UNIVERSIDADE ESTADUAL DE LONDRINA

BIANCA DE FÁTIMA GARCIA

PEPTÍDEOS BIOATIVOS DO FEIJÃO COM POTENCIAL PARA DIMINUIR O RISCO DE DESENVOLVIMENTO DE DOENÇAS CRÔNICAS NÃO TRANSMISSÍVEIS

Londrina
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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

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Londrina
2019

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

G216p Garcia, Bianca de Fátima.
Peptídeos bioativos do feijão com potencial para diminuir o risco de desenvolvimento de doenças crônicas não transmissíveis / Bianca de Fátima Garcia. - Londrina, 2019.
80 f. : il.

Orientador: Thaís Rocha.
Coorientador: Mauro Cezar Barbosa.
Dissertação (Mestrado em Ciência de Alimentos) - Universidade Estadual de Londrina, Centro de Ciências Agrárias, Programa de Pós-Graduação em Ciência de Alimentos, 2019.
Inclui bibliografia.

1. Peptídeos bioativos - Tese. 2. Diabetes - Tese. 3. Hipertensão - Tese. 4. Bioinformática - Tese. I. Rocha, Thaís. II. Barbosa, Mauro Cezar. III. Universidade Estadual de Londrina. Centro de Ciências Agrárias. Programa de Pós-Graduação em Ciência de Alimentos. IV. Título.

CDU 641.1

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Londrina, 10 de setembro de 2019

À todos que de alguma forma estão incapacitados de realizar seu trabalho.

AGRADECIMENTOS

Agradeço à Deus, por ter me capacitado para realizar este trabalho.

Agradeço à minha orientadora, professora Thaís, pelo apoio nos momentos difíceis, compreensão, ensinamentos, empenho e dedicação.

Aos professores que fizeram parte da minha formação, pelo suporte e ensinamentos.

Aos colegas de curso, pelo companheirismo, ajuda e amizade.

Ao meu esposo, Cezar, pelo constante apoio e parceria.

Aos meus pais Jaime e Izabel, e minhas irmãs Rebeca e Raquel pelo apoio e carinho.

Nem os longes mais longes do mundo são intransponíveis ao que pretende chegar.

Pe Fábio de Melo.

GARCIA, Bianca de Fátima. **Peptídeos bioativos do feijão com potencial para diminuir o risco de desenvolvimento de doenças crônicas não transmissíveis**. 2019. 80 f. Dissertação (Mestrado em Ciência de Alimentos) – Universidade Estadual de Londrina, Londrina, 2019.

RESUMO

Devido à possibilidade de obter-se peptídeos com atividade biológica a partir da hidrólise de proteínas presentes no feijão, este tem se tornado objeto de diversas pesquisas atualmente. Peptídeos bioativos são sequências de aproximadamente 2-20 aminoácidos, que podem apresentar atividade moduladora em processos metabólicos que ocorrem no organismo humano, como por exemplo, a via de absorção da glicose. No entanto, a hidrólise de proteínas pode gerar peptídeos com alta diversidade estrutural, dificultando a seleção das fontes proteicas mais adequadas para obter-se peptídeos candidatos a exercerem atividade biológica *in vivo*. Nesse sentido, a bioinformática pode ser uma ferramenta simples e barata para auxiliar este processo. Portanto, o objetivo do presente estudo foi identificar *in silico* a possibilidade de obtenção de peptídeos bioativos a partir das proteínas de reserva, especificamente as globulinas, das espécies de feijão *Phaseolus vulgaris* (L.), *Vigna angularis* (Willd.), *Vigna radiata* (L.), e *Vigna unguiculata* (L.) Walp., utilizando-se para isso as bases de dados UniProt, BIOPEP e PeptideRanker. Foram avaliados o perfil de potencial atividade biológica das globulinas das espécies de feijão mencionadas, e a obtenção de fragmentos bioativos por meio da simulação da hidrólise proteica por dois processos distintos: a digestão gastrointestinal com pepsina, tripsina e quimiotripsina e a hidrólise pela ação da enzima subtilisina. A frequência de ocorrência de peptídeos com atividades específicas foi calculada através da seguinte equação: ‘’A’’ = a/At, onde ‘’a’’ corresponde à quantidade de peptídeos com determinada atividade na sequência da proteína e ‘’At’’ é o número total de resíduos de aminoácidos na proteína. Para todas as espécies estudadas a maior frequência de ocorrência de peptídeos com potencial atividade biológica encontrada foi para a atividade de inibição da enzima dipeptidil peptidase-IV, seguida pela inibição da enzima conversora de angiotensina-I, e pela atividade antioxidante, sendo que as duas primeiras enzimas são alvos terapêuticos no tratamento de doenças crônicas como a diabetes mellitus tipo 2 (DM2), e a hipertensão arterial respectivamente. A digestão gastrointestinal pareceu ser suficiente para liberar fragmentos com potencial atividade biológica das proteínas globulinas das espécies estudadas, indicando que o consumo diário destas leguminosas pode ser considerado uma estratégia para diminuir o risco de desenvolvimento de DM2 e hipertensão. No entanto, a hidrólise com a subtilisina pode ser utilizada para potencializar a quantidade de fragmentos potencialmente bioativos obtidos, visto que este processo gerou diferentes fragmentos do que os encontrados quando simulada a digestão com enzimas gastrointestinais, possibilitando o desenvolvimento de produtos funcionais a partir das fontes proteicas estudadas.

Palavras-chave: Diabetes melittus tipo 2. Hipertensão. BIOPEP. Bioinformática.

GARCIA, Bianca de Fátima. **Bioactive peptides from beans with the potencial to decrease the risk of developing nontransmissible chronic diseases**. 2019. 80 p. Dissertation (Master's degree in Food Science) – Universidade Estadual de Londrina, Londrina, 2019.

ABSTRACT

Due to the possibility of obtaining peptides with biological activity from the hydrolysis of proteins present in beans, this has become the object of several researches currently. Bioactive peptides are sequences of approximately 2-20 amino acids, which may exhibit modulatory activity in metabolic processes occurring in the human body, such as the glucose uptake pathway. However, the hydrolysis of proteins can generate peptides with high structural diversity, making it difficult to select the most adequate protein sources to obtain peptides candidates to exert biological activity *in vivo.* In this sense, the bioinformatic can be a simple and inexpensive tool to aid this process. Therefore, the objective of this study was to identify *in silico* the possibility of obtaining peptides with potential biological activity from the storage proteins, specifically the globulins, of the bean species *Phaseolus vulgaris* (L.), *Vigna angularis* (Willd.), *Vigna radiata* (L.), and *Vigna unguiculata* (L.) Walp., using the UniProt, BIOPEP and PeptideRanker databases. It was evaluated the profile of potential biological activity of the globulin proteins of the afore mentioned bean species and the obtention of bioactive fragments by the protein hydrolysis simulation by two different processes: gastrointestinal digestion with pepsin, trypsin and chymotrypsin and the hydrolysis by the action of enzyme subtilisin. The frequency of peptide occurrence with specific activities was calculated by the following equation: ''A'' = a / At, where ''a'' corresponds to the number of peptides with certain activity in the protein sequence and ''At'' is the total number of amino acids residues. For all the species studied, the highest frequency of peptides occurrence with potential biological activity was found for inhibiting dipeptidyl peptidase-IV (DPP-IV) activity, followed by the inhibit angiotensin-I converting enzyme (ACE) activity, followed by antioxidant activity. The inhibition of DPP-IV and ACE are therapeutic targets in the treatment of noncommunicable diseases such as diabetes mellitus type 2 (T2D) and arterial hypertension respectively. The gastrointestinal digestion appears to be enough to release fragments with potential biological activity from the globulin proteins of the studied species, indicating that the daily consumption of this legumes can be considered a strategy to reduce the risk of developing T2D and hypertension. However, hydrolysis with subtilisin can be used to potentiate the amount of potentially bioactive fragments obtained, since this process generated different fragments of those found when digestion with gastrointestinal enzymes was simulated, making possible the development of functional products from the protein sources studied.

Keywords: Diabetes mellittus type 2. Hypertension. BIOPEP. Bioinformatic.

LISTA DE ABREVIATURAS E SIGLAS

ADA	*American Diabetes Association*
ANVISA	Agência Nacional de Vigilância Sanitária
CDC	Centers for Disease Control and Prevetion
CONAB	Companhia Nacional de Abastecimento
COX2	Ciclooxigenase-2
DM2	Diabetes melittus tipo 2
DPP-IV	Dipeptidil peptidase-IV
ECA	Enzima conversora de angiotensina-I
GIP	*Glucose-dependent insulinotropic peptide*
GLP-1	*Glucagon-like peptide*
HDL-c	Colesterol de lipoproteína de alta densidade
HMG-CoAr	3-hidroxi-3-metil-glutaril-CoA redutase
IC50	Concentração necessária para inibir 50% de atividade enzimática
iNS-1E	Insulin secreting beta cell derived line
LDL-c	Colesterol de lipoproteína de baixa densidade
NF-κB	Fator de transcrição nuclear kappa B
SBC	Sociedade Brasileira de Cardiologia
SBD	Sociedade Brasileira de Diabetes
SEAB	Secretaria de Estado da Agricultura e do Abastecimento

LISTA DE AMINOÁCIDOS

A	Alanina
R	Arginina
N	Asparagina
D	Aspartato
C	Cisteína
Q	Glutamina
E	Ácido glutâmico
G	Glicina
H	Histidina
I	Isoleucina
L	Leucina
K	Lisina
M	Metionina
F	Fenilalanina
P	Prolina
S	Serina
T	Treonina
W	Triptofano
Y	Tirosina
V	Valina

SUMÁRIO

1. INTRODUÇÃO

Devido à fatores como alimentação inadequada, obesidade, tabagismo e sedentarismo, a incidência de doenças crônicas não transmissíveis como a hipertensão arterial, diabetes melittus tipo 2 (DM2), dislipidemia, câncer, entre outras, tem aumentado consideravelmente, acompanhando as mudanças que ocorreram nos hábitos de vida da população do Brasil e de outros países.

Dentre essas doenças, a hipertensão e a DM2 estão em constante associação, devido à alta frequência em que ocorrem, e, portanto, são consideradas problemas de saúde pública mundial (BRASIL. Secretária de Saúde do Tocantins, 2017). Estima-se que 13 milhões de pessoas sejam portadoras de diabetes no Brasil (SBD-SOCIEDADE BRASILEIRA DE DIABETES, 2017). No que diz respeito à hipertensão, este número é ainda maior, atingindo 36 milhões de adultos (SOCIEDADE BRASILEIRA DE CARDIOLOGIA, 2016).

Apesar da existência de protocolos estabelecidos para o tratamento da DM2, e hipertensão, há uma busca por alternativas que diminuam os efeitos adversos causados pelos medicamentos e que auxiliem no tratamento. Portanto, é de interesse o desenvolvimento de ingredientes e produtos alimentícios que contenham moléculas bioativas com potencial atuação nos alvos terapêuticos dessas doenças, e que possam ser inseridos na alimentação de indivíduos em curso de desenvolvimento.

Compostos biotivos são componentes obtidos de origem animal e vegetal, e que podem ter uma influência positiva na saúde humana, ao agir em tecidos específicos ou células. Dentre eles, encontram-se os peptídeos bioativos, os quais são definidos como sequências de aproximadamente 2-20 aminoácidos (ASTLEY; FINGLAS, 2016); (LI; YU, 2015). Devido à possibilidade de obter essas moléculas a partir das proteínas de reserva presentes no feijão, este tem se tornado objeto de inúmeras pesquisas atualmente.

Foi demonstrado que a hidrólise enzimática *in vitro* pela ação da Alcalase® e/ou simulação da digestão gastrointestinal de proteínas presentes nos feijões -azuki, -caupi, -comum e -mungo-verde, produziu peptídeos bioativos com

potencial antioxidante e atividade para inibir a enzima dipeptidil peptidase-IV (DPP-IV) (ROCHA et al., 2014; ROCHA et al., 2015; MOJICA; GONZALEZ DE MEJÍA, 2015), alvo terapêutico no tratamento da DM2 e para inibir a enzima conversora de angiotensina-I (ECA) (LI et al., 2006; MOJICA; GONZALEZ DE MEJÍA, 2015), alvo molecular de medicações anti-hipertensivas.

No entanto, a produção industrial de peptídeos bioativos e ingredientes que contenham estes compostos ainda é incipiente, devido à dificuldade em selecionar moléculas adequadas e à falta de tecnologias para processos como hidrólise de proteínas, separação e purificação, além de faltarem estudos que caracterizem a biodisponibilidade dessas moléculas (RIZZELLO et al., 2016). Atualmente, muitos estudos a respeito da ação dos peptídeos bioativos são realizados por meio de ensaios *in vitro*, constatando atividade dessas moléculas sobre biomarcadores como as enzimas, sendo que apenas uma pequena quantidade é realizada em animais ou humanos.

Diante da possibilidade de se obter uma grande variedade estrutural de peptídeos bioativos a partir de diversos alimentos, a bioinformática pode ser uma ferramenta útil para auxiliar na seleção de fontes proteicas adequadas para obter-se essas moléculas e na produção de ingredientes funcionais a partir de concentrados proteicos ou de peptídeos isolados candidatos a exercerem atividade *in vivo*.

Bases de dados como a UniProt (THE UNIPROT CONSORTIUM, 2017); BIOPEP-UWM (MINKIEWICZ et al., 2008) e PeptideRanker (MOONEY et al., 2012) têm sido muito utilizadas em estudos científicos para confirmar sequências e predizer a bioatividade de peptídeos *in silico* utilizando-se da bioinformática (LUNA VITAL et al., 2014; MOJICA; GONZALEZ DE MEJÍA, 2015; OSEGUERA-TOLEDO; GONZALEZ DE MEJÍA; AMAYA-LLANO, 2015; ROCHA et al., 2015; YU et al., 2018). Estudos *in silico* são simulações computacionais utilizadas para caracterizar experimentos biológicos conduzidos inteiramente em um computador por meio de softwares, sendo que as principais vantagens deste tipo de abordagem são, a rapidez para obter-se resultados e a diminuição de custos (MARSHALL, 2018).

2. OBJETIVO

2.1 OBJETIVO GERAL

Identificar *in silico* peptídeos bioativos das proteínas de reserva, especialmente as globulinas, das espécies *Phaseolus vulgaris* (L.) (feijão-comum), *Vigna angularis* (Willd.) (feijão-azuki), *Vigna radiata* (L.) (feijão-mungo-verde), e *Vigna unguiculata* (L.) Walp. (feijão-caupi), e revisar estudos científicos que demonstraram o potencial destas moléculas na diminuição do risco de desenvolvimento de doenças crônicas não transmissíveis.

2.2 OBJETIVOS ESPECÍFICOS

- Realizar um levantamento sobre o sequenciamento de aminoácidos das proteínas de reserva da classe das globulinas das espécies de feijões mencionadas.

- Avaliar a similaridade entre diferentes sequências de aminoácidos reportadas para a mesma proteína.

- Avaliar *in silico* o perfil de potencial atividade biológica das proteínas globulinas.

- Realizar uma predição científica quanto ao potencial de obtenção de peptídeos bioativos das globulinas dos feijões mencionados, por meio da simulação de dois processos de hidrólise distintos, sendo eles a digestão gastrointestinal com pepsina, tripsina e quimiotripsina e a hidrólise pela enzima subtilisina.

- Realizar uma predição científica quanto ao potencial dos peptídeos obtidos a partir dos dois objetivos anteriores serem bioativos.

- Confrontar os fragmentos obtidos com potencial para serem bioativos no presente estudo com sequências peptídicas identificadas por outros autores com a mesma atividade em estudos científicos realizados *in vitro.*

- Levantar a quantidade de estudos científicos disponíveis sobre peptídeos bioativos do feijão.

3. REVISÃO BIBLIOGRÁFICA

3.1 DOENÇAS CRÔNICAS NÃO TRANSMISSÍVEIS

Em geral as doenças crônicas estão relacionadas a causas múltiplas; são caracterizadas por início gradual, de prognóstico geralmente incerto, com longa ou indefinida duração. Seu desenvolvimento clínico oscila ao longo do tempo, podendo haver períodos de agudização que gerem incapacidades. Requerem intervenções com o uso de tecnologias, associadas a mudanças de estilo de vida em processo de cuidado contínuo, e que nem sempre leva à cura (BRASIL. Ministério da Saúde, 2013).

Doenças cardiovasculares, cânceres, doenças respiratórias crônicas e a diabetes melittus são as principais doenças crônicas não transmissíveis, e foram responsáveis por 51,6% do total de óbitos na população de 30 a 69 anos no Brasil em 2015 (BRASIL. Ministério da Saúde, 2019).

Estima-se que há mais de 13 milhões de pessoas vivendo com diabetes no Brasil, sendo que aproximadamente 90% dos casos são do tipo 2, e o restante do tipo 1 e gestacional (SBD-SOCIEDADE BRASILEIRA DE DIABETES, 2017). Em relação à hipertensão, este número é ainda maior, atingindo 36 milhões de adultos, contribuindo direta e indiretamente com 50% das mortes por doença cardiovascular (SBC-SOCIEDADE BRASILEIRA DE CARDIOLOGIA, 2016).

No paciente com DM2 o corpo não responde adequadamente à insulina, o que é chamado de resistência à ação da insulina. Como consequência, há elevação da glicose sanguínea, chamada de hiperglicemia. O critério de diagnóstico para esta doença é a glicemia de jejum ≥126 mg/dL. Com o passar do tempo, o pâncreas não consegue mais produzir insulina suficiente para manter os níveis de glicose normais (ADA-AMERICAN DIABETES ASSOCIATION, 2017; 2019).

A dipeptidil peptidase-IV (DPP-IV) é uma enzima que participa da inativação das incretinas, hormônios que estimulam a secreção de insulina, sendo os principais o GLP-1 (glucagon-like peptide) e o GIP (glucose-dependent insulinotropic peptide), portanto, a inibição da DPP-IV é um mecanismo utilizado para controle da DM2 (OSEGUERA-TOLEDO et al., 2014).

A hipertensão é uma condição multifatorial, caracterizada por elevação sustentada dos níveis pressórios >130 e/ou 80mmHg, e pode ser agravada por intolerância à glicose e diabetes (AMERICAN HEART ASSOCIATION, 2019).

A inibição da enzima conversora de angiotensina-I (ECA) é um alvo terapêutico no tratamento da hipertensão, pois esta catalisa a conversão da angiotensina I em II, a qual é um potente vasoconstritor, que leva à elevação da pressão sanguínea (ARIZA-ORTEGA et al., 2014).

A oxidação é um processo vital no organismo humano, no qual ocorre a produção de radicais livres (moléculas altamente reativas que contém um elétron desemparelhado em sua última camada eletrônica). Em circunstâncias normais, a oxidação é um balanço dinâmico e contínuo entre produção e eliminação de radicais livres, porém o excesso de produção destas moléculas pode causar dano contínuo às células, o que é conhecido como stress oxidativo (LI; YU, 2015). Este processo pode ocasionar danos e mutações no DNA, o que pode ser um fator de risco para o desenvolvimento de câncer e doenças relacionadas à idade como doenças cardiovasculares, autoimunes e diabetes. Por esta razão, o consumo regular de compostos antioxidantes tem sido recomendado (KHANSARI, SHAKIBA & MAHMOUDI, 2009).

3.2 FEIJÃO

De acordo com a estimativa publicada pela CONAB-Companhia Nacional de Abastecimento em janeiro de 2019, a produção total de feijão no Brasil na safra 18/19 foi de 3,099 milhões de toneladas. Sendo que 498,0 mil toneladas foram de feijão-comum preto, 1,810 milhão de toneladas de feijão-comum cores e 791,0 mil toneladas de feijão-caupi.

Originário da América Central o feijão-comum (*Phaseolus vulgaris* (L.)) está entre as principais leguminosas comestíveis (BRASIL, SEAB-Secretaria de Estado da Agricultura e do Abastecimento, 2016).

De acordo com estudo de Perazzini et al. (2008) que avaliou 8 cultivares de feijão-comum, o conteúdo de proteína variou entre 21.8% a 29.2%, sendo que após fracionamento e extração das proteínas de reserva, o conteúdo de

albuminas variou entre 14.8% a 20.8%, e o de globulinas entre 33.1% a 45.1%, representando a maioria do total de proteínas. Por outro lado, o conteúdo de glutelinas variou entre 12.8% a 41.2% enquanto que para as prolaminas, em apenas 3 cultivares os valores foram maiores que 1% do conteúdo total de proteínas.

De acordo com estudo de Mojica e Gonzalez de Mejía (2015) que avaliou 15 cultivares diferentes de feijão-comum provenientes do México e Brasil, a faseolina foi a proteína mais abundante, representando de 30.2% a 53.5% do total do conteúdo de proteínas. A faseolina (7S globulina) pertence à fração proteica das globulinas, e é uma proteína oligomérica, que consiste em duas ou três subunidades polipeptídicas denominadas α-, β-, e γ- faseolina, com pesos moleculares que variam entre 43 a 53 kDa (ROMERO et al., 1975).

As espécies de feijão do gênero Vigna são cultivadas em diversas regiões do mundo, entre elas, destacam-se o feijão-caupi (*Vigna unguiculata* (L.) Walp.), também denominado de feijão-de-corda e feijão-fradinho, *Vigna radiata* (L.), (feijão-mungo-verde) e *Vigna angularis* (Willd.) (feijão-azuki) (BELL et al., 2011).

O feijão-caupi, cultivado na Ásia, África e na América do Sul, é uma leguminosa de grande importância nas regiões tropicais e subtropicais do mundo (BRASIL, SEAB-Secretaria de Estado da Agricultura e do Abastecimento, 2016).

Segundo Awika e Duodu (2017) o feijão-caupi possui de 22-30% de proteínas em base seca, sendo que a maioria são proteínas de reserva. De acordo com estudo de Gupta et al. (2010) que avaliou 21 genótipos de feijão-caupi, o conteúdo total de proteínas variou de 22.4% a 27.9%. Dentre eles, foram selecionados os 7 genótipos com maior conteúdo de proteínas, nos quais as proteínas de reserva, entre elas as globulinas, representaram o maior conteúdo variando de 55,6% a 58.8% do total de proteínas, em seguida vieram as glutelinas (14.4% a 15.6%), as albuminas (8.2% a 11.9%) e as prolaminas (2.3% a 5.0%).

Dentre as globulinas, o feijão-caupi é composto em menor quantidade pelas leguminas (11 S globulina) e em sua maioria pelas vicilinas (FOTSO et al., 1994). As vicilinas são proteínas de reserva da classe 7 S globulina, com alta massa molecular (150 kDa) (GOMES et al., 1998).

O feijão-azuki é uma espécie oriunda da China, tradicionalmente consumida no leste da Ásia, sendo muito popular no Japão onde é consumido na forma de grãos e utilizado como ingrediente essencial em diversas preparações. Nos últimos anos, essa leguminosa também tem sido consumida em países europeus na

forma de grãos e brotos. No Brasil, é cultivada principalmente pelos colonos de origem japonesa (GUARESCHI et al., 2009; SATO et al., 2016). O feijão-azuki possui aproximadamente 55% de amido, 0,45% de gordura e 25% de proteína (YOUSIF; DEETH; CAFFIN, 2002). Na fração proteica composta pelas globulinas, a proteína de reserva 7S globulina (vicilinas) é a que está presente em maior quantidade nos grãos de feijão-azuki, correspondendo a aproximadamente 78%, enquanto a 11S globulina (leguminas) possui valores próximos a 12% (MENG e MA, 2001).

O feijão-mungo-verde é originário da região nordeste da Índia e Birmânia (Myanmar), localizados na Ásia. É cultivado na Ásia, África, América do Sul e do Norte e Austrália, e tem seu perfil de aminoácidos essenciais comparáveis ao da soja. No Brasil, a recorrente utilização de broto de feijão na culinária está associada ao aumento do consumo de feijão-mungo-verde (LI et al., 2010; LIMA et al., 2004).

O conteúdo de proteínas do feijão-mungo-verde varia entre 17-26% (MENDOZA et al., 2001), o qual é composto por 62% de globulinas, 16.3% de albuminas, 13.3% de glutelinas e 0.9% de prolaminas (AMARAL et al., 2017). Dentre as globulinas, a vicilina (8 S) corresponde a 89% do total de globulinas, enquanto que as leguminas (11 S) e as tipo básicas (7 S) correspondem a 7.6% e 3.4%, respectivamente (MENDOZA et al., 2001).

O conteúdo de proteína dos feijões pode ser influenciado por fatores como as condições ambientais do local de crescimento e de maturação das sementes, o genótipo da planta mãe e a expressão de genes que regulam a síntese e acumulação de frações proteicas e não proteicas na semente (PERAZZINI et al., 2008).

3.3 PEPTÍDEOS BIOATIVOS

Alguns componentes obtidos de origem animal e vegetal, como os carotenoides, polifenóis, fitoesteróis, ácidos graxos e peptídeos, podem ter uma influência positiva na saúde humana, ao agir em tecidos específicos ou células, e, portanto, são denominados de compostos bioativos (ASTLEY; FINGLAS, 2016).

Peptídeos bioativos são sequências de aproximadamente 2-20 aminoácidos, obtidos de proteínas de origem animal e vegetal (LI; YU, 2015). Estes compostos são liberados por processos como fermentação por micro-organismos proteolíticos, especialmente para liberar peptídeos de produtos lácteos, hidrólise por enzimas gastrointestinais, e por enzimas obtidas de plantas ou micro-organismos. Os peptídeos bioativos podem ser absorvidos no intestino, e exercerem atividades em diversas vias metabólicas, como por exemplo, as vias envolvidas na absorção de glicose e da produção de colesterol (AGYEI et al., 2016).

Algumas pesquisas têm avaliado e caracterizado peptídeos bioativos presentes em alimentos, demonstrando potencial atividade farmacológica, o que abre a possibilidade de termos alimentos funcionais para prevenção de doenças. O consumo destes alimentos pode ter um impacto positivo na saúde quando combinados com hábitos saudáveis (ARIZA-ORTEGA et al., 2014; LI; YU, 2015).

Atualmente, a maioria dos estudos caracterizando peptídeos bioativos são realizados por meio de ensaios *in vitro*. Dentre os métodos mais utilizados para a obtenção de peptídeos está a simulação da digestão gastrointestinal. As proteases mais utilizadas neste processo são a pepsina, a pancreatina e a quimiotripsina.

A pepsina é uma endopeptidase, a qual age no estômago ao hidrolisar proteínas em peptídeos, sendo responsável por menos que 20% da digestão proteica no trato gastrointestinal (SMITH, 2010). A pancreatina é uma mistura de enzimas obtidas do pâncreas, dentre elas, está presente a tripsina, a qual é uma serina endopeptidase que hidrolisa ligações peptídicas após resíduos de arginina e lisina (CHEN; RADISKY; FÉREC, 2013). Além da tripsina, o pâncreas também secreta quimiotripsina, uma endoprotease que age no duodeno clivando proteínas que possuem resíduos aromáticos na extremidade (PRASAD; HOLLINS; LAMBERT, 2010).

Durak et al. (2013) evidenciaram que peptídeos obtidos das frações proteicas de grãos de feijão-azuki através da hidrólise enzimática *in vitro*, pela simulação das condições gastrointestinais com as enzimas α-amilase, pepsina e pancreatina, demonstraram atividade inibitória da ECA, sendo que na fração composta por globulinas, o valor de IC_{50} obtido foi de 1,03 mg/mL. A IC_{50} é definida como a concentração de inibidor necessária para inibir 50% de atividade enzimática, portanto, quanto menor o seu valor, mais potente será o inibidor.

Segundo Mojica e Gonzalez de Mejía (2015), além das já conhecidas propriedades nutricionais do feijão-comum, após hidrólise do isolado proteico através da simulação da digestão gastrointestinal com as enzimas α-amilase, pepsina e pancreatina de diferentes cultivares provenientes do Brasil e do México, foram identificadas sequências peptídicas com potencial bioativo antioxidante e para inibir a ECA e DPP-IV, as quais estão envolvidas com a fisiopatologia das doenças crônicas hipertensão e DM2, respectivamente.

Outro método bastante utilizado para a obtenção de peptídeos com potencial atividade biológica a partir de proteínas presentes em alimentos, é a hidrólise com enzimas comerciais. De acordo com estudo realizado por Segura-Campos; Chel-Guerrero e Betancur-Ancona (2011), após hidrólise das proteínas presentes no feijão-caupi com a enzima Flavourzyme® (complexo de proteases fúngica que cataboliza proteínas através da hidrólise de ligações peptídicas) a fração peptídica < 1 kDa obteve a maior atividade no ensaio de inibição da ECA in vitro, com IC_{50} = 0,04 µg/mL.

A utilização de peptídeos bioativos inibidores da ECA derivados do feijão, pode ser uma alternativa para minimizar efeitos adversos causados por medicamentos sintéticos, como rash cutâneo, proteinúria e distúrbios na percepção do gosto (ARIZA-ORTEGA et al., 2014).

Estudo realizado por Oseguera-Toledo; Gonzalez de Mejía; Amaya-Llano (2015) demonstrou que após a simulação da digestão gastrointestinal do hidrolisado proteico de feijão-comum obtido pela ação da Alcalase® (forma comercial da subtilisina, obtida do *Bacillus licheniformis*), a qual é uma endopeptidase com atividade em ésteres de peptídeos e aminoácidos, produziu peptídeos bioativos com potencial para inibir as enzimas DPP-IV e aumentar a secreção de insulina pelas células iNS-1E (*insulin secreting beta cell derived line*).

Li et al. (2006) isolaram e sequenciaram três tipos de peptídeos inibidores da ECA a partir da hidrólise pela Alcalase durante 2 h do isolado proteico de feijão-mungo-verde, sendo eles: KDYRL; VTPALR; KLPAGTLF, com valores de IC_{50} = 26,5 µM, 82,4 µM e 13,4 µM respectivamente.

Rocha et al. (2015) demonstraram que a hidrólise enzimática pela ação da Alcalase do concentrado proteico obtido da farinha de feijão-comum germinado, gerou peptídeos bioativos com alta capacidade antioxidante, além de identificar a sequência peptídica RGPLVNPDPKPFL presente na proteína faseolina,

a qual tem potencial predito para interagir com o sítio ativo da enzima DPP-IV. Os resultados descritos, indicam que o feijão-comum tem potencial para dar origem a ingredientes para utilização no controle da DM2.

Devido ao potencial de peptídeos bioativos encontrados no feijão de inibir enzimas como a DPP-IV, e ECA, envolvidas com o aparecimento de doenças crônicas, o consumo de feijão como suplemento dietético pode contribuir para o controle da DM2 e hipertensão (MOJICA; LUNA-VITAL; GONZALEZ DE MEJÍA, 2017).

Apesar de serem realizados em menor quantidade, há também na literatura estudos realizados *in vivo* para caracterizar peptídeos bioativos obtidos a partir de alimentos. Ariza-Ortega et al. (2014) concluiu que após hidrólise proteica com Alcalase a partir do feijão-comum, a fração peptídica de 3 -10 kDa possuiu atividade inibitória da ECA *in vitro* e atividade anti-hipertensiva *in vivo* na dose de 4 mg/kg, ao identificar a diminuição da pressão sistólica em ratos hipertensos naturalmente após duas horas de administração intraperitoneal.

Li et al. (2006) concluíram que a hidrólise enzimática em diferentes tempos pela Alcalase do isolado proteico de feijão-mungo-verde, gerou peptídeos com atividade inibitória da ECA, sendo que a maior atividade foi detectada após 2 h de hidrólise, IC_{50} = 0,64 mg proteína/mL, e que após administração oral deste hidrolisado em ratos hipertensivos naturalmente, a uma dose de 600 mg/kg, houve diminuição na pressão sistólica após 2, 4, 6 e 8 h da administração, sendo que a redução máxima de 30,8 mmHg foi observada após 6 h.

Estudo de Yao; Cheng e Ren (2014) realizado com ratos diabéticos alimentados durante 42 dias com extratos ricos em proteínas (86,04%), obtidos a partir do extrusado de feijão-azuki, concluiu que a concentração de glicose sanguínea após 30, 60, 90 e 120 min da administração de glicose oral, foi menor nos ratos diabéticos que receberam o extrato rico em proteínas, quando comparados com o grupo controle, sugerindo que o consumo de proteínas obtidas a partir do extrusado de feijão-azuki pode auxiliar na modulação da DM2 e suas complicações.

Estudo clínico duplo-cego, placebo-controlado realizado com 22 indivíduos entre eles homens e mulheres, com idade variando entre 21 à 55 anos, nos Estados Unidos e Canadá, avaliou o efeito do consumo do isolado comercial de proteínas do feijão-mungo-verde consistindo de 92% de proteína (GLUCODIA™), no metabolismo de glicose e lipídico. O principal componente do isolado proteico é a

proteína de reserva 8 S globulina, a qual representa 80% do total. À uma dose diária de 3.0 g, foi observado diminuição significativa na média do modelo de avaliação homestática de resistência à insulina, do nível médio de triacilgliceróis, e aumento nos níveis de adiponectina sérica, quando em comparação com o grupo controle, sugerindo que o GLUCODIA™ pode ser útil na prevenção de resistência à insulina e do acúmulo de gordura visceral (KOHNO et al., 2017).

Além da possibilidade de peptídeos obtidos do feijão de atuaram em alvos terapêuticos da DM2 e hipertensão, as quais as propriedades tem sido extensivamente estudadas, há também na literatura outras atividades biológicas descritas para estas moléculas. Estudo de Amaral et al. (2017) após isolar a proteína vicilina do feijão-mungo-verde, e submetê-la à hidrólise enzimática *in vitro* com pepsina e pancreatina simulando as condições da digestão gastrointestinal, concluiu que as frações peptídicas de 10, 12, 14, 22 e 32 kDa foram responsáveis por reduções de 63,7%, 64,8%, 62,6%, 67% e 65,5%, respectivamente, de atividade da enzima HMG-CoAr, a qual participa da via metabólica que produz colesterol e é alvo de fármacos como as estatinas, indicando possível atividade anticolesterolêmica.

A partir da fração não digerível de diferentes cultivares de feijão-comum, Luna Vital et al., (2014) evidenciaram que após simulação da digestão gastrointestinal com pepsina e pancreatina do isolado proteico obtido, as sequências peptídicas formadas (GLTSK, LSGNK, GEGSGA, MPACGSS e MTEEY) representaram 70% do total de proteínas, sendo que estas contribuíram para o efeito antiproliferativo de células de câncer colorretal, por meio da modificação de moléculas envolvidas no ciclo de aprisionamento ou apoptose celular.

Estudo dos peptídeos bioativos obtidos a partir do feijão-comum, pela hidrólise proteica enzimática com Alcalase, realizado por Ariza-Ortega et al. (2014), identificou que a fração peptídica <1 kDa apresentou atividade antioxidante e antimicrobiana ao inibir o crescimento de micro-organismos patógenos como a *Shigella dysenteriae.*

Oseguera-Toledo et al. (2011) concluíram que após hidrólise com Alcalase e simulação da digestão gastrointestinal com pepsina e pancreatina do isolado proteíco de feijão-comum, os peptídeos formados inibiram a expressão das enzimas ciclooxigenase-2, (COX2) e oxido nítrico sintase, e a produção de prostaglandinas E2, oxido nítrico, e a transativação de NF-κB, sendo estes, importantes marcadores e mediadores envolvidos em processos inflamatórios.

Portando, hidrolisados proteicos obtidos a partir do feijão-comum podem auxiliar no manejo de doenças associadas a processos inflamatórios crônicos como o câncer.

3.3.1 Mecanismo de ação dos peptídeos bioativos

3.3.1.1 Inibição de enzimas

De acordo com Mojica; Chen e Gonzalez de Mejía (2015) a sequência peptídica e o tipo de aminoácido são determinantes no potencial de interação entre os grupos funcionais dos peptídeos bioativos, com os aminoácidos presentes no sítio ativo das enzimas, os quais vão determinar a afinidade pelo inibidor. As interações dependem da distância e dos grupos funcionais presentes nas cadeias laterais, portanto, para que ocorra a inibição competitiva dessas enzimas, os compostos bioativos precisam se posicionar de maneira adequada para interagir com os aminoácidos presentes no sítio catalítico. As principais interações preditas a partir das sequências peptídicas encontradas são ligações de hidrogênio, interações polares e hidrofóbicas.

Biologicamente, a angiotensina I é convertida em II no sítio catalítico da ECA, ocorrendo elevação da pressão sanguínea devido ao efeito vasoconstritor da molécula formada. O sítio catalítico da ECA é formado por três subunidades, nas quais, a angiotensina I irá interagir por meio de três aminoácidos hidrofóbicos presentes em sua região C-terminal, sendo eles, a prolina, histidina e fenilalanina. Portanto, peptídeos capazes de inibir a ECA mais potentemente, preferencialmente possuem aminoácidos como tirosina, prolina, triptofano, fenilalanina e leucina em sua extremidade C-terminal, havendo uma correlação positiva entre a hidrofobicidade dos aminoácidos presentes na extremidade C-terminal e atividade inibitória da ECA. Aminoácidos carregados positivamente na região C-terminal, como arginina e lisina também promovem atividade inibitória da ECA. Aminoácidos aromáticos ou alcalinos na extremidade N-terminal de peptídeos inibidores da ECA podem aumentar a atividade inibitória, como arginina, glicina, valina, alanina e isoleucina. Em geral, os peptídeos inibidores da ECA apresentam baixa massa molecular (LI; YU, 2015).

Em relação à DPP-IV, a interação de peptídeos inibidores com o sítio ativo da enzima ainda não está totalmente compreendida. Em geral, bons inibidores contêm de 2 a sete aminoácidos, com prolina ou alanina na penúltima posição da extremidade N-terminal (POWER et al., 2014).

3.3.1.2 Atividade antioxidante

Vias antioxidantes podem se dar por meio da inativação de oxigênio ativo, neutralização de radicais livres, quelação de íons metálicos e da redução da formação de peróxido de hidrogênio. Peptídeos bioativos com aminoácidos hidrofóbicos possuem alta correlação com atividade antioxidante, quanto mais aminoácidos hidrofóbicos e aromáticos, maior a atividade antioxidante, devido ao fato de que estes tendem a se combinar primeiro com os radicais livres (LI; YU, 2015).

3.4 ESTUDOS *IN SILICO*

A produção industrial de peptídeos bioativos ainda é limitada, devido à falta de tecnologias adequadas para processos como a hidrólise de proteínas, separação e purificação. Embora na última década a produção de peptídeos tenha sido explorada extensivamente em termos de fontes de proteínas de origem animais, especialmente do leite, atualmente a obtenção de peptídeos de fontes vegetais se tornou interessante devido ao seu custo inferior e à variedade de plantas disponíveis (RIZZELLO et al., 2016).

Atualmente muitos dos estudos a respeito de peptídeos bioativos obtidos de fontes vegetais são realizados por meio de ensaios *in vitro*, constatando a atividade dessas moléculas em marcadores biológicos, como por exemplo as enzimas. Estudos *in vivo* são mais escassos, sendo que não é comum que estes tenham uma abordagem farmacológica, em termos de biodisponibilidade e interação com outros componentes alimentares (LI; YU, 2015).

Estudos *in silico* são simulações computacionais utilizadas para caracterizar experimentos biológicos conduzidos inteiramente em um computador por meio de softwares (MARSHALL, 2018). Bases de dados como a UniProt (The UniProt Consortium, 2017), BIOPEP-UWM (MINKIEWICZ et al. 2008) e PeptideRanker (MOONEY et al., 2012) são gratuitas e têm sido muito utilizadas em estudos científicos para confirmar sequências e predizer a bioatividade de peptídeos (LUNA VITAL et al., 2014; MOJICA; GOZALEZ DE MEJÍA, 2015; OSEGUERA-TOLEDO; GONZALELZ DE MEJÍA; AMAYA-LLANO, 2015; ROCHA et al., 2015; YU et al., 2018).

A base de dados UniProt provê informações sobre a sequência proteica das mais diversas proteínas, por meio da compilação de dados obtidos em estudos científicos. A partir da sequência proteica obtida, pode-se utilizar a base de dados BIOPEP-UWM para avaliar o perfil de potencial atividade biológica e para obter-se peptídeos bioativos através da simulação de processos proteolíticos. Posteriormente, os fragmentos peptídicos obtidos na base de dados BIOPEP, podem ser avaliados na base de dados PeptideRanker, a qual realiza uma predição de classes de peptídeos por meio de um rank que representa a probabilidade de que o peptídeo será bioativo.

Apesar de não avaliar com precisão a estrutura tridimensional de proteínas, este tipo de estudo oferece a vantagem de usar processos de digestão proteica *in silico*, para uma triagem de proteínas e proteases, prevendo as melhores combinações para obtenção de peptídeos bioativos a partir de matérias-primas animais e vegetais, evitando desperdícios (UDENIGWE; FOGLIANO, 2017).

Diante da possibilidade de obtenção de uma vasta quantidade de peptídeos com alta diversidade estrutural, a bioinformática pode ser uma ferramenta útil na seleção de potenciais moléculas a exercerem atividade biológica *in vivo* de acordo com o efeito desejado, facilitando a seleção das melhores fontes proteicas e de processos adequados, visando também a produção de ingredientes e produtos com atividade farmacológica.

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4. MATERIAL E MÉTODOS

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

ARTIGO CIENTÍFICO: BIOACTIVE PEPTIDES FROM BEANS WITH THE POTENTIAL TO DECREASE THE RISK OF DEVELOPING NONCOMMUNICABLE CHRONIC DISEASES.

5. RESULTADOS E DISCUSSÃO

5.1 ARTIGO CIENTÍFICO: BIOACTIVE PEPTIDES FROM BEANS WITH THE POTENTIAL TO DECREASE THE RISK OF DEVELOPING NONCOMMUNICABLE CHRONIC DISEASES.

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Word count: 10,251.

Abstract: Type 2 diabetes mellitus (T2D) and hypertension are highly associated diseases and are among the most important noncommunicable chronic diseases (NCDs) currently. The treatment of these diseases is based on medications and changes in lifestyle, however, it does not always lead to a cure. Currently, several studies demonstrated that peptides obtained from proteins of different bean species have the potential to act on therapeutic targets of NCDs. Because of the possibility of obtaining peptides with high structural diversity from the hydrolysis of proteins present in foods, bioinformatics may be a simple and inexpensive tool for selecting suitable protein sources and candidate peptides to exert biological activity *in vivo*. Therefore, the objective of this study was to identify *in silico,* the possibility of obtaining peptides with potential biological activity from the globulin storage proteins of the bean species *Phaseolus vulgaris* (L.), *Vigna angularis* (Willd.), *Vigna radiata* (L.) and *Vigna unguiculata* (L.) Walp., using the UniProt, BIOPEP and PeptideRanker databases, as well as reviewing available researches that evidenced through *in vitro* assays bioactive properties of peptides obtained from beans. For all the studied species, the highest frequency of bioactive fragments occurrence was found for the dipeptidyl peptidase-IV inhibition, followed by angiotensin-converting enzyme inhibition and antioxidant activity. Both enzymes are therapeutic targets of drugs used for T2D and hypertension treatment, respectively. These results suggest that the daily consumption of this legume may be considered a strategy to reduce the risk of developing T2D and hypertension, besides the possibility of obtaining functional products from these protein sources.

Keywords: Type 2 diabetes mellitus, hypertension, bioinformatics, UniProt, BIOPEP.

Introduction

According to the World Health Organization (WHO, 2018), an estimated 41 million deaths occurred due to noncommunicable chronic diseases (NCDs) in 2016, accounting for 71% of the Mundial total of 57 million deaths. Cardiovascular disease, cancer, chronic respiratory disease, and diabetes are the four main diseases with the majority of deaths, accounting for 17.9 million (44%), 9.0 million (22%), 3.8 million (9%) and 1.6 million (4%) of deaths, respectively.

Generally, the NCDs are related to multiples causes, and they are characterized by gradual initiation, with an uncertain prognosis, and with a long or indefinite duration. Their clinic development oscillates over time, and there may be periods of exacerbation that generate disabilities. These diseases require interventions with the use of medications, associated with changes in lifestyle, in a process of continuous care that does not always lead to a cure (Brazil, Health Ministry, 2013).

Diabetes and hypertension are highly associated and they are considered global public health problems due to their high incidence. According to the International Diabetes Federation (IDF, 2017), there are 425 million people with diabetes in the world. Gestational diabetes, type 1 diabetes and type 2 diabetes (T2D) are the three main types of this disease. The last one is responsible for about 90% of all cases. In relation to hypertension, the incidence is even higher, reaching 1.13 billion in 2015 (NCD Risk Factor Collaboration [NCD-RisC], 2017).

Diabetes can lead to cardiovascular diseases, amputation, kidney failure, and blindness, this happens because of the persistently high blood glucose levels which cause generalized vascular damage, affecting the heart, nerves, kidney, and eyes (IDF, 2017). Hypertension is also an important risk factor for cardiovascular diseases and chronic kidney disease (NCD-RisC, 2017).

Some vegetal and animal compounds may have a positive influence on human health, acting in specific tissues or cells, and therefore they are

denominated bioactive compounds (Astley & Finglas, 2016). Among them, there are the bioactive peptides, which are sequences of approximately 2-20 amino acids (Li & Yu, 2015).

Due to the possibility of obtaining bioactive peptides from the hydrolysis of the bean's storage proteins, this legume is the aim of several researches currently. It was demonstrated that *in vitro* enzymatic hydrolysis by Alcalase® and/or by the simulation of gastrointestinal digestion with pepsin and pancreatin of the bean's protein from species such as *Phaseolus vulgaris* (L.) (common bean), *Vigna angularis* (Willd.) (adzuki bean), *Vigna radiata* (L.) (mung bean), and *Vigna unguiculata* (L.) Walp. (cowpea), generated bioactive peptides with antioxidant potential and inhibition activity of dipeptidyl peptidase-IV enzyme (DPP-IV), which is a molecular target in the treatment of T2D *(*Mojica & Gonzalez de Mejía, 2015; Rocha, Hernandez, Chang & Gonzalez de Mejía, 2014; Rocha et al., 2015), to inhibit the angiotensin-converting enzyme (ACE), which is a molecular target of antihypertensive medications (Li, Wan, Le & Shi 2006; Mojica & Gonzalez de Mejía, 2015) and to decrease inflammatory markers and mediators (Oseguera-Toledo, Gonzalez de Mejía, Dia and Amaya-Llano, 2011).

Despite the existence of well-established protocols for T2D treatment and hypertension, there is a search for alternatives that reduce the adverse effects caused by medications and that aid in the treatment. Therefore, it is of interest the development of ingredients and food products that contain bioactive molecules with potential action in the therapeutic targets of these diseases, and that can be inserted in the diet of individuals in the developmental stage.

However, the industrial production of bioactive peptides is still incipient, due to the difficulty in selecting suitable molecules and the lack of technologies for processes such as proteins hydrolysis, separation, and purification, besides the lack of studies that characterize the bioavailability of these molecules (Rizzello et al., 2016).

Currently, many of the studies about bioactive peptides are performed by *in vitro* assays, evidencing the activity of these molecules in biological markers, such as enzymes. *In vivo* studies are more scarce, and it is not common for these to have a pharmacological approach in terms of bioavailability and interaction with other food components (Li & Yu, 2015).

In silico studies are computational simulations used to characterize

biological experiments conducted entirely on a computer through softwares (Marshall, 2018). Given the possibility of obtaining peptides with high structural diversity from the hydrolysis of proteins present in foods, bioinformatics can be a useful tool in the selection of molecules with the potential to exert biological activity according to the desired effect, facilitating the selection of adequate protein sources, and supporting the selection of peptides for realize *in vivo* studies that could be good candidates for industrial production.

Nowadays, databases such as UniProt (The UniProt Consortium, 2017), BIOPEP-BWM (Minkiewicz, Dziuba, Iwaniak, Dziuba & Darewicz, 2008) and PeptideRanker (Mooney, Haslam, Pollastri & Shields, 2012) have been used by studies to confirm sequences and to predict peptide bioactivity (Luna Vital, Gonzalez de Mejía, Dia & Loarca-Piña, 2014; Mojica & Gonzalez de Mejía, 2015; Oseguera-Toledo, Gonzalez de Mejía, Amaya-Llano, 2015; Rocha et al., 2015; Yu et al., 2018). Thus, the aim of this study was to realize a scientific prediction by identifying *in silico,* peptides with potential bioactivity of the bean's storage proteins, specifically the globulins, from the species *Phaseolus vulgaris* (L.) (common bean), *Vigna angularis* (Willd.) (adzuki bean), *Vigna radiata* (L.) (mung bean), and *Vigna unguiculata* (L.) Walp. (cowpea) using the databases UniProt (The UniProt Consortium, 2017), BIOPEP-BWM (Minkiewicz et al., 2008) and PeptideRanker (Mooney et al., 2012). Another objective was to review the available research demonstrating that the proteins and peptides from the bean species studied have bioactive properties with the potential to decrease the risk of developing NCDs.

Beans

According to the Food And Agriculture Organization of the United Nations (FAO, 2019), the production of dry beans in 2017 was bigger than 31 million tonnes. Brazil was the third major producer, with approximately 3 million tonnes. Similar to other legumes, beans accumulate a large amount of protein in their seeds, the most part of them are storage proteins, which receive this name because they are mobilized from specialized subcellular compartments to provide nutrients for the growth of a new plant during the germination process (Argos, Narayana & Nielsen, 1985).

The seed storage proteins can be classified into fractions according to their solubility. Most of them are globulins, which are soluble in neutral saline solutions, but there are also the albumins which are soluble in water, the glutelins which are soluble in alkaline or acids diluted solutions, and the prolamins which are soluble in alcohol (Osborne, 1912).

According to Osborne's criteria based on the solubility and coagulation properties, Danielsson (1949) used ammonium sulphate at three different degrees of saturation (15, 40 and 70%) and dialysis to precipitate the globulins present in the sodium chloride extract of ground seeds of different legumes, including the bean species *Phaseolus vulgaris* (L.), to isolate two globulin major fractions, the legumin (doesn't coagulate on heating at 100 °C) and vicilin (coagulates on heating at 95-100 °C and is soluble in salt solutions more dilute than legumin). It was found that vicilin and legumin occur in the seeds of practically all the studied species, and they have sedimentation constant in Svedberg units varying from 6.77 to 8.69 S for vicilin and 10.10 to 13.67 S for legumin.

Originated in Central América, the common bean (*Phaseolus vulgaris* (L.)) is among the main edible legume (BRAZIL. State Secretariat of Agriculture and Food Supply [SEAB], 2016). According to a study by Perazzini et al. (2008) that evaluated 8 common bean cultivars, the total protein content varied from 21.8 to 29.2%, and after fractionation and extraction of the storage proteins according to their solubility, the globulins corresponded for 33.1 to 45.1%, representing the majority of the total protein content. The glutelins varied from 12.8 to 41.2%, and the albumins varied from 14.8 to 20.8%, while the prolamins obtained values above 1.0% only in three of the studied cultivars.

Mojica & Gonzalez de Mejía (2015) evaluated 15 different cultivars of common bean from México and Brazil, and the phaseolin (vicilin 7 S globulin) from the globulin fraction, represent 30.2 to 53.5% of the total protein content. On the other hand, Muhling, Gilroy & Croy (1997) partially purified another globulin protein from *Phaseolus vulgaris* (L.), the legumin (11 S globulin), which accounted for 3% of the total content of protein in the seeds.

The bean species of the genus Vigna are cultivated in several regions of the world, among them, stand out the *Vigna unguiculata* (L.) (cowpea), *Vigna radiata* (L.) (mung bean) and *Vigna angularis* (Willd.) (adzuki bean) (Bell, Bennett, Ryan & Clarke, 2010).

The cowpea is cultivated in Ásia, África, and South América, and it is a legume with high importance in the tropical and subtropical regions of the world (BRAZIL. SEAB, 2016). According to Awika & Duodu (2016), the total protein content of the cowpea varies from 22 to 30%, the majority are storage proteins. Gupta, Singh, Malhotra, Boora & Singal (2010) evaluated 21 genotypes of cowpea and the total protein content varied from 22.4 to 27.9%, among them, 7 genotypes with the major protein content were selected, in which the globulins represent the major fraction of the total protein content, accounting for 55.6 to 58.8%, followed by the glutelins (14.4 to 15.6%), the albumins (8.2 to 11.9%) and the prolamins (2.3 to 5.0%). Among the globulins, the cowpea is composed in minor quantity by the legumin (11 S globulin), and in major quantity by the vicilin (7 S globulin) (Fotso, Azanza, Pasquet & Raymond, 1994).

The adzuki bean is a species native from China, traditionally consumed in the east of Asia, very popular in Japan, where it is consumed in the form of grains and used as an essential ingredient in various preparations. In recent years, this legume has also been consumed in European countries in the form of grains and bean sprout. In Brazil, it is cultivated mainly by colonists of Japanese origin (Guareschi, Araujo, Gazolla & Rocha, 2009; Sato, Mukai, Kataoka & Kurasaki, 2016). The adzuki bean has approximately 25% of proteins (Yousif, Deeth & Caffin, 2002). The globulin fraction of the adzuki bean is composed in majority by the storage protein vicilin (7 S globulin), representing 78%, and in minority by the legumin (11 S globulin), accounting for 12% (Meng & Ma, 2001).

The mung bean is native from the northeastern region of India and Myanmar, both located in Asia. It is cultivated in Asia, Africa, South, and North America and Australia, and has its essential amino acid profile comparable to that of soybeans (Li, Shu, Yan & Shen, 2010). In Brazil, the recurrent use of bean sprouts in cooking is associated with increased consumption of mung bean (Lima et al., 2004). The mung bean content of protein varies from 17 to 26% (Mendoza, Adachi, Bernardo & Utsumi, 2001), the globulins correspond for the major fraction, accounting for 62%, followed by albumins (16.3%), the glutelins (13.3%) and the prolamins (0.9%) (Amaral, Ferreira, Silva, Neves & Demonte, 2017). Among the globulins, the vicilin (8 S globulin) corresponds to 89% of the total, while the legumin (11 S globulin), and the basic 7 S globulin, correspond for 7.6% and 3.4% respectively (Mendoza et al., 2001).

The environmental conditions of the seed growth and maturation site, the expression of genes that regulate the synthesis and accumulation of protein and nonprotein fractions in the seed, and the genotype of the maternal plant are key factors that influence the content of proteins in beans (Perazzini et al., 2008).

Type Two Diabetes Mellitus and Arterial Hypertension

In the patient with T2D, the body does not respond adequately to the action of the insulin, therefore there is an elevation of the blood glucose level named as hyperglycemia. The diagnostic criterion for this disease is fasting glucose ≥126 mg /dL. Because of this inefficient action of the insulin, the production of this hormone increases as a response trying to reduce de glucose levels, but over time the pancreas cannot produces enough insulin to maintain the blood glucose levels normal (American Diabetes Association [ADA], 2017, 2019; IDF, 2017).

The DPP-IV is an enzyme that participates in the inactivation of the incretins, which are hormones that stimulate the secretion of insulin. The main incretins are the GLP-1 (glucagon-like peptide) and GIP (glucose-dependent insulinotropic peptide), therefore the inhibition of the DPP-IV is a mechanism used for controlling the T2D (Oseguera-Toledo, Gonzalez de Mejía, Reynoso-Camacho, Cardador-Martínez & Amaya-Llano, 2014).

The arterial hypertension is a multifactorial condition characterized by sustained elevation of the blood pressure levels >130 and/or 80 mmHg and may be aggravated by glucose intolerance and diabetes (American Heart Association, 2019). The inhibition of ACE is a therapeutic target in the treatment of hypertension, since it catalyzes the conversion of the angiotensin I in II, which is a potent vasoconstrictor, leading to elevated blood pressure (Ariza-Ortega et al., 2014).

Bioactive Peptides

The bioactive peptides can be released by processes such as fermentation by proteolytic microorganisms, especially to release peptides from dairy products, hydrolysis by gastrointestinal enzymes and by enzymes obtained from plants and microorganisms. After ingestion, the bioactive peptides can be absorbed

in the intestine and perform activities in various metabolic pathways, such as the pathways involved in glucose uptake and elevation of blood pressure (Agyei, Ongkudon, Wei, Chan & Danquah, 2016).

Although peptide production has been extensively explored in the last decade in terms of animal source of proteins, especially milk, plant-derived peptides have now become interesting because of their lower cost and the variety of plants available (Rizzello et al., 2016).

Some researches have evaluated and characterized bioactive peptides from foods, demonstrating potential pharmacological activity, which opens the possibility of obtaining therapeutically functional foods. The consumption of these foods may have a positive impact on human health when combined with healthy habits (Ariza-Ortega et al., 2014; Li, Yu, 2015).

Currently, most studies characterizing bioactive peptides are performed by *in vitro* assays. Among the methods most used to obtain these peptides, there is the simulation of gastrointestinal digestion. The most commonly used proteases in this process are pepsin, pancreatin, and chymotrypsin.

Pepsin is an endopeptidase that degrades proteins into peptides, which is responsible for less than 20% of the protein digestion in the gastrointestinal tract, acting on the stomach (Smith & Morton, 2010). Pancreatin contains several enzymatic components from the pancreas, including trypsin, which is a serine endopeptidase which hydrolyzes peptide bonds after arginine or lysine residues (Chen, Radisky & Férec, 2013). Besides trypsin, during the gastrointestinal digestion, the pancreas also secrets chymotrypsin, an endoprotease, which cleaves proteins with aromatic amino acid residues, acting on duodenum (Prasad, Hollins & Lambert, 2010).

Durak, Baraniak & Jakubczyk, (2013) evidenced that bioactive peptides obtained from the protein fractions of the adzuki bean by *in vitro* enzymatic hydrolysis through the simulation of gastrointestinal conditions with the enzymes α-amylase, pepsin and pancreatin, demonstrated inhibitory activity of the ACE, and, in the fraction composed by globulins, the IC_{50} value was 1,03 mg/mL. The IC_{50} is defined as the inhibitor concentration required to inhibit 50% of enzyme activity, therefore, the lower the value, more potent the inhibitor.

According to Mojica & Gonzalez de Mejía (2015), in addition to the well-known nutritional properties of common bean, after hydrolysis of the protein

isolate through the simulation of gastrointestinal digestion with α-amylase, pepsin and pancreatin, of different cultivars from Brazil and Mexico, it was identified peptide sequences with antioxidant bioactive potential and for inhibiting the ACE and DPP-IV enzymes, which are therapeutic targets in the treatment of hypertension and T2D, respectively.

The hydrolysis with commercial enzymes is another method widely used to obtain peptides with potential biological activity from proteins present in foods. According to study carried out by Segura-Campos, Chel-Guerrero & Betancur-Ancona (2011) after hydrolysis of the proteins present in cowpea with Flavourzyme® (fungal protease complex which catabolizes proteins by hydrolysis of peptide bonds), the peptide fraction < 1 kDa obtained the highest activity in the *in vitro* assay of inhibition of ACE, with IC_{50} = 0.04 µg/mL.

The use of bioactive peptides inhibitors of ACE derived from bean may be an alternative to minimize adverse effects caused by synthetic drugs, such as skin rash, proteinuria, and taste perception disorders (Ariza-Ortega et al., 2014).

A study conducted by Oseguera-Toledo et al. (2015) demonstrated that the common bean protein hydrolysate obtained through the action of Alcalase®, possessed bioactive peptides with potential to inhibit the enzymes DPP-IV and increasing the insulin secretion by iNS-1E cells. Alcalase® is a commercial form of subtilisin, obtained from *Bacillus licheniformis.* Subtilisins are serine proteases which catalyze the hydrolysis of proteins with broad specificity for peptide bonds (Hera, 2007).

Li et al. (2006) isolated and sequenced three types of ACE inhibitory peptides from the mung bean protein isolate hydrolyzed by Alcalase for 2 h: KDYRL, VTPALR, KLPAGTLF, with IC_{50} values of 26.5 µM, 82.4 µM and 13.4 µM respectively.

Rocha et al. (2015) demonstrated that the enzymatic hydrolysis by the action of Alcalase of the protein concentrate obtained from germinated common bean flour, generate bioactive peptides with high antioxidant capacity, besides identifying the peptide sequence RGPLVNPDPKPFL present in the phaseolin protein, which has predicted potential for interacting with the active site of the DPP-IV enzyme. These results indicate that the common bean has the potential to originate ingredients for use in the control of T2D.

Due to the potential of bioactive peptides obtained from beans for inhibiting enzymes such as DPP-IV and ACE, involved in chronic diseases, bean consumption as a dietary supplement may improve the quality of life of patients with T2D and hypertension (Mojica, Luna-Vital & Gonzalez de Mejía, 2017).

Although they are performed in less quantity, there are also in the literature *in vivo* studies to characterize bioactive peptides obtained from foods. Ariza-Ortega et al. (2014) concluded that after protein hydrolysis with Alcalase from common bean, the peptide fraction of 3 -10 kDa had ACE inhibitory activity *in vitro* and antihypertensive activity *in vivo* at 4 mg/kg dose, by identifying the decrease in systolic blood pressure in naturally hypertensive rats after two hours of intraperitoneal administration.

The study of Li et al. (2006) concluded that enzymatic hydrolysis at different times by the Alcalase of the mung bean protein isolate, generate peptides with ACE inhibitory activity. The highest activity was detected after 2 h of hydrolysis, IC_{50} = 0,64 mg/mL. In addition, after the oral administration of this hydrolysate in hypertensive rats at a dose of 600 mg/kg, there was a decrease in systolic blood pressure after 2, 4, 6 and 8 of administration, the maximum decrease of 30,8 mmHg was observed after 6 h (Li, Shi, Liu, Le, 2006).

Study of Yao, Cheng & Ren (2014) with diabetic rats fed for 42 days with protein-rich extracts (86.04%) obtained from the adzuki bean extrusion, concluded that blood glucose concentration after 30, 60, 90 and 120 min of oral glucose administration was lower in diabetic rats that received the protein-rich extract when compared to the control group, besides decreasing the serum triglyceride level, the blood urea nitrogen level in 19.9%, which is an important marker of renal dysfunction, and increasing the content of high-density lipoprotein cholesterol (HDL-c). The results suggest that consumption of proteins obtained from the extrusion of adzuki bean may aid in the modulation of T2D and its complications.

A double-blind, placebo-controlled clinical study of 22 subjects, among them male and female, aged 21 to 55 years, in the United States and Canada, evaluated the effect of consumption of commercial mung bean protein isolate, consisting of 92% protein (GLUCODIA ™) on glucose and lipid metabolism. The major component of the protein isolate is the 8 S storage protein globulin, which accounts for 80% of the total. At a daily dose of 3.0 g, there was a significant decrease in the mean homostatic insulin resistance assessment model, the mean

triacylglycerol level, and an increase in serum adiponectin levels, when compared with the control group, suggesting that GLUCODIA may be useful in preventing insulin resistance and visceral fat accumulation (Kohno et al., 2017).

Besides the possibility of peptides obtained from bean act in therapeutic targets of DM2 and hypertension, which properties have been extensively studied, there are also in the literature other biological activities described for these molecules. Study of Amaral et al. (2017) concluded that after isolating the vicilin protein from mung bean and subjecting it to *in vitro* enzymatic hydrolysis with pepsin and pancreatin, simulating gastrointestinal digestion conditions, the peptide fractions of 10, 12, 14, 22 and 32 kDa were responsible for reductions of 63.7%, 64.8%, 62.6%, 67% and 65.5%, respectively of the 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoAr) enzyme activity. This enzyme participates in the metabolic pathway that produces cholesterol and it is a molecular target of drugs such as statins, indicating possible anticholesterolemic activity. Together with hypertension, hypercholesterolemia is also a risk factor for the development of cardiovascular diseases (SBC, 2017).

From the non-digestible fraction of different common bean cultivars, Luna Vital et al. (2014) evidenced that after simulation of gastrointestinal digestion with pepsin and pancreatin of the protein isolate obtained, the peptide sequences formed (GLTSK, LSGNK, GEGSGA, MPACGSS, and MTEEY) represented 70% of the total proteins, which contributed to the antiproliferative effect of colorectal cancer cells by modifying the molecules involved in the cycle of cell trapping or apoptosis.

A study by Oseguera-Toledo et al. (2011) concluded that after hydrolysis with Alcalase and simulation of gastrointestinal digestion with pepsin and pancreatin of the common bean protein isolate, the peptides formed inhibited the expression of cyclooxygenase-2 (COX2) and nitric oxide synthase enzymes, the production of prostaglandins E2 and the NF-κB transactivation. These are important markers and mediators involved in inflammatory processes, therefore, protein hydrolysates obtained from common bean may help in the management of diseases associated with chronic inflammatory processes such as T2D and cancer.

A study of the bioactive peptides obtained from common bean by enzymatic protein hydrolysis with Alcalase performed by Ariza-Ortega et al. (2014) identified that the peptide fraction <1 kDa presented antioxidant activity and

antimicrobial activity by inhibiting the growth of pathogenic microorganisms such as *Shigella dysenteriae.*

It is important to highlight that for the peptides to carry out a biological activity, they must reach their molecular target intact. Although gastrointestinal enzymes are extremely important in releasing peptide sequences with a potential biological activity, which were previously inactive in the core of the source protein, during the digestion process the released peptides can also be inactivated during further digestion by peptidases present on the brush border and on the cytoplasm of the intestinal epithelial cells (Segura-Campos, Chel-Guerrero, Betancur-Ancona & Hernandez-Escalante, 2011).

In the case of molecules formed by more than three amino acids, they may be hydrolyzed in the extracellular space by enzymes present on the brush border membrane of the intestinal epithelium, and the dipeptides and tripeptides are preferably absorbed intact and then hydrolyzed. Some peptides may be resistant to hydrolysis by gastrointestinal enzymes and arrive intact in greater amounts in the bloodstream. This feature is related to amino acid composition, in which peptides with proline and hydroxyproline residues, in general, are able to withstand degradation by gastrointestinal enzymes (Segura-Campos et al., 2011).

For peptides to reach the bloodstream, they need to cross the intestinal barrier, so the permeability of these molecules through the enterocytes depends on their physicochemical properties, the composition of intestinal fluid, characteristics of the gastrointestinal barrier and the transport mechanism (Segura-Campos et al., 2011).

Two major mechanisms are responsible for the uptake of peptides in the intestinal epithelium, the receptor-mediated and non-receptor mediated transport. In receptor-mediated transport, hydrolize-resistant peptides are transported from the intestinal lumen to the cells by a peptide transport system, such as PepT1, which uses a transmembrane electrochemical proton gradient maintained by the Na+ /H+ exchanger which gets energy from Na+ /K+-ATPase and then crosses the basolateral cell membrane into the bloodstream (Segura-Campos et al., 2011).

Peptides can also be absorbed intact by paracellular and transcellular transport. Paracellular transport is favorable for the absorption of small hydrophilic solutes through passive diffusion since this is an aqueous pathway that passes through the intracellular space between adjacent cells, which is restricted by

tight junctions in the apical portion of the cells along the intestinal wall (Segura-Campos et al., 2011).

On the other hand, the transcellular transport takes place in the enterocytes by endocytosis through the apical membrane in the brushed border and the movement of the peptides through the basolateral membrane, in which the molecules pass by facilitated diffusion or active transport to the bloodstream (Segura-Campos et al., 2011).

The peptide uptake capacity decreases with increasing chain size and increases with increasing hydrophobicity. Once absorbed, the peptides may also undergo inactivation by enzymes present in the blood and undergo liver extraction, although the transport time is short and the enzyme capacity is lower than that present in the gastrointestinal tract (Segura-Campos et al., 2011).

Therefore, further *in vivo* studies are important to begin understanding how different factors may affect the bioavailability of these molecules to be used as therapeutic agents.

Tools in Bioinformatics

The tools used in this study are simple to use and they are open access. The UniProt database provides information on the protein sequence of the most diverse proteins through the compilation of data obtained in scientific studies. It is also possible to compare different protein sequences reported for the same protein in this database.

From the protein sequence obtained trough the UniProt, the BIOPEP-UWM database can be used to evaluate the profile of potential biological activity and simulating the obtention of bioactive peptides trough proteolytic processes. This database is designed to interlink three databases of protein sequences, bioactive peptides, and proteolytic enzymes.

Posteriorly, the peptides fragments obtained trough the BIOPEP database can be evaluated at the database PeptideRanker, to identify among a set of peptides, those that are more likely to be bioactive, based on the impact of extracellular status and amino acid composition of the fragments (Mooney et al.,

2012).

Although not accurately assessing the three-dimensional structure of proteins, bioinformatics studies offer the advantage of using *in silico* protein digestion processes for protein and protease screening, providing the best combinations for obtaining bioactive peptides from animal and plant raw materials, avoiding waste (Udenigwe & Fogliano, 2017).

Bioactive Peptides Scientific Prediction From the Bean's Storage Proteins

Storage proteins amino acids sequence

The database UniProt was used to obtain the storage proteins amino acids sequence of the bean species *Phaseolus vulgaris* (L.) (common bean), *Vigna angularis* (Willd.) (adzuki bean), *Vigna radiata* (L.) (mung bean), and *Vigna unguiculata* (L.) Walp. (cowpea). In the tool UniProtKB (https://www.uniprot.org/uniprot/), it was inserted the protein's name and its respective species and realize the search. Only the proteins classified as globulins were included in this study because they are the majority of the storage proteins in beans. In order to verify all the globulin proteins amino acid sequence present in the database for each studied species, the search was performed according to the names and synonyms of each protein and its respective species, as an example, ''7 S globulin *Vigna angularis''* and ''Vicilin *Vigna angularis''*.

According to the database classification, only sequences with protein and transcription evidence level were collected. When obtained more than one sequence for the same protein name, the tool BLAST (https://www.uniprot.org/blast/) was used to find the identity between the sequences, and in case it was less than 90% identical, they were considered different proteins.

According to the bean studied species, Table 1 presents the proteins identified in the UniProt database and the percentage of identity obtained in the BLAST tool when more than one sequence was collected for the same protein. In none of the species was found protein sequences with protein and/or transcript evidence level for the proteins named as legumin (11 S globulin), therefore, they were not selected. In the species *Vigna angularis* (Willd.), three polypeptide chains

were found for the 7 S globulin protein. The species *Phaseolus vulgaris* (L.) and *Vigna radiata* (L.) had four sequences for the phaseolin and 8 S globulin respectively. The species *Vigna unguiculata* (L.) Walp. obtained the highest number of sequences for the same protein, with a total of five sequences for the vicilin protein.

Chart 1 exemplifies how the percentage of identity between two different sequences obtained for the phaseolin present in common bean was performed. The negative signal in one of the sequences indicates that it does not have amino acids in that position, and the positive sign between the sequences indicates that they have different amino acids but with similar properties in the same position, contributing to the growth of the percentage of identity between them.

In the species *Phaseolus vulgaris* (L.), *Vigna angularis* (Willd.) and *Vigna unguiculata* (L.) Walp., the different protein sequences obtained for phaseolin, 7 S globulin and vicilin, respectively, had more than 90% of identity to each other in the BLAST tool. Therefore, these were considered identical, being selected randomly one protein of each species to be presented in the scientific prediction regarding the bioactive potential in the database BIOPEP. The codes of the proteins selected were P07219, A4PI98 and A0A2U96L2 respectively (Charts 2, 3 and 5).

For the species *Vigna radiata* (L.), the proteins Q198W4, B1NPN8 and Q198W5 presented identity values above 90% among them (Table 1), thus, one of them (B1NPN8) was randomly selected to carry out the scientific prediction in the BIOPEP database (Chart 4). The protein Q198W3 presented percentages below 90% of identity in relation to all the proteins mentioned above and, therefore, this one was considered different from the others, being also used in the scientific prediction in the database BIOPEP (Chart 4).

Scientific prediction regarding the potential of obtaining bioactive peptides and analysis of bioactivity

In the database BIOPEP-UWM, in the tool Bioactive peptides, the access was: Analysis ➔ Profile of Potential Biological Activity ➔ For Your Sequence (http://www.uwm.edu.pl/biochemia/index.php/en/biopep), it was evaluated *in silico* the profiles of potential biological activity by inserting the proteins sequence obtained

previously in the UniProt, according to each bean species.

Posteriorly, in the tool Bioactive Peptides, the access was: Analysis ➔ Enzyme Action ➔ For Your Sequence (http://www.uwm.edu.pl/biochemia/index.php/en/biopep), the protein hydrolysis was simulated by two different processes: the gastrointestinal digestion with pepsin (EC 3.4.23.1), trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) and the hydrolysis with the enzyme subtilisin (EC 3.4.21.62). From the results of enzyme action, it was performed the search for bioactive fragments. The frequency of bioactive fragments occurrence (A) with specific activity (ACE and DPP-IV inhibition, antioxidant, etc.) in the protein sequence, was calculated according to the BIOPEP definition by the following equation: ‘’A’’ = a/N, where “a” was the number of fragments with given activity in the storage protein sequence, and “N” was the number of amino acid residues of the storage protein.

Posteriorly, the bioactive fragments obtained from BIOPEP database were analyzed trough the database PeptideRanker (Mooney et al., 2012) (http://distilldeep.ucd.ie/PeptideRanker/), which ranks peptides by the predicted probability that the peptide will be bioactive by attributing a score from 0 to 1 for each peptide, the closer the value to 1, more likely it is that the peptide will be bioactive. According to the database, any peptide predicted over a 0.5 threshold is labeled as bioactive, however, choosing a threshold of 0.8 it will reduce the false positive rate from 11% and 16%, at 0.5 threshold, to 2% and 6%, for long and short peptides, respectively. Thus, it was chosen to present only the peptides with a score of 0.8 on PeptideRanker in this work. In this step, peptide sequences identified in other scientific studies that have the same bioactive fragments as those identified in the present study with antioxidant potential and inhibition activity of ECA and DPP-IV enzymes were compared to each other.

Through the analysis of the profile of potential biological activity from the BIOPEP, it is possible to predict any type of peptide fragment with potential biological activity that can be obtained from proteins, since it does not use a specific mechanism to simulate the hydrolysis of the polypeptide chain. Thus, this profile presents a large possibility of obtaining different peptides fragments with the most diverse potential biological activities. Because of this, many activities have a low frequency of occurrence (Chart 2).

In this study, all the studied species presented a similar profile of

potential biological activities between each other, as can be observed in Chart 2 for common bean. Some of the potential activities were ACE and DPP-IV inhibitors, antithrombotic effect, hypotensive effect, stimulating vasoactive substance release peptides and glucose uptake stimulating peptides. The exception was that the adzuki bean and cowpea also presented antibacterial activity, both with frequency of bioactive fragments occurrence of 0.0023 (data not shown). Based on that, it was chosen to present all the activities found in the profile of potential biological activity only for the species *Phaseolus vulgaris* (L.) as an example (Chart 2), and for the other bean species it was presented only the biological activities that had a frequency of bioactive fragment occurrence above 0.05 in this profile (Charts 3, 4 and 5).

As an example, Figure 1 shows the fragments obtained after the simulation of the hydrolysis of phaseolin (P07219) trough the action of gastrointestinal enzymes: pepsin, trypsin, and chymotrypsin. Since the enzymes act at specific sites of the polypeptide chain according to their affinity for the substrate, the simulation of protein hydrolysis by gastrointestinal enzymes or by the subtilisin action, shows a decrease in the amount of different fragments that can be obtained when compared with the profile of potential biological activity (Chart 2), and consequently evidences a decrease at the potential biological activities observed.

The phaseolin is highly resistant to proteolysis due to its compact and rigid structure, thus factors such as the enzyme specificity, the enzyme/substrate ratio, time of hydrolysis and the own structural heterogeneity of phaseolin among different cultivars, impact its proteolytic rate, leading to finding different biological activities (Garcia-Mora et al., 2015).

Observing the profile of potential biological activity and the two process of hydrolysis simulated, in general, all the proteins of the four bean studied species had a higher frequency of bioactive fragment occurrence for DPP-IV inhibition followed by ACE inhibition and by antioxidant activity (Charts 2, 3, 4 and 5).

The majority of bioactive fragments obtained in all the studied species were dipeptides, followed by tripeptides (Charts 2, 3, 4 and 5). Larger fragments were only observed in the profile of potential biological activity. The gastrointestinal digestion appears to be enough to release bioactive fragments from the globulins present in the common bean, adzuki bean, mung bean, and cowpea. Therefore, the consumption of this legume in a daily basis could be considered a promising strategy to decrease the risk of developing T2D and hypertension,

according to the higher frequency of bioactive fragments occurrence found for DPP-IV and ACE inhibition activities, respectively. However, it was observed that the hydrolysis by the subtilisin also can generate different fragments from those obtained only by gastrointestinal digestion, indicating that this process can be used in the formulation of health-enhancing foods to increase the amount of different bioactive fragments potentializing its effect.

Tables 2, 3 and 4 present the score (only above 0.8) on PeptideRanker of the peptides obtained from the BIOPEP database with the potential to inhibit the DPP-IV and ACE enzymes, and with antioxidant activity, respectively. These tables also present peptide sequences identified in scientific studies, which evidenced the inhibition of DPP-IV and ACE enzymes and antioxidant activity by *in vitro* assays.

It may be noted that the bioactive fragments identified in the *in vitro* studies of inhibition of ACE and DPP-IV (Table 2 and 3) were present in different peptide sequences, most of them with more than 4 amino acid residues. This may occur due to factors such as the enzyme specificities and different time of hydrolysis applied in the different studies. Garcia-Mora et al. (2015) evaluated the obtention of bioactive peptides from common bean by hydrolysis with two different subtilisins, the Alcalase® and Savinase®, the authors concluded that the hydrolysates obtained by Alcalase treatment for 120 min and Savinase for 90 min had the higher biological potential, including anti-inflammatory, ACE inhibitory and antioxidant activities. The bioactive fragments with the bigger score on PeptideRanker in the present study that was also observed in the peptide sequences identified in the *in vitro* studies, were FP, PF, and FL for DPP-IV inhibition (Table 2) and FFL, FP, and FG for ACE inhibition (Table 3).

Since in this study the majority of the peptides with good possibility of being bioactive had the potential to inhibit DPP-IV and ACE, there are two different mechanisms to explain the inhibition of these enzymes: peptides bound to the enzyme and alters its shape, which will incapacitate the binding between the enzyme and substrate, or by competitive inhibition, where the peptides will compete with the substrate for the enzyme catalytic site (Ngoh & Gan, 2017).

According to Mojica et al. (2015), the peptide sequence and the amino acid type are determinant in the interaction potential between the functional groups of the bioactive peptides with the amino acids present in the active site of the

enzymes, which will determine the affinity for the inhibitor. The interactions depend on the distance and the functional groups present in the side chains, so for the competitive inhibition of these enzymes to occur, the bioactive compounds need to position themselves adequately to interact with the amino acids present in the catalytic site. The main interactions predicted from the peptide sequences found were hydrogen bonds, polar and hydrophobic interactions.

The interaction of DPP-IV inhibitory peptides with the active site of the enzyme is not yet fully understood. In general, good inhibitors contain from 2 to 7 amino acids, with proline or alanine in the penultimate position of the N-terminal extremity (Power, Nongonierma, Jakeman & Fitzgerald, 2014). Also, a study of Lan et al. (2015) which analyzed 337 dipeptides evidenced the presence of a tryptophan residue at the N-terminal position of the most potent peptides. In the present study, many peptide fragments from all the studied species with a score above 0.8 on PeptideRanker also contained proline, alanine or tryptophan residues (WF, FP, PF, WG, RW, WY, WN, SW, AF, FA) (Table 2) confirming the potential to exert bioactivity. Also, many of these potential bioactive fragments were identified in the peptide sequences obtained from the analysis of *in vitro* studies.

When it comes to the inhibition of ACE, its catalytic site is formed by three subunits, in which, angiotensin I will interact trough three hydrophobic amino acids present in its C-terminal region, being proline, histidine, and phenylalanine. Therefore, potent peptides capable of inhibiting the ACE preferentially will have amino acids such as tyrosine, proline, tryptophan, phenylalanine, and leucine at the C-terminal extremity, having a positive correlation between their hydrophobicity and inhibitory activity. Positively charged amino acids such as arginine and lysine also promote ACE inhibitory activity. Aromatic or alkaline amino acids at the N-terminal extremity of inhibitors peptides can increase the inhibitory activity, such as arginine, glycine, valine, alanine, and isoleucine. In general, the ACE inhibitors peptides present low molecular weight (Li & Yu, 2015).

Similar to the profile found previously for the fragments with the potential to inhibit DPP-IV, it was also found peptide fragments with the potential to inhibit ACE which contains amino acids with the characteristics cited above in all the bean species (Table 3), also many of these bioactive fragments are present in the peptide sequences obtained at the studies which realized the *in vitro* inhibition of ACE assay.

Study of Ngoh & Gan (2017) used the database PeptideRanker to selected five peptide fragments from common bean with score above 0.8, PPHMLP, LSSLEMGSLGALFVCM, PPHMGGP, PLPLHMLP, PLPWGAGF, the authors concluded that these fragments demonstrated ACE inhibition activity by *in vitro* assays with IC_{50} values of 1.52 μM, 1,84 μM, 11,04 μM, 27,32 μM, and 31,88 μM, respectively. From these sequences, four of them had bioactive fragments also found in this work (Table 3), being that GG, GP (PPHM**GGP**), PL (**PLPL**HMLP) and WG (**PL**P**WG**AG).

When it comes to antioxidant activity (Table 4), a small number of potential bioactive fragments with a score above 0.8 was observed when compared with the number found for the other two studied activities, and only two amino acids (RW) from a tripeptide (RWY) were identified in the peptide sequences (**RW**AEK) present in the *in vitro* studies analyzed (Mojica, Chen & Gonzalez de Mejía, 2015).

Oxidation is a vital process in the human organism, in which the production of free radicals (highly reactive molecules that contain an unpaired electron in its last electron layer) occurs. Under normal circumstances, oxidation is a dynamic and continuous balance between free radical production and elimination, but overproduction of these molecules can cause damage to cells, which is known as oxidative stress. This process can lead to diseases such as cancer, high blood pressure and inflammation (LI; YU, 2015).

Antioxidant pathways can occur through the inactivation of active oxygen, neutralization of free radicals, chelating metallic ions, and reducing the formation of hydrogen peroxide. Bioactive peptides with hydrophobic amino acids have a high correlation with antioxidant activity, the more hydrophobic and aromatic amino acids, the greater the antioxidant activity, since these tend to combine first with free radicals (Li & Yu, 2015). According to that, the majority of the peptide fragment found in this study with good potential to be bioactive in PeptideRanker had hydrophobic and aromatic amino acids such as WG, RW, WY, RWY, and ADF (Table 4).

The score on PeptideRanker can be used for the prediction or optimal design of bioactive peptides, leading investigators to initiate analyses on peptides that are most favored by the software, facilitating experimental decision and improving efficiency (Mooney et al., 2012).

The results found in this work evidence that the bioinformatics can be an efficient,

simplified and cost-effective tool to be used in the design, synthesis and selection of food-derived bioactive peptides to realize *in vivo* studies, regarding its potential bioactive and to decrease the risk of developing T2D and hypertension.

Scientific Studies

The number of scientific studies that demonstrated biological activity from the bean's proteins was raised in the databases Wiley, Medline/Pubmed and others available in the CAPES portal of periodicals (Fig. 2).

It was observed that the common bean is the bean species most explored by the studies that have as its theme the bioactive peptides, followed by cowpea, mung bean and adzuki bean. Probably the common bean may be the most studied because it is the most important legume for human consumption, with approximately 12 million tons produced annually (Consultative Group on International Agricultural Research [CGIAR], 2019).

For common bean, cowpea, and mung bean species, the inhibition of ACE and antioxidant activity were the most studied, followed by inhibition of DPP-IV, this goes according to our results, since the bigger frequency of bioactive fragments occurrence also was found for these activities. However, as mentioned previously, other activities are possible according to the profile of potential bioactive peptides (Chart 02), besides antioxidant and inhibition of ACE and DPP-IV. Some of these different activities were identified by *in vitro* studies, such as antithrombotic (Basha, Maheswaraiah & Rao, 2017), stimulation of glucose uptake (Oseguera-Toledo, Gonzalez de Mejía, Sivaguru & Amaya-Llano 2016), hypotensive (Cú-Cañetas, Ancona, Tintoré, Peraza & Guerrero, 2015) and antimicrobial (Ariza-Ortega et al. (2014).

The Chart 2 shows that common bean possesses potential to release fragments with antithrombotic activity (A = 0.0023), the same profile was also observed for *Vigna radiata* (L.), (A = 0.0022, data not shown). Basha et al. (2017) studied the bioactive compounds such as polyphenols, peptides and proteins in the seed exudate of mung bean which is obtained from the first step in the germination process which involves soaking the seeds for 4-24 h in water for imbibition, during this process, the influx of water into cells of dry seeds causes cracks in the seed coat

which causes the leakage of the endogenous substances of the seed into the water used for soaking. The authors found that the protein seed exudate showed inhibition of platelet aggregation, with the low molecular fraction 12.4 kDa (1.0 mg/mL) being responsible for 36,49% of inhibition of platelet aggregation.

Thrombosis occurs when a blood clot forms due to platelet aggregation in one or more large veins of the legs and thighs. This clot blocks the flow of blood and causes swelling and pain in the area. The biggest problem is when a clot detaches and moves in the bloodstream, in a process called embolism. An embolism may be stuck in the brain, lungs, heart, or another area, leading to serious injury (BRAZIL. MS, 2019).

In this study, all the studied species presented peptides fragments with potential hypotensive, especially for the cowpea, the frequency of occurrence was 0.0208 (data not shown). A study that evaluated the hypotensive effect of peptide fractions obtained by the Flavourzyme hydrolysis of cowpea proteins, concluded that the fraction <1 kDa decreased the systolic pressure in Wistar rats by 8.61% and the diastolic pressure by 14.09%, while the control with Captopril decreased by 9.84% and 11.14%, respectively (Cú-Cañetas et al., 2015).

Oseguera-Toledo et al. (2016) found that the protein fraction <1 kDa obtained by the action of Alcalase on common bean's proteins, generated the LL peptide, and by the action of bromelain generated the peptides VL, LV, LL, after simulation of gastrointestinal digestion. All the fragments had biological activity to increase glucose uptake. The same bioactive fragments were observed in the present study for common bean (Chart 2) cowpea, mung bean and adzuki bean (data not shown).

According to the BIOPEP database, only the adzuki bean and cowpea had the potential to obtain peptide fragments with antibacterial activity (A = 0.0023, data not shown), however, in the literature, this activity was found for common bean. A study by Ariza-Ortega et al. (2014) found that the common bean protein hydrolysate obtained by Alcalase® action had antibacterial activity (fraction <1 kDa) against *Shigella dysenteriae*.

Although they did not appear in the profile of potential bioactivity performed for the evaluated bean species in this study, other different activities were found in the literature, such as the inhibition of the α-glucosydase and α-amylase, increase in the secretion of the insulin by INS-1 cells (Oseguera-Toledo et al., 2015),

antifungal, antiinflamatory (Oseguera-Toledo et al., 2011), anticancer (Luna Vital et al, 2014), anticholesterolemic (Amaral et al., 2017) and others, most of them also using common bean. These findings open the possibility of obtaining other health benefits from the regular consumption of the bean studied species, in addition to the activities more extensively studied as antioxidant, inhibition of ACE and DPP-IV.

Conclusion

Our results suggest that the globulins present in the common bean, adzuki bean, mung bean, and cowpea are a good source of proteins to release potential bioactive peptides to act inhibiting the ACE and DPP-IV enzymes and with antioxidant activity, from gastrointestinal digestion. Therefore, the consumption of this legume in a daily basis could be considered a promising strategy to decrease the risk of developing T2D, hypertension, and several other diseases related to oxitative stress, such as cancer and inflamations. In addition, the hydrolysis with subtilisin can be used to potentialize this effect and to origin health-enhanced ingredients from the bean's protein. Also, it shows that the computational prediction of bioactive peptides can be a useful and cost-effective tool to direct the design and synthesis of new food-derived peptide to perform *in vivo* studies.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Author Contributions

B. F. Garcia wrote the initial and final drafts. T. S. Rocha provided the major headings for the review and evaluated the preliminary drafts.

Author Disclosures

The authors declare no conflict of interest.

Nomenclature

ACE	Angiotensin-converting enzyme
CAPES	Coordination of Improvement of Higher Level Personnel
COX2	Ciclooxygenase-2
DPP-IV	Dipeptidyl peptidase-IV
GIP	*Glucose-dependent insulinotropic peptide*
GLP-1	*Glucagon-like peptide*
HDL-c	High-density lipoprotein
HMG-CoAr	3-hydroxy-3-methyl-glutaryl-CoA reductase
IC_{50}	Inhibitor concentration required to inhibit 50% of enzyme activity
iNS-1E	Insulin secreting beta cell derived line
NF-κB	Nuclear factor kappa light chain enhancer of activated B cells
PepT1	Human peptide transporter 1
T2D	Type 2 Diabetes melittus

List of amino acids: A, Alanine; R, Arginine; N, Asparagine; D, Aspartic acid; C, Cysteine; Q, Glutamine; E, Glutamic acid; G, Glycine; H, Histidine; I, Isoleucine; L, Leucine; K, Lysine; M, Methionine; F, Phenylalanine; P, Proline; S, Serine; T, Threonine; W, Tryptophan; Y, Tyrosine; V, Valine.

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Table 1 - Globulin storage proteins obtained from UniProtKB according to the bean species and Identity between the sequences (BLAST).

Protein name	UniProt code	Length	Identity (BLAST)					
Phaseolus vulgaris								
Alpha phaseolin	P07219	436		P07219	P02853	Q43632	Q43633	
Phaseolin beta-type	P02853	421	P07219		94.70%	94.00%	97.00%	
Phaseolin	Q43632	421	P02853			98.80%	95.80%	
Phaseolin	Q43633		Q43632				95.60%	
			Q43633					
Vigna angularis								
7S globulin-3	A4PIA0	433		A4PIA0	A4PI98	A4PI99		
7S globulin-1	A4PI98	434	A4PIA0		95.20%	96.80%		
7S globulin-2	A4PI99	434	A4PI98			98.40%		
			A4PI99					
Vigna radiata								
8S globulin beta isoform	Q198W3	453		Q198W3	Q198W4	B1NPN8	Q198W5	
8S globulin alpha' isoform	Q198W4	453	Q198W3		85.00%	84.40%	84.70%	
8S globulin alpha subunit	B1NPN8	454	Q198W4			90.00%	90.30%	
8S globulin alpha isoform	Q198W5	454	B1NPN8				99.80%	
			Q198W5					
Vigna unguiculata								
Vicilin	A0A2U9K6L2	432		A0A2U9K6L2	A0A2U9K6K6	A0A2U9K6K9	A0A2U9K6L4	A0A2U9K6K8
Vicilin	A0A2U9K6K6	432	A0A2U9K6L2		97.70%	97.50%	97.50%	98.40%
Vicilin	A0A2U9K6K9	429	A0A2U9K6K6			98.40%	98.40%	99.30%
Vicilin	A0A2U9K6L4	432	A0A2U9K6K9				97.70%	98.60%
Vicilin	A0A2U9K6K8	432	A0A2U9K6L4					99.10%
			A0A2U9K6K8					

Table 2 - Score at PeptideRanker of the Bioactive Peptide Fragments with Potential to Inhibit DPP-IV obtained from BIOPEP and Peptide Sequences with the same bioactive fragment identified in scientific studies at the literature.

Score	Bioactive fragment	Bean specie	Peptide Sequence	Reference	Species studied
0.9988	WF	mung bean, cowpea			
0.9966	MF	mung bean			
0.9947	GF	adzuki bean, mung bean, cowpea			
0.9939	FP	adzuki bean, mung bean, cowpea	SAK**FP**PAGGK, VDT**FP**A, **FP**LV, LTT**FP**E, DVT**FP**A, EVT**FP**A, VA**FP**GSSVE, FDD**FP**W, VDT**FP**A, DLT**FP**A, **FP**NGGSL, **FP**VTPF, LTT**FP**E	Rocha et al.(2014) Rocha et al. (2015) Mojica and Gonzalez de Mejía (2015) Mojica and Gonzalez de Mejía (2016)	cowpea, common bean
0.9934	PF	common bean, adzuki bean, mung bean, cowpea	SKDGG**PF**, QT**PF**, SG**PF**GPK, LPPSPERTAAP**PF**, LT**PF**A, DV**PF**VS, FPVT**PF**, SSKAGD**PF**, YLAGN**PF**APPHGGK	Rocha et al.(2014) Mojica and Gonzalez de Mejía (2015)	cowpea, common bean
0.9924	WG	adzuki bean			
0.9896	FL	common bean, adzuki bean, mung bean, cowpea	YV**FL**S, VY**FL**S, V**FL**PA, **FL**PTGGL, F**FL**, DF**FL**, DF**FL**S **FL**EMLLLD**FL**, F**FL**LEQLAATT, LE**FL**LLMLDF, Q**FL**LQMLALRK, TELELL**FL**EF, RYA**FL**ELLTQ, E**FL**DLMLLLF	Rocha et al., (2014) Mojica & Gonzalez de Mejía (2015) Oseguera-Toledo, Gonzalez de Mejía & Amaya-Llano (2015) Mojica & Gonzalez de Mejía (2016)	cowpea common bean
0.9857	FR	common bean, adzuki bean, mung bean, cowpea			
0.9818	YF	common bean, adzuki bean, mung bean, cowpea	V**YF**LS	Mojica & Gonzalez de Mejía (2016)	common bean

Table 2 - (Continued)

0.9784	RW	adzuki bean, mung bean, cowpea			
0.9749	WY	mung bean			
0.9733	AF	common bean, adzuki bean, mung bean, cowpea	V**AF**PGSSVE, F**AF**GLN, FFAAAFT, RLLFNLML**AF**, F**AF**QFT, RY**AF**LELLTQ,	Rocha et al. (2014) Mojica & Gonzalez de Mejía (2015) Oseguera-Toledo, Gonzalez de Mejía & Amaya-Llano (2015)	cowpea common bean
0.9713	MM	common bean	L**MM**LMYLLLL	Mojica & Mejia (2015)	common bean
0.9601	MP	common bean, cowpea	**MP**HLK, **MP**PM	Mojica & Gonzalez de Mejía (2015) Mojica, Luna-Vital & Gonzalez de Mejía (2016)	common bean
0.9558	FA	common bean, adzuki bean, mung bean	**FA**TGT, LTP**FA**, Q**FA**DG, **FA**FGLN, F**FA**AAFT, YLAGNP**FA**PPHGGK, **FA**FQFT, F**FA**QFT, **FA**AG	Rocha et al. (2014) Mojica & Gonzalez de Mejía (2015) Mojica, Chen & Gonzalez de Mejía (2015) Oseguera-Toledo, Gonzalez de Mejía & Amaya-Llano (2015)	cowpea common bean
0.9512	FN	common bean, adzuki bean, mung bean, cowpea	LS**FN**T, RLL**FN**LMLAF	Mojica & Gonzalez de Mejía (2015) Mojica, Luna-Vital & Gonzalez de Mejía (2016)	common bean
0.9510	HF	adzuki bean, mung bean, cowpea			
0.9488	SF	common bean, adzuki bean, mung bean, cowpea	L**SF**NT	Mojica, Luna-Vital & Gonzalez de Mejía (2016)	common bean
0.9461	QF	mung bean	**QF**ADG, FAF**QF**T, FFA**QF**T	Rocha et al. (2014) Mojica & Gonzalez de Mejía (2015)	cowpea common bean
0.9411	NF	common bean, adzuki bean, mung bean, cowpea			
0.9391	WN	common bean			

Table 2 - (Continued)

0.9339	SW	common bean, mung bean, cowpea			
0.9068	KF	common bean, adzuki bean, mung bean, cowpea	SA**KF**PPAGGK, V**KF**MT	Rocha et al., (2015) Mojica; Luna-Vital, Mejia (2016)	common bean
0.9055	GP	common bean, mung bean, cowpea	SKDG**GP**F, C**GP**HGA, K**GP**ASK, S**GP**F**GP**K, SAK**GP**PMGAK, SAK**GP**PTSAK, SRSPA**GP**PPTEK, FDD**GP**EF, FDD**GP**EF, LE**GP**KRAGW, SAK**GP**PTSAQ, S**GP**TLSK, SAK**GP**PMGAK, **GP**ALP, S**GP**AKWKW	Rocha et al., (2014) Rocha et al., (2015) Mojica and Mejia (2015) Mojica and Mejia (2016) Mojica; Luna-Vital, Mejia (2016)	cowpea common bean
0.8874	GG	adzuki bean, mung bean, cowpea	SAKFPPA**GG**K, S**GG**YKLLLLK, SKD**GG**PF, **GG**GLHK, **GG**DEAG, **GG**NEGA, GSL**GG**H, LKE**GG**K, TT**GG**K**GG**K, SKGS**GG**GKL, SLPA**GG**NRYGK, TPKNSDPLPGV**GG**SELSKEV, EKAS**GGGG**LS, FLPT**GG**L, SRGVA**GG**AGV, TD**GG**LLE, FPN**GG**SL, LTGATLEPPKP**GG**GL, FA**GG**TSGSGV, DK**GG**LL, YLAGNPFAPPH**GG**K, ASK**GG**GVAGKK, SKA**GG**V**GG**LSK	Rocha et al., (2014) Rocha et al., (2015) Mojica and Mejia (2015) Oseguera-Toledo; Mejia; Amaya-Llano (2015) Mojica and Mejia (2016) Mojica; Luna-Vital, Mejia (2016)	cowpea common bean

Table 2 - (Continued)

0.8642	LM	adzuki bean, cowpea	LRENNK**LM**LLELK, A**LM**LEEYLLE, RA**LM**PN, RLLFN**LM**LAF, LEFLL**LM**LDF, RRKA**LM**RGQNK, **LM**MLMYLLLL, EFLD**LM**LLLF, DLLLL**LM**GESLLF	Rocha et al., (2014) Mojica and Mejia (2015) Mojica, Chen and Mejía (2015) Mojica and Mejia (2016)	cowpea common bean
0.8491	MR	common bean	RKLK**MR**Q, RRKAL**MR**GQNK	Mojica and Mejia (2015) Mojica and Mejia (2016)	common bean
0.8267	TF	common bean, mung bean, cowpea	VD**TF**PA, LT**TF**PE, DV**TF**PA, EV**TF**PA, VD**TF**PA, DL**TF**PA, LT**TF**PE	Rocha et al., (2014)	cowpea
0.8154	VF	common bean, adzuki bean, mung bean, cowpea	LLYEVEL**VF**E, LSG**VF**, Y**VF**LS, **VF**LPA, KRKRYEKLLLLREEGRS**VF**Q	Rocha et al., (2014) Mojica and Mejia (2015) Mojica and Mejia (2015) Mojica and Mejia (2016) Mojica; Luna-Vital, Mejia (2016)	cowpea common bean
0.8111	PL	common bean, mung bean, cowpea	ATN**PL**F, AKS**PL**F, TTN**PL**F, TPKNSD**PL**PGVGGSELSKEV, A**PL**GKP, **PL**LELELVEAAG, F**PL**V, RQRL**PL**	Rocha et al., (2014) Mojica and Mejia (2015) Mojica and Mejia (2016)	cowpea common bean
0.8088	GL	common bean, adzuki bean, mung bean, cowpea	GG**GL**HK, KTY**GL**, E**GL**ELLLLLLAG, TAT**GL**LE, EKASGGG**GL**S, ART**GL**AP, FLPTG**GL**, TTA**GL**LE, FAF**GL**N, TDG**GL**LE, LTGATLEPPKPGG**GL**, DKG**GL**L, **GL**SLELLLLL, Y**GL**VAGK, **GL**ASK, ER**GL**AGS, SKAGGVG**GL**SK	Rocha et al., (2014) Mojica and Mejia (2015) Oseguera-Toledo; Mejia; Amaya-Llano (2015) Mojica and Mejia (2016) Mojica; Luna-Vital, Mejia (2016)	cowpea common bean

It was presented only the fragments with score above 0.8.
Bold: bioactive fragment.
A, Alanine; R, Arginine; N, Asparagine; D, Aspartic acid; C, Cysteine; Q, Glutamine; E, Glutamic acid; G, Glycine; H, Histidine; I, Isoleucine; L, Leucine; K, Lysine; M, Methionine; F, Phenylalanine; P, Proline; S, Serine; T, Threonine; W, Tryptophan; Y, Tyrosine; V, Valine.

Table 3 - Score at PeptideRanker of the Bioactive Peptide Fragments with Potential to Inhibit ACE obtained from BIOPEP and Peptide Sequences with the same bioactive fragment identified in scientific studies at the literature.

Score	Bioactive fragment	Bean specie	Peptide Sequence	Reference	Species studied
0.9966	MF	mung bean	AMPVNNPQIHD**FFL**S**,** AMPVNNPQIHE**FFL**S**,** **FFL**LEQLAATT, LL**FFL**E	Garcia-Mora et al. (2015) Mojica & Gonzalez de Mejía (2015)	common bean
0.9955	FFL	common bean, adzuki bean			
0.9947	GF	adzuki bean, mung bean, cowpea	**FP**LV, **FP**VL	Mojica & Gonzalez de Mejía (2015)	common bean
0.9939	FP	adzuki bean, mung bean, cowpea	KVDN**FG**	Mojica & Gonzalez de Mejía (2015)	common bean
0.9931	FG	adzuki bean, mung bean, cowpea	RL**LF**NLMLAF, L**LF**FLE, PLTA**LF**V, TELEL**LF**LEF, EFLDLMLL**LF**, DLLLLLMGESL**LF**, KLPAGT**LF**	Li et al. (2006) Mojica & Gonzalez de Mejía (2015)	common bean mung bean
0.9924	WG	adzuki bean			
0.9869	LF	common bean, adzuki bean, mung bean, cowpea			
0.9866	RF	common bean, adzuki bean, mung bean, cowpea	V**RF**V, LV**RF**, VL**RF**, **RF**KL,	Mojica & Gonzalez de Mejía (2015)	common bean
0.9857	FR	common bean, adzuki bean, mung bean, cowpea			
0.9824	FY	common bean, adzuki bean, mung bean, cowpea			
0.9784	RW	adzuki bean, mung bean, cowpea	SGKKPT**RW**, **RW**AEK	Mojica & Gonzalez de Mejía (2015) Mojica, Chen & Gonzalez de Mejía (2015)	common bean
0.9733	AF	common bean, adzuki bean, mung bean, cowpea	RLLFNLML**AF**, F**AF**QFT, RY**AF**LELLTQ, ER**AF**	Mojica & Gonzalez de Mejía (2015) Mojica, Chen & Gonzalez de Mejía (2015)	common bean

Table 3 - (Continued)

0.9713	MM	common bean	L**MM**LMYLLLL	Mojica & Gonzalez de Mejía (2015)	common bean
0.9492	lF	common bean			
0.9488	SF	common bean, adzuki bean, mung bean, cowpea	EGG**SF**, L**SF**NT	Mojica, Chen & Gonzalez de Mejía (2015) Mojica, Luna-Vital & Gonzalez de Mejía (2016)	common bean
0.9424	DF	adzuki bean, mung bean, cowpea			
0.9411	NF	common bean, adzuki bean, mung bean, cowpea	KVD**NF**G	Mojica & Gonzalez de Mejía (2015)	common bean
0.9395	LFR	mung bean, cowpea			
0.9161	FQ	adzuki bean, mung bean, cowpea	FA**FQ**FT, YEKLLLLREEGRSV**FQ**	Mojica & Gonzalez de Mejía (2015)	common bean
0.9068	KF	common bean, adzuki bean, mung bean, cowpea	V**KF**MT	Mojica, Luna-Vital & Gonzalez de Mejía (2016)	common bean
0.9055	GP	common bean, mung bean, cowpea	K**GP**ASK, C**GP**HGA, LE**GP**KRAGW, SAK**GP**PTSAQ, S**GP**TLSK, **GP**KVGWAVSG, **GP**ALP, S**GP**AKWKW	Mojica & Gonzalez de Mejía (2015) Mojica, Luna-Vital & Gonzalez de Mejía (2016)	common bean
0.8953	RFH	mung bean			
0.8882	GPL	cowpea			
0.8874	GG	adzuki bean, mung bean, cowpea	**GG**GLHK, **GG**DEAG, **GG**NEGA, E**GG**SF, YLAGNPFAPPH**GG**K, C**GG**E, SGKAPPTS**GG**T, TAK**GG**VGAAKN, KGA**GG**AAAH, ASK**GG**GVAGKK, SKA**GG**VGGLSK	Mojica & Gonzalez de Mejía (2015) Mojica, Chen & Gonzalez de Mejía (2015) Mojica, Luna-Vital & Gonzalez de Mejía (2016)	common bean
0.8358	LNF	mung bean, cowpea			

Table 3 - (Continued)

0.8279	FVP	common bean	SIEMKEGAL**FVP**H, SIEMEEGAL**FVP**H, ISSIEMKEGAL**FVP**H, SIEMKEGA**LFVP**HYYSK, ISSIEMEEGAL**FVP**H, ISSIEMKEGAL**FVP**HYYSK, SIEMKEGAL**FVP**HYYSK, SIEMEEGAL**FVP**HYYSKAIVIL	Garcia-Mora et al., (2015)	common bean
0.8267	TF	common bean, mung bean, cowpea	TT**TF**, F**TF**FNLET,	Mojica & Gonzalez de Mejía (2015)	common bean
0.8154	VF	common bean, adzuki bean, mung bean, cowpea	LSG**VF**, YEKLLLLREEGRS**VF**Q,	Mojica & Gonzalez de Mejía (2015) Mojica, Luna-Vital & Gonzalez de Mejía (2016)	common bean
0.8111	PL	common bean, mung bean, cowpea	**PL**EAL, **PL**LELELVEAAG, F**PL**V, **PL**TALFV, RQRL**PL**,	Mojica & Gonzalez de Mejía (2015)	common bean
0.8088	GL	common bean, adzuki bean, cowpea	GG**GL**HK, KTY**GL**, PNLL**GL**SLELLLLL, Y**GL**VAGK, **GL**ASK, ER**GL**AGS, SKAGGVG**GL**SK,	Mojica & Gonzalez de Mejía (2015) Mojica, Luna-Vital & Gonzalez de Mejía (2016)	common bean

It was presented only the fragments with score above 0.8.
Bold: bioactive fragment.
A, Alanine; R, Arginine; N, Asparagine; D, Aspartic acid; C, Cysteine; Q, Glutamine; E, Glutamic acid; G, Glycine; H, Histidine; I, Isoleucine; L, Leucine; K, Lysine; M, Methionine; F, Phenylalanine; P, Proline; S, Serine; T, Threonine; W, Tryptophan; Y, Tyrosine; V, Valine.

Table 4 - Score at PeptideRanker of the Bioactive Peptide Fragments with Potential Antioxidant Activity obtained from BIOPEP and Peptide Sequences with the same bioactive fragment identified in scientific studies at the literature.

Score	Bioactive fragment	Bean specie	Peptide Sequence	Reference	Species studied
0.9924	WG	adzuki bean			
0.9784	RW	adzuki bean, mung bean			
0.9749	WY	mung bean			
0.9713	MM	common bean			
0.9216	RWY	mung bean	**RW**AEK	Mojica, Chen & Gonzalez de Mejía (2015)	common bean
0.8062	ADF	adzuki bean, mung bean			

It was presented only the fragments with score above 0.8.

Bold: bioactive fragment.

A, Alanine; R, Arginine; N, Asparagine; D, Aspartic acid; C, Cysteine; Q, Glutamine; E, Glutamic acid; G, Glycine; H, Histidine; I, Isoleucine; L, Leucine; K, Lysine; M, Methionine; F, Phenylalanine; P, Proline; S, Serine; T, Threonine; W, Tryptophan; Y, Tyrosine; V, Valine.

P07219 - Protein sequence: MMRARVPLLL LGILFLASLS ASFATSLREE EESQDNPFYF NSDNSWNTLFKNQYGHIRVLQRFDQQSKRLQNLEDYRLVEFRSKPETLLLQQADAELLLV VRSGSAILVLVKPDDRREYFFLTQGDNPIFSDNQKIPAGIFYLVNPDPKEDLRIIQLAMPVN NPQIHEFFLSSTEAQQSYLQEFSKHILEASFNSKFEEINRVLFEEEGQQEEGQQEGVIVNI DSEQIEELSKHAKSSSRKSHSKQDNTIGNEFGNLTERTDNSLNVLISSIEMKEGALFVPHY YSKAIVILVVNEGEAHVELVGPKGNKETLEFESYRAELSKDDVFVIPAAYPVAIKATSNVNF TGFGINANNNNRNLLAGKTDNVISSIGRALDGKDVLGLTFSGSGEEVMKLINKQSGSYFV DG HHHQQEQQKG SHQQEQQKGR GAFVY

Enzyme action: M - M - R - **AR** - **VPL** - L - L - L - GIL - F - L - **ASL** - S ASF - AT**SL** - R - EE EESQDN - **PF** - Y - F - N - SDN - **SW** - N - **TL** - F - K - N - **QY** - **GH** - **IR** - **VL** - QR - F - DQQ**SK** - R - L - QN - L - EDY - R - L - VE F - R - **SK** - PE**TL** - L - L - PQQAD**AEL** - L - L - VVR - SGSAIL - V L - **VK** - PDDR - R - **EY** - F - F - L - TQGDN - PI F - SDN - **QK** - IPAG TIF - Y - L - **VN** - PDP K - EDL - R - IIQL - A M - P**VN** - N - PQIH - E F - F - L - SSTEAQQ SY - L - Q**EF** - **SK** - H - I L - EASF - N - **SK** - F - E E**IN** - R - **VL** - F - EEE GQQEEGQQEG VI**VN** - IDSEQI EEL - **SK** - H - AK - SS SR - K - **SH** - **SK** - QDN - TIGN - **EF** - GN - L - T ER - TDN - **SL** - N - **VL** - ISSIEM - K - EGA L - F - VPH - Y - Y - **SK** - A IVIL - V**VN** - EGE AH - VEL - VGPK - G N - K - E**TL** - **EF** - ESY - R - **AEL** - **SK** - DDVF - VIPAAY - PVAI K - ATSN - **VN** - F - TG F - G**IN** - AN - N - N - N - R - N - L - L - AGK - TDN - V ISSI**GR** - **AL** - DG K - D**VL** - **GL** - **TF** - SG SGEEVM - K - L - **IN** - K - QSGSY - F - VDG H - H - H - QQEQ**QK** - G **SH** - QQEQ**QK** - **GR** - K - GAF - **VY**

Figure 1 - Fragments obtained after the simulation of the hydrolysis of phaseolin (P07219) trough the action of gastointestinal enzymes: pepsin, trypsin and quimiotrypsin.
Bold: bioactive fragments.

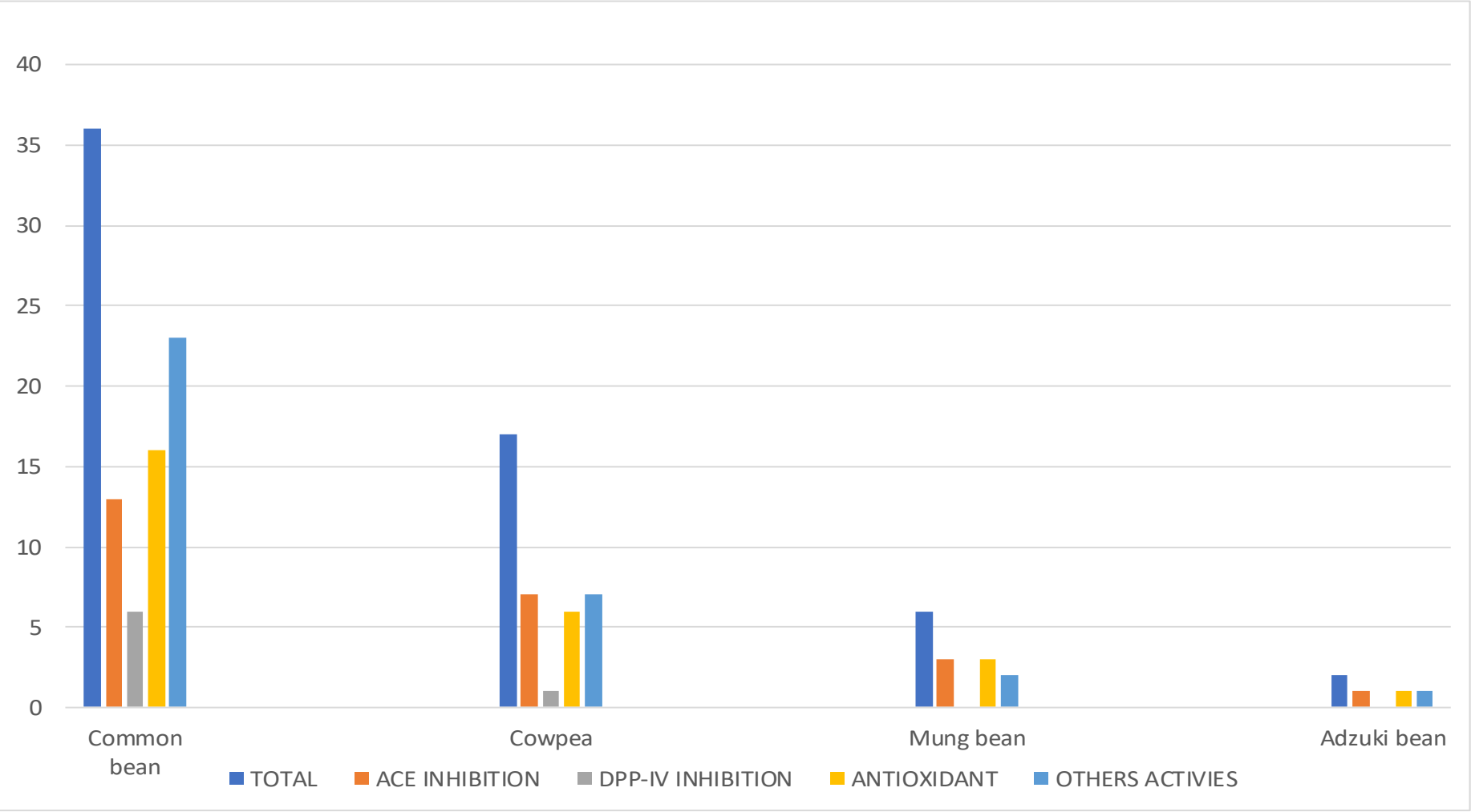

Figure 2 - Amount of studies about bioactive peptides according to the biological activity.
Other activities: Antihypertensive in vivo, anticancer, antibacterial, antiinflamatory, antifungal, hypotensive, inhibition of alfa-amylase and alfa-glucosidase, inhibition of PG2 and TNF production, inhibition of platelet agregation, inhibition of acetilcolinesterase, increase of insulin scretion by INS1 cells.

P07219: P02853
Identity: 94.70%

P07219	1	MMRARVPLLLLGILFLASLSASFATSLREEEESQDNPFYFNSDNSWNTLFKNQYGHIRVL	60
		MMRARVPLLLLGILFLASLSASFATSLREEEESQDNPFYFNSDNSWNTLFKNQYGHIRVL	
P02853	1	MMRARVPLLLLGILFLASLSASFATSLREEEESQDNPFYFNSDNSWNTLFKNQYGHIRVL	60
P07219	61	QRFDQQSKRLQNLEDYRLVEFRSKPETLLLPQQADAELLLVVRSGSAILVLVKPDDRREY	120
		QRFDQQSKRLQNLEDYRLVEFRSKPETLLLPQQADAELLLVVRSGSAILVLVKPDDRREY	
P02853	61	QRFDQQSKRLQNLEDYRLVEFRSKPETLLLPQQADAELLLVVRSGSAILVLVKPDDRREY	120
P07219	121	FFLTQGDNPIFSDNQKIPAGTIFYLVNPDPKEDLRIIQLAMPVNNPQIHEFFLSSTEAQQ	180
		FFLT DNPIFSD+QKIPAGTIFYLVNPDPKEDLRIIQLAMPVNNPQIHEFFLSSTEAQQ	
P02853	121	FFLTS-DNPIFSDHQKIPAGTIFYLVNPDPKEDLRIIQLAMPVNNPQIHEFFLSSTEAQQ	179
P07219	181	SYLQEFSKHILEASFNSKFEEINRVLFEEEGQQEEGQQEGVIVNIDSEQIEELSKHAKSS	240
		SYLQEFSKHILEASFNSKFEEINRVLFEEEGQQE GVIVNIDSEQI+ELSKHAKSS	
P02853	180	SYLQEFSKHILEASFNSKFEEINRVLFEEEGQQE-----GVIVNIDSEQIKELSKHAKSS	234
P07219	241	SRKSHSKQDNTIGNEFGNLTERTDNSLNVLISSIEMKEGALFVPHYYSKAIVILVVNEGE	300
		SRKS SKQDNTIGNEFGNLTERTDNSLNVLISSIEM+EGALFVPHYYSKAIVILVVNEGE	
P02853	235	SRKSLSKQDNTIGNEFGNLTERTDNSLNVLISSIEMEEGALFVPHYYSKAIVILVVNEGE	294
P07219	301	AHVELVGPKGNKETLEFESYRAELSKDDVFVIPAAYPVAIKATSNVNFTGFGINANNNNR	360
		AHVELVGPKGNKETLE+ESYRAELSKDDVFVIPAAYPVAIKATSNVNFTGFGINANNNNR	
P02853	295	AHVELVGPKGNKETLEYESYRAELSKDDVFVIPAAYPVAIKATSNVNFTGFGINANNNNR	354
P07219	361	NLLAGKTDNVISSIGRALDGKDVLGLTFSGSGEEVMKLINKQSGSYFVDGHHHQQEQQKG	420
		NLLAGKTDNVISSIGRALDGKDVLGLTFSGSG+EVMKLINKQSGSYFVD HH	
P02853	355	NLLAGKTDNVISSIGRALDGKDVLGLTFSGSGDEVMKLINKQSGSYFVDAHH---------	406
P07219	421	SHQQEQQKGRKGAFVY	436
		HQQEQQKGRKGAFVY	
P02853	407	-HQQEQQKGRKGAFVY	421

Chart 1 - BLAST: example of (%) Identity between two sequences of the same protein.
Legend: PA07219: Alpha phaseolin. P02853 Phaseolin beta-type.

+ there is similarity between the two amino acids
- gap

***Phaseolus vulgaris* (L.) - Phaseolin**						
P07219 - Protein sequence: MMRARVPLLL LGILFLASLS ASFATSLREE EESQDNPFYF NSDNSWNTLF KNQYGHIRVL QRFDQQSKRL QNLEDYRLVE FRSKPETLLL PQQADAELLL VVRSGSAILV LVKPDDRREY FFLTQGDNPI FSDNQKIPAG TIFYLVNPDP KEDLRIIQLA MPVNNPQIHE FFLSSTEAQQ SYLQEFSKHI LEASFNSKFE EINRVLFEEE GQQEEGQQEG VIVNIDSEQI EELSKHAKSS SRKSHSKQDN TIGNEFGNLT ERTDNSLNVL ISSIEMKEGA LFVPHYYSKA IVILVVNEGE AHVELVGPKG NKETLEFESY RAELSKDDVF VIPAAYPVAI KATSNVNFTG FGINANNNNR NLLAGKTDNV ISSIGRALDG KDVLGLTFSG SGEEVMKLIN KQSGSYFVDG HHHQQEQQKG SHQQEQQKGR KGAFVY					
	Profiles of potential		**GI: pepsin, trypsin,**		**Subtilisin**	
Activity	*Bioactive fragment*	A	*Bioactive fragment*	A	*Bioactive fragment*	A
Antiamnestic (prolyl endopeptidase inhibitor)	VPL, GP	0.0046	VPL	0.002	VPL	0.0020
ACE inhibitor	RL, IR, VF, HIR, RF, VY, HY, IPA, YL, LF, YG, FY, AY, YP, GP, PL, VK, FFL, IP, AF, LA, KR, VP, RA, AA, FR, IF, VG, IG, GI, GA, GL, AG, GH, GR, KG, FG, DA, GS, GQ, GK, GE, QG, SG, LG, GD, TG, EG, EA, VR, LTF, QK, DG, NF, SY, SF, KF, KL, NK, RR, AR, KA, LVE, EY, KP, EI, IE, EV, VE, TE, LQ, LN, TQ, AH, PQ, KE, PH, TF, AI, VNP, VKP, LEF, FVP, PVNNPQIH, ASL, LGI, VGP, DY, IL, MM, AEL, ST, LR	0.2133	IR, ASL, AEL, VY, VK, GL, GH, GR, QK, AR, EY, TF	0.0240	RL, VF, VY, GL, GS, KF, TF	0.0160
Antithrombotic	GP	0.0023	-	-	-	-
Immunomodulating	YG	0.0023	-	-	-	-
Stimulating (stimulating vasoactive substance release)	VPL, EEE, LLL, EE, SE	0.0275	VPL	0.008	VPL	0.0100
Stimulating (glucose uptake stimulating peptide)	VL, LV, IV, IL, LI, II, LL		VL		VL	
Neuropeptide	GQ, YL	0.0046	-	-	-	-

Chart 2 - Predicted Potential of Obtaining Bioactive Peptides for Phaseolin.

Chart 2 - (Continued)

Regulating (peptide regulating ion flow)	DY	0.0046	-	-	-	-
Regulating (peptide regulating the stomach mucosal membrane activity)	GP					
Antioxidative	HH, AY, AH, EL, HYY, YYS, HHH, PHY, KD, IR, KP, VY, SWN, VKP, FVPH, MM	0.0367	IR, VY	0.0040	VY	0.0020
Inhibitor (CaMPDE inhibitor)	IR, KF, EF	0.0046	IR, EF	0.0060	KF, EF	0.0040
Hypotensive (renin inhibitor)	FT, LR, IR, KF, EF, NR, SF, TF	0.0183	IR, EF, TF	0.0080	KF, EF, TF	0.0060
Activating ubiquitin-mediated proteolysis	RA, LA	0.0046	-	-	-	-
Dipeptidyl peptidase IV inhibitor	GP, MP, VA, KA, LA, FA, PA, VP, LL, VV, HA, IPA, VPL, IP, KP, YP, GA, RA, NP, FL, AL, SL, GL, VR, AA, PL, WN, AD, AE, AF, AG, AH, AS, AT, AY, DN, DP, DQ, DR, EG, EI, ES, ET, EV, EY, FN, FR, GE, GH, GI, HE, HH, HI, HS, HV, HY, IH, II, IL, IN, IQ, IR, KE, KF, KG, KH, KI, KR, KS, KT, LI, LN, LT, LV, MK, MM, MR, NA, NE, NF, NL, NN, NQ, NR, NT, NV, PF, PH, PI, PK, PQ, PV, QA, QD, QE, QG, QI, QL, QN, QQ, QS, QY, RI, RK, RL, RR, SF, SH, SI, SK, SW, SY, TD, TE, TF, TG, TI, TL, TQ, TS, VD, VE, VF, VG, VI, VK, VL, VM, VN, VY, YF, YG, YL, YR, YS, YY	0.3119	VPL, AL, SL, GL, EY, GH, IN, IR, PF, QY, SH, SK, SW, TF, TL, VK, VL, VN, VY	0.0561	VPL, GL, AS, ES, HS, KF, RL, TF, VF, VI, VL, VL, VL, VL, VY	0.0301

Protein sequence obtained from Uniprot database; Bioactive peptides fragments obtained from BIOPEP database.
A: frequency of occurence; GI: gastrointestinal digestion.
A, Alanine; R, Arginine; N, Asparagine; D, Aspartic acid; C, Cysteine; Q, Glutamine; E, Glutamic acid; G, Glycine; H, Histidine; I, Isoleucine; L, Leucine; K, Lysine; M, Methionine; F, Phenylalanine; P, Proline; S, Serine; T, Threonine; W, Tryptophan; Y, Tyrosine; V, Valine.

Vigna angularis (Willd.) - 7S globulin						
A4PI98 - Protein sequence: IVHREHHESR EEVSVSSGKN NPFYFNSDRW FRTLYRNEWG HIRVLQRFDQ RSKQMQNLEN YRVVEFKSKP NTLLLPHHAD ADFLLVVLNG TAVLTLVNPD SRDSYILEQG HAQKIPAGTT FFLVNPDDNE NLRIIKLAIP VNNPHRFQDF FLSSTEAQQS YLRGFSKNIL EASFDSDFKE INRVLFGEER QQQQGEESRE EGVIVELKRE QIQELMKHAK SSSRKELSSQ DEPFNLRNSK PIYSNKFGRW YEMTPEKNPQ LKDLDVFISS VDMKEGALLL PHYSSKAIVI MVINEGEAKI ELVGLSDQQQ QKQQEESLEV QRYRAELSED DVFVIPAAYP VAINATSNLN FFAFGINAEN NRRNFLAGGK DNVMSEIPTE VLEVSFPASG KKVEKLIKKQ SESHFVDAQP EQQQREEGHK GRKGSLSSIL GSLY						
	Profiles of potential biological activity		**GI: pepsin, trypsin, chymotrypsin**		**Subtilisin**	
Activity	*Bioactive fragment*	A	*Bioactive fragment*	A	*Bioactive fragment*	A
ACE inhibitor	IR, RY, LY, IY, VF, HIR, RF, HY, FP, IPA, YL, LF, FY, AY, AIP, YP, LLP, FFL, RW, IP, AF, LA, KR, RA, AA, GF, FR, VG, GI, GA, GL, AG, GH, GR, KG, FG, DA, GS, GV, GK, GT, WG, GE, GG, QG, SG, EG, EA, NG, QK, NF, SY, SF, KF, KL, NK, RR, KA, KP, EI, EV, VE, TE, LQ, LN, PT, PQ, EW, EK, KE, PH, HK, AI, VNP, AV, AVL, TP, DF, DM, FQ, YE, IL, AEL, RG, ST, LR	0.1982	IR, AF, GF, GR, EW, PH, IL, AEL	0.0201	VF, AF, DF, IL	0.0121
Antioxidative	PHH, LLPH, LPHH, HH, LLPHH, IKK, AY, ADF, SDF, LY, IY, EL, EHH, PHR, PHY, IKL, EAK, KAI, KD, RW, IR, LK, KP, WG	0.0553	EL, IR	0.0040	KAI	0.0020
Dipeptidyl peptidase IV inhibitor	VA, KA, LA, FA, PA, LP, LL, VV, HA, IPA, IP, TP, FP, KP, YP, GA, RA, EP, NP, TA, QP, FL, EK, AL, SL, GL, AA, VGL, WG, AD, AE, AF, AG, AS, AT, AV, AY, DN, DQ, DR, EG, EH, EI, ES, EV, EW, FN, FQ, FR, GE, GF,GG, GH, GI, GV, HE, HF, HH, HI, HR, HY, II, IL, IN, IQ, IR, KE, KF, KG, KH, KI, KK, KR, KS, KV, LI, LM, LN, LT, LV, MK, MQ, MV, NA, NE, NF, NG, NL, NN, NR, NT, NV, PF, PH, PI, PQ, PT, QD, QE, QG, QI, QN, QQ, QS, RG, RI, RK, RN, RR, RW, SF, SH, SI, SK, SV, SY, TE, TL, TS, TT, VD, VE, VF, VG, VH, VI, VL, VM, VN, VS, YE, YF, YI, YL, YR, YS	0.3134	VGL, AF, EH, EW, GF, IL, IR, PF, PH, QN, SK, TL, VL, VM, VN	0.0483	VGL, AF, ES, HF, IL, KS, NL, TL, VF, VI, VL, VS	0.0423

Chart 3 - Predicted Potential of Obtaining Bioactive Peptides for 7S globulin.
Protein sequence obtained from Uniprot database; Bioactive peptides fragments obtained from BIOPEP database.
The chart only presents the activities with A>0.05 at the profile of potencial biological activity.
A: frequency of occurence; GI: gastrointestinal digestion.
A, Alanine; R, Arginine; N, Asparagine; D, Aspartic acid; C, Cysteine; Q, Glutamine; E, Glutamic acid; G, Glycine; H, Histidine; I, Isoleucine; L, Leucine; K, Lysine; M, Methionine; F, Phenylalanine; P, Proline; S, Serine; T, Threonine; W, Tryptophan; Y, Tyrosine; V, Valine.

***Vigna radiata* (L.) - 8 S globulin**						
Q198W3 - Protein sequence: MVRARVQLLL GILFLASLSV SFGIVHREHQ ESQEESDSRG QNNPFYFNSD RRFHTLFKNQ YGHLRVIHRF DQRSKQIQNL ENYRVVEFKS KPNTLLLPHH ADADFLLVVL NGRAILTLVN PDGRDSYILE QGHAQKIPAG TTFFLVNPND NDNLRIIKLA IPVNNPHRFQ NFFLSSTEAQ QSYLRGFSKN ILEASFDSDF KEIDRVLFGE ERQQQHGEES QEEGVIVELK REQIRELIKH AKSSSRKELS SQDEPFNLRN SNPIYSNKFG RWYEITPEKN PQLKDLDVFI SSVDMKEGGL LLPHYNSKAI VILVINEGEA KIELVGPSDQ QQQDESLEVQ RYRAELSEDD VFVIPAAYPV AINATSNLNF FAFGINAENN QRNFLAGEKD NVMSEIPTEV LDVSFPASGN KVEKLIKKQS ESHFVDAQPE QQQREEGHKG RKGSLSSILG SLY						
	Profiles of potential biological activity		**GI: pepsin, trypsin, chymotrypsin**		**Subtilisin**	
Activity	*Bioactive fragment*	A	*Bioactive fragment*	A	*Bioactive fragment*	A
ACE inhibitor	IR, RY, LY, IY, VF, RFH, RF, HY, FP, IPA, YL, LF, YG, FY, AY, YP, LLP, GP, FFL, RW, IP, AF, LA, RA, AA, GF, VG, GI, AG, GH, HL, GR, KG, FG, DA, GS, GV, HG, GE, GG, QG, SG, LG, EG, EA, NG, VR, QK, DG, NY, NF, SY, SF, KF, KL, NK, RR, AR, KA, KP, EI, IE, EV, VE, TE, LN, PT, PQ, EK, KE, PH, HK, TF, AI, VNP, LNF, ASL, VGP, TP, DF, DM, FQ, YE, IL, AEL, RG, ST, YN, LR	0.1987	AF, GF, GH, GR, VR, AR, PH, PH, PH, ASL, IL, AEL	0.0231	VF, AF, GI, NF, DF, IL	0.0154
Antioxidative	PHH, LLPH, HL, LPHH, HH, LLPHH, IKK, AY, ADF, SDF, LY, IY, EL, WY, PHR, PHY, RWY, IKL, KAI, KD, RW, IR, LK, KP	0.0530	EL	0.0040	PHY	0.0020
Dipeptidyl peptidase IV inhibitor	GP, KA, LA, FA, PA, LP, LL, VV, HA, IPA, IP, TP, FP, KP, YP, RA, EP, NP, QP, FL, HL, EK, SL, GL, VR, AA, WY, AD, AE, AF, AG, AS, AT, AY, DN, DQ, DR, EG, EH, EI, ES, EV, FN, FQ, GE, GF, GG, GH, GI, GV, HF, HH, HR, HT, HY, IH, II, IL, IN, IQ, IR, KE, KF, KG, KH, KI, KK, KS, KV, LI, LN, LT, LV, MK, MV, NA, ND, NE, NF, NG, NL, NN, NQ, NT, NV, NY, PF, PH, PI, PN, PQ, PS, PT, PV, QD, QE, QG, QH, QI, QL, QN, QQ, QS, RG, RI, RK, RN, RR, RW, SF, SH, SI, SK, SV, SY, TE, TF, TL, TS, TT, VD, VE, VF, VG, VH, VI, VL, VM, VN, VQ, VS, YE, YF, YG, YI, YL, YN, YR, YS	0.3068	VR, AF, DN, EH, GF, GH, IL, PF, PH, PN, SK, TL, VL, VM, VN	0.0442	AF, AS, GI, HF, IL, KS, NF, NL, TL,VF, VF, VI, VL, VS	0.0327

Chart 4 - Predicted Potential of Obtaining Bioactive Peptides for 8S globulin.

Chart 4 (Continued)

B1NPN8 - Protein sequence: MVRARIPLLL LLGILFLASL SVSFGIVHRE NIDGAEVSVS RGKNNPFYFN SDRWFHTLFR NQFGHLRVLQ RFDQRSKQMQ NLENYRVVEL MSKPNTLLLP HHADADFLLV VLNGRAVLTL VNPDGRDSNI LEQGHAQKIP AGTTFFLVNP DDNENLRIIK LAVPVNNPHR FQDFFLSSTE AQQSYLQGFS KNILEASFDS DIKEISRVLF GEEGQQQQQG QESQQEGVIV ELKREQIREL TKHAKSSSKK SLSSEDQPFN LRNQKPIYSN KLGRWFEITP EKNPQLRDLD MFIRSVDMKE GSLLLPHYNS KAIVILVINE GKANIELVGQ REQQKQQEEQ EESWEVQRYR AELSEDDVFI IPATYPVAIN ATSNLNFFAF GINAENNQRN FLAGEKDNVI SEIPTEVLDV TFPASGEKVK KLIKKQSESQ FVDAQPEQQE REEARKGGKG PFVY

	Profiles of potential biological activity		**GI: pepsin, trypsin, chymotrypsin**		**Subtilisin**	
Activity	*Bioactive fragment*	A	*Bioactive fragment*	A	*Bioactive fragment*	A
ACE inhibitor	IR, AVP, RY, IY, VF, MF, RF, VY, HY, FP, IPA, YL, LF, FY, YP, LLP, PL, VK, FFL, RW, IP, AF, LA, KR, VP, RA, GF, FR, VG, GI, GA, AG, GH, HL, GR, KG, FG, DA, GS, GV, GQ, GK, GT, GE, GG, QG, SG, LG, EG, EA, NG, VR, QK, DG, NY, NF, SY, SF, KL, AR, KA, KP, EI, IE, EV, VE, TE, LQ, LN, PT, PQ, EK, KE, PH, TF, AI, VNP, LNF, AV, ASL, AVL, LGI, TP, DF, DM, FQ, IL, AEL, RG, ST, YN, LFR, LR	0.2048	IR, VY, VK, AF, GH, GR, GK, VR, QK, AR, PH, ASL, AVL, IL	0.0307	VF, VY, AF, VP, GI, NF	0.0115
Dipeptidyl peptidase IV inhibitor	VA, KA, LA, FA, PA, LP, VP, LL, VV, HA, IPA, IP, TP, FP, KP, YP, GA, RA, NP, QP, FL, HL, EK, SL, VR, PL, WE, WF, AD, AE, AF, AG, AS, AT, AV, DN, DQ, DR, EG, EI, ES, EV, FN, FQ, FR, GE, GF, GG, GH, GI, GV, HH, HR, HT, HY, II, IL, IN, IR, KE, KG, KH, KI, KK, KR, KS, KV, LI, LN, LT, LV, MF, MK, MQ, MV, NA, NE, NF, NG, NL, NN, NQ, NT, NV, NY, PF, PH, PI, PN, PQ, PT, PV, QD, QE, QF, QG, QI, QL, QQ, QS, RG, RI, RK, RN, RW, SF, SK, SV, SW, SY, TE, TF, TK, TL, TS, TT, TY, VD, VE, VF, VG, VH, VI,VK, VL, VN, VQ, VS, VY, YF, YL, YN, YR, YS	0.2952	VR, AF, DN, GH, IL, IR, PF, PH, PN, QF, SK, TL, VK, VL, VN, VY	0.0403	VP, AF, AS, ES, GI, NF, NL, TL, VF, VI, VL, VS, VY	0.0365

Chart 4 - Predicted Potential of Obtaining Bioactive Peptides for 8S globulin.
Protein sequence obtained from Uniprot database; Bioactive peptides fragments obtained from BIOPEP database.
The chart only presents the activities with A>0.05 at the profile of potencial biological activity.
A: frequency of occurence; GI: gastrointestinal digestion.
A, Alanine; R, Arginine; N, Asparagine; D, Aspartic acid; C, Cysteine; Q, Glutamine; E, Glutamic acid; G, Glycine; H, Histidine; I, Isoleucine; L, Leucine; K, Lysine; M, Methionine; F, Phenylalanine; P, Proline; S, Serine; T, Threonine; W, Tryptophan; Y, Tyrosine; V, Valine.

***Vigna unguiculata (L.) Walp* - Vicilin**						
A0A2U9K6L2 - Protein sequence: IVHREHQESQ EESEPRGQNN PFYFDSDRWF HTLFRNQYGH LRVLQRFDQR SKQIQNLENY RVVEFKSKPN TLLLPHHADA DFLLVVLNGR AILTLVNPDG RDSYILEEGH AQKIPAGTTF FLVNPDDNEN LRIVKLAVSV NNPHRFQDFF LSSTEAQQSY LQGFSKNILE ASFGSDCKEI NRVLFGEEEQ QQQDEESQQE GVIVQLKREQ IRELMKHAKS TSKKSLSSQN EPFNLRSQKP IYSNKFGRLH EITPEKNPQL RDLDVFLTSV DMKEGGLFMP NYNSKAIVIL VVNKGEANIE LVGQREQQQQ QQEESWEVQR YRAEVSEDDV FVIPASYPVA ITATSNLNFI AFGINAESNQ RNFLAGEEDN VMSEIPTEVL DVTFPASGEK VEKLINKQSD SHFTDAQPEQ QQREEDRKGR KGPLSSILDS LY					
	Profiles of potential biological activity		**GI: pepsin, trypsin, chymotrypsin**		**Subtilisin**	
Activity	*Bioactive fragment*	A	*Bioactive fragment*	A	*Bioactive fragment*	A
ACE inhibitor	RL, IR, LY, IY, VF, RF, FP, IPA, PR, LF, YG, FY, YP, LLP, GPL, GP, PL, VK, RW, IP, AF, LA, KR, RA, GF, FR, VG, GI, GL, AG, GH, GR, KG, FG, DA, GS, GV, GQ, GT, GE, GG, QG, SG, EG, EA, NG, QK, DG, NY, NF, SY, SF, KF, KL, NK, KA, KP, EI, IE, EV, VE, TE, LQ, LN, PT, PQ, EK, KE, PH, TF, AI, VNP, LNF, AV, LEE, IVQ, TP, DF, DM, FQ, IL, KGP, RG, ST, YN, LFR, LR	0.2014	GPL, GH, GR, PH, IL	0.0182	VF, GS, NF, IL	0.0101
Dipeptidyl peptidase IV inhibitor	GP, MP, VA, KA, LA, PA, LP, LL, VV, HA, IPA, IP, TP, FP, KP, YP, RA, EP, NP, TA, QP, FL, EK, SL, GL, PL, DVTFPA, WE, WF, AD, AE, AF, AG, AS, AT, AV, DN, DQ, DR, EG, EH, EI, ES, EV, FN, FQ, FR, GE, GF, GG, GH, GI, GV, HF, HH, HR, HT, IL, IN, IQ, IR, KE, KF, KG, KH, KI, KK, KR, KS, LH, LI, LM, LN, LT, LV, MK, NA, NE, NF, NG, NL, NN, NQ, NR, NY, PF, PH, PN, PQ, PT, PV, QD, QE, QG, QI, QL, QN, QQ, QS, QY, RG, RI, RK, RL, RN, RW, SF, SH, SI, SK, SV, SW, SY, TD, TE, TF, TL, TS, TT, VE, VF, VG, VH, VI, VK, VL, VM, VN, VQ, VS, VT, YF, YG, YI, YN, YR, YS	0.3171	SL, EH, GH, IL, IN, PH, PN, QY, SK, TL, VL, VN	0.0384	HF, IL, KS, NF, NL, RI, TL, TS, VF, VI, VL, VS	0.0364

Chart 5 - Predicted Potential of Obtaining Bioactive Peptides for Vicilin.
Protein sequence obtained from Uniprot database; Bioactive peptides fragments obtained from BIOPEP database.
The chart only presents the activities with A>0.05 at the profile of potencial biological activity.
A: frequency of occurence; GI: gastrointestinal digestion.
A, Alanine; R, Arginine; N, Asparagine; D, Aspartic acid; C, Cysteine; Q, Glutamine; E, Glutamic acid; G, Glycine; H, Histidine; I, Isoleucine; L, Leucine; K, Lysine; M, Methionine; F, Phenylalanine; P, Proline; S, Serine; T, Threonine; W, Tryptophan; Y, Tyrosine; V, Valine.