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INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM MICROBIOLOGIA AGRÍCOLA E DO AMBIENTE

CARACTERIZAÇÃO DE COMPOSTOS BIOATIVOS RESULTANTES DA BIOTRANSFORMAÇÃO DE FARINHETA DE TRIGO POR *Bacillus* spp. E BACTÉRIAS ÁCIDO LÁCTICAS

PRISCILLA MAGRO REQUE

Orientador: Prof. Dr. Adriano Brandelli

Porto Alegre
Novembro/2018

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“Nenhuma grande vitória é possível sem que tenha sido precedida de pequenas vitórias sobre nós mesmos.”

Leonid M. Leonov

“A experiência humana não seria tão rica e gratificante se não existissem obstáculos a superar. O cume ensolarado de uma montanha não seria tão maravilhoso se não existissem vales sombrios a atravessar.”

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CARACTERIZAÇÃO DE COMPOSTOS BIOATIVOS RESULTANTES DA BIOTRANSFORMAÇÃO DE FARINHETA DE TRIGO POR *Bacillus* spp. E BACTÉRIAS ÁCIDO LÁCTICAS[1]

Autor: Priscilla Magro Reque

Orientador: Prof. Dr. Adriano Brandelli

RESUMO

Este trabalho visou avaliar e caracterizar os compostos bioativos e suas propriedades em um resíduo da produção de farinha de trigo, a farinheta, após o seu cultivo com isolados de *Bacillus* spp. e bactérias ácido lácticas (LAB). Para isso, foram verificados e comparados o conteúdo de compostos fenólicos e as atividades antioxidante e antimicrobiana dos diferentes cultivos de *Bacillus* spp.; determinou-se a curva de crescimento, as atividades anti-hipertensiva, enzimáticas e prebióticas, a formação de açúcares, bem como o perfil de compostos fenólicos e xilooligossacarídeos (XOS), do cultivo com *Bacillus* que apresentou as melhores características bioativas (*B. subtilis* FTC01); e avaliou-se e comparou-se as atividades antioxidante, antimicrobiana, prebiótica e antiescurecimento, o conteúdo de substâncias fenólicas, mudanças na composição química, assim como a identificação e quantificação dos ácidos orgânicos e açúcares produzidos pelas fermentações com LAB. Os farelos de trigo bioprocessados por 72h com *Bacillus* spp. exibiram um aumento na capacidade antioxidante e compostos fenólicos totais. Alterações no perfil fenólico, com identificação de alguns ácidos hidroxibenzóico e hidroxicinâmico, também foram observadas por análise de HPLC-DAD, além da inibição do crescimento de bactérias Gram-positivas (*L. monocytogenes* e *B. cereus*) e negativas (*E. coli*). A amostra cultivada por 72h com *B. subtilis* FTC01 promoveu o crescimento de uma cepa probiótica (*L. acidophilus* LA-5) e mostrou uma alta atividade inibitória da enzima conversora da angiotensina-I. A atividade de enzimas xilanolíticas aumentou continuamente durante o cultivo do *B. subtilis* FTC01 por 72h, dentre as quais a β-xilosidase apresentou o maior valor (70.31 U/mL). O perfil e a concentração de XOS variaram consideravelmente entre amostras controle e bioprocessadas, e entre estas em diferentes tempos. O escore máximo de atividade prebiótica foi encontrado para as amostras bioprocessadas por 24h e 72h. Com relação aos cultivos 24h com LAB, as amostras apresentaram crescimento das linhagens LAB e diminuição do pH, provavelmente devido à produção de ácido láctico. Os açúcares apresentaram um padrão de redução após 24h de fermentação, exceto a maltose, produzida a partir da degradação do amido. Observou-se que a farinheta de trigo fermentada apresentou propriedades antioxidantes, antiescurecimento, antibacteriana (contra *S. aureus* e *B. cereus*) e prebiótica, além do aumento no teor total de fenólicos. Assim, o uso de técnicas de bioprocessamento demonstrou ser uma abordagem interessante, por tratar-se de uma ferramenta eficiente e ecologicamente sustentável, a fim de melhorar a bioacessibilidade e a biodisponibilidade de compostos com propriedades promotoras de saúde a partir de resíduos lignocelulósicos.

[1] Tese de Doutorado em Microbiologia Agrícola e do Ambiente – Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (135 p.) Novembro, 2018.

BIOATIVE COMPOUNDS CHARACTERIZATION RESULTING FROM WHEAT MIDDLINGS BIOTRANSFORMATION BY *Bacillus* spp. AND LACTIC ACID BACTERIA[1]

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Advisor: Prof. Dr. Adriano Brandelli

ABSTRACT

This work aimed to evaluate and characterize the bioactive compounds and their properties in a residue of wheat flour production, wheat middlings, after its cultivation with *Bacillus* spp. and lactic acid bacteria (LAB). For this, the phenolic compounds content and the antioxidant and antimicrobial activities of different *Bacillus* spp. cultures were analyzed and compared; the growth curve, the antihypertensive, enzymatic and prebiotic activities, the reducing sugars content, as well as the profile of phenolic compounds and xylooligosaccharides (XOS) of the *Bacillus* culture that presented the best bioactive characteristics (*B. subtilis* FTC01); and the antioxidant, antimicrobial, prebiotic and anti-browning activities, the phenolic substances content, changes in chemical composition, as the identification and quantification of organic acids and sugars produced by LAB fermentations, were evaluated and compared. Wheat middlings bioprocessed for 72h with *Bacillus* spp. exhibited an increase in antioxidant capacity and total phenolic compounds. Phenolic profile changes, with the identification of some hydroxybenzoic and hydroxycinnamic acids, were also observed by HPLC-DAD analysis. Furthermore, growth inhibition of Gram-positive (*L. monocytogenes* and *B. cereus*) and Gram-negative (*E. coli*) bacteria were also observed. The 72h cultured sample with *B. subtilis* FTC01 promoted the growth of a probiotic strain (*L. acidophilus* LA-5) and showed a high inhibitory activity against the angiotensin-I converting enzyme. The activity of xylanolytic enzymes increased continuously during the cultivation of *B. subtilis* FTC01 for 72h, where β-xylosidase had the highest value (70.31 U/mL). The XOS profile and concentration varied considerably between control and bioprocessed samples, and between these at different times. Maximum prebiotic activity score was found for the 24h and 72h bioprocessed samples with *B. subtilis* FTC01. Regarding the 24h LAB fermentations, all samples presented LAB strain growth and decrease of the pH, probably due to the production of lactic acid. Sugars showed a reduction pattern after 24h fermentation, except maltose, produced from starch degradation. It was observed that the processed wheat middlings presented antioxidant, anti-browning, antibacterial (against *S. aureus* and *B. cereus*) and prebiotic properties, also with an increase in total phenolic content. Thus, the use of bioprocessing techniques has been proven to be an interesting approach, as it is an efficient and ecologically sustainable tool, in order to improve bioaccessibility and bioavailability of compounds with health promoting properties from lignocellulosic waste.

[1] Doctoral Dissertation in Agricultural and Environmental Microbiology – Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (135 p.) November, 2018.

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LISTA DE ABREVIATURAS E SIGLAS

AACC	American Association of Cereal Chemists
ABITRIGO	Associação Brasileira das Indústrias do Trigo
ANVISA	Agência Nacional de Vigilância Sanitária
AX	Arabinoxilanos(as)
AXOS	Arabinoxiloligossacarídeos
CONAB	Companhia Nacional de Abastecimento
DCNT	Doenças crônicas não transmissíveis
DP	Degree of polymerization
FDA	Food and Drug Administration
FOS	Frutooligossacarídeos
GABA	Ácido gama-aminobutírico
GOS	Galactooligossacarídeos
GRAS	Generally recognized as safe
HMOs	Oligossacarídeos de leite humano
IMO	Isomaltooligossacarídeo
ISAPP	International Association for Scientific Prebiotics and Probiotics
LAB	Bactérias ácido láticas
LDL	Lipoproteína de baixa densidade
MOS	Mananoligossacarídeos
NPX	Nitrophenyl β-D-xylopyranoside
NPG	Nitrophenyl β-D-glucopyranoside
OSD	Oligossacarídeos dietéticos
SFCA	Ácido graxo de cadeia curta
UFC	Unidade formadora de colônia
USDA	United States Department of Agriculture
XOS	Xilooligossacarídeos
WGO	World Gastroenterology Organisation
WHO	World Health Organization

1. INTRODUÇÃO

Atualmente, as doenças crônicas não transmissíveis (DCNT), como diabetes, câncer, doenças cardiovasculares e demência, representam o maior problema global de saúde, causando inúmeras mortes prematuras e perda de qualidade de vida, além de gerarem grandes impactos econômicos tanto para as famílias quanto para a sociedade em geral (Malta et al., 2014). Como principal causa de morte no mundo, as DCNT foram responsáveis por 38 milhões (68 %) dos óbitos registrados em 2012, sendo mais de 40 % mortes prematuras de indivíduos com menos de 70 anos (WHO, 2014). No Brasil, estima-se que 72 % das mortes sejam causadas por DCNT, principalmente doenças cardiovasculares e respiratórias, câncer e diabetes (Malta et al., 2014).

Cada vez mais se reconhece que o modelo de saúde do século XXI vem sendo constituído tanto por estilos de vida preventivos quanto por processos terapêuticos, com a dieta desempenhando um papel importante (Blatchford et al., 2013). Com o aumento da preocupação e conscientização do consumidor em relação à saúde e a sua alimentação, o mercado de produtos funcionais tem crescido consideravelmente (Singh et al., 2015).

O farelo de trigo pode ser considerado um subproduto bastante versátil. Diversos compostos e produtos podem ser gerados a partir desse resíduo lignocelulósico, oferecendo uma ampla variedade de aplicações, tanto no setor industrial de alimentos e rações, quanto na indústria química (Apprich et al., 2014). Cerca de 90 % desse resíduo é aproveitado para uso na alimentação animal, enquanto apenas 10 % é usado pela indústria alimentícia, sendo considerada uma fonte barata e abundante de fibra alimentar. Mesmo assim, a produção de farelo de trigo para consumo humano está estimada em cerca de 90 milhões de toneladas/ano (Onipe, Jideani e Beswa, 2015).

O aproveitamento de resíduos agroindustriais tem sido amplamente utilizado na formulação de rações para alimentação animal como uma alternativa sustentável de baixo custo, minimizando o impacto ambiental gerado pelo acúmulo destes e pela própria cadeia produtiva de alimentos, agregando valor a tais subprodutos (Brandelli et al., 2012). Segundo Prückler et al. (2014), aproximadamente 150 milhões de toneladas de farelo de trigo são produzidas anualmente pela indústria mundial de moagem e usadas principalmente como

ração animal.

Além disso, o uso desses resíduos possibilita a criação de novos produtos alimentícios com características funcionais, devido a sua composição bioativa, tal como os compostos com potencial prebiótico e antioxidante (Charalampopoulos et al., 2002; Apprich et al., 2014). Entretanto, existem algumas desvantagens com relação à utilização de farelos de cereais pela indústria agroalimentar, como sabor amargo e alta capacidade de reter água. Assim, são necessárias pesquisas adicionais sobre a aplicabilidade do farelo no setor de alimentos, bem como uma melhor avaliação de sua viabilidade econômica (Prückler et al., 2014).

Diante do exposto, este trabalho visa avaliar e caracterizar os compostos bioativos e suas propriedades presentes em um resíduo da produção de farinha de trigo, a farinheta, após o seu cultivo com diferentes isolados de *Bacillus* spp. e bactérias ácido lácticas. A utilização de técnicas de bioprocessamento tem demonstrado ser uma interessante abordagem por tratar-se de uma ferramenta eficiente e ecologicamente sustentável para obtenção de modificações positivas na matriz de alimentos, aumentando, assim, a bioacessibilidade e a biodisponibilidade de substâncias associadas à geração de efeitos benéficos à saúde.

Nesse sentido, devido à falta de dados bibliográficos consistentes com relação ao efeito do bioprocessamento de farelo de trigo em sua composição e propriedades bioativas, o presente trabalho justifica-se por aprofundar o conhecimento sobre o melhoramento da bioacessibilidade e biodisponibilidade de compostos com alegações de promoção de saúde, através do uso de cepas bacterianas não patogênicas, cuja maioria das espécies são reconhecidamente consideradas probióticas. Ademais, além de agregar valor e minimizar o impacto ambiental causado pela acumulação de resíduos agroindustriais, subprodutos de cereais podem ser utilizados no desenvolvimento de novos produtos com propriedades funcionais, representando uma fonte potencialmente útil a ser explorada.

2. OBJETIVOS

2.1 Objetivo Geral

Caracterização e propriedades dos compostos bioativos de farinheta de trigo, um resíduo da produção de farinha, obtidos através da biotransformação desse subproduto por isolados de *Bacillus* spp. e bactérias ácido láticas (LAB).

2.2 Objetivos Específicos

2.2.1 Avaliar e comparar as atividades antioxidante e antimicrobiana, além do conteúdo de compostos fenólicos dos diferentes cultivos de isolados de *Bacillus* spp. (*B.* spp. P7, *B.* spp. P45, *B. amyloliquefaciens* I3, *B. subtilis* FTC01 e *B. subtilis* ATCC19659);

2.2.2 Determinar as atividades anti-hipertensiva e prebiótica, bem como caracterizar o perfil de compostos fenólicos do cultivo com *Bacillus* que apresentar as melhores características bioativas;

2.2.3 Analisar, ao longo do tempo, a curva de crescimento, atividades enzimáticas e prebióticas, a formação de açúcares e identificar e quantificar os oligossacarídeos do cultivo com *Bacillus* que apresentar as melhores características bioativas;

2.2.4 Avaliar e comparar as atividades antioxidante, prebiótica, antimicrobiana e antiescurecimento, bem como o conteúdo de substâncias fenólicas dos diferentes cultivos dos isolados de LAB (*Streptococcus thermophilus* 985, *Lactobacillus plantarum* DSM20174, *L. fabifermentans* T30PCM38 e *L. fermentum* LM7);

2.2.5 Investigar as mudanças na composição química, assim como identificar e quantificar os ácidos orgânicos e açúcares produzidos pelas fermentações com LAB.

3. REVISÃO DA LITERATURA

3.1 Trigo (*Triticum* spp.)

O trigo corresponde, atualmente, a uma das culturas de cereais mais importantes, juntamente com o milho e o arroz, atingindo cerca de 30 % da produção total de grãos e 17 % da terra cultivável no mundo. Acredita-se que sua domesticação tenha iniciado por volta de 10.000 a.C., às margens dos rios Tigre e Eufrates. Há 5.000 anos, os egípcios já utilizavam o processo de fermentação de grãos para produzirem pão. Com relação ao Brasil, existem relatos de que o cultivo do trigo foi introduzido pelos colonizadores europeus em 1534, na capitania de São Vicente, tendo sido pioneiro nas Américas na exportação deste cereal em 1680 (De Mori, 2015).

Dados recentes reportam que a produção global de trigo alcançou a marca de 758 milhões de toneladas entre os anos de 2017 a 2018, tendo sido consumidas cerca de 740 milhões de toneladas no mesmo período, das quais aproximadamente 70 % para consumo humano e 20 % para uso em ração animal. Atualmente, os maiores produtores e consumidores são União Europeia, China e Índia. (IGC, 2018; USDA, 2018)

No Brasil, a Companhia Nacional de Abastecimento (CONAB) estima para este ano uma produção de cerca de 5 milhões de toneladas de trigo. O Estado do Paraná lidera como maior produtor brasileiro, com aproximadamente 1 milhão de hectares destinados ao cultivo do trigo, contribuindo com 65,5 % deste total, seguido pelo Rio Grande do Sul, com 700 mil hectares de área cultivada e 31 % do abastecimento desse cereal (CONAB, 2018). Entretanto, tal produção não é capaz de suprir a demanda do mercado nacional, gerando a necessidade de importação do grão, estimada em 6,7 milhões de toneladas/ano (USDA, 2018).

O trigo é uma gramínea extremamente adaptável, crescendo em uma ampla variedade de condições, o que permite seu cultivo em larga escala (Cracknell, 2016). Além disso, possui alto rendimento e seus grãos podem ser armazenados a longo prazo, desde que o teor de água se mantenha abaixo de 15 % e as pragas estejam controladas (Shewry, 2009). Tais características favorecem o cultivo da planta, promovendo sua produtibilidade e comercialização.

As espécies de trigo mais importantes são a hexaploide *Triticum aestivum* L. e a tetraploide *Triticum turgidum* L. subsp. *durum* (Peña-Bautista et al., 2017). A

primeira é conhecida como trigo comum e a mais cultivada ao redor do mundo, correspondendo a cerca de quatro quintos da produção mundial, sendo bastante utilizada para fabricação de pães, enquanto que a segunda é mais indicada na preparação de massas, na medida em que forma um glúten mais resistente, permitindo uma textura firme após seu cozimento (ABITRIGO, 2016).

Os grãos de trigo são amplamente consumidos, especialmente na forma de produtos derivados da farinha, como pães, massas, biscoitos e bolos, representando um dos principais componentes da dieta. De acordo com Peña-Bautista et al. (2017), este cereal corresponde ao grão mais versátil na produção de diferentes tipos de preparações, fornecendo grande quantidade de calorias e proteínas a diversas populações ao redor do mundo, sendo de extrema importância para a segurança alimentar. Além do seu uso na alimentação humana, o trigo e alguns de seus compostos (tais como amido, gérmen e glúten) possuem também diversas aplicações em produtos não-alimentícios e na alimentação animal, além da produção de bioetanol e cerveja pela fermentação dos grãos (De Mori, 2015).

As frações que compõem os grãos desse cereal (figura 1) correspondem ao endosperma, gérmen e farelo, sendo esta última caracterizada pelas camadas exteriores (aleurona, hialina, testa, pericarpo interno e externo) e liberada através do processo de moagem (Onipe, Jideani e Beswa, 2015). Por sua vez, o farelo de trigo, o principal subproduto da produção de farinha, corresponde a um conjunto de frações com diferentes tamanhos de partículas, denominadas farinheta de trigo, farelo de trigo fino e grosso (Wesendonck et al., 2013).

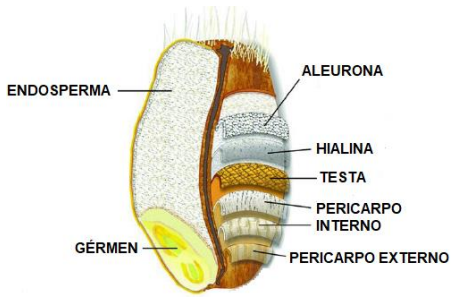

Figura 1. Estrutura do grão de trigo. Adaptado de: Onipe, Jideani e Beswa, 2015.

O farelo extraído pode ser utilizado como suplemento alimentar e ração animal, bem como ser moído com o restante dos componentes do grão para a

produção da farinha de trigo integral. Com relação à composição deste subproduto, aproximadamente 50 % é representada por fibras dietéticas, tais como xilana, lignina e celulose, possuindo entre 8 e 13 % de umidade, 10-19 % de proteína e de 9 a 40 % de amido (Onipe, Jideani e Beswa, 2015).

Além de vitaminas e minerais, uma grande variedade de compostos bioativos encontra-se presente, como os ácidos fenólicos e outros polifenóis, alquilresorcinóis e carotenoides, com reconhecidas atividades antioxidante e anti-inflamatória (Anson et al., 2012). Os compostos fenólicos do farelo de trigo, em sua maioria, correspondem a fenóis contendo um anel aromático, representados pelos alquilresorcinóis, ácidos fenólicos (ferúlico, sinápico e cumárico) e vitamina E (Apprich et al., 2014).

A porção do farelo com maior capacidade antioxidante é a camada aleurona, a qual contém elevado conteúdo fenólico, especialmente ácido ferúlico (Anson et al., 2008; Prückler et al., 2014). A maioria das proteínas encontra-se localizada na camada aleurona, representada principalmente por albuminas e globulinas, ao invés de gluteninas e gliadinas, que são mais abundantes no endosperma. Essa diferença também se reflete na composição de aminoácidos, onde as proteínas apresentam maior concentração de lisina, arginina, alanina, asparagina e glicina e reduzido teor de glutamina, prolina, fenilalanina e enxofre, possuindo assim alto valor biológico e nutricional. (Arte et al., 2015)

Diversas propriedades benéficas à saúde têm sido associadas aos compostos bioativos e às fibras alimentares encontradas no farelo de trigo. Estas incluem a prevenção contra danos oxidativos e, portanto, redução do risco de desenvolvimento de doenças crônicas, incluindo alguns tipos de câncer (particularmente colorretal), doenças cardiovasculares, diabetes tipo 2 e obesidade; inibição da oxidação da LDL-colesterol; proteção de eritrócitos humanos contra radicais livres; promoção da saúde mental; prevenção de doenças gastrointestinais; e atividade prebiótica (Poutanen, 2012; Stevenson et al., 2012). Além disso, foi relatado que o farelo de trigo possui atividade anti-biofilme e propriedades anti-adesivas contra patógenos entéricos (González-Ortiz et al., 2014).

3.2 Resíduos lignocelulósicos

Devido a seu elevado conteúdo de celulose e hemicelulose, os materiais

residuais lignocelulósicos de origem agroindustrial podem ser convertidos em produtos de alto valor agregado, como bioetanol e outros biocompostos, formados pela fermentação dos seus açúcares. Sua utilização, além de limitar a polêmica do uso de alimentos para geração de combustível, evita o deslocamento de culturas alimentares e o desmatamento de novas áreas, reduzindo os impactos negativos causados ao meio ambiente (Liguori et al., 2016).

Segundo Kahra et al. (2013) e Ogeda e Petri (2010), esses resíduos são formados por três principais compostos: celulose (homopolímero de glicose ligadas através de ligações β-1,4-glicosídicas), hemicelulose (heteropolímero de xilose, arabinose, glicose, galactose, manose e ácidos glucurônico e manurônico) e lignina (polímero aromático com unidades fenilpropano interligadas). Os polissacarídeos encontrados na parede celular correspondem, majoritariamente, a arabinoxilanos (AX) e β-glucanos. O teor de açúcares, bem como a quantidade e o tamanho do pericarpo, aleurona e a espessura da parede celular, variam entre os diferentes tipos de cereais originários (Coda, Katina e Rizello, 2015).

As ligninas podem ser utilizadas na produção de espumas de poliuretanas, resinas fenólicas e epóxi, como fontes de fenol e etileno, e também para conversão em fibras de carbono. A hidrólise de celulose gera glicose e celobiose, enquanto que a hidrólise de ligninas e hemicelulose gera açúcares e subprodutos (como difenóis, derivados de fenilpropano, cetonas, furfural e ácido acético), os quais podem acabar inibindo a fermentação microbiana (Ogeda e Petri, 2010). Com relação ao amido, este pode ser hidrolisado em glicose e assim ser utilizado em processos fermentativos, produzindo substâncias de interesse comercial, tais como ácido láctico, ácido succínico, etanol e/ou butanol (Apprich et al., 2014).

O processo de fermentação do material lignocelulósico é geralmente caracterizado por três fases. A primeira corresponde ao pré-tratamento da biomassa, a fim de remover a lignina e liberar os polissacarídeos, e representa a etapa de maior impacto ambiental por conta do alto gasto energético. Após, os polissacarídeos são hidrolisados em monossacarídeos, principalmente através do uso de enzimas hidrolíticas, sendo a etapa mais dispendiosa devido ao alto custo das enzimas. Na última fase, os açúcares são então convertidos nos bioprodutos de valor agregado (Liguori et al., 2016).

Existe uma demanda crescente por combustíveis de transporte

alternativos e sustentáveis, baseados em recursos renováveis. Atualmente, os biocombustíveis são produzidos a partir do amido de milho ou cana-de-açúcar (bioetanol) ou a partir de óleos vegetais e gordura animal (biodiesel). No entanto, estes biocombustíveis de primeira geração fazem concorrência com a indústria de alimentos e rações animais. Nesse sentido, os resíduos lignocelulósicos apresentam-se como uma alternativa de matéria-prima. Porém, o processo de conversão da biomassa lignocelulósica em biocombustíveis é muito mais complexo, sendo necessário um pré-tratamento termoquímico a fim de decompor suas estruturas, aumentando a acessibilidade enzimática. Após esse passo, são realizadas as etapas de hidrólise e fermentação. (Weber et al., 2010)

Cerca de 95 % da produção de etanol nos EUA é de milho e o restante de sorgo, enquanto que, no Reino Unido e em outros países europeus, o cereal predominante é o trigo (Du et al., 2009). No entanto, o uso do farelo de trigo nas biorrefinarias de cereais é considerado problemático em termos econômicos. Nesse sentido, a utilização dos AX pode ser considerada promissora como um potencial coproduto importante, que proporcionaria uma integração de processos. Os coprodutos são essenciais para a economia das biorrefinarias e, portanto, para a sustentabilidade do processo de obtenção do bioetanol (Misailidis et al., 2009).

Outras aplicações possíveis, interessantes e inovadoras são o uso desse material fibroso natural para o desenvolvimento de biocompósitos, em substituição aos polímeros de petróleo (Alemdar e Sain, 2008; Masłowski et al., 2017). Ainda, diversas substâncias de interesse comercial podem ser obtidas a partir de compostos do farelo de trigo, não somente dos açúcares, tais como ácido láctico, vanilina, proteínas e aminoácidos, GABA, β-glucano, xilano, xilose e xilitol (Apprich et al., 2014).

3.2.1 Farinheta de trigo

O processamento dos grãos de trigo para a obtenção da farinha gera cerca de 25 % de resíduo, majoritariamente representado pelo farelo de trigo, que correspondeu a 2,655 mil toneladas produzidas pela indústria brasileira no ano de 2017 (ABITRIGO, 2018). A produção de farinha branca refinada envolve um processo caracterizado pelas etapas de limpeza, condicionamento, moagem e peneiração dos grãos e resíduos (Brandelli et al., 2012), cujo fluxograma

encontra-se apresentado na figura 2. As quantidades específicas de cada subproduto gerado dependerão das propriedades físicas do trigo, da operação de moagem e dos produtos finais desejados (Blasi et al., 1998).

O conjunto de resíduos formam o farelo de trigo, o qual é composto por três frações, representadas pelos subprodutos farinheta, farelo fino e farelo grosso. Como anteriormente mencionado, a maioria desses subprodutos são aproveitados pela indústria de rações para uso na alimentação animal. No entanto, animais não-ruminantes, como aves e suínos, possuem uma baixa capacidade para digerir o conteúdo fibroso desses resíduos, constituindo uma dieta de baixo valor energético e, portanto, de baixa valorização econômica. Uma alternativa simples e viável seria a separação destas diferentes frações resultantes do processamento dos grãos de trigo, ao invés de mesclá-las, como comumente é feito pelos moinhos, e a posterior utilização daquelas com menor teor de fibras para os animais não-ruminantes e, para os ruminantes e equinos, o uso dos resíduos fibrosos. (Brandelli et al., 2012)

A farinheta é um subproduto composto por cerca de 5 % de fibra bruta e possui boa aceitabilidade tanto por aves quanto por suínos, apresentando maior energia metabolizável entre as frações que compõem o farelo de trigo, sendo assim recomendado seu uso na alimentação de não-ruminantes (Brandelli et al., 2012). No caso de animais ruminantes, sua fibra é altamente digerível, porém, com reduzido tamanho de partícula, o que a torna menos eficaz na estimulação e tamponamento do rúmen (Dhuyvetter, Hoppe e Anderson, 1999).

Apresenta como características partículas finas, textura aveludada e coloração parda clara, devido à presença de resquícios de cascas dos grãos de trigo (Brandelli et al., 2012). Contém cerca de 18 % de proteína e altas concentrações de macro e microminerais, com exceção do cálcio, sendo considerada uma boa fonte de fósforo, potássio, cobre, zinco, magnésio e selênio (Dhuyvetter, Hoppe e Anderson, 1999). O teor de nutrientes da farinheta pode ser influenciado por diferentes fatores, tais como tipo e variedade do trigo e fatores ambientais durante a produção e armazenamento dos grãos (Blasi et al., 1998).

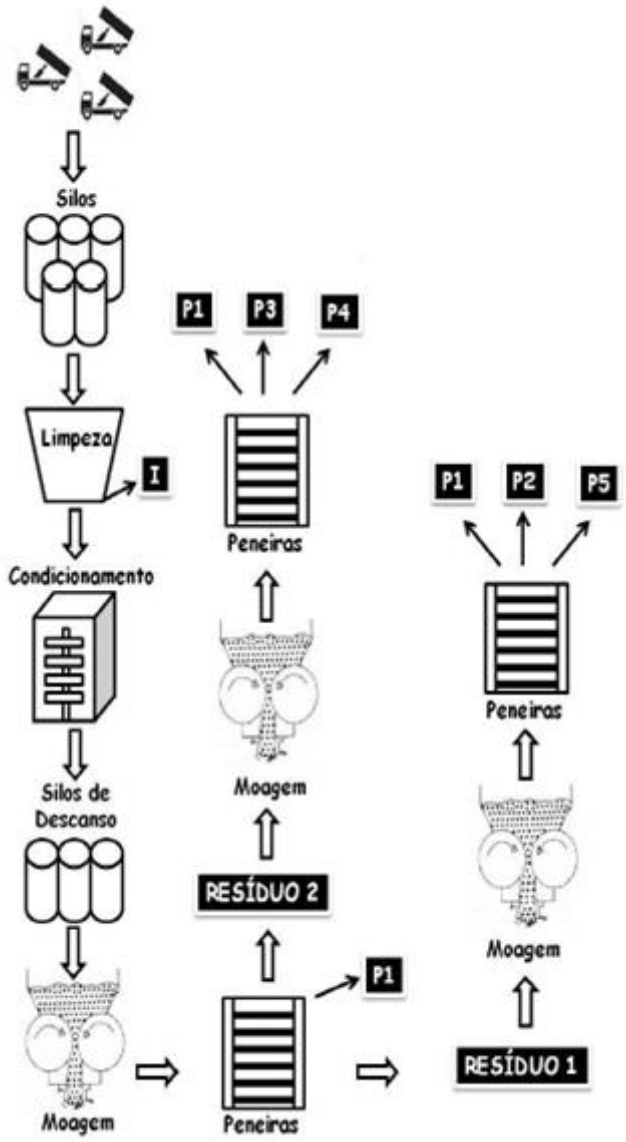

Figura 2. Fluxograma do processamento dos grãos de trigo. Fonte: Brandelli et al. (2012); onde I, P1, P2, P3, P4 e P5 correspondem, respectivamente, a impurezas, farinha, farelo fino, farinheta, farelo grosso e gérmen.

3.3 *Bacillus* spp.

Bacillus spp. são bastonetes Gram-positivos, formadores de esporos resistentes a condições adversas, aeróbicos ou anaeróbicos facultativos, com algumas espécies descritas como estritamente anaeróbicas. Pertencem ao filo Firmicutes, classe Bacilli, ordem Bacillales e família Bacillaceae, podendo ser isolados de diversos ambientes, como ar, água, intestino de homens e animais, vegetais entre outros alimentos. A maioria das espécies tem pouco ou nenhum potencial patogênico e raramente está associada a doenças em humanos ou outros animais, com exceção do *Bacillus anthracis*, o agente do antraz. Algumas

são causadoras de intoxicação alimentar e infecções oportunistas enquanto outras podem ser consideradas probióticas. (Logan e De Vos, 2015; Elshaghabee et al., 2017)

Esse gênero corresponde ao grupo de maior heterogeneidade com relação a características genéticas e fenotípicas (Elshaghabee et al., 2017). Tais bactérias exibem uma ampla diversidade de propriedades: possuem colônias muito variáveis quanto à morfologia e ao tamanho, inter e intraespecífica; podem ser psicrofílicas ou termofílicas, acidofílicas ou alcalifílicas, halofílicas ou tolerantes ao sal, catalase e oxidase positivas ou negativas; são quimiorganotróficas, porém com duas espécies quimiolitotróficas facultativas (Logan e De Vos, 2015).

No que diz respeito a potenciais funcionalidades probióticas, diversas cepas já foram testadas em modelos *in vitro* e *in vivo* e, além de apresentarem os pré-requisitos necessários para a caracterização de um microrganismo probiótico, oferecem maior tolerância a ácidos e melhor estabilidade durante o processamento térmico e armazenamento a baixas temperaturas. Tais propriedades probióticas podem ser relacionadas à capacidade de produzir peptídeos antimicrobianos, bem como interagir com o hospedeiro através de fatores de adesão. Além disso, possuem também propriedades de exclusão competitiva, antioxidantes, antimicrobianas, imunomoduladoras e fermentativas. (Elshaghabee et al., 2017)

Segundo Schulz, Bonelli e Batista (2005), o gênero *Bacillus* pode ser considerado a maior fonte industrial de enzimas, como fitase, xilanase, ciclodextrinase e queratinase, sendo a espécie *B. amyloliquefaciens* uma das mais utilizadas na produção de amilases e proteases. Essas últimas possuem aplicação na fabricação de queijos, a fim de melhorar o sabor, bem como na indústria panificadora, sendo usadas para hidrólise parcial do glúten.

Bacillus produzem várias substâncias antimicrobianas, como antibióticos peptídicos e lipopeptídicos, e bacteriocinas, peptídeos de baixa massa molecular. A maioria das bacteriocinas faz parte do grupo dos lantibióticos, categoria de peptídeos modificados pós-traducionalmente, e encontram-se classificadas conforme a Tabela 1. As bacteriocinas produzidas por membros desse gênero vêm ganhando importância devido ao seu amplo espectro de inibição, que inclui bactérias Gram-positivas e negativas, leveduras entre outros fungos (Abriouel et

al., 2011). Mais de 700 antibióticos já foram identificados, sendo o *B. subtilis* a espécie mais produtiva (Sorokulova, 2013).

Tabela 1. Classificação das bacteriocinas de espécies de *Bacillus* e sua comparação com LAB.

Bacteriocinas de *Bacillus* spp.	Exemplos	Bacteriocinas de LAB
Classe I. Peptídeos pós-traducionalmente modificados		**Classe I.** Lantibióticos
Subclasse I.1. Peptídeos lantibióticos alongados	Subtilina, Ericina S e A	
Subclasse I.2. Outros peptídeos lantibióticos	Sublancina 168, Mersacidina, Paenibacillina	
Subclasse I.3. Dipeptídeos lantibióticos	Haloduracina, Lichenicidina	
Subclasse I.4. Outros peptídeos pós-traducionalmente modificados	Subtilisina A	
Classe II. Peptídeos não modificados		**Classe II.** Peptídeos pequenos e lineares
Subclasse II.1. Peptídeos tipo Pediocina	Coagulina, SRCAM 37, 602 e 1580	**Classe IIa**
Subclasse II.2. Peptídeos tipo Turicina	Turicina H, S e 17, Bacturicina F4, Cereína, MRX1	
Subclasse II.3. Outros peptídeos lineares	Cereína 7A e 7B, Lichenina, Turicina 439	
Classe III. Proteínas grandes	Megacina A-216 e A-19213	**Classe III.** Bacteriocinas termolábeis grandes

Fonte: adaptado de Abriouel et al. (2011).

Cepas de *Bacillus* produtoras de bacteriocinas possuem potencial aplicabilidade como probióticos, devido à sua atividade inibitória contra patógenos intestinais, e também como contraceptivos naturais, tal como exemplo da bacteriocina subtilisina A, que apresenta atividade espermicida contra espermatozoides de humanos e animais de criação (Abriouel et al., 2011). A subtilisina, formada por *B. subtilis*, também possui aplicação em alimentos, com o objetivo de hidrolisar proteínas de soja para fabricação do seu molho, aumentar a capacidade emulsificante na produção de salsichas e mortadelas, bem como melhorar o sabor de carnes curadas. (Schulz, Bonelli e Batista, 2005)

Além de possuírem ação contra patógenos humanos, os *Bacillus* também

agem como biocontroladores de fitopatógenos, devido principalmente à produção de antibióticos peptídicos antifúngicos e antibacterianos. Um exemplo desses compostos é a iturina A, produzida por *B. amyloliquefaciens*, sendo utilizada no controle do fitopatógeno *Rhizoctonia solani* na produção de soja. (Schulz, Bonelli e Batista, 2005). Assim, bioprotetores baseados em *Bacillus* produtores de bacteriocinas podem ser aplicados para melhorar a saúde de plantas e também evitar a decomposição pós-colheita de frutas e vegetais (Abriouel et al., 2011). Diversas cepas de *B. subtilis* produzem lipopeptídeos cíclicos, como a surfactina, considerado um dos mais potentes biossurfactantes conhecidos, por conta de sua natureza anfifílica, exercendo importante papel na biorremediação (Barros et al., 2007; Carvalho et al, 2010).

Cepas de *Bacillus* administradas oralmente favoreceram a redução significativa dos níveis de LDL-colesterol plasmático, colesterol total hepático e triglicerídeos, em estudos com animais (Sorokulova, 2013). De acordo com Cutting (2011), as espécies de *Bacillus* que foram mais extensivamente estudadas quanto a seu potencial probiótico são *B. subtilis*, *B. clausii*, *B. cereus*, *B. coagulans* e *B. licheniformis*. Diversas cepas vêm sendo utilizadas como probióticos em rações animais, suplementos alimentares humanos e vários medicamentos registrados. Ainda segundo o mesmo autor, esse gênero possui vantagens em relação a outros microrganismos não esporulados, tais como *Lactobacillus* spp., na medida em que seus esporos são estáveis ao calor e sobrevivem ao baixo pH da barreira gástrica, o que torna seu uso bastante interessante como aditivo alimentar.

Bacillus também podem ser aplicados no controle de mastite animal, para inibição de patógenos entéricos em aves, bem como para melhorar a fermentação ruminal por inibição seletiva de microbiota indesejada e proliferação de cepas com atividades hidrolíticas (Abriouel et al., 2011). Segundo Cutting (2011), em estudos realizados com aves, a administração oral de esporos de *B. subtilis* foi capaz de reduzir a infecção por *Salmonella* Enteritidis, *Clostridium perfringens* e *Escherichia coli* O78:K80.

Apesar da diversidade de bacteriocinas produzidas por *Bacillus* com propriedades tecnológicas atrativas, sua importância e valor industrial são amplamente subestimados, havendo poucos relatos de aplicações em alimentos. Isso se deve ao fato da ausência do reconhecimento de segurança GRAS

(“Generally Recognized As Safe”) pelo órgão norte-americano “Food and Drug Administration” (FDA) para muitas das espécies, com exceção de alguns representantes, como *B. subtilis* e *B. licheniformis*. Além disso, algumas espécies são altamente patogênicas ou produzem toxinas capazes de contaminar alimentos. Portanto, um rigoroso processo se faz necessário para seleção e desenvolvimento de candidatos probióticos ou culturas iniciadoras de *Bacillus*, considerando-se as características de virulência divergentes intraespecíficas. (Abriouel et al., 2011)

De qualquer forma, diversos microrganismos desse gênero são utilizados na fermentação de alimentos, atuando na hidrólise de proteínas a aminoácidos e peptídeos. A oxidação dos aminoácidos pela bactéria resulta em aumento do pH, gerando produtos com odor característico de amônia (Schulz, Bonelli e Batista, 2005). *B. subtilis* possui metabolismo anaeróbico através de amonificação de nitrato e vários processos de fermentação, onde lactato, acetato e butanodiol são os principais produtos formados (Ramos et al., 2000). De acordo com Abriouel et al. (2011), as bactérias desse gênero desempenham um papel importante na produção de alimentos e bebidas de fermentação alcalina.

B. subtilis var. *natto* é uma linhagem utilizada na fermentação da soja para preparar o alimento japonês conhecido como “natto”. A serino protease nattokinase, secretada a partir de células vegetativas de *B. subtilis* e, na cepa *natto*, em altos níveis, tem o status de enzima GRAS nos EUA, sendo purificada e vendida como suplemento de saúde em todo o mundo (Cutting, 2011).

Algumas cepas de *B. subtilis* são capazes de produzir ácido γ-poliglutâmico (PGA), um polímero de aminoácido comumente encontrado nos alimentos fermentados de soja (Tamang, Watanabe e Holzapfel, 2016). Além disso, cepas específicas de *B. subtilis* também são utilizadas para a fermentação da soja no tradicional condimento africano “dawadawa” ou para a fermentação de sementes de algaroba na produção do condimento nigeriano “okpehe”. Um *B. cereus* sp. t*oyoi*, não tóxico e com propriedades probióticas, é usado como aditivo para ração animal. (Abriouel et al., 2011)

3.4 Bactérias ácido láticas (LAB)

As bactérias ácido láticas, ou como são também chamadas por sua sigla em inglês LAB (“lactic acid bacteria”), possuem esse nome por produzirem ácido

lático como principal produto de seu metabolismo. São encontradas em diversos habitats, como alimentos e rações, água, solo, membranas mucosas, trato respiratório, gastrointestinal e genital de humanos e animais. Essas bactérias são bastonetes e cocos não-esporulados, Gram-positivas, catalase negativas, anaeróbicas ou microaerofílicas, majoritariamente mesófilas, com algumas linhagens termófilas, podendo crescer em temperaturas entre 5 e 45 ºC e pHs ácidos. (Forsythe, 2002; Liu et al., 2014)

Dentro do filo Firmicutes, as LAB pertencem à ordem Lactobacillales e incluem diversos gêneros: *Aerococcus, Alloiococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Symbiobacterium, Tetragenococcus, Vagococcus* e *Weissella*. Com relação ao filo Actinobacteria, são consideradas LAB somente as espécies do gênero *Bifidobacterium*. O maior gênero, *Lactobacillus*, inclui mais de 100 espécies, que foram listadas e divididas em três grupos: grupo I (homofermentativas obrigatórias), grupo II (heterofermentativas facultativas) e grupo III (heterofermentativas obrigatórias). (Liu et al., 2014)

As LAB do tipo homofermentativas possuem a enzima aldolase e são capazes de fermentar os açúcares mais diretamente ao ácido lático do que as heterofermentativas, gerando duas moléculas de lactato a partir de uma molécula de glicose. Já as heterofermentativas, que usam a via alternativa pentose monofosfato, convertendo hexoses a pentoses pela enzima fosfoquetolase, produzem uma molécula de lactato e outra de etanol ou acetato, além de gás carbônico (CO_2). (Carr, Chill e Maida, 2002; Forsythe, 2002)

Durante a fermentação, a conversão dos açúcares disponíveis em ácido láctico via piruvato pode gerar ainda outros metabólitos, como acetato, etanol, diacetil e acetaldeído. Essas substâncias contribuem para o sabor típico dos produtos fermentados, como “kefir” e “koumiss” (etanol), manteiga (diacetil) e iogurte (acetaldeído). Através do uso de linhagens específicas e otimização do processo, a atividade da LAB pode ser modificada a fim de aumentar o conteúdo de compostos bioativos, bem como remover substâncias tóxicas ou antinutricionais. (Leroy e De Vuyst, 2004)

Tais bactérias possuem grande relevância para a indústria de alimentos e bebidas, sendo bastante utilizadas como culturas iniciais na fabricação de produtos lácteos, como iogurte, manteiga e diversos tipos de queijo. Também

exercem papel importante no processamento de carnes, bebidas alcoólicas e alguns vegetais, cujos produtos incluem salsicha, presunto, vinho, cerveja, “pickles” e “saukerkraut”. (Carr, Chill e Maida, 2002)

A combinação entre *Streptococcus thermophilus* e *Lactobacillus delbrueckii* subsp. *bulgaricus, Lb. lactis* e/ou *Lb. helveticus*, é amplamente utilizada como cultura inicial para fabricação de iogurte e outros produtos lácteos fermentados, como queijos suíços e italianos. Existe uma relação especial entre *S. thermophilus* e *Lb. delbrueckii* subsp. *bulgaricus*, onde a produção de ácido fórmico pelo primeiro promove o crescimento das outras espécies, as quais, por sua vez, fornecem compostos aromáticos (acetaldeído) e atividade proteolítica para permitir que o *S. thermophilus* possa crescer no leite. (Liu et al., 2014)

As LAB podem também ser consideradas e utilizadas como culturas iniciadoras funcionais, na medida em que contribuem para a segurança alimentar e/ou oferecem vantagens organolépticas, tecnológicas, nutricionais ou de saúde. Possuem a capacidade de gerar substâncias antimicrobianas, enzimas, exopolissacarídeos, adoçantes, compostos aromáticos e nutracêuticos ou LAB com propriedades promotoras de saúde, as chamadas cepas probióticas. Como exemplo de iniciadores funcionais produtores de exopolissacarídeos, *Lb. delbrueckii* subsp. *bulgaricus* e *S. thermophilus* são promissores. (Leroy e De Vuyst, 2004)

Além de ácidos orgânicos (lático, acético, fórmico, propiônico), diacetil, etanol, CO_2 e peróxido de hidrogênio, as principais substâncias antimicrobianas produzidas por LAB correspondem às bacteriocinas, cujo exemplo mais importante é a nisina (única bacteriocina permitida para uso em alimentos no Brasil), produzida pelo *Lactococcus lactis* subsp. *lactis*, capaz de inibir diversas bactérias Gram-positivas. (Forsythe, 2002; Leroy e De Vuyst, 2004). A nisina age inativando os grupos sulfidrila da membrana citoplasmática, causando perda do conteúdo celular, bem como previne a germinação do endósporo. Sua eficácia pode ser expandida contra bactérias Gram-negativas através da inclusão de agentes quelantes. (Carr, Chill e Maida, 2002)

3.5 Bioprocessamento de resíduos agroindustriais

Alguns compostos bioativos presentes na camada externa de grãos de cereais, como fibras e substâncias fenólicas, encontram-se ligados em estruturas

complexas da parede celular, que podem resistir ao processo de moagem convencional, possuindo assim baixa bioacessibilidade (Delcour et al., 2012; Stevenson et al., 2012).

A bioacessibilidade reduzida, por sua vez, corresponde a um dos fatores limitantes da biodisponibilidade de tais compostos, na medida em que se tornam indisponíveis para absorção no trato gastrointestinal. Assim, o uso de técnicas de bioprocessamento, que se refere à aplicação de enzimas e/ou cultivo microbiano para obtenção de modificações desejáveis em uma matriz, pode ser uma estratégia interessante a ser aplicada a fim de melhorar a biodisponibilidade de substâncias com alegações de promoção da saúde (Coda, Katina e Rizello, 2015).

Alterações em estruturas específicas dos cereais e nas interações entre diferentes moléculas proporcionam um aumento da qualidade destes produtos, tanto com relação ao seu potencial funcional e nutricional quanto ao aspecto tecnológico. Portanto, a utilização de novas técnicas de moagem, em conjunto com o bioprocessamento por fermentação e/ou tecnologias enzimáticas, representam uma boa alternativa de melhoramento dos alimentos integrais (Coda, Katina e Rizello, 2015). Assim, além de uma boa fonte de fibra dietética, farelos de cereais podem ser explorados como um componente nutricional e funcional a fim de melhorar a qualidade da dieta (Nordlung et al., 2013).

No farelo de trigo, a adição da enzima fitase é aconselhável para limitar o efeito prejudicial do ácido fítico na disponibilidade de minerais. Porém, enzimas comerciais são muitas vezes economicamente inviáveis. Assim, o uso de microrganismos produtores da enzima desejada para ação no substrato pode oferecer uma via tecnologicamente possível (Prückler et. al, 2014). Além disso, germinação, fermentação e cozimento, que produzem a hidrólise do fitato, também podem gerar efeitos benéficos sobre a biodisponibilidade mineral (Stevenson et al., 2012).

Com relação aos processos fermentativos, dependendo da origem dos microrganismos envolvidos, é possível classificá-los em dois tipos: fermentações naturais, que ocorre através da microbiota autóctone, e inoculadas, onde um ou mais microrganismos são adicionados. Além disso, de acordo com o teor de água no sistema, o processamento pode ser do tipo fermentação em estado sólido ou fermentação em estado líquido. Diversos fatores influenciam e afetam a eficiência

do bioprocessamento, devendo, portanto, serem otimizados (Gan et al., 2017).

O uso de processos fermentativos através de LAB já está bem estabelecido e reconhecido pelo aumento das propriedades nutricionais, bem como melhora na textura e palatabilidade de produtos ricos em grãos e fibras. Além disso, aumenta a concentração de peptídeos bioativos, a solubilidade das fibras alimentares, a biodisponibilidade mineral e diminui o índice glicêmico. O incremento no teor de aminoácidos livres acontece por conta da alta atividade proteolítica das bactérias e da ativação endógena da protease em pH ácido. Com relação ao aumento das propriedades nutricionais, este pode estar relacionado com o menor tamanho de partícula dos farelos. Tal característica gera uma maior liberação dos compostos pela ruptura das células, bem como uma superfície mais acessível para microrganismos e atividade enzimática, devido a uma maior área de contato para acessar os carboidratos fermentáveis. (Coda et al., 2014)

A degradação da parede celular de farelo pelo seu bioprocessamento foi capaz de aumentar a solubilização e digestão das proteínas, além do catabolismo de carboidratos complexos e do ácido ferúlico, em modelos de digestão *in vitro* e no cólon (Nordlung et al., 2013). Anson et al. (2009) utilizaram técnicas de bioprocessamento para liberar compostos fenólicos em farelo de trigo, como o uso de enzimas visando ligações específicas ou de sistemas fermentativos como fontes dessas enzimas. Os autores verificaram um aumento do conteúdo de ácidos fenólicos nos pães contendo farelo processado, concluindo que o bioprocessamento do farelo de trigo foi capaz de melhorar significativamente a bioacessibilidade dos ácidos fenólicos em pães integrais, bem como aumentar a sua liberação no intestino e conversão em seus metabólitos.

De acordo com Gan et al. (2017), sementes comestíveis fermentadas e seus produtos derivados apresentam maior conteúdo de compostos bioativos, como algumas vitaminas, GABA, fenólicos e peptídeos, em comparação com materiais não fermentados. Além disso, exibem diversas bioatividades, como propriedades antioxidantes, anti-hipertensivas e anticancerígenas, sugerindo que seu consumo possa gerar benefícios para a saúde. A capacidade antioxidante de cereais está especialmente correlacionada com o seu conteúdo de compostos fenólicos, sendo possível, portanto, ser influenciada por processos como moagem, extrusão, germinação e fermentação (Hole et al., 2012).

O bioprocessamento, quando realizado por cultivo microbiano

concomitante com o uso enzimas hidrolíticas, produz um efeito ainda maior no melhoramento do potencial bioativo de farelo de trigo. A ação conjunta de enzimas adicionadas, endógenas e microbianas leva a uma ruptura parcial das estruturas das diferentes camadas, especialmente a aleurona, por conta da degradação das estruturas de suas paredes celulares (Coda, Katina e Rizello, 2015).

Essa estratégia de tratamento com enzimas e microrganismos também tem sido utilizada no melhoramento das propriedades tecnológicas do farelo na massa e no pão de trigo, atribuindo maior qualidade ao produto final, em características como volume, textura e prazo de validade. O bioprocessamento do farelo com enzimas e fermentação de levedura permite um processo eficiente e microbiologicamente seguro (Nordlung et al., 2013; Savolainen et al., 2014).

A fermentação de cereais é realizada principalmente por espécies de LAB e leveduras. Os microrganismos comumente associados são *Enterococcus, Lactococcus, Lactobacillus, Leuconostoc, Pediococcus, Streptococcus* e *Weissella*, como representantes das bactérias, e *Candida, Debaryomyces, Hansenula, Kazachstania, Pichia, Saccharomyces, Trichosporon* e *Yarrowia*, com relação às leveduras (Tamang, Watanabe e Holzapfel, 2016). O uso de LAB e leveduras para a fermentação da farinha para a fabricação de produtos de panificação é conhecido como “sourdough”. As espécies de LAB e levedura mais utilizadas nessa fermentação são *Lactobacillus sanfranciscensis* e *Candida humilis*, respectivamente. Essa técnica pode variar, tanto de acordo com a cultura iniciadora, quanto com base em seu sistema tecnológico. As principais características geradas são a acidificação e a formação de sabores nos alimentos produzidos por esse processo. (De Vuyst, Van Kerrebroeck e Leroy, 2017)

Além de melhorar os aspectos sensoriais, a fermentação “sourdough” pode retardar a digestibilidade do amido, diminuindo o índice glicêmico, bem como aumentar a bioacessibilidade de minerais e compostos bioativos e produzir oligossacarídeos prebióticos através da ação de enzimas endógenas. Utilizando esse processo fermentativo com a adição de um consórcio enzimático (α-amilase, xilanase e lipase) à massa mãe contendo 20 % de farelo, o pão resultante apresentou melhor volume, textura e prazo de validade (Prückler et al., 2014).

A fermentação do tipo “sourdough” também vem sendo utilizada para a degradação do glúten, ao passo que a acidificação e redução das ligações

dissulfeto do glúten por lactobacilos heterofermentativos aumentam a atividade das proteases de cereais e a acessibilidade ao substrato. Neste contexto, a proteólise gerada por LAB poderia ser considerada uma nova ferramenta para o processamento de alimentos para pessoas celíacas. No entanto, estudos clínicos com o consumo de produtos derivados a partir do uso de tais técnicas são previamente requeridos e necessários (Poutanen, Flander e Katina, 2009).

3.6 Compostos bioativos

Compostos bioativos são substâncias que possuem propriedades benéficas à saúde humana, porém não são considerados elementos nutricionais essenciais ao organismo, sendo normalmente encontrados em pequenas quantidades nos alimentos (Gani et al., 2012). De acordo com a Resolução RDC 02/2002 da Agência Nacional de Vigilância Sanitária (ANVISA), as substâncias bioativas são aquelas que possuem ações metabólicas ou fisiológicas específicas, podendo ser consideradas ou não como nutrientes (BRASIL, 2002).

Os compostos bioativos obtidos através da dieta possuem diferentes mecanismos de ação e alvos fisiológicos. Por exemplo, a ação antioxidante desses compostos está relacionada ao potencial de óxido-redução de determinadas moléculas, à capacidade dessas moléculas em competir por sítios ativos e receptores nas estruturas celulares, bem como à modulação da expressão de genes que codificam proteínas envolvidas em mecanismos intracelulares de defesa contra processos oxidativos degenerativos. (Bastos, Rogero e Arêas, 2009)

O efeito de promoção da saúde das substâncias bioativas de ocorrência natural deve ser fundamentalmente preventivo, distinguindo-as das drogas curativas. Esse efeito de tais compostos no organismo pode ser pequeno a curto prazo, porém pode contribuir significativamente para a saúde quando consumidos ao longo da vida como parte da dieta diária. Estudos de intervenção devem ser realizados a fim de demonstrar a eficácia de determinada substância bioativa em seres humanos, com dosagem específica e sob condições precisamente controladas. (Biesalski et al., 2009)

Por outro lado, diversos fatores são capazes de afetar a biodisponibilidade dos compostos bioativos, fazendo com que não sejam totalmente absorvidos/metabolizados. É o caso da complexidade da matriz

(alimento); da estrutura química da substância; da estrutura e quantidade de outros compostos presentes na dieta; da massa da mucosa e o tempo de trânsito intestinal; da taxa de esvaziamento gástrico; do metabolismo e do grau de conjugação e ligação com as proteínas de transporte no sangue e nos tecidos. (Bastos, Rogero e Arêas, 2009)

As principais substâncias bioativas abrangem os grupos dos carotenóides, fitoesteróis, flavonóides, fosfolipídeos, organossulfurados e polifenóis. Podem ser extraídas de fontes alimentares ou não alimentares, bem como obtidas de forma sintética, conforme os seguintes exemplos: substâncias bioativas de fontes alimentares (licopeno do tomate e fitoesteróis de óleos vegetais); substâncias bioativas de fontes não alimentares, mas presentes em alimentos (fitoesteróis extraídos de árvores coníferas, luteína e zeaxantina extraídas da planta *Tagetes erecta*); e substâncias bioativas que podem ser sintetizadas (licopeno e resveratrol sintéticos). (ANVISA, 2013)

3.6.1 Substâncias fenólicas

Os compostos fenólicos são metabólitos secundários sintetizados pelas plantas durante o seu desenvolvimento normal ou em resposta a condições de estresse, como radiação UV, infecções, poluição do ar e exposição a temperaturas extremas (Naczk e Shahidi, 2006). Esses compostos desempenham um papel importante no seu crescimento e reprodução, proporcionando proteção contra patógenos e predadores, além de contribuir para as características sensoriais de frutas e vegetais, como amargura, adstringência, cor, sabor e odor (Balasundram, Sundram e Samman, 2006; Naczk e Shahidi, 2006). O conteúdo de fenólicos de fontes vegetais depende de diversos fatores, como condições e técnicas de cultivo, cultivar, processo de maturação e condições de processamento e armazenamento (Naczk e Shahidi, 2006).

As substâncias fenólicas podem ser moléculas simples ou polímeros, apresentando, em sua estrutura química, hidroxilas e anéis aromáticos. Encontram-se presentes nos vegetais na forma livre ou ligadas a açúcares (glicosídeos) e proteínas. Os ácidos fenólicos, flavonoides e taninos são considerados os principais compostos fenólicos obtidos a partir de fontes naturais. (Angelo e Jorge, 2007)

Os ácidos fenólicos consistem em dois subgrupos: hidroxibenzóicos

(ácidos gálico, *p*-hidroxibenzóico, protocatecóico, vanílico e siríngico) e hidroxicinâmicos (ácidos caféico, ferúlico, *p*-cumárico e sinápico). Os flavonoides constituem o maior grupo de fenólicos vegetais, cujas principais classes estão representadas por flavonóis, flavonas, flavanonas, flavanóis (ou catequinas), isoflavonas, flavanonóis e antocianidinas. Os taninos correspondem a compostos de maior peso molecular e podem ser subdivididos em hidrolisáveis e condensados. (Balasundram, Sundram e Samman, 2006)

Os flavonoides são compostos largamente distribuídos no Reino Vegetal (Plantae), sendo encontrados em frutas, folhas, sementes, entre outras partes da planta na forma de glicosídeos ou agliconas (Angelo e Jorge, 2007). A quercetina é o flavonoide predominante na dieta e é encontrada em frutas, legumes, nozes, sementes, flores e cascas (Kris-Etherton et al., 2002).

Os cereais também apresentam uma variedade de compostos fenólicos, como flavonoides, ácidos fenólicos e proantocianidinas, localizados principalmente nas camadas mais externas do grão. Esses podem ser encontrados nas formas livres ou conjugadas, sendo um exemplo representado pelo ácido ferúlico (Figura 3), que consiste em ésteres ligados a polímeros da parede celular (Acquistucci et al., 2013). As principais fontes alimentares dos compostos fenólicos estão apresentadas na Tabela 2.

Algumas das propriedades fisiológicas associadas a esses compostos são antialérgicas, anti-inflamatórias, antimicrobianas, antioxidantes, antiaterogênicas, antitrombóticas, cardioprotetoras e vasodilatadoras. Os efeitos benéficos à saúde derivados dos compostos fenólicos da dieta têm sido atribuídos à sua capacidade antioxidante. Essa atividade antioxidante se deve especialmente à sua capacidade de sequestrar radicais livres, doar átomos de hidrogênio ou elétrons, e quelar cátions metálicos. (Balasundram, Sundram e Samman, 2006)

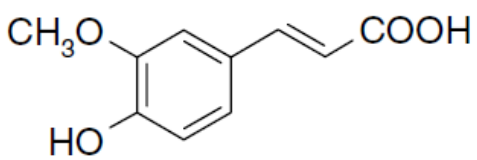

Figura 3. Estrutura molecular do ácido ferúlico. Fonte: Balasundram, Sundram e Samman (2006).

Tabela 2. Fontes alimentares dos principais compostos fenólicos.

FENÓLICO	FONTES ALIMENTARES
Ácidos fenólicos	Damasco, mirtilo, cenoura, cereais, pera, cereja, frutas cítricas, ameixa sementes oleaginosas, pêssego, espinafre, tomate, berinjela.
Flavonóides	
Antocianinas	Mirtilo, cereja, uva, morango.
Chalconas	Maçã
Flavanóis	Maçãs, mirtilos, uvas, cebolas, alface.
Flavanonóis	Uvas
Flavanonas	Frutas cítricas
Flavonóis	Maçã, feijão, mirtilo, trigo, alho-poró, alface, cebola, pimenta, tomate.
Flavonas	Frutas cítricas, aipo, salsa, espinafre.
Isoflavonas	Soja
Xantonas	Manga
Taninos	Maçã, uva, pêssego, ameixa, pera, romã, framboesa.

Fonte: Naczk e Shahidi (2006).

Estudos epidemiológicos demonstram uma associação inversa entre ingestão de flavonoides e risco de desenvolvimento de doenças coronarianas e câncer. Os efeitos antitrombóticos dos compostos fenólicos parecem estar relacionados à redução da agregação plaquetária, síntese reduzida de mediadores pró-trombóticos e pró-inflamatórios, diminuição da expressão de moléculas de adesão e atividade do fator tecidual. Além disso, existem evidências de que os polifenóis do vinho podem ser capazes de modular a produção de óxido nítrico pelo endotélio vascular, gerando vasorelaxamento (Kris-Etherton et al., 2002).

3.6.2 Oligossacarídeos

De acordo com a definição da “American Association of Cereal Chemists” (AACC, 2001), as fibras alimentares correspondem a partes comestíveis de plantas ou carboidratos resistentes ao processo de digestão e absorção no intestino delgado humano, com fermentação completa ou parcial no intestino grosso, tais como polissacarídeos, oligossacarídeos e lignina. Por sua vez, os oligossacarídeos são considerados carboidratos de cadeia curta, com grau de polimerização (DP) entre 3 e 10, exibindo algumas das mesmas propriedades biológicas que os polissacarídeos. No entanto, outras definições classificam os

oligossacarídeos como açúcares contendo de 3 a 19 unidades monossacarídicas, assim como alguns dissacarídeos – lactulose e xilobiose – igualmente fazem parte desse grupo (Mussatto e Mancilha, 2007).

Tais compostos podem ser sintetizados *in vivo*, pela ação da enzima glicosiltransferase, ou ainda formados *in vivo*/*in vitro* através da hidrólise de outros oligossacarídeos, polissacarídeos, glicoproteínas e glicolipídeos (Eggleston e Côté, 2003). O bioprocessamento de cereais, através de reações enzimáticas ou por uso de microrganismos na fermentação, é capaz de produzir uma grande variedade de oligossacarídeos. Etapas anteriores à hidrólise do amido, como moagem, também podem gerar um efeito no processo de biotransformação e devem ser consideradas. (Charalampopoulos et al., 2002)

Os oligossacarídeos possuem como características alta capacidade de retenção de umidade, solubilidade em água, sabor adocicado e baixo valor calórico (de 0,3 a 0,6 vezes mais doces e com cerca de 50 % menos calorias do que a sacarose). Por esta razão, são bastante utilizados pela indústria alimentícia como agentes de volume juntamente com adoçantes artificiais, para alterar a temperatura de congelamento de produtos congelados ou controlar a intensidade do escurecimento causado por reações de Maillard. (Mussatto e Mancilha, 2007; Singh et al., 2015)

Assim, devido à ampla variedade de propriedades, há um crescente interesse no uso dos oligossacarídeos pelas indústrias de produtos alimentícios e farmacêuticos (Menezes e Durrant, 2008). As principais fontes naturais de diferentes oligossacarídeos obtidos pela dieta, bem como suas estruturas químicas e processos de produção estão apresentados na Tabela 3.

Dentre esses compostos, encontram-se os xilooligossacarídeos (XOS). XOS são prebióticos emergentes derivados da hidrólise de arabinoxilanos (AX), polissacarídeos não amiláceos presentes nos cereais (Manisseri e Gudipati, 2010). O grupo enzimático mais importante ligado à clivagem de AX são as endoxilanases, produzidas por algumas bactérias da microbiota intestinal (Broekaert et al., 2011).

Segundo Samanta et al. (2015), XOS são oligômeros de xilose (2 a 10) unidos por meio de ligações β-1,4 (Figura 4). Os substituintes mais frequentes de AX de cereais são L-arabinofuranose, L-arabinose, D-xilose, D-galactose, D-glicose, ácidos glucurônico e hidroxicinâmicos (principalmente ácido ferúlico),

sendo os arabinoxiloligossacarídeos (AXOS) feruloilados os únicos compostos com propriedades antioxidantes e prebióticas (Broekaert et al., 2011). Os XOS podem ser encontrados em frutas, legumes, mel, leite, bem como em material lignocelulósico rico em xilano, obtido a partir de resíduos agrícolas, florestais e industriais (Singh et al., 2015).

Tabela 3. Oligossacarídeos dietéticos (OSD) e suas fontes naturais, estruturas químicas e processos de obtenção.

OSD	ESTRUTURA	FONTES	PRODUÇÂO
Lactulose	Ga-Fr	Leite de vaca	Isomerização alcalina de lactose
Inulina	GuFr*n*	Raízes de chicória, cebola, espargos, alcachofra	Difusão em água quente, refino e *spray drying*
FOS	(Fr)*n*–Gu	Alcachofra, alho, cebola, aspargos, chicória	Hidrólise de inulina, síntese de sacarose
GOS	(Ga)*n*–Gu	Leite humano e de vaca	Transglicosilação enzimática da lactose
IMO	(Gu)*n*	Amido de trigo, cevada, batata, arroz, aipim, mel	Transformação enzimatica do amido
OSD de soja	Rafinose, Estaquiose	Soja	Extraído do soro de soja
XOS	Xy*n*	Madeira, sabugo de milho, palha de trigo e cevada, casca de arroz	Hidrólise de xilanos

Fonte: adaptado de Singh et al. (2015).

FOS: frutooligossacarídeo, GOS: galactooligossacarídeo, IMO: isomaltooligossacarídeo, XOS: xilooligossacarídeo, Gu: glicose, Fr: frutose, Ga: galactose, Xy: xilose, *n*: número de resíduos.

Esses oligossacarídeos são estáveis em uma ampla faixa de pH (2,5 a 8,0) e a temperaturas de até 100 ºC. XOS com DP entre 3 e 5 são mais sensíveis a meios alcalinos quando comparados com os de cadeia longa, enquanto os com DP entre 2 e 4 são preferíveis para uso em alimentos. Ainda, possuem maior estabilidade térmica durante a pasteurização e a esterilização em pH baixo em relação aos FOS. Além disso, apresentam propriedades prebióticas e atividades antioxidante, antialérgica, antimicrobiana, imunomoduladora e citotóxica. Nesse sentido, a utilização dos XOS é bastante atrativa no processamento de alimentos, como, por exemplo, dos sucos ácidos. Por serem obtidos de fontes abundantes e descartáveis, como os resíduos lignocelulósicos, podem ser considerados ainda

uma matéria-prima de baixo custo. (Menezes e Durrant, 2008; Singh et al., 2015)

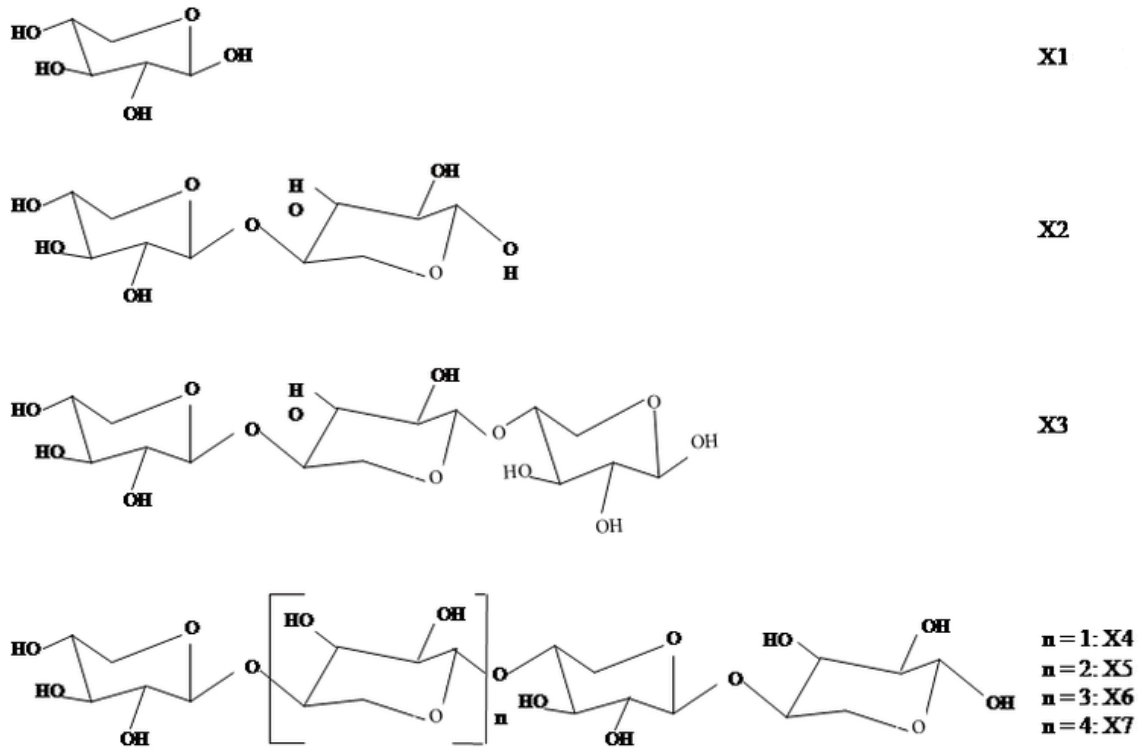

Figura 4. Estrutura química de xilose e XOS. Fonte: Brienzo et al., 2016.

X1: xilose, X2: xilobiose, X3: xilotriose, X4: xilotetrose, X5: xilopentose, X6: xilohexose e X7: xiloheptose.

Além disso, a maioria desses carboidratos possuem propriedades benéficas para a saúde dos consumidores, sendo considerados prebióticos e, portanto, ingredientes funcionais. Devido à sua estrutura química, os oligossacarídeos não digeríveis são substratos consumidos por microrganismos do trato gastrointestinal, como as bifidobactérias e os lactobacilos, estimulando assim seu crescimento. A fermentação desses compostos por tais bactérias pode gerar os seguintes efeitos benéficos à saúde do hospedeiro: modificação significativa da microbiota intestinal; diminuição do pH no cólon; produção de nutrientes, como vitaminas do complexo B, ácidos nicotínico e fólico; alívio da constipação; inibição de diarreia; efeito protetor contra infecções nos tratos gastrointestinal, respiratório e urogenital; aumento na absorção de minerais; incremento do metabolismo de carboidratos e lipídios; e redução do risco de câncer intestinal (Mussatto e Mancilha, 2007).

3.7 Probióticos e Prebióticos

O termo probióticos foi definido pelas organizações internacionais “Food and Agricultural Organization of the United Nations” e “World Health Organization” (FAO/WHO, 2001) e revisado pela “International Association for Scientific Prebiotics and Probiotics” (ISAPP), abrangendo o seguinte conceito: “microrganismos vivos que, quando administrados em quantidades adequadas, conferem um benefício à saúde do hospedeiro” (Hill et al., 2014).

Os microrganismos probióticos devem apresentar certas características a fim de serem incluídos nessa categoria, tais como: serem seguros para consumo (ou seja, considerados GRAS) e passíveis de processamentos industriais necessários para a sua comercialização, permanecerem viáveis nos produtos e durante seu armazenamento e persistirem no trato gastrointestinal pelo tempo suficiente para gerar efeitos benéficos à saúde do hospedeiro (Blatchford et al., 2013). Para serem utilizados em alimentos ou como suplementos com alegação específica de saúde, é necessária comprovação prévia através de estudos conduzidos em humanos, como meta-análises positivas, ensaios clínicos randomizados ou fortes evidências de estudos observacionais (Hill et al., 2014).

No Brasil, o uso de probióticos em alimentos requer uma prévia avaliação pela ANVISA. De acordo com a Resolução RDC 02/2002, probióticos são definidos como “microrganismos vivos capazes de melhorar o equilíbrio microbiano intestinal produzindo efeitos benéficos à saúde do indivíduo” (BRASIL, 2002). A avaliação realizada pelo referido órgão deve contemplar três fatores principais: comprovação da identidade da linhagem do microrganismo, de sua segurança e de seu efeito benéfico (ANVISA, 2017).

O principal benefício gerado pelos probióticos, através de mecanismos compartilhados pela maioria desses microrganismos, seria o desenvolvimento de um ambiente intestinal mais favorável e de sistemas digestivo e imunológico saudáveis. Alguns efeitos são específicos de cada linhagem, enquanto outras propriedades são comuns entre diferentes gêneros de microrganismos probióticos, tais como resistência à colonização, produção de ácidos graxos de cadeia curta (SCFAs), regulação do trânsito intestinal, normalização da microbiota intestinal, maior rotatividade de enterócitos e exclusão competitiva de patógenos. (Hill et al., 2014)

Diversos estudos já relataram que os probióticos são capazes de

estimular o sistema imunológico, diminuir o colesterol sérico, aliviar a intolerância à lactose, diminuir a incidência de diarreia, controlar infecções, agir como antibióticos, suprimir tumores e proteger contra cânceres de cólon e bexiga (O'Bryan et al., 2013).

De acordo com a Organização Mundial de Gastroenterologia (WGO, 2017), a dose necessária de microrganismos probióticos varia em função da cepa e do produto. Embora muitos produtos disponíveis no comércio forneçam entre 1 e 10 bilhões de UFC/dose, alguns demonstraram eficácia com níveis mais baixos, enquanto outros requerem quantidades maiores. Os lactobacilos e as bifidobactérias são os microrganismos mais importantes dentre os probióticos (Roberfroid et al., 2010).

A indústria de probióticos pode ser categorizado em cinco diferentes grupos: *Lactobacillus*, *Bifidobacterium*, microrganismos formadores de esporos, leveduras e outros. Em níveis globais, este mercado foi avaliado em US$ 36,6 bilhões em 2015, com perspectiva de mais de 7 % de taxa composta de crescimento anual até 2023. O maior participante do setor corresponde ao Pacífico Asiático, especialmente Índia, China e Japão, representando cerca de 40 % do total mundial. O crescente interesse e a preocupação com saúde e estilo de vida, além de questões relacionadas a distúrbios metabólicos e digestivos, são fatores importantes que contribuem para a ampliação do mercado probiótico. (Elshaghabee et al., 2017)

Prebióticos foram previamente definidos por Roberfroid et al. (2010) como substâncias capazes de estimular o crescimento e/ou a atividade de microrganismos probióticos, favorecendo a microbiota intestinal benéfica e as funções intestinais. Recentemente, um painel de especialistas promovido pela ISAPP atualizou o conceito de prebiótico para "um substrato que é utilizado seletivamente por microrganismos do hospedeiro, conferindo-lhe benefícios à saúde", sendo incapaz de ser degradado pelas enzimas do organismo (Gibson et al., 2017).

A fermentação prebiótica aumenta a diversidade e a atividade da microbiota intestinal através de mecanismos de "cross-feeding", promovendo o crescimento de microrganismos probióticos (Valcheva e Dieleman, 2016). Diversos fatores podem influenciar a taxa fermentativa de oligossacarídeos: DP, ligação glicosídica e grau de ramificação, sinergia entre bactérias, relação entre

bactéria-substrato e produtos da fermentação, natureza das fermentações e capacidade sacarolítica (Mussatto e Mancilha, 2007).

As propriedades promotoras de saúde dos prebióticos estão especialmente ligadas à produção de SCFAs durante sua fermentação pela microbiota intestinal (Florowska et al., 2016), induzindo diminuição do pH, melhoria da biodisponibilidade de minerais e inibição de bactérias patogênicas (Broekaert et al., 2011). A maioria desses benefícios à saúde pela ingestão de prebióticos está relacionada ao trato gastrointestinal, ao metabolismo cardíaco e às saúdes mental e óssea (Gibson et al., 2017).

O efeito prebiótico já configura um fato científico bem estabelecido (Roberfroid et al., 2010). Algumas dessas propriedades benéficas associadas aos prebióticos são: redução da prevalência e duração de diarreia, alívio de sintomas associados à síndrome do intestino irritável, prevenção do câncer de cólon, aumento da biodisponibilidade e absorção de minerais, redução de fatores de risco para doenças cardiovasculares e promoção da saciedade e perda de peso, prevenindo assim a obesidade (Pandey, Naik e Vakil, 2015).

Os critérios para a classificação de determinado composto como prebiótico são: resistência ao processo de digestão; ser fermentado pela microbiota intestinal; estimular seletivamente o crescimento e/ou atividade de bactérias intestinais benéficas (Blatchford et al., 2013). Embora ensaios *in vitro* possam ser usados para triagem de possíveis candidatos, o crescimento dos microrganismos deve ser quantificado em estudos clínicos, após um período de alimentação com níveis aceitáveis de consumo, além de serem necessários estudos epidemiológicos que comprovem os efeitos benéficos à saúde. (Binns, 2013)

Frutooligossacarídeos (FOS), galactooligossacarídeos (GOS) e inulina são os principais compostos com efeitos prebióticos bem estabelecidos (Roberfroid et al., 2010). Além destes, oligossacarídeos de leite humano (HMOs), mananoligossacarídeos (MOS) e xiloligossacarídeos (XOS) também são considerados prebióticos por muitos autores. Outros compostos, como polifenóis e ácidos graxos poliinsaturados, pelo novo conceito ISAPP, igualmente poderiam ser classificados como tal (Gibson et al., 2017). Algumas das maiores fontes alimentares de prebióticos são alho, cebola, banana, alcachofra, chicória, farelo de trigo e cevada (Florowska et al., 2016).

Os prebióticos podem ser obtidos pela extração de plantas, como a inulina (extraída da chicória); por hidrólise enzimática de polissacarídeos, como, por exemplo, os XOS (produção pela hidrólise enzimática de xilanas de cereais); ou ainda através de reações de transgalactosilação catalisadas por uma enzima, utilizando mono ou dissacarídeos como substrato, tal como no caso dos GOS (produzidos a partir da lactose com β-galactosidase como biocatalisador). Após, podem ser formulados como pó ou xarope e comercializados como suplementos, bem como incorporados em produtos alimentícios, mais comumente iogurtes, sucos de frutas e pães. (Charalampopoulos e Rastall, 2012)

4. RESULTADOS

Os resultados estão apresentados e discutidos na forma de três artigos científicos, cuja formatação encontra-se de acordo com as normas das revistas em que foram publicados ou submetidos.

4.1. Artigo 1

Biological activities of wheat middlings bioprocessed with *Bacillus* spp.

4.2. Artigo 2

Prebiotic xylooligosaccharides produced from wheat middlings bioprocessed with *Bacillus subtilis*

4.3. Artigo 3

Lignocellulosic biomass utilization by lactic acid bacteria: prospective analysis of the characteristics and properties resulting from its biotransformation

ARTIGO 1

Biological activities of wheat middlings bioprocessed with *Bacillus* spp.[1]

[1] Artigo publicado na revista “LWT – Food Science and Technology”, volume 77, no ano de 2017.

Biological activities of wheat middlings bioprocessed with *Bacillus* spp.

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Abstract

Wheat middlings were cultivated with five different *Bacillus* spp. strains in order to evaluate their effect on the phenolic composition and bioactive properties of this agro-industrial waste. Total phenolic content and phenolic profile as well as antioxidant, antibacterial, antihypertensive and prebiotic activities were determined. The bioprocessed wheat middlings exhibited an increase in antioxidant capacity (ABTS and DPPH assays) and total phenolic compounds. Changes in the phenolic profile, with identification of some hydroxybenzoic and hydroxycinnamic acids were also observed by HPLC-DAD analysis. Selected samples inhibited the growth of Gram-positive (*L. monocytogenes* and *B. cereus*) and Gram-negative (*E. coli*) bacteria. The sample cultured for 72 h with *B. subtilis* FTC01 also promoted the growth of a probiotic strain (*Lactobacillus acidophilus*) and showed a high angiotensin-I converting enzyme-inhibitory activity. Thus, the use of bioprocessing techniques can be an interesting approach to improve the bioavailability of compounds with health-promoting properties from wheat coproducts.

Keywords: *Bacillus*; biological activities; phenolic compounds; wheat coproducts

1. Introduction

Wheat (*Triticum* spp.) is one of the most important cereal crops, along with maize and rice, reaching about 30% of total grain production around the world. The annual production of European Union, China and India sums 375 million tons, while the global production exceeds 730 million tons/year (USDA, 2016). Wheat grains are widely consumed, especially in the form of products derived from wheat flour, representing a major component of the human diet.

Wheat grain milling process gives rise to wheat bran, a coproduct of flour production that corresponds to a set of fractions with different particle sizes (wheat middlings, fine and coarse wheat bran). Wheat bran has a range of bioactive compounds in its composition, such as phenolic acids and other polyphenols, alkylresorcinols and carotenoids, which are thought to have antioxidant and anti-inflammatory activities (Anson, Hemery, Bast, & Haenen, 2012). The wheat bran portion with higher antioxidant capacity is the aleurone layer, which contains elevated phenolic content, especially ferulic acid (Anson, van den Berg, Havenaar, Bast, & Haenen, 2008). Moreover, it has been reported that wheat bran could have anti-biofilm activity and anti-adhesive properties against enteric pathogens (González-Ortiz et al., 2014).

Several beneficial properties have been associated to bioactive compounds and dietary fibers found in wheat bran. These include prevention of oxidative damage and thus reducing the risk of chronic diseases, including some type of cancers (particularly colorectal), cardiovascular diseases and obesity; inhibition of LDL oxidation; protection of human erythrocytes against free radicals; prevention of gastrointestinal diseases; and prebiotic activity (Stevenson, Phillips, O'Sullivan, & Walton, 2012). Prebiotics are defined as substances that stimulate the growth/activity of probiotic microorganisms, thus improving the beneficial gut microbiota and intestinal function. The main compounds with established prebiotic effects are fructooligosaccharides (FOS), galactooligosaccharides (GOS) and inulin,

whereas *Lactobacilli* and *Bifidobacteria* are the most important species of probiotic microorganisms (Roberfroid et al., 2010). Xylooligosaccharides (XOS) are considered emerging prebiotics derived from hydrolysis of arabinoxylans, non-starch polysaccharides that are present in cereals (Manisseri & Gudipati, 2010).

Nevertheless, some bioactive compounds of the outer layer of cereal grains are trapped in resilient cell wall structures that can resist conventional milling, thus owning low bioaccessibility (Delcour, Rouau, Courtin, Poutanen, & Ranieri, 2012; Stevenson et al., 2012). The use of bioprocessing techniques, which refers to the application of enzymes and/or microbial cultivation to obtain desirable changes in a matrix, can be an interesting approach to improve the bioavailability of compounds with health-promoting claims (Coda, Katina, & Rizello, 2015). However, few studies compare antioxidant phytochemicals from fermented and non-fermented cereals and therefore more studies are needed to enhance the bioactive potential of such products (Masisi, Beta, & Moghadasian, 2016). Besides adding value and minimizing the environmental impact caused by the accumulation of agro-industrial wastes, cereal coproducts can be used in the development of novel foods with functional properties, representing a potentially useful resource to be explored.

The aim of the present study was to investigate the effect of bioprocessing of wheat middlings, a coproduct of wheat flour production. This agro-industrial waste was used as substrate for cultivation of different isolates of *Bacillus* spp., and the bioactive properties of the resulting cultures were examined.

2. Materials and methods

2.1. Sample preparation and bioprocessing procedure

Wheat middlings were obtained from a local milling company (Moinho Estrela, Porto Alegre, Brazil). The sample had a mean particle size of 257 μm and 88.4 g/100g dry matter, and its

composition was determined by standard methods (Van Soest, Robertson, & Lewis, 1991) as (g/100g, dry basis): protein (17.9), ash (3.5), crude fiber (5.4), neutral detergent fiber (24.9) and acid detergent fiber (5.9). Samples of approximately 250 g were stored in polypropylene bags at -20°C.

Bacillus sp. P7 and P45 were isolated from fish intestines (Motta, Cladera-Olivera & Brandelli, 2004), *B. amyloliquefaciens* I3 was a soil isolate (Lisboa, Bonato, Bizani, Henriques, & Brandelli, 2006), *B. subtilis* FTC01 was obtained from the probiotic product Biotop (Fatec, Arujá, Brazil) and *B. subtilis* ATCC 19659 was from American Type Culture Collection (Manassas, VA, USA). Stock cultures were maintained at -20°C in brain heart infusion broth (BHI, Oxoid, Basingstoke, UK) containing 20% (v/v) glycerol.

For bacterial cultivation, vials containing 5 g of wheat middlings and 100 mL phosphate saline buffer pH 7.4 were autoclaved and then inoculated with 1 mL of each strain at a concentration of 10^8 CFU/mL in 8.5 g/L NaCl solution, or NaCl solution as control. Then, samples were incubated at 37°C for 72 h in an orbital shaker (Marconi, Piracicaba, Brazil) at 125 rpm. Aliquots of 10 mL were aseptically collected every 24 h, centrifuged at 3000 *g* (Megafuge 16R, Thermo Scientific, Waltham, USA) for 15 min at 4°C, then filtered by 0.22 µm membranes (Sartorius, São Bernardo do Campo, Brazil) and frozen at -20°C. This experiment was carried out in triplicate at three different times. The pH was measured in triplicate using a pH meter (PHtek, Curitiba, Brazil) and average values were 6.02 ± 0.01, 5.89 ± 0.02, 5.85 ± 0.01, 5.83 ± 0.01, 5.86 ± 0.01, 5.86 ±0.00, and 5.80 ± 0.02 for starting control sample, 72 h control sample and 72 h cultivated samples with *Bacillus* sp. P7, *Bacillus* sp. P45, *B. amyloliquefaciens* I3, *B. subtilis* FTC01, and *B. subtilis* ATCC 19659, respectively.

2.2. Antioxidant activity

The antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Brand-Williams, Cuvelier, & Berset, 1995), based on the capture of free radicals by antioxidants, and the 2,2'-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) method (Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, 1999), in which the free radical is generated by a chemical reaction with potassium persulfate.

For the DPPH method, a 100 µL aliquot of each sample was added to 3.9 mL of DPPH solution (Sigma Aldrich, St. Louis, USA) and after 45 min the absorbance at 515 nm was measured in a spectrophotometer (Shimadzu, Kyoto, Japan), using methanol as the blank. For the ABTS method, an aliquot of 10 µL of each sample was added to 1.0 mL ABTS radical (Sigma Aldrich), and the sample was read at 734 nm after 6 min of reaction, using ethanol as the blank and Trolox (Sigma Aldrich) to generate a standard curve. The analyses were carried out in triplicate, and the results were presented as µmol of DPPH or as Trolox equivalents (TE) per gram of sample dry weight (DW).

2.3. Phenolic compounds

Total phenolic compounds analyses were carried out in triplicate, using the Folin-Ciocalteau reagent (Swain & Hillis, 1959), and the results were expressed as mg of gallic acid equivalents (GAE) per g of sample DW.

For HPLC-DAD analysis, phenolic compounds were extracted in duplicate from the freeze-dried samples (Rodrigues, Mariutti, & Mercadante, 2013). Samples (20 mg) were mixed with 1 mL of a 8:2 (v/v) methanol/water mixture, vortexed for 5 min and centrifuged at 5000 *g* for 5 min at 15°C. The extraction was repeated three times; the supernatants were combined, filtered by 0.22 µm membranes and then injected into the chromatograph (Shimadzu, Kyoto, Japan). The soluble phenolic compounds were separated on a Synergi Hydro-RP C18 column (4 µm, 250 mm × 4.6 mm, Phenomenex, Torrance, CA, USA) at a

flow rate of 0.7 mL/min and column temperature of 29°C, using a mobile phase consisting of a 99.5:0.5 (v/v) water/formic acid mixture (solvent A) and a 99.5:0.5 (v/v) acetonitrile/formic acid mixture (solvent B) in a linear gradient of 99:1 (v/v) A/B for 50 min, from 99:1 (v/v) A/B to 50:50 (v/v) A/B over 5 min, and from 50:50 (v/v) A/B to 1:99 (v/v) A/B over 5 min. The UV-vis spectra were obtained between 200 and 600 nm, and the chromatograms were processed at 280, 320 and 360 nm. The identification was performed by comparisons with standards and their retention times (Rt) and λmax.

2.4. Antibacterial activity

Antibacterial activity was determined according to Motta and Brandelli (2002), in duplicate assays. The indicator strains tested were *Listeria monocytogenes* ATCC 7644, *Bacillus cereus* ATCC 9634, *Staphylococcus aureus* ATCC 1901, *Salmonella* Enteritidis SE86, and *Escherichia coli* ATCC 8739. Indicator microorganisms, at a concentration of 10^8 CFU/mL in 8.5 g/L NaCl, were inoculated with a swab onto BHI agar plates. Samples of bioprocessed wheat middlings were freeze-dried and suspended in phosphate buffered saline pH 7.4. These samples were sonicated for 10 min, centrifuged at 10000 *g* for 10 min, and then filtered through 0.22 µm membranes. Dilutions were prepared with concentrations of 50, 100, 150 and 200 mg/mL. Aliquots of 20 µL of each dilution were spotted on the freshly prepared lawn of indicator strain, and plates were incubated at 37°C for 18 h (*B. cereus* and *S. aureus*) or 24 h (*Salmonella*, *L. monocytogenes* and *E. coli*). Subsequently, the diameter of growth inhibition was measured with a digital caliper and expressed as inhibition zone (mm).

2.5. Prebiotic activity

Two commercial probiotic cultures, *Lactobacillus acidophilus* LA-5® and *Bifidobacterium lactis* BB-12® (Chr. Hansen, Valinhos, Brazil), were employed to determine the prebiotic

activity. *E. coli* ATCC 8739 was used as an enteric culture. The analyses were performed as described by Huebner, Wehling, and Hutkins (2007). For the tests, 0.05 g of freeze-dried BB-12 and 100 µL of LA-5 (from frozen stock at -20ºC in milk) were activated in 10 mL MRS broth (Himedia, Mumbai, India), during 24 h at 37ºC, under anaerobic conditions (GasPak, BD Diagnostics, Sparks, USA). Thereafter, 500 µL of BB-12 culture or 100 µL of LA-5 culture were transferred to 10 mL fresh MRS broth and incubated anaerobically for 24 h at 37ºC. The bacteria *E. coli*, kept frozen at -20ºC in BHI containing 20% glycerol (v/v), was activated by transferring an aliquot of 100 µL to 10 mL BHI and incubating at 37ºC for 24 h. An additional transfer to BHI broth was performed prior to use. The initial bacterial counts were 10^6 CFU/mL.

Tests were performed by placing 1% (v/v) of each activated culture in individual sterile vials containing MRS broth for LA-5 and BB-12 or M9 Minimal Medium (33.9 g/L Na_2HPO_4, 15 g/L KH_2PO_4, 5 g/L NH_4Cl and 2.5 g/L NaCl) for *E. coli*, added with 10 g/L of either glucose (control) or bioprocessed samples. The prebiotic activity score was determined by cell density (CD) immediately after bacterial inoculation (time 0) and after 24 h incubation at 37ºC according to this equation:

$$\textit{Prebiotic activity score} = CD\ (24\ h) - CD\ (0\ h)$$

where the cell density was measured by optical density at 600 nm in a spectrophotometer.

2.6. Angiotensin I-converting enzyme (ACE)-inhibitory activity

Inhibition of ACE was assayed by the method of Cushman and Cheung (1971) with some modifications, in duplicate. Each sample (20 µL) was mixed with 100 µL buffered substrate solution: 3 mg/mL hippuryl-L-histidyl-L-leucine (HHL) in 50 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-HCl buffer with 300 mmol/L NaCl, pH 8.3 at 37°C. After adding 40 µL ACE (Sigma Aldrich), the reaction mixture was incubated at 37°C for 30

min, and stopped with 150 μL of 1 mol/L HCl. Then, the hippuric acid released was extracted with 1 mL ethyl acetate, shaken vigorously for 60 s, and centrifuged at 3000 *g* (model 5415R, Eppendorf, Hamburg, Germany) for 10 min. The organic phase was then transferred to a glass tube to be heat evaporated. The residue was dissolved with 800 μL distilled water and measured spectrophotometrically at 228 nm. The ACE-inhibitory activity was expressed as a percentage, according to the following equation:

$$\% \text{ of inhibitory activity} = (A - B)/(A - C) \times 100$$

where A is the absorbance without sample, B is the absorbance without ACE, and C is absorbance in the presence of both ACE and sample.

2.7. Data analysis

The coefficient of variation was below 5% for each analysis and below 10% among the bioprocessing experiments. The results were analyzed by one-way ANOVA and the Tukey means comparison test, and the correlation test was performed by Person correlation, both at 5% significance level, using the Statistica® 13 software (StatSoft, Inc., Tulsa, USA).

3. Results and discussion

3.1. Antioxidant activity and phenolic compounds

DPPH and ABTS assays were used to assess the antioxidant capacity of bioprocessed wheat middlings. These two assays were chosen because they are simple and fast methods to be applied, and widely used in antioxidant screening (Prior, Wu, & Schaich, 2005). The results, along with the analysis of phenolic concentration, are presented in Table 1. The bioprocessed wheat middlings exhibited improved antioxidant activity and higher content of phenolic compounds. The control sample also presented a significant increase (p<0.05) after 72 h, but not as much as most of the bacterial cultured samples.

By the DPPH method, it was observed that all *Bacillus*-treated samples showed an increase in their antioxidant activity after 48 h processing, but none of them presented a significant difference by additional incubation until 72 h (p>0.05). The samples bioprocessed with *Bacillus* sp. P45, *Bacillus* sp. P7 and *B. amyloliquefaciens* I3 showed the major antioxidant values, which were significantly higher (p<0.05) than the 72 h control sample.

Regarding the ABTS assay, all the 72 h bacterial cultured samples showed a significant increase (p<0.05) in their antioxidant activities comparing to the starting sample and their respectively 24 h cultivated sample. Most of the samples presented a high antioxidant value after 48 h, with the exception of the bioprocessed samples with *Bacillus* sp. P45 and *B. amyloliquefaciens* I3, which showed the highest antioxidant values after 72 h.

Salar, Certik, and Brezova (2012) also observed an increase in the antioxidant capacity of solid-state fermented maize with a filamentous fungus (*Thamnidium elegans*) after 120 h at 25ºC, when evaluated by ABTS and DPPH assays. Zhang, Gao, Chen, and Wang (2014) tested eight microbial strains including *Bacillus* species, yeasts, and filamentous fungi for wheat bran fermentation during 144 h at 30ºC. The authors showed that the antioxidant activity of bioprocessed wheat bran might significantly differ depending on the strain and the assay used for evaluating the activity.

The amounts of total phenolics were higher for the samples cultured 72 h with *B. amyloliquefaciens* I3, *B. subtilis* FTC01 and *B. subtilis* ATCC 19659 (Table 1). Every *Bacillus*-treated sample after 72 h presented a total phenolic content significantly higher (p<0.05) than the 72 h control sample, as well as their respectively 24 h cultured sample. Đorđević, Šiler-Marinković, and Dimitrijević-Branković (2010) found a slight increase in the phenolic content of wheat fermented with *L. rhamnosus* (16.2 to 20.7 mg GAE/g DW) or *S. cerevisiae* (16.2 to 18.4 mg GAE/g DW) for 24 h at 30ºC. The amount of total phenolics was

enhanced by 15.66 and 67.82% in wheat bran treated with *B. amyloliquefaciens* and *Mucor circinelloides*, respectively (Zhang et al., 2014).

In this work, the total phenolic content showed a strong correlation with the DPPH and ABTS values (R^2 = 0.80). The degree of antioxidant activity and total phenolic content obtained in bioprocessed wheat bran is often influenced by the microbial species utilized (Đorđević et al., 2010; Coda, Rizello, Curiel, Poutanen, & Katina, 2014). According to Anson et al. (2008), the antioxidant capacity of wheat grain is mainly related to its phenolic content, in particular ferulic acid. The antioxidant property of ferulic acid is then especially related to its ability to donate electrons and transfer hydrogen atoms to free radicals (Anson et al., 2012). This phenolic acid is the most important and abundant phenolic compound of wheat bran, but is mostly found esterified to arabinoxylan-type polysaccharides, presenting low bioaccessibility and bioavailability (Coda et al., 2015).

From HPLC-DAD analysis, ferulic acid was not found in large amounts. The extraction protocol releases soluble phenolics and ferulic acid is mostly found in its bound form, which corresponds to about 95% of its concentration in the wheat grain (Wang et al., 2013). The control samples at 0 and 72 h showed similar patterns and major compounds were found at 280 nm (hydroxybenzoic acids) and 320 nm (hydroxycinnamic acids). Only coumaric and ferulic acids were identified in the controls (Fig. 1). In contrast, the 72 h *Bacillus* treated samples presented more phenolic substances at 320 nm, with the identification of gallic, caffeic, coumaric and ferulic acids and possibly their derived compounds (Fig. 1). A major peak was observed at Rt 8.6 min and λmax 283 nm, but it could not be identified since the Rt was not in agreement with those of standard phenolics. However, this peak decreased in 72 h *Bacillus* treated samples, especially for *B. subtilis* FTC01, where an increase in two other peaks was observed. At 360 nm (flavonoids), 1 or 2 unidentified peaks were found in most of samples (data not shown).

A synergistic action of endoxylanase and esterase enzymes in xylan hydrolysis could release the phenolic acids (Reddy & Krishnan, 2013), and food fermentation can improve the health functionality of conjugated phenolic compounds by bioconversion to their free forms (Torino et al., 2013). Bioprocessing of cereal bran can induce a partial disruption of cell wall structures, mostly in the aleurone layer, related to the combined activities of added, endogenous, and microbial enzymes (Coda et al., 2015). Therefore, hydrolytic enzymes produced by *Bacillus* spp. may be useful to release antioxidant phenolics during bioprocessing.

3.2. Antibacterial activity

The ability of bioprocessed wheat middlings to inhibit the growth of Gram-positive and Gram-negative bacteria was investigated. The results are summarized in Table 2. Only the samples from 72 h cultivation showed activity against the indicator microorganisms. Control and *Bacillus* sp. P7 treated samples did not inhibit bacterial growth.

The indicator microorganisms *S. aureus* and *S.* Enteritidis were not inhibited by bioprocessed samples. The bacterium *L. monocytogenes* was inhibited by the sample cultured with *Bacillus* sp. P45, showing a MIC of 50 mg/mL. *B. cereus* was inhibited by samples cultured with *Bacillus* sp. P45, *B. amyloliquefaciens* I3, *B. subtilis* FTC01 and ATCC19659. Another study using different strains of *B. subtilis* concluded that the inhibition of *B. cereus* was influenced by both the substrate and the strain during fermentation of baobab seeds (Kaboré et al., 2013).

The Gram-negative bacteria *E. coli* had their growth inhibited by the sample treated with *B. subtilis* FTC01, but only at highest concentration. It is well known that Gram-negative bacteria are often more resistant than Gram-positive bacteria (Jemil et al., 2014). Chi, Rong,

Li, Tang, and Chi (2015) observed that biosurfactins produced by *B. amyloliquefaciens* were capable to inhibit the growth of pathogenic *E. coli* with multi-drug resistant profile.

Many antimicrobial compounds are produced by the genus *Bacillus*, and there is an increased interest in their bacteriocins because the ability to inhibit Gram-negative bacteria, yeasts and fungi, in addition to Gram-positive species (Abriouel, Franz, Omar, & Gálvez, 2011). Thus, it is feasible that antibacterial properties present in fermented products could be related to the production of a bacteriocin or peptides derived from protein hydrolysis (Jemil et al., 2014). In this regard, production of antimicrobial peptides by *B. amyloliquefaciens* I3 (Lisboa et al., 2006) and *Bacillus* sp. P45 (Sirtori, Cladera-Olivera, Lorenzini, Tsai, & Brandelli, 2006) have been previously demonstrated.

3.3. Prebiotic activity

Wheat middlings bioprocessed with *B. subtilis* FTC01 was selected for evaluation of prebiotic activity. This strain was isolated from a probiotic product used in animal feed and showed inhibitory activity against *E.coli*. The prebiotic activity scores obtained for two commercial probiotic strains and *E. coli* showed positive values (Fig. 2), indicating that both bioprocessed wheat middlings and glucose (used as control) were able to promote the growth of the microorganisms. This increase in cell density was dependent on the sample and the strain tested, since each bacterium has different metabolic responses.

The sample cultured for 72 h with *B. subtilis* FTC01 presented the highest prebiotic activity score for *L. acidophilus* and this growth promotion was statistically different ($p<0.05$) from the other samples and glucose. These results suggest that the bioprocessing of wheat middlings with *B. subtilis* FTC01 probably formed some prebiotic carbohydrates. Xylooligosaccharides (XOS) are considered emerging prebiotics with many applications as well as potential health benefits (Patel & Goyal, 2012). These non-digestible oligosaccharides

are derived from endoxylanase hydrolysis of arabinoxylans, non-starch polysaccharides that are present in cereals (Manisseri & Gudipati, 2010). Xylanases produced by *B. subtilis* are able to form XOS with low degree of polymerization (Reddy & Krishnan 2013), which are preferable for growth stimulation of probiotic bacteria (Samanta et al., 2015). Thus, it is conceivable that *B. subtilis* FTC01 enzymes could hydrolyze arabinoxylans and other polysaccharides from wheat middlings, producing oligosaccharides that are beneficial to lactobacilli.

On the other hand, bioprocessed wheat middlings was not capable to stimulate the *B. lactis* growth in comparison to glucose ($p<0.05$), and its prebiotic activity scores were lower than those observed for *E. coli*. Huebner et al. (2007) also found that *Bifidobacterium* strains often present less growth on commercial prebiotics (FOS, inulin and GOS) than by glucose. In that study, *L. paracasei* grown on inulin showed the highest score, while the lowest score was found for *B. bifidum* grown on GOS.

In respect to the increase in cell density for the *E. coli* strain, a common bacterium of the enteric microbiota, it is possible to infer that wheat middlings was a better growth substrate than glucose. However, it is noteworthy that the sample cultured 72 h with *B. subtilis* FTC01 presented a lower score ($p<0.05$) than the control samples. This result suggests that the sample treated with *B. subtilis* FTC01 had partially impaired the growth of the *E.coli*, in agreement with that observed for antimicrobial activity.

3.4. ACE-inhibitory activity

The renin-angiotensin-aldosterone system is considered the main biological regulation of blood pressure, in which the angiotensin converting enzyme (ACE) converts angiotensin I into angiotensin II, a potent vasoconstrictor (Patten, Abeywardena, & Bennett, 2016). This enzyme also inactivates the vasodilator peptide bradykinin, and therefore ACE inhibitors are

involved in the control of blood pressure, and can be used in the treatment of hypertension. Bioactive compounds with ACE-inhibitory function derived from food sources are considered safer, effective, and economical compared to synthetic drugs (Huang, Davidge, & Wu, 2013).

A significant increase ($p<0.05$) in the ACE-inhibitory activity can be clearly observed when the samples were treated with *B. subtilis* FTC01 (Fig. 3). The percentage of ACE inhibition increased from 15.7 to 45.1% for control sample after 72 h, but comparing with the sample cultured 72 h with *B. subtilis* FTC01, the increase was much higher, reaching 94.9%. This result suggests that the *Bacillus* strain tested in this study might have contributed to the formation of ACE-inhibitory substances during the wheat middlings processing.

The protein hydrolysis into short chain peptides is considered one of the main reasons for the increase in ACE-inhibitory capacity of fermented foods, and *B. subtilis* is recognized by producing proteases (Juan, Wu, & Chou, 2010). Possibly, native enzymes from the wheat middlings were also responsible for the increased ACE-inhibitory activity observed for the control sample after 72 h. Good yields of ACE-inhibitory peptides can be produced from wheat coproducts by autolysis reaction, which can be achieved at an optimal temperature of 40ºC (Nogata, Nagamine, Yanaka, & Ohta, 2009). This temperature was similar to that employed in the present work, where the incubation was at 37ºC. Torino et al. (2013) found lower ACE inhibition for solid-state fermented lentils with *B. subtilis* comparing with spontaneously or with *Lactobacillus plantarum* liquid-state fermentation.

4. Conclusion

This work indicates that bioprocessing techniques are useful to enhance the biological properties of wheat coproducts. Bioprocessed wheat middlings exhibited an increase in antioxidant capacity and phenolic content. Most of cultured samples showed antibacterial activity and the sample cultured for 72 h with *B. subtilis* FTC01 also presented prebiotic and

antihypertensive potential. Thus, it could be considered a good raw material for developing optimized cereal products, as well as improved animal feed. Bioprocessing is an efficient and environmentally friendly tool to obtain some positive modifications in the wheat bran matrix and thus enhancing the bioaccessibility and bioavailability of its health-promoting compounds. The use of enzymes is expensive, thus using non-pathogenic microbial strains would be an economic alternative. It is necessary to investigate the mechanisms involved in the bioprocessing with these *Bacillus* strains as perform the further characterization of substances with biological activities, such as phenolic compounds, oligosaccharides and peptides, especially the prebiotic compounds, to the extent that they have great commercial appeal.

Acknowledgments

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Table 1. Antioxidant activity (ABTS and DPPH assays) and phenolic compounds (PC) of bioprocessed wheat middlings.

Treatment	Time (h)	ABTS (µM TE/ g DW)[a]	DPPH (µM DPPH/ g DW)[a]	PC (mg GAE/ g DW)[a]
Starting sample	0	307.47 ± 8.31^{fg}	12.80 ± 0.05^{g}	34.65 ± 0.52^{g}
Control	24	347.06 ± 2.72^{defg}	13.61 ± 0.64^{g}	34.58 ± 0.21^{g}
	48	376.15 ± 30.73^{cdef}	15.43 ± 0.44^{fg}	45.55 ± 0.4^{ef}
	72	412.36 ± 8.75^{bcde}	21.61 ± 1.73^{e}	47.40 ± 0.93^{def}
B. subtilis FTC01	24	365.84 ± 11.24^{def}	14.00 ± 0.63^{g}	33.66 ± 3.12^{g}
	48	433.59 ± 18.19^{abcd}	23.49 ± 2.27^{cde}	59.07 ± 4.55^{bc}
	72	467.58 ± 38.41^{abc}	21.56 ± 0.34^{e}	79.20 ± 5.79^{a}
B. subtilis ATCC 19659	24	365.66 ± 13.05^{def}	15.45 ± 0.40^{fg}	34.63 ± 0.71^{g}
	48	425.45 ± 8.59^{abcd}	22.35 ± 1.35^{de}	60.72 ± 3.66^{bc}
	72	467.50 ± 0.53^{abc}	24.56 ± 1.16^{bcde}	68.91 ± 0.78^{ab}
B. amyloliquefaciens I3	24	273.09 ± 25.11^{g}	17.53 ± 0.74^{f}	36.57 ± 2.95^{fg}
	48	369.13 ± 9.07^{def}	24.34 ± 0.28^{cde}	58.85 ± 2.11^{bc}
	72	517.53 ± 20.40^{a}	25.74 ± 0.52^{abcd}	68.28 ± 3.46^{ab}
Bacillus sp. P7	24	308.66 ± 9.44^{fg}	17.44 ± 0.52^{f}	33.33 ± 1.01^{g}
	48	474.37 ± 45.88^{ab}	25.98 ± 0.63^{abc}	53.73 ± 2.67^{cde}
	72	501.08 ± 42.89^{ab}	26.72 ± 0.25^{abc}	61.83 ± 1.60^{bc}
Bacillus sp. P45	24	302.65 ± 1.12^{fg}	17.16 ± 1.05^{f}	30.57 ± 2.72^{g}
	48	325.03 ± 5.39^{efg}	27.68 ± 0.51^{ab}	56.93 ± 4.96^{bcd}
	72	506.20 ± 45.66^{a}	28.10 ± 0.33^{a}	64.15 ± 4.49^{b}

[a] Values are the means ± standard deviation of three independent experiments. Different superscript letters within each column represent significant differences (p<0.05).

Table 2. Antibacterial activity of the bioprocessed wheat middlings after 72 h of culture.

Indicator microorganism	Inhibitory culture	MIC (mg/mL)[b]	Inhibition zone (mm)[c]
L. monocytogenes ATCC7644	*Bacillus* sp. P45	50	10.2 ± 0.1
B. cereus ATCC9634	*Bacillus* sp. P45	100	9.8 ± 0.4
	B. amyloliquefaciens I3	150	9.9 ± 0.2
	B. subtilis FTC01	50	9.5 ± 0.03
	B. subtilis ATCC19659	100	8.8 ± 0.1
S. aureus ATCC1901	NI [a]		
S. Enteritidis SE86	NI [a]		
E. coli ATCC8739	*B. subtilis* FTC01	200	9.1 ± 0.1

[a] Not inhibited by any *Bacillus*-treated wheat middlings.

[b] Minimum Inhibitory Concentration

[c] Values are the mean ± standard deviations of three independent experiments.

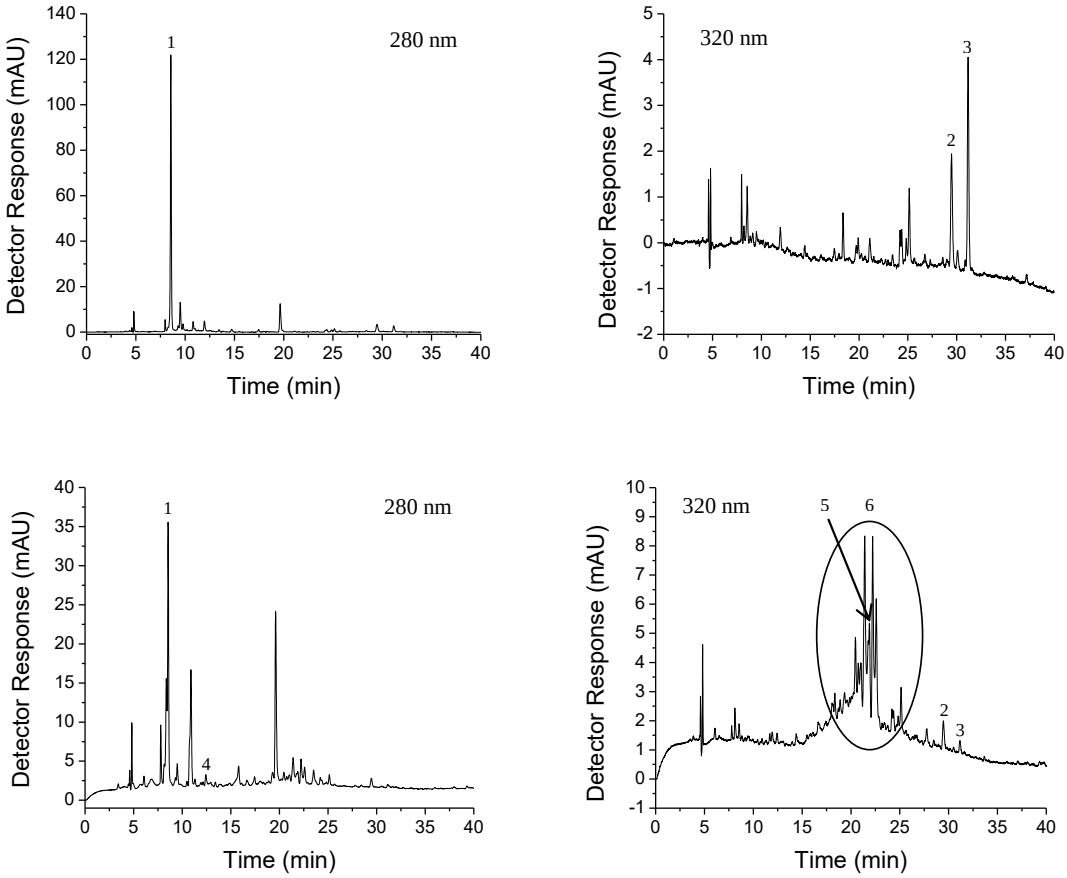

Fig. 1. HPLC chromatograms of control sample (A,B) and sample treated with *B. subtilis* FTC01 for 72 h (C,D), monitored at 280 nm (A,C) and 320 nm (B,D). Peaks: 1- Unidentified compound; 2- coumaric acid; 3- ferulic acid; 4- gallic acid; 5- caffeic acid; 6- coumaric acid and/or ferulic acid derived compounds.

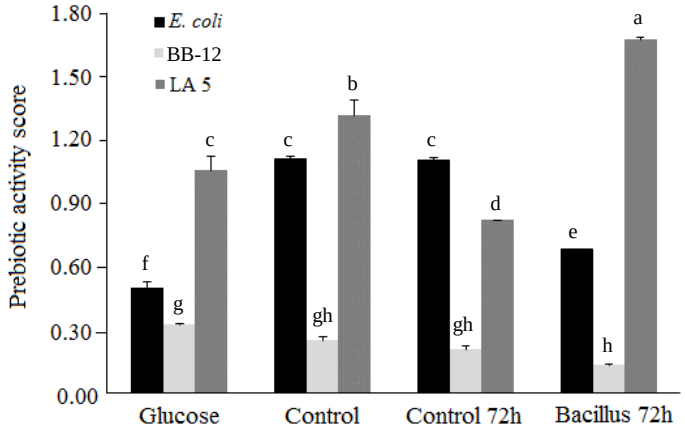

Fig. 2. Prebiotic activity score of the 72 h bioprocessed wheat middlings inoculated with *B. subtilis* FTC01 compared with control and glucose. Samples were incubated for 24 h with *Bifidobacterium lactis* BB-12, *Lactobacillus acidophilus* LA-5 or *E. coli*. Bars are the means ± standard deviation of two independent experiments. Different letters represent significant differences (p<0.05). Control, untreated wheat middlings (time 0); Control 72 h, control after 72h; Bacillus 72h, samples treated with *B. subtilis* FTC01 for 72 h.

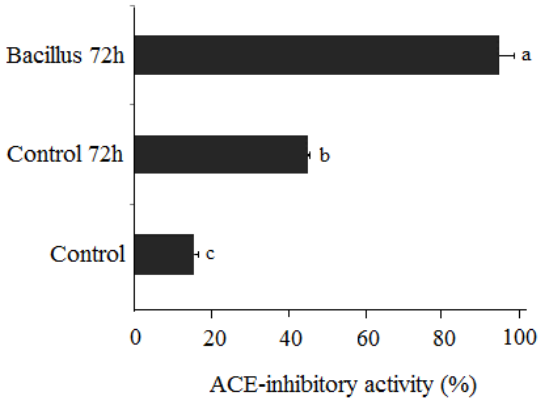

Fig. 3. ACE-inhibitory activity of 72 h bioprocessed wheat middlings inoculated with *B. subtilis* FTC01. Bars are the means ± standard deviation of two independent experiments. Different letters represent significant differences ($p<0.05$). Control, untreated wheat middlings (time 0); Control 72 h, control after 72h; Bacillus 72h, samples treated with *B. subtilis* FTC01 for 72 h.

ARTIGO 2

Prebiotic xylooligosaccharides produced from wheat middlings bioprocessed with *Bacillus subtilis*[2]

[2] Artigo submetido na revista “Food Research International”, em processo de revisão.

Prebiotic xylooligosaccharides produced from wheat middlings bioprocessed with *Bacillus subtilis*

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Abstract

Prebiotic compounds are substrates not metabolized by host enzymes, but by its microbiota, providing health benefits. Xylooligosaccharides (XOS) are emerging prebiotics derived from arabinoxylans, which are polysaccharides present in cereals. This study aimed to investigate the bioprocessing of wheat middlings, a coproduct of wheat flour, using a probiotic *Bacillus subtilis*. The identification of XOS and the enzymatic and prebiotic activities of resulting cultures were evaluated. The activity of xylanolytic enzymes continuously enhanced during the 72 h bacterial growth, where β-xylosidase presented the highest value (70.31 U/mL). XOS profile and concentration varied considerably between control and bioprocessed samples and among these at different times. Maximum prebiotic activity score was found for the 24 h and 72 h bioprocessed samples (1.73 and 1.61, respectively). Wheat middlings showed to be a promising substrate for production of prebiotics like XOS and *B. subtilis* FTC01 appears to be a good source of xylanolytic enzymes.

Keywords: *Bacillus subtilis*; wheat middlings; lignocellulosic biomass; bioprocessing; xylanolytic enzymes; prebiotic compounds; XOS.

1. Introduction

Prebiotics were previously defined by Roberfroid et al. (2010) as substances that stimulate growth and/or activity of probiotic microorganisms, improving beneficial gut microbiota and intestinal functions. In December 2016, a panel of experts promoted by the International Association for Scientific Prebiotics and Probiotics (ISAPP) updated the prebiotic concept for "a substrate that is selectively utilized by host microorganisms conferring a health benefit", and it must not be degraded by the enzymes of the host organism (Gibson et al., 2017).

Prebiotic fermentation increases diversity and activity of intestinal microbiota through cross-feeding mechanisms that promote the growth of probiotic microorganisms (Valcheva & Dieleman, 2016). Health-promoting properties of prebiotics are especially linked to the short-chain fatty acids (SCFAs) production during their fermentation by gut microbiota (Florowska et al., 2016), engendering a pH decrease, improvement of minerals bioavailability, and inhibition of pathogenic bacteria (Broekaert et al., 2011). Most of these prebiotic health benefits are related with the gastrointestinal tract, cardiac metabolism, mental and bone health (Gibson et al., 2017).

Fructooligosaccharides (FOS), galactooligosaccharides (GOS) and inulin are main compounds with established prebiotic effects, whereas *Lactobacilli* and *Bifidobacteria* are the most important species of probiotic microorganisms (Roberfroid et al., 2010). Apart from these, human milk oligosaccharides (HMOs), mannanoligosaccharides (MOS) and xylooligosaccharides (XOS) are also recognized prebiotics. Other compounds, such as polyphenols and polyunsaturated fatty acids, by the new ISAPP concept, could also be classified as prebiotics (Gibson et al., 2017). Some of the dietary sources of prebiotics are garlic, onion, banana, artichoke, chicory, wheat bran and barley (Florowska et al., 2016).

XOS are emerging prebiotics derived from the hydrolysis of arabinoxylans (AX), non-starch polysaccharides that are present in cereals (Manisseri & Gudipati, 2010). The most

important enzymes group linked to cleavage of AX are the endoxylanases, produced by some bacteria from colonic microbiota (Broekaert et al., 2011). According to Samanta et al. (2015), XOS are sugar oligomers (2 to 10) of xylose united through β-1,4 linkages, that are heat-resistant and acid-stable. The most frequent substituents of cereal AX are L-arabinofuranose, L-arabinose, D-xylose, D-galactose, D-glucose, glucuronic acid and hydroxycinnamic acids (mainly ferulic acid), being the feruloylated arabinoxylooligosaccharides (AXOS) the only prebiotic compound with both antioxidant and prebiotic properties (Broekaert et al., 2011).

The aim of the present study was to investigate the effect of bioprocessing wheat middlings, a coproduct of wheat flour, on the production of prebiotic compounds. This agro-industrial byproduct was used as substrate for cultivation of a probiotic *B. subtilis* strain FTC01, and the enzymatic activity, XOS formation and identification, and the prebiotic activity of resulting cultures were examined.

2. Materials and methods

2.1. Sample preparation and bioprocessing procedure

The wheat middlings were obtained from a local milling company (Moinho Estrela, Porto Alegre, Brazil) and have a mean particle size of 257 μm and 88.4 g/100 g dry matter. The sample composition was determined as (g/100 g, dry basis): protein (17.9), ash (3.5), crude fiber (5.4), neutral detergent fiber (24.9) and acid detergent fiber (5.9) (Van Soest et al., 1991). The sample was separated into aliquots of approximately 250 g each, and then stored in polypropylene bags at -20°C.

Bacillus subtilis FTC01 was isolated from the probiotic product Biotop (Fatec, Arujá, Brazil) and then maintained at -20°C in brain heart infusion broth (BHI, Oxoid, Basingstoke, UK) medium containing 20% (v/v) glycerol. The strain was inoculated in BHI broth and

incubated at 37ºC for 24 h under shaking (125 rpm), then maintained at 4°C in BHI agar plates, and subcultured periodically.

Vials containing 10 g of wheat middlings and 200 mL phosphate saline buffer pH 7.4 were autoclaved and then inoculated with 1 mL of bacterial strain at a concentration of 10^8 CFU/mL in 8.5 g/L NaCl solution, or 1 mL saline solution as control. After that, samples were incubated at 37°C for 72 h in an orbital shaker (model MA410, Marconi, Piracicaba, Brazil) at 125 rpm. Aliquots were aseptically collected after inoculation (time 0) and 12, 24, 36, 48, and 72 h of cultivation, then centrifuged at 10000 *g* (Megafuge 16R, Thermo Scientific, Waltham, MA, USA) for 15 min at 4°C, filtered by 0.22 µm membranes (Sartorius, São Bernardo do Campo, Brazil) and frozen at -20°C. This experiment was carried out in triplicate.

The pH was measured in triplicate using a pH meter (PHtek, Curitiba, Brazil) right after collection of the sample aliquots. For the bacterial growth verification, counts were estimated at points 0, 2, 4, 6, 8, 10, 28, 32, 48, 52, and 72 h of cultivation. The quantification of viable cells was performed through the drop culture method as described previously (Naghili et al., 2013). BHI plates were incubated at 37°C and colonies were enumerated after 18 h.

2.2. Enzymatic activity and reducing sugars determination

The endo-β-1,4-xylanase activity was verified according to the method of Bailey, Biely and Poutanen (1992) using 1% (w/v) of birchwood xylan (Sigma Aldrich, St. Louis, USA) in 50 mM sodium citrate buffer pH 5.3. The mixture consisting of 0.9 mL of the substrate solution and 0.1 mL of suitably diluted enzyme extract was incubated at 50°C for 5 min. Then, it was added 1 mL of 3,5-dinitrosalicyclic acid (DNS) reagent and the released reducing sugars were measured as described by Miller (1959), using xylose as standard. An enzyme activity unit (U) was defined as the amount of enzyme capable of releasing 1 µmol xylose per minute.

The enzymatic activities of β-xylosidase and β-glucosidase were determined, respectively, using 0.2% (w/v) of the synthetic substrates ρ-nitrophenyl β-D-xylopyranoside (ρ-NPX) and ρ-nitrophenyl β-D-glucopyranoside (ρ-NPG) from Sigma Aldrich, prepared in 50 mM sodium acetate buffer pH 4.8, according to methodology of Tan, Mayers and Saddler (1987). The mixture consisting of 0.8 mL of the substrate solution and 0.2 mL of suitably diluted enzyme extract was incubated at 50°C for 30 min. After, the reaction was stopped by the addition of 2 mL sodium carbonate (10% w/v) and ρ-nitrophenol (ρ-NP) released was then quantified at 410 nm in an UV-visible spectrophotometer (Shimadzu, Kyoto, Japan). In this assay, enzyme activity units (U) were defined as the amount of enzyme capable of releasing 1 μmol ρ-NP per minute.

2.3. Xylooligosaccharides and xylose analysis

The yield and identification of XOS and xylose content of samples were determined using a liquid chromatograph (Shimadzu, Kyoto, Japan) with refractive index detector (RID-10A). 20 μl aliquots of samples previously filtered at 0.22 μm were injected and eluted on an Aminex HPX-42A column (25 μm, 300 mm × 7.8 mm, Bio-Rad, Hercules, USA) using ultra-pure water (Milli-Q, Millipore, Barueri, Brazil) as mobile phase with a constant flow of 0.4 mL/min in a 40 min isocratic run. The column temperature was maintained at 40ºC. Xylose (Sigma Aldrich) - X1 - and XOS (Megazyme, Bray, Ireland) - xylobiose, xylotriose, xylotetrose and xylopentose (X2, X3, X4 and X5, respectively) - were used as reference standards by preparing calibration curves under the same conditions that were described for the samples.

2.4. Prebiotic activity

In order to verify the prebiotic activity, a commercial probiotic culture, *Lactobacillus acidophilus* LA-5® (Chr. Hansen, Valinhos, Brazil), was chosen because it was demonstrated in a previously study that this strain presented the best activity using the wheat middlings bioprocessed with *B. subtilis* FTC01 as substrate (Reque et al., 2017). The analyses were performed following to the method previously described by Huebner et al. (2007), where 100 µL of LA-5 (from frozen stock at -20ºC in milk) was activated in 10 mL of MRS broth (Himedia, Mumbai, India) after 24 h at 37ºC under anaerobic conditions (GasPak, BD Diagnostics, Sparks, USA). Thereafter, 100 µL of this culture was transferred to 10 mL of fresh MRS broth and incubated anaerobically for additionally 24 h at 37ºC.

Tests were performed by placing 1% (v/v) of LA-5 activated culture at 10^6 UFC/mL in individual sterile vials containing MRS broth, added with 10 mg/mL xylose, initial control or bioprocessed samples. The prebiotic activity score was calculated using the value of cell density (CD) immediately after bacterial inoculation (time 0) and after 24 h incubation at 37ºC according to this equation:

$$\textit{Prebiotic activity score} = CD\ (24\ h) - CD\ (0\ h)$$

where the cell density was measured by optical density (O.D.) at 600 nm in an UV-visible spectrophotometer.

2.7. Data analysis

The coefficient of variation was below 5%, except for prebiotic activity and XOS concentration analyses, that was below 10%. The results were analyzed by one-way ANOVA and Tukey means comparison test, and the correlation test was performed by Person correlation, both at 5% significance level, using the STATISTICA® program (StatSoft. Inc., Tulsa, USA) version 10.

3. Results and discussion

3.1. Bacterial growth, enzymatic activity and reducing sugars concentration

XOS can be obtained from the lignocellulosic biomass auto hydrolysis and acid hydrolysis, however, these methods have disadvantages such as undesired by-products production, high amounts of monosaccharides, as well as requiring a special equipment utilization, being, therefore, more advantageous the use of hydrolytic enzymes (Broekaert et al., 2011). Therefore, a *Bacillus subtilis* strain (FTC01) was used for the wheat middlings processing, insofar as *Bacillus* species are known to produce xylanolytic enzymes capable of releasing XOS into the medium (Reddy & Krishnan, 2013).

The growth curve of the *B. subtilis* FTC01 using wheat middlings as substrate is shown in Figure 1. In the first 12 h, the exponential phase was observed, and then a stationary phase with a slow multiplication rate from 12 to 72 h, and an increment of 1 CFU/mL. A maximum cell count around 8 CFU/mL was reached in 48 h and maintained until the end. No tropophase was observed during the 72 h incubation. The culture medium presented a slight alkalinization, where the pH value varied from 5.10 at 12 h to 6.27 at 48 h, and decreased again to 5.89 at 72 h. Analyzing xylanases production by *B. subtilis* using wheat bran as substrate, Ho (2015) reported maximum cell counts of 2.50×10^9 cells/mL, maximum xylanase activity, and the highest pH (6.52) at 48 h. Similar pH trend was verified by Nawawi et al. (2017) using an isolated *Bacillus* strain for obtention of extracelular xylanases during cultivation on rice bran. They observed an increment of enzymatic activity in direct correlation with the alkalinization of the culture media during the 144 h growth. Battan et al. (2007) reported no bacterial xylanase production from *Bacillus pumilus* ASH at pH 4, but when pH changed to 5, xylanase activity started and achieved its maximum activity at pH 8. Our results indicated a similar behavior, where the increase of endo-β-1,4-xylanase and β-

glucosidase activities follows the increment of pH value in the culture media (Figures 2A and 2C).

The enzymatic activities of endo-β-1,4-xylanase, β-xylosidase and β-glucosidase, as the concentration of reducing sugars, were monitored during bacterial incubation (Figure 2). The results showed a dynamic process where, in the early stages of culture, β-glucosidase and endo-β-1,4-xylanase increase their activities hydrolyzing the wheat middlings, but the probable presence of other monosaccharides such as glucose and arabinose in the medium and the low pH may have affected the production of β-xylosidase in the first 24 h. It has been reported that wheat bran contains arabinose and xylose as main sugar components, and also rhamnose, mannose, galactose and glucose in minor amounts (Manisseri & Gudipati, 2010).

Low activity of β-xylosidase at the first 36 h of culture (Figure 2B) could be related to a lack of β-xylosidase synthesis during bacterial growth attributable to carbon catabolite repression. According to Lindner et al. (1994), β-xylosidase from *B. subtilis* is strongly repressed by glucose, where there is no β-xylosidase synthesis until glucose concentration in the medium is as low as 0.01%. Interestingly, the β-xylosidase activity increases from 21.0 to 70.3 U/mL between 48 and 72 h of culture, when the bacterial cells are still in the stationary phase (Figure 1), showing non-repressing conditions, which is in agreement with the significant reduction in the reducing sugars concentration at the same time (Figure 2D).

The β-glucosidase activity also increased during the incubation period (Figure 2C), with a maximum enzymatic activity of 21.44 U/mL at 72 h of bacterial growth. Tiwari et al. (2017) found a maximum β-glucosidase activity of 57.31 U/g at 72 h culture at 40°C, using *B. subtilis* RA10 under optimized conditions with paddy straw as main component. They also reported an optimal temperature of 50°C, best activity at pH 5 and minimal effect of glucose concentration in the purified β-glucosidase. The results indicated that *B. subtilis* FTC01 could be a promising producer of β-glucosidase, given that new potential β-glucosidase enzymes

with enhanced activities to obtain efficient enzymatic hydrolysis of biomass is an important industry concern (Singhania et al., 2013).

Analyzing the endo-1,4-β-xylanase activity, that randomly cleaves the β-1,4-linked backbone of xylans, it was observed a remarkable increase from 24 to 48 h of growth, reaching 18.72 U/mL at 72 h (Figure 2A). This value is closer to other studies of xylanase activity using agro-industrial waste as substrates. Ho (2015) reported the maximum xylanase activity of 22.07 U/mL at 48 h, using a *B. subtilis* strain growing in optimized culture media containing wheat bran, peptone, yeast extract, KH_2PO_4, and $MgSO_4.7H_2O$. Also, when used barley husk, the maximum xylanase activity found was 11.64 U/mL at 84 h. Soliman et al. (2012), showed a maximum xylanase activity of 12.5 and 11.0 U/g produced from barley bran by *Aspergillus niger* and *Trichoderma viride*, respectively. In addition, a maximum xylanase activity of 18.66 U/g was reported by *Bacillus mojavensis* A21 using barley bran (Haddar et al., 2012). Based on our results, wheat middlings was found to be an optimum carbon source for *B. subtilis*, with high xylanase production.

In general, the enzymatic activity profile coincided with the cellular growth on stationary stage with a high enzymatic synthesis attaining the maximum activity at 72 h of bioprocess (Figure 2). During this phase, it was produced different XOS (Table 1) and other sugars, which maintain the bacteria in continuous growth. No reduction in the enzymatic production was observed. The enzymatic activity increment even in a low reducing sugars concentration (Figure 2D), can be due to the presence of wheat middlings components in the medium that promoted *B. subtilis* growth and enzyme production. Such substances may have originated from despolimerization caused by endo-β-1,4-xylanase that produce XOS, the removal of the chain substituents of xylan by the β-glucosidase and the hydrolysis of non-reducing xylose residues from XOS by the β-xylosidase.

In this biotransformation, XOS (Table 1) and reducing sugars (Figure 2D) composition and concentration in the media change continuously during the hydrolysis by enzymatic activity. This occurs because the bacteria consume carbohydrates, in a dynamic process where the microorganism probably uses a cluster of enzymes, each with a specific function in the process that brings efficient wheat middlings hydrolysis and allows a continue availability of carbohydrates for its growth.

Lignocellulosic material, including wheat bran, is formed by cellulose, hemicellulose and lignin, wherein the hemicellulose fraction is basically composed by xylan (Reddy and Krishnan, 2016), which is formed by a ß-D-(1,4)-linked xylopyranosyl backbone in wheat bran (Immerzeel et al., 2014), thus being a rich source of biopolymers with high added value, such as the prebiotic compounds XOS.

3.2. Xylooligosaccharides and xylose production and prebiotic activity analysis

Concerning to the XOS and xylose production, it was possible to observe that the control samples, that is, those were not inoculated with *Bacillus*, did not undergo alterations during the 72 h incubation at 37ºC (data not shown). However, when compared to the bioprocessed samples, both XOS profile and concentration varied considerably (Table 1, Figure 3).

With respect to the profile, control sample showed the presence of xylose and XOS with degree of polymerization (DP) between 2 and 4, whereas XOS with higher DP (4-5) were present in greater amount after 36 h of bioprocessing. Xylopentose (X5) appears to be three times more concentrated in 72 h than in 12 h of cultivation and was not even identified in control sample. Xylotriose (X3) and xylotetrose (X4) were present in significantly higher concentrations (p<0.05) in the 48 h of bacterial bioprocessing sample. However, xylobiose (X2) and xylose (X1) were found in low amount, where the bioprocessed samples had significantly lower concentrations (p<0.05) than control. Those differences are probably due

to the fact that the *B. subtilis* FTC01 is continuously producing xylanolytic enzymes, but also consuming the sugars, as was previously observed. Besides, the presence of xylose in control samples may be caused by the autoclaving pretreatment.

According to Immerzeel et al. (2014), pretreatment intensity can affect the xylan backbone degree of substitution, where the increase in pretreatment severity factor shows a trend to have a relative higher monosaccharides amount. However, although the presence of xylose in the medium is not desirable, since it is not a XOS, this sugar is fermentable and can be converted into xylitol, a natural sweetener used as a substitute for sucrose (Kocabas and Ozben, 2014).

The insoluble xylan fraction from wheat bran, hydrolyzed with a purified xylanase from *Aspergillus niger* for 24 h at 40ºC, presented a very low xylose concentration (0.137 mg/mL) and concentrations of XOS with DP between 2-6 in the order of 0.358, 0.551, 0.493, 0.535 and 0.376 mg/mL, respectively (Dai et al., 2011). These values were higher than those found in this study for the 24 h bacterial bioprocessed sample, but we did not used any purified enzyme and, at 24 h of cultivation, the xylanase activity was low. Meanwhile, using a recombinant *Bacillus amyloliquefaciens* xylanase, expressed in *Pichia pastoris*, for treatment of different waste materials, Liu et al. (2017) found lower XOS concentrations (X2, X3 and X5) for the 24 h wheat bran hydrolyzed at 40ºC in comparison with our study.

Regarding the total amount of the analyzed sugars, calculated by the sum of the concentrations for each sample and each sugar, the XOS with the highest concentration found was X3 (3.13 mg/mL) and the sample was that one bioprocessed for 48 h (3.25 mg/mL). Kocabas and Ozben (2014), using different types of residues as substrate for XOS production through alkaline hydrolysis and subsequent enzymatic treatment with a five days culture of the fungus *Scytalidium thermophilum*, found that wheat bran had a low total maximum

concentration (approximately 2 mg/mL), represented by the sum of X1, X2, X3 and X4 amount.

Concerning the prebiotic activity, it was evaluated by the increase in the growth of *Lactobacillus acidophilus* LA-5, a commercial probiotic strain, using the wheat middlings bioprocessed samples as substrate. The prebiotic activity reflects the ability of a given substrate to improve the growth of a beneficial microorganism relative to a non-prebiotic substrate (Huebner et al., 2007).

The prebiotic activity score of the bioprocessed wheat middlings are shown in Figure 4. Among the wheat and control (xylose) samples tested, maximum prebiotic activity values were 1.73 and 1.61, obtained by the fractions B24 and B72, respectively, that represent the mixture of XOS and other compounds produced by the *Bacillus* at 24 and 72 h of bioprocess. Thitiratsakul and Anprung (2014) reported prebiotic activity scores of 1.69 and 1.44 for *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB-12, respectively, growth on longan fruit hydrolysate, and values of 1.20 and 1.69, respectively, using inulin as substrate, which are very similar results to those found in our study, demonstrating that the XOS formed by wheat middlings bioprocessing present prebiotic potential.

Observing the composition of XOS in those fractions (Table 1), the hydrolyzed B24 presented low concentration of X1 (0.10 mg/mL) and absence of X4, whereas the B72 sample showed the highest X5 concentration and the other XOS were not identified. On the other hand, the lowest values of prebiotic activity score were found for xylose, used as control, and in the 36 and 48 h cultured samples (0.93, 0.89 and 0.82, respectively). These fractions presented the highest total concentrations of XOS and the sugar X4 was present as main XOS component in B36 and as 45% of total XOS in B48. A strong negative correlation was found between the prebiotic activity and X3 and X4 of 0.70 and 0.85, respectively. According to

Immerzeel et al. (2014), both the DP and the type and degree of XOS substituents can influence the rate of *in vitro* fermentation.

The growth of some bifidobacteria and lactobacilli strains and their capability to metabolize XOS from corncob auto hydrolyzed fractions were analyzed by Moura et al. (2007). *B. adolescentis* and *L. brevis* were the ones that grew better in the hydrolysates and commercial XOS. *L. brevis* showed a tendency to prefer pentose containing media over glucose, as we noted for the *L. acidophilus* LA-5 consumption of the 72 h bioprocessed sample (which is composed mostly by X5), while *L. fermentum* efficiently grew only in glucose and xylose (Moura et al., 2007).

Microorganism growth patterns on a particular oligosaccharide is believed to be specific for each strain due to differences in their transport systems (Wang et al., 2010). According to Madhukumar and Muralikrishna (2012), the mechanisms that are involved can be (a) transport and metabolism of monosaccharides after the action of extracellular enzymes on the oligosaccharides, or (b) hydrolysis of the oligosaccharides that were specifically transported into the cell by intracellular enzymes.

In a study with several bifidobacteria and lactobacilli strains, Li et al. (2015) did not observed any growth for *Lactobacillus acidophilus*, ATCC or isolated from stool, in a substrate with different concentrations of XOS. They found that all *Bifidobacterium* strains were capable of grew in the XOS medium against only 69% of lactobacilli. From those, 91% of bifidobacterial and 28% of lactobacilli presented a strong growth stimulation by XOS, being that the *Bifidobacterium bifidum* species showed the weakest growth stimulation, as observed by our previously work (Reque et al., 2017).

4. Conclusion

Wheat middlings appears to be a good substrate for production of prebiotic compounds like XOS. The profile and concentration of those oligosaccharides varied considerably between control and bioprocessed samples and among these at different culture times. *B. subtilis* FTC01 appears to be a great source of xylanolytic enzymes, that presented significant enzymatic activities, and its use for bioprocessing the wheat middlings was able to improve this waste material by given it potential prebiotic claims and value-added. Thus, processed wheat middlings could be considered a promising material for developing optimized cereal foods and new ingredients, as well as improved animal feed.

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Conflict of interest

Authors declare no conflicts of interest.

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Table 1. Xylooligosaccharides and xylose retention time (Rt, min) and concentration (mg/mL) in control and bioprocessed samples determined by HPLC-RID analysis [a].

	X1	X2	X3	X4	X5	Total
Rt (min)	26.3-26.9	23.6-24.2	21.5-22.1	19.8-20.3	18.5-18.9	-----
Control	0.41±0.01^{a}	0.37±0.00^{a}	0.31±0.01^{b}	0.10±0.00^{c}	ND	1.21
B12h	0.32±0.03^{b}	0.23±0.01^{c}	0.30±0.02^{b}	ND	0.31±0.00^{c}	1.17
B24h	0.10±0.01^{d}	0.28±0.02^{b}	0.27±0.02^{b}	ND	0.33±0.01^{c}	0.98
B36h	0.15±0.01^{c}	NQ	0.47±0.02^{b}	0.98±0.03^{b}	0.48±0.04^{b}	2.08
B48h	NQ	NQ	1.78±0.10^{a}	1.47±0.03^{a}	ND	3.25
B72h	ND	ND	ND	ND	0.90±0.06^{a}	0.90
Total	0.99	0.89	3.13	2.55	2.02	8.38

[a] Sugars concentration is the mean ± standard deviation of three independent experiments. Different superscript letters within each column represent significant differences (p<0.05). Total are the sum of the quantified sugars. X1, X2, X3, X4 and X5 correspond to xylose, xylobiose, xylotriose, xylotetrose and xylopentose, respectively. B12h, B24h, B36h, B48h and B72h are wheat middlings samples after 12, 24, 36, 48 and 72 h of bacterial bioprocessing, respectively. NQ means not possible to quantify and ND means not detected.

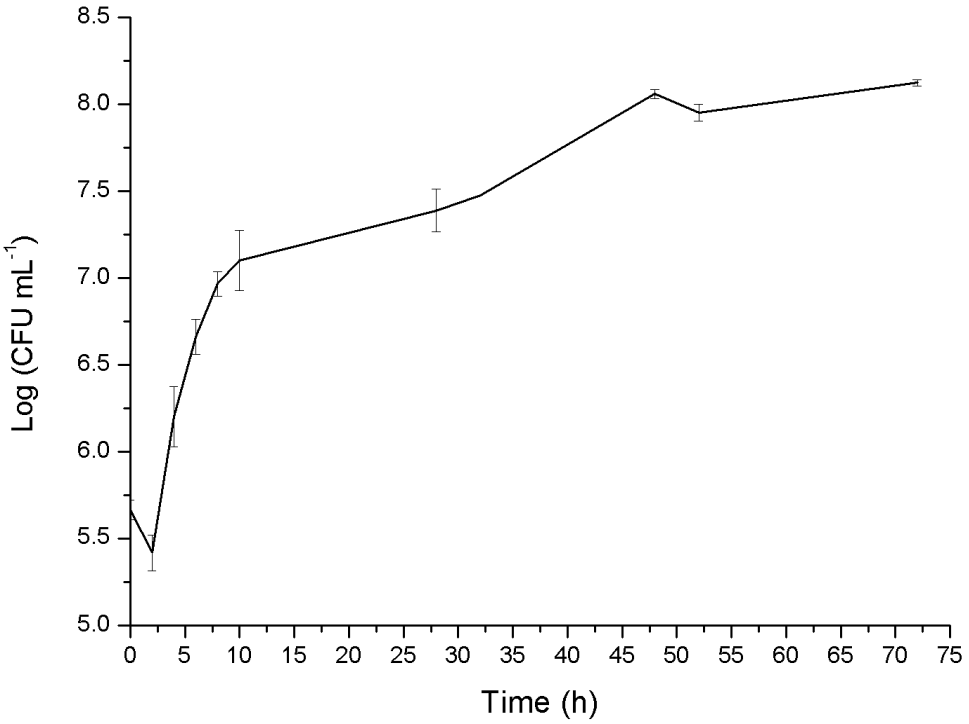

Figure 1. *Bacillus subtilis* FTC01 growth pattern in wheat middlings during 72 h at 37ºC. Values are the means ± standard deviation of two independent experiments.

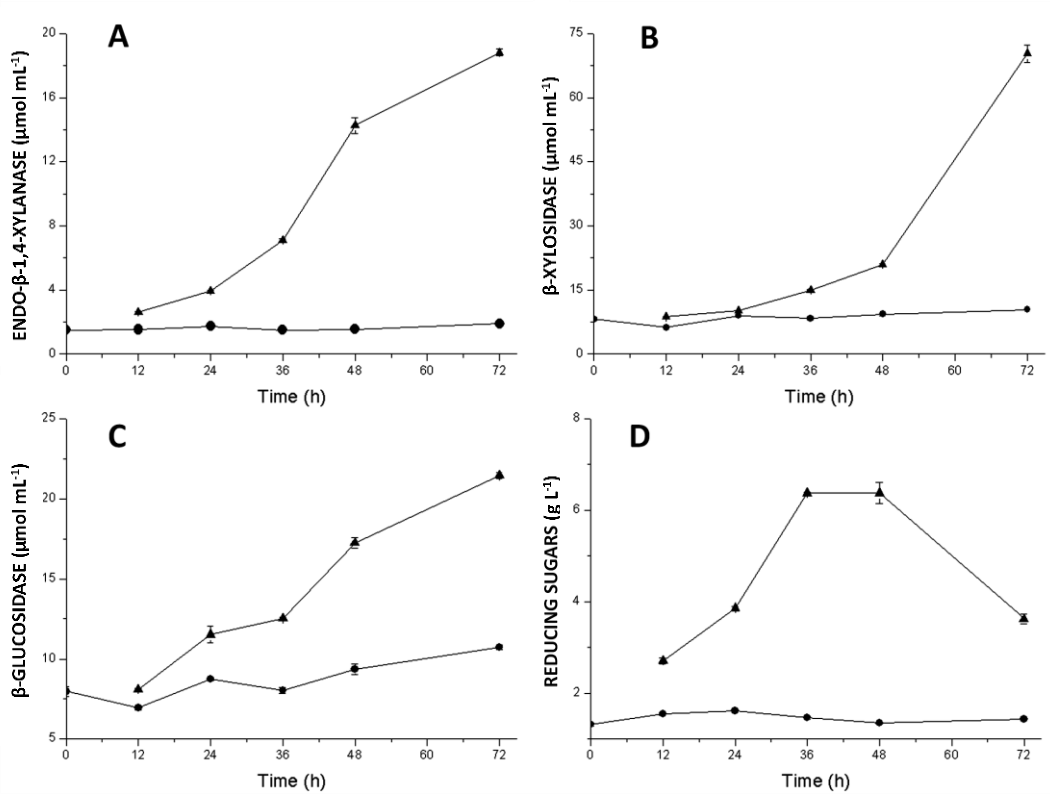

Figure 2. Enzymatic activity and reducing sugars concentration in control (●) and bacterial bioprocessed samples (▲) over time. (A) Endo-β-1,4-xylanase; (B) β-xylosidase; (C) β-glucosidase and (D) reducing sugars. Bars are the means ± standard deviation of three independent experiments.

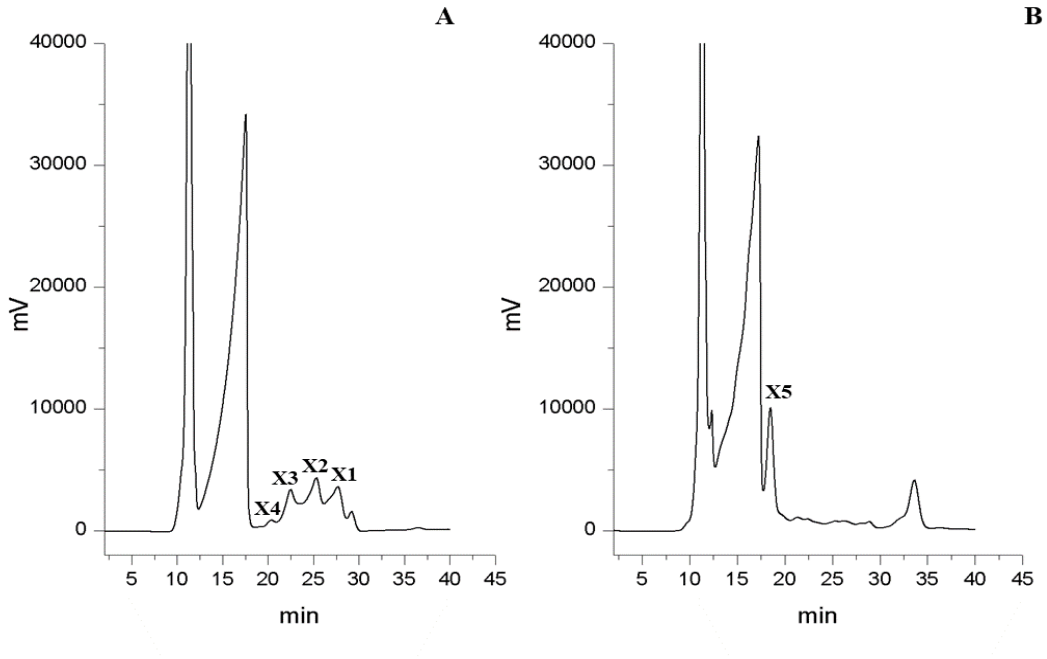

Figure 3. HPLC-RID chromatograms of the control (A) and the 72 h bacterial bioprocessed wheat middlings (B) samples. X1, X2, X3, X4 and X5 are xylose, xylobiose, xylotriose, xylotetrose and xylopentose, respectively.

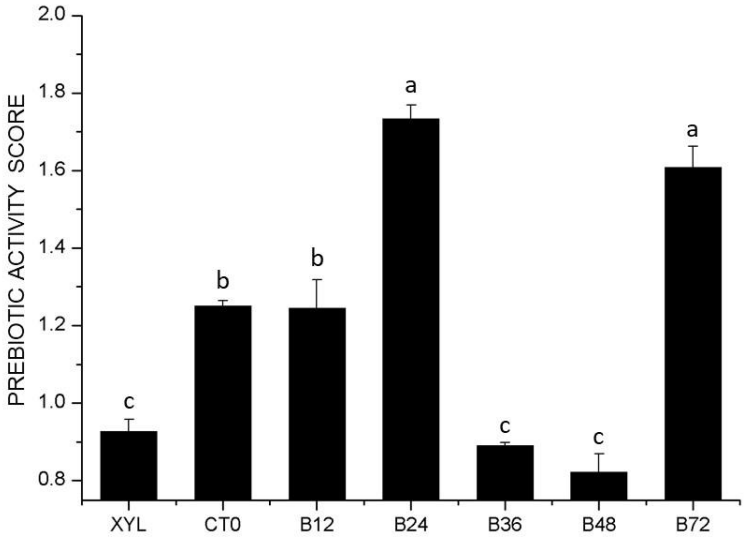

Figure 4. Prebiotic activity score of the *B. subtilis* bioprocessed wheat middlings compared with control and xylose. Samples were incubated at 37ºC for 24 h with *Lactobacillus acidophilus*. Bars are the means ± standard deviation of two independent experiments. Different letters represent significant differences (p<0.05). XYL, CT0, B12, B24, B36, B48 and B72 are xylose, control at initial time and wheat middlings samples after 12, 24, 36, 48 and 72 h of bacterial bioprocessing, respectively.

ARTIGO 3

Lignocellulosic biomass utilization by lactic acid bacteria: prospective analysis of the characteristics and properties resulting from its biotransformation[3]

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Lignocellulosic biomass utilization by lactic acid bacteria: prospective analysis of the characteristics and properties resulting from its biotransformation

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Abstract

The present study aimed to investigate the effect of bioprocessing of wheat middlings, a byproduct of wheat flour production, with different lactic acid bacteria (LAB), in order to evaluate the potential use of such waste for the production of bioactive compounds and the biological properties of resulting cultures. Therefore, lactic acid and reducing sugars concentration, total phenolic content, characterization and differentiation of fermented samples, as well as antioxidant, antibacterial, prebiotic and anti-browning activities were analyzed. All samples showed LAB strains growth and pH medium decrease, probably due to lactic acid production. Sugars showed a reduction pattern after 24 h LAB fermentation, except maltose, produced from starch degradation. It was observed that processed wheat middlings exhibited antioxidant, anti-browning, antibacterial and prebiotic properties as total phenolic content increase. Thus, bioprocessing techniques can be an interesting approach to improve the bioavailability of compounds with health-promoting properties from lignocellulosic material.

Keywords: Lactic acid bacteria; lignocellulosic biomass; bioprocessing; phenolic content; biological activities.

1. Introduction

Recent data reported that global wheat production reached the milestone of 758 million tons between the years 2017-2018, have being consumed about 740 million tons in the same period, of which approximately 70% is for human consumption and 20% for animal feed use. Currently, the largest producers and consumers are European Union, China and India. (IGC, 2018; USDA, 2018)

These cereal grains have fractions that correspond to endosperm, germ and bran, which is characterized by the outer layers (aleurone, hyaline, internal and external pericarp) and are released through the milling process (Onipe, Jideani and Beswa, 2015). Wheat bran, the main byproduct of flour production, is composed by a set of residues with different particle sizes named wheat middlings, fine and coarse wheat bran.

Due to its high content of cellulose and hemicellulose, agroindustrial lignocellulosic waste can be converted into products of high added value, such as bioethanol and other biocomposites, formed by the fermentation of their sugars. Its use, besides limiting the controversy of the food use, avoids the displacement of food crops and the deforestation of new areas, thus reducing the negative impacts caused to the environment (Liguori et al., 2016).

In addition to vitamins and minerals, a wide variety of bioactive compounds are present in wheat bran, such as phenolic acids and other polyphenols, alkylresorcinols and carotenoids, which have antioxidant and anti-inflammatory activities (Anson et al., 2012). The fraction with higher antioxidant capacity is the aleurone layer, which has high phenolic content, especially ferulic acid (Anson et al., 2008).

The bioactive compounds as well as the dietary fiber content found in wheat bran have been associated with several beneficial properties to human health. These include: oxidative damage prevention, reducing the risk of developing chronical diseases, like cancer (especially colorectal), cardiovascular disease, type 2 diabetes and obesity; human erythrocytes protection

against free radicals; mental health promotion; gastrointestinal diseases prevention; and prebiotic activity (Poutanen, 2012; Stevenson et al., 2012).

However, these bioactive compounds present in the outer layer of cereal grains are bound in complex structures of the cell wall, which can withstand the conventional milling process, thus owning low bioaccessibility (Delcour et al., 2012; Stevenson et al., 2012). So, the use of bioprocessing techniques can be an interesting approach to improve the bioavailability of compounds with health-promoting claims (Coda et al., 2014).

Cereal fermentations are carried out mainly by lactic acid bacteria (LAB) species and yeasts. LAB have this name because lactic acid is the main product of their metabolism, and are found in several habitats, such as food and feed, water, soil, respiratory, gastrointestinal and genital tract of humans and animals (Liu et al., 2014). LAB can be used as functional starter cultures insofar as their contribution to food safety and/or offer organoleptic, technological, nutritional or health advantages. Such bacteria are able to produce antimicrobial substances, enzymes, exopolysaccharides, sweeteners, aromatic compounds and nutraceuticals or even have health promoting properties, the so-called probiotic strains (Leroy and De Vuyst, 2004).

The aim of the present study was to investigate the effect of bioprocessing of wheat middlings, a byproduct of wheat flour production. This lignocellulosic biomass was used as substrate for cultivation of LAB isolates, in order to evaluate the potential use of such waste for the production of compounds of interest, as the biological properties of the resulting cultures. Thereby, lactic acid and reducing sugars concentration, total phenolic content, characterization and differentiation of fermented samples, as well as antioxidant, antibacterial, prebiotic and anti-browning activities were analyzed.

2. Materials and methods

2.1. Sample preparation and bioprocessing procedure

The wheat middlings were obtained from a local milling company (Moinho Estrela, Porto Alegre, Brazil). The sample has a mean particle size of 257 μm and 88.4 g/100g dry matter. The sample composition was determined as (g/100 g, dry basis): protein (17.9), ash (3.5), crude fiber (5.4), neutral detergent fiber (24.9) and acid detergent fiber (5.9) (Van Soest et al., 1991). The sample was separated into aliquots of approximately 250 g each, and then stored in polypropylene bags at -20°C.

For wheat middlings bioprocessing, were used the following strains: heterofermentative LAB *Lactobacillus plantarum* DSM20174 (DSMZ, Braunschweig, Germany), *L. fabifermentans* T30PCM38 and *L. fermentum* LM7, and homofermentative LAB *Streptococcus thermophilus* 985, isolates from the DAFNAE laboratory collection. Stock cultures maintained at -20°C in 8.5 g L^{-1} NaCl solution containing 20% (v/v) glycerol were used for bacterial cultivation. Lactobacilli were grown in Man, Rogosa and Sharp medium (MRS, Oxoid, Rodano, Italy) and *Streptococcus* in M17 medium (Oxoid) supplemented with 0.5% of lactose at 10% concentration, and then incubated at 37ºC overnight twice. On the third day, 1 mL of each strain at stationary phase was centrifuged at 5000 *g* for 6 min and then the pellet was resuspended in the same volume of 8.5 g L^{-1} NaCl solution.

For the fermentation, performed in triplicate, vials containing 5% (w/v) of wheat middlings in demineralized water were autoclaved at 121ºC for 15 min and then inoculated with 1% (v/v) of each strain (at an initial concentration of 10^{7-8} CFU mL^{-1}) in saline solution, and also the co-fermentation of *Streptococcus* with each Lactobacilli strain (1:1), or saline as control. Samples were incubated at 37°C for 24 h in microaerophilic conditions, then centrifuged at 5000 *g* for 15 min at 4°C. After pH measurement with a Mettler Toledo MP225 pHmeter (Columbus, USA), samples were centrifuged again at 16000 *g* for 10 min, then filtered in 0.22 μm cellulose acetate membranes (Sartorius, São Bernardo do Campo, Brazil), aliquoted and frozen at -20°C.

For the reducing sugars, antimicrobial and prebiotic activities analyses, samples were prior freeze-dried and then stored at -20°C.

LAB growth in the fermentations was estimated by spread plate method, where 0.1 mL of each serial dilution for the inoculum and 24 h cultures were added to the plates and then spread. The plates were incubated at 37°C for 48 h for subsequent counting of the colonies. The appropriated medium used for counting of *Streptococcus*, Lactobacilli and co-fermentation was, respectively: M17 with 0.5% lactose, MRS and homofermentative-heterofermentative differential (HHD) medium. This HHD medium was adapted from McDonald et al. (1987) and prepared as follows (g L^{-1}): 2.5 fructose, 2.5 KH_2PO_4, 10 casein hydrolysate, 1.5 tryptone from casein, 3 protease peptone, 1 yeast extract, 1 Tween 80 and 20 mL of a Bromocresol green solution (0.1 g in 30 mL 0.01 N NaOH), with the pH prior adjusted to 7.

2.2. Antioxidant activity

The *in vitro* antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Brand-Williams, Cuvelier, & Berset, 1995), based on the capture of free radicals by antioxidants, and the Ferric Ion Reducing Antioxidant Power (FRAP) assay, based on reduce (electron transfer) Fe^{3+} to Fe^{2+} ions in the presence of 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), forming a complex with a maximum absorption at 593 nm (Stratil et al., 2006). All the analyses were carried out in triplicate. The results were presented as µg Trolox equivalents (TE) mL^{-1} sample.

2.3. Anti-browning activity

The anti-browning activity of the bioprocessed samples was examined by the analysis of the inhibition of tyrosinase (TYR), an important group of enzymes that catalyzes phenolic compounds oxidation and is responsible for the enzymatic browning of natural products, as fruits

and vegetables. It was utilized the methodology described in Zocca, Lomolino and Lante (2011), with some adaptations.

The commercial tyrosinase (Sigma Aldrich, St. Louis, USA) was dissolved in 0.1 M sodium citrate buffer (pH 6.0) to a final concentration of 9390 U mL^{-1}. The reaction mixture included 1.0 mL of 10 mM catechol in buffer with 200 μL of samples or sodium citrate buffer (control) and 5 μL of enzyme. Absorbance at 420 nm was monitored continuously at 25°C for 5 min using a JASCO 7800 UV–Vis spectrophotometer (Tokyo, Japan). One unit of enzyme activity (UEA) is herein defined as the amount of enzyme that cause an increase of 0.001 in absorbance per minute, at 420 nm and 25°C. The percent of inhibition of TYR was calculated as follows:

$$\% \text{ inhibition TYR} = [(\text{UEA control} - \text{UEA sample})/ \text{UEA control}] \times 100$$

2.4. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was used to observe changes in the chemical composition of the samples. The FTIR spectra was obtain using KBr compression method in a Perkin Elmer Spectrum 400 spectrometer (São Paulo, Brazil), recorded in the range of 4000–650 cm^{-1}. Scanning was performed from 4000 cm to 400cm^{1} (resolution = 4 cm).

2.5. Total phenolic, Lactic acid and Sugars concentration

Phenolic compounds analysis was carried out in triplicate, using the Folin-Ciocalteau reagent (Swain & Hillis, 1959), and the results expressed as mg of gallic acid equivalents (GAE) mL^{-1}.

Lactic acid content was determined using a Thermo Finnigan chromatographer (San Jose, USA) with a Waters 410 differential refractometer detector, and an Aminex HPX-87H (300 x

7.8mm) column (Bio-Rad, Hercules, USA) maintained at 60 ºC. The mobile phase consisted of H_2SO_4 0.0025 N on isocratic elution mode for 45 min, flow rate of 0.6 mL min^{-1} and volume of injection of 10 µL. Sugars (glucose, maltose and xylose) concentration was determined by an HPLC system with diode array detection and an Aminex HPX-87C anion exchange column at 85°C. The mobile phase was deionized water and the flow rate was 0.6 mL min^{-1}. Sugars and lactic acid were identified by comparisons with a standard calibration curve and their retention times (Rt).

For reducing sugars analysis, it was added 1 mL of 3,5-dinitrosalicyclic acid (DNS) reagent to samples and the released sugars were determined according to Miller (1959), using glucose as standard.

2.6. Antibacterial activity

Antibacterial activity was tested by well diffusion technique, in triplicate, against the indicator strains *Staphylococcus aureus* ATCC1901 and *Bacillus cereus* ATCC14575 from American Type Culture Collection (Manassas, VA, USA). These microorganisms, at a concentration of 10^8 UFC mL^{-1} in saline solution (8.5 g L^{-1} NaCl), were inoculated with a swab onto Brain Heart Infusion (BHI, Kasvi, São José dos Pinhais, Brazil) agar plates. Lyophilized samples were suspended in purified water, sonicated for 10 min, centrifuged at 10000 *g* for 10 min twice, and then filtered through 0.22 µm membranes. 20 µL aliquots of each sample at 25, 50, 75 and 100 mg mL^{-1} concentrations were spotted on discs into the freshly prepared lawn of the indicator strain, and plates were incubated at 37°C overnight. Subsequently, the diameter of growth inhibition was measured with a digital caliper and expressed as inhibition zone (mm).

2.7. Prebiotic activity

In order to verify the prebiotic activity, a commercial probiotic culture, *Lactobacillus acidophilus* LA-5® (Chr. Hansen, Valinhos, Brazil), was chosen because it was demonstrated in a previously study that this strain presented the best activity using the wheat middlings bioprocessed with *B. subtilis* FTC01 as substrate (Reque et al., 2017). The analyses were performed following to the method previously described by Huebner, Wehling and Hutkins (2007), where 100 µL of LA-5 (from frozen stock at -20ºC in milk) was activated in 10 mL of MRS broth (Himedia, Mumbai, India) after 24 h at 37ºC under anaerobic conditions (GasPak, BD Diagnostics, Sparks, USA). Thereafter, 100 µL of this culture was transferred to 10 mL of fresh MRS broth and incubated anaerobically for additionally 24 h at 37ºC.

Tests were performed by placing 1% (v/v) of LA-5 activated culture at 10^6 UFC mL^{-1} in individual sterile vials containing MRS broth, added with 10 mg mL^{-1} glucose, initial control or bioprocessed samples. The prebiotic activity score was calculated using the value of cell density (CD) immediately after bacterial inoculation (time 0) and after 24 h incubation at 37ºC according to this equation:

$$\textit{Prebiotic activity score} = CD\ (24\ h) - CD\ (0\ h)$$

where the cell density was measured by optical density (OD) at 600 nm in an UV-visible spectrophotometer.

2.8. Data analysis

The coefficient of variation was below 5% for the analyses and below 10% among the bioprocessing experiments. The results were analyzed by one-way ANOVA and Tukey means comparison test at 5% significance level, using the STATISTICA® program (StatSoft. Inc., Tulsa, USA) version 10.

3. Results and Discussion

3.1. Growth, pH, Lactic acid production and Sugars concentration after LAB fermentation

With respect to growth analysis of LAB strains in the co-fermentations, carried out between the different isolates of Lactobacilli and *S. thermophilus*, it was observed the formation of only Lactobacilli colonies in the HHD medium. It is possible that these microorganisms inhibited their growth either by competing for the same carbon and nitrogen sources present in the fermentation medium or by accumulating inhibitory compounds. According to Gobbetti et al. (2005), the use of co-fermentations can be an interesting approach for improving LAB strains adaptability to the utilization of non-fermentable substrates as a metabolic alternative.

Optimum temperature and pH values in LAB fermentations are commonly 37-43°C and 5.0-7.0, respectively (Abdel-Rahman, Tashiro & Sonomoto, 2011), that were the initial conditions of wheat middlings bioprocessing. All fermentations and co-fermentations showed LAB strains growth and pH medium reduction, probably due to the organic acids formation, particularly lactic acid. As can be seen in table 1, samples inoculated with *L. plantarum*, alone and/or with *S. thermophilus*, presented the highest values of growth (2.84±0.11 log CFU mL^{-1} for LP) and lactic acid concentration (2.03±0.10 and 2.14±0.06 mg mL^{-1} for LP and STLP, respectively) as well as reduction of the pH.

Charalampopoulos, Pandiella and Webb (2002), working with *L. fermentum*, *L. reuteri*, *L. acidophilus* and *L. plantarum*, cultured in different cereal substrates, reported that Lactobacilli *fermentum* and *plantarum* reached high cell populations growing in barley and wheat media, while the other strains did not grow well. These authors also found, for the *L. plantarum* fermentation, similar patterns for pH reduction in wheat medium (from 5.85±0.09 to 3.83±0.09) and higher values for lactic acid production in malt (5.74±0.1), compared to our study.

According to Charalampopoulos et al. (2002), lactobacilli strains have complex nutritional requirements such as carbohydrates, amino acids, peptides, fatty esters, salts, nucleic acid

derivatives, and vitamins, which can vary considerably between different species. *L. plantarum* is considered a good species for bioprocessing lignocellulosic materials due to its high metabolic variability, as the ability of fermenting both pentoses and hexoses and its high acid tolerance (Moraïs et al., 2013). The main carbohydrates present in cereals are starch, dietary fibre components, and free sugars, like glucose, xylose, fructose, maltose, sucrose, and arabinose (Charalampopoulos et al., 2002).

However, fermentable carbohydrates and reducing sugars concentration is relatively low in wheat flour, requiring starch degradation by amylase activity, producing maltodextrins, maltose, and glucose during fermentation. Nonetheless, only few lactobacilli exhibit extracellular amylase activities, like *L. plantarum* (Gänzle, 2014). In relation to the wheat middlings carbohydrates amounts and total reducing sugars concentration, all reducing sugars analyzed and quantified showed a reduction pattern after 24 h fermentation by all LAB strains, with the exception of maltose (Table 1).

Maltose is a reducing disaccharide obtained from the starch hydrolysis by the β-amylase, being converted into glucose. The ST and STFE samples exhibited significantly higher maltose concentration ($p<0.05$) than control samples, probably produced by the substrate starch metabolization, in order to release glucose for their consumption. Glucose was consumed by all microorganisms and their combinations, and together with total reducing sugars content, presented significative amount reduction ($p<0.05$) comparing to control samples.

Xylose concentration of ST sample showed no significant difference ($p>0.05$) to CTO sample, but lower than CT24 sample. This 24h control sample presented significantly higher reducing sugars amounts ($p<0.05$) than initial control sample, possibly released from starch by its disruption and water solubility at 37°C. The starch gelatinization, produced by heating its granules in aqueous medium, generates the breakdown of its molecular order and causes irreversible changes (Morales-Sanchez, Figueroa & Gaytan-Martínez, 2009).

According to Imran et al. (2016), the decreasing in total reducing sugars content may be related to the fermentable carbohydrates consumption by the microorganisms, which contributes to increasing growth, higher cell viability and lactic acid production. These authors reported a reducing sugar consumption by two *L. plantarum* strains (NTMI05 and NTMI20) of 10.5±0.2 and 9.0±0.1 mg mL^{-1}, respectively. In the present study, the lower consumption of sugars had been shown by the ST sample (7.53 mg g^{-1} DW), followed by LP (10.39 mg g^{-1} DW), however, when co-inoculated, STLP sample showed the higher consumption of these carbohydrates (18.44 mg g^{-1} DW).

Charalampopoulos, Pandiella and Webb (2002) emphasized that *L. plantarum* strains have preference for metabolizing glucose, whereas *L. fermentum* from sourdough fermentation tends to prefer to use maltose. In relation to the *L. plantarum* strain of our study, such characteristic was observed, however, with regard to *L. fermentum*, our strain did not showed preference for maltose. The referred authors have found, in malt 48 h fermentation with Lactobacilli *fermentum* and *plantarum*, maltose content of 2.10 and 2.25 g L^{-1} and glucose amount of 0.03 and 0.17 g L^{-1}, respectively.

Working with quinoa and wheat slurries fermentation with a *L. plantarum* strain during 24 h at 30ºC, Dallagnol et al. (2013) observed that, although there was no marked decrease in pH, this bacterium grew better and produced a higher amount of lactic acid in the quinoa medium than in wheat, probably due to the fact that quinoa contains higher fermentable sugars content compared to wheat, which contributes to bacterial growth.

3.2. Antioxidant and Anti-browning activities and Phenolic content

The antioxidant capacity of the bioprocessed wheat middlings was evaluated by the DPPH and FRAP methods, in order to verify the samples ability to scavenge free radicals and reduce iron. Both assays presented a significant increase (p<0.05) in the antioxidant activity of the

LAB-fermented material compared to control (CT0 and CT24), but not for every sample (Figure 1). The ST and LP samples showed the highest antioxidant activity values by both methodologies, whereas STLP and STFE, in DPPH assay, as FA and STFA, by FRAP test, did not had any significant difference with the control samples (p>0.05).

Wheat antioxidant capacity can mainly be related to its phenolic content, especially ferulic acid, which is able to donate electrons and transfer hydrogen atoms to free radicals (Anson et al., 2008 & 2012). Both antioxidant capacity and phenolic compounds can often be influenced by the microorganism species used in cereal bioprocessing (Coda et al., 2014). In addition, antioxidant activity increase in plant-based foods by fermentation may be related to several other factors, including pH, temperature, water content, fermentation time, type of food and aerobic conditions (Hur et al., 2014).

Regarding phenolic compounds (Figure 1), processed samples increased their concentrations in comparison to CT0 (200.1±2.0 µg GAE mL^{-1}), with only the STFA sample being statistically similar (p>0.05). However, the CT24 sample had higher values than initial control (252.8±2.5 µg GAE mL^{-1}) and was statistically equal to or greater than all other samples. This difference could also be related to phenolic substances releasing from cells/granules disruption and higher solubility after 24 h at 37ºC but did not had influence in the antioxidant capacity.

Lactobacilli species have been reported to degrade phenolic compounds, with several proteins involved in their metabolism already well characterized. *L. plantarum* is able to metabolize some phenolic acids, such as coumaric, caffeic, ferulic, gallic and protocatechuic acids (Rodríguez et al., 2009). LAB phenolic acids metabolism is mediated by reductases and decarboxylases, and their bioconversion is strain specific (Gänzle, 2014), which could explain the differences between fermented samples and CT24.

According to Gan et al. (2017), most studies show an increase in total phenolic compounds in fermentations, as soluble phenolic substances can be released during cultivation. But also, other studies have found that fermentation did not increase, or may even reduce, the phenolic content in some products, like wheat, probably related to the degradation of such compounds. Working with 24 h cereals fermentation at 30ºC by using yeast and lactic acid bacteria, Đorđević, Šiler-Marinković and Dimitrijević-Branković (2010) did not found any significant effect in the analysis of antioxidant activity by FRAP and DPPH for processed wheat, showing only a slight increase in the phenolic content of wheat fermented with *L. rhamnosus* (16.2 to 20.7 mg GAE/g DW) or *S. cerevisiae* (16.2 to 18.4 mg GAE/g DW).

Tyrosinases (TYR), beloing to the polyphenol oxidases (PPO) enzyme family, are known to catalyze the hydroxylation of monophenols to diphenols, followed by the oxidation of diphenol to its corresponding quinone, which are subsequently polymerized, thus forming dark pigments. Such enzymes are related to the browning caused by the transformation of phenolic compounds into dark pigments with the presence of oxygen and water in fermentations (Zhao, Guo & Zhu, 2017).

So, it was also evaluated the anti-browning capacity of the LAB-processed wheat middlings (Figure 1), measured by TYR inhibition. It was observed that all fermented samples had significantly higher inhibitory activity ($p<0.05$) compared to control samples, apart from FE (21.0%), which was shown to be statistically similar to CT24 (17.4%). The highest values were presented by the STLP and LP samples, with about 25% of TYR inhibition. Wang et al. (2017) analyzed the TYR inhibitory and antioxidant activities of plant extracts fermented by probiotic bacteria. They have found an anti-TYR activity of 44.5% for the *L. acidophilus* fermentation and 8.0% for the cultivation with *L. brevis*.

3.3. Antibacterial activity

The inhibitory activity of the bioprocessed wheat middlings cultures against indicator strains of *Bacillus cereus* and *Staphylococcus aureus* was investigated (Table 2). Inhibition zones against *B. cereus* ATCC14575 were detected for the 24 h bioprocessed samples LP, ST, STLP, STFA and STFE, but not for the FE and FA or control samples. The highest inhibition halo (8.64±0.23 mm), as well as the lowest MIC value (50 mg mL^{-1}), were found for the STLP sample. These findings are in agreement with the antibacterial activity against *S. aureus* ATCC1901, where the fermented samples LP and STLP presented a significantly higher activity (p<0.05), with inhibition halos of 12.89±0.34 and 13.37±0.57 mm, respectively, comparing to the other samples. The lowest MIC (50 mg mL^{-1}) was found for the STLP sample. Only control samples did not exhibit inhibitory activity against *S. aureus* strain. Working with chicory fermentation using the same *L. plantarum* strain of the present study (DSM 20174), Kagkli et al. (2016) also reported inhibition halos larger than 10 mm for the indicator microorganisms *P. fluorescens, E. coli, B. amyloliquefaciens, B. subtilis, S. xylosus* and *L. innocua*.

The results may indicate a synergistic effect of LP and ST, probably due to nutrient competition, on the production of metabolic antimicrobial compounds as bacteriocins or lactic acid and other organic acids, that could be related to the growth inhibition of the pathogenic bacteria *B. cereus* and *S. aureus*. Organic acids are able to inhibit microbial growth in their undissociated form, dissociated form or by releasing proton H^+ in the medium. Is well recognized that lactic acid is the major metabolic product of carbohydrates fermentation by LAB and such characteristic is widely used in food fermentations since acidification inhibits spoilage microorganisms (Imran et al., 2016).

3.4. Fourier Transform Infrared Spectroscopy (FTIR)

Bioprocessed wheat middlings were also analyzed by FTIR (Figure 2) in order to determine the specific absorption bands present in each lyophilized sample. Spectra of all

samples showed typical absorption peaks of polysaccharides at 1045 cm^{-1}, attributed to ring vibration, absorption bands at 1401 cm^{-1} (C-C) and 1664 cm^{-1} (C-C group) (Imran et al., 2016; Cao et al., 2011). Additionally, was observed the symmetric and antisymmetric stretching vibrations of acyl chains with frequency values around 2850 cm^{-1} and 2920 cm^{-1}, respectively. These absorption peaks are representative of the principal chemical groups present in all samples.

The C=O stretching band at 1730 cm^{-1}, characteristic of carboxylic acid groups as lactic acid, and absorption bands at 1237 cm^{-1}, that usually represent the stretching vibrations of C-O alcohols, carboxylic acids, esters and ethers (Păucean et al., 2017) were present in the bioprocessed wheat middlings with LAB (FE, FA, LP, ST and its co-fermentations) and absent in control samples (CTO and CT24). Differences of intensity on the broad band around 3395 cm^{-1} due to the O–H stretching was observed in the samples.

In addition, different patterns were observed at the wave number region from 1200 to 800 cm^{-1}, that is the fingerprint region and can be used to characterize diverse polysaccharides (Imran et al., 2016). Also, specific peaks around 770 and 864 cm^{-1}, that are related to the stretching vibration of C–O–C and C–O–H from glycosidic bonds of α-glycosidic and β-glycosidic linkages (Capek, Drábik & Turjan, 2010; Bernardino-Nicanor et al., 2017), were observed in the fermented samples (FE, FA, LP, ST and its combinations), which could indicate a high level of starch hydrolysis.

3.5. Prebiotic activity

With respect to the prebiotic potential of the wheat middlings before and after LAB fermentations (Figure 3), it was observed that the highest prebiotic activity scores were presented by the ST, LP, STFE and STFA samples, together with the untreated sample (CT0), with no statistically significant difference between them, whereas the bioprocessed FA and STLP

samples were statistically similar to the CT24 sample. All samples had statistically higher values (p<0.05) than those presented by the glucose control (GLU), except for the FE sample, whose mean is statistically similar to GLU, indicating that wheat middlings represents a good substrate for fermentation by probiotic bacteria, such as LA-5. However, as there was practically no difference between the LAB treated and untreated samples (CT0 and CT24), the use of such bacterial group, that can also be considered probiotic, in the processing of wheat middlings was not able to increase prebiotic substances content.

In a previously study (Reque et al., 2017), working with wheat middlings cultured for 72h with a *B. subtilis* strain, the treated sample presented the highest prebiotic activity score for LA-5 (1.67), and such growth promotion was statistically different (p<0.05) from the untreated samples CT0 and CT72 (1.31 and 0.82, respectively) and glucose (1.05). The genus *Bacillus* is a recognized producer of xylanolytic enzymes, favoring XOS production and, consequently, of potentially prebiotic compounds (Reddy & Krishnan, 2013). On the other hand, according to Moraïs et al. (2013), *L. plantarum* strains have several genes encoding glycoside hydrolase families, but none are strict cellulase or xylanase.

Also, both the degree of polymerization as the type and degree of XOS substituents can influence *in vitro* fermentation rate (Immerzeel et al., 2014). Microorganisms growth patterns on oligosaccharides may be specific for each strain owing to differences in their transport systems (Wang et al., 2010). The mechanisms that are involved can be transport and metabolism of monosaccharides after the action of extracellular enzymes on the oligosaccharides, or hydrolysis of the oligosaccharides that were specifically transported into the cell by intracellular enzymes (Madhukumar & Muralikrishna, 2012).

4. Conclusion

This work indicates that bioprocessing techniques are useful to enhance the biological properties of lignocellulosic materials. All samples showed LAB strains growth and pH medium decrease, probably due to lactic acid production. Sugars showed a reduction pattern after 24 h LAB fermentation, except maltose, produced from starch degradation. It was also observed that processed wheat middlings exhibited antioxidant, anti-browning, antibacterial and prebiotic properties as total phenolic content increase. Thus, wheat middlings could be considered a good raw material for developing optimized cereal products, as well as improved animal feed. So, bioprocessing is an efficient and environmentally friendly tool to obtain some positive modifications in the wheat bran matrix and thus enhancing the bioaccessibility and bioavailability of its health-promoting compounds and biological properties.

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Table 1. Growth, pH, lactic acid concentration and sugars patterns of wheat middlings control samples and fermented by LAB.[a]

Samples	Growth (log CFU mL^{-1})	Ph	Latic acid ($mg\ mL^{-1}$)	Maltose ($mg\ mL^{-1}$)	Glucose ($mg\ mL^{-1}$)	Xylose ($mg\ mL^{-1}$)	Reducing sugars ($mg\ g^{-1}$ DW)
CT0	---	5.73 ± 0.05^{a}	0	1.29 ± 0.11^{cd}	1.42 ± 0.15^{b}	0.71 ± 0.07^{b}	26.47 ± 0.69^{b}
CT24	---	5.72 ± 0.02^{a}	0	1.56 ± 0.05^{ab}	1.80 ± 0.02^{a}	0.89 ± 0.03^{a}	32.38 ± 0.49^{a}
ST	2.31 ± 0.01^{b}	4.29 ± 0.10^{b}	1.27 ± 0.02^{e}	1.68 ± 0.09^{a}	1.03 ± 0.03^{c}	0.67 ± 0.01^{b}	18.94 ± 0.03^{c}
LP	2.84 ± 0.11^{a}	3.85 ± 0.15^{d}	2.03 ± 0.10^{a}	1.25 ± 0.03^{cd}	0.72 ± 0.03^{ef}	0	16.08 ± 0.03^{d}
FE	1.98 ± 0.06^{bc}	4.11 ± 0.04^{bc}	1.23 ± 0.01^{e}	1.41 ± 0.04^{bc}	0.82 ± 0.03^{de}	0	11.82 ± 0.04^{f}
FA	1.45 ± 0.30^{d}	3.95 ± 0.07^{cd}	1.63 ± 0.01^{c}	1.28 ± 0.02^{cd}	0.80 ± 0.03^{de}	0	11.68 ± 0.06^{f}
STLP	1.56 ± 0.06^{cd}	3.83 ± 0.01^{d}	2.14 ± 0.06^{a}	1.18 ± 0.05^{d}	0.56 ± 0.03^{f}	0	8.03 ± 0.07^{h}
STFE	1.00 ± 0.18^{e}	4.03 ± 0.12^{cd}	1.79 ± 0.01^{b}	1.59 ± 0.03^{a}	0.89 ± 0.03^{cd}	0	9.12 ± 0.05^{g}
STFA	1.51 ± 0.21^{d}	3.97 ± 0.09^{cd}	1.40 ± 0.03^{d}	1.28 ± 0.03^{cd}	0.73 ± 0.05^{de}	0.34 ± 0.03^{c}	13.87 ± 0.02^{e}

[a] Values as the mean ± standard deviation of three independent experiments. Different superscript letters within each column represent significant differences ($p<0.05$). CT0, CT24, ST, LP, FE, FA, STLP, STFE, STFA, CFU, and DW represents, respectively: initial control sample; 24 h control sample; 24 h fermented sample with *S. thermophilus, L. plantarum, L. fermentum, L. fabifermentans, S. thermophilus* and *L. plantarum, S. thermophilus* and *L. fermentum, S. thermophilus* and *L. fabifermentans*; colony forming unit; dry weight.

Table 2. Antimicrobial activity of the control samples and 24 h bioprocessed wheat middlings against the pathogenic bacteria *Bacillus cereus* and *Staphylococcus aureus*.[a]

Sample	*B. cereus* ATCC14575		*S. aureus* ATCC1901	
	Inhibition zone (mm)[b]	**MIC (mg mL^{-1})**	**Inhibition zone (mm)**[b]	**MIC (mg mL^{-1})**
CT0	0	0	0	0
C24	0	0	0	0
FE	0	0	8.24±0.34^{c}	75
FA	0	0	6.11±0.17^{d}	100
LP	5.74±0.22^{c}	100	12.89±0.34^{a}	75
ST	5.09±0.21^{d}	100	8.735±0.02^{c}	75
STFA	5.33±0.13^{cd}	100	10.37±0.25^{b}	75
STLP	8.64±0.23^{a}	50	13.37±0.57^{a}	50
STFE	6.17±0.16^{b}	75	9.04±0.33^{c}	100

[a] Values as the mean ± standard deviation of three independent experiments. Different superscript letters within each column represent significant differences (p<0.05). CT0, CT24, ST, LP, FE, FA, STLP, STFE, STFA, and MIC represents, respectively: initial control sample; 24 h control sample; 24 h fermented sample with *S. thermophilus*, *L. plantarum, L. fermentum*, *L. fabifermentans*, *S. thermophilus* and *L. plantarum*, *S. thermophilus* and *L. fermentum*, *S. thermophilus* and *L. fabifermentans*; minimum inhibitory concentration.

[b] Inhibition halos are presented for the samples concentration of 100 mg mL^{-1}.

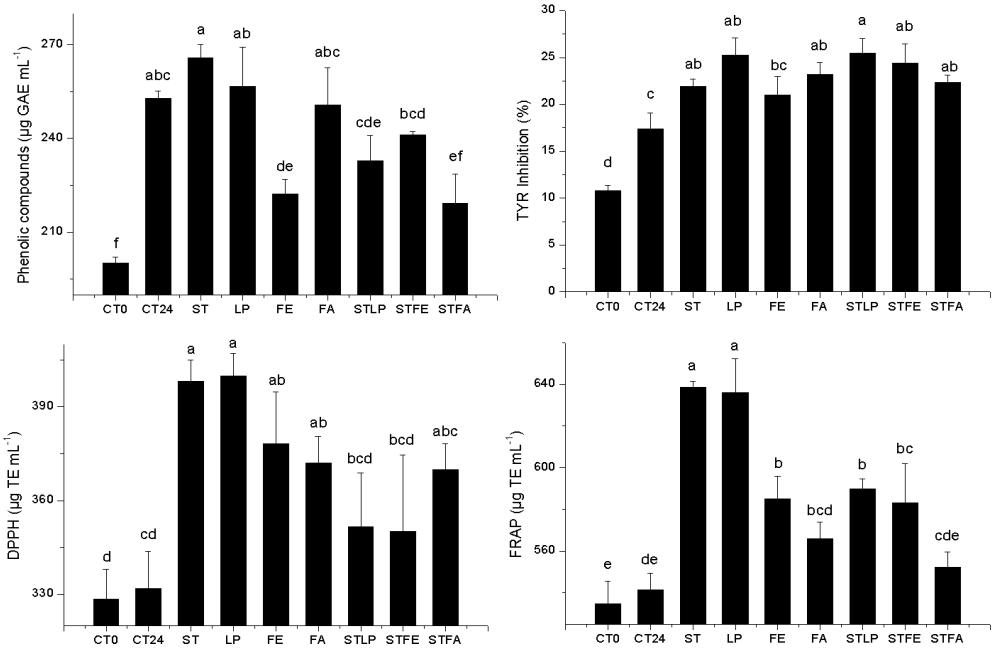

Figure 1. Antioxidant and anti-browning activities and total phenolic content of control samples and bioprocessed wheat middlings with the LAB strains and its co-inoculations. CT0, CT24, ST, LP, FE, FA, STLP, STFE and STFA represents, respectively: initial control sample; 24h control sample; 24h fermented sample with *S. thermophilus, L. plantarum, L. fermentum, L. fabifermentans, S. thermophilus* and *L. plantarum, S. thermophilus* and *L. fermentum, S. thermophilus* and *L. fabifermentans*. Bars are the means ± standard deviation of three independent experiments. Different letters represent significant differences (p<0.05).

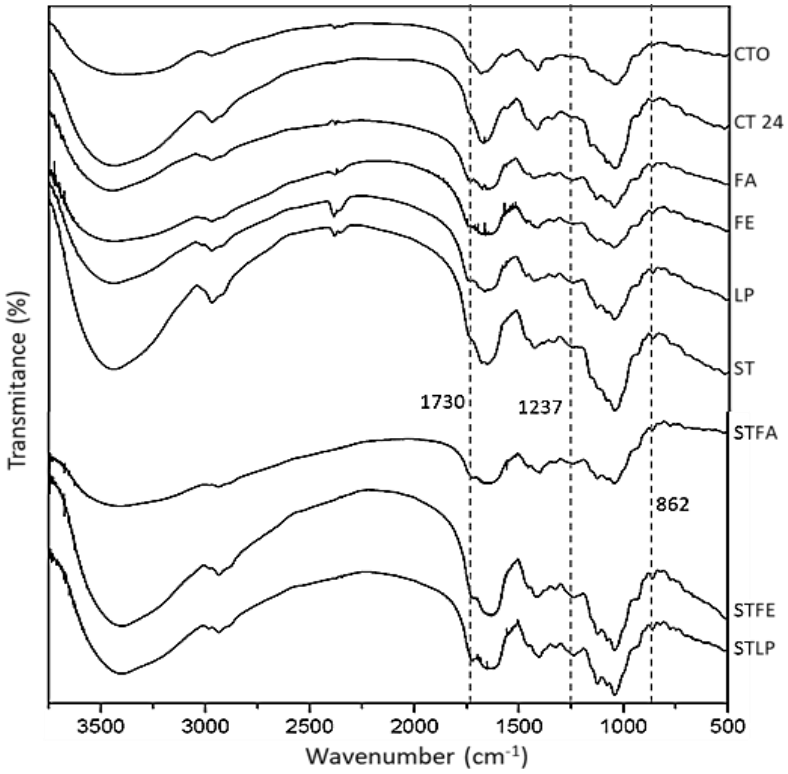

Figure 2. FTIR spectra of control samples and bioprocessed wheat middlings with the LAB strains and its co-inoculations. CT0, CT24, ST, LP, FE, FA, STLP, STFE and STFA represents, respectively: initial control sample; 24h control sample; 24h fermented sample with *S. thermophilus*, *L. plantarum*, *L. fermentum*, *L. fabifermentans*, *S. thermophilus* and *L. plantarum*, *S. thermophilus* and *L. fermentum*, *S. thermophilus* and *L. fabifermentans*.

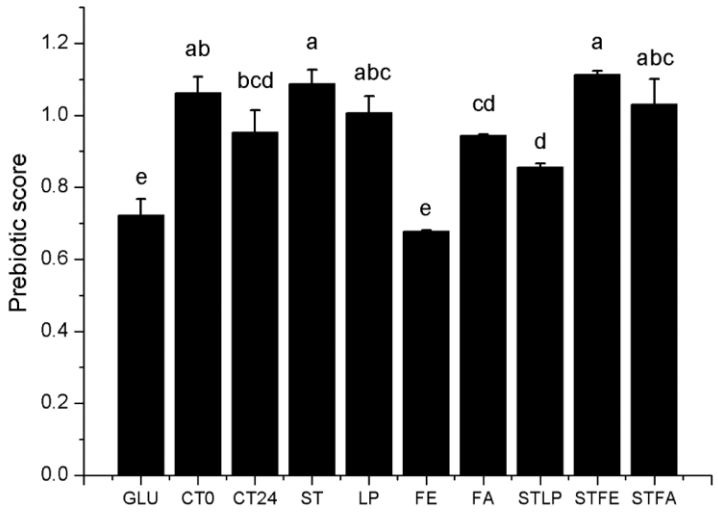

Figure 3. Prebiotic activity score of the bioprocessed wheat middlings compared with control and glucose. Samples were incubated at 37ºC for 24 h with *Lactobacillus acidophilus* LA-5. Bars are the means ± standard deviation of three independent experiments. Different letters represent significant differences (p<0.05). GLU, CT0, CT24, ST, LP, FE, FA, STLP, STFE and STFA represents, respectively: glucose; initial control sample; 24h control sample; 24h fermented sample with *S. thermophilus, L. plantarum, L. fermentum, L. fabifermentans, S. thermophilus* and *L. plantarum, S. thermophilus* and *L. fermentum, S. thermophilus* and *L. fabifermentans*.

5. DISCUSSÃO GERAL

O presente trabalho foi desenvolvido com o intuito de avaliar e caracterizar os compostos bioativos e suas propriedades presentes em um resíduo da produção de farinha de trigo, a farinheta, após o seu bioprocessamento com diferentes isolados de *Bacillus* spp. e bactérias ácido lácticas (LAB). O uso de microrganismos não-patogênicos foi uma alternativa encontrada para o processamento desse subproduto, na medida em que a utilização de enzimas específicas apresenta um custo bastante elevado.

Nesse sentido, na revisão bibliográfica, foram abordados os principais aspectos referentes à cultura do trigo, sua importância histórica e nutricional, características e propriedades dos grãos. Tratou-se ainda sobre os resíduos lignocelulósicos, de maneira geral, e, mais especificamente, da farinheta de trigo, bem como sobre o bioprocessamento desse tipo de material através da utilização de microrganismos e/ou enzimas. Além disso, foram apresentados conceitos e características das bactérias do gênero *Bacillus* e também com relação ao grupo de microrganismos conhecido como LAB. Por fim, discutiu-se a respeito dos compostos bioativos, sendo abordados, em particular, as substâncias fenólicas e os oligossacarídeos, além dos conceitos de probióticos e prebióticos.

Com relação aos resultados da tese, estes foram apresentados no formato de artigos, os quais foram separados em três capítulos. No primeiro, foi possível observar que as amostras de farinheta de trigo, bioprocessadas com diferentes cepas de *Bacillus* por 72 horas, apresentaram atividade inibitória contra as bactérias *L. monocytogenes*, *B. cereus* e *E. coli*, aumento significativo da capacidade antioxidante e dos compostos fenólicos totais, além de alterações no perfil fenólico, com identificação de alguns ácidos hidroxibenzóico e hidroxicinâmico. A amostra cultivada com *B. subtilis* FTC01 exibiu ainda potencial prebiótico e anti-hipertensivo.

O segundo artigo foi realizado objetivando o processamento da farinheta de trigo com a cepa considerada mais promissora (*B. subtilis* FTC01), a partir dos resultados obtidos no primeiro artigo, a fim de avaliar a produção de compostos com propriedades prebióticas. Assim, verificou-se que a atividade de enzimas xilanolíticas aumentou continuamente durante o crescimento bacteriano, especialmente da β-xilosidase. Além disso, o perfil e a concentração de XOS

variaram consideravelmente entre as amostras durante as 72 horas de cultivo, sendo os maiores valores de atividade prebiótica encontrados nas amostras processadas por 24 e 72 horas. Tais resultados, provavelmente, devem-se ao fato de a taxa de fermentação *in vitro* ser influenciada tanto pelo DP quanto pelo tipo e grau dos substituintes dos XOS, que variaram ao longo do tempo. Assim, a farinheta de trigo mostrou-se um substrato promissor para a produção de prebióticos como XOS e a cepa *B. subtilis* FTC01 demonstrou ser uma boa fonte de enzimas xilanolíticas.

Com relação ao terceiro artigo, realizou-se o processamento da farinheta de trigo através do uso de bactérias do tipo LAB, na medida em que estas são, em sua maioria, consideradas probióticas e/ou GRAS. Todas as amostras apresentaram crescimento das cepas LAB e diminuição do pH, provavelmente devido à produção de ácido láctico. Os açúcares apresentaram um padrão de redução após 24 horas de fermentação, exceto a maltose, produzida a partir da degradação do amido. As amostras fermentadas apresentaram ainda propriedades antioxidantes, anti-escurecimento, antibacterianas e prebióticas, com aumento no teor de fenólicos totais.

Por fim, mais estudos devem continuar sendo realizados, a fim de ampliar o conhecimento a respeito das cepas mais promissoras com relação às características bioativas da farinheta de trigo, em especial, o isolado *B. subtilis* FTC01, que apresentou os melhores resultados. Apesar dessa espécie possuir várias linhagens consideradas GRAS e de tal cepa ser caracterizada como probiótica, é necessário comprovar sua segurança para o consumo humano, antes de sua utilização em produtos alimentícios. Além disso, deve-se otimizar seu cultivo nesse tipo de substrato, levando em consideração o composto ou propriedade que se deseja obter.

6. CONCLUSÃO

A utilização de técnicas de bioprocessamento tem demonstrado ser uma interessante abordagem por tratar-se de uma ferramenta eficiente e ecologicamente sustentável para obtenção de modificações positivas na matriz de alimentos, aumentando, assim, a bioacessibilidade e a biodisponibilidade de

substâncias associadas à geração de efeitos benéficos à saúde. Além de agregar valor e minimizar o impacto ambiental causado pelo acúmulo de resíduos agroindustriais, os subprodutos de cereais podem ser utilizados no desenvolvimento de novos alimentos com propriedades funcionais, representando um recurso potencialmente útil a ser explorado.

A aplicação de cepas produtoras de bacteriocinas em substratos alimentícios também oferece novas oportunidades na biopreservação de alimentos. No entanto, deve-se ressaltar que existem ainda poucos estudos considerando os potenciais problemas de segurança dos produtos fermentados. Faz-se necessário atentar ao fato de que estes podem conter bactérias patogênicas e aumentar o risco de intoxicação alimentar, bem como pode ocorrer a liberação de substâncias tóxicas ou nocivas durante a fermentação, sendo prejudiciais à saúde. Assim, o uso de cepas reconhecidamente seguras, com ausência de características de virulência, é essencial para seu uso em produtos alimentícios.

Por último, a obtenção de compostos com potencial prebiótico mostrou-se bastante promissora. Mais pesquisas são necessárias para estabelecer a funcionalidade prebiótica da farinheta de trigo bioprocessada, e avaliar seu uso prático como ingrediente na alimentação animal e humana. Portanto, o aproveitamento de resíduos lignocelulósicos de origem agroindustrial, através do seu bioprocessamento com microrganismos, para obtenção de substâncias bioativas com apelo comercial e características funcionais, apresenta-se como uma alternativa viável e interessante ao uso de enzimas.

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