ISSN: 2347-467X, Vol. 12, No. (1) 2024, Pg. 29-40

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

A Scoping Review on The Relationship between Pulse Protein Consumption and its Effect on Human Gastrointestinal Tract and its Microbiome

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Abstract

The gastrointestinal tract (GIT) is a very intricate set of organs of the human body due to the diversity of microorganisms that inhabit it. The relationship between food intake and GIT health is known to food science and nutrition. However, the effect on the human gastrointestinal tract and its microbiome of legume proteins is not yet clearly understood. The objective of this review was documenting the effects of the consumption of legume proteins and their derived peptides on the GIT, in order to elucidate, based on the available scientific evidence, the relationship of this consumption with the microbiota and the prevention of diseases. A scoping review was carried out based on a search of articles published in four databases: Web of Science, Scopus, PubMed, and Google Academic, published between 1992 and 2022, in English or Spanish, excluding research carried out on animals. From a collection of 715 initially-selected articles, 13 met the eligibility criteria. Research confirmed that legume proteins and peptides go beyond simply providing amino acids for the development and repair of body tissues. In fact, numerous proteins, including lectins and enzyme inhibitors considered antinutrients, together with peptides, such as lunasin, have exhibited anticancer, anti-inflammatory, and immunostimulatory properties. Also, they might regulate the microbiota flora and the manufacture of metabolites.

The need for further research in humans is highlighted to understand the specific effect that these proteins and peptides have on the GIT and its microbiota, as well as the mechanisms behind their positive properties in inflammatory processes and diseases such as cancer. A knowledge gap

Article History

Received: 2 November 2023

Accepted: 20 March 2024

Keywords

Gastrointestinal Tract;
Health;
Legumes;
Proteins;
Peptides;

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Doi: https://dx.doi.org/10.12944/CRNFSJ.12.1.03

is identified in the mechanisms of action of these proteins in the GIT, which represents an opportunity to assess the nutraceutical potential of legume proteins and their derived peptides.

Introduction

The gastrointestinal tract (GIT) is a very intricate set of organs of the human body and is made up of a microbial ecosystem that spans from the upper part of the intestine to the colon.[1] The intestinal microbiota (IM) is an assemblage of living, heterogeneous microorganisms, composed mostly of bacteria, with a minority of viruses, fungi, and eukaryotic cells.[1] It includes native species that permanently colonize the[2] GIT and a series of diverse microorganisms that temporarily transit through the digestive tract (DT).[1] This intestinal barrier is mainly composed of *Lactobacillus* and *Bifidobacterium* species, and opportunistic pathogens from the *Enterobacteraceae, Desulfovibrionaceae*, and *Streptococcaceae* families.[3] The health of the human body depends significantly on a healthy and functional GIT, being closely related to the IM, the good health and care of the GIT will depend on its state. Under normal conditions, MI affects the anatomical and physiological structure of the GIT, increasing the absorption surface, promoting villous cell renewal, increasing intraluminal content and accelerating intestinal transit, also acting as a defense, performing metabolic and behavioral functions.[4] Due to the unusually high turnover rate and metabolic requirements of the digestive tract (DT), the cells lining it are more sensitive than most tissues to micronutrient deficiencies, protein and calorie malnutrition, injury from toxins, drugs, irradiation and allergic reactions to food. In addition, the body is exposed daily to various polluting chemicals in the environment that can affect IM and therefore the health of the individual. For this reason, it is important to pay attention to the care of this barrier housed in the GIT through adequate food intake and its composition.[5]

In relation to food intake, nutrients are not only essential for human health, but especially for the health of the MI and therefore of the GIT. The metabolic functions of MI are linked to the digestion of complex polysaccharides, production of short-chain fatty acids (SCFA), metabolism of bile acids, production of vitamins, and others.[5,6] In particular, various authors have pointed out that proteins and legumes are of great benefit for the health of the GIT, positively affecting the proliferation of protective bacteria.[7,8] Protein digestion begins in the stomach, where some of the protein is hydrolyzed to generate proteases, peptones, and large polypeptides. Inactive pepsinogen is converted to the enzyme pepsin when it comes into contact with hydrochloric acid and other pepsin molecules. Unlike any of the other proteolytic enzymes, pepsin digests collagen, the main protein in connective tissue. Most protein digestion occurs in the upper small intestine, although it continues throughout the DT. All residual protein fractions are fermented by colonic microorganisms.[8] In turn, proteins also modulate the composition of the microbiota and the production of metabolites. On the other hand, legumes usually contain about twice the amount of protein found in whole grain cereals such as wheat,[7] proving to be especially helpful for people with diabetes, risk of heart disease and pregnant women. It should be noted that legumes feed useful bacteria located in the GIT, therefore, in the dietary guide of most countries it is recommended to consume them twice a week.[9,10]

Additionally, the combination of legumes with other foods, especially cereals, increases the nutritional value and contributes to the security that the individual will obtain a large part of the necessary amino acids.[7] Amino acids are vital for certain functions developed in the human body. Some of them cannot be produced by the body, and for that reason they are called essential amino acids. These are only acquired thanks to the daily intake of food.[8] There are various tests carried out in studies on the benefits of legumes, proteins and amino acids playing a fundamental role within the host biodiversity of the GIT.[5,6] However, it is important to note that protein consumption can also lead to some complications. For example, the presence of molecules considered antinutrients because they interrupt the digestion process and which, in the case of those from legumes, can produce severe effects on human health.[11]

Despite mounting evidence supporting the effect of legume proteins on gastrointestinal health, there is a lack of research on this specific relationship. A preliminary search made it possible to verify that there were no literature reviews on the research topic. A landscape review could help identify gaps in knowledge and highlight areas for future research. For this reason, the objective of this review is to analyze the relationship between the consumption of legume proteins and derived peptides and their effect on the GIT and its microbiota based on information published in the last 30 years, in order to identify the main scientific evidence found, and highlight the possible opportunities for knowledge development in this area.

Methods

This scoping review was prepared following the PRISMA-ScR guidelines for pscoping reviews, adapted to achieve the objectives of this article.[8] Four databases were used: PubMed, Web of Science (WoS), Scopus, and Google Academic (the latter as an additional source for manual search). The search in each base was performed using a search equation with Boolean operators. For WoS the equation used was:

TS=((proteins OR peptides) AND (pulses OR grains) AND ("gastrointestinal tract" OR "digestive system" OR intestines OR "biliary tract" OR "lower gastrointestinal tract" OR "upper gastrointestinal tract"))

For PubMed and Scopus, the same equation was used, which is given below:

(proteins OR petides) AND ((pulses OR grains) AND NOT(electric)) AND (health OR wellness) AND ("gastrointestinal tract" OR "digestive system" OR intestines OR "biliary tract" OR "lower gastrointestinal tract" OR "upper gastrointestinal tract").

Keywords were obtained from the Descriptors in Health Sciences/Medical Subject Headings (DeCS/MeSH) thesaurus. The search in Google Academic was carried out in order to delve into some specific research topics.

The eligibility criteria used to search for the information sources included: year of publication between 1992 and 2022. Initially, an unlimited search of years was carried out in order to identify the range of years in which there were publications on the subject and the number of them. The result showed that prior to 2010, the number of publications was less than 10, so it was decided to set the lower time limit to 1992 to include them in the initial selection of the study. The languages selected for the search were limited to English and Spanish. Due to the scope of the study, it was decided to exclude from the review any publications related to research in animal models.

Two reviewers independently searched and assessed articles by reading their titles, abstracts and the full article when deemed potentially eligible. Article inclusion was also performed independently, results were compared and discrepancies resolved by consensus between the two reviewers. In case of disagreement between pairs, a third reviewer was consulted. Duplicate studies were discarded in the first screening stage, after reading the titles of the selected articles in each database.

Results and Discussion

Seven hundred fifteen (715) studies were identified after the initial search: 21 results in PubMed, 137 in Scopus, and 557 in WoS. According to the inclusion and exclusion criteria, plus reading the title, 93 articles were considered adequate (after eliminating 1 duplicate between the databases). The summary was read and, based on this reading, 85 were discarded, as they were studies carried out on animal models. These 8 studies were fully read and selected for inclusion in the review as they met the inclusion criteria. Additionally, and based on the bibliographic references of these articles, 5 manually selected studies from Google Academic were included, considering that they contained relevant information for the topic under study. These include information on studies of proteins and peptides from legumes with important results for the review. Thus, 13 articles were finally included in the scoping review, published between 1992 and 2022, all of them in English (see Figure 1).

A synthesis of the selected studies can be found in Table 1. The analysis of the information found in the selected publications is presented below, however, it follows a structure that has been considered useful to facilitate the understanding of the topic under study. Additionally, it has been considered pertinent to add some publications that support the development of the topic as they provide information that clarifies or defines some concepts that are mentioned in the selected articles.

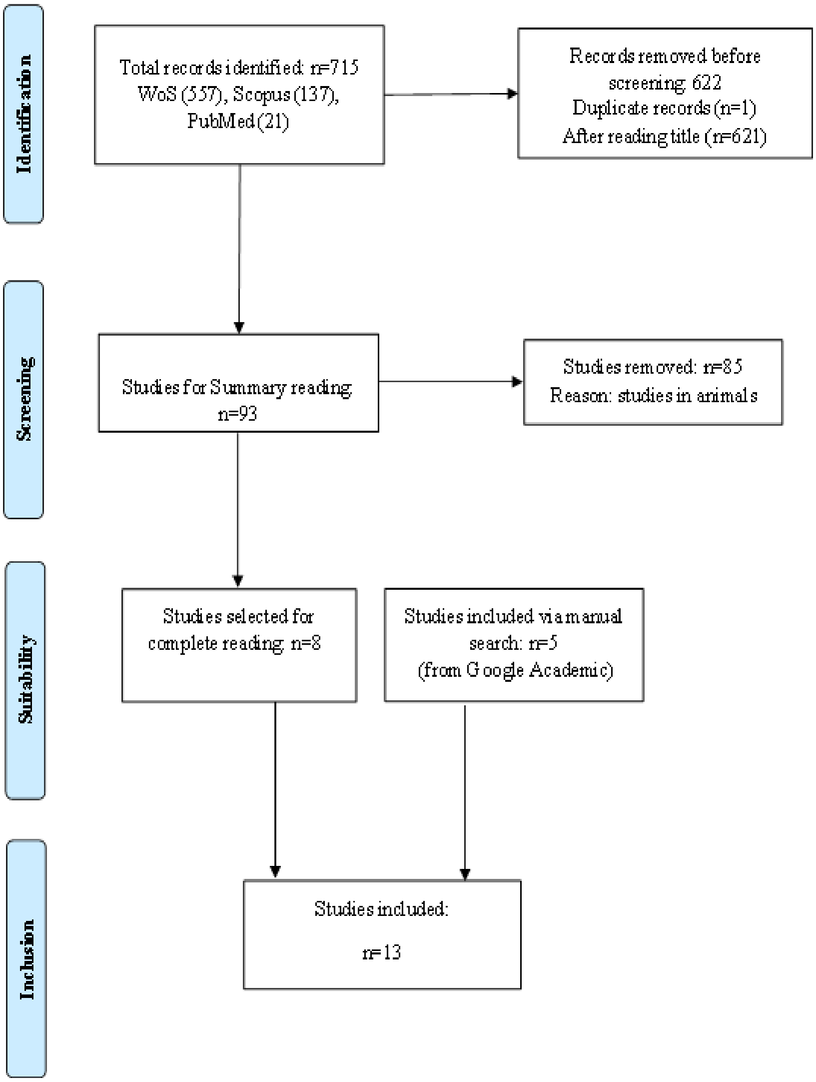

Fig. 1: PRISMA flowchart for studies selection

Microbiota, Food and Health of the GIT

The presence of microorganisms in the human body can be counted in the millions. Among these, the intestinal microbiota (IM), or microorganisms that inhabit the intestine, carry out various physiological processes of different complexity, such as somatic development, nutrition, and immunity. They also perform other essential functions such as providing genes (microbiome).[5] There are different factors, such as age, type and/or eating habits, whether the person suffers from any disease and therefore also whether they are being treated with antibiotics, among others; all these factors have a powerful influence on the composition of the intestinal microbiota (IM), such that it can be composed of various microorganisms such as yeasts, phages and protists.[12] Due to this diversity of factors that are undoubtedly influential, there is a distinctive composition of the IM in each individual.

Table 1. Summary of selected studies related to proteins, peptides and amino acids from legumes

Authors	Title	Type of study
Aranda-Olmedo I., Rubio L. (2020)[6]	Dietary legumes, intestinal microbiota, inflammation and colorectal cancer.	Review
Roy F., *et al*. (2010)[11]	Bioactive proteins and peptides in pulse crops: Pea, chickpea and lentil	Review
Carbonaro M., Nucara A. (2022)[16]	Legume Proteins and Peptides as Compounds in Nutraceuticals: A Structural Basis for Dietary Health Effects	Review
Zhang H., *et al* (2015)[17]	Bioactive dietary peptides and amino acids in inflammatory bowel disease	Review
Shigeru K., *et al*. (2007)[18]	Antioxidative activity of amino acids on tissue oxidative stress in human intestinal epithelial cell model	In vitro
López L., *et al*. (2014)[19]	Bioactive peptides and hydrolysates from pulses and their potential use as functional ingredients	Review
Lee I., *et al*. (2011)[20]	Effect of mung bean ethanol extract on pro-inflammtory cytokines in LPS stimulated macrophages	*In vitro* In vitro
Fernández S., *et al*. (2019)[21]	Novel immunomodulatory role of food bioactive peptide lunasin in the healthy human intestinal mucosa	*In vitro*
Kovacs-Nolan, *et al*. (2012)[22]	The PepT1-transportable soy tripeptide VPY reduces intestinal inflammation	*In vitro*
Girón-Calle J, *et al*. (2010)[23]	Effect of chickpea aqueous extracts, organic extracts, and protein concentrates on cell proliferation	*In vitro*
Niehues M., *et al* (2010)[24]	Peptides from Pisum sativum L. enzymatic protein digest with anti-adhesive activity against Helicobacter pylori: structure-activity and inhibitory activity against BabA, SabA, HpaA and a fibronectin-binding adhesin	*In vitro*
Clemente A., (2014)25	Bowman-Birk inhibitors from legumes as colorectal chemopreventive agents	Review
Lichtenstein G., *et al* (2008)[26]	Bowman-Birk inhibitor concentrate: a novel therapeutic agent for patients with active ulcerative colitis	Human
Valadez-Vega C., *et al* (2011)[27]	Detection of Cytotoxic Activity of Lectin on Human Colon Adenocarcinoma (Sw480) and Epithelial Cervical Carcinoma (C33-A)	*In vitro*

However, generally speaking, there are patterns that are repeated in various individuals, which are called "enterotypes". There are 3 of them: in enterotype 1, *Bacteroides* microorganisms dominate, while in enterotype 2, the presence of *Prevotella* dominates; on the other hand, *Ruminococcus* or *Bifidobacterium* are characteristic of enterotype.[35] One of the main determinants of enterotypes is diet. Due to this, the food industry together with the pharmacological industry are responsible for alterations produced in IM as a result of the invasion of substances used as additives in food or as remedies to combat diseases. With this, the IM has been damaged, for example, by increasing its permeability.[13]

A person's usual diet is made up of macro and micro nutrients. The group of macronutrients includes carbohydrates, lipids and proteins, while micronutrients are made up of minerals and vitamins. Each of these compounds has specific functions for the development and functioning of the human body.[8]

When referring to the relationship between diet and GIT health, it has been found that some types of diets promote GIT health. Among these, the vegetarian and the Mediterranean stand out, mainly because they are rich in bioactive nutrients; as such, a beneficial impact on the intestinal microbiota is expected.

Some studies such as that of Gentile C. and Weir T.[14] cited by Álvarez *et al*.,[5] have shown that vegetarian and vegan diets produce changes at the level of different taxa, which, although minimal, may be sufficient to justify the benefits in SCFA production that is increased in the vegetarian population. It is also likely that the benefits of vegetarian diets derive from the presence of phytochemical products, such as isoflavones, which due to their antioxidant effect have demonstrated vast benefits on population health, reducing the risk of mortality and many chronic diseases.[15]

This effect is also observed in the Mediterranean diet, where this type of molecule abounds.[14] Studies suggest that in dietary intervention, the inclusion of quantity and variety of plant foods should be prioritized rather than the exclusion of foods of animal origin, supporting the concept that dietary diversity favors the stability of the microbiota.[5]

On the other hand, it has been seen that in the diet of Western communities, which is characterized by a high intake of fats and proteins of animal origin and a low fiber content, the IM is affected, since a considerable increase in *Bacteroides* can be observed, (such as *Alistipes* and *Bilophila*, which are tolerant to bile salts) and it also produces a decrease in the presence of Firmicutes that degrade complex plant polysaccharides, such as Roseburia, Eubacterium and Ruminococcus and other fermentative species.[5,8,28]

The effect of intestinal dysbiosis (alteration in the composition and/or functions of the microorganisms that inhabit the GIT) is also related to changes in bacterial metabolism. For example, changes in the intestinal microbiota related to COVID-19 involve a decrease in SCFA-producing bacteria and an increase in opportunistic pathogens. These changes in SCFA have been related to an increased susceptibility to secondary infections, which increases mortality from other infections such as respiratory infections.[29] Likewise, when this imbalance is generated in MI, the human body is susceptible to suffering from any disease, such as diverticulitis (DD). This condition occurs when small pouches or sacs known as diverticula form, protruding through weak points in the wall of the colon, mainly in the lower part, known as the sigmoid colon.[30] This can develop due to different factors, among them a low intake of dietary fiber. In fact, colonic DD is the result of complex interactions between dietary fiber, colonic wall structure, imbalance of colonic microflora, and intestinal motility.[15] Along with this, the intake of fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) contribute to irritable bowel syndrome (IBS) and likewise the large intestine.[31] All these negative aspects of the Western diet are contrasted with the benefits of consuming a Mediterranean diet, which is characterized by the diversity and high intake of foods such as vegetables, legumes, fruits, nuts, cereals and extra virgin olive oil, among others.[14] In relation to legumes, the Mediterranean diet usually consumes chickpeas, lentils and beans, which are rich in protein, with a content of more than double that of cereals.[11,16] In various meta-analyses of prospective observational studies and randomized controlled trials, legume intake has been associated with a lower risk of mortality from all causes under study and coronary heart disease, in addition to showing beneficial effects on body weight, total cholesterol, LDL cholesterol, systolic blood pressure and fasting glucose.[14]

As mentioned above, diet is closely related to the health of the gastrointestinal GIT. Because of this, it is important to maintain a balance in food consumption, such that the daily diet must include a high consumption of legumes, fiber, short-chain polysaccharides and taking caution regarding the use of additives contained in foods and the consumption of drugs. All this, in order to maintain a healthy IM and, consequently, a fully functional GIT.

Proteins and GIT Health

The composition of the microbiota and the production of metabolites in the GIT can be positively or negatively modified by the action of proteins in the different tissues and components thereof .[6] It is known that diets rich in certain proteins (especially of animal origin) and low in carbohydrates alter the colon microbiome, favoring a potentially pathogenic and pro-inflammatory microbiota profile, decreasing the production of SCFAs and increasing concentrations of ammonia, phenols and sulfur. of hydrogen.[32] The generation of genotoxic nitrous compounds that promote colorectal carcinogenesis may also occur, as a result of microbial catabolism of amino acids. This process generates indoles, phenols, ammonia and amines, which when combined with nitric oxide, form these compounds.[17,33]

Despite this, the bioactive properties of proteins and peptides obtained from the hydrolysis of proteins ingested through the diet can also act to benefit health and have clinical importance for the therapeutic approach to problems related to inflammatory bowel disease. and colorectal cancer.[6] Depending on the size, net charge, amino acid composition and solubility of the peptide, these can interact with target cells found in the intestinal mucosa or with colon epithelial cells to modify proinflammatory processes and reduce inflammation, as well as exert immunoregulatory effects.[22] Similarly, microbial metabolism of amino acids helps protect the GIT. For example, tryptophan metabolism generates indole-propionic acid and indole-3-acetate, which prevent colitis and/or reduce hepatocyte inflammation by maintaining intestinal homeostasis.[5,22] In the study by Katayama and Mine (2007), the *in vitro* effect of oxidative stress produced by H_2O_2 was investigated in human intestinal epithelial cells Caco-2, when they were pretreated with amino acids at different concentrations. As an indicator of oxidative stress, the secretion of the proinflammatory molecule IL-8 was measured. The results indicated that pre-treatment with amino acids such as Cysteine, Valine, Isoleucine, Leucine, Tryptophan, Histidine, Lysine and Alanine exert a protective effect against oxidative stress in epithelial tissue. This effect, which depends on the type of amino acid, is related to the improvement of glutathione (GHS) biosynthesis and activity.[18,34] However, it is important to remember that since this is an *in vitro* study, the results must be tested in studies on human beings before being conclusive.

The bioactive properties of proteins are of great relevance for the GIT, preventing oxidative stress of the epithelial tissue, as well as exerting therapeutic effects related to different problems, such as colorectal cancer and inflammation of the GIT. This is why it is suggested that the choice of proteins should be varied with respect to their origin, and thus maintain non-pathogenic GIT health by maintaining a positive amino acid load.

Proteins, peptides and amino acids from legumes

Legumes are a considerable and important source of dietary proteins that contain a high amount of amino acids such as lysine, leucine, aspartic acid, glutamic acid, and arginine.[24] The main proteins present in legumes are globulins and albumins.[35] Albumins are water-soluble and include enzyme proteins, protease inhibitors, amylase inhibitors, and lectins. On the other hand, globulins are soluble in salt and mostly comprise storage proteins.[11]

Several proteins (enzyme inhibitors, lectins, storage globulins) and peptides derived from them (lunasin, hydrophobic peptides) have shown anticancer, hypocholesterolemic, hypoglycemic, antioxidant, antimicrobial and immunostimulant properties.[11] A greater understanding of how the structural characteristics of legume proteins affect digestion and the production of bioactive sequences represents a key step in valorizing the nutraceutical potential of legume proteins and peptides derived from them.

Including proteins from legumes in the diet results in a series of benefits both for the human body and at the GIT level. The proteins and peptides in legumes have been found to play a role far beyond providing amino acids for the growth and maintenance of body tissues. Hydrolysis of these proteins in the human body produces peptides with important bioactivity, such as angiotensin I-converting enzyme inhibitory activity and antioxidant activity.[18,36] Also, studies carried out *in vitro* in human epithelial tissue cells, with protein hydrolysates from various legumes, have shown bioactivity of peptides against cancer, cardiovascular diseases or their physiological elements such as oxidative damage, inflammation, hypertension and high cholesterol.[11,23] Additionally, in another study carried out on protein sequence data from common bean (Phaseolus vulgaris), Carrasco-Castilla *et al*. (2012), identified up to 12 different biological activities in 15 proteins of this legume.[19,37] Those with the highest abundance of bioactive peptides were phytohemagglutinin and phaseolin. However, it should be noted that, as these are studies carried out on protein fractions produced by enzymatic hydrolysis, it is likely that not all biological activities and active peptides will be detected, since this is conditioned by the type of enzyme used.

At the GIT level, studies in the last decade have found a relationship between some protein fractions of legumes, the composition of the intestinal microbiota and the prevention of intestinal inflammation.[6] For example, in a study by Lee *et al*. (2011), about the effect of proteins found in the methanolic extract of Mung bean or green soybean (Vigna radiata) on proinflammatory cytokines in macrophages stimulated by lipopolysaccharides (LPS), it was found that these have anti-inflammatory functions and can suppress the proinflammatory processes caused by LPS20. Similarly, a study by Fernández-Tomé *et al*. (2019), showed that lunasin abrogates the production of proinflammatory cytokines in the presence of LPS by expanding the production of interleukin-10 (tolerogenic IL-10) and transforming growth factor beta (TGFβ), which produces an anti-inflammatory effect in the human intestinal mucosa.[21] Lunasin is a 43 amino acid peptide derived from soy protein recognized for its biological activity in *in vitro* assays and cell cultures. Likewise, the tripeptide Valine-Proline-Tyrosine (VPY) obtained from the hydrolysis of soy protein has been identified as an agent with anti-inflammatory activity. In an *in vitro* assay, Kovacs-Nolan *et al*. (2012) examined the transport of VPY using the peptide transporter PepT1 through intestinal epithelial cells Caco-2 and THP-122. They discovered that the secretion of IL-8 and TNF-α in both cell types is inhibited in the presence of VPY, indicating its anti-inflammatory capacity. When comparing the transport of VPY using glycylsarcosine as a carrier, the anti-inflammatory effects are lower. This indicates that the inhibitory activity of the production of proinflammatory molecules by the VPY tripeptide is mediated by the PepT1 transporter.[22]

On the other hand, the research by Girón-Calle *et al*. (2010) on chickpea protein hydrolysates from the action of pepsin and pancreatin, showed its potential as an agent against colon cancer. In this study, peptides produced by peptidase hydrolysis affected *in vitro* cell proliferation of Caco-2 and THP-1 cells by 45 and 78%, respectively. The above suggests that they could inhibit the growth of tumors in the colon. Additionally, an immunomodulatory activity of the peptides is suggested when performing bioavailability experiments using Caco-2 cells as an intestinal barrier in co-culture with THP-1 cells.[23]

Another study conducted by Niehues *et al*. (2010)[24] with peptides produced by the hydrolysis of pea proteins with trypsin, allowed the identification of an 11 amino acid peptide with antiadhesive activity against Helicobacter pylori. This bacterium is considered a group I carcinogenic agent due to the risk of infection and inflammation associated with its adhesion to stomach epithelial tissue. First, the research team tested the inhibitory efficacy of the peptide against H. pylori adhesion through an *in vitro* flow cytometry assay in human epithelial adherent gastric adenocarcinoma (AGS) cells. Then, in an in-situ assay, they tested bacterial adhesion in sections of human gastric mucosa using H. pylori labeled with fluorescein isothiocyanate (FITC). Both tests allowed us to verify the effectiveness of the undecapeptide as an antiadhesive agent while elucidating the mechanism by which it acts. In this particular case, the peptide from the pea protein interacts with the outer membrane proteins of H. pylori, preventing it from adhering to certain gastric epithelial cells, specifically the adhesins Baba, SabA and HpaA.[33] So, proteins and peptides derived from legumes have a positive impact on human health, including the prevention of inflammatory diseases, cancer and other disorders. Its inclusion in the diet can provide a number of benefits for the health of the gastrointestinal tract. However, it is important to highlight that the studies found do not include in vivo or clinical studies, which are necessary to better understand the efficacy or action of peptides and proteins under conditions that fully reflect the complexity of the interactions in a real biological environment.

Protein Antinutrients from Legumes and their Effect on the Git

The benefits provided by foods such as legumes can be diminished by the presence of compounds called antinutrients. These compounds significantly reduce the nutritional contribution of the food through various mechanisms. In the case of legumes, it is important to consider that they have various antinutritional factors (AF) both protein and non-protein.[11,26,33] In relation to the protein AFs of legumes, there are two important groups: i) trypsin and chymotrypsin inhibitors and ii) lectins. These AFs exist mainly when legumes are raw, so the way to inactivate them or reduce their concentration is based mainly on culinary techniques such as fermentation, heat treatments or soaking in water.[19] However, studies carried out for at least a decade show that it is possible to take advantage of the inhibition mechanisms of these molecules to benefit health.

Trypsin and chymotrypsin inhibitors (Bowman-Birk): Bowman-Birk family inhibitors (BBI) are mainly present in the protein fraction of albumins of legumes such as lentils, chickpeas, peas and soybeans. They are capable of inhibiting the activity of enzymes of the serine protease type, particularly trypsin and chymotrypsin. The inhibition mechanism lies in its interaction with the active site of the enzymes. As they are not affected by gastric acid or proteolytic enzymes, they reach the large intestine in active form and in high quantities.[6,38] For this reason, research has focused on its use as a therapeutic strategy in the treatment of GIT diseases and also on its possible anti-inflammatory and anticancer properties.

Studies by Clemente *et al*. (2014, 2017)[25,39] and Caccialupi *et al*. (2010)40 have shown that treatment with BBI from peas, lentils and soy significantly decreases the spread of human colorectal adenocarcinoma cells (HT29, Caco 2 and LoVo). Concentrations between 19 and 73 μmol/L produce a significant decrease in cell viability of up to 50%.[25,41] In another investigation, Lichtenstein *et al*. (2008)[26] evaluated the safety and efficacy of using soy BBI in patients with active ulcerative colitis (UC). Using a randomized, double-blind, placebo-controlled design, a 12-week treatment was applied to patients with UC. Treatment consisted of a daily dose of 800 units of chymotrypsin inhibitor concentrate (BBIC). The effect on patients was assessed by measuring the Sutherland disease activity index, that is, stool frequency, rectal bleeding, mucosal appearance, and physician rating of disease activity. At the end of treatment, approximately 50% of patients responded clinically and 36% showed disease remission, in contrast to 29% and 7.1%, respectively, in the placebo group. Additionally, patients showed no adverse side effects or apparent toxicity.[36]

Lectins

Lectins are protein compounds found in most plants, where they are involved in plant defense,[11] and generally occur in the form of glycoproteins that have the ability to bind to certain carbohydrate molecules without altering the covalent structure.[6] The toxic effect of lectins has been partially explained by their ability to modify the antigenic structure of the intestinal mucosa. This knowledge has allowed to explore the manipulation of IM to prevent the growth of pathogenic microorganisms such as E. coli using non-toxic lectins such as Galantus nivalis.[11] There are also some studies related to the use of soy proteins and various varieties of beans, especially the tepary variety as a potential adjunctive treatment for cancer due to its cytotoxic and antiproliferative effect.[11,27] The study by Valadez-Vega *et al*. (2011) showed that tepary bean lectin has cytotoxic effects on 2 human malignant cell lines, namely colon cancer and cervical cancer. This lectin produces a decrease in cell viability of both types of cancer by 30% and 90%, respectively. It is suggested that this effect is due to the inhibition of DNA synthesis, especially by a decrease in thymidine incorporation.[38]

Therapeutically, there is scientific evidence that these can contribute to the prevention or treatment of various diseases at the GIT level.[10,33,34] However, although the aforementioned studies provide promising information on the effects of Bowman-Birk family inhibitors (BBI) and lectins in the prevention and treatment of gastrointestinal diseases and cancer, it is essential to recognize the limitations and consider the need for additional research is needed to validate and fully understand its potential benefit in clinical and nutritional contexts. For example, the proposed mechanisms must be validated through additional research and the doses or concentrations of compounds indicated as effective are not necessarily equivalent to those that can be achieved in the human intestine due to the bioavailability or absorption that is achieved after ingestion. food. This reinforces the need to expand research through clinical studies.

Conclusions

The review has confirmed that legume proteins and peptides have a role far beyond the supply of amino acids for the growth and maintenance of body tissues. The proteins in legumes are closely related to the composition of the IM and the prevention of intestinal inflammation. In addition, they have been found to have other beneficial properties, such as anti-cancer, anti-inflammatory, and immunostimulant.

Various legume proteins, considered antinutrients (enzyme inhibitors, lectins), and peptides derived from them (lunasin, among others) are also associated with the reduction of certain forms of cancer due to their ability to modify the antigenic structure of the intestinal mucosa, which allows this action to be regulated in the MI and thus prevent the growth of pathogenic microorganisms. They may also exert a protective and/or suppressive effect on cancer development and inflammatory processes within the GIT.

However, more research is still needed on the effect of consuming legume proteins in humans, since most related studies have been carried out in *in vitro* or animal models. A knowledge gap has also been identified regarding the mechanisms by which these proteins and their derived peptides produce a positive effect on inflammatory processes and diseases related to the GIT and its microbiota, such as cancer. The available scientific evidence demonstrates that proteins and peptides from legumes can contribute to the prevention or treatment of various diseases at the GIT level. However, it is essential to validate the studies carried out by continuing research in clinical and nutritional contexts, considering aspects related to the variability between individuals or populations, and the phenomena of absorption and bioavailability of nutrients after ingestion.

Funding

This research has been financed by the Universidad Adventista de Chile through the Internal Research Funds, under project code: PIR 78

Conflict of Interest

Authors declare that they have no conflict of interest.

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