ANDRESSA MARIA SUZIN

EFEITO DA GERMINAÇÃO DE FEIJÃO COMUM (*Phaseolus vulgaris* L.) NA GERAÇÃO DE PEPTÍDEOS BIOATIVOS COM CAPACIDADE ANTIOXIDANTE E ANTI-HIPERTENSIVA

Londrina
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Centro de Ciências Agrárias
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Programa de Pós-Graduação em Ciência de Alimentos

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Dissertação apresentada ao Programa de Pós-Graduação em Ciência de Alimentos, nível Mestrado, da Universidade Estadual de Londrina, como requisito parcial à obtenção do Título de Mestre em Ciência de Alimentos

Orientador: Profa. Dra. Thaís de Souza Rocha
Coorientador: Dr. Pedro Henrique Freitas Cardines

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RESUMO

O feijão é um grão consumido mundialmente, sendo de grande importância, especialmente para países em desenvolvimento, pois representa uma fonte de proteína barata quando comparada a fontes de proteína animal. A capacidade de obtenção de peptídeos bioativos a partir da hidrólise ou germinação do feijão, faz com que este grão seja amplamente estudado. Com isso, há um crescente interesse no desenvolvimento de alimentos funcionais elaborados a partir do feijão. O presente estudo teve o objetivo de avaliar o efeito da germinação no perfil proteico da farinha de feijão e o potencial de formação de peptídeos bioativos com capacidades antioxidantes e anti-hipertensivas. Feijões comuns (*Phaseolus vulgaris* L.), foram submetidos a germinação por 48 h a 25 ºC, foram secos, moídos e padronizados quanto ao tamanho de partícula (0,355 mm). As farinhas de feijão foram submetidas à digestão gastrointestinal in vitro e as amostras digeridas e não digeridas foram avaliadas quanto a solubilidade das proteínas e digestibilidade, mudanças nas massas moleculares das proteínas foram avaliadas por eletroforese (SDS-PAGE) e cromatografia líquida por exclusão de tamanho (SE-HPLC). Ainda foram avaliados o poder antioxidante de redução do ferro (FRAP), a capacidade de absorção do radical de oxigênio (ORAC) e a atividade anti-hipertensiva através capacidade de inibição da enzima conversora de angiotensina (ECA). A germinação provocou aumento da solubilidade das proteínas de 86,26 para 92,67% e a sua digestibilidade aumentou de 68,37 para 71,42%. Por SDS-PAGE não houve alterações na intensidade das bandas de faseolina e legumina após a germinação, porém, foi observado diminuição dessas bandas após a digestão. A abundância relativa de proteínas avaliada por SE-HPLC mostrou que a fração com massa molecular de 450-100 kDa diminuiu após a digestão, gerando aumento nas frações < 10 kDa, que correspondem a peptídeos e aminoácidos com potencial bioativo. Foi observada uma nova fração proteica de massa molecular variando de 230 kDa à 270 kDa. Essa nova fração gerada foi atribuída à hidrólise de agregados solúveis e frações de leguminas. A atividade

inibitória da ECA foi melhorada pela germinação, e após a digestão, a atividade inibitória da ECA aumentou, mas sem diferença entre amostras germinadas e não germinadas. Não foi observada diferenças no poder de redução do ferro por FRAP. A atividade antioxidante avaliada por ORAC aumentou após a digestão gastrointestinal in vitro, sugerindo que peptídeos antioxidantes poderiam ter sido formados pela ação de enzimas proteolíticas no trato gastrointestinal humano. A germinação provocou alterações nas estruturas químicas das proteínas do feijão comum, diminuindo sua a massa molecular e aumentando sua solubilidade e digestibilidade. Apesar da germinação de não ter propiciado aumento nas atividades bioativas após a digestão, pode-se inferir que a germinação é capaz de melhorar a bioacessividade dos peptídeos bioativos gerados, uma vez que estarão mais solúveis e as chances de serem absorvidos pelo trato gastrointestinal é maior.

Palavras-chave: Leguminosas, Digestibilidade, Hipertensão, Estresse Oxidativo.

SUZIN, Andressa Maria. **Effects of Germination of Common Beans (*Phaseolus vulgaris* L.) on the Generation of Bioactive Peptides with Antioxidant and Antihypertensive capacities.** 2024. 65p. Dissertação (Mestrado em Ciência de Alimentos) – Universidade Estadual de Londrina, Londrina, 2024.

ABSTRACT

Beans are consumed worldwide, being of great importance, especially for developing countries, as they represent a cheap source of protein when compared to animal protein sources. The ability to obtain bioactive peptides from hydrolysis or germination is the reason why beans are widely studied. As a result, there is a growing interest in the development of functional foods made from beans. The present study aimed to evaluate the effect of germination on the protein profile of bean flour and the formation of bioactive peptides with antioxidant and antihypertensive capabilities. Common beans (*Phaseolus vulgaris L.*) were subjected to germination for 48 h at 25 ºC, dried, ground and standardized for particle size (0.355 mm). Bean flours were subjected to in vitro gastrointestinal digestion and samples were evaluated for protein solubility, digestibility, changes in protein molecular masses by electrophoresis (SDS-PAGE) and size exclusion liquid chromatography (SE-HPLC). The antioxidant activities by FRAP and ORAC and the ability to inhibit ACE were also evaluated. Germination decreased the crude protein content, but the protein solubility increased from 86.26 to 92.67% and the protein digestibility increased from 68.37 to 71.42%. No changes in the intensity of phaseolin and legumin bands after germination were observed by SDS-PAGE, however, a considerable decrease in these bands was observed after digestion. SE-HPLC showed that the relative abundance of proteins with a molecular mass of 450-100 kDa decreased after digestion, generating an increase in the < 10 kDa fractions, which correspond to peptides and amino acids with bioactive potential. A new protein fraction with molecular mass ranging from 230 kDa to 270 kDa was observed. This new fraction was attributed to the hydrolysis of soluble aggregates and legumin fractions. The ACE inhibitory activity was improved by germination, and after digestion, the ACE inhibitory activity increased but there was no difference between germinated and non-germinated samples. No differences were observed in the ferric reducing antioxidant power (FRAP). The antioxidant activity assessed by ORAC increased after in vitro gastrointestinal digestion, suggesting that antioxidant peptides

could have been formed by the action of proteolytic enzymes present in the human gastrointestinal tract. Germination caused changes in the chemical structures of common bean proteins, reducing their molecular mass and increasing their solubility and digestibility. Although no increase in bioactive activities was observed after digestion, caused by germination, it can be inferred that germination is capable of improving the bioaccessibility of peptides with bioactive potential since they will be more soluble and the chances of being absorbed by the digestive tract gastrointestinal is larger.

Keywords: Pulses, Digestibility, Hypertension, Oxidative stress.

LISTA DE ABREVIATURAS E SIGLAS

CONAB	Companhia Nacional de Abastecimento
DDW	*Deionized distilled Water* – Água deionizada e destilada
ACE/ECA	*Angiotensin converting enzyme* – Enzima conversora de angiotensina
IC_{50}	Concentração necessária para inibir 50% de atividade enzimática
SSF	*Simulated Salivary Fluid* – Simulação de Fluido Salivar
SGF	*Simulated Gastric Fluid* – Simulação de Fluido Gástrico
SIF	*Simulated Intestinal Fluid* – Simulação de Fluido Intestinal

SUMÁRIO

1 INTRODUÇÃO

O feijão comum (*Phaseolus vulgaris* L.) é considerado como pulses e constituído por sementes secas comestíveis que pertencem à família Leguminosae (Rawal; Navarro, 2019). O feijão comum tem origem na América Central e do Sul (Siddiq; Uebersax, 2013) e é amplamente consumido em todo o mundo com uma produção total de 28,3 milhões de toneladas, cultivadas em 36,8 milhões de hectares em 2022 (FAO, 2024). Alimentações à base de plantas e promotoras da saúde e bem-estar das pessoas, é uma macrotendência duradoura que continuará a impulsionar o mercado de alimentos (Sloan, 2021). Nesse contexto, vários estudos estão sendo desenvolvidos de forma a buscar novos compostos benéficos à saúde. Vários estudos têm demostrado que peptídeos obtidos a partir de diversas fontes de feijão apresentam potencial poder de diminuição do risco de desenvolvimento de doenças crônicas não transmissíveis (DCNT), como a diabetes e a hipertensão (Garcia; de Barros; Rocha, 2021). Processos como germinação e hidrólise podem gerar fragmentos peptídicos com atividade biológica, ou também chamados, peptídeos bioativos, que consistem em sequências de 2 a 20 aminoácidos e podem ser obtidos de origem animal ou vegetal. Durante a germinação do feijão, proteases são ativadas para quebrar grandes proteínas de reserva formando peptídeos menores e mais solúveis. Além disso, pode ajudar a reduzir os níveis de inibidores de protease (frequentemente chamados de antinutrientes). Esses peptídeos com atividade biologica podem ainda ser formados durante a digestão gastrointestinal ou por hidrólise enzimática in vitro (Phelan e Kerins, 2011). Métodos tradicionais de preparação do feijão em países africanos e asiáticos, como a germinação e fermentação, encontram grande aceitação, e são reconhecidos por melhorar a digestibilidade do feijão e reduzir os fatores antinutricionais (Uebersax, 2006).

As farinhas de feijão podem ser utilizadas como ingrediente no desenvolvimento de alimentos benéficos à saúde. Podem ser utilizadas no preparo de biscoitos mais saudáveis, por exemplo, com maior teor de proteínas, menores teores de amido rapidamente digerível e maiores teores de amido resistente (Cappa; Kelly; Ng, 2020), com efeitos antidiabéticos (Pérez-Ramírez et al., 2018) e anti-hipertensivos (Sarmadi e Ismail, 2010).

Os objetivos deste estudo foram 1) avaliar as alterações no perfil de massa molecular da proteína durante a germinação e digestão gastrointestinal in vitro;

e 2) determinar se esses tratamentos são capazes de gerar peptídeos bioativos com propriedades antioxidantes e anti-hipertensivas.

2 OBJETIVO

2.1 Objetivo Geral

Avaliar as alterações no perfil de massa molecular da proteína durante a germinação e digestão gastrointestinal in vitro, e determinar se esse tratamento é capaz de gerar peptídeos bioativos com propriedades antioxidantes e anti-hipertensivas.

2.2 Objetivos Específicos

- Produzir farinhas de feijão germinado com potencial bioatividade anti-hipertensiva e antioxidante;

- Realizar a simulação da digestão gastrointestinal in vitro das farinhas de feijão germinado e não germinado;

- Verificar se as atividades biológicas e propriedades estruturais das farinhas foram alteradas após a germinação e simulação da digestão gastrointestinal in vitro.

3 REVISÃO BIBLIOGÁFICA

3.1 Feijão

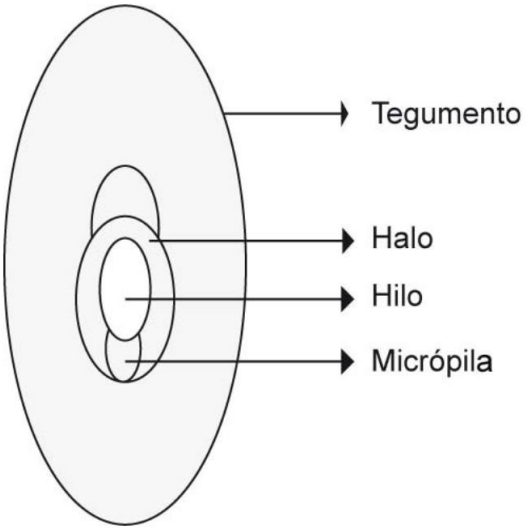
De acordo com a CONAB (2023), o Brasil teve uma produção de 3,04 milhões de toneladas de feijão durante o período da safra 2022/23 que representa 1,7% de aumento em relação à safra passada. O feijão comum (*Phaseolus vulgaris* (L.)) é considerado a variedade mais amplamente cultivada, estando a frente de mais de 30 outras espécies de Phaseolus descritas na literatura (Siddiq e Uebersax, 2013). O feijão comum é cultivado em clima temperado, assim como em regiões temperadas subtropicais. É originário da América Central e do Sul (Siddiq et al. 2013) e é amplamente consumido em todo o mundo com uma produção total de 28,3 milhões de toneladas, cultivadas em 36,8 milhões de hectares em 2022 (FAO, 2024).

O feijão é considerado como pulses, que são uma das culturas alimentares mais importantes devido à sua acessibilidade, nutritividade e sustentabilidade nos sistemas agrícolas (CALLES et al., 2019). Muitos tipos diferentes de pulses são cultivados em todo o mundo para consumo humano e alimentação animal. Os pulses são um subgrupo de leguminosas, constituído por sementes secas comestíveis que pertencem à família Leguminosae (RAWAL; NAVARRO, 2019). Alguns exemplos de leguminosas comumente consumidas são feijão branco (*Phaseolus vulgaris* L.), feijão faba (*Vicia faba* L.), grão de bico (*Cicer arietinum* L.), ervilha seca ou partida (*Pisum sativum* L.), e diversas variedades de lentilhas (*Lens culinaris* Medik.). Segundo a Organização das Nações Unidas (FAO), a definição de pulses não inclui espécies de leguminosas utilizadas como vegetais, para extração de óleo e para fins de semeadura (FAO, 2021).

O feijão se destaca entre uma ampla e diversa classe de leguminosas. Botanicamente, as leguminosas são sementes dicotiledôneas (bivalvuladas) de plantas pertencentes à família *Leguminosae* (Uebersax, 2006). As sementes de leguminosas se diferem muito na aparência geral (cor, tamanho, forma). A estrutura da semente do feijão compreende um tegumento e um cotilédone embrionário. As características estruturais dos tecidos das sementes (tegumento, cotilédone, eixo embrionário) e os componentes celulares subcelulares (células de paliçada, ampulheta e parênquima, e paredes celulares constituintes da lamela média e outras

organelas), influenciam muito na hidratação, cozimento, e desempenho do processamento do grão. No geral, as sementes de leguminosas possuem uma estrutura de semente semelhante, compreendendo um tegumento (ou testa), cotilédones e partes embrionárias. De acordo com a Figura 1, o tegumento da semente compreende a parte mais externa da semente e serve como um protetor da estrutura embrionária. Os cotilédones consistem na maior área das sementes e servem como armazenamento de energia (amido e proteína). Já as estruturas embrionárias variam em tamanho e possuem alto teor lipídico e fornecem os locais de germinação para a semente. Na estrutura externa, podemos observar o hilo e a micrópila que desempenham um papel na absorção de água e troca gasosa da semente (Siddiq e Uebersax, 2013).

Figura 1 – Esquema de um grão e feijão



Fonte: (DA COSTA, 2023).

Os nutrientes e a biodisponibilidade dos mesmos no feijão são influenciados por fatores como tipo e cultivar, ambiente e sistemas de cultivo, condições de armazenamento, manuseio, processamento e preparação do produto final (Siddiq e Uebersax, 2013). Os hábitos de consumo do feijão variam significativamente entre regiões e culturas, e são determinados pelas interações sociais e tradições. Dessa forma, criam-se preferências por certas cores, tamanhos e formatos de feijão, assim como meios de preparo e uso; e estes padrões de uso geram impactos significativos na saúde pública (Uebersax, 2006). O feijão comum possui alto valor nutricional sendo fonte de energia, proteínas, fibras, vitaminas e minerais.

Os principais objetivos dos programas de melhoramento do feijão está

voltada a fatores como fixação biológica de nitrogênio, resistência às pragas e doenças, interação genótipo x ambiente e facilidade de cultivo incluindo viabilidade de mecanização. Programas de melhoramento no Brasil têm contribuído para o aumento da produtividade com melhores adaptações às condições climáticas do país. Um examplo disso é a cultivar BRS Ártico, que é uma cultivar de feijão branco, originária do cruzamento (G13922 x G13145) x {(G13922 x [G07930 x (A156 x A494)]}, realizado em 1985, no CIAT (Centro Internacional de Agricultura Tropical, Cali, Colômbia). Essa cultivar possui um bom potencial produtivo e grãos que atendem às exigências do mercado interno e externo. É apropriada para o cultivo na região central do Brasil e no estado do Paraná. Além disso, apresenta resistência moderada à ferrugem e à murcha de Curtobacterium (EMBRAPA, 2016).

3.2 Composição Centesimal e Aminoácidos do Feijão

As leguminosas incluindo o feijão comum, são componentes muito importantes na dieta em países em desenvolvimento como África, América Latina e Ásia e são fontes de proteínas complementares às dos cereais, raízes e tubérculos. O teor de proteína das leguminosas fica entre 20-30% e são geralmente ricas em lisina e restritas em aminoácidos sulfurados (metionina e cisteína). As leguminosas são ainda valiosas fontes de fibras, vitaminas, e minerais incluindo folato, tiamina e riboflavina (Phillips et al., 2003).

Mesquita et al. (2007) avaliaram a composição química de 21 linhagens de feijão comum fornecidas pelo Banco de Germoplasma do Setor de Genética e Melhoramento de Plantas do Departamento de Biologia da Universidade Federal de Lavras. Foram encontrados valores de proteína bruta variando de 22,34 a 36,28 g/100 g (matéria seca). A umidade variou entre 13,76 a 18,26 g/100 g. O extrato etéreo variou de 0,53 a 2,55 g/100 g e o teor de cinzas de 2,97 a 4,87 g/100 g. Outra variedade de feijão muito consumida no Brasil é o feijão-caupi (*Vigna unguiculata*). Medeiros et al. (2019) avaliaram a composição centesimal de 8 variedades de feijão-caupi *in natura* e encontrou valores de umidade entre 7,12 e 11,32%, para cinzas de 3,20 a 5,31%, para lipídeos de 0,40 a 1,09%, para proteínas de 20,66 a 26,06% e para carboidratos de 60,33 a 67,46%. Além disso, os valores energéticos das variedades do feijão-caupi variaram entre 340,28 e 357,56 Kcal/100 g. Contudo, os autores verificaram também que esses valores são afetados pelo processo de cozimento,

causando diminuição nos teores de proteínas, carboidratos e valor energético no grão cozido.

Globulinas e albuminas são as duas principais proteínas de armazenamento nas leguminosas. No feijão comum, as globulinas representam 54-79% da proteína total. A faseolina 7S é a principal fração de globulina com 40-50% de seu nitrogênio total, seguida pela legumina (11S) com 11-30%. (Montoya et al., 2010). O valor nutricional, ou a qualidade proteica dos alimentos, depende do seu teor de aminoácidos e da adequada utilização fisiológica de aminoácidos específicos após sua digestão e absorção (Elharadallou et al., 2015). A concentração de aminoácidos no feijão pode variar de acordo com fatores genéticos, ambientais, interações de genótipos com ambientes, manejo da cultura, origem do germoplasma e processamento pós-colheita (Flores-Sosa et al., 2020). De acordo com o estudo feito por Ribeiro et al. (2007), que avaliou a composição de aminoácidos de 19 cultivares de feijão cultivados em dois municípios do estado do Rio Grande do Sul, verificou-se que houve variações para os teores de aminoácidos essenciais e não essenciais de acordo com cada cultivar. De modo geral, o aminoácido essencial mais abundante nos grãos foi a leucina, seguido por lisina, fenilalanina, valina, isoleucina, treonina, histidina e metionina. Quanto aos aminoácidos não-essenciais, verificou-se que os grãos são constituídos na ordem decrescente de ácido glutâmico, ácido aspártico, arginina, serina, alanina, glicina, tirosina, prolina e cisteína. Sendo que, todas as cultivares apresentaram teores de aminoácidos superiores ao padrão apropriado para suprir as necessidades diárias de um indivíduo adulto segundo a Organização das Nações Unidas para Agricultura e Alimentação (FAO), o que indica alta qualidade da proteína do feijão.

Flores-Sosa et al., (2020) identificaram, por HPCL, 16 aminoácidos presentes em diferentes variedades nativas de feijão *Phaseolus vulgaris* (*L.*) e *Phaseolus coccineus (L)*) cultivadas por pequenos agricultores em dois municípios do estado de Oaxaca no México. Através da análise de variância, os autores verificaram diferenças significativas nos teores de proteínas e aminoácidos das variedades de diferentes municípios. As variedades do município de Santa Lucia Miahuatlan apresentaram maiores teores dos aminoácidos estudados (ácido aspártico, ácido glutâmico, serina, histidina, glicina, treonina, arginina, alanina, tirosina, cisteína, valina, metionina, isoleucina, fenilalanina, leucina e lisina), exceto de histidina, que foi maior nas variedades do município de Coatecas Altas. Ao fazer um comparativo com

as variedades padrão de feijão comercial, estas apresentaram a maior média de teor de proteína (20,8%), seguidas pelas variedades de Coatecas Altas (17,6%) e Santa Lucia Miahuatlan (16,4%). Os autores atribuíram as variações nos teores de proteínas e aminoácidos a fatores como características genéticas das variedades cultivadas, influências agroecológicas-ambientais e regime de cultivo.

3.3 Peptídeos Bioativos

Peptídeos bioativos são produtos da degradação de proteínas por proteases presentes do trato gastrointestinal que terão funções específicas somente após liberadas da matriz proteica original. Portanto, são definidos como fragmentos de proteínas específicos que causam impactos positivos no funcionamento do organismo e consequentemente na saúde humana (Tranter; Board, 1982 apud Kitts; Weiler, 2003). A atividade peptídica depende da composição e sequência dos aminoácidos, que podem variar entre 2 a 20 resíduos (Phelan; Kerins, 2011). Existem algumas exceções, como por exemplo o lunasin, que é composto por 43 resíduos de aminoácidos, e é conhecido por possuir propriedades anti-hipertensivas, antioxidantes, anti-inflamatórias, anti-obesidade, além de auxiliar na imunomodulação, prevenção do câncer, e possuir atividade hipocolesterolêmica (Liu et al., 2014). Os peptídeos bioativos podem ser derivados de várias fontes de proteína animal e vegetal, como por exemplo, do ovo, leite (caseína e proteína do soro do leite), carne, soja, aveia, leguminosas (feijão, grão de bico, ervilha e lentilha), canola, trigo, linhaça, sementes de cânhamo, além disso, podem também ser encontrados em proteínas de origem marinha, como peixe, lula, salmão, ouriço-do-mar, ostra, cavalo-marinho e caranguejo das neves (Chakrabarti; Guha; Majumder, 2018).

A formação de peptideos bioativos a partir da sua proteina original pode ocorrer por hidrólise in vitro, pela utilização de enzimas endógenas ou exógenas para simular o que ocorre in vivo durante a digestão gastrointestinal (DG) através da ação das enzimas salivares, estomacais, intestinais e pancreáticas. Peptideos bioativos podem também serem formados por fermentação microbiana (Samaranayaka; Li-chan, 2011; Toldrá et al., 2018). Além destas, a hidrólise química é outra forma que já foi muito utilizada no passado, onde ácidos ou bases são usados para clivar ligações peptídicas gerando peptídeos e aminoácidos livres. Porém, esta

tecnologia tem muitos fatores limitantes, como ser um processo de difícil controle, tendência a fornecer aminoácidos modificados, e gerar produtos com composições químicas e propriedades funcionais variáveis (Wang et al., 2017). A maioria dos estudos que envolvem caracterização de peptídeos bioativos são geralmente realizados in vitro, por hidrólise com enzimas comerciais. As enzimas selecionadas para liberação de peptídeos possuem diferentes especificidades, e as mais comuns são: pepsina, pancreatina, tripsina, alcalase e papaína (Lorenzo et al., 2018).

Vários novos métodos têm surgido como alternativa aos métodos convencionais para obtenção de peptídeos bioativos. Tratamento por alta pressão hidrostática tem sido empregado recentemente para facilitar a hidrólise de proteínas provenientes de várias fontes como feijão (*Phaseolus vulgaris L.*) (Al-ruwaih et al., 2019), soja (*Glycine max* L. Merril) (Guan et al., 2018; Kim et al., 2017), soro do leite (Piccolomini et al., 2012; Landim et al., 2021) e ervilha (Chao et al., 2013). Esse tipo de tratamento é capaz de modificar a estrutura e conformação das proteínas, causando seu desdobramento parcial, que facilita o acesso de enzimas nos locais de clivagem, aumentando a atividade enzimática e, consequentemente, a extensão de hidrólise (Bonomi et al., 2003). Além deste, outros tratamentos como campo elétrico pulsado (Lin et al., 2013), hidrólise por água subcrítica (Rivas-vela et al., 2021) e ultrassom Wu et al. (2018), também têm sido aplicados para melhorar a produção de potenciais peptídeos bioativos.

3.4 Germinação do Feijão

A germinação é um processo biológico que ocorre naturalmente nas plantas e faz com que as sementes saiam do estado de latência. Para que a germinação ocorra são necessárias condições ambientais favoráveis como umidade, temperatura e nutrientes. A germinação pode afetar a composição de nutrientes e antinutrientes presentes nas sementes do feijão (Siddiq; Uebersax, 2013). A presença de fatores antinutricionais (inibidores de proteases, lectinas e ácido fítico) é uma questão preocupante e exige condições de processamento adequadas para amenizar seus efeitos. A germinação e a fermentação são utilizadas como alternativas capazes de reduzir ou inativar fatores antinutricionais (Uebersax, 2006). Sangronis & Machado (2007) avaliaram os efeitos da germinação em alguns nutrientes e fatores antinutricionais do feijão. Os grãos higienizados foram deixados de molho em água

destilada por 5 horas e então submetidos a germinação. Os autores perceberam uma diminuição de 52,5, 26,6 e 41% de unidades inibidoras de tripsina/mg nos feijões germinados comum branco (*Phaseolus vulgaris* L.), comum preto (*Phaseolus vulgaris* L.) e guandu (*Cajanus cajan* L. Millsp.) respectivamente. Da mesma forma, perceberam uma diminuição no conteúdo de ácido fítico de 44,7, 52,9 e 40,7% e de taninos de 36,2, 19 e 14,3% para o feijão comum branco, feijão comum preto e feijão guandu, respectivamente. Além disso, no estudo in vitro da digestibilidade da proteína, percebeu-se um aumento de 2% para o feijão comum branco, 3% para o feijão comum preto e 4% para o feijão guandu. Esse aumento poderia ser explicado pela redução no conteúdo de ácido fítico e taninos, uma vez que estes compostos interagem com as moléculas de proteína, formando complexos e reduzindo a susceptibilidade das proteínas à ação enzimática.

As endopeptidases são responsáveis por degradar proteínas de armazenamento produzindo oligopeptídeos, que são posteriormente degradados por exopeptidases formando aminoácidos livres (Sangronis et al., 2006; Zakharov et al., 2004). O processo de germinação aumenta a biodisponibilidade de nutrientes pelo fato de que alguns componentes são hidrolisados a moléculas menores, que tornam a sua digestão e absorção pelo corpo humano mais fácil. Além disso, a germinação aumenta a concentração de alguns compostos desejáveis como os compostos fenólicos, minerais como o cálcio, magnésio, zinco e ferro (Atudorei; Atudorei; Codină, 2021).

3.5 Farinha de Feijão e sua Aplicação em Alimentos

O crescimento da população mundial traz a necessidade de utilização de fontes alternativas de proteína para atender a demanda de produção de alimentos. A preocupação com o bem-estar animal e meio ambiente, crescimento da população vegana, flexitariana e vegetariana, além da preocupação da população com a saúde faz com o que mercado de alimentos à base de plantas continue crescendo (Markets and Markets, 2023). As farinhas de feijão podem ser utilizadas para substituir parcialmente ou até totalmente a farinha de trigo na formulação de produtos de panificação como bolos, pães e biscoitos. Antes da incorporação de grãos de leguminosas germinadas devem ser levados em conta fatores como, preferências sensoriais dos consumidores, perfil nutricional dos grãos e seus impactos do ponto de

vista tecnológico. Por meio da germinação, a atividade enzimática dos grãos é aumentada, o que facilita a digestão de compostos como amido e proteínas, permitindo que as leguminosas germinadas sejam utilizadas com sucesso em alimentos onde a atividade enzimática é necessária (Atudorei; Atudorei; Codină, 2021).

Através de uma revisão sistemática, os autores Oliveira et al., (2021) concluíram que a farinha de feijão-caupi (*Vigna unguiculata* L.) possui alta versatilidade podendo ser aplicado em diversas formulações como pães (Cavalcante et al., 2016), cookies (Landim et al., 2019), hambúrguer (Lima et al., 2018) e biscoitos (SOUZA, 2018). Portanto, possui um alto potencial como matéria-prima com relativa significância e agregação de valor nutricional aos produtos.

Ukeyima; Dendegh e Isusu (2019) avaliaram as características de qualidade do pão produzido com farinha de trigo e de feijão-comum vermelho (*Phaseolus vulgaris* L.) e concluiram que as amostras compostas dessas farinhas apresentaram alto teor de cinzas, gordura, fibra e proteína quando comparado com o controle. Além disso, a capacidade de absorção de água, capacidade de absorção de óleo, densidade aparente e índice de intumescimento diminuem com o aumento da farinha de feijão. Por outro lado, a capacidade de formação de espuma aumenta com o aumento da adição de farinha de feijão. A análise sensorial mostrou que a amostra com 85% de farinha de trigo e 15% de farinha de feijão vermelho foi a preferida pelos provadores.

3.6 Propriedades Anti-hipertensivas

A hipertensão é um grande fator de risco que favorece o desenvolvimento de doenças cardiovasculares como doenças cardíacas e acidente vascular cerebral (Yamamoto, 2010). De acordo com a Organização Mundial da Saúde (WHO - World Health Organization, 2021) as doenças crônicas não transmissíveis (DCNT) representaram 73,6% de todas as mortes em todo o mundo em 2019 (dados prévios à pandemia de Covid-19). Essas mortes foram causadas pelas quatro principais DCNT: Doença cardiovascular (17,9 milhões), câncer (9,3 milhões), doença respiratória crônica (4,1 milhões) e diabetes (2,0 milhões). Para prevenir essas doenças, podem ser usadas substâncias farmacológicas que

diminuem a pressão artérial para uma faixa normal. Existem 5 categorias de produtos anti-hipertensivos, sendo eles: enzima conversora de angiotensina - I (inibidores de ECA), antagonistas dos canais de cálcio (ACC), bloqueadores beta e alfa, agentes diuréticos e antagonista dos receptores de endotelina (Yamamoto, 2010). Por mais que inibidores sintéticos de ECA sejam eficazes e amplamente utilizados para o controle da pressão arterial, eles podem causar efeitos colaterais como tosse, proteinúria, erupção cutânea e alteração no paladar, entre outras (Jackson et al., 1988,Harnedy; Fitzgerald, 2012). Portanto, a busca por medidas preventivas a hipertensão relacionada a dieta é de grande interesse, e os peptídeos bioativos inibidores de ECA aparecem como potenciais componentes em alimentos funcionais para este fim.

Peptídeos bioativos com propriedades anti-hipertensivas, conhecidos como inibidores da enzima conversora de angiotensina I (ECA) podem ser derivados de fontes de proteína de leite, milho e peixe (Kitts; Weiler, 2003). O feijão também é uma fonte de peptídeos bioativos dos quais podem ser encontrados peptídeos responsáveis pela atividade inibitória da ECA. No estudo feito por LI et al., (2006) foram identificados três tipos de peptídeos inibidores de ECA, obtidos da proteína isolada do feijão-mungo verde (*Phaseolus radiatus* L.), hidrolisada pela enzima alcalase. Ainda, segundo os autores, os isolados de proteína que não foram submetidos a hidrólise, não apresentaram atividade inibitória da ECA, e a maior porcentagem de inibição da ECA foi com o valor de IC_{50} sendo de 0,64 mg de proteína/ml, obtido por isolados da proteína hidrolisados por 2 h pela enzima alcalase.

Muitos estudos mostram a possibilidade de incorporação da farinha de feijão em alimentos com o objetivo de diminuir o risco de desenvolvimento de DCNT através da ação dos peptídeos bioativos. Estudos sobre as bioatividades dos peptídeos após digestão gastrointestinal são fundamentais para acessar a viabilidade do uso destas farinhas. Ainda são necessários estudos para verificar se o processamento dos alimentos iria influenciar as bioatividades detectadas.

3.7 Propriedades Antioxidantes

Radicais livres podem ser gerados através de reações normais do corpo humano durante a respiração (Sarmadi e Ismail, 2010). Os radicais livres podem

ser formado ainda por fontes exógenas e hábitos como exposição a processos radioativos, tabaco, poluentes atmosféricos, consumo de alimentos com alto teor de gordura, baixo consumo de alimentos naturais, e dependendo da velocidade na qual são formados, o sistema antioxidante endógeno do organismo pode não ser suficiente para neutralização de todos os radicais livres. Esses radicais livres podem reagir quimicamente com proteínas, lipídeos e DNA, causando várias modificações celulares. Esse processo é chamado de estresse oxidativo e é responsável pela degeneração celular. Os radicais livres, quando atacam o DNA, danificam os genes que codificam as proteínas, o que leva à falha de execução das funções celulares, mutações, rearranjos cromossômicos e a ativação ou inativação de genes, os quais afetam a biossíntese de cadeias de DNA. O stress oxidativo, que tem sido associado à obesidade, doenças crônicas degenerativas, doenças neurodegenerativas, doenças cardiovasculares, diabetes mellitus e cancro, bem como danos celulares graves (López-García et al., 2022).

Os antioxidantes exógenos, como os peptídeos bioativos, que podem ser obtidos através da dieta, para atuar na inibição da oxidação através da inativação de espécies reativas de oxigênio, eliminação de radicais livres, quelação de metais de transição pró-oxidativos, redução de hidroperóxidos e eliminação enzimática de oxidantes específicos (Elias; Kellerby; Decker, 2008). De acordo com Zhu et al. (2022), a avaliação química da capacidade antioxidante dos peptídeos é essencial para a compreensão dos seus mecanismos moleculares e ação biológica, porém, não existe uma ferramenta única de avaliação para descrever a capacidade antioxidante global dos peptídeos antioxidantes. Dessa forma, os métodos de avaliação química e mecanismos de atividade mais estudados são: inibição dos radicais livres através das reações de transferência de átomo de hidrogênio (HAT) e transferência de elétrons (SET), atividade quelante do íon de ferro e inibição da peroxidação lipídica.

3.8 Digestão in vitro

Métodos in vitro que simulam processos de digestão são amplamente utilizados para estudar o comportamento gastrointestinal de alimentos ou produtos farmacêuticos. A utilização de métodos in vitro permite um estudo mais rápido, menos caro e não possue restrições éticas, permitindo a análise de um número maior de

amostras em paralelo, além de boa reprodutibilidade e escolha de condições controladas (Jones; Caballero; Davidov-Pardo, 2019). O método descrito por Minekus et al. (2014) inclui a utilização de fluidos que simulam os fluidos digestivos da via oral, fases gástrica e intestinal. Este método simula as condições fisiológicas in vivo, tendo em conta a presença de enzimas digestivas e suas concentrações, pH, tempo de digestão, e concentrações de sal, entre outros fatores.

Peptídeos bioativos podem ser liberados das proteínas de reserva das plantas através de hidrólise in vitro usando enzimas endógenas ou exógenas, ou in vivo durante a digestão gastrointestinal (GI) devido à ação de enzimas salivares, estomacais, intestinais e pancreáticas, bem como fermentação microbiana (Samaranayaka; Li-chan, 2011; Toldrá et al., 2018) resultando em peptídeos com diferentes sequências e comprimentos. Porém, para exercerem sua atividade biológica, eles devem passar pela barreira intestinal e permanecerem intactos até chegarem à corrente sanguínea de forma ativa (Xu et al., 2019). No organismo humano, a digestão inicia-se na boca com a mastigação, onde se forma o bolo alimentar devido à degradação mecânica e enzimática. A digestão das proteínas inicia-se após o bolo alimentar chegar ao estômago devido à ação das pepsinas presentes no suco gástrico. Após a quebra das ligações peptídicas, são formadas partículas menores, como polipeptídeos, oligopeptídeos e aminoácidos. Essas partículas são bombeadas para o duodeno, onde são posteriormente hidrolisadas pelas enzimas pancreáticas (tripsina, quimotripsina, elastase e carboxipeptidases A e B) e intestinais (enteroquinase e erepsina). Uma vez digeridos, aminoácidos e pequenos peptídeos podem ser absorvidos pelas células do epitélio e entrar na corrente sanguínea (Amigo; Hernández-ledesma, 2020; Zhang; Zhang; Mcclements, 2017). Proteínas de diferentes fontes são hidrolisadas em diferentes locais, que podem ser no estômago, por enzimas gástricas, ou no duodeno, por enzimas pancreáticas. Como cada sítio contém diferentes grupos de enzimas com diferentes especificidades de clivagem, elas determinam o tipo e a composição dos peptídeos produzidos durante a hidrólise e, consequentemente, suas propriedades fisiológicas (Jahan-mihan et al., 2011). As propriedades estruturais dos peptídeos, como tamanho, composição de aminoácidos, sequência e carga, desempenham um papel importante em suas atividades biológicas (Akbarian et al., 2022). Assim, a caracterização estrutural dos peptídeos gerados após digestão gastrointestinal pode ajudar a elucidar informações a respeito da biodisponibilidade dos peptídeos bioativos gerados.

REFERÊNCIAS

AKBARIAN, M. et al. Bioactive Peptides: Synthesis, Sources, Applications, and Proposed Mechanisms of Action. **International Journal of Molecular Sciences**, v. 23, n. 3, p. 1445, 27 jan. 2022.

AMIGO, L.; HERNÁNDEZ-LEDESMA, B. Current Evidence on the Bioavailability of Food Bioactive Peptides. **Molecules**, v. 25, n. 19, p. 4479, 29 set. 2020.

AL-RUWAIH, N. et al. High-pressure assisted enzymatic proteolysis of kidney beans protein isolates and characterization of hydrolysates by functional, structural, rheological and antioxidant properties. **LWT – Food Science and Technology**, v. 100, p. 231–236, 1 fev. 2019.

ATUDOREI, D.; ATUDOREI, O.; CODINĂ, G. G. Dough rheological properties, microstructure and bread quality of wheat-germinated bean composite flour. **Foods**, v. 10, n. 7, 1 jul. 2021.

BONOMI, F. et al. Reduction of immunoreactivity of bovine β-lactoglobulin upon combined physical and proteolytic treatment. **Journal of Dairy Research**, v. 70, n. 1, p. 51–59, 17 fev. 2003.

CALLES, T.; XIPSITI, M.; DEL CASTELLO, R. Legacy of the International Year of Pulses. **Environmental Earth Sciences**, v. 78, n. 5, p. 1–8, 1 mar. 2019.

CAPPA, C.; KELLY, J. D.; NG, P. K. W. Baking performance of 25 edible dry bean powders: Correlation between cookie quality and rapid test indices. **Food Chemistry**, v. 302, 1 jan. 2020.

CAVALCANTE, R. B. M. et al. Cheese bread enriched with biofortified cowpea flour. **Ciência e Agrotecnologia**, v. 40, n. 1, p. 97–103, 2 jan. 2016.

CHAO, D. et al. Effect of pressure or temperature pretreatment of isolated pea protein on properties of the enzymatic hydrolysates. **Food Research International**, v. 54, n. 2, p. 1528–1534, 1 dez. 2013.

CHAKRABARTI, S.; GUHA, S.; MAJUMDER, K. Food-Derived Bioactive Peptides in Human Health: Challenges and Opportunities. **Nutrients**, v. 10, n. 11, p. 1738, 12 nov. 2018.

CONAB. **Perspectivas para a Agropecuária** - Safra 2023/24. 19 set. 2023.

DA COSTA, J. G. C. **Morfologia.** Embrapa. Disponível em: <https://www.embrapa.br/agencia-de-informacao-tecnologica/cultivos/feijao/pre-producao/morfologia#:~:text=Tegumento%3A%20%C3%A9%20a%20capa%20externa,realiza%20a%20absor%C3%A7%C3%A3o%20da%20%C3%A1gua.>. Acesso em: 7 jan. 2024.

DE FÁTIMA GARCIA, B.; DE BARROS, M.; DE SOUZA ROCHA, T. Bioactive peptides

from beans with the potential to decrease the risk of developing noncommunicable chronic diseases. **Critical Reviews in Food Science and Nutrition.** Bellwether Publishing, Ltd., 2021.

ELHARADALLOU, S. B. et al. Amino Acid Composition of Cowpea (Vigna ungiculata L. Walp) Flour and Its Protein Isolates. **Food and Nutrition Sciences**, v. 06, n. 09, p. 790–797, 2015.

ELIAS, R. J.; KELLERBY, S. S.; DECKER, E. A. Antioxidant Activity of Proteins and Peptides. **Critical Reviews in Food Science and Nutrition**, v. 48, n. 5, p. 430–441, 8 maio 2008.

ELIZABETH SLOAN. Top 10 Food Trends of 2021. **Food Technology Magazine**, 2021.

EMBRAPA. **BRS Ártico: Cultivar de Feijão comum de Grãos Brancos com Padrão para Exportação**. Comunicado Técnico 234, p. 1–4, jun. 2016.

FAO, 2024. **Crop Production and Trade Data**. Disponível em: http://www.fao.org/fao stat/en/#data. Acesso em 15, Jan, 2024.

FAO, 2021. **Nutritional benefits of pulses**. Rome. Disponível em: <https://www.fao.org/documents/card/en/c/7737e426-5e65-4883-bd7f 8bd57b3fb87f/>. Acesso em: 5 abr. 2022.

FLORES-SOSA, Á. R. et al. Variation in protein and amino acids content among landraces of common bean (Phaseolus vulgaris L.). **Emirates Journal of Food and Agriculture**, v. 32, n. 10, p. 750–760, 8 nov. 2020.

GUAN, H. et al. The enzymatic hydrolysis of soy protein isolate by Corolase PP under high hydrostatic pressure and its effect on bioactivity and characteristics of hydrolysates. **Food Chemistry**, v. 245, p. 89–96, 15 abr. 2018.

HARNEDY, P. A.; FITZGERALD, R. J. Bioactive peptides from marine processing waste and shellfish: A review. **Journal of Functional Foods**, v. 4, n. 1, p. 6–24, 1 jan. 2012.

JACKSON, B. et al. Lack of Cross Sensitivity Between Captopril and Enalapril. **Australian and New Zealand Journal of Medicine**, v. 18, n. 1, p. 21–27, fev. 1988.

JAHAN-MIHAN, A. et al. Dietary Proteins as Determinants of Metabolic and Physiologic Functions of the Gastrointestinal Tract. **Nutrients**, v. 3, n. 5, p. 574–603, 11 maio 2011.

JONES, D.; CABALLERO, S.; DAVIDOV-PARDO, G. Bioavailability of nanotechnology-based bioactives and nutraceuticals. *In* LOONG-TAK L.; ROGERS, M. **Food Applications in Nanotechnology**. v. 88, 2019. p. 235–273.

KITTS, D. D.; WEILER, K. Bioactive Proteins and Peptides from Food Sources. Applications of Bioprocesses used in Isolation and Recovery. **Current**

Pharmaceutical Design, v. 9, p. 1309–1323, 2003.

KIM, M. Y. et al. Characteristics and in vitro anti-inflammatory activities of protein extracts from pre-germinated black soybean [Glycine max (L.)] treated with high hydrostatic pressure. **Innovative Food Science & Emerging Technologies**, v. 43, p. 84–91, 1 out. 2017.

LANDIM, A. P. M. et al. Effect of high hydrostatic pressure on the antioxidant capacity and peptic hydrolysis of whey proteins. **Ciência Rural**, v. 51, n. 4, p. 1–12, 2021.

LANDIM, L. A. DOS S. R. et al. **Composição centesimal de cookies a base de farinhas variadas. 2019.** Disponível em: <. https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1117885/composicao-centesimal-de-cookies-a-base-de-farinhasvariadas>. Acesso em: 6 fev. 2022.

LIMA, J. R. et al. Vegetal burgers of cashew fiber and cowpea: formulation, characterization and stability during frozen storage. **Revista Ciência Agronômica**, v. 49, n. 4, p. 708–714, 2018.

LI, G.-H. et al. Novel angiotensin I-converting enzyme inhibitory peptides isolated from Alcalase hydrolysate of mung bean protein. **Journal of Peptide Science**, v. 12, n. 8, p. 509–514, ago. 2006.

LIN, S. et al. Research on the preparation of antioxidant peptides derived from egg white with assisting of high-intensity pulsed electric field. **Food Chemistry**, v. 139, n. 1–4, p. 300–306, 15 ago. 2013.

LIU, J. et al. Lunasin as a promising health-beneficial peptide. **European review for medical and pharmacological sciences**, v. 18, n. 14, p. 2070–2075, 2014.

LORENZO, J. M. et al. Bioactive peptides as natural antioxidants in food products – A review. **Trends in Food Science & Technology**, v. 79, p. 136–147, 1 set. 2018.

LÓPEZ-GARCÍA, G. et al. Antioxidant and Antimicrobial Peptides Derived from Food Proteins. **Molecules**, v. 27, n. 4, p. 1343, 16 fev. 2022.

MARKETSANDMARKETS. **Protein Ingredients Market - Global Forecast to 2028**. 2023, p.1-251.

MESQUITA RIVELLI, F. et al. Linhagens de Feijão (Phaseolus vulgaris L.): Composição Química e Digestibilidade Proteica. **Ciência e Agrotecnologia**, v. 31, n. 4, p. 1114–1121, 2007.

MINEKUS, M. et al. A standardised static in vitro digestion method suitable for food – an international consensus. **Food & Function.**, v. 5, n. 6, p. 1113–1124, 2014.

NAOYUKI YAMAMOTO. Functional Food Products with Antihypertensive Effects. In: YOSHINORI MINE; EUNICE LI-CHAN; BO JIANG (Eds.). **Bioactive proteins and peptides as functional foods and nutraceuticals**. 1. ed. [s.l.] Blackwell Publishing

Ltd. and Institute of Food Technologists, 2010. p. 169–177.

PHELAN, M.; KERINS, D. The potential role of milk-derived peptides in cardiovascular disease. **Food & Function**, v. 2, n. 3–4, p. 153–167, 2011.

PHILLIPS, R. D. et al. Utilization of Cowpeas for Human Food. **Field Crops Research**, v. 82, n. 2–3, p. 193–213, 2003.

PÉREZ-RAMÍREZ, I. F. et al. Cookies elaborated with oat and common bean flours improved serum markers in diabetic rats. **Journal of the Science of Food and Agriculture**, v. 98, n. 3, p. 998–1007, 1 fev. 2018.

PICCOLOMINI, A. et al. High hydrostatic pressure pre-treatment of whey proteins enhances whey protein hydrolysate inhibition of oxidative stress and IL-8 secretion in intestinal epithelial cells. **SNF Swedish Nutrition Foundation**, v. 56, 2012.

POU, K. R. J. Applications of high pressure technology in food processing. **International Journal of Food Studies**, v. 10, n. 1, p. 248–281, 16 abr. 2021.

RAWAL, V.; NAVARRO, D. K. **The Global Economy of Pulses**. Rome: FAO, 2019.
REDDY, N. R. et al. Dry bean tannins: A review of nutritional implications. **Journal of the American Oil Chemists Society**, v. 62, n. 3, p. 541–549, mar. 1985.

RIBEIRO, N. D. et al. Composição de aminoácidos de cultivares de feijão e aplicações para o melhoramento genético. **Pesquisa Agropecuária Brasileira**, v. 42, n. 10, out. 2007.

RIVAS-VELA, C. I. et al. Protein Hydrolysis by Subcritical Water: A New Perspective on Obtaining Bioactive Peptides. **Molecules 2021, Vol. 26, Page 6655**, v. 26, n. 21, p. 6655, 3 nov. 2021.

SANGRONIS, E.; MACHADO, C. J. Influence of germination on the nutritional quality of Phaseolus vulgaris and Cajanus cajan. **LWT - Food Science and Technology**, v. 40, n. 1, p. 116–120, jan. 2007.

SARMADI, B. H.; ISMAIL, A. Antioxidative peptides from food proteins: A review. **Peptides**, v. 31, n. 10, p. 1949–1956, 1 out. 2010.

SAMARANAYAKA, A. G. P.; LI-CHAN, E. C. Y. Food-derived peptidic antioxidants: A review of their production, assessment, and potential applications. **Journal of Functional Foods**, v. 3, n. 4, p. 229–254, 1 out. 2011.

SIDDIQ, M.; UEBERSAX, A. M. Dry Beans and Pulses Production and Consumption—An Overview. Em: **Dry Beans and Pulses Production, Processing and Nutrition**. [s.l.] John Wiley & Sons, Inc, 2013. p. 3–22.

SOUZA, E. J. D. **Propriedades tecnológicas, nutricionais e sensoriais de biscoito tipo cookie sem glúten, desenvolvido com arroz de diferentes teores de amilose e feijão-caupi**. Pelotas: Universidade Federal de Pelotas, 14 jun. 2018.

TOLDRÁ, F. et al. Generation of bioactive peptides during food processing. **Food Chemistry**, v. 267, p. 395–404, 30 nov. 2018.

UEBERSAX, M. Dry Edible Beans: Indigenous Staple and Healthy Cuisine. **Forum on Public Policy: A Journal of the Oxford Round Table**, out. 2006.

ULUG, S. K.; JAHANDIDEH, F.; WU, J. Novel technologies for the production of bioactive peptides. **Trends in Food Science & Technology**, v. 108, p. 27–39, fev. 2021.

UKEYIMA, M. T.; DENDEGH, T. A.; ISUSU, S. E. Quality Characteristics of Bread Produced from Wheat and White Kidney Bean Composite Flour. **European Journal of Nutrition & Food Safety**, p. 263–272, 31 out. 2019.

WANG, X. et al. Characterization, Preparation, and Purification of Marine Bioactive Peptides. **BioMed Research International**, v. 2017, p. 1–26, 2017.

WU, Q. et al. Effect of ultrasonic pretreatment on whey protein hydrolysis by alcalase: Thermodynamic parameters, physicochemical properties and bioactivities. **Process Biochemistry**, v. 67, p. 46–54, 1 abr. 2018.

WHO - WORLD HEALTH ORGANIZATION. **World health statistics 2021: monitoring health for the SDGs, sustainable development goals**. p. 1–119, 2021.

ZAKHAROV, A. et al. A comparative study of the role of the major proteinases of germinated common bean (Phaseolus vulgaris L.) and soybean (Glycine max (L.) Merrill) seeds in the degradation of their storage proteins. **Journal of Experimental Botany**, v. 55, n. 406, p. 2241–2249, 10 set. 2004.

ZHANG, Z.; ZHANG, R.; MCCLEMENTS, D. J. Control of protein digestion under simulated gastrointestinal conditions using biopolymer microgels. **Food Research International**, v. 100, p. 86–94, out. 2017.

ZHU, Y. et al. Food Protein-Derived Antioxidant Peptides: Molecular Mechanism, Stability and Bioavailability. **Biomolecules**, v. 12, n. 11, p. 1622, 3 nov. 2022.

XU, Q. et al. Bioavailability of bioactive peptides derived from food proteins across the intestinal epithelial membrane: A review. **Trends in Food Science & Technology**, v. 86, p. 399–411, abr. 2019.

4 MATERIAL E MÉTODOS

O item 4 foi contemplado com o desenvolvimento de um artigo científico que será apresentado no item 5 RESULTADOS E DISCUSSÃO.

Artigo Científico: *Effects of Germination of Common Beans (Phaseolus vulgaris L.) on the Generation of Bioactive Peptides with Antioxidant and Antihypertensive Activities.*

5 RESULTADOS E DISCUSSÃO

5.1 Artigo Científico

Effects of Germination of Common Beans (Phaseolus vulgaris L.) on the Generation of Bioactive Peptides with Antioxidant and Antihypertensive Activities.

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Abstract:

This study evaluated the effect of germination and the in vitro simulated gastrointestinal digestion in the protein molecular mass profile of bean flour (Phaseolus vulgaris L.) and the generation of bioactive peptides. Protein solubility and digestibility increased from 86.3 to 92.7%, and from 68.4 to 71.4%, respectively, after 48 h germination. SDS-PAGE indicated that the germination changed the molecular conformation, and consequently, the accessibility of proteolytic enzymes to the storage proteins. SE-HPLC showed that the relative abundance of proteins with 450-100 kDa decreased after digestion, increasing the fractions < 10 kDa, corresponding to peptides and amino acids with potential bioactive properties. ACE inhibitory activity was

improved by germination, and the antioxidant capacity by ORAC increased after digestion, suggesting that antioxidant peptides were formed by the action of proteolytic enzymes. The increased digestibility and solubility of proteins due to germination may improve the bioaccessibility of the bioactive peptides generated after digestion.

Keywords: Pulses, Digestibility**,** Hypertension, Oxidative stress.

1. Introduction

Common beans (*Phaseolus vulgaris* L.) are considered pulses, a subgroup of legumes, consisting of edible dried seeds that belong to the Leguminosae family (RAWAL et al. 2019). These beans represent one of the most important food crops due to their accessibility, nutritional quality, and sustainability in agricultural systems (Calles et al. 2019). Common beans originated from Central and South America (Siddiq et al. 2013) and are now widely consumed worldwide with a total production of 28.3 million tons, cultivated on 36.8 million ha in 2022 (FAO, 2024).

Common beans are a good source of proteins, dietary fiber, phenolic compounds, and slowly digestible starch. Specifically, the protein component constitutes mainly globulin proteins. Globulins are the main storage proteins in common beans, representing 54-79% of the total proteins (Montoya et al. 2010; Osborne, 1912). Phaseolin, which belongs to the 7S vicilin group, is the major subunit of globulins in beans (40-50%), while legumins (11S) are present in lesser amounts (11-30%) (García-Cordero et al., 2021; Mariniello et al. 2007; Mühling et al., 1997). Proteins in common beans can release biologically active peptides that have a significant impact on human health (Ovando-Martínez et al. 2011; de Fátima Garcia et al., 2021). Studies have reported that bioactive peptides from beans can exert antioxidant (Matemu et al. 2021), antihypertensive (Sonklin et al. 2020), antidiabetic (Rahmi & Arcot, 2023), anticancer (Rao et al. 2018), antimicrobial (Osman et al. 2021), antiobesity (Wang et al. 2020), and anti-inflammatory (Xue et al. 2018) activities.

Germination of pulses is being explored, since it has the potential to generate a new functional food with physiological benefits against health issues, mainly non-communicable chronic diseases. According to Bewley et al. (2013), the germination process can be divided into phase I, which refers to the initial absorption

of water and the initiation of physiological activities within minutes of a cell becoming hydrated; phase II is where the major metabolic reactions occur such as hydrolysis and transcription of new genes; at this point phase III starts and the embryo growth is initiated, the young seedling are established, and the major nutrient reservoir is utilized. The duration of these phases is dependent on the temperature and source of water.

The germination process can help reduce the levels of protease inhibitors (considered as anti-nutrients), enabling the activation of proteases to break down large storage proteins into smaller and more soluble peptides. The bioactivity of these peptides is based on their inherent amino acid composition and the length of their sequence.

The digestion of proteins in the gastrointestinal tract can also generate bioactive peptides that are readily absorbed (Nong & Hsu, 2022). Protein digestion starts in the stomach where the acidic environment causes denaturation and activates pepsin, breaking down proteins into smaller peptides. Then the gastric chyme, containing a mix of peptides, reaches the duodenum where the intestinal enzymes (trypsin/chymotrypsin) continue to break down the peptides. Brush border enzymes hydrolyze peptides into di or tri peptides and amino acids ready to be absorbed. However, before entering the bloodstream they can still be further degraded by intracellular proteases (Tapal et al. 2018).

Therefore, the objectives of this study were to 1) evaluate the changes in protein molecular mass profile during germination and in vitro gastrointestinal digestion; and 2) determine if these treatments are able to generate bioactive peptides with antioxidant and antihypertensive properties.

2. Material and Methods

2.1 Material

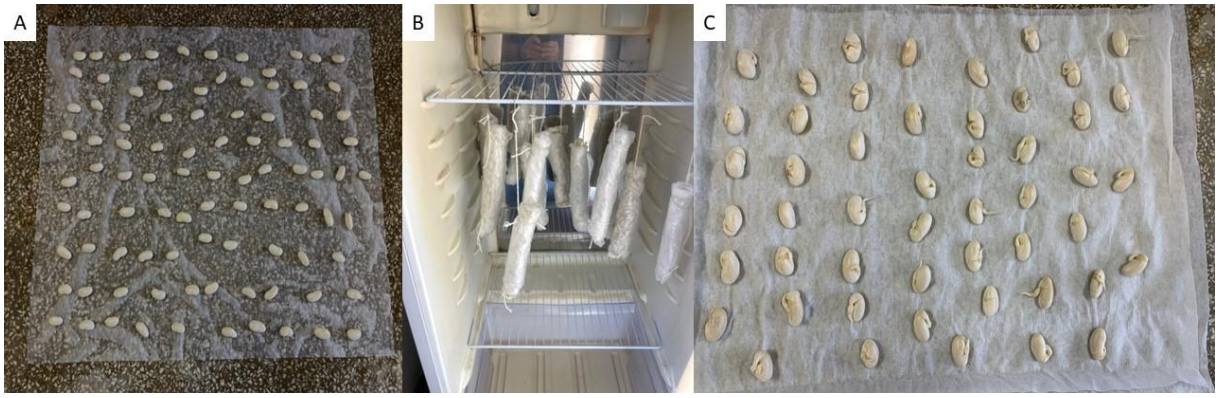
Common bean (*Phaseolus vulgaris* L., cultivar BRS Ártico) of white tegument, cultivated in the winter of 2019 (Apr-Jun) at the research center of EMBRAPA (Brazilian Agricultural Research Corporation) Rice and Beans - Santo Antônio de Goiás, GO, Brazil, was donated by EMBRAPA. Germination paper Germitest (28 x 38 cm) was purchased from Germilab. Thermostable α-amylase (3,000 units/mL) was purchased from Megazyme Dietary Fibre Kit (200A). Porcine pepsin (EC

3.4.23.1), pancreatin (EC 232-468-9), angiotensin I-converting enzyme from rabbit lung (EC 3.4.15.1, 0.25 U, Sigma A-6778) and N-Hippuryl-His-Leu hydrate (HHL) were purchased from Sigma-Aldrich (St. Louis, MO, EUA). Imperial™ Protein Stain, was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Criterion™ TGX™ 4-20% precast gels, Laemmli sample buffer, 10X Tris/Glycine/sodium dodecyl sulfate running buffer, and Precision Plus Protein™ molecular mass marker were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). A Superdex™ 200 Increase 10/300 GL Prepacked Tricorn™ Column, gel filtration low molecular weight (LMW) calibration kit, and gel filtration high molecular weight (HMW) calibration kit for size-exclusion high performance liquid chromatography (SE-HPLC) were purchased from Cytiva (Marlborough, MA, USA). All other chemicals used were of analytical grade.

2.2 Preparation of common bean germinated flour

Common bean (1 kg) was sanitized for 10 min with sodium hypochlorite (0.1%), rinsed three times with distilled water and spread on a tray to dry at room temperature for 4 h. Preliminary trials determined the germination setup, and the condition that resulted in higher protein solubility was chosen for this study. Germination papers were sprayed with 2.5% weight with distilled water and one hundred bean seeds were distributed on the paper (Fig. 1A). Germination papers were rolled up, tied with rubber bands, and placed inside plastic bags (low density polyethylene), to prevent moisture loss (Fig. 1B). Rolls were placed in a germination chamber (TE-402, TECNAL) for 48 h at 25 °C in a light–darkness cycle (12/12 h) at a light intensity of 2077 ± 428 lumens. After germination (Fig. 1C), the beans were dried in an air-circulating oven (TE394/2, TECNAL) at 40 ºC until the moisture content fell below 12%. Both germinated and ungerminated sanitized common beans were ground using an analytical mill (IKA A11, Staufen, Germany) and their particle size was standardized to 0.355 mm. The bean flours were stored at -20 °C.

Fig. 1. Germination process of common bean seeds. A. Common beans were distributed on top of germination paper and rolled up. B. Rolls were placed into a germination chamber for 48 h at 25 °C in a light–darkness cycle (12/12 h). C. Germinated common beans after 48 h.



2.3 Compositional of common bean flours

Protein was determined by the Dumas method (AOAC 990.03) using LECO® FP828 nitrogen analyzer (LECO, St. Joseph, MI, USA), with a conversion factor of 6.25. Fat, moisture and ash contents were measured following the Mojonnier method of analysis (AOAC 992.06), the vacuum oven method (AOAC 926.08), and the dry ashing method (AOAC 942.05), respectively. Total carbohydrate content was determined by difference. Total dietary fiber was determined by Megazyme Kit (K-TDFR-200A).

2.4 Simulated gastrointestinal digestion

Simulated gastrointestinal digestion was performed in triplicate as reported by Minekus et al. (2014) with modifications. For the oral phase, 5 g of bean flour was mixed with 3.5 mL of pre-warmed simulated salivary fluid (SSF) with addition of 150 µL of thermostable α-amylase (3,000 units/mL), 25 µL of 0.3 M CaCl2 and 975 µL of deionized distilled water (DDW). The mixture was incubated in a shaking incubator (at 37 ºC and 200 rpm) for 2 min. For the gastric phase, the sample from the oral phase was mixed with 7.5 mL of pre-warmed simulated gastric fluid (SGF), 5 µL of 0.3 M CaCl2, 0.695 µL of DDW, and the pH was adjusted to 3 with 6 M HCl. Pepsin from porcine gastric mucosa stock solution (25,000 U/mL) was prepared in SGF and 1.25 mL was added to the mixture, which was incubated in a shaking incubator (at 37 ºC and 200 rpm) for 2 h. For the intestinal phase, the sample from the gastric phase was mixed with 11 mL of pre-warmed simulated intestinal fluid (SIF), 40 µL of 0.3 M CaCl2, 2.5 mL of bile (160 mg/mL), 1.31 mL of DDW, and the pH was adjusted to 7

with 1 M NaOH and 5 mL of pancreatin solution (800 U/mL) was added to each sample. The mixture was incubated in a shaking incubator (at 37 ºC and 200 rpm) for 2 h. To stop the reaction, the samples were placed in a water bath at 75 ºC for 20 min and centrifuged at 2061 g for 20 min. The precipitate was considered as the insoluble part that is potentially non-absorbable, while the supernatant potentially contained the soluble and absorbable peptides. Both fractions were lyophilized and kept at -20 ºC until further use.

2.5 Digestibility

The digestibility of germinated and ungerminated bean flour was calculated based on the relation between the lyophilized soluble part mass and the initial sample mass. The results were expressed as percentages.

The protein digestibility of germinated and ungerminated bean flour was calculated according to Nehi, Boora & Khetarpaul (2001) using the following equation (1):

Protein digestibility [%] = [(Digested protein)/(Total protein)] *100.

2.6 Protein solubility

The solubility of bean flours, before and after simulated gastrointestinal digestion (soluble fractions), was determined, in triplicate, using the method described by Bu et al. (2022), with modifications. Protein solutions were prepared with nondigested and digested (soluble fraction) bean samples (2% protein in DDW, w/v), in triplicate, and stirred for one hour at room temperature. The pH was adjusted to 7 using 2 M HCl and allowed to stir for another hour at room temperature. The pH was checked again before placing aliquots (1 mL) into microcentrifuge tubes. Aliquots were assessed at room temperature. Samples were centrifuged at 13,000 g for 10 min. The protein content of the supernatants and initial protein solutions was determined following the Dumas method. Protein solubility was expressed as the percentage of soluble protein (present in the supernatant) compared to the total protein (present in the initial sample).

2.7 Electrophoretic protein/peptide profile

Protein/peptide profiling, before and after simulated gastrointestinal digestion (soluble and insoluble fractions) of the bean flour, was performed using sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) according to Boyle et al. (2018). Briefly, protein molecular mass marker and samples (5 µL, ~0.05 mg protein) were loaded into a Criterion TM TGX TM 4-20% precast Tris-HCl gradient gel and ran at 200V. The gel was stained with Imperial TM Protein Stain (Coomassie brillian blue R-250) and imaged using the Molecular Imager Gel XR system (Bio-Rad Laboratories).

2.8 Molecular mass distribution by size exclusion high performance liquid chromatography

The molecular mass distribution of proteins/peptides, before and after simulated gastrointestinal digestion (soluble fractions) of the bean flour, was evaluated, in duplicate, by size-exclusion high performance chromatography (SE-HPLC), according to the method reported by Brückner-Gühmann et al. (2018) and modified by Bu et al. (2022), using a Shimadzu HPLC system (Shimadzu Scientific Instruments, Colombia, MD, USA) LC- 2050 C 3D equipped with Superdex 200 Increase 10/300 GL Tricorn™ (10 × 300 mm) column. Briefly, samples were solubilized for 2 h (1% protein, w/v) in three different sample buffers: (a) phosphate buffer (0.05 M sodium phosphate buffer and 0.1M NaCl adjusted to pH 7), (b) SDS phosphate buffer (0.05 M sodium phosphate buffer, 0.1 M NaCl and 0.1% SDS), (c) SDS/β-mercaptoethanol (BME) buffer (0.05 M sodium phosphate buffer, 0.1 M NaCl, 0.1% SDS and 2.5% β-mercaptoethanol). After solubilization, all samples were filtered through a 0.45 µm filter and automatically injected (75 µL). The mobile phase used was phosphate buffer at pH 7 and at a flow rate of 0.5 mL per minute with total run time of 80 min. Data analysis was performed at 220 nm and the molecular masses were calculated by running gel filtration calibration standards (HMW and LMW kits). The differences in molecular mass distribution among the samples were discussed using the relative peak areas (ratio of the area of a single peak to total peak area).

2.9 Antioxidant capacity

The antioxidant capacity, before and after simulated gastrointestinal

digestion (soluble fractions) of the bean flour, was measured in aqueous extracts (in triplicate) by ferric reducing antioxidant power (FRAP) assay (Sigma-Aldrich Assay Kit, MAK 369), and the oxygen radical absorbance capacity (ORAC) assay (Dávalos et al., 2004).

2.9.1 Ferric reducing antioxidant power (FRAP) assay.

To obtain the sample extracts, before and after simulated gastrointestinal digestion (soluble fractions), bean flour samples were diluted in DDW to the final concentration of 2 mg/mL. Germinated and ungerminated bean flours were shaken for 30 min, centrifuged at 3220 g for 10 min, and the supernatants were filtered using Whatman paper no.1. Samples and standards were pipetted (in triplicate) in a clear flat bottom 96-well plate (10 µL per well) with 190 µL of the reaction mixture. The absorbance at 594 nm was measured at the end of 60 min incubation at 37 ºC. Results were expressed as µM Fe2+ equivalents per g of sample using a standard curve (y = 0.058x - 0.0071, R2 = 0.9988).

2.9.2 Oxigen radical absorbance capacity (ORAC) assay

Bean flour samples, before and after simulated, gastrointestinal digestion (soluble fractions) were diluted in 75 mM phosphate buffer, pH 7.4, to the final concentration of 2 mg/mL. The samples were shaken for 30 min, centrifuged at 3220 g for 10 min, and the supernatant was filtered using Whatman paper no.1. Briefly, 150 µL of sodium fluorescein solution (8.1x10-5 mM) and 25 µL of sample, Trolox (as standard [0 to 10 µM]), and phosphate buffer (as blank), were added to each well of a microplate in triplicate. The microplate was incubated for 10 min at 37 ºC, after which 25 µL of 2,2’ Azobis (2-methylpropionamidine) dihydrochloride (AAPH) was added to each well. Fluorescence measurements were taken every min for 80 min with an excitation wavelength of 485 nm and an emission wavelength of 528 nm. Gene5TM software, version 5.0 (BioTek Instruments) was used for data acquisition. The areas under the fluorescence decay curve (AUC) were calculated and the AUC of blanks was subtracted. Results were expressed as µmol Trolox equivalents (TE)/g sample using a standard curve (y = 5.7748x + 2.3981, R2 = 0.99).

2.10 Angiotensin I-converting enzyme (ACE) inhibition

The ACE inhibition activity was evaluated according to the method outlined by Cushman & Cheung (1971) and modified by Ribeiro et al. (2021). N-hippuryl-histidyl-leucine (HHL) was used as a substrate, which liberates hippuric acid (HA) by the action of the ACE enzyme. HA formed in the reaction was determined by spectrophotometry. To prepare the extracts, germinated and ungerminated bean flours, as well as the soluble fractions of the digested bean flours, were solubilized (in triplicate), in 0.1 M sodium borate buffer, containing 0.3 M NaCl, adjusted to pH 8.3, shaken for 2 h, centrifuged at 3220 g for 10 min, and the supernatant filtered using Whatman paper no. 1. Briefly, an aliquate (25 µL) of each bean sample extract (2.5 mg/mL) was mixed with 250 µL of the reaction substrate, HHL (5mM in 0.1M sodium borate buffer, containing 0.3M NaCl adjusted to pH 8.3) and incubated for 10 min at 37ºC and 250 rpm. To start the reaction, an aliquot (40 µL) of ACE (100 mU/mL) was added to the mixture, which was incubated for 30 min at 37 ºC while stirring at 250 rpm. The reaction was stopped by the addition of 70 µL of 1 M HCl, and the HA formed was extracted into 1.5 mL of ethyl acetate, followed by centrifugation at 3220 g for 10 minutes. An aliquot (1 mL) of the extracted phase was transferred to a 2 mL centrifuge tube and dried in an oven at 100 ºC. The HA residue was solubilized in 1 mL of DDW, and the absorbance was read at 228 nm. A control was prepared containing all the reagents except the sample, which was replaced by sodium borate buffer, and the blank contained sodium borate buffer and substrate (HHL). The absorbance of the blank was subtracted from that of each sample and that of the control.

The ACE inhibition (%) was calculated according to the following equation: (2)

$$ACEi\ (\%) = (C-A)/(C-B)\ X\ 100$$

Where: A = sample absorbance minus that of the blank; B = blank absorbance; C = control absorbance.

2.11 Statistical Analysis

JMP Pro (version 17) was used to perform analysis of variance (ANOVA) and t-tests. Tukey-Kramer Honest Significant Difference (HSD) multiple means comparison test was used to determine significant differences among the

means under a completely randomized design and the significance level was set at 5% (P value < 0.05). Two-sample, unpaired t-test was used to determine significant differences between the means of two different samples (P value < 0.05).

3. Results and Discussion

3.1 Proximate composition of flours affected by germination

Digestive enzymes such as amylases, proteases, and lipases are activated during germination, and the hydrolysis of starch, proteins, and lipids takes place to enable the growth of the new plant (Akkad et al., 2021). This process could affect the proximate composition of pulses (Xu et al., 2019). Although the protein content of the germinated bean flour decreased after 48 h of germination (Table 1), the difference was not prominent. Other studies reported an increase in protein content. Atudorei et al. (2021) observed a significant increase in crude protein of common beans from 22.6 to 24.1% after 2 days of germination and to 26% after 4 days. However, according to Xu et al. (2019) an increase in protein during germination with pure water is not expected since pure water is a negligible source of nitrogen, and no net gain or loss should be observed. An increase in crude protein could occur if the process was carried out with a nitrogen source supplement.

The lipid content of germinated and ungerminated bean flours showed no significant difference between them. Ash content decreased during germination, similar to the observation by Atudorei et al. (2021). According to Erba et al. (2019), such observation can be attributed to the fact that soluble minerals could leach out from the plant tissue in soaking water. Germination did not cause a difference in total dietary fiber. Other studies reported a decrease in fiber after germination of flour samples, which was attributed to fiber breakdown and utilization during germination (Oskaybaş-Emlek et al., 2021). However, according to Martincabrejas et al. (2008), changes in dietary fiber content during germination depend on the type of legume being studied and the germination conditions such as the presence of light. Potentially, that is the reason why no difference was observed in this study.

Table 1. Composition (on dry basis) of ungerminated and germinated bean flours (Phaseolus vulgaris L.).

Sample	Sample Composition (g/100g)					
	Moisture	Protein	Lipids	Ash	Fiber	Total Carbohydrates
BF	9.39 ± 0.12[b]	22.8 ± 0.05[a]	2.13 ± 0.11[a]	3.26 ± 0.04[a]	17.9 ± 1.25[a]	71.8
GBF (48h)	10.5 ± 0.15[a]	22.5 ± 0.07[b]	1.94 ± 0.03[a]	3.06 ± 0.01[b]	14.9 ± 0.65[a]	72.5

[1] Different lower-case letters in the same column indicate significant differences ($P < 0.05$). BF = ungerminated bean flour; GBF = germinated bean flour after.

3.2 Protein solubility and in vitro digestibility

The protein solubility of germinated bean flour (*Phaseolus vulgaris* L.) was higher than the ungerminated bean flour at pH 7.0 prior to in vitro gastrointestinal digestion (Table 2). Similar results were found by de Souza Rocha et al. (2015), who observed an increase of soluble proteins after 72 h of germination in common beans, ranging from 73.0% to 80.2%. The protein solubility also increased after the in vitro simulated gastrointestinal digestion. The treatment with digestive enzymes such as pepsin and pancreatin, to mimic physiological conditions in vivo, promotes the breakdown of proteins into small peptides and/or free amino acids that are more soluble and can be easily absorbed. The increased solubility of the germinated bean flour after digestion indicated that the germination process was able to modify the structure of the proteins which could potentially improve its bioaccessibility and absorption in the gastrointestinal tract.

The digestibility of the samples was higher for the germinated bean flour than the ungerminated bean flour, (Table 2). This observation can be attributed to the release and/or formation of soluble components post the hydrolysis of macronutrients such as proteins and carbohydrates, and the breakdown of complexes, such as with minerals and phytates. Germinated bean flour also showed an increase in protein digestibility. Similar results were found by Kalpanadevi & Mohan (2013), who observed an increase of 11% in protein in vitro digestibility of germinated Vigna unguiculata subsp. unguiculata, ranging from 71.3 g/100 g in raw seeds to 79.5 g/100 g after 48h of germination. The improvement of protein digestibility post germination could also be attributed to the reduction or elimination of complexes between proteins and different antinutrients such as phytic acid, tannins, and polyphenols. (Alonso et al., 2000). Tannins, for instance, have a strong tendency to form insoluble complexes with proteins due to their great affinity to carboxyl oxygens of the peptide group. These complexes play a role in the inhibition of digestive enzymes, which, consequently, results in low protein digestibility and amino acid availability (Reddy et al., 1985). Thus, germination can be used to improve protein digestibility and enhance the bioacessibility of peptides that can exert biological and beneficial physiological functions.

Table 2. Solubility (%) at pH 7.0 and 2% protein concentration, in vitro protein digestibility (%) and antioxidant activity of germinated and ungerminated bean flours (Phaseolus vulgaris L.), before and after simulated in vitro gastrointestinal digestion.

Sample	Solubility (%)	Protein Digestibility (%)	Sample Digestibility (%)	Antioxidant Activity	
				ORAC (μM Eq trolox/g)	FRAP (μM Fe^{2+} equivalents/g)
BF	86.3 ± 0.60[d]	N/A	N/A	2.99 ± 0.18[b]	0.120± 0.01[a]
GBF (48h)	92.7 ± 0.23[c]	N/A	N/A	3.05 ± 0.20[b]	0.125 ± 0.002[a]
BF After Digestion	96.6 ± 0.54[b]	68.4 ± 0.39[b]	52.8[b]	5.21 ± 0.19[a]	0.151± 0.02[a]
GBF (48h) After Digestion	100 ± 1.4[a]	71.4 ± 0.73[a]	55.7[a]	5.15 ± 0.11[a]	0.152 ± 0.01[a]

[1] Different lowercase letters indicate significant differences among the means (n = 2) in each column, according to the Tukey-Kramer multiple means comparison test ($P < 0.05$). BF = ungerminated bean flour. GBF (48h) = germinated bean flour after 48h.

3.3 Protein/peptide electrophoretic profile

Fig. 2 shows the protein profile by SDS-PAGE of the common bean samples. Protein bands corresponding to subunits of phaseolin (40-47 kDa) and legumin (~75 kDa) with similar intensity were identified for both germinated and ungerminated bean flours under non-reducing conditions (Fig. 2). Phaseolins form trimeric complexes with molecular mass of 145-190 kDa, and because of the absence of cysteine in this fraction, its polypeptides are not linked by disulfide linkages. Legumins form hexameric complexes with high molecular mass (320-400 kDa), and their subunits, acidic (α, 33-43 kDa) and basic (β, 19-23 kDa) polypeptides, are linked by disulfide linkages (Bewley & Black, 1994). Accordingly, the band corresponding to legumin at ~75 kDa (Fig. 2, lanes 2 and 3) was not apparent under reducing conditions (Fig. 2, lanes 8 and 9), due to breakage of disulfide linkages. Reducing conditions also resulted in a decrease in the intensity of polypeptides bands with < 15 kDa, suggesting presence of disulfide linkages holding individual subunits. A dark smearing in the upper region of lanes 2 and 3, 6 and 7 indicated the presence of large molecular mass polymers, consisting of both insoluble and soluble aggregates, which were still apparent under reducing conditions. Smearing was not present in the soluble fraction of digested flours. The bands at the top of the stacking gel also indicated the presence of high molecular mass aggregates in germinated and ungerminated bean flours. These bands disappeared under reducing conditions for soluble fractions but not for insoluble fractions, indicating the presence of poorly soluble proteins (Pietrysiak et al., 2018), which could resist digestion.

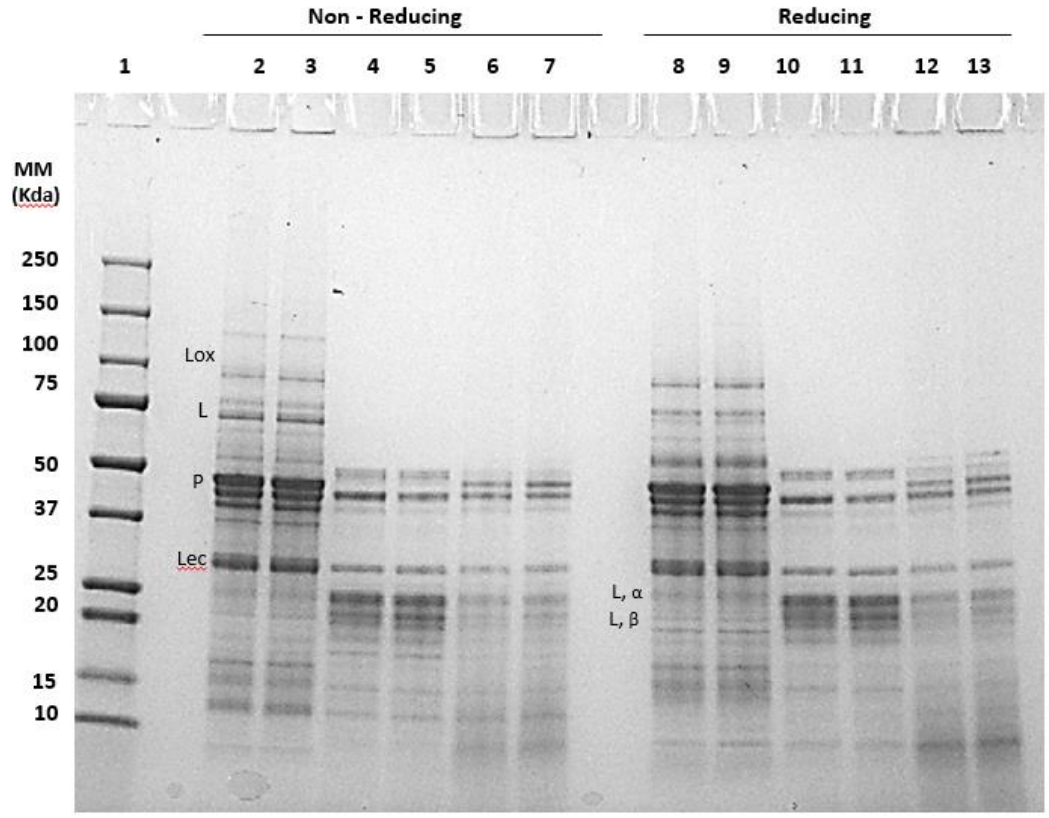
When we look at the soluble fractions of the bean flours after digestion, a band at ~47 kDa, corresponding to phaseolin subunits appeared more intense in the ungerminated sample under both reducing and non-reducing conditions. This observation could be attributed to the fact that germination changed the molecular conformation and consequently the accessibility of proteolytic enzymes to the storage proteins. The band at ~30 kDa (Fig. 2, lanes 2 and 3), corresponding to subunits of lectins, remained intact under reducing conditions (Fig. 2, lanes 8 and 9), but was less intense after digestion (Fig. 2, lanes 4 to 7 and 10 to 13). This observation can be associated with the fact that plant lectins showed high resistance to proteolysis both in vivo and in vitro (Vasconcelos & Oliveira, 2004).

The enzymes involved in gastrointestinal digestion have different

specificities and the site of cleavage determines the type of peptides formed and, consequently, their biological properties. During the in vitro protocol used to mimic human gastrointestinal digestion, pepsin and pancreatin were used. Pepsin breaks peptide linkages next to aromatic amino acids such as phenylalanine, tryptophan, and tyrosine (Amigo & Hernández-Ledesma, 2020). Pancreatin is a mix of enzymes containing amylase, protease, and lipase and represents the most relevant pancreatic enzymes present in human gastrointestinal tract (Salhi et al., 2020). Among the proteolytic enzymes, pancreatin contains trypsin, α-chymotrypsin, and carboxypeptidase (Gray et al., 2014). While trypsin cleaves the carboxyterminal of Arg and Lys residues, resulting in a positive charge at the C terminal of the peptide, α-chymotrypsin cleaves substrates with peptide bonds where the N-terminal contains a hydrophobic and aromatic ring (Phe, Trp, Tyr), and carboxypeptidase cleaves the C-terminal peptide bonds releasing free amino acids individually (Dau et al., 2020; Munasinghe et al., 2020; Song et al., 2021).

After the *in vitro* simulated gastrointestinal digestion, a reduction in the intensity of protein bands was noticeable for both soluble and insoluble fractions of germinated and ungerminated bean flours (Fig. 2. Lanes 4 to 7 and 10 to 13). This observation can be attributed to the action of proteases during gastric and intestinal phases of that digestion that resulted in the formation of low molecular mass peptides.

Fig. 2. SDS-PAGE gel visualization of the protein/peptide electrophoretic profiles of the different protein fractions and treatments under non-reducing (Lanes 2 to 7) and reducing conditions (Lanes 8 to 13). Lane 1: molecular weight standard; lane 2 and 8: ungerminated bean flour; lane 3 and 9: germinated bean flour; lane 4 and 10: digested ungerminated bean flour (soluble fraction); lane 5 and 11: digested germinated bean flour (soluble fraction); lane 6 and 12: digested ungerminated bean flour (insoluble fraction); lane 7 and 13: digested germinated bean flour (soluble fraction). Lox: lipoxygenase; L: legumin subunits; P: Phaseolin subunits; Lec: Lectin; L, α: acidic subunits of legumin; L, β basic subunits of legumin.



3.4 Protein/peptide molecular mass distribution by size exclusion HPLC

The relative abundance of each protein fraction in germinated and ungerminated bean flours, before and after simulated gastrointestinal digestion, was evaluated (Table 3 and Fig. 3). When the samples were solubilized in phosphate buffer alone, non-covalent interactions and disulfide linkages remained intact due to the absence of SDS and a reducing agent. Thus, to obtain further information regarding protein profile and molecular mass distribution of each sample, they were also solubilized under SDS and BME conditions. Peak identities were assigned based on reported molecular masses: soluble aggregates, >450 kDa (since samples were soluble in buffer and passed filtration (0.45 μm) prior to injection on the column);

hexameric legumin (11S), 320-400 kDa; soluble aggregates/legumin polymers, 200-300 kDa; trimeric phaseolin (7S), 145-190 kDa; and albumin polymers, 125-100 kDa (Bewley & Black, 1994; Montoya et al., 2010; Zhang et al., 2008).

Among all samples, ungerminated and germinated bean flour had the highest abundance of soluble aggregates (Table 3). In the presence of SDS, the abundance of soluble aggregates was the lowest in the ungerminated bean flour. Theoretically, SDS breaks accessible non-covalent interactions, primarily hydrophobic interactions, however, restricted accessibility could limit its capacity to dissociate large protein aggregates. Accordingly, there was no difference in relative abundance of soluble aggregates in germinated bean flour solubilized in buffer and buffer + SDS. Germination may have changed the configuration of proteins, limiting the access of SDS. In the presence of SDS + BME, soluble aggregates were only observed in the ungerminated bean flour. This observation may indicate that during the germination process, the disulfide linkages within the soluble aggregates proteins were dissociated and it may have influenced the increased protein solubility (Table 3).

Phaseolin was the most abundant fraction observed in all samples and all conditions, as expected, since phaseolin is the major protein fraction present in beans (García-Cordero et al., 2021). Protein fractions between 109 -111 kDa were attributed to albumin polymers, the second major component of common bean proteins (Montoya et al., 2010), and might have included lectins, α-amylase inhibitors, and trypsin and chymotrypsin inhibitors (Kunitz and Bowman-birk) (Tan et al., 2022). Germinated and ungerminated bean flours showed the highest abundances of albumin polymers when solubilized in phosphate buffer, with no difference between them, and the lowest abundance when solubilized in phosphate buffer + SDS + BME, suggesting that proteins were further modified due to the breakage of accessible disulfide interactions along with non-covalent interactions.

A new protein fraction, with molecular mass ranging from 230 to 270 kDa, was observed after simulated gastrointestinal digestion. This could be a result of the hydrolysis of soluble aggregates and legumin fractions by the digestive enzymes. Both samples showed the highest relative abundance of SA/legumin polymers when solubilized in the presence of SDS + BME, suggesting that these individual polymers were initially associated by disulfide linkages.

After digestion, the relative abundance of phaseolin was lower, in both phosphate buffer and buffer + SDS, for the germinated sample, indicating that the

germination process caused changes in the structure of proteins, which might have influenced the cleaving sites of the digestive enzymes. This occurrence could result in the formation of peptides with different chemical structures. Also, the relative abundance of albumin polymers decreased after digestion, but with no difference between germinated and ungerminated samples, suggesting that they were equally hydrolyzed by digestive enzymes.

The highest relative abundance of fractions with molecular mass <10 kDa was observed in the presence of SDS+BME (Fig. 3c), and it increased with germination, confirming that proteolysis occurred during this process. Also, the germination improved the solubility of proteins, which could make these fractions more bioaccessible in the human gastrointestinal tract. No difference was observed in the abundance of fractions with <10 kDa after the germination, except for bean flours solubilized in SDS + BME (Fig. 3a, b, c)

After the *in vitro* gastrointestinal digestion, the soluble fractions of germinated and ungerminated samples showed lower relative abundance of soluble aggregates, potentially due to action of digestive enzymes that hydrolyzed these polymers into smaller molecular size, more soluble. Such soluble components could contribute to enhanced bioactivity in vivo.

In general, the relative abundance of proteins with molecular mass of 450-100 kDa decreased significantly after the simulated gastrointestinal digestion, contributing to an increase in the fractions with less than 10 kDa (Fig. 3a, b, c), which corresponds to peptides and amino acids with potential bioactive properties. Many bioactive peptides are encrypted within the protein molecule. Once these peptides are released due to enzymatic action, these peptides can begin to perform various biological functions such as antioxidant and antihypertensive actions.

Table 3. Molecular mass and relative abundance of soluble aggregates, legumin, soluble aggregates/legumin polymers, phaseolin and albumin polymers present in germinated and ungerminated bean flours (Phaseolus vulgaris L.) before and after simulated in vitro gastrointestinal digestion as analyzed by size-exclusion high-performance liquid chromatography (SE-HPLC).

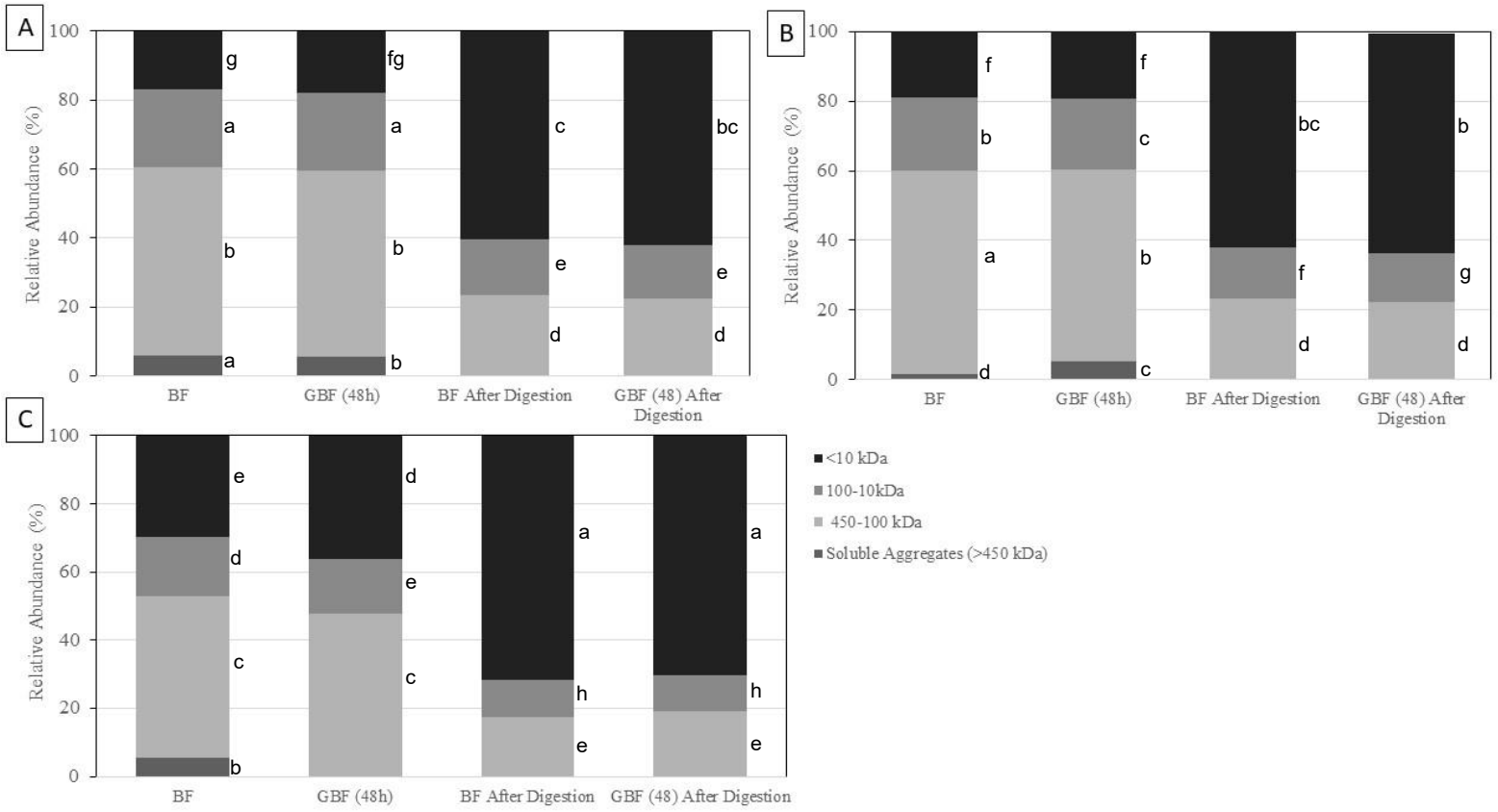
	[1]Relative Abundance (%) of Protein Fractions[2]														
	Phosphate Buffer					Phosphate Buffer (0.1% SDS)					Phosphate Buffer (0.1% SDS + 2.5% BME)				
Samples	SA	Legumin	SA/ Legumin Polymers	Phaseolin	[3]Albumin Polymers	SA	Legumin	SA/ Legumin Polymers	Phaseolin	[3]Albumin Polymers	SA	Legumin	SA/ Legumin Polymers	Phaseolin	[3]Albumin Polymers
	(>450 kDa)	(~ 320-440 kDa)	(~ 230-270 kDa)	(~ 168-195 kDa)	(~ 109-111 kDa)	(>450 kDa)	(~ 320-440 kDa)	(~ 230-270 kDa)	(~ 168-195 kDa)	(~ 109-111 kDa)	(>450 kDa)	(~ 320-440 kDa)	(~ 230-270 kDa)	(~ 168-195 kDa)	(~ 109-111 kDa)
BF	5.92 ± 0.08 [a]	6.54 ± 0.01 [b]	N/A	34.07 ± 0.06 [bc]	13.92 ± 0.02 [a]	1.45 ± 0.02 [d]	7.67 ± 0.01 [a]	N/A	37.62 ± 0.00 [a]	13.35 ± 0.00 [b]	5.62 ± 0.02 [b]	6.38 ± 0.04 [b]	N/A	30.10 ± 0.02 [d]	10.74 ± 0.05 [e]
GBF (48h)	5.69 ± 0.14 [b]	6.41 ± 0.05 [b]	N/A	33.72 ± 0.22 [c]	13.75 ± 0.08 [a]	5.26 ± 0.04 [c]	7.46 ± 0.08 [a]	N/A	35.08 ± 0.09 [b]	12.72 ± 0.00 [c]	N/A	5.90 ± 0.25 [c]	N/A	30.85 ± 0.24 [d]	11.08 ± 0.02 [d]
BF After Digestion	0.25 ± 0.00 [e]	1.32 ± 0.00 [d]	0.88 ± 0.03 [b]	16.92 ± 0.01 [e]	4.03 ± 0.03 [f]	0.24 ± 0.00 [e]	1.33 ± 0.00 [d]	1.02 ± 0.04 [b]	16.78 ± 0.02 [ef]	3.98 ± 0.02 [f]	N/A	0.58 ± 0.01 [fg]	13.41 ± 0.10 [a]	2.02 ± 0.39 [h]	3.02 ± 0.12 [g]
GBF (48h) After Digestion	0.18 ± 0.03 [e]	0.91 ± 0.05 [e]	1.51 ± 0.22 [b]	15.54 ± 0.00 [fg]	4.17 ± 0.15 [f]	0.19 ± 0.02 [e]	0.89 ± 0.07 [ef]	2.06 ± 0.27 [b]	15.08 ± 0.00 [g]	4.01 ± 0.00 [f]	N/A	0.39 ± 0.04 [g]	11.61 ± 1.92 [a]	2.56 ± 0.64 [h]	3.01 ± 0.14 [g]

[1] Relative abundance (%) is the area of a specific peak divided by the total peak area for that sample.

[2] Data is presented as means (n =2). Different letters indicate significant differences among the means of each protein fraction (SA, Legumin, SA/Legumin Polymers, Phaseolin, Albumin Polymers) for all buffer system used, according to the Tukey-Kramer multiple means comparison test ($P < 0.05$). BF = ungerminated bean flour; GBF = germinated bean flour after 48h; SA = Soluble aggregates; N/A = not available.

[3]Albumin polymers might include lectins, α-amylase inhibitors and trypsin and chymotrypsin inhibitors (Kunitz and Bowman-birk).

Fig. 3. Percent relative abundance of different protein fractions in germinated and ungerminated bean flours (Phaseolus vulgaris L.) before and after simulated in vitro gastrointestinal digestion. Samples were dissolved in (A) pH 7 phosphate buffer, (B) pH 7 phosphate buffer with 0.1% SDS, and (C) pH 7 phosphate buffer with 0.1% SDS and 2.5% BME and analyzed by SE-HPLC. Data is presented as means (n =2). Different letters indicate significant differences among the means of each molecular mass fraction (Soluble Aggregates, 450-100 kDA, 100-10 kDa and <10 kDa) of for all buffer system used, according to the Tukey-Kramer multiple means comparison test ($P < 0.05$). BF = ungerminated bean flour; GBF = germinated bean flour after 48h.



3.5 Antioxidant Capacity

The reaction environment used to access the antioxidant capacity was hydrophilic. Therefore, the antioxidant capacity of the tested components was dependent on their solubility in the aqueous media. Accordingly, there was no interference from the antioxidant activity of hydrophobic components, such as terpenes. However, polyphenols and flavonoids, that are commonly extracted using aqueous alcohol (Yang et al., 2018), may be present to a certain extent in the water extracts.

FRAP determines the reduction ability of Fe^{3+} to Fe^{2+}. This assay is a relevant antioxidant measure, since Fe contributes to harmful oxidation processes in vivo (Yamamoto et al., 2002). The FRAP values obtained for germinated and ungerminated bean flours did not statistically differ (Table 2), indicating no apparent effect of germination on the capacity of the sample to reduce Fe^{3+}. In contrast, Wongsiri et al. (2015) detected an increase in both DPPH radical scavenging activity and FRAP after 24 h of germination of mung beans, which was attributed to the increase in the amount of total phenolic content, antioxidative amino acids, and certain peptides. However, according to Xu et al. (2020), the antioxidant activity of phenolic compounds can either increase or decrease after germination depending on the seed species and germination conditions that will determine the changes in the phenolic compounds metabolism during germination, which comprises: a) The synthesis or transformation of aromatic amino acids into phenolic acids and posterior formation of compounds that can form complexes with macromolecules and remain stored in cell walls or vacuoles. b) The enzymatic hydrolysis of macromolecules resulting in the release of these phenolic compounds from their bound state; and c) Utilization of phenolic compounds to eliminate free radicals.

No significant difference was observed in FRAP values after gastrointestinal digestion of the samples (Table 2). Thus, germination did not appear to contribute to the generation of bioactive peptides with ferric-reducing antioxidant activity.

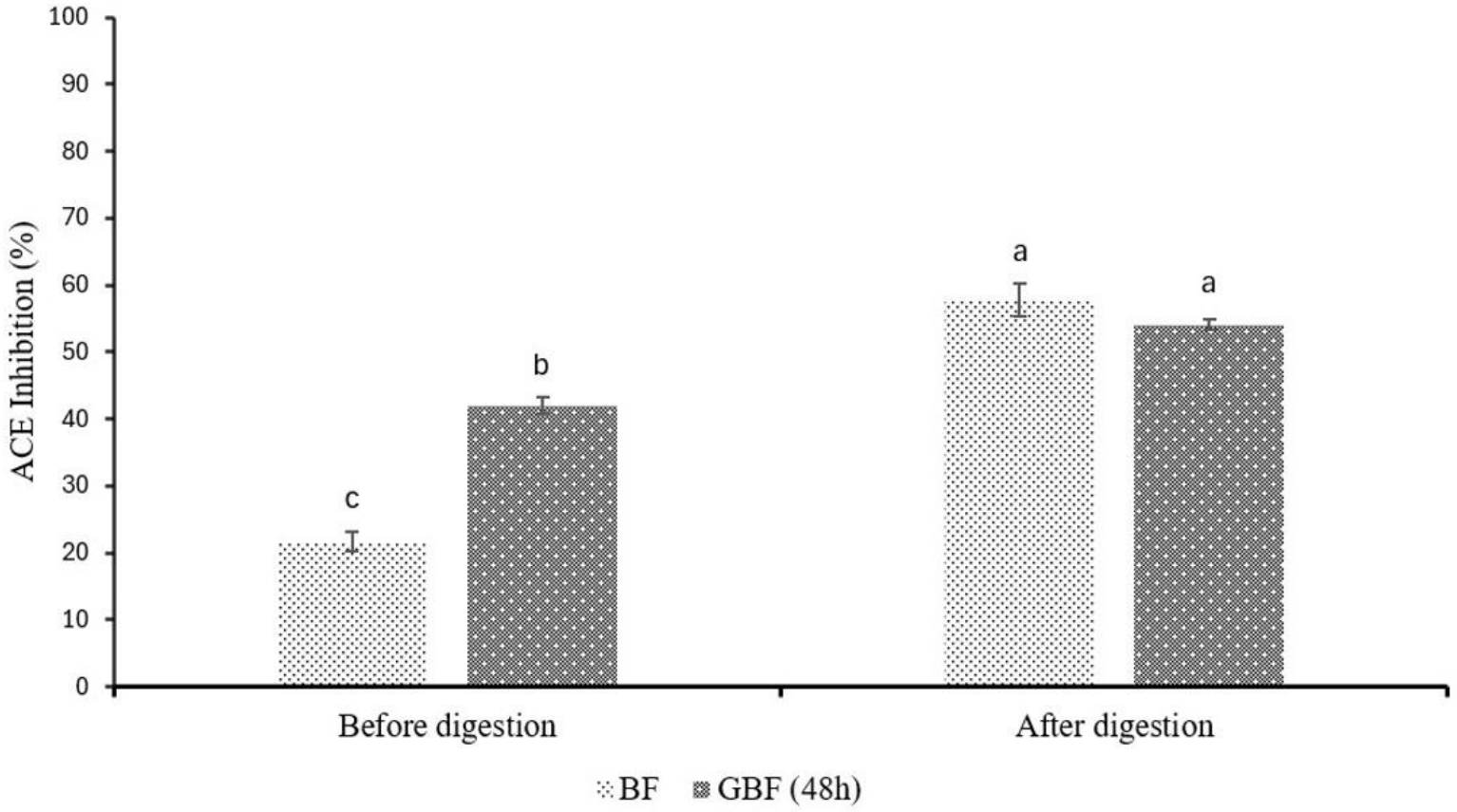
On the other hand, The ORAC values for the germinated and ungerminated bean flours increased after the simulated gastrointestinal digestion, but with no apparent impact of germination (Table 2). The observed ORAC values were in

the range obtained by Wu et al. (2004) for different varieties of common beans.

Differences can be found in ORAC values among different varieties of common beans according to Xu et al. (2007). The authors found values ranging widely from 13.3 μmol of TE/g to 92.7 μmol of TE/g, which was attributed to differences in raw material. In comparison to literature, the lower antioxidant values obtained in this study could be attributed to water-based extraction, which will extract less phenolics compounds compared to organic solvent extraction. Also, common beans with white tegument are reported to have lower phenolic content compared to colored common beans (Ombra et al., 2016). Obtained results may indicate that the ORAC antioxidant capacity is more related to phenolic compounds or phenolic compounds associated with proteins than bioactive peptides released upon protein hydrolysis.

Antioxidant peptides found in literature typically have more than three amino acid residues with molecular masses lower than 1 kDa. Gly (G), Pro (P), Leu (L) and hydrophobic amino acids are observed in more quantity compared to other hydrophilic amino acids (Sudhakar & Nazeer, 2015, Zou et al., 2016). Therefore, the effect of digestive enzymes changing the protein configuration and exposing internal hydrophobic amino acids during hydrolysis enables the formation of antioxidant peptides. Since in human organism there is a hydrolytic environment, it affects the exposition of hydrophobic amino acids.

Fig. 4. ACE inhibitory activity of germinated and ungerminated bean flours (Phaseolus vulgaris L.) before and after simulated in vitro gastrointestinal digestion.



3.6 Angiotensin converting enzyme (ACE) inhibition

Before digestion, ungerminated bean flour showed a significantly lower ACE inhibition when compared to the germinated bean flour (Fig. 4). This observation indicated that germination generated bioactive peptides with ACE inhibitory capacity. The values obtained match previous reported results, such as those reported by Mamilla & Mishra (2017). The authors reported 31.7% and 25.3% of ACE inhibition from ungerminated kidney bean and mung bean extracts, respectively, while extracts from germinated bean flours showed inhibition ranging from 31.1% to 82.3%. According to the authors, germination enhanced production of low molecular mass ACE inhibitory peptides and concluded that it can be used as an inexpensive method for production of functional foods to support clinical management of hypertension. Based on SDS-PAGE and Se-HPLC (Fig. 2 and 3) observations, phaseolin, the major storage protein of common bean, was most likely the main protein fraction responsible for the observed ACE inhibition activity.

After digestion, the percentage of ACE inhibition increased for both ungerminated and germinated bean flours with no significant difference between them (Figure 4). Even though the increased protein solubility (Table 2) and the higher degradation of phaseolin for the germinated sample (Table 3) suggested that germination could induce the formation of peptides with different chemical structure after digestion, this different chemical structure did not exert additional ACE inhibition activity. Therefore, it is possible to infer that the germination did not change the capacity of the simulated in vitro gastrointestinal digestion to release additional peptides with ACE inhibitory capacity.

4. Conclusion

Germination led to an increase in protein solubility, which contributed to an increase in protein digestibility. While ACE inhibitory activity was improved by germination, antioxidant activity was not impacted. In contrast to ACE inhibition, the antioxidant activity, as evaluated by ORAC increased post-simulated in vitro gastrointestinal digestion of germinated bean flour caused an increase in their values, suggesting that antioxidant peptides can potentially be formed by the action of proteolytic enzymes present in human gastrointestinal tract. Our results indicate that

while there were structural and chemical modifications in common bean proteins upon germination, the noted change did not affect the release of peptides with ACE-inhibitory activity or antioxidant activity. Further studies are required to evaluate the action of isolated peptides and their in vivo bioavailability and bioaccessibility.

Credit authorship contribution statement

Andressa Maria Suzin – Conceptualization, Investigation, Formal analysis, Visualization, Writing – original draft. **Pedro Henrique Freitas Cardines** – Writing – review and editing, Visualization. **Baraem P. Ismail** – Conceptualization, Writing – review and editing, funding acquisition. **Thais de Souza Rocha** – Conceptualization, Supervision, Project Administration, Writing – review and editing, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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References

Akkad, R., Buchko, A., Johnston, S. P., Han, J., House, J. D., & Curtis, J. M. (2021). Sprouting improves the flavour quality of faba bean flours. Food Chemistry, 364, 130355. https://doi.org/10.1016/J.FOODCHEM.2021.130355.

Alonso, R., Aguirre, A., & Marzo, F. (2000). Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. Food Chemistry, 68(2), 159–165. https://doi.org/10.1016/S0308-8146(99)00169-7.

Al-Ruwaih, N., Ahmed, J., Mulla, M. F., & Arfat, Y. A. (2019). High-pressure assisted enzymatic proteolysis of kidney beans protein isolates and characterization of hydrolysates by functional, structural, rheological and antioxidant properties. LWT, 100, 231–236.https://doi.org/10.1016/J.LWT.2018.10.074.

Amigo, L., & Hernández-Ledesma, B. (2020). Current Evidence on the Bioavailability of Food Bioactive Peptides. Molecules 2020, Vol. 25, Page 4479, 25(19), 4479. https://doi.org/10.3390/MOLECULES25194479.

Atudorei, D., Atudorei, O., & Codină, G. G. (2021). Dough rheological properties, microstructure and bread quality of wheat-germinated bean composite flour. Foods, 10(7). https://doi.org/10.3390/foods10071542.

Atudorei, D., Stroe, S. G., & Codină, G. G. (2021). Impact of Germination on the Microstructural and Physicochemical Properties of Different Legume Types. Plants (Basel, Switzerland), 10(3), 1–19. https://doi.org/10.3390/PLANTS10030592.

AOAC. (1990). Official methods of analysis of the AOAC, 15th ed. Methods 934.01, 923.03, 920.86, 922.06 920.87. Association of official analytical chemists. Arlington, VA, USA.

Barbana, C., & Boye, J. I. (2010). Angiotensin I-converting enzyme inhibitory activity of chickpea and pea protein hydrolysates. Food Research International, 43(6), 1642–1649. https://doi.org/10.1016/j.foodres.2010.05.003.

Ben Romdhane, M., Krichen, F., Ghazala, I., Ellouz-Chaabouni, S., & Haddar, A. (2017). Effect of Extraction Methods on Chemical Composition, Angiotensin I-Converting Enzyme Inhibitory and Antioxidant Activity of Coffee Residue. Journal of Food Processing and Preservation, 41(2), e12768. https://doi.org/10.1111/JFPP.12768.

Benzie, I. F. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "'Antioxidant Power'": The FRAP Assay. In ANALYTICAL BIOCHEMISTRY (Vol. 239).

Bewley, J. D., & Black, M. (1994). Seeds. Seeds, 1–33. https://doi.org/10.1007/978-1-4899-1002-8_1.

Bonomi, F., Fiocchi, A., Frøkiær, H., Gaiaschi, A., Iametti, S., Poiesi, C., Rasmussen, P., Restani, P., & Rovere, P. (2003). Reduction of immunoreactivity of bovine β-lactoglobulin upon combined physical and proteolytic treatment. Journal of Dairy Research, 70(1), 51–59. https://doi.org/10.1017/S0022029902005678.

Boyle, C., Hansen, L., Hinnenkamp, C., & Ismail, B. P. (2018). Emerging Camelina Protein: Extraction, Modification, and Structural/Functional Characterization. Journal of the American Oil Chemists' Society, 95(8), 1049–1062. https://doi.org/10.1002/AOCS.12045.

Brückner-Gühmann, M., Heiden-Hecht, T., Sözer, N., & Drusch, S. (2018). Foaming characteristics of oat protein and modification by partial hydrolysis. European Food Research and Technology, 244(12), 2095–2106. https://doi.org/10.1007/S00217-018-3118-0/METRICS.

Bu, F., Nayak, G., Bruggeman, P., Annor, G., & Ismail, B. P. (2022). Impact of plasma reactive species on the structure and functionality of pea protein isolate. Food Chemistry, 371, 131135. https://doi.org/10.1016/j.foodchem.2021.131135.

Calles, T., Xipsiti, M., & del Castello, R. (2019). Legacy of the International Year of Pulses. Environmental Earth Sciences, 78(5), 1–8. https://doi.org/10.1007/S12665-019-8106-6/FIGURES/1.

Cavalcante, R. B. M., Morgano, M. A., e Silva, K. J. D., Rocha, M. D. M., Araújo, M. A. D. M., & Moreira-Araújo, R. S. D. R. (2016). Cheese bread enriched with biofortified cowpea flour. Ciência e Agrotecnologia, 40(1), 97–103. https://doi.org/10.1590/S1413-70542016000100009.

Dau, T., Bartolomucci, G., & Rappsilber, J. (2020). Proteomics Using Protease Alternatives to Trypsin Benefits from Sequential Digestion with Trypsin. Analytical Chemistry, 92(14), 9523–9527. https://doi.org/10.1021/acs.analchem.0c00478.

Dave, L. A., Montoya, C. A., Rutherfurd, S. M., & Moughan, P. J. (2014). Gastrointestinal endogenous proteins as a source of bioactive peptides--an in

silico study. PloS One, 9(6). https://doi.org/10.1371/JOURNAL.PONE.0098922.

de Fátima Garcia, B., de Barros, M., & de Souza Rocha, T. (2021). Bioactive peptides from beans with the potential to decrease the risk of developing noncommunicable chronic diseases. In Critical Reviews in Food Science and Nutrition (Vol. 61, Issue 12, pp. 2003–2021). Bellwether Publishing, Ltd. https://doi.org/10.1080/10408398.2020.1768047.

de Souza Rocha, T., Hernandez, L. M. R., Mojica, L., Johnson, M. H., Chang, Y. K., & González de Mejía, E. (2015). Germination of Phaseolus vulgaris and alcalase hydrolysis of its proteins produced bioactive peptides capable of improving markers related to type-2 diabetes in vitro. Food Research International, 76(P1), 150–159. https://doi.org/10.1016/j.foodres.2015.04.041.

Erba, D., Angelino, D., Marti, A., Manini, F., Faoro, F., Morreale, F., Pellegrini, N., & Casiraghi, M. C. (2019). Effect of sprouting on nutritional quality of pulses. International Journal of Food Sciences and Nutrition, 70(1), 30–40. https://doi.org/10.1080/09637486.2018.1478393.

García-Cordero, J., Martínez-Palma, N., Madrigal-Bujaidar, E., Jiménez-Martínez, C., Madrigal-Santillán, E., Morales-González, J., Paniagua-Pérez, R., & Álvarez-González, I. (2021). Phaseolin, a Protein from the Seed of Phaseolus vulgaris, Has Antioxidant, Antigenotoxic, and Chemopreventive Properties. Nutrients, 13(6), 1750. https://doi.org/10.3390/nu13061750.

Gray, V. A., C. Marques, M. R., Cole, E., Riva Toma, J. M. D., Ghidorsi, L., Guo, J.-H., Han, J.-H., Han, F., Hosty, C. T., Kochling, J. D., Kraemer, J., Langdon, T., Leinbach, S. R., Martin, G. P., Meyerhoffer, S. M., Moreton, R. C., Raghaven, K. S., Shneyvas, E., Suggett, J. A.,.Eranui, T. (2014). Use of Enzymes in the Dissolution Testing of Gelatin Capsules and Gelatin-Coated Tablets-Revisions to Dissolution; and Disintegration and Dissolution of Dietary Supplements; Dissolution Technologies, 21(4), 6–18. https://doi.org/10.14227/DT210414P6.

Guo, Y., Zhang, T., Jiang, B., Miao, M., & Mu, W. (2014). The effects of an antioxidative pentapeptide derived from chickpea protein hydrolysates on oxidative stress in Caco-2 and HT-29 cell lines. Journal of Functional Foods, 7, 719–726. https://doi.org/10.1016/j.jff.2013.12.013.

Kalpanadevi, V., & Mohan, V. R. (2013). Effect of processing on antinutrients and in vitro protein digestibility of the underutilized legume, Vigna

unguiculata (L.) Walp subsp. unguiculata. LWT, 51(2), 455–461. https://doi.org/10.1016/J.LWT.2012.09.030.

Lucia Medeiros Bernussi, A. (1996). Efeito da combinação do assamento convencional e energia de microondas na qualidade tecnologica de biscoito semi-doce duro [Universidade Estadual de Campinas]. https://doi.org/10.47749/T/UNICAMP.1996.113521.

Mariniello, L., Giosafatto, C. V. L., Di Pierro, P., Sorrentino, A., & Porta, R. (2007). Synthesis and Resistance to in Vitro Proteolysis of Transglutaminase Cross-Linked Phaseolin, the Major Storage Protein from Phaseolus vulgaris. Journal of Agricultural and Food Chemistry, 55(12), 4717–4721. https://doi.org/10.1021/jf0637269.

Martincabrejas, M., Diaz, M., Aguilera, Y., Benitez, V., Molla, E., & Esteban, R. (2008). Influence of germination on the soluble carbohydrates and dietary fibre fractions in non-conventional legumes. Food Chemistry, 107(3), 1045–1052. https://doi.org/10.1016/j.foodchem.2007.09.020.

Matemu, A., Nakamura, S., & Katayama, S. (2021). Health Benefits of Antioxidative Peptides Derived from Legume Proteins with a High Amino Acid Score. https://doi.org/10.3390/antiox10020316.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., … Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food – an international consensus. Food Funct., 5(6), 1113–1124. https://doi.org/10.1039/C3FO60702J.

Montoya, C. A., Lallès, J.-P., Beebe, S., & Leterme, P. (2010). Phaseolin diversity as a possible strategy to improve the nutritional value of common beans (Phaseolus vulgaris). Food Research International, 43(2), 443–449. https://doi.org/10.1016/j.foodres.2009.09.040.

Mühling, M., Gilroy, J., & Croy, R. R. D. (1997). Legumin proteins from seeds of Phaseolus vulgaris L. Journal of Plant Physiology, 150(4), 489–492. https://doi.org/10.1016/S0176-1617(97)80103-4.

Munasinghe, A., Baker, S. L., Lin, P., Russell, A. J., & Colina, C. M. (2020). Structure–function–dynamics of α-chymotrypsin based conjugates as a function of polymer charge. Soft Matter, 16(2), 456–465.

https://doi.org/10.1039/C9SM01842E.

Nong, N. T. P., & Hsu, J.-L. (2022). Bioactive Peptides: An Understanding from Current Screening Methodology. Processes, 10(6), 1114. https://doi.org/10.3390/pr10061114.

Ombra, M. N., d'Acierno, A., Nazzaro, F., Riccardi, R., Spigno, P., Zaccardelli, M., Pane, C., Maione, M., & Fratianni, F. (2016). Phenolic Composition and Antioxidant and Antiproliferative Activities of the Extracts of Twelve Common Bean (Phaseolus vulgaris L.) Endemic Ecotypes of Southern Italy before and after Cooking. Oxidative Medicine and Cellular Longevity, 2016, 1–12. https://doi.org/10.1155/2016/1398298.

Osborne, T. B. (1912). The Vegetable Proteins. Monographs on Biochemistry, pp. 1- 121. Research Chemist in the Connecticut Agricultural Experiment Station, New Haven, Connecticut, Research Associate of the Carnegie Institution of Washington, D.C.

Osman, A., Enan, G., Al-Mohammadi, A.-R., Abdel-Shafi, S., Abdel-Hameid, S., Sitohy, M. Z., & El-Gazzar, N. (2021). Antibacterial Peptides Produced by Alcalase from Cowpea Seed Proteins. Antibiotics, 10(7), 870. https://doi.org/10.3390/antibiotics10070870.

Oskaybaş-Emlek, B., Özbey, A., & Kahraman, K. (2021). Effects of germination on the physicochemical and nutritional characteristics of lentil and its utilization potential in cookie-making. Journal of Food Measurement and Characterization, 15(5), 4245–4255. https://doi.org/10.1007/s11694-021-00958-y.

Ovando-Martínez, M., Bello-Pérez, L. A., Whitney, K., Osorio-Díaz, P., & Simsek, S. (2011). Starch characteristics of bean (Phaseolus vulgaris L.) grown in different localities. Carbohydrate Polymers, 85(1), 54–64. https://doi.org/10.1016/j.carbpol.2011.01.043.

Pietrysiak, E., Smith, D. M., Smith, B. M., & Ganjyal, G. M. (2018). Enhanced functionality of pea-rice protein isolate blends through direct steam injection processing. Food Chemistry, 243, 338–344. https://doi.org/10.1016/j.foodchem.2017.09.132.

Rahmi, A., & Arcot, J. (2023). In Vitro Assessment Methods for Antidiabetic Peptides from Legumes: A Review. https://doi.org/10.3390/foods12030631.

Rao, S., Chinkwo, K., Santhakumar, A., & Blanchard, C. (2018).

Inhibitory Effects of Pulse Bioactive Compounds on Cancer Development Pathways. Diseases, 6(3), 72. https://doi.org/10.3390/diseases6030072.

Rawal, V., & Navarro, D. K. (2019). The Global Economy of Pulses. FAO. https://doi.org/10.4060/I7108EN.

Reddy, N. R., Pierson, M. D., Sathe, S. K., & Salunkhe, D. K. (1985). Dry bean tannins: A review of nutritional implications. Journal of the American Oil Chemists Society, 62(3), 541–549. https://doi.org/10.1007/BF02542329.

Ribeiro, E., Rocha, T. de S., & Prudencio, S. H. (2021). Potential of green and roasted coffee beans and spent coffee grounds to provide bioactive peptides. Food Chemistry, 348. https://doi.org/10.1016/J.FOODCHEM.2021.129061.

Salhi, A., Amara, S., Mansuelle, P., Puppo, R., Lebrun, R., Gontero, B., Aloulou, A., & Carrière, F. (2020). Characterization of all the lipolytic activities in pancreatin and comparison with porcine and human pancreatic juices. Biochimie, 169, 106–120. https://doi.org/10.1016/J.BIOCHI.2019.07.004.

Siddiq, M., & Uebersax, A. M. (2013). Dry Beans and Pulses Production and Consumption—An Overview. In Dry Beans and Pulses Production, Processing and Nutrition (pp. 3–22). John Wiley & Sons, Inc.

Sonklin, C., Alashi, M. A., Laohakunjit, N., Kerdchoechuen, O., & Aluko, R. E. (2020). Identification of antihypertensive peptides from mung bean protein hydrolysate and their effects in spontaneously hypertensive rats. Journal of Functional Foods, 64, 103635. https://doi.org/10.1016/j.jff.2019.103635.

Song, P., Xu, W., Zhang, Y., Wang, F., Zhou, X., Shi, H., & Feng, W. (2021). A new carboxypeptidase from Aspergillus niger with good thermostability, pH stability and broad substrate specificity. Scientific Reports, 11(1), 18745. https://doi.org/10.1038/s41598-021-98003-x.

Sudhakar, S., & Nazeer, R. A. (2015). Preparation of potent antioxidant peptide from edible part of shortclub cuttlefish against radical mediated lipid and DNA damage. LWT - Food Science and Technology, 64(2), 593–601. https://doi.org/10.1016/j.lwt.2015.06.031.

Tan, X., Zhang, S., Malde, A. K., Tan, X., & Gilbert, R. G. (2022). Effects of chickpea protein fractions on α-amylase activity in digestion. Food Hydrocolloids, 133, 108005. https://doi.org/10.1016/j.foodhyd.2022.108005.

Tapal, A., & Tiku, P. K. (2018). Nutritional and nutraceutical improvement by enzymatic modification of food proteins. Enzymes in Food

Biotechnology: Production, Applications, and Future Prospects, 471–481. https://doi.org/10.1016/B978-0-12-813280-7.00027-X.

Vasconcelos, I. M., & Oliveira, J. T. A. (2004). Antinutritional properties of plant lectins. Toxicon, 44(4), 385–403. https://doi.org/10.1016/j.toxicon.2004.05.005.

Wang, S., Chen, L., Yang, H., Gu, J., Wang, J., & Ren, F. (2020). Regular intake of white kidney beans extract (Phaseolus vulgaris L.) induces weight loss compared to placebo in obese human subjects. Food Science and Nutrition, 8(3), 1315–1324. https://doi.org/10.1002/FSN3.1299.

Wongsiri, S., Ohshima, T., & Duangmal, K. (2015). Chemical Composition, Amino Acid Profile and Antioxidant Activities of Germinated Mung Beans (V igna radiata). Journal of Food Processing and Preservation, 39(6), 1956–1964. https://doi.org/10.1111/jfpp.12434.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. Journal of Agricultural and Food Chemistry, 52(12), 4026–4037. https://doi.org/10.1021/JF049696W.

Xu, B. J., Yuan, S. H., & Chang, S. K. C. (2007). Comparative Analyses of Phenolic Composition, Antioxidant Capacity, and Color of Cool Season Legumes and Other Selected Food Legumes. Journal of Food Science, 72(2), S167–S177. https://doi.org/10.1111/J.1750-3841.2006.00261.X.

Xu, M., Jin, Z., Simsek, S., Hall, C., Rao, J., & Chen, B. (2019). Effect of germination on the chemical composition, thermal, pasting, and moisture sorption properties of flours from chickpea, lentil, and yellow pea. Food Chemistry, 295, 579–587. https://doi.org/10.1016/j.foodchem.2019.05.167.

Xu, M., Rao, J., & Chen, B. (2020). Phenolic compounds in germinated cereal and pulse seeds: Classification, transformation, and metabolic process. Critical Reviews in Food Science and Nutrition, 60(5), 740–759. https://doi.org/10.1080/10408398.2018.1550051.

Xue, Z., Wang, R., Wen, H., Yu, W., & Kou, · Xiaohong. (2018). Biochanin A and CPe-III Peptide Improved Hepatic Inflammation by Regulating the Hepatic Lipid Metabolic Pathways in Diet-Induced Obese Mice. Transactions of Tianjin University, 24, 234–243. https://doi.org/10.1007/s12209-018-0117-y.

Yamamoto, A., Shin, R., Hasegawa, K., Naiki, H., Sato, H.,

Yoshimasu, F., & Kitamoto, T. (2002). Iron (III) induces aggregation of hyperphosphorylated tau and its reduction to iron (II) reverses the aggregation: implications in the formation of neurofibrillary tangles of Alzheimer's disease. Journal of Neurochemistry, 82(5), 1137–1147. https://doi.org/10.1046/J.1471-4159.2002.T01-1-01061.X.

Zhang, J., Shi, J., Ilic, S., Jun Xue, S., & Kakuda, Y. (2008). Biological Properties and Characterization of Lectin from Red Kidney Bean (Phaseolus Vulgaris). Food Reviews International, 25(1), 12–27. https://doi.org/10.1080/87559120802458115.

Zou, T.-B., He, T.-P., Li, H.-B., Tang, H.-W., & Xia, E.-Q. (2016). The Structure-Activity Relationship of the Antioxidant Peptides from Natural Proteins. Molecules, 21(1), 72. https://doi.org/10.3390/molecules21010072.

AACC. American Association of Cereal Chemists. 10th ed., St. Paul: AACC, 2000.

Web References:

EMBRAPA, 2016. BRS Ártico: Common bean cultivar with export-standard white grain, 163-166. Retreived from https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1053340/brs-artico-common-bean-cultivar-with-export-standard-white-grain (02/29/2024 acessed).

FAO, 2024. Crop Production and Trade Data. Retrived from http://www.fao.org/faostat/en/#data (01/15/2024 acessed).