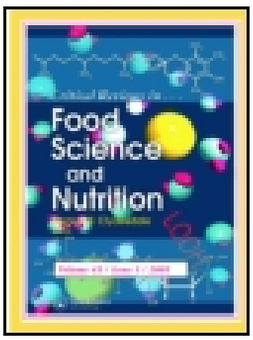

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REVIEW

Bioactive peptides from beans with the potential to decrease the risk of developing noncommunicable chronic diseases

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ABSTRACT

Several studies have demonstrated that peptides obtained from the proteins of different bean species have the potential to act on therapeutic targets of noncommunicable chronic diseases or NCDs. However, peptides with great structural diversity can be obtained from the hydrolysis of proteins present in foods. Therefore, the present review had the objective of identifying, *in silico,* the possibility of obtaining peptides with potential biological activity from the storage globulin proteins of the bean species *Phaseolus vulgaris* (L.), *Vigna angularis* (Willd.), *Vigna radiata* (L.) and *Vigna unguiculata* (L.) Walp., using the UniProtKB, BIOPEP and PeptideRanker databases, as well as reviewing available research reports that showed evidence bioactive properties of peptides obtained from beans via *in vitro* assays. For all the species studied, the highest frequency of the occurrence of bioactive fragments was found for the inhibition of dipeptidyl peptidase-IV, followed by the inhibition of the angiotensin-converting enzyme and by antioxidant activity. The inhibition of the two enzymes is the therapeutic target of drugs used for type 2 diabetes mellitus (T2DM) and for hypertension, respectively, while the antioxidant activity can prevent the development of several chronic diseases related to oxidative stress.

KEYWORDS

Arterial hypertension; type 2 diabetes mellitus; public health; protein; bioinformatics

Introduction

According to the World Health Organization (WHO 2018), an estimated 41 million deaths occurred in 2016 due to noncommunicable chronic diseases (NCDs), accounting for 71% of the worldwide total of 57 million deaths. Cardiovascular disease, cancer, chronic respiratory disease and diabetes are the four main diseases accounting for the majority of deaths, with 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 multiple causes, and are characterized by a gradual initiation with an uncertain prognosis, and a long or indefinite duration. Their clinic development oscillates with time, and there may be periods of exacerbation that generate disabilities. These diseases require interventions with the use of medications and are 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 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 throughout the world, and gestational diabetes, type 1 diabetes and type 2 diabetes (T2DM) are the three main types of this disease, the latter being responsible for about 90% of all cases. With respect to hypertension, the incidence is even higher, reaching 1.13 billion in 2015 (NCD Risk Factor Collaboration (NCD-RisC) 2017). Furthermore, diabetes can lead to cardiovascular diseases, amputation, kidney failure and blindness, due to the persistently high blood glucose levels which cause generalized vascular damage, affecting the heart, nerves, kidneys and eyes (IDF 2017). Hypertension is also an important risk factor for cardiovascular diseases and chronic kidney disease (NCD-RisC 2017).

In this context, it is worth mentioning that some vegetable and animal compounds may have a positive influence on human health, acting in specific tissues or cells, and are therefore denominated as bioactive compounds (Astley and Finglas 2016), including the bioactive peptides, which are sequences of approximately 2–20 amino acids (Li and Yu 2015). These compounds can be obtained by hydrolyzing the storage proteins of beans, a legume which has been the focus of much research recently. It has been demonstrated that the *in vitro* enzymatic hydrolysis by Alcalase® of the bean 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), and/or by the simulation of gastrointestinal digestion with pepsin and pancreatin, generated bioactive peptides with antioxidant potential and inhibitory activity against the dipeptidyl peptidase-IV enzyme (DPP-IV), which is a molecular target in the treatment of T2DM (Mojica and González de Mejía 2015; Rocha et al. 2014; Rocha et al.

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2015), and against the angiotensin-converting enzyme (ACE), which is a molecular target of antihypertensive medications (Li, Shi, et al. 2006; Mojica and González de Mejía 2015) and also to decrease inflammatory markers and mediators (Oseguera-Toledo et al. 2011).

Despite the existence of well-established protocols for the treatment of T2DM and hypertension, there is a search for alternatives that reduce the adverse effects caused by the medications and that aid in the treatment. 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, is therefore of interest. However, the industrial production of bioactive peptides is still incipient, due to the difficulty of selecting suitable molecules and the lack of technologies for processes such as protein hydrolysis, separation and purification, besides a lack of studies that characterize the bioavailability of these molecules (Rizzello et al. 2016).

Currently, many of the studies concerning bioactive peptides are carried out using *in vitro* assays, evidencing the activity of these molecules on biological markers such as enzymes. In vivo studies are scarce and do not usually have a pharmacological approach in terms of bioavailability and interaction with other food components (Li and Yu 2015).

In silico studies are computational simulations used to characterize biological experiments, carried out entirely on a computer by way of software (Marshall 2018). Considering the possibility of obtaining peptides with great structural diversity from the hydrolysis of proteins present in foods, bioinformatics could 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 that could be good candidates for industrial production, to carry out *in vivo* studies.

Recently, databases such as UniProtKB (The UniProt Consortium 2019), BIOPEP-UWM (Minkiewicz, Iwaniak, and Darewicz 2019) and PeptideRanker (Mooney et al. 2012) have been used by studies to confirm sequences and to predict peptide bioactivity (Luna Vital et al. 2014; Mojica and González de Mejía 2015; Oseguera-Toledo, González de Mejía, and 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 in the storage proteins of beans, 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 UniProtKB (The UniProt Consortium 2019), BIOPEP-UWM (Minkiewicz, Iwaniak, and Darewicz 2019) 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 had 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 greater than 31 million tons, and Brazil was the third major producer, with approximately 3 million tons. Similar to other legumes, beans accumulate large amounts of protein in their seeds, the majority of which are storage proteins, which receive this name because they are mobilized from specialized subcellular compartments to provide nutrients for the growth of new plants during the germination process (Argos, Narayana, and Nielsen 1985). These 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 albumins which are soluble in water, glutelins which are soluble in dilute alkaline or acid solutions, and prolamins which are soluble in alcohol (Osborne 1912).

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

The common bean (*Phaseolus vulgaris* (L.)) originated in Central America and is among the main edible legumes (BRAZIL. State Secretariat of Agriculture and Food Supply (SEAB)) 2016). According to a study by Perazzini et al. (2008), who 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 to 33.1–45.1%, representing the majority of the total protein content. The glutelins varied from 12.8 to 41.2% and the albumins from 14.8 to 20.8%, while the prolamins only reached values above 1.0% in three of the cultivars studied.

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

Bean species of the genus *Vigna* are cultivated in several regions of the world, and amongst them, *Vigna unguiculata* (L.) (cowpea), *Vigna radiata* (L.) (mung bean) and *Vigna angularis* (Willd.) (adzuki bean) stand out (Bell et al. 2011). The cowpea is cultivated in Asia, Africa, and South America, and is a very important legume in the tropical and subtropical regions of the world (BRAZIL. State Secretariat of Agriculture and Food Supply (SEAB)) 2016). According to Awika and Duodu (2017), the total protein content of the cowpea varies from 22 to 30%, the majority of which is

storage protein. Gupta et al. (2010) evaluated 21 cowpea genotypes and the total protein content varied from 22.4 to 27.9%. They selected the 7 genotypes with the highest protein contents and found that the globulins represented the major fraction of the total protein content, accounting for 55.6–58.8%, followed by the glutelins (14.4–15.6%), the albumins (8.2–11.9%) and the prolamins (2.3–5.0%). Of the globulins, legumin (11 S globulin) represented a minor fraction and vicilin (7 S globulin) the major fraction (Fotso et al. 1994).

The adzuki bean is native to China, traditionally consumed in the east of Asia and 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 sprouts. In Brazil, it is cultivated mainly by colonists of Japanese origin (Guareschi et al. 2009; Sato et al. 2016). The adzuki bean contains approximately 25% of protein (Yousifet al. 2002), the majority of the globulin fraction being composed of the storage protein vicilin (7 S globulin), representing 78%, with legumin (11 S globulin) as a minor component, accounting for 12% (Meng and Ma 2001).

The mung bean (*Vigna radiata)* is native to 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 an essential amino acid profile comparable to that of soybeans (Li et al. 2010). In Brazil, the recurrent use of bean sprouts in cooking is associated with an increased consumption of mung beans (Lima et al. 2004). The protein content of mung beans varies from 17 to 26% (Mendoza et al. 2001), the globulins representing the major fraction, accounting for 62%, followed by the albumins (16.3%), the glutelins (13.3%) and the prolamins (0.9%) (Amaral et al. 2017). Of the globulins, vicilin (8 S globulin) corresponded to 89% of the total, while legumin (11 S globulin) and the basic 7 S globulin corresponded to 7.6% and 3.4%, respectively (Mendoza et al. 2001).

The environmental conditions of the locality used for seed growth and maturation, the gene expressions regulating the synthesis and accumulation of the protein and nonprotein fractions in the seed, and the genotype of the maternal plant are key factors that influence the protein contents of beans (Perazzini et al. 2008).

Type two diabetes mellitus, arterial hypertension and oxidative stress

In a patient with T2DM, the body does not respond adequately to the action of the insulin and hence the blood glucose level rises, a condition known as hyperglycemia. The diagnostic criterion for this disease is a fasting glucose level $\geq$126 mg/dL. Due to the inefficient action of the insulin, the production of this hormone increases in an attempt to reduce the glucose levels, but eventually the pancreas is unable to produce enough insulin to maintain normal blood glucose levels (American Diabetes Association (ADA)), 2017, 2019; IDF, 2017).

DPP-IV is an enzyme that takes part in the inactivation of the incretins, which are hormones that stimulate insulin secretion. The main incretins are GLP-1 (glucagon-like peptide) and GIP (glucose-dependent insulinotropic peptide), and hence the inhibition of DPP-IV is a mechanism used to control T2DM (Oseguera-Toledo et al. 2014).

On the other hand, arterial hypertension is a multifactorial condition characterized by a sustained elevation of the blood pressure levels at >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 angiotensin I to angiotensin II, which is a potent vasoconstrictor, leading to elevated blood pressure (Ariza-Ortega et al. 2014).

Considering the chronic diseases, it is worth highlighting the oxidation process, the resulting products of which can contribute to the emergence of degenerative diseases. Oxidation is a vital process in the human body in which the production of free radicals (highly reactive molecules that contain an unpaired electron in their last electron shell) occurs. Under normal circumstances, oxidation is a dynamic and continuous balance between the production and elimination of free radicals, but the excess production of these molecules can cause continuous damage to cells, known as oxidative stress (Li and Yu 2015; Liguori et al. 2018; Zuo et al. 2019). This process can cause DNA damage and mutations, which can be a risk factor for the development of several acute and chronic pathological processes such as cardiovascular diseases, chronic obstructive pulmonary disease, chronic kidney disease, neurodegenerative diseases, cancer and diabetes (Liguori et al. 2018). For this reason, the regular consumption of antioxidant compounds has been recommended (Khansari, Shakiba, and Mahmoudi 2009).

Bioactive peptides

Bioactive peptides are fragments obtained from proteins, which promote health benefits when consumed by humans by acting positively on the nutrition and demonstrating numerous physiological functions in the body (Bhandari et al. 2020). After ingestion, bioactive peptides can be absorbed in the intestine and carry out activities in various metabolic pathways, such as the pathways involved in glucose uptake, and also modulate the blood pressure by inhibiting enzymes like ACE (Agyei et al. 2016).

Some researchers have evaluated and characterized the bioactive peptides obtained 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 and Yu 2015).

Obtention of bioactive peptides

Bioactive peptides can be produced by fermentation processes using proteolytic microorganisms, especially those which release peptides from dairy products, hydrolysis by gastrointestinal enzymes and by enzymes obtained from

plants and microorganisms. According to Rizzello et al. (2016) peptide production has been extensively explored in the last decade in terms of animal protein sources, especially milk, but nowadays, plant-derived peptides have become interesting because of their lower cost and the variety of plants available (Rizzello et al. 2016).

Most studies characterizing bioactive peptides are carried out by *in vitro* assays, and the simulation of gastrointestinal digestion is amongst the methods most used to obtain these peptides. Pepsin, pancreatin and chymotrypsin are the most commonly used proteases for this process (Wang et al. 2018). Pepsin is an endopeptidase that degrades proteins into peptides and is responsible for less than 20% of the protein digestion in the gastrointestinal tract, acting in the stomach (Smith and Morton 2010). Pancreatin contains several enzymatic components produced in the pancreas, including trypsin, which is a serine endopeptidase that hydrolyzes the peptide bonds after arginine or lysine residues (Chen, Radisky, and Férec 2013). Besides trypsin, during gastrointestinal digestion, the pancreas also secretes chymotrypsin, an endoprotease, which cleaves proteins with aromatic amino acid residues, acting in the duodenum (Prasad, Hollins, and Lambert 2010).

Hydrolysis with commercial enzymes is another method widely used to obtain peptides with potential biological activity from the proteins present in foods. The use of Flavourzyme®, a fungal protease complex that catabolizes proteins by hydrolyzing the peptide bonds has been reported for the obtaining of peptides from bean proteins (Segura-Campos, Chel-Guerrero, and Betancur-Ancona 2011), and also Alcalase® (Oseguera-Toledo, González de Mejía, and Amaya-Llano 2015; Li, Shi, et al. 2006; Rocha et al. 2015), which is a commercial form of subtilisin obtained from *Bacillus licheniformis*. Subtilisins are serine proteases which catalyze protein hydrolysis and show broad specificity for peptide bonds (HERA 2007).

In vitro evidences of the health benefits of *bioactive peptides* from beans

Several *in vitro* studies have demonstrated the health benefit potentials of the bioactive peptides obtained from bean sources. The anti-hypertensive potential was studied by Durak et al. (2013), who obtained bioactive peptides from the protein fractions of the adzuki bean by *in vitro* enzymatic hydrolysis via the simulation of gastrointestinal conditions with the enzymes α-amylase, pepsin and pancreatin. These authors verified the inhibitory activity of the peptides obtained on ACE, and the fraction composed of globulins showed an IC50 value of 1.03 mg/mL. The IC50 value is defined as the inhibitor concentration required to inhibit 50% of the enzyme activity, and hence the lower the value, the more potent the inhibitor. In addition to the previous findings and to the well-known nutritional properties of common beans, after hydrolysis of the protein isolates from different cultivars from Brazil and Mexico via the simulation of gastrointestinal digestion with α-amylase, pepsin and pancreatin. Mojica and González de Mejía (2015) identified peptide sequences with antioxidant bioactive potential and also the potential to inhibit the ACE and DPP-IV enzymes, which are the therapeutic targets in the treatment of hypertension and T2DM, respectively.

Moreover, according to the study carried out by Segura-Campos, Chel-Guerrero, and Betancur-Ancona (2011), after hydrolysis of the proteins present in cowpea with Flavourzyme, the peptide fraction < 1 kDa showed the highest activity in the *in vitro* assay for the inhibition of ACE, with an IC50 = 0.04 µg/mL. The use of bioactive peptide ACE inhibitors derived from beans may be an alternative to minimize the adverse effects of synthetic drugs, such as skin rash, proteinuria, and taste perception disorders (Ariza-Ortega et al. 2014). Oseguera-Toledo, González de Mejía, and Amaya-Llano (2015) demonstrated that the common bean protein hydrolysate obtained through the action of Alcalase, possessed bioactive peptides with the potential to inhibit the enzyme DPP-IV and increase insulin secretion by the iNS-1E cells.

In another study, Li, Shi, et al. (2006) isolated and sequenced three types of ACE inhibitory peptides from mung bean protein isolates hydrolyzed by Alcalase for 2 h: KDYRL, VTPALR, KLPAGTLF, with IC50 values of 26.5 μM, 82.4 μM and 13.4 μM respectively. In addition, Rocha et al. (2015) demonstrated that the enzymatic hydrolysis by Alcalase of the protein concentrate obtained from germinated common bean flour, generated bioactive peptides with high antioxidant capacity. The authors also identified the peptide sequence RGPLVNPDPKPFL in the phaseolin protein, which has the predicted potential to interact with the active site of the DPP-IV enzyme and inhibit its activity. This interaction was demonstrated by computational docking. These results indicated that the common bean has the potential to originate ingredients for use in the control of T2DM and hence the consumption of beans as a dietary supplement may improve the quality of life of patients with T2DM and hypertension (Mojica, Luna-Vital, and González de Mejía 2017).

Besides the possibility of the peptides obtained from beans acting on the therapeutic targets of DM2 and hypertension, the properties of which have been extensively studied, other biological activities have been described in the literature for these molecules. Amaral et al. (2017) concluded that after isolating the protein vicilin from mung beans and subjecting it to *in vitro* enzymatic hydrolysis with pepsin and pancreatin, simulating gastrointestinal digestion, the peptide fractions obtained with 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) activity. This enzyme takes part 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) showed evidence that after the 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, and contributed to the antiproliferative effect of colorectal cancer cells by modifying the molecules involved in the cycle of cell trapping or apoptosis.

Oseguera-Toledo et al. (2011) concluded that after hydrolysis with Alcalase and the simulation of gastrointestinal digestion with pepsin and pancreatin of the common bean protein isolate, the peptides formed inhibited the expression of the enzymes cyclooxygenase-2 (COX2) and nitric oxide synthase, the production of prostaglandins E2 and NF-κB transactivation. These are important markers and mediators involved in inflammatory processes, and hence protein hydrolysates obtained from common beans may help in the management of diseases associated with chronic inflammatory processes such as T2DM and cancer. In addition, a study of the bioactive peptides obtained from common beans by enzymatic protein hydrolysis with Alcalase carried out by Ariza-Ortega et al. (2014), showed that the peptide fraction <1 kDa presented antioxidant activity and antimicrobial activity by inhibiting the growth of pathogenic microorganisms such as *Shigella dysenteriae*.

In vivo studies concerning the health benefits of *bioactive peptides* from beans

Although less in vivo studies are carried out, there are reports in the literature of *in vivo* studies aimed at characterizing bioactive peptides obtained from foods. Ariza-Ortega et al. (2014) concluded that after hydrolysis of the common bean protein with Alcalase, the peptide fraction with 3–10 kDa showed ACE inhibitory activity *in vitro* and antihypertensive activity *in vivo* with doses of 4 mg/kg, identifying a decrease in systolic blood pressure of naturally hypertensive rats after two hours of intraperitoneal administration. Li, Shi, et al. (2006) concluded that the enzymatic hydrolysis of the mung bean protein isolate by Alcalase for different times generated peptides with ACE inhibitory activity. The highest activity was detected after 2 h of hydrolysis, with an IC50 = 0.64 mg/mL. In addition, after the oral administration of this hydrolysate to 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 being observed after 6 h (Li, Shi, et al. 2006).

In another study with diabetic rats fed for 42 days on protein-rich extracts (86.04%) obtained from the extrusion of adzuki beans, Yao, Cheng, and Ren (2014) concluded that after 30, 60, 90 and 120 min of oral glucose administration, the blood glucose concentration was lower in the diabetic rats that received the protein-rich extract than in the control group, besides decreasing the serum triglyceride level, the blood urea nitrogen level by 19.9%, which is an important marker of renal dysfunction, and increasing the high-density lipoprotein cholesterol (HDL-c) content. The results suggest that the consumption of proteins obtained from the extrusion of adzuki beans may aid in the modulation of T2DM and its complications.

A double-blind, placebo-controlled clinical study of 22 subjects, including both males and females aged 21–55 years in the United States and Canada, evaluated the effect of consuming a commercial mung bean protein isolate consisting of 92% protein (GLUCODIA ^{TM}), on the glucose and lipid metabolisms. The major component of the protein isolate was the 8 S storage protein globulin, which accounted for 80% of the total. With a daily dose of 3.0 g, there were significant decreases in the mean homeostatic insulin resistance assessment model and the mean triacylglycerol levels, and increases in the serum adiponectin levels, when compared with the control group, suggesting that GLUCODIA might be useful in preventing insulin resistance and visceral fat accumulation (Kohno et al. 2017).

It is important to highlight that for the peptides to carry out a biological activity they must reach their molecular target intact, and must therefore cross the intestinal barrier. The permeability of these molecules through the enterocytes depends on their physicochemical properties, the composition of the intestinal fluid, the characteristics of the gastrointestinal barrier and the transport mechanism (Segura-Campos, Chel-Guerrero, and Betancur-Ancona 2011). Although gastrointestinal enzymes are extremely important in releasing peptide sequences with potential biological activity, which were previously inactive in the core of the source protein, during the digestion process the peptides released can also be inactivated during further digestion by peptidases present in the brush border and in the cytoplasm of the intestinal epithelial cells (Segura-Campos, Chel-Guerrero, and Betancur-Ancona 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.

Bioinformatic tools for proteins and peptides

There are several databases that can be used for proteins and peptides in *in silico* analyses. Some examples are the UniProtKB (The UniProt Consortium 2019), RCSB PDB (Protein Data Bank) (Berman et al. 2000), NCBI (National Center for Biotechnology Information) (Geer et al. 2010), BRENDA (The Comprehensive Enzyme Information System) (Jeske et al. 2019), BIOPEP-UWM (Minkiewicz, Iwaniak, and Darewicz 2019), PeptideRanker (Mooney et al. 2012), PEPstrMOD (Singh et al. 2015) AAIndex (Kawashima et al. 2008), CancerPPD (Database of Anticancer Peptides & Proteins) (Tyagi et al. 2015), PeptideCutter – ExPASy (Gasteiger et al. 2005), and MEROPS (The Peptidase Database) (Rawlings et al. 2018). There are also more than 60 other different softwares and tools that can be used to simulate molecular docking. A few examples that are used more widely are the Rosetta FlexPepDock (London et al. 2011), CABS-dock (Kurcinski et al. 2020), and ClusPro server (Kozakov et al. 2017).

The tools used in this study were UniProtKB (The UniProt Consortium 2019), BIOPEP-UWM (Minkiewicz, Iwaniak, and Darewicz 2019) and PeptideRanker (Mooney et al. 2012). These are simple to use and of open access. The UniProtKB database (The UniProt Consortium 2019) 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 using the UniProtKB (The UniProt Consortium 2019), the BIOPEP-UWM database can be used to evaluate the profile of potential biological activity and simulate the obtaining of bioactive peptides through proteolytic processes (Minkiewicz, Iwaniak, and Darewicz 2019). This database is designed to interlink three databases of protein sequences, bioactive peptides and proteolytic enzymes. Posteriorly, the peptide fragments obtained using the BIOPEP-UWM database can be evaluated using the PeptideRanker database, to identify those that are more likely to be bioactive amongst a set of peptides, based on properties such as the charge distribution on the peptide and the amino acid sequence (Mooney et al. 2012).

Although not accurately assessing the three-dimensional structure of proteins, bioinformatic 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 raw animal and plant materials, avoiding waste (Udenigwe and Fogliano 2017).

Scientific prediction of the bioactive peptides from the bean storage proteins

Amino acid sequences of the storage proteins

The UniProtKB database (The UniProt Consortium 2019) was used to determine the amino acid sequences of the storage proteins 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). The database was accessed between June and December 2019. The name of the protein, its respective species and the search carried out were inserted in the tool UniProtKB (https://www.uniprot.org/uniprot/). Only the proteins classified as globulins were included in this study because they represent the majority of the bean storage proteins. In order to verify the amino acid sequences of all the globulin proteins present in the database for each species studied, the search was carried out according to the names and synonyms of each protein and its respective species, such as, for example, "7 S globulin *Vigna angularis*" and "Vicilin *Vigna angularis*."

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

According to the bean species studied (Supplementary material Table 1) presents the proteins identified using the UniProtKB database and the percentage of identity obtained according to the tool BLAST when more than one sequence was collected for the same protein. The protein sequences with protein and/or transcript evidence levels for the proteins named as legumin (11 S globulin) were not found in any of the species, therefore they were not selected. Three polypeptide chains were found for the 7S globulin protein in the species *Vigna angularis* (Willd.). The species *Phaseolus vulgaris* (L.) and *Vigna radiata* (L.) had four sequences for phaseolin and 8 S globulin respectively. The species *Vigna unguiculata* (L.) Walp. showed the highest number of sequences for the same protein, with a total of five sequences for the protein vicilin.

The examples show how the percentage of identity between two different sequences obtained for the phaseolin present in the common bean was determined (Supplementary material, Figure 1). 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, each had more than 90% of identity according to the tool BLAST. Therefore, they were considered identical, one protein of each species being selected at random to be presented in the scientific prediction regarding the bioactive potential according to the BIOPEP-UWM database. The codes of the proteins selected were P07219, A4PI98 and A0A2U96L2, respectively (Tables 1, 2 and 4).

For the species *Vigna radiata* (L.), the proteins Q198W4, B1NPN8 and Q198W5 presented identity values above 90% amongst them (Supplementary material Table 1), so one of them (B1NPN8) was randomly selected to carry out the scientific prediction according to the BIOPEP-UWM database (Table 3). The protein Q198W3 presented percentages below 90% of identity in relation to all the proteins mentioned above, and was therefore considered different from the others, also being used in the scientific prediction according to the BIOPEP-UWM database (Table 3).

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

In the BIOPEP-UWM database, the access for the tool Bioactive peptides was: Analysis, followed by the Profile of Potential Biological Activity, followed by For Your Sequence (http://www.uwm.edu.pl/biochemia/index.php/en/biopep). The profiles of potential biological activity were evaluated *in silico* by inserting the protein sequences obtained previously in the UniProtKB according to each bean species. The database was accessed between June and December 2019.

Posteriorly, in the tool Bioactive Peptides, the access was: Analysis, followed by Enzyme Action, followed by For Your Sequence (http://www.uwm.edu.pl/biochemia/index.php/en/biopep), and the protein hydrolysis was simulated twice. Gastrointestinal digestion was simulated first by selecting the enzymes pepsin (EC 3.4.23.1), trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1). Secondly, hydrolysis with the enzyme subtilisin (EC 3.4.21.62) was simulated by selecting only this enzyme. The results of the enzyme actions show the peptide fragments formed after simulating the hydrolysis processes. With these results, the search for bioactive fragments was selected, where the database shows which of the fragments formed in the hydrolysis processes has the potential to be

Table 1. Predicted potential of obtaining bioactive peptides for phaseolin.

Phaseolus vulgaris (L.) - Phaseolin

P07219 - Protein sequence: MMRARVPLLLLGILFLASLSASFATSLREEEESQDNPFYFNSDNSWNTLFKNQYGHIRVLQRFDQQSKRLQNLEDYRLVEFRSKPETLLLPQQADAELLLVVRSGSAILVLVKPDDRREYFFLTQGDNPIFSDNQKIPAGTIFYLVNPDPK EDLRIIQLAMPVNNPQIHEFFLSSTEAQQSYLQEFSKHILEASFNSKFEEINRVLFEEEGQQEEGQQEGVIVNIDSEQIEELSKHAKSSSRKSHSKQDNTIGNEFGNLTERTDNSLNVLISSIEMKEGALFVPHYYSKAIVILVVNEGEAHVELVGPKGNKETLEFESYRAELSKDD VFVIPAAYPVAIKATSNVNFTGFGINANNNNRNLLAGKTDNVISSIGRALDGKDVLGLTFSGSGEEVMKLINKQSGSYFVDGHHHQQEQQKGSHQQEQQKGRKGAFVY

	Profiles of potential biological activity		GI: pepsin, trypsin, chymotrypsin		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	–	–	–	–
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 UniProtKB database. The profile of potential bioactives fragments was obtained at BIOPEP-UWM database by selecting Profile of Potential Biological Activity, For your Sequence. The other bioactive fragments were obtained at BIOPEP-UWM database by selecting Bioactive Peptide and choosing the enzyme. A: frequency of bioactive occurrence; 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.

Table 2. Predicted potential of obtaining bioactive peptides for 7S globulin.

Vigna angularis (Willd.) - 7S globulin

A4PI98 - Protein sequence: IVHREHHESREEVSVSSGKNNPFYFNSDRWFRTLYRNEWGHIRVLQRFDQRSKQMQNLENYRVVEFKSKPNTLLLPHHADADFLLVVLNGTAVLTLVNPDSRDSYILEQGHAQKIPAGTTFFLVNPDDNENLRIIKLAIPVNNPHRFQ
DFFLSSTEAQQSYLRGFSKNILEASFDSDFKEINRVLFGEERQQQQGEESREEGVIVELKREQIQELMKHAKSSSRKELSSQDEPFNLRNSKPIYSNKFGRWYEMTPEKNPQLKDLDVFISSVDMKEGALLLPHYSSKAIVMVINEGEAKIELVGLSDQQQQKQQEESLEVQ
RYRAELSEDDVFVIPAAYPVAINATSNLNFFAFGINAENNRRNFLAGGKDNVMSEIPTEVLEVSFPASGKKVEKLIKKQSESHFVDAQPEQQQREEGHKGRKGSLSSILGSLY

Activity	Profiles of potential biological activity		Gl: pepsin, trypsin, chymotrypsin		Subtilisin	
	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

Protein sequence obtained from UniProtKB database. The profile of potential bioactives fragments was obtained at BIOPEP-UWM database by selecting Profile of Potential Biological Activity, For your Sequence. The other bioactive fragments were obtained at BIOPEP-UWM database by selecting Bioactive Peptide and choosing the enzyme. A: frequency of bioactive occurrence; Gl: 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.

bioactive, according to each activity. The frequency of the occurrence of bioactive fragments (A) with specific activity (ACE and DPP-IV inhibition, antioxidant, etc.) in the protein sequence, was calculated according to the BIOPEP-UWM definition using 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 in the storage protein (Minkiewicz, Iwaniak, and Darewicz 2019).

Posteriorly, the bioactive fragments obtained from the BIOPEP-UWM database were analyzed using the PeptideRanker database (Mooney et al. 2012) (http://distill-deep.ucd.ie/PeptideRanker/), which ranks peptides according to the predicted probability that they will be bioactive by attributing a score from 0 to 1 for each peptide: the closer the value is to 1, the more likely it is that the peptide will be bioactive. The PeptideRanker database was accessed between June and December 2019. According to the database, any peptide predicted to have a threshold over 0.5 is labeled as bioactive, but by choosing a threshold of 0.8 this will reduce the false positive rate from 11% and 16% with a threshold of 0.5, to 2% and 6%, for long and short peptides, respectively. Thus, for this study, only peptides with a score of 0.8 on the PeptideRanker were presented. In this step, peptide sequences identified in other scientific studies as having the same bioactive fragments as those identified in the present study and showing antioxidant potential and inhibitory activity of the ACE and DPP-IV enzymes, were compared to each other.

Using the analysis of the potential biological activity profile from the BIOPEP-UWM, one can 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 peptide fragments with the most diverse potential biological activities. Due to this, many activities have a low occurrence frequency (Table 1).

In the present study, all the species studied presented similar profiles of their potential biological activities. Table 1 shows the results obtained for the common bean, including the following potential activities: ACE and DPP-IV inhibitors, antithrombotic effect, hypotensive effect, stimulating vasoactive substance release peptides and glucose uptake stimulating peptides. In comparison with the other beans, one difference was that the adzuki bean and the cowpea also presented antibacterial activity, both with frequencies of bioactive fragment occurrence of 0.0023 (data not shown). Thus it was decided only to present all the activities found in the potential biological activity profile for the species *Phaseolus vulgaris* (L.), as an example (Table 1), and for the other bean species, only the biological activities that had a bioactive fragment occurrence frequency above 0.05 in this profile were presented (Tables 2–4).

As an example, Supplementary material Figure 2 shows the fragments obtained after simulation of the hydrolysis of phaseolin (P07219) using the gastrointestinal enzymes: pepsin, trypsin and chymotrypsin. Since the enzymes act at

Table 3. Predicted potential of obtaining bioactive peptides for 8S globulin.

Vigna radiata (L.) - 8 S globulin

Q198W3 - Protein sequence: MVRARVQLLLGILFLASLSVSFGIVHREHQESQEESDSRGQNNPFYFNSDRRFHTLFKNQYGHLRVIHRFDQRSKQIQNLENYRVVEFKSKPNTLLLPHHADADFLLVVLNGRAILTLVNPDGRDSYILEQGHAQKIPAGTTFFLVNP
NDNDNLRIIKLAIPVNNPHRFQNFFLSSTEAQQSYLRGFSKNILEASFDSDFKEIDRVLFGEERQQQHGEESQEEGVIVELKREQIRELIKHAKSSSRKELSSQDEPFNLRNSNPIYSNKFGRWYEITPEKNPQLKDLDVFISSVDMKEGGLLLPHYNSKAIVILVINEGEAKIEL
VGPSDQQQQDESLEVQRYRAELSEDDVFVIPAAYPVAINATSNLNFFAFGINAENNQRNFLAGEKDNVMSEIPTEVLDVSFPASGNKVEKLIKKQSESHFVDAQPEQQQREEGHKGRKGSLSSILGSLY

	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

B1NPN8 - Protein sequence: MVRARIPLLLLLGILFLASLSVSFGIVHRENIDGAEVSVSRGKNNPFYFNSDRWFHTLFRNQFGHLRVLQRFDQRSKQMQNLENYRVVELMSKPNTLLLPHHADADFLLVVLNGRAVLTLVNPDGRDSNILEQGHAQKIPAGTT
FFLVNPDDNENLRIIKLAVPVNNPHRFQDFFLSSTEAQQSYLQGFSKNILEASFDSDIKEISRVLFGEEGQQQQQQGQESQQEGVIVELKREQIRELTKHAKSSSKKSLSSEDQPFNLRNQKPIYSNKLGRWFEITPEKNPQLRDLDMFIRSVDMKEGSLLLPHYNSKAIV
ILVINEGKANIELVGQREQQKQQEEQEESWEVQRYRAELSEDDVFIIPATYPVAINATSNLNFFAFGINAENNQRNFLAGEKDNVISEIPTEVLDVTFPASGEKVKKLIKKQSESQFVDAQPEQQEREEARKGGKGPFVY

	Profiles of potential biological activity		GI: pepsin, trypsin, chymotrypsin		Subtilisin	
	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

Protein sequence obtained from UniProtKB database. The profile of potential bioactives fragments was obtained at BIOPEP-UWM database by selecting Profile of Potential Biological Activity, For your Sequence. The other bioactive fragments were obtained at BIOPEP-UWM database by selecting Bioactive Peptide and choosing the enzyme. A: frequency of bioactive occurrence; 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.

Table 4. Predicted potential of obtaining bioactive peptides for vicilin.

Vigna unguiculata (L.) Walp - Vicilin

A0A2U9K6L2 - Protein sequence:
IVHREHQESQEESEPRGQNNPFYFDSDRWFHTLFRNQYGHLRVLQRFDQRSKQIQNLENYRVVEFKSKPNTLLLPHHADADFLLVVLNGRAILTLVNPDGRDSYILEEGHAQKIPAGTTFFLVNPDDNENLRIVKLAVSVNNPHRFQDFFLSSTEAQQSYLQGFSKNILEA
SFGSDCKEINRVLFGEEEQQQQDEESQQEGVIVQLKREQIRELMKHAKSTSKKSLSSQNEPFNLRSQKPIYSNKFGRLHEITPEKNPQLRDLDVFLTSVDMKEGGLFMPNYNSKAIVLVVNKGEANIELVGQREQQQQQQEESWEVQRYRAEVSEDDVFVIPASYPVAITAT
SNLNFIAFGINAESNQRNFLAGEEDNVMSEIPTEVLDVTFPASGEKVEKLINKQSDSHFTDAQPEQQQREEDRKGRKGPLSSILDSLY

Activity	Profiles of potential biological activity		Gi: pepsin, trypsin, chymotrypsin		Subtilisin	
	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

Protein sequence obtained from UniProtKB database. The profile of potential bioactives fragments was obtained at BIOPEP-UWM database by selecting Profile of Potential Biological Activity, For your Sequence. The other bioactive fragments were obtained at BIOPEP-UWM database by selecting Bioactive Peptide and choosing the enzyme. A: frequency of bioactive occurrence; 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.

specific sites of the polypeptide chain according to their affinity for the substrate, the simulation of protein hydrolysis by gastrointestinal enzymes or by the action of subtilisin shows a decrease in the amount of different fragments that can be obtained when compared with the profile of potential biological activity (Table 1), and consequently evidences a decrease in the potential biological activities observed.

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

Observing the potential biological activity profile and the two hydrolysis processes simulated, in general, all the proteins of the four bean species studied had a higher frequency of bioactive fragment occurrence for DPP-IV inhibition followed by ACE inhibition and then antioxidant activity (Tables 1–4).

Most of the bioactive fragments obtained in all the species studied were dipeptides, followed by tripeptides (Tables 1–4). Larger fragments were only observed in the potential biological activity profiles. Gastrointestinal digestion appears to be sufficient to release bioactive fragments from the globulins present in the common bean, adzuki bean, mung bean and cowpea. Therefore, the daily consumption of this legume could be considered a promising strategy to decrease the risk of developing T2DM and hypertension, according to the higher bioactive fragment occurrence frequencies found for the DPP-IV and ACE inhibitory activities, respectively. However, it was observed that hydrolysis by subtilisin can also generate different fragments from those obtained by gastrointestinal digestion, indicating that this process could be used in the formulation of health-enhancing foods to increase the amount of different bioactive fragments potentializing its effect.

Tables 5–7 present the scores (only above 0.8) according to the PeptideRanker for the peptides obtained from the BIOPEP-UWM 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 inhibition of the DPP-IV and ACE enzymes and antioxidant activity by *in vitro* assays.

It can be seen that the bioactive fragments identified in the *in vitro* studies for the inhibition of ACE and DPP-IV (Tables 5 and 6) were present in different peptide sequences, most of which had more than 4 amino acid residues. This may have occurred due to factors such as the enzyme specificities and different hydrolysis times applied in the different studies. Garcia-Mora et al. (2015) evaluated the obtaining of bioactive peptides from the common bean by hydrolysis with two different subtilisins, Alcalase® and Savinase®. The authors concluded that the hydrolysates obtained by treating with Alcalase for 120 min and Savinase for 90 min showed the highest biological potentials, including anti-inflammatory, ACE inhibitory and antioxidant activities. The bioactive fragments with the highest scores according to the PeptideRanker

Table 5. Scores at PeptideRanker of the bioactive peptide fragments with potential to inhibit DPP-IV obtained from BIOPEP-UWM and peptide sequences with the same bioactive fragment identified in scientific studies at the literature.

Score[a]	Bioactive fragment	Bean specie	Peptide Sequence[b]	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 González de Mejía (2015) Mojica and González de Mejía (2015)	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 González 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 and González de Mejía (2015) Oseguera-Toledo, González de Mejía and Amaya-Llano (2015) Mojica and González de Mejía (2015)	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 and González de Mejía (2016)	common bean
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 and González de Mejía (2015) Oseguera-Toledo, González de Mejía and Amaya-Llano (2015)	cowpea common bean
0.9713	MM	common bean	L**MM**LMYLLLL	Mojica and Mejía (2015)	common bean
0.9601	MP	common bean, cowpea	**MP**HLK, **MP**PM	Mojica and González de Mejía (2015) Mojica, Luna-Vital and González de Mejía (2017)	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 and González de Mejía (2015) Mojica, Chen and González de Mejía (2015) Oseguera-Toledo, González de Mejía and Amaya-Llano (2015)	cowpea common bean
0.9512	FN	common bean, adzuki bean, mung bean, cowpea	LS**FN**T, RLL**FN**LMLAF	Mojica and González de Mejía (2015) Mojica, Luna-Vital and González de Mejía (2017)	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 and González de Mejía (2017)	common bean
0.9461	QF	mung bean	**QF**ADG, FAF**QF**T, FFA**QF**T	Rocha et al. (2014) Mojica and González de Mejía (2015)	cowpea common bean
0.9411	NF	common bean, adzuki bean, mung bean, cowpea			
0.9391	WN	common bean			
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 and González de Mejía (2017)	common bean

(*continued*)

Table 5. Continued.

Score[a]	Bioactive fragment	Bean specie	Peptide Sequence[b]	Reference	Species studied
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 Mejía (2015) Mojica and Mejía (2016) Mojica, Luna-Vital and González de Mejía (2017)	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 Mejía (2015) Oseguera-Toledo, Mejía, Amaya-Llano (2015) Mojica and Mejía (2016) Mojica, Luna-Vital and González de Mejía (2017)	cowpea common bean
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 Mejía (2015) Mojica, Chen and Mejía (2015) Mojica and Mejía (2016)	cowpea common bean
0.8491	MR	common bean	RKLK**MR**Q, RRKAL**MR**GQNK	Mojica and Mejía (2015) Mojica and Mejía (2015)	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 Mejía (2015) Mojica and Mejía (2015) Mojica and Mejía (2015) Mojica, Luna-Vital and González de Mejía (2017)	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 Mejía (2015) Mojica and Mejía (2015)	cowpea common bean
0.8088	GL	common bean, adzuki bean, mung bean, cowpea	GG**GL**HK, KTY**GL**, E**GL**ELLLLLAG, 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**SLELLLL, Y**GL**VAGK, **GL**ASK, ER**GL**AGS, SKAGGVG**GL**SK	Rocha et al. (2014) Mojica and Mejía (2015) Oseguera-Toledo, Mejía, Amaya-Llano (2015) Mojica and Mejía (2015) Mojica, Luna-Vital and González de Mejía (2017)	cowpea common bean

[a]It was presented only the fragments with score above 0.8;
[b]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. 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.

in the present study, that were also found in the peptide sequences identified in the *in vitro* studies, were FP, PF and FL for DPP-IV inhibition (Table 5) and FFL, FP and FG for ACE inhibition (Table 6).

Since in the present study the majority of the peptides showing good possibility of being bioactive, had the potential to inhibit DPP-IV and ACE, two different mechanisms were raised to explain the inhibition of these enzymes: the peptides bound to the enzyme hence altering its shape, making it impossible for the enzyme to bind to the substrate, or competitive inhibition, where the peptides competed with the substrate for the catalytic sites on the enzyme (Ngoh and Gan 2018).

According to Mojica, Chen, and González de Mejía (2015), the peptide sequence and amino acid type are determinant in the interaction potential between the functional groups of the bioactive peptides and 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 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 et al. 2014). Lan et al. (2015), who 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 species studied with scores above 0.8 according to the PeptideRanker, also contained proline, alanine or tryptophan residues (WF, FP, PF, WG, RW, WY, WN, SW, AF, FA) (Table 5) confirming their potential to exert bioactivity. Also, many of these potentially bioactive fragments were identified in the peptide sequences obtained from the analysis of the *in vitro* studies.

When considering the inhibition of ACE, its catalytic site is formed of three subunits, whereby angiotensin I interacts via three hydrophobic amino acids present in its C-terminal region, these being proline, histidine and phenylalanine. Therefore, potent peptides capable of inhibiting ACE will preferentially 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, such as arginine, glycine, valine, alanine and isoleucine at the N-terminal extremity of inhibitor peptides, can increase the inhibitory activity. In general, the ACE inhibitor peptides have low molecular weights (Li and Yu 2015).

Similar to the profiles previously found for the fragments with the potential to inhibit DPP-IV, peptide fragments with the potential to inhibit ACE containing amino acids with the above-cited characteristics were found in all the bean species (Table 6), and many of these bioactive fragments were present in the peptide sequences found in the *in vitro* ACE inhibitory assays.

Ngoh and Gan (2018) used the database PeptideRanker to select the following five peptide fragments from the common bean with scores above 0.8: PPHMLP, LSSLEMGSLGALFVCM, PPHMGGP, PLPLHMLP and PLPWGAGF. The authors concluded that these fragments demonstrated ACE inhibitory activity in in vitro assays with IC50 values of 1.52 μM, 1.84 μM, 11.04 μM, 27.32 μM and 31.88 μM, respectively. Of these sequences, four had bioactive fragments also found in the present work (Table 6), as follows: GG, GP (PPHMGGP), PL (PLPLHMLP) and WG (PLPWGAG).

When one considers antioxidant activity (Table 7), a smaller number of potentially bioactive fragments with a score above 0.8 was observed when compared with the number found for the other two activities studied, and only two amino acids (RW) from a tripeptide (RWY) were identified in the peptide sequences (RWAEK) present in the *in vitro* studies analyzed (Mojica, Chen, and González 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 their 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 cell damage, which is known as oxidative stress. This process can lead to diseases such as cancer, high blood pressure and inflammation (Li and Yu 2015).

Antioxidant pathways can occur through the inactivation of active oxygen, neutralization of free radicals, chelation of metallic ions and by reducing the formation of hydrogen peroxide. Bioactive peptides with hydrophobic amino acids are highly correlated with antioxidant activity, the more hydrophobic and aromatic the amino acids, the greater their antioxidant activity, since these tend to combine first with the free radicals (Li and Yu 2015). Considering this, the majority of the peptide fragments found in the present study with good potential for bioactivity according to the PeptideRanker, had hydrophobic and aromatic amino acids such as WG, RW, WY, RWY and ADF (Table 7).

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

The results found in the present work provide evidence that bioinformatics can be an efficient, simple and cost-effective tool to be used in the design, synthesis and selection of food-derived bioactive peptides to carry out *in vivo* studies with respect to their bioactive potential in decreasing the risk of developing T2DM and hypertension.

Scientific studies

The number of scientific studies that demonstrated biological activity obtained from bean proteins was raised in

Table 6. Scores at PeptideRanker of the bioactive peptide fragments with potential to inhibit ACE obtained from BIOPEP-UWM and peptide sequences with the same bioactive fragment identified in scientific studies at the literature.

Score[a]	Bioactive fragment	Bean specie	Peptide Sequence[b]	Reference	Specie studied
0.9966	MF	mung bean			
0.9955	FFL	common bean, adzuki bean	AMPVNNPQIHD**FFL**S, AMPVNNPQIHE**FFL**S, **FFL**LEQLAATT, LL**FFL**E	Garcia-Mora et al. (2015) Mojica and González de Mejía (2015)	common bean
0.9947	GF	adzuki bean, mung bean, cowpea			
0.9939	FP	adzuki bean, mung bean, cowpea	**FP**LV, **FP**VL	Mojica and González de Mejía (2015)	common bean
0.9931	FG	adzuki bean, mung bean, cowpea	KVDN**FG**	Mojica and González de Mejía (2015)	common bean
0.9924	WG	adzuki bean			
0.9869	LF	common bean, adzuki bean, mung bean, cowpea	RL**LF**NLMLAF, L**LF**FLE, PLTA**LF**V, TELEL**LF**LEF, EFLDLML**LF**, DLLLLLMGESL**LF**, KLPAGT**LF**	Li, Shi, et al. (2006) Mojica and González de Mejía (2015)	common bean mung bean
0.9866	RF	common bean, adzuki bean, mung bean, cowpea	V**RF**V, LV**RF**, VL**RF**, **RF**KL,	Mojica and González 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 and González de Mejía (2015) Mojica, Chen and González 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 and González de Mejía (2015) Mojica, Chen and González de Mejía (2015)	common bean
0.9713	MM	common bean	L**MM**LMYLLLL	Mojica and González de Mejía (2015)	common bean
0.9492	IF	common bean			
0.9488	SF	common bean, adzuki bean, mung bean, cowpea	EGG**SF**, L**SF**NT	Mojica, Chen and González de Mejía (2015) Mojica, Luna-Vital and González de Mejía (2017)	common bean
0.9424	DF	adzuki bean, mung bean, cowpea			
0.9411	NF	common bean, adzuki bean, mung bean, cowpea	KVD**NF**G	Mojica and González 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 and González de Mejía (2015)	common bean
0.9068	KF	common bean, adzuki bean, mung bean, cowpea	V**KF**MT	Mojica, Luna-Vital and González 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 and González de Mejía (2015) Mojica, Luna-Vital and González de Mejía (2017)	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 and González de Mejía (2015) Mojica, Chen and González de Mejía (2015) Mojica, Luna-Vital and González de Mejía (2017)	common bean
0.8358	LNF	mung bean, cowpea			
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	TTTF, FTFFNLET,	Mojica and González de Mejía (2015)	common bean
0.8154	VF	common bean, adzuki bean, mung bean, cowpea	LSG**VF**, YEKLLLLREEGRS**VF**Q,	Mojica and González de Mejía (2015) Mojica, Luna-Vital and González de Mejía (2017)	common bean
0.8111	PL	common bean, mung bean, cowpea	**PL**EAL, **PL**LELELVEAAG, F**PL**V, **PL**TALFV, RQR**PL**,	Mojica and González de Mejía (2015)	common bean
0.8088	GL	common bean, adzuki bean, cowpea	GGG**GL**HK, KTY**GL**, PNL**GL**SLELLLLL, Y**GL**VAGK, **GL**ASK, ER**GL**AGS, SKAGGVG**GL**SK,	Mojica and González de Mejía (2015) Mojica, Luna-Vital and González de Mejía (2017)	common bean

[a]It was presented only the fragments with score above 0.8;
[b]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. 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.

the databases Wiley, Medline/Pubmed and others available in the CAPES periodical portal (Supplementary material, Figure 3).

The common bean was found to be the most explored bean species in the studies targeted at bioactive peptides, followed by the cowpea, mung bean and adzuki bean. The common bean was probably 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 the common bean, cowpea and mung bean species, the inhibition of ACE and antioxidant activity were the factors most studied, followed by the inhibition of DPP-IV, in agreement with the results of the present study, where the greatest frequency of bioactive fragments also occurred for these activities. However, as mentioned previously, other activities are possible according to the profiles of the potentially bioactive peptides (Table 1), besides antioxidant activity and the inhibition of ACE and DPP-IV. Some of these different activities were identified by the *in vitro* studies, such as antithrombotic activity (Basha, Maheswaraiah, and Rao 2017), the stimulation of glucose uptake (Oseguera-Toledo et al. 2016), and anti-hypotensive (Cú-Cañetas et al. 2015) and antimicrobial (Ariza-Ortega et al. 2014) activities.

Table 1 shows that the common bean possesses the potential to release fragments with antithrombotic activity ($A = 0.0023$), and the same profile was also observed for *Vigna radiata* (L.), ($A = 0.0022$, data not shown). Basha, Maheswaraiah, and Rao (2017) studied bioactive compounds such as polyphenols, peptides and proteins in the seed exudate from the mung bean, which is obtained in the first step of the germination process, which involves soaking the seeds for 4–24 h in water for imbibition. During this process, the influx of water into the cells of the dry seeds causes cracks in the seed coat which causes the leakage of endogenous seed substances 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 the inhibition of platelet aggregation.

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

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

Table 7. Scores at PeptideRanker of the bioactive peptide fragments with potential antioxidant activity obtained from BIOPEP-UWM and peptide sequences with the same bioactive fragment identified in scientific studies at the literature.

Score [a]	Bioactive fragment	Bean specie	Peptide Sequence [b]	Reference	Specie 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, and González de Mejía (2015)	common bean
0.8062	ADF	adzuki bean, mung bean			

[a] It was presented only the fragments with score above 0.8;

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

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

Although they did not appear in the profiles of potential bioactivity carried out for the bean species evaluated in this study, other different activities were found in the literature, such as the inhibition of α-glucosidase and α-amylase, an increase in the secretion of insulin by INS-1 cells (Oseguera-Toledo, González de Mejía, and Amaya-Llano 2015), antifungal, anti-inflammatory (Oseguera-Toledo et al. 2011), anticancer (Luna Vital et al. 2014) and anticholesterolemic (Amaral et al. 2017) activities and others, most of them also using the common bean. These findings raise the possibility of obtaining other health benefits from the regular consumption of the bean species studied, in addition to the activities more extensively studied such as antioxidant activity and the inhibition of ACE and DPP-IV.

Conclusions

The present results suggest that the globulins present in the common bean, adzuki bean, mung bean and cowpea are good sources of proteins to release, by gastrointestinal digestion, potentially bioactive peptides which can act by inhibiting the ACE and DPP-IV enzymes and also showing antioxidant activity. Therefore, the daily consumption of this legume could be considered a promising strategy to decrease the risk of developing T2DM, hypertension and several other diseases related to oxidative stress, such as cancer and inflammations. In addition, the hydrolysis with subtilisin can be used to potentialize this effect and to originate health-enhanced ingredients from the bean proteins. The present study also shows that the computational prediction of bioactive peptides can be a useful and cost-effective tool to aid in the design and synthesis of new food-derived peptides to use in *in vivo* studies.

Author contributions

B. F. Garcia wrote the initial and final drafts. M. Barros reviewed the content and helped to organize the structure of the manuscript. T. S. Rocha provided the major headings for the review and evaluated the preliminary drafts.

Disclosure statement

The authors declare no conflicts of interest.

Abbreviations

A	Alanine
ACE	Angiotensin-converting enzyme
C	Cysteine
CAPES	Coordination of the Improvement of Higher Level Personnel
COX2	Ciclooxygenase-2
D	Aspartic acid
DPP-IV	Dipeptidyl peptidase-IV
E	Glutamic acid
F	Phenylalanine
G	Glycine
GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide
H	Histidine
HDL-c	High-density lipoprotein-cholesterol
HMG-CoAr	3-hydroxy-3-methyl-glutaryl-CoA reductase
I	Isoleucine
IC50	Inhibitor concentration required to inhibit 50% of enzyme activity
iNS-1E	Insulin secreting beta cell derived line
K	Lysine
L	Leucine
M	Methionine
N	Asparagine
NF-κB	Nuclear factor kappa light chain enhancer of activated B cells
P	Proline
PepT1	Human peptide transporter 1
Q	Glutamine
R	Arginine
S	Serine
T	Threonine
T2DM	Type 2 Diabetes mellitus
V	Valine
W	Tryptophan
Y	Tyrosine

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