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POTENCIAL PREBIÓTICO DE FARINHAS DE DIFERENTES CULTIVARES DE BATATA-DOCE (*Ipomoea batatas* L.) EM SISTEMAS DE FERMENTAÇÃO *IN VITRO*

JOÃO PESSOA
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Orientador: Prof. Dr. Evandro Leite de Souza

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RESUMO

A microbiota intestinal exerce um papel importante sobre aspectos fisiológicos e metabólicos do hospedeiro, sofrendo influência, sobretudo, dos componentes dietéticos. Alimentos prebióticos fornecem substratos necessários para garantir o equilíbrio e manutenção de microrganismos benéficos encontrados no trato gastrointestinal de seres humanos. A batata-doce (*Ipomoea batatas* L.) é um alimento de elevado valor nutritivo e, devido à quantidade de diferentes carboidratos, particularmente fibras e amido, pode apresentar propriedades prebióticas. O objetivo desse estudo é avaliar o potencial prebiótico de farinhas obtidas de diferentes cultivares de raízes de batata-doce em sistemas de fermentação *in vitro*. As farinhas foram elaboradas a partir das cultivares Rainha-branca (WSPRB), Campina-branca (WSPCB), Vitória (PSPV) e Lagoinha (PSPL), e caracterizadas quanto a sua composição nutricional e parâmetros físico-químicos. Cada farinha elaborada foi submetida ou não a uma digestão gastrointestinal *in vitro* a fim de avaliar seu potencial de utilização como fonte de carbono para o crescimento das cepas probióticas *Lactobacillus acidophilus* LA-05, *L. casei* L-26 e *Bifidobacterium animalis* subsp. *lactis* BB-12 utilizando ensaio de contagem de células viáveis, bem como avaliar as mudanças na microbiota fecal humana usando a técnica de fluorescência de hibridização *in situ* acoplada com citometria de fluxo multiparamétrica durante 48 horas de fermentação *in vitro*. Escores de atividade prebiótica e atividade metabólica bateriana (valores de pH, conteúdo de açúcares e ácidos orgânicos) foram determinados nos diferentes sistemas de cultivo. Os resultados foram expressos como a média ± desvio padrão e submetidos ao teste *t* de Student ou análise de variância (ANOVA), seguida pelo teste de Tukey usando $p \leq 0,05$. Os resultados obtidos para WSPRB, WSPCB, PSPV e PSPL apresentaram altas contagens de células viáveis de cepas probióticas e induziu mudanças desejáveis nos grupos bacterianos formadores da microbiota intestinal, como aumento abundância relativa das populações de *Lactobacillus–Enterococcus* e *Bifidobacterium*, e diminuição da abundância relativa de *Bacteroides–Prevotella*, *C. histolyticum* e *E. rectall–C. coccoides* durante a fermentação colônica. O aumento da atividade metabólica microbiana foi evidenciado por meio da diminuição do pH do meio, aumento do consumo de açúcares (frutose, glicose e maltose) e aumento da quantidade de diferentes ácidos orgânicos (fórmico, lático, málico, succínico e tartárico) e ácidos graxos de cadeia curta (acético, butírico e propiônico) ao longo do tempo. Esses achados demonstram intensa atividade metabólica bacteriana, indicando a capacidade de WSPRB, WSPCB, PSPV e PSPL de modular a composição e a atividade metabólica da microbiota intestinal humana, as quais devem estar ligadas a propriedades prebióticas. Escores de atividade prebiótica positivos foram encontrados em ambas fermentações *in vitro*, indicando desejável atividade seletiva fermentável pelas bactérias benéficas formadoras da microbiota intestinal em relação a competidores entéricos. Esses resultados mostram que farinhas de diferentes cultivares de batata-doce cultivadas no Nordeste do Brasil poderiam ser consideradas potenciais ingredientes prebióticos para serem usadas em preparações domésticas, bem como, formulações de alimentos funcionais ou suplementos dietéticos.

Palavras-chave: Alimentos funcionais. Inóculo fecal. Microbiota intestinal. Probióticos. Propriedades prebióticas. Tubérculos.

ABSTRACT

The intestinal microbiota plays an important role on the physiological and metabolic aspects of the host, being influenced, above all, by dietary components. Prebiotic foods provide necessary substrates to ensure the balance and maintenance of beneficial microorganisms found in the gastrointestinal tract of humans. Sweet potato (*Ipomoea batatas* L.) is a food of high nutritional value and, due to the amount of different carbohydrates, particularly fiber and starch, can have prebiotic properties. The aim of this study is to evaluate the prebiotic potential of flours obtained from different sweet potato root cultivars in *in vitro* fermentation systems. The flours were made from the cultivars Rainha-branca (WSPRB), Campina-branca (WSPCB), Vitória (PSPV) and Lagoinha (PSPL), and characterized in terms of their nutritional composition and physical-chemical parameters. Each elaborated flour was submitted or not to an *in vitro* gastrointestinal digestion in order to evaluate its potential use as a carbon source for the growth of the probiotic strains *Lactobacillus acidophilus* LA-05, *L. casei* L-26 and *Bifidobacterium animalis* subsp. *lactis* BB-12 using viable cell count assay, as well as assessing changes in the human fecal microbiota using the fluorescence *in situ* hybridization technique coupled with multiparametric flow cytometry during 48 hours of *in vitro* fermentation. Scores of prebiotic activity and bacterial metabolic activity (pH values, sugar content and organic acids) were determined in the different culture systems. The results were expressed as the mean ± standard deviation and submitted to Student's t test or analysis of variance (ANOVA), followed by the Tukey test using $p \leq 0.05$. The results obtained for WSPRB, WSPCB, PSPV and PSPL showed high viable cell counts of probiotic strains and induced desirable changes in the bacterial groups forming the intestinal microbiota, such as increase in the relative abundance of the *Lactobacillus–Enterococcus* and *Bifidobacterium* populations, and decrease in the relative abundance of *Bacteroides–Prevotella*, *C. histolyticum* and *E. rectall–C. coccoides* during colonic fermentation. Increase in microbial metabolic activity was evidenced by decreasing the pH of the medium, increasing the consumption of sugars (fructose, glucose and maltose) and increasing the amount of different organic acids (formic, lactic, malic, succinic and tartaric) and acids short-chain fatty acids (acetic, butyric and propionic) over time. These findings demonstrate intense bacterial metabolic activity, indicating the ability of WSPRB, WSPCB, PSPV and PSPL to modulate the composition and metabolic activity of the human intestinal microbiota, which must be linked to prebiotic properties. Positive prebiotic activity scores were found in both *in vitro* fermentations, indicating desirable selective fermentable activity by beneficial bacteria that form the intestinal microbiota in relation to enteric competitors. These results show that flours from different sweet potato root varieties grown in Northeastern Brazil could be considered potential prebiotic ingredients to be used in domestic preparations, as well as functional food formulations or dietary supplements.

Keywords: Fecal inoculum. Functional foods. Intestinal microbiota. Prebiotic properties. Probiotic. Tubers.

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Figure 1. Abundance of different bacterial groups as measured by fluorescence *in situ* hybridization (FISH) combined with multiparametric flow cytometry in an *in vitro* colonic fermentation system with digested flours from different sweet potato root varieties (WSPRB, WSPCB, PSPV and PSPL) and fructooligosaccharides (FOS), as well as in a negative control (no added fermentable substrate) inoculated with a human fecal inoculum, at time zero (baseline) and after 24 and 48 h of incubation (37 °C). Results are expressed as percentage (average ± standard deviation, n = 3) of hybridized cells (Lab 158 specific to *Lactobacillus – Enterococcus*, Bif 164 specific to *Bifidobacterium*, Bac 303 specific to *Bacteroides – Prevotella*, Chis 150 specific to *Clostridium histolyticum* and Erec 482 specific to *Eubacterium rectall – Clostridium coccoides*). WSPRB: flour from sweet potato root variety Rainha Branca (white peel); WSPCB: flour from sweet potato root variety Campina Branca (white peel); PSPV: flour from sweet potato root variety Vitória (purple peel); PSPL: flour from sweet potato root variety Lagoinha (purple peel). a - c: Different superscript small letters for a same cultivation media at different fermentation times denote difference ($p \leq 0.05$), based on Tukey's test. A - E: different superscript capital letters at the same fermentation time denote difference ($p \leq 0.05$) among treatments, based on Tukey's test. 113

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1 INTRODUÇÃO

O trato gastrointestinal humano representa um ecossistema complexo, onde os nutrientes disponíveis influenciam a diversidade da microbiota e suas atividades metabólicas, de modo que o cólon é reconhecido como o compartimento mais metabolicamente ativo no corpo humano (LABARTHE et al., 2019). Um crescente número de evidências sugere que a microbiota intestinal pode afetar vários processos fisiológicos e metabólicos no hospedeiro, como função de barreira e moduladora da resposta imunológica, vindo a atuar na prevenção de diversas doenças, além de impactar na susceptibilidade frente a agentes patogênicos intestinais (DESAI et al., 2016; LLEWELLYN et al., 2018; GHOLIZADEH et al., 2019).

Grande parte do impacto na composição da microbiota intestinal é induzido pela dieta e pelo consumo de alimentos específicos, tais como prebióticos, criando uma demanda de investigação sobre o efeito modulador de padrões dietéticos e de ingredientes que possam modificar a composição e atividade desta microbiota (TEIXEIRA et al., 2016; D'HOE et al., 2018). Os prebióticos são componentes alimentares que proporcionam fonte seletiva de carbono fermentável para o crescimento e/ou atividade metabólica de microrganismos benéficos no intestino, particularmente bactérias probióticas, conferindo um benefício de saúde ao hospedeiro associado com a modulação da microbiota (FAO, 2008; GIBSON et al., 2017). Probióticos são definidos como microrganismos vivos que, quando administrados em quantidades adequadas, conferem benefícios à saúde do hospedeiro (FAO, 2006).

Abordagens com foco no estudo da modulação da composição da microbiota intestinal têm utilizado culturas anaeróbias de origem fecal em ensaios de fermentação de substratos em condições colônicas experimentais, as quais simulam o ambiente encontrado no cólon distal humano (JUÁNIZ et al., 2017; CONTERNO et al., 2019). Os componentes finais do metabolismo microbiano de prebióticos são, principalmente, ácidos graxos de cadeia curta (AGCC), como ácido acético, propiônico e butírico, os quais são utilizados pelo organismo hospedeiro como fontes de energia, além de atuarem na inibição de patógenos entéricos e no aumento da biodisponibilidade de minerais (AMBALAM et al., 2015; WEI et al., 2018).

Nos últimos anos tem ocorrido crescente número de estudos na busca de novos ingredientes com potencial prebiótico (MORO et al., 2018; LAM et al., 2019; MASSA et al., 2020). A batata-doce (BD, *Ipomoea batatas* L.) é considerada um alimento de elevado valor nutritivo por apresentar uma variedade de vitaminas, aminoácidos, minerais, fibras dietéticas, ácidos fenólicos, antocianinas, tocoferol e betacaroteno, além de elevada quantidade de

carboidratos (TRANCOSO-REYES et al., 2016; CARTIER et al., 2017; KHANH et al., 2018). Assim, a BD é reconhecida mundialmente como uma das culturas alimentares mais importantes para o consumo humano (ESATBEYOGLU et al., 2017; ZHANG et al., 2018a).

A região Nordeste é responsável por aproximadamente 317 265 toneladas da produção nacional de BD, ocupando o primeiro lugar (IBGE, 2020). A alta capacidade de resistência a diferentes condições ambientais, de solo e de temperatura das culturas de BD permite satisfatório cultivo em diferentes regiões, facilitando o seu aproveitamento e utilização (MARCZAK; SAWICKA; SALACH, 2018; SANTOS et al., 2019). Diversas cultivares de BD podem ser encontradas, as quais variam de acordo com suas características físico-químicas, de coloração e valor nutricional (SHEKHAR et al., 2015; CARTIER et al., 2017).

Estudos recentes têm mostrado diversos benefícios associados ao consumo de BD, tais como efeito antioxidante, antitumorais, anti-inflamatório, antidiabético, antiobesidade, imunomodulador e hepatoprotetor (KIM et al., 2015; WANG; NIE; ZHU, 2016; JU et al., 2017; TANG et al., 2019; JIANG et al., 2020). Compostos bioativos presentes na BD, como fibras, compostos fenólicos e diferentes polissacarídeos têm demonstrado propriedades benéficas à saúde (LESTARI et al., 2013; ZHANG et al., 2016; TANG et al., 2018). O amido presente na BD, o qual apresenta diferentes propriedades físico-químicas, tem sido particularmente valorizado como ingrediente alimentar funcional (MENON; PADMAJA; SAJEEV, 2015; ZHENG et al., 2016; GUO et al., 2019a). Além disso, a farinha de BD tem sido empregada na formulação de diferentes alimentos, tais como pães, biscoitos e massas, em substituição parcial da farinha de trigo, com a finalidade de melhorar a qualidade nutricional dos produtos (PÉREZ et al., 2017; SALEH; LEE; OBEIDAT, 2018). Dessa forma, há um crescente interesse para o uso da BD, tanto por seu valor nutritivo e possíveis aplicações tecnológicas, quanto por suas potenciais atividades biológicas benéficas à saúde do consumidor.

Devido ao consumo crescente de BD nos últimos anos pela população e à sua elevada produção na região Nordeste do Brasil, pesquisas têm buscado agregar valor ao produto, impulsionando sua cultura, a qual se apresenta principalmente vinculada às atividades do agronegócio familiar (EMBRAPA, 2015; CAVALCANTI et al., 2019; SANTOS et al., 2019). A identificação de componentes com ação prebiótica em farinhas de BD, bem como dos seus efeitos quando utilizados em processos fermentativos sob condições que se aproximem daquelas encontradas no ambiente intestinal, ainda são escassos. Assim, o objetivo desse estudo foi avaliar o potencial prebiótico de farinhas obtidas de diferentes cultivares de raízes de BD em sistemas de fermentação *in vitro*.

2 REFERENCIAL TEÓRICO

2.1 MICROBIOTA INTESTINAL: DESENVOLVIMENTO E COMPOSIÇÃO

A microbiota intestinal é considerada um ecossistema complexo, composto por diferentes microrganismos, como bactérias, fungos e vírus (DERRIEN; ALVAREZ; de VOS, 2019). Esse ecossistema denso, dominado principalmente por bactérias, tem atraído interesse nos últimos anos pela descoberta das diferentes interações entre a microbiota e o seu hospedeiro e, com isso, a possível solução de diversos problemas fisiopatológicos por meio da modulação da microbiota intestinal (TILG et al., 2018).

Desde a formação fetal, diferentes fatores podem influenciar o desenvolvimento e composição da microbiota intestinal e nas suas relações com o sistema imunológico do hospedeiro (Figura 1). A simbiose da microbiota intestinal infantil é modulada durante os primeiros anos de vida e co-evolui com o hospedeiro e com sua programação metabólica e neurológica (GENSOLLEN et al., 2016). Durante esse período, os bebês crescem rapidamente, sendo também um período fundamental para o estabelecimento da microbiota intestinal, a qual influencia diretamente a maturação do sistema imunológico, absorção de nutrientes e metabolismo, e evita a colonização por patógenos (BELKAID; HAND, 2014). Diversos estudos destacam a natureza crucial do desenvolvimento dessa simbiose no início da vida para a saúde infantil e suas consequências ao longo dos anos (DOGRA et al., 2015; KORPELA; de VOS, 2016; BERNSTEIN et al., 2019).

A microbiota intestinal é relativamente estável e resiliente em adultos na ausência de estressores externos extremos (por exemplo, mudanças na dieta ou tratamento com antibióticos) (FAITH et al., 2013). Essa considerável resiliência permite retornar ao seu estado original quando um desafio cessa (PALLEJA et al., 2018). Nessa fase da vida, a microbiota possui mais de 100 trilhões de microrganismos, com a maior densidade presente no íleo terminal e intestino grosso, sendo caracterizada pela alta variabilidade interindividual (MAYNARD et al., 2012; CHENG et al., 2020). A microbiota do trato gastrointestinal contém mais de mil espécies, com aproximadamente 75% da composição bacteriana já elucidada, sendo composta por Firmicutes e Bacteroidetes como filos dominantes, além de Actinobacteria e Proteobacteria (D'ARGENIO; SALVATORE, 2015). Uma microbiota intestinal saudável é caracterizada pela predominância de bactérias anaeróbias obrigatórias dos filos Firmicutes e Bacteroidetes, que devem impedir a expansão da população de aeróbios facultativos e membros potencialmente patogênicos da família Enterobacteriaceae

pertencentes ao filo Proteobacteria (KRISS et al., 2018). Estimativas sugerem que o trato gastrointestinal contém cerca de 10 vezes mais bactérias do que células que compõem o corpo humano (ORIACH et al., 2016).

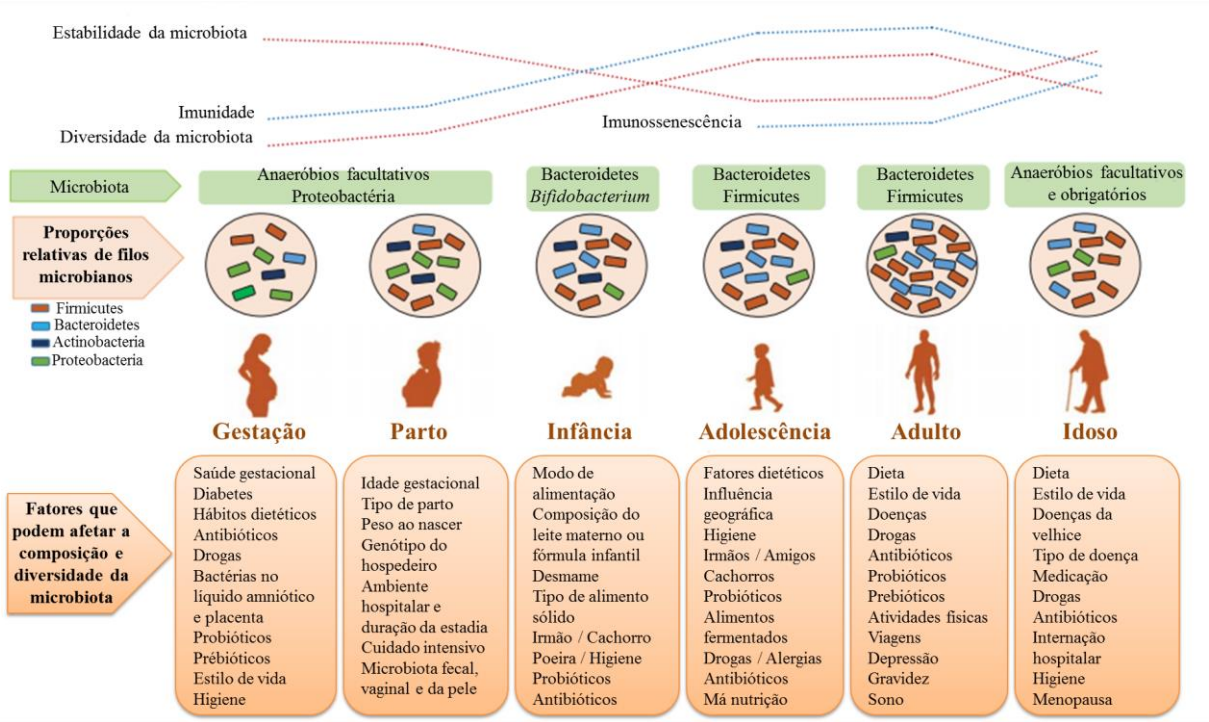

Figura 1. Fatores potenciais para afetar a composição e diversidade da microbiota intestinal e sua correlação com o sistema imunológico humano em diferentes estágios da vida.
Fonte: adaptado de Nagpal et al. (2018).

Descobertas recentes relacionadas à evolução da microbiota intestinal em idosos forneceram informações adicionais sobre as múltiplas trajetórias da microbiota durante o curso de uma vida humana (BIAGI et al., 2016; CLEMENTS; CARDING, 2018; NAGPAL et al., 2018). O comprometimento da saúde como um todo em indivíduos com idade avançada também tem sido associado à composição da microbiota intestinal inerente a esse estágio da vida (O'TOOLE; JEFFERY, 2018).

A comunidade microbiana intestinal co-existe por meio da composição de bactérias benéficas (aquelas que oferecem algum benefício à saúde do hospedeiro), bactérias comensais (aquelas que não demonstram malefício ou benefício óbvio) e bactérias patogênicas (aquelas que, em situação de desequilíbrio, causam danos à saúde do hospedeiro) (BÄUMLER; SPERANDIO, 2016). O desequilíbrio da microbiota intestinal é chamado de disbiose e tem sido associado com uma variedade de distúrbios relacionados ao estilo de vida humano, como obesidade, diabetes, alergias, dermatite atópica, doenças autoimunes e doença inflamatória intestinal (KRISS et al., 2018; GHOLIZADEH et al., 2019). O impacto da disbiose ainda

influencia o desenvolvimento de doenças cardiovasculares, câncer, gota (acúmulo de ácido úrico) e artrite, os quais estão associados à presença de metabólitos microbianos indesejáveis (DING et al., 2019).

Além das doenças sistêmicas, tem sido reportada forte relação entre cérebro e microbiota intestinal, chamado de eixo cérebro-intestino-microbiota, sendo responsável por atuar diretamente na saúde emocional do indivíduo, onde a disbiose pode ser manifestada por meio de sinais e sintomas como depressão, ansiedade, estresse, mau humor, perda de memória, medo e síndrome do pânico (ORIACH et al., 2016; DING et al., 2019). Em contrapartida, essas condições emocionais despertadas por outros gatilhos, também podem ocasionar disbiose, exercendo, assim, uma comunicação cérebro-intestino-microbiota de forma bidirecional (CRYAN; DINAN, 2012). Os mecanismos relacionados com a restauração dessas condições emocionais estão associados à produção de metabólitos microbianos benéficos, como ácido gama-aminobutírico (GABA), dopamina, noraepinefrina, acetilcolina, AGCC e 5-hidroxitriptofano. O triptofano é conhecido como precursor da serotonina, a qual tem aproximadamente 90% de sua produção no intestino (ORIACH et al., 2016; DANNESKIOLD-SAMSØE et al., 2019).

A disbiose pode envolver um aumento considerável de bactérias potencialmente patogênicas, bem como uma proporção reduzida e/ou uma diminuição da diversidade de espécies de bactérias benéficas (ELSON; ALEXANDER, 2015). Assim, existem várias maneiras de restaurar ou manter um equilíbrio favorável para o hospedeiro, tais como a ingestão de microrganismos benéficos vivos, a estimulação do crescimento seletivo de microrganismos intestinais nativos e/ou a aplicação combinada de ambas as estratégias. Esses três métodos, correspondem, respectivamente, ao consumo de probióticos, prebióticos e simbióticos (KIM; KEOGH; CLIFTON, 2018).

A Organização Mundial de Saúde define probióticos como sendo microrganismos vivos que, quando administrados em quantidades adequadas, conferem benefícios à saúde do hospedeiro (FAO/WHO, 2006). As espécies de *Lactobacillus* e *Bifidobacterium* são amplamente estudadas como microrganismos probióticos, e, frequentemente, estão envolvidas na avaliação de novos ingredientes prebióticos por serem os principais grupos microbianos com efeitos benéficos presentes no trato gastrointestinal de mamíferos (AMBALAM et al., 2015; SARKAR; MANDAL, 2016; ZHANG et al., 2018b). Esses microrganismos são eficazes na restauração da homeostase intestinal, modulam o sistema imunológico, melhoram a função de barreira intestinal, além de disponibilizarem nutrientes essenciais e energia para as células epiteliais do hospedeiro (KRISS et al., 2018).

Os principais mecanismos de ação de *Lactobacillus* e *Bifidobacterium* para promoção de benefícios à saúde do hospedeiro envolvem a produção de enzimas, produção de mucina, produção de ácidos orgânicos, diminuição do pH intestinal, exclusão competitiva, alimentação cruzada, produção de substâncias antimicrobianas e de metabólitos bioativos, além das interações diretas probiótico-hospedeiro mediada pela superfície celular intestinal (SANDERS et al., 2019; PEREDO-LOVILLO, ROMERO-LUNA; JIMÉNEZ-FERNÁNDEZ, 2020). As contagens de *Lactobacillus* e *Bifidobacterium* encontradas no trato gastrointestinal humano saudável variam de acordo com a idade do hospedeiro, da localização dentro do intestino, do tipo de dieta, do uso de antibióticos e dos hábitos de vida (ZHANG et al., 2018c).

O gênero *Lactobacillus*, pertencente taxonomicamente ao filo Firmicutes, classe Bacilli, ordem Lactobacillales e família Lactobacillaceae, é composto por mais de 170 espécies de bastonetes anaeróbios facultativos, catalase negativos, Gram-positivos e não formadores de esporos (KLEEREBEZEM; VAUGHAN, 2009). Apesar de serem encontrados em fezes adultas em aproximadamente 0,01 a 0,06% (10^5 a 10^8 UFC/g) de todas as espécies bacterianas, *Lactobacillus* exercem vários efeitos promotores da saúde quando submetidos a diferentes circunstâncias. Os benefícios para a saúde induzidos por essas bactérias são significativos em diversos campos e continuam a se expandir com conhecimento e avanços científicos (ZHANG et al., 2018c).

O gênero *Bifidobacterium,* incluído no filo e classe Actinobacteria, ordem Bifidobacteriales e família Bifidobacteriaceae, são Gram-positivos, estritamente anaeróbicos, e contém mais de 50 espécies e elevado número de subespécies (HIDALGO-CANTABRANA et al., 2017). *Bifidobacterium* são bactérias dominantes da microbiota intestinal em mamíferos, incluindo humanos desde a infância, representando, portanto, um importante padrão microbiano para investigar a modulação da microbiota intestinal sob variados aspectos (MILANI et al., 2017). Durante a vida adulta, a população de *Bifidobacterium* estabilizada representa de 3% a 6% da população microbiana total do intestino, enquanto nos idosos (> 65 anos) os números geralmente diminuem (O'CALLAGHAN; SINDEREN, 2016).

Embora se possa descrever a grande diversidade da microbiota intestinal humana, o papel da maioria das espécies bacterianas na saúde e na doença permanece amplamente desconhecido devido à complexidade desta microbiota, com uma infinidade de interações entre microrganismos e hospedeiro, microrganismos e fatores ambientais, e microrganismos entre si (TILG et al., 2018; SANDERS et al., 2019). Esses estudos abriram o caminho para o entendimento da variação da microbiota em indivíduos saudáveis e com diferentes doenças,

bem como do planejamento de intervenções baseadas em microrganismos ao longo da vida de um indivíduo. Portanto, o comportamento de espécies de bactérias benéficas formadoras da microbiota intestinal ou do seu ecossistema frente a diferentes fatores, a exemplo dos hábitos alimentares, e o seu impacto na homeostase do hospedeiro são temas de investigação de interesse atual.

2.2 COMPONENTES PREBIÓTICOS

Prebióticos são definidos como substratos seletivamente utilizados por microrganismos que formam a microbiota do hospedeiro, conferindo uma variedade de benefícios para a saúde. Essa definição expande o conceito de prebióticos para incluir outras substâncias além de carboidratos, outros sítios de aplicações no corpo além do trato gastrointestinal e diversas outras categorias além de alimentos (GIBSON et al., 2017). Porém, esses substratos ainda atuam principalmente no intestino grosso, estimulando a proliferação e a atividade da microbiota intestinal (MOHANTY et al., 2018).

Atualmente, a dieta é considerada um dos mais importantes moduladores da composição da microbiota intestinal, podendo ser responsável por aproximadamente 60% das alterações, enquanto os genes do hospedeiro são responsáveis por menos do que 12% dessas alterações (CLARK; MACH, 2016; NA ZHANG; JU; ZUO, 2018). A ingestão de carboidratos complexos, proteínas, fibras, polifenóis e prebióticos podem estar envolvidos nessa modulação de forma positiva, enquanto que gorduras, aditivos alimentares e outros componentes menores, incluindo contaminantes, podem estar envolvidos de forma negativa (ROCA-SAAVEDRA et al., 2018; DANNESKIOLD-SAMSØE et al., 2019). No entanto, o equilíbrio geral entre os macronutrientes proteínas, carboidratos e gorduras é determinante para influenciar a composição e o potencial funcional da microbiota intestinal, uma vez que uma dieta onívora tem ocasionado maior diversidade de bactérias quando comparada à dieta vegetariana (MADSEN et al., 2017). Dessa forma, a dieta representa o principal fator impulsionador de mudanças na relação funcional entre microbiota e hospedeiro (D'HOE et al., 2018; ASHWINI et al., 2019).

Diversos substratos têm sido amplamente estudados como possíveis prebióticos, incluindo desde ingredientes isolados a alimentos. Oligossacarídeos são as fibras dietéticas consideradas como a principal classe de prebióticos e os primeiros a serem reconhecidos comercialmente, a exemplo dos frutooligossacarídeos (FOS), galactooligossacarídeo (GOS) e inulina (AL-SHERAJI et al., 2013; GUO et al., 2019b). Esses oligossacarídeos são

relativamente produzidos com baixo custo e facilmente isolados de fontes naturais de alimentos (BAJURY et al., 2018).

Atualmente, a legislação brasileira reconhece apenas FOS e inulina como ingredientes prebióticos com a alegação de contribuírem para o equilíbrio da microbiota intestinal, desde que a recomendação de consumo diário do produto pronto para consumo forneça no mínimo 5 g do ingrediente prebiótico (BRASIL, 2016). Uma dose apropriada deve ser suficiente para gerar um efeito prebiótico, mas não muito alta para induzir efeitos indesejáveis ou adversos, como formação excessiva de gás ou utilização não seletiva. A dose "adequada" irá variar dependendo do ecossistema microbiano e dos efeitos metabólicos associados (GIBSON et al., 2017). A maioria dos prebióticos requer uma dose oral de mais de 3 g por dia para provocar efeitos (ROBERFROID et al., 2010; WANG et al., 2020). Produtos que contenham doses inferiores a este nível não devem ser chamados de prebióticos, a menos que tal dose tenha sido comprovada para provocar efeitos seletivos sobre a microbiota e aspectos de saúde concomitantes (GIBSON et al., 2017).

Além de oligossacarídeos, outras fibras dietéticas, como pectina e beta-glucanas, também têm sido estudadas como prebióticos (ZHANG et al., 2018b; LAM et al., 2019). O amido resistente presente em raízes e tubérculos tem sido amplamente valorizado como ingrediente prebiótico por apresentar diversos benefícios à saúde (MENON; PADMAJA; SAJEEV, 2015). Compostos fenólicos têm demonstrado efeitos promissores na modulação da microbiota intestinal e atualmente têm sido aceitos como prebióticos em potencial (GIBSON et al., 2017; OWOLABI et al., 2020). Assim, a ocorrência natural de prebióticos pode ser encontrada em vários alimentos, estando sua ingestão associada com hábitos alimentares saudáveis (BRASIL, 2016; DUARTE et al., 2017; MORO et al., 2018).

Nos últimos anos, pesquisas têm demandado empenho em avaliar o impacto de alimentos vegetais integrais na composição de espécies microbianas do intestino, o que não apenas aumentará as possíveis fontes de prebióticos, mas também reduzirá resíduos industriais para produção de alimentos mais sustentáveis (JUÁNIZ et al., 2017; PARKAR et al., 2018; GONG et al., 2019). Para avaliar o potencial prebiótico de um substrato é necessário inicialmente realizar a caracterização físico-química completa desse componente, seguido da caracterização funcional, padronização da formulação do produto cujo substrato prebiótico estará presente, se for o caso, além de garantir aspectos de segurança (FAO, 2008).

As principais características funcionais dos prebióticos à nível intestinal são a capacidade de resistir à acidez gástrica e não sofrer hidrólise ou absorção no intestino delgado, alcançando o cólon como um substrato seletivamente fermentável pela microbiota

intestinal benéfica (GIBSON et al., 2017; ASHWINI et al., 2019). Os microrganismos são capazes de biotransformar componentes alimentares para exercer efeitos benéficos no hospedeiro e obter energia por meio do processo de fermentação (SENGHOR et al., 2018). As estruturas e comprimentos de cadeia de prebióticos (incluindo candidatos a prebióticos) são determinantes críticos da fermentabilidade, uma vez que o tipo de ligação glicosídica e a composição do monômero podem afetar a resistência dos prebióticos à hidrólise no intestino delgado, além de causar diferença na fermentação seletiva pela microbiota no intestino grosso (FERREIRA-LAZARTE et al., 2019).

Prebióticos têm a habilidade de melhorar a sobrevivência, crescimento e metabolismo de microrganismos benéficos, permitindo que estes desenvolvam melhor as suas propriedades promotoras de saúde no sistema digestivo (MOHANTY et al., 2018). Alguns mecanismos de ação propostos para os prebióticos estão apresentados na Figura 2.

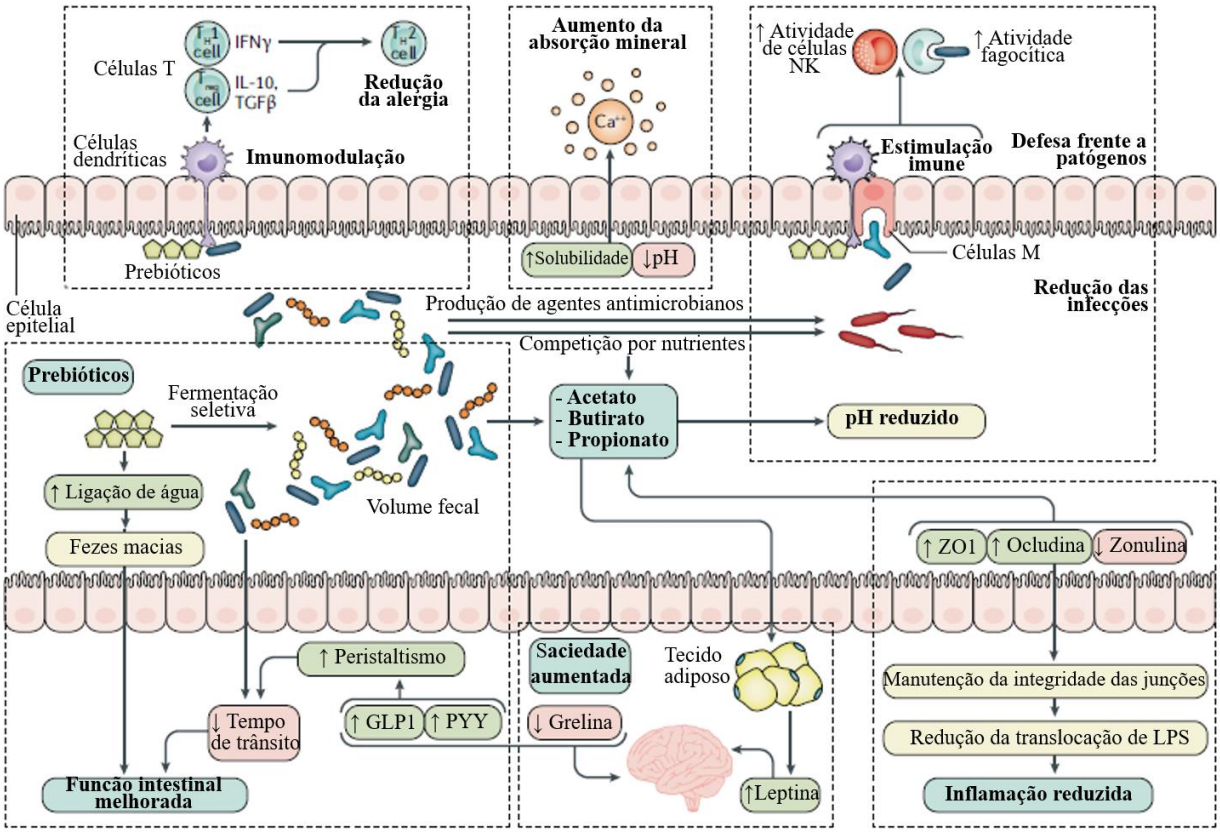

Figura 2. Mecanismos de ação de prebióticos.
Célula M: célula microfenestradas; Célula NK: célula *natural killer*; Célula TH1: célula T auxiliar tipo 1; Célula TH2: célula T auxiliar tipo 2; Célula Treg: célula T reguladora; GLP1: peptídeo semelhante a glucagon 1; LPS: Lipopolissacarídeo; PYY: peptídeo YY; TGFβ: fator de transformação do crescimento β; ZO1: zonula ocludente 1.
Fonte: adaptado de Sanders et al. (2019).

A fermentação seletiva de componentes prebióticos resulta no aumento da produção de AGCC, como acetato, butirato e propionato, os quais atuam na redução do pH, redução de infecções, na integridade epitelial prevenindo o câncer de cólon, na regulação lipídica e na redução da inflamação (WU et al., 2017; WANG et al., 2020). A fermentação de ingredientes prebióticos ainda causam efeitos significativos no sistema imunológico, o qual é influenciado pelo aumento da biomassa fecal e dos componentes da parede celular das bactérias, causando efeito imunomodulatório e de defesa contra patógenos (WEI et al., 2018; ASHWINI et al., 2019). Além de modificar a abundância ou a atividade dos microrganismos, a presença de prebióticos favorece o funcionamento do intestino pela sua capacidade umectante, o que reduz os efeitos de gastroenterites agudas, aumenta a saciedade pela regulação hormonal e melhora a absorção mineral pelo aumento da solubilidade (AL-SHERAJI et al., 2013; MOHANTY et al., 2018; SANDERS et al., 2019).

Embora diversos efeitos benéficos pelo consumo de prebióticos sejam conhecidos, deve-se observar que cada candidato a ingrediente prebiótico tem características e comportamentos diferentes que devem ser validados para avaliar corretamente sua funcionalidade (HURTADO-ROMERO et al., 2020). A fim de confirmar a seletividade de um candidato a uso como prebiótico, métodos *in vitro* e *in vivo* tem sido propostos com o intuito de monitorar com precisão as alterações na microbiota fecal durante a suplementação com o componente de estudo (BAJURY et al., 2018; CONTERNO et al., 2019). Dessa forma, a busca por novos ingredientes prebióticos tem sido uma alternativa promissora para a área de alimentos funcionais e suplementos dietéticos.

2.3 BATATA-DOCE: CULTIVO E CARACTERÍSTICAS NUTRICIONAIS

A BD é um dos mais importantes alimentos cultivados no mundo, com sua produção alcançando aproximadamente 100 milhões de toneladas por ano, tendo a China como principal produtor. Os Estados Unidos e o Brasil têm representatividade na produção de BD no continente americano, ocupando o primeiro e o segundo lugar, respectivamente (FAOSTAT, 2019). A produção de BD no Brasil tem aumentado ao longo dos anos, sendo responsável por produzir 805 412 toneladas de BD em 2019, com a região Nordeste entregando cerca de 40% dessa produção, seguido pela região Sul com 31%, Sudeste com 27%, Centro-Oeste com 1% e Norte com 1% (IBGE, 2020). A produção regional de BD no Brasil nos últimos anos está apresentada na Figura 3.

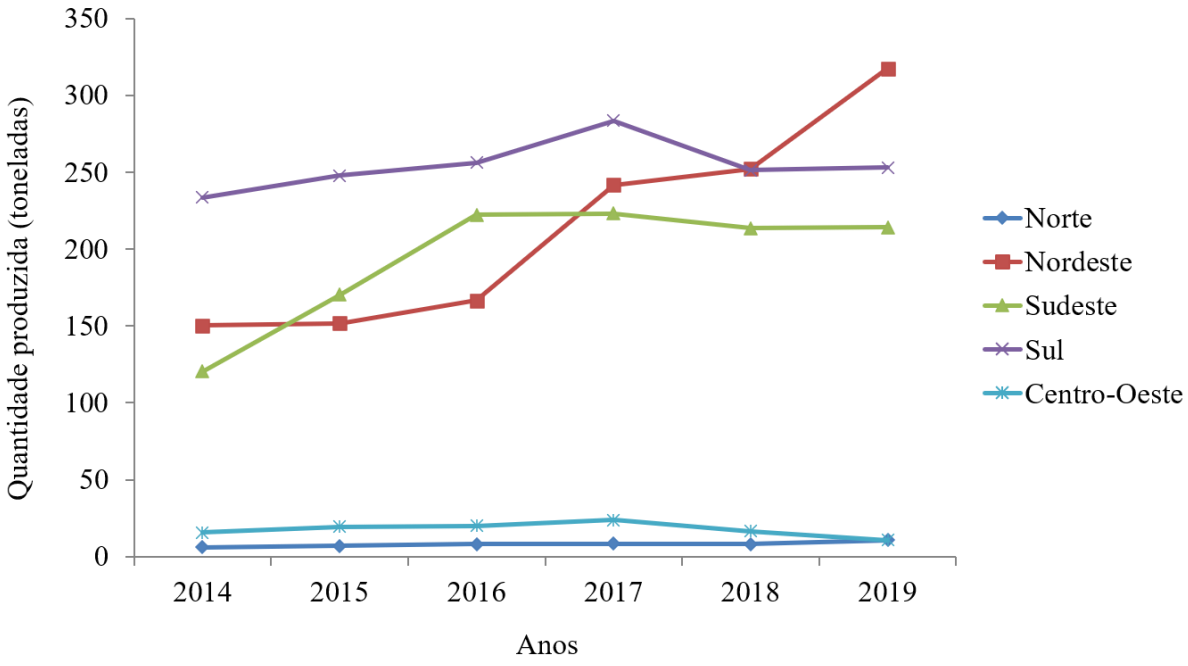

Figura 3. Produção regional de batata-doce no Brasil de 2014 a 2019 (IBGE, 2020).

No Brasil, a BD é uma planta de grande importância socioeconômica, contribuindo para a fixação do homem no campo por seu cultivo estar principalmente associado à subsistência dos produtores (CAVALCANTI et al., 2019). No entanto, o excedente produzido é geralmente comercializado em mercados locais ou exportado para Estados não produtores, podendo-se obter um significativo retorno financeiro, o qual resulta na geração de emprego e renda e, consequentemente, no impulsionamento do agronegócio familiar (MELO, 2009; EMBRAPA, 2012).

O plantio de BD no Brasil ocupa uma área de aproximadamente 57 486 hectares, sendo caracterizada pela facilidade de cultivo adaptada aos sistemas de baixo nível tecnológico, baixo custo de produção, com um ciclo de 90 a 120 dias e ampla utilização em diversas áreas. Aproximadamente 50% da produção total de BD é voltada para a alimentação humana, enquanto aproximadamente 40% para a alimentação animal, embora possa ser utilizada também para fins industriais, o que propicia fluxo regular de capital (EMBRAPA, 2012; IBGE, 2020).

Devido à sua rusticidade, a BD apresenta raízes tuberosas com grande resistência a pragas, sendo pouco exigente quanto à fertilidade do solo e com fácil adaptação climática (MARCZAK; SAWICKA; SALACH, 2018). A capacidade da BD para se adaptar aos solos de baixa fertilidade permite a conversão eficiente de energia solar (que é abundante nos climas trópicos) em carboidratos, sem competir com outras culturas que demandam uma

quantidade maior de nutrientes do solo. Além disso, o uso eficiente da água presente no solo permite a exploração de BD em zonas de estação seca prolongada, como algumas regiões do Norte e Nordeste do Brasil e em outros continentes, como a África (EMBRAPA, 2015; SANTOS et al., 2019).

A parte folhosa da BD possui caule herbáceo, sendo de hábito de crescimento rasteiro, com ramificações que podem variar em tamanho, cor e recortes (Figura 4). Essa parte aérea também é constituída por uma vegetação agressiva, com grande massa foliar, a qual forma boa cobertura do solo e compete vantajosamente com plantas invasoras (SHEKHAR et al., 2015). As folhas de BD apresentam uma variedade de aminoácidos essenciais, vitaminas e minerais, podendo ser preparadas como qualquer outra hortaliça folhosa para uso na alimentação, embora seu consumo ainda seja pouco difundido no Brasil (FU et al., 2016; SU et al., 2019).

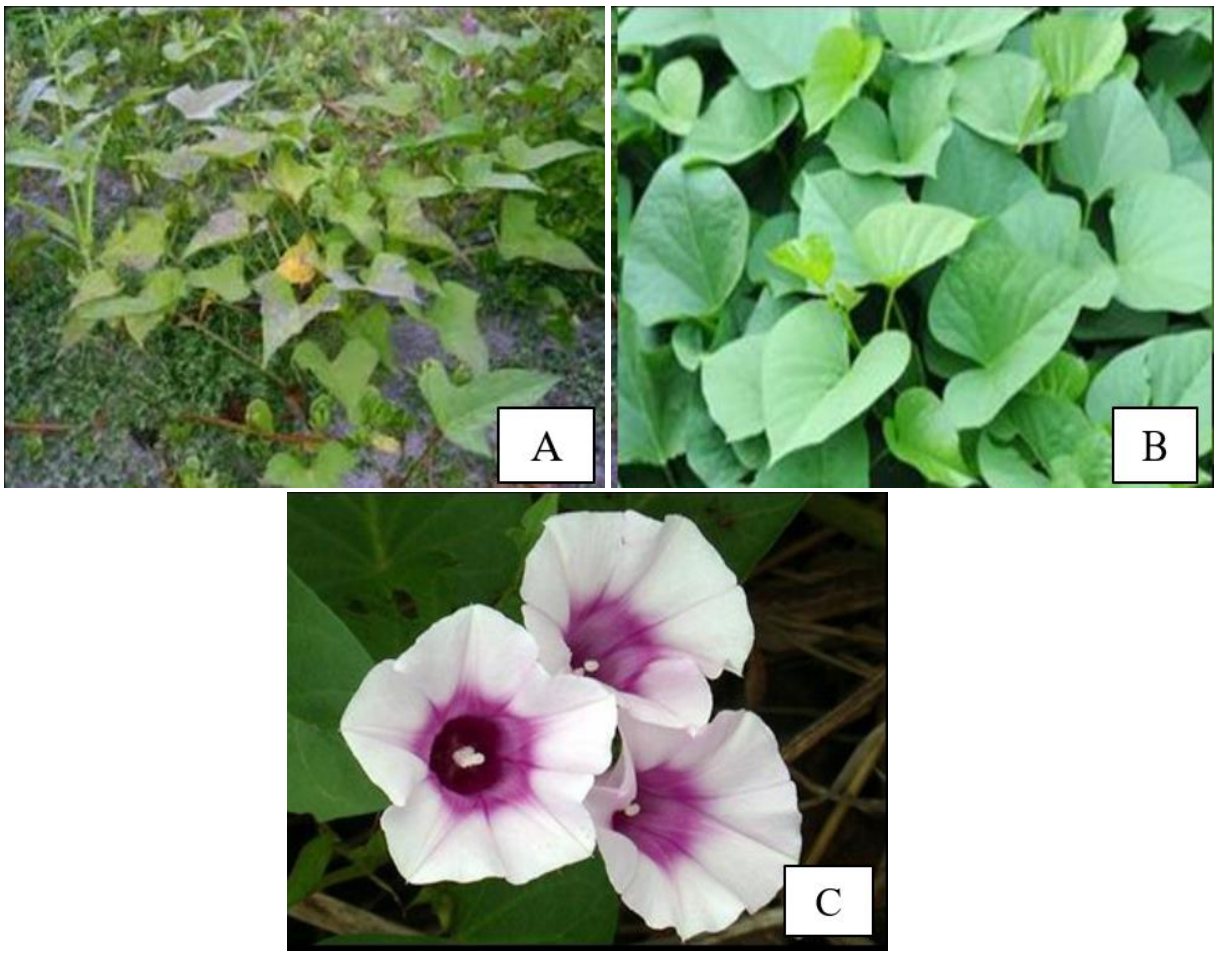

Figura 4. Plantação rasteira (A), tipo de folhagem (B) e floração (C) da batata-doce (*Ipomoea batatas* L.).
Fonte: Salasar et al. (2019).

Um elevado número de variedades de BD tem sido cultivado em diferentes lugares, a exemplo do relato de cultivo de mais de 2000 variedades na China (ZHANG et al., 2018a). O

Brasil também apresenta uma grande diversidade genética de BD, sendo mantidas por pequenos agricultores, comunidades indígenas e, até mesmo, em hortas domésticas. Normalmente, os municípios brasileiros possuem cultivares locais, podendo ser usualmente encontrado nomes diferentes para a mesma cultivar, como também, o mesmo nome para diferentes cultivares (EMBRAPA, 2015; AMARO et al., 2017). A propagação desta planta pode ser feita por meio de mudas, estacas, sementes botânicas e cultura de tecidos. Porém, as ramas constituem o meio de propagação mais recomendado para cultivo comercial por ser mais econômico e manter as características genéticas do vegetal (EMBRAPA, 2005).

Além da cor da casca, as cultivares de BD podem diferir também na cor da polpa, proporcionando as diferentes características físico-químicas encontradas, o que pode fornecer efeitos benéficos distintos à saúde do consumidor (CARTIER et al., 2017). A composição nutricional e efeitos benéficos associados à BD e seus subprodutos tem demonstrado ser cultivar-dependente (JU; MU; SUN, 2017). As cultivares de BD de casca branca Rainha-branca e Campina-branca, e de casca roxa Vitória e Lagoinha são comumente encontradas no Nordeste do Brasil (GUIMARÃES et al., 2018; CAVALCANTI et al., 2019).

A tuberização é um processo complexo que envolve várias vias metabólicas, levando ao acúmulo maciço de amido, proteínas e outros componentes. Os tubérculos, os quais são órgãos de armazenamento, agem como um reservatório e competem pelos fotoassimilados disponíveis (SHEKHAR et al., 2015). Raízes de BD são consideradas de elevado valor energético, fornecendo aproximadamente 86 kcal/100 g, tendo o amido como principal componente, sendo que o seu conteúdo na raiz varia de 50 a 80% entre diferentes variedades (MOHANRAJ; SIVASANKAR, 2014; ZHANG et al., 2018a). Ao longo do armazenamento, ocorre atividade enzimática (α-amilase) de degradação desse amido, o que diminui sua concentração no tubérculo e aumenta o conteúdo de sólidos solúveis devido ao aumento de açúcares simples (SANCHEZ et al., 2020). A composição de carboidratos da BD, encontrados principalmente na forma de fibras e amido, possui baixo índice glicêmico, e seu consumo pode minimizar o risco de doenças como diabetes e obesidade, além de controlar o apetite e atuar na saúde intestinal como um todo (MOSER et al., 2018).

Baixos teores de proteínas (< 5%) e lipídios (< 3%), além de diferentes vitaminas e minerais também compõem as raízes de BD (MOHANRAJ; SIVASANKAR, 2014; JU; MU; SUN, 2017). As variações no teor de proteínas em BD foram atribuídas a sua carga genética e nicho ambiental durante a produção, onde o aumento da biossíntese de proteínas é uma consequência do aumento da taxa de fotossíntese, a qual é eventualmente um fator crucial para maior rendimento (AGRAWAL et al., 2013; SHEKHAR et al., 2015). As principais

vitaminas encontradas em raízes de BD são as vitaminas A, C, E e do complexo B (IKANONE; OYEKAN, 2014; MOHANRAJ; SIVASANKAR et al., 2014).

A composição centesimal de BD orgânicas e convencionais não apresentaram diferenças significativas, embora as cultivares orgânicas ofereçam concentrações mais elevadas de minerais (SANTOS et al., 2019). Raízes de BD cultivadas no Nordeste do Brasil apresentaram maior valor nutricional em relação aos macrominerais magnésio, sódio e potássio do que a cenoura, quiabo e repolho, e em fósforo, magnésio e sódio do que a berinjela. Considerando os microminerais, tem sido relatado que a BD apresenta maiores teores de cobre e manganês do que a cenoura, e teores semelhantes de ferro que o repolho (SANTOS et al., 2019), bem como maior conteúdo de cálcio do que qualquer outro tubérculo e maior conteúdo mineral do que a batata comum (IKANONE; OYEKAN, 2014). Além disso, a presença de diferentes compostos bioativos em raízes de BD a tornam um alimento saudável e seguro para todas as idades (ALBUQUERQUE; SAMPAIO; SOUZA, 2019).

Dessa forma, nos últimos anos, raízes de BD têm se apresentado como um alimento promissor para realização de estudos de investigação sobre diferentes formas de utilização e de possíveis efeitos benéficos relacionados ao seu consumo devido, principalmente, seu cultivo ser produzido de forma sustentável e ser destacável fonte de nutrientes essenciais à saúde.

2.4 BATATA-DOCE COMO ALTERNATIVA PARA MELHORAR A QUALIDADE NUTRICIONAL DE PRODUTOS

Atualmente, a indústria de alimentos enfrenta o desafio de atender à demanda dos consumidores por alimentos que proporcionam benefícios adicionais à saúde e, ao mesmo tempo, atendam às necessidades nutricionais (ZOU et al., 2018). Algumas das razões para este aumento na demanda incluem a influência dos estilos de vida modernos, o aumento dos custos dos cuidados de saúde devido à maior expectativa de vida e o interesse natural das pessoas idosas em aumentar sua qualidade de vida (GONZÁLEZ-HERRERA et al., 2015).

Nesse contexto surge a BD, que pode ser consumida de várias formas, entre elas, coccionadas por meio de ebulição ou fritura e desidratadas, os quais representam os principais métodos utilizados em todo o mundo (MOSER et al., 2018; SHEN et al., 2018), embora *chips* de BD sem óleo tenha sido uma tendência de consumo (MONTEIRO et al., 2020). A utilização da BD logo após a sua colheita é essencial para minimizar as mudanças fitoquímicas associadas ao armazenamento (GRACE et al., 2014). A BD não é apenas uma fonte rica de energia, mas também pode gerar produtos derivados, tal como a farinha

(MUSSOLINE; WILKIE, 2017; PEREIRA et al., 2019). Farinha pode ser definida como o produto obtido de partes comestíveis de uma ou mais espécies de cereais, leguminosas, frutos, sementes, tubérculos e rizomas por meio da moagem e/ou outros processos tecnológicos considerados seguros para produção de alimentos (BRASIL, 2005).

Além da variedade, diversos outros fatores podem influenciar as características físico-químicas e nutricionais durante a elaboração das farinhas de BD, como o descascamento, pré-tratamento e método de secagem (AHMED; AKTER; EUN, 2010; OLATUNDE et al., 2016). A produção de farinha de BD oferece algumas facilidades, como armazenamento mais longo e estabilidade ao transporte, redução do volume e das perdas devido à perecibilidade da BD *in natura*, aumento da vida de prateleira e aumento do valor nutritivo devido ao fato de carboidratos, proteínas, gorduras e minerais estarem concentrados nos produtos alimentícios secos (KAMAL; ISLAM; AZIZ, 2013). Farinhas de BD encapsuladas podem ser uma alternativa de consumo por apresentarem maior conteúdo fenólico total, capacidade antioxidante e índice de solubilidade em água do que a farinha não encapsulada (AHMED et al., 2010).

A farinha de BD possui propriedades essenciais para agregar valor na formulação de diferentes produtos, além de serem utilizadas em substituição parcial da farinha de trigo (PÉREZ et al., 2017; SALEH; LEE; OBEIDAT, 2018). A elaboração de pães contendo 5, 10 ou 15% de farinha de BD, em substituição a farinha de trigo, mostrou que a cor, sabor, textura, aroma e aceitabilidade geral foram igualmente aceitáveis em relação ao pão controle (KAMAL; ISLAM; AZIZ, 2013). Produtos de panificação sem glúten têm sido uma inovação para atender aos consumidores com essa exigência, buscando manter ou melhorar a qualidade daqueles elaborados com farinha de trigo. Para isso, farinha de arroz foi utilizada como alternativa, porém o pão, também sem aditivos, apresentou-se com firmeza indesejável, a qual pôde ser reduzida substituindo parcialmente a farinha de arroz pela farinha de BD com alta atividade de β-amilase (AOKI, 2018).

A maltose é um dos principais açúcares predominantes na BD cozida (MEI; MU; HAN, 2010; DINCER et al., 2011). O teor de maltose foi correlacionado com a doçura da BD cozida e com a hidrólise do amido (WARAMBOI et al., 2011; LAURIE et al., 2013). A produção de biscoitos com farinha de BD, principalmente do tipo *cookies*, tem atraído interesse. A mistura de 40% de farinha de BD e 60% de farinha de trigo ou farinha de milho foi a mais adequada para a formulação de biscoitos compostos com melhores qualidades físicas, nutricionais e sensoriais dentro dos padrões aceitos (ADEYEYE; AKINGBALA, 2016; JEMZIYA; MAHENDRAN, 2017). Além disso, a produção bem-sucedida de biscoitos

com farinha de BD não é recomendada apenas pela substituição parcial ou total de farinha de trigo, mas devido a sua doçura natural, a qual possibilita reduzir satisfatoriamente até 30% da quantidade de açúcar na elaboração dos biscoitos (SILVA et al., 2017).

Dessa forma, produtos à base de farinha de BD também têm alcançado o público infantil. A formulação de bolo contendo 40% de farinha de BD de polpa alaranjada teve alta aceitabilidade por escolares. Ainda, uma porção deste bolo (60 g) pode contribuir com até 22% das necessidades diárias de vitamina A (RANGEL et al., 2011). Atualmente, outras alternativas e produtos utilizando BD têm sido desenvolvidos por países com altos índices de crianças com deficiência de vitamina A (LAURIE; FABER; CLAASEN, 2018).

Amidos de BD comercialmente processados (MENON; PADMAJA; SAJEEV, 2015) e bioprodutos (EL SHEIKHA; RAY, 2017) também foram avaliados. A compreensão das características do amido de BD, o qual é o seu componente majoritário, é fundamental na formulação de novos produtos contendo essa matriz (XIANG et al., 2018). Resíduos de BD originados da extração do amido também podem ser aproveitados na forma de farinhas e apresentam elevado valor nutricional e benefícios à saúde (JU; MU; SUN, 2017). Macarrão desenvolvido com o uso da mistura de farinha de trigo com farinha de BD apresentou características sensoriais de firmeza e aparência superiores quando comparados com macarrão preparado com farinha de colocasia e castanha-d'água (YADAV et al., 2014).

A farinha de BD foi adicionada de fontes de fibra alimentar, como farelo de aveia, farelo de trigo e farelo de arroz, com o objetivo de aumentar o valor funcional da massa. O aumento do conteúdo de fibra de 10 para 20% diminuiu o índice de intumescimento e perda de cozimento, resultante possivelmente do elevado conteúdo de proteína na massa fortificada a 20%. A digestibilidade lenta da massa de BD contendo fibras, juntamente com o alto nível de amido residual não digerido, torna essas massas alimentícias recomendáveis para consumo por pessoas com diabetes e obesidade (KRISHNAN et al., 2012).

A exploração de propriedades particulares de algumas bactérias láticas em um bioprocessamento ofereceu oportunidades para desenvolver alimentos fermentados novos ou melhorados a partir de matérias-primas amiláceas, como novas bebidas funcionais sem glúten baseadas em culturas tropicais amiláceas (HAYDERSAH et al., 2012). Extrato herbal rico em fenólicos composto de mate verde (*Ilex paraguariensis*), cravo (*Syzygium aromaticum*) e capim cidreira (*Cymbopogon citratus*) foram adicionados a leites fermentados com ou sem polpa de BD. Após avaliação dos atributos sensoriais, leites fermentados adicionados de polpa de BD tiveram a melhor aceitação sensorial, mostrando que extratos herbais e polpa de BD podem ser usados para desenvolver novos produtos lácteos com potenciais propriedades

funcionais (RAMOS et al., 2017). A elaboração de um iogurte probiótico de Moringa adicionado de BD teve boa aceitação sensorial, podendo ser comparável ao iogurte probiótico sem BD, o que pode oferecer benefícios adicionais à saúde devido aos nutrientes adicionados, dando origem a um produto simbiótico (KUIKMAN; O'CONNOR et al., 2015).

O suco de BD funcional também pode ser uma alternativa para ampliar a variedade de produtos alimentícios derivados desse vegetal (GRACE et al., 2015). Néctar de BD roxa foi desenvolvido com sabor agradável, fornecendo possibilidade de uso para a comercialização de bebidas derivadas de BD. A aplicação de temperatura ultra alta (140 °C por 10 segundos) não alterou o pH, acidez titulável, viscosidade, distribuição granulométrica, conteúdo de açúcares redutores, fenólicos totais e capacidade antioxidante na bebida, embora tenha reduzido o grau de escurecimento e conteúdo de ácido ascórbico e antocianinas totais (ZOU et al., 2018).

A cor representa característica fundamental quando os consumidores avaliam a qualidade de alimentos. Corantes naturais e antioxidantes presentes em BD roxa e vermelha podem ser usados para o desenvolvimento de alimentos funcionais (OKE; WORKNEH, 2013). A extração comercial de pigmentos de BD pode ser um desafio devido à textura firme e alta conteúdo da enzima polifenoloxidase; entretanto, métodos para melhorar a extração de antocianinas de BD têm sido estudados (CIPRIANO et al., 2015). As antocianinas de BD roxa possuem grupos glicosil aromáticos acilados e exibem tolerância ao pH ácido e termoestabilidade relativamente alta (KIM et al., 2012). A estabilidade das antocianinas depende da temperatura, pH e matriz solvente (JIE et al., 2013). Antocianinas de BD apresentaram boa estabilidade em pH 2 – 6, enquanto a cor do extrato da BD manteve-se estável durante 30 dias de armazenamento a 20 °C no escuro. Tanto a exposição a luz ultra violeta quanto a fluorescente enfraqueceram a estabilidade da cor do extrato de BD roxa, embora a exposição a luz ultra violeta mostrou um efeito mais drástico (HE et al., 2015).

Em contraste com seus homólogos sintéticos, corantes alimentares naturais exibem estabilidade substancialmente diminuída durante o processamento e vida de prateleira. No entanto, métodos para modular a cor de antocianinas de BD roxa pela adição de ácidos fenólicos e extratos vegetais fenólicos de grau alimentício têm apresentado resultados satisfatórios em relação a estabilização, melhoramento e modulação da cor resultante (FERNÁNDEZ-LÓPEZ et al., 2013; GRAS et al., 2017). A BD roxa apresentou melhor estabilidade em comparação com os pigmentos de morango, repolho roxo, perilla e outras plantas (ZHANG et al., 2009). Ácido acético, ácido málico ou outros ácidos orgânicos produzidos durante a fermentação da BD também podem aumentar a estabilidade das

antocianinas (WU et al., 2012). Antocianinas mono-aciladas e cianidinas predominantes em BD de polpa roxa P40 mostraram-se estáveis a tratamentos térmicos (XU et al., 2015). A BD roxa já foi utilizada como corante alimentar natural com altas concentrações de antocianinas aciladas, nos quais nenhum efeito tóxico foi observado (CIPRIANO et al., 2015).

As diferentes formas de utilização da BD e seu potencial tecnológico, além de sua importância nutricional e benefícios à saúde, permitem que esse tubérculo seja considerado uma alternativa promissora para melhorar a qualidade nutricional, sensorial e tecnológica de diferentes produtos alimentícios. Estudos envolvendo a investigação de farinha de BD como substrato prebiótico ainda são escassos ou inexistentes, embora componentes isolados da BD, tais como fibras (LESTARI et al., 2013), antocianinas (ZHANG et al., 2016), amido resistente (ZHENG et al., 2016), polissacarídeos (TANG et al., 2019) e oligossacarídeos (GUO et al., 2019b), já tenham demonstrado efeitos prebióticos.

2.5 ARTIGO DE REVISÃO DE LITERATURA: “Sweet potato roots: Unrevealing an old food as a source of health promoting bioactive compounds – A review”

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Review

Sweet potato roots: Unrevealing an old food as a source of health promoting bioactive compounds – A review

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ABSTRACT

Background: Sweet potato (SP) roots play an old and important role in food culture worldwide, although only in recent years this food has been research focus as a source of bioactive compounds.

Scope and approach: This review provides an update and discusses available literature concerning bioactive compounds naturally present in SP roots and their potential beneficial effects on human health. Available literature reveals that, in addition to the valuable nutritional composition, SP roots contain a variety of constituents capable of promoting health and preventing lifestyle-related diseases. Functionalities of polyphenols, carotenoids, polysaccharides, dietary fiber and proteins/peptides commonly found in SP roots have been confirmed in *in vitro* and *in vivo* investigations, which include: antioxidant, prebiotic, anti-hypertensive and anti-proliferative effects. Several SP varieties can be found, including purple, orange, white and yellow-fleshed SP, which differ in amounts of nutrients and bioactive compounds, and, consequently, in their ability to exert beneficial effects to consumers.

Key findings and conclusions: SP roots should be considered a healthy food option for use in different domestic meal preparations as well as for use by food industry as a functional ingredient for formulation of added-value food products.

1. Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam.) is an old and important food crop in many countries, ranking the third most important crop in production value and the fifth in caloric contribution to human diet in the world (Faostat, 2015; Muñoz-Rodríguez et al., 2018). Sweet potato (SP) is originated from Latin America and nowadays is an important economic crop not only in Latin American countries but also in many Asian, especially in China and Japan, and African countries (Zhang et al., 2018). SP is cultivated worldwide in both tropical and subtropical climates, growing well during all the year, even in nitrogen poor soil, requiring minimal crop supervision. Full maturity of SP roots is overall achieved in a maximum period of four months and the time from harvest to consumption range from days to months depending on the crop variety (Ellong, Billard, & Adenet, 2014; Grace et al., 2014). SP is considered a food with high nutritional value because the presence of a variety of vitamins, amino acids and minerals, tocopherol and beta-carotene, in addition to be a rich source of carbohydrates (Dako, Retta, & Desse, 2016; Dong, Mu, & Sun, 2017).

SP roots contain simple fermentable sugars (e.g., glucose, fructose and sucrose) and dietary fibers and minimal amounts of proteins and lipids (Marques et al., 2014). Compared to the ordinary potatoes, SP roots have a much better nutritional composition, presenting overall energy value and vitamin C levels at least two-fold higher than the formers (Ellong et al., 2014). Artificial selection as well as the occurrence of natural hybrids and mutations have resulted in the existence of a number of SP cultivars (Guo, Liu, Lian, Li, & Wu, 2014). Orange, yellow, purple and white SP varieties can differ not only on their skin or flesh color but also on their nutritional composition and profile of bioactive compounds. These compositional differences among SP varieties have been exploited in different studies, some of them approaching selected factors that could affect the development of these distinct characteristics (Andre et al., 2018; Shekhar, Mishra, Buragohain, Chakraborty, & Chakraborty, 2015; Wang et al., 2017a).

Recently, the occurrence of bioactive compounds in SP roots, such as phenolics, carotenoids, polysaccharides and peptides, have received an interest for human nutrition and renewed the attention of researchers and agro-food sector to this food (Ogutu & Mu, 2017; Tanaka, Ishiguro, Oki, & Okuno, 2017). These compounds are directly responsible for a variety of potential health promoting effects exerted by

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SP roots, to cite: antioxidant, anti-inflammatory, immunomodulatory, anticancer/antitumour, antimicrobial, antiulcer, antidiabetic, anti-obesity and hepatoprotective (Ayeleso, Ramachela, & Mukwevho, 2016; Hermes et al., 2013; Kim et al., 2015; Shen et al., 2018; Shin et al., 2013; Wang, Nie, & Zhu, 2016; Wu et al., 2015).

This review provides an update and discusses available literature concerning bioactive compounds naturally present in SP roots and their potential beneficial effects on human health.

2. Bioactive compounds in SP roots

2.1. Polyphenols

Polyphenols mainly include phenolic acids, flavonoids, stilbenes and lignans (Neveu et al., 2010). Results of epidemiological studies suggest an inverse relationship between consumption of polyphenol-rich foods and incidence of a variety of chronic diseases (Cooke, Steward, Gescher, & Marczylo, 2005; Kaur, Agarwal, & Agarwal, 2009). Recent interest in polyphenols and their antioxidant properties have prompted studies to exploit beneficial properties to human health in plant foods, including SP (Wang et al., 2018). Current available studies on the related health-promoting effects of polyphenols of different SP varieties are summarized in Table 1.

2.1.1. Phenolic acids

Curiously, varieties of SP with the same flesh color may differ in total phenolic content, individual phenolic acid profile and antioxidant activity (Andre et al., 2018; Koala et al., 2013). Among the different varieties, purple SP overall has greater commercial representativeness, being the most commercially SP exploited variety, mainly because of its high polyphenol content (Zhao, Yan, Xue, Zhang, & Zhang, 2014). Total phenolics, individual phenolic acids (e.g., chlorogenic, caffeic and di-caffeoylquinic) as well as total anthocyanins are highly concentrated in purple SP varieties compared to yellow, white, red or orange SP varieties (Grace et al., 2014; Ji, Zhang, Li, & Li, 2015). However, not only the intake levels but also their bioavailability is necessary to assess the biological significance of polyphenols on human health. Heat treatments widely used in SP roots can induce changes in their chemical composition, impacting on the concentration and bioavailability of polyphenols. This is an important aspect because part of the polyphenols present in SP roots are actually bind to fiber (bound form) and not in free form (Donado-Pestana, Salgado, Rios, Santos, & Jablonski, 2012).

Caffeoylquinic acid derivatives, including 5-caffeoylquinic acid (chlorogenic acid), 6-ocaffeoyl- β-D-fructofuranosyl-(2–1)-α-D-glucopyranoside, *trans*-4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid, represent the major types of polyphenols in purple SP roots; these compounds have shown to possess strong antioxidant and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities (Zhao et al., 2014). A phenolic acid derivative (4,5-di-O-caeffeoyldaucic acid) was firstly identified in an extract obtained from a specific SP variety collected in Peru. Antioxidant activities of 4,5-di-O-caeffeoyldaucic acid were found higher than those presented by a variety of well-known standard antioxidants when used at the same molar concentration (Dini, Tenore, & Dini, 2006).

A study for identification and quantification of polyphenols responsible for the antioxidant activity of SP roots with different flesh colors showed that purple-fleshed SP presented the highest caffeoylquinic acid content (749.7 μg/g) (Lebot, Michalet, & Legendre, 2016). Orange-fleshed SP varieties presenting the highest total polyphenol contents (2.43 mg GAE/g) also presented the highest total antioxidant activities (4.12 mg TE/g) (Koala et al., 2013). Caffeoylquinic acid-rich purple SP extract, with or without anthocyanin, imparted neuroprotection on $A\beta_{1-42}$-treated SH-SY5Y cells and contributed to the improvement of spatial learning and memory of senescence-accelerated prone mouse receiving 20 mg/kg/day of SP extract for 30 days. Tested SP extract also increased cell proliferation and intracellular ATP production, as well as inhibited intracellular ROS production (Sasaki, Han, Shimozono, Villareal, & Isoda, 2013).

Chlorogenic acid is the caffeoylquinic acid derivative present in the highest amounts in different SP varieties. Chlorogenic acid is capable of preventing hydroxyl radical formation, scavenging free radicals and avoiding oxidative activities, in addition to exert anti-mutagenic and anti-carcinogenic effects *in vitro* and *in vivo* (Feng et al., 2005). However, the iron chelating function of chlorogenic acid may also cause reduced bioaccessibility of iron from SP roots, which are considered moderate sources of dietary iron (approximately 8.5 μg/g) (Andre et al., 2018).

Amounts of polyphenols are commonly higher at the stem end of SP roots, being the chlorogenic acids 5-caffeoylquinic and 3,5-di-caffeoylquinic the most predominant (Jung, Lee, Kozukue, Levin, & Friedman, 2011). A study that evaluated the content of phenolic acids in three commercially important SP varieties grown in the United States found chlorogenic acid as the most predominant phenolic acid in SP roots, and 3,5-di-O-caffeoylquinic acid and/or 4,5-di-O-caffeoylquinic acids being predominant in SP leaves. Moreover, the cooking of SP roots did not increase the amounts of total phenolics and individual phenolic acids, as well as did not increase total phenolic content and antioxidant activity in purees made with whole or peeled SP roots (Truong, McFeeters, Thompson, Dean, & Shofran, 2007).

Fermentation of mashed SP roots by *Lactobacillus acidophilus* has shown to change SP roots composition. Amounts of caffeic acid or 3,5-dicaffeoylquinic acids increased four-fold in fermented mashed SP roots compared to raw or boiled SP roots. Two phenolics, namely p-coumaric acid and ferulic acid, not detected in raw or boiled SP roots were found in fermented SP roots. Additionally, SP roots fermented by *L. acidophilus* showed increased amounts of free antioxidant phenolics and enhanced cancer cell proliferation inhibitory activity (Shen et al., 2018).

2.1.2. Anthocyanins

Anthocyanins belong to a class of flavonoids synthesized via the phenylpropanoid pathway. Although a variety of flavonoids are present in SP roots (e.g., quercetin, myricetin, kaempferol and luteolin) (Cai et al., 2016; Park et al., 2016), anthocyanins are the main responsible for color ranging from pale pink to purple and deep blue in SP (Tanaka et al., 2017). Purple-fleshed SP roots typically present higher anthocyanin contents, whereas white-, yellow- or orange-fleshed SP roots contain little or no anthocyanin content (Grace et al., 2014; Ji et al., 2015). Contents of flavonoids in purple-fleshed SP roots (579.5 μg/g) ranked the highest, followed by orange-fleshed (127.12 μg/g) and white-fleshed SP roots (45.41 μg/g) (Park et al., 2016). Anthocyanin contents in SP roots have been reported to vary from around 32 to 1390 mg/100 g fresh weight (Xu et al., 2015).

Thirteen different anthocyanins were identified in purple-fleshed SP roots (He et al., 2016). The anthocyanins found in the highest amounts in purple-fleshed SP roots are 3,5-diglucoside derivatives from cyanidin and peonidin acylated with p-hydroxybenzoic acid, ferulic acid or caffeic acid, respectively (Esatbeyoglu, Rodríguez-Werner, Schlösser, Winterhalter, & Rimbach, 2017). Purple SP roots (namely, Majesty and Mackintosh varieties) already showed stronger antioxidant activity than other vegetables (e.g., red onion, purple asparagus and eggplant) because of their higher contents of anthocyanins (Li et al., 2012). Cyanidin-type anthocyanins have been closely associated with the radical scavenging activity against DPPH in purple-fleshed SP roots (Takahata, Kai, Tanaka, Nakayama, & Yoshinaga, 2011).

Concentrated anthocyanin extracts obtained from vegetables have gained attention for use as natural alternatives to synthetic red dyes and even to other natural colorant sources (Garzón, 2008). In addition to color contribution, anthocyanins of SP have shown a variety of health-promoting properties, such as reduction of reactive oxygen species, inflammatory response, proliferation of hepatic stellate cells, neuroprotective effects and improvements of visual acuity (Choi et al., 2011;

Table 1
Polyphenols, carotenoids and related health-promoting effects in different sweet potato varieties.

Reference	Origin	Variety	Color	Bioactive compounds	Health-promoting effects
				Polyphenols	
Koala et al. (2013)	Africa	TIB-440060, Caromex, BF82xCIP-13, BF82xCIP-18, BF82xTIB-4, F82xTIB-6, BF92xResisto-14, F92xCIP-6	Orange-fleshed	(1.062–2.432 mg/GAE dry weight)	Antioxidant activity
Phenolic acids					
Sasaki et al. (2013)	Japan	Ayamurasaki	Purple	5-caffeoylquinic acids: 3,4-, 4,5-, 3,5-dicaffeoylquinic acids (10.7–53.3 mg/100 g)	Neuroprotective effect
Grace et al. (2014)	United States of America	NC07-847, NCPUR06-020, Covington, Yellow Covington	Purple, orange, light yellow, yellow	Chlorogenic acid, caffeic acid, 3,4-, 4,5-, 3,5-dicaffeoylquinic acids (0.34–16.76 mg/g)	Antioxidant activity, anti-inflammatory capacity
Lebot et al. (2016)	Vanuatu	Baby, Salili, Bankis, Teouma	White, yellow, orange and purple flesh	Chlorogenic acid, 3,4-, 4,5-, 3,5-dicaffeoylquinic acids (0–7609.8 μg/g fresh weight)	Antioxidant activity
Esatbeyoglu et al. (2017)	China	n.s.	Purple	5-caffeoylquinic acids, 5-feruloylquinic acid and derivative, 3,4-, 4,5-, 3,5-dicaffeoylquinic acids (3.54–107 mg/100 g)	Enzyme inhibitory (α-amylase, α-glucosidase and xanthine oxidase), antioxidant activity, decreased lipid peroxidation in cultured hepatocytes
Anthocyanins					
Lim et al. (2013)	United States of America	P40	Purple-fleshed	Cyanidin-3-sophoroside-5-glucoside, peonidin-3-sophoroside-5-glucoside, cyandin-3-p-hydroxybenzoylsophoroside-5-glucoside, peonidin-3-p-hydroxybenzoylsophoroside-5-glucoside, peonidin-3-glucoside, cyandin-3-caffeoyl-p-hydroxybenzoylsophoroside-5-glucoside, pelargodinin-3-sophoroside-5-glucoside, cyanidin-3-(6''-feruloylsophoroside)-5-glucose, peonidin-3-caffeoyl-p-hydroxybenzoyl-sophoroside-5-glucoside, (7.5 mg P3GE/g dry weight)	Protection against colorectal cancer
Zhang et al. (2013)	China	n.s.	Purple	n.s.	Improvement of the fasting blood glucose level, glucose and insulin tolerance; increase of antioxidant enzymes activity
Wang et al. (2013)	China	Ayamurasaki	Purple-fleshed	n.s. (~0.4–2.75 ab/g)	Antioxidant properties
Zhao et al. (2014)	China	Ayamurasaki	Purple	Cyanidin-3-(6″,‴-dicaffeoylsoph)-5- glucoside, cyanidin -3-(6″-caffeoyl-6‴-feruloylsoph)-5- glucoside, peonidin-3-(6″,6‴-dicaffeoylsoph)-5- glucoside, peonidin-3-(6″-caffeoyl-6‴-p-hydroxybenzoylsoph)-5- glucoside, peonidin-3-(6″-caffeoyl-6‴-feruloylsoph)-5-glucoside	Antioxidant activity; radical scavenging activity
Grace et al. (2014)	United States of America	NC07-847; NCPUR06-020; Covington; Yellow Covington	Purple, orange, light yellow, yellow	Cyandin-3-caffeoyl-p-hydroxybenzoylsophoroside-5-glucoside, cyanidin 3-caffeoylsophoroside-5-glucoside; cyanidin 3-(600-caffeoyl-6000-feruloylsophoroside)-5-glucoside, peonidin 3-caffeoyl-p-hydroxybenzoylsophoroside-5-glucoside, (n.d. – 8.49 mg/g)	Antioxidant activity; anti-inflammatory capacity
Ji et al. (2015)	China	Jizi 01, Xi-nong 431, Beijing 553, Shangshu 19	Purple, red, yellow and white fleshed	n.s. (n.d. – 6.23 mg PN3GE/g)	Antioxidant activity
Sun et al. (2015a)	China	n.s.	Purple pigments	cyanidin acylglucosides and peonidin acylglucosides (> 85%)	Improvement of visual acuity; attenuation of vascular vessel deterioration; inhibition of endothelial senescence
Lebot et al. (2016)	Vanuatu	Baby,Salili, Bankis, Teouma	White, yellow, orange and purple flesh	Cyanidin-3-sophoroside-5-glucoside, peonidin-3-sophoroside-5-glucoside, canidin-3-(6''-p-hydroxybenzoylsophoroside)-5-glucoside, peonidin-3-(6''-p-hydroxybenzoylsophoroside)-5-glucoside, cyanidin-3-(6''-feruloylsophoroside)-5-glucoside, cyanidin-3-(6''-caffeoylsophoroside)-5-glucoside, cyanidin-3-(6''-caffeoyl-6‴-p-hydroxybenzoylsophoroside)-5-glucoside, peonidin-3-(6''-caffeoylsophoroside)-5-glucoside, cyanidin-3-(6''-caffeoyl-6''-feruloylsophoroside)-5-glucoside, peonidin-3-(6''-caffeoyl-6''-p-hydroxybenzoylsophoroside)-5-glucoside, peonidin-3-(6''-caffeoyl-6''-feruloylsophoroside)-5-glucoside, (0–13583 AU)	Antioxidant activity

(*continued on next page*)

Table 1 (*continued*)

Reference	Origin	Variety	Color	Bioactive compounds	Health-promoting effects
				Polyphenols	
Cai et al. (2016)	China	n.s.	Purple	Cyanidin-3-sophoroside-5-glucoside, peonidin-3-sophoroside-5-glucoside, cyanidin-3-p-hydroxybenzoylsophoroside-5-glucoside, peonidin-3-p-hydroxybenzoylsophoroside)-5-glucoside, cyanidin-3-feruloylsophoroside-5-glucoside, cyanidin-3-caffeoylsophoroside-5-glucoside, peonidin-3-p-feruloylsophoroside-5-glucoside, peonidin-3-p- caffeoylsophoroside-5-glucoside, cyanidin-3-caffeoyl-6-p-hydroxybenzoylsophoroside-5-glucoside, cyanidin-3-caffeoyl-feruloylsophoroside-5-glucoside, peonidin-3-caffeoyl-p-hydroxybenzoylsophoroside)-5-glucoside, peonidin-3-caffeoyl-feruloylsophoroside-5-glucoside, (mean 218–244 mg/100 g dry weight)	Antioxidant activity
Esatbeyoglu et al. (2017)	China	n.s.	Purple	Peonidin-3-soph-5- glucose, cyanidin-3-p-hydroxybenzoylsoph-5- glucose, cyanidin-3-(6‴caffeoylsoph)-5- glucose, peonidin-derivative, peonidin-3-p-hydroxybenzoylsoph-5- glucose, cyanidin-3-feruloylsoph-5- glucose, peonidin-3-feruloylsoph-5- glucose, cyanidin-3-(6‴caffeoylsoph)-5-glucose, cyanidin-3-(6‴,6‴-dicaffeoylsoph)-5- glucose, cyanidin-3-(6‴-caffeoyl-6‴-p-hydroxybenzoylsoph)-5- glucose, peonidin-3-(6‴caffeoylsoph)-5- glucose, peonidin-3-(6‴,6‴-dicaffeoylsoph)-5- glucose, peonidin-3-(6‴-caffeoyl-6‴-p-hydroxybenzoylsoph)-5- glucose, peonidin-3-(6‴-caffeoyl-6‴-feruloylsoph)-5- glucose, (2.4–68.4 mg/100 g)	Enzyme inhibitory, antioxidant activity
Wang et al. (2017b)	China	Eshu No. 8	Purple	Peonidin-3-O-(6-O-(E)-caffeoyl-2-O-β-D-glucopyranosyl-β-D-glucopyranoside)-5-O-β-D-glucoside, peonidin 3-O-(2-O-(6-O-(E)-caffeoyl-β-D-glucopyranosyl)-6-O-(E)-caffeoyl-β-Dglucopyranoside)-5-O-β-D-glucopyranoside, peonidin3-O-(2-O-(6-O-(E)-feruloyl-β-D-glucop yranosyl)-6-O-(E)-caffeoyl-β-D-glucopyranoside)-5-O-β-D-glucopyranoside, cyanidin 3-O-(6-O-p-coumaroyl)-β-D-glucopyranoside)	Improvement of metabolic parameters closely related to obesity, decrease in liver injury and attenuation of hepatic steatosis in high-fat-diet treated mice
Carotenoids					
Koala et al. (2013)	Africa	TIB-440060; Caromex; BF82xCIP-13; BF82xCIP-18; BF82xTIB-4; F82xTIB-6; BF92xResisto-14; F92xCIP-6	Orange-fleshed	n.s. (0.016–0.180 mg BCE/g)	Antioxidant activity
Grace et al. (2014)	United States	NC07-847; NCPUR06-020; Covington; Yellow Covington	Purple, orange, light yellow, yellow	β-caroteno (1–253 μg/g; total carotenoids 2–281.9 μg/g)	Antioxidant and anti-inflammatory activity

ns.: non specified; nd: not detected.

Lu et al., 2012; Sun et al., 2015a).

Purple SP pigments (named SP color), a class of naturally occurring anthocyanins, showed protective effect to hepatic insulin resistance via blocking oxidative-stress-mediated endoplasmic reticulum stress in the liver of high-fat-diet treated mice (700 mg/kg/day during 20 weeks). Additionally, purple SP pigments improved the fasting blood glucose level, glucose and insulin tolerance by suppressing the production of reactive oxygen species and restoring glutathione content and activity of antioxidant enzymes in mice (Zhang et al., 2013). Hypoglycemic effects of anthocyanins from purple SP roots were also associated with the inhibitory effects toward α-amylase and α-glucosidase activity, which may decrease blood glucose levels (Esatbeyoglu et al., 2017). Additionally, purple SP pigments (700 mg/kg/day for 20 weeks) were capable of improving metabolic parameters closely related to obesity, decreasing liver injury and attenuating hepatic steatosis in mice fed a high-fat-diet (Wang et al., 2017b).

Purple SP pigments (200 mg/kg for 4 weeks) were effective to attenuate domoic acid (a natural neurotoxin produced by marine algae) induced cognitive deficits in mice by promoting estrogen receptor-α-mediated mitochondrial biogenesis signaling. These results indicated that SP pigments could be possible candidates for the prevention and treatment of cognitive deficits in excitotoxic and other brain disorders (Lu et al., 2012). Purple SP pigments (700 mg/kg/day for 20 weeks) also attenuated the deterioration of vascular vessel and inhibited the endothelial senescence by blocking the NLRP3 inflammasome in pre-diabetic mice, revealing a potential target for the prevention of endothelial senescence-related cardiovascular diseases (Sun et al., 2015b).

Anthocyanin-enriched purple-fleshed SP clone P40 was investigated considering potential anticancer effects of their roots in both *in vitro* cell culture and *in vivo* animal model. In addition to a high level of total phenolics and antioxidant capacity, SP P40 roots possess a high content of anthocyanins (7.5 mg/g). Treatment of human colonic SW480 cancer cells with P40 roots anthocyanin extracts (0–40 μM of peonidin-3-glucoside equivalent) caused a dose-dependent decrease in colonic cell number due to cytostatic arrest of cell cycleat G1 phase. These results indicate the capacity of anthocyanin-enriched SP P40 of protecting against colorectal cancer by inducing cell-cycle arrest, antiproliferative and apoptotic mechanisms (Lim et al., 2013).

Polyphenols have also shown to exert effects similar to well-known prebiotic compounds (Souza, Albuquerque, Santos, Massa, & Alves, 2018). A prebiotic is a nonviable food component that confers a health benefit on the host associated with modulation of microbiota (FAO, 2008). During the digestion process, which involves pH changes, action of enzymes and microbial metabolism, anthocyanins are released from the food matrix and undergo extensive degradation that may affect their bioactivities. Digestion of anthocyanins present in purple-fleshed SP roots using a dynamic human gastrointestinal model to evaluate bioaccessibility and biotransformation found that the release of anthocyanins from this matrix is an accession dependent process. Microbial metabolism can account for anthocyanin degradation and differences in components present in roots of different SP varieties should affect anthocyanin release during gastrointestinal digestion, impacting their antioxidant capacities and prebiotic effects in the small intestine (Kubow et al., 2016).

Anthocyanins from purple SP roots (1% w/v) increased the population of *Bifidobacterium* spp. and *Lactobacillus/Enterococcus* spp. besides to decrease the population of *Bacteroides-Prevotella* spp. and *Clostridium histolyticum* during *in vitro* fermentation using faecal inocula. Total short-chain fatty acids (SCFAs) amounts in cultures were increased by purple SP anthocyanins. Furthermore, SP anthocyanins were partially converted to phenolic acids during fermentation, which commonly exert superior effect on intestinal microecology than parental dietary anthocyanins (Zhang, Yang, Wu, & Weng, 2016).

Peonidin-based anthocyanins extracted from roots of a Chinese purple SP variety increased the population of *Bifidobacterium bifidum*, *Bifidobacterium adolescentis*, *Bifidobacterium infantis* and *L. acidophilus in vitro*. Additionally, they decreased the population of *Staphylococcus aureus* and *Salmonella typhimurium*, indicating that tested anthocyanins might exert prebiotic-like activity through a positive modulation of intestinal microbiota (Sun, Zhang, Zhu, Lou, & He, 2018).

2.2. Carotenoids

There is strong evidence that the regular consumption of carotenoids can reduce the risk for lifestyle related diseases (Laurie, Faber, & Claasen, 2018). Current available studies on the related health-promoting effects of carotenoids of different SP varieties are summarized in Table 1. SP roots can be excellent source of carotenoids, although the content and profile of carotenoids in SP roots vary among different varieties. Contents of carotenoids are typically higher in peeled and lyophilized orange-fleshed SP roots (281.9 μg/g), followed by yellow-fleshed (~26.2 μg/g), light yellow-fleshed (~16.9 μg/g) and purple-fleshed SP roots (~2.0 μg/g), being β-carotene predominant in all of these varieties (Grace et al., 2014).

However, a study analyzing the contents of carotenoids in cooked roots of 11 SP varieties, including five orange-fleshed, two yellow-fleshed and four white-fleshed varieties, found values ranging from 0.4 to 72.5 μg/g fresh weight – recently harvested (Tomlins, Owori, Bechoff, Menya, & Westby, 2012). The application of a blanching pre-treatment to orange-fleshed SP roots during flour production, either with steam (78.21–134.19 μg/g of β-carotene) or microwaves (119.80–163.57 μg/g of β-carotene), did not affect the bioaccessibility of β-carotene after an *in vitro* digestion when compared to those non-blanched (137.49 μg/g of β-carotene) (Trancoso-Reyes et al., 2016).

Orange-fleshed SP is the less common SP variety in many countries, but it is one of the best sources of β-carotene in the nature, with superior ability to raise vitamin A blood levels (Donado-Pestana et al., 2012; Mohanraj & Sivasankar, 2014). SP cultivars in the eastern Caribbean Sea were characterized as rich sources of minerals and vitamins, with up to 63-fold more β-carotene than ordinary potatoes (Ellong et al., 2014). SP breeders have been engaged to develop new varieties of SP with deep-orange or yellow flesh, which present attractive color to use in the formulation of processed foods, in addition to contribute adding nutritional and functional value to these products because their high amounts of carotenoids (Tanaka et al., 2017).

Roots of orange-fleshed SP varieties have the potential to contribute ≥100% of the recommended dietary allowance of 4–8 years-old children for vitamin A, 27% for magnesium, 15% for zinc and 11% for iron. Orange-fleshed SP varieties were superior to cream-fleshed SP varieties in calcium and magnesium contents. *Trans*-β-carotene contents in roots of orange-fleshed and cream-fleshed SP vary over the geographical location, which could be ascribed to differences in soil mineral content and pH (Laurie, Jaarsveld; Faber, Philpott, & Labuschagne, 2012).

Six orange-fleshed SP varieties were developed in order to present superior β-carotene contents and be adapted to the agro-ecological characteristics of Burkina Faso (Africa), as a well-accepted dietary alternative to combat malnutrition, especially in mothers and their children. Total carotenoid contents in roots of developed SP varieties ranged from 0.016 to 0.18 mg of β-carotene equivalent (BCE)/g of dried weight, representing more than 11-fold increase in β-carotene contents when compared to other SP varieties early introduced in that country (Koala et al., 2013).

A study demonstrated that purees from orange fleshed SP roots may have prebiotic potential because modulated positively faecal microbiota of humans by increasing the population of *Bifidobacterium* spp., besides to stimulate the production of SCFAs, especially butyric acid, a well-known component favourable to gut health (Muchiri & McCartney, 2017).

Table 2
Carbohydrates in roots of different sweet potato varieties and related health-promoting effects.

Reference	Origin	Variety	Color	Bioactive compounds	Health-promoting effects
				Polysaccharides in general	
Wu et al. (2015)	China	NING No. 1	Purple	Polysaccharide composed by rhamnose, xylose, glucose and/or galactose	Antioxidant and antitumour activities
Guo et al. (2017)	China	n.s.	Purple	n.s.	Free radical scavenging activity and inhibition of albumin non-enzymatic glycosylation
Yuan et al. (2017)	China	Tongshan, Xuzhou, 90 Jiangsu Province	n.s.	Water soluble polysaccharide composed by rhamnose, glucose and galactose modified by selenylation (Se-SWP)	Antioxidant activity, inhibition of tumor growth and adjustment of immune factor levels
Tang et al. (2018a)	China	Xuzi Three	Purple	Water-soluble polysaccharide, diluted alkali-soluble polysaccharide and concentrated alkali-soluble polysaccharide	Immunomodulatory activity
Tang et al. (2018b)	China	Xuzi Three	Purple	Water-soluble polysaccharide, diluted alkali-soluble polysaccharide and concentrated alkali-soluble polysaccharide	Immunomodulatory activity and improvement of gut microbiota composition
Sun, Zhang, et al. (2018) and Sun, Zhou, et al. (2018)	China	Xushu No. 3	Purple	Water soluble and alkaline soluble polysaccharides composed by arabinose, glucose, galactose and rhamnose	Antioxidant activity, hepatoprotective effect, protective effects to acute liver damage
Dietary fiber					
Ji et al. (2015)	China	Jizi 01, Xinong 431, Beijing 553, Shangshu 19	Purple, red, yellow and white-fleshed	n.s. (1.85–2.35%)	Antioxidant activity
Ogutu and Mu (2017)	China	n.s.	n.s.	Pectin	Antioxidant activity of extracted pectin.

n.s.: non specified.

2.3. Carbohydrates

2.3.1. Polysaccharides in general

Recently, polysaccharides have attracted increasing attention because of their beneficial bioactivities and nontoxic side effects (Ghazala et al., 2015; Sheng et al., 2017). Current available studies on the related health-promoting effects of carbohydrates of different SP varieties are summarized in Table 2. Purple SP roots present around 5.5% of water-soluble polysaccharides (Qu, Wu, & Jia, 2014). Available literature concerning the characterization and biological activities of polysaccharides from SP roots is still scarce and most of the available studies have evaluated these aspects in polysaccharides from purple SP. A recent study found that polysaccharides extracted from purple SP comprised rhamnose, xylose, glucose and/or galactose residues (Wu et al., 2015).

A water soluble polysaccharide, composed by rhamnose, glucose and galactose, isolated from SP roots was modified by selenylation (a key constituent of Se-dependent enzymes) to develop a new derivative polysaccharide (named Se-SWP) using microwave synthesis. This Se-SWP showed strong antioxidant properties toward scavenging free radical DPPH and reducing activity. Se-WSP (100 mg/kg/day) was effective to inhibit tumor growth and adjust immune factor levels (e.g., IL-2, TNF-α and VEGF) in tumor-induced mice. Se-SWP also reduced the levels of malondialdehyde and increased the antioxidant activity in organs of streptozocin (STZ)-induced diabetic rats. These results indicate that selenylation should improve the biological activities and therapeutic potential of SP polysaccharides (Yuan et al., 2017).

Three different polysaccharides (being one water soluble polysaccharide and two alkaline soluble polysaccharides – 1 and 2) were isolated from purple SP roots and characterized as being β-type polysaccharides with different contents of uronic acid, proteins and polyphenols. The water soluble polysaccharide and alkaline soluble polysaccharide-1 were composed by arabinose, glucose and galactose, whereas alkaline soluble polysaccharide-2 was composed by arabinose, rhamnose and glucose. All the three polysaccharides exhibited moderate DPPH radical scavenging and reducing activity. The administration of these polysaccharides (100, 200 or 400 mg/kg/day for 30 days) to mice with CCl_4-induced hepatic injury decreased the levels of serum enzymes (alkaline phosphatase, alanine transaminase and aspartate transaminase) and hepatic lipid peroxidation, whereas increased the levels of hepatic antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic antioxidant (glutathione). Overall, water soluble polysaccharide displayed stronger antioxidant activity and hepatoprotective effect than alkaline soluble polysaccharides (Sun et al., 2018). These results reveal that polysaccharides from purple SP roots present potential antioxidant properties and protective effects to acute liver damage.

A study reported that polysaccharides extracted from purple SP roots using optimized ultrasonic conditions presented free radical scavenging activity and inhibited albumin non-enzymatic glycosylation (Guo, Kong, & Yan, 2017). Immunomodulatory effects of water-soluble polysaccharide, diluted alkali-soluble polysaccharide and concentrated alkali-soluble polysaccharide from purple SP roots were evaluated using lipopolysaccharide treated RAW264.7 macrophage cell models. Water-soluble polysaccharide, diluted alkali-soluble polysaccharide and concentrated alkali-soluble polysaccharide (100, 200 and 400 μg/mL) were shown to suppress abnormal phagocytosis, overproduction of TNF-alfa and IL-6 as well as abnormal apoptosis caused by lipopolysaccharide. The highest tested doses (200 and 400 μg/mL) of diluted alkali-soluble polysaccharide and concentrated alkali-soluble polysaccharide markedly reduced excessive nitric oxide production in macrophage cells. Additionally, 400 μg/mL of water-soluble polysaccharide, diluted alkali-soluble polysaccharide and concentrated alkali-soluble polysaccharide sharply decreased the expression of IL-1b stimulated by lipopolysaccharide as well as improved the expression of anti-inflammatory cytokine IL-10 (Tang et al., 2018a).

The effects of water-soluble polysaccharide, diluted alkali-soluble polysaccharide and concentrated alkali-soluble polysaccharide from SP roots (400 mg/kg for 30 days) were also studied in normal and cyclophosphamide (CTX) treated mice. Water soluble polysaccharide and concentrated alkali-soluble polysaccharide restored spleen index and immune cytokine (IL-2 and IL-6) levels in CTX-treated mice, indicating that these polysaccharides could enhance the levels of TNF-α, IL-2 and IL-6 and assist the immune response *in vivo*. These ppolysaccharides also increased the faecal populations of *Bacteroidetes*, Ruminococcaceae, Lachnospiraceae, *Ruminococcus* and *Oscillospira* besides to decrease the populations of *Firmicutes*, *Proteobacteria*, *Alcaligenaceae* and *Sutterella* in CTX-treated mice, as well as increased the intestinal amounts of acetic, propionic and butyric acid in healthy mice. Only water soluble polysaccharide increased the intestinal production of these acids in CTX-treated mice (Tang et al., 2018b). These findings indicate that SP polysaccharides could positively modulate host intestinal microbiota.

2.3.2. Dietary fiber

Dietary fiber (DF) is a collective term employed for a variety of plant constituents resistant to digestion by gastrointestinal enzymes (Theuwissen & Mensink, 2008). Plant cell wall is mainly composed by three major polysaccharides classes, namely cellulose, hemicellulose and pectin. These polysaccharides and lignin form the plant DF components (Salvador, Suganuma, Kitahara, Tanoue, & Ichiki, 2000).

DF content in SP roots is variable and depends on genetic characteristics and growing conditions of crops (Wang et al., 2016). DF extracted from residues obtained after starch extraction in ten different SP varieties using a sieving method presented an average DF content of 75.19% in dry matter. Most prevalent polysaccharides in SP agro-industrial residues were cellulose > lignin > pectin > hemicellulose (Mei, Mu, & Han, 2010). Another study found DF contents in the range of 1.85–2.35% in freeze-dried SP roots with different colors using an enzymatic gravimetric extraction method (Ji et al., 2015).

Pectin is a heteropolysacharide found in plant cell wall. Pectin extracted from SP roots presented a good antioxidant activity, which was enhanced after applying a sonication treatment that decreased the molecular weight of SP pectin fragments. Ultrasonic application was cited as a useful treatment to cause pectin depolymerization and enhance its antioxidant properties (Ogutu & Mu, 2017). SP pectin commonly presents low degree of methyl esterification, being rich in galactose and arabinose, with technological properties similar to apple pectin (Takamine et al., 2007).

The prebiotic components fructooligosaccharides (FOS), inulin and raffinose were found in the composition of Bestak SP variety from Indonesian origin. Bestak SP fiber extract presented prebiotic activity score similar to FOS but higher than inulin toward *Lactobacillus plantarum* Mut7 and *Bifidobacterium longum* JCM 1217, indicating that this extract could act as a prebiotic component (Lestari, Soesatyo, Iravati, & Harmayani, 2013).

2.3.3. Starch

Starch is the component found in the highest amounts in SP roots, presenting distinct physicochemical characteristics and valued functional properties. Starch content in roots of SP varieties commonly varies from 50 to 80% (Abegunde, Mu, Chen, & Deng, 2013; Zhang et al., 2018; Zhu & Wang, 2014).

Starch is composed by two polymers, namely amylose and amylopectin, which can be only evidenced after solubilization and separation of starch granules. Most important properties influencing starch nutritional value include the rate of digestion along the gastrointestinal tract and the metabolism of absorbed monomers (Abegunde et al., 2013). Rapidly digestible starch can be hydrolyzed to glucose within 20 min after initiation of treatment with amylase and other enzymes, while slowly digestible starch is hydrolyzed to glucose within 20–120 min. Resistant starch (RS) is digested after 120 min, being typically fermented by colonic microbiota (Keenan et al., 2015). RS ranged from 13.2 to 17.2% in flours from roots of five different SP cultivars commonly available in Sri Lanka (Senanayake, Ranaweera, Gunaratne, & Bamunuarachchi, 2013). Consumption of RS has been associated with improvements in insulin resistance, reduced accumulation of adipose tissue and decreased risk for metabolic diseases (Bindels, Walter, & Ramer-Tait, 2015; Harazaki, Inoue, Imai, Mochizuki, & Goda, 2014). However, available literature regarding the characterization of RS from SP roots is still limited.

Starch of SP commonly presents higher rates of amylose to amylopectin when compared to ordinary potatoes. Amylose has shown capable of raising blood sugar levels slower than simple sugars, being recommended as a healthy food choice even for patients with diabetes (Mohanraj & Sivasankar, 2014). Reported average amounts of amylose and amylopectin in SP starches used in Chinese starch industry was of 22 and 78%, respectively (Abegunde et al., 2013).

The digestibility of starch in SP root flours typically increases when the starch particle size is reduced by milling (Hung, My, & Phi, 2014). Studies evaluating the optimum size and particle size distribution for SP starch digestibility could help to improve the processing of SP roots as well as increase their use to formulate specialized products with proven nutritional and health benefits (Chen & Sopade, 2013). Alternative procedures for obtaining RS from SP roots have been a topic of interest to researchers and food industry (Hung et al., 2014; Xia, Li, & Gao, 2016).

Resistant starch can reach the large intestine where is fermented by colonic bacteria, being also considered a prebiotic (Zaman & Sarbini, 2016). Fermentation of resistant starch typically causes production of SCFAs and reduces pH in the proximal large intestine (Keenan et al., 2015). A study evaluated the efficacy of heat-moisture treatment (HMT) and enzyme debranching combined with heat-moisture treatment (EHMT) to improve the technological and prebiotic properties of resistant starch from purple SP roots. Modified resistant starch from both HMT and EHMT treated SP roots was capable of increasing the population of *Bifidobacterium* spp. during fermentation, in addition to decrease the pH of cultivation media due to the accumulation of metabolites. These results indicate the use of HMT and EHMT-treated resistant starch from purple SP roots as a strategy to produce prebiotic ingredients (Zheng et al., 2016).

The ability of different lactic acid bacteria to hydrolyze starch of SP roots has been also investigated. The amylolytic lactic acid bacteria *L. plantarum* A6 and *Lactobacillus fermentum* Ogi E1 were capable of altering the consistency of thick sticky gelatinized SP starch slurries (semiliquid) to liquid products. These changes besides to the production of maltooligosaccharides confirmed SP starch hydrolysis by tested lactic acid bacteria. Amounts of rapidly digestible starch and resistant starch in SP roots was decreased and increased after fermentation by tested lactic acid bacteria, respectively (Haydersah et al., 2012). Overall, the presence of anthocyanins, phenolics acid, oligosaccharides, polysaccharides and resistant starch in SP roots can exert important role on the composition and function of gut microbiota, characterizing this food as an important source of prebiotic ingredients.

2.4. Proteins

Despite of the small protein fraction commonly presents in SP roots (1.61–3.98%), these components may exert multiple biological functions (Dong et al., 2017). Current available studies on the related health-promoting effects of proteins and peptides of different SP varieties are summarized in Table 3. Sporamin is the major storage protein in SP, being account for around 80% of the total protein in SP roots (Senthilkumar & Yeh, 2012). Several peptides from SP roots have already been isolated and characterized (Zhang & Mu, 2017). Proteins from SP roots are typically rich in threonine, valine, tryptophan and aromatic amino acids, but are deficient in lysine (Sun, Mu, Zhang, & Arogundade, 2012).

Table 3
Proteins and peptides in roots of different sweet potato varieties and related health-promoting effects.

Reference	Origin	Variety	Color	Bioactive compounds	Health-promoting effects
				Proteins	
Li et al. (2013)	China	n.s.	n.s.	n.s. (2–40 μmol/L)	Anticancer effects
Huang et al. (2014)		Tainong 57	n.s.	MT-I and MT-II	Antioxidative activity, Fe2 + -chelating ability and protection of calf thymus DNA
Zhang and Mu (2016)	China	Mixuan No. 1	n.s.	Protein hydrolysates	Antioxidant activity
Zhang and Mu (2018)	China	Mixuan No. 1	n.s.	Protein hydrolysates	Antiproliferative effects on HT-29 colon cancer cells
Peptides					
Huang et al. (2014)		Tainong 57	n.s.	MSSGCK, CGSDCK, LTLEGSSEK, ATEGGHACK, CGNGCGGCK	Antioxidative activity
Ishiguro et al. (2016)	Japan	Shiroyutaka	n.s.	n.s. (0.5% or 5%, w/w)	Decreased blood levels of triglyceride, cholesterol and leptin
Zhang and Mu (2017)	China	Shangshu 19	n.s.	HDSASGQY, YYMVSA, HDSESGQY, YYIVS, RYYDPL	Antioxidant activity

n.s.: non specified.

A peptide from SP roots (100 and 500 mg/kg) was orally administered to spontaneously hypertensive rats, being effective to decrease systolic blood pressure 4 or 8 h after administration. Diastolic blood pressure was also decreased in SP peptide-treated rats. In this study, a peptide with strong angiotensin I-converting enzyme inhibitory activity was purified from SP roots. These results indicate that peptides from SP roots may be effective to prevent or treat hypertension (Ishiguro et al., 2012).

Peptides from SP roots were also evaluated for their impacts on the lipid metabolism in mice. Body and liver weight as well as epididymal and mesenteric fat were lowered in mice fed a high-fat diet containing 0.5 or 5% SP roots peptides during 28 days. Mice fed high-fat diet containing SP roots peptides also presented decreased blood levels of triglycerides and cholesterol (VLDL and LDL) and leptin. SP roots peptides were suggested to suppress lipogenesis in mice by increasing adiponectin levels and decreasing TNF-α levels in adipocytes (Ishiguro, Kurata, Shimada, Sameshima, & Kume, 2016).

A study showed that free radical-scavenging activity of proteins from SP roots and their hydrolysates were affected by single or combined enzymatic treatments. Hydrolysates presented higher radical-scavenging activity than whole SP roots proteins. Hydrolysates obtained from SP roots proteins through a single enzyme treatment presented higher radical-scavenging activity than those obtained through a treatment with combined enzymes, which presented higher degree of hydrolysis than the former. These results indicate that moderate hydrolysis with single enzymes could increase antioxidant properties of SP roots proteins (Zhang, Mu, Wang, & Sun, 2012).

A simulated *in vitro* gastrointestinal digestion modified molecular weight, increased fractions concentration and retained antioxidant activity of hydrolysates from SP roots proteins, revealing that this treatment could be a strategy to obtain antioxidant peptides from SP roots (Zhang & Mu, 2016). Hydrolysates from SP roots proteins obtained after treatment with six proteases showed antiproliferative effects on HT-29 colon cancer cells. Compared with other five proteases, hydrolysates obtained after treatment with alcalase exhibited the highest antiproliferation effects; particularly, small fractions (< 3 kDa) showed stronger antiproliferation effects than larger fractions (Zhang & Mu, 2018).

Defensin protein from SP Tainong 57 roots decreased the production of intracellular peroxide in HepG2 cells in *ex vivo* experiments. Four peptides derived from this defensin through tryptic hydrolysis simulation, namely GFR, GPCSR, CFCTKPC and MCESASSK, were tested for their antioxidative activity. CFCTKPC showed the best trolox equivalent antioxidant capacity and the highest DPPH radical scavenging activity and protection against lipid peroxidation; the presence of cysteine was considered a key factor for remarkable antioxidative effects of CFCTKPC (Huang et al., 2012). Similar results were found with two recombinant proteins of MT-I and MT-II from SP Tainong 57 roots, which exhibited strong trolox equivalent antioxidative capacity, DPPH radical scavenging activity, Fe2 + -chelating ability and protection of calf thymus DNA against hydroxyl radical-induced damage. Peptides obtained after hydrolysis of MT-I and MT-II by tripsin also presented strong DPPH radical scavenging activity; the presence of cysteine residue in tested peptides was also considered positive for their antiradical activities (Huang, Deng, Chen, Lin, & Huang, 2014).

SP roots protein (2 μmol/L per kg/day administered intraperitoneally or 80 μmol/L per kg/day administered intragastrically; for 9 days) exerted anticancer effects on human colorectal cancer cells. Proteins purified from SP roots inhibited the proliferation of human colorectal cancer SW480 cells *in vitro* in a dose-dependent manner. SP protein (2 μmol/L per kg/d administered intraperitoneally or 120 μmol/L per kg/d administered intragastrically, for 25 days) also decreased the spontaneous formation of pulmonary metastatic nodule in murine Lewis lung carcinoma 3LL cells subcutaneously transplanted in C57 BL/6 mice (Li, Mu, & Deng, 2013).

3. Conclusion

Considering the large production in different countries, the consume all over the world and the available literature reporting their high nutritional value and presence of a variety of bioactive compounds, SP roots are indeed a good food choice with great potential to be exploited by agro-food sector. Roots of different SP varieties possess a range of compounds (e.g., polyphenols, vitamins, proteins and polysaccharides) capable of exerting a key role in health promotion and prevention of diseases related to human nutrition and life-style. However, the nutritional composition and presence and amounts of bioactive compounds in SP roots are variety-dependent. Finally, SP roots should be considered a healthy food option for use by consumers in different domestic meal preparations as well as for use by food industry as an ingredient for formulation of added-value functional food products.

Conflicts of interest

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

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3 MATERIAL E MÉTODOS

3.1 AQUISIÇÃO DAS AMOSTRAS DE DIFERENTES CULTIVARES DE BATATA-DOCE

Para o desenvolvimento desse estudo, foram utilizadas quatro cultivares de raízes de BD (Figura 5), sendo duas de casca branca: Rainha-branca (WSPRB) e Campina-branca (WSPCB); e duas de casca roxa: Vitória (PSPV) e Lagoinha (PSPL). As amostras de raízes de BD foram cedidas pela Estação Experimental de Itapirema (07º38’24.1’’ latitude e 34º57’22.0’’ longitude), pertencente ao Instituto Agronômico de Pernambuco, Goiana – PE, Brasil. As batatas-doces foram transportadas logo após a colheita para o Laboratório de Microbiologia e Bioquímica de Alimentos (LMBA), pertencente ao Departamento de Nutrição (DN), Centro de Ciências da Saúde (CCS), Universidade Federal da Paraíba (UFPB).

Figura 5. Cultivares de batata-doce (*Ipomoea batatas* L.) Rainha-branca (A), Campina-branca (B), Vitória (C) e Lagoinha (D) produzidas no Nordeste do Brasil.
Fonte: Elaborada pelo autor.

3.2 DELINEAMENTO EXPERIMENTAL

A partir das quatro cultivares de BD foram elaboradas suas respectivas farinhas e realizada a determinação da composição nutricional e parâmetros físico-quimicos, seguido por uma digestão gastrointestinal *in vitro*. As farinhas de BD foram testadas em ensaios de fermentação com microrganismos probióticos, sendo contadas em placas as células viáveis. Em outro momento, as farinhas foram testadas em ensaios de fermentação com inóculo fecal humano, com enumeração de grupos bacterianos utilizando a técnica de fluorescência de hibridização *in situ* (FISH), seguido por citometria de fluxo multiparamétrica (CFM). Ambas as fermentações foram realizadas no LMBA CCS/UFPB. O monitoramento de indicadores de atividade metabólica (pH, açúcares e ácidos orgânicos) presentes no meio fermentado foi realizado ao longo do tempo. Escores prebióticos foram determinados para cada fermentação *in vitro*. O desenho do estudo é apresentado na Figura 6.

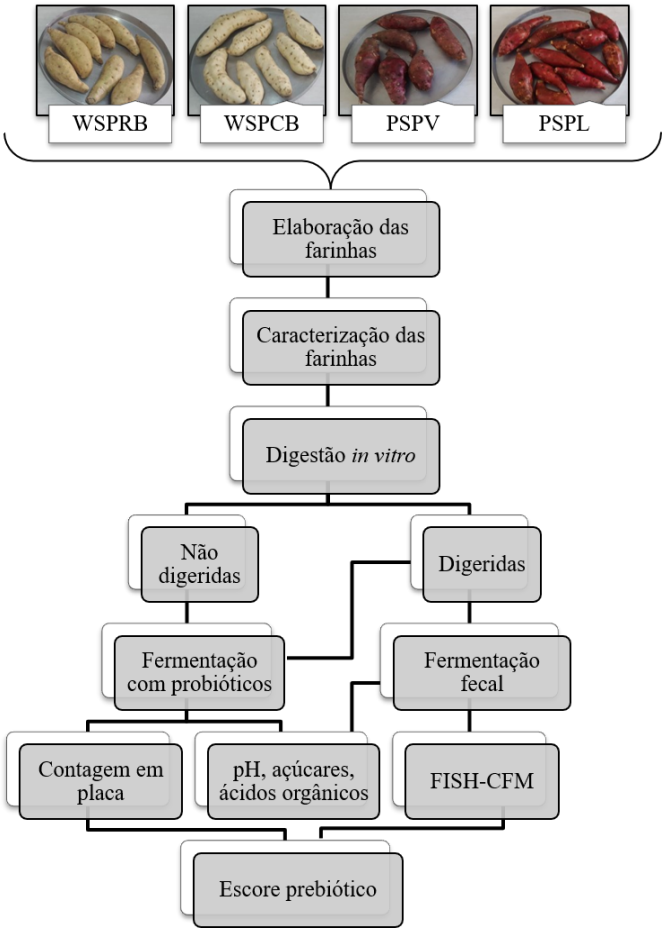

Figura 6. Desenho do estudo de potencial prebiótico de farinhas de diferentes cultivares de batata-doce (*Ipomoea batatas* L.) em sistemas de fermentação *in vitro*.
Batata-doce cultivar Rainha-branca (WSPRB), Campina-branca (WSPCB), Vitória (PSPV) e Lagoinha (PSPL).

3.3 ELABORAÇÃO DAS FARINHAS DE BATATA-DOCE

Após a aquisição, as raízes de BD foram selecionadas, lavadas em água corrente e imergidas em solução de hipoclorito de sódio (Lafepe, Pernambuco, Brasil) a 200 ppm por 15 min, seguido por enxágue em água corrente. As raízes foram descascadas manualmente e cortadas em rodelas de aproximadamente 3 mm. O branqueamento para inativação das enzimas foi realizado em água quente (100 ºC) por 2 segundos, seguido por um banho frio (7 °C). As farinhas foram elaboradas por meio de secagem em estufa com circulação de ar (American Lab, São Paulo, Brasil) a 55 °C por aproximadamente 6 horas a fim de alcançar um teor de umidade <10%. As rodelas foram trituradas em processador doméstico (baixa velocidade, 10 min; Philips Walita, Minas Gerais, Brasil), e os fragmentos foram passados em peneiras de 0,25 mm (60 mesh) para obter um fino pó (KHANH et al., 2018; PEREIRA et al., 2019). O armazenamento das farinhas foi feito em sacos de polietileno (Zip Lock, Brazilian Plast, São Paulo, Brasil) hermeticamente fechados, armazenadas em local fresco e seco, sob temperatura ambiente, por até 6 meses. Cada farinha foi testada isoladamente em todos os ensaios. As farinhas elaboradas estão apresentadas na Figura 7.

Figura 7. Farinhas de batata-doce (*Ipomoea batatas* L.) variedade Rainha-branca (A), Campina-branca (B), Vitória (C) e Lagoinha (D) utilizadas nos experimentos.
Fonte: Autor.

3.4 DETERMINAÇÃO DA COMPOSIÇÃO NUTRICIONAL E PARÂMETROS FÍSICO-QUÍMICOS

As farinhas de BD foram avaliadas quanto aos teores de umidade por secagem direta em estufa estabilizada a 105 °C até obtenção de peso constante; cinzas por carbonização seguida de incineração em forno mufla estabilizado a 550 °C; determinação de proteína pelo

método de Kjeldahl, cujo fator 5,75 foi utilizado; determinação de gordura pela extração de lipídeos ligados às proteínas e carboidratos através da utilização de solventes polares; e pH por leitura em potenciômetro (IAL, 2008; AOAC, 2016). Os conteúdos de fibras dietéticas solúveis e insolúveis e amido resistente foram determinados usando um kit de ensaio enzimático-gravimétrico (Sigma Aldrich, St. Louis, EUA) e um kit de ensaio enzimático-colorimétrico (Megazyme International Ireland Ltd., Bray, Irlanda), respectivamente (AOAC 1995; AOAC 2005; TOBARUELA et al., 2018).

Extratos aquosos das farinhas foram preparados para determinar as quantidades de açúcares (frutose, glicose e maltose), ácidos orgânicos (acético, fórmico, lático, málico, succínico e tartárico) e oligossacarídeos (rafinose e FOS: kestose e nistose). Para isso, 2 g de cada farinha foram misturados com 10 mL de água ultrapurificada (Milli-Q® Sistema de Purificação Integral de Água, EMD Millipore Corporation, Billerica, EUA) por 10 min usando um aparelho turratec (TE-102, Tecnal, São Paulo, Brasil). A mistura foi centrifugada (centrífuga MPW-351R, MPW Medical Instruments, Warsaw, Poland) (4000 × *g*, 15 min, 24 °C) e o sobrenadante foi filtrado duas vezes (papel de filtro qualitativo e membrana de celulose regenerada, tamanho de poro de 0,45 μm; Whatman®, GE Healthcare, Chicago, EUA) (COELHO et al., 2018; LIMA et al., 2019).

Extratos metanólicos (Neon, São Paulo, Brazil) das farinhas foram preparados para determinação dos compostos fenólicos (antocianinas: cianidina 3,5-diglucosídeo, pelargonidina 3,5-diglucosídeo, delfinidina 3-glicosídeo, malvidina 3,5-diglucosídeo, cianidina 3-glicosídeo, pelargonidina 3-glicosídeo, peonidina 3-glicosídeo, malvidina 3-glicosídeo, petunidina 3-glicosídeo; ácidos fenólicos: ácido cafeico, ácido caftárico, ácido clorogênico, ácido gálico, ácido p-cumárico, ácido siríngico; flavanóis: catequina, epigalocatequina galato, epicatequina, epicatequina galato, procianidina A2, procianidina B1, procianidina B2; flavonóis: miricetina, kaempferol 3-glicosídeo, quercetina 3-glicosídeo; flavanonas: hesperidina, rutina, naringenina; estilbenos: cis-resveratrol, trans-resveratrol). Para isso, 2 g de cada farinha foram acidificados com 0,1 M HCl (Neon, São Paulo, Brazil) para atingir pH 2 e misturados a 10 mL de metanol:água (70:30 v/v) a 30 °C durante 60 min com uso de ultrassonicador (Ultrasonic, Tecnal, São Paulo, Brazil). As misturas foram centrifugadas (4000 × *g*, 15 min, 24 °C) e os sobrenadantes foram filtrados (membrana de celulose regenerada, tamanho de poro de 0,45 μm) (PADILHA et al., 2017; MORO et al., 2018).

As determinações de açúcares, ácidos orgânicos, oligossacarídeos e compostos fenólicos nas farinhas foram realizadas por cromatografia líquida de alta eficiência (CLAE) utilizando um cromatógrafo Agilent (modelo 1260 Infinity LC, Agilent Technologies, St. Clara, EUA) equipado com bomba de solvente quaternário (modelo G1311C), desgaseificador, compartimento da coluna termostática (modelo G1316A) e amostrador automático (modelo G1329B), acoplado a um detector de arranjo de diodos (DAD) (modelo G1315D) e Detector de Índice de Refração (RID) (modelo G1362A). As outras condições analíticas foram as seguintes: coluna Hydro-RP C18 (150 x 4.6 mm, 4 micrômetros); fase móvel H_2SO_4 4 mM/L em água ultrapura e taxa de fluxo 0,7 mL/min. Os dados foram processados usando o software OpenLAB CDS ChemStation Edition^{TM} (Agilent Technologies, St. Clara, EUA). Os picos da amostra observados na CLAE foram identificados comparando seus tempos de retenção com os respectivos padrões para açúcares, ácidos orgânicos, oligossacarídeos e compostos fenólicos (Sigma-Aldrich, St. Louis, MO, EUA).

3.5 DIGESTÃO GASTROINTESTINAL *IN VITRO*

Cada farinha de BD foi submetida a uma digestão *in vitro* a fim de serem transformadas em materiais com características semelhantes àqueles que alcançam o cólon humano. Inicialmente, 10 g de WSPRB, WSPCB, PSPV ou PSPL foram misturadas com 50 mL de água destilada esterilizada em frascos de vidro com tampa e homogeneizadas manualmente por 5 min. A mistura de α-amilase (3.33 mg) e $CaCl_2$ 1 mM (1.04 mL, pH 7) foi adicionada a solução das farinhas e mantidas a 37 ºC por 30 min sob agitação de 130 rpm. Posteriormente, o pH da mistura foi ajustado para 2 – 2.5 usando HCl 1 M, adicionado de pepsina (0,45 g em 0,1 M HCl, 4,16 mL) e mantida a 37 ºC for 2 horas sob agitação de 130 rpm. Bile (0,58 g) e pancreatina (0,93 g) misturados com 20,8 mL de $NaHCO_3$ 0,5 M foram incorporados a mistura. O pH foi ajustado para 6,5 – 7 usando $NaHCO_3$ 1 M e mantidos a 37 ºC por 2 horas sob agitação de 45 rpm. Todas as enzimas e reagentes usados para digestão gastrointestinal *in vitro* foram adquiridos na Sigma-Aldrich.

O material resultante do processo digestivo foi submetido à diálise em membrana de celulose regenerada de 1 kDa de peso molecular (Spectra/Por® 6, Spectrum Europe BV, Breda, Holanda), previamente hidratada por 30 min em água destilada aquecida (60 °C). As membranas contendo o material foram imergidas em NaCl 0,01 M e incubadas a 5 ± 0.5 ºC, para remover produtos da digestão de baixo peso molecular. Após 15 horas, os fluidos de

diálise foram trocados e a diálise continuou por mais 2 horas. As amostras dializadas foram congeladas a -80 °C por 24 horas, liofilizadas (temperatura -55 ± 2 ºC, pressão do vácuo <138 µHg, velocidade 1 mm/h) por 14 - 16 horas usando liofilizador de bancada (modelo L-101, LIOTOP, São Carlos, Brasil) e armazenadas sob refrigeração (5 ± 0.5 °C) em sacos de polietileno hermeticamente fechados por até 4 semanas (RODRIGUES et al., 2016).

3.6 AVALIAÇÃO DOS EFEITOS PREBIÓTICOS SOBRE CEPAS DE *Lactobacillus* E *Bifidobacterium* PROBIÓTICAS

3.6.1 Microrganismos e condições de cultura

Foram utilizadas cepas probióticas de *Lactobacillus acidophilus* LA-05, *Lactobacillus casei* L-26 e *Bifidobacterium animalis* subsp. *lactis* BB-12 (LU; PUTRA; LIU, 2018; MORAIS et al., 2019), fornecidas pela Coleção de Microrganismos do Colégio de Biotecnologia, Universidade Católica Portuguesa (Porto, Portugal). Estas espécies também têm sido reportadas como sendo parte da microbiota intestinal humana (DAVID et al., 2014; SUN et al., 2018). Para uso nos ensaios, cada cepa foi cultivada duas vezes em caldo de Man, Rogosa e Sharpe (MRS; HiMedia, Mumbai, Índia) a 37 °C por 20 – 24 horas, centrifugadas (4500 × *g*, 15 min, 4 °C), lavadas duas vezes e ressuspendidas em solução salina esterilizada (NaCl 8,5 g/L; FMaia Ltd., Minas Gerais, Brasil) para obter uma suspensão de células com leitura de densidade óptica (DO; Espectrofotômetro, Bel Photonics, São Paulo, Brasil) a 655nm de 0,8. Esta suspensão forneceu, aproximadamente, 7 log de unidades formadoras de colônias por mL (UFC/mL) quando inoculadas em ágar MRS (HiMedia) (SOUSA et al., 2015). Cada cepa probiótica foi analisada separadamente como inóculo simples.

As cepas de *Escherichia coli* ATCC 8739 e *E. coli* ATCC 25922, gentilmente fornecidas pela Coleção de Referência em Microrganismos, Instituto Nacional de Controle de Qualidade em Saúde, Fundação Oswaldo Cruz (Rio de Janeiro, Brasil), foram usadas para preparar o inóculo da mistura entérica utilizada na determinação dos escores de atividade prebiótica. Para uso nos ensaios, estas cepas foram cultivadas duas vezes em caldo infusão cérebro-coração (BHI; HiMedia) a 37 °C por 18 – 20 horas. Cada cultura foi centrifugada (4500 × *g*, 15 min, 4 °C), lavada duas vezes e ressuspendida em solução salina esterilizada (NaCl 8,5 g/L) para obter uma suspensão de células com DO_{655nm} 0,1. Esta suspensão forneceu, aproximadamente, 7 log UFC/mL quando cultivada em ágar BHI (HiMedia). O

inóculo da mistura entérica foi obtido pela mistura das duas diferentes suspensões de *E. coli*, com uma proporção de 1:1.

Os estoques de todas as cepas foram mantidos em caldo MRS ou BHI contendo glicerol (150 g/L; Sigma-Aldrich) a –20 ºC. Os meios usados para o cultivo de *B. animalis* subsp. *lactis* BB-12 foram sempre suplementados com solução de 0.5 g/L L-cisteina·HCl (Fluka, St. Gallen, Suíça) e incubados sob condições anaeróbicas usando um gerador de gás anaeróbico (AnaeroGen, Oxoid, Basingstoke, Inglaterra).

3.6.2 Preparação dos meios de cultivo

O meio de cultivo utilizado inicialmente para avaliar o potencial efeito prebiótico das farinhas de BD, submetidas ou não a uma digestão gastrointestinal *in vitro*, foi o caldo MRS preparado com uma mistura de diferentes compostos de modo a permitir a substituição da sua fonte de carbono (SOUSA et al., 2015; DUARTE et al., 2017). A composição base do caldo MRS modificado foi a seguinte: 10 g/L de triptona, 8 g/L de extrato de carne, 4 g/L de extrato de levedura, 2 g/L de fosfato de di-potássio de hidrogênio, 1 g/L de Tween 80, 5 g/L de acetato de sódio, 2 g/L de citrato de amônio tribásico, 0,2 g/L de sulfato de magnésio, 0,04 g/L de sulfato de manganês e 20 g/L da respectiva fonte de carbono. Para o crescimento das cepas probióticas, foram preparados quatro caldos de cultivo distintos: i) caldo contendo 20 g/L de glicose (ingrediente não prebiótico padrão do caldo MRS; ZHANG et al., 2018b), ii) caldo contendo 20 g/L de FOS (ingrediente prebiótico reconhecido, GIBSON et al., 2017), iii) caldo contendo 20 g/L de WSPRB, WSPCB, PSPV ou PSPL não submetida à digestão *in vitro*, e iv) caldo contendo 20 g/L de WSPRB, WSPCB, PSPV ou PSPL submetida à digestão *in vitro*.

Todos os ingredientes usados para preparar os meios de cultivo foram obtidos da Sigma-Aldrich, com exceção do FOS que foi obtido da empresa Galena Ltd. (Campinas, Brasil). Os meios usados para o cultivo de *B. animalis* subsp. *lactis* BB-12 foram sempre suplementados com solução de 0,5 g/L L-cisteina·HCl e incubados sob condições anaeróbicas usando um gerador de gás anaeróbico (AnaeroGen).

3.6.3 Enumeração de contagens de células viáveis

Inicialmente, o inóculo de cada cepa probiótica foi adicionado (0,2 mL) em frascos estéreis contendo 10 mL de cada caldo de cultivação elaborado (contagem final de células viáveis de aproximadamente 6 log UFC/mL). As misturas foram homogeneizadas por 30

segundos e incubadas a 37 ºC. Em diferentes intervalos de tempos (zero hora, ou seja, logo após a homogeneização e após 6, 12, 18, 24 e 48 horas pós-incubação), alíquotas de 100 µL de cada mistura foram seriadamente diluídas em solução salina esterilizada e, subsequentemente, 20 µL de cada diluição foram plaqueados em ágar MRS. As placas foram incubadas a 37 ºC por 24 – 48 horas. Os resultados das contagens de células viáveis foram expressos como log UFC/mL (MOUSAVI; MOUSAVI, 2019).

3.6.4 Determinação dos escores de atividade prebiótica

Para avaliar a capacidade de cada farinha de estimular seletivamente o crescimento das cepas probióticas, foram determinados os escores de atividade prebiótica dessas farinhas quando não submetidas e quando submetidas à digestão gastrointestinal *in vitro*. Inicialmente, o inóculo (0,2 mL) de cada cepa probiótica testada foi adicionado a frascos esterilizados contendo 10 mL de caldo MRS com glicose, FOS ou WSPRB, WSPCB, PSPV ou PSPL não digerida ou digerida. Em paralelo, o inóculo (0,2 mL) da mistura entérica foi adicionado a frascos esterilizados contendo 10 mL de caldo M9 (Sigma-Aldrich) com glicose, FOS ou WSPRB, WSPCB, PSPV ou PSPL não digerida ou digerida (20 g/L). As misturas foram homogeneizadas usando um Vórtex (AP 56, Phoenix Luferco, São Paulo, Brasil) por 30 segundos e incubadas a 37 ºC.

Em dois diferentes intervalos de tempos (zero hora, ou seja, logo após-homogeneização e após 48 horas de incubação), alíquotas de 100 µL de cada mistura foram seriadamente diluídas em solução salina esterilizada e, subsequentemente, 20 µL de cada diluição foram plaqueados, com a técnica de microgota, em ágar MRS ou ágar eosina azul de metileno (HiMedia) para as cepas probióticas e mistura entérica, respectivamente. As placas foram incubadas a 37 ºC por 24 – 48 horas. Após o período de incubação, as células viáveis foram contadas (log UFC/mL) e os valores adicionados à seguinte equação:

$$\text{Escore de atividade prebiótica} = \left[\frac{\text{probiótico log UFC/mL no prebiótico à 48 h} - \text{probiótico log UFC/mL no prebiótico à 0 h}}{\text{probiótico log UFC/mL na glicose à 48 h} - \text{probiótico log UFC/mL na glicose à 0 h}}\right] - \left[\frac{\text{mix entérico log UFC/mL no prebiótico à 48 h} - \text{mix entérico log UFC/mL no prebiótico à 0 h}}{\text{mix entérico log UFC/mL na glicose à 48 h} - \text{mix entérico log UFC/mL na glicose à 0 h}}\right]$$

Por definição, um escore prebiótico positivo é obtido se as cepas probióticas testadas crescerem melhor na presença do prebiótico do que na presença da glicose e/ou tiverem

melhor crescimento na presença do prebiótico quando comparado com a mistura entérica, refletindo um crescimento seletivo e uma potencial atividade prebiótica (ZHANG et al., 2018b).

3.7 AVALIAÇÃO DOS EFEITOS PREBIÓTICOS UTILIZANDO INÓCULO FECAL

3.7.1 Procedimentos éticos

Considerando as exigências do Conselho Nacional de Saúde, este estudo foi submetido à apreciação e aprovação pelo Comitê de Ética em Pesquisa com Seres Humanos do Centro de Ciências da Saúde/UFPB, tendo em vista a utilização de material biológico proveniente de fezes humanas, baseado na Resolução n° 466/12 (BRASIL, 2012), que aprova as diretrizes e normas regulamentadoras de pesquisas envolvendo seres humanos. O protocolo experimental foi aprovado sob o número 3.489.789, de acordo com o parecer consubstanciado (ANEXO A). Para participar, os voluntários assinaram um Termo de Consentimento Livre e Esclarecido (APÊNDICE A) antes do início dos experimentos.

3.7.2 Coleta das amostras fecais

As amostras fecais frescas foram doadas por quatro voluntários adultos saudáveis, sendo dois homens e duas mulheres, entre 19 e 36 anos. Como critérios de inclusão, os doadores seguiam uma dieta onívora, sem precedentes de doença no intestino grosso, sem o uso de alimentos probióticos ou prebióticos concentrados, além de não ter feito uso de antibióticos ou qualquer outro medicamento de uso controlado por, pelo menos, seis meses antes da coleta (GUERGOLETTO et al., 2016). Um kit para coleta foi disponibilizado para cada doador, contendo frascos coletores esterilizados, luvas, prato e espátula descartável, além de álcool 70%, sendo instruídos quanto ao manuseio asséptico das amostras.

As amostras foram coletadas em até 30 min após a excreção, sendo transportadas em jarra de anaerobiose (Anaerojar, Oxoid, Basingstoke, Inglaterra) contendo gerador de gás anaeróbico (AnaeroGen) e utilizadas imediatamente (RODRIGUES et al., 2016). Parte das amostras fecais coletadas de cada doador foram direcionadas para um laboratório privado local para realização de parasitológico de fezes e avaliação de sangue oculto nas fezes para atestar a normalidade do material fecal dos doadores.

3.7.3. Preparação das amostras fecais e pré-cultura

As amostras fecais frescas coletadas de cada doador foram misturadas em igual quantidade (1:1:1:1), diluídas (1:10 p/v) em tampão fosfato salino (PBS 0,1 M; pH 7,4) esterilizados e homogeneizadas por 2 min sob agitação (200 rpm). A mistura fecal (120 g) foi pré-cultivada (37 ± 0,5 °C) em 1 L de meio composto por 10 g de triptona, 5 g de extrato de levedura, 10 g de NaCl, 5 g de glicose, 6 g de lactose e água destilada, previamente estilizado em autoclave (121 °C, 1 atm, 15 min). Após 18 horas de cultivo em anaerobiose (AnaeroGen), a pré-cultura foi filtrada com o uso de uma camada tripla de gazes esterilizadas para remoção de partículas grandes e armazenadas (37 ± 0,5 °C) em frascos esterilizados sob condições anaeróbicas (AnaeroGen) (HU et al., 2013; MENEZES et al., 2020). Os ingredientes usados para preparar o meio de pré-cultura foram obtidos da Sigma-Aldrich.

3.7.4 Preparação do meio de fermentação

Para o processo de fermentação foi utilizado 1 L de meio de crescimento esterilizado em autoclave (121°C, 1 atm, 15 min), com a seguinte composição: 4,5 g de NaCl, 4,5 g de KCl, 1,5 g de $NaHCO_3$, 0,69 g de $MgSO_4$, 0,8 g de L-cisteína HCl, 0,5 g de KH_2PO_4, 0,5 g K_2HPO_4, 0,4 g de sal biliar, 0,08 g $CaCl_2$, 0,005 g de $FeSO_4$, 1 mL de Tween 80 e 4 mL de solução de resazurina (0,025%, como um indicador anaeróbico) e água destilada. As farinhas de BD digeridas foram submetidas à fermentação com o pré-cultivo da microbiota fecal humana. O volume final da fermentação foi de 40% do meio de fermentação, 40% da pré-cultura fecal e 20% de WSPRB, WSPCB, PSPV ou PSPL digerida, incubados anaerobicamente a 37 °C por 48 horas (HU et al., 2013; MENEZES et al., 2020). Um meio de fermentação contendo FOS (20%) e sem substrato adicionado foram utilizados como controle positivo e negativo, respectivamente, sendo submetidos aos mesmos procedimentos citados acima. Os ingredientes usados para preparar o meio de fermentação foram obtidos da Sigma-Aldrich.

3.7.5 Enumeração bacteriana por fluorescência de hibridização *in situ* acoplado com citometria de fluxo multiparamétrica

A técnica de fluorescência de hibridização *in situ* (FISH) usando sondas de oligonucleotídeos designadas para regiões alvo específicas do gene 16S rRNA de distintos grupos bacterianos em combinação com citometria de fluxo multiparamétrica (CFM) foram utilizadas para avaliar a capacidade de WSPRB, WSPCB, PSPV e PSPL digerida de induzir

mudanças na composição da população bacteriana colônica (CONTERNO et al., 2019; MENEZES et al., 2020;). Cinco diferentes sondas (Lab 158 específica para *Lactobacillus–Enterococcus*, Bif 164 específica para *Bifidobacterium*, Bac 303 específica para *Bacteroides–Prevotella*, Chis 150 específica para *Clostridium histolyticum* e Erec 482 específica para *Eubacterium rectale–Clostridium coccoides*) sintetizadas comercialmente e marcadas com o fluoróforo Cy3 (Sigma-Aldrich) foram utilizadas (RODRIGUES et al., 2016; MENEZES et al., 2020;). O marcador SYBR Green (Molecular Probes, Invitrogen, Carlsbad, CA, EUA) foi usado para enumerar a população total de bactérias por meio da marcação da fita dupla de DNA (CONTERNO et al., 2019).

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

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

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

≥99.9%, Sigma-Aldrich), incubadas por 10 min no escuro sob temperatura ambiente (25 ± 0,5 °C), centrifugadas (4000 × *g*, 15 min, 25 °C) e ressuspendidas com 200 µL de PBS 1X.

Uma amostra em branco (sem a sonda oligonucleotídica e sem SYBR Green), e uma amostra marcada somente com SYBR Green, foram preparadas para todas as amostras, seguindo os mesmos passos das amostras hibridizadas, como um controle para definir o limiar das comportas do citômetro de fluxo (BD Accuri C6, New Jersey, EUA), o que permite revelar a potencial autofluorescência das amostras, excluindo os falsos positivos. Na análise de CFM, os sinais fluorescentes das células individuais passam através de uma zona de laser, sendo coletados como sinais logarítmos. Os sinais fluorescentes (mensuramento da área de pulso) foram coletados pelos canais FL1 (SYBR Green) e FL2 (Lab 158, Bif 164, Bac 303, Chis 150 e Erec 482). A aquisição das amostras foi configurada para passar em um baixo fluxo, com nível limite para dispersão direta (FSC) ajustado para 30000 e com total de 10000 eventos coletados para cada amostra. Os citogramas de emissão de fluorescência foram registrados com o software BD Accuri C6 (Becton Dickinson and Company). Os resultados foram expressos como abundância (porcentagem relativa) de células hibridizadas com cada sonda Cy3 específica de grupo bacteriano (registrados como eventos fluorescentes) em relação ao total de bactérias enumeradas com coloração SYBR Green (CONTERNO et al., 2019).

3.7.6 Determinação de índices prebióticos

Para obter uma medida comparativa quantitativa geral do equilíbrio entre diferentes populações bacterianas nas amostras de fermentação colônica, bem como para comparar a influência das diferentes farinhas de BD examinadas na modulação microbiana seletiva durante a fermentação colônica, um índice prebiótico foi calculado com dados obtidos na análise de FISH-CFM. A equação usada foi adaptada (PALFRAMAN; GIBSON; RASTALL, 2003; OWOLABI et al., 2020), uma vez que este estudo é o primeiro a relatar um índice prebiótico obtido de uma fermentação colônica em que diferentes populações bacterianas foram enumeradas com cinco sondas oligonucleotídicas usando a técnica de FISH-CFM. Após o cálculo da abundância relativa (porcentagem relativa), onde foi feita uma correlação com o número total (abundância, %) de bactérias enumeradas, a seguinte equação foi aplicada:

$$\text{Índice prebiótico} = \%\text{Lab} + \%\text{Bif} - \%\text{Bac} - \%\text{Chis} - \%\text{Erec}$$

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

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

3.8 MONITORAMENTO DE INDICADORES DE ATIVIDADE METABÓLICA

As análises de indicadores físico-químicos de atividade metabólica foram realizadas nos diferentes sistemas de cultivo descritos nos tópicos 3.6 e 3.7. Os valores de pH nos meios de cultivo fermentados por microrganismos probióticos foram mensurados nos tempos zero e após 6, 12, 18, 24 e 48 horas de incubação, enquanto os meios de fermentação fecal foram mensurados nos tempos zero e após 24 e 48 de incubação, utilizando pHmetro digital de bancada (Q400AS, Quimis, São Paulo, Brasil), seguindo o procedimento padrão (AOAC, 2016).

A determinação e quantificação de açúcares e ácidos orgânicos nos meios de cultivo fermentados por microrganismos probióticos foram mensurados nos tempos zero e após 6, 12,

18, 24 e 48 horas de incubação, enquanto nos meios de fermentação fecal os teores de açúcares e ácidos orgânicos foram mensurados nos tempos zero e após 24 e 48 horas de incubação, usando a técnica de CLAE conforme descrito na seção 3.4 (COELHO et al., 2018; LIMA et al., 2019).

3.9 ANÁLISES ESTATÍSTICAS

Os ensaios de fermentação utilizando microrganismos probióticos foram realizados em triplicata e em três experimentos distintos, enquanto que os ensaios de fermentação utilizando inóculo fecal foram realizados em duplicata em dois experimentos distintos. Os resultados de todos os ensaios foram expressos como média ± desvio padrão. Os dados foram submetidos ao teste de Shapiro-Wilk para normalidade e para o teste *t* de Student ou análises de variância (ANOVA), seguido pelo teste de Tukey, considerando $p \leq 0,05$. Para o tratamento estatístico, foi utilizado o software GraphPad Prism 7.0 (GraphPad Software, San Diego, Califórnia, EUA).

4 RESULTADOS

O artigo intitulado *Potential prebiotic properties of flours from different varieties of sweet potato (Ipomoea batatas* L.*) roots cultivated in Northastern Brazil* (APÊNDICE B) foi publicado na revista Food Bioscience, fator de impacto 3.067 (2019). Os resultados demonstraram a presença de nutrientes passíveis de serem utilizados como substratos potencialmente prebióticos pelas bactérias testadas, tais como fibras solúveis, FOS, amido resistente e compostos fenólicos. Os resultados encontrados para WSPRB, WSPCB, PSPV e PSPL digeridas e não digeridas mostraram escores de atividade prebiótica positivos para todas as cepas probióticas testadas, altas contagens de células viáveis, além de diminuição do pH, consumo de açúcares (frutose, glicose e maltose) e aumento da quantidade de diferentes ácidos orgânicos (ácidos acético, fórmico, lático, málico, succínico e tartárico) ao longo do tempo, revelando intensa atividade metabólica.

O artigo intitulado *Flours from different sweet potato (Ipomoea batatas L.) root varieties modulate positively the composition and metabolic activity of human colonic microbiota in vitro* (APÊNDICE C) foi submetido a revista Food Chemistry, fator de impacto 6.306 (2019). Os resultados desse estudo indicam que WSPRB, WSPCB, PSPV e PSPL digeridas aumentaram a abundância relativa das populações de *Lactobacillus–Enterococcus* e *Bifidobacterium*, enquanto diminuíram a abundância relativa de *Bacteroides–Prevotella*, *C. histolyticum* e *E. rectall–C. coccoides* durante a fermentação colônica. WSPRB, WSPCB, PSPV e PSPL apresentaram índices prebióticos positivos após 24 e 48 h de fermentação colônica. O aumento da atividade metabólica microbiana foi evidenciado por meio da diminuição do pH e aumento do consumo de açúcares (frutose, glicose e maltose) e produção de ácido lático e AGCC (acético, butírico e propiônico) durante 48 h de fermentação colônica. Esses resultados indicam a capacidade das farinhas de BD testadas de modular positivamente a composição e a atividade metabólica da microbiota intestinal humana, o que deve estar relacionado às propriedades prebióticas. Dessa forma, farinhas de raízes de BD são ingredientes com potenciais propriedades prebióticas para serem usados na formulação de suplementos dietéticos ou alimentos funcionais com alto valor agregado.

A execução desse estudo também possibilitou a elaboração de um pedido de patente intitulado “Farinha de batata-doce prebiótica, processo e produto”, o qual foi depositado no Instituto Nacional de Propriedade Intelectual sob o nº BR 1020190223901, conforme comprovante anexado (ANEXO B).

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APÊNDICES

APÊNDICE A

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

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Prezado(a) Senhor(a),

Esta pesquisa é intitulada como AVALIAÇÃO DO POTENCIAL PREBIÓTICO DE FARINHAS DE DIFERENTES CULTIVARES DE BATATA-DOCE (*Ipomoea batatas* L.) EM SISTEMAS DE FERMENTAÇÃO *IN VITRO*, a qual verificará o efeito na modulação da microbiota intestinal frente às farinhas de batata-doce utilizando inóculo fecal. Esta pesquisa está sendo desenvolvida pela pesquisadora THATYANE MARIANO RODRIGUES DE ALBUQUERQUE, aluna do Programa de Pós-Graduação em Ciências da Nutrição, da Universidade Federal da Paraíba, sob a orientação do Prof. Dr. Evandro Leite de Souza.

A condução desta proposta trará informações científicas atualmente escassas no campo de estudo de componentes com propriedades prebióticas, em especial provenientes de farinhas de batata-doce utilizando inóculo fecal de doadores saudáveis, o que irá colaborar para a formulação de estratégias de utilização desses produtos de forma a agregar valor e, consequentemente, auxiliar no incremento do agronegócio familiar.

Solicitamos a sua colaboração para coletar amostras fecais, como também sua autorização para apresentar os resultados deste estudo em eventos da área da saúde e publicar em revista científica. Por ocasião da publicação dos resultados, seu nome será mantido em sigilo. Informamos que essa pesquisa não oferece riscos previsíveis para a sua saúde.

Esclarecemos que sua participação no estudo é voluntária e, portanto, o (a) senhor (a) não é obrigado (a) a fornecer as informações e/ou colaborar com as atividades solicitadas pela pesquisadora. Caso decida não participar do estudo, ou resolver a qualquer momento desistir do mesmo, não sofrerá nenhum dano. Os pesquisadores estarão a sua disposição para qualquer esclarecimento que considere necessário em qualquer etapa da pesquisa.

Diante do exposto, declaro que fui devidamente esclarecido (a) e dou o meu consentimento para participar da pesquisa e para publicação dos resultados.

João Pessoa,_____/_____/_____

_____
Assinatura do Participante da Pesquisa

Caso necessite de maiores informações sobre o presente estudo, favor ligar para a pesquisadora: Thatyane Mariano Rodrigues de Albuquerque – (83) 98827-6775
Laboratório de Microbiologia e Bioquímica de Alimentos/CCS/UFPB - ☎ (83) 3216-7807

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Assinatura do Pesquisador Responsável

APÊNDICE B – ARTIGO I

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Potential prebiotic properties of flours from different varieties of sweet potato (*Ipomoea batatas* L.) roots cultivated in Northeastern Brazil

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ABSTRACT

The chemical composition and potential prebiotic effects of 4 different flours from varieties of sweet potato roots (SPR), including two with white peel and two with purple peel, cultivated in Northeastern Brazil were studied. SPR flours (SPRF) showed variable contents of soluble (1.8–3.0%) and insoluble (4.6–5.8%) fiber, resistant starch (6.2–10.3%), nystose (not found up to 7.1 g/l), kestose (1.7–5.4 g/l), phenolic compounds (phenolic acids: not found up to 16.9 mg/l, flavanols: not found up to 20.4 mg/l, flavanones: not found up to 26.0 mg/l) and sugars (fructose: 4.6–6.1 g/l, glucose: 5.4–8.7 g/l, maltose: 3.2–27.2 g/l). SPRF (20 g/l) with and without a simulated gastrointestinal digestion had an average positive prebiotic activity score (0.11–0.55) with probiotics *Lactobacillus acidophilus, L. casei* and *Bifidobacterium animalis* to the detriment of enteric competitors, showing selective stimulatory effects on beneficial bacteria species that are found as part of the gut microbiota. Cultivation of these probiotics in media with or without simulated digestion (SPRF, 20 g/l) showed high bacterial counts (8.2–9.7 log CFU/ml), decreased pH, increased acetic, formic, lactic, malic, succinic and tartaric acids and decreased fructose, glucose and maltose over time, which are indicative of strong metabolic activity. These results showed that flours from different SPR varieties are potential prebiotic ingredients for use in the formulation of functional foods or dietary supplements.

1. Introduction

Sweet potato (*Ipomoea batatas* L.) in the Convolvulaceae family, is the third most important crop in production value and the fifth in caloric contribution to global human diets (Esatbeyoglu, Rodríguez-Werner, Schlösser, Winterhalter, & Rimbach, 2017; Zhang et al., 2018a). Sweet potato originated from Latin America but it is an important crop also in many Asian and African countries (Zhang et al., 2018a). The annual production of sweet portato roots (SPR) in Brazil has been around 740 tonnes, with the Brazilian Northeastern region accounts for ~34% of this production (IBGE, 2019).

Artificial selection and occurrence of natural hybrids and mutations have resulted in different SP varieties, which differ not only on their peel and/or flesh color, but also on their nutritional composition, sensory characteristics and bioactive compounds profile (Trancoso-Reyes et al., 2016). The economic value of SPR has been related to the presence of a variety of vitamins, amino acids and minerals, being a good source of starch, dietary fiber and phenolics (Dako, Retta, & Desse, 2016; Wang et al., 2018). Although SPR are mainly consumed in the cooked form, SPR flours (SPRF) might be accepted as a healthy ingredient for use in domestic meal preparations and food formulations by industry (Zhu & Sun, 2019).

SPR have been considered foods with potential health-promoting properties, including possible prebiotic effects primarily related to their high amounts of fermentable carbohydrates (Guo, Zhao, Li, & Miao, 2019; Shen et al., 2018). Prebiotics are substrates selectively used by microorganisms that are part of the gut microbiota, conferring a variety of health benefits to the host (Gibson et al., 2017). Criteria widely applied for the selection of prebiotics include the resistance to gastric acidity and hydrolysis by digestive enzymes, as well as non-

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absorbability and fermentability by beneficial intestinal microorganisms (David, Maurice, Button, & Turnbaugh, 2014; Gibson et al., 2017). Prebiotics have the ability to improve the growth and metabolism of probiotics in the large intestine (Mohanty, Misra, Mohapatra, & Sahu, 2018). *Lactobacillus* and *Bifidobacterium* species are often studied as probiotics, being the main microbial groups forming the gut microbiota of mammals and effective in gut homeostasis (Kerry et al., 2018).

Studies on the potential prebiotic effects of SPR and SPRF are still limited. Only one study evaluated *in vtiro* the prebiotic properties of an orange fleshed SPR puree, and reported stimulatory effects on the growth of *Bifidobaterium* spp. and production of short-chain fatty acids favorable to human gut health (Muchiri & McCartney, 2017). Considering these aspects, this study evaluated the potential prebiotic properties of flours from two white and two purple peel varieties of SPR cultivated in Northeastern Brazil. The chemical composition and prebiotic activity scores of flours on probiotic *Lactobacillus* and *Bifidobacterium* strains were determined. The viable counts of probiotics, as well as the ability to produce organic acids and metabolize sugars in cultivation media were evaluated over time.

2. Materials and methods

2.1. Preparation of SPRF

Roots of 4 different varieties of sweet potato (*Ipomoea batatas* L., white flesh), two with white peel, namely Rainha branca (WSPRB) and Campina branca (WSPCB), and two with purple peel, namely Vitória (PSPV) and Lagoinha (PSPL), cultivated in Northeastern Brazil were obtained from an experimental station of the Agronomic Institute of Pernambuco located in the municipality of Itapirema (07°38′24.1″ latitude and 34°57′22.0″ longitude, Pernambuco, Brazil). SPR were washed in running water, immersed in sodium hypochlorite (Lafepe, Pernambuco, Brazil) aqueous solution (200 ppm) for 15 min at room temperature (25 ± 1 °C), manually peeled and sliced (~3 mm thick). Blanching for inactivation of enzymes was done by immersion of SPR slices in hot water (100 °C) for 2 s followed by a cold bath (7 °C). Drying of SPR slices was carried out in an oven (American Lab, São Paulo, Brazil) with forced air circulation (55 °C, 6 h) to reach a moisture < 10%. Dried SPR slices were grounded using a food processor (low speed, 10 min; Philips Walita, Varginha, Minas Gerais, Brazil) and sieved through a 0.25 mm (60 mesh) to obtain a fine powder (Khanh, Chittrakorn, Rutnakornpituk, Tai, & Ruttarattanamongkol, 2018; Pereira et al., 2019). SRPF were stored at room temperature in hermetically sealed polyethylene bags (Zip Lock, Brazilian Plast, São Paulo, Brazil) for up to 6 months. SPRF was tested individually in all assays.

2.2. Microorganisms and culture conditions

Lactobacillus acidophilus LA-05, *Lactobacillus casei* L-26 and *Bifidobacterium animalis* subsp. *lactis* BB-12 were supplied by the Collection of Microorganisms, College of Biotechnology, Portuguese Catholic University (Porto, Portugal). These *Lactobacillus* and *Bifidobacterium* species have been reported as being part of the human gut microbiota (David et al., 2014; Sun et al., 2018). Each strain was cultivated two times in de Man, Rogosa and Sharpe (MRS) broth (HiMedia, Mumbai, India) at 37 °C for 20–24 h, centrifuged (4500 × *g* 15 min, 4 °C; centrifuge MPW-351R, MPW Medical Instruments, Warsaw, Poland), washed two times and re-suspended in sterile saline solution (NaCl 8.5 g/l; FMaia Ltd., Minas Gerais, Brazil) to obtain cell suspensions with an opitical density (OD; Spectrophotometer, Bel Photonics, São Paulo, Brazil) at 655 nm of 0.8. These suspensions provided viable cell counts of ~7 log colony forming units (CFU)/ml when plated on MRS agar (Sousa et al., 2015). Each probiotic strain was tested as a single inoculum.

Escherichia coli ATCC 8739 and *E. coli* ATCC 25922, supplied by the Collection of Reference Microorganisms, National Institute for Control Quality in Heath, Oswaldo Cruz Foundation (Rio de Janeiro, Brazil), were used to prepare the mixed enteric inoculum used in assays for determination of prebiotic activity scores. These strains were cultivated two times in brain-heart infusion (BHI) broth (HiMedia) at 37 °C for 18–20 h. Each culture was centrifuged (4500 × *g* 15 min, 4 °C), washed two times and re-suspended in sterile saline solution to obtain a cell suspension with an OD reading of 0.1 at 655 nm (OD_{655}). These suspensions provided viable cell counts of ~7 log CFU/ml when plated on BHI agar. The enteric mixture inoculum was obtained by mixing the two different *E. coli* suspensions with a ratio of 1:1.

Stocks of all strains were maintained in MRS or BHI broth with glycerol (150 g/l; Sigma-Aldrich, St. Louis, MO, USA) at −20 °C for a maximum of 4 wk. MRS broth and MRS agar used for cultivation of *B. animalis* were always supplemented with 0.5 g/l l-cysteine·HCl (Fluka, St. Gallen, Switzerland) and incubated under anaerobic conditions using an anaerobic gas generator (AnaeroGen™, Oxoid, Basingstoke, England).

2.3. Determination of chemical composition of SPRF

Moisture (method 925.10), ash (method 923.03), crude protein (method 920.87; N x 5.75) and fat contents (method 940.26), as well as pH values (method 981.12) of different SPRF were determined using standard procedures (AOAC, 2016). Dietary fibers (insoluble and soluble) contents (method 991.43) and resistant starch contents (method 2002.02) were determined using an enzymatic-gravimetric (Sigma-Aldrich) and an enzymatic-colorimetric assay kit (Megazyme International Ireland Ltd., Bray, Ireland), respectively (Tobaruela et al., 2018).

Aqueous extracts of SPRF were prepared to determine the contents of sugars (fructose, glucose and maltose), organic acids (acetic, formic, lactic, malic, succinic and tartaric) and oligosaccharides (raffinose and fructooligosaccharides (FOS): kestose and nystose). For extraction, 2 g of SPRF were mixed with 10 ml of ultrapurified water (Milli-Q® Integral Water Purification System, EMD Millipore Corp., Billerica, MA, USA) for 10 min using a Turratec apparatus (TE-102, Tecnal, São Paulo, Brazil). The mixture was centrifuged (4000 × *g* 15 min, 24 °C) and the supernatant was filtered two times (qualitative filter paper and regenerated cellulose membrane, 0.45 μm pore size; Whatman®, GE Healthcare, Chicago, IL, USA). Methanol (Neon, São Paulo, Brazil) extracts of SPRF were prepared to determine the phenolic compounds (anthocyaninins: cyanidin 3,5-diglucoside, pelargonidin 3,5-diglucoside, delphinidin 3-glucoside, malvidin 3,5-diglucoside, cyanidin 3-glucoside, pelargonidin 3-glucoside, peonidin 3-glucoside, malvidin 3-glucoside, petunidin 3-glucoside; phenolic acids: caffeic acid, caftaric acid, chlorogenic acid, gallic acid, p-coumaric acid, syringic acid; flavanols: catechin, epigallocatechin gallate, epicatechin, epicatechin gallate, procyanidin A2, procyanidin B1, procyanidin B2; flavonols: myricetin, kaempferol 3-glucoside, quercitin 3-glucoside; flavanones: hesperidin, rutin, naringenin; and stilbenes: cis-resveratrol, trans-resveratrol). SPRF (2 g) were acidified with 0.1 M HCl to reach pH 2 and mixed using an ultrasound bath (110 W, 37 kHz, Ultrasonic, Tecnal) with 10 ml of methanol:water (70:30 v/v) at 30 °C for 60 min. The mixtures were centrifuged (4000 × *g* 15 min, 24 °C) and the supernatants were membrane filtered (Moro et al., 2018).

The determinations of the sugars, organic acids, oligosaccharides and phenolic compounds in SPRF were done using high-performance liquid chromatography (HPLC) using an Agilent chromatograph (model 1260 Infinity LC, Agilent Technologies, St. Clara, CA, USA) equipped with a quaternary solvent pump (G1311C model), degasser, thermostatic column compartment (G1316A model) and automatic auto sampler (G1329B model), coupled with a diode array detector (DAD) (G1315D model) and refractive index detector (RID) (G1362A model). The other analytic conditions were as follows: an Agilent Hi-Plex H column (7.7 × 300 mm, 8 μ); mobile phase H_2SO_4 4 mM/l in ultrapure water; and flow rate 0.7 ml/min. The data were processed using the OpenLAB CDS ChemStation Edition™ software (Agillent Technologies).

The HPLC sample peaks were measured by comparing their retention times with the previously mentioned standards of sugars, organic acids, oligosaccharides and phenolic compounds (Sigma-Aldrich). Duplicate injections were done and average peak areas were used for quantification using the standards (Coelho et al., 2018; Lima et al., 2019; Padilha et al., 2017).

2.4. In vitro digestion of SPRF

SPRF were used for a simulated human digestion to be transformed by solutions in materials with characteristics similar to those that reach the human colon. Initially, 10 g of the flours were mixed with 50 ml of sterile distilled water and manually stirred for 5 min. A mixture of α-amylase (3.33 mg) and 1 M $CaCl_2$ (1.04 ml, pH 7) was added to the SPRF solution and maintained at 37 °C for 30 min with stirring (130 rpm). The pH of the mixture was adjusted to 2–2.5 using 1 M HCl, pepsin added (0.45 g in 0.1 M HCl, 4.16 ml) and maintained at 37 °C for 2 h with stirring (130 rpm). Bile (0.58 g) and pancreatin (0.93 g) mixed with 20.8 ml of 0.5 M $NaHCO_3$ were incorporated into the mixture, the pH was adjusted to 6.5–7 using 1 M $NaHCO_3$ and maintained at 37 °C for 2 h with stirring (45 rpm). The mixture was transferred to one kDa nominal molecular weight cut-off regenerated cellulose dialysis tubing (Spectra/Por® 6, Spectrum Europe BV, Breda, Netherlands) and dialysed against 0.01 M NaCl at 5 ± 0.5 °C for 15 h. The dialysis fluids were replaced and the process continued for an additional 2 h (Rodrigues et al., 2016). All the enzymes and reagents used to simulate the gastrointestinal digestion were purchased from Sigma-Aldrich. The dialyzed pre-digested samples were freeze-dried (temperature −55 ± 2 °C, vacuum pressure < 138 μH g, freeze-drying speed 1 mm/h) for 14–16 h using a bench top liophylizer (L-101 model, Liotop, São Carlos, Brazil) and stored at 5 ± 0.5 °C in hermetically sealed polyethylene bags for a maximum of 4 wk. Each SPRF was assayed individually as a single carbon source.

2.5. Preparation of cultivation media

MRS broth with a modified carbon source was used as a basal medium to evaluate the potential prebiotic effects of the SPRF with and without *in vitro* digestion (Duarte et al., 2017; Sousa et al., 2015). The composition of the modified MRS broth used in these assays were: tryptone 10 g/l, meat extract 8 g/l, yeast extract 4 g/l, di-potassium hydrogen phosphate 2 g/l, tween 80 1 g/l, sodium acetate 5 g/l, tribasic ammonium citrate 2 g/l, magnesium sulfate 0.2 g/l, manganese sulfate 0.04 g/l and their respective carbon source 20 g/l. To monitor the growth of the probiotic strains, 4 different cultivation media were prepared: 1) glucose (non-prebiotic ingredient; Zhang, Hu, Wang, Liu, & Pan, 2018b): 20 g/l (standard MRS broth); 2) fructooligosaccharides (FOS, a prebiotic ingredient; Gibson et al., 2017): 20 g/l; 3) not digested WSPRB, WSPCB, PSPV and PSPL: 20 g/l; and 4) digested WSPRB, WSPCB, PSPV and PSPL: 20 g/l. All of the ingredients used to prepare the cultivation media were obtained from Sigma-Aldrich, with the exception of FOS that was obtained from Galena Ltd. (Campinas, Brazil). Media used for *B. animalis* cultivation were supplemented with 0.5 g/l l-cysteine·HCl (Fluka) and incubated under anaerobic conditions.

2.6. Determination of prebiotic activity scores of SPRF

To assess the ability of SPRF to selectively stimulate the growth of the probiotics, the prebiotic activity scores of these flours were determined. Initially, the inoculum (0.2 ml) of each of the probiotics was put in sterile flasks containing 10 ml of basal MRS broth with glucose (20 g/l), FOS (20 g/l) or either not digested or digested WSPRB, WSPCB, PSPV or PSPL (20 g/l). In parallel, the inoculum (0.2 ml) of the enteric mixture of two *E. coli* strains were added into sterile flasks with containing 10 ml of M9 broth (Sigma-Aldrich) with glucose (20 g/l), FOS (20 g/l) or either not digested or digested WSPRB, WSPCB, PSPV or PSPL (20 g/l). The mixtures were homogenized using a Vortex mixer (AP 56, Phoenix Luferco, São Paulo, Brazil) for 30 s and, subsequently, incubated statically at 37 °C. At two different time intervals (0 – just after homogenization and 48 h of incubation), 100 μl aliquots of each mixture were serially diluted in sterile saline solution, subsequently, 20 μl aliquots of each dilution were plated on MRS agar or eosin methylene blue agar for enumeration of the probiotic strains and enteric mixture, respectively. The plates were incubated at 37 °C for 24–48 h. The viable cells were counted and the average were expressed as log CFU/ml. Media used for the cultivation of *B. animalis* were supplemented with 0.5 g/l l-cysteine·HCl and incubated under anaerobic conditions.

The prebiotic activity score of SPRF on each probiotic was determined using the following equation:

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

By definition, if a substance can be metabolized by probiotics but not by enteric mixture, a positive prebiotic activity score will be obtained. A higher score indicates higher prebiotic acitivity (Zhang et al., 2018b).

2.7. Measurements of metabolic activity of probiotic Lactobacillus and Bifidobacterium strains in media with SPRF

The metabolic activity of probiotics in media with the different SPRF, glucose or FOS was measured through the enumeration of the viable counts of the inoculated probiotics, as well as the measurements of the organic acids production and sugars consumption in cultivation media over time.

2.7.1. Enumeration of viable cell counts

The inoculum of the probiotic strain was dispensed (20 ml/l) in sterile flasks containing 10 ml of the specific cultivation broth (final viable cell counts of ~6 log CFU/ml). The mixtures were gently hand-shaken for 30 s and subsequently incubated at 37 °C. At different incubation times (0 – just after homogenization and 6, 12, 18, 24 and 48 h of incubation), 100 μl aliquots of each mixture were serially diluted in sterile saline solution, and, subsequently, 20 μl aliquots of each dilution were plated on MRS agar and subsequently counted. The plates were incubated at 37 °C for 24–48 h and the viable cells were counted (log CFU/ml) (Mousavi & Mousavi, 2019).

2.7.2. Measurements of sugar consumption and organic acid production

The pH values of the cultivation media were measured at time zero (baseline - just after homogenization), 6, 12, 18, 24 and 48 h of incubation, while the sugar and organic acid contents were measured at 12, 24 and 48 h of incubation. The pH values (AOAC method 981.12) were measured using a digital potentiometer (Q400AS, Quimis, São Paulo, Brazil) following standard procedures (AOAC, 2016). Sugars (glucose, fructose and maltose) and organic acids (acetic, formic, lactic, malic, succinic and tartaric) were simultaneously measured using HPLC as described in section 2.3.

2.8. Statistical analysis

All the analyses were done in triplicate in three different experiments and the results were expressed as the average of these assays. Data were submitted to a Student *t*-test or analysis of variance (one way ANOVA) followed by Tukey *post-hoc* test using $p \leq 0.05$. For these analysis, the computational software GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA) was used.

Table 1
Chemical composition of flours from different sweet potato roots used in assays to evaluate potential prebiotic properties.

Parameters	Flours			
	WSPRB	WSPCB	PSPV	PSPL
Moisture (%)	9.3 ± 1.6^{A}	9.1 ± 1.2^{A}	7.3 ± 1.8^{A}	7.4 ± 1.8^{A}
Ash (%)	2.3 ± 0.3^{A}	2.2 ± 0.2^{A}	2.1 ± 0.3^{A}	2.1 ± 0.2^{A}
pH	5.7 ± 0.2^{A}	5.9 ± 0.3^{A}	5.6 ± 0.2^{A}	5.8 ± 0.2^{A}
Gross composition (%)				
Crude protein	3.6 ± 0.1^{D}	1.8 ± 0.2^{A}	3.4 ± 0.1^{C}	2.4 ± 0.1^{B}
Lipid	1.1 ± 0.1^{A}	1.1 ± 0.1^{A}	1.7 ± 0.1^{B}	1.3 ± 0.1^{A}
Resistant starch	6.7 ± 0.4^{A}	10.3 ± 0.2^{C}	6.2 ± 0.2^{A}	9.2 ± 0.3^{B}
Soluble fiber	1.8 ± 0.2^{A}	2.3 ± 0.3^{AB}	2.7 ± 0.4^{B}	3.0 ± 0.6^{B}
Insoluble fiber	5.8 ± 1.2^{A}	5.2 ± 1.4^{A}	5.5 ± 1.3^{A}	4.6 ± 1.2^{A}
Total dietary fiber	7.6 ± 0.9^{A}	7.4 ± 1.1^{A}	8.2 ± 1.3^{A}	7.6 ± 0.9^{A}
Carbohydrates (g/l)				
Fructose	4.6 ± 0.5^{A}	5.1 ± 0.7^{AB}	6.1 ± 0.4^{B}	4.9 ± 0.4^{A}
Glucose	5.4 ± 0.3^{A}	6.9 ± 0.9^{BC}	8.7 ± 0.5^{C}	6.5 ± 0.7^{AB}
Maltose	3.2 ± 0.1^{A}	8.8 ± 0.7^{B}	27 ± 1^{D}	22 ± 2^{C}
Kestose	2.0 ± 0.2^{BC}	5.4 ± 0.5^{D}	2.2 ± 0.2^{C}	1.7 ± 0.1^{A}
Nystose	NF	NF	7.1 ± 0.8^{B}	1.6 ± 0.2^{A}
Organic acids (g/l)				
Formic acid	2.0 ± 0.3^{A}	6.7 ± 0.4^{C}	6.4 ± 0.5^{C}	3.2 ± 0.2^{B}
Malic acid	7.5 ± 0.6^{BC}	3.9 ± 0.2^{A}	6.8 ± 0.3^{B}	7.9 ± 0.6^{C}
Succinic acid	1.2 ± 0.1^{AB}	1.2 ± 0.2^{A}	1.0 ± 0.1^{A}	1.7 ± 0.3^{B}
Tartaric acid	NF	1.6 ± 0.2^{A}	9.7 ± 0.9^{B}	NF

WSPRB: flour from sweet potato root variety Rainha Branca (white peel); WSPCB: flour from sweet potato root variety Campina Branca (white peel); PSPV: flour from sweet potato root variety Vitória (purple peel); PSPL: flour from sweet potato root variety Lagoinha (purple peel).
NF = not found.
A – D: Different superscript capital letters in the same row indicate a significant difference ($p \leq 0.05$) among samples of flours from roots of different sweet potato varieties, based on Tukey's test.

3. Results

3.1. Chemical composition of SPRF

The chemical composition of samples are shown in Table 1. Moisture values were < 10% in all cases. The amounts of crude proteins differed ($p \leq 0.05$) among the tested SPRF. The highest and lowest contents ($p \leq 0.05$) of crude proteins were found in WSPRB and WSPCB, respectively. Lipids were found in low amounts in WSPRB, WSPCB, PSPV and PSPL. The highest contents ($p \leq 0.05$) of resistant starch were found in WSPCB and PSPL. Similar ($p > 0.05$) contents of total dietary fiber and soluble and insoluble fiber were observed among the tested SPRF. The contents of maltose were higher ($p \leq 0.05$) in PSPV and PSPL. Nystose was found only in PSPV and PSPL.

Formic, malic and succinic acids were found in variable contents in WSPRB, WSPCB, PSPV and PSPL, while tartaric acid was found only in WSPCB and PSPV. Rhamnose, raffinose, acetic acid and lactic acid were not found in any flours.

The contents of phenolic compounds in samples are shown in Table 2. Chlorogenic acid was found in WSPRB and WSPCB; caftaric acid was found in PSPV and PSPL; and p-coumaric acid was found in all SPRF. PSPV showed the highest ($p \leq 0.05$) amounts of catechin and naringenin, while WSPCB showed the highest ($p \leq 0.05$) amounts of procyanidin B2. Anthocyanins, flavonols and stilbenes were not found in any flours.

3.2. Prebiotic activity scores of SPRF

Prebiotic activity scores of FOS and samples of flours on *L. acidophilus*, *L. casei* and *B. animalis* are shown in Table 3. Not digested and digested WSPRB, WSPCB, PSPV and PSPL showed positive prebiotic activity scores on the three tested strains, although these values varied with the flour and probiotic strain.

Table 2
Contents (mg/l) of phenolic compounds in flours from different sweet potato roots used in assays to evaluate potential prebiotic properties.

Phenolic compounds	Flours			
	WSPRB	WSPCB	PSPV	PSPL
Phenolic acids				
Caftaric acid	NF	NF	8.5 ± 0.8^{A}	10.6 ± 0.5^{B}
Chlorogenic acid	9.2 ± 0.5^{B}	5.0 ± 0.3^{A}	NF	NF
p-Coumaric acid	10 ± 1^{A}	16 ± 1^{B}	17 ± 1^{B}	11 ± 1^{A}
Flavanols				
Catechin	7.6 ± 0.4^{A}	7.1 ± 0.5^{A}	24 ± 2^{B}	6.8 ± 0.6^{A}
Epigallocatechin gallate	5.7 ± 0.3^{B}	3.0 ± 0.5^{A}	3.3 ± 0.2^{A}	NF
Procyanidin A2	13.7 ± 0.2^{A}	16 ± 1^{B}	NF	14 ± 1^{A}
Procyanidin B2	5.5 ± 0.3^{A}	20 ± 2^{D}	14 ± 1^{C}	11 ± 1^{B}
Flavanones				
Rutin	NF	6.1 ± 0.4^{A}	NF	8.6 ± 0.6^{B}
Naringenin	14 ± 1^{B}	16 ± 1^{B}	26 ± 2^{C}	3.4 ± 0.2^{A}

WSPRB: flour from sweet potato root variety Rainha Branca (white peel); WSPCB: flour from sweet potato root variety Campina Branca (white peel); PSPV: flour from sweet potato root variety Vitória (purple peel); PSPL: flour from sweet potato root variety Lagoinha (purple peel).
A – D: Different superscript capital letters in the same row indicate a significant difference ($p \leq 0.05$) among samples of flours from roots of different sweet potato varieties, based on Tukey's test.
NF = not found.
Anthocyanins, flavonols and stilbenes were not found in WSPRB, WSPCB, PSPV and PSPL.

Prebiotic activity scores were similar ($p > 0.05$) or higher ($p \leq 0.05$) for digested WSPRB, WSPCB, PSPV and PSPL with *B. animalis* when compared to FOS. Similar prebiotic activity scores were found for digested WSPRB, digested WSPCB and FOS with *L. acidophilus*, as well as not digested PSPV, not digested PSPL, digested WSPRB, digested WSPCB and FOS with *L. casei*. Digested SPRF showed overall higher prebiotic activity scores ($p \leq 0.05$) on the tested strains than not digested SPRF. The only exception was digested PSPV which showed a lower prebiotic activity score with *L. casei* than not digested PSPV.

3.3. Measurements of metabolic activity of probiotics in media with SPRF

3.3.1. Enumeration of probiotic viable counts and measurement of pH values

The viable counts of the three probiotics in media with not digested and digested WSPRB, WSPCB, PSPV and PSPL, as well as with glucose and FOS during 48 h of cultivation are shown in Fig. 1 (A–F). The viable counts of the probiotics in different media during the cultivation period were ≥6.6 log CFU/ml. For all the cultivation media and inoculated probiotic strains, there was no clear *lag* phase and the length of the exponential growth phase varied from 6 to 12 h, resulting in viable count increases of > 2 log CFU/ml when compared to the initial bacterial population.

The viable counts of *L. acidophilus* and *L. casei* at the end of the incubation period were as high as > 9 log CFU/ml and similar ($p > 0.05$) in media with not digested and digested WSPRB, WSPCB, PSPV and PSPL (Fig. 1A–D). The highest viable counts of *B. animalis* were observed after 24 h of cultivation, being followed by a decrease in viable counts after 48 h of cultivation, with the exception of media with not digested PSPL and FOS (Fig. 1E and F). The viable counts of the probiotics during the incubation period were similar ($p > 0.05$) overall in media with not digested and digested WSPRB, WSPCB, PSPV and PSPL, as well as with FOS and glucose.

The viable counts of the tested probiotics at the end of the cultivation period were higher ($p \leq 0.05$) in media with not digested PSPV and not digested PSPL when compared to those found in media with not

Table 3
Prebiotic activity scores (mean ± standard deviation, n = 3) of fructooligossaccharides (FOS) and flours from different sweet potato roots (20 g/l) not digested and digested *in vitro* on *L. acidophilus*, *L. casei* or *B. animalis*.

Strains	Carbon source									
	FOS	Not digested				Digested				
		WSPRB	WSPCB	PSPV	PSPL	WSPRB	WSPCB	PSPV	PSPL	
L. acidophilus	0.28 ± 0.02^{Aa*}	0.11 ± 0.03^{Aa}	0.18 ± 0.02^{Ba}	0.12 ± 0.03^{Aa}	0.19 ± 0.01^{Ba}	0.26 ± 0.01^{Ba*}	0.32 ± 0.05^{Ba*}	0.16 ± 0.04^{Aa}	0.21 ± 0.01^{Aa}
L. casei	0.37 ± 0.03^{Cc*}	0.18 ± 0.03^{Ab}	0.28 ± 0.01^{Bb}	0.32 ± 0.04^{BCb*}	0.40 ± 0.05^{Cb*}	0.36 ± 0.02^{Bb*}	0.39 ± 0.03^{BCab*}	0.25 ± 0.04^{Ab}	0.41 ± 0.02^{Cb*}
B. animalis	0.32 ± 0.05^{Bb*}	0.12 ± 0.02^{Aa}	0.34 ± 0.01^{Bc*}	0.14 ± 0.03^{Aa}	0.38 ± 0.05^{Bb*}	0.41 ± 0.03^{Ac}	0.42 ± 0.02^{Ab}	0.42 ± 0.03^{Ac}	0.55 ± 0.03^{Bc}

WSPRB: flour from sweet potato root variety Rainha Branca (white peel); WSPCB: flour from sweet potato root variety Campina Branca (white peel); PSPV: flour from sweet potato root variety Vitória (purple peel); PSPL: flour from sweet potato root variety Lagoinha (purple peel).
A – C: Different superscript capital letters in the same row indicate a significant difference ($p \leq 0.05$) among samples not digested *in vitro*, based on Tukey's test. A – C: Different superscript capital letters in the same row indicate a significant difference ($p \leq 0.05$) among samples digested *in vitro*, based on Tukey's test. a-c: Different superscript small letters in the same column denote a significant difference ($p \leq 0.05$) among different strains, based on Tukey's test.
*: Superscript asterisk on the same row indicates no significant difference ($p > 0.05$) among FOS and samples not digested or digested, based on Student's t-test.

digested WSPRB, not digested WSPCB, glucose and FOS (Fig. 1A, C and 1E). However, when considering only digested SPRF, the highest viable counts ($p \leq 0.05$) of the probiotics at the end of the cultivation period were found in media with WSPCB and PSPV. The viable counts of the probiotics did not difer ($p > 0.05$) in media with digested WSPRB and digested PSPL at the end of the cultivation period (Fig. 1B, D and 1F).

The cultivation of the three probiotics in media with the different carbon sources caused decreases in pH values over time (Fig. 1A–F). The lowest pH values in the different cultivation media were observed after 18 or 24 h of incubation, with no significant changes ($p > 0.05$) up to 48 h of incubation regardless of the probiotic strain (Fig. 1A – F). These decreases were up to ~ pH 3.5 in media with glucose and FOS, and to pH 4–5 in media with not digested or digested WSPRB, WSPCB, PSPL and PSPV, following the overall decreasing rank of final pH value in media with WSPRB > WSPCB > PSPL > PSPV. Specifically, the lowest pH value at the end of the cultivation period was found in media with not digested and digested PSPV and inoculated with *B. animalis*.

3.3.2. Measurements of sugar consumption and organic acid production

The contents of fructose, glucose and maltose during 48 h of cultivation are shown in Table 4. Overall, the contents of fructose, glucose and maltose decreased during the incubation period in media with any of the carbon sources and inoculated probiotic strains. As expected, the contents of fructose and glucose were higher ($p \leq 0.05$) over time in media with FOS and glucose, respectively, when compared to media with not digested or digested WSPRB, WSPCB, PSPV and PSPL regardless of the inoculated probiotic strain. The lower glucose contents ($p \leq 0.05$) during the 48 h of incubation were found in media with not digested or digested WSPRB, WSPCB, PSPV and PSPL regardless of the inoculated probiotic strain. Maltose was found in media with not digested and digested WSPRB, WSPCB, PSPV and PSPL, being consumed over time by all probiotics.

Contents of acetic, formic, lactic, malic, succinic and tartaric acids during 48 h of cultivation are shown in Table 5. Acetic and lactic acids were the organic acids produced in the highest amounts in media with not digested and digested WSPRB, WSPCB, PSPV and PSPL regardless of the inoculated probiotic strain. The contents of acetic and lactic acids overall increased ($p \leq 0.05$) during the incubation period in media with any of the carbon sources and inoculated probiotic strains. Acetic acid contents were higher ($p \leq 0.05$) in media with not digested and digested WSPRB, WSPCB, PSPV and PSPL compared to media with FOS and glucose when inoculated with *L. casei*. Overall, acetic acid contents were similar in media with not digested and digested WSPRB, WSPCB, PSPV and PSPL compared to media with FOS and glucose when inoculated with *L. acidophilus* and *B. animalis*.

Lactic acid contents were higher in media with not digested PSPV when compared to the other cultivation media regardless of the inoculated probiotic strain. The highest contents of lactic acid were found in media with not digested and digested WSPRB, WSPCB, PSPV and PSPL and inoculated with *B. animalis*. Additionally, lactic acid was the organic acid found in the highest contents ($p \leq 0.05$) in media with glucose and FOS.

Formic and malic acids were not found or were found at low levels over time in media with glucose and FOS regardless of the inoculated probiotic strain. Overall, the contents of formic acid increased over time in media with digested and not digested WSPRB, WSPCB, PSPV and PSPL and inoculated with *L. acidophilus* and *L. casei*. Formic acid was not found after 24 h in media with not digested and digested WSPRB, WSPCB, PSPV and PSPL and inoculated with *B. animalis*. Malic acid contents did not differ ($p > 0.05$) over time in media with not digested or digested WSPRB, WSPCB, PSPV and PSPL regardless of the inoculated probiotic strain.

The contents of succinic and tartaric acids overall remained similar ($p > 0.05$) or decreased ($p \leq 0.05$) in media with not digested and digested WSPRB, WSPCB, PSPV and PSPL regardless of the inoculated probiotic strain. Tartaric acid was not found in media with glucose and

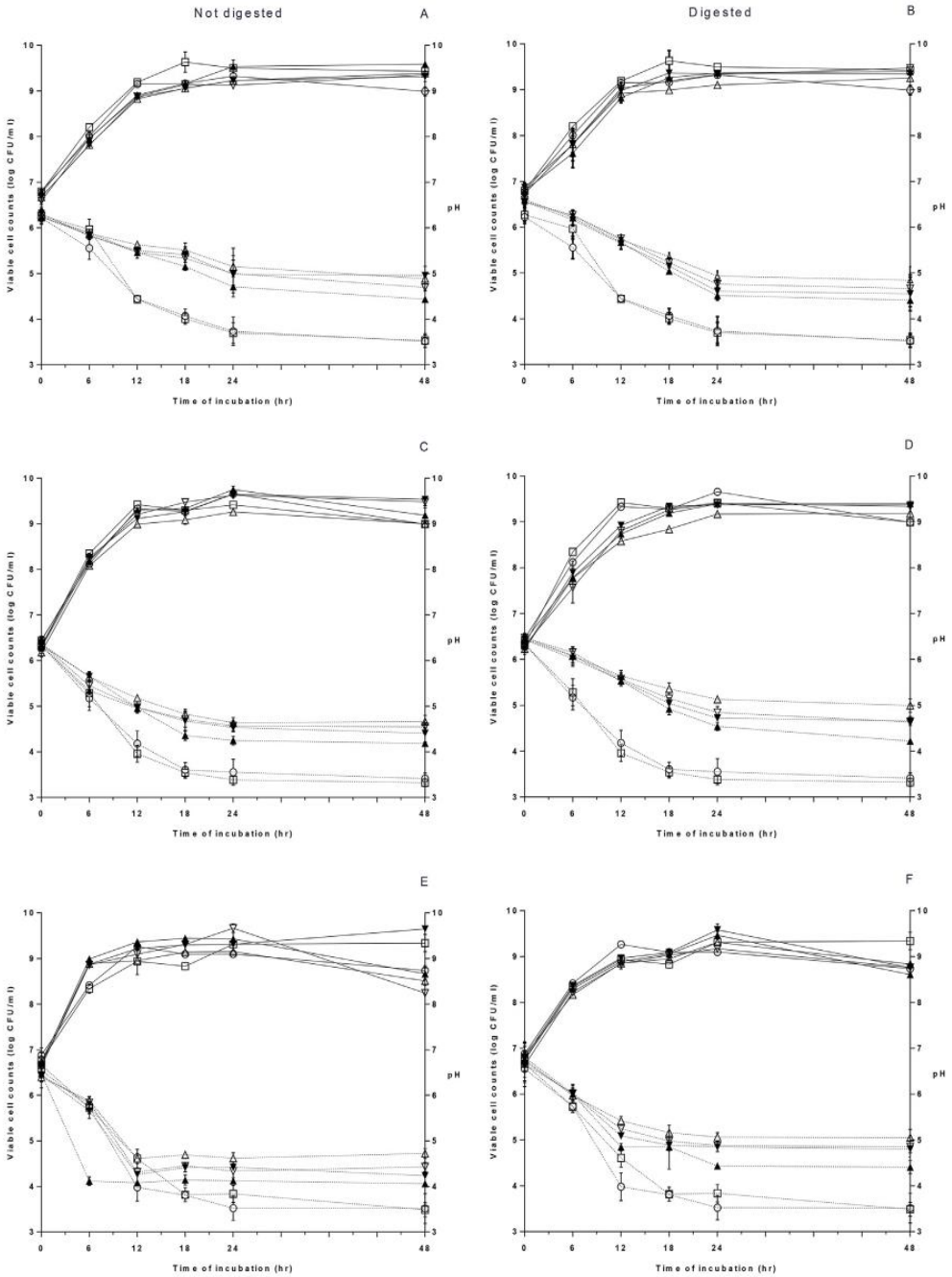

Fig. 1. Viable cell counts (—) of *L. acidophilus* (A, B), *L. casei* (C, D) and *B. animalis* (E, F) and pH values (—) in broth with glucose (20 g/l, ○), fructooligosaccharides (20 g/l, □) or sweet potato root flour (20 g/l; WSPRB, Δ; WSPCB, ∇; PSPV, ▲; or PSPL, ▼) not digested (A, C, E) and digested (B, D, F) during 48 h of incubation. WSPRB: flour from sweet potato root variety Rainha Branca (white peel); WSPCB: flour from sweet potato root variety Campina Branca (white peel); PSPV: flour from sweet potato root variety Vitória (purple peel); PSPL: flour from sweet potato root variety Lagoinha (purple peel).

FOS.

4. Discussion

The positive prebiotic activity scores found for all samples both digested and not indicated that flours had the ability to selectively stimulate the growth of beneficial microorganisms rather than of enteric competitors (Duarte et al., 2017; Lestari, Soesatyo, Iravati, & Harmayani, 2013). The different positive values of prebiotic activity scores found for the samples as well as FOS could be associated with a strain-dependent effect. The different metabolic characteristics (*e.g.*, production of specific enzymes and specific transport systems) of the three probiotics could result in different abilities to use the nutrients available in cultivation media (Zhang et al., 2018b).

The detection of high viable counts of the probiotics in parallel to decreases in pH values over time in media with not digested and digested WSPRB, WSPCB, PSPV and PSPL indicated intense bacterial metabolic activities (Zakaria, Afandi, Noor, Hussin, & Shahidan, 2018).

Table 4
Contents (g/l) of sugars in media with glucose (20 g/l), fructooligosaccharides (20 g/l) or flours from different sweet potato roots (20 g/l) not digested and digested *in vitro* and inoculated with *L. acidophilus*, *L. casei* or *B. animalis* during 48 h of incubation.

Sugars	Carbon source	Strains								
		L. acidophilus			*L. casei*			*B. animalis*		
		12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h
Fructose	Glucose	NF	NF	NF	NF	NF	NF	NF	NF	NF
	FOS	4.1 ± 0.1^{Cb}	1.48 ± 0.01^{Bb}	0.47 ± 0.03^{Ab}	2.58 ± 0.05^{Cc}	0.88 ± 0.02^{Bb}	0.39 ± 0.03^{Ab}	4.1 ± 0.1^{Bb}	2.96 ± 0.04^{Ab}	1.89 ± 0.5^{Ab}
	Not digested									
	WSPRB	0.08 ± 0.01^{Ba}	0.05 ± 0.02^{Ba}	0.01 ± 0.00^{Aa}	0.06 ± 0.02^{Bab}	0.03 ± 0.01^{ABa}	0.02 ± 0.01^{Aa}	0.06 ± 0.03^{Aa}	0.03 ± 0.02^{Aa}	0.03 ± 0.01^{Aa}
	WSPCB	0.09 ± 0.01^{Ba}	0.03 ± 0.01^{Aa}	0.02 ± 0.00^{Aa}	0.05 ± 0.01^{Aa}	0.03 ± 0.02^{Aa}	0.04 ± 0.01^{Aa}	0.05 ± 0.02^{Aa}	0.03 ± 0.01^{Aa}	0.03 ± 0.02^{Aa}
	PSPV	0.10 ± 0.01^{Ba}	0.06 ± 0.01^{Aa}	0.01 ± 0.00^{Aa}	0.08 ± 0.01^{Bb}	0.03 ± 0.02^{Aa}	0.04 ± 0.02^{Aa}	0.08 ± 0.01^{Ba}	0.04 ± 0.02^{Aa}	0.04 ± 0.01^{Aa}
	PSPL	0.11 ± 0.02^{Ba}	0.05 ± 0.01^{Aa}	0.02 ± 0.00^{Aa}	0.04 ± 0.03^{Aa}	0.02 ± 0.01^{Aa}	0.04 ± 0.02^{Aa}	0.05 ± 0.03^{Aa}	0.03 ± 0.01^{Aa}	0.02 ± 0.00^{Aa}
	Digested									
	WSPRB	0.14 ± 0.02^{Bab}	0.12 ± 0.01^{Ba}	0.08 ± 0.02^{Aa}	0.19 ± 0.02^{Bb}	0.14 ± 0.02^{Ba}	0.07 ± 0.01^{Ab}	0.06 ± 0.01^{Ba}	0.02 ± 0.02^{Aa}	0.03 ± 0.01^{Aa}
	WSPCB	0.16 ± 0.02^{Bb}	0.14 ± 0.03^{ABab}	0.09 ± 0.02^{Aa}	0.14 ± 0.02^{Ba}	0.11 ± 0.03^{Ba}	0.04 ± 0.02^{Aa}	0.08 ± 0.01^{Ba}	0.03 ± 0.01^{Aa}	0.07 ± 0.02^{Bb}
	PSPV	0.21 ± 0.04^{Bc}	0.18 ± 0.03^{Bb}	0.10 ± 0.03^{Aa}	0.13 ± 0.02^{Ba}	0.10 ± 0.02^{Ba}	0.02 ± 0.00^{Aa}	0.07 ± 0.02^{Ba}	0.03 ± 0.01^{Aa}	0.02 ± 0.01^{Aa}
	PSPL	0.11 ± 0.02^{Ba}	0.13 ± 0.02^{Bab}	0.06 ± 0.01^{Aa}	0.18 ± 0.01^{Cb}	0.10 ± 0.02^{Ba}	0.06 ± 0.01^{Ab}	0.05 ± 0.02^{Aa}	0.02 ± 0.02^{Aa}	0.02 ± 0.01^{Aa}
Glucose	Glucose	8.1 ± 0.1^{Cd}	3.08 ± 0.05^{Bd}	0.72 ± 0.02^{Ae}	16.9 ± 0.02^{Cd}	7.94 ± 0.01^{Bd}	2.94 ± 0.03^{Ad}	13.9 ± 0.02^{Cc}	8.35 ± 0.01^{Bd}	7.06 ± 0.02^{Ae}
	FOS	0.43 ± 0.02^{Bc}	0.38 ± 0.02^{Ac}	0.36 ± 0.01^{Ad}	0.45 ± 0.01^{Ba}	0.47 ± 0.03^{Bc}	0.24 ± 0.02^{Ac}	0.50 ± 0.01^{Bb}	0.47 ± 0.02^{Ac}	0.45 ± 0.01^{Ad}
	Not digested									
	WSPRB	0.08 ± 0.02^{Aa}	0.06 ± 0.02^{Aa}	0.05 ± 0.01^{Ab}	0.17 ± 0.04^{Ba}	0.06 ± 0.01^{Aa}	0.05 ± 0.02^{Aa}	0.11 ± 0.02^{Aa}	0.06 ± 0.03^{Aa}	0.03 ± 0.01^{Aa}
	WSPCB	0.09 ± 0.01^{ABa}	0.05 ± 0.02^{ABa}	0.02 ± 0.01^{Aa}	0.22 ± 0.02^{Ba}	0.12 ± 0.02^{Ab}	0.09 ± 0.02^{Aa}	0.09 ± 0.02^{Aa}	0.10 ± 0.02^{Aab}	0.05 ± 0.02^{Aab}
	PSPV	0.12 ± 0.01^{Bb}	0.05 ± 0.03^{Ab}	0.06 ± 0.01^{Ab}	0.23 ± 0.03^{Bc}	0.11 ± 0.01^{Ab}	0.06 ± 0.01^{Aa}	0.07 ± 0.02^{Aa}	0.07 ± 0.01^{Aa}	0.07 ± 0.02^{Aab}
	PSPL	0.14 ± 0.02^{Bb}	0.11 ± 0.03^{ABa}	0.08 ± 0.02^{Ab}	0.18 ± 0.02^{Cb}	0.09 ± 0.01^{Ab}	0.13 ± 0.03^{Bb}	0.14 ± 0.03^{Ab}	0.12 ± 0.02^{Ab}	0.11 ± 0.03^{Abc}
	Digested									
	WSPRB	0.16 ± 0.04^{Ba}	0.11 ± 0.02^{AB}	0.07 ± 0.02^{Aa}	0.31 ± 0.03^{Cb}	0.21 ± 0.02^{Bb}	0.15 ± 0.03^{Ab}	0.04 ± 0.03^{Aab}	0.03 ± 0.01^{Aa}	0.03 ± 0.01^{Aa}
	WSPCB	0.49 ± 0.05^{Cd}	0.23 ± 0.02^{Bb}	0.19 ± 0.01^{Ab}	0.25 ± 0.03^{Bab}	0.26 ± 0.02^{Bb}	0.10 ± 0.01^{Aa}	0.07 ± 0.01^{Ba}	0.05 ± 0.02^{Aa}	0.02 ± 0.01^{Ab}
	PSPV	0.35 ± 0.05^{Cc}	0.21 ± 0.04^{Bb}	0.12 ± 0.02^{Ab}	0.21 ± 0.05^{Ba}	0.17 ± 0.04^{Ba}	0.09 ± 0.01^{Aa}	0.08 ± 0.03^{Bab}	0.07 ± 0.02^{Bb}	0.03 ± 0.01^{Aa}
	PSPL	0.25 ± 0.03^{Bb}	0.12 ± 0.03^{Aa}	0.09 ± 0.02^{Aa}	0.32 ± 0.05^{Cb}	0.23 ± 0.03^{Bb}	0.12 ± 0.02^{Aab}	0.07 ± 0.03^{Aab}	0.05 ± 0.01^{Aab}	0.04 ± 0.01^{Aa}

(*continued on next page*)

Table 4 (*continued*)

Sugars	Carbon source	Strains								
		L. acidophilus			*L. casei*			*B. animalis*		
		12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h
Maltose	Glucose	NF	NF	NF	NF	NF	NF	NF	NF	NF
	FOS	NF	NF	NF	NF	NF	NF	NF	NF	NF
	Not digested									
	WSPRB	0.08 ± 0.01^{Bab}	0.04 ± 0.01^{Aa}	0.02 ± 0.01^{Aa}	0.08 ± 0.01^{Ba}	0.05 ± 0.01^{Aa}	0.04 ± 0.02^{Aa}	0.17 ± 0.01^{Bb}	0.05 ± 0.01^{Aa}	0.04 ± 0.03^{Aa}
	WSPCB	0.06 ± 0.01^{Ba}	0.03 ± 0.02^{ABa}	0.01 ± 0.00^{Aa}	0.16 ± 0.03^{Bbc}	0.08 ± 0.01^{Ab}	0.07 ± 0.03^{Aa}	0.09 ± 0.01^{Ba}	0.04 ± 0.02^{Aa}	0.04 ± 0.01^{Aa}
	PSPV	0.09 ± 0.04^{Bab}	0.08 ± 0.02^{Bb}	0.04 ± 0.02^{Aa}	0.10 ± 0.02^{Bab}	0.08 ± 0.02^{ABab}	0.06 ± 0.01^{Aa}	0.20 ± 0.03^{Bb}	0.17 ± 0.03^{Bb}	0.05 ± 0.02^{Aa}
	PSPL	0.10 ± 0.01^{Bb}	0.09 ± 0.01^{Ba}	0.02 ± 0.01^{Aa}	0.12 ± 0.02^{Bbc}	0.06 ± 0.01^{Aa}	0.05 ± 0.04^{Aa}	0.21 ± 0.04^{Bb}	0.18 ± 0.03^{Bb}	0.05 ± 0.02^{Aa}
	Digested									
	WSPRB	0.18 ± 0.02^{Ca}	0.10 ± 0.02^{Ba}	0.06 ± 0.01^{Aa}	0.13 ± 0.03^{Ba}	0.08 ± 0.02^{Ba}	0.03 ± 0.01^{Aa}	0.10 ± 0.01^{Ba}	0.05 ± 0.02^{Aa}	0.03 ± 0.01^{Aa}
	WSPCB	0.15 ± 0.03^{Bb}	0.12 ± 0.04^{ABb}	0.07 ± 0.02^{Aa}	0.27 ± 0.03^{Cc}	0.22 ± 0.02^{Bc}	0.07 ± 0.01^{Ab}	0.09 ± 0.01^{Ba}	0.04 ± 0.01^{Aa}	0.02 ± 0.03^{Aa}
	PSPV	0.13 ± 0.04^{Bd}	0.09 ± 0.05^{ABc}	0.05 ± 0.03^{Ac}	0.33 ± 0.04^{Cd}	0.17 ± 0.03^{Bd}	0.09 ± 0.03^{Ac}	0.17 ± 0.03^{Bb}	0.06 ± 0.01^{Aa}	0.06 ± 0.02^{Aa}
	PSPL	0.15 ± 0.02^{Bc}	0.07 ± 0.03^{Ab}	0.09 ± 0.02^{Ab}	0.20 ± 0.03^{Bb}	0.14 ± 0.04^{Ab}	0.08 ± 0.02^{Ab}	0.11 ± 0.03^{Ba}	0.04 ± 0.01^{Aa}	0.03 ± 0.01^{Aa}

WSPRB: flour from sweet potato root variety Rainha Branca (white peel); WSPCB: flour from sweet potato root variety Campina Branca (white peel); PSPV: flour from sweet potato root variety Vitória (purple peel); PSPL: flour from sweet potato root variety Lagoinha (purple peel).

NF: Not found.

A – C: Different superscript capital letters in the same row for the same cultivation media and probiotic strain denote differences ($p \leq 0.05$), based on Tukey's test; a-e: different superscript small letters in the same column at a same time interval and measured sugar denote difference ($p \leq 0.05$) among glucose, FOS and samples not digested *in vitro*, based on Tukey's test; a-d: different superscript small letters in the same column at a same time interval and measured sugar denote difference ($p \leq 0.05$) among samples digested *in vitro*, based on Tukey's test.

Table 5
Contents (g/l) of organic acids in media with glucose (20 g/l), fructooligosaccharides (20 g/l) or flours from different sweet potato roots varieties (20 g/l) not digested and digested *in vitro* and inoculated with *L. acidophilus*, *L. casei* or *B. animalis* during 48 h of incubation.

Organic acids	Carbon source	Strains								
		L. acidophilus			L. casei			B. animalis		
		12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h
Acetic	Glucose	2.22 ± 0.02^{Ab}	2.25 ± 0.02^{Ac}	2.23 ± 0.01^{Ac}	1.49 ± 0.02^{Ab}	1.58 ± 0.03^{Bb}	1.54 ± 0.02^{Ba}	2.28 ± 0.03^{Aa}	2.45 ± 0.01^{Ba}	2.46 ± 0.02^{Ba}
	FOS	2.20 ± 0.01^{Bb}	2.15 ± 0.01^{Ab}	2.12 ± 0.02^{Ab}	1.27 ± 0.03^{Aa}	1.40 ± 0.02^{Ba}	1.53 ± 0.02^{Ca}	2.52 ± 0.03^{Ab}	2.64 ± 0.04^{Bb}	2.83 ± 0.01^{Cb}
	Not digested									
	WSPRB	2.42 ± 0.02^{Ac}	3.64 ± 0.05^{Be}	3.93 ± 0.01^{Ca}	2.31 ± 0.02^{Ad}	2.92 ± 0.03^{Be}	3.2 ± 0.1^{Cc}	2.61 ± 0.02^{Ac}	2.76 ± 0.03^{Bc}	2.88 ± 0.03^{Cc}
	WSPCB	3.4 ± 0.1^{Ae}	3.70 ± 0.04^{Be}	3.50 ± 0.03^{Ae}	1.97 ± 0.01^{Ac}	2.36 ± 0.02^{Bc}	3.8 ± 0.1^{Cd}	2.91 ± 0.03^{Ad}	3.1 ± 0.1^{Bd}	2.95 ± 0.03^{Ad}
	PSPV	2.02 ± 0.02^{Aa}	2.42 ± 0.02^{Cd}	2.90 ± 0.02^{Bd}	2.92 ± 0.04^{Ae}	3.56 ± 0.05^{Cf}	3.20 ± 0.03^{Bb}	2.60 ± 0.01^{Ac}	2.79 ± 0.03^{Bc}	3.02 ± 0.02^{Ce}
	PSPL	3.29 ± 0.05^{Ad}	3.83 ± 0.01^{Ba}	3.96 ± 0.02^{Cc}	2.86 ± 0.03^{Be}	2.54 ± 0.02^{Ad}	4.3 ± 0.1^{Ce}	3.20 ± 0.05^{Ae}	3.27 ± 0.05^{Ae}	3.2 ± 0.1^{Af}
	Digested									
	WSPRB	2.00 ± 0.02^{Ac}	2.30 ± 0.02^{Cb}	2.14 ± 0.01^{Ba}	2.70 ± 0.02^{Ac}	2.85 ± 0.03^{Bc}	3.08 ± 0.03^{Cb}	2.00 ± 0.02^{Aa}	2.55 ± 0.03^{Ba}	3.9 ± 0.1^{Cd}
	WSPCB	1.94 ± 0.02^{Ab}	2.36 ± 0.03^{Bc}	2.57 ± 0.03^{Cc}	2.63 ± 0.02^{Ab}	2.70 ± 0.02^{Bb}	3.12 ± 0.03^{Cb}	2.14 ± 0.05^{Ac}	2.64 ± 0.02^{Cb}	2.54 ± 0.01^{Ba}
	PSPV	2.32 ± 0.03^{Cd}	2.17 ± 0.01^{Aa}	2.34 ± 0.02^{Bb}	2.60 ± 0.02^{Ab}	2.84 ± 0.03^{Cc}	2.70 ± 0.02^{Ba}	2.88 ± 0.03^{Bb}	3.03 ± 0.04^{Cc}	2.73 ± 0.02^{Ab}
	PSPL	1.84 ± 0.01^{Aa}	2.48 ± 0.02^{Bd}	2.96 ± 0.04^{Cd}	2.13 ± 0.01^{Aa}	2.30 ± 0.02^{Ba}	3.10 ± 0.04^{Cb}	2.88 ± 0.04^{Bb}	2.63 ± 0.02^{Ab}	2.80 ± 0.04^{Bc}
Formic	Glucose	0.09 ± 0.01^{Ab}	0.11 ± 0.02^{Aab}	NF	0.20 ± 0.02^{Ab}	NF	NF	NF	NF	NF
	FOS	0.11 ± 0.02^{Ab}	0.10 ± 0.03^{Aab}	NF	0.16 ± 0.02^{Ab}	NF	NF	0.21 ± 0.01^{Ab}	0.34 ± 0.02^{B}	NF
	Not digested									
	WSPRB	0.05 ± 0.00^{Aa}	0.14 ± 0.02^{Cb}	0.09 ± 0.01^{Ba}	0.08 ± 0.01^{Aa}	0.14 ± 0.03^{Ba}	0.15 ± 0.02^{Ba}	0.17 ± 0.04^{Ab}	NF	NF
	WSPCB	0.08 ± 0.03^{Aab}	0.20 ± 0.02^{Cc}	0.15 ± 0.02^{Bb}	0.07 ± 0.02^{Aa}	0.11 ± 0.02^{Aa}	0.19 ± 0.02^{Ba}	0.15 ± 0.03^{Aa}	NF	NF
	PSPV	0.04 ± 0.01^{Aa}	0.09 ± 0.01^{Ba}	0.11 ± 0.01^{Ba}	0.07 ± 0.02^{Aa}	0.10 ± 0.02^{Aa}	0.13 ± 0.01^{Bb}	0.11 ± 0.02^{Aa}	NF	NF
	PSPL	0.07 ± 0.02^{Aab}	0.08 ± 0.02^{Aa}	0.12 ± 0.01^{Bab}	0.11 ± 0.02^{Aa}	0.13 ± 0.01^{Aa}	0.20 ± 0.03^{Ba}	0.19 ± 0.03^{Ab}	NF	NF
	Digested									
	WSPRB	0.04 ± 0.01^{Aa}	0.12 ± 0.02^{Bc}	0.12 ± 0.01^{Bc}	0.06 ± 0.02^{Aa}	0.10 ± 0.02^{Aa}	0.08 ± 0.01^{Aa}	0.13 ± 0.04^{a}	NF	NF
	WSPCB	0.02 ± 0.01^{Aa}	0.12 ± 0.02^{Bc}	0.14 ± 0.02^{Bc}	0.05 ± 0.01^{Aa}	0.06 ± 0.02^{Aa}	0.07 ± 0.02^{Aa}	0.12 ± 0.03^{a}	NF	NF
	PSPV	0.04 ± 0.01^{Aa}	0.07 ± 0.01^{Bb}	0.05 ± 0.00^{Ab}	0.05 ± 0.01^{Aa}	0.08 ± 0.01^{Ba}	ND	0.09 ± 0.02^{a}	NF	NF
	PSPL	0.02 ± 0.01^{Aa}	0.02 ± 0.01^{Aa}	0.01 ± 0.00^{Aa}	0.04 ± 0.00^{Aa}	0.07 ± 0.01^{Ba}	ND	0.10 ± 0.03^{a}	NF	NF

(*continued on next page*)

Table 5 (*continued*)

Organic acids	Carbon source	Strains								
		L. acidophilus			L. casei			B. animalis		
		12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h
Lactic	Glucose	11.5 ± 0.02^{Ae}	17.6 ± 0.01^{Bf}	22.0 ± 0.01^{Cf}	22.5 ± 0.03^{Ce}	18.6 ± 0.01^{Af}	22.3 ± 0.02^{Bf}	12.1 ± 0.01^{Ae}	17.4 ± 0.01^{Bf}	19.6 ± 0.03^{Cf}
	FOS	10.9 ± 0.02^{Ad}	16.5 ± 0.01^{Be}	18.7 ± 0.03^{Ce}	17.9 ± 0.03^{Bd}	11.5 ± 0.01^{Ae}	20.1 ± 0.02^{Ce}	1.49 ± 0.01^{Aa}	1.67 ± 0.01^{Ba}	9.98 ± 0.01^{Ce}
	Not digested									
	WSPRB	2.20 ± 0.02^{Aa}	3.05 ± 0.02^{Bb}	3.56 ± 0.02^{Ca}	1.66 ± 0.02^{Aa}	2.73 ± 0.04^{Bb}	2.90 ± 0.02^{Ca}	2.98 ± 0.03^{Cb}	2.52 ± 0.03^{Ab}	2.78 ± 0.03^{Ba}
	WSPCB	3.9 ± 0.1^{c}	3.68 ± 0.03^{Bc}	2.18 ± 0.03^{Ac}	1.40 ± 0.02^{Aa}	2.37 ± 0.03^{Ba}	4.17 ± 0.03^{Cb}	4.37 ± 0.04^{Cc}	3.82 ± 0.03^{Bc}	3.55 ± 0.04^{Ab}
	PSPV	3.29 ± 0.05^{Ab}	5.7 ± 0.1^{Cd}	4.6 ± 0.1^{Bd}	3.28 ± 0.04^{Ac}	6.6 ± 0.1^{Cd}	4.33 ± 0.04^{Bc}	6.4 ± 0.1^{Bd}	5.1 ± 0.1^{Ae}	6.7 ± 0.1^{Cd}
	PSPL	3.33 ± 0.02^{Bb}	1.96 ± 0.03^{Aa}	1.91 ± 0.02^{Ab}	1.95 ± 0.03^{Ab}	3.01 ± 0.04^{Bc}	4.6 ± 0.1^{Cd}	4.45 ± 0.05^{Ac}	4.41 ± 0.05^{Ad}	4.8 ± 0.1^{Bc}
	Digested									
	WSPRB	1.40 ± 0.02^{Ab}	2.10 ± 0.02^{Ba}	2.07 ± 0.02^{Ba}	1.80 ± 0.02^{Ab}	2.65 ± 0.03^{Ca}	1.97 ± 0.02^{Ba}	4.7 ± 0.1^{Cd}	2.37 ± 0.02^{Aa}	2.77 ± 0.03^{Ba}
	WSPCB	1.37 ± 0.02^{Ab}	2.40 ± 0.02^{Bb}	3.35 ± 0.04^{Bb}	1.90 ± 0.03^{Ac}	3.58 ± 0.05^{Bc}	3.66 ± 0.04^{Bc}	4.0 ± 0.1^{Cb}	3.39 ± 0.04^{Ac}	3.65 ± 0.01^{Bc}
	PSPV	1.80 ± 0.03^{Ac}	3.42 ± 0.04^{Bd}	4.8 ± 0.1^{Cc}	1.90 ± 0.04^{Ac}	5.0 ± 0.1^{Bd}	5.1 ± 0.1^{Bd}	4.42 ± 0.05^{Ac}	5.74 ± 0.05^{Cd}	4.68 ± 0.05^{Bd}
	PSPL	1.21 ± 0.01^{Aa}	3.22 ± 0.05^{Bc}	4.81 ± 0.04^{Cc}	1.53 ± 0.02^{Aa}	3.18 ± 0.04^{Bb}	3.50 ± 0.03^{Cb}	3.71 ± 0.05^{Ca}	3.23 ± 0.03^{Bb}	3.05 ± 0.04^{Ab}
Malic	Glucose	0.31 ± 0.01^{Ac}	NF	NF	0.22 ± 0.02^{Ac}	NF	NF	0.08 ± 0.02^{Aa}	0.11 ± 0.02^{Ab}	NF
	FOS	0.09 ± 0.01^{Ab}	NF	NF	0.13 ± 0.02^{Ab}	0.22 ± 0.02^{Bb}	0.23 ± 0.03^{Bb}	0.06 ± 0.02^{Aa}	0.05 ± 0.02^{Aa}	NF
	Not digested									
	WSPRB	NF	0.04 ± 0.02^{Aa}	0.02 ± 0.01^{Aa}	0.04 ± 0.02^{Aa}	0.05 ± 0.02^{Aa}	0.06 ± 0.02^{Aa}	0.08 ± 0.03^{Aa}	0.04 ± 0.01^{Aa}	0.04 ± 0.02^{Aa}
	WSPCB	0.05 ± 0.02^{Aa}	0.05 ± 0.02^{Aa}	0.03 ± 0.01^{Aa}	0.03 ± 0.01^{Aa}	0.04 ± 0.01^{Aa}	0.08 ± 0.02^{Ba}	0.07 ± 0.03^{Aa}	0.05 ± 0.02^{Aa}	0.05 ± 0.02^{Aa}
	PSPV	0.03 ± 0.01^{Aa}	0.04 ± 0.02^{Aa}	0.04 ± 0.02^{Aa}	0.05 ± 0.02^{Aa}	0.07 ± 0.03^{Aa}	0.04 ± 0.02^{Aa}	0.06 ± 0.02^{Aa}	0.04 ± 0.01^{Aa}	0.05 ± 0.02^{Aa}
	PSPL	NF	0.02 ± 0.01^{Aa}	0.03 ± 0.01^{Aa}	0.05 ± 0.02^{Aa}	0.04 ± 0.01^{Aa}	0.08 ± 0.03^{Aa}	0.07 ± 0.03^{Aa}	0.05 ± 0.02^{Aa}	0.05 ± 0.01^{Aa}
	Digested									
	WSPRB	0.11 ± 0.02^{Aa}	0.10 ± 0.01^{Aa}	0.11 ± 0.02^{Aa}	NF	0.06 ± 0.01^{Aa}	0.05 ± 0.01^{Aa}	0.03 ± 0.00^{Aa}	0.04 ± 0.01^{Aa}	0.06 ± 0.02^{Ba}
	WSPCB	0.14 ± 0.02^{Aa}	0.13 ± 0.02^{Aa}	0.13 ± 0.03^{Aa}	NF	0.16 ± 0.04^{Bb}	0.06 ± 0.01^{Aa}	0.06 ± 0.03^{Aa}	0.05 ± 0.02^{Aa}	0.05 ± 0.02^{Aa}
	PSPV	0.13 ± 0.03^{Aa}	0.13 ± 0.02^{Aa}	0.11 ± 0.01^{Aa}	NF	0.16 ± 0.03^{Bb}	0.05 ± 0.02^{Aa}	0.04 ± 0.01^{Aa}	0.05 ± 0.02^{Aa}	0.04 ± 0.01^{Aa}
	PSPL	0.12 ± 0.01^{Aa}	0.14 ± 0.03^{Aa}	0.13 ± 0.02^{Aa}	NF	0.14 ± 0.02^{Bb}	0.05 ± 0.02^{Aa}	0.05 ± 0.01^{Aa}	0.04 ± 0.01^{Aa}	0.04 ± 0.01^{Aa}
Succinic	Glucose	1.55 ± 0.05^{Cb}	1.40 ± 0.02^{ABa}	1.43 ± 0.04^{Bc}	1.33 ± 0.03^{Ad}	1.39 ± 0.04^{Af}	1.37 ± 0.03^{Ae}	0.43 ± 0.02^{Ca}	0.40 ± 0.01^{BCa}	0.33 ± 0.01^{Aa}
	FOS	1.6 ± 0.1^{Bb}	1.42 ± 0.02^{Aa}	1.44 ± 0.04^{Ac}	1.28 ± 0.02^{Bc}	1.21 ± 0.04^{Ae}	1.26 ± 0.01^{Bd}	0.52 ± 0.02^{Bb}	0.42 ± 0.02^{Aa}	0.44 ± 0.02^{Ab}
	Not digested									
	WSPRB	1.41 ± 0.02^{Ba}	1.53 ± 0.01^{Cb}	0.80 ± 0.01^{Aa}	1.06 ± 0.02^{Cb}	0.50 ± 0.01^{Ab}	0.56 ± 0.02^{Bb}	0.51 ± 0.01^{Ab}	0.54 ± 0.02^{Ac}	0.60 ± 0.03^{Bcd}
	WSPCB	2.44 ± 0.05^{Cd}	2.16 ± 0.03^{Bd}	1.46 ± 0.03^{Ac}	0.84 ± 0.01^{Ca}	0.34 ± 0.01^{Aa}	0.65 ± 0.02^{Bc}	0.53 ± 0.02^{Ab}	0.56 ± 0.03^{Ac}	0.56 ± 0.02^{Ac}
	PSPV	2.07 ± 0.01^{Cc}	1.61 ± 0.02^{Bc}	1.11 ± 0.02^{Ab}	1.10 ± 0.03^{Bb}	1.05 ± 0.03^{Bd}	0.46 ± 0.01^{Aa}	0.58 ± 0.02^{Bc}	0.48 ± 0.02^{Ab}	0.61 ± 0.02^{Bd}
	PSPL	2.77 ± 0.03^{Ce}	1.60 ± 0.01^{Bc}	1.47 ± 0.03^{Ac}	1.11 ± 0.03^{Bb}	0.70 ± 0.02^{Ac}	1.92 ± 0.04^{Cf}	0.60 ± 0.03^{Ac}	0.64 ± 0.03^{Bd}	0.66 ± 0.04^{Bd}
	Digested									
	WSPRB	2.05 ± 0.04^{Cb}	1.45 ± 0.05^{Ba}	1.31 ± 0.02^{Ab}	3.12 ± 0.05^{Cd}	1.65 ± 0.02^{Ba}	1.55 ± 0.02^{Ac}	4.03 ± 0.02^{Ab}	4.75 ± 0.02^{Ba}	5.94 ± 0.03^{Cc}
	WSPCB	1.93 ± 0.03^{Ca}	1.61 ± 0.04^{Bb}	1.33 ± 0.02^{Ab}	2.96 ± 0.04^{Cc}	1.84 ± 0.03^{Bc}	0.59 ± 0.01^{Aa}	0.55 ± 0.03^{Bc}	0.50 ± 0.03^{Ba}	0.27 ± 0.01^{Aa}
	PSPV	2.36 ± 0.04^{Cc}	1.85 ± 0.06^{Bc}	1.03 ± 0.02^{Aa}	2.84 ± 0.04^{Cb}	2.01 ± 0.03^{Bd}	0.77 ± 0.01^{Ab}	0.37 ± 0.03^{Aa}	0.50 ± 0.03^{Ba}	0.42 ± 0.02^{Ab}
	PSPL	1.93 ± 0.03^{Ca}	1.60 ± 0.02^{Bb}	1.44 ± 0.03^{Ac}	2.71 ± 0.04^{Ca}	1.70 ± 0.02^{Ab}	1.97 ± 0.03^{Bd}	0.55 ± 0.03^{Bc}	0.46 ± 0.02^{Aa}	0.44 ± 0.02^{Ab}

(*continued on next page*)

Table 5 (*continued*)

Organic acids	Carbon source	Strains								
		L. acidophilus			L. casei			B. animalis		
		12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h
Tartaric	Glucose	NF	NF	NF	NF	NF	NF	NF	NF	NF
	FOS	NF	NF	NF	NF	NF	NF	NF	NF	NF
	Not digested									
	WSPRB	0.04 ± 0.01^{Aa}	NF	NF	0.02 ± 0.00^{Aa}	0.02 ± 0.00^{Aa}	0.03 ± 0.01^{Aa}	NF	NF	NF
	WSPCB	0.02 ± 0.01^{Aa}	NF	NF	0.02 ± 0.00^{Aa}	0.02 ± 0.00^{Aa}	0.02 ± 0.01^{Aa}	NF	NF	NF
	PSPV	0.01 ± 0.00^{Aa}	NF	NF	0.03 ± 0.01^{Aa}	0.02 ± 0.00^{Aa}	0.02 ± 0.00^{Aa}	0.06 ± 0.03^{Ba}	0.03 ± 0.01^{Aa}	0.04 ± 0.02^{A}
	PSPL	0.02 ± 0.01^{Aa}	NF	NF	0.02 ± 0.00^{Aa}	0.02 ± 0.01^{Aa}	0.02 ± 0.00^{Aa}	0.08 ± 0.01^{Ba}	0.03 ± 0.02^{Aa}	ND
	Digested									
	WSPRB	0.01 ± 0.00^{Aa}	0.01 ± 0.00^{Aa}	0.01 ± 0.00^{Aa}	0.05 ± 0.03^{Aa}	0.02 ± 0.01^{Aa}	0.03 ± 0.00^{Aa}	0.02 ± 0.00^{Aa}	NF	0.02 ± 0.01^{Aa}
	WSPCB	0.01 ± 0.00^{Aa}	0.01 ± 0.00^{Aa}	0.01 ± 0.00^{Aa}	0.03 ± 0.02^{Aa}	0.01 ± 0.00^{Aa}	0.03 ± 0.02^{Aa}	0.02 ± 0.00^{Aa}	0.02 ± 0.00^{Aa}	0.03 ± 0.02^{Bb}
	PSPV	0.02 ± 0.00^{Aa}	0.01 ± 0.00^{Aa}	NF	0.02 ± 0.01^{Aa}	0.01 ± 0.00^{Aa}	0.03 ± 0.02^{Aa}	0.01 ± 0.00^{Aa}	0.05 ± 0.01^{Bb}	0.02 ± 0.01^{Aa}
	PSPL	0.02 ± 0.00^{Aa}	0.01 ± 0.00^{Aa}	NF	0.01 ± 0.00^{Aa}	0.01 ± 0.00^{Aa}	0.03 ± 0.02^{Aa}	0.04 ± 0.02^{Aa}	0.02 ± 0.01^{Aa}	0.01 ± 0.01^{Aa}

WSPRB: flour from sweet potato root variety Rainha Branca (white peel); WSPCB: flour from sweet potato root variety Campina Branca (white peel); PSPV: flour from sweet potato root variety Vitória (purple peel); PSPL: flour from sweet potato root variety Lagoinha (purple peel).
NF: Not found.
A – C: different superscript capital letters in the same row for the same cultivation media and probiotic strain denote differences ($p \leq 0.05$), based on Tukey's test; a-f: different superscript small letters in the same column at a same time interval and measured organic acid denote difference ($p \leq 0.05$) among glucose, FOS and samples not digested *in vitro*, based on Tukey's test; a-d: different superscript small letters in the same column at a same time interval and measured organic acid denote difference ($p \leq 0.05$) among samples digested *in vitro*, based on Tukey's test.

The presence of resistant starch, soluble fibers, insoluble fibers and kestose in WSPRB, WSPCB, PSPV and PSPL showed the availability of carbohydrates resistant to digestion and absorption in the human small intestine, which can reach the colon to be metabolized by gut microbiota (Guo et al., 2019; Teixeira et al., 2016; Zhang et al., 2018a). The highest viable counts of the probiotics as well as the lowest pH values were observed overall in media with not digested PSPV and PSPL. These findings could be related to the higher contents of maltose and nystose in PSPV and PSPL when compared to WSPRB and WSPCB, allowing PSPV and PSPL to exert higher growth-promoting effects on *Lactobacillus* and *Bifidobacterium*.

Lactobacillus and *Bifidobacterium* strains showed overall similar growth behavior in media with not digested and digested WSPRB, WSPCB, PSPV and PSPL, indicating that the observed prebiotic effects were promoted by non-absorbable nutrients that remain available in the colon (Espirito-Santo et al., 2014; Huang et al., 2019). The available literature presents evidence that phenolic compounds (*e.g.*, catechin, epigallocatechin gallate and rutin) identified in WSPRB, WSPCB, PSPV and PSPL can selectively stimulate the growth of beneficial colonic microbiota, including *Lactobacillus* and *Bifidobacterium* species (Albuquerque et al., 2019; Sun et al., 2018). Although some phenolic compounds (*e.g.*, chlorogenic acid, p-coumaric acid and procyanidin) found in WSPRB, WSPCB, PSPV and PSPL are typically found conjugated to fibers or starch in tubers (Mousavi & Mousavi, 2019; Velderrain-Rodríguez et al., 2016), conditions mimicking the gastrointestinal digestion and fermentation have been shown to be capable of releasing conjugated forms of phenolic compounds, increasing their availability for use by gut microbiota (Giusti, Capuano, Sagratini, & Pellegrini, 2019; Juániz et al., 2017). The presence of soluble and insoluble fibers, resistant starch, FOS and a variety of phenolic compounds in WSPRB, WSPCB, PSPV and PSPL should be associated with their potential prebiotic properties.

Lactobacillus and *Bifidobacterium* strains were capable of metabolizing glucose, fructose and maltose in media with not digested and digested WSPRB, WSPCB, PSPV and PSPL to produce acetic, formic, lactic, malic, succinic and tartaric acids. Lactic and acetic acids were produced at the highest levels in different cultivation media with SPRF regardless of the inoculated probiotic strain. Lactic acid is the main end product of the metabolism of *Lactobacillus* species (Zhao & Ganzle, 2018), being an important fermentation end product of *Bifidobacteirum* species (Kaprasob, Kerdchoechuen, Laohakunjit, & Somboonpanyakul, 2018). Acetic acid is a short chain fatty acid produced by *Lactobacillus* and *Bifidobacterium* during carbohydrates colonic fermentation (Patrignani et al., 2018).

The detection of formic, malic, succinic and tartaric acids in variable amounts during the incubation period in media with SPRF could be related to the presence of these organic acids in SPRF and/or with the capability of the inoculated probiotic strains of producing these organic acids during the fermentation of carbohydrates present in SPRF (Dessie et al., 2018; Sousa et al., 2015). The enhanced production of organic acids in the colon has been typically associated with the inhibition of enteric pathogens, lowering the occurrence of subclinical infections and secretion of immune mediators, as well as with increased mineral bioavailability, water and sodium intestinal absorption (Ambalam et al., 2015; Wei et al., 2018).

Results showing the potential prebiotic properties of SPRF when obtained using *in vitro* tests indicative of selective stimulatory effects on *Lactobacillus* and *Bifidobacterium* species known to form beneficial microbial groups of gut microbiota should present limitations to be extrapolated to the achievement of prebiotic effects under *in vivo* conditions. A number of factors, such as diet composition, abundance and types of microorganisms forming the host gut microbiota, peristaltic flow and individual host response, could affect the prebiotic effects from the consumption of prebiotics and, ultimately, the outcomes on the host health (Modrackova et al., 2019; Seong et al., 2019; Souza, Jonathan, Saad, Schols, & Venema, 2019). However, results of *in vitro* assays, such as those used in this investigation, have been considered important evidence of the potential prebiotic properties of foods and ingredients from conventional and non-conventional sources (Duarte et al., 2017; Gómez et al., 2019; Huang et al., 2019; Martinez-Gutierrez et al., 2017; Sousa et al., 2015; Zhang et al., 2018b).

5. Conclusion

The results showed that flours from WSPRB, WSPCB, PSPV and PSPL have variable amounts of fiber, resistant starch, FOS, phenolic compounds and sugars. These flours led to positive prebiotic activity scores with probiotic strains, indicating their ability to selectively stimulate beneficial bacterial while limiting enteric competitors. The cultivation of *L. acidophilus*, *L. casei* and *B. animalis* in media with either not digested or digested WSPRB, WSPCB, PSPV and PSPL resulted in high bacterial counts, decreased pH values, increased production of organic acids and consumption of sugars over time, which are indicative of intense bacterial metabolic activities on these substrates, either forming or using various compounds. Flours from varieties of white and purple peel SPR cultivated in Northeastern Brazil should be considered potential prebiotic ingredients for use in the formulation of functional foods or dietary supplements.

CRediT authorship contribution statement

Thatyane Mariano Rodrigues de Albuquerque: Conceptualization, Methodology, Investigation, Validation, Writing - review & editing. **Camyla Wanderley Pereira Borges:** Methodology, Investigation, Validation. **Mônica Tejo Cavalcanti:** Methodology, Investigation, Validation. **Marcos dos Santos Lima:** Methodology, Investigation, Validation. **Marciane Magnani:** Writing - review & editing. **Evandro Leite de Souza:** Conceptualization, Methodology, Investigation, Validation, Writing - review & editing.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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APÊNDICE C – ARTIGO II

Flours from different sweet potato (*Ipomoea batatas* L.) root varieties modulate positively the composition and metabolic activity of human colonic microbiota *in vitro*

Abstract

The effects of flours from four different sweet potato root (SPR) varieties on human colonic microbiota during a 48 h-*in vitro* fermentation were evaluated. Examined SPR flours (SPRF) increased the relative abundance of *Lactobacillus* - *Enterococcus* and *Bifidobacterium* populations and decreased the relative abundance of *Bacteroides* - *Prevotella*, *C. histolyticum* and *E. rectall* - *C. coccoides* populations during the colonic fermentation. Different examined SPRF had positive prebiotic indexes after 24 and 48 h of colonic fermentation. Increased microbial metabolic activity was evidenced with decreased pH and increased sugar consumption and lactic acid and short chain fatty acids production in media during the 48 h-colonic fermentation. These results indicate the ability of the examined SPRF to modulate positively the composition and metabolic activity of human colonic microbiota, which should be linked to their prebiotic properties. SPRF are potential prebiotic ingredients to be used in the formulation of added-value dietary supplements or functional foods.

Keywords: Tuber, gut microbiota, colonic fermentation, modulatory effects, prebiotic properties.

1. Introduction

Sweet potato (*Ipomoea batatas* L.) is one of the most important plant foods in the world, producing more biomass and nutrients than any other food crop (Wang, Nie, & Zhu, 2016). Sweet potato (SP) is cultivated in tropical and warm temperature regions because of the high capacity of resistance to different environmental, soil and temperature conditions (Marczak, Sawicka, & Salach, 2018; Santos et al., 2019), with a worldwide annual production around 100 million tonnes (FAOSTAT, 2019).

The occurrence of a great genetic diversity has resulted in different SP varieties, which have shown roots, as the traditional SP edible part, with distinct physical (*e.g.*, peel and flesh color) and nutritional characteristics (Cartier et al., 2017). Differences in profile of health-related bioactive compounds in sweet potato roots (SPR) and their by-products have been also found to be variety-dependent (Albuquerque, Sampaio, & Souza, 2019; Albuquerque et al., 2020; Ju, Mu, & Sun, 2017). SPR flours (SPRF) have been an alternative ingredient to formulate different foods, such as bread, cookies and pasta, in partial or total replacement of wheat flour in order to improve nutritional, sensory and technological characteristics of these products (Saleh, Lee, & Abeidat, 2018; Zhu & Sun, 2019).

Potential health-promoting properties found in SPR include prebiotic effects primarily related to their high contents of non-digestible fermentable carbohydrates (Guo, Zhao, Li, & Miao, 2019; Zheng et al., 2016). Prebiotics have been defined as substrates fermented selectively by host microorganisms conferring a health benefit (Gibson et al., 2017). Interactions between human colon microbiota and non-digestible dietary components exert important role in human health (Singh et al., 2017; Wang et al., 2020). Abundance and diversity of intestinal microbiota increase gradually from small intestine, cecum to colon due to varying physiological environments, such as chemical and nutrient gradients, pH, host immune activity and intestinal content transit time (Cheng et al., 2020; Singh et al., 2017;

Wang et al., 2020). Positive impacts on health caused by prebiotics have been linked specifically to fermentation of substrate in the colon, inducing alterations in gut microbiota due to different mechanisms, including: (i) competition of gut microorganisms for prebiotics, resulting in selective colonization by beneficial microorganisms and exclusion of pathogens in gut epithelial cells; (ii) production of short chain fatty acids (SCFA); and (iii) modulation of immune response, enhancing the intestinal barrier function (Gibson et al., 2017; Sanders, Merenstein, Reid, Gibson, & Rastall, 2019; Wang et al., 2020).

Considering the reported outstanding nutritional value, bioactive compound profile and health-related properties of different SPR varieties (Albuquerque et al., 2019; Albuquerque et al., 2020), this study hypothesized that SPRF could modulate positively the composition and metabolic activity of human colonic microbiota. To test this hypothesis, this study evaluated the effects of flours from two white and two purple peel varieties of SPR in an *in vitro* colonic fermentation system with measurements of the relative abundance of specific bacterial groups forming the human gut microbiota, as well as of the variation of pH values and organic acid and sugar contents in fermentation medium over time.

2. Materials and methods

2.1 Preparation of SPRF

Sweet potato (*Ipomoea batatas* L., white flesh) roots used were two with white peel, namely Rainha branca (WSPRB) and Campina branca (WSPCB), and two with purple peel, namely Vitória (PSPV) and Lagoinha (PSPL). These SPR were obtained from an experimental station of the Agronomic Institute of Pernambuco located in the municipality of Itapirema (07°38′24.1″ latitude and 34°57′22.0″ longitude, Pernambuco, Brazil). SPR were washed in running water, immersed in sodium hypochlorite (Lafepe, Pernambuco, Brazil) aqueous solution (200 ppm) for 15 min at room temperature (25 ± 1 °C), manually peeled and

sliced (~3 mm thick). Blanching for inactivation of enzymes was done by immersion of SPR slices in hot water (100 °C) for 2 s followed by a cold bath (7 °C). SPRF were produced by drying the SPR slices in an oven (American Lab, São Paulo, Brazil) with forced air circulation (55 °C, 6 h), followed by grounding with a domestic mixer (Philips Walita, Minas Gerais, Brazil) to obtain a fine powder (Albuquerque et al., 2020; Khanh, Chittrakorn, Rutnakornpituk, Tai, & Ruttarattanamongkol, 2018). SPRF were stored at room temperature in hermetically sealed polyethylene bags (Zip Lock, Brazilian Plast, São Paulo, Brazil) for up to six months. SPRF was tested individually in all experiments. The physicochemical characteristics of examined SPRF were reported in a previously published study, as shown in Table 1 (Albuquerque et al., 2020).

2.2 In vitro digestion of SPRF

The SPRF were submitted to an *in vitro* digestion to be transformed by solutions in materials with characteristic similar to those that reach the human colon. Initially, 10 g of the flours were mixed with 50 mL of sterile distilled water and manually stirred for 5 min. A mixture of α-amylase (3.33 mg) and 1 M $CaCl_2$ (1.04 mL, pH 7) was added to SPRF solution and maintained at 37 °C for 30 min with stirring (130 rpm). pH of the mixture was adjusted to 2 – 2.5 with 1 M HCl, pepsin added (0.45 g in 0.1 M HCl, 4.16 mL) and maintained at 37 °C for 2 h with stirring (130 rpm). Bile (0.58 g) and pancreatin (0.93 g) mixed with 20.8 mL of 0.5 M $NaHCO_3$ were incorporated into the mixture, the pH was adjusted to 6.5 – 7 with 1 M $NaHCO_3$ and maintained at 37 °C for 2 h with stirring (45 rpm). Enzymes and reagents used to simulate the gastrointestinal digestion were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Final digested SPRF solutions were transferred to 1 kDa nominal molecular weight cut-off regenerated cellulose dialysis tubing (Spectra/Por® 6, Spectrum Europe BV, Breda,

Netherlands) and dialyzed against 0.01 M NaCl at 5 ± 0.5 °C to remove low molecular mass digestion products. After 15 h, dialyzed fluids were replaced and process continued for 2 h. Dialyzed digested SPRF was frozen (−18 °C), freeze-dried (temperature −55 ± 2 °C, vacuum pressure <138 μHg, freeze-drying speed 1 mm/h) for 14 – 16 h with a liophylizer (Liotop, São Paulo, Brazil) and stored (5 ± 0.5 °C) in hermetically sealed polyethylene bags for a maximum period of four weeks (Albuquerque et al., 2020; Rodrigues et al., 2016).

2.3 Preparation of human fecal inoculum

Fecal samples were collected from four healthy adult volunteers (two men and two women, 19- to 36-years old), which declared not suffering from any known colonic conditions. Volunteers ingested an omnivorous diet and had not ingested antibiotics or other medicines known to affect the microbiota for at least 6 months. Volunteers were not regular consumers of concentrated prebiotics or probiotics. Fecal samples were collected into sterile tubes arranged in an anaerobic jar with an anaerobiosis generator system (AnaeroGen, Oxoid, Basingstoke, England). Fecal samples had absence of parasites and blood, being used within 30 min of collect. Fresh fecal samples were mixed with an equal amount of feces from each volunteer (1:1:1:1, w/w), diluted (1:10, w/v) in sterile phosphate-buffered saline (PBS; 0.1 M; pH 7.4) and homogenized for 2 min with stirring (200 rpm) (Rodrigues et al., 2016).

Fecal slurry (120 g) was pre-cultured (37 ± 0.5 °C) under anaerobic conditions (AnaeroGen) in a pre-culture medium (1 L) composed of 10 g tryptone, 5 g yeast extract, 10 g NaCl, 5 g glucose, 6 g lactose and distilled water previously sterilized by autoclavation (121°C, 1 atm, 15 min). After an overnight cultivation, pre-culture was filtered with a triple layer of sterile gauze to remove large particles and stored (37 ± 0.5 °C) in sterile vials under anaerobic conditions (AnaeroGen) (Hu, Nie, Li, & Xie, 2013; Menezes et al., 2020). Ingredients used to prepare pre-culture medium were obtained from Sigma-Aldrich.

2.4 Preparation of fermentation media

A culture medium (1 L) composed of 4.5 g NaCl, 4.5 g KCl, 1.5 g $NaHCO_3$, 0.69 g $MgSO_4$, 0.8 g L-cysteine, 0.5 g KH_2PO_4, 0.5 g K_2HPO_4, 0.4 g bile salt, 0.08 g $CaCl_2$, 0.005 g $FeSO_4$, 1 mL Tween 80 and 4 mL resazurin solution (0.025%, w/v, as an anaerobic indicator) in distilled water and sterilized by autoclavation (121 °C, 1 atm, 15 min) was used to comprise single batch-culture fermentation systems. Digested WSPRB, WSPCB, PSPV and PSPL were used for fermentation with pre-cultured human faecal microbiota. Final fermentation volume was composed of 40% of fermentation medium (v/v), 40% of human fecal pre-culture (v/v) and 20% of digested WSPRB, WSPCB, PSPV or PSPL (w/v) and incubated anaerobically (AnaeroGen) at 37 °C for 48 h. Fermentation media with fructooligosaccharides (FOS, a well-known prebiotic, 20%, w/v) and with no added substrate were tested as positive and negative controls, respectively (Hu et al., 2013; Menezes et al., 2020). Ingredients used to prepare fermentation media were obtained from Sigma-Aldrich.

2.5 Enumeration of bacterial population by fluorescent in situ hybridization coupled with multiparametric flow cytometry

Fluorescent *in situ* hybridization (FISH) technique using selected oligonucleotide probes designed to target specific regions of 16S rRNA gene of distinct bacterial groups combined with multiparametric flow cytometry (MFC) was used to evaluate the capability of digested WSPRB, WSPCB, PSPV and PSPL of inducing changes in composition of colonic bacterial population (Menezes et al., 2020; Conterno et al. 2019). Five different probes (Bif 164 specific to *Bifidobacterium*, Lab 158 specific to *Lactobacillus -Enterococcus*, Bac 303 specific to *Bacteroides - Prevotella*, Erec 482 specific to *Eubacterium rectall - Clostridium coccoides* and Chis 150 specific to *Clostridium histolyticum*) commercially synthesized and

fluorescent Cy3-labelled (Sigma-Aldrich) were used (Menezes et al., 2020; Rodrigues et al., 2016). SYBR Green (Molecular Probes, Invitrogen, Carlsbad, CA, USA) was used to enumerate the total bacterial population by staining double stranded DNA (Conterno et al. 2019).

At the time zero (*i.e.*, just after homogenization of batch-culture medium components) and after 24 and 48 h of fermentation, 375 µL aliquots of cultures were fixed overnight (4 ± 0.5 °C) with 1125 µL of 4% filtered paraformaldehyde solution, centrifuged (10,000 × *g*, 5 min, 4 °C), washed twice (10,000 × *g*, 5 min, 4 °C) with sterile PBS 1 M, resuspended in 300 µL PBS:ethanol 99% (1:1 v/v), filtered with a 0.45 µm-pore size filter (Whatman®, GE Healthcare, Chicago, EUA) and stored (-20 °C). Ten µL aliquots of fixed cells were resuspended in 190 µL of sterile PBS 1X (Gibco®, Gaithersburg, USA; pH 7.2), centrifuged (4,000 × *g*, 15 min, 4 °C), resuspended in 200 µL of Tris-EDTA buffer (100 mM Tris-HCl and 50 mM EDTA; pH 8) and centrifuged (4,000 × *g*, 15 min, 4 °C).

Samples were treated with 200 µL of Tris-EDTA with lysozyme (1 mg/mL) and incubated for 10 min in the dark at room temperature (25 ± 0.5 °C) to permeabilize cells for use with probes Lab 158 and Bif 164, being followed by centrifugation (4,000 × *g*, 15 min, 4 °C). Samples were resuspended in 45 µL of hybridization buffer [(0.9 M NaCl, 20 mM Tris–HCl (pH 7.5), 0.1% (w/v) sodium dodecyl sulphate (SDS)] and 5 µL of Cy3-labelled fluorescent oligonucleotide probe (50 ng/µL) and maintained under appropriate hybridization temperature (45 or 50 °C) in the dark for 4 h. After this hybridization step, samples were centrifuged (4,000 × *g*, 15 min, 25 °C), resuspended with 200 µL of hybridization buffer without SDS and maintained under appropriate wash temperature (45 or 50 °C) in the dark for 30 min. After this wash step, samples were centrifuged (4,000 × *g*, 15 min, 25 °C), resuspended with 200 µL PBS 1X and 20 µL SYBR Green (1:1000 stock diluted in dimethyl

sulfoxide ≥99.9%, Sigma-Aldrich), incubated for 10 min in the dark under room temperature (25 ± 0.5 °C), centrifuged (4,000 × *g*, 15 min, 25 °C) and resuspended with 200 µL PBS 1X.

A blank sample (without the fluorescently Cy3-labelled oligonucleotide probe and without the SYBR Green) and a sample only with SYBR Green were prepared for every sample, following the same steps as per the hybridized sample, as control to set the threshold of flow cytometer gates, which permits the revelation of microbial species and exclude false positives due to potential sample autofluorescence. Multiparametric flow cytometry measurements were done with a flow cytometer (BD Accuri C6, New Jersey, USA) with 488 nm excitation from a blue solid-state laser. Dispersion and fluorescence signals from individual cells passing through the laser zone were collected as logarithmic signals. Fluorescence signals (pulse area measurements) were collected by FL1 (SYBR Green) and FL2 (Lab 158, Bif 164, Bac 303, Chis 150, Erec 482) channels. Sample acquisition was operated at a low flow rate, threshold level was adjusted for forward scatter (FSC) of 30,000 and a total of 10,000 events were collected for each sample. Fluorescence emission cytograms were recorded with BD Accuri C6 Software (Becton Dickinson and Company). Results were expressed as abundance (relative percentage) of cells hybridized with each bacterial group-specific Cy3 probe (recorded as fluorescent events) in relation to total bacteria enumerated with SYBR Green staining (Conterno et al., 2019; Menezes et al., 2020).

2.6 Determination of prebiotic index of SPRF

To obtain a general quantitative comparative measure of the balance between distinct bacterial populations of colonic fermentation samples, as well as to compare the influence of different examined SPRF on the selective microbial modulation during colonic fermentation, a prebiotic index was calculated with data obtained from FISH-MFC technique. The equation used was adapted (Owolabi, Dat-arun, Yupanqui, & Wichienchot, 2020; Palframan, Gibson,

& Rastall, 2003) because this study would be the first to report a prebiotic index obtained of a colonic fermentation in which different bacterial populations were enumerated with five oligonucleotide probes using FISH-MFC technique. After the calculation of the relative abundance (percentage), where a correlation is made with the total number (abundance, %) of enumerated bacteria, the following equation was applied:

$$\text{Prebiotic index} = \%Lab + \%Bif - \%Bac - \%Chis - \%Erec \quad \text{(Eq. 1)}$$

Where: %Lab = abundance found for Lab after 24 or 48 h – abundance found for Lab at time zero; %Bif = abundance found for Bif after 24 or 48 h – abundance found for Bif at time zero; %Bac = abundance found for Bac after 24 or 48 h – abundance found for Bac at time zero; %Chis = abundance found for Chis after 24 or 48 h – abundance found for Chis at time zero; and %Erec = abundance found for Erec after 24 or 48 h – abundance found for Erec at time zero.

The equation assumes that an increase in population of *Lactobacillus* - *Enterococcus* (hybridized by probe Lab 158) and/or *Bifidobacterium* (hybridized by probe Bif 164) is a positive effect, while an increase in population of *Bacteroides* - *Prevotella* (hybridized by probe Bac 303), *C. histolyticum* (hybridized by probe Chis 150) and *E. rectall* - *C. coccoides* (hybridized by probe Erec 482) is a negative effect. Changes in abundance of these groups are entered into the equation as related to their starting levels (abundance). If a bacterial group shows negative differences between a fermentation time and time zero, the signal (positive or negative) in the equation immediately prior to that probe is changed. This allows to normalize the use of the equation for a varied number of probes. A positive prebiotic index indicates a beneficial balance of the bacterial groups identified in colonic fermentation induced by

examined substrate and, consequently, a potential prebiotic activity. A negative prebiotic index indicates an undesirable modulation of the microbiota by examined substrate.

2.7 Measurement of microbial metabolic activity parameters

The metabolic activity of human fecal inoculum in media with different digested SPRF, as well as with FOS (positive control) and negative control (medium with no added fermentable substrate) was measure with determination of pH values and organic acid and sugar contents in different fermentation media over time. pH values (method 981.12) of fermentation media were measured at time zero (baseline - just after homogenization) and after 24 and 48 h of fermentation with a digital potentiometer (Quimis, Diadema, São Paulo, Brazil) (AOAC, 2016).

Sugar (glucose, fructose and maltose) and organic acid contents [lactic acid and short-chain fatty acids (SCFA), namely propionic, acetic and butyric acid] were measured at time zero (baseline - just after homogenization) and after 24 and 48 h of fermentation with high performance liquid chromatography (HPLC) with an Agilent chromatograph (model 1260 Infinity LC, Agilent Technologies, St. Clara, CA, USA) equipped with a quaternary solvent pump (G1311C model), degasser, thermostatic column compartment (G1316A model) and automatic auto sampler (G1329B model), coupled with a diode array detector (DAD) (G1315D model) and refractive index detector (RID) (G1362A model). Analytical conditions were: an Agilent Hi-Plex H column (7.7 × 300 mm, 8 μ); mobile phase H_2SO_4 4 mM/L in ultrapure water; and flow rate of 0.7 mL/min. Data were processed with OpenLAB CDS ChemStation Edition^{TM} software (Agillent Technologies). HPLC sample peaks were identified by comparing their retention times with those of standards of sugars and organic acids (Sigma Aldrich) and average peak areas were used for quantification (Coelho et al., 2018; Lima et al., 2019).

2.8 Statistical analysis

All analyses were done in triplicate in three different experiments and results were expressed as average ± standard deviation. Data were submitted to a Student t test or analysis of variance (one-way ANOVA) followed by Tukey post-hoc test with $p \leq 0.05$, using the computational software GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA).

3. Results

3.1 Abundance of bacterial populations during in vitro colonic fermentation

Relative abundance (percentage) of colonic bacterial groups enumerated with specific oligonucleotide probes in fermentation media with digested WSPRB, WSPCB, PSPV and PSPL, as well as with FOS (positive control) and negative control (medium with no added fermentable substrate) during a 48 h-*in vitro* fermentation is shown in Fig. 1. Differences in abundance of measured bacterial groups varied overall with examined fermentation medium and incubation period.

Increases ($p \leq 0.05$) in abundance of *Lactobacillus* - *Enterococcus* (hybridized by probe Lab 158) were found in all examined fermentation media over time. The highest abundance of *Lactobacillus* - *Enterococcus* was found after 48 h in medium with WSPCB (4.48 ± 0.18%), followed by media with FOS (2.25 ± 0.13%), PSPV (2.20 ± 0.14%), negative control (1.96 ± 0.16%), PSPL (1.65 ± 0.11%) and WSPCB (0.84 ± 0.09%). However, media with WSPRB, WSPCB and PSPL had a two-fold increase in abundance of *Lactobacillus* - *Enterococcus* after 48 h when compared to time zero.

The highest abundance ($p \leq 0.05$) of *Bifidobacterium* (hybridized by probe Bif 164) was found in medium with WSPCB (2.18 ± 0.18%) after 24 h, which was similar ($p > 0.05$) to the abundance found in medium with FOS (2.28 ± 0.15%). The highest abundance of

Bifidobacterium after 48 h was found in media with FOS (3.29 ± 0.19%) and WSPCB (3.27 ± 0.17%), followed by media with PSPV (1.50 ± 0.20%), PSPL (1.03 ± 0.08%) and WSPRB (0.33 ± 0.13%). Abundance of *Bifidobacterium* decreased ($p \leq 0.05$) after 24 and 48 h in negative control (0.79 ± 0.09% and 0.16 ± 0.05%, respectively) when compared to time zero.

Bacteroides - Prevotella (hybridized by probe Bac 303) had the lowest abundance ($p \leq 0.05$) in media with WSPRB (0.29 ± 0.09 %), WSPCB (0.48 ± 0.08%), PSPL (0.88 ± 0.15%), PSPV (5.03 ± 0.23%) and FOS (5.55 ± 0.25%) after 24 h. An increase in abundance ($p \leq 0.05$) of *Bacteroides - Prevotella* in media with WSPRB, WSPCB, PSPL and PSPV and FOS was found after 48 h, with the highest abundance being found in media with PSPV (7.49 ± 0.20%) and FOS (5.99 ± 0.25%). There was no alteration ($p > 0.05$) in abundance of *Bacteroides - Prevotella* in negative control over time.

Changes in abundance of *C. histolyticum* (hybridized by probe Chis 150) were found after 24 and 48 h. Abundance of *C. histolyticum* decreased ($p \leq 0.05$) in examined fermentation media after 24 h, with the exception of medium with PSPL and negative control. Lowest ($p \leq 0.05$) abundance of *C. histolyticum* was found after 48 h in medium with WSPRB (0.15 ± 0.05%), followed by media with PSPV (0.21 ± 0.08%), PSPL (0.68 ± 0.10%) and WSPCB (0.73 ± 0.13 %). Abundance of *C. histolyticum* increased in medium with FOS (3.35 ± 0.15%) and negative control (0.53 ± 0.10 %) after 48 h. The highest decreases in abundance of *C. histolyticum* when compared to initial abundance (time zero) after 24 and 48 h were found in medium with PSPV.

Abundance of *E. rectall - C. coccoides* (hybridized by probe Erec 482) decreased ($p \leq 0.05$) after 24 h in media with WSPRB (0.28 ± 0.10%), FOS (2.83 ± 0.20%) and negative control (0.13 ± 0.07%), as well as after 48 h in media with PSPV (2.19 ± 0.22%) and PSPL (0.86 ± 0.20%). The highest abundance ($p \leq 0.05$) of *E. rectall - C. coccoides* was found in

medium with FOS (4.85 ± 0.26%) after 48 h. Abundance of *E. rectall* - *C. coccoides* increased ($p \leq 0.05$) in medium with WSPCB after 24 and 48 h.

3.2 Measurements of prebiotic index of SPRF during colonic fermentation

Prebiotic indexes found for WSPRB, WSPCB, PSPV, PSPL and FOS were positive after 24 and 48 h of *in vitro* colonic fermentation, while negative control had a negative prebiotic index (Table 2). Prebiotic index found for medium with PSPV (7.46 ± 0.25) after 24 h was higher ($p \leq 0.05$) than that found for medium with FOS (6.40 ± 0.35). The highest ($p \leq 0.05$) prebiotic index after 48 h was found in medium with PSPV (5.99 ± 0.32), followed by media with WSPCB (5.05 ± 0.17), FOS (4.64 ± 0.22), PSPL (2.98 ± 0.15) and WSPRB (2.74 ± 0.05).

3.3 Measurements of bacterial metabolic activity during colonic fermentation in media with SPRF

The cultivation of a human fecal inoculum in media with digested WSPRB, WSPCB, PSPV and PSPL, as well as with FOS caused a decrease ($p \leq 0.05$) in pH values after 24 and 48 h, while no alteration in pH values was found in negative control over time (Table 3). The lowest pH values ($p \leq 0.05$) after 24 h were found in media with PSPV (3.69 ± 0.03) and FOS (3.68 ± 0.02). pH values in media with PSPV (3.20 ± 0.03), WSPCB (3.24 ± 0.01) and PSPL (3.30 ± 0.02) after 48 h were similar ($p > 0.05$) or lower ($p \leq 0.05$) than those found in medium with FOS (3.68 ± 0.02 and 3.32 ± 0.01, respectively). The highest ($p \leq 0.05$) pH values after 48 h were found in medium with WSPRB (3.48 ± 0.02) and negative control (4.63 ± 0.04).

Contents of fructose, glucose and maltose in media with WSPRB, WSPCB, PSPV and PSPL, as well as with FOS and negative control during 48 h of *in vitro* colonic fermentation

are shown in Table 4. Overall, the contents of fructose, glucose and maltose decreased ($p \leq 0.05$) during the measured fermentation period in media with examined SPRF.

Fructose contents were higher ($p \leq 0.05$) during colonic fermentation in medium with FOS (10.69 ± 0.05 – 12.40 ± 0.05 g/L) when compared to media with WSPRB, WSPCB, PSPV and PSPL. Glucose content decreased ($p \leq 0.05$) during colonic fermentation in media with WSPCB (1.14 ± 0.02 – 0.30 ± 0.02), PSPV (3.99 ± 0.04 – 0.71 ± 0.03) and PSPL (0.81 ± 0.01 – 0.25 ± 0.02). Contents of maltose increased ($p \leq 0.05$) after 24 h in media with WSPRB, WSPCB, PSPV and PSPL, being followed by a decrease ($p \leq 0.05$) after 48 h. Fructose and maltose were not found in negative control. No alteration in glucose content was found during colonic fermentation in negative control.

Contents of lactic acid and SCFA (propionic, acetic and butyric acid) in media with WSPRB, WSPCB, PSPV and PSPL, as well as with FOS and negative control during 48 h of colonic fermentation are shown in Table 5. Lactic acid ($p \leq 0.05$) content increased during colonic fermentation, with the highest content being found in medium with PSPV (19.14 ± 0.08), followed by media with WSPCB (16.66 ± 0.04), PSPL (15.26 ± 0.06), WSPRB (12.55 ± 0.05) and FOS (11.57 ± 0.05). No alteration in lactic and butyric acid contents in negative control was found over time.

Contents of acetic acid in media with WSPCB, PSPV, PSPL and FOS increased ($p \leq 0.05$) after 24 h. However, all examined colonic fermentation media had an increase ($p \leq 0.05$) in acetic acid content after 48 h, including negative control (0.49 ± 0.02). Higher contents ($p \leq 0.05$) of acetic acid were found in medium with WSPCB (0.67 ± 0.03) and PSPV (0.67 ± 0.02) after 48 h. Contents of acetic acid were similar ($p > 0.05$) in media with WSPRB (0.57 ± 0.02), PSPL (0.59 ± 0.03) and FOS (0.58 ± 0.02) after 48 h.

The highest contents ($p \leq 0.05$) of butyric acid were found in media with FOS (0.40 ± 0.03) and WSPCB (0.38 ± 0.04) after 48 h. Propionic acid contents decreased ($p > 0.05$) or

were similar ($p > 0.05$) in different examined fermentation media over time, with the exception of medium with FOS (1.60 ± 0.04) after 24 h, which had the highest ($p \leq 0.05$) propionic acid content.

4. Discussion

The four examined SPRF induced increases in abundance of *Lactobacillus-Enterococcus* and *Bifidobacterium* populations in colonic fermentation media over time, which should indicate high amounts of fermentable carbohydrates in these substrates (Albuquerque et al., 2020). These microorganisms are recognized as the most important bacterial groups associated with human health, providing beneficial effects in large intestine (Heeney, Gareau, & Marco, 2018; O'Callaghan & van Sinderen, 2016).

Bacteroides - Prevotella is one of the predominant bacterial groups found in the human gut microbiota. Alterations in abundance of *Bacteroides - Prevotella* were induced by examined SPRF, causing a decrease after 24 h of colonic fermentation, which was followed by an increase after 48 h. These bacteria are strongly affected by substrates available in fermentation medium and could be considered as having ability to initiate breakdown of high molecular weight substrates, such as resistant starch found in SRPF (Flint, Scott, Duncan, Louis, & Forano, 2012; Rodrigues et al., 2016).

Decrease in abundance of *C. histolyticum* during colonic fermentation could confirm selective stimulatory effects of examined SPRF on beneficial microorganisms forming intestinal microbiota, because *C. histolyticum* is a well-known enteric pathogen (Liu, Kolida, Charalampopoulos, & Rastall, 2020). Decrease in abundance of *C. histolyticum* could be a consequence of the decreased pH in colonic fermentation media due to the production of organic acids, which is a limiting factor for *C. histolyticum* growth (Menezes et al., 2020). *E. rectall - C. coccoides* group is a major anaerobic population in human intestine (Rodrigues et

al., 2016). Abundance of *E. rectall* - *C. coccoides* varied in colonic fermentation with examined SPRF over time, which reinforces the presence of high amounts of fermentable carbohydrates in these flours (Albuquerque et al., 2020; Guo et al., 2019). Carbohydrates, such as soluble and insoluble fibers, resistant starch and FOS found in WSPRB, WSPCB, PSPV and PSPL, have been considered the main prebiotic components due to their association with improvements of different metabolic disorders through the modulation of intestinal microbiota (Cheng et al., 2020).

Positive prebiotic indexes found to examined SPRF during colonic fermentation demonstrate their potential ability to exert a desirable modulation of intestinal microbiota. These positive prebiotic indexes reinforce the results found in FISH-MCF assays showing their positive effects on modulation of human colonic microbiota. Furthermore, these results also corroborate with previous investigations showing that SPRF stimulated selectively the growth of probiotic *Lactobacillus* and *Bifidobacterium* rather than of enteric competitors in laboratory media (Albuquerque et al., 2020). WSPCB and PSPV had the highest prebiotic indexes after 24 and 48 h of colonic fermentation. Prebiotic index results in a quantitative score describing the prebiotic effect of a food or bioactive compounds (Owolabi et al., 2020). Although prebiotic index has a well-reported use with oligonucleotide probes (Palframan et al., 2003), the equation developed in this study is the first to consider five bacterial groups enumerated by FISH-MCF technique.

Fecal microbiota was capable of metabolizing glucose, fructose and maltose in media with WSPRB, WSPCB, PSPV and PSPL to produce organic acids in parallel to cause a decrease in pH values over time. Media with WSPCB and PSPV had the lowest pH values after 48 h of colonic fermentation, indicating intense bacterial metabolic activities (Mousavi & Mousavi, 2019). High production of lactic acid during colonic fermentation could be related to increased abundance of *Lactobacillus* - *Enterococcus* because these species are the

main lactic acid producers, although lactic acid could also be produced in lesser amounts by *Bifidobacterium* (Liu et al., 2020).

Lactic acid and SCFA, the main products from microbial carbohydrate fermentation, can modulate cholesterol and lipid metabolism, suppress pathogenic intestinal bacteria and regulate gut hormones, which modulate the local motor responses of the gut, besides to improve intestinal barrier function and immune system (Kanauchi, Andoh, & Mitsuyama, 2013; Rodrigues et al., 2016; Sanders et al., 2019). In addition, SCFA can act as electron sinks of anaerobic respiration in the gut and decrease intestinal pH, enhancing the bioavailability of minerals, such as calcium and magnesium (Gullón et al., 2014; Sanders, et al., 2019).

Acetic acid is produced in intestine by *Lactobacillus* and *Bifidobacterium* during fermentation of non-digestible carbohydrates (Rios-Covian et al., 2016; Owolabi et al., 2020). Increase in acetic acid contents has been associated with increased abundance of *Bifidobacterium* and *Lactobacillus* - *Enterococcus* during colonic fermentation of SPRF, which exert important role in controlling inflammation and combatting pathogen invasion (Sanders et al., 2019). Butyric acid is an energy source for colonic epithelial cells (Koh, De Vadder, Kovatcheva-Datchary, & Backhed, 2016). Increase in butyric acid contents could be related to variations in abundance of *E. rectall* - *C. coccoides* during colonic fermentation of SPRF. *Lactobacillus* and *Bifidobacterium* do not produce butyrate, but through cross-feeding of other commensal microbiota, the contents of butyric acid in gut can increase and influence many physiological aspects (Canfora, Jocken, & Blaak, 2015; Souza, Jonathan, Saad, Schols, & Venema, 2019).

Media with WSPCB and PSPV had the highest contents of lactic, acetic and butyric acids during colonic fermentation. Low propionic acid contents found in colonic fermentation of SPRF could be related to predominance of *Bacteroides* in detriment to *Prevotella* in fecal

inoculum. *Prevotella* could ferment carbohydrates more rapidly and produce higher ratios of propionate to acetate and butyrate than *Bacteroides*-dominant microbiota (Chen, Long, Zhang, Liu, & Zhao, 2017).

Although there might be a huge diversity of individual taxa in human gut microbiota, a high level of functional redundancy and specific ecological functions are provided by a range of bacteria across different individuals (Sanders et al., 2019; Moya & Ferrer, 2016). It is important to note that before evaluation of the performance in *in vitro* colonic fermentation each examined SPRF was submitted to a simulated gastrointestinal digestion because the resistance to gastric acidity and hydrolysis by mammalian enzymes are limiting factors to be assured in order to enable the substrate to reach the colon and be fermented by intestinal microbiota, meeting the pre-requisite for a prebiotic effect or gut modulation effect (Gibson et al., 2017; Menezes et al., 2020; Rodrigues et al., 2016). Furthermore, the assessment of the prebiotic properties of foods or bioactive compounds by *in vitro* fermentation with human fecal microbiota enumerated by FISH-MFC techniques should provide an effective and rapid alternative to assess the modulation capacity of different substrates on a laboratory scale comparative basis (Menezes et al., 2020; Rodrigues et al., 2016).

5. Conclusion

Digested WSPRB, WSPCB, PSPV and PSPL promoted an increase in relative abundance of *Lactobacillus* - *Enterococcus* and *Bifidobacterium* populations during *in vitro* colonic fermentation, in addition to cause a decrease in relative abundance of *Bacteroides* - *Prevotella*, *C. histolyticum* and *E. rectall* - *C. coccoides* populations. WSPRB, WSPCB, PSPV and PSPL achieved positive prebiotic indexes during *in vitro* colonic fermentation, besides to induce a decrease in pH values and an increase in sugar consumption and lactic acid and SCFA production over time. These results, although under *in vitro* but controlled

conditions, indicate the ability of examined SPRF to modulate selectively the composition and metabolic activity of human colonic microbiota, which should be linked to their prebiotic effects and ability to improve gut health. Finally, these results characterize SPRF as potential prebiotic ingredients to be used in the formulations of added-value dietary supplements or functional foods.

Conflicts of interest

Authors confirm no conflicts of interest with respect to the work described in this manuscript.

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Author contribution

Conceptualization: TMRA, ELS. Data curation: TMRA, ELS; Formal analysis: TMRA, MSL, MM, LRCC, ELS; Funding acquisition: ELS; Investigation: TMRA, MSL, LRCC; Methodology: TMRA, MSL, MM, LRCC, ELS; Project administration: ELS; Resources: TMRA, MM, MSL, ELS; Supervision: ELS; Validation: TMRA, MSL, MM, LRCC, ELS; Visualization; Writing – original draft: TMRA, MM, ELS; Writing – review & editing: TMRA, ELS.

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Figure caption

Figure 1. Abundance of different bacterial groups as measured by fluorescence *in situ* hybridization (FISH) combined with multiparametric flow cytometry in an *in vitro* colonic fermentation system with digested flours from different sweet potato root varieties (WSPRB, WSPCB, PSPV and PSPL) and fructooligosaccharides (FOS), as well as in a negative control (no added fermentable substrate) inoculated with a human fecal inoculum, at time zero (baseline) and after 24 and 48 h of incubation (37 °C). Results are expressed as percentage (average ± standard deviation, n = 3) of hybridized cells (Lab 158 specific to *Lactobacillus – Enterococcus*, Bif 164 specific to *Bifidobacterium*, Bac 303 specific to *Bacteroides – Prevotella*, Chis 150 specific to *Clostridium histolyticum* and Erec 482 specific to *Eubacterium rectall – Clostridium coccoides*). WSPRB: flour from sweet potato root variety Rainha Branca (white peel); WSPCB: flour from sweet potato root variety Campina Branca (white peel); PSPV: flour from sweet potato root variety Vitória (purple peel); PSPL: flour from sweet potato root variety Lagoinha (purple peel). a - c: Different superscript small letters for a same cultivation media at different fermentation times denote difference ($p \leq 0.05$), based on Tukey's test. A - E: different superscript capital letters at the same fermentation time denote difference ($p \leq 0.05$) among treatments, based on Tukey's test.

Table 1. Physicochemical parameters of flours from different sweet potato root varieties used in assays to evaluate potential prebiotic properties (Albuquerque et al., 2020).

Parameters	Flours			
	WSPRB	WSPCB	PSPV	PSPL
Gross composition (g/100 g)				
Crude protein	3.6 ± 0.1	1.8 ± 0.2	3.4 ± 0.1	2.4 ± 0.1
Lipid	1.1 ± 0.1	1.1 ± 0.1	1.7 ± 0.1	1.3 ± 0.1
Resistant starch	6.7 ± 0.4	10.3 ± 0.2	6.2 ± 0.2	9.2 ± 0.3
Soluble fiber	1.8 ± 0.2	2.3 ± 0.3	2.7 ± 0.4	3.0 ± 0.6
Insoluble fiber	5.8 ± 1.2	5.2 ± 1.4	5.5 ± 1.3	4.6 ± 1.2
Total dietary fiber	7.6 ± 0.9	7.4 ± 1.1	8.2 ± 1.3	7.6 ± 0.9
Fructooligosaccharide (g/L)				
Kestose	2.0 ± 0.2	5.4 ± 0.5	2.2 ± 0.2	1.7 ± 0.1
Nystose	Nd	Nd	7.1 ± 0.8	1.6 ± 0.2

WSPRB: flour from sweet potato root variety Rainha Branca (white peel); WSPCB: flour from sweet potato root variety Campina Branca (white peel); PSPV: flour from sweet potato root variety Vitória (purple peel); PSPL: flour from sweet potato root variety Lagoinha (purple peel).
Nd = not detected.

Table 2. Prebiotic index (average ± standard deviation, n=3) of digested flours from different sweet potato root varieties (WSPRB, WSPCB, PSPV and PSPL) and fructooligosaccharides (FOS), as well as in a negative control (no added fermentable substrate) after 24 and 48 h of *in vitro* colonic fermentation.

Flours	Prebiotic index	
	24 h	48 h
WSPRB	3.01 ± 0.14^{Bc}	2.74 ± 0.05^{Ab}
WSPCB	3.02 ± 0.08^{Ac}	5.05 ± 0.17^{Be}
PSPV	7.46 ± 0.25^{Be}	5.99 ± 0.32^{Af}
PSPL	0.38 ± 0.10^{Ab}	2.98 ± 0.15^{Bc}
FOS	6.40 ± 0.35^{Bd}	4.64 ± 0.22^{Ad}
Negative control	-0.34 ± 0.06^{Ba}	-1.23 ± 0.11^{Aa}

WSPRB: flour from sweet potato root variety Rainha Branca (white peel); WSPCB: flour from sweet potato root variety Campina Branca (white peel); PSPV: flour from sweet potato root variety Vitória (purple peel); PSPL: flour from sweet potato root variety Lagoinha (purple peel).

A – B: Different superscript capital letters in the same row for the same cultivation media denote differences ($p \leq 0.05$), based on Student's t test;

a-f: different superscript small letters in the same column at a same time interval denote difference ($p \leq 0.05$) among cultivation media, based on Tukey's test.

Table 3. pH measurement (mean ± standard deviation, n=3) in media with digested flours from different sweet potato roots (WSPRB, WSPCB, PSPV and PSPL) and fructooligosaccharides (FOS), as well as in a negative control (no added fermentable substrate) at time zero (baseline) and after 24 and 48 h of *in vitro* colonic fermentation.

Flours	pH values		
	0 h	24 h	48 h
WSPRB	4.62 ± 0.02^{Cb}	3.83 ± 0.04^{Bb}	3.48 ± 0.02^{Ac}
WSPCB	4.72 ± 0.04^{Cc}	3.77 ± 0.03^{Bb}	3.24 ± 0.01^{Aa}
PSPV	4.71 ± 0.03^{Cc}	3.69 ± 0.03^{Ba}	3.20 ± 0.03^{Aa}
PSPL	4.75 ± 0.04^{Cc}	3.80 ± 0.04^{Bb}	3.30 ± 0.02^{Ab}
FOS	4.55 ± 0.02^{Ca}	3.68 ± 0.02^{Ba}	3.32 ± 0.01^{Ab}
Negative control	4.70 ± 0.03^{Ac}	4.65 ± 0.02^{Ac}	4.63 ± 0.04^{Ad}

WSPRB: flour from sweet potato root variety Rainha Branca (white peel); WSPCB: flour from sweet potato root variety Campina Branca (white peel); PSPV: flour from sweet potato root variety Vitória (purple peel); PSPL: flour from sweet potato root variety Lagoinha (purple peel).

A – C: Different superscript capital letters in the same row for the same cultivation media denote differences ($p \leq 0.05$), based on Tukey's test;

a-d: different superscript small letters in the same column at a same time interval denote difference ($p \leq 0.05$) among cultivation media, based on Tukey's test.

Table 4. Contents of sugars (g/L) in media with digested flours from different sweet potato root varieties (WSPRB, WSPCB, PSPV and PSPL) and fructooligosaccharides (FOS), as well as in a negative control (no added fermentable substrate) at time zero (baseline) and after 24 and 48 h of *in vitro* colonic fermentation.

Sugars	Flours	Time of incubation		
		0 h	24 h	48 h
Fructose	WSPRB	0.24 ± 0.02^{Ab}	Nd	Nd
	WSPCB	0.22 ± 0.01^{Ab}	Nd	Nd
	PSPV	0.26 ± 0.03^{Ab}	Nd	Nd
	PSPL	0.13 ± 0.02^{Aa}	Nd	Nd
	FOS	10.69 ± 0.05^{Ac}	11.89 ± 0.04^{Ba}	12.40 ± 0.05^{Ca}
	Negative control	Nd	Nd	Nd
Glucose	WSPRB	8.70 ± 0.03^{Ae}	8.66 ± 0.05^{Ad}	8.66 ± 0.04^{Ad}
	WSPCB	1.14 ± 0.02^{Bc}	0.33 ± 0.01^{Ab}	0.30 ± 0.02^{Ab}
	PSPV	3.99 ± 0.04^{Cd}	1.26 ± 0.02^{Bc}	0.71 ± 0.03^{Ac}
	PSPL	0.81 ± 0.01^{Cb}	0.34 ± 0.01^{Bb}	0.25 ± 0.02^{Aa}
	FOS	Nd	Nd	Nd
	Negative control	0.17 ± 0.01^{Aa}	0.15 ± 0.02^{Aa}	0.20 ± 0.03^{Aa}
Maltose	WSPRB	0.05 ± 0.01^{Ba}	0.10 ± 0.02^{Ca}	0.01 ± 0.01^{Aa}
	WSPCB	0.08 ± 0.02^{Aa}	0.65 ± 0.03^{Cb}	0.22 ± 0.02^{Bb}
	PSPV	0.27 ± 0.03^{Ab}	2.30 ± 0.05^{Bd}	2.22 ± 0.04^{Bc}
	PSPL	0.05 ± 0.01^{Aa}	0.94 ± 0.03^{Cc}	0.22 ± 0.02^{Bb}
	FOS	Nd	Nd	Nd
	Negative control	Nd	Nd	Nd

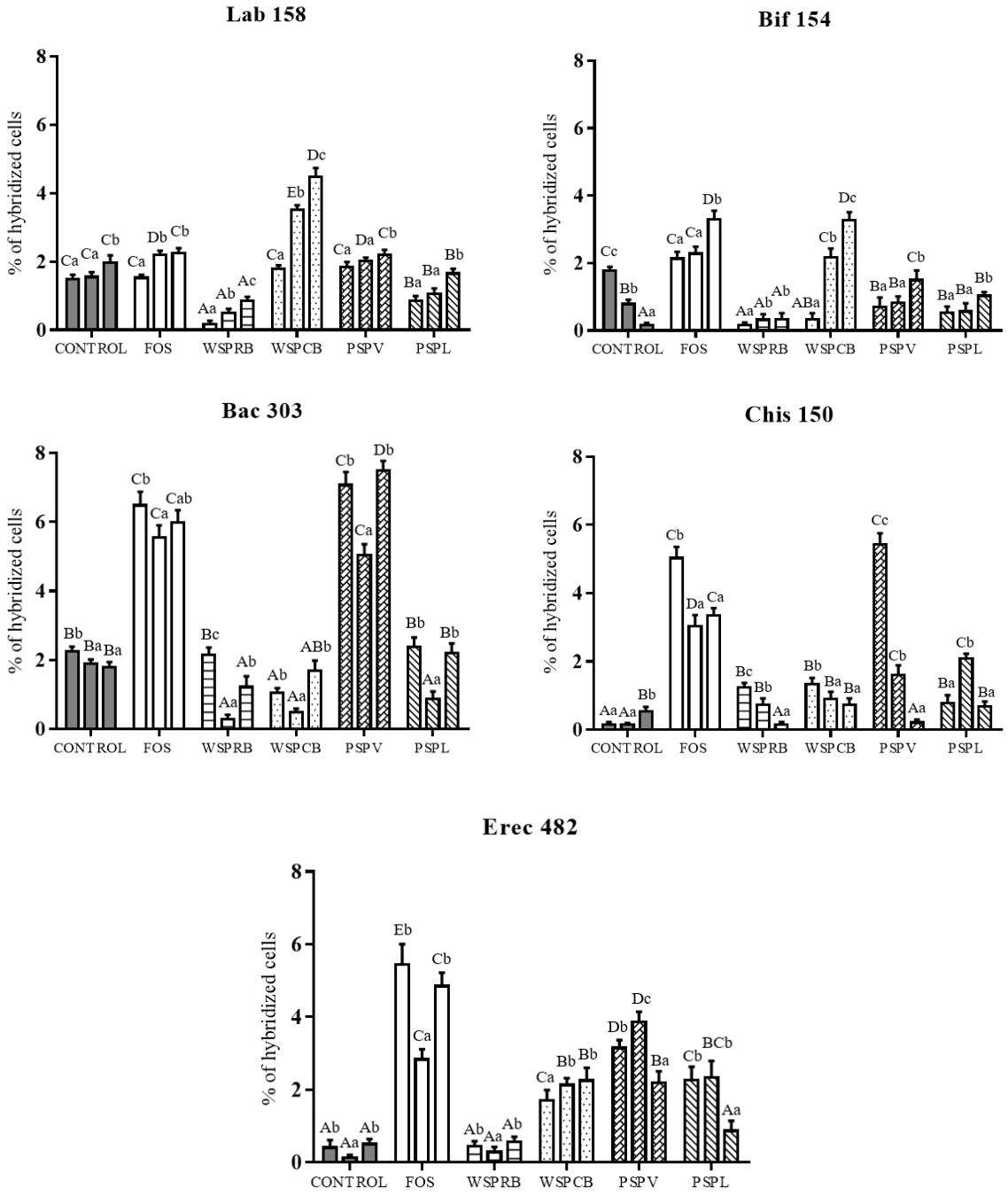
WSPRB: flour from sweet potato root variety Rainha Branca (white peel); WSPCB: flour from sweet potato root variety Campina Branca (white peel); PSPV: flour from sweet potato root variety Vitória (purple peel); PSPL: flour from sweet potato root variety Lagoinha (purple peel).
Nd: Not detected.
A – C: Different superscript capital letters in the same row for the same cultivation media denote differences ($p \leq 0.05$), based on Tukey's test;
a-e: different superscript small letters in the same column at a same time interval and measured sugar denote difference ($p \leq 0.05$) among cultivation media, based on Tukey's test.

Table 5. Contents (g/L) of lactic acid and short-chain fatty acids (SCFA) during *in vitro* colonic fermentation with a human fecal inoculum of digested flours from different sweet potato roots (WSPRB, WSPCB, PSPV and PSPL) and fructooligosaccharides (FOS), as well as in a negative control (no added fermentable substrate) at time zero (baseline) and after 24 and 48 h of incubation.

Organic acids	Flours	Time of incubation		
		0 h	24 h	48 h
Lactic acid	WSPRB	8.01 ± 0.05^{Ad}	10.95 ± 0.04^{Bd}	12.55 ± 0.05^{Cc}
	WSPCB	6.27 ± 0.04^{Ab}	13.10 ± 0.05^{Be}	16.66 ± 0.04^{Ce}
	PSPV	8.29 ± 0.05^{Ae}	10.27 ± 0.03^{Bc}	19.14 ± 0.08^{Cf}
	PSPL	7.46 ± 0.03^{Ac}	13.21 ± 0.06^{Be}	15.26 ± 0.06^{Cd}
	FOS	2.57 ± 0.01^{Aa}	8.52 ± 0.05^{Bb}	11.57 ± 0.05^{Cb}
	Negative control	2.57 ± 0.02^{Ba}	1.99 ± 0.02^{Aa}	2.00 ± 0.01^{Aa}
Acetic acid	WSPRB	0.46 ± 0.03^{Ab}	0.49 ± 0.04^{Ab}	0.57 ± 0.02^{Bb}
	WSPCB	0.37 ± 0.02^{Aa}	0.57 ± 0.05^{Bc}	0.67 ± 0.03^{Cc}
	PSPV	0.36 ± 0.01^{Aa}	0.45 ± 0.03^{Bb}	0.67 ± 0.02^{Cc}
	PSPL	0.38 ± 0.02^{Aa}	0.58 ± 0.03^{Bc}	0.59 ± 0.03^{Bb}
	FOS	0.36 ± 0.01^{Aa}	0.50 ± 0.02^{Bb}	0.58 ± 0.02^{Bb}
	Negative control	0.36 ± 0.03^{Aa}	0.40 ± 0.01^{Aa}	0.49 ± 0.02^{Ba}
Butyric acid	WSPRB	0.14 ± 0.01^{Aa}	0.17 ± 0.03^{Aa}	0.31 ± 0.01^{Bb}
	WSPCB	0.19 ± 0.03^{Abc}	0.29 ± 0.02^{Bc}	0.38 ± 0.04^{Cc}
	PSPV	0.15 ± 0.04^{Aab}	0.18 ± 0.01^{Aab}	0.33 ± 0.02^{Bb}
	PSPL	0.18 ± 0.02^{Abc}	0.18 ± 0.02^{Aab}	0.20 ± 0.02^{Aa}
	FOS	0.21 ± 0.01^{Ac}	0.23 ± 0.03^{Ab}	0.40 ± 0.03^{Bc}
	Negative control	0.21 ± 0.02^{Ac}	0.20 ± 0.02^{Aab}	0.22 ± 0.03^{Aa}
Propionic acid	WSPRB	1.47 ± 0.05^{Cd}	0.70 ± 0.02^{Ac}	0.82 ± 0.03^{Be}
	WSPCB	1.17 ± 0.04^{Bc}	0.72 ± 0.03^{Ac}	0.77 ± 0.02^{Ae}
	PSPV	0.74 ± 0.02^{Ca}	0.47 ± 0.01^{Ba}	0.42 ± 0.01^{Aa}
	PSPL	0.97 ± 0.03^{Cb}	0.57 ± 0.02^{Bb}	0.50 ± 0.02^{Ab}
	FOS	1.45 ± 0.03^{Bd}	1.60 ± 0.04^{Ce}	0.65 ± 0.03^{Ad}
	Negative control	1.44 ± 0.02^{Cd}	0.79 ± 0.02^{Bd}	0.57 ± 0.02^{Ac}

WSPRB: flour from sweet potato root variety Rainha Branca (white peel); WSPCB: flour from sweet potato root variety Campina Branca (white peel); PSPV: flour from sweet potato root variety Vitória (purple peel); PSPL: flour from sweet potato root variety Lagoinha (purple peel).

A – C: Different superscript capital letters in the same row for the same cultivation media denote differences ($p \leq 0.05$), based on Tukey's test; a-e: different superscript small letters in the same column at a same time interval and measured organic acid denote difference ($p \leq 0.05$) among cultivation media, based on Tukey's test.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Bif 154
Bac 303
Chis 150
Erec 482
% of hybridized cells
CONTROL
FOS
WSPRB
WSPCB
PSPV
PSPL

Fig. 1

ANEXOS

ANEXO A – CERTIDÃO DO COMITÊ DE ÉTICA EM PESQUISA

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

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO DO POTENCIAL PREBIÓTICO DE FARINHAS DE DIFERENTES CULTIVARES DE BATATA-DOCE (Ipomoea batatas L.) EM SISTEMAS DE FERMENTAÇÃO IN VITRO

Pesquisador: THATYANE MARIANO RODRIGUES DE ALBUQUERQUE

Área Temática:

Versão: 2

CAAE: 12880518.5.0000.5188

Instituição Proponente: Centro De Ciências da Saúde

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.489.789

Apresentação do Projeto:

O trato gastrointestinal humano representa um ecossistema complexo, onde os nutrientes disponíveis influenciam a diversidade da microbiota e suas atividades metabólicas, de modo que o cólon é reconhecido como o compartimento mais metabolicamente ativo no corpo humano (ROBERFROID et al., 2010; LIU et al., 2014). Um crescente número de evidências sugere que a microbiota intestinal pode afetar vários processos fisiológicos e metabólicos no hospedeiro, como função de barreira e moduladora da resposta imunológica, vindo a atuar na prevenção de diversas doenças, como doença inflamatória intestinal, diarreia e câncer colorretal, além de impactar na susceptibilidade a agentes patogênicos intestinais (FLINT et al., 2012; MCKENNEY; PAMER, 2015; CAMERON; SPERANDIO, 2015; DESAI et al., 2016).

Objetivo da Pesquisa:

Avaliar o potencial prebiótico de farinhas obtidas de diferentes cultivares de batata-doce utilizando sistemas de fermentação in vitro

Objetivo Secundário:- Elaborar farinhas a partir das cultivares de batata-doce Rainha-branca, Campina-branca, Vitória e Lagoinha; - Caracterizar os aspectos físico-químicos das diferentes farinhas de batata-doce, incluindo umidade, cinzas, pH, oligossacarídeos, açúcares, amido resistente, proteínas, lipídios, fibra total, fibra solúvel e insolúvel e compostos fenólicos; - Expôr cada farinha de batata-doce a condições simuladas do trato gastrointestinal humano; - Avaliar o

Endereço: UNIVERSITARIO S/N
Bairro: CASTELO BRANCO **CEP:** 58.051-900
UF: PB **Município:** JOAO PESSOA
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potencial de utilização das farinhas de batata-doce, submetidas ou não ao processo de digestão simulada, como fontes de carbono para o crescimento de cepas de bactérias probióticas; - Realizar um escore de atividade prebiótica utilizando cepas de E. coli; - Verificar o efeito bifidogênico e o comportamento de outras bactérias.

Avaliação dos Riscos e Benefícios:

Esses aspectos foram considerados pela pesquisadora.

Comentários e Considerações sobre a Pesquisa:

Estudo de interesse científico.

Considerações sobre os Termos de apresentação obrigatória:

Em conformidade com as comendações deste CEP.

Recomendações:

Recomendamos sua aprovação.

Conclusões ou Pendências e Lista de Inadequações:

Nada mais a registrar.

Considerações Finais a critério do CEP:

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

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1236080.pdf	19/06/2019 20:10:49		Aceito
Outros	Informacoes.docx	19/06/2019 20:09:10	THATYANE MARIANO RODRIGUES DE ALBUQUERQUE	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Thatyane_CEP.doc	19/06/2019 20:03:16	THATYANE MARIANO RODRIGUES DE ALBUQUERQUE	Aceito
Declaração de	Termo_de_Anuencia_IPA.PDF	19/06/2019	THATYANE	Aceito

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Continuação do Parecer: 3.489.789

Instituição e Infraestrutura	Termo_de_Anuencia_IPA.PDF	19:31:01	MARIANO RODRIGUES DE ALBUQUERQUE	Aceito
Declaração de Instituição e Infraestrutura	Termo_de_Anuencia_IFSertao.pdf	19/06/2019 19:30:20	THATYANE MARIANO RODRIGUES DE ALBUQUERQUE	Aceito
Declaração de Instituição e Infraestrutura	Certidao_de_aprovacao.PDF	23/04/2019 20:57:57	THATYANE MARIANO RODRIGUES DE ALBUQUERQUE	Aceito
Declaração de Instituição e Infraestrutura	Termo_de_Anuencia.pdf	23/04/2019 20:56:23	THATYANE MARIANO RODRIGUES DE ALBUQUERQUE	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.doc	23/04/2019 20:54:39	THATYANE MARIANO RODRIGUES DE ALBUQUERQUE	Aceito
Orçamento	Orcamento.docx	23/04/2019 20:54:16	THATYANE MARIANO RODRIGUES DE ALBUQUERQUE	Aceito
Cronograma	Cronograma.docx	23/04/2019 20:51:34	THATYANE MARIANO RODRIGUES DE ALBUQUERQUE	Aceito
Folha de Rosto	Folha_de_rosto_assinada.PDF	23/04/2019 20:50:56	THATYANE MARIANO RODRIGUES DE ALBUQUERQUE	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

JOAO PESSOA, 07 de Agosto de 2019

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

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ANEXO B – COMPROVANTE DE DEPÓSITO DE PATENTE

INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

25/10/2019 870190108322 10:23

29409161910772797

Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

Número do Processo: BR 10 2019 022390 1

Dados do Depositante (71)

Depositante 1 de 1

Nome ou Razão Social: UNIVERSIDADE FEDERAL DA PARAIBA
Tipo de Pessoa: Pessoa Jurídica
CPF/CNPJ: 24098477000110
Nacionalidade: Brasileira
Qualificação Jurídica: Instituição de Ensino e Pesquisa
Endereço: Cidade Universitária
Cidade: João Pessoa
Estado: PB
CEP: 58059-900
País: Brasil
Telefone: (83) 32167558
Fax:
Email: inova@reitoria.ufpb.br

PETICIONAMENTO ELETRÔNICO Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 25/10/2019 às 10:23, Petição 870190108322

Dados do Pedido

Natureza Patente: 10 - Patente de Invenção (PI)

Título da Invenção ou Modelo de Utilidade (54): FARINHA DE BATATA-DOCE PREBIÓTICA, PROCESSO E PRODUTO

Resumo: A presente invenção tem aplicação na área de alimentos funcionais e refere-se ao uso da farinha de batata-doce como ingrediente prebiótico para consumo em preparações domésticas, bem como para a formulação de alimentos funcionais, suplementos alimentares ou ração animal. O uso da farinha de batata-doce objetiva estimular o crescimento seletivo de bactérias benéficas presentes no trato gastrointestinal de mamíferos, conferindo uma variedade de benefícios para a saúde do hospedeiro. As farinhas de batata-doce apresentam ação comprovada estimulante seletiva de espécies de Lactobacillus e Bifidobacterium, as quais demonstraram intensa atividade metabólica na presença desse substrato. Assim, a obtenção das farinhas de batata-doce se deu pelo processo de secagem e caracterizam-se como uma alternativa para melhorar a qualidade nutricional de preparações e capaz de promover a saúde intestinal.

Figura a publicar: 1

PETICIONAMENTO ELETRÔNICO Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 25/10/2019 às 10:23, Petição 870190108322

Dados do Inventor (72)

Inventor 1 de 2

Nome: THATYANE MARIANO RODRIGUES DE ALBUQUERQUE

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Nacionalidade: Brasileira

Qualificação Física: Doutorando

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Cidade: João Pessoa

Estado: PB

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País: BRASIL

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Inventor 2 de 2

Nome: EVANDRO LEITE DE SOUZA

CPF: 03247278443

Nacionalidade: Brasileira

Qualificação Física: Professor do ensino superior

Endereço: Rua Antonio Gama, 80 - Ap 1102 - Tambauzinho

Cidade: João Pessoa

Estado: PB

CEP: 58042-005

País: BRASIL

Telefone: (83) 999 058809

Fax:

Email: evandroleitesouza@ccs.ufpb.br

Documentos anexados

Tipo Anexo	Nome
Comprovante de pagamento de GRU 200	00_Comprovante de pagamento.pdf
Relatório Descritivo	01_Relatorio_descritivo.pdf
Reivindicação	02_Reivindicacoes.pdf
Desenho	03_Desenho.pdf
Resumo	04_Resumo.pdf
Sisgen	05_Sisgen.pdf

Acesso ao Patrimônio Genético

☑ Declaração Positiva de Acesso - Declaro que o objeto do presente pedido de patente de invenção foi obtido em decorrência de acesso à amostra de componente do Patrimônio Genético Brasileiro, realizado a partir de 30 de junho de 2000, e que foram cumpridas as determinações da Lei 13.123 de 20 de maio de 2015, informando ainda:

Número da Autorização de Acesso: AF83AAD
Data da Autorização de Acesso: 20/07/2018

Origem do material genético e do conhecimento tradicional associado, quando for o caso

Goiana - PE.

Declaração de veracidade

☑ Declaro, sob as penas da lei, que todas as informações acima prestadas são completas e verdadeiras.