UNIVERSIDADE FEDERAL DE GOIÁS
FACULDADE DE NUTRIÇÃO
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ALINE MEDEIROS ALVES SANTOS

POTENCIAL PREBIÓTICO DA POLPA DE BARU

Goiânia
2022

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Tese apresentada à Coordenação do Programa de Pós-Graduação em Nutrição e Saúde da Faculdade de Nutrição da Universidade Federal de Goiás, como exigência para obtenção do título de Doutor em Nutrição e Saúde.

Orientador: Profª Drª Maria Margareth Veloso Naves

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ATA DE DEFESA DE TESE

Ata número **UM** da sessão de Defesa de **Tese** de **Aline Medeiros Alves Santos**, que confere o título de **Doutora** em **Nutrição e Saúde**, na área de concentração em **Nutrição e Saúde**.

Aos **vinte e nove de agosto de dois mil e vinte e dois**, a partir das **quatorze horas**, **via webconferência**, realizou-se a sessão **Pública** de Defesa de Tese intitulada **"Potencial prebiótico da polpa de baru (*Dipteryx alata Vog.*)"**. Os trabalhos foram instalados pela Orientadora, Professora Doutora **Maria Margareth Veloso Naves (UFG),** com a participação dos demais membros da Banca Examinadora: Professora Doutora **Roberta Melquiades Silva de Andrade (UFRJ)**, membro titular externo; Professora Doutora **Ângela Giovana Batista (UFJF)**, membro titular externo**;** Professora Doutora **Mariana Buranelo Egea (IFGoiano)**, membro titular externo**; e** Professora Doutora **Simone Mendonça (Embrapa Agroenergia)**, membro titular interno. A Banca Examinadora reuniu-se em sessão secreta a fim de concluir o julgamento da Tese, tendo sido a candidata **aprovada** pelos seus membros. Proclamados os resultados pela Professora Doutora **Maria Margareth Veloso Naves**, Presidente da Banca Examinadora, foram encerrados os trabalhos e, para constar, lavrou-se a presente ata que é assinada pelos Membros da Banca Examinadora.

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RESUMO

O baru (*Dipteryx alata* Vog.) é um fruto nativo do bioma Cerrado, constituído por uma casca fina, uma polpa fibrosa e um endocarpo lenhoso, que reveste a castanha. Durante o processamento do fruto para extração da castanha, a polpa de baru não é aproveitada pela agroindústria, e sua composição química e os efeitos para a saúde são pouco conhecidos. Alguns estudos indicam que a polpa de baru é fonte de fibra alimentar e polifenóis, os quais possuem atividade prebiótica. A demanda crescente por alimentos de fontes alternativas promotores de saúde e bem-estar, como os subprodutos agroindustriais, e os impactos social, econômico e ambiental gerados pelo aproveitamento desses subprodutos justificam a busca por novos prebióticos. Assim, os objetivos deste estudo foram: elaborar e publicar uma revisão sistemática sobre o efeito prebiótico dos polifenóis; elaborar e publicar uma revisão crítica sobre as características nutricionais e funcionais do baru; avaliar a composição química da polpa de baru e investigar os seus efeitos sobre o crescimento e o metabolismo de probióticos e sobre a microbiota colonica humana. Segundo a revisão sistemática, existem fortes evidências em estudos pré-clínicos de que alguns polifenóis podem estimular o crescimento de bactérias intestinais benéficas e aumentar a produção de ácidos graxos de cadeia curta. A revisão crítica sobre o baru evidenciou o potencial nutricional e funcional da polpa de baru, assim como as lacunas existentes na literatura sobre sua composição química. Quanto aos resultados do estudo empírico, a polpa de baru apresentou altos teores de fibras insolúveis e compostos fenólicos, sobretudo hesperidina, e estimulou o crescimento e metabolismo de *Bifidobacterium animalis* subsp. *lactis* BB-12, *Lactobacillus acidophilus* LA-05, e *Lacticaseibacillus casei* L-26. Além disso, mudanças benéficas significativas foram induzidas pela polpa de baru digerida sobre a microbiota colonica, com aumento da abundância de *Lactobacillus-Enterococcus*, *Bifidobacterium* e *Bacteroides-Prevotella*, e da produção de lactato e ácidos graxos de cadeia curta, e redução do pH. Portanto, a polpa de baru possui propriedades prebióticas potenciais a serem confirmadas em estudos pré-clínicos e clínicos, e exploradas como ingrediente na formulação de novos alimentos promotores de saúde.

Palavras-chave: alimentos funcionais, subproduto de fruto, fitoquímicos, digestão gastrointestinal *in vitro*, inoculo fecal, microbiota intestinal.

ABSTRACT

Prebiotic potential of baru pulp

Baru (*Dipteryx alata* Vog.) is a fruit native to the Cerrado biome consisting of a thin peel, a fibrous pulp and a woody endocarp, which covers the nut. During the processing of the fruit for nut extraction, the baru pulp is not used by the agro-industry, and its chemical composition and health effects are poorly known. Some studies indicate that baru pulp has dietary fiber and polyphenols, which have prebiotic activity. The growing interest in health and well-being promoting foods from alternative sources, such as agro-industrial by-products, and the social, economic and environmental impacts generated by the use of these by-products justify the search for new prebiotics. Thus, the aims of this study were: to elaborate and publish a systematic review on the prebiotic effect of polyphenols; to carry out and publish a critical review of the literature on the nutritional and functional characteristics of baru; in addition to evaluate the chemical composition of baru pulp and to investigate its effects on the growth and metabolism of probiotics, and on the human colonic microbiota. According to the systematic review, there is strong evidence from preclinical studies that some polyphenols can stimulate the growth of beneficial gut bacteria and increase the production of short-chain fatty acids. The critical review on baru showed the nutritional and functional potential of baru pulp, as well as the gaps in the literature on its chemical composition. Results of the empirical research show that baru pulp has a high content of insoluble fibers and phenolic compounds, mainly hesperidin. Baru pulp stimulated the growth and metabolism of *Bifidobacterium animalis* subsp. *lactis* BB-12, *Lactobacillus acidophilus* LA-05 and *Lacticaseibacillus casei* L-26. Furthermore, significant beneficial changes were induced by the digested baru pulp on colonic microbiota, with an increase in the abundance of *Lactobacillus-Enterococcus*, *Bifidobacterium* and *Bacteroides-Prevotella*, and the production of lactate and short-chain fatty acids, and pH reduction. Therefore, baru pulp has potential prebiotic properties to be confirmed in preclinical and clinical studies, and to be explored as an ingredient in the formulation of new health-promoting foods.

Key words: functional foods, fruit by-product, phytochemicals, *in vitro* gastrointestinal digestion, fecal inoculum, gut microbiota

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CAPÍTULO 1

1 INTRODUÇÃO

O baru (*Dipteryx alata* Vog.), fruto nativo do bioma Cerrado, é constituído por uma casca fina (epicarpo), pela polpa (mesocarpo fibroso), e por um endocarpo rígido que reveste a semente, parte comestível do fruto mais conhecida como castanha ou amêndoa[1]. A castanha de baru tem um importante valor comercial pelas suas características sensoriais, nutricionais e pelos efeitos benéficos para saúde[1], sobretudo para o metabolismo lipídico[2,3]. Durante o processamento do fruto para extração da castanha, em geral, a polpa de baru, que representa cerca de 40% da massa do fruto[4], não é aproveitada pela agroindústria[1]. Pouco se conhece sobre a composição em nutrientes e em substâncias bioativas desse subproduto, mas os estudos disponíveis relatam teores elevados de açúcares e fibra alimentar total, e conteúdo considerável de compostos fenólicos totais[5,6]. Não há relatos sobre os efeitos do seu consumo para saúde, especialmente, sobre a microbiota intestinal.

A microbiota intestinal é composta por uma ampla diversidade de micro-organismos que colonizam o trato gastrointestinal, e possui uma importância relevante para a saúde do hospedeiro, participando de diversos processos fisiológicos, tais como o metabolismo de nutrientes e compostos bioativos que não são digeridos no intestino delgado e chegam intactos no cólon humano[7]. A dieta é um dos principais fatores ambientais que influenciam a composição e funcionalidade da microbiota intestinal[8,9]. Nesse sentido, o consumo de prebióticos constitui uma abordagem acessível e segura para melhorar a saúde intestinal[10].

Prebióticos são substratos utilizados seletivamente por micro-organismos do hospedeiro proporcionando benefícios à saúde. Dentre os benefícios, destaca-se a regulação do metabolismo lipídico e glicídico[11]. Os principais gêneros de micro-organismos reconhecidos como alvos prebióticos são *Lactobacillus* e *Bifidobacterium*, além de *Roseburia*, *Eubacterium*, *Faecalibacterium* e *Akkermansia*, mas não se limitam a esses[10,11]. Os prebióticos mais evidenciados pela literatura são inulina, fruto- e galacto-oligossacarídeos[11], os quais são classificados como fibras alimentares, embora nem todas as fibras alimentares sejam prebióticos[12]. Estudos recentes

mostram que os polifenóis e seus metabólitos também podem se enquadrar na definição atual de prebióticos[11,13,14].

Fibras alimentares e polifenóis são encontrados em concentrações elevadas nos subprodutos do processamento de frutos, como goiaba e acerola[15], jabuticaba[16], jerivá e macaúba[17], os quais têm sido investigados quanto ao potencial prebiótico. Os resultados desses estudos mostraram que os subprodutos de frutos estimulam o crescimento e o metabolismo de probióticos, como *Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *Bifidobacterium animalis* e *Bifidobacterium lactis*. Além disso, uma farinha obtida de subprodutos de frutos (laranja, maracujá e melão) e vegetais (alface, abobrinha, cenoura, espinafre, hortelã, inhame, pepino e rúcula) promoveu efeitos significativos sobre a abundância de bactérias benéficas (*Lactobacillus* e *Bifidobacterium*) na microbiota colonica humana *in vitro*[18]. Esses achados indicam o potencial dos subprodutos de frutos para melhorar a saúde intestinal. No entanto, ainda não há relatos na literatura sobre o potencial prebiótico da polpa de baru.

A crescente demanda por produtos funcionais e pelo consumo sustentável têm impulsionado as pesquisas por novos ingredientes prebióticos, especialmente, subprodutos do processamento de frutos, o que contribui para aumentar o valor agregado aos frutos, e reduzir os impactos ambientais, sociais e econômicos gerados pela perda e desperdício de alimentos. Além disso, a escassez de dados na literatura sobre a composição química da polpa de baru, e a ausência de informação sobre seu potencial prebiótico constituem as justificativas para a realização do presente estudo.

2 REVISÃO DA LITERATURA

2.1 BARU

O baruzeiro (*Dipteryx alata* Vog.) é uma espécie arbórea nativa do bioma Cerrado (Figura 1), pertencente à família *Fabaceae*, possui altura entre 15 e 25 m, floresce de novembro a fevereiro, e os frutos maduros podem ser encontrados entre os meses de julho e outubro, produzindo cerca de 2.000 a 6.000 frutos por planta[19]. No entanto, essa produção não é regular e pode variar conforme o ano e a região de plantio[19]. De acordo com informações adquiridas das principais agroindústrias que processam baru no Estado de Goiás, em 2021, a produção de fruto foi cerca de 250 toneladas. A distribuição dessa espécie é ampla no Brasil, com ocorrência registrada nos Estados de Goiás, Tocantins, Mato Grosso, Mato Grosso do Sul, Pará, Bahia, Maranhão, Minas Gerais, São Paulo, e Distrito Federal. Além disso, há relatos da ocorrência de baruzeiro no Paraguai, Peru e Bolívia[19].

Figura 1. Baruzeiro (*Dipteryx alata Vog*.) e o seu fruto, o baru.
Fotos registradas nas fazendas “Dr Jamar Urias Mendonça” e “Três Jotas” (Caiapônia/GO, 2022)

Os frutos do baruzeiro (Figura 1) são conhecidos como baru, mas em alguns Estados brasileiros também podem ser chamados de cumaru ou cumbaru. Os frutos são do tipo drupa (apenas uma semente), ovoides, levemente achatados, de coloração marrom, com comprimento e largura variando de 3,5 a 5,0 cm e de 2,0 a 3,5 cm, respectivamente[19]. O baru é constituído por uma casca fina (epicarpo), pela

polpa (mesocarpo fibroso), e por um endocarpo rígido que reveste a semente, parte comestível do fruto mais conhecida como castanha ou amêndoa[1]. A casca e a polpa do baru representam cerca de 40% da massa do fruto, e a castanha compreende apenas 4% do fruto[4]. A castanha possui comprimento entre 1,8 e 2,3 cm, largura média de 1,0 cm e massa média de 1,25 g[1].

A castanha torrada é muito consumida na região Centro-Oeste do Brasil, e, atualmente, tem sido exportada para outros países, como Estados Unidos, Canadá e Coreia do Sul. O importante valor comercial da castanha de baru é atribuído às suas características sensoriais e nutricionais, tais como teor considerável de fibra alimentar (6 a 16 g/100 g), alto conteúdo de ácidos graxos monoinsaturados (45 a 54%) e concentração elevada de compostos bioativos, como fenólicos totais (388 a 1.306 mg GAE/100 g) e tocoferóis totais (11 a 13 mg/100 g)[1]. A película da castanha de baru também se destaca pelo teor elevado de compostos fenólicos totais (9.730 mg GAE/100 g)[20]. A castanha de baru pode ser usada para substituir outras nozes ou enriquecer formulações de novos produtos com farinha de trigo (cupcakes, cookies, barras de cereais, e pães), ou como ingrediente adicional para agregar valor à produtos tradicionais, como manteigas, sorvetes, e sobremesas diversas[1]. O óleo extraído da castanha de baru também tem potencial para ser utilizado em novos produtos funcionais[1]. Estudos com suplementação da castanha ou do óleo de baru constataram redução do estresse oxidativo em ratos tratados com dieta hiperlipidica[3], melhora do perfil lipídico sérico em indivíduos moderadamente hipercolesterolêmicos[2], redução da adiposidade abdominal em mulheres com sobrepeso e obesidade[21], e da proteína C reativa ultrassensível em pacientes renais crônicos em tratamento de hemodiálise[22].

A polpa de baru possui sabor adocicado e, por isso, é bastante consumida pelo gado e por animais silvestres em períodos de seca[23]. Existem relatos de seu consumo na alimentação humana como substituto da farinha de trigo em formulações de cookies[24,25], do farelo de aveia em barras de cereais[26] e do farelo de trigo em pães integrais[27]. Os autores concluíram que a inclusão da polpa de baru proporcionou formulações com índices elevados de aceitabilidade. Além disso, a polpa de baru apresenta grande potencial para produção de doces, geleias, farinhas, sorvetes e licores, representando uma possível fonte de renda para a população local. O uso da polpa em conjunto com o endocarpo lenhoso foi relatado como sendo de grande potencial energético para produção de biocarvão por seus teores elevados de lignina,

hemicelulose e celulose[28]. Entretanto, em geral, durante o processamento do fruto para extração da castanha, a polpa de baru não é aproveitada pela agroindústria.

Pouco se conhece sobre a composição nutricional e funcional da polpa de baru, mas alguns estudos ressaltam alto teor de fibra alimentar (18-42 g/100 g)[1] e açúcares totais[24], e conteúdo considerável de compostos fenólicos totais (292 mg GAE/100 g)[5]. Ácidos fenólicos, flavonoides, terpenos, ácidos orgânicos e derivados de ácidos graxos foram identificados na polpa de baru[29]. Fatores antinutricionais, como ácido fítico (0,27%) e inibidor de tripsina (0,67 UTI/mg) foram reportados em baixas concentrações na polpa de baru[30]. O extrato da casca e polpa de baru exibiu efeito citotóxico contra linhagens de câncer de colo de útero (SiHa e C33A), e citotoxicidade fraca contra linhagens de células não tumorais (HaCaT e L929), sugerindo sua segurança e fornecendo evidências preliminares de seu potencial anticancerígeno[31]. Além disso, *in vivo*, a polpa de baru não apresentou efeitos tóxicos e promoveu resistência ao estresse oxidativo em nematoides expostos a um agente químico oxidante[29]. No entanto, mais estudos são necessários para investigar o perfil de carboidratos, de oligossacarídeos e de polifenóis, e os efeitos do consumo da polpa de baru na saúde, especialmente sobre a microbiota intestinal.

2.2 MICROBIOTA INTESTINAL

O conjunto de micro-organismos que colonizam o trato gastrointestinal, especialmente o cólon, é definido como microbiota intestinal, a qual é caracterizada pela composição (diversidade de micro-organismos e abundância relativa) e pela funcionalidade (capacidade metabólica e efeitos no hospedeiro)[32,33]. Estima-se que a microbiota intestinal contenha mais de um trilhão de células de diferentes espécies de bactérias, fungos, arqueias e protistas[32].

Em relação às bactérias, a microbiota intestinal humana é constituída, predominantemente, pelos filos *Bacillota* (antigo nome "*Firmicutes*"), *Bacteroidota* (antigo nome "*Bacteroidetes*"), *Actinomycetota* (antigo nome "*Actinobacteria*"), *Verrucomicrobiota* (antigo nome "*Verrucomicrobia*") e *Pseudomonadota* (antigo nome "*Proteobacteria*")[34,35]. *Bacillota* e *Bacteroidota* representam 90% da microbiota intestinal. O filo *Bacillota* é composto por cerca de 250 gêneros, incluindo *Lactobacillus*, *Enterococcus*, *Eubacterium*, *Clostridium*, *Roseburia*, *Ruminococcus* e

Faecalibacterium. O gênero *Clostridium* é considerado o mais abundante desse filo. O filo *Bacteroidota* é representado, principalmente, pelos gêneros *Bacteroides* e *Prevotella*. *Actinomycetota* é um dos filos menos abundante na microbiota intestinal, cerca de 5%, e tem como principal representante, o gênero *Bifidobacterium*. O filo *Verrucomicrobiota* é representado pelo gênero *Akkermansia*, e o filo *Pseudomonadota* é representado pelos gêneros *Escherichia* e *Helicobacter*[34].

Fatores genéticos e ambientais, como dieta, idade e estilo de vida influenciam a composição da microbiota intestinal[5,6], a qual pode ser avaliada por diferentes métodos, variando desde técnicas de cultivo em placas à ferramentas moleculares, tais como a hidridização *in situ* por fluorescência (FISH) e o sequenciamento do gene RNAr 16S[8,33]. A técnica FISH permite a identificação de ácidos nucleicos alvos por meio da hibridização com sondas de oligonucleotídeos complementares específicas, marcadas de forma fluorescente. Em combinação com outras técnicas, como microscopia de fluorescência ou citometria de fluxo, possibilita a caracterização de populações microbianas filogeneticamente definidas em ambientes complexos, como a microbiota intestinal[36]. A técnica FISH acoplada à citometria de fluxo (FISH-FC) tem sido amplamente utilizada para enumeração rápida e com alto rendimento de populações bacterianas fecais[37]. No entanto, uma desvantagem desta técnica é a identificação limitada de novas populações bacterianas, uma vez que as sondas são projetadas para gêneros e espécies mais prevalentes e conhecidos[38].

A microbiota intestinal está envolvida em várias funções no hospedeiro, tais como metabolismo de nutrientes, síntese de vitaminas, metabolismo de drogas, modulação do sistema imune, regulação da integridade da barreira intestinal, e proteção antimicrobiana[8,9,39]. Nutrientes e outros compostos que resistem à digestão no intestino delgado e atingem o cólon são metabolizados pela microbiota intestinal por meio de duas vias principais[7,40]. Na primeira via, os carboidratos não digeríveis, presentes em uma dieta rica em fibras, são metabolizados pela microbiota no intestino grosso, produzindo ácidos graxos de cadeia curta (AGCC) e reduzindo o pH intestinal[9,40]. A segunda via do metabolismo de nutrientes com participação da microbiota intestinal é representada pela fermentação de proteínas[9]. Essa via é estimulada, principalmente, por dietas ricas em proteína e gordura de origem animal e pobres em fibras e substâncias bioativas, as quais têm sido associadas a um tempo prolongado de trânsito intestinal, o que resulta em aumento da proteólise pelos micro-organismos[32,40]. Com isso, ácidos graxos de cadeia ramificada, gases, fenóis, aminas

e amônia são produzidos promovendo aumento do pH intestinal e produção reduzida de AGCC[40].

Os AGCC mais abundantes no cólon são acetato, propionato e butirato, representando cerca de 95%[41]. As vias de produção de acetato são amplamente distribuídas entre os grupos bacterianos, enquanto as vias de produção de propionato e butirato parecem mais conservadas e específicas do substrato[42]. A maior parte do acetato é produzida a partir da fermentação de carboidratos pela maioria das bactérias entéricas[41]. Outra parte é sintetizada a partir de hidrogênio e dióxido de carbono ou por bactérias acetogênicas, como algumas espécies do gênero *Clostridium*[41]. A via do succinato é dominante na microbiota intestinal para formação de propionato. Essa via está presente em vários micro-organismos pertencentes aos filos *Bacillota* (antigo nome *"Firmicutes"*) *e Bacteroidota* (antigo nome *"Bacteroidetes"*)[35], e a abundância de *Bacteroidota* tem sido associada ao conteúdo de propionato nas fezes[41]. A produção de acetato e propionato também é mediada por algumas espécies de *Bifidobacterium* e por *Akkermansia muciniphila*[43]. A via butiril-CoA: acetato CoA-transferase, na qual butiril-CoA é convertido em butirato, é utilizada pela maioria dos produtores intestinais de butirato, incluindo *Faecalibacterium*, *Eubacterium*, *Clostridium* e *Roseburia*[41,43].

Os AGCC ativam os receptores acoplados à proteína G (GPR41, GPR43) nas células L enteroendócrinas, estimulando a secreção do peptídeo semelhante ao glucagon 1 (GLP-1) e o peptídeo YY (PYY), que induzem a secreção de insulina, reduzem os níveis séricos de glicose e regulam o apetite[44]. O GPR43 também pode ser ativado no tecido adiposo branco reduzindo a lipólise. O receptor ativado por proliferadores de peroxissoma gama (PPARγ), por sua vez, pode ser estimulado pelos AGCC aumentando a sensibilidade à insulina no tecido adiposo branco, e reduzindo a esteatose hepática[44]. Além dos metabolismos lipídico e glicídico, os AGCC parecem modular o sistema imunológico, a resposta inflamatória, e a manutenção e reparação da integridade epitelial[42]. O butirato tem uma relevância particular pelo efeito anti-inflamatório, além de ser uma fonte de energia importante para os colonócitos[33,39]. Um dos efeitos à saúde atribuídos à produção de AGCC é a concomitante redução do pH luminal, que por si só inibe micro-organismos patogênicos e aumenta a absorção de alguns minerais, como o cálcio[10].

A dieta é um dos principais fatores ambientais que influenciam a composição e funcionalidade da microbiota intestinal e, consequentemente, afetam as concentrações circulantes dos AGCC[45]. Um estudo de meta-análise, envolvendo 64

estudos e 2.099 participantes, concluiu que intervenções com fibra dietética, particularmente frutanos e galacto-oligossacarídeos, resultam em maior abundância de *Bifidobacterium* e *Lactobacillus*, e maior concentração fecal de butirato, em comparação com grupos placebos ou com dieta com baixo teor de fibra em adultos saudáveis[39]. Dos 64 estudos, 20 utilizaram a técnica FISH para caracterizar a microbiota fecal[39]. Uma dieta com baixo teor de carboidratos à base de amêndoa aumentou significativamente as bactérias produtoras de AGCC, incluindo *Roseburia*, *Ruminococcus* e *Eubacterium* em pacientes com diabetes tipo 2 durante três meses[46]. Pacientes com diabetes tipo 2 suplementados com alimentos ou compostos funcionais (inulina, chia, proteína de soja, cacto nopal, ácido graxo ômega-3, proteínas vegetais, polifenóis, fibras solúveis e insolúveis), durante três meses, apresentaram aumento da diversidade da microbiota fecal e da atividade antioxidante, e redução nas concentrações séricas de colesterol total, LDL e triacilgliceróis[47]. Nesse sentido, os prebióticos constituem uma abordagem interessante para modulação da microbiota intestinal visando melhoras à saúde do hospedeiro, uma vez que são eficazes, acessíveis e seguros[10].

2.3 PREBIÓTICOS

2.3.1 Definição, compostos prebióticos e efeitos na saúde

Os prebióticos são substratos utilizados seletivamente por micro-organismos do hospedeiro proporcionando benefícios à saúde[11]. Um composto para ser classificado como prebiótico deve resistir à acidez gástrica, hidrólise enzimática e absorção gastrointestinal, demonstrar capacidade de ser fermentado pela microbiota intestinal, e estimular seletivamente o crescimento e/ou metabolismo de micro-organismos associados à saúde e bem-estar[11]. Além disso, os prebióticos não devem causar efeitos colaterais ao hospedeiro, como por exemplo, produção excessiva de gás[11]. Em geral, os prebióticos são consumidos, principalmente, por via oral, mas também podem ser administrados diretamente em locais extra intestinais, como a pele e o trato urogenital[11].

Os efeitos dos prebióticos na saúde incluem benefícios para o trato gastrointestinal (inibição de patógenos, estimulação imunológica), o sistema cardiometabólico (redução nos níveis séricos de lipídios e da resistência à insulina), a

saúde mental (metabólitos que influenciam a função cerebral, energia e cognição), e a saúde óssea (biodisponibilidade mineral)[11]. Esses efeitos devem ser confirmados no hospedeiro-alvo e mediados pela microbiota intestinal. Além disso, o efeito prebiótico de um substrato deve ser confirmado em uma avaliação completa da microbiota intestinal, por meio de técnicas moleculares, que são capazes de detectar e quantificar as alterações na composição da microbiota, juntamente com a avaliação da funcionalidade pela análise de metabólitos[11,48].

Os principais gêneros de micro-organismos reconhecidos como alvos prebióticos são *Lactobacillus* e *Bifidobacterium*, além de *Roseburia*, *Eubacterium*, *Faecalibacterium* e *Akkermansia*, mas não se limitam a esses[10,11]. O gênero *Lactobacillus* compreende mais de 170 espécies de bactérias produtoras de lactato, Gram-positivas, anaeróbicas facultativas, catalase-negativas, e bastonetes não formadores de esporos[49]. Os *Lactobacillus* são micro-organismos importantes da microbiota intestinal humana, e têm sido estudados pelos efeitos benéficos à saúde do hospedeiro, uma vez que possuem muitos genes envolvidos no transporte e metabolismo de carboidratos, contemplando espécies capazes de utilizar carboidratos complexos, como prebióticos. Dependendo da disponibilidade de fontes de carbono, os Lactobacilos podem variar entre as vias catabólicas para hexoses e pentoses[50]. Por exemplo, *Lacticaseibacillus casei* podem degradar açúcares por meio da via da glicólise ou da via da pentose fosfato[51]. Os *Lactobacillus* estão entre os probióticos mais utilizados na indústria de alimentos[49]. O gênero *Bifidobacterium* também tem sido muito utilizado na indústria de alimentos e muito investigado em relação aos benefícios para saúde[52]. As bifidobactérias metabolizam carboidratos por meio de uma única via chamada “bifid shunt”, na qual a enzima frutose-6-fosfato fosfocetolase desempenha um papel fundamental[53]. Embora muitos micro-organismos possam metabolizar um substrato, é o benefício para saúde do hospedeiro resultante de sua utilização seletiva pelo micro-organismo que confere a denominação de prebiótico[11].

Os prebióticos mais documentados na literatura são inulina, fruto-oligossacarídeos (FOS) e galacto-oligossacarídeos (GOS), os quais são classificados como fibras alimentares[10,11]. A inulina está presente em alimentos como chicória, cebola, alcachofra, trigo e banana; o FOS pode ser encontrado, principalmente, em aspargos, alho e alho poro; e o GOS é produzido enzimaticamente a partir da lactose[48]. As fibras alimentares são polímeros de carboidratos resistentes à digestão e absorção no intestino delgado, com fermentação parcial ou completa no intestino

grosso. Elas podem ser classificadas em polissacarídeos não amiláceos (celulose, hemicelulose, pectina), oligossacarídeos resistentes (rafinose, estaquiose, fruto-oligossacarídeos), amidos resistentes (tipos 1 ao 4) e substâncias associadas, não carboidratos (lignina e quitina)[54]. No entanto, nem todas as fibras alimentares são prebióticos. Algumas fibras, tais como pectina e amido resistente, são consideradas candidatas a prebióticos, enquanto outras, como a celulose, parece não ter ação prebiótica[55].

Entretanto, evidências recentes têm mostrado que as fibras insolúveis, como a celulose, podem modular a microbiota intestinal com produção de AGCC. Em um estudo de fermentação fecal *in vitro*, a fibra alimentar insolúvel do bambu, constituída por 43% de celulose, 17% de hemicelulose e 28% de lignina, alterou a composição da microbiota intestinal, com aumento na abundância de *Bacteroides*, e aumentou a produção de AGCC totais após 24 h de fermentação[56]. Intervenção com fibra alimentar insolúvel de soja em camundongos tratados com dieta hiperlipídica, por 20 semanas, aumentou a abundância de *Lactobacillus*, e reduziu a abundância de *Bacteroides acidifaciens*, bactéria potencialmente patogênica correlacionada com a obesidade. Além disso, a fermentação da fibra insolúvel de soja aumentou o conteúdo de AGCC e promoveu a secreção de hormônios da saciedade (PYY e GLP-1)[57]. O maior tempo de interação das fibras insolúveis com a microbiota intestinal, e a redução do tamanho das partículas por meio do processamento dos alimentos podem favorecer a fermentação das fibras insolúveis pela microbiota intestinal, promovendo assim alterações benéficas para o hospedeiro[57]. Os gêneros *Bacteroides*, *Eubacterium* e *Roseburia* degradam xilanas, enquanto os gêneros *Ruminococcus* e *Faecalibacterium* podem degradar hemiceluloses ou pectina[58].

Outras substâncias não carboidratos também podem se enquadrar na definição de prebiótico, como os polifenóis e ácidos graxos[11,59]. Os polifenóis são metabólitos secundários de plantas, caracterizados pela presença de anéis aromáticos com um ou mais grupos hidroxila em sua estrutura química, podendo variar de uma simples molécula a um polímero complexo de alto peso molecular. De acordo com a base de dados Phenol-Explorer[60], os polifenóis podem ser classificados em flavonoides, ácidos fenólicos, estilbenos, lignanas, outros polifenóis, e metabólitos não fenólicos. A maioria dos polifenóis ocorre, naturalmente, conjugados a um açúcar. Isso dificulta a absorção desses compostos no intestino delgado, os quais chegam intactos no cólon, onde se tornam substratos para a microbiota intestinal, produzindo metabólitos

que são melhores absorvidos[7]. Estima-se que uma pequena porção de polifenóis é absorvida (5-10%), e uma grande proporção (90-95%) atinge o cólon, onde interagem com a microbiota intestinal[58].

Existem evidências de que os polifenóis possuem propriedades antioxidante, anti-inflamatória[61], anti-obesogênica e antilipidêmica[62], e mais recentemente, que podem modular positivamente a microbiota intestinal[63]. Segundo Rodríguez-Daza et al.[58], o impacto dos polifenóis na microbiota intestinal está associado a dois principais efeitos: antimicrobiano e prebiótico. Assim, os polifenóis são capazes de inibir a colonização de bactérias patogênicas, restabelecer a função de barreira epitelial da mucosa e sua resposta imunológica, e reduzir as espécies reativas de oxigênio e radicais livres. Essas três atividades favorecem o crescimento de bactérias intestinais benéficas à saúde e ao bem-estar do hospedeiro. No entanto, o efeito prebiótico dos polifenóis pode ser influenciado pela fonte alimentar, pela estrutura química do composto e pelas diferenças individuais na composição da microbiota intestinal[63].

Os polifenóis podem ser metabolizados por diversas espécies bacterianas como *L. acidophilus*, *B. animalis*, *A. muciniphila*, *Bacteroides thetaiotaomicrom* e *Faecalibacterium prausnitzii*. Essas espécies são capazes de produzir enzimas envolvidas no metabolismo de polifenóis, tais como *β*-glicosidases e tanases[58]. A fermentação *in vitro* pela microbiota fecal humana de um polifenol do chá preto (teaflavina 3,3'-digalato) demonstrou efeitos promotores de crescimento em *Bacteroides*, *Faecalibacterium* e *Bifidobacterium*, e efeitos inibitórios em *Prevotella* e *Fusobacterium*[64]. A incubação de flavonoides, sobretudo catequina e epicatequina, com bactérias fecais aumentou o crescimento de *Bifidobacterium*, e inibiu o crescimento de *C. histolyticum*. A epicatequina aumentou significativamente o crescimento do grupo *C. coccoides-E. rectale*[65]. O consumo de polifenóis do chá verde aumentou a abundância de *Bifidobacterium* e *Akkermansia*, e reduziu as concentrações séricas de LDL-c, glicose e insulina em camundongos tratados com dieta hiperlipídica[13,14]. Em indivíduos com sobrepeso e obesidade, a ingestão de extrato de romã, rico em ácidos hidroxibenzóicos, aumentou a abundância de *Faecalibacterium*[66].

Metabólitos de polifenóis gerados pela microbiota intestinal têm sido investigados em relação aos seus efeitos na saúde. Por exemplo, o equol, metabólito das isoflavonas de soja, possui atividade estrogênica e antioxidante[67]. As urolitinas, metabólitos derivados da microbiota intestinal a partir dos polifenóis elagitaninos e ácido elágico, possuem efeitos em diferentes tecidos e órgãos, como neuro proteção,

melhora da integridade da barreira intestinal, da força muscular e da longevidade, além de reduzir o acúmulo de lipídios e a intolerância a glicose[68]. No entanto, ainda são escassos os estudos *in vivo* que investigam as alterações na composição da microbiota intestinal, os metabólitos gerados pela metabolização microbiana dos polifenóis e seus efeitos na saúde.

2.3.2 Potencial prebiótico de subprodutos de frutos

Conteúdos elevados de fibra alimentar e polifenóis são encontrados em subprodutos do processamento de frutos, como cascas, sementes, folhas, caules, raízes, e bagaço[15,16,18]. Na maioria dos casos, a concentração de substâncias bioativas no subproduto é maior do que na parte comestível do fruto[69]. Os nutrientes e outros compostos presentes nesses subprodutos podem enriquecer produtos alimentícios, melhorando suas propriedades físicas e químicas[70], como aroma, sabor, cor, capacidade antioxidante e potencial prebiótico. Além disso, o aumento crescente na demanda por produtos naturais com importância nutricional e terapêutica, e os impactos econômico, social e ambiental gerados pelas perdas nas indústrias de processamento de frutos, têm estimulado as pesquisas com subprodutos[71].

Vale ressaltar que esses impactos gerados pelo descarte de subprodutos e resíduos do processamento de alimentos têm sido alvos de políticas ambientais e de segurança alimentar[72]. Os princípios de reutilização, redução e reciclagem apoiam o conceito de uma economia circular, e a busca pela transição de uma economia linear para circular reforça a necessidade de recuperar recursos de resíduos orgânicos. No entanto, essa transição requer novos estudos, novas políticas, estrutura financeira, e conscientização da população[73].

Nesse sentido, subprodutos de frutos brasileiros têm sido investigados em relação ao potencial prebiótico *in vitro*. O crescimento seletivo de probióticos *L. acidophilus*, *L. casei e Lacticaseibacillus paracasei* foi estimulado pelo subproduto de caju[74]. Observou-se também o crescimento de *L. casei*, *L. paracasei* e *B. animalis* subsp. *lactis* em meios de cultivo enriquecidos com subprodutos de acerola e goiaba[15]. A adição de subproduto digerido de jabuticaba no meio de cultivo induziu o crescimento de *Lactobacillus* e *Bifidobacterium*, reduziu o pH e aumentou a produção de lactato e AGCC[16]. Os subprodutos da extração do óleo de jerivá e macaúba estimularam o crescimento de probióticos, como *L. casei*, *L. acidophilus* e *B. lactis*, e não promoveram o crescimento de *Escherichia coli*[17]. O albedo de laranja também

estimulou o crescimento e o metabolismo de *L. acidophilus* e *B. animalis*, com melhor potencial prebiótico do que fibras comerciais[75]. Os resultados deste estudo indicam que fibras intrínsecas ao alimento são mais efetivas na modulação positiva da microbiota do que fibras isoladas.

Em ensaios de fermentação colonica *in vitro*, a farinha digerida de subprodutos de frutas (laranja, maracujá, melão) e vegetais (alface, abobrinha, cenoura, espinafre, hortelã, inhame, pepino e rúcula) demonstrou efeito positivo no crescimento de *Bifidobacterium* e *Lactobacillus* em amostras fecais de doadores, igual ou melhor que o FOS (prebiótico padrão), com produção de butirato após 24 h de fermentação[18]. Os autores sugerem que a composição da farinha, rica em fibras insolúveis (39%), além de um teor significativo de proteína (9,5%) e fibra solúvel (9,5%), possa estar associada a esse efeito[18]. Os subprodutos digeridos de acerola (35% fibra insolúvel, 13% fibra solúvel) e goiaba (41% fibra insolúvel, 3% fibra solúvel) promoveram alterações benéficas na composição da microbiota fecal, com aumento da abundância de *Bifidobacterium*, *Lactobacillus–Enterococcus*, *E. rectale–C. coccoides* e *Bacteroides–Prevotella*, e aumento da produção de AGCC durante 24 h de fermentação[15]. O subproduto digerido da jabuticaba (56% de fibra solúvel, 28% de fibra insolúvel, 8% de umidade) também aumentou a abundância de *Lactobacillus–Enterococcus* e *Bifidobacterium* e a produção de AGCC, diminuiu a abundância de *E. rectale–C. cocoides* e *C. histolyticum*, e reduziu os valores de pH após 48 h de fermentação, indicando seu potencial para melhorar a saúde intestinal[76]. Os estudos realizados com os subprodutos de acerola e goiaba[15], e de jabuticaba[76] avaliaram a composição da microbiota fecal pela técnica FISH-FC.

Quanto ao efeito prebiótico avaliado *in vivo*, o consumo de subproduto de acerola por ratas dislipidêmicas aumentou a contagem fecal de *Bifidobacterium* e *Lactobacillus* e a produção de ácidos orgânicos no intestino, e reduziu o acúmulo de gordura visceral e hepática e os níveis de lipídios séricos[77]. A ingestão de dieta hiperlipídica suplementada com casca de jabuticaba aumentou a excreção de triacilgliceróis, reduziu esteatose hepática e estimulou a produção de AGCC em camundongos[78]. A suplementação com extrato de casca de pêssego em camundongos tratados com dieta hiperlipídica aumentou a abundância de *Lactobacillus*, *Bacteroides*, *Akkermansia*, *Roseburia* e *Ruminococcus*, além de aumentar a produção de AGCC e melhorar o metabolismo lipídico[79]. A ingestão de biscoitos enriquecidos com bagaço de azeitona por indivíduos moderadamente hipercolesterolêmicos, durante oito

semanas, resultou em uma tendência de aumento na abundância de bifidobactérias, avaliada pela técnica FISH-FC[80]. Contudo, não há relatos na literatura de estudos sobre o potencial prebiótico da polpa de baru.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar a composição química da polpa de baru e seus efeitos sobre o crescimento e o metabolismo de probióticos e sobre a microbiota colonica humana.

3.2 OBJETIVOS ESPECÍFICOS

- Elaborar e publicar revisões de literatura sobre o efeito prebiótico de polifenóis e sobre o potencial nutricional e funcional do baru.
- Determinar o conteúdo de fibra alimentar total, solúvel e insolúvel, de frutanos e de compostos bioativos, e identificar e quantificar açúcares, ácidos orgânicos, oligossacarídeos e polifenóis na polpa de baru.
- Avaliar a capacidade antioxidante da polpa de baru.
- Avaliar os efeitos da polpa de baru sobre o crescimento e metabolismo de cepas probióticas.
- Avaliar os efeitos da polpa de baru digerida sobre a composição e atividade metabólica da microbiota colonica humana.

4 MATERIAL E MÉTODOS

4.1 ESTUDO DE REVISÃO SISTEMÁTICA

Uma busca sistemática da literatura foi realizada nas bases de dados *PubMed*, *Scopus* e *Web of Science* até 2 de agosto de 2019, utilizando termos relacionados a prebiótico, microbiota intestinal e polifenóis. Os critérios de inclusão utilizados foram os seguintes: (1) estudo controlado randomizado com animais e/ou humanos; (2) intervenção com polifenol isolado ou extrato alimentar; (3) perfil de polifenóis do extrato alimentar disponível; (4) desfechos relacionados à composição da microbiota intestinal, com aumento da abundância de micro-organismos reconhecidos como alvos prebióticos (*Lactobacillus*, *Bifidobacterium*, *Roseburia*, *Eubacterium* e *Faecalibacterium* spp.), incluindo *Akkermansia* spp., e/ou aumento na produção de AGCC, incluindo butirato; e (5) publicação em inglês. Estudos com número inferior a oito animais por grupo, estudos agudos, patologias induzidas por drogas, avaliação da composição da microbiota intestinal por métodos de cultivo e sem a identificação dos gêneros dos micro-organismos foram excluídos da seleção. De acordo com a estratégia de busca inicial, foram recuperados 4.025 artigos, dos quais 1.685 foram excluídos como duplicados. Após análise de títulos e resumos, e revisão de texto completo, 24 artigos preencheram os critérios de elegibilidade e foram incluídos na revisão sistemática (Capítulo 2 desta tese).

4.2 ESTUDO DE REVISÃO CRÍTICA

Revisão crítica da literatura foi realizada a partir de buscas bibliográficas nas bases de dados Scopus, ScienceDirect, SciELO, PubMed, Google Scholar utilizando os seguintes descritores: *Dipteryx alata* Vog., baru, baru fruit (nut, oil, skin, peel and pulp), baru almond, nutritional and bioactive compounds, health effects, functional foods. Diferentes combinações foram realizadas com os descritores utilizando os operadores booleanos. A partir dos documentos recuperados, como resultado da busca realizada entre julho e dezembro de 2020, foram selecionados 50 artigos. Além desses, foram incluídos 37 artigos considerados pertinentes para discussão do

assunto revisado. Ao final, foram incluídos 87 artigos neste estudo de revisão (Capítulo 3 desta tese).

4.3 ESTUDO EMPÍRICO

4.3.1 Aquisição e processamento da amostra

A polpa (epicarpo e mesocarpo) de baru (Figura 2) foi adquirida *in natura* de uma agroindústria no município de Caiapônia (Goiás, Brasil) que processa frutos nativos do bioma Cerrado. Após a aquisição, a amostra foi congelada (-80 ºC), liofilizada em liofilizador de bancada (Liobras, L-108, temperatura: -50 ± 5 ºC, pressão: < 100 µHg, tempo: 48 h), triturada em moinho analítico (A-11, IKA, Campinas, Brasil) para obtenção de partículas com tamanho inferior a 1,0 mm (16 mesh; Bertel, Caieiras, Brasil), embalada a vácuo com proteção à luz, e armazenada a 4 ºC por até 6 meses.

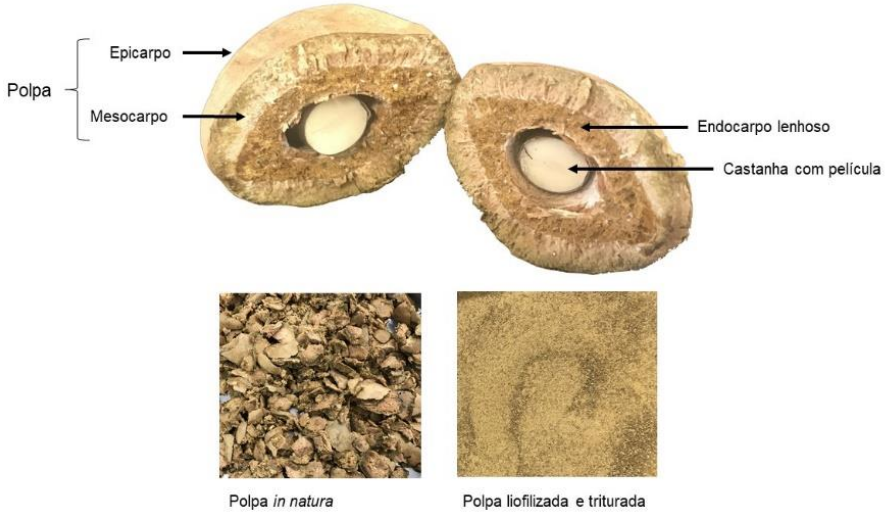

Figura 2. Visão geral do fruto baru e da polpa *in natura* e liofilizada.

4.3.2 Reagentes químicos e padrões externos

Os reagentes Folin-Ciocalteau, Folin-Dennis, 2,2-difenil-1-picrilhidrazila (DPPH), 2,4,6-tris(2-piridil)-s-triazina (TPTZ), trolox, bem como os padrões externos de glicose, frutose, oligossacarídeos (1-kestose, nistose, rafinose), e de alguns compostos fenólicos (ácidos gálico, clorogênico, *p*-cumárico, siríngico, caftárico e caféico, hesperidina, naringenina, procianidina B1, procianidina B2, catequina, epicatequina, cianidina-3,5-diglicosídeo, malvidina-3,5-diglicosídeo, pelargonidina-3,5-diglicosídeo) foram adquiridos da Sigma-Aldrich (St Louis, EUA). Maltose e

ramnose foram adquiridas da Chem Service (West Chester, EUA). Procianidina A2, epigalocatequina galato, epicatequina galato, kaempferol-3-glicosídeo, rutina, quercetina-3-glicosídeo, miricetina, peonidina-3-glicosídeo, petunidina-3-glicosídeo, delfinidina-3-glicosídeo, malvidina-3-glicosídeo, cianidina-3-glicosídeo, pelargonidina -3-glicosídeo foram adquiridos da Extrasynthese (Genay, França). *cis*-Resveratrol e *trans*-resveratrol foram adquiridos da Cayman Chemical Company (Ann Arbor, EUA). Os padrões de ácidos orgânicos (cítrico, tartárico, málico, succínico, lático, fórmico, acético, propionico e butírico) foram adquiridos da Química Vetec (Rio de Janeiro, Brasil). Ácido tânico foi adquirido da Neon (Suzano, Brasil).

O caldo e o ágar de Man, Rogosa e Sharpe (MRS), triptona, e os extratos de carne e de levedura foram adquiridos da Kasvi (São José dos Pinhais, Brasil). Citrato de amônio tribásico, caldo Infusão Cérebro e Coração (BHI), caldo M9, α-amilase, sal biliar, pancreatina e membrana de diálise foram adquiridos da Sigma-Aldrich (St. Louis, EUA). Os seguintes reagentes foram adquiridos de: ágar Eosina Azul de Metileno (EMB) - Merck (Darmstadt, Alemanha); frutooligossacarídeos (FOS, Orafti P95) - Orafti (Oreye, Bélgica); L-cisteína-HCl - Êxodo Científica (Sumaré, Brasil); e pepsina - Dinâmica (Indaiatuba, Brasil).

4.3.3 Caracterização química da polpa de baru

4.3.3.1 Composição centesimal, fibra alimentar e frutanos

A composição centesimal foi realizada por meio das análises de umidade (método 934.06), cinzas (método 940.26), nitrogênio total (método 920.152)[81] e conversão em proteína bruta utilizando o fator de 5,75[82]. Os lipídios totais foram analisados conforme Bligh e Dyer[83]. O teor de carboidratos foi estimado por diferença, subtraindo-se de 100 os valores de umidade, cinzas, proteínas, lipídios e fibra alimentar total. O valor energético foi mensurado por meio dos valores de conversão de Atwater, de 4, 4 e 9 kcal/g para proteínas, carboidratos e lipídios, respectivamente. Os teores de fibra alimentar total, insolúvel e solúvel foram analisados pelo método enzimático-gravimétrico (método 985.29)[81]. Os teores de lignina, hemicelulose e celulose foram determinados pelo método de fibra em detergente ácido e neutro[84]. O conteúdo de frutanos foi determinado pelo método enzimático-espectrofotométrico[81].

4.3.3.2 Análises de açúcares, ácidos orgânicos e oligossacarídeos

Açúcares e ácidos orgânicos foram analisados simultaneamente em um cromatógrafo líquido (Agilent, modelo 1260 Infinity LC) acoplado a um detector de índice de refração (RID) e um detector de arranjo de diodos (DAD). As amostras foram previamente extraídas em água ultrapura a 50 ºC (1:20), homogeneizadas por 5 min, centrifugadas (3000 *x* g, 5 min) e filtradas em membrana de nylon de 0,45 µm (Unifil, Alvorada, RS, Brasil). O volume injetado foi de 10 µL. Uma coluna de troca iônica (Agilent Hi-Plex H; 300 x 7,7 mm) com partículas internas de 8 µm foi utilizada e protegida por uma pré-coluna (PL Hi-Plex H; 5 x 3 mm). A temperatura do compartimento da coluna foi mantida a 70 °C. O fluxo foi de 0,6 mL/min. A fase móvel foi uma solução de H_2SO_4 (4,0 mmol/L). A detecção dos açúcares foi realizada por RID, e a determinação dos ácidos orgânicos foi realizada a 210 nm no DAD[85]. Para todos os compostos analisados, o limite de detecção (LOD) foi inferior a 0,044 g/L, e o limite de quantificação (LOQ) foi inferior a 0,151 g/L.

A determinação de oligossacarídeos foi realizada por Cromatografia Líquida de Alta Eficiência (HPLC) acoplada a RID[86]. As amostras foram extraídas (1:5) em água ultrapura e filtradas em membrana de nylon de 0,45 µm (Unifil, Alvorada, RS, Brasil). O volume de injeção foi de 20 µL. A separação dos compostos foi realizada em uma coluna Synergi™ Hydro-RP C18 com *endcapping* polar (150 x 4,6 mm, 4 µm; Phenomenex, Torrance, EUA) a 35 °C. O fluxo de solvente foi de 0,7 mL/min. Os gradientes utilizados para os solventes A (água ultrapura) e B (acetonitrila) foram: 0 - 8 min: 100% A; 8 - 9 min: 80% B; 9 - 12 min: 80% B; 12 - 13 min: 100% A; 13 - 20 min: 100% A (fluxo de 1,5 mL/min). Para todos os compostos analisados, o LOD foi inferior a 0,042 g/L, e o LOQ foi inferior a 0,109 g/L.

4.3.3.3 Compostos fenólicos e capacidade antioxidante

Para obtenção dos extratos para análise de compostos fenólicos totais, taninos totais e capacidade antioxidante, a polpa de baru liofilizada (500 mg) foi homogeneizada com metanol 50% (25 mL) em banho ultrassônico (USC 2800, Unique, Indaiatuba, Brasil) a 25 °C por 60 min[87]. Em seguida, a mistura foi centrifugada (350-R, MPW Instruments, Warszawa, Polônia) a 4000 rpm por 10 min a 25 °C. Para separação, identificação e quantificação de compostos fenólicos, a polpa de baru liofilizada foi diluída em metanol 70% (1:10), homogeneizada em banho

ultrassônico por 60 min, centrifugada a 3000 *x* g por 5 min e filtrada em membrana de 0,45 µm (Unifil, Alvorada, RS, Brasil)[75].

O conteúdo de compostos fenólicos totais foi determinado de acordo com Singleton e Rossi[88] a 725 nm em espectrofotômetro UV/visível (V-630, Jasco, Indaiatuba, Brasil), utilizando uma curva padrão de ácido gálico (Concentração = [absorbância + 0,0438] / 0,0078; r = 0,9991). Os resultados foram expressos em mg de equivalentes de ácido gálico por 100 g de amostra (mg GAE/100 g). Taninos totais foram analisados pela redução do reagente Folin-Dennis[81] a 760 nm. O ácido tânico foi utilizado como padrão para a curva de calibração (Concentração = [absorbância + 0,0005] / 0,0952; r = 0,9999), e os resultados foram expressos em mg de equivalentes de ácido tânico por 100 g de amostra (mg TAE/100 g).

A separação e quantificação de compostos fenólicos foram realizadas utilizando um cromatógrafo líquido (1260 Infinity LC, Agilent Technologies, St. Clara, EUA) acoplado a um DAD[89,90]. As condições analíticas foram as seguintes: coluna Zorbax Eclipse Plus RP-C18 (100 x 4,6 mm; 3,5 µm), pré-coluna Zorbax C18 (12,6 x 4,6 mm; 5 µm), temperatura de 35 °C e volume de injeção de 20 µL da amostra previamente diluída e filtrada. O fluxo de solvente foi de 0,8 mL/min. Os gradientes utilizados para os solventes A (solução de ácido fosfórico) e B (metanol acidificado com H_3PO_4) foram: 0-5 min - 5% B; 5-14 min - 23% B; 14-30 min - 50% B; 30-33 min - 80% B. A detecção dos compostos foi realizada por comparação com padrões externos. Para todos os compostos analisados, o LOD foi inferior a 0,17 mg/L, e o LOQ foi inferior a 1,41 mg/L.

A capacidade de sequestro do radical livre DPPH foi determinada conforme Brand-Williams, Cuvelier e Berset[91]. Alíquotas (0,1 mL) dos extratos foram adicionadas a 3,9 mL de solução de DPPH (25 mg/L) e mantidas em luminosidade reduzida a 25 ºC por 120 min. A absorbância foi mensurada a 517 nm, e a curva padrão foi preparada com solução de trolox (Concentração = [absorbância + 0,6296] / 0,0006; r = - 0,9989). O ensaio da capacidade de redução do íon férrico (FRAP - Ferric Reducing Antioxidant Power) foi conduzido de acordo com Pulido, Bravo e Saura-Calixto[92]. O reagente FRAP foi obtido a partir de uma mistura de tampão acetato, solução de cloreto férrico e solução de TPTZ. Alíquotas (90 µL) dos extratos foram homogeneizadas com água ultrapura (270 µL) e reagente FRAP (2,7 mL), e mantidas a 37 °C por 30 min. A absorbância da mistura foi medida a 595 nm. A curva padrão foi preparada com solução de trolox (Concentração = [absorbância + 0,0293]

/ 0,0011; r = 0,9979). Os resultados dos ensaios DPPH e FRAP foram expressos em µmol de equivalente de trolox por grama de amostra (µmol TE/g).

4.3.4 Potencial prebiótico *in vitro*

4.3.4.1 Micro-organismos e condições de cultivo

Os probióticos utilizados neste estudo (*Bifidobacterium animalis* subsp. *lactis* BB-12, *Lactobacillus acidophilus* LA-05 e *Lacticaseibacillus casei* L-26) foram fornecidos pela Escola Superior de Biotecnologia, da Universidade Católica Portuguesa (Porto, Portugal). As culturas estoque foram mantidas em caldo MRS contendo glicerol (150 g/L) a -80 °C. Para os ensaios, cada cepa foi cultivada, inicialmente, em caldo MRS a 37 °C, por 20-24 h, para obter suspensões de células com densidade óptica de 0,8 a 655 nm (DO_{655}), e contagem de células viáveis de, aproximadamente, 6 log unidades formadoras de colônias (UFC)/mL em ágar MRS. O caldo e o ágar MRS utilizados para o cultivo de *B. animalis* subsp. *lactis* BB-12 foram suplementados com L-cisteína-HCl (0,5 g/L) e incubados em anaerobiose[93].

As cepas de *Escherichia coli* CCCD-E004 (Cefar Diagnóstica Ltda.) e *E. coli* ATCC 25922 (fornecida pelo Laboratório de Controle Higiênico-Sanitário de Alimentos, da Faculdade de Nutrição, UFG) constituíram o inóculo da mistura entérica. As cepas foram cultivadas em caldo BHI a 37 °C, por 18-20 h, para obter suspensão celular com uma DO_{655} de 0,1 e contagem de células viáveis de, aproximadamente, 6 log UFC/mL em ágar EMB. O inóculo da mistura entérica foi obtido pela mistura das duas diferentes suspensões de *E. coli* (1:1).

4.3.4.2 Preparação dos meios de cultivo bacteriano

O caldo MRS com modificações na composição da fonte de carbono padrão (glicose) foi utilizado como meio para avaliar o potencial prebiótico da polpa de baru[93]. A composição dos diferentes caldos MRS utilizados para cultivar os probióticos foi triptona (10 g/L), extrato de carne (8 g/L), extrato de levedura (4 g/L), fosfato de dipotássio de hidrogênio (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 a sua respectiva fonte de carbono. As fontes de carbono foram glicose, FOS (prebiótico padrão) e polpa de baru, cujas concentrações foram definidas de acordo com a concentração de glicose (20 g/L) do caldo MRS comercial. Os meios foram

submetidos à homogeneização em banho ultrassônico a 37 ºC por 60 min, e esterilizados em autoclave a 120 ºC por 15 min.

4.3.4.3 Enumeração de células viáveis de probióticos

O inóculo de cada probiótico testado foi dispensado (2%, v/v) em frascos estéreis contendo os respectivos caldos de cultivo (10 mL). As misturas foram homogeneizadas e incubadas a 37 °C. Em diferentes intervalos de tempo (0 h – após a homogeneização, 12, 18, 24 e 48 h após a incubação), alíquotas (100 µL) de cada mistura foram diluídas em série em solução salina estéril (8,5 g/L). Alíquotas (20 µL) de cada diluição foram plaqueadas em ágar MRS, utilizando a técnica de inoculação de microgotas. As placas foram incubadas a 37 °C por 48 h, e os resultados foram expressos em log UFC/mL[93].

O inóculo da mistura entérica (2%, v/v) foi adicionado ao caldo M9 contendo glicose (20 g/L), FOS (20 g/L), ou polpa de baru (20 g/L). As misturas foram homogeneizadas e incubadas em condições aeróbicas a 37 °C. Em diferentes intervalos de tempo (0 h e 48 h), alíquotas (100 µL) de cada mistura foram diluídas em série em solução salina estéril (8,5 g/L). Em seguida, alíquotas (20 µL) de cada diluição foram plaqueadas em ágar EMB utilizando a técnica de microgotas. As placas foram incubadas em condições aeróbicas a 37 °C por 24 h, e os resultados foram expressos em log UFC/mL.

4.3.4.4 Escore de atividade prebiótica

O escore de atividade prebiótica foi determinado de acordo com a equação proposta por Huebner, Wehling e Hutkins[94], considerando o crescimento de cada probiótico e da mistura entérica durante a fermentação ($\Delta_{\log UFC/mL}$ = log $UFC/mL_{t=48}$ – log $UFC/mL_{t=0}$) utilizando os substratos (glicose, FOS e polpa de baru), conforme descrita a seguir: Escore de atividade prebiótica = probiótico ($\Delta_{\log UFC/mL}$ prebiótico/$\Delta_{\log UFC/mL}$ glicose) – mistura entérica ($\Delta_{\log UFC/mL}$ prebiótico/$\Delta_{\log UFC/mL}$ glicose). Um escore de atividade prebiótica positivo indica que o substrato foi metabolizado seletivamente pelo probiótico, mas não pela mistura entérica[94].

4.3.4.5 Avaliação da atividade metabólica dos probióticos

A atividade metabólica dos probióticos foi avaliada por meio da determinação do pH, e das concentrações de açúcares e ácidos orgânicos nos diferentes meios de

cultivo. Os valores de pH foram mensurados de acordo com AOAC[81], usando um medidor de pH digital (Adwa AD100, Szeged, Hungria) em diferentes intervalos de tempo. Os açúcares e ácidos orgânicos foram quantificados simultaneamente por HPLC[85], conforme descrito na seção 4.3.3.2.

4.3.5 Digestão gastrointestinal *in vitro*

A digestão da polpa de baru liofilizada foi realizada em quatro fases para simular a digestão oral, gástrica e intestinal seguida por diálise[76]. Alíquotas (10 g) da polpa de baru foram homogeneizadas com água destilada estéril (50 mL). A fase oral foi simulada pela adição de 1,2 mL de α-amilase (100 U/mL em $CaCl_2$ 1 Mm) a solução inicial. O pH da solução foi ajustado para 5,6–6,9 com $NaHCO_3$ 1 M e incubada por 2 min a 37 ºC, 130 rpm em incubadora refrigerada com agitação (TE-424, Tecnal, Piracicaba, Brasil). Na fase gástrica, a pepsina (25 mg/mL em HCl 0,1 M) foi adicionada a mistura (0,05 mL/mL), o pH foi ajustado para 2,0–2,5 com HCl 1 M, e incubada por 90 min a 37 ºC, 130 rpm. Para simular a fase intestinal, uma solução composta por sais biliares e pancreatina (12 g/L e 2 g/L, respectivamente, em $NaHCO_3$ 0,1 M) foi adicionada à mistura (0,25 mL/mL), o pH foi ajustado para 6,5–7,0 com $NaHCO_3$ 1 M, e a mistura foi incubada por 90 min a 37 ºC, 45 rpm. Em seguida, a amostra foi submetida à diálise em membrana de celulose previamente hidratada, a qual foi imersa em solução salina estéril a 4 ºC por 18 h. A amostra digerida e dialisada foi congelada a -80 °C por 24 h, liofilizada e armazenada a 4 ºC.

4.3.6 Fermentação colonica humana *in vitro*

4.3.6.1 Procedimentos éticos

Este estudo foi submetido à apreciação e aprovação pelo Comitê de Ética em Pesquisa da Universidade Federal de Goiás. O protocolo experimental foi aprovado sob o número 4.970.001, de acordo com o parecer consubstanciado (ANEXO A). Os indivíduos selecionados e aptos a participarem da pesquisa foram informados sobre o estudo por meio do Termo de Consentimento Livre e Esclarecido - TCLE (ANEXO B).

4.3.6.2 Coleta das amostras fecais

Os doadores de amostras fecais foram selecionados de acordo com os seguintes critérios de inclusão: idade entre 18 e 65 anos, sem dieta restritiva, sem histórico de intolerâncias alimentares, alergias alimentares ou outras doenças

gastrointestinais e sem uso de probióticos, prebióticos, antibióticos ou medicamentos controlados por pelo menos seis meses antes da coleta[18,95]. Foram selecionados cinco voluntários adultos saudáveis (três homens e duas mulheres) com idade entre 20 e 45 anos. Além do TCLE e das orientações dos pesquisadores, os doadores receberam frascos coletores estéreis e materiais descartáveis necessários para coleta e manuseio asséptico das amostras. Os frascos com as amostras fecais frescas foram transportados para o laboratório de análise em condições anaeróbicas.

4.3.6.3 Fermentação fecal e determinação de pH, açucares, lactato e AGCC

Logo após a coleta (cerca de 60 min), as amostras fecais frescas foram misturadas em quantidades iguais, diluídas (1:10) em solução salina estéril (8,5 g/L) com adição de L-cisteína-HCl (0,5 g/L), homogeneizadas e filtradas em uma camada tripla de gaze estéril para obtenção do *pool* de inoculo fecal fresco. Para a fermentação, o meio de crescimento utilizado foi composto por NaCl (4,5 g/L), KCl (4,5 g/L), $NaHCO_3$ (1,5 g/L), $MgSO_4$ (0,70 g/L), L-cisteína-HCl (0,8 g/L), KH_2PO_4 (0,5 g/L), K_2HPO_4 (0,5 g/L), sais biliares (0,4 g/L), $CaCl_2$ (0,08 g/L), $FeSO_4$ (0,005 g/L), Tween 80 (1 mL/L) e solução de resazurina a 0,025% (4 mL/L) em água destilada estéril. O pH do meio foi ajustado para 6,8 com HCl 1 M[76].

A amostra digerida e dialisada da polpa de baru (20%) foi submetida à fermentação com o *pool* de inoculo fecal fresco (40%) e o meio de crescimento (40%). Além disso, um meio contendo FOS (20%) e um meio sem adição de substrato fermentável (controle negativo) também foram submetidos a fermentação a 37 °C por 48 h em condições anaeróbicas[18,76]. Nos tempos zero e após 24 e 48 h de fermentação, alíquotas foram coletadas para monitorar a atividade metabólica bacteriana por meio da determinação de pH[78] e dos conteúdos de açúcares, lactato e AGCC[85], conforme descrito na seção 4.3.3.2.

4.3.6.4 Hibridização *in situ* por fluorescência (FISH) com citometria de fluxo

A análise de FISH foi realizada com sondas oligonucleotídicas sintetizadas comercialmente (Eurofins Genomics, Ebersberg, Alemanha), marcadas com Cy3 fluorescente, e capazes de emparelhar com a sequência alvo do gene 16S rRNA dos seguintes grupos bacterianos (Tabela 1): Bac 303 - *Bacteroides* e *Prevotella*[96]; Bif 164 – *Bifidobacterium*[97]; Chis 150 - *Clostridium histolyticum*[98]; Erec 482 - *Eubacterium rectale* e *Clostridium coccoides*[98]; e Lab 158 - *Lactobacillus* e *Enterococcus*[99]. As

sondas foram selecionadas para os principais grupos bacterianos frequentemente encontrados no intestino humano (Tabela 1). SYBR Green foi usado para enumerar células totais[80].

Nos diferentes intervalos de tempo (0, 24 e 48 h de fermentação), alíquotas (375 µL) dos meios de fermentação foram fixadas com 1125 µL de solução filtrada (membrana de 0,22 µm e 30 mm) de paraformaldeído (4%) e incubadas *overnight* a 4 ºC. Em seguida, as alíquotas foram centrifugadas (9000 rpm, 5 min, 4 ºC), lavadas duas vezes com PBS 1 M estéril (1000 µL), ressuspensas com PBS 1 M (300 µL) e etanol absoluto (300 µL), filtradas em membrana estéril de 0,45 µm e 33 mm (Merck Millipore, Burlington, EUA), e armazenadas a -20 ºC por 24 h.

Tabela 1. Sondas de oligonucleotídeos de rRNA 16S e condições de hibridização utilizadas na análise de FISH.

Sonda	Grupo bacteriano	Sequência (5' – 3')	Temperatura (ºC)	
			Hibridização	lavagem
Bac 303	*Bacteroides* e *Prevotella*	CCAATGTGGGGGACCTT	45	45
Bif 164	*Bifidobacterium*	CATCCGGCATTACCACCC	50	50
Chis 150	*Clostridium histolyticum* (*Clostridium* clusters I e II)	TTATGCGGTATTAATCTYCCTTT	50	50
Erec 482	*Eubacterium rectale* - *Clostridium coccoides* (*Clostridium* clusters XIVa e XIVb)	GCTTCTTAGTCARGTACCG	50	50
Lab 158	*Lactobacillus* e *Enterococcus*	GGTATTAGCAYCTGTTTCCA	50	50

Para hidridização, as células fixadas (10 µL) foram ressuspensas em 190 µL PBS 1X estéril (Gibco, Gaithersburg, EUA), centrifugadas (4000 rpm, 15 min, 4 ºC), ressuspensas em 200 µL Tris-EDTA (Tris-HCl 100 mM e EDTA 50 mM; pH 8) e centrifugadas (4000 rpm, 15 min, 4 ºC). Amostras foram tratadas com Tris-EDTA (200 µL) e lisozima (1 mg/mL), incubadas em luminosidade reduzida a ± 25 °C por 10 min para permeabilizar as células que receberão as sondas Lab 158 e Bif 164, e centrifugadas (4000 rpm, 15 min, 25 ºC). Posteriormente, todas as amostras foram ressuspendidas com 45 µL de tampão de hibridização (NaCl 0,9 M; Tris-HCl 20 mM [pH 7,5]; 0,1% dodecil sulfato de sódio) e com 5 µL da sonda oligonucleotídica (50 ng/µL) e incubadas com luminosidade reduzida por 4 h em temperatura de hibridização específica para cada sonda (Tabela 1). Após essa etapa, as amostras foram centrifugadas (4000 rpm, 4 min, 25 ºC), ressuspensas em tampão de lavagem (NaCl 0,9 M e Tris-HCl 20 mM; pH 7,5) e incubadas por 30 min em temperatura de

lavagem específica para cada sonda (Tabela 1). As amostras foram centrifugadas (4000 rpm, 15 min, 25 ºC), ressuspensas em PBS 1X (200 µL) e 20 µL de SYBR Green (1:1000 em dimetil sulfóxido ≥ 99,9%), incubadas por 10 min a 25 °C, centrifugadas (4000 rpm, 15 min, 25 ºC) e ressuspendidas com PBS 1X (200 µL). Uma amostra sem a sonda oligonucleotídica e sem SYBR Green (branco) e uma amostra somente com SYBR Green foram preparadas para todas as amostras, seguindo os mesmos procedimentos anteriores.

Os sinais de fluorescência das células foram coletados em um citômetro de fluxo (BD Accuri C6, New Jersey, EUA) pelos canais FL1 (SYBR Green) e FL2 (Bac 303, Bif 164, Chis 150, Erec 482 e Lab 158), e os citogramas de emissão de fluorescência foram registrados usando o software BD Accuri C6. As condições de leituras foram fluxo médio, limite para dispersão direta (FSC) de 30000, e total de 1000 eventos coletados por amostra. Os resultados foram expressos como abundância (%) de células hibridizadas com cada sonda específica em relação ao número total de células enumeradas por SYBR Green[80].

4.3.7 Análise estatística

As análises da composição química foram realizadas em triplicata. Os ensaios *in vitro* de avaliação do potencial prebiótico e da fermentação colonica foram realizados em duplicata, em três experimentos diferentes. Desvios da normalidade dos resíduos foram avaliados pelo teste Shapiro-Wilk. Os dados foram submetidos à análise de variância (ANOVA one-way), seguida pelo teste de Tukey. A análise de componentes principais e as análises de correlação de Pearson foram realizadas usando os valores médios obtidos da fermentação colonica *in vitro*. Todos os testes foram realizados utilizando um nível de significância de 0,05. As análises estatísticas foram conduzidas no software R, versão 4.1.0.

REFERÊNCIAS

1. Alves-Santos AM, Fernandes DC, Naves MMV. Baru (*Dipteryx alata* Vog.) fruit as an option of nut and pulp with advantageous nutritional and functional properties: a comprehensive review. NFS J. 2021;24:26–36.
2. Bento APN, Cominetti C, Simões Filho A, Naves MMV. Baru almond improves lipid profile in mildly hypercholesterolemic subjects: a randomized, controlled, crossover study. Nutr Metab Cardiovasc Dis. 2014;24(12):1330–6.
3. Fernandes DC, Alves AM, Castro GSF, Jordao Junior AA, Naves MMV. Effects of baru almond and Brazil nut against hyperlipidemia and oxidative stress *in vivo*. J Food Res. 2015;4(4):38–46.
4. Lima DC, Alves MR, Noguera NH, et al. A review on Brazilian baru plant (*Dipteryx alata* Vogel): morphology, chemical composition, health effects, and technological potential. Future Foods 2022;5:100146.
5. Santiago GL, Oliveira IG, Horst MA, Naves MMV, Silva MR. Peel and pulp of baru (*Dipteryx alata* Vog.) provide high fiber, phenolic content and antioxidant capacity. Food Sci Technol. 2018;38(2):244–9.
6. Silva D, Oliveira D, Resende O, Silva M, Barcelos K. Nutritional quality of the epicarp and mesocarp flours of baru fruits submitted to drying. Rev Bras Eng Agric Ambient. 2019;23(1):65–70.
7. Shortt C, Hasselwander O, Meynier A, Nauta A, Fernández EN, Putz P, et al. Systematic review of the effects of the intestinal microbiota on selected nutrients and non-nutrients. Eur J Nutr. 2018;57(1):25–49.
8. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN. Role of the normal gut microbiota. World J Gastroenterol. 2015;21(29):8836–47.
9. Tang WHW, Kitai T, Hazen SL. Gut microbiota in cardiovascular health and disease. Circ Res. 2017;120(7):1183–96.
10. Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nat Rev Gastroenterol Hepatol. 2019;16(10):605–16.
11. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol. 2017;14(8):491–502.
12. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes. 2017;8(2):172–84.
13. Ma H, Zhang B, Hu Y, Wang J, Liu J, Qin R, et al. Correlation analysis of intestinal redox state with the gut microbiota reveals the positive intervention of tea polyphenols on hyperlipidemia in high fat diet fed mice. J Agric Food Chem. 2019;67(26):7325–35.
14. Wang L, Zeng B, Liu Z, Liao Z, Zhong Q, Gu L, et al. Green tea polyphenols modulate colonic microbiota diversity and lipid metabolism in high-fat diet treated HFA mice. J Food Sci. 2018;83(3):864–73.
15. Menezes FNDD, Melo FHC, Vieira ARS, Almeida ETC, Lima MS, Aquino J, 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. J Appl Microbiol. 2021;130(4):1323–36.
16. Massa NML, Menezes FNDD, Albuquerque TMR, Oliveira SPA, Lima MS,

Magnani M, 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 Sci Technol. 2020;131:109766.
17. Andrade AC, Marinho JFU, Souza AC, Tavares TS, Dias DR, Schwan RF, et al. Prebiotic potential of pulp and kernel cake from jerivá (*Syagrus romanzoffiana*) and macaúba palm fruits (*Acrocomia aculeata*). Food Res Int. 2020;136:109595.
18. Andrade RMS, Silva S, Costa CMSF, Veiga M, Costa E, Ferreira MSL, et al. Potential prebiotic effect of fruit and vegetable byproducts flour using *in vitro* gastrointestinal digestion. Food Res Int. 2020;137:109354.
19. Sano S, Brito M, Ribeiro J. *Dipteryx alata*: baru. In: Vieira RF, Camillo J, Coradin L, editores. Espécies nativas da flora brasileira de valor econômico atual ou potencial: plantas para o futuro – região Centro-Oeste. Brasília: MMA, 2018. p. 203–15.
20. Silva PN, Dias T, Borges LL, Alves-Santos AM, Horst MA, Silva MR, et al. Total phenolic compounds and antioxidant capacity of baru almond and by-products evaluated under optimizing extraction conditions. Rev Bras Ciênc Agrar. 2020;15(4):1–6.
21. Souza RGM, Gomes AC, Castro IA, Mota JF. A baru almond–enriched diet reduces abdominal adiposity and improves high-density lipoprotein concentrations: a randomized, placebo-controlled trial. Nutrition. 2018;55–56:154–60.
22. Schincaglia RM, Cuppari L, Neri HFS, Cintra DE, Sant'Ana MR, Mota JF. Effects of baru almond oil (*Dipteryx alata* Vog.) supplementation on body composition, inflammation, oxidative stress, lipid profile, and plasma fatty acids of hemodialysis patients: a randomized, double-blind, placebo-controlled clinical trial. Complement Ther Med. 2020;52:102479.
23. Lorenzi H. Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil (volume 1). 8ª ed. Plantarum; 2020. 384 p.
24. Alves AM, Mendonça AL, Caliari M, Cardoso-Santiago RA. Avaliação química e física de componentes do baru (*Dipteryx alata* Vog.) para estudo da vida de prateleira. Pesq Agropec Trop. 2010;40(3):266–73.
25. Ferreira THB, Silva SR, Munhoz CL, Argandoña EJS. Elaboration of biscuits type cookies with pre-treated baru (*Dipteryx alata* Vog.) pulp flour. J Food Meas Charact. 2020;14:3156–62.
26. Lima JCR, Freitas JB, Czeder LP, Fernandes DC, Naves MMV. Qualidade microbiológica, aceitabilidade e valor nutricional de barras de cereais formuladas com polpa e amêndoa de baru. Bol Cent Pesqui Process Aliment. 2010;28(2):331–43.
27. Rocha LS, Cardoso-Santiago RA. Implicações nutricionais e sensoriais da polpa e casca de baru (*Dipterix alata* vog.) na elaboração de pães. Ciênc Tecnol Aliment. 2009;29(4):820–5.
28. Rambo MKD, Rambo MCD, Melo PM, Oliveira NML, Nemet YKS, Scapin E, et al. Sustainability of biorefinery processes based on baru biomass waste. J Braz Chem Soc. 2020;31(2):273–9.
29. Leite NR, Araújo LCA, Rocha PS, Agarrayua DA, Ávila DS, Carollo CA, et al. Baru pulp (*Dipteryx alata* vogel): fruit from the Brazilian savanna protects against oxidative stress and increases the life expectancy of *Caenorhabditis elegans* via SOD-3 and DAF-16. Biomolecules. 2020;10(8):1–22.
30. Togashi M, Sgarbieri VC. Caracterização química parcial do fruto do baru (*Dipteryx alata*, Vog.). Food Sci Technol. 1994;14:85–95.

31. Barizao EO, Boeing JS, Rotta EM, et al. Phenolic Composition of *Dipteryx alata* Vogel Pulp + peel and its antioxidant and cytotoxic properties. J Braz Chem Soc 2021;32(12):2206–14.
32. Martinez KB, Leone V, Chang EB. Western diets, gut dysbiosis, and metabolic diseases: are they linked? Gut Microbes. 2017;8(2):130–42.
33. Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J. 2017;474(11):1823–36.
34. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, et al. What is the healthy gut microbiota composition? a changing ecosystem across age, environment, diet, and diseases. Microorganisms. 2019;7:1–22.
35. Oren A, Garrity GM. Valid publication of the names of forty-two phyla of prokaryotes. Int J Syst Evol Microbiol. 2021;71(10):005056.
36. Huber D, Voith von Voithenberg L, Kaigala GV. Fluorescence *in situ* hybridization (FISH): history, limitations and what to expect from micro-scale FISH? Micro Nano Eng. 2018;1:15–24.
37. Namsolleck P, Thiel R, Lawson P, Holmstrøm K, Rajilic M, Vaughan EE, et al. Molecular methods for the analysis of gut microbiota. Microb Ecol Health Dis. 2004;16(2–3):71–85.
38. Sampaio KB, Nascimento DS, Garcia EF, et al. 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. Arch Microbiol 2022;204:469.
39. So D, Whelan K, Rossi M, Morrison M, Holtmann G, Kelly JT, et al. Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. Am J Clin Nutr. 2018;107(6):965–83.
40. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. Nat Rev Microbiol. 2020;19:55–71.
41. Ríos-Covián D, Ruas-Madiedo P, Margolles A, Gueimonde M, de los Reyes-Gavilán CG, Salazar N. Intestinal short chain fatty acids and their link with diet and human health. Front Microbiol. 2016;7:1–9.
42. Morrison DJ, Preston T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microbes. 2016;7(3):189–200.
43. Venegas DP, De la Fuente MK, Landskron G, González MJ, Quera R, Dijkstra G, et al. Short chain fatty acids (SCFAs) mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. Front Immunol. 2019;10:1–16.
44. Schoeler M, Caesar R. Dietary lipids, gut microbiota and lipid metabolism. Rev Endocr Metab Disord. 2019;20(4):461–72.
45. O'Riordan KJ, Collins MK, Moloney GM, Knox EG, Aburto MR, Fülling C, et al. Short chain fatty acids: microbial metabolites for gut-brain axis signalling. Mol Cell Endocrinol. 2022;546:1–18.
46. Ren M, Zhang H, Qi J, Hu A, Jiang Q, Hou Y, et al. An almond-based low carbohydrate diet improves depression and glycometabolism in patients with type 2 diabetes through modulating gut microbiota and GLP-1: a randomized controlled trial. Nutrients. 2020;12(10):1–21.
47. Medina-Vera I, Sanchez-Tapia M, Noriega-López L, Granados-Portillo O, Guevara-Cruz M, Flores-López A, et al. A dietary intervention with functional foods reduces metabolic endotoxaemia and attenuates biochemical abnormalities by modifying faecal microbiota in people with type 2 diabetes. Diabetes Metabol. 2019;45(2):122–31.

48. Scott KP, Grimaldi R, Cunningham M, Sarbini SR, Wijeyesekera A, Tang MLK, et al. Developments in understanding and applying prebiotics in research and practice—an ISAPP conference paper. J Appl Microbiol. 2020;128(4):934–49.
49. Zhang Z, Lv J, Pan L, Zhang Y. Roles and applications of probiotic Lactobacillus strains. Appl Microbiol Biotechnol. 2018;102(19):8135–43.
50. De Angelis M, Calasso M, Cavallo N, et al. Functional proteomics within the genus *Lactobacillus*. Proteomics. 2016;16(6):946–62.
51. 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 Food Sci. Technol. 2021;115:486–99.
52. Jungersen M, Wind A, Johansen E, Christensen J, Stuer-Lauridsen B, Eskesen D. The science behind the probiotic strain *Bifidobacterium animalis* subsp. *lactis* BB-12®. Microorganisms. 2014;2(2):92–110.
53. Kelly SM, Munoz-Munoz J, van Sinderen D. Plant glycan metabolism by Bifidobacteria. Frontiers Microbiol. 2021;12:609418.
54. Stephen AM, Champ MMJ, Cloran SJ, Fleith M, Van Lieshout L, Mejborn H, et al. Dietary fibre in Europe: current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. Nutr Res Rev. 2017;30:149–90.
55. Rezende ESV, Lima GC, Naves MMV. Dietary fibers as beneficial microbiota modulators: a proposal classification by prebiotic categories. Nutrition. 2021;89:111217.
56. Ge Q, Li H, Zheng Z, Yang K, Li P, Xiao Z, et al. *In vitro* fecal fermentation characteristics of bamboo insoluble dietary fiber and its impacts on human gut microbiota. Food Res Int. 2022;156:111173.
57. Wang B, Yu H, He Y, Wen L, Gu J, Wang X, et al. Effect of soybean insoluble dietary fiber on prevention of obesity in high-fat diet fed mice: via regulation of the gut microbiota. Food Funct. 2021;12(17):7923–37.
58. Rodríguez-Daza MC, Pulido-Mateos EC, Lupien-Meilleur J, Guyonnet D, Desjardins Y, Roy D. Polyphenol-mediated gut microbiota modulation: toward prebiotics and further. Front Nutr. 2021;8:1–24.
59. Cunningham M, Azcarate-Peril MA, Barnard A, et al. Shaping the future of probiotics and prebiotics. Trends Microbiol. 2021;29(8):667–85.
60. Rothwell JA, Perez-Jimenez J, Neveu V, Medina-Remon A, M'Hiri N, Garcia-Lobato P, et al. Phenol-Explorer 3.0: a major update of the Phenol-Explorer database to incorporate data on the effects of food processing on polyphenol content. Database. 2013;2013:bat070.
61. Chai SC, Davis K, Zhang Z, Zha L, Kirschner KF. Effects of tart cherry juice on biomarkers of inflammation and oxidative stress in older adults. Nutrients. 2019;11(2):1–10.
62. Fang C, Kim H, Yanagisawa L, Bennett W, Sirven MA, Alaniz RC, et al. Gallotannins and *Lactobacillus plantarum* WCFS1 mitigate high-fat diet-induced inflammation and induce biomarkers for thermogenesis in adipose tissue in gnotobiotic mice. Mol Nutr Food Res. 2019;63(9):1–12.
63. Alves-Santos AM, Sugizaki CSA, Lima GC, Naves MMV. Prebiotic effect of dietary polyphenols: a systematic review. J Funct Foods. 2020;74:104169.
64. Liu Z, Bruijn WJC, Bruins ME, Vincken JP. Microbial metabolism of theaflavin-3,3′-digallate and its gut microbiota composition modulatory effects. J Agric Food Chem. 2021;69(1):232–45.

65. Tzounis X, Vulevic J, Kuhnle GGC, George T, Leonczak J, Gibson GR, et al. Flavanol monomer-induced changes to the human faecal microflora. Br J Nutr. 2008;99(4):782–92.
66. González-Sarrías A, Romo-Vaquero M, García-Villalba R, Cortés-Martín A, Selma MV, Espín JC. The endotoxemia marker lipopolysaccharide-binding protein is reduced in overweight-obese subjects consuming pomegranate extract by modulating the gut microbiota: a randomized clinical trial. Mol Nutr Food Res. 2018;62(11):1–10.
67. Mayo B, Vázquez L, Flórez AB. Equol: a bacterial metabolite from the daidzein isoflavone and its presumed beneficial health effects. Nutrients. 2019;11(9):1–20.
68. D'Amico D, Andreux PA, Valdés P, Singh A, Rinsch C, Auwerx J. Impact of the natural compound urolithin A on health, disease, and aging. Trends Mol Med. 2021;27(7):687–99.
69. Silva LMR, Figueiredo EAT, Ricardo NMPS, Vieira IGP, Figueiredo RW, Brasil IM, et al. Quantification of bioactive compounds in pulps and by-products of tropical fruits from Brazil. Food Chem. 2014;143:398–404.
70. Sagar NA, Pareek S, Sharma S, Yahia EM, Lobo MG. Fruit and vegetable waste: bioactive compounds, their extraction, and possible utilization. Compr Rev Food Sci Food Saf. 2018;17(3):512–31.
71. Majerska J, Michalska A, Figiel A. A review of new directions in managing fruit and vegetable processing by-products. Trends Food Sci Technol. 2019;88:207–19.
72. Oliveira MM, Lago A, Dal' Magro GP. Food loss and waste in the context of the circular economy: a systematic review. J. Clean. Prod. 2021;294:126284.
73. Cruz IA, Andrade LRS, Bharagava RN, et al. Valorization of cassava residues for biogas production in Brazil based on the circular economy: an updated and comprehensive review. Clean. Eng. Technol. 2021;4:100196.
74. Duarte FND, Rodrigues JB, Lima MC, Lima MS, Pacheco MTB, Pintado MME, et al. Potential prebiotic properties of cashew apple (*Anacardium occidentale* L.) agro-industrial byproduct on *Lactobacillus* species. J Sci Food Agric. 2017;97(11):3712–9.
75. Rezende ESV, Lima GC, Lima MS, Coelho ASG, Naves MMV. Prebiotic potential of isolated commercial dietary fibres compared to orange albedo in *Lactobacillus* and *Bifidobacterium* species. Bioact Carbohydr Diet Fibre. 2022;28:100316.
76. Massa NML, Oliveira SPA, Rodrigues NPA, Menezes FNDD, Lima MS, Magnani M, et al. *In vitro* colonic fermentation and potential prebiotic properties of pre-digested jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg) by-products. Food Chem. 2022;388:133003.
77. Batista KS, Alves AF, Lima MDS, Silva LA, Lins PP, Gomes JAS, et al. Beneficial effects of consumption of acerola, cashew or guava processing by-products on intestinal health and lipid metabolism in dyslipidaemic female Wistar rats. Br J Nutr. 2018;119:30–41.
78. Batista AG, Silva-Maia JK, Mendonça MCP, Soares ES, Lima GC, Bogusz Junior S, et al. Jaboticaba berry peel intake increases short chain fatty acids production and prevent hepatic steatosis in mice fed high-fat diet. J Funct Foods. 2018;48:266–74.
79. Kan J, Chen C, Huo T, Xie W, Hui Y, Liu J, et al. Polyphenolic-enriched peach peels extract regulates lipid metabolism and improves the gut microbiota composition in high fat diet-fed mice. J Funct Foods. 2020;72:104082.

80. Conterno L, Martinelli F, Tamburini M, Fava F, Mancini A, Sordo M, et al. Measuring the impact of olive pomace enriched biscuits on the gut microbiota and its metabolic activity in mildly hypercholesterolaemic subjects. Eur J Nutr. 2019;58(1):63–81.
81. AOAC - Association of Official Analytical Chemists. Official Methods of Analysis of AOAC International 20 th ed. 2016. 3100 p.
82. Brasil. Agência Nacional de Vigilância Sanitária. Resolução RDC nº 360, de 23 de dezembro de 2003. Aprova o regulamento técnico sobre rotulagem nutricional de alimentos embalados. Brasília (DF): ANVISA; 2003. Disponível em: https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2003/res0360_23_12_2003.html
83. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 1959;37(8):911–7.
84. Trujillo AI, Marichal MJ, Carriquiry M. Comparison of dry matter and neutral detergent fibre degradation of fibrous feedstuffs as determined with *in situ* and *in vitro* gravimetric procedures. Anim. Feed Sci. Technol. 2010;161(1–2):49–57.
85. Coelho EM, Padilha CVS, Miskinis GA, Sá AGB, Pereira GE, Azevêdo LC, 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. J Food Compos Anal. 2018;66:160–7.
86. Lima MS, Nunes PC, Silva BLA, Padilha CVS, Bonfim THF, Stamford TLM, 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. J Food Sci Technol. 2019;56(10):4575–84.
87. Xu K, Alves-Santos AM, Dias T, et al. Grumixama (*Eugenia Brasiliensis* lam.) cultivated in the cerrado has high content of bioactive compounds and great antioxidant potential. Ciênc Rural 2020; 50(4): e20190630.
88. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16:144–58.
89. Padilha CVS, Miskinis GA, Souza MEAO, Pereira GE, Oliveira D, Bordignon-Luiz MT, 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 Chem. 2017;228:106–15.
90. Dutra MCP, Rodrigues LL, Oliveira D, Pereira GE, Lima MS. Integrated analyses of phenolic compounds and minerals of Brazilian organic and conventional grape juices and wines: validation of a method for determination of Cu, Fe and Mn. Food Chem. 2018;269:157–65.
91. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci Technol. 1995;28:25–30.
92. Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J Agric Food Chem. 2000;48:3396–402.
93. Albuquerque TMR, Borges CWP, Cavalcanti MT, Lima MS, Magnani M, Souza EL. Potential prebiotic properties of flours from different varieties of sweet potato (*Ipomoea batatas* L.) roots cultivated in Northeastern Brazil. Food Biosci. 2020;36:100614.
94. Huebner J, Wehling RL, Hutkins RW. Functional activity of commercial prebiotics. Int Dairy J. 2007;17(7):770–5.
95. Guergoletto KB, Costabile A, Flores G, Garcia S, Gibson GR. *In vitro* fermentation of juçara pulp (*Euterpe edulis*) by human colonic microbiota. Food Chem.

2016;196:251–8.
96. Manz W, Amann R, Ludwig W, Vancanneyt M, Schleifer KH. Application of a suite of 16s rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment. Microbiology. 1996;142(5):1097–106.
97. Langendijk PS, Jansen GJ, Raangs GC, Kamphuis GERR, Wilkinson MHF, Welling GW. Quantitative fluorescence *in situ* hybridization of *Bifidobacterium* spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. Appl Environ Microbiol. 1995;61(8):3069–75.
98. Franks AH, Harmsen HJM, Raangs GC, Jansen GJ, Schut F, Welling GW. Variations of bacterial populations in human feces measured by fluorescent *in situ* hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. Appl Environ Microbiol. 1998;64(9):3336–45.
99. Harmsen HJM, Elfferich P, Schut F, Welling GW. A 16S rRNA-targeted probe for detection of Lactobacilli and Enterococci in faecal samples by fluorescent *in situ* hybridization. Microb Ecol Health Dis. 1999;11(1):3–12.

CAPÍTULO 2

ARTIGO CIENTÍFICO I (REVISÃO SISTEMÁTICA)

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Prebiotic effect of dietary polyphenols: A systematic review

Aline Medeiros Alves-Santos, Clara Sandra Araújo Sugizaki, Glaucia Carielo Lima, Maria Margareth Veloso Naves*

School of Nutrition, Federal University of Goiás (UFG), Goiânia, Goiás, Brazil

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ABSTRACT

Prebiotics are substrates selectively metabolized by hindgut microorganisms conferring health benefits. Recent studies suggest polyphenols as candidate to prebiotics. Thus, this systematic review aimed to investigate the prebiotic effect of dietary polyphenols in preclinical and clinical studies. Animal studies demonstrated that the consumption of polyphenols, especially catechins, anthocyanins and proanthocyanidins, increases the abundance of *Lactobacillus*, *Bifidobacterium*, *Akkermansia*, *Roseburia*, and *Faecalibacterium* spp. Moreover, polyphenols supplementation increased the production of short-chain fatty acids (SCFA), including butyrate. The included clinical trials showed an increased abundance of *Lactobacillus acidophilus*, *Bifidobacterium* and *Faecalibacterium* spp., and a reduction in plasma lipopolysaccharide-binding protein after the consumption of anthocyanins and ellagic acid. In conclusion, there is strong evidence in preclinical studies that dietary polyphenols can stimulate both the growth of microorganisms identified as prebiotic targets and an increase in the production of SCFA. Therefore, clinical trials are warranted to investigate the prebiotic effect of dietary polyphenols on humans.

1. Introduction

Prebiotics are defined as substrates selectively utilized by the host's microorganisms resulting in benefits for metabolic health, gastrointestinal system, bone health and mental health. Some dietary fibers, especially resistant oligosaccharides (inulin, fructo-oligosaccharides and galacto-oligosaccharides), are well-recognized in literature as prebiotics (Gibson et al., 2017). Besides dietary fibers, recent studies have shown the interaction between polyphenols and the gut microbiota, suggesting them as candidate compounds to prebiotics (Sanders, Merenstein, Reid, Gibson, & Rastall, 2019; Shortt et al., 2018; Singh, Cabral, Kumar, Ganguly, & Pandey, 2019).

Polyphenols are secondary metabolites of plants, characterized by aromatic rings bearing one or more hydroxyl groups in their chemical structure, ranging from that of a simple phenolic molecule to that of a complex high-molecular mass polymer (Mojzer et al., 2016). These compounds have low bioavailability and extensive metabolism in the large intestine, favoring interactions with intestinal microorganisms (Bian, Wei, Zhao, & Li, 2020). Actually, there is a bidirectional interaction, in which polyphenols modulate the gut microbiota and, conversely, microorganisms can modulate the activity of the phenolic compounds. This interaction can regulate the metabolism and the bioavailability of polyphenols, converting them into metabolites, which may have different effects on the host health (Singh et al., 2019).

Dietary polyphenols are associated with a reduced risk of cardiometabolic diseases when regularly consumed (Noad et al., 2016). Studies show that polyphenols have antioxidant, anti-inflammatory (Chai, Davis, Zhang, Zha, & Kirschner, 2019), anti-obesogenic, antilipidemic (Fang et al., 2019) and anti-diabetic (Paquette et al., 2017) activities. However, the role of dietary polyphenols in health largely depends on their metabolism, absorption and bioavailability processes which are, in turn, related to the gut microbiota modulation, in terms of composition and functionality. Although polyphenols are currently recognized as modulators of the gut microbiota composition, there is still no conclusive evidence of their prebiotic effect (Cueva, Silva, Pinillos, Bartolomé, & Moreno-Arribas, 2020). The prebiotic effect of each polyphenol can be influenced by the food source and the chemical structure of the compound, along with the individual differences in the gut microbiota composition (Serreli & Deiana, 2019). Therefore, establishing a relationship among the polyphenols consumption, the growth of microorganisms recognized as prebiotic targets, the metabolites generated and the effect on health is somewhat of a challenge. This systematic review aimed to investigate the available evidence of the prebiotic effect of dietary polyphenols.

* Corresponding author at: School of Nutrition, UFG, Rua 227, Setor Leste Universitário, 74605-080 Goiânia, Goiás, Brazil.
E-mail addresses: alinemedeiros@ufg.br (A.M. Alves-Santos), maria_margareth_veloso@ufg.br (M.M.V. Naves).

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Table 1
Detailed search terms in the databases (August 2, 2019).

Database	Search term	N° of items found (total = 4025)
PubMed	(polyphenols[MeSH Terms] or flavonoids [MeSH Terms] or tannins [MeSH Terms] or lignans [MeSH Terms] or stilbenes [MeSH Terms] or curcumin [MeSH Terms] or "phenolic acids" [Title/Abstract]) AND (microbiota [MeSH Terms] or "human microbiome" [MeSH Terms] or "gut microbiota" [Title/Abstract] or "gut microbiome" [Title/Abstract] or prebiotic [MeSH Terms] or firmicutes [Title/Abstract] or bacteroidetes [MeSH Terms])	796
Scopus	(TITLE-ABS-KEY ((polyphenols OR flavonoids OR tannins OR lignans OR stilbenes OR curcumin OR "phenolic acids")) AND TITLE-ABS-KEY ((microbiota OR "human microbiome" OR "gut microbiota" OR "gut microbiome" OR prebiotic OR firmicutes OR bacteroidetes)))	1633
Web of Science	#1 TS = (polyphenols OR flavonoids OR tannins OR lignans OR stilbenes OR curcumin OR "phenolic acids") AND #2 TS = (microbiota OR "human microbiome" OR "gut microbiota" OR "gut microbiome" OR prebiotic OR firmicutes OR bacteroidetes)	1596

2. Material and methods

2.1. Search strategy and study selection

In accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2015), a systematic literature search was carried out through the PubMed, Scopus and Web of Science databases up to August 02, 2019, using terms related to prebiotic, gut microbiota and polyphenols (Table 1). After the removal of duplicates using a bibliographic reference manager, two authors independently reviewed the titles and abstracts of each paper identified in the search. This procedure was performed using a systematic review software. The selected studies were retrieved for full-text analysis and eligible articles were identified. Afterwards, they were reanalyzed for details of the study design and their outcome to select the papers included in this review. Any disagreements in this regard were solved by the means of discussion with a third author.

2.2. Inclusion and exclusion criteria

The following criteria were applied for inclusion: (1) a randomized controlled study design using animals and/or humans; (2) intervention with either isolated polyphenol or food extract; (3) phenolic compounds profile of the food extract available; (4) outcomes related to changes in the gut microbiota composition, with stimulation of microorganisms recognized as prebiotic targets (*Lactobacillus, Bifidobacterium, Roseburia, Eubacterium,* and *Faecalibacterium* spp.) (Gibson et al., 2017), including the *Akkermansia* spp. (Sanders et al., 2019), and/or an increase in the production of short-chain fatty acids (SCFA), including butyrate; and (5) publishing in English. Studies with a lower than eight number of animals per group, acute studies, drug-induced pathologies, gut microbiota composition assessment by culture plate methods, and without the microorganism genera identification were excluded.

2.3. Data extraction

The included studies were reviewed and the following data were abstracted: sample source, exposure dosage, animal model or study population (subjects and sample size), intervention period, diet type, outcomes related to gut microbiota and host health, paper's first author and date of publication.

2.4. Quality assessment

The quality assessment of animal studies was conducted using the SYRCLE's risk of bias tool (Hooijmans et al., 2014). Cochrane Collaboration's tool (Higgins et al., 2011) was used for the quality assessment of clinical trials.

3. Results

3.1. Description of studies

The initial search strategy yielded 4025 articles, out of which 1685 were excluded as duplicates. After analysis of titles and abstracts, 2044 records were excluded, including *in vitro* studies, review articles, conference abstracts, letters, protocols, editorials, and unavailable full-texts, and 296 were selected for full-text review. Among these, 242 studies were excluded according to the exclusion criteria. Thus, full-text review of 54 eligible studies were made, excluding 30 articles for different reasons: there was no growth of microorganisms recognized as prebiotic targets (Casanova-Marti et al., 2018; Cheng, Chen, Zhang, et al., 2019; Cires et al., 2019; Collins et al., 2016; Cowan et al., 2014; González-Sarrías et al., 2017; Griffin et al., 2017; Guo, Tang, et al., 2018; Li, Liu, Liu, Liao, & Zou, 2019; Most, Penders, Lucchesi, Goossens, & Blaak, 2017; Porras et al., 2017; Remely et al., 2017; Shen, Wan, Wang, & Jiang, 2019; Sung et al., 2017; Tan et al., 2018; Unno & Osakabe, 2018; Wang et al., 2019; Yu et al., 2019; Yuan et al., 2018; Zhang, Dong, et al., Zhang, Zhang, et al., 2018; Zhang, Wu, Li, Xin, & Liu, 2019; Zhou, Zhang, Arikawa, & Chen, 2019); there was no increase in the production of butyrate (Ginés et al., 2019; Grzelak-Błaszczyk et al., 2018; Zhou, Tang, Shen, & Wang, 2018); and extracts contained other compounds such as oligosaccharides, polysaccharides or dietary fiber (Chiu et al., 2017; Gao et al., 2018; Garcia-Mazcorro et al., 2018; Romo-Vaquero et al., 2014). Finally, 24 articles (22 animal studies and 2 clinical trials) fulfilled the eligibility criteria and were included in this systematic review (Fig. 1). The animal studies were organized into two sections, according to polyphenol classes and considering the majority compound of the polyphenols profile available in the studies: flavonoids and other polyphenols (lignans, phenolic acids, stilbenes and vanillin) (Table 2). Data on the polyphenol class and subclass were extracted from the Phenol-Explorer database (www.phenol-explorer.eu).

3.2. Prebiotic effect of dietary polyphenols

3.2.1. Animal studies

3.2.1.1. Flavonoids. The flavonoids were the most investigated phenolic compounds in relation to the effects on the gut microbiota composition and the benefits to the host health. Prebiotic effect of anthocyanins, a subclass of the flavonoids, was evaluated in three animal studies (Anhê et al., 2018; Li, Wu, et al., 2019; Van Hul et al., 2018). In a study with Arctic berry extracts (Anhê et al., 2018), and another with cinnamon bark and grape pomace extracts (Van Hul et al., 2018), the most abundant polyphenols present in the extracts were anthocyanins and proanthocyanidins. However, the authors associated the main outcomes with the presence of the proanthocyanidins. The proanthocyanidins or procyanidins effects on the gut microbiota were investigated in other three studies. A marked increase in the abundance of *Akkermansia* was observed after the consumption of polymeric

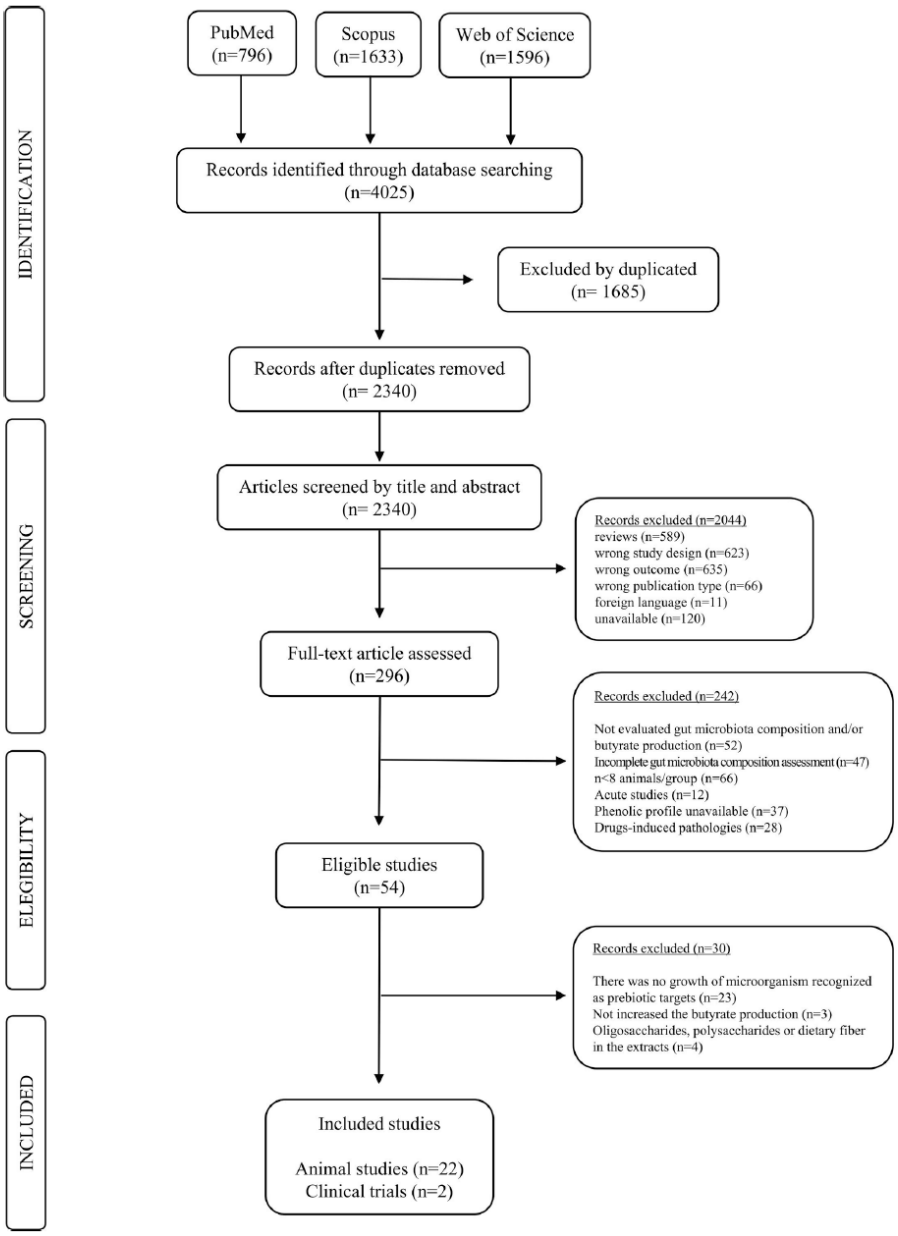

Fig. 1. Flowchart of the systematic review.

procyanidins from apple (Masumoto et al., 2016), and cranberry extract rich in proanthocyanidins (Anhê et al., 2015, 2017). Moreover, body weight gain reduction, insulin sensitivity improvement, upregulation of genes involved in lipid catabolism, and downregulation of pro-inflammatory genes in the liver were the health benefits associated with the consumption of proanthocyanidins (Anhê et al., 2015, 2017; Masumoto et al., 2016).

The prebiotic effect of the flavanols (epigallocatechin gallate, epigallocatechin, epicatechin gallate, epicatechin), a flavonoid subclass, was characterized in seven animal studies. The main food sources investigated were green tea, black tea, oolong tea, and Pu-erh tea. The intervention doses were very different among studies, and intervention periods ranged from 28 to 196 days (Dey et al., 2019; Henning et al., 2018; Liu et al., 2019; Lu et al., 2019; Ma et al., 2019; Wang et al., 2018; Xia et al., 2019). An increase in the abundance of *Akkermansia* and *Bifidobacterium* was observed in mice fed a high-fat diet after the consumption of green tea polyphenols (Dey et al., 2019; Ma et al., 2019; Wang et al., 2018). Supplementation of the oolong tea extract and decaffeinated black tea extract increased the butyrate production (Liu et al., 2019; Henning et al., 2018), while the Pu-erh tea extract consumption stimulated the *Akkermansia* and *Roseburia* growth (Lu et al., 2019; Xia et al., 2019).

Table 2
Characteristics of included studies on prebiotic effect of dietary polyphenols (n = 24).

Compound (food)	Dose	Animal/Human	Study design		Main outcome		Reference
			Period (days)	Diet	Gut microbiota	Health	
			Animal studies				
Flavonoids							
Anthocyanins (bilberry extract)	10, 20 or 40 mg extract/kg bw/day (IG)	Sprague-Dawley rats (n = 10/group)	70	STD	↑ *Lactobacillus* ↑ cecal SCFA ↓ *Firmicutes/Bacteroidetes* ratio	↓ digestive enzymes activity	Li, Wu, et al. (2019)
Anthocyanins and proanthocyanidins (Arctic berry extracts)	200 mg extract/kg bw/day (water)	Male C57BL/6J mice (n = 12/group)	56	HFHS	↑ *Akkermansia muciniphila* (NS) cecal SCFA	↑ insulin sensitivity	Anhê et al. (2018)
Anthocyanins (grape pomace extract) and proanthocyanidin A (cinnamon bark extract)	grape pomace: 2 g extract/kg diet cinnamon bark: 8.2 g extract/kg diet	Male C57BL/6J mice (n = 14/group)	56	HFD	↑ *Roseburia* (NS) cecal SCFA	↓ fat mass gain ↓ liver steatosis	Van Hul et al. (2018)
Catechins (green tea extract)	2% of extract (diet)	Male C57BL/6J mice (n = 10/group)	56	HFD	↑ *Bifidobacterium* and *Akkermansia muciniphila* ↓ *Firmicutes/Bacteroidetes* ratio	↓ adiposity	Dey et al. (2019)
Catechins (green tea polyphenols)	0.05, 0.2 or 0.8% of tea polyphenols (diet)	HFA C57BL/6J mice (n = 8/group)	56	HFHS	↑ fecal acetate and butyrate ↓ *Firmicutes/Bacteroidetes* ratio	↓ bw gain ↓ serum lipid profile, glucose and insulin	Wang et al. (2018)
Catechins (green tea polyphenols)	100, 200 or 400 mg of green tea polyphenols/kg bw/day (IG)	SPF C57BL/6 mice (n = 8/group)	84	HFD	↑ *Bifidobacterium*, *Akkermansia*, *Roseburia* (the highest dose)	↑ expression of hepatic lipid metabolism genes	Ma et al. (2019)
Catechins and caffeine (green tea, black tea and oolong tea water extracts)	1% tea extract (diet)	Male C57BL/6J mice (n = 12/group)	196	HFD	↑ fecal SCFA (oolong tea) ↓ plasma LPS	↑ glucose tolerance ↓ bw gain	Liu et al. (2019)
Catechins (decaffeinated green and black tea polyphenol extracts)	0.5 g extract/100 g diet	Male C57BL/6J Mice (n = 12/group)	28	HFHS	↑ fecal propionate and butyrate (black tea) ↓ *Firmicutes* ↑ *Bacteroidetes*	Induced bw loss	Henning et al. (2018)
Catechins (ripened Pu-erh tea extract)	0.1, 0.2, or 0.4% of extract (water)	Male C57BL/6N mice (n=10/group)	56	HFD	↑ *Roseburia* and *Akkermansia* ↓ *Firmicutes/Bacteroidetes* ratio ↓ serum LPS	↓ bw gain and metabolic endotoxemia	Lu et al. (2019)
Catechins and caffeine (aqueous raw and ripe Pu-erh tea extracts)	0.15 or 0.4 mg extract/kg bw/day (diet)	Male Wistar rats (n=18/group)	77	HFHS	↑ *Akkermansia muciniphila* ↑ *Firmicutes* ↓ *Bacteroidetes*	↑ glycolysis	Xia et al. (2019)
Genistein	3 mg/kg bw/day (diet)	Male C57BL6 mice (n=8/group)	180	HFD	↑ *Faecalibacterium* ↓ serum LPS	↓ serum triglycerides	López et al. (2018)
Hydroxysafflor yellow A	200 mg/kg bw/day (IG)	SPF C57BL/6J mice (n=8/group)	84	HFD	↑ *Akkermansia* ↑ cecal SCFA ↓ *Firmicutes/Bacteroidetes* ratio	↓ inflammation	Liu et al. (2018)
Isoflavones (soy extract)	150 or 450 mg extract/kg bw/day (diet)	Male Sprague Dawley rats (n=16/group)	91	HFD	↑ *Faecalibacterium* ↑ fecal butyrate and ↓ serum LPS ↓ *Firmicutes/Bacteroidetes* ratio	↓ oxidative damage and inflammation	Luo et al. (2019)
Naringenin (*S. chinensis* pollen extract)	7.86 or 15.72 g extract/kg bw/day (IG)	Male C57BL/6 mice (n=12/group)	112	HFD	↑ *Lactobacillus*	↓ fasting blood glucose	Cheng, Chen, Liu, Zhao, and Cao (2019)

(*continued on next page*)

Table 2 (*continued*)

Compound (food)	Dose	Animal/Human	Study design		Main outcome		Reference
			Period (days)	Diet	Gut microbiota	Health	
Polymeric and oligomeric procyanidin (apple)	0.5% procyanidins (diet)	C57BL/6J male mice (n = 10/group)	140	HFHS	↑ *Akkermansia* (eight times) ↓ *Firmicutes/Bacteroidetes* ratio ↓ serum LPS	Attenuate bw gain	Masumoto et al. (2016)
Proanthocyanidin (cranberry extract)	200 mg extract/kg bw/day (water)	Male C57Bl/6J mice (n = 8–11/group)	147	HFHS	↑ *Akkermansia muciniphila* ↓ *Firmicutes/Bacteroidetes ratio*	↑ insulin sensitivity	Anhê et al. (2017)
Proanthocyanidin (cranberry extract)	200 mg extract/kg bw/day (IG)	Male C57Bl/6J mice (n = 12/group)	56	HFHS	↑ *Akkermansia* spp. ↓ plasma LPS	↓ bw gain ↑ insulin sensitivity	Anhê et al. (2015)
Other polyphenols							
Lignans - syringaresinol	10 or 50 mg/kg bw/day (diet)	Male C57BL/6 mice (n = 12/group)	70	STD	↑ *Lactobacillus* and *Bifidobacterium* ↓ serum LPS-binding protein	↑ immune system	Cho et al. (2016)
Phenolic acids - dicaffeoylquinic acids (*Ilex kudingcha)*	3.3 or 10 mg/mouse (IG)	SPF male C57BL/6 mice (n = 8/group)	56	HFD	↑ *Bifidobacterium* and *Akkermansia* ↓ serum LPS	↓ inflammation	Xie et al. (2019)
Stilbenes - pterostilbene	15 mg/kg bw/day (IG)	Zucker (*fa/fa*) rats (n = 10/group)	42	STD	↑ *Akkermansia muciniphila* ↓ *Firmicutes*	↑ insulin sensitivity	Etxeberria et al. (2017)
Stilbenes - resveratrol	200 mg/kg bw/day (diet)	Male Kunming mice (n = 8/group)	84	HFD	↑ *Lactobacillus* and *Bifidobacterium* ↓ *Firmicutes* ↑ *Bacteroidetes*	↓ bw ↓ blood glucose and lipid	Qiao et al. (2014)
Other polyphenols - vanillin	0.1% diet	Male C57BL/6J mice (n = 7–8/group)	98	HFD	↓ *Firmicutes* ↑ cecal SCFA	↑ insulin sensitivity ↓ inflammation	Guo, Han, Zhan, You, and Huang (2018)
		Clinical trials					
Anthocyanins (wild blueberry drink)	25 g/250 mL água	20 healthy male individuals	crossover, placebo controlled (6 weeks)		↑ *Lactobacillus acidophilus* ↑ *Bifidobacterium* spp.	NR	Vendrame et al. (2011)
Punicalagins and ellagic acid (pomegranate extract)	0.45 g or 1.8 g extract (capsules)	49 overweight-obese subjects (mild hyperlipidemia)	double-blind, crossover, placebo controlled (9 weeks)		↑ *Faecalibacterium* ↓ plasma LBP	NR	González-Sarrías et al. (2018)

bw: body weight, SCFA: short-chain fatty acids, STD: standard, chow and normal diet, HFD: high-fat diet, HFHS: high-fat/high-sucrose diet, IG: intragastric, LPS: lipopolysaccharides, HFA: human flora-associated, SPF: specific-pathogen-free, (NS): not significant or not affected, NR: not reported, LBP: lipopolysaccharide-binding protein, ↑ and ↓: increase and decrease in the specific parameter, respectively.

The intervention with soy isoflavone extract containing 51% daidzin, 30% glycitin and 9% genistein increased the abundance of *Faecalibacterium* and the fecal butyrate content in obese rats, and improved intestinal barrier function through increasing the expressions of zonula occludens 1 (ZO-1), occludin and mucin 2 (Muc-2) genes (Luo et al., 2019). An increase of the *Faecalibacterium* genus was also observed after the consumption of genistein (López et al., 2018).

The effects of other flavonoid subclasses were investigated in two studies, in which the intervention samples were hydroxysafflor yellow A (HSYA) (Liu et al., 2018) and *Schisandra chinensis* pollen extract (Cheng et al., 2019). The HSYA supplementation, a compound with a mono-chalcone glycoside structure, increased the abundance *of Akkermansia*, and the production of acetate, propionate, and butyrate. Moreover, the number of goblet cells and the expression of tight junction proteins ZO-1 were increased after intragastric supplementation with HSYA (Liu et al., 2018). Regarding the *S. chinensis* pollen extract, the most abundant phenolic compound was naringenin (1.89 mg/g), a flavanone. Treatment with pollen extract increased *Lactobacillus* in obese mice with a dose-effect relationship (Cheng, Chen, Liu, et al., 2019).

3.2.1.2. Other polyphenols. Prebiotic effect of other phenolic compounds, like lignans, phenolic acids, stilbenes and vanillin, was evaluated in five animal studies (Cho et al., 2016; Etxeberria et al., 2017; Guo et al., 2018; Qiao et al., 2014; Xie et al., 2019). In summary, there was an increase in the abundance of *Lactobacillus*, *Bifidobacterium* and *Akkermansia*, and a reduction in serum concentrations of pro-inflammatory cytokines and expression of lipid synthesis related genes.

The consumption of syringaresinol, a lignan present in oilseeds, cereal brans and berry seeds, enhanced the population of *Lactobacillus* and *Bifidobacterium*, and reduced serum lipopolysaccharide-binding protein (LBP) concentration (Cho et al., 2016). Supplementation with aqueous kudingcha extract, composed by 3,4-di-*O*-caffeoylquinic acids (3,4-diCQAs – 26.9%), 3,5-diCQAs (42.3%) and 4,5-diCQAs (30.8%), increased the abundance of *Bifidobacterium* and *Akkermansia*, and decreased the concentrations of serum interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF-α) and lipopolysaccharides (LPS) and the hepatic expression of lipid synthesis related genes (Xie et al., 2019). The supplementation with resveratrol, the largest representative of the stilbenes class, increased the abundance of *Lactobacillus* and *Bifidobacterium*, and decreased the genes expression related to fatty acids synthesis, adipogenesis and lipogenesis in mice fed a high-fat diet (Qiao et al., 2014). Animals treated with a standard diet supplemented with pterostilbene, a dimethoxy resveratrol derivative, showed an increase in the abundance of *Akkermansia muciniphila*, and an improvement in insulin sensitivity (Etxeberria et al., 2017). An increase in the production of acetate, propionate and butyrate and a decrease in the high concentrations of inflammatory factors (LPS, IL-6, and TNF-α) were observed in mice fed a high-fat diet after the supplementation with vanillin (Guo, Han, et al., 2018).

3.2.2. Clinical trials

The effect of anthocyanins was also evaluated in a crossover clinical trial, in which the consumption of a wild blueberry drink (375 mg of anthocyanins) by healthy adults increased *Bifidobacterium* spp. and *Lactobacillus acidophilus* (Vendrame et al., 2011). In another clinical trial with overweight-obese individuals, the consumption of pomegranate extract (1.8 g/day, 656 mg of phenolics) rich in hydroxybenzoic acids (punicalagins and free ellagic acid) increased *Faecalibacterium* and decreased the concentration of plasma LBP (González-Sarrías et al., 2018).

3.3. Methodological quality assessment of studies

The animal studies were generally classified as low or unclear regarding the risk of bias (Table S1). None of the included studies satisfied all areas established by the SYRCLE's tool for methodological quality assessment of animal studies. Data on selection bias (baseline characteristics and allocation concealment), performance bias (random housing and blinding), and detection bias (random outcome assessment and blinding) were unavailable in all studies. The clinical trials (González-Sarrías et al., 2018; Vendrame et al., 2011), evaluated by Cochrane Collaboration's tool, were at low-risk for selection bias (random sequence generation), performance bias (blinding of participants and personnel), reporting bias (selective reporting), and other bias. In addition, these studies were unclear for selection bias (allocation concealment) and detection bias (blinding of outcome assessment) and showed high-risk for attrition bias (incomplete outcome data).

4. Discussion

To our knowledge, this is the first systematic review aiming to explore the prebiotic effect of dietary polyphenols investigated in preclinical and clinical studies. We found 24 studies of which: 8 showed an increased abundance of *Lactobacillus* spp. and/or *Bifidobacterium* spp. (Cheng, Chen, Liu, et al., 2019; Cho et al., 2016; Dey et al., 2019; Li, Wu, et al., 2019; Ma et al., 2019; Qiao et al., 2014; Vendrame et al., 2011; Xie et al., 2019); 10 showed an increased abundance of *Akkermansia* spp. (Anhê et al., 2015, 2017; Dey et al., 2019; Etxeberria et al., 2017; Liu et al., 2018; Lu et al., 2019; Ma et al., 2019; Masumoto et al., 2016; Xia et al., 2019; Xie et al., 2019); 3 showed an increased abundance of *Faecalibacterium* spp. (González-Sarrías et al., 2018; López et al., 2018; Luo et al., 2019); and 3 showed an increased abundance of *Roseburia* spp. (Lu et al., 2019; Ma et al., 2019; Van Hul et al., 2018). Seven studies reported an increase in the production of SCFA, including butyrate (Guo, Han, et al., 2018; Henning et al., 2018; Li, Wu, et al., 2019; Liu et al., 2018, 2019; Luo et al., 2019; Wang et al., 2018).

The current definition of prebiotic recognizes that prebiotic targets extend beyond the stimulation of *Bifidobacterium* and *Lactobacillus*, and include other microorganisms such as *Roseburia*, *Eubacterium* and *Faecalibacterium* spp., but are not limited to these (Gibson et al., 2017). Thereby, the inclusion criteria applied in this systematic review is in accordance with the current concept of prebiotics. The health benefits associated with prebiotics are immune modulation, increased mineral absorption, improved bowel function, and a positive effect on glucose homeostasis, inflammation, blood lipid profile, satiety and defense against pathogens (Sanders et al., 2019). Although these effects cannot be easily extrapolated to the human gut microbiota, many of them seem to be mediated by SCFA, especially acetate, propionate, and butyrate.

The pathway that explains how polyphenols increase the production of SCFA is not yet fully understood. It is believed that the increase of anaerobic microorganisms, such as *Lactobacillus*, *Lachnospiraceae* and *Ruminococcaceae* can promote the increase of SCFA, especially of butyrate (Li, Wu, et al., 2019; Liu et al., 2018, 2019). Another possible explanation is associated with decafeinated green tea and black tea polyphenols that have been shown to inhibit α-amylase and α-glucosidase in saliva and small intestine, which may lead to residual carbohydrate in the large intestine providing substrate for the SCFA production (Henning et al., 2018). It is worth mentioning that SCFA are the major mediators among nutrition, gut microbiota, physiology, and pathology (Ríos-Covián et al., 2016). The butyrate has been investigated most extensively, and it is recognized that butyrate-producing bacteria and butyrate per se may be beneficial for human health (Koh, De Vadder, Kovatcheva-Datchary, & Bäckhed, 2016), suggesting it as a biomarker of prebiotic effect.

The studies reviewed here reported that the health benefits associated with the consumption of catechins were reduced concentrations of serum low-density lipoprotein cholesterol (LDL-c) (Ma et al., 2019), glucose and insulin (Wang et al., 2018). Studies also found that catechins could prevent an increase in toll-like receptor 4 (TLR4)/ nuclear factor kappa B (NFκB)-dependent inflammatory genes (Dey et al., 2019), and increase the hepatic adenosine monophosphate-activated

protein kinase (AMPK) phosphorylation (Henning et al., 2018). Besides, the consumption of green tea extract containing 48% epigallocatechin gallate prevented from a decrease in the expression of intestinal tight junction proteins induced by a high-fat diet (Dey et al., 2019). The treatment with soy isoflavone and HSYA improved intestinal barrier function through increasing the number of goblet cells and the expression of tight junction proteins ZO-1, occludin and Muc-2 (Liu et al., 2018; Luo et al., 2019). Digestive enzymes activities (β-glucosidase, β-galactosidase and β-glucuronidase) in the cecal environment were increased by the supplementation with anthocyanins (Li, Wu, et al., 2019). The health effects of proanthocyanidins consumption were improved glucose tolerance and insulin sensitivity, upregulation of lipid catabolism genes (PPARα), and downregulation of pro-inflammatory genes (COX2, TNFα) in the liver (Anhê et al., 2015, 2017; Masumoto et al., 2016).

Dietary flavonoids are consumed predominantly as glycosides (conjugated to sugar), which hinders the absorption of these compounds by the small intestine (Kawabata, Yoshioka, & Terao, 2019). Glycosylated flavonoids can serve as the sole source of carbon and energy for some microorganisms in the gut microbiota, which preferentially ferment the sugars linked to flavonoids. Thus, it could explain the prebiotic effect observed for these compounds (Braune & Blaut, 2016). For instance, aqueous extract of jaboticaba, a Brazilian native fruit rich in cyanidin-3-O-glucoside and delphinidin-3-O-glucoside, modulated the abundance of *Lactobacillus* and *Bifidobacterium* in rats with induced-colitis (Silva-Maia et al., 2019). Cyanidin-3-O-glucoside ameliorated gut microbial dysbiosis caused by 3-chloro-1,2-propanediol (chemical food contaminant) in rats (Chen et al., 2019). In other study, the Quzhou Fructus Aurantii extract, rich in naringin, a natural flavanone glycoside, increased the genus *Akkermansia* and the expression of tight junction proteins, and reduced metabolic endotoxemia in mice fed high-fat diet (Bai et al., 2019).

Most dietary polyphenols arrive intact in the colon, where they become substrates for the gut microbiota, producing better-absorbing metabolites (Mojzer et al., 2016; Kawabata et al., 2019). The flavonoids, such as epicatechin, catechin, procyanidin and quercetin, when metabolized by gut microbiota, generate hydroxy phenylacetic and hydroxyphenyl propionic acids (Shortt et al., 2018). Equol and O-desmethylangolensin (ODMA) are active metabolites produced by the action of colonic bacteria on soy isoflavones (Mayo, Vázquez, & Flórez, 2019). The urolithins, in turn, are metabolites produced from ellagitannins and ellagic acid by the human gut microbiota (Tomás-Barberán et al., 2017). These metabolites also have beneficial health effects, such as estrogenic and antioxidant activity (Mayo et al., 2019), anti-inflammatory and antioxidant effects (Lee, Park, Lee, Ahn, & Kim, 2019), and hepatoprotective effect (Zhao et al., 2018). Thus, further studies are warranted to investigate the potential of these metabolites as prebiotic effect markers, such as the SCFA modulation.

Studies with syringaresinol in male C57BL/6 mice (Cho et al., 2016) and pomegranate extract in overweight-obese individuals (González-Sarrías et al., 2018) demonstrated a decrease in serum LBP, indicating that polyphenols could modulate metabolic endotoxemia (Fuke, Nagata, Suganuma, & Ota, 2019). LBP is a glycoprotein mainly synthesized in the hepatocytes with long half-life in the blood and can bind to LPS promoting an LPS-induced immune response via toll-like receptors in macrophages (Jamar, Ribeiro, & Pisani, 2020). LPS, known as endotoxin, is a breakdown product present in the outer membrane of gram-negative bacteria, composed by an O-antigen portion in its outer part and by a lipid-A portion in its inner part. The lipid-A portion exerts most of the immunogenic effects, such as the activation of TLR4, through the formation of the complex containing LBP and the CD14 co-receptor, which signals the NFκB activation to upregulate pro-inflammatory mediators causing low-grade inflammation. The O-antigen portion activates components of the adaptive immunity, intending to induce the production of antibodies. Therefore, plasma LBP may be an inflammation marker caused by endotoxins (Jamar et al., 2020).

The dysbiosis of the gut microbiota caused by a high-fat diet has been considered as a possible cause for metabolic endotoxemia (Fuke et al., 2019). Thus, it is expected that the consumption of polyphenols may also improve the metabolic endotoxemia associated to dysbiosis, since the consumption of catechins (Liu et al., 2019; Lu et al., 2019), isoflavones (López et al., 2018; Luo et al., 2019), proanthocyanidins (Anhê et al., 2015; Masumoto et al., 2016), and dicaffeoylquinic acids (Xie et al., 2019) decreased plasma or serum LPS in animals fed a high-fat diet. Circulating LPS is released by lysis of a fraction of the bacterial cell wall and flows into the blood by increasing intestinal permeability. The excess intestinal LPS itself, caused by dysbiosis, destroys the narrow junction of intestinal epithelial cells via TLR4 and inhibits mRNA expression of factors related to the restricted junction, such as ZO-1 and occludin, in the intestinal epithelial cell. Therefore, LPS is considered an inflammation marker for dysbiosis (Fuke et al., 2019).

The improvement of the intestinal barrier function through increasing the expressions of ZO-1, occludin and Muc-2 genes, and of the mucin-producing goblet cells number is one of the possible mechanisms associated with the prebiotic effects of catechins (Dey et al., 2019), soy isoflavones (Luo et al., 2019) and HSYA (Liu et al., 2019). Kruppel-like factor 4 (KLF4), a marker of goblet cells, and Muc2 mRNA expression in the proximal colon were also associated with the administration of proanthocyanidins rich-cranberry extract, supporting that these polyphenols could be able to stimulate mucus production (Fig. 2), and therefore create an ecological niche for the *Akkermansia* spp., a mucus-degrading bacterium (Anhê et al., 2015). Xia et al. (2019) reported an increased abundance of *Akkermansia* spp. through an increase in other markers, such as type II and III secretion system proteins, the elongation factor thermo unstable, and a glyceraldehyde-3-phosphate dehydrogenase. This methodology was not observed in other studies. In this review, *Akkermansia* spp. was included as prebiotic target along with the bacteria recognized as probiotics by the Consensus Statement on the Definition and Scope of Prebiotics (Gibson et al., 2017), considering the recent evidences in literature about the relationship among *Akkermansia* spp., gut microbiota and human health (Cani & de Vos, 2017; Jayachandran, Chung, & Xu, 2019; Sanders et al., 2019). *Akkermansia* spp. and *Propionibacterium* spp. are promising candidates among the next generation of microorganisms to be recognized as prebiotic targets (Sanders et al., 2019).

There is also evidence of an additional mechanism by which polyphenols protect the intestinal barrier against oxidative stress. A decrease in the oxidative stress in the liver and ileum was observed in specific pathogen-free mice after a lower-dose consumption of green tea polyphenols (100 mg/kg body weight/day). The improvement of intestinal oxidative stress is supposed to be a potential mechanism for the modulation of tea polyphenols in the gut microbiota (Ma et al., 2019), since excess reactive oxygen species will damage the cell membrane and disrupt the tight junctions leading to an increased intestinal permeability and the development of metabolic disorders (Qiao, Sun, Ding, Le, & Shi, 2013). The tight junctions are multiprotein complexes that maintain barrier function between the enterocytes, creating paracellular barrier properties, which are composed by transmembrane proteins which control the transport across the intercellular space between adjacent cells and cytoplasmic plaque (Costea et al., 2019). The main components of the cytoplasmic plaque are claudin and zonula occludens proteins. In oxidative stress, the interactions of occludin with claudins or proteins of the zonula family are affected directly influencing the formation and function of the tight junctions. Oxidative stress downregulates occludin, reduces its specific membrane localization and regulatory contribution to barrier tightness via multiple signaling pathways (Costea et al., 2019). Nonetheless, most of the evidence is based *in vitro* and animal studies, and then further studies in humans are needed to clarify these mechanisms.

Regarding the methodological quality of the included papers, none of the studies satisfied all criteria established by the SYRCLE and Cochrane Collaboration's tool. No preclinical studies provide detailed descriptions of the methods used for allocation concealment, random

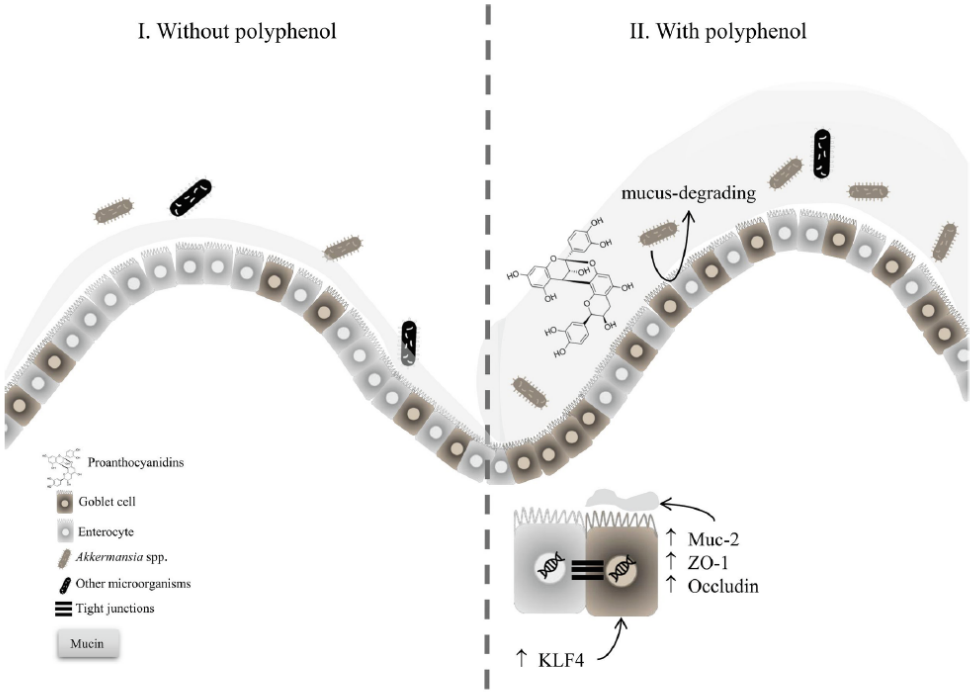

Fig. 2. Mechanism of mucin synthesis modulation by proanthocyanidins in the hindgut. KLF4: kruppel-like factor 4, Muc-2: mucin 2, ZO-1: zonula occludens 1. The consumption of polyphenols, represented in the figure by proanthocyanidins, may increase the number of goblet cells, according to the Kruppel-like factor 4 (KLF4) marker, and consequently, the mucin production; so the enhanced mucin provides a favorable environment for the proliferation of *Akkermansia* spp., a mucus-degrading bacterium (Anhê et al., 2015; Xia et al., 2019). In addition, increased expression of ZO-1 and occludin can be observed after the consumption of polyphenols, decreasing the intestinal permeability through the junction of epithelial cells (tight junctions) (Dey et al., 2019; Luo et al., 2019; Liu et al., 2019).

housing and random outcome assessment, or blinding researchers and outcome assessors. The clinical trials did not provide details on allocation concealment and blinding of outcome assessment. The absence of descriptions regarding sample losses in the preclinical and clinical studies also was noted. Therefore, we suggest the use of these tools in experimental planning, as a reference for well-designed studies aiming to reduce the risk of bias and allow more consistent conclusions.

It is noteworthy that the included studies had quite heterogeneous designs, including differences in the intervention period, the methodology for gut microbiome analysis, and the supplementation with polyphenol (food sample and dosage), as well as the polyphenols profile of the samples. Additional studies with isolated polyphenols are needed to eliminate the effects of other compounds in the extract, especially in clinical trials. Moreover, we suggest that further studies aiming to evaluate the prebiotic effect of dietary polyphenols should be carried out according to the internationally accepted prebiotics definition, with complete assessment of the gut microbiota, including the microorganism's genera. The low number of studies on humans is also a limitation in this systematic review.

5. Conclusion

The prebiotic effect of dietary polyphenols, especially catechins, anthocyanins, and proanthocyanidins, has strong evidence based on preclinical studies. Despite the limitations of these studies, it is evident that polyphenols can stimulate the growth of microorganisms recognized as prebiotic targets (*Lactobacillus* spp., *Bifidobacterium* spp., *Akkermansia* spp., *Roseburia* spp., and *Faecalibacterium* spp.), and increase the production of SCFA, including butyrate. Nevertheless, well-designed clinical trials are warranted to prove the prebiotic effect of polyphenols on humans.

Ethics statement

This research did not include any human subjects and animal experiments.

CRediT authorship contribution statement

Aline Medeiros Alves-Santos: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing - original draft, Writing - review & editing. **Clara Sandra Araújo Sugizaki:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing - original draft, Writing - review & editing. **Glaucia Carielo Lima:** Conceptualization, Data curation, Supervision, Visualization, Writing - review & editing. **Maria Margareth Veloso Naves:** Conceptualization, Data curation, Supervision, Visualization, 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.

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Appendix A. Supplementary data

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References

Anhê, F. F., Nachbar, R. T., Varin, T. V., Vilela, V., Dudonné, S., Pilon, G., ... Marette, A. (2017). A polyphenol-rich cranberry extract reverses insulin resistance and hepatic steatosis independently of body weight loss. *Molecular Metabolism, 6*(12), 1563–1573. https://doi.org/10.1016/j.molmet.2017.10.003.

Anhê, F. F., Roy, D., Pilon, G., Dudonné, S., Matamoros, S., Varin, T. V., ... Marette, A. (2015). A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased *Akkermansia* spp. population in the gut microbiota of mice. *Gut, 64*(6), 872–883. https://doi.org/10.1136/gutjnl-2014-307142.

Anhê, F. F., Varin, T. V., Le Barz, M., Pilon, G., Dudonné, S., Trottier, J., ... Marette, A. (2018). Arctic berry extracts target the gut–liver axis to alleviate metabolic endotoxaemia, insulin resistance and hepatic steatosis in diet-induced obese mice. *Diabetologia, 61*(4), 919–931. https://doi.org/10.1007/s00125-017-4520-z.

Bai, Y., Wang, S., Wang, X., Weng, Y., Fan, X., Sheng, H., ... Zhang, F. (2019). The flavonoid-rich Quzhou Fructus Aurantii extract modulates gut microbiota and prevents obesity in high-fat diet-fed mice. *Nutrition and Diabetes, 9*(1), 1–11. https://doi.org/10.1038/s41387-019-0097-6.

Bian, Y., Wei, J., Zhao, C., & Li, G. (2020). Natural polyphenols targeting senescence: A novel prevention and therapy strategy for cancer. *International Journal of Molecular Sciences, 21*(2), 1–17. https://doi.org/10.3390/ijms21020684.

Braune, A., & Blaut, M. (2016). Bacterial species involved in the conversion of dietary flavonoids in the human gut. *Gut Microbes, 7*(3), 216–234. https://doi.org/10.1080/19490976.2016.1158395.

Cani, P. D., & de Vos, W. M. (2017). Next-generation beneficial microbes: The case of *Akkermansia muciniphila*. *Frontiers in Microbiology, 8*, 1–8. https://doi.org/10.3389/fmicb.2017.01765.

Casanova-Marti, A., Serrano, J., Portune, K. J., Sanz, Y., Blay, M. T., Terra, X., ... Pinent, M. (2018). Grape seed proanthocyanidins influence gut microbiota and enteroendocrine secretions in female rats. *Food and Function, 9*(3), 1672–1682. https://doi.org/10.1039/c7fo02028g.

Chai, S. C., Davis, K., Zhang, Z., Zha, L., & Kirschner, K. F. (2019). Effects of tart cherry juice on biomarkers of inflammation and oxidative stress in older adults. *Nutrients, 11*(2), 1–10. https://doi.org/10.3390/nu11020228.

Chen, G., Wang, G., Zhu, C., Jiang, X., Sun, J., Tian, L., & Bai, W. (2019). Effects of cyanidin-3-O-glucoside on 3-chloro-1,2-propanediol induced intestinal microbiota dysbiosis in rats. *Food and Chemical Toxicology, 133*, 110767. https://doi.org/10.1016/j.fct.2019.110767.

Cheng, L., Chen, Y., Zhang, X., Zheng, X., Cao, J., Wu, Z., ... Cheng, K. (2019). A metagenomic analysis of the modulatory effect of *Cyclocarya paliurus* flavonoids on the intestinal microbiome in a high-fat diet-induced obesity mouse model. *Journal of the Science of Food and Agriculture, 99*(8), 3967–3975. https://doi.org/10.1002/jsfa.9622.

Cheng, N., Chen, S., Liu, X., Zhao, H., & Cao, W. (2019). Impact of SchisandraChinensis bee pollen on nonalcoholic fatty liver disease and gut microbiota in highfat diet induced obese mice. *Nutrients, 11*(2), 1–16. https://doi.org/10.3390/nu11020346.

Chiu, H. F., Chen, Y. J., Lu, Y. Y., Han, Y. C., Shen, Y. C., Venkatakrishnan, K., & Wang, C. K. (2017). Regulatory efficacy of fermented plant extract on the intestinal microflora and lipid profile in mildly hypercholesterolemic individuals. *Journal of Food and Drug Analysis, 25*(4), 819–827. https://doi.org/10.1016/j.jfda.2016.10.008.

Cho, S. Y., Kim, J., Lee, J. H., Sim, J. H., Cho, D. H., Bae, I. H., ... Kim, H. R. (2016). Modulation of gut microbiota and delayed immunosenescence as a result of syringaresinol consumption in middle-aged mice. *Scientific Reports, 6*, 1–14. https://doi.org/10.1038/srep39026.

Cires, M. J., Navarrete, P., Pastene, E., Carrasco-Pozo, C., Valenzuela, R., Medina, D. A., ... Gotteland, M. (2019). Effect of a proanthocyanidin-rich polyphenol extract from avocado on the production of amino acid-derived bacterial metabolites and the microbiota composition in rats fed a high-protein diet. *Food and Function, 10*(7), 4022–4035. https://doi.org/10.1039/c9fo00700h.

Collins, B., Hoffman, J., Martinez, K., Grace, M., Lila, M. A., Cockrell, C., ... McIntosh, M. (2016). A polyphenol-rich fraction obtained from table grapes decreases adiposity, insulin resistance and markers of inflammation and impacts gut microbiota in high-fat-fed mice. *Journal of Nutritional Biochemistry, 31*, 150–165. https://doi.org/10.1016/j.jnutbio.2015.12.021.

Costea, L., Mészáros, A., Bauer, H., Bauer, H. C., Traweger, A., Wilhelm, I., ... Krizbai, I. A. (2019). The blood–brain barrier and its intercellular junctions in age-related brain disorders. *International Journal of Molecular Sciences, 20*(21), 1–28. https://doi.org/10.3390/ijms20215472.

Cowan, T. E., Palmnäs, M. S. A., Yang, J., Bomhof, M. R., Ardell, K. L., Reimer, R. A., ... Shearer, J. (2014). Chronic coffee consumption in the diet-induced obese rat: Impact on gut microbiota and serum metabolomics. *Journal of Nutritional Biochemistry, 25*(4), 489–495. https://doi.org/10.1016/j.jnutbio.2013.12.009.

Cueva, C., Silva, M., Pinillos, I., Bartolomé, B., & Moreno-Arribas, M. V. (2020). Interplay between dietary polyphenols and oral and gut microbiota in the development of colorectal cancer. *Nutrients, 12*(3), 1–19. https://doi.org/10.3390/nu12030625.

Dey, P., Sasaki, G. Y., Wei, P., Li, J., Wang, L., Zhu, J., ... Bruno, R. S. (2019). Green tea extract prevents obesity in male mice by alleviating gut dysbiosis in association with improved intestinal barrier function that limits endotoxin translocation and adipose inflammation. *Journal of Nutritional Biochemistry, 67*, 78–89. https://doi.org/10.1016/j.jnutbio.2019.01.017.

Etxeberria, U., Hijona, E., Aguirre, L., Milagro, F. I., Bujanda, L., Rimando, A. M., ... Portillo, M. P. (2017). Pterostilbene-induced changes in gut microbiota composition in relation to obesity. *Molecular Nutrition and Food Research, 61*(1), 1–12. https://doi.org/10.1002/mnfr.201500906.

Fang, C., Kim, H., Yanagisawa, L., Bennett, W., Sirven, M. A., Alaniz, R. C., ... Mertens-Talcott, S. U. (2019). Gallotannins and *Lactobacillus plantarum* WCFS1 mitigate high-fat diet-induced inflammation and induce biomarkers for thermogenesis in adipose tissue in gnotobiotic mice. *Molecular Nutrition and Food Research, 63*(9), 1–12. https://doi.org/10.1002/mnfr.201800937.

Fuke, N., Nagata, N., Suganuma, H., & Ota, T. (2019). Regulation of gut microbiota and metabolic endotoxemia with dietary factors. *Nutrients, 11*(10), 1–38. https://doi.org/10.3390/nu11102277.

Gao, X., Xie, Q., Kong, P., Liu, L., Sun, S., Xiong, B., ... Xiang, H. (2018). Polyphenol- and caffeine-rich postfermented Pu-erh tea improves dietinduced metabolic syndrome by remodeling intestinal homeostasis in mice. *Infection and Immunity, 86*(1), 1–16. https://doi.org/10.1128/IAI.00601-17.

Garcia-Mazcorro, J. F., Pedreschi, R., Chew, B., Dowd, S. E., Kawas, J. R., & Noratto, G. (2018). Dietary supplementation with raspberry extracts modifies the fecal microbiota in obese diabetic db/db mices. *Journal of Microbiology and Biotechnology, 28*(8), 1247–1259. https://doi.org/10.4014/jmb.1803.03020.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., ... Reid, G. (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 and Hepatology, 14*(8), 491–502. https://doi.org/10.1038/nrgastro.2017.75.

Ginés, I., Gil-Cardoso, K., Terra, X., Blay, M., Pérez-Vendrell, A. M., Pinent, M., & Ardévol, A. (2019). Grape seed proanthocyanidins target the enteroendocrine system in cafeteria-diet-fed rats. *Molecular Nutrition and Food Research, 63*(11), 1–7. https://doi.org/10.1002/mnfr.201800912.

González-Sarrías, A., García-Villalba, R., Romo-Vaquero, M., Alasalvar, C., Örem, A., Zafrilla, P., ... Espín, J. C. (2017). Clustering according to urolithin metabotype explains the interindividual variability in the improvement of cardiovascular risk biomarkers in overweight-obese individuals consuming pomegranate: A randomized clinical trial. *Molecular Nutrition and Food Research, 61*(5), 1–14. https://doi.org/10.1002/mnfr.201600830.

González-Sarrías, A., Romo-Vaquero, M., García-Villalba, R., Cortés-Martín, A., Selma, M. V., & Espín, J. C. (2018). The endotoxemia marker lipopolysaccharide-binding protein is reduced in overweight-obese subjects consuming pomegranate extract by modulating the gut microbiota: A randomized clinical trial. *Molecular Nutrition and Food Research, 62*(11), 1–10. https://doi.org/10.1002/mnfr.201800160.

Griffin, L. E., Witrick, K. A., Klotz, C., Dorenkott, M. R., Goodrich, K. M., Fundaro, G., ... Neilson, A. P. (2017). Alterations to metabolically active bacteria in the mucosa of the small intestine predict anti-obesity and anti-diabetic activities of grape seed extract in mice. Food and Function, 8(10), 3510–3522. 10.1039/c7fo01236e.

Grzelak-Błaszczyk, K., Milala, J., Kosmala, M., Kołodziejczyk, K., Sójka, M., Czarnecki, A., ... Jurgoński, A. (2018). Onion quercetin monoglycosides alter microbial activity and increase antioxidant capacity. *Journal of Nutritional Biochemistry, 56*, 81–88. https://doi.org/10.1016/j.jnutbio.2018.02.002.

Guo, J., Han, X., Zhan, J., You, Y., & Huang, W. (2018). Vanillin alleviates high fat diet-induced obesity and improves the gut microbiota composition. *Frontiers in Microbiology, 9*, 1–12. https://doi.org/10.3389/fmicb.2018.02733.

Guo, X., Tang, R., Yang, S., Lu, Y., Luo, J., & Liu, Z. (2018). Rutin and its combination with inulin attenuate gut dysbiosis, the inflammatory status and endoplasmic reticulum stress in Paneth cells of obese mice induced by high-fat diet. *Frontiers in Microbiology, 9*, 1–11. https://doi.org/10.3389/fmicb.2018.02651.

Henning, S. M., Yang, J., Hsu, M., Lee, R. P., Grojean, E. M., Ly, A., ... Li, Z. (2018). Decaffeinated green and black tea polyphenols decrease weight gain and alter microbiome populations and function in diet-induced obese mice. *European Journal of Nutrition, 57*(8), 2759–2769. https://doi.org/10.1007/s00394-017-1542-8.

Higgins, J. P. T., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., ... Sterne, J. A. C. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ, 343*(7829), 1–9. https://doi.org/10.1136/bmj.d5928.

Hooijmans, C. R., Rovers, M. M., De Vries, R. B. M., Leenaars, M., Ritskes-Hoitinga, M., & Langendam, M. W. (2014). SYRCLE's risk of bias tool for animal studies. *BMC Medical Research Methodology, 14*(1), 1–9. https://doi.org/10.1186/1471-2288-14-43.

Jamar, G., Ribeiro, D. A., & Pisani, L. P. (2020). High-fat or high-sugar diets as trigger inflammation in the microbiota-gut-brain axis. *Critical Reviews in Food Science and Nutrition, 1–19*. https://doi.org/10.1080/10408398.2020.1747046.

Jayachandran, M., Chung, S. S. M., & Xu, B. (2019). A critical review of the relationship between dietary components, the gut microbe *Akkermansia muciniphila*, and human health. *Critical Reviews in Food Science and Nutrition, 1–12*. https://doi.org/10.1080/10408398.2019.1632789.

Kawabata, K., Yoshioka, Y., & Terao, J. (2019). Role of intestinal microbiota in the bioavailability and physiological functions of dietary polyphenols. *Molecules, 24*(2), 1–25. https://doi.org/10.3390/molecules24020370.

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell, 165*(6),

1332–1345. https://doi.org/10.1016/j.cell.2016.05.041.
Lee, G., Park, J. S., Lee, E. J., Ahn, J. H., & Kim, H. S. (2019). Anti-inflammatory and antioxidant mechanisms of urolithin B in activated microglia. *Phytomedicine, 55*, 50–57. https://doi.org/10.1016/j.phymed.2018.06.032.
Li, J., Wu, T., Li, N., Wang, X., Chen, G., & Lyu, X. (2019). Bilberry anthocyanin extract promotes intestinal barrier function and inhibits digestive enzyme activity by regulating the gut microbiota in aging rats. *Food and Function, 10*(1), 333–343. https://doi.org/10.1039/c8fo01962b.
Li, Q., Liu, F., Liu, J., Liao, S., & Zou, Y. (2019). Mulberry leaf polyphenols and fiber induce synergistic antiobesity and display a modulation effect on gut microbiota and metabolites. *Nutrients, 11*(5), 1–19. https://doi.org/10.3390/nu11051017.
Liu, J., Hao, W., He, Z., Kwek, E., Zhao, Y., Zhu, H., ... Chen, Z. Y. (2019). Beneficial effects of tea water extracts on the body weight and gut microbiota in C57BL/6J mice fed with a high-fat diet. *Food and Function, 10*(5), 2847–2860. https://doi.org/10.1039/c8fo02051e.
Liu, J., Yue, S., Yang, Z., Feng, W., Meng, X., Wang, A., ... Yan, D. (2018). Oral hydroxysafflor yellow A reduces obesity in mice by modulating the gut microbiota and serum metabolism. *Pharmacological Research, 134*, 40–50. https://doi.org/10.1016/j.phrs.2018.05.012.
López, P., Sánchez, M., Perez-Cruz, C., Velázquez-Villegas, L. A., Syeda, T., Aguilar-López, M., ... Tovar, A. R. (2018). Long-term genistein consumption modifies gut microbiota, improving glucose metabolism, metabolic endotoxemia, and cognitive function in mice fed a high-fat diet. *Molecular Nutrition and Food Research, 62*(16), 1–12. https://doi.org/10.1002/mnfr.201800313.
Lu, X., Liu, J., Zhang, N., Fu, Y., Zhang, Z., Li, Y., ... Cao, Y. (2019). Ripened Pu-erh tea extract protects mice from obesity by modulating gut microbiota composition. *Journal of Agricultural and Food Chemistry, 67*(25), 6978–6994. https://doi.org/10.1021/acs.jafc.8b04909.
Luo, Q., Cheng, D., Huang, C., Li, Y., Lao, C., Xia, Y., ... Chen, Z. (2019). Improvement of colonic immune function with soy isoflavones in high-fat diet-induced obese rats. *Molecules, 24*(6), 1–17. https://doi.org/10.3390/molecules24061139.
Ma, H., Zhang, B., Hu, Y., Wang, J., Liu, J., Qin, R., ... Wang, S. (2019). Correlation analysis of intestinal redox state with the gut microbiota reveals the positive intervention of tea polyphenols on hyperlipidemia in high fat diet fed mice. *Journal of Agricultural and Food Chemistry, 67*(26), 7325–7335. https://doi.org/10.1021/acs.jafc.9b02211.
Masumoto, S., Terao, A., Yamamoto, Y., Mukai, T., Miura, T., & Shoji, T. (2016). Non-absorbable apple procyanidins prevent obesity associated with gut microbial and metabolomic changes. *Scientific Reports, 6*, 1–10. https://doi.org/10.1038/srep31208.
Mayo, B., Vázquez, L., & Flórez, A. B. (2019). Equol: A bacterial metabolite from the daidzein isoflavone and its presumed beneficial health effects. *Nutrients, 11*(9), 1–20. https://doi.org/10.3390/nu11092231.
Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., ... PRISMA-P Group (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews, 4*(1), 1–9. https://doi.org/10.1186/2046-4053-4-1.
Mojzer, E. B., Knez Hrnčič, M., Škerget, M., Knez, Ž., & Bren, U. (2016). Polyphenols: Extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. *Molecules, 21*(7), 1–38. https://doi.org/10.3390/molecules21070901.
Most, J., Penders, J., Lucchesi, M., Goossens, G. H., & Blaak, E. E. (2017). Gut microbiota composition in relation to the metabolic response to 12-week combined polyphenol supplementation in overweight men and women. *European Journal of Clinical Nutrition, 71*(9), 1040–1045. https://doi.org/10.1038/ejcn.2017.89.
Noad, R. L., Rooney, C., McCall, D., Young, I. S., McCance, D., McKinley, M. C., ... McKeown, P. P. (2016). Beneficial effect of a polyphenol-rich diet on cardiovascular risk: A randomised control trial. *Heart, 102*(17), 1371–1379. https://doi.org/10.1136/heartjnl-2015-309218.
Paquette, M., Larqué, A. S. M., Weisnagel, S. J., Desjardins, Y., Marois, J., Pilon, G., ... Jacques, H. (2017). Strawberry and cranberry polyphenols improve insulin sensitivity in insulin-resistant, non-diabetic adults: A parallel, double-blind, controlled and randomised clinical trial. *British Journal of Nutrition, 117*(4), 519–531. https://doi.org/10.1017/S0007114517000393.
Porras, D., Nistal, E., Martínez-Flórez, S., Pisonero-Vaquero, S., Olcoz, J. L., Jover, R., ... Sánchez-Campos, S. (2017). Protective effect of quercetin on high-fat diet-induced non-alcoholic fatty liver disease in mice is mediated by modulating intestinal microbiota imbalance and related gut-liver axis activation. *Free Radical Biology and Medicine, 102*, 188–202. https://doi.org/10.1016/j.freeradbiomed.2016.11.037.
Qiao, Y., Sun, J., Ding, Y., Le, G., & Shi, Y. (2013). Alterations of the gut microbiota in high-fat diet mice is strongly linked to oxidative stress. *Applied Microbiology and Biotechnology, 97*(4), 1689–1697. https://doi.org/10.1007/s00253-012-4323-6.
Qiao, Y., Sun, J., Xia, S., Tang, X., Shi, Y., & Le, G. (2014). Effects of resveratrol on gut microbiota and fat storage in a mouse model with high-fat-induced obesity. *Food and Function, 5*(6), 1241–1249. https://doi.org/10.1039/c3fo60630a.
Remely, M., Ferk, F., Sterneder, S., Setayesh, T., Roth, S., Kepcija, T., ... Haslberger, A. G. (2017). EGCG prevents high fat diet-induced changes in gut microbiota, decreases of DNA strand breaks, and changes in expression and DNA methylation of Dnmt1 and MLH1 in C57BL/6J male mice. *Oxidative Medicine and Cellular Longevity, 2017*, 1–17. https://doi.org/10.1155/2017/3079148.
Ríos-Covián, D., Ruas-Madiedo, P., Margolles, A., Gueimonde, M., de los Reyes-Gavilán, C. G., & Salazar, N. (2016). Intestinal short chain fatty acids and their link with diet and human health. Frontiers in Microbiology, 7, 1–9. 10.3389/fmicb.2016.00185.
Romo-Vaquero, M., Selma, M. V., Larrosa, M., Obiol, M., García-Villalba, R., González-Barrio, R., ... García-Conesa, M. T. (2014). A rosemary extract rich in carnosic acid selectively modulates caecum microbiota and inhibits β-glucosidase activity, altering fiber and short chain fatty acids fecal excretion in lean and obese female rats. *PLoS ONE, 9*(4), e94687. https://doi.org/10.1371/journal.pone.0094687.
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 and Hepatology, 16*(10), 605–616. https://doi.org/10.1038/s41575-019-0173-3.
Serreli, G., & Deiana, M. (2019). In vivo formed metabolites of polyphenols and their biological efficacy. *Food and Function, 10*(11), 6999–7021. https://doi.org/10.1039/c9fo01733j.
Shen, C. Y., Wan, L., Wang, T. X., & Jiang, J. G. (2019). *Citrus aurantium* L. var. *amara* Engl. inhibited lipid accumulation in 3T3-L1 cells and *Caenorhabditis elegans* and prevented obesity in high-fat diet-fed mice. *Pharmacological Research, 147*, 104347. https://doi.org/10.1016/j.phrs.2019.104347.
Shortt, C., Hasselwander, O., Meynier, A., Nauta, A., Fernández, E. N., Putz, P., ... Antoine, J. M. (2018). Systematic review of the effects of the intestinal microbiota on selected nutrients and non-nutrients. *European Journal of Nutrition, 57*(1), 25–49. https://doi.org/10.1007/s00394-017-1546-4.
Silva-Maia, J. K., Batista, A. G., Cazarin, C. B. B., Soares, E. S., Bogusz Junior, S., Leal, R. F., ... Maróstica Junior, M. R. (2019). Aqueous extract of Brazilian berry (*Myrciaria jaboticaba*) peel improves inflammatory parameters and modulates *Lactobacillus* and *Bifidobacterium* in rats with induced-colitis. *Nutrients, 11*, 1–13. https://doi.org/10.3390/nu11112776.
Singh, A. K., Cabral, C., Kumar, R., Ganguly, R., & Pandey, A. K. (2019). Beneficial effects of dietary polyphenols on gut microbiota and strategies to improve delivery efficien. *Nutrients, 11*, 1–21. https://doi.org/10.3390/nu11092216.
Sung, M. M., Kim, T. T., Denou, E., Soltys, C. L. M., Hamza, S. M., Byrne, N. J., ... Dyck, J. R. B. (2017). Improved glucose homeostasis in obese mice treated with resveratrol is associated with alterations in the gut microbiome. *Diabetes, 66*(2), 418–425. https://doi.org/10.2337/db16-0680.
Tan, S., Caparros-Martin, J. A., Matthews, V. B., Koch, H., O'Gara, F., Croft, K. D., & Ward, N. C. (2018). Isoquercetin and inulin synergistically modulate the gut microbiome to prevent development of the metabolic syndrome in mice fed a high fat diet. *Scientific Reports, 8*(1), 1–13. https://doi.org/10.1038/s41598-018-28521-8.
Tomás-Barberán, F. A., González-Sarrías, A., García-Villalba, R., Núñez-Sánchez, M. A., Selma, M. V., García-Conesa, M. T., & Espín, J. C. (2017). Urolithins, the rescue of "old" metabolites to understand a "new" concept: Metabotypes as a nexus among phenolic metabolism, microbiota dysbiosis, and host health status. *Molecular Nutrition and Food Research, 61*(1), 1–35. https://doi.org/10.1002/mnfr.201500901.
Unno, T., & Osakabe, N. (2018). Green tea extract and black tea extract differentially influence cecal levels of short-chain fatty acids in rats. *Food Science and Nutrition, 6*(4), 728–735. https://doi.org/10.1002/fsn3.607.
Van Hul, M., Geurts, L., Plovier, H., Druart, C., Everard, A., Ståhlman, M., ... Cani, P. D. (2018). Reduced obesity, diabetes, and steatosis upon cinnamon and grape pomace are associated with changes in gut microbiota and markers of gut barrier. *American Journal of Physiology - Endocrinology and Metabolism, 314*(4), E334–E352. https://doi.org/10.1152/ajpendo.00107.2017.
Vendrame, S., Guglielmetti, S., Riso, P., Arioli, S., Klimis-Zacas, D., & Porrini, M. (2011). Six-week consumption of a wild blueberry powder drink increases Bifidobacteria in the human gut. *Journal of Agricultural and Food Chemistry, 59*(24), 12815–12820. https://doi.org/10.1021/jf2028686.
Wang, L., Zeng, B., Liu, Z., Liao, Z., Zhong, Q., Gu, L., ... Fang, X. (2018). Green tea polyphenols modulate colonic microbiota diversity and lipid metabolism in high-fat diet treated HFA mice. *Journal of Food Science, 83*(3), 864–873. https://doi.org/10.1111/1750-3841.14058.
Wang, W., Pan, Y., Wang, L., Zhou, H., Song, G., Wang, Y., ... Li, A. (2019). Optimal dietary ferulic acid for suppressing the obesity-related disorders in leptin-deficient obese C57BL/6J-*ob*/*ob* mice. *Journal of Agricultural and Food Chemistry, 67*(15), 4250–4258. https://doi.org/10.1021/acs.jafc.8b06760.
Xia, Y., Tan, D., Akbary, R., Kong, J., Seviour, R., & Kong, Y. (2019). Aqueous raw and ripe Pu-erh tea extracts alleviate obesity and alter cecal microbiota composition and function in diet-induced obese rats. *Applied Microbiology and Biotechnology, 103*(4), 1823–1835. https://doi.org/10.1007/s00253-018-09581-2.
Xie, M., Chen, G., Wan, P., Dai, Z., Zeng, X., & Sun, Y. (2019). Effects of dicaffeoylquinic acids from *Ilex kudingcha* on lipid metabolism and intestinal microbiota in high-fat-diet-fed mice. *Journal of Agricultural and Food Chemistry, 67*(1), 171–183. https://doi.org/10.1021/acs.jafc.8b05444.
Yu, J., Guo, H., Xie, J., Luo, J., Li, Y., Liu, L., ... Peng, X. (2019). The alternate consumption of quercetin and alliin in the traditional Asian diet reshaped microbiota and altered gene expression of colonic epithelial cells in rats. *Journal of Food Science, 84*(3), 678–686. https://doi.org/10.1111/1750-3841.14473.
Yuan, L., Li, X., He, S., Gao, C., Wang, C., & Shao, Y. (2018). Effects of natural flavonoid isoorientin on growth performance and gut microbiota of mice. *Journal of Agricultural and Food Chemistry, 66*(37), 9777–9784. https://doi.org/10.1021/acs.jafc.8b03568.
Zhang, C., Wu, W., Li, X., Xin, X., & Liu, D. (2019). Daily supplementation with fresh *Angelica ketsket* Juice alleviates high-fat diet-induced obesity in mice by modulating gut microbiota composition. *Molecular Nutrition and Food Research, 63*(14), 1–9. https://doi.org/10.1002/mnfr.201900248.
Zhang, L., Dong, M., Xu, G., Tian, Y., Tang, H., & Wang, Y. (2018). Metabolomics reveals that dietary ferulic acid and quercetin modulate metabolic homeostasis in rats.

CAPÍTULO 3

ARTIGO CIENTÍFICO II (REVISÃO CRÍTICA)

O artigo intitulado “*Baru (Dipteryx alata) fruit as an option of nut and pulp with advantageous nutritional and functional properties: a comprehensive review*” foi publicado no periódico NFS Journal (Qualis: A2, CiteScore: 5.9, Percentil 83%). Até o momento, este artigo recebeu 5 citações na base científica Scopus.

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REV

Baru (*Dipteryx alata* Vog.) fruit as an option of nut and pulp with advantageous nutritional and functional properties: A comprehensive review

Aline Medeiros Alves-Santos [a], Daniela Canuto Fernandes [b], Maria Margareth Veloso Naves [a,*]

[a] Federal University of Goiás, School of Nutrition, Goiânia, Goiás, Brazil
[b] Pontifical Catholic University of Goiás, School of Social and Health Sciences, Goiânia, Goiás, Brazil

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ABSTRACT

Baru (*Dipteryx alata* Vog.) is a native Brazilian fruit consisting of a thin peel, a fibrous pulp, and a woody endocarp, which coats the nut covered by skin. The baru nut has relevant commercial value, and nutritional and functional properties. The peel and pulp are residues generated during the extraction of the nut in the agro-industrial processing of baru, and their chemical composition is poorly known. However, some studies indicate that they are a source of phytochemicals, such as dietary fiber and polyphenols, which have several health benefits, including prebiotic activity. This review presents an overview of the nutritional and functional attributes of the components of the baru fruit (nut, oil, skin, peel, and pulp); the health effects of the consumption of baru nut evidenced in preclinical and clinical studies; and the potential use of the different fractions of the baru fruit in functional food products.

1. Introduction

The baru tree (*Dipteryx alata* Vog.) is a species native to the Brazilian Cerrado, and its fruit, called baru, has an oval shape, slightly flattened and brown in color [1,2]. The baru fruit is composed of a thin peel, a fibrous pulp, and a woody endocarp that coats the nut, the edible fraction of the fruit [3]. Roasted baru nut is mild in flavor, whose taste is between peanut and almond. It is widely consumed in the Brazilian Midwest and found in the big markets worldwide, like in the United States [4,5]. In addition to its unique flavor, the commercial value of the baru nut is related to its nutritional and functional properties, such as high content of quality protein [6,7] and lipids, mainly oleic acid [8]; dietary fiber [6]; and phenolic compounds, and great antioxidant capacity [3,9,10]. The thick skin that coats the baru nut, in turn, is a rich source of polyphenols [9].

The baru peel and pulp are solid residues wasted in the agro-industrial fruit processing. The baru pulp is still poorly used in the human diet, but it is consumed by the cattle when the fruit falls down from the tree, for its aromatic and sweet flavor [5]. The pulp (with peel) has a high content of carbohydrates, with almost 25% of total sugars, and a remarkably high concentration of dietary fiber [11]. It is also source of calcium, magnesium, and zinc [12], and polyphenols [3]. However, several nutritional and bioactive parameters of these baru wastes remain unknown.

Studies with supplementation of baru nut in the diet reported oxidative stress reduction [13,14] and lipid profile improvement [15,16] in animal models. In clinical studies, the baru nut consumption decreased the serum concentration of total cholesterol, low-density lipoprotein cholesterol (LDL-c) [17], and the abdominal adiposity [18], as well as the baru nut oil reduced the ultra-sensitive C-reactive protein [19]. To our knowledge, the effects of the consumption of baru peel and pulp have not yet been described in literature.

The nut and the by-products of the baru fruit (peel and pulp) have been used to replace other nuts or wheat flour in processed foods, like cupcakes [20], cereal bars [21], cookies [11,22], breads [23] and dairy desserts [24], with good acceptability in terms of appearance and flavor [11,22]. Therefore, baru fruit as a whole (nut and residues) has a great potential to be exploited by the food industry in several added value products, by increasing the nutritional, technological and sensory properties.

This comprehensive review provides an overview of the available evidence on: 1) the nutritional and bioactive composition of the whole baru fruit (nut, oil, skin, peel and pulp); 2) the health effects of the consumption of baru nut evidenced in preclinical and clinical studies;

* Corresponding author at: Rua 227, quadra 68, Setor Leste Universitário, Goiânia, Goiás CP 74605-080, Brazil.
E-mail address: maria_margareth_veloso@ufg.br (M.M.V. Naves).

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and 3) the potential use of the different fractions of the baru fruit in functional food products.

2. Baru fruit

Baru fruit consists of the epicarp (peel), a thin layer; the mesocarp (pulp), a fibrous layer; and the endocarp, a hard and woody layer that coats a seed called almond or nut (Fig. 1) [1]. The fruits have an oval shape, dark beige to reddish-brown color, with an average 26 g of mass, 5 cm of length, and 4 cm of width [25]. The baru nut has an oval to elliptical shape and is coated by a thick brown skin [1,26]. Physical characteristics of the baru fruits, as well as of the baru nuts show significant differences among native regions and among fruits of plants native to the same region [27,28]. The nut showed 1.2 to 1.9 g of mass, which comprises 5% of the total fruit mass [25,29,30]; 1.8 to 2.4 cm of length, and 0.7 to 1.0 cm of width [25,26]. Data on physical characteristics of the baru peel and pulp, such as mass and yield, are scarce in literature.

3. Nutritional and functional properties and antioxidant potential of baru fruit

3.1. Baru nut, oil and skin

Proximate composition of raw and roasted baru nut stands out for its high content of protein (20 to 31 g/100 g) and lipids (32 to 46 g/100 g) (Table 1). The first study published on the proximate composition of the baru nut reported 30% of protein and 40% of lipids [36], which are values compatible with the results of later studies (Table 1). However, according to Table 1, Gonçalves et al. [35] found a relatively low lipid content in roasted baru nut, of 24 g/100 g. This finding could be partially explained by the different method used to analyze the lipids, the Goldfish method [35], *versus* Soxhlet method, or extraction with methanol and chloroform [37], used in other studies. The methodology chosen for lipid extraction can affect the amount of lipids extracted, and the solvents and temperature significantly influence the efficiency of the extraction [38]. Regarding dietary fiber, high contents were found in the baru nut (from 9 to 17 g/100 g), mainly insoluble fiber (Table 1), which are higher than those of other nuts, such as peanut (5.2 g/100 g) [7] and cashew nut (6.5 g/100 g) [33]. Oliveira-Alves et al. [34] reported the lowest dietary fiber content in roasted baru nut (6.1 g/100 g), although the nut was analyzed with skin and the methodology used was the enzymatic-gravimetric method, like in other studies. It is also worth highlighting in Table 1 that the sums of the values of the proximate composition in two studies [31,34] were higher than 100, which probably happened because the authors did not discount the total dietary fiber content from the carbohydrate contents. Thus, if our hypothesis is correct, the adjusted values of carbohydrate content should be 22.69 g/100 g in the study of Campidelli et al. [31], and 14.61 g/100 g in the report of Oliveira-Alves et al. [34]. These adjusted values of carbohydrate content are within the range reported for those shown in Table 1 (9.20–23.02 g/100 g), except that of the study of Gonçalves et al. [35], of 29.4 g/100 g, which value might be explained by the low lipid content found in that study, as formerly highlighted.

The protein content in baru nut is higher than those of other nuts, such as pecan (7 to 9 g/100 g) [39], Brazil nut (15 g/100 g), cashew nut (18 g/100 g), pistachio (15 g/100 g), macadamia (9 g/100 g) and walnut (14 g/100 g) [40]. A serving of 20 g of roasted baru nut contains from 4.6 g to 6.2 g of quality protein, according to the values presented in Table 1. Therefore, its consumption should be encouraged in healthy and plant-based diets [6,32,33]. On the other hand, the lipid and energy concentrations are lower than those of other nuts, such as Brazil nut (lipid: 66 g/100 g; energy: 693 kcal/100 g), macadamia (lipid: 76 g/100 g; energy: 741 kcal/100 g) and walnut (lipid: 60 g/100 g; energy: 631 kcal/100 g) [40].

Concerning the mineral composition, baru nut has high content of potassium, magnesium and iron, and considerable amounts of calcium and zinc. However, there is a large range in the mineral concentration among the studies (Table 1), for instance: Fe – from 3.0 to 19.8 mg/100 g; Ca – from 82 to 300 mg/100 g; and Zn – from 1.04 to 6.74 mg/100 g. Nevertheless, the iron contents are higher than those of Brazil nut (2.2 mg/100 g), hazelnut (2.5 mg/100 g) and walnut (2.1 mg/100 g) [41]. The minimum Ca concentration found in baru nut is higher than that found for cashew nuts (25 mg/100 g) and walnuts (73 mg/100 g) [41]. The contents of Zn observed in Brazil nut (2.4 mg/100 g), hazelnut (1.5 mg/100 g), walnut (1.8 mg/100 g) and cashew nut (3.0 mg/100 g) [41] are within the range reported for the baru nut (Table 1). In addition, the potassium and magnesium contents in baru nut are remarkably higher than those of peanut (K: 242–294 mg/100 g, Mg: 25–30 mg/100 g) [42].

The total phenolic content in raw and roasted baru nut ranged from 390 to 1300 mg GAE/100 g (Table 2), higher than those of roasted hazelnut (17 mg GAE/100 g) [46] and almond (87.5 mg GAE/100 g)

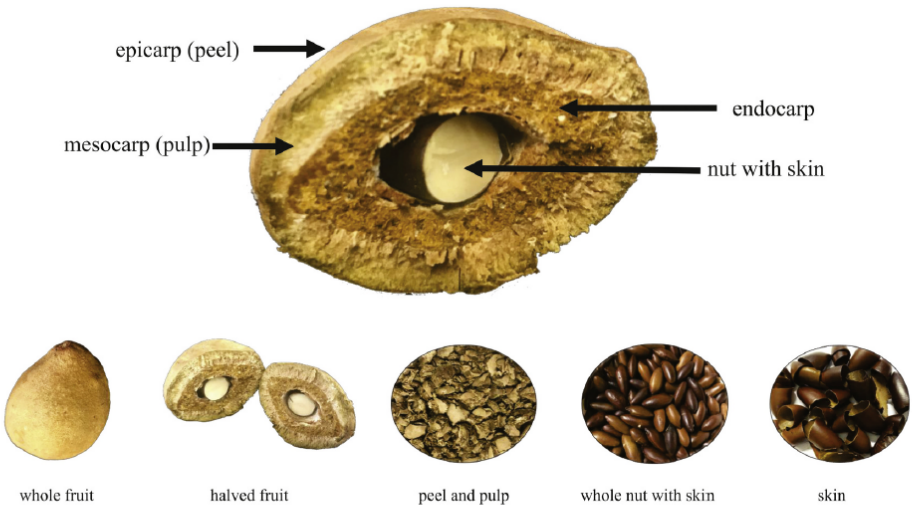

Fig. 1. Overview of baru (*Dipteryx alata* Vog.) fruit.

Table 1
Proximate composition and minerals of the baru nut.

Component	Raw nut (with skin)					Roasted nut[a]							
	Vallilo et al. [1]	Takemoto et al. [30]	Siqueira et al. [10]	Campidelli et al. [31]	Vera et al. [2]	Fernandes et al. [6]	Sousa et al. [7]	Czeder et al. [32]	Freitas et al. [33]	Fernandes et al. [13]	Santiago et al. [3]	Oliveira-Alves et al. [34]	Gonçalves et al. [35]
Proximate composition (g/100 g)													
Moisture	5.80	6.1	7.38	6.63	3.99	3.71	3.49	3.58	1.98	3.17	6.8	3.20	2.17
Protein	23.45	23.9	19.72	22.96	26.25	25.81	29.92	30.92	27.96	28.94	22.9	27.06	24.3
Lipid	41.65	38.2	38.37	31.73	33.28	41.97	41.95	41.25	42.69	42.40	40.6	45.80	24.2
Ash	2.85	2.70	2.46	1.55	–	3.32	3.18	2.98	3.08	3.01	3.1	3.24	3.06
Total dietary fiber	–	13.4	12.60	14.44	–	11.59	9.21	12.08	14.26	11.70	16	6.10	16.6
Soluble fiber	–	2.5	–	–	–	–	2.03	1.31	0.90	2.40	–	–	–
Insoluble fiber	–	10.9	–	–	–	–	7.18	10.77	13.35	9.30	–	–	–
Carbohydrate	23.02	15.8	19.47	37.13	–	13.61	12.25	9.20	10.03	10.79	11	20.71	29.4
Energy value (kcal/100 g)	561	502	502	–	–	535	546	532	536	574	501	603	–
Minerals (mg/100 g)													
Ca	82	140	88	240	300	110–149	111	130	–	–	–	–	–
Cu	1.08	1.45	–	2.8	1.67	–	–	–	–	–	–	–	1.80
Fe	5.35	4.24	3	6.5	19.81	3.9–5.9	3.57	3.18	–	–	–	–	8.65
K	811	827	–	1810	920	–	980	–	–	–	–	–	–
Mg	143	178	107	330	130	–	165	–	–	–	–	–	–
Mn	9.14	4.9	–	6.4	5.72	–	–	–	–	–	–	–	8.85
Na	3.30	–	2	–	–	–	7.46	9.83	–	–	–	–	–
P	317	358	–	–	730	–	833	–	–	–	–	–	–
Se (μg/100 g)	–	–	0.1	–	–	–	0.37	–	–	0.26	–	–	–
Zn	1.04	4.1	2	–	2.36	4.1–5.4	4.29	3.46	–	6.74	–	–	4.83

[a] With skin: Santiago et al. [3], Oliveira-Alves et al. [34]; without skin: Czeder et al. [32], Fernandes et al. [13]; not mentioned or not clear: Fernandes et al. [6], Sousa et al. [7], Freitas et al. [33], Gonçalves et al. [35].

[47], but lower than that of pecan (1988–4525 mg GAE/100 g) [39]. The main phenolic compounds identified in baru nut were gallic acid, ferulic acid, caffeic acid, catechin and rutin (Table 2) [43,45]. Siqueira et al. [10] found high tannin content (563 mg/100 g) in raw baru nut (with skin). The antioxidant capacity of baru nut, expressed in μmol TE/g, ranged from 67 [10] to 259 [9], regardless of the analysis method. By DPPH method, the antioxidant capacity of baru nut was higher than those of raw and roasted pistachio (8 μmol TE/g and 9.8–11.5 μmol TE/g, respectively) [48]. Concerning ORAC method, baru nut showed an antioxidant capacity (Table 2) higher than those of natural and roasted hazelnut (34.5 μmol TE/g and 9.5 μmol TE/g, respectively) [46]. Based on the studies available in literature, there is a wide range of results on the bioactive compounds and antioxidant capacity of the baru nut (Table 2). This fact could be partially explained either by the different methods used for the extraction of bioactive compounds [49] or the different measurement units used to express the results. Among tocopherols, α-tocopherol is the most abundant in the baru nut oil (Table 2), which concentration is similar to that in soybean oil (8.72 mg/100 g) [44].

The essential amino acids concentrations of the protein in the baru nut overall meet the dietary recommendations, except for the amino acids methionine + cysteine, valine, and lysine, and its amino acid score (AAS) ranged from 75% to 105% (Table 3). This considerable range suggests that the essential amino acid profile of the protein in the baru nut is particularly influenced by the native region of the fruits [6,32]. Deficiency of these amino acids is common in other nuts, such as peanut, almond, and cashew nut [51], and pequi almond and Cerrado cashew nut [7]. The protein digestibility-corrected amino acid score (PDCAAS) values reported for the protein in the baru nut, of 73% [6] and 91% [7], are higher than those of other oilseeds, such as peanut (70%) [6], Brazil nut (63%) [33], and almonds (44% to 48%) [52]. The PDCAAS is the recommended method for estimating the nutritional quality of protein in foods and diets [53]. Based on the PDCAAS values, the baru nut has a good quality protein [6,7]. So, its consumption is recommended for replacing animal protein or as an alternative source of protein aiming to reduce the risk factors for cardiovascular diseases [54].

The baru nut oil has about 50% monounsaturated fatty acids and 30% polyunsaturated fatty acids, with high concentration of oleic acid (C18:1) and linoleic acid (C18:2ω6), respectively (Table 4). The linoleic acid content in the baru nut oil is higher than that in pecan and pistachio oil, but the oleic acid concentration is lower than those in these oilseeds [39,55]. Lemos et al. [56] reported that the roasting process did not significantly change the fatty acid composition in baru nuts, with or without skin. The fatty acid profile of the baru nut oil is favorable for health, considering that a diet rich in unsaturated fatty acids reduces risk factors, as well as the morbidity and mortality related to cardiovascular diseases [57]. The atherogenicity index (AI), thrombogenic index (TI) and the hypocholesterolemic fatty acid/hypercholesterolemic fatty acid (h/H) ratio of baru nut oil are better than those of other native Brazilian fruits, such as the giant pequi pulp oil (AI = 1.92, TI = 1.88, h/H = 1.04) [58], and the Brazil nut oil (AI = 0.20, TI = 0.42) [59]. It is worth highlighting that the h/H ratio of baru nut oil is higher than that of olive oil (6.14) [60]. Low values of AI and TI suggest high concentration of antiatherogenic fatty acids in oil; and high values of h/H ratio, as found in baru nut oil, indicate that the oil may have a hypocholesterolemic effect [61]. This effect can be enhanced by the phytosterols in the baru nut oil, such as campesterol, stigmasterol, β-sitosterol, and cycloartenol [62]. However, further studies are warranted to quantify these compounds in the baru nut.

The baru nut skin stands out for its remarkably high content of total phenolic compounds (9730 mg GAE/100 g) and antioxidant capacity (1620 μmol TE/g, by ABTS assay) [9]. The polyphenols concentration of the baru nut skin is higher than that in hazelnut skin (7225 mg GAE/100 g) [46] and almond skin (703 mg GAE/100 g) [63]. Thus, these data suggest the importance of ingesting the baru nut with skin, and the use of its skin as an ingredient in functional food products.

Table 2
Bioactive compounds and antioxidant capacity of the baru nut (with skin).

Component	Raw nut					Roasted nut				
	Takemoto et al. [30]	Lemos et al. [43]	Borges, Malheiro, Souza, Casal, and Pereira [44]	Siqueira et al. [10]	Campidelli et al. [45]	Lemos et al. [43]	Santiago et al. [3]	Campidelli et al. [45][a]	Oliveira-Alves et al. [34]	Silva et al. [9][b]
Bioactive compounds (mg/100 g)										
Total tocopherols	–	–	**13.09**	**11.61**	–	–	–	–	–	–
α-tocopherol	5.0	–	7.63	–	0.05	–	–	0.09	–	–
β-tocopherol	–	–	0.52	–	–	–	–	–	–	–
γ-tocopherol	4.3	–	4.94	–	0.15	–	–	–	–	–
Total phenolics (mgGAE/100 g)	–	569	–	388	1254	532	728	1306	492–574	562
Total tannins	–	–	–	563	–	–	–	–	–	–
p-Coumaric acid	–	14.3	–	–	0.43	3.8	–	0.30	–	–
Ellagic acid	–	8.5	–	–	–	4.9	–	–	–	–
Caffeic acid	–	6.3	–	–	19.89	2.3	–	11.68	–	–
Gallic acid	–	224	–	–	45.83	170.9	–	48.90	–	–
Chlorogenic acid	–	–	–	–	7.33	–	–	6.80	–	–
Hydroxybenzoic acid	–	2.3	–	–	–	0.6	–	–	–	–
Ferulic acid	–	45.4	–	–	1.17	17.5	–	0.30	–	–
trans-Cinnamic acid	–	–	–	–	9.12	–	–	10.12	–	–
Catechin	–	87.2	–	–	9.01	45.6	–	11.06	–	–
Epicatechin	–	23.9	–	–	–	4.8	–	–	–	–
Vanillin	–	–	–	–	7.56	–	–	10.17	–	–
Quercetin	–	–	–	–	1.60	–	–	1.45	–	–
Rutin	–	–	–	–	17.81	–	–	18.40	–	–
Antioxidant capacity (μmol TE/g)										
DPPH	–	2.88	–	67	–	1.49	76	–	–	259
FRAP	–	–	–	–	–	–	126.8	–	–	144
ABTS	–	–	–	–	–	–	77	–	–	171
ORAC	–	–	–	–	4.06[c]	–	–	2.96[c]	89–110	–

[a] Sample T3 (baru nut submitted to 105 °C per 30 min) - not mentioned if nut is with or without skin.
[b] Total phenolic compounds of the roasted nut skin: 9730 mg GAE/100 g.
[c] μM TE/g. GAE: gallic acid equivalents; TE: trolox equivalents; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power; ABTS: 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonate); ORAC: Oxygen radical absorbance capacity.

Table 3
Amino acids composition (mg/g protein) of the baru nut.

Amino acid	WHO/FAO/UNU requirement pattern [50]	Roasted nut			
		Fernandes et al. [6]	Sousa et al. [7]	Czeder et al. [32]	Freitas et al. [33]
Essentials					
His	16.0	23.4	23.4	22–26	25.7
Ile	31.0	37.5	32.5	**25–28**	29.8
Leu	61.0	77.8	74.4	78–79	83.0
Lys	48.0	48.4	66.4	51–54	**36.2**
Met + Cys	24.0	**22.0**	29.8	**21–27**	**21.2**
Phe + Tyr	41.0	77.2	88.5	76–80	79.9
Thr	25.0	44.9	55.3	41–45	43.4
Trp	6.6	20.2	11.2	16–20	13.9
Val	40.0	51.8	55.6	**31–36**	38.3
TOTAL	292.6	403.2	437.1	368–384	371.3
AAS (%)	100	92 (Met + Cys)	105	77–89 (Val)	75 (Lys)
Non-Essentials					
Asp	–	101.6	91.4	102–107	–
Glu	–	216.8	176.9	214–223	–
Ala	–	46.1	42.6	45–50	–
Arg	–	85.6	151.4	92–96	–
Gly	–	47.2	41.7	48–52	–
Pro	–	55.3	3.8	57–59	–
Ser	–	44.1	58.4	49–49.2	–
TOTAL	–	596.7	566.2	607–636.2	–

Bold values indicate limiting amino acids. AAS: amino acid score.

3.2. Baru peel and pulp

The baru peel and pulp are discarded as solid residues during the extraction of the nut. Although there is little information on the chemical composition of the baru peel and pulp, some studies indicate that the baru pulp has a remarkably high content of dietary fiber (18%–42%), and a considerable concentration of sugars (20%–30%) (Table 5). Therefore, further studies are warranted to investigate the profile of sugars and other oligosaccharides in the baru pulp. Alves et al. [11] observed a significant increase in the content of dietary fiber and total

Table 4
Fatty acid composition of the baru nut oil.

Fatty acid (g/100 g lipid)	Raw nut						Roasted nut			
	Vallilo et al. [1]	Togashi and Sgarbieri [36]	Takemoto et al. [30]	Vera et al. [2]	Borges et al. [44]	Campidelli et al. [45]	Fernandes et al. [13]	Alves et al. [8]	Oliveira-Alves et al. [34]	Campidelli et al. [45][a]
C12:0	–	–	–	–	–	0.24	0.12	–	–	0.08
C14:0	–	–	–	0.03	0.03	0.16	0.18	–	–	0.05
C16:0	7.40	7.16	7.6	6.10	6.32	6.59	5.94	7.16	6.80	6.16
C17:0	–	–	–	0.06	0.08	0.09	0.04	0.06	–	0.08
C18:0	3.12	5.33	5.4	5.27	4.88	4.39	5.42	4.97	4.70	5.22
C20:0	0.82	1.40	1.07	1.39	1.26	1.06	1.27	0.86	1.20	1.21
C22:0	2.12	3.19	2.6	4.39	4.16	3.47	–	0.51	3.40	3.73
Total SFA	**13.46**	**17.08**	**16.67**	**22.92**	**21.72**	**16.32**	**12.98**	**15.47**	**21.10**	**16.79**
C16:1	–	–	–	–	0.08	0.08	–	0.11	–	0.07
C17:1	–	–	–	–	–	0.15	0.05	–	–	0.13
C18:1	50.17	44.53	50.4	47.15	45.81	48.99	41.41	51.45	47.20	51.01
C20:1	–	–	2.7	2.71	2.83	2.40	0.13	–	2.70	2.56
C22:1	–	–	–	0.05	0.36	0.26	–	–	0.30	0.29
Total MUFA	**50.17**	**44.53**	**53.10**	**49.91**	**49.07**	**51.91**	**45.49**	**51.57**	**50.65**	**54.08**
C18:2 ω6	30.70	31.70	28.0	25.51	28.95	27.28	24.40	28.57	28.20	26.89
C18:3 ω3	–	2.22	–	0.13	0.21	0.14	–	3.14	0.05	0.12
C20:2	–	–	–	–	–	0.08	0.10	–	–	0.05
Total PUFA	**30.70**	**33.92**	**28.00**	**25.64**	**29.16**	**31.50**	**24.79**	**31.71**	**28.25**	**31.25**
Ratios										
ω6/ω3	–	14	–	196	138	195	–	9	564	224
MUFA/SFA	3.73	2.61	3.19	2.18	2.26	3.18	3.50	3.33	2.40	3.22
UFA/SFA	6.01	4.59	4.87	3.30	3.60	5.11	5.41	5.38	3.74	5.08
Quality indexes of the baru nut oil										
AI	0.09	0.09	0.09	0.08	0.08	0.09	0.10	0.09	0.09	0.08
TI	0.26	0.28	0.32	0.30	0.28	0.28	0.33	0.24	0.29	0.28
h/H	10.93	10.96	10.67	12.32	12.25	11.68	10.77	11.61	11.49	12.59
COX	3.66	4.19	3.39	3.13	3.49	3.33	2.93	4.14	3.39	3.31

[a] Sample T3 (baru nut submitted to 105 °C for 30 min). SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; AI: atherogenicity index = (C12:0 + 4 × C14:0 + C16:0)/[ΣMUFA + ΣPUFA(n − 6) and (n − 3)]; TI: thrombogenic index = (C14:0 + C16:0 + C18:0)/[0.5 × ΣMUFA +0.5 × ΣPUFA(n − 6) + 3 × ΣPUFA(n − 3) + Σ(n − 3)/(n − 6)]; h/H: hypocholesterolemic fatty acid/hypercholesterolemic fatty acid ratio = [C18:1(n − 9) + C18:2(n − 6) + C18:3(n − 3) + C20:1(n − 9) + C20:3(n − 9)]/(C14:0 + C16:0); COX: calculated oxidability value = (C18:1 + 10.3 × C18:2 + 21.6 × C18:3)/100.

Table 5
Chemical composition and antioxidant capacity of the baru peel and pulp.

Component	Peel	Peel and pulp		Pulp					
	Santiago et al. [3]	Rocha and Cardoso-Santiago [23]	Silva et al. [12]	Vallilo et al. [1]	Togashi and Sgarbieri [36][a]	Alves et al. [11][b]	Lima et al. [21]	Almeida et al. [64]	Santiago et al. [3]
Macronutrients and energy (g/100 g)									
Moisture	16.3	21.05	9.64	20.23	–	13.76	24.45	17.1	14.9
Protein	2.5	4.45	3.19	5.0	5.59	4.17	5.88	5.0	3.2
Lipid	2.7	3.30	4.82	4.13	3.46	3.73	3.57	0.9	3.7
Ash	2.9	1.79	4.44	1.7	2.99	4.34	2.00	1.8	3.1
Crude fiber	–	4.39	–	5.71	–	–	–	–	–
Total dietary fiber	24.1	–	–	–	29.50	19.1	41.6	–	18
Insoluble fiber	–	–	–	–	28.20	–	39.5	–	–
Soluble fiber	–	–	–	–	1.30	–	2.1	–	–
Carbohydrates	51.5	65.01	77.43	–	–	54.9	22.5	75.4	57
Total sugars	–	–	–	30.80	20.45	23.0	–	–	–
Glucose	–	–	–	23.09	–	–	–	–	–
Sucrose	–	–	–	7.71	–	–	–	–	–
Starch	–	–	–	32.38	38.01	–	–	–	–
Energy (kcal/100 g)	240	–	366	310	–	270	146	329	276
Minerals (mg/100 g)									
Ca	–	–	116	75.2	–	–	–	–	–
Cu	–	–	3.38	3.54	–	–	–	–	–
Fe	–	–	3.59	5.94	–	–	–	–	–
K	–	–	1187	572	–	–	–	–	–
Mg	–	–	80	3.9	–	–	–	–	–
Mn	–	–	1.01	3.84	–	–	–	–	–
Na	–	–	–	1.74	–	–	–	–	–
P	–	–	114	82.2	–	–	–	–	–
Zn	–	–	8.75	1.08	–	–	–	–	–
Bioactive compounds									
Total tannins (mg/100 g)	–	–	–	–	3112	–	–	–	–
Phytic acid (%)	–	–	–	–	0.27		–	–	–
Total phenolics (mg GAE/100 g)	477	–	186	–	–	–	–	–	292
Antioxidant capacity (μmol TE/g)									
ABTS	60	–	14	–	–	–	–	–	49
DPPH	45	–	9.5	–	–	–	–	–	21
FRAP	50	–	–	–	–	–	–	–	24

[a] Values on dry basis. [b] Pulp with 1 day of storage. GAE: gallic acid equivalents; TE: trolox equivalents; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonate); FRAP: Ferric Reducing Antioxidant Power.

Table 6
Summary of preclinical and clinical studies investigating the effects of baru nut (whole nut, extract or oil) supplementation on health.

Reference	Intervention	Models/Subjects	Study design	Main outcomes
In vitro study				
Oliveira-Alves et al. [34]	crude and hydrolyzed methanolic extract of baru nut (6.25–200 mg/mL)	human colon cancer cell lines (HT29 and Caco-2)	cytotoxicity assay in Caco-2 cell model, and antiproliferative assay in HT29 monolayers and spheroids	baru extracts have the potential to inhibit colorectal cancer cell proliferation in HT29 cell spheroids
Animal studies				
Fernandes et al. [6]	baru nut (418 g/kg diet) and peanut (298 g/kg diet)	30 male Wistar rats (n = 6/group)	5 groups (14 days): two casein diets (one with 7% or 17% lipids); two experimental diets with 17% lipids (baru nut or peanut diets); and a protein-free diet	baru nut is a food with high content of quality protein
Czeder et al. [32]	baru nut (317–332 g/kg diet)	36 male Wistar rats (n = 6/group)	6 groups (14 days): two casein diets with 7% or 14% lipids; three experimental diets with 14% lipids (baru nut from East, Southeast or West region); and a protein-free diets	the region of the Brazilian Savanna does not influence the protein quality of baru nuts
Freitas et al. [33]	baru nut (36% diet), peanut (31% diet), Brazil nut (61% diet), cashew nut (43% diet)	42 male Wistar rats (n = 6/group)	7 groups (14 days): two casein diets with 7% or 14% lipids; four experimental diets with 10% proteins (baru nut, peanut, Brazil nut or cashew nut diets); and a protein-free diet	the cashew nut has the best quality protein among these nuts
Ravagnani et al. [69]	baru ether extract (22% diet, replacing the lipid of the diet)	42 Wistar rats (n = 4–10/group)	chocolate-based diet (2 months): to induce obesity 6 groups (8 weeks): sedentary control; trained control; sedentary baru; trained baru; sedentary chocolate and trained chocolate	the exercise training seemed more effective than the diet in attenuating lipid tissue accumulation
Siqueira et al. [70]	baru nut (100 g/kg diet)	30 adult male Wistar rats (n = 6/group)	5 groups (17 days): AIN-93G diet; AIN-93G diet + baru nut; AIN-93G + $FeSO_4$ oral supplementation; AIN-93G diet + $FeSO_4$ + baru nut; AIN-93G diet + phytic acid, inositol hexaphosphate	↓ carbonyl levels in the liver, heart and spleen of the iron-supplemented rats
Fernandes et al. [13]	baru nut (35% diet) or Brazil nut (23% diet)	young adult male Wistar rats	4 groups (9 weeks): high-fat diets (0.1% cholic acid +1% cholesterol +5% lard +15% of lipid from lard, baru nut or Brazil nut), and reference diet (7% soybean oil)	↓ serum total cholesterol, triacylglycerols, and total malondialdehyde ↑ hepatic vitamin E Only baru nut: ↑ HDL-c
Araujo et al. [15]	baru nut (82 g/kg diet)	28 obese male Swiss mice (n = 7/group)	high-glucose diet (60 days): to induce obesity 4 groups (8 weeks): control diet, high-glucose diet, high-glucose with baru nut or soybean oil	↓ weight gain, glucose and TG
Fiorini et al. [16]	baru nut (20, 30 or 40% diet)	40 male Wistar rats (n = 10/group)	4 groups (40 days): commercial diet or commercial diet with baru nut	↓ TG, VLDL-c, LDL-c, ultra-sensitive C-reactive protein ↑ HDL-c
Reis et al. [14]	baru nut oil (1 g/kg/day)	40 male Wistar rats (n = 10/group)	4 groups (15 weeks): control; baru nut oil; lipid emulsion; lipid emulsion + baru nut oil (intragastric)	↓ lipid peroxidation (MDA), and liver damage
Clinical studies				
Bento et al. [17]	20 g/day of baru nuts or placebo (1 corn starch capsule/day)	20 mildly hypercholesterolemic subjects (total cholesterol mean ± SEM = 5.8 ± 0.2 mmol/L)	randomized, crossover, placebo controlled (6 weeks)	↓ total cholesterol, low-density lipoprotein cholesterol and nonhigh-density lipoprotein cholesterol
Souza et al. [18]	Baru nut-enriched diet (20 g baru nut)	46 overweight and obese women	randomized, placebo-controlled (8 weeks)	↓ abdominal adiposity and improved high-density lipoprotein
Souza et al. [71]	Normocaloric and isoenergetic diet with placebo or similar advice plus 20 g baru nut	46 overweight and obese women (age: 40 ± 11 years; body mass index: 33.3 ± 4.3)	randomized placebo-controlled (8 weeks)	↑ the activity of glutathione peroxidase and plasma copper concentration
Schincaglia et al. [19]	5 g of baru nut oil or 5 g of mineral oil (placebo)	29 patients with chronic kidney disease under hemodialysis treatment	randomized, double-blind, placebo-controlled (12 weeks)	↓ ultra-sensitive C-reactive protein

sugars in baru pulp after 136 days of storage. In addition, the baru peel and pulp have high concentration of minerals, especially potassium, calcium, magnesium, zinc and iron (Table 5). Potassium and calcium are also the major minerals in the jatobá-do-cerrado pulp, another native Brazilian fruit [65].

Regarding the bioactive compounds and antioxidant capacity, few studies reported that the baru peel and pulp have a considerable content of total phenolics, and that the baru pulp has high total tannins content (Table 5). The total phenolic concentration in the baru pulp is higher than that in the flour of jatobá-do-cerrado pulp (125 mg GAE/100 g; 13% moisture) [65], and of the pequi pulp (198 mg GAE/100 g; 12% of moisture) [66]. The total phenolic content and the antioxidant capacity of baru peel are higher than those of the pulp (Table 5). Fruit peels usually have high total phenolics content, since these compounds are secondary metabolites of plants, which protect the fruit against ultra-violet light, pathogens, parasites, and predators [67]. Silva et al. [12] showed the total phenolic content and antioxidant capacity of the peel with pulp lower than that of the peel or pulp reported by another study

[3]. This difference can be partially explained by the solvent used to obtain the extracts. One of the studies used 70% acetone plus 50% methanol [12], and the other used 50% ethanol [3]. Polyphenols such as protocatechuic acid, coumaric acid derivative, luteolin, vicenin 2, and di-O-methoxy dihydroxy isoflavone have been identified in baru pulp [68].

Concerning the anti-nutritional factors, the phytic acid and trypsin inhibitor contents are very low, and hemagglutinating activity was not detected in the baru pulp [36]. So, the consumption of raw baru pulp may not be deleterious for human health considering its negligible amounts of anti-nutritional factors. Further studies are needed to investigate the profile of carbohydrates, including oligosaccharides, and polyphenols, and the health benefits of the baru peel and pulp consumption in preclinical and clinical studies.

4. Health effects of the consumption of baru nut and oil

4.1. Effects on serum lipid profile

The health benefits of the baru nut have been investigated in both animal models and humans (Table 6). Concerning the effects on the lipid profile, the consumption of baru nut decreased serum concentration of triacylglycerols (TG) [13,15,16], total cholesterol (TC) [13] and low-density lipoprotein cholesterol (LDL-c) [16] (Table 6). In a randomized, placebo controlled and crossover study, the intervention with 20 g of baru nut for six weeks reduced serum concentration of TC, LDL-c, and non-high-density lipoprotein cholesterol (non-HDL-c) of mildly hypercholesterolemic individuals [17]. Moreover, the consumption of 20 g of baru nut for eight weeks decreased TC, LDL-c, TG, and non-HDL-c serum concentrations in a randomized clinical trial with overweight or obese women (Table 6) [18].

The effects of the baru nut consumption on the lipid profile can be explained by the high contents of unsaturated fatty acids, mainly oleic fatty acid, with low values of atherogenicity index and thrombogenic index (Table 4), which indicate that the oil has a hypocholesterolemic effect [61]. These benefits for lipid profile are probably optimized by the dietary fibers [72] and phytosterols [62], because their cholesterol-lowering effect [72], and by other bioactive compounds present in the baru nut (Tables 1 and 2). A systematic review and meta-analysis concluded that the consumption of tree nuts reduce TC, LDL-c, TG, and Apolipoprotein B (ApoB), with decreasing effects on the TC and LDL-c by the intake of a daily serving of 60 g [73]. Considering that a high-unsaturated fatty acids diet can decrease risk factors to cardiovascular diseases [57], baru nut represents a promising dietary source of these fatty acids.

4.2. Effects on body mass

Preclinical and clinical studies did not report body weight gain after baru nut consumption (Table 6), even though a serving of 20 g of baru nut can offer from 100 to 120 kcal, a relatively high-calorie value. The baru nut supplementation improved the serum lipid profile without promoting body mass gain, both in animal models [13,15,16], and in mildly hypercholesterolemic individuals [17]. Furthermore, a study with overweight or obese women supplemented with baru nut reported a reduction in body fat mass [18]. Indeed, as reviewed by a meta-analysis of 62 clinical trials [74], nut consumption had positive effects on body weight, waist circumference and body mass index compared with a control diet.

The positive effects of the baru nut on body weight control can be related to the synergic effects of its high contents of bioactive compounds and fibers. The satiety improvement due to the intake of the dietary fibers is a possible mechanism for maintaining the body weight [75]. In addition, the prebiotic effect of some fibers and phenolic compounds, which undergo bacterial fermentation in the gut, generate metabolites that may have favorable effects on lipid and energy metabolism [76]. Studies have highlighted the importance of fermentable polysaccharides and polyphenols present in nuts, which causes favorable alterations in the gut microbiota and possible effect on the body weight control [76–78]. It has been suggested that polyphenols may act as prebiotics, and this mechanism would explain part of the health benefits of these compounds [78,79]. Thus, baru nut is a potential functional food, which may favorably modulate the gut microbiota and the body mass. However, this topic requires further studies, especially concerning the intake of the other fractions of baru fruit.

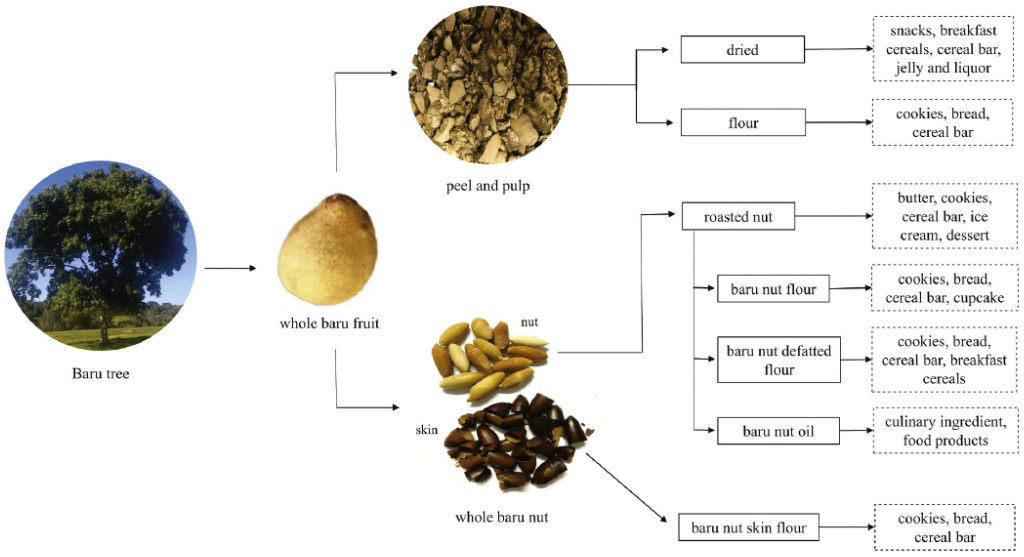

Fig. 2. Potential applications of baru fruit in functional food products.

4.3. Effects on oxidative stress and lipid peroxidation

Supplementation with baru nut or baru nut oil reduced oxidative stress in animal models [13,14,70] (Table 6). Baru nut was effective in reducing carbonyl concentrations in the liver, heart and spleen of iron-supplemented rats [70]. Moreover, the roasted baru nut intake reduced lipid peroxidation (estimated by malondialdehyde - MDA) on the liver tissue of rats fed a high-fat diet [13]. Baru nut oil also was effective in reducing the vascular lipid peroxidation and hepatic morphological damage in a murine model of dyslipidemia and lipotoxicity [14]. It is worth adding that baru oil is rich in linoleic acid (Table 4), which may be related to the positive effects on energy metabolism and prevention of lipoperoxidation [80].

A daily intake of 20 g of baru nut increased glutathione peroxidase (GPx) activity and plasmatic copper levels in overweight or obese women, but no statistical difference was observed in MDA and cytokines concentrations [71]. However, the intake of the same serving of baru nut by mildly hypercholesterolemic individuals did not change thiobarbituric acid reactive substances (TBARS) concentration, superoxide dismutase (SOD) activity and the serum antioxidant status evaluated by the ferric reducing antioxidant potential (FRAP) assay [17]. Also, the supplementation with 5 g of baru nut oil in patients with chronic hemodialysis did not influence the MDA concentration [19]. On the other hand, the median value of the servings of nuts supplemented in clinical trials was 56 g/d [73], a much larger value than the serving of baru nut consumed in clinical trials (Table 6). Therefore, the daily intake of at least 20 g of baru nut, as part of a healthy diet, could promote an improvement in the oxidative stress biomarkers.

The effects on oxidative biomarkers and lipid peroxidation can be explained by the high antioxidant potential of the baru nut, such as its content in tocopherols and phenolic compounds (Table 2). Gallic acid was the main phenolic compound identified in baru nut [45], which is favorable to health especially for its anti-oxidative activity [81]. Other phenolic compounds found in baru nut were ferulic acid, caffeic acid, catechin and rutin [43,45]. However, studies on a broader profile of polyphenols in the baru nut are needed. The health benefits of phenolic compounds have been widely reported, which include antioxidant [82] and anti-inflammatory properties [83]. Furthermore, dietary polyphenols have been associated with a decrease in the risk factors for cardiovascular diseases [84].

5. Baru nut and baru residues as ingredients for functional food products

The use of baru nut and the baru residues in food products is an alternative with great potential to be exploited by agro-food industries, because this fruit is available in many regions of the Brazilian Cerrado and has a remarkable nutritional and functional composition. Furthermore, the baru by-products constitute alternatives for fortification, innovation, and sustainability in food technology since using the waste is a strategy to reduce residues and add value to new products.

The baru nut can replace other nuts or wheat flour in new formulations or be used as an additional ingredient in traditional food products. The roasted baru nut had a shelf life that ranged from 90 to 120 days, depending on the packaging used, and unsaturated oleic and linoleic fatty acids were preserved during the storage period [85]. The baru nut flour was used in a dairy dessert with chocolate and the authors reported a considerable antioxidant capacity of the product, evaluated by FRAP [24]. The baru nut flour was also used in reduced-fat cupcakes, in which 30% wheat flour was replaced by baru nut flour [20]. The authors reported that although the dough development time increased, baru can be used in the formulation of cupcakes adding nutritional, technological and sensory properties.

The baru nut oil obtained by superfluid critical extraction showed high contents of tocopherols (6 to 11 mg/100 g), total phenolic compounds (685 to 1390 mg GAE/100 g), and oleic (50% to 54%) and linoleic (23% to 25%) unsaturated fatty acids [86]. Thus, the baru nut oil has potential application in functional food products. The partially defatted baru nut flour is another baru by-product with remarkable attributes, such as its high contents of good quality protein, dietary fiber and bioactive compounds [10]. This flour may be used in functional foods with low calories and high density in nutrients and bioactive compounds.

The baru peel and pulp are not usually consumed in human diet, but there are reports of the use of the pulp in processed foods, such as cookies [11,22], cereal bars [21] and breads [23]. The baru pulp has high tannin content, which is related to the antioxidant capacity in pulp, as well as to its bitter taste and astringency. Tannins accumulate during early developing stages of the fruit, and then decrease continuously in baru pulp, mainly after 15 days of post-harvest storage [11]. Cookies with partial replacement of wheat flour (25%) by baru pulp flour had good acceptance in terms of appearance and flavor, and the baru pulp increased the fiber and reduced the fat content in the product [11]. Another study used pre-treated baru pulp flour (with acetic acid solution to tannin extraction) in cookies and the acceptability index was higher than 70% [22]. A cereal bar made with baru nut and pulp resulted in a formulation with high content of protein (10.64 g/100 g) and fiber (15.7 g/100 g), when compared to a commercial cereal bar [21]. The content of dietary fiber in the baru cereal bar [21] was twice the amount of fiber found in cereal bars made with banana and murici [87].

Therefore, the baru fruit by-products – nut, nut skin, nut oil, defatted nut, peel and pulp – can be used in several food products, as shown in Fig. 2. They have a wide potential to be used by the food agroindustry as promising nutritious and functional ingredients in the formulation of new products, including plant-based products. Thus, research is warranted on new processed foods with the baru by-products and their role in health promotion.

6. Conclusions

Baru nut is an energy-dense food with high contents of good quality protein and lipids, dietary fiber and polyphenols. The consumption of baru nut contributes to improving lipid profile, body mass control, and the oxidative stress. The baru peel and pulp, residues from baru fruit processing, have a remarkably high content in dietary fiber, and is source of polyphenols. Baru nut and baru residues represent alternatives for the development of food products with functional and nutraceutical properties. Moreover, the use of the baru by-products as ingredients in food products and nutraceuticals could have a positive impact on the environment and add value to this native Brazilian fruit. However, *in vivo* studies are needed to investigate the effect of consuming different fractions of the fruit on cardiometabolic biomarkers and on the gut microbiota.

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.

References

[1] M.I. Vallilo, M.T.A. Tavares, S. Aued, Composição química da polpa e da semente do fruto do cumbaru (*Dipteryx alata* Vog.) - caracterização do óleo da semente, Rev. Inst. Flor. 2 (1990) 115–125.

[2] R. Vera, M.S. Soares Junior, R.V. Naves, E.R.B. Souza, E.P. Fernandes, M. Caliari, W.M. Leandro, Características químicas de amêndoas de barueiros (*Dipteryx alata* Vog.) de ocorrência natural no Cerrado do estado de Goiás, Brasil, Rev. Bras. Frutic. 31 (2009) 112–118, https://doi.org/10.1590/s0100-29452009000100017.

[3] G.L. Santiago, I.G. Oliveira, M.A. Horst, M.M.V. Naves, M.R. Silva, Peel and pulp of baru (*Dipteryx alata* Vog.) provide high fiber, phenolic content and antioxidant capacity, Food Sci. Technol. 38 (2018) 244–249, https://doi.org/10.1590/1678-457X.36416.

[4] A.M. Castro, Flavors from the Cerrado. https://sistemas.mre.gov.br/kitweb/datafiles/KualaLumpur/en-us/file/revistaing13-mat13.pdf, 2021 (accessed 10 march 2021).

[5] S. Sano, M. Brito, J. Ribeiro, *Dipteryx alata*: baru, in: R.F. Vieira, J. Camillo, L. Coradin (Eds.), Espécies nativas da flora Brasileira de valor econômico atual ou potencial: Plantas para o futuro – região Centro-Oeste, Ministério do Meio Ambiente, Brasília, 2018, pp. 203–215.

[6] D.C. Fernandes, J.B. Freitas, L.P. Czeder, M.M.V. Naves, Nutritional composition and protein value of the baru (*Dipteryx alata* Vog.) almond from the Brazilian Savanna, J. Sci. Food Agric. 90 (2010) 1650–1655, https://doi.org/10.1002/jsfa.3997.

[7] A.G.O. Sousa, D.C. Fernandes, A.M. Alves, J.B. Freitas, M.M.V. Naves, Nutritional quality and protein value of exotic almonds and nut from the Brazilian Savanna compared to peanut, Food Res. Int. 44 (2011) 2319–2325, https://doi.org/10.1016/j.foodres.2011.02.013.

[8] A.M. Alves, D.C. Fernandes, J.F. Borges, A.G.O. Sousa, M.M.V. Naves, Oilseeds native to the Cerrado have fatty acid profile beneficial for cardiovascular health, Rev. Nutr. 29 (2016) 859–866, https://doi.org/10.1590/1678-98652016000600010.

[9] P.N. Silva, T. Dias, L.L. Borges, A.M. Alves-Santos, M.A. Horst, M.R. Silva, M.M. V. Naves, Total phenolic compounds and antioxidant capacity of baru almond and by-products evaluated under optimizing extraction conditions, Rev. Bras. Cienc. Agrar. 15 (2020) 1–6, https://doi.org/10.5039/agraria.v15i4a8530.

[10] A.P.S. Siqueira, M.T.B. Pacheco, M.M.V. Naves, Nutritional quality and bioactive compounds of partially defatted baru almond flour, Food Sci. Technol. 35 (2015) 127–132, https://doi.org/10.1590/1678-457X.6532.

[11] A.M. Alves, A.L. Mendonça, M. Caliari, R.A. Cardoso-Santiago, Avaliação química e física de componentes do baru (*Dipteryx alata* Vog.) para estudo da vida de prateleira, Pesq. Agropec. Trop. 40 (2010) 266–273, https://doi.org/10.5216/pat.v40i3.6343.

[12] D. Silva, D. Oliveira, O. Resende, M. Silva, K. Barcelos, Nutritional quality of the epicarp and mesocarp flours of baru fruits submitted to drying, Rev. Bras. Eng. Agric. Ambient. 23 (2019) 65–70, https://doi.org/10.1590/1807-1929/agriambi.v23n1p65-70.

[13] D.C. Fernandes, A.M. Alves, G.S.F. Castro, A.A. Jordao Junior, M.M.V. Naves, Effects of baru almond and Brazil nut against hyperlipidemia and oxidative stress in vivo, J. Food Res. 4 (2015) 38–46, https://doi.org/10.5539/jfr.v4n4p38.

[14] M.A. Reis, R.D. Novaes, S.R. Baggio, A.L.M. Viana, B.C.C. Salles, S.M.S. Duarte, F.B. A. Paula, Hepatoprotective and antioxidant activities of oil from baru almonds (*Dipteryx alata* Vog.) in a preclinical model of lipotoxicity and dyslipidemia, Evid-Based Compl. Alt. Med. 2018 (2018) 1–11, https://doi.org/10.1155/2018/8376081.

[15] A. Araujo, J. Rocha, A. Paraiso, A.V.M. Ferreira, S.H.S. Santos, L. Pinho, Consumption of baru nuts (*Dipteryx alata*) in the treatment of obese mice, Cienc. Rural 47 (2017) 2015–2018, https://doi.org/10.1590/0103-8478cr20151337.

[16] A.M.R. Fiorini, S.M. Barbalho, E.L. Guiguer, M. Oshiiwa, C.G. Mendes, R.L. Vieites, A.G. Chies, P.B. Oliveira, M.S.S. Souza, C.C.T. Nicolau, *Dipteryx alata* Vogel may improve lipid profile and atherogenic indices in Wistar rats *Dipteryx alata* and atherogenic indices, J. Med. Food 20 (2017) 1121–1126, https://doi.org/10.1089/jmf.2017.0052.

[17] A.P.N. Bento, C. Cominetti, A. Simões Filho, M.M.V. Naves, Baru almond improves lipid profile in mildly hypercholesterolemic subjects: a randomized, controlled, crossover study, Nutr. Metab. Cardiovasc. Dis. 24 (2014) 1330–1336, https://doi.org/10.1016/j.numecd.2014.07.002.

[18] R.G.M. Souza, A.C. Gomes, I.A. Castro, J.F. Mota, A baru almond–enriched diet reduces abdominal adiposity and improves high-density lipoprotein concentrations: a randomized, placebo-controlled trial, Nutrition 55–56 (2018) 154–160, https://doi.org/10.1016/j.nut.2018.06.001.

[19] R.M. Schincaglia, L. Cuppari, H.F.S. Neri, D.E. Cintra, M.R. Sant'Ana, J.F. Mota, Effects of baru almond oil (*Dipteryx alata* Vog.) supplementation on body composition, inflammation, oxidative stress, lipid profile, and plasma fatty acids of hemodialysis patients: a randomized, double-blind, placebo-controlled clinical trial, Complement. Ther. Med. 52 (2020) 102479, https://doi.org/10.1016/j.ctim.2020.102479.

[20] C.S. Paglarini, M.S. Queirós, S.S. Tuyama, A.C.V. Moreira, Y.K. Chang, C.J. Steel, Characterization of baru nut (*Dipteryx alata* Vog) flour and its application in reduced-fat cupcakes, J. Food Sci. Technol. 55 (2018) 164–172, https://doi.org/10.1007/s13197-017-2876-1.

[21] J.C.R. Lima, J.B. Freitas, L.P. Czeder, D.C. Fernandes, M.M.V. Naves, Qualidade microbiológica, aceitabilidade e valor nutricional de barras de cereais formuladas com polpa e amêndoa de baru, Bol. Cent. Pesqui. Process. Aliment. 28 (2010) 331–343, https://doi.org/10.5380/cep.v28i2.20450.

[22] T.H.B. Ferreira, S.R. Silva, C.L. Munhoz, E.J.S. Argandoña, Elaboration of biscuits type cookies with pre-treated baru (*Dipteryx alata* Vog.) pulp flour, J. Food Meas. Charact. 14 (2020) 3156–3162, https://doi.org/10.1007/s11694-020-00557-3.

[23] L.S. Rocha, R.A. Cardoso-Santiago, Implicações nutricionais e sensoriais da polpa e casca de baru (*Dipterix alata* Vog.) na elaboração de pães, Food Sci. Technol. 29 (2009) 820–825, https://doi.org/10.1590/s0101-20612009000400019.

[24] P.N. Cruz, L.A. Gama, M.F. Américo, P.B. Pertuzatti, Baru (*Dipteryx alata* Vogel) almond and dairy desserts with baru regulates gastrointestinal transit in rats, J. Food Process. Preserv. 43 (2019) 1–8, https://doi.org/10.1111/jfpp.14167.

[25] S.M. Sano, L.J. Vivaldi, C.R. Spehar, Diversidade morfológica de frutos e sementes de baru (*Dipteryx alata* Vog.), Pesqu. Agropecu. Bras. 34 (1999) 513–518, https://doi.org/10.1590/s0100-204x1999000400001.

[26] R. Ferreira, S. Botelho, A. Davide, M. Malavasi, Caracterização morfológica de fruto, semente, plântula e muda de *Dipteryx alata* Vogel - baru (Leguminosae Papilionoideae), Cerne 4 (1998) 73–87.

[27] G.C. Corrêa, R.V. Naves, M.R. Rocha, L.F. Zica, Caracterização física de frutos de baru (*Dipteryx alata* Vog.) em três populações nos Cerrados do Estado de Goiás, Pesq. Agropec. Trop. 30 (2000) 5–11.

[28] A. Zuffo, F. Andrade, J. Zuffo Júnior, Caracterização biométrica de frutos e sementes de baru (*Dipteryx alata* Vog.) na região leste de Mato Grosso, Brasil, Rev. Cienc. Agrar. 37 (2014) 463–471.

[29] L. Botezelli, A.C. Davide, M.M. Malavasi, Características dos frutos e sementes de quatro procedências de *Dipteryx alata* Vogel (baru), Cerne 6 (2000) 9–18.

[30] E. Takemoto, I.A. Okada, M.L. Garbelotti, M. Tavares, S. Aued-Pimentel, Chemical composition of seeds and oil of baru (*Dipteryx alata* Vog.) native from Pirenópolis, State of Goiás, Brazil, Rev. Inst. Adolfo Lutz 60 (2001) 113–117.

[31] M.L.L. Campidelli, J.D.S. Carneiro, E.C. Souza, M.L. Magalhães, E.E.C. Nunes, P. B. Faria, M. Franco, E.V.B. Villas Boas, Effects of the drying process on the fatty acid content, phenolic profile, tocopherols and antioxidant activity of baru almonds (*Dipteryx alata* Vog.), Grasas Aceites 71 (2020) 1–11, https://doi.org/10.3989/gya.1170182.

[32] L.P. Czeder, D.C. Fernandes, J.B. Freitas, M.M.V. Naves, Baru almonds from different regions of the Brazilian Savanna: implications on physical and nutritional characteristics, Agric. Sci. 3 (2012) 745–754, https://doi.org/10.4236/as.2012.35090.

[33] J.B. Freitas, D.C. Fernandes, L.P. Czeder, J.C.R. Lima, A.G.O. Sousa, M.M.V. Naves, Edible seeds and nuts grown in Brazil as sources of protein for human nutrition, Food Nutr. Sci. 2012 (2012) 857–862, https://doi.org/10.4236/fns.2012.36114.

[34] S.C. Oliveira-Alves, R.S. Pereira, A.B. Pereira, A. Ferreira, E. Mecha, A.B. Silva, M. R. Bronze, Identification of functional compounds in baru (*Dipteryx alata* Vog.) nuts: nutritional value, volatile and phenolic composition, antioxidant activity and antiproliferative effect, Food Res. Int. 131 (2020) 109026, https://doi.org/10.1016/j.foodres.2020.109026.

[35] T.O. Gonçalves, G.S. Filbido, A.P.O. Pinheiro, P.D.P. Piereti, R.D. Villa, A. P. Oliveira, In vitro bioaccessibility of the Cu, Fe, Mn and Zn in the baru almond and bocaiúva pulp and, macronutrients characterization, J. Food Compos. Anal. 86 (2020) 103356, https://doi.org/10.1016/j.jfca.2019.103356.

[36] M. Togashi, V.C. Sgarbieri, Caracterização química parcial do fruto do baru (*Dipteryx alata*, Vog.), Food Sci. Technol. 14 (1994) 85–95.

[37] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917, https://doi.org/10.1139/y59-099.

[38] A.A.S. Brum, L.F. Arruda, M.A.B. Regitano-d'Arce, Métodos de extração e qualidade da fração lipídica de matérias-primas de origem vegetal e animal, Quim Nova 32 (2009) 849–854, https://doi.org/10.1590/S0100-40422009000400005.

[39] S.R. Ribeiro, B. Klein, Q.M. Ribeiro, I.D. Santos, A.L.G. Genro, D.F. Ferreira, R. Wagner, Chemical composition and oxidative stability of eleven pecan cultivars produced in southern Brazil, Food Res. Int. 136 (2020) 109596, https://doi.org/10.1016/j.foodres.2020.109596.

[40] B.R. Cardoso, G.B.S. Duarte, B.Z. Reis, S.M.F. Cozzolino, Brazil nuts: nutritional composition, health benefits and safety aspects, Food Res. Int. 100 (2017) 9–18, https://doi.org/10.1016/j.foodres.2017.08.036.

[41] J. Suliburska, Z. Krejpcio, Evaluation of the content and bioaccessibility of iron, zinc, calcium and magnesium from groats, rice, leguminous grains and nuts, J. Food Sci. Technol. 51 (2014) 589–594, https://doi.org/10.1007/s13197-011-0535-5.

[42] M. Gülfen, A. Özdemir, Analysis of dietary minerals in selected seeds and nuts by using ICP-OES and assessment based on the recommended daily intakes, Nutr. Food Sci. 46 (2016) 282–292, https://doi.org/10.1108/NFS-11-2015-0138.

[43] M.R.B. Lemos, E.M.A. Siqueira, S.F. Arruda, R.C. Zambiazi, The effect of roasting on the phenolic compounds and antioxidant potential of baru nuts [*Dipteryx alata* Vog.], Food Res. Int. 48 (2012) 592–597.

[44] T.H. Borges, R. Malheiro, A.M. Souza, S. Casal, J.A. Pereira, Microwave heating induces changes in the physicochemical properties of baru (*Dipteryx alata* Vog.) and soybean crude oils, Eur. J. Lipid Sci. Technol. 117 (2015) 503–513, https://doi.org/10.1002/ejlt.201400351.

[45] M. Campidelli, J.D. Carneiro, E.C. Souza, M. Magalhães, I. Konig, M. Braga, T. Orlando, S.D. Simão, L. Lima, E.V.B. Villas Boas, Impact of the drying process on the quality and physicochemical and mineral composition of baru almonds (*Dipteryx alata* Vog.) impact of the drying process on baru almonds, J. Culin. Sci. Technol. 18 (2020) 231–243, https://doi.org/10.1080/15428052.2019.1573710.

[46] E. Pelvan, E.O. Olgun, A. Karadağ, C. Alasalvar, Phenolic profiles and antioxidant activity of Turkish Tombul hazelnut samples (natural, roasted, and roasted hazelnut skin), Food Chem. 244 (2018) 102–108, https://doi.org/10.1016/j.foodchem.2017.10.011.

[47] H.K. Ozer, Phenolic compositions and antioxidant activities of Maya nut (*Brosimum alicastrum*): comparison with commercial nuts, Int. J. Food Prop. 20 (2017) 2772–2781, https://doi.org/10.1080/10942912.2016.1252389.

[48] J.J. Rodríguez-Bencomo, H. Kelebek, A.S. Sonmezdag, L.M. Rodríguez-Alcalá, J. Fontecha, S. Selli, Characterization of the aroma-active, phenolic, and lipid profiles of the pistachio (*Pistacia vera* L.) nut as affected by the single and double roasting process, J. Agric. Food Chem. 63 (2015) 7830–7839, https://doi.org/10.1021/acs.jafc.5b02576.

[49] T.W. Caldas, K.E.L. Mazza, A.S.C. Teles, G.N. Mattos, A.I.S. Brígida, C.A. Conte-Junior, R.G. Borguini, R.L.O. Godoy, L.M.C. Cabral, R.V. Tonon, Phenolic compounds recovery from grape skin using conventional and non-conventional extraction methods, Ind. Crop. Prod. 111 (2018) 86–91, https://doi.org/10.1016/j.indcrop.2017.10.012.

[50] World Health Organization (WHO), Protein and Amino Acid Requirements in Human Nutrition, WHO, Geneva, 2007.

[51] J.B. Freitas, M.M.V. Naves, Chemical composition of nuts and edible seeds and their relation to nutrition and health, Rev. Nutr. 23 (2010) 269–279, https://doi.org/10.1590/S1415-52732010000200010.

[52] J.D. House, K. Hill, J. Neufeld, A. Franczyk, M.G. Nosworthy, Determination of the protein quality of almonds (*Prunus dulcis* L.) as assessed by in vitro and in vivo methodologies, Food Sci. Nutr. 7 (2019) 2932–2938, https://doi.org/10.1002/fsn3.1146.

[53] Food and Agriculture Organization (FAO) of the United Nations, Dietary Protein Quality Evaluation in Human Nutrition, FAO, Rome, 2013.

[54] R.G.M. Souza, A.C. Gomes, M.M.V. Naves, J.F. Mota, Nuts and legume seeds for cardiovascular risk reduction: scientific evidence and mechanisms of action, Nutr. Rev. 73 (2015) 335–347, https://doi.org/10.1093/nutrit/nuu008.

[55] M. Esteki, P. Ahmadi, Y.V. Heyden, J. Simal-Gandara, Fatty acids-based quality index to differentiate worldwide commercial pistachio cultivars, Molecules 24 (2019) 1–16, https://doi.org/10.3390/molecules24010058.

[56] M.R.B. Lemos, R.C. Zambiazi, E.M.S. Almeida, E.R. Alencar, Tocopherols and fatty acid profile in baru nuts (*Dipteryx alata* Vog.), raw and roasted: important sources in nature that can prevent diseases, Food Sci. Nutr. Technol. 1 (2016) 1–11, https://doi.org/10.1016/j.foodres.2012.05.027.

[57] H.E. Billingsley, S. Carbone, C.J. Lavie, Dietary fats and chronic noncommunicable diseases, Nutrients 10 (2018) 1–16, https://doi.org/10.3390/nu10101385.

[58] M.S. Silva, A.M. Alves-Santos, I.D. Santos, R. Wagner, M.M.V. Naves, M.W. S. Cordeiro, A new population of pequi (*Caryocar* spp.) developed by Brazilian indigenous people has agro-industrial and nutraceutical advantages, Eur. Food Res. Technol. 246 (2020) 1715–1724, https://doi.org/10.1007/s00217-020-03525-9.

[59] E. Pereira, M.C. Ferreira, K.A. Sampaio, R. Grimaldi, A.J.A. Meirelles, G.J. Maximo, Physical properties of Amazonian fats and oils and their blends, Food Chem. 278 (2019) 208–215, https://doi.org/10.1016/j.foodchem.2018.11.016.

[60] F. Hashempour-Baltork, M. Torbati, S. Azadmard-Damirchi, G.P. Savage, Chemical, rheological and nutritional characteristics of sesame and olive oils blended with linseed oil, Adv. Pharm. Bull. 8 (2018) 107–113, https://doi.org/10.15171/apb.2018.013.

[61] R.C.T. Barbi, A.R.C. Souza, F. Hamerski, G.L. Teixeira, M.L. Corazza, R.H. Ribani, Subcritical propane extraction of high-quality inajá (*Maximiliana maripa*) pulp oil, J. Supercrit. Fluids 153 (2019) 104576, https://doi.org/10.1016/j.supflu.2019.104576.

[62] F.G. Marques, J.R. Oliveira Neto, L.C. Cunha, J.R. Paula, M.T.F. Bara, Identification of terpenes and phytosterols in *Dipteryx alata* (baru) oil seeds obtained through pressing, Rev. Bras. Farmacogn. 25 (2015) 522–525, https://doi.org/10.1016/j.bjp.2015.07.019.

[63] A. Smeriglio, G. Mandalari, C. Bisignano, A. Filocamo, D. Barreca, E. Bellocco, D. Trombetta, Polyphenolic content and biological properties of Avola almond (*Prunus dulcis* Mill. D.A. Webb) skin and its industrial byproducts, Ind. Crop. Prod. 83 (2016) 283–293, https://doi.org/10.1016/j.indcrop.2015.11.089.

[64] A.B. Almeida, A.K.C. Silva, A.R. Lodete, M.B. Egea, M.C.P.M. Lima, F.G. Silva, Assessment of chemical and bioactive properties of native fruits from the Brazilian Cerrado, Nutr. Food Sci. 49 (2019) 381–392, https://doi.org/10.1108/NFS-07-2018-0199.

[65] C.P. Silva, M.S.B. Sousa, E.S. Siguemoto, R.A.M. Soares, J.A.G. Arêas, Chemical composition and antioxidant activity of jatobá-do-cerrado (*Hymenaea stigonocarpa* Mart.) flour, Food Sci. Technol. 34 (2014) 597–603, https://doi.org/10.1590/1678-457x.6405.

[66] N.R.R. Nascimento, A.M. Alves, M.R. Silva, M.M.V. Naves, Antioxidant capacity of pequi (*Caryocar brasiliense* Camb.) pulp is preserved by freeze-drying and light-resistant packaging, Rev. Bras. Frutic. 39 (2017) e150, https://doi.org/10.1590/0100-29452017.

[67] E.B. Mojzer, M.K. Hrnčič, M. Škerget, Z. Knez, U. Bren, Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects, Molecules 21 (2016) 901, https://doi.org/10.3390/molecules21070901.

[68] N.R. Leite, L.C.A. Araújo, P.S. Rocha, D.A. Agarrayua, D.S. Ávila, C.A. Carollo, D. B. Silva, L.M. Estevinho, K.P. Souza, E.L. Santos, Baru pulp (*Dipteryx alata* Vogel): fruit from the Brazilian savanna protects against oxidative stress and increases the life expectancy of *Caenorhabditis elegans* via SOD-3 and DAF-16, Biomolecules 10 (2020) 1106, https://doi.org/10.3390/biom10081106.

[69] F.C.P. Ravagnani, C.F.C. Ravagnani, J.A. Braga Neto, F.A. Voltarelli, A.A.Z. Zavala, C.A. Habitante, C.M. Inouye, Effects of high fat diets with baru extract and chocolate on adipocyte area of rats subjected to physical exercise, Rev. Bras. Med. Esporte 18 (2012) 190–194, https://doi.org/10.1590/S1517-86922012000300011.

[70] E.M.A. Siqueira, A.M.F. Marin, M.S.B. Cunha, A.M. Fustinoni, L.P. Sant'Ana, S. F. Arruda, Consumption of baru seeds [*Dipteryx alata* Vog.], a Brazilian savanna nut, prevents iron-induced oxidative stress in rats, Food Res. Int. 45 (2012) 427–433, https://doi.org/10.1016/j.foodres.2011.11.005.

[71] R.G.M. Souza, A.C. Gomes, A.M. Navarro, L.C. Cunha, M.A.C. Silva, F. Barbosa Junior, J.F. Mota, Baru almonds increase the activity of glutathione peroxidase in overweight and obese women: a randomized, placebo-controlled trial, Nutrients 11 (2019) 1–9, https://doi.org/10.3390/nu11081750.

[72] S. Fuller, E. Beck, H. Salman, L. Tapsell, New horizons for the study of dietary fiber and health: a review, Plant Foods Hum. Nutr. 71 (2016) 1–12, https://doi.org/10.1007/s11130-016-0529-6.

[73] L.C. Del Gobbo, M.C. Falk, R. Feldman, K. Lewis, D. Mozaffarian, Effects of tree nuts on blood lipids, apolipoproteins, and blood pressure: systematic review, meta-analysis, and dose-response of 61 controlled intervention trials, Am. J. Clin. Nutr. 102 (2015) 1347–1356, https://doi.org/10.3945/ajcn.115.110965.

[74] H. Li, X. Li, S. Yuan, Y. Jin, J. Lu, Nut consumption and risk of metabolic syndrome and overweight/obesity: a meta-analysis of prospective cohort studies and randomized trials, Nutr. Metab. 15 (2018) 1–10, https://doi.org/10.1186/s12986-018-0282-y.

[75] R.D. Mattes, P.M. Kris-Etherton, G.D. Foster, Impact of peanuts and tree nuts on body weight and healthy weight loss in adults, J. Nutr. 138 (2008) 1741–1745, https://doi.org/10.1093/jn/138.9.1741s.

[76] C.S.A. Sugizaki, M.M.V. Naves, Potential prebiotic properties of nuts and edible seeds and their relationship to obesity, Nutrients 10 (2018) 1645, https://doi.org/10.3390/nu10111645.

[77] R.M. Lamuel-Raventos, M.P.S. Onge, Prebiotic nut compounds and human microbiota, Crit. Rev. Food Sci. Nutr. 57 (2017) 3154–3163, https://doi.org/10.1080/10408398.2015.1096763.

[78] C. Lordan, D. Thapa, R.P. Ross, P.D. Cotter, Potential for enriching next-generation health-promoting gut bacteria through prebiotics and other dietary components, Gut Microbes 11 (2020) 1–20, https://doi.org/10.1080/19490976.2019.1613124.

[79] A.M. Alves-Santos, C.S.A. Sugizaki, G.C. Lima, M.M.V. Naves, Prebiotic effect of dietary polyphenols: a systematic review, J. Funct. Foods 74 (2020) 104169, https://doi.org/10.1016/j.jff.2020.104169.

[80] M. Mota, B.A. Banini, S.C. Cazanave, A.J. Sanyal, Molecular mechanisms of lipotoxicity and glucotoxicity in nonalcoholic fatty liver disease, Metab. Clin. Exp. 65 (2016) 1049–1061, https://doi.org/10.1016/j.metabol.2016.02.014.

[81] M. Suwalsky, J. Colina, M.J. Gallardo, M. Jemiola-Rzeminska, K. Strzalka, M. Manrique-Moreno, B. Sepúlveda, Antioxidant capacity of gallic acid in vitro assayed on human erythrocytes, J. Membr. Biol. 249 (2016) 769–779, https://doi.org/10.1007/s00232-016-9924-z.

[82] Y. Shen, H. Zhang, L. Cheng, L. Wang, H. Qian, X. Qi, In vitro and in vivo antioxidant activity of polyphenols extracted from black highland barley, Food Chem. 194 (2016) 1003–1012, https://doi.org/10.1016/j.foodchem.2015.08.083.

[83] F. Van de Velde, D. Esposito, M.H. Grace, M.E. Pirovani, M.A. Lila, Anti-inflammatory and wound healing properties of polyphenolic extracts from strawberry and blackberry fruits, Food Res. Int. 121 (2019) 453–462, https://doi.org/10.1016/j.foodres.2018.11.059.

[84] D. Del Rio, A. Rodriguez-Mateos, J.P.E. Spencer, M. Tognolini, G. Borges, A. Crozier, Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases, Antioxid. Redox Signal. 18 (2013) 1818–1892, https://doi.org/10.1089/ars.2012.4581.

[85] D.S. Fernandes, J.R. Donadon, T.F. Rangel, R.C.A. Guimarães, R.P. Campos, L. B. Lima, P.A. Hiane, Quality of roasted baru almonds stored in different packages, Food Sci. Technol. (2020), https://doi.org/10.1590/fst.10720.

[86] D.L. Fetzer, P.N. Cruz, F. Hamerski, M.L. Corazza, Extraction of baru (*Dipteryx alata* vogel) seed oil using compressed solvents technology, J. Supercrit. Fluids 137 (2018) 23–33, https://doi.org/10.1016/j.supflu.2018.03.004.

[87] M.M. Guimarães, M.S. Silva, Qualidade nutricional e aceitabilidade de barras de cereais adicionadas de frutos de murici-passa, Rev. Inst. Adolfo Lutz 68 (2009) 426–433.

CAPÍTULO 4

MANUSCRITO CIENTÍFICO (ESTUDO EMPÍRICO)

O manuscrito intitulado “*Chemical composition and prebiotic activity of baru (Dipteryx alata Vog.) pulp on probiotic strains and human colonic microbiota*” foi submetido ao periódico Food Research International (Qualis: A1 - CiteScore: 11.1, Percentil 96%; Fator de impacto: 7.425, Percentil: 91%). A formatação do manuscrito está de acordo com as Instruções aos autores do referido periódico (ANEXO C).

Chemical composition and prebiotic activity of baru (*Dipteryx alata* Vog.) pulp on probiotic strains and human colonic microbiota

Aline Medeiros Alves-Santos[1], Karoliny Brito Sampaio[2], Marcos dos Santos Lima[3], Alexandre Siqueira Guedes Coelho[4], Evandro Leite de Souza[2], Maria Margareth Veloso Naves[1*]

[1] Laboratory of Experimental Nutrition, School of Nutrition, Federal University of Goiás (UFG), Goiânia, Brazil

[2] Laboratory of Food Microbiology, Department of Nutrition, Federal University of Paraíba, João Pessoa, Brazil

[3] Department of Food Technology, Federal Institute of Sertão de Pernambuco, Petrolina, Brazil

[4] Laboratory of Plant Genetics and Genomics, School of Agronomy, UFG, Goiânia, Brazil

* Corresponding author: maria_margareth_veloso@ufg.br

ABSTRACT

Little knowledge is available in literature regarding the chemical composition and health-promoting effects of baru (*Dipteryx alata* Vog.) pulp, a by-product not used by the agro-industry during the processing of baru fruit. This study evaluated the chemical composition of baru pulp and investigated its prebiotic activity on distinct probiotic strains and human colonic microbiota with *in vitro* assays. Baru pulp had high contents of insoluble dietary fibers (mostly hemicellulose) and phenolic compounds (mainly hesperidin). Baru pulp stimulated the growth and metabolism of the probiotics *Bifidobacterium animalis* subsp. *lactis* BB-12, *Lactobacillus acidophilus* LA-05, and *Lacticaseibacillus casei* L-26. In addition, digested baru pulp induced significant benefits on the human colonic microbiota, increasing the relative abundance of *Lactobacillus-Enterococcus*, *Bifidobacterium*, and *Bacteroides-Prevotella*, as well as the production of lactate, acetate, propionate, and butyrate. The results show that baru pulp has potential prebiotic properties to be explored in the formulation of new health-promoting foods.

Keywords: Cerrado biome; baru; fruit by-product; functional foods; gut microbiota; modulatory effects.

1. Introduction

Baru (*Dipteryx alata* Vog.) is a native fruit of the Cerrado biome, consisting of a thin peel (epicarp), pulp (fibrous mesocarp), and a woody endocarp coating a seed. The edible fraction of the fruit is known as baru nut (Alves-Santos et al., 2021). The baru peel and pulp represent 42% of the fruit mass, and the nut comprises only 4% of the fruit (Lima et al., 2022). However, baru nut has an important commercial value in the Brazilian Cerrado regions due to its sensory attributes and beneficial health effects, also being exported to other countries (Alves-Santos et al., 2021).

During the processing of the fruit for nut extraction, baru pulp is not used by the agro-industry. Nevertheless, there are reports regarding the use of baru pulp as a substitute for wheat flour and oat bran in the formulation of cookies (Alves et al., 2010; Ferreira et al., 2020) and cereal bars (Lima et al., 2010). Little is known about the nutritional and bioactive composition of baru pulp, but some studies reported high contents of dietary fibers and sugars (Alves et al., 2010; Gadioli et al., 2021), and phenolic compounds (Santiago et al., 2018). Phenolic acids, flavonoids, terpenes, organic acids, and fatty acids were also identified in baru pulp (Leite et al., 2020). Antinutritional factors such as phytic acid and trypsin inhibitor were reported in low concentrations in baru pulp (Togashi & Sgarbieri, 1994). Baru peel and pulp extract exhibited a cytotoxic effect against cervical cancer cell lines, and weak cytotoxicity against non-tumor cell lines, suggesting its safety and anticancer potential (Barizão et al., 2021). Furthermore, baru pulp had *in vivo* action against oxidative stress and did not present toxic effects (Leite et al., 2020), indicating its potential safe use as a functional ingredient.

There has been great interest in investigating food by-products as prebiotic ingredients due to their high contents of carbohydrates and phytochemicals, high abundance, low cost, and environmental, economic and social impacts generated by food loss and waste (Abedi & Hashemi, 2020). This is driven by environmental and food safety policies that encourage

sustainable consumption (Oliveira et al., 2021), and contribute to adding value to fruits aligned with circular economy practices in the agro-industry. In this sense, previous research has shown that by-products of acerola and guava (Menezes et al., 2021), by-product of jabuticaba (Massa et al., 2022), and orange albedo (Rezende et al., 2022) have potential prebiotic properties.

Prebiotics are substrates selectively metabolized by host microorganisms conferring beneficial health effects such as improvements on gut barrier function, immunity, inflammation, mineral absorption, and satiety (Gibson et al., 2017; Cunningham et al., 2021). The prebiotics which are documented in literature the most are inulin, fructo- and galacto-oligosaccharides (Gibson et al., 2017), classified as dietary fibers (Rezende et al., 2021). Some dietary polyphenols and their metabolites can also modulate the gut microbiota, being considered candidates to prebiotics (Alves-Santos et al., 2020; Cunningham et al., 2021).

The high content of fibers and polyphenols reported in baru pulp suggests prebiotic properties. However, there hasn´t been found any report in literature regarding the prebiotic properties of the baru pulp, and studies on its carbohydrates, oligosaccharides and polyphenols profile are scarce and with limited information. Thus, this study evaluated the chemical composition of baru pulp and investigated its prebiotic activity on distinct probiotic strains and human colonic microbiota.

2. Material and methods

2.1. Sample

Baru (*Dipteryx alata* Vog.) fruit pulp (epicarp and mesocarp) was purchased *in natura* from an agro-industry in the municipality of Caiapônia (Goiás, Brazil) that processes native fruits from Cerrado biome. The sample was frozen (-80 ºC), freeze-dried (temperature: -50 ± 5 ºC, pressure: < 100 µHg, time: 48 h), milled (sieve number and size: mesh 16 and < 1.0 mm), vacuum packed with light protection, and kept at 4 ºC for up to 6 months.

2.2. External standards and chemicals

The external standards (glucose, fructose, 1-kestose, nystose, raffinose, gallic acid, *p*-coumaric acid, chlorogenic acid, syringic acid, *trans*-caftaric acid, caffeic acid, hesperidin, naringenin, procyanidin B1, procyanidin B2, catechin, epicatechin, cyanidin-3,5-diglucoside, malvidin-3,5-diglucoside, pelargonidin-3,5-diglucoside), as well as the Folin-Ciocalteau, Folin-Dennis, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), and trolox reagents were purchased from Sigma-Aldrich (St Louis, USA). Maltose and rhamnose were purchased from Chem Service (West Chester, USA). Procyanidin A2, epigallocatechin gallate, epicatechin gallate, kaempferol-3-glucoside, rutin, quercetin-3-glucoside, myricetin, peonidin-3-glucoside, petunidin-3-glucoside, delphinidin-3-glucoside, malvidin-3-glucoside, cyanidin-3-glucoside, and pelargonidin-3-glucoside were purchased from Extrasynthese (Genay, France). *cis*-Resveratrol and *trans*-resveratrol were purchased from Cayman Chemical Company (Ann Arbor, USA). Citric, tartaric, malic, succinic, lactic, formic, acetic, propionic, and butyric acids were purchased from Química Vetec (Rio de Janeiro, Brazil). Tannic acid was purchased from Neon (Suzano, Brazil).

The de Man, Rogosa, and Sharpe (MRS) broth and agar, tryptone, and meat and yeast extracts were purchased from Kasvi (São José dos Pinhais, Brazil). Tribasic ammonium citrate, brain heart infusion (BHI) broth, M9 broth, α-amylase, bile salt, pancreatin, and dialysis tubing cellulose membrane were purchased from Sigma-Aldrich (St. Louis, USA). Other chemicals were acquired as follows: eosin methylene blue (EMB) agar from Merck (Darmstadt, Germany); fructooligosaccharides (FOS) from Orafti (Orafti P95, Oreye, Belgium); L-cysteine-HCl from Êxodo Científica (Sumaré, Brazil); and pepsin from Dinâmica (Indaiatuba, Brazil).

127 *2.3. Chemical characterization of the baru pulp*

128 *2.3.1. Proximate composition, dietary fiber, and fructans*

129 Proximate composition was determined by analyzing moisture (method 934.06), ash
130 (method 940.26), total nitrogen (method 920.152), and conversion to crude protein (AOAC,
131 2016). Total lipid was determined according to Bligh and Dyer (1959). Carbohydrate content
132 was estimated by subtracting the values of the mentioned components from 100. Total energy
133 value was estimated using Atwater conversion factors of 4, 4, and 9 kcal/g to protein,
134 carbohydrate, and lipid, respectively. The contents of total, insoluble and soluble dietary fibers
135 were determined with an enzymatic-gravimetric method (method 985.29), and the fructan
136 content was determined by the enzymatic-spectrophotometric method (AOAC, 2016). The
137 contents of lignin, hemicellulose, and cellulose were determined according to the acid and
138 neutral detergent fiber method (Trujillo et al., 2010).

139

140 *2.3.2. Analyzes of sugars, organic acids and oligosaccharides*

141 Sugars and organic acids were determined simultaneously on a liquid chromatograph
142 (Agilent, model 1260 Infinity LC) coupled to a refractive index detector (RID) and a diode
143 array detector (DAD). Freeze-dried baru pulp was diluted (1:20) in ultrapure water at 50 ºC,
144 centrifuged, and filtered through a 0.45 µm nylon membrane. The analytical conditions were
145 performed according to Coelho et al. (2018). The detection of sugars was performed by RID,
146 and the determination of organic acids was performed on the DAD at 210 nm. The limit of
147 detection (LOD) was lower than 0.044 g/L, and the limit of quantification (LOQ) was lower
148 than 0.151 g/L for all analyzed compounds. The determination of oligosaccharides was
149 performed by High Performance Liquid Chromatography (HPLC) with a RID. Freeze-dried
150 baru pulp was diluted (1:5) in ultrapure water, and filtered through a 0.45 µm nylon membrane.

151 The separation of compounds was performed as described by Lima et al. (2019). For all
152 analyzed compounds, the LOD was lower than 0.042 g/L, and the LOQ was lower than 0.109 g/L.
153
154 *2.3.3. Phenolic compounds and antioxidant capacity*
155 Freeze-dried baru pulp (500 mg) was homogenized with 50% methanol to obtain the
156 extracts for the determination of total phenolic compounds, total tannins, and antioxidant
157 capacity (Xu et al., 2020). The freeze-dried baru pulp was diluted in 70% methanol (1:10),
158 homogenized in an ultrasonic bath, centrifuged, and filtered through a 0.45 μm nylon membrane
159 so the phenolic compounds could be separated and quantified (Rezende et al., 2022).
160 The content of total phenolic compounds was determined according to Singleton and
161 Rossi (1965) using a gallic acid standard curve (Concentration = [absorbance + 0.0438] /
162 0.0078; r = 0.9991). The results were expressed in mg of equivalent gallic acid per 100 g of
163 sample (mg GAE/100 g). Total tannins were analyzed by reducing the Folin-Dennis reagent
164 (AOAC, 2016). Tannic acid was used as a standard to the calibration curve (Concentration =
165 [absorbance + 0.0005] / 0.0952; r = 0.9999), and the results were expressed in mg of equivalent
166 tannic acid per 100 g of sample (mg TAE/100 g). The separation and quantification of phenolic
167 compounds were performed using a liquid chromatograph coupled to a DAD. The analytical
168 conditions were performed as described by Padilha et al. (2017) with adaptations by Dutra et
169 al. (2018). The detection of compounds was performed by comparison with external standards.
170 The LOD was lower than 0.17 mg/L and the LOQ was lower than 1.41 mg/L for all analyzed
171 compounds.
172 The free-radical scavenging capacity of DPPH was determined according to Brand-
173 Williams, Cuvelier and Berset (1995). The Ferric Reducing Antioxidant Power (FRAP) was
174 conducted according to Pulido, Bravo, and Saura-Calixto (2000). Trolox solution was used as
175 a standard to the calibration curves of the DPPH (Concentration = [absorbance +

0.6296]/0.0006, r = - 0.9989) and FRAP (Concentration = [absorbance + 0.0293]/0.0011, r = 0.9979), and the results were expressed as μmol trolox equivalents per gram of sample (μmol TE/g).

2.4. In vitro prebiotic potential

2.4.1. Microorganisms and cultivation conditions

The probiotics used in this study were *Bifidobacterium animalis* subsp. *lactis* BB-12, *Lactobacillus acidophilus* LA-05, and *Lacticaseibacillus casei* L-26, which were supplied by the College of Biotechnology, Portuguese Catholic University (Porto, Portugal). Stock cultures were maintained in MRS broth containing glycerol (150 g/L) at -80 °C. For the assays, each strain was initially cultivated in MRS broth at 37 °C for 20-24 h to obtain cell suspensions with an optical density at 655 nm (OD_{655}) of 0.8, and viable cell counts of approximately 6 log colony forming units (CFU) per mL on MRS agar. The MRS broth and agar used to the *B. animalis* growth were supplemented with L-cysteine hydrochloride (0.5 g/L) and incubated under anaerobic conditions (Albuquerque et al., 2020). *Escherichia coli* CCCD-E004 and *E. coli* ATCC 25922 constituted the enteric mixture inoculum. Strains were cultured in BHI broth at 37 °C for 18-20 h to obtain cell suspension with an OD_{655} of 0.1, and viable cell count of approximately 6 log CFU per mL on EMB agar. The inoculum of the enteric mixture was obtained by mixing the two different *E. coli* suspensions (1:1).

2.4.2. Preparation of bacterial cultivation medium

MRS broth with modifications in the composition of the standard carbon source (glucose) was used as a medium to evaluate the prebiotic potential of the baru pulp (Albuquerque et al., 2020). The composition of the different broths used to cultivate the probiotics was tryptone (10 g/L), meat extract (8 g/L), yeast extract (4 g/L), dipotassium

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 respective carbon sources. The carbon sources were glucose, FOS (standard prebiotic) and baru pulp, whose concentrations were defined according to the glucose concentration (20 g/L) of commercial MRS broth.

2.4.3. Enumeration of probiotic viable cell counts

The inoculum of each probiotic was added (2%, v/v) into sterile tubes containing the respective cultivation medium. The mixtures were homogenized and incubated at 37 °C. At different time intervals (0 - after homogenization, 12, 18, 24, and 48 h after incubation), aliquots (100 μL) of each mixture were serially diluted in sterile saline (8.5 g/L). Aliquots (20 μL) of each dilution were inoculated using the microdroplet technique on MRS agar. The plates were incubated at 37 °C for 48 h, and the results were expressed as log CFU per mL (Albuquerque et al., 2020).

Enteric mixture inoculum (2%, v/v) was added to M9 broth containing glucose (20 g/L), FOS (20 g/L), or baru pulp (20 g/L). The mixtures were homogenized and incubated under aerobic conditions at 37 °C. At 0 and 48 h, aliquots (100 μL) of each mixture were serially diluted in sterile saline. Then, aliquots (20 μL) of each dilution were inoculated using the microdroplet technique on EMB agar. The plates were incubated under aerobic conditions at 37 °C for 24 h, and the results were expressed as log CFU per mL.

2.4.4. Prebiotic activity score

The prebiotic activity score was estimated according to the equation proposed by Huebner, Wehling, and Hutkins (2007), considering the growth of each probiotic and the enteric mixture during the fermentation ($\Delta_{\log\ CFU/mL}$ = log $CFU/mL_{t=48}$ – log $CFU/mL_{t=0}$) of the

substrates (glucose, FOS, and baru pulp) as follows: Prebiotic activity score = probiotic ($\Delta_{\log \text{CFU/mL}}$ prebiotic/$\Delta_{\log \text{CFU/mL}}$ glucose) – enteric mixture ($\Delta_{\log \text{CFU/mL}}$ prebiotic/$\Delta_{\log \text{CFU/mL}}$ glucose). A positive prebiotic activity score indicates that the substrate was selectively metabolized by the probiotic, but not by enteric mixture (Huebner et al., 2007).

2.4.5. Evaluation of probiotic metabolic activity

The metabolic activity of the probiotics was evaluated by determining the pH and the concentrations of sugars and organic acids in the cultivation media. The pH values were measured according to AOAC (2016) using a digital pH meter (Adwa AD100, Szeged, Hungary) at different time intervals (0, 12, 18, 24, and 48 h). The sugars and organic acids, at 0, 12, and 48 h, were quantified simultaneously by HPLC, as described in the section 2.3.2.

2.5. In vitro gastrointestinal digestion

The digestion of the freeze-dried baru pulp was performed in four phases to simulate oral, gastric, and intestinal digestion, followed by dialysis (Massa et al., 2022). The oral phase was simulated by adding α-amylase (100 U/mL in $CaCl_2$ 1 Mm) to the initial solution (baru pulp with sterile distilled water). In the gastric phase, pepsin (25 mg/mL in HCl 0.1 M) was added to the mixture and incubated for 90 min at 37 °C and 130 rpm. To simulate the intestinal phase, a bile salt (12 g/L) and pancreatin (2 g/L) solution (in $NaHCO_3$ 0.1 M) was added to the mixture, the pH was adjusted to 6.5 - 7.0, and incubated for 90 min at 37 °C and 45 rpm. Then, the sample was submitted to dialysis in a previously hydrated dialysis tubing cellulose membrane. Digested and dialyzed sample was freeze-dried and kept at 4 °C.

2.6. In vitro human colonic fermentation

2.6.1. Fecal sample collection

The fecal sample donors were selected according to the following inclusion criteria: age between 18-65 years old, no restrictive diet, no history of food intolerances and allergies, or other gastrointestinal diseases, and no use of probiotics, prebiotics, antibiotics, or controlled drugs for at least six months prior to collection (Andrade et al., 2020). Five healthy adult volunteers (three men and two women) aged between 20-45 years were selected and instructed about the aseptic collection and handling of the samples. The bottles with fecal samples were transported to the laboratory for analysis under anaerobic conditions. The study protocol was approved by the Research Ethics Committee of the Federal University of Goiás (UFG), protocol nº 4.970.001/2021, and all donors signed a formal consent form.

2.6.2. Fecal fermentation and determination of pH, sugars, lactate, and short-chain fatty acids

Immediately after collection (about 60 min), the fresh fecal samples were mixed (1:1:1:1:1, w/w), diluted in NaCl (8.5 g/L) with L-cysteine hydrochloride (0.5 g/L), homogenized and filtered with sterile triple-layer gauze to obtain the fresh pooled fecal inoculum. The digested and dialyzed baru pulp (20% w/v) was subjected to fermentation with the mixture of fresh pooled fecal inoculum (40% v/v) and the fermentation medium (40% v/v). In addition, a medium containing FOS (20% w/v) and a medium without addition of fermentable substrate (negative control) were also subjected to fermentation for 48 h at 37 °C under anaerobic conditions (Massa et al., 2022). At time zero and after 24 and 48 h of fermentation, aliquots were collected to monitor the bacterial metabolic activity by determining the pH (AOAC, 2016) and the contents of sugars, lactate, and short-chain fatty acids (SCFA) (Coelho et al., 2018). The composition of the fermentation medium is available in the supplementary material data (Table S1).

2.6.3. Fluorescent in situ hybridization (FISH) coupled with flow cytometry

The FISH analysis was performed with commercially synthesized oligonucleotide probes (Eurofins Genomics, Ebersberg, Germany), fluorescent Cy3-labelled, and capable of pairing with the target sequence of 16S rRNA gene from the following bacterial groups (Table S2): Bac 303 - *Bacteroides* and *Prevotella* (Manz et al., 1996); Bif 164 - *Bifidobacterium* (Langendijk et al., 1995); Chis 150 - *Clostridium histolyticum* (Franks et al., 1998); Erec 482 - *Eubacterium rectale* and *Clostridium coccoides* (Franks et al., 1998); and Lab 158 - *Lactobacillus* and *Enterococcus* (Harmsen et al., 1999). Probes were selected for major bacterial groups usually found in the human gut (Supplementary material data, Table S2). SYBR Green was used to enumerate total cells (Conterno et al., 2019).

At different time intervals (0, 24, and 48 h of fermentation), aliquots of fermentation media were fixed with paraformaldehyde (4%) and incubated overnight at 4 ºC. The fixed cells were centrifuged, washed with PBS 1 M, resuspended with PBS 1 M and absolute ethanol, filtered in 0.45 µm membrane and kept at -20 ºC. For hybridization, fixed cells were resuspended in PBS 1X (Gibco, Gaithersburg, USA), centrifuged, resuspended in Tris-EDTA (Tris-HCl 100 mM, EDTA 50 mM; pH 8), and centrifuged. Samples were treated with Tris-EDTA and lysozyme (1 mg/mL) and incubated at 25 °C for 10 min in a dim light environment to permeabilize the cells for use with probes Lab 158 and Bif 164, and centrifuged. Subsequently, all samples were resuspended with hybridization buffer (NaCl 0.9 M, Tris-HCl 20 mM, 0.1% sodium dodecyl sulfate; pH 7.5) and with the oligonucleotide probe (50 ng/µL), and incubated for 4 h at 45-50 °C (Table S2) in a dim light environment. After that, the samples were centrifuged, resuspended in washing buffer (NaCl 0.9 M, Tris-HCl 20 mM; pH 7.5), and incubated for 30 min at 45-50 °C (Table S2). Samples were centrifuged, resuspended in PBS 1X and SYBR Green (1:1000 in dimethyl sulfoxide ≥ 99.9%), incubated for 10 min at 25 °C, centrifuged, and resuspended with PBS 1X.

The fluorescence signals of the samples were collected in a flow cytometer (BD Accuri C6, New Jersey, USA) by FL1 (SYBR Green) and FL2 (Bac 303, Bif 164, Chis 150, Erec 482 and Lab 158) channels, and the fluorescence emission cytograms were recorded using BD Accuri C6 software. Results were expressed as the abundance (%) of cells hybridized with each specific probe, relative to the total number of cells enumerated by SYBR Green (Conterno et al., 2019).

2.7. Statistical analysis

Chemical composition analyses were performed in three replicates. *In vitro* assays to evaluate the prebiotic potential and the relative abundance of bacterial groups during human colonic fermentation were performed in two replicates in three different experiments. Deviations from the normality of the residuals were evaluated using the Shapiro-Wilk's test. The data were submitted to analysis of variance, followed by Tukey's test. Principal Component Analysis and Pearson's correlation were carried out using the mean values obtained from *in vitro* human colonic fermentation. All tests were performed by adopting a significance level of 0.05 ($p < 0.05$). The statistical analysis was performed using R software (R Foundation for Statistical Computing, Vienna, Austria), version 4.1.0.

3. Results and discussion

3.1. Chemical composition and antioxidant capacity of baru pulp

Baru pulp had a high content of total dietary fiber, mainly insoluble fiber, and low content of moisture, ash, lipid, and total energy value (Table 1). The content of total dietary fiber was in the range of values (29-39 g/100 g) reported in baru pulp flour from three different regions of the Cerrado (Gadioli et al., 2021). In relation to insoluble fibers, baru pulp had a high content of hemicellulose (61%), cellulose (45%), and lignin (31%). Fructose was the

predominant sugar in baru pulp, and the organic acid found in the highest content was succinic acid (Table 1). Representative chromatograms of the sugars and organic acids analyzes are available in supplementary material data (Fig. S1). Fructans and oligosaccharides (1-kestose, nystose, and raffinose) were not detected.

According to Phenol-Explorer Database (Rothwell et al., 2013), the classes of phenolic compounds identified in baru pulp were phenolic acids (caffeic, *p*-coumaric, chlorogenic, syringic), flavonoids (catechin, epicatechin gallate, epigallocatechin gallate, hesperidin, myricetin, procyanidin B1, procyanidin B2, quercetin-3-glucoside, rutin), and stilbenes (*cis*-resveratrol) (Table 1). The phenolic compound found in the highest content was hesperidin, one of the main flavonoids (flavanones) in citrus fruits, with important beneficial health effects, such as antioxidant and anti-inflammatory activities (Hajialyani et al., 2019). Moreover, evidence suggests that hesperidin and its aglycones may also improve gastrointestinal health (Stevens et al., 2019). Coumaric acid derivatives have also been reported in baru pulp from the State of Mato Grosso do Sul (Brazil) (Leite et al., 2020). Representative chromatograms of the phenolic compounds analysis are available in supplementary material data (Fig. S2).

The content of total phenolic compounds (Table 1) was higher than that found in baru pulp from the State of Mato Grosso do Sul (Brazil), of 262 mg GAE/100 g (Leite et al., 2020), as well as in pulp (292 mg GAE/100 g; 15% moisture) and peel (477 mg GAE/100 g; 16% moisture) of baru from the State of Goiás (Brazil) (Santiago et al., 2018). High contents of phenolic compounds contribute to add commercial and industrial value to the baru fruit, as well as to define intervention doses in future pre-clinical and clinical studies.

Considering the diversity of antioxidant substances in the food matrix, such as vitamins, minerals, and polyphenols, two methods were used to estimate the antioxidant capacity of baru pulp. The antioxidant capacity evaluated by the DPPH and FRAP methods (Table 1) was higher than the values reported in baru pulp (Goiás State, Brazil), which were of 21 μmol TE/g (DPPH)

351 and 24 μmol TE/g (FRAP) (Santiago et al., 2018). These data indicate the potential of the baru
352 pulp as a functional ingredient, and suggest its use in new formulations of functional foods with
353 high density of nutrients and bioactive compounds, low fat content and low energy value.
354
355 *3.2. Effects of baru pulp on probiotics*
356 As far as we know, this is the first study that evaluated the prebiotic potential of baru
357 pulp on the growth and metabolism of gut bacteria related to health and well-being, such as
358 *Lactobacillus* and *Bifidobacterium* (Gibson et al., 2017). In all tests, the viable counts of
359 *Lacticaseibacillus casei* L-26 (*L. casei*) in baru pulp medium were similar to glucose and FOS
360 media ($p > 0.05$), and the viable counts of *L. acidophilus* LA-05 (*L. acidophilus*) in baru pulp
361 medium were higher ($p < 0.05$) than glucose and FOS media at 48 h of fermentation (Fig. 1).
362 In addition, at 12 and 18 h, the viable counts of *B. animalis* BB-12 (*B. animalis*) in baru pulp
363 medium was higher than FOS medium ($p < 0.05$) (Fig. 1). This result may be linked to FOS
364 polymerization degree, which influences the bifidobacteria capabilities to metabolize these
365 oligosaccharides (Scott et al., 2020). Therefore, the quantification of viable cells indicates that
366 baru pulp was used as a carbon source by the probiotics in similar or better way than FOS, and
367 it may be explained by the fermentation of the available pulp compounds, such as dietary fibers
368 and polyphenols (Table 1). However, baru pulp was unable to maintain the growth of *B.*
369 *animalis* until the end of the assay, as a reduction in the viable cell counts was observed in the
370 respective medium at 48 h of fermentation (Fig. 1). This finding may be related to the carbon
371 sources in the baru pulp (insoluble dietary fibers and polyphenols) and the capacity of
372 bifidobacteria to use them as *B. animalis* subsp. *lactis* has a smaller number of genes involved
373 in the use of carbon sources, which may interfere with its ability to adapt in monoculture assays
374 (Pokusaeva et al., 2011).

375 The use of baru pulp, glucose, and FOS as carbon sources by the probiotics decreased
376 the pH of the cultivation media at 48 h compared to the initial pH (Fig. 1). The lowest pH values
377 were found in the media with glucose and *B. animalis* (3.57), FOS and *L. acidophilus* (3.65),
378 and FOS and *L. casei* (3.66) at 48 h of fermentation. The decrease in pH values is related to the
379 production of organic acids generated from the fermentation of substrates by beneficial bacteria
380 and, therefore, to the growth of these bacteria (Gibson et al., 2017).
381 Overall, the contents of fructose, glucose, and maltose were reduced in cultivation media
382 with different substrates and probiotics (Fig. 2) at the end of the fermentation. Lactate-
383 producing bacteria, such as *Lactobacillus* and *Bifidobacterium*, metabolize glucose to lactate
384 through the glycolysis or phosphoketolase pathways (Abedi & Hashemi, 2020). Indeed, lactate
385 was the organic acid present in the highest concentration in all tested media at 48 h, except in
386 the FOS and *B. animalis* medium (Fig. 2).
387 In addition to lactate, the fermentation of some non-digestible carbohydrates produces
388 other metabolites, such as SCFA (acetate, propionate, and butyrate) (Scott et al., 2020). In this
389 study, the content of acetate increased in baru pulp medium with *B. animalis* ($p < 0.05$), and it
390 was higher ($p < 0.05$) in baru pulp medium with *L. acidophilus* than in FOS medium with *L.*
391 *acidophilu*s at 48 h of fermentation (Fig. 2). The contents of propionate in the baru pulp media
392 with *L. acidophilus* and *L. casei* were similar to those of FOS media with *L. acidophilus* and *L.*
393 *casei* ($p > 0.05$) at 48 h (Fig. 2). It is noteworthy that the production of organic acids, including
394 lactate and SCFA and hence the low pH impair the growth of potentially pathogenic bacteria
395 (Vieco-Saiz et al., 2019).
396 The prebiotic activity score of baru pulp on *B. animalis* was higher than that of FOS
397 (baru pulp = 0.12 ± 0.02; FOS = - 0.24 ± 0.13). Negative values of prebiotic activity score were
398 reported to FOS on *L. acidophilus* and *L. plantarum*, and to inulin on *B. bifidum* (Huebner et
399 al., 2007). The baru pulp also had a positive score on *L. casei* (baru pulp = 0.24 ± 0.06; FOS =

400 0.34 ± 0.10) and *L. acidophilus* (baru pulp = 0.18 ± 0.02; FOS = 0.26 ± 0.08), indicating a
401 selective consumption of baru pulp by the probiotics and a putative prebiotic effect.
402
403 *3.3. Effects of baru pulp on human colonic microbiota*
404 Regarding the *in vitro* colonic fermentation assay, it is important to emphasize that the
405 baru pulp was previously subjected to a simulated gastrointestinal digestion to present
406 characteristics similar to those when reaching the human colon. The predominant bacterial
407 groups in the initial fecal microbiota of the donors were *E. rectale-C. coccoides* and *C.*
408 *histolyticum*. *Lactobacillus-Enterococcus* and *Bifidobacterium* showed lower relative
409 abundance than the other examined bacterial groups (Fig. 3).
410 At the end of fermentation, an increase in the relative abundance of *Lactobacillus-*
411 *Enterococcus*, *Bifidobacterium* and *Bacteroides-Prevotella*, and a decrease of *E. rectale-C.*
412 *coccoides* and *C. histolyticum* were observed in FOS and digested baru pulp media (Fig. 3).
413 Digested guava by-product, with 41% insoluble fiber and 3% soluble fiber (Menezes et al.,
414 2021), also increased the abundance of *Lactobacillus-Enterococcus* and *Bifidobacterium* during
415 *in vitro* human colonic fermentation. These two microbial groups are the main targets of
416 prebiotics in the gut microbiota (Gibson et al., 2017) and related to several beneficial effects on
417 human gut health (Cunningham et al., 2021). In addition, these bacterial genera have inhibitory
418 mechanisms against potentially pathogenic bacteria, such as lactate and SCFA production, and
419 competition for nutrients (Vieco-Saiz et al., 2019). This inhibitory action of *Lactobacillus* and
420 *Bifidobacterium* may partially explain the reduction in the relative abundance of *E. rectale-C.*
421 *coccoides* and *C. histolyticum*, observed in this study after 48 h of fermentation (Fig. 3). The
422 highest relative abundance of *Bacteroides-Prevotella* was found in the digested baru pulp
423 medium (Fig. 3) at 48 h of fermentation. This finding is in accordance with reports indicating
424 that some species of *Bacteroides* are involved in the degradation of insoluble fibers, such as

425 hemicellulose and cellulose (Flint et al., 2012), and in the bacterial metabolism of flavanones,
426 such as hesperidin (García-Villalba et al., 2022).

427 The sugars contents were decreased in FOS and digested baru pulp media at the end of
428 the colonic fermentation (Fig. 4). Sugars were not detected in the negative control medium
429 (without substrate). An increase in the production of lactate, and SCFA (Fig. 4), as well as a
430 significant decrease of pH values (Fig. 3) were observed in FOS and digested baru pulp media
431 after 48 h of fermentation. The increase in the lactate and acetate production may be associated
432 with an increase in the relative abundance of *Lactobacillus-Enterococcus* and *Bifidobacterium*,
433 which can convert glucose and fructose into lactate and acetate by different metabolic pathways
434 (Abedi & Hashemi, 2020). Increased propionate content may be related to an increase in the
435 *Bacteroides-Prevotella* relative abundance (Chen et al., 2017), especially in the baru pulp
436 medium.

437 Lactate and acetate are the main end products of *Lactobacillus* and *Bifidobacterium*
438 metabolism, and can be metabolized by other microorganisms to produce propionate and
439 butyrate (Scott et al., 2020). However, high concentrations of lactate and acetate were observed
440 at 48 h of fermentation, which may partially result from the decrease of butyrate-producing
441 bacteria, such as *Eubacterium rectale* (Gibson et al., 2017). Moreover, the metabolism of
442 propionate-producing bacteria, as *Bacteroides-Prevotella*, may be influenced by relatively low
443 pH observed in the *in vitro* assays compared to human intestinal mucosa and, thus, affect the
444 production of propionate (Chen et al., 2017; Holscher, 2017).

445 Regarding the Principal Component Analysis (Fig. 5A), the first and second components
446 explained 61% and 21% of the observed total variation, respectively. The analysis indicates that
447 FOS and digested baru pulp (at 48 h) have the best behavior, according to its high values of
448 abundance for *Lactobacillus-Enterococcus*, *Bifidobacterium*, *Bacteroides-Prevotella*, and
449 SCFA contents, and low values of abundance for *E. rectale-C. coccoides, C. histolyticum,*

sugars and pH. The heatmap (Fig. 5B) shows the significant correlations ($p < 0.05$) between the abundance of the bacterial groups, the SCFA and sugars concentrations, and pH values. SCFA concentrations correlated positively to abundance of *Lactobacillus-Enterococcus*, *Bifidobacterium* and *Bacteroides-Prevotella*. Fructose concentration correlated positively to lactate content. The pH values correlated negatively to the SCFA concentrations and abundance of *Lactobacillus-Enterococcus*, *Bifidobacterium*, *Bacteroides-Prevotella*, and correlated positively to abundance of *E. rectale-C. coccoides* and *C. histolyticum.* Principal Component Analysis and the Pearson's correlation summarize and validate the results of this study.

The positive impacts of digested baru pulp on the human colonic microbiota are consonant with the results showing its selective stimulatory effect on the tested probiotic strains, and they are probably associated with the contents of sugars, insoluble fibers, and flavonoids of the baru pulp. Insoluble fibers do not meet the definition of prebiotics, but there is evidence that these components may be eventually metabolized in the distal colon, where microbial density is higher and intestinal transit time is slower (Holscher, 2017). Insoluble fibers from soybean (Wang et al., 2021) and bamboo (Ge et al., 2022) promoted significant changes on fecal microbiota, increasing the abundance of *Bacteroides* and the production of SCFA, confirming that some *Bacteroides* species can degrade hemicellulose (Flint et al., 2012). The prebiotic effect of dietary fibers, in turn, may be influenced by the phenolic compounds bound to them (García-Villalba et al., 2022). In addition, dietary polyphenols are also prebiotic candidates, as they interact with the hindgut microbiota selectively stimulating the growth of potentially beneficial bacteria (Alves-Santos et al., 2020). For instance, the oral administration of hesperidin, flavonoid found at highest concentration in the baru pulp, increased the relative abundance of *Lactobacillus*, and decreased the relative abundance of *E. rectale-C. coccoides* in the feces of rats (Estruel-Amades et al., 2019). Some *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* species have α-rhamnosidase and β-glucosidase activity, and are involved in the

metabolism of flavanone, such as hesperidin (García-Villalba et al., 2022; Rodríguez-Daza et al., 2021).

The limited number of probes for the quantification of bacterial targets of the human colonic microbiota does not reflect a community-wide study, which could limit the inference on the results of this study. However, the results provide consistent evidence of the baru pulp potential as a prebiotic ingredient, as well as relevant information to guide pre-clinical and clinical studies in the investigation of the *in vivo* effects of baru pulp on the composition and function of the gut microbiota.

4. Conclusion

Baru pulp has a high content of insoluble dietary fibers and polyphenols, and low lipid content and energy value. Hesperidin was the most relevant phenolic compound identified in the baru pulp. The prebiotic potential of the baru pulp was evidenced by increasing growth and metabolism of probiotics (*B. animalis* BB-12, *L. acidophilus* LA-05 and *L. casei* L-26), as well as by the positive impact on the abundance of *Lactobacillus-Enterococcus*, *Bifidobacterium*, and *Bacteroides-Prevotella* in human colonic microbiota, and on the production of lactate and SCFA. The synergic effect among dietary fibers and phenolic compounds possibly contributed to the prebiotic activity of the baru pulp. *In vivo* studies are warranted to confirm the prebiotic effect of baru pulp and to encourage the use of this baru by-product in the formulation of new functional foods and nutraceuticals.

CRediT authorship contribution statement

Aline M. Alves-Santos: Data curation, Formal analysis, Methodology, Software, Investigation, Resources, Project administration, Writing – original draft, Writing – review & editing. **Karoliny B. Sampaio**: Formal analysis, Methodology, Resources, Writing – review. **Marcos S. Lima**: Data curation, Formal analysis, Methodology, Writing – review. **Alexandre S. G. Coelho**: Software, Validation, Writing – review. **Evandro L. Souza**:

Conceptualization, Writing – review. **Maria Margareth V. Naves**: Conceptualization, Resources, Supervision, Writing – critical 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.

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References

Abedi, E., & Hashemi, S. M. B. (2020). Lactic acid production – producing microorganisms and substrates sources-state of art. *Heliyon*, *6*(10), e04974. https://doi.org/10.1016/j.heliyon.2020.e04974

Albuquerque, T. M. R., Borges, C. W. P., Cavalcanti, M. T., Lima, M. S., Magnani, M., & 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*, 100614. https://doi.org/10.1016/j.fbio.2020.100614

Alves-Santos, A. M., Fernandes, D. C., & Naves, M. M. V. (2021). Baru (*Dipteryx alata* Vog.) fruit as an option of nut and pulp with advantageous nutritional and functional properties: a comprehensive review. *NFS Journal*, *24*, 26–36. https://doi.org/10.1016/j.nfs.2021.07.001

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*, 104169. https://doi.org/10.1016/j.jff.2020.104169

Alves, A. M., Mendonça, A. L., Caliari, M., & Cardoso-Santiago, R. A. (2010). Avaliação química e física de componentes do baru (*Dipteryx alata* Vog.) para estudo da vida de prateleira. *Pesquisa Agropecuaria Tropical*, *40*(3), 266–273. https://doi.org/10.5216/pat.v40i3.6343

Andrade, R. M. S., Silva, S., Costa, C. M. S. F., Veiga, M., Costa, E., Ferreira, M. S. L., Gonçalves, E. C. B. A., & Pintado, M. E. (2020). Potential prebiotic effect of fruit and vegetable byproducts flour using *in vitro*

gastrointestinal digestion. *Food Research International*, *137*, 109354. https://doi.org/10.1016/j.foodres.2020.109354

AOAC - Association of Official Analytical Chemists. (2016). *Official Methods of Analysis of AOAC International 20 th ed.*

Barizão, E. O., Boeing, J. S., Rotta, E. M., Volpato, H., Nakamura, C. V, Maldaner, L., & Visentainer, J. V. (2021). Phenolic composition of *Dipteryx alata* Vogel pulp + peel and its antioxidant and cytotoxic properties. *Journal of the Brazilian Chemical Society*, *32*(12), 2206–2214. https://dx.doi.org/10.21577/0103-5053.20210112

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917. https://doi.org/https://doi.org/10.1139/y59-099

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

Chen, T., Long, W., Zhang, C., Liu, S., Zhao, L., & Hamaker, B. R. (2017). Fiber-utilizing capacity varies in *Prevotella*- versus *Bacteroides*-dominated gut microbiota. *Scientific Reports*, *7*(1), 2594. https://doi.org/10.1038/s41598-017-02995-4

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

Conterno, L., Martinelli, F., Tamburini, M., Fava, F., Mancini, A., Sordo, M., Pindo, M., Martens, S., Masuero, D., Vrhovsek, U., Dal Lago, C., Ferrario, G., Morandini, M., & Tuohy, K. (2019). Measuring the impact of olive pomace enriched biscuits on the gut microbiota and its metabolic activity in mildly hypercholesterolaemic subjects. *European Journal of Nutrition*, *58*(1), 63–81. https://doi.org/10.1007/s00394-017-1572-2

Cunningham, M., Azcarate-Peril, M. A., Barnard, A., Benoit, V., Grimaldi, R., Guyonnet, D., Holscher, H. D., Hunter, K., Manurung, S., Obis, D., Petrova, M. I., Steinert, R. E., Swanson, K. S., van Sinderen, D., Vulevic, J., & Gibson, G. R. (2021). Shaping the future of probiotics and prebiotics. *Trends in Microbiology*, *29*(8), 667–685. https://doi.org/10.1016/j.tim.2021.01.003

Dutra, M. C. P., Rodrigues, L. L., Oliveira, D., Pereira, G. E., & Lima, M. S. (2018). Integrated analyses of phenolic compounds and minerals of Brazilian organic and conventional grape juices and wines: validation of a

559 method for determination of Cu, Fe and Mn. *Food Chemistry*, *269*, 157–165.
560 https://doi.org/10.1016/j.foodchem.2018.07.014
561 Estruel-Amades, S., Massot-Cladera, M., Pérez-Cano, F. J., Franch, À., Castell, M., & Camps-Bossacoma, M.
562 (2019). Hesperidin effects on gut microbiota and gut-associated lymphoid tissue in healthy rats. *Nutrients*,
563 *11*(2), 324. https://doi.org/10.3390/nu11020324
564 Ferreira, T. H. B., Silva, S. R., Munhoz, C. L., & Argandoña, E. J. S. (2020). Elaboration of biscuits type cookies
565 with pre-treated baru (*Dipteryx alata* Vog.) pulp flour. *Journal of Food Measurement and Characterization*,
566 *14*, 3156–3162. https://doi.org/10.1007/s11694-020-00557-3
567 Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex
568 carbohydrates in the gut. *Gut Microbes*, *3*(4), 289–306.https://doi.org/10.4161/gmic.19897
569 Franks, A. H., Harmsen, H. J. M., Raangs, G. C., Jansen, G. J., Schut, F., & Welling, G. W. (1998). Variations of
570 Bacterial Populations in human feces measured by fluorescent in situ hybridization with group-specific 16S
571 rRNA-targeted oligonucleotide probes. *Applied and Environmental Microbiology*, *64*(9), 3336–3345.
572 https://doi.org/10.1128/aem.64.9.3336-3345.1998
573 Gadioli, I. L., Asquieri, E. R., Oliveira, L. L., Figueiredo, L. F. A., Celestino, S. M. C., Batista, R. D., & Asquieri,
574 E. M. A. R. (2021). Influence of climatic and geographical variations on the nutritional and antioxidant
575 properties of baru mesocarp (*Dipteryx alata* Vog.) and the crystallization of sucrose by secondary nucleation.
576 *Sugar Tech*. https://doi.org/10.1007/s12355-021-01042-x
577 García-Villalba, R., González-Sarrías, A., Giménez-Bastida, J. A., Selma, M. V., Espín, J. C., & Tomás-Barberán,
578 F. A. (2022). Metabolism of dietary (poly)phenols by the gut microbiota. In M. Glibetic (Ed.),
579 *Comprehensive Gut Microbiota* (pp. 149–175). Elsevier. https://doi.org/10.1016/b978-0-12-819265-
580 8.00091-7
581 Ge, Q., Li, H., Zheng, Z., Yang, K., Li, P., Xiao, Z., Xiao, G., & Mao, J. (2022). *In vitro* fecal fermentation
582 characteristics of bamboo insoluble dietary fiber and its impacts on human gut microbiota. *Food Research*
583 *International*, *156*, 111173. https://doi.org/10.1016/j.foodres.2022.111173
584 Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C.,
585 Swanson, K. S., Cani, P. D., Verbeke, K., & Reid, G. (2017). Expert consensus document: The International
586 Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope
587 of prebiotics. *Nature Reviews Gastroenterology and Hepatology*, *14*(8), 491–502.
588 https://doi.org/10.1038/nrgastro.2017.75

589 Hajialyani, M., Farzaei, M. H., Echeverría, J., Nabavi, S. M., Uriarte, E., & Eduardo, S. S. (2019). Hesperidin as
590 a neuroprotective agent: a review of animal and clinical evidence. *Molecules*, *24*(3), 648.
591 https://doi.org/10.3390/molecules24030648
592 Harmsen, H. J. M., Elfferich, P., Schut, F., & Welling, G. W. (1999). A 16S rRNA-targeted probe for detection of
593 Lactobacilli and Enterococci in faecal samples by fluorescent *in situ* hybridization. *Microbial Ecology in*
594 *Health and Disease*, *11*(1), 3–12. https://doi.org/10.1080/089106099435862
595 Holscher, H. D. (2017). Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes*, *8*(2), 172–
596 184. https://doi.org/10.1080/19490976.2017.1290756
597 Huebner, J., Wehling, R. L., & Hutkins, R. W. (2007). Functional activity of commercial prebiotics. *International*
598 *Dairy Journal*, *17*(7), 770–775. https://doi.org/10.1016/j.idairyj.2006.10.006
599 Langendijk, P. S., Jansen, G. J., Raangs, G. C., Kamphuis, G. E. R. R., Wilkinson, M. H. F., & Welling, G. W.
600 (1995). Quantitative fluorescence in situ hybridization of *Bifidobacterium* spp. with genus-specific 16S
601 rRNA-targeted probes and its application in fecal samples. *Applied and Environmental Microbiology*, *61*(8),
602 3069–3075.
603 Leite, N. R., Araújo, L. C. A., Rocha, P. D. S., Agarrayua, D. A., Ávila, D. S., Carollo, C. A., Silva, D. B.,
604 Estevinho, L. M., Souza, K. P., & Santos, E. L. (2020). Baru pulp (*Dipteryx alata* vogel): fruit from the
605 Brazilian savanna protects against oxidative stress and increases the life expectancy of *Caenorhabditis*
606 *elegans* via SOD-3 and DAF-16. *Biomolecules*, *10*(8), 1–22. https://doi.org/10.3390/biom10081106
607 Lima, D. C., Alves, M. R., Noguera, N. H., & Nascimento, R. P. (2022). A review on Brazilian baru plant (*Dipteryx*
608 *alata* Vogel): morphology, chemical composition, health effects, and technological potential. *Future Foods*,
609 5, 100146. https://doi.org/10.1016/j.fufo.2022.100146
610 Lima, J. C. R., Freitas, J. B., Czeder, L. P., Fernandes, D. C., & Naves, M. M. V. (2010). Qualidade microbiológica,
611 aceitabilidade e valor nutricional de barras de cereais formuladas com polpa e amêndoa de baru. *Boletim*
612 *Centro de Pesquisa de Processamento de Alimentos*, *28*(2), 331–343.
613 Lima, M. S., Nunes, P. C., Silva, B. L. A., Padilha, C. V. S., Bonfim, T. H. F., Stamford, T. L. M., Vasconcelos,
614 M. A. S., & Aquino, J. S. (2019). Determining 1-kestose, nystose and raffinose oligosaccharides in grape
615 juices and wines using HPLC: method validation and characterization of products from Northeast Brazil.
616 *Journal of Food Science and Technology*, *56*(10), 4575–4584. https://doi.org/10.1007/s13197-019-03936-x
617 Manz, W., Amann, R., Ludwig, W., Vancanneyt, M., & Schleifer, K. H. (1996). Application of a suite of 16s
618 rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-

bacteroides in the natural environment. *Microbiology*, *142*(5), 1097–1106. https://doi.org/10.1099/13500872-142-5-1097

Massa, N. M. L., Oliveira, S. P. A., Rodrigues, N. P. A., Menezes, F. N. D. D., Lima, M.S., Magnani, M., & 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, 133003. https://doi.org/10.1016/j.foodchem.2022.133003

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

Oliveira, M. M., Lago, A., & Dal' Magro, G. P. (2021). Food loss and waste in the context of the circular economy: a systematic review. *Journal of Cleaner Production*, *294*, 126284. https://doi.org/10.1016/j.jclepro.2021.126284

Padilha, C. V. S., Miskinis, G. A., Souza, M. E. A. O., Pereira, G. E., Oliveira, D., Bordignon-Luiz, M. 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

Pokusaeva, K., Fitzgerald, G. F., & van Sinderen, D. (2011). Carbohydrate metabolism in Bifidobacteria. *Genes and Nutrition*, *6*(3), 285–306. https://doi.org/10.1007/s12263-010-0206-6

Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, *48*, 3396–3402. https://doi.org/10.1021/jf9913458

Rezende, E. S. V., Lima, G. C., & Naves, M. M. V. (2021). Dietary fibers as beneficial microbiota modulators: a proposal classification by prebiotic categories. *Nutrition*, *89*, 111217. https://doi.org/10.1016/j.nut.2021.111217

Rezende, E. S. V, Lima, G. C., Lima, M. S., Coelho, A. S. G., & Naves, M. M. V. (2022). Prebiotic potential of isolated commercial dietary fibres compared to orange albedo in *Lactobacillus* and *Bifidobacterium* species. *Bioactive Carbohydrates and Dietary Fibre*, *28*, 100316. https://doi.org/10.1016/j.bcdf.2022.100316

Rodríguez-Daza, M. C., Pulido-Mateos, E. C., Lupien-Meilleur, J., Guyonnet, D., Desjardins, Y., & Roy, D. (2021). Polyphenol-mediated gut microbiota modulation: toward prebiotics and further. *Frontiers in Nutrition*, 8, 1–24. https://doi.org/10.3389/fnut.2021.689456

Rothwell, J. A., Pérez-Jiménez, J., Neveu, V., Medina-Remón, A., M'Hiri, N., García-Lobato, P., Manach, C., Knox, K., Eisner, R., Wishart, D., & Scalbert, A. (2013). Phenol-Explorer 3.0: a major update of the Phenol-Explorer database to incorporate data on the effects of food processing on polyphenol content. *Database*, *2013*, bat070. https://doi.org/10.1093/database/bat070

Santiago, G. L., Oliveira, I. G., Horst, M. A., Naves, M. M. V, & Silva, M. R. (2018). Peel and pulp of baru (*Dipteryx Alata* Vog.) provide high fiber, phenolic content and antioxidant capacity. *Food Science and Technology*, *38*(2), 244–249. https://doi.org/10.1590/1678-457X.36416

Scott, K. P., Grimaldi, R., Cunningham, M., Sarbini, S. R., Wijeyesekera, A., Tang, M. L. K., Lee, J. C. Y., Yau, Y. F., Ansell, J., Theis, S., Yang, K., Menon, R., Arfsten, J., Manurung, S., Gourineni, V., & Gibson, G. R. (2020). Developments in understanding and applying prebiotics in research and practice—an ISAPP conference paper. *Journal of Applied Microbiology*, *128*(4), 934–949. https://doi.org/10.1111/jam.14424

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144–158.

Stevens, Y., Rymenant, E. V., Grootaert, C., Camp, J. V., Possemiers, S., Masclee, A., & Jonkers, D. (2019). The intestinal fate of citrus flavanones and their effects on gastrointestinal health. *Nutrients*, *11*(7), 1464. https://doi.org/10.3390/nu11071464

Togashi, M., & Sgarbieri, V. C. (1994). Caracterização química parcial do fruto do baru (*Dipteryx alata*, Vog.). *Food Science and Technology*, *14*(1), 85–95.

Trujillo, A. I., Marichal, M. de J., & Carriquiry, M. (2010). Comparison of dry matter and neutral detergent fibre degradation of fibrous feedstuffs as determined with *in situ* and *in vitro* gravimetric procedures. *Animal Feed Science and Technology*, *161*(1–2), 49–57. https://doi.org/10.1016/j.anifeedsci.2010.08.001

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*, *10*, 1–17. https://doi.org/10.3389/fmicb.2019.00057

Wang, B., Yu, H., He, Y., Wen, L., Gu, J., Wang, X., Miao, X., Qiu, G., & Wang, H. (2021). Effect of soybean insoluble dietary fiber on prevention of obesity in high-fat diet fed mice: via regulation of the gut microbiota. *Food and Function*, *12*(17), 7923–7937. https://doi.org/10.1039/d1fo00078k

Xu, K., Alves-Santos, A. M., Dias, T., & Naves, M. M. V. (2020). Grumixama (*Eugenia Brasiliensis* lam.) cultivated in the cerrado has high content of bioactive compounds and great antioxidant potential. *Ciência Rural*, *50*(4), 1–7. https://doi.org/10.1590/0103-8478cr20190630

Table 1

Chemical composition and antioxidant capacity of freeze-dried baru pulp.

Parameter	Concentration
Proximate composition (g/100 g)	
Moisture	10.28 ± 0.39
Ash	4.56 ± 0.03
Protein	8.84 ± 0.22
Lipid	3.54 ± 0.08
Carbohydrate	39.87 ± 0.39
Total dietary fiber	32.90 ± 0.42
insoluble dietary fiber	32.90 ± 0.42
soluble dietary fiber	< 0.10
Energy value (kcal/100 g)	226.73 ± 1.73
Sugars and fructans (g/100 g)	
Glucose	6.32 ± 0.70
Fructose	7.76 ± 0.82
Fructans	< 0.10
Organic acids (g/100 g)	
Citric	0.88 ± 0.11
Malic	0.70 ± 0.08
Succinic	3.75 ± 0.41
Phenolic compounds (mg/100 g)	
Caffeic acid	0.20 ± 0.00
Chlorogenic acid	0.46 ± 0.01
p-Coumaric acid	0.29 ± 0.01
Syringic acid	0.08 ± 0.00
Catechin	0.43 ± 0.00
Epicatechin gallate	0.49 ± 0.01
Epigallocatechin gallate	0.20 ± 0.00
Procyanidin B1	0.15 ± 0.00
Procyanidin B2	0.53 ± 0.00
Hesperidin	19.34 ± 0.10
Myricetin	0.96 ± 0.01
Quercetin-3-glucoside	0.14 ± 0.00
Rutin	0.09 ± 0.01
cis-Resveratrol	0.18 ± 0.02
Total phenolics (mg GAE/100 g)	507.01 ± 15.41
Total tannins (mg TAE/100 g)	142.91 ± 7.16
Antioxidant capacity (µmol TE/g)	
DPPH	23.91 ± 0.82
FRAP	31.60 ± 1.85

Values are expressed as mean ± standard deviation.

GAE: gallic acid equivalents, TAE: tannic acid equivalents, DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: ferric reducing antioxidant power, TE: trolox equivalents.

Figure captions

Fig. 1. Viable cell counts (log CFU/mL) of *Bifidobacterium animalis* subsp. *lactis* BB-12, *Lactobacillus acidophilus* LA-05, and *Lacticaseibacillus casei* L-26, and pH values in MRS broth with glucose (GLU), fructooligosaccharides (FOS) or baru pulp (BAR) during 48 h of fermentation.

Fig. 2. Sugars (g/L) and organic acids (g/L) contents in MRS broth with glucose (GLU), fructooligosaccharides (FOS) or baru pulp (BAR) during 48 h of fermentation with *Bifidobacterium animalis* subsp. *lactis* BB-12, *Lactobacillus acidophilus* LA-05, and *Lacticaseibacillus casei* L-26. a,b: different small letters for a same treatment at different fermentation times denote difference (Tukey's test; $p < 0.05$). A,B: different capital letters at the same fermentation time denote difference among treatments (Tukey's test; $p < 0.05$).

Fig. 3. Relative abundance (%) of the bacterial groups, measured by fluorescence *in situ* hybridization with flow cytometry, and pH values during the *in vitro* human colonic fermentation of digested baru pulp (diBAR), fructooligosaccharides (FOS) and negative control (NC).

Fig. 4. Concentrations of sugars (g/L), lactate (g/L), and short chain fatty acids (g/L) during the *in vitro* human colonic fermentation of digested baru pulp (diBAR), fructooligosaccharides (FOS) and negative control (NC). a,b: different small letters for a same treatment at different fermentation times denote difference (Tukey's test; $p < 0.05$). A,B: different capital letters at the same fermentation time denote difference among treatments (Tukey's test; $p < 0.05$).

Fig. 5. Principal Component Analysis (A) and Pearson's correlation (B) between the relative abundance of different bacterial groups, short chain fatty acids and sugars concentrations, and pH values in an *in vitro* human colonic fermentation of digested baru pulp (diBAR), fructooligosaccharides (FOS) and negative control (NC).
PC1: first principal component, PC2: second principal component, bac.303: *Bacteroides-Prevotella*, bif.164: *Bifidobacterium* spp., lab.158: *Lactobacillus-Enterococcus*, chis.150: *Clostridium histolyticum*, erec.482: *Eubacterium rectale-Clostridium coccoides*.

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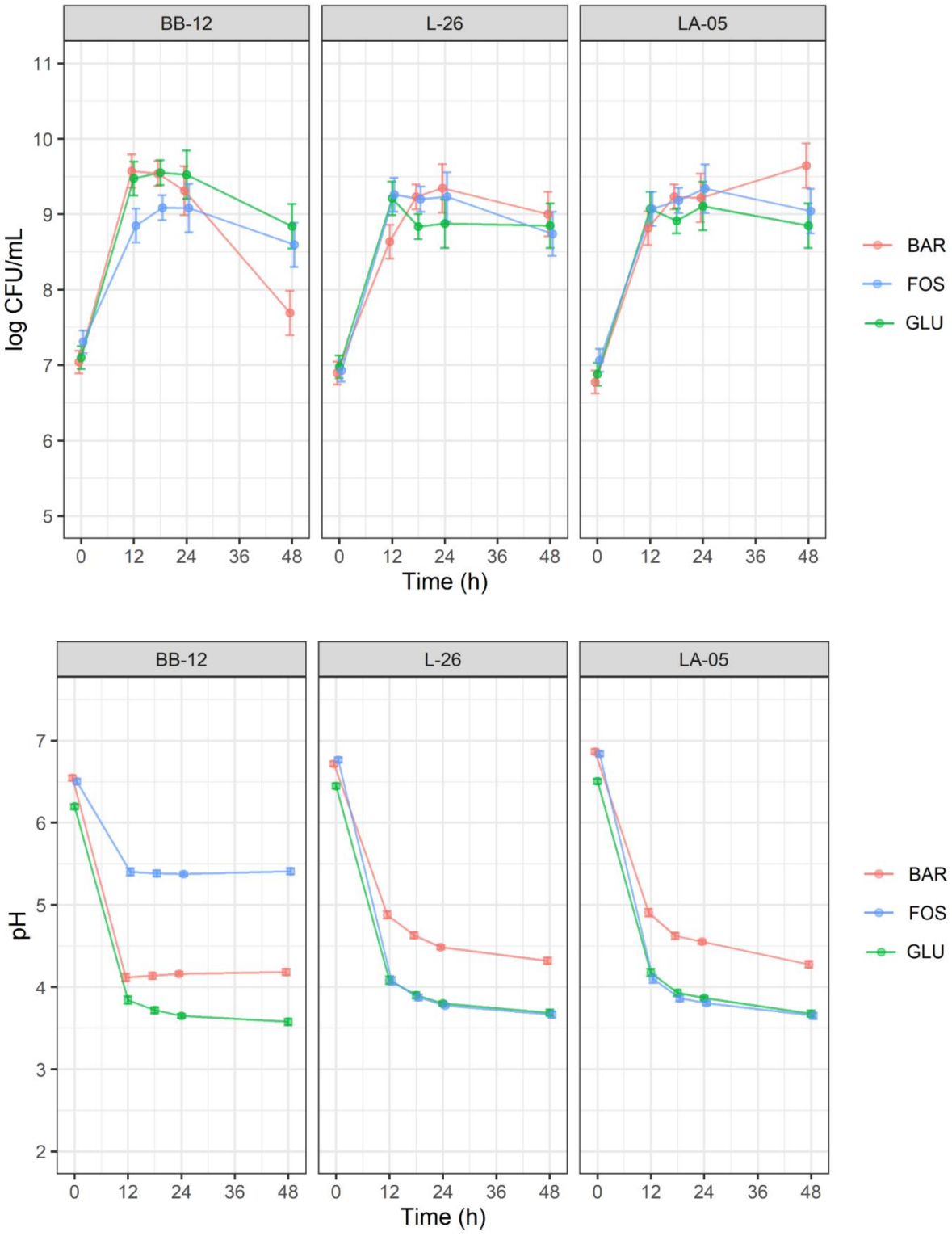

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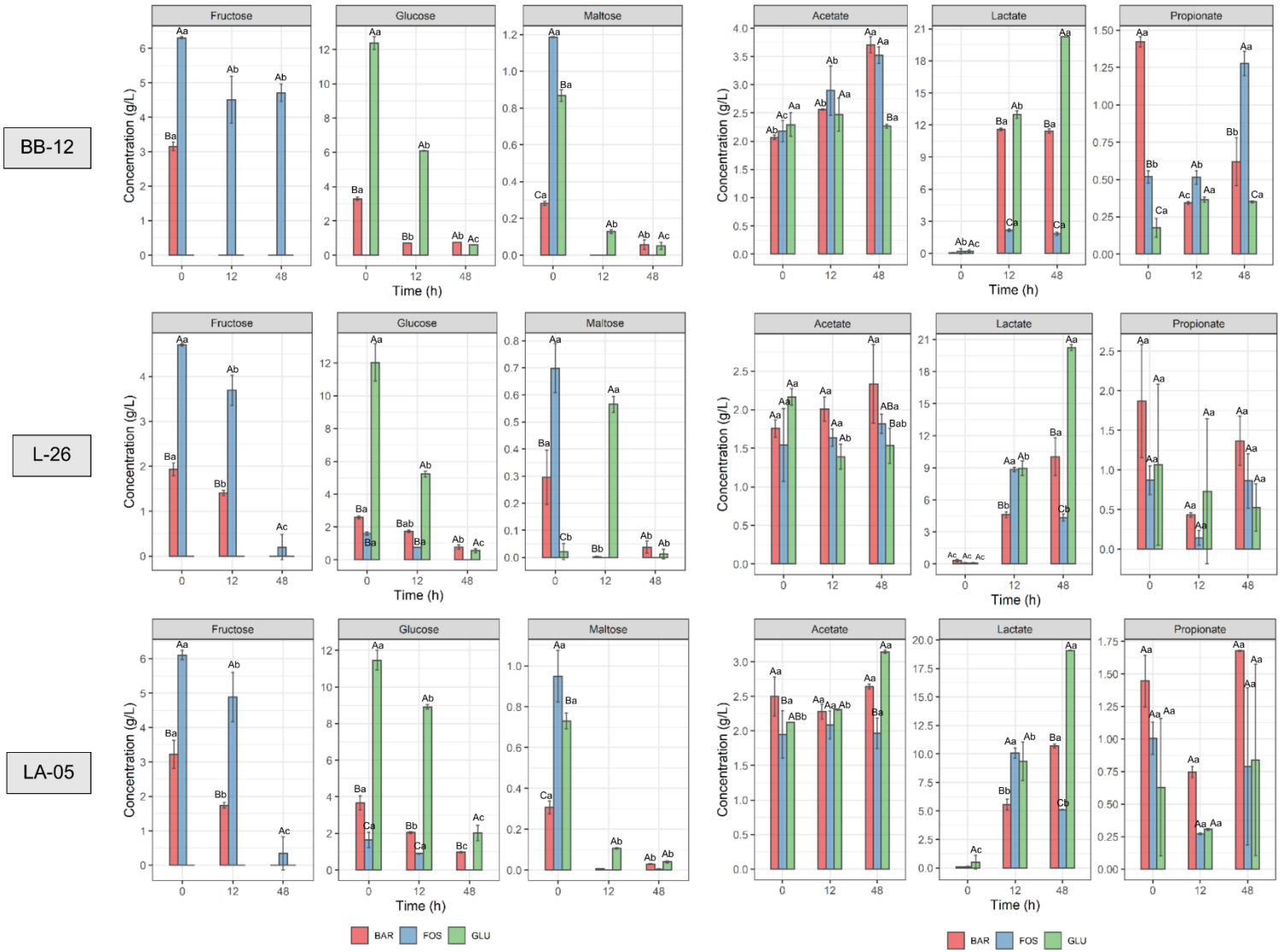

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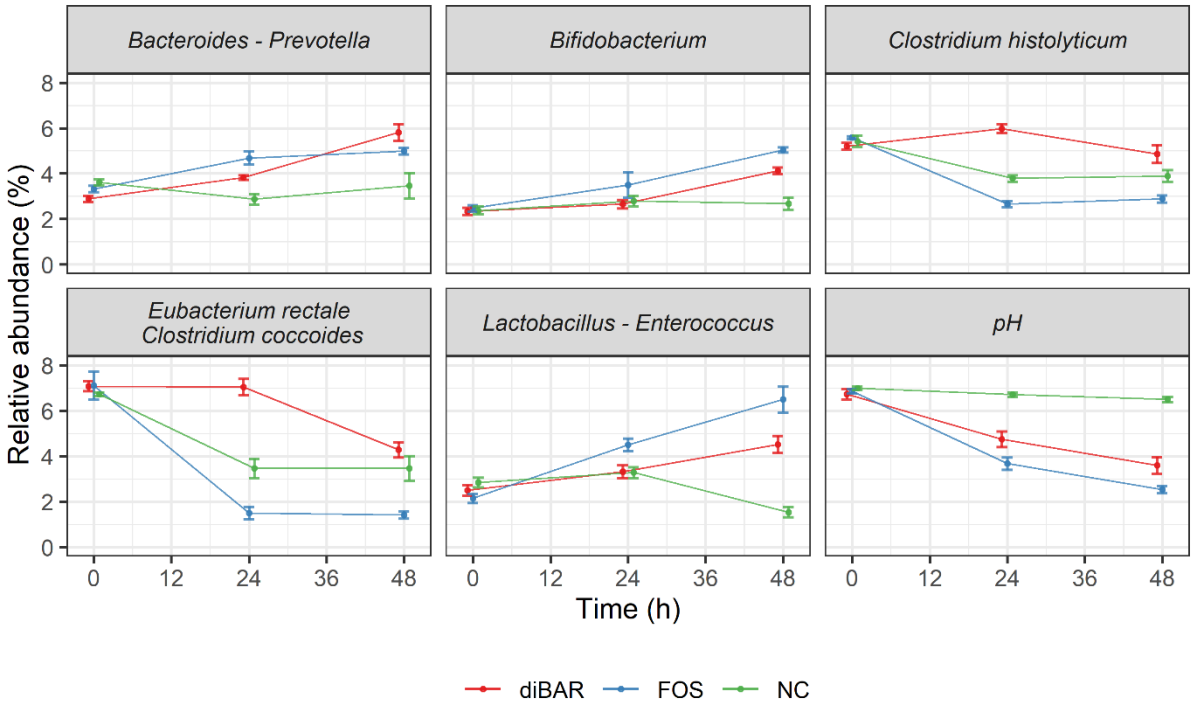
Figure 2


Bacteroides - Prevotella
Bifidobacterium
Clostridium histolyticum
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Clostridium coccoides
Lactobacillus - Enterococcus
pH
Relative abundance (%)
Time (h)
diBAR
FOS
NC

Figure 3

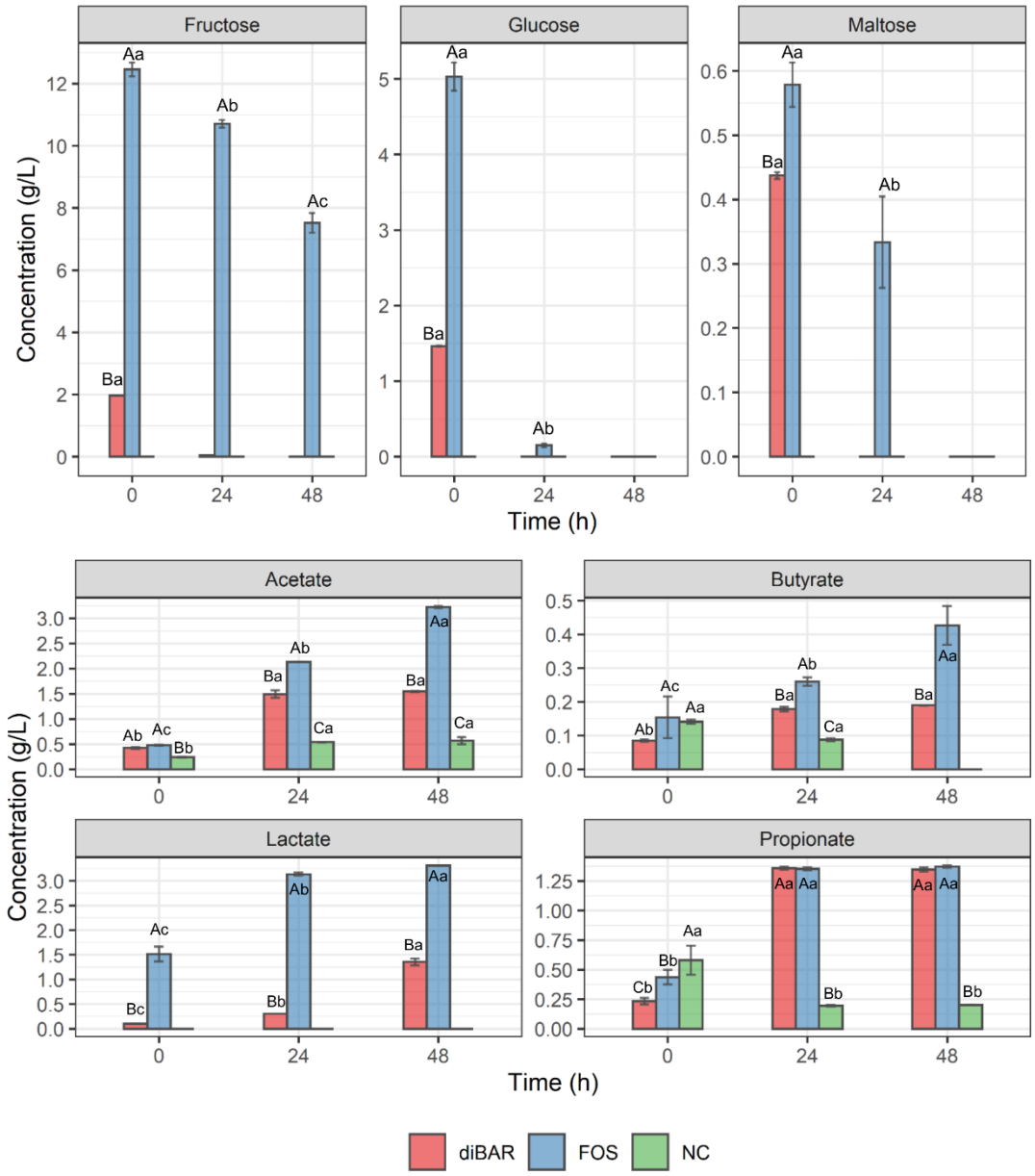
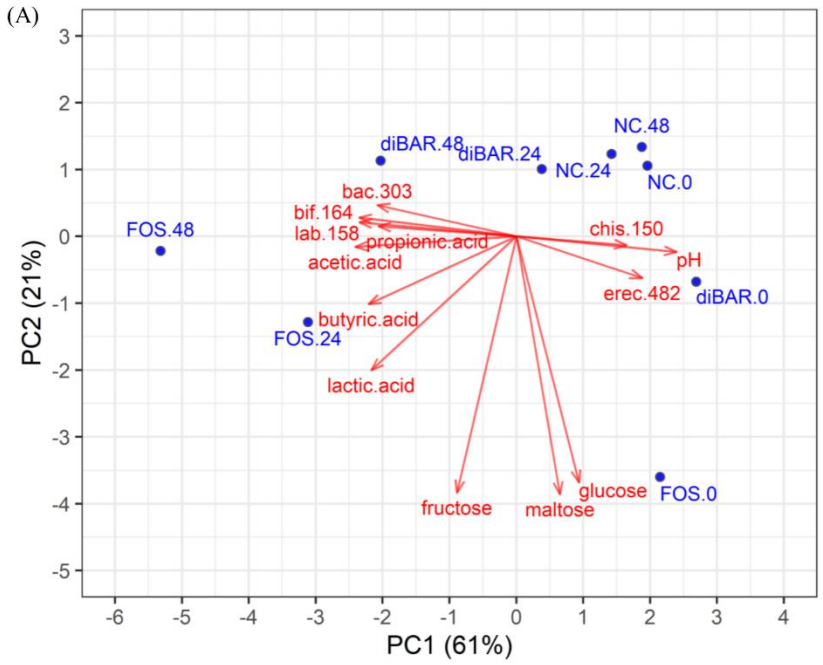

Fructose
Glucose
Maltose
Concentration (g/L)
Time (h)
Acetate
Butyrate
Lactate
Propionate
diBAR
FOS
NC

Figure 4


(A)
PC2 (21%)
PC1 (61%)
diBAR.48
diBAR.24
NC.48
NC.24
NC.0
bac.303
bif.164
lab.158
propionic.acid
acetic.acid
chis.150
pH
erec.482
diBAR.0
FOS.48
butyric.acid
FOS.24
lactic.acid
fructose
maltose
glucose
FOS.0

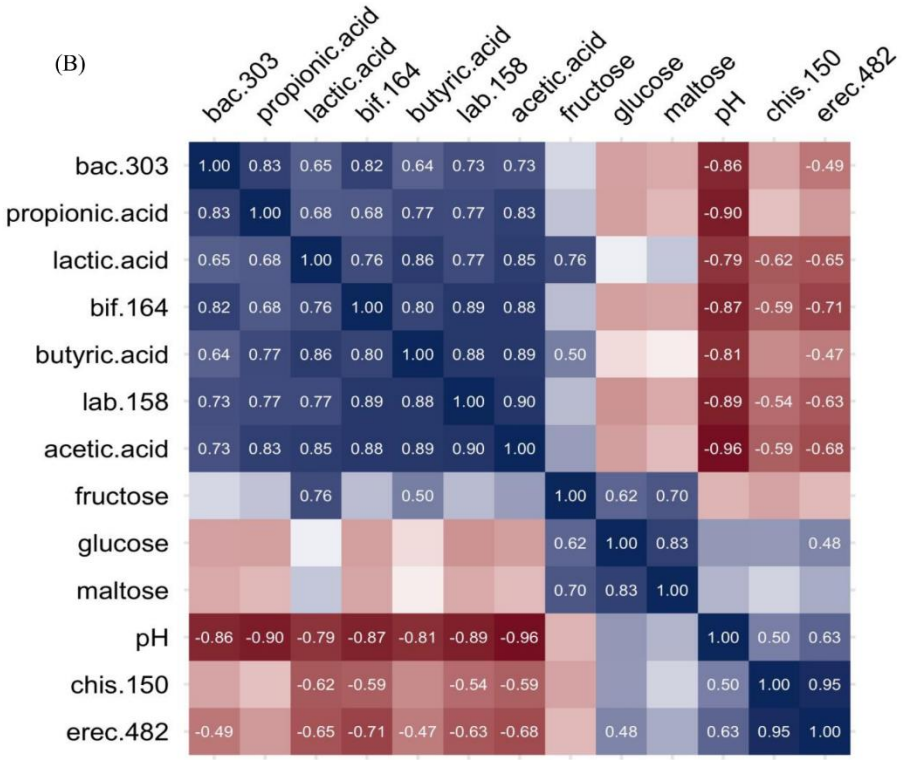

(B)
bac.303
propionic.acid
lactic.acid
bif.164
butyric.acid
lab.158
acetic.acid
fructose
glucose
maltose
pH
chis.150
erec.482

Figure 5

SUPPLEMENTARY MATERIAL

Table S1

Composition of *in vitro* human colonic fermentation medium.

Compounds	Concentration (g/L)
NaCl	4.5
KCl	4.5
$NaHCO_3$	1.5
$MgSO_4$	0.7
L-cysteine hydrochloride	0.8
KH_2PO_4	0.5
K_2HPO_4	0.5
Bile salt	0.4
$CaCl_2$	0.08
$FeSO_4$	0.005
Tween 80	1 mL/L
Resazurin (0.025%)	4 mL/L

Bile salt and resazurin were purchased from Sigma-Aldrich (St. Louis, USA), L-cysteine-HCl was purchased from Êxodo Científica (Sumaré, Brazil), and the remaining reagents were purchased from Dinâmica (Indaiatuba, Brazil).

Table S2

Oligonucleotide probes and hybridization conditions used in FISH analysis.

Probe	Sequence (5' – 3')	Target group	Temperature (ºC)	
			hybridization	washing
Bac 303	CCAATGTGGGGGACCTT	*Bacteroides* spp. - *Prevotella* spp.	45	45
Bif 164	CATCCGGCATTACCACCC	*Bifidobacterium* spp.	50	50
Chis 150	TTATGCGGTATTAATCTYCCTTT	*Clostridium histolyticum*	50	50
Erec 482	GCTTCTTAGTCARGTACCG	*Eubacterium rectale* - *Clostridium coccoides*	50	50
Lab 158	GGTATTAGCAYCTGTTTCCA	*Lactobacillus* spp. - *Enterococcus* spp.	50	50

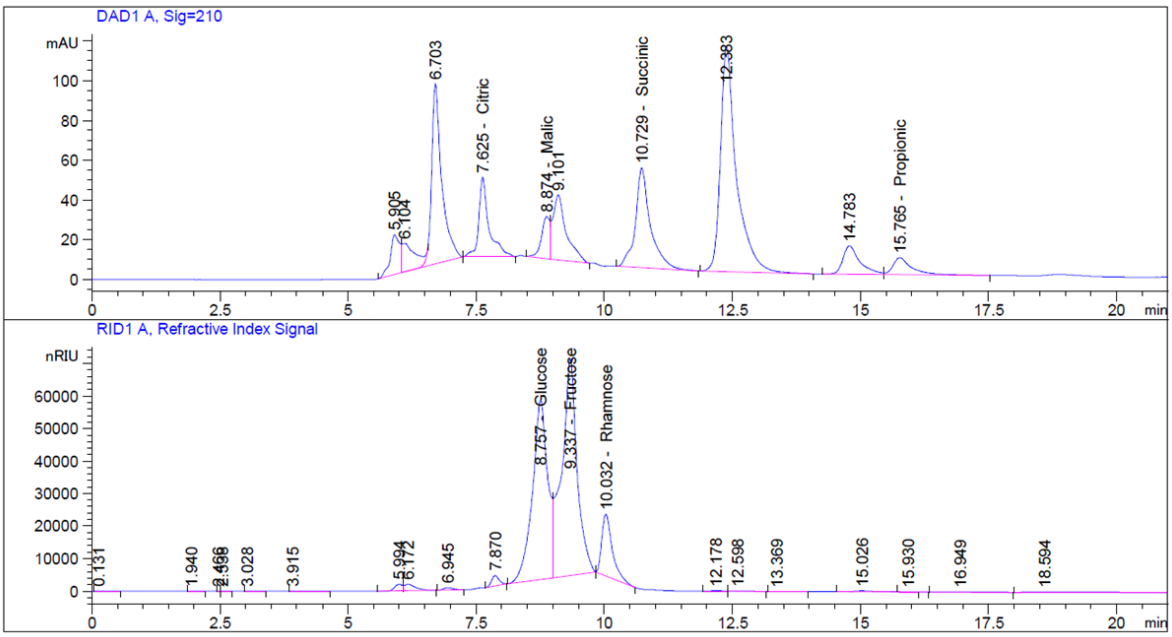


Fig. S1. Representative chromatograms of the analysis of organic acids and sugars in the baru pulp.

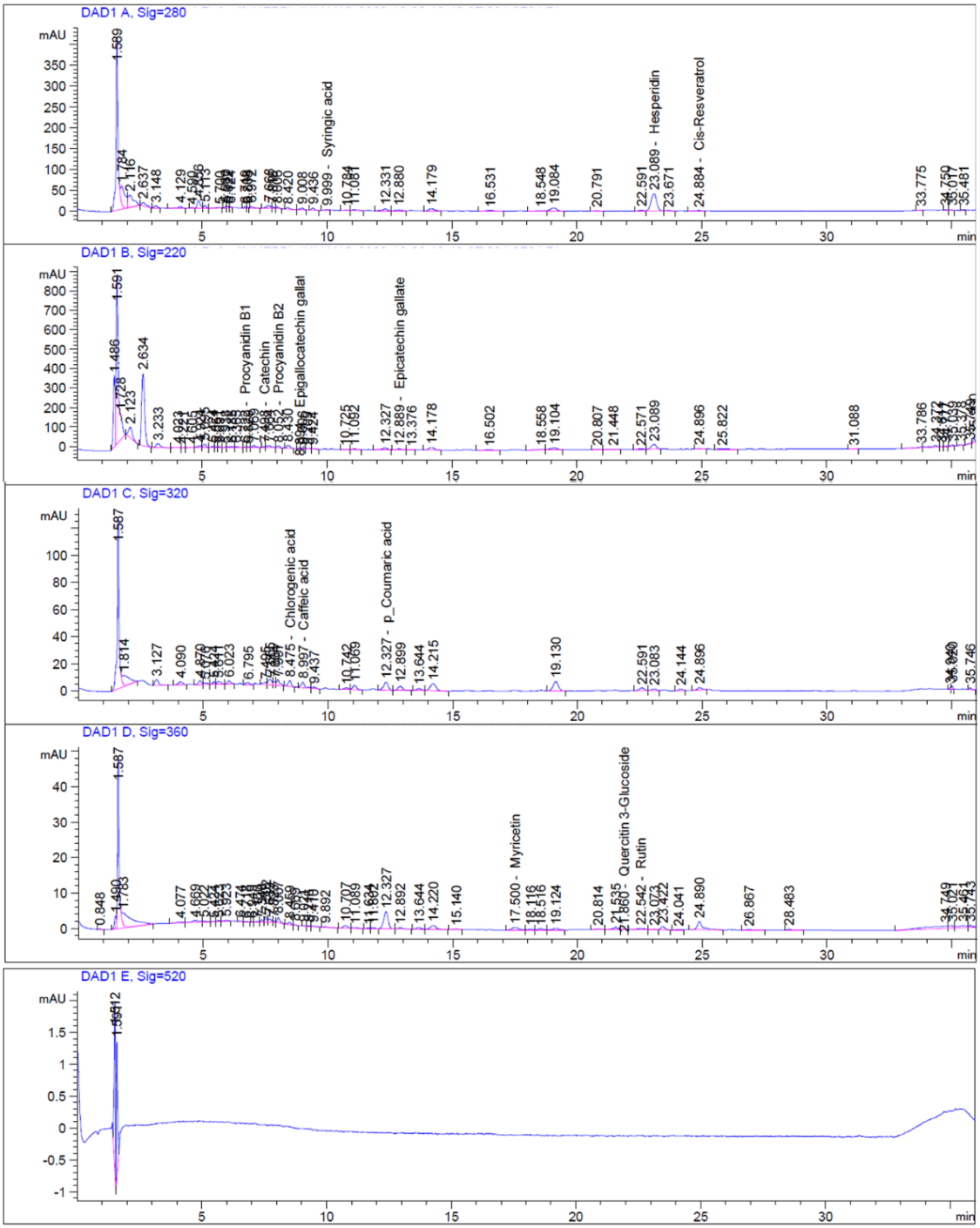


Fig. S2. Representative chromatograms of the analysis of phenolic compounds in the baru pulp.

959 GRAPHICAL ABSTRACT
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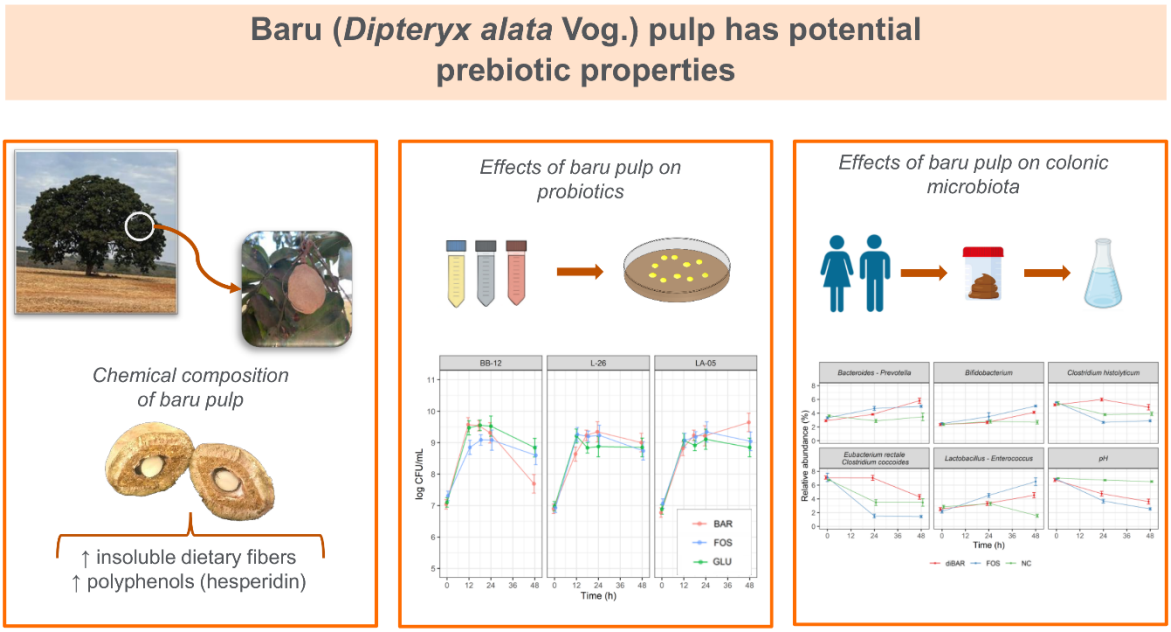

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CONCLUSÃO

Com base nas revisões de literatura, é possível concluir que alguns polifenóis dietéticos são capazes de estimular o crescimento e o metabolismo de bactérias intestinais benéficas, e que a polpa de baru representa uma alternativa para o desenvolvimento de produtos com propriedades funcionais e nutracêuticas, apesar de ainda ser pouco explorada pela agroindústria. De acordo com o estudo empírico, a polpa de baru possui concentração elevada de fibras insolúveis e compostos fenólicos, baixo teor de lipídios e reduzido valor energético. A hesperidina foi o polifenol encontrado em maior concentração na polpa de baru. O potencial prebiótico dessa polpa foi evidenciado pelo aumento do crescimento e metabolismo de probióticos, e pelo impacto positivo sobre a abundância e atividade metabólica de grupos bacterianos benéficos na microbiota colonica humana. O efeito sinérgico entre fibras alimentares e polifenóis possivelmente contribuiu para a atividade prebiótica da polpa de baru.

Embora os protocolos *in vitro* utilizados neste estudo tenham mostrado resultados consistentes indicando propriedades prebióticas, algumas limitações podem ser citadas, como: a dificuldade de reproduzir as interações diretas com o hospedeiro; o número limitado de sondas de oligonucleotídeos para avaliação da composição da microbiota intestinal; a falta de dados relacionados ao conteúdo de carboidratos estruturais da polpa de baru; e a falta do perfil de metabólitos de polifenóis nos produtos finais, após os experimentos. Sugere-se para estudos futuros a inclusão de sondas de oligonucleotídeos específicas para *Akkermansia*, *Ruminococcus* e *Escherichia coli*, ou a associação do protocolo de fermentação colonica humana ao sequenciamento do gene 16S RNAr para avaliação da microbiota intestinal. O estudo de metabólitos de polifenóis pode aumentar a consistência dos resultados sobre o potencial prebiótico da polpa de baru e direcionar novos estudos. O uso da polpa de baru no desenvolvimento de novos alimentos com propriedades funcionais e nutracêuticas, e a possível aplicação em biofilmes e embalagens ativas também são recomendados. Além disso, estudos *in vivo* são necessários para investigar o efeito prebiótico de polifenóis dietéticos, como a hesperidina, para confirmar o potencial prebiótico da polpa de baru e para investigar os efeitos do seu consumo sobre a saúde do hospedeiro.

ANEXOS

ANEXO A – Parecer consubstanciado do Comitê de Ética em Pesquisa

UFG - UNIVERSIDADE FEDERAL DE GOIÁS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: POTENCIAL NUTRICIONAL E PREBIÓTICO IN VITRO E EFEITOS DO CONSUMO DE SUBPRODUTOS E RESÍDUOS DO PROCESSAMENTO DE BARU SOBRE MARCADORES DE RISCO CARDIOMETABÓLICO E MICROBIOTA INTESTINAL IN

Pesquisador: MARIA MARGARETH VELOSO NAVES

Área Temática:

Versão: 2

CAAE: 49689321.8.0000.5083

Instituição Proponente: Faculdade de Nutrição

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.970.001

Apresentação do Projeto:

Estudo vinculado a Faculdade de Nutrição da UFG, sob coordenação da Profa Dra Maria Margareth Veloso Naves. Busca identificar uma mistura de subprodutos e resíduos de baru com alto valor nutricional e funcional, que possa ser utilizado em diferentes sistemas alimentares visando à prevenção de doenças.O baru é composto por uma casca fina (epicarpo) que reveste a polpa (mesocarpo), e por um endocarpo lenhoso que contém a amêndoa. A castanha de baru se destaca pelo valor nutricional, sobretudo por seu perfil de ácidos graxos insaturados, principalmente os monoinsaturados, assim como pela qualidade proteica e fibras alimentares, resultados que impulsionaram o consumo desta semente. O óleo da amêndoa de baru possui principalmente os ácidos graxos oleico e linoleico, além de tocoferóis e fenólicos totais. A casca e a polpa do fruto são descartadas durante o processamento e ainda são pouco estudadas. Neste sentido, este estudo visa contribuir para o aproveitamento sustentável do baruzeiro e aumento do valor agregado dos frutos nativos do Cerrado, por meio da investigação do valor nutricional e funcional e do potencial prebiótico in vitro de subprodutos e resíduos do processamento de baru, bem como os efeitos do consumo destes nos marcadores de risco cardiometabólico e na microbiota intestinal in vivo. Serão determinadas a composição centesimal e os teores de fibra dietética, açúcares, oligossacarídeos, compostos fenólicos totais, além do perfil químico (especiação) destes compostos. O potencial prebiótico será avaliado em culturas de bactérias lácteas. Será realizado

Endereço: Alameda Flamboyant, Qd. K, Edifício K2, sala 110
Bairro: Campus Samambaia, UFG **CEP:** 74.690-970
UF: GO **Município:** GOIANIA
Telefone: (62)3521-1215 **E-mail:** cep.prpi@ufg.br

um ensaio clínico para avaliação dos efeitos do consumo dos subprodutos e resíduos do processamento de baru nos marcadores de risco cardiometabólico e na microbiota intestinal. Espera-se, com este estudo, identificar uma mistura de subprodutos e resíduos de baru com alto valor nutricional e funcional, que possa ser utilizado em diferentes sistemas alimentares visando à prevenção de doenças.

Objetivo da Pesquisa:

OBJETIVO GERAL - Investigar os potenciais nutricional e prebiótico in vitro do consumo de subprodutos e resíduos do processamento do baru, bem como os efeitos do consumo destes nos marcadores de risco cardiometabólico e na microbiota intestinal in vivo.

OBJETIVOS ESPECÍFICOS - Analisar a composição centesimal e o conteúdo de compostos bioativos dos subprodutos e resíduos do processamento do baru. - Avaliar o potencial prebiótico in vitro de misturas de subprodutos e resíduos do processamento do baru por meio do uso de bactérias lácteas. - Avaliar o efeito do consumo dos subprodutos e resíduos do processamento do baru nos marcadores de risco cardiometabólico. Avaliar o efeito do consumo dos subprodutos e resíduos do processamento do baru na microbiota intestinal in vivo.

Avaliação dos Riscos e Benefícios:

Segundo os pesquisadores:

Riscos: A análise sensorial de produtos alimentícios formulados com a mistura de subprodutos e resíduos do baru oferece riscos mínimos ao participante. Serão excluídos do teste participantes que possuam alergia a nozes e sementes comestíveis. Para minimizar o risco de contaminação, os produtos serão submetidos previamente à análise microbiológica. No início e ao final de cada teste, as cabines serão higienizadas, conforme os protocolos de biossegurança vigentes. Os utensílios utilizados na análise sensorial serão descartáveis. Os participantes serão orientados a realizar a higienização das mãos no início da análise sensorial. A avaliação do efeito prebiótico pelo inóculo fecal também envolve riscos mínimos ao participante. Os voluntários serão orientados quanto à coleta e entrega de amostras fecais frescas, de modo a minimizar o risco de contaminação, e estas serão recebidas pelos pesquisadores e acondicionadas até o momento das análises. A entrega das amostras cedidas pelos participantes ocorrerá em local reservado para minimizar os desconfortos ou constrangimentos. O participante será orientado quanto a possibilidade de retirada a qualquer tempo e sem quaisquer ônus ou prejuízos, do consentimento para utilização do material biológico coletado e armazenado para a análise. Para a condução do ensaio clínico, os pesquisadores serão treinados a fim de minimizar os riscos possíveis em cada

etapa. Após a padronização da mistura de subprodutos e resíduos do processamento do baru, esta será submetida à análise microbiológica. Após a análise serão acondicionadas em recipientes porcionados, seguindo-se as boas práticas de manipulação de alimentos, para entrega aos participantes. Os participantes serão orientados quanto à forma correta de armazenamento das porções para consumo semanal, a fim de minimizar os riscos de contaminação. Estes participantes serão monitorados semanalmente quanto ao consumo da porção e a sintomas gastrointestinais. O ensaio clínico envolve ainda etapas de avaliações antropométrica e bioquímica. A avaliação antropométrica oferece riscos mínimos ao participante, visto que os procedimentos utilizados não são invasivos. Na avaliação bioquímica, os riscos inerentes envolvem a dor e o desconforto durante a coleta ou a possibilidade de surgimento de hematoma no local de coleta de sangue. A coleta de sangue será realizada por equipe treinada para minimizar os riscos.

Benefícios: A pesquisa trará inúmeros benefícios, pois a mistura de subprodutos e resíduos do processamento de baru é fonte de nutrientes e compostos bioativos que poderão auxiliar na redução do risco cardiometabólico, bem como trazer benefícios para a microbiota intestinal, por seu potencial prebiótico. Outrossim, a divulgação dos resultados desta pesquisa possivelmente promoverá maior adesão ao consumo integral do fruto do baru, valorizando assim os frutos nativos do Cerrado brasileiro. Nesse sentido, poderá contribuir para o desenvolvimento socioeconômico e sustentabilidade do Cerrado, bem como para a melhoria do potencial econômico da região Centro-Oeste.

Comentários e Considerações sobre a Pesquisa:

Trata-se de ensaio clínico com 124 participantes, que visa investigar os potenciais nutricional e prebiótico in vitro do consumo de subprodutos e resíduos do processamento do baru, bem como os efeitos do consumo destes nos marcadores de risco cardiometabólico e na microbiota intestinal in vivo. Para isso o estudo envolverá diversas etapas, sendo elas: análises químicas do baru, elaboração dos produtos e análise sensorial (com 60 participantes). O ensaio clínico é composto pelas fases: avaliação in vitro do prebiótico, avaliação do efeito do prébiótico através de inóculo fecal (com 4 participantes) e o ensaio clínico (com 60 participantes) que contará com análises bioquímicas (coleta de sangue periférico) e antropométricas. Os participantes adultos com sobrepeso e obesidade, levemente hipercolesterolêmicos serão alocados aletoriamente em grupos, recebendo uma das seguintes intervenções: dieta habitual suplementada ou não com misturas de subprodutos e resíduos do processamento do baru. As abordagens serão feitas na FANUT e possui aval do conselho diretor. As abordagens de coleta de sangue periférico será realizada no Laboratório Rômulo Rocha (UFG), por profissional habilitado disponibilizado pelo próprio

laboratório. O recrutamento dos participantes se dará por meio da divulgação da pesquisa em mídias digitais e folders, contendo as informações para esclarecimento do projeto, tanto para a comunidade interna da UFG quanto para a comunidade externa.

Considerações sobre os Termos de apresentação obrigatória:

Foram apresentados:

- Projeto de pesquisa atualizado, contendo a descrição da forma de recrutamento, bem como a anuência do Laboratório Rômulo Rocha para a realização da pesquisa;
- Declaração do Laboratório Rômulo Rocha atestando que a coleta de sangue será realizada por profissional habilitado - devidamente preenchida e assinada;
- Carta de encaminhamento detalhada sobre os atendimentos das pendências anteriores;
- TCLE do ensaio clínico alterado de acordo com as pendências anteriores;
- Anuência do Laboratório Rômulo Rocha devidamente preenchida e assinada;
- Termo de Compromisso adequado;
- TCLE efeito prebiótico e inóculo fecal - adequado;
- TCLE Análise sensorial - Adequado;
- Folha de Rosto devidamente preenchida e assinada.

Conclusões ou Pendências e Lista de Inadequações:

Diante do atendimento de pendências e esclarecimentos quanto ao recrutamento dos participantes, bem como a anuência e responsabilidade técnica assumida pelo laboratório Rômulo Rocha sobre a coleta de sangue dos participantes de pesquisa, o presente protocolo de pesquisa segue em conformidade com as Resoluções CNS n. 466/12 e CNS n. 510/16, estando portanto APROVADO por este comitê de ética em pesquisa.

Considerações Finais a critério do CEP:

Informamos que o Comitê de Ética em Pesquisa / CEP-UFG considera o presente protocolo APROVADO e lembramos que o pesquisador responsável deverá encaminhar ao CEP-UFG o Relatório Final baseado na conclusão do estudo e na incidência de publicações decorrentes deste, de acordo com o disposto na Resolução CNS n. 466/12. O prazo para entrega do Relatório é de 30 dias após o encerramento da pesquisa.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação

Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1790502.pdf	02/09/2021 09:37:26		Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_BARU_CEP_ALTERADO.docx	02/09/2021 09:35:31	MARIA MARGARETH VELOSO NAVES	Aceito
Outros	Declaracao_Laboratorio.pdf	02/09/2021 09:16:50	MARIA MARGARETH	Aceito
Outros	Carta_encaminhamento_parecer_4918895.pdf	02/09/2021 09:16:03	MARIA MARGARETH	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_ENSAIO_CLINICO_ALTERADO.doc	02/09/2021 09:12:18	MARIA MARGARETH VELOSO NAVES	Aceito
Outros	TERMO_DE_ANUENCIA_EM_PESQUISA.pdf	13/07/2021 16:55:21	MARIA MARGARETH	Aceito
Outros	TERMO_DE_COMPROMISSO.docx	13/07/2021 16:54:57	MARIA MARGARETH	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_EFEITO_PREBIOTICO_INOCULO_FECAL.doc	13/07/2021 16:52:10	MARIA MARGARETH VELOSO NAVES	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_ANALISE_SENSORIAL.doc	13/07/2021 16:52:02	MARIA MARGARETH VELOSO NAVES	Aceito
Folha de Rosto	FOLHA_DE_ROSTO.pdf	13/07/2021 16:48:03	MARIA MARGARETH	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

GOIANIA, 13 de Setembro de 2021

Assinado por:
Rosana de Morais Borges Marques
(Coordenador(a))

ANEXO B – Termo de Consentimento Livre e Esclarecido

UNIVERSIDADE FEDERAL DE GOIÁS
FACULDADE DE NUTRIÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM NUTRIÇÃO E SAÚDE

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO - TCLE

Você está sendo convidado (a) a participar, como voluntário (a), da pesquisa intitulada "**POTENCIAL NUTRICIONAL E PREBIÓTICO *IN VITRO* E EFEITOS DO CONSUMO DE SUBPRODUTOS E RESÍDUOS DO PROCESSAMENTO DE BARU SOBRE MARCADORES DE RISCO CARDIOMETABÓLICO E MICROBIOTA INTESTINAL *IN VIVO***". Meu nome é Aline Medeiros Alves Santos, sou a pesquisadora responsável, e minha área de atuação é Nutrição. Após receber os esclarecimentos e as informações a seguir, se você aceitar fazer parte do estudo, assine ao final deste documento, que está impresso em duas vias, sendo que uma delas é sua e a outra ficará comigo. Esclareço que em caso de recusa na participação, em qualquer etapa da pesquisa, você não será penalizado (a) de forma alguma. Mas se aceitar participar, as dúvidas sobre a pesquisa poderão ser esclarecidas pelo (a) pesquisador (a) responsável, via e-mail (alinemedeiros@ufg.br) e, por meio do seguinte contato telefônico: (62) 98130-3800, inclusive com possibilidade de ligação a cobrar. Ao persistirem as dúvidas sobre os seus direitos como participante desta pesquisa, você também poderá fazer contato com o **Comitê de Ética em Pesquisa** da Universidade Federal de Goiás, pelo telefone (62) 3521-1215, que é a instância responsável por dirimir as dúvidas relacionadas ao caráter ético da pesquisa. O Comitê de Ética em Pesquisa da Universidade Federal de Goiás (CEP-UFG) é independente, com função pública, de caráter consultivo, educativo e deliberativo, criado para proteger o bem-estar dos participantes da pesquisa, em sua integridade e dignidade, visando contribuir no desenvolvimento da pesquisa dentro de padrões éticos vigentes.

Este estudo visa avaliar os benefícios do consumo de subprodutos do processamento de baru para os microrganismos intestinais. Você será solicitado a ceder amostras fecais. Você deverá coletar as fezes no frasco descartável codificado que será entregue a você. As amostras cedidas serão armazenadas até o momento das análises.

Esta pesquisa permitirá a avaliação do potencial prebiótico de subprodutos e resíduos do processamento de baru. Os prebióticos são utilizados pela nossa microbiota intestinal trazendo vários benefícios para a nossa saúde. Além disso, a pesquisa contribuirá para o aproveitamento sustentável do baruzeiro e aumento do valor agregado aos frutos nativos do Cerrado, assim como promover a redução do desperdício e do impacto ambiental causado no processamento deste fruto.

Você tem direito ao ressarcimento das despesas decorrentes da cooperação com a pesquisa, inclusive transporte e alimentação, se for o caso.

Em caso de danos, você tem o direito de pleitear indenização, conforme previsto em Lei.

Se você não quiser que seu nome seja divulgado, está garantido o sigilo que assegure a privacidade e o anonimato. As informações desta pesquisa serão confidenciais e serão divulgadas apenas em eventos ou publicações científicas. Existe o risco de constrangimento e desconforto na entrega das amostras fecais. Para minimizar o constrangimento, a entrega das amostras ocorrerá em local reservado ou os pesquisadores responsáveis pela pesquisa farão a coleta individualmente.

Durante todo o período da pesquisa e na divulgação dos resultados, sua privacidade será respeitada, ou seja, seu nome ou qualquer outro dado ou elemento que possa, de alguma forma, identificar-lhe, será mantido em sigilo. Todo material ficará sob minha guarda por um período mínimo de cinco anos. O seu consentimento é necessário para condução da coleta. Faça uma rubrica entre os parênteses da opção que valida sua decisão.

Pode haver também a necessidade de utilizarmos sua opinião em publicações, faça uma rubrica entre os parênteses da opção que valida sua decisão:

( ) Permito a divulgação da minha opinião nos resultados publicados da pesquisa.
( ) Não Permito a divulgação da minha opinião nos resultados publicados da pesquisa.

UNIVERSIDADE FEDERAL DE GOIÁS
FACULDADE DE NUTRIÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM NUTRIÇÃO E SAÚDE

Pode haver necessidade de utilização dos dados coletados em pesquisas futuras, desde que seja feita nova avaliação pelo CEP/UFG. Assim, solicito a sua autorização, validando a sua decisão com uma rubrica entre os parênteses abaixo:

( ) Declaro ciência de que os meus dados coletados podem ser relevantes em pesquisas futuras e, portanto, autorizo a guarda do material em banco de dados e/ou biobancos e biorrepositórios.

( ) Declaro ciência de que os meus dados coletados podem ser relevantes em pesquisas futuras, mas não autorizo a guarda do material em banco de dados e/ou biobancos e biorrepositórios.

Consentimento da Participação da Pessoa como Sujeito da Pesquisa

Eu, ..., abaixo assinado, concordo em participar do estudo intitulado "**POTENCIAL NUTRICIONAL E PREBIÓTICO *IN VITRO* E EFEITOS DO CONSUMO DE SUBPRODUTOS E RESÍDUOS DO PROCESSAMENTO DE BARU SOBRE MARCADORES DE RISCO CARDIOMETABÓLICO E MICROBIOTA INTESTINAL *IN VIVO***". Informo ter mais de 18 anos de idade e destaco que minha participação nesta pesquisa é de caráter voluntário. Fui devidamente esclarecido (a) pela pesquisadora responsável Aline Medeiros Alves Santos sobre a pesquisa, os procedimentos e métodos envolvidos, assim como os possíveis riscos e benefícios decorrentes de minha participação no estudo. Foi-me garantido que posso retirar meu consentimento a qualquer momento, sem que isto leve a qualquer penalidade. Declaro, portanto, que concordo com a minha participação no projeto de pesquisa acima descrito.

Goiânia, ... de ..... de 2021

_____
Assinatura por extenso do(a) participante

_____
Assinatura por extenso da pesquisadora responsável

ANEXO C – Instruções aos autores do periódico Food Research International

FOOD RESEARCH INTERNATIONAL

AUTHOR INFORMATION PACK

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- **Editorial Board** **p.2**
- **Guide for Authors** **p.7**

ISSN: 0963-9969

DESCRIPTION

Food Research International provides a forum for the rapid dissemination of significant novel and high impact research in food science, technology, engineering and nutrition. The journal only publishes novel, high quality and high impact review papers, original research papers and letters to the editors, in the various disciplines encompassing the science and technology of food. It is journal policy to publish special issues on topical and emergent subjects of food research or food research-related areas. Special issues of selected, peer-reviewed papers from scientific meetings, workshops, conferences on the science, technology and engineering of foods will be also published.

Food Research International is the successor to the Canadian Institute of Food Science and Technology Journal. Building on the quality and strengths of its predecessor, *Food Research International* has been developed to create a truly international forum for the communication of research in **food science**.

Topics covered by the journal include:
food chemistry food microbiology and **safety** microbiome food **toxicology materials** science of foods **food engineering** physical **properties** of foods **sensory science food quality** health and **nutrition food biophysics** analysis of foods **food nanotechnology emerging technologies**

Subjects that ***will not*** be considered for publication in *Food Research International*, and will be rejected as being outside of scope, include :
Studies testing different formulations and ingredients leading to the choice of the best formulation or ingredient to be used in the manufacture of a specified food; Optimization studies aiming to determine processing conditions and/or raw materials that increase the yield of a production process or improve nutritional and sensorial qualities; Studies describing the production of ingredients and only their characterization without a strong mechanistic emphasis; Studies describing the biological activity of foods lacking identification of the compounds responsible for the reported activity will not be published. This is also valid for any other chemical compounds such as phytochemicals and minor components of foods. Compounds of interest need to be characterized at least by mass spectrometry-based methods. Studies that do not clearly prove the relationship between the structure of the compounds and their activity; Fingerprinting studies lacking molecular insights and validation sets; Studies on antimicrobial compounds that do not consider a validation step in foods, lacking full data on chemical composition indicating the compounds responsible for the inhibitory activity and, when appropriate, the use of molecular biology approaches to support the findings; Development of analytical methods not comprising a validation step in situ that represent the range of conditions faced during their application will not be considered; Surveys of chemical, nutritional, physical and microbiological hazards will not be considered. Only papers presenting a significant data set, wide

coverage, novel and supported by adequate chemical or microbiological techniques will be considered; Pharmacology and nutritional studies papers focusing in hosts rather than in foods. Pharmacology and nutritional studies that do not contain bioavailability or biofunctionality. Engineering studies lacking of mathematical verification or validation in situ, when appropriate; Fragmented studies, of low scientific quality, or poorly written. Studies with no food component.

IMPACT FACTOR

2020: 6.475 © Clarivate Analytics Journal Citation Reports 2021

ABSTRACTING AND INDEXING

CAB International
EMBiology
AGRICOLA
BIOSIS Citation Index
Elsevier BIOBASE
FSTA (Food Science and Technology Abstracts)
International Packaging Abstracts
Science Citation Index
Publications in Food Microbiology
Index to Scientific Reviews
Current Packaging Abstracts
Chemical Abstracts
Current Contents - Agriculture, Biology & Environmental Sciences
Scopus
PubMed/Medline

EDITORIAL BOARD

GUIDE FOR AUTHORS

INTRODUCTION

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Food Research International does not publish papers with a product development emphasis, statistical optimizations of processes or surveys. This is based on the editorial policy of the journal to publish more fundamental work with a strong quantitative emphasis and of a general nature.

Topics covered by the journal include:
Emerging Technologies Sensory Aspects of Foods Food Toxicology Food Chemistry and Analysis Food Omics Nutrition, health and food digestion Food Engineering and Materials Science of Foods Functional Foods Food Microbiology, Safety and Quality

Please also refer to the list of subjects not considered in *Food Research International* before you submit your paper. These topics can be found in the full aims and scope of the journal.

Types of paper

Research papers - original full-length research papers which have not been published previously, except in a preliminary form. It is preferable that manuscripts do not exceed 6,000 words. The word count refers to the text of the manuscript per se, i.e., references, figures and tables are not considered. This limit might be exceeded as required for manuscripts to be as complete as possible towards ensuring quality, novelty and impact. There are no limits on the figures and tables to ensure manuscripts are thorough. Review articles - will be accepted in all areas of food science covered by the scope of the journal. Review articles focused on recent literature published (for example, over the previous 2-5 years) as well as comprehensive and definitive reviews will be considered.Review papers must contain critical assessment of literature and may also contains author's views on the subject. There are no word counts and reference numbers limit for review papers. Short communications - Food Research International does not publish short communication papers. Letters to the Editor - Letters are published from time to time on matters of topical interest. Book Reviews

Food Research International is concerned with safeguarding the rights and welfare of animals and human research subjects. Authors must provide a letter with the approval from the ethics committee from the respective University or research center where the study was performed.

The list of references must be as updated as possible. Making reference to recent work in the field is particularly key to highlight the current context of the manuscript and to make it more comprehensive, to highlight the novelty to the readers as well as its contribution to the field.

Contact details for submission

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