity. *Food Sciences and Nutrition, 9*(11). https://doi.org/10.1002/fsn3.2577

Chhikara, N., Kushwaha, K., Jaglan, S., Sharma, P., & Panghal, A. (2019a). Nutritional, physicochemical, and functional quality of beetroot (*Beta vulgaris* L.) incorporated in Asian noodles. *Cereal Chemistry, 96*. https://doi.org/10.1002/cche.10126

Chhikara, N., Kushwaha, K., Sharma, P., Gat, Y., & Panghal, A. (2019b). Bioactive compounds of beetroot and utilization in food processing industry: A critical review. *Food Chemistry, 272*. https://doi.org/10.1016/j.foodchem.2018.08.022

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

Cui, Y., & Qu, X. (2021). Genetic mechanisms of prebiotic carbohydrate metabolism in lactic acid bacteria: Emphasis on *Lacticaseibacillus casei* and *Lacticaseibacillus paracasei* as flexible, diverse and outstanding prebiotic carbohydrate starters. *Trends in Food Science & Technology, 115*, 486–499. https://doi.org/10.1016/J.TIFS.2021.06.058

Desseva, I., Stoyanova, M., Petkova, N., & Mihaylova, D. (2020). Red beetroot juice phytochemicals bioaccessibility: An *in vitro* approach. *Polish Journal of Food and Nutrition Sciences, 70*(1). https://doi.org/10.31883/pjfns/116590

Duarte, F. N. D., Rodrigues, J. B., da Costa Lima, M., Lima, M. dos S., Pacheco, M. T. B., Pintado, M. M. E., de Souza Aquino, J., & de Souza, E. L. (2017). Potential prebiotic properties of cashew apple (*Anacardium occidentale* L.) agro-industrial byproduct on *Lactobacillus* species. *Journal of the Science of Food and Agriculture, 97*(11). https://doi.org/10.1002/jsfa.8232

von Elbe, J. H. (2001). Betalains. *Current Protocols in Food Analytical Chemistry, 1*, 311–317. https://doi.org/10.1002/0471142913.faf0301s00, 00.

Fernandez, M. V., Bengardino, M., Jagus, R. J., & Agüero, M. V. (2020). Enrichment and preservation of a vegetable smoothie with an antioxidant and antimicrobial extract obtained from beet by-products. *LWT - Food Science and Technology, 117*, Article 108622. https://doi.org/10.1016/J.LWT.2019.108622

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

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

Gómez-García, R., Vilas-Boas, A. A., Oliveira, A., Amorim, M., Teixeira, J. A., Pastrana, L., Pintado, M. M., & Campos, D. A. (2022). Impact of simulated human gastrointestinal digestion on the bioactive fraction of upcycled pineapple by-products. *Foods, 11*(1). https://doi.org/10.3390/FOODS11010126

Gómez, B., Gullón, B., Yáñez, R., Schols, H., & Alonso, J. L. (2016). Prebiotic potential of pectins and pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp: A comparative evaluation. *Journal of Functional Foods, 20*, 108–121. https://doi.org/10.1016/j.jff.2015.10.029

Gómez, B., Peláez, C., Martínez-Cuesta, M. C., Parajó, J. C., Alonso, J. L., & Requena, T. (2019). Emerging prebiotics obtained from lemon and sugar beet byproducts: Evaluation of their *in vitro* fermentability by probiotic bacteria. *LWT–Food Science and Technology, 109*. https://doi.org/10.1016/j.lwt.2019.04.008

Gong, X., Li, X., Bo, A., Shi, R. Y., Li, Q. Y., Lei, L. J., Zhang, L., & Li, M. H. (2020). The interactions between gut microbiota and bioactive ingredients of traditional Chinese medicines: A review. *Pharmacological Research, 157*. https://doi.org/10.1016/j.phrs.2020.104824

Guldiken, B., Toydemir, G., Nur Memis, K., Okur, S., Boyacioglu, D., & Capanoglu, E. (2016). Home-processed red beetroot (*Beta vulgaris* L.) products: Changes in antioxidant properties and bioaccessibility. *International Journal of Molecular Sciences, 17*(6). https://doi.org/10.3390/ijms17060858

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

Holck, J., Hjernø, K., Lorentzen, A., Vigsnæs, L. K., Hemmingsen, L., Licht, T. R., Mikkelsen, J. D., & Meyer, A. S. (2011). Tailored enzymatic production of oligosaccharides from sugar beet pectin and evidence of differential effects of a single DP chain length difference on human faecal microbiota composition after *in vitro* fermentation. *Process Biochemistry, 46*(5). https://doi.org/10.1016/j.procbio.2011.01.013

Hotchkiss, A. T., Chau, H. K., Strahan, G. D., Nuñez, A., Simon, S., White, A. K., Dieng, S., Heuberger, E. R., Yadav, M. P., & Hirsch, J. (2022). Structural characterization of red beet fiber and pectin. *Food Hydrocolloids, 129*, Article 107549. https://doi.org/10.1016/J.FOODHYD.2022.107549

Howarth, G. S., & Wang, H. (2013). Role of endogenous microbiota, probiotics and their biological products in human health. *Nutrients, 5*, 58–81. https://doi.org/10.3390/nu5010058

Huang, R., Wu, F., Zhou, Q., Wei, W., Yue, J., Xiao, B., & Luo, Z. (2022). *Lactobacillus* and intestinal diseases: Mechanisms of action and clinical applications. *Microbiological Research, 260*, Article 127019. https://doi.org/10.1016/J.MICRES.2022.127019

Huebner, J., Wehling, R. L., & Hutkins, R. W. (2007). Functional activity of commercial prebiotics. *International Dairy Journal, 17*(7). https://doi.org/10.1016/j.idairyj.2006.10.006

Jakobek, L., & Matić, P. (2019). Non-covalent dietary fiber - polyphenol interactions and their influence on polyphenol bioaccessibility. *Trends in Food Science & Technology, 83*, 235–247. https://doi.org/10.1016/J.TIFS.2018.11.024

Ketnawa, S., Reginio, F. C., Sukanya, T., & Ogawa, Y. (2021). Changes in bioactive compounds and antioxidant activity of plant-based foods by gastrointestinal digestion: A review. *Critical Reviews in Food Science and Nutrition*. https://doi.org/10.1080/10408398.2021.1878100

Lee, S. Y., Lee, S. J., Yim, D. G., & Hur, S. J. (2020). Changes in the content and bioavailability of onion quercetin and grape resveratrol during *in vitro* human digestion. *Foods, 9*(6). https://doi.org/10.3390/foods9060694

Lillo-Pérez, S., Guerra-Valle, M., Orellana-Palma, P., & Petzold, G. (2021). Probiotics in fruit and vegetable matrices: Opportunities for nondairy consumers. *LWT–Food Science and Technology, 151*, Article 112106. https://doi.org/10.1016/J.lwt.2021.112106

Li, Z., Teng, J., Lyu, Y., Hu, X., Zhao, Y., & Wang, M. (2019). Enhanced antioxidant activity for apple juice fermented with *Lactobacillus plantarum* ATCC14917. *Molecules, 24*(1). https://doi.org/10.3390/MOLECULES24010051

Malik, M., Bora, J., & Sharma, V. (2019). Growth studies of potentially probiotic lactic acid bacteria (*Lactobacillus plantarum, Lactobacillus acidophilus*, and *Lactobacillus casei*) in carrot and beetroot juice substrates. *Journal of Food Processing and Preservation, 43*(11). https://doi.org/10.1111/jfpp.14214

Massa, N. M. L., Dantas Duarte Menezes, F. N., de Albuquerque, T. M. R., de Oliveira, S. P. A., Lima, M. dos S., Magnani, M., & de Souza, E. L. (2020). Effects of digested jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg) by-product on growth and metabolism of *Lactobacillus* and *Bifidobacterium* indicate prebiotic properties. *LWT - Food Science and Technology, 131*. https://doi.org/10.1016/j.lwt.2020.109766

Minj, J., Chandra, P., Paul, C., & Sharma, R. K. (2021). Bio-functional properties of probiotic *Lactobacillus*: Current applications and research perspectives. *Critical*

Reviews in Food Science and Nutrition, 61(13). https://doi.org/10.1080/10408398.2020.1774496

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

Moumita, S., & Das, B. (2022). Assessment of the prebiotic potential and bioactive components of common edible mushrooms in India and formulation of synbiotic microcapsules. *LWT–Food Science and Technology, 156*, Article 113050. https://doi.org/10.1016/J.LWT.2021.113050

Nilsson, T. (1970). Studies into the pigments in beetroot (Beta vulgaris L. ssp. vulgaris var. rubra L.). *Lantbrukshogskolans Annaler, 36*.

Okoye, C. O., Dong, K., Wang, Y., Gao, L., Li, X., Wu, Y., & Jiang, J. (2022). Comparative genomics reveals the organic acid biosynthesis metabolic pathways among five lactic acid bacterial species isolated from fermented vegetables. *New Biotech, 70*, 73–83. https://doi.org/10.1016/J.NBT.2022.05.001

de Oliveira, S. P. A., do Nascimento, H. M. A., Sampaio, K. B., & de Souza, E. L. (2020). A review on bioactive compounds of beet (*Beta vulgaris* L. subsp. *vulgaris*) with special emphasis on their beneficial effects on gut microbiota and gastrointestinal health. *Critical Reviews in Food Science and Nutrition, 61*(12). https://doi.org/10.1080/10408398.2020.1768510

Ozcan, T., Ozdemir, T., & Avci, H. R. (2021). Survival of *Lactobacillus casei* and functional characteristics of reduced sugar red beetroot yoghurt with natural sugar substitutes. *International Journal of Dairy Technology, 74*(1). https://doi.org/10.1111/1471-0307.12741

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

Panghal, A., Jaglan, S., Sindhu, N., Anshid, V., Charan, M. V. S., Surendran, V., & Chhikara, N. (2019). Microencapsulation for delivery of probiotic bacteria. In R. Prasad, V. Kumar, M. Kumar, & D. Choudhary (Eds.), *Nanobiotechnology in bioformulations. Nanotechnology in the life sciences*. Cham: Springer. https://doi.org/10.1007/978-3-030-17061-5_6.

Panghal, A., Virkar, K., Dhull, S., Gat, Y., & Chhikara, N. (2017). Development of probiotic beetroot drink. *Current Research in Nutrition and Food Science, 5*(3). https://doi.org/10.12944/CRNFSJ.5.3.10

Płatosz, N., Sawicki, T., & Wiczkowski, W. (2020). Profile of phenolic acids and flavonoids of red beet and its fermentation products. Does long-term consumption of fermented beetroot juice affect phenolics profile in human blood plasma and urine? *Polish Journal of Food and Nutrition Sciences, 70*(1). https://doi.org/10.31883/pjfns/116613

Ranganna, S. (1979). *Manual analysis of fruits and vegetables product*. New Delhi: Tata McGraw Hill Co. Ltd., -10: 0-7-451851-8.

Rasika, D. M. D., Vidanarachchi, J. K., Luiz, S. F., Azeredo, P., Cruz, A. G., & Ranadheera, C. S. (2021). Probiotic delivery through non-dairy plant-based food matrices. *Agriculture, 11*, 599. https://doi.org/10.3390/agriculture11070599

Rodrigues, N. P. A., Garcia, E. F., Sampaio, K. B., do Nascimento, H. M. A., de Sousa Guedes, J. P., & de Souza, E. L. (2022). Dynamics of physiological responses of potentially probiotic fruit-derived *Limosilactobacillus fermentum* in apple and orange juices during refrigeration storage and exposure to simulated gastrointestinal conditions. *Archives of Microbiology, 204*(1). https://doi.org/10.1007/s00203-021-02672-1

Sampaio, K. B., do Nascimento, Y. M., Tavares, J. F., Cavalcanti, M. T., de Brito Alves, J. L., Garcia, E. F., & de Souza, E. L. (2021). Development and *in vitro* evaluation of novel nutraceutical formulations composed of *Limosilactobacillus fermentum*, quercetin and/or resveratrol. *Food Chemistry, 342*, Article 128264. https://doi.org/10.1016/J.FOODCHEM.2020.128264

Sawicki, T., Bączek, N., & Wiczkowski, W. (2016). Betalain profile, content and antioxidant capacity of red beetroot dependent on the genotype and root part. *Journal of Functional Foods, 27*, 249–261. https://doi.org/10.1016/J.JFF.2016.09.004

Šeremet, D., Durgo, K., Jokić, S., Huđek, A., Cebin, A. V., Mandura, A., Jurasović, J., & Komes, D. (2020). Valorization of banana and red beetroot peels: Determination of basic macrocomponent composition, application of novel extraction methodology and assessment of biological activity *in vitro*. *Sustainability, 12*(11). https://doi.org/10.3390/su12114539

Shehata, M. G., Awad, T. S., Asker, D., el Sohaimy, S. A., Abd El- Aziz, N. M., & Youssef, M. M. (2021). Antioxidant and antimicrobial activities and UPLC-ESI-MS/MS polyphenolic profile of sweet orange peel extracts. *Current Research in Food Science, 4*. https://doi.org/10.1016/j.crfs.2021.05.001

de Souza, E. L., de Albuquerque, T. M. R., dos Santos, A. S., Massa, N. M. L., & de Brito Alves, J. L. (2019). Potential interactions among phenolic compounds and probiotics for mutual boosting of their health-promoting properties and food functionalities–A review. *Critical Reviews in Food Science and Nutrition, 59*(10). https://doi.org/10.1080/10408398.2018.1425285

Suissa, R., Oved, R., Jankelowitz, G., Turjeman, S., Koren, O., & Kolodkin-Gal, I. (2022). Molecular genetics for probiotic engineering: Dissecting lactic acid bacteria. *Trends in Microbiology, 30*(3), 293–306. https://doi.org/10.1016/J.TIM.2021.07.007

Takács-Hájos, M., & Vargas-Rubóczki, T. (2022). Evaluation of bioactive compounds in leaf and root of five beetroot varieties. *Journal of Agriculture and Food Research, 7*. https://doi.org/10.1016/j.jafr.2022.100280

Tobaruela, E. C., Santos, A. O., Almeida-Muradian, L. B., Araujo, E. S., Lajolo, F. M., & Menezes, E. W. (2018). Application of dietary fiber method AOAC 2011.25 in fruit and comparison with AOAC 991.43 method. *Food Chemistry, 238*. https://doi.org/10.1016/j.foodchem.2016.12.068

Wang, T., Liu, L., Rakhmanova, A., Wang, X., Shan, Y., Yi, Y., Liu, B., Zhou, Y., & Lü, X. (2020). Stability of bioactive compounds and *in vitro* gastrointestinal digestion of red beetroot jam: Effect of processing and storage. *Food Bioscience, 38*, Article 100788. https://doi.org/10.1016/J.FBIO.2020.100788

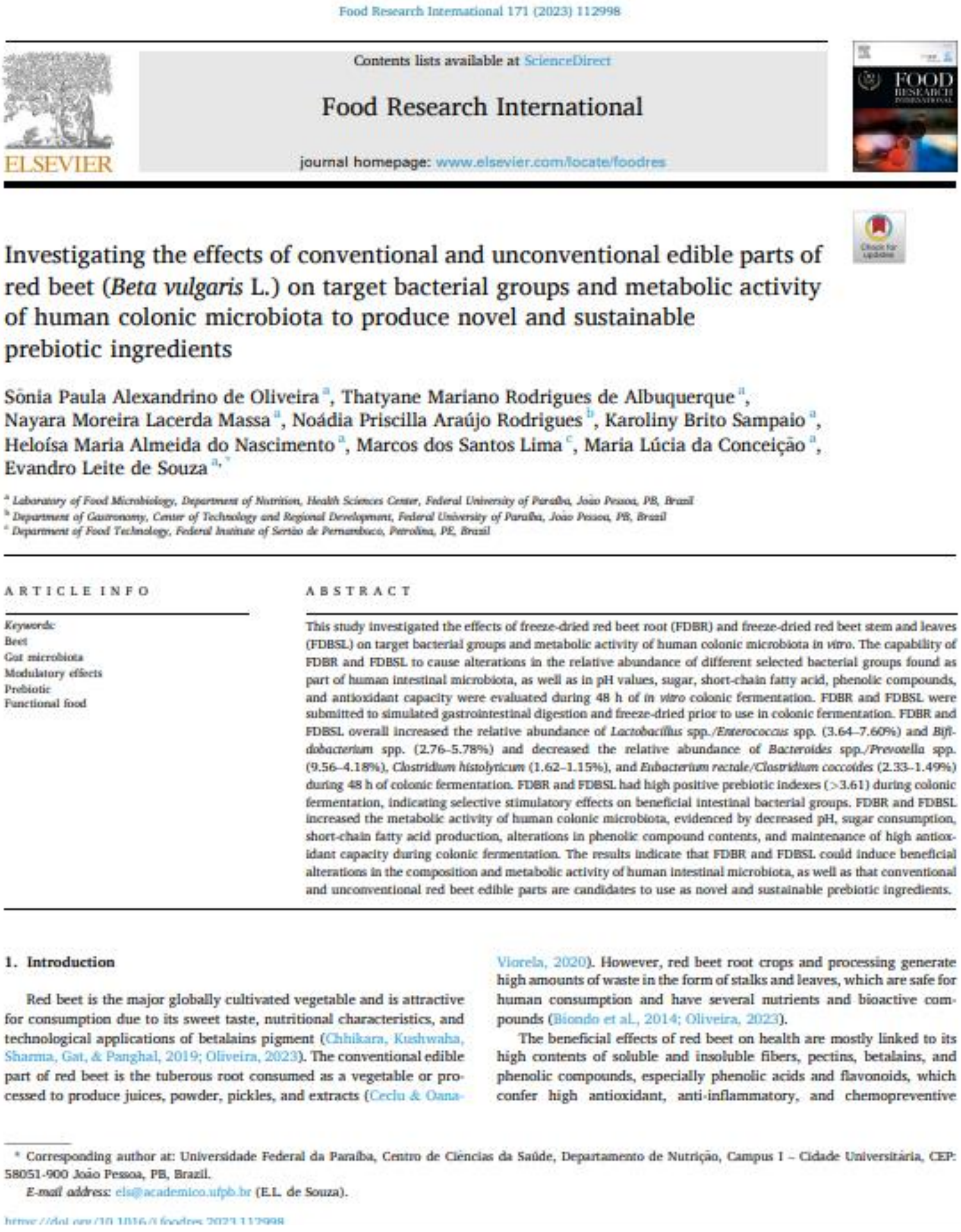
Yan, W., Jia, X., Zhang, Q., Chen, H., Zhu, Q., & Yin, L. (2021). Interpenetrating polymer network hydrogels of soy protein isolate and sugar beet pectin as a potential carrier for probiotics. *Food Hydrocolloids, 113*, Article 106453. https://doi.org/10.1016/J.FOODHYD.2020.106453

Yu, C., Ahmadi, S., Shen, S., Wu, D., Xiao, H., Ding, T., Liu, D., Ye, X., & Chen, S. (2022). Structure and fermentation characteristics of five polysaccharides sequentially extracted from sugar beet pulp by different methods. *Food Hydrocolloids, 126*, Article 107462. https://doi.org/10.1016/J.FOODHYD.2021.107462

Zhang, X. H., Ahmad, W., Zhu, X. Y., Chen, J., & Austin, B. (2021). Viable but nonculturable bacteria and their resuscitation: Implications for cultivating uncultured marine microorganisms. *Marine Life Science and Technology, 3*(2). https://doi.org/10.1007/s42995-020-00041-3

Zhang, N., Jin, M., Wang, K., Zhang, Z., Shah, N. P., & Wei, H. (2022). Functional oligosaccharide fermentation in the gut: Improving intestinal health and its determinant factors-a review. *Carbohydrate Polymers, 284*, Article 119043. https://doi.org/10.1016/J.CARBPOL.2021.119043

APÊNDICE D – ARTIGO ORIGINAL 2 (https://doi.org/10.1016/j.foodres.2023.112998).



Food Research International 171 (2023) 112998

Contents lists available at ScienceDirect

Food Research International

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

Investigating the effects of conventional and unconventional edible parts of red beet (*Beta vulgaris* L.) on target bacterial groups and metabolic activity of human colonic microbiota to produce novel and sustainable prebiotic ingredients

Sônia Paula Alexandrino de Oliveira[a], Thatyane Mariano Rodrigues de Albuquerque[a], Nayara Moreira Lacerda Massa[a], Noádia Priscilla Araújo Rodrigues[b], Karoliny Brito Sampaio[a], Heloísa Maria Almeida do Nascimento[a], Marcos dos Santos Lima[c], Maria Lúcia da Conceição[a], Evandro Leite de Souza[a,*]

[a] *Laboratory of Food Microbiology, Department of Nutrition, Health Sciences Center, Federal University of Paraíba, João Pessoa, PB, Brazil*
[b] *Department of Gastronomy, Center of Technology and Regional Development, Federal University of Paraíba, João Pessoa, PB, Brazil*
[c] *Department of Food Technology, Federal Institute of Sertão de Pernambuco, Petrolina, PE, Brazil*

ARTICLE INFO

Keywords:
Beet
Gut microbiota
Modulatory effects
Prebiotic
Functional food

ABSTRACT

This study investigated the effects of freeze-dried red beet root (FDBR) and freeze-dried red beet stem and leaves (FDBSL) on target bacterial groups and metabolic activity of human colonic microbiota *in vitro*. The capability of FDBR and FDBSL to cause alterations in the relative abundance of different selected bacterial groups found as part of human intestinal microbiota, as well as in pH values, sugar, short-chain fatty acid, phenolic compounds, and antioxidant capacity were evaluated during 48 h of *in vitro* colonic fermentation. FDBR and FDBSL were submitted to simulated gastrointestinal digestion and freeze-dried prior to use in colonic fermentation. FDBR and FDBSL overall increased the relative abundance of *Lactobacillus* spp./*Enterococcus* spp. (3.64–7.60%) and *Bifidobacterium* spp. (2.76–5.78%) and decreased the relative abundance of *Bacteroides* spp./*Prevotella* spp. (9.56–4.18%), *Clostridium histolyticum* (1.62–1.15%), and *Eubacterium rectale*/*Clostridium coccoides* (2.33–1.49%) during 48 h of colonic fermentation. FDBR and FDBSL had high positive prebiotic indexes (>3.61) during colonic fermentation, indicating selective stimulatory effects on beneficial intestinal bacterial groups. FDBR and FDBSL increased the metabolic activity of human colonic microbiota, evidenced by decreased pH, sugar consumption, short-chain fatty acid production, alterations in phenolic compound contents, and maintenance of high antioxidant capacity during colonic fermentation. The results indicate that FDBR and FDBSL could induce beneficial alterations in the composition and metabolic activity of human intestinal microbiota, as well as that conventional and unconventional red beet edible parts are candidates to use as novel and sustainable prebiotic ingredients.

1. Introduction

Red beet is the major globally cultivated vegetable and is attractive for consumption due to its sweet taste, nutritional characteristics, and technological applications of betalains pigment (Chhikara, Kushwaha, Sharma, Gat, & Panghal, 2019; Oliveira, 2023). The conventional edible part of red beet is the tuberous root consumed as a vegetable or processed to produce juices, powder, pickles, and extracts (Ceclu & Oana-Viorela, 2020). However, red beet root crops and processing generate high amounts of waste in the form of stalks and leaves, which are safe for human consumption and have several nutrients and bioactive compounds (Biondo et al., 2014; Oliveira, 2023).

The beneficial effects of red beet on health are mostly linked to its high contents of soluble and insoluble fibers, pectins, betalains, and phenolic compounds, especially phenolic acids and flavonoids, which confer high antioxidant, anti-inflammatory, and chemopreventive

---

[*] Corresponding author at: Universidade Federal da Paraíba, Centro de Ciências da Saúde, Departamento de Nutrição, Campus I – Cidade Universitária, CEP: 58051-900 João Pessoa, PB, Brazil.

E-mail address: els@academico.ufpb.br (E.L. de Souza).

https://doi.org/10.1016/j.foodres.2023.112998

properties. Red beet consumption has been associated with positive impacts on gastrointestinal and cardiovascular systems and endurance exercise performance, offering a source of high-value compounds for the food industry (Babarykin et al., 2019; Domínguez et al., 2020; Oliveira, 2020).

There has been continuing interest in the food industry to identify new natural sources of bioactive components capable of promoting health, besides having potential added-value technological applications (Ghasempour et al., 2020). In this perspective, the investigation of components with prebiotic properties, i.e., substrates selectively utilized by host microorganisms conferring a health benefit (Gibson, Hutkins, Sanders, Prescott, Reimer, Salminen, & Reid, 2017), in complex food matrices as well as in food by-products notably rich in fibers and phenolic compounds has been a research focus (Awasthi et al., 2022; Massa et al., 2022; Silva et al., 2023). Prebiotic substrates traditionally refer to non-digestible carbohydrates. However, other classes of substances, including phenolic compounds, have been shown to exert this function (Gibson et al., 2017; Massa et al., 2022). Recent studies have pointed out the effects of non-digestible fermentable carbohydrates, phenolic acids, and betalains found in red beet root on intestinal microbial populations, emphasizing the possible beneficial impacts of this vegetable on intestinal and systemic health (Capper et al., 2020; Oliveira, 2023; Wang, Do, Marshall, & Boesch, 2023).

Although different experimental protocols have been proposed to investigate the effects of foods or food ingredients as prebiotics on human intestinal microbiota, validated *in vitro* protocols using human feces as inoculum are simpler and reproducible systems, allowing the study of their fermentation and impacts on intestinal microbiota composition and metabolite production (Menezes et al., 2021). Furthermore, the use of pooled human fecal inoculum to ferment these substrates enables the reduction of inter-donor variability by having a more homogeneous and balanced mixture of intestinal microbiota (Aguirre, Ramiro-Garcia, Koenen, & Venema, 2014; de Carvalho, Oliveira, Dib Saleh, Pintado, & Madureira, 2021). The fluorescence *in situ* hybridization coupled to flow cytometry (FISH-FC) technique is based on the use of fluorescent probes designed for specific bacterial groups/species, being successfully used to measure alterations in the abundance of several bacterial groups considered as the main fermenting and representative of the human intestinal microbiota, including *Lactobacillus* spp./*Enterococcus* spp., *Bifidobacterium* spp., *Bacteroides* spp./*Prevotella* spp., *Clostridium histolyticum*, and *Eubacterium rectale*/*Clostridium coccoides* as target bacterial groups (Albuquerque, Magnani, Lima, Castellano, & de Souza, 2021; Macêdo et al., 2023; Massa et al., 2022; Menezes et al., 2021; Medeiros et al., 2021; Sampaio et al., 2022a; Wang et al., 2020), and being recognized as an effective technique to indicate potential impacts of prebiotic candidates on human intestinal microbiota composition (Sampaio, Nascimento, Garcia, & Souza, 2022b).

However, integrative investigations to evaluate the functional properties and the impacts of conventional (root) and unconventional (stem and leaves) edible red beet parts on human intestinal microbiota aligned with a sustainable and circular agro-food perspective are still scarce. Therefore, this study investigated the effects of root and stem and leaves of red beet on the relative abundance of target bacterial groups and metabolic activity of human intestinal microbiota during an *in vitro* colonic fermentation using a pooled human fecal inoculum to envisage their potential use as novel prebiotic ingredients.

2. Materials and methods

2.1. Preparation of red beet samples

Roots, stalks, and leaves samples of red beet (*Beta vulgaris* L., early wonder cultivar) were obtained from three different suppliers (10 samples from each supplier) in local agroecological markets (João Pessoa, PB, Brazil), washed with running potable water and sanitized with sodium hypochlorite solution (150 ppm, 15 min). Roots, stalks, and leaves were processed with a domestic electronic processor (1.2 mm grater disc), frozen (−18 ± 2 °C, 24 h), freeze-dried (−55 ± 2 °C, <138μHg, 1 mm/h, 24 h) with a bench-freeze-dryer (model L-101, Liotop, São Carlos, SP, Brazil), sieved to obtain a powder (particle size < 1 mm), packed in laminated metalized bi-axially oriented polypropylene bags, and stored under refrigeration (4 ± 0.5 °C, maximum period of one month) (Oliveira, 2023; Massa et al., 2022). Freeze-dried red beet root (FDBR) and freeze-dried red beet stalks + freeze-dried red beet leaves (FDBSL; 1:1, w/w) were tested separately in the experiments. The physicochemical characteristics of FDBR and FDBSL examined in this study were previously reported (Oliveira, 2023), standing out for their high contents of soluble and insoluble fibers, betalains, and a variety of phenolic compounds.

2.2. *In vitro* digestion of FDBR and FDBSL

FDBR and FDBSL were submitted (10 g diluted in 50 mL of sterile distilled water) separately to an *in vitro* gastrointestinal digestion using a well-known validated protocol (Albuquerque et al., 2021; Massa et al., 2022; Menezes et al., 2021) simulating the different compartments of the human gastrointestinal tract, i.e., mouth (3.33 mg of α-amylase in 1.04 mL of 10 mM $CaCl_2$, pH 7, 130 rpm, 30 min), stomach (0.45 g of pepsin in 4.16 mL 10 mM HCl, pH 2–2.5, 130 rpm, 120 min), and intestine (0.58 g of bovine bile salts and 0.93 g of pancreatin in 20.8 mL of 0.5 M $NaHCO_3$, pH 6.5–7, 45 rpm, 120 min) to obtain materials like those reaching the human colon. Mechanical orbital agitation simulated the peristaltic movements. The temperature was controlled and adjusted to maintain at human temperature (37 ± 0.5 °C). Alterations in pH were performed using 0.5 M $NaHCO_3$ or 0.01 M HCl.

The suspensions containing the final digestion phase were dialyzed (1 kDa nominal molecular weight cut-off, Spectra/Por 6, Spectrum Europe BV, Breda, Netherlands) with 0.01 mol/L NaCl (5 ± 0.5 °C) to eliminate low molecular mass digestion products. After 15 h, the dialysis fluid was replaced, and the process continued for an additional 2 h. The dialyzed material was frozen (−18 °C), freeze-dried, and stored (5 ± 0.5 °C) in hermetically sealed polyethylene bags for a maximum period of four weeks (Guergoletto, Costabile, Flores, Garcia, & Gibson, 2016). The cellulose dialysis tubing, enzymes, bile salts, and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Fecal sample preparation

Considering the requirements of the National Health Council (Resolution 466, 2012), this study was approved (protocol number 4.251.958) by an institutional ethics committee for research with human beings (Federal University of Paraíba, João Pessoa, PB, Brazil). The fecal samples were obtained from four healthy adult volunteers (two men and two women, 18 to 40 years old) declaring eating an omnivorous diet, having unprecedented large bowel disease, not using concentrated probiotics or prebiotics, and not using antibiotics or any other controlled medication for at least six months prior to collection (Albuquerque et al., 2021).

Fresh fecal samples were collected in sterile tubes arranged in an anaerobic jar containing an anaerobiosis generator system (AnaeroGen, Oxoid, Basingstoke, UK), mixed (1:1:1:1, w/w), diluted (1:10, w/v) using a reduced physiological salt solution (0.5 g/L of cysteine-HCl + 8.5 g/L of NaCl; Sigma-Aldrich), homogenized (2 min, 200 rpm), filtered using sterile triple-layer gauze, and kept (37 ± 1 °C) under anaerobiosis (AnaeroGen) for immediate use in the colonic fermentation (de Andrade, Silva, Costa, Veiga, Costa, Ferreira, & de Gonçalves, 2020; Massa et al., 2022).

2.4. Colonic fermentation system

The colonic fermentation system was composed of 40% (v/v) of the fermentation medium [4.5 g NaCl, 4.5 g KCl, 1.5 g $NaHCO_3$, 0.69 g

$MgSO_4$, 0.8 g L-cysteine, 0.5 g KH_2PO_4, 0.5 g K_2HPO_4, 0.4 g bile salt, 0.08 g $CaCl_2$, 0.005 g $FeSO_4$, 1 mL Tween 80, 4 mL resazurin solution (0.25 g/L, as an anaerobic indicator) and 1 L of distilled water] autoclaved (121 °C, 1 atm, 15 min), 40% (v/v) of the pooled human fecal inoculum, and 20% (v/v) of the digested FDBR or FDBSL in sterile phosphate-buffered saline (0.1 M PBS; pH 7.4) under a density of 0.11 (Menezes et al., 2021). The fermentation system underwent pH adjustment to 6.8 using 1 M $NaHCO_3$ to simulate colonic conditions and was kept (37 ± 1 °C, 48 h) under anaerobiosis (AnaeroGen). Fermentation medium containing the well-known prebiotic fructooligosaccharides (FOS; 20%, w/v) and with no substrate added were included in the experiment as positive and negative controls, respectively (Massa et al., 2022). Ingredients to prepare the fermentation media were purchased from Sigma-Aldrich.

2.5. *Enumeration of target intestinal bacterial populations during colonic fermentation*

An aliquot (375 μL) of each medium collected at zero (immediately after homogenization of the fermentation medium components), 24, and 48 h of colonic fermentation were fixed overnight (4 ± 0.5 °C) with filtered paraformaldehyde (4% w/v, 1.125 μL), centrifuged (10,000g, 5 min, 4 °C), and washed two times with sterile PBS (1 mL, 1 M, pH 7.0). The cell pellet was resuspended in PBS-ethanol 99% mixture (1:1, 300 μL), filtered (0.45 μm pore size filter, Whatman, GE Healthcare, Chicago, IL, USA), and stored (−20 °C) until used for hybridization (Albuquerque et al., 2021; Menezes et al., 2021).

The fluorescent *in situ* hybridization technique was performed for four h using five commercially synthesized oligonucleotide probes (Lab 158, Bif 164, Bac 303, Chis 150, and Erec 482) designed to target specific regions of 16S rRNA gene and labeled with the fluorescent dye Cy3 (Sigma-Aldrich) using hybridization conditions described in Table 1. An aliquot (10 μL) of fixed cells was resuspended in PBS (190 μL, 0.1 M PBS, pH 7.4) and repeated centrifugation, washing, and buffering procedures were performed as previously described (Massa et al., 2022; Albuquerque et al., 2021). The samples were treated with lysozyme (1 mg/mL, 10 min, 25 ± 0.5 °C) under light protection to permeabilize cells for use with probes Lab 158 and Bif 164. SYBR Green staining (Molecular Probes, Invitrogen, Carlsbad, CA, USA) was used to enumerate the total bacterial population (Conterno et al., 2019).

Multiparametric flow cytometry measurements were performed using a flow cytometer (BD Accuri C6, BD Biosciences, East Rutherford, NJ, USA). The fluorescence signals were collected with FL1 (SYBR Green) and FL2 channels (Lab 158, Bif 164, Bac 303, Chis 150, and Erec 482). Sample acquisition was performed at a low flow rate, a threshold level was set for a forward scatter (FSC) of 30,000, and a total of 10,000 events were collected. Channel compensation eliminated false positive results (autofluorescence). Fluorescence emission cytograms were recorded with BD Accuri C6 Software (BD Biosciences). Results were expressed as relative abundance (percentage) of cells hybridized with each bacterial group specific Cy3 probe (fluorescent events) in relation to total bacteria enumerated with SYBR Green staining (Albuquerque et al., 2021; Medeiros et al., 2021).

2.6. *Determination of prebiotic index during colonic fermentation*

After calculating the relative abundance of each measured bacterial group, the prebiotic index of FDBR, FDBSL, FOS, and negative control was calculated using the equation (Albuquerque et al., 2021):

$$\text{Prebiotic index} = \%\text{Lab} + \%\text{Bif} - \%\text{Bac} - \%\text{Chis} - \%\text{Erec} \tag{1}$$

where %Lab is the percentage hybridized for Lab 158 at 24 or 48 h – percentage hybridized at time zero; %Bif is the percentage hybridized for Bif 164 at 24 or 48 h – the percentage hybridized for Bif 164 at time zero; % Bac is the percentage hybridized for Bac at 24 or 48 h – the percentage hybridized for Bac 303 at time zero; %Chis is the percentage hybridized for Chis 150 at 24 or 48 h – the percentage hybridized for Chis 150 at time zero; and %Erec is the percentage hybridized for Erec 482 at 24 or 48 h – the percentage hybridized for Erec 482 at time zero. A positive prebiotic index indicates a beneficial balance of the intestinal microbiota during colonic fermentation induced by the examined substrate, resulting in a potential prebiotic effect. A negative prebiotic index indicates an undesirable modulation of the microbiota by the examined substrate (Albuquerque et al., 2021).

2.7. *Measurements of microbial metabolic activity during colonic fermentation*

The microbiota metabolic activity in media with digested FDBR, FDBSL, FOS (positive control), and negative control (medium with no added fermentable substrate) was evaluated with measurements of pH values and contents of sugars, SCFA (short-chain fatty acids), and phenolic compounds at zero, 24, and 48 h of colonic fermentation. The pH values (method 981.12) were measured with a digital potentiometer (Quimis, Diadema, SP, Brazil) (AOAC, 2016). Contents of sugars (glucose and fructose), SCFA (acetic, propionic, and butyric acids), and phenolic compounds were measured with high-performance liquid chromatography (HPLC) using an Agilent chromatograph (model 1260 Infinity LC, Agilent Technologies, St. Clara, CA, USA) coupled to a diode array and a refractive index detector using analytical conditions previously described (Oliveira, 2023). Data were processed with OpenLAB CDS ChemStation Edition software (Agilent Technologies). The peaks of the measured compounds were identified by comparing their retention times with those of external standards (Sigma Aldrich) according to previously validated methods (Coelho et al., 2018; Padilha et al., 2017). The limit of detection and limit of quantification of the measured compounds are shown in supplementary material data (Table S1).

2.8. *Measurement of antioxidant capacity during colonic fermentation*

The antioxidant capacity in media with FDBR, FDBSL, FOS, and negative control were measured with DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) methods at zero, 24, and 48 h of colonic fermentation. Samples (0.05 mL) were reacted in the dark with DPPH solution in methanol (0.1 mL), shaken vigorously, and left to rest (30 min). Measurements of DPPH scavenging activity were performed at 517 nm. Controls were prepared with water to replace a sample. DPPH radical-scavenging activity (%) was determined using the equation (Brand-Williams, Cuvelier, & Berset, 1995):

$$\text{DPPH radical scavenging activity}(\%) = [(\text{ABScontrol} - \text{ABSsample})/(\text{ABScontrol})] \times 100 \tag{2}$$

where ABScontrol is the absorbance of the DPPH radical + water, and ABSsample is the absorbance of the DPPH radical + tested sample.

The ABTS radical cation (ABTS•+) was generated with the reaction of 5 mL of aqueous ABTS solution (7 mM) + 88 μL of potassium persulfate solution (140 mM). The mixture was kept in the dark (14 h, 28 ± 0.5 °C) before use and diluted with ethanol to reach an absorbance of 0.7 ± 0.02 units at 734 nm. Tested samples (0.05 mL) were allowed to react in the dark with the resulting blue green ABTS radical solution (0.1 mL). Decreases of absorbance at 734 nm were measured after 6 min. The percentage inhibition was calculated using the equation (Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, 1999):

$$\text{ABTS radical scavenging activity}(\%) = [(\text{ABScontrol} - \text{ABSsample})/(\text{ABScontrol})] \times 100 \tag{3}$$

where ABScontrol is the absorbance of ABTS radical + water, and ABSsample is the absorbance of ABTS radical + tested sample.

Table 1
Oligonucleotide probes and hybridization conditions used in analyzes with fluorescent *in situ* hybridization coupled to multiparametric flow cytometry.

Probe	Bacterial group	Genetic sequence	Lysozyme	Temperature
Lab 158	*Lactobacillus* spp./*Enterococcus* spp.	GGTATTAGCAYCTGTTTCCA	+	50 °C
Bif 164	*Bifidobacterium* spp.	CATCCGGCATTACCACCC	+	50 °C
Bac 303	*Bacteroides* spp. /*Prevotella* spp.	CCAATGTGGGGGACCTT	–	45 °C
Chis 150	*Clostridium histolyticum*	TTATGCGGTATTAATCTYCCTTT	–	50 °C
Erec 482	*Clostridium coccoides/ Eubacterium rectale*	GCTTCTTAAGTCARGTACCG	–	50 °C

2.9. *Statistical analysis*

The experiments were performed in triplicate on three independent occasions. The results were expressed as average ± standard deviation. The Kolmogorov-Smirnov normality test was run to check the data normal distribution. Data were submitted to Student's *t*-test or analysis of variance (one-way ANOVA) followed by Tukey's test. Pearson's correlation test assessed the relationship among the relative abundance of *Lactobacillus* spp./*Enterococcus* spp., *Bifidobacterium* spp., *Bacteroides* spp./*Prevotella* spp., *C. histolyticum*, and *E. rectale/C. coccoides* with pH values and SCFA contents. Principal component analysis (PCA) was run with data of the relative abundance of the measured bacterial groups, pH values, SCFA contents, and prebiotic index at three distinct colonic fermentation times (zero, 24, and 48 h). A p-value of <0.05 was considered statistically significant. R software (version 2.15.3, Ross Ihaka and Robert Gentleman, University of Auckland, New Zealand) and GraphPad Prism 7.0 software (GraphPad Software, La Jolla, CA, USA) were used to run the statistical analysis.

3. Results

3.1. *Relative abundance of target bacterial populations during colonic fermentation*

The relative abundances of the measured target human intestinal bacterial groups in media with FDBR, FDBSL, and FOS (positive control), as well as in the negative control during 48 h of colonic fermentation are shown in Fig. 1. Increases ($p \leq 0.05$) in the relative abundance of *Lactobacillus* spp./*Enterococcus* spp. were found in the different fermentation media over time. The highest relative abundance of *Lactobacillus* spp./*Enterococcus* spp. at 24 h was found in medium with FDBSL (7.03 ± 0.25%), followed by medium with FDBR (5.91 ± 0.12%), FOS (4.55 ± 0.11%), and negative control (2.35 ± 0.14%). The highest relative abundance of *Lactobacillus* spp./*Enterococcus* spp. was found at 48 h of fermentation in medium with FDBR (7.60 ± 0.28%) when compared to time zero, which was similar ($p > 0.05$) to the relative abundance found in medium with FOS (7.32 ± 0.19%). The relative abundance of all measured bacterial groups decreased ($p \leq 0.05$) in negative control during colonic fermentation.

The relative abundance of *Bifidobacterium* spp. increased ($p \leq 0.05$) in media with FDBR, FDBSL, and FOS at 24 h of fermentation when compared to time zero, while it increased in medium with FDBR only at 48 h of fermentation. Media with FDBSL and FOS had a two-fold increase in the relative abundance of *Bifidobacterium* spp. at 48 h of fermentation when compared to time zero. The highest relative abundance ($p \leq 0.05$) of *Bifidobacterium* spp. was found in medium with FOS (8.47 ± 0.31%) at 48 h of fermentation, followed by medium with FDBR (5.24 ± 0.27%), FDBSL (5.17 ± 0.14%), and negative control (1.18 ± 0.13%).

The relative abundance of *Bacteroides* spp./*Prevotella* spp. was halved (4.18 ± 0.17%, $p \leq 0.05$) in medium with FDBSL at 24 h of fermentation when compared to time zero, reaching the lowest percentage. The relative abundance of *Bacteroides* spp./*Prevotella* spp. was reduced (4.65 ± 0.13%, $p \leq 0.05$) by more than half in medium with FOS at 48 h of fermentation when compared to time zero, but it did not differ ($p > 0.05$) in relation to medium with FDBSL (4.27 ± 0.18%). The relative abundance ($p \leq 0.05$) of *Bacteroides* spp./*Prevotella* spp. decreased in medium with FDBR (6.62 ± 0.15%) at 48 h of fermentation, as well as in negative control (9.15 ± 0.37%) at 24 h of fermentation.

The relative abundance of *C. histolyticum* did not differ ($p > 0.05$) during fermentation in medium with FDBR (1.40 ± 0.12–1.62 ± 0.16%), while it increased in medium with FDBSL (1.37 ± 0.19%) and decrease in medium with FOS (1.06 ± 0.12%) ($p \leq 0.05$), which had the lowest relative abundance at 24 h of fermentation. Negative control had the highest reduction in relative abundance of *C. histolyticum* during fermentation, reaching the lowest relative abundance (0.15 ± 0.08%) at 24 and 48 h when compared to time zero.

The relative abundance of *E. rectale/C. coccoides* decreased ($p \leq 0.05$) in medium with FDBR (1.60 ± 0.12%) and negative control (2.12 ± 0.11%) at 24 h of fermentation, while increased ($p \leq 0.05$) in medium with FOS (3.88 ± 0.16%). The relative abundance of *E. rectale/C. coccoides* increased in medium with FDBSL (3.77 ± 0.17%) during colonic fermentation. The relative abundance of *E. rectale/C. coccoides* decreased ($p \leq 0.05$) in media with FDBR and FOS as well as in negative control at 48 h of fermentation, with the lowest ($p \leq 0.05$) relative abundance being found in medium with FDBR (1.49 ± 0.13%) and negative control (0.80 ± 0.1%).

3.2. *Measurements of the prebiotic index during colonic fermentation*

Prebiotic indexes calculated for fermentation media with FDBR, FDBSL, and FOS, as well as in the negative control at 24 and 48 h of colonic fermentation are shown in Table 2. Media with FDBR, FDBSL, and FOS had positive prebiotic indexes at 24 and 48 h of fermentation, while negative control had negative prebiotic indexes. The highest prebiotic indexes were found for media with FDBSL (7.92 ± 0.23) and FOS (7.94 ± 0.41) at 24 h of fermentation. Medium with FOS (14.81 ± 1.62) had a higher prebiotic index ($p \leq 0.05$) than media with FDBR (7.68 ± 0.25) and FDBSL (5.82 ± 0.32) at 48 h of fermentation.

3.3. *Measurements of metabolic activity during colonic fermentation*

Media with FDBR, FDBSL, and FOS had a decrease ($p \leq 0.05$) in pH values during 48 h of colonic fermentation, while negative control had no change in pH value ($p < 0.05$) (Table 3). The lowest pH value ($p \leq 0.05$) at 24 h of fermentation was found in medium with FOS (3.92 ± 0.11), followed by media with FDBR (4.99 ± 0.11) and FDBSL (5.95 ± 0.17). pH values were higher ($p \leq 0.05$) in media with FDBR (4.66 ± 0.15) and FDBSL (5.57 ± 0.13) than in medium with FOS (3.70 ± 0.15) at 48 h of fermentation. The highest ($p \leq 0.05$) pH value at 48 h of fermentation was found in the negative control (6.79 ± 0.22).

Glucose content decreased ($p \leq 0.05$) during 48 h of colonic fermentation in media with FDBR (0.79 ± 0.08 g/L) and FDBSL (0.04 ± 0.01 g/L), while it was not detected in medium with FOS (<LOD) (Table 3). Fructose content was higher ($p \leq 0.05$) during fermentation in medium with FOS (12.20 ± 0.21 g/L) than in media with FDBR (5.02 ± 0.16 g/L) and FDBSL (<LOD). Negative control had no change ($p > 0.05$) in glucose and fructose contents during fermentation.

Acetic acid content increased ($p \leq 0.05$) overall during 48 h of colonic fermentation, with the highest contents being found in medium

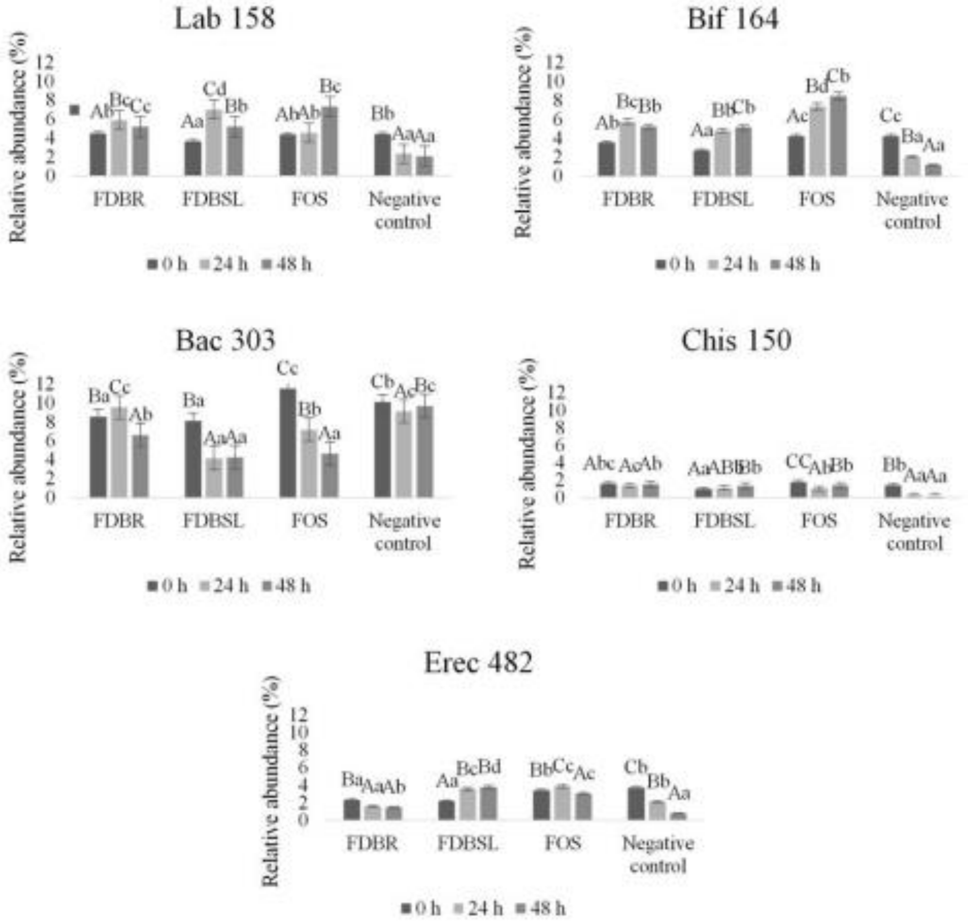


Fig. 1. Relative abundance (% average ± standard deviation; n = 3) of different bacterial groups in media with digested freeze-dried red beet root (FDBR), stems and leaves (FDBSL), and fructooligosaccharides (FOS), as well as in the negative control (without fermentable substrate) at zero, 24, and 48 h of colonic fermentation. Lab 158: *Lactobacillus* spp./*Enterococcus* spp.; Bif 164: *Bifidobacterium* spp.; Bac 303: *Bacteroides* spp./*Prevotella* spp.; Chis 150: *Clostridium histolyticum*); Erec 482: *Clostridium coccoides*/*Eubacterium rectale*. A–C: Different superscript capital letters for the same fermentation medium and bacterial group at different fermentation periods denote differences ($p \leq 0.05$), based on Tukey's test; a–d: Different superscript small letters at the same fermentation period and bacterial group at different fermentation media denote difference ($p \leq 0.05$), based on Tukey's test.

with FDBSL (0.99 ± 0.11 g/L), followed by media with FDBR (0.62 ± 0.03 g/L) and FOS (0.58 ± 0.04 g/L) (Table 3). Propionic acid content did not change ($p > 0.05$) in the different media at 24 h of fermentation, except for a decrease in negative control. Propionic acid contents decreased in media with FDBR and FDBSL at 48 h of fermentation. Media with FDBR (1.63 ± 0.13 g/L) and FOS (1.85 ± 0.16) had similar propionic acid contents ($p > 0.05$) at 48 h of fermentation. Propionic acid content decreased ($p \leq 0.05$) in negative control over time. Butyric acid content decreased ($p \leq 0.05$) in media with FDBR and FDBSL at 24 h of fermentation, while increased ($p \leq 0.05$) in medium with FOS. The highest ($p \leq 0.05$) butyric acid content at 48 h of fermentation was found in medium with FDBR (0.42 ± 0.03 g/L), while butyric acid content did not differ ($p > 0.05$) in media with FDBSL (0.35 ± 0.02 g/L) and FOS (0.36 ± 0.02 g/L). No alteration in acetic and butyric acid content was found in negative control during fermentation.

3.4. Changes in phenolic compound contents and antioxidant capacity during colonic fermentation

High contents of catechin (5.10 ± 0.18 mg/L), procyanidin A2 (1.21 ± 0.14 mg/L), and procyanidin B2 (6.90 ± 0.14 mg/L) were found in medium with FDBR during 48 h of colonic fermentation, while high contents of procyanidin A2 (3.73 ± 0.22 mg/L), procyanidin B2 (1.47 ± 0.16 mg/L), kaempferol 3-glucoside (3.25 ± 0.21 mg/L), and *cis*-resveratrol (1.10 ± 0.04 mg/L) were found in medium with FDBSL (Table 4). The contents of catechin and procyanidin A2 increased in media with FDBR and FDBSL during fermentation, while the contents of caftaric acid, procyanidin B1, procyanidin B2, kaempferol 3-glucoside, and quercitin 3-glucoside decreased.

The media with FDBR and FDBSL kept high antioxidant capacities during 48 h of colonic fermentation (Table 4). The highest ($p \leq 0.05$) antioxidant capacity in medium with FDBR was found at 24 h of

Table 2
Prebiotic index (mean ± standard deviation; n = 3) calculated for media with digested freeze-dried red beet root (FDBR), stems and leaves (FDBSL), and fructooligosaccharides (FOS), as well as in the negative control (without fermentable substrate) at 24 and 48 h of colonic fermentation.

Fermentation medium	Prebiotic index	
	24 h	48 h
FDBR	3.61 ± 0.12^{Ab}	7.68 ± 0.25^{Bc}
FDBSL	7.92 ± 0.23^{Bc}	5.82 ± 0.32^{Ab}
FOS	7.94 ± 0.41^{Ac}	14.81 ± 1.62^{Bd}
Negative control	-0.54 ± 0.08^{Aa}	-0.78 ± 0.17^{Ba}

A – B: Different superscript capital letters in the same row for the same fermentation media denote differences ($p \leq 0.05$), based on Student's *t* test; a-d: different superscript small letters in the same column at the same time interval denote difference ($p \leq 0.05$), based on Tukey's test.

Table 3
pH values and contents of sugars and short chain fatty acid (SCFA; g/L; average ± standard deviation; n = 3) in media with digested freeze-dried red beet root (FDBR), stems and leaves (FDBSL), and fructooligosaccharides (FOS), as well as in the negative control (without fermentable substrate) at zero, 24, and 48 h of colonic fermentation.

Parameter	Fermentation medium	Time of fermentation		
		0 h	24 h	48 h
pH values				
pH	FDBR	6.90 ± 0.12^{Ca}	4.99 ± 0.11^{Bb}	4.66 ± 0.15^{Ab}
	FDBSL	6.90 ± 0.16^{Ca}	5.95 ± 0.17^{Bc}	5.57 ± 0.13^{Ac}
	FOS	6.80 ± 0.10^{Ba}	3.92 ± 0.11^{Aa}	3.70 ± 0.15^{Aa}
	Negative control	6.91 ± 0.16^{Aa}	6.86 ± 0.18^{Ad}	6.79 ± 0.22^{Ad}
Sugars				
Glucose	FDBR	7.95 ± 0.25^{Cb}	2.75 ± 0.14^{Bc}	0.79 ± 0.08^{Ac}
	FDBSL	0.13 ± 0.02^{Ba}	0.05 ± 0.01^{Aa}	0.04 ± 0.01^{Aa}
	FOS	<LOD	<LOD	<LOD
	Negative control	0.17 ± 0.03^{Aa}	0.15 ± 0.02^{Ab}	0.19 ± 0.03^{Ab}
Fructose	FDBR	7.66 ± 0.23^{Cb}	6.59 ± 0.01^{Bb}	5.02 ± 0.16^{Aa}
	FDBSL	0.70 ± 0.09^{Ca}	0.35 ± 0.05^{Ba}	<LOD
	FOS	10.49 ± 0.25^{Ac}	11.69 ± 0.22^{Bc}	12.20 ± 0.21^{Cb}
	Negative control	<LOD	<LOD	<LOD
SCFA				
Acetic acid	FDBR	0.52 ± 0.05^{Ab}	0.52 ± 0.03^{Ac}	0.62 ± 0.03^{Bb}
	FDBSL	0.68 ± 0.03^{Ac}	0.61 ± 0.05^{Ad}	0.99 ± 0.11^{Bc}
	FOS	0.36 ± 0.04^{Aa}	0.45 ± 0.02^{Bb}	0.58 ± 0.04^{Cb}
	Negative control	0.30 ± 0.02^{Aa}	0.35 ± 0.07^{ABa}	0.39 ± 0.06^{Ba}
Propionic acid	FDBR	3.21 ± 0.16^{Bd}	3.13 ± 0.20^{Bd}	1.63 ± 0.13^{Ab}
	FDBSL	2.79 ± 0.13^{Bc}	2.72 ± 0.18^{Bc}	0.75 ± 0.08^{Aa}
	FOS	1.46 ± 0.11^{Ab}	1.50 ± 0.12^{Ab}	1.85 ± 0.16^{Bb}
	Negative control	1.34 ± 0.15^{Ca}	0.82 ± 0.04^{Ba}	0.67 ± 0.03^{Aa}
Butyric acid	FDBR	0.55 ± 0.06^{Cc}	0.24 ± 0.01^{Ab}	0.42 ± 0.03^{Bc}
	FDBSL	0.34 ± 0.02^{Bb}	0.24 ± 0.02^{Ab}	0.35 ± 0.02^{Bb}
	FOS	0.23 ± 0.01^{Aa}	0.28 ± 0.03^{Bb}	0.36 ± 0.02^{Cb}
	Negative control	0.21 ± 0.02^{Aa}	0.20 ± 0.01^{Aa}	0.23 ± 0.01^{Aa}

<LOD: below the limit of detection. A–C: Different superscript capital letters in the same row for the same fermentation medium denote differences ($p \leq 0.05$), based on Tukey's test; a–d: different superscript small letters in the same column at a same time interval and measured parameter denote difference ($p \leq 0.05$), based on Tukey's test.

fermentation when measured by either DPPH (93.81 ± 1.21%) or ABTS (82.60 ± 1.15%) method. The highest ($p \leq 0.05$) antioxidant capacity in medium with FDBSL was found at 24 h of fermentation when measured by ABTS method (79.70 ± 1.11%) and at 48 h of fermentation when measured by DPPH method (90.70 ± 1.76%).

3.5. Chemometric analysis

The PCA results located the media with FDBR, FDBSL, and FOS at 24 and 48 h of colonic fermentation in the upper quadrants with the higher relative abundance of *Lactobacillus* spp./*Enterococcus* spp. and *Bifidobacterium* spp., higher SCFA content, and higher antioxidant capacity (DPPH and ABTS methods) (Fig. 2). The negative control at time zero, 24, and 48 h of colonic fermentation was located at the lower quadrants with lower results.

Pearson's correlation test showed that relative abundance of *Lactobacillus* spp./*Enterococcus* spp. and *Bifidobacterium* spp. correlated positively ($p < 0.001$) with prebiotic index, SCFA content, and antioxidant capacity (DPPH and ABTS methods), while correlated negatively with pH value (Fig. 3). Antioxidant capacity correlated positively ($p < 0.001$) with SCFA content, while prebiotic index correlated negatively ($p < 0.001$) with pH value and SCFA content.

4. Discussion

The examined conventional and non-conventional edible red beet parts induced an increase in the relative abundance of *Lactobacillus* spp./*Enterococcus* spp. and *Bifidobacterium* spp. populations during colonic fermentation, indicating the availability of components (*e.g.*, insoluble and soluble fibers, pectin, and phenolic compounds) capable of reaching

Table 4

Contents of phenolic compounds (mg/L) and antioxidant capacity (%) (average ± standard deviation; n = 3) in media with digested freeze-dried red beet root (FDBR), stems and leaves (FDBSL), and fructooligosaccharides (FOS), as well as in the negative control (without fermentable substrate) at zero, 24 and 48 h of colonic fermentation.

Phenolic compound (mg/L)	Fermentation medium					
	FDBR			FDBSL		
	0 h	24 h	48 h	0 h	24 h	48 h
Phenolic acids						
Caftaric acid	0.43 ± 0.06^{Bc}	0.20 ± 0.02^{Ab}	0.21 ± 0.03^{Aa}	0.29 ± 0.04^{Cb}	0.18 ± 0.02^{B}	0.09 ± 0.01^{A}
Gallic acid	0.26 ± 0.05^{b}	<LOD	<LOD	0.25 ± 0.04^{ab}	<LOD	<LOD
Syringic acid	0.12 ± 0.03^{Aa}	0.12 ± 0.04^{Aa}	0.10 ± 0.02^{Aa}	0.20 ± 0.03^{a}	<LOD	<LOD
Flavanols						
Catechin	0.41 ± 0.05^{Ab}	1.07 ± 0.12^{Bd}	5.10 ± 0.18^{Ce}	0.81 ± 0.06^{Bc}	0.76 ± 0.04^{Bc}	0.64 ± 0.03^{Ad}
Epigallocatechin gallate	<LOD	<LOD	<LOD	<LOD	0.06 ± 0.01^{Aa}	0.09 ± 0.02^{Ab}
Epicatechin	0.12 ± 0.03^{Aa}	0.15 ± 0.01^{Aa}	0.24 ± 0.04^{Bb}	0.23 ± 0.01^{Aa}	0.13 ± 0.02^{Ab}	<LOD
Epicatechin gallate	0.37 ± 0.04^{Bb}	0.20 ± 0.03^{Ab}	<LOD	0.40 ± 0.02^{Ab}	<LOD	0.37 ± 0.03^{Ac}
Procyanidin A2	0.72 ± 0.06^{Ac}	0.73 ± 0.08^{Ac}	1.21 ± 0.14^{Bc}	0.76 ± 0.04^{Ac}	3.66 ± 0.25^{Be}	3.73 ± 0.22^{Be}
Procyanidin B1	0.12 ± 0.03^{Aa}	0.12 ± 0.02^{Aa}	0.10 ± 0.01^{Aa}	0.20 ± 0.03^{Ca}	0.07 ± 0.01^{Ba}	0.04 ± 0.01^{Aa}
Procyanidin B2	11.94 ± 0.22^{Cd}	6.90 ± 0.14^{Be}	2.11 ± 0.19^{Ad}	16.16 ± 0.32^{Bd}	1.47 ± 0.16^{Ad}	<LOD
Flavanones						
Hesperidin	<LOD	<LOD	<LOD	<LOD	0.52 ± 0.04^{A}	0.69 ± 0.04^{B}
Flavonols						
Kaempferol 3-glucoside	<LOD	<LOD	<LOD	<LOD	3.25 ± 0.21^{Bb}	1.87 ± 0.16^{Ab}
Quercitin 3-glucoside	0.12 ± 0.03	<LOD	<LOD	<LOD	0.16 ± 0.04^{Aa}	0.14 ± 0.02^{Aa}
Rutin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Stilbenes						
Cis-Resveratrol	0.47 ± 0.04	<LOD	<LOD	0.57 ± 0.04^{A}	1.10 ± 0.04^{B}	0.56 ± 0.04^{A}
Antioxidant activity (%)						
DPPH	73.50 ± 1.36^{Ab}	93.81 ± 1.21^{Cb}	89.07 ± 2.23^{Bb}	74.90 ± 1.45^{Aa}	83.50 ± 2.08^{Bb}	90.70 ± 1.76^{Cb}
ABTS	70.90 ± 1.16^{Ba}	82.60 ± 1.15^{Ca}	60.00 ± 1.03^{Aa}	81.10 ± 1.31^{Bb}	79.70 ± 1.11^{Ba}	63.20 ± 1.08^{Aa}

<LOD: below the limit of detection. A–C: different superscript capital letters in the same row for the same fermentation medium and phenolic compound or antioxidant capacity denote differences ($p < 0.05$), based on Tukey's test or Student's *t* test; a–e: different superscript small letters in the same column at a same time interval and class of phenolic compounds or antioxidant activity denote difference ($p < 0.05$), based on Tukey's test or Student's *t* test.

the colon to be fermented by intestinal microbiota (Oliveira, 2023; Zhang et al., 2022). Pectic polysaccharides from sugar beet pulp are promising prebiotic candidates due to their reported capability of modulating the intestinal microbiota (Gómez et al., 2019; Prandi et al., 2018; Yu et al., 2022). In addition to non-digestible carbohydrates, phenolic compounds have shown growing evidence of their stimulatory properties on intestinal microbiota linked to prebiotic effects (Alves-Santos, Sugizaki, Lima, & Naves, 2020; Massa et al., 2022; de Souza, de Albuquerque, dos Santos, Massa, & de Brito Alves, 2018).

Bacteroides spp./*Prevotella* spp. was the bacterial group with the highest population during colonic fermentation regardless of the fermentable substrate in the medium. These genera are part of the phylum Bacteroidetes that together with Firmicutes comprise about 90% of the adult human intestinal microbiota (Gómez et al., 2014; Nagpal et al., 2018). *Bacteroides* spp./*Prevotella* spp. degrade complex carbohydrates, such as pectin found in red beet parts, producing acetic acid and mainly propionic acid (Diotallevi et al., 2021). Alterations in the relative abundance of *Bacteroides* spp./*Prevotella* spp. were induced by FDBR and FDBSL, with a decrease at 48 h of colonic fermentation. It could be considered a positive effect since *Bacteroides* spp./*Prevotella* spp. are commonly reported as opportunistic microorganisms and the increase in their populations has been typically linked to undesirable effects on colon health, intestinal dysbiosis, and metabolic disorders (Iljazovic, Amend, Galvez, Oliveira, & Strowing, 2021).

In general, Clostridia is a class of bacteria considered harmful to health (Liu, Kolida, Charalampopoulos, & Rastall, 2020). Decrease in the relative abundance of *C. histolyticum* and *E. rectale*/*C. coccoides* in medium with FDBR demonstrates that red beet root is an ingredient selectively fermented by intestinal microbiota. This could be justified by the higher amounts of specific phenolic compounds (*e.g.*, catechin and procyanidin B2) and betalains in FDBR compared to FDBSL, as well as by the lowering pH effect in the colonic fermentation medium, which are limiting factors for pathogenic bacteria growth (Molinari, Merendino, & Costantini, 2021; Oliveira Filho et al., 2022; Oliveira, 2023). Although the increases in *C. histolyticum* and *E. rectale*/*C. coccoides* populations induced by FDBSL are undesirable, the positive prebiotic index found for FDBSL indicates an overlap of beneficial bacteria rather than pathogenic bacteria during colonic fermentation (Albuquerque et al., 2021; Massa et al., 2022).

The prebiotic index has emerged as a valuable tool to putatively indicate the potential prebiotic properties of different foods (Albuquerque et al., 2021; Medeiros et al., 2021; Owolabi, Dat-arun, Yupanqui, & Wichienchot, 2020). Positive prebiotic indexes were found for FDBR and FDBSL during colonic fermentation, which agrees with previous *in vitro* investigations showing that different anatomical red beet parts stimulated selectively the growth of probiotics to the detriment of enteric competitors (Oliveira, 2023). FDBSL had a prebiotic index like FOS at 24 h of colonic fermentation, although having a lower positive prebiotic index than FDBR and FOS at 48 h of colonic fermentation. The positive prebiotic indexes in media with FDBSL and FDBR could be linked to the most significant relative abundance of *Lactobacillus* spp./*Enterococcus* spp. and *Bifidobacterium* found therein, besides the lowest

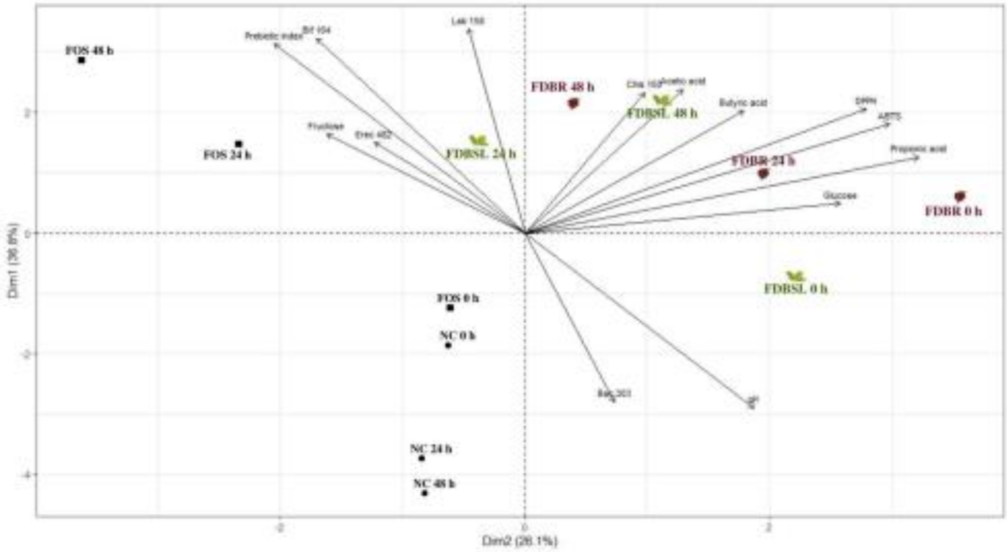


Fig. 2. Principal Component Analysis (PCA) run for media with different red beet parts (FDBR and FDBSL), and fructooligosaccharides (FOS), as well as in negative control (no added fermentable substrate) at zero, 24, and 48 h of colonic fermentation (variables: relative abundance of distinct bacterial groups, contents of sugars and short chain fatty acids, pH values, prebiotic index, and antioxidant capacity).

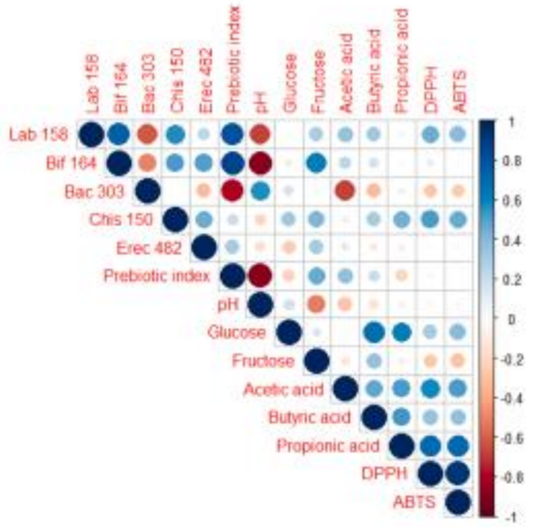


Fig. 3. Correlation coefficients indicate the associations among the relative abundance of distinct bacterial groups, contents of sugars and short chain fatty acids, and prebiotic index values in media with different red beet parts (FDBR and FDBSL) and fructooligosaccharides (FOS), as well as in the negative control (no added fermentable substrate) at zero, 24, and 48 h of colonic fermentation. The white cell represents no significant correlation.

relative abundance of *Bacteroides* spp./*Prevotella* spp., *C. histolyticum*, and *E. rectale/C. coccoides* (Medeiros et al., 2021). *Lactobacillus* and *Bifidobacterium* species typically exert antagonist action against potentially pathogenic bacteria due to SCFA and bacteriocin production, besides competing for nutrients (Alves-Santos et al., 2023; Vieco-Saiz et al., 2019). These results could still be related to the presence of betalains and phenolic compounds in FDBR and FDBSL with reported antimicrobial effects against pathogens (Oliveira Filho et al., 2022; Sadowska-Bartosz & Bartosz, 2021), as well as of insoluble and soluble fibers, pectin, and phenolic compounds that reach the colon as a substrate selectively fermentable by beneficial microorganisms forming the human intestinal microbiota (Gómez et al., 2019; Massa et al., 2022). *Lactobacillus* spp./*Enterococcus* spp. and *Bifidobacterium* spp. are considered the major microbial targets for prebiotic action due to their beneficial effects on intestinal health with systemic outcomes (Gibson et al., 2017; Sanders, Merenstein, Reid, Gibson, & Rastall, 2019).

Glucose and fructose were metabolized by intestinal microbiota in media with FDBR and FDBSL to produce SCFA and decrease the pH values during colonic fermentation (Menezes et al., 2021), as reinforced by Pearson's correlation results showing a negative correlation between pH values and relative abundance of *Lactobacillus* spp./*Enterococcus* spp., *Bifidobacterium* spp., and prebiotic index. FDBR induced a lower pH value at 48 h of colonic fermentation than FDBSL, indicating a faster metabolization of nutrients present in red beet root (Albuquerque et al., 2021), besides being a possible condition to cause the lowest *E. rectale/C. coccoides* relative abundance in medium with FDBR during colonic fermentation.

Many beneficial effects induced by intestinal microbiota modulation are associated with SCFA production (Gómez et al., 2019). The approximate SCFA proportions in the large intestine are acetic acid (65%), propionic acid (25%), and butyric acid (15%) (Tingirikari, 2018), although acetic acid could be utilized by different microorganisms to produce propionic and butyric acids (Hosseini, Grootaert, Verstraete, & Van de Wiele, 2011; Riviere, Selak, Lantin, Leroy, & De Vuyst, 2016). The highest production of acetic acid occurred in media with FDBR and FDBSL, which is associated with cholesterol metabolism, lipogenesis, and hormonal appetite regulation in humans (Rowland et al., 2018). *Lactobacillus* and *Bifidobacterium* genera produce acetic acid as one of the main metabolic end products, which when produced *in situ* can lower luminal pH and suppress pathogen growth (Sanders et al., 2019; Yu et al., 2022). These results agree with Pearson's correlation test where the SCFA content correlated positively with the relative abundance of *Lactobacillus* spp./*Enterococcus* spp. and *Bifidobacterium* spp.

Propionic acid plays an important role in energy metabolism homeostasis, especially stimulating gluconeogenesis that acts as a satiety signal (De Vadder et al., 2014). Considering the contents of the measured SCFA contents during colonic fermentation, there were far more propionic acid-producing bacteria in the tested fecal inoculum than those producing acetic and butyric acids, corroborating with the detected high relative abundance of *Bacteroides* spp./*Prevotella* spp. This indicates the predominance of *Prevotella* genus since *Prevotella*-dominant cultures produce substantially higher ratios of propionic acid to acetic and butyric acids than *Bacteroides*-dominant cultures (Hosseini et al., 2011; Sanders et al., 2019). However, propionic acid decreased overall in media with FDBR and FDBSL during colonic fermentation, agreeing with early studies showing that the presence of pectin causes a decrease in propionic acid during colonic fermentation (Bang et al., 2018; Ferreira-Lazarte, Kachrimanidou, Villamiel, Rastall, & Moreno, 2018; Yang, Martínez, Walter, Keshavarzian, & Rose, 2013).

Butyric acid is very important to human health, attracting attention despite of its lowest ratio in the large intestine (Yu et al., 2022). Besides causing beneficial effects on carcinogenesis, inflammation, and oxidative stress, butyric acid is known to affect several components of the colonic defense barrier, resulting in enhanced protection against luminal antigens (Rowland et al., 2018). Variations in butyric acid contents during FDBR and FDBSL colonic fermentation could be linked to the relative abundance of *E. rectale* spp./*C. coccoides* spp. since *E. rectale* spp. is of special interest due to its ability to produce butyric acid (Gómez, Gullón, Yáñez, Schols, & Alonso, 2016).

The decrease of phenolic compounds during colonic fermentation indicates their metabolization by the intestinal microbiota and potential prebiotic effects (Macêdo et al., 2023; Sampaio et al., 2022a). Metabolites derived from colonic fermentation of carbohydrates and associated phenolic compounds have been related to beneficial health effects (Tabernero & Cedrón, 2017). There was an increase in procyanidin A2 content during FDBR and FDBSL colonic fermentation and a sharp decrease in procyanidin B2 content. These results could be associated with the alterations in catechin and epigallocatechin contents since procyanidins are oligomers and polymers of (+)-catechins, (-)-epicatechins, and their derivatives (Masumoto, Aoki, Miura, & Shoji, 2018). The differences in the physiological activities of procyanidins A2 and B2 are probably related to their different microbial metabolic pathways and resultant phenolic and aromatic catabolites (Chen et al., 2021).

An early study reported high antioxidant capacity in FDBR and FDBSL, which decreased after reaching the intestine in a simulated gastrointestinal digestion model (Oliveira, 2023). However, the antioxidant capacity of media with FDBR and FDBSL increased overall during the 48 h of colonic fermentation when measured by DPPH method, while decreased at 48 h of colonic fermentation when measured by ABTS method. These distinct trends in the scavenging abilities of the two free radicals could be due to the difference in chemical properties of the compounds, principles, and reaction conditions (Fan et al., 2022). Considering the different types of free radicals and their different action modes there is no simple and universal method by which the antioxidant capacity can be accurately and quantitatively determined. However, ABTS and DPPH method are reported as fast and efficient method to measure *in vitro* the antioxidant capacity of foods rich in phenolic compounds and betalains (Sampaio et al., 2022a; Oliveira, 2023). The high antioxidant capacities in media with FDBR and FDBSL could be due to the presence of phenolic compounds, which in the large intestine protect cells against oxidative stress, preventing colon cancer, and favoring beneficial bacteria growth (Fidelis et al., 2021; Burgos-Edwards, Jiménez-Aspee, Theoduloz, & Schmeda-Hirschmann, 2018). It was reinforced by the positive correlation among the antioxidant capacity and the relative abundance of *Lactobacillus* spp./*Enterococcus* spp. and *Bifidobacterium* spp.

The results correlating the relative abundance of the different measured bacterial groups, pH values, sugar consumption, SCFA production, alterations in phenolic compounds, and antioxidant capacity in fermentation media with FDBR and FDBSL reveal the importance of a balanced microbial ecosystem for the survival of beneficial bacterial species and, consequently, for the promotion of desirable effects on human health during colonic fermentation of ingredients with prebiotic activity (Albuquerque et al., 2021; Alves-Santos et al., 2023; Macêdo et al., 2023). An overview of the data obtained in this study summarized by PCA analysis showed that colonic fermentation medium with FDBR and FDBSL had superior results in several analyses, confirming the capability of the bioactive compounds found in different anatomical red beet parts of exerting beneficial effects on intestinal microbiota (Oliveira, 2020).

Although the use of *in vitro* colonic fermentation systems and FISH-FC technique have been shown effective methodological approaches to indicate the potential modulatory effects of pre-digested FDBR and FDBSL on human intestinal microbiota, some possible limitations of this study could be considered: i) difficulties in mimicking some physicochemical and physiological events that occur during human gastrointestinal digestion; ii) the complexity of the human colon and host-gut microbiota interactions cannot be estimated accurately; iii) difficulties in reproducing a continuous and anaerobic fermentation system at the same time; and iv) the use of more probes to measure other important bacterial groups found as part of the human intestinal microbiota (*e.g.*, *Ruminococcus* spp. and *Akkermansia muciniphila*). However, the use of a validated *in vitro* digestion methodology, fresh pooled fecal inoculum, anaerobic generating system, and measurement of representative target groups forming the human intestinal microbiota and metabolite production has shown significant results to assess the effects of prebiotic candidates on intestinal microbiota (Albuquerque et al., 2021; Macêdo et al., 2023; Massa et al., 2022; Medeiros et al., 2021; Menezes et al., 2021; Sampaio et al., 2022a,2022b).

5. Conclusion

The examined red beet parts promoted an increase in the relative abundance of *Lactobacillus* spp./*Enterococcus* spp. and *Bifidobacterium* spp. populations during colonic fermentation and decreased the relative abundance of *Bacteroides* spp./*Prevotella* spp., *C. histolyticum*, and *E. rectale*/*C. coccoides* populations. FDBR and FDBSL achieved positive prebiotic indexes during colonic fermentation and induced decreased pH values, sugars consumption, increased SCFA production, alterations in phenolic compound contents, and high antioxidant capacity during colonic fermentation. These results indicate that FDBR and FDBSL caused beneficial alterations in the composition and metabolic activity of human intestinal microbiota during colonic fermentation, besides demonstrating that conventional and unconventional red beet parts have potential use as novel prebiotic ingredients. These results should encourage the consumption of red beet root, stems, and leaves as functional foods in a healthy diet as well as sources of added-value ingredients for the food industry, being a strategy for the whole valorization of red beet linked to a sustainable and circular economy perspective in the agri-food sector. Further studies using advanced molecular technologies (*e.g.*, next-generation sequencing – NGS) and whole metabolic profiling could be warranted to deepen the knowledge of the impacts of FDBR and FDBSL on the colonic microbiome and their repercussions in human health.

Ethical statement

Ethical approval for the involvement of human subjects in this study was granted by the Ethics Committee for Research with Human Beings of the Federal University of Paraíba (João Pessoa, PB, Brazil) with Protocol number 4.251.958.

CRediT authorship contribution statement

Sonia Paula Alexandrino de Oliveira: Conceptualization, Data curation, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Thatyane Mariano Rodrigues de Albuquerque:** Writing – original draft, Writing – review & editing. **Nayara Moreira Lacerda Massa:** Investigation. **Noádia Priscilla Araújo Rodrigues:** Investigation. **Karoliny Brito Sampaio:** Investigation, Writing – original draft. **Heloísa Maria Almeida do Nascimento:** Formal analysis, Investigation. **Marcos dos Santos Lima:** Investigation, Methodology. **Maria Lúcia da Conceição:** Investigation, Methodology. **Evandro Leite de Souza:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgments

The authors thank the Coordenaçào de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) for partial funding of this research (Finance code 001), as well as the Universidade Federal da Paraíba (Brazil) for the funding support for this research (Grant number PVG14839-2021).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.112998.

References

Aguirre, M., Ramiro-Garcia, J., Koenen, M., & Venema, K. (2014). To pool or not to pool? Impact of the use of individual and pooled fecal samples for in vitro fermentation studies. *Journal of Microbiological Methods, 107*, 1–7. https://doi.org/10.1016/j.mimet.2014.08.022

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

Alves-Santos, A. M., Sampaio, K. B., Lima, M. S., Coelho, A. S. G., Souza, E. L., & Naves, M. M. V. (2023). Chemical composition and prebiotic activity of baru (*Dipteryx alata* Vog.) pulp on probiotic strains and human colonic microbiota. *Food Research International, 164*, Article 112366. https://doi.org/10.1016/j.foodres.2022.112366

Alves-Santos, A. M., Sugizaki, C. S. A., Lima, G. C., & Naves, M. M. V. (2020). Prebiotic effect of dietary polyphenols: A systematic review. *Journal of Functional Foods, 74*, Article 104169. https://doi.org/10.1016/j.jff.2020.104169

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

Association of Official Analytical Chemists – AOAC (2016). Official methods of analysis of the AOAC International, 20th ed. Rockville, MD, USA. ISBN: 97-8-093558487-5.

Awasthi, M. K., Tarafdar, A., Gaur, V. K., Amulya, K., Narisetty, V., Yadav, D. K., … Sirohi, R. (2022). Emerging trends of microbial technology for the production of oligosaccharides from biowaste and their potential application as prebiotic. *International Journal of Food Microbiology, 368*, Article 109610. https://doi.org/10.1016/j.ijfoodmicro.2022.109610

Babarykin, D., Smirnova, G., Pundinsh, I., Vasiljeva, S., Krumina, G., & Agejchenko, V. (2019). Red beet (*Beta vulgaris*) impact on human health. *Journal of Biosciences and Medicines, 07*, 61–79. https://doi.org/10.4236/jbm.2019.73007

Bang, S. J., Kim, G., Lim, M. Y., Song, E. J., Jung, D. H., Kum, J.-S., … Seo, D. H. (2018). The influence of in vitro pectin fermentation on the human fecal microbiome. *AMB Express, 8*, 1–9. https://doi.org/10.1186/s13568-018-0629-9

Biondo, P. B. F., Boeing, J. S., Barizão, É. O., de Souza, N. E., Matsushita, M., de Oliveira, C. C., Boroski, M., & Visentainer, J. V. (2014). Evaluation of beetroot (*Beta vulgaris* L.) leaves during its developmental stages: A chemical composition study. *Food Science and Technology, 34*, 94–101. https://doi.org/10.1590/S0101-20612014005000007

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology, 28*, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Burgos-Edwards, A., Jiménez-Aspee, F., Theoduloz, C., & Schmeda-Hirschmann, G. (2018). Colonic fermentation of polyphenols from Chilean currants (*Ribes* spp.) and its effect on antioxidant capacity and metabolic syndrome-associated enzymes. *Food Chemistry, 258*, 144–155. https://doi.org/10.1016/j.foodchem.2018.03.053

Capper, T. E., Houghton, D., Stewart, C. J., Blain, A. P., McMahon, N., Siervo, M., West, D. J., & Stevenson, E. J. (2020). Whole beetroot consumption reduces systolic blood pressure and modulates diversity and composition of the gut microbiota in older participants. *NFS Journal, 21*, 28–37. https://doi.org/10.1016/J.NFS.2020.08.001

Ceclu, L., & Oana-Viorela, N. (2020). Red beetroot: Composition and health effects – A review. *Journal of Nutritional Medicine and Diet Care*, 6, 043. doi:10.23937/2572-3278.1510043.

Chen, W., Zhang, L., Zhao, L., Yan, F., Zhu, X., Lu, Q., & Liu, R. (2021). Metabolomic profiles of A-type procyanidin dimer and trimer with gut microbiota in vitro. *Journal of Functional Foods, 85*, Article 104637. https://doi.org/10.1016/j.jff.2021.104637

Chhikara, N., Kushwaha, K., Sharma, P., Gat, Y., & Panghal, A. (2019). Bioactive compounds of beetroot and utilization in food processing industry: A critical review. *Food Chemistry, 272*, 192–200. https://doi.org/10.1016/j.foodchem.2018.08.022

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

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

de Carvalho, N. M., Oliveira, D. L., Dib Saleh, M. A., Pintado, M., & Madureira, A. R. (2021). Preservation of human gut microbiota inoculum for in vitro fermentations studies. *Fermentation, 7*, 14. https://doi.org/10.3390/fermentation7010014

de Souza, E. L., de Albuquerque, T. M. R., dos Santos, A. S., Massa, N. M. L., & de Brito Alves, J. L. (2018). Potential interactions among phenolic compounds and probiotics for mutual boosting of their health-promoting properties and food functionalities – A review. *Critical Reviews in Food Science and Nutrition, 59*, 1645–1659. https://doi.org/10.1080/10408398.2018.1425285

De Vadder, F., Kovatcheva-Datchary, P., Goncalves, D., Vinera, J., Zitoun, C., Duchampt, A., & Mithieux, G. (2014). Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell, 156*, 84–96. https://doi.org/10.1016/j.cell.2013.12.016

Diotallevi, C., Gaudioso, G., Fava, F., Angeli, A., Lotti, C., Vrhovsek, U., … Tuohy, K. (2021). Measuring the effect of Mankai® (*Wolffia globosa*) on the gut microbiota and its metabolic output using an in vitro colon model. *Journal of Functional Foods, 84*, Article 104597. https://doi.org/10.1016/j.jff.2021.104597

Domínguez, R., Munekata, P. E. S., Pateiro, M., Maggiolino, A., Bohrer, B., & Lorenzo, J. M. (2020). Red beetroot. A potential source of natural additives for the meat industry. *Applied Sciences, 10*, 8340. https://doi.org/10.3390/app10238340

Fan, S., Li, J., Zhang, X., Xu, D., Liu, X., Dias, A. C. P., … Chen, C. (2022). A study on the identification, quantification, and biological activity of compounds from *Cornus officinalis* before and after in vitro gastrointestinal digestion and simulated colonic fermentation. *Journal of Functional Foods, 98*, Article 105272. https://doi.org/10.1016/j.jff.2022.105272

Ferreira-Lazarte, A., Kachrimanidou, V., Villamiel, M., Rastall, R. A., & Moreno, F. J. (2018). In vitro fermentation properties of pectins and enzymatic-modified pectins obtained from different renewable bioresources. *Carbohydrate Polymers, 199*, 482–491. https://doi.org/10.1016/j.carbpol.2018.07.041

Fidelis, M., Santos, J. S., Escher, G. B., Rocha, R. S., Cruz, A. G., Cruz, T. M., … Granato, D. (2021). Polyphenols of jabuticaba [*Myrciaria jaboticaba* (Vell.) O.Berg] seeds incorporated in a yogurt model exert antioxidant activity and modulate gut microbiota of 1,2-dimethylhydrazine-induced colon cancer in rats. *Food Chemistry, 334*, Article 127565. https://doi.org/10.1016/j.foodchem.2020.127565

Ghasempour, Z., Javanmard, N., Langroodi, A. M., Alizadeh-Sani, M., Ehsani, A., & Kia, E. M. (2020). Development of probiotic yogurt containing red beet extract and basil seed gum; techno-functional, microbial and sensorial characterization. *Biocatalysis and Agricultural Biotechnology, 29*, Article 101785. https://doi.org/10.1016/j.bcab.2020.101785

Gibson, R. G., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., … & Reid, G. (2017). The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatolology, 14*, 491–502. doi:10. 1038/nrgastro.2017.75.

Gómez, B., Gullón, B., Remoroza, C., Schols, H. A., Parajó, J. C., & Alonso, J. L. (2014). Purification, characterization and prebiotic properties of pectic oligosaccharides from orange peel wastes. *Journal of Agricultural and Food Chemistry, 62*, 9769–9782. https://doi.org/10.1021/jf503475b

Gómez, B., Gullón, B., Yáñez, R., Schols, H., & Alonso, J. L. (2016). Prebiotic potential of pectins and pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp: A comparative evaluation. *Journal of Functional Foods, 20*, 108–121. https://doi.org/10.1016/j.jff.2015.10.029

Gómez, B., Peláez, C., Martínez-Cuesta, M. C., Parajó, J. C., Alonso, J. L., & Requena, T. (2019). Emerging prebiotics obtained from lemon and sugar beet byproducts: Evaluation of their *in vitro* fermentability by probiotic bacteria. *LWT - Food Science and Technology, 109*, 17–25. https://doi.org/10.1016/j.lwt.2019.04.008

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

Hosseini, E., Grootaert, C., Verstraete, W., & Van de Wiele, T. (2011). Propionate as a health- promoting microbial metabolite in the human gut. *Nutrition Reviews, 69*, 245–258. https://doi.org/10.1111/j.1753-4887.2011.00388.x

Iljazovic, A., Amend, L., Galvez, E. J. C., Oliveira, R., & Strowing, T. (2021). Modulation of inflammatory responses by gastrointestinal *Prevotella* spp. – From associations to functional studies. *International Journal of Medical Microbiology, 311*, Article 151472. https://doi.org/10.1016/j.ijmm.2021.151472

Liu, C., Kolida, S., Charalampopoulos, D., & Rastall, R. A. (2020). An evaluation of the prebiotic potential of microbial levans from *Erwinia* sp. 10119. *Journal of Functional Foods, 64*, Article 103668. https://doi.org/10.1016/j.jff.2019.103668

Macêdo, E. L. C., Pimentel, T. C., Morais, J. S., Melo, D. S., Souza, A. C., Lima, M. S., … Magnani, M. (2023). Effects of yeast fermented soursop and umbu-cajá pulps on the colonic microbiota of middle-aged hypertensive adults. *Food Bioscience, 51*, Article 102309. https://doi.org/10.1016/j.fbio.2022.102309

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

Masumoto, S., Aoki, S., Miura, T., & Shoji, T. (2018). Flavan-3-ol/procyanidin metabolomics in rat urine using HPLC-quadrupole TOF/MS. *Molecular Nutrition & Food Research, 62*, e1700867.

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

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

Molinari, R., Merendino, N., & Costantini, L. (2021). Polyphenols as modulators of pre-established gut microbiota dysbiosis: State-of-the-art. *BioFactors, 48*, 255–273. https://doi.org/10.1002/biof.1772

Nagpal, R., Mainali, R., Ahmadi, S., Wang, S., Singh, R., Kavanagh, K., … de Yadav, H. (2018). Gut microbiome and aging: Physiological and mechanistic insights. *Nutrition and Healthy Aging, 15*, 267–285. https://doi.org/10.3233/NHA-170030

Oliveira Filho, J. G., Bertolo, M. R. V., Rodrigues, M.Á. V., Silva, G. C., Mendonça, G. M. N., Junior, S. B., … Egea, M. B. (2022). Recent advances in the development of smart, active, and bioactive biodegradable biopolymer-based films containing betalains. *Food Chemistry, 390*, Article 133149. https://doi.org/10.1016/j.foodchem.2022.133149

Oliveira, S. P. A., do Nascimento, H. M. A., Rodrigues, N. P. A., Sampaio, K. B., Lima, M. dos S., da Conceição, M. L., & de Souza, E. L. (2023). Different parts from the whole red beet (*Beta vulgaris* L.) valorization with stimulatory effects on probiotic lactobacilli and protection against gastrointestinal conditions. *Food Bioscience, 52*, 102439. doi:10.1016/J.FBIO.2023.102439.

Oliveira, S. P. A., do Nascimento, H. M. A., Sampaio, K. B., & de Souza, E. L. (2020). A review on bioactive compounds of beet (*Beta vulgaris* L. subsp. *vulgaris*) with special emphasis on their beneficial effects on gut microbiota and gastrointestinal health. *Critical Reviews in Food Science and Nutrition, 61*, 2022–2033. doi:10.1080/10408398.2020.1768510.

Owolabi, I. O., Dat-arun, P., Yupanqui, C. T., & Wichienchot, S. (2020). Gut microbiota metabolism of functional carbohydrates and phenolic compounds from soaked and germinated purple rice. *Journal of Functional Foods, 66*, Article 103787. https://doi.org/10.1016/j.jff.2020.103787

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

Prandi, B., Baldassarre, S., Babbar, N., Bancalari, E., Vandezande, P., Hermans, D., … Sforza, S. (2018). Pectin oligosaccharides from sugar beet pulp: Molecular characterization and potential prebiotic activity. *Food & Function, 9*, 1557–1569. https://doi.org/10.1039/c7fo01182b

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237. doi:10.1016/S0891-5849(98)00315-3.

Riviere, A., Selak, M., Lantin, D., Leroy, F., & De Vuyst, L. (2016). Bifidobacteria and butyrate- producing colon bacteria: Importance and strategies for their stimulation in the human gut. *Frontiers in Microbiology, 7*, 979. https://doi.org/10.3389/fmicb.2016.00979

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., & Tuohy, K. (2018). Gut microbiota functions: Metabolism of nutrients and other food components. *European Journal of Nutrition, 57*, 1–24. https://doi.org/10.1007/s00394-017-1445-8

Sadowska-Bartosz, I., & Bartosz, G. (2021). Biological properties and applications of betalains. *Molecules, 26*, 2520. https://doi:10.3390/molecules26092520.

Sampaio, K. B., Alves, J. L. B., Nascimento, Y. M., Tavares, J. F., Silva, M. S., Nascimento, D. S., … de Souza, E. L. (2022a). Nutraceutical formulations combining *Limosilactobacillus fermentum*, quercetin, and or resveratrol with beneficial impacts on the abundance of intestinal bacterial populations, metabolite production, and antioxidant capacity during colonic fermentation. *Food Research International, 161*, Article 111800. https://doi.org/10.1016/j.foodres.2022.111800

Sampaio, K. B., Nascimento, D. S., Garcia, E. F., & Souza, E. L. (2022b). An outlook on fluorescent in situ hybridization coupled to flow cytometry as a versatile technique to evaluate the effects of foods and dietary interventions on gut microbiota. *Archives of Microbiology, 204*, 469. https://doi.org/10.1007/s00203-022-03090-7

Sanders, M. E., Merenstein, D. J., Reid, G., Gibson, G. R., & Rastall, R. A. (2019). Probiotics and prebiotics in intestinal health and disease: From biology to the clinic. *Nature Reviews Gastroenterology & Hepatology, 16*, 605–616. https://doi.org/10.1038/s41575-019-0173-3

Silva, F. A., Queiroga, R. C. R. E., Souza, E. L., Voss, G. B., Pintado, M. M. E., & Vasconcelos, M. A. S. (2023). Ingredients from integral valorization of Isabel grape to formulate goat yogurt with stimulatory effects on probiotics and beneficial impacts on human colonic microbiota in vitro. *Food Science and Human Wellness, 12*, 1331–1342. https://doi.org/10.1016/j.fshw.2022.10.034

Tabernero, M., & Cedrón, M. G. (2017). Microbial metabolites derived from colonic fermentation of non-digestible compounds. *Current Opinion in Food Science, 13*, 91–96. https://doi.org/10.1016/j.cofs.2017.03.005

Tingirikari, J. M. R. (2018). Microbiota-accessible pectic poly- and oligosaccharides in gut health. *Food & Function, 9*(10), 5059–5073. https://doi.org/10.1039/c8fo01296b

Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Gancel, F., Kempf, I., & Drider, D. (2019). Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Frontiers in Microbiology, 11*, 57. https://doi.org/10.3389/fmicb.2019.00057

Wang, S., Xiao, Y., Tian, F., Zhao, J., Zhang, H., Zhai, Q., & Chen, W. (2020). Rational use of prebiotics for gut microbiota alterations: Specific bacterial phylotypes and related mechanisms. *Journal of Functional Foods, 66*, Article 103838. https://doi.org/10.1016/J.JFF.2020.103838

Wang, Y., Do, T., Marshall, L. J., & Boesch, C. (2023). Effect of two-week red beetroot juice consumption on modulation of gut microbiota in healthy human volunteers – A pilot study. *Food Chemistry, 406*, Article 134989. https://doi.org/10.1016/J.FOODCHEM.2022.134989

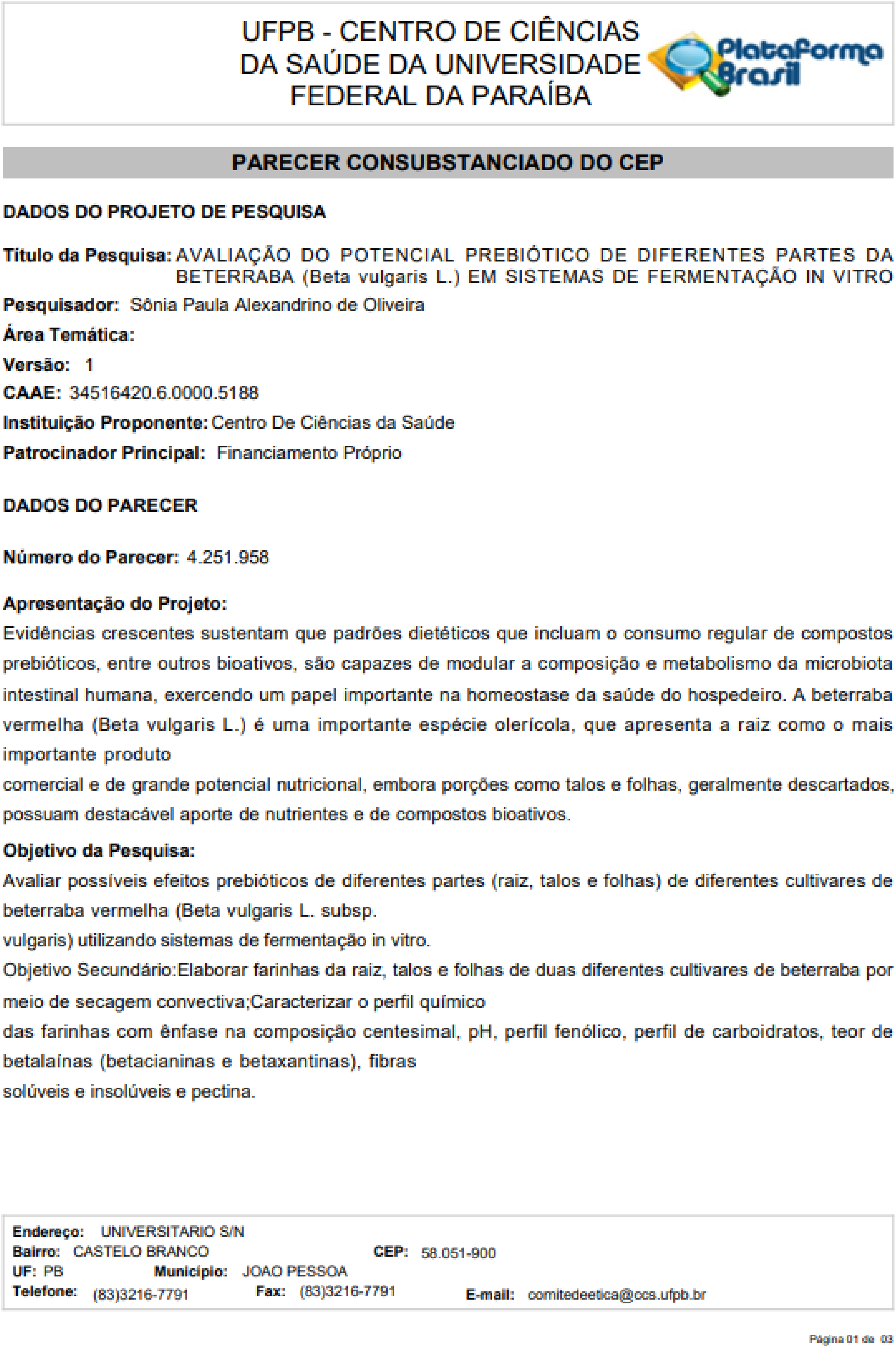
Yang, J., Martínez, I., Walter, J., Keshavarzian, A., & Rose, D. J. (2013). In vitro characterization of the impact of selected dietary fibers on fecal microbiota composition and short chain fatty acid production. *Anaerobe, 23*, 74–81. https://doi.org/10.1016/j.anaerobe.2013.06.012

Yu, C., Ahmadi, S., Shen, S., Wu, D., Xiao, H., Ding, T., … Chen, S. (2022). Structure and fermentation characteristics of five polysaccharides sequentially extracted from sugar beet pulp by different methods. *Food Hydrocolloids, 126*, Article 107462. https://doi.org/10.1016/j.foodhyd.2021.107462

Zhang, N., Jin, M., Wang, K., Zhang, Z., Shah, N. P., & Wei, H. (2022). Functional oligosaccharide fermentation in the gut: Improving intestinal health and its determinant factors - a review. *Carbohydrate Polymers, 284*, Article 119043. https://doi.org/10.1016/J.CARBPOL.2021

ANEXOS

ANEXO A – Parecer Consubstanciado do Comitê de Ética



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

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO DO POTENCIAL PREBIÓTICO DE DIFERENTES PARTES DA BETERRABA (Beta vulgaris L.) EM SISTEMAS DE FERMENTAÇÃO IN VITRO

Pesquisador: Sônia Paula Alexandrino de Oliveira

Área Temática:

Versão: 1

CAAE: 34516420.6.0000.5188

Instituição Proponente: Centro De Ciências da Saúde

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.251.958

Apresentação do Projeto:

Evidências crescentes sustentam que padrões dietéticos que incluam o consumo regular de compostos prebióticos, entre outros bioativos, são capazes de modular a composição e metabolismo da microbiota intestinal humana, exercendo um papel importante na homeostase da saúde do hospedeiro. A beterraba vermelha (Beta vulgaris L.) é uma importante espécie olerícola, que apresenta a raiz como o mais importante produto
comercial e de grande potencial nutricional, embora porções como talos e folhas, geralmente descartados, possuam destacável aporte de nutrientes e de compostos bioativos.

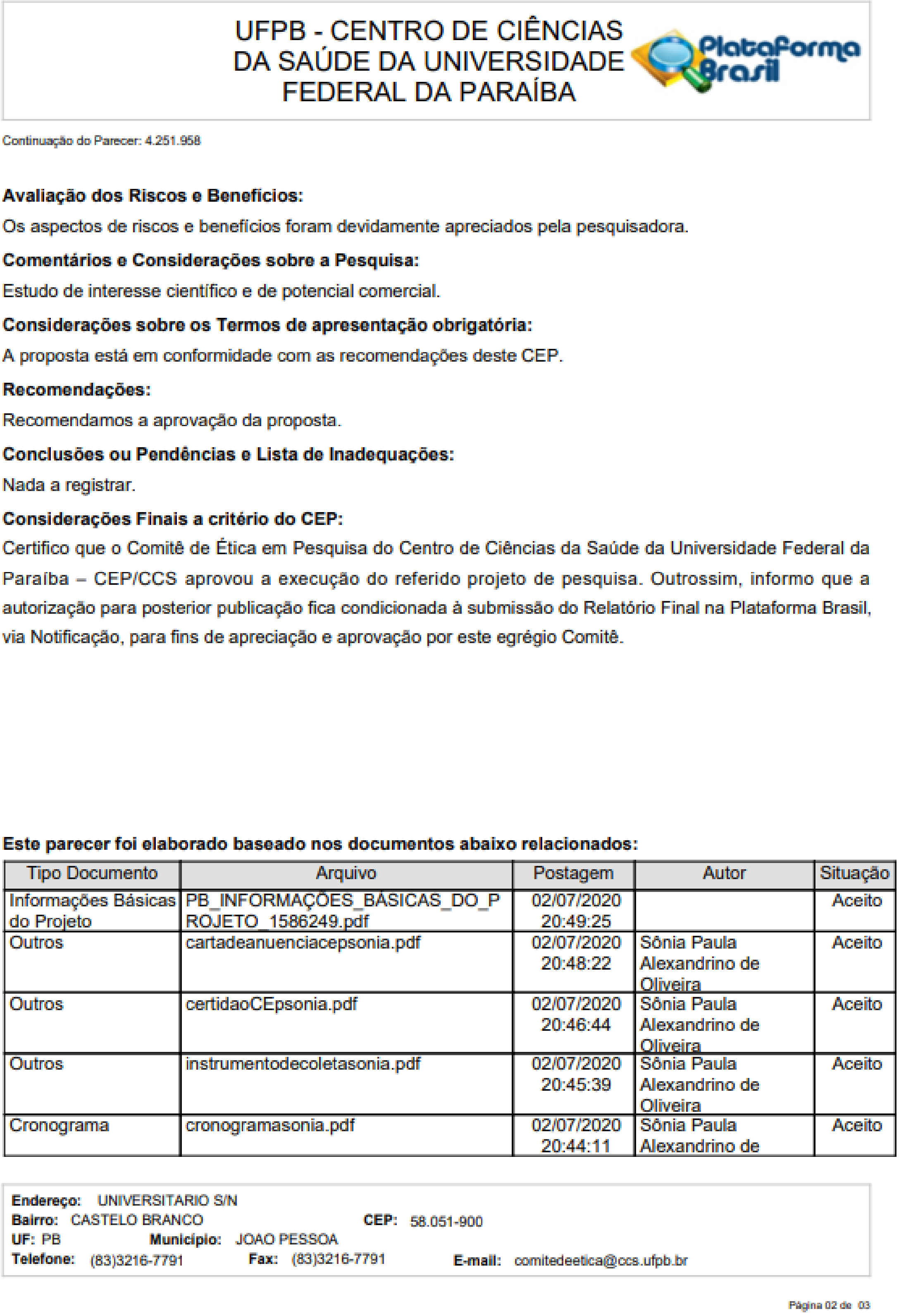
Objetivo da Pesquisa:

Avaliar possíveis efeitos prebióticos de diferentes partes (raiz, talos e folhas) de diferentes cultivares de beterraba vermelha (Beta vulgaris L. subsp.
vulgaris) utilizando sistemas de fermentação in vitro.

Objetivo Secundário:Elaborar farinhas da raiz, talos e folhas de duas diferentes cultivares de beterraba por meio de secagem convectiva;Caracterizar o perfil químico
das farinhas com ênfase na composição centesimal, pH, perfil fenólico, perfil de carboidratos, teor de betalaínas (betacianinas e betaxantinas), fibras
solúveis e insolúveis e pectina.

Endereço: UNIVERSITARIO S/N
Bairro: CASTELO BRANCO CEP: 58.051-900
UF: PB Município: JOAO PESSOA
Telefone: (83)3216-7791 Fax: (83)3216-7791 E-mail: comitedeetica@ccs.ufpb.br

Página 01 de 03



Continuação do Parecer: 4.251.958

Avaliação dos Riscos e Benefícios:

Os aspectos de riscos e benefícios foram devidamente apreciados pela pesquisadora.

Comentários e Considerações sobre a Pesquisa:

Estudo de interesse científico e de potencial comercial.

Considerações sobre os Termos de apresentação obrigatória:

A proposta está em conformidade com as recomendações deste CEP.

Recomendações:

Recomendamos a aprovação da proposta.

Conclusões ou Pendências e Lista de Inadequações:

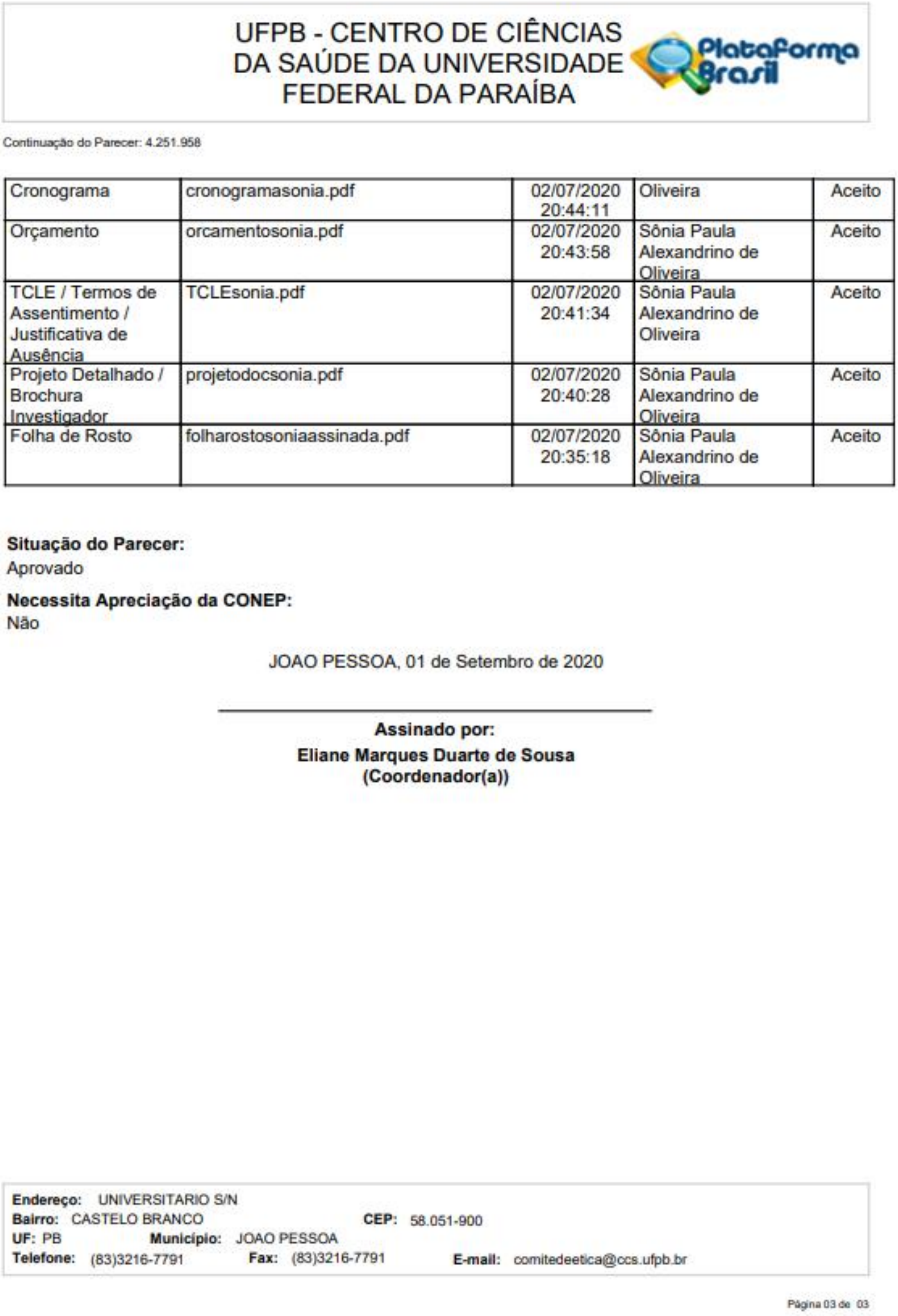
Nada a registrar.

Considerações Finais a critério do CEP:

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

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1586249.pdf	02/07/2020 20:49:25		Aceito
Outros	cartadeanuenciacepsonia.pdf	02/07/2020 20:48:22	Sônia Paula Alexandrino de Oliveira	Aceito
Outros	certidaoCEpsonia.pdf	02/07/2020 20:46:44	Sônia Paula Alexandrino de Oliveira	Aceito
Outros	instrumentodecoletasonia.pdf	02/07/2020 20:45:39	Sônia Paula Alexandrino de Oliveira	Aceito
Cronograma	cronogramasonia.pdf	02/07/2020 20:44:11	Sônia Paula Alexandrino de	Aceito



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

Continuação do Parecer: 4.251.958

Cronograma	cronogramasonia.pdf	02/07/2020 20:44:11	Oliveira	Aceito
Orçamento	orcamentosonia.pdf	02/07/2020 20:43:58	Sônia Paula Alexandrino de Oliveira	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEsonia.pdf	02/07/2020 20:41:34	Sônia Paula Alexandrino de Oliveira	Aceito
Projeto Detalhado / Brochura Investigador	projetodocsonia.pdf	02/07/2020 20:40:28	Sônia Paula Alexandrino de Oliveira	Aceito
Folha de Rosto	folharostosoniaassinada.pdf	02/07/2020 20:35:18	Sônia Paula Alexandrino de Oliveira	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

JOAO PESSOA, 01 de Setembro de 2020

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

Endereço: UNIVERSITARIO S/N
Bairro: CASTELO BRANCO **CEP:** 58.051-900
UF: PB **Município:** JOAO PESSOA
Telefone: (83)3216-7791 **Fax:** (83)3216-7791 **E-mail:** comitedeetica@ccs.ufpb.br

Página 03 de 03