Chapter

Digestibility of Proteins in Legumes

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Abstract

Legume proteins have recently attracted interest from the food industry. Indeed, they are economical and have good nutritional and functional attributes. In addition to being important for growth and maintenance, they also provide antioxidant peptides, and are hence gaining importance for these additional health benefits. The nutritional benefits of leguminous seeds, are linked to the digestibility of the proteins into peptides and amino acids. Seed proteins have a complex structure. Coexisting with these proteins in the seed matrix, are other components that interfere with protein digestibility. Among them, are the antinutritional factors (ANFs), like trypsin inhibitors, which are also significant in animal nutrition. Thus, improving access to legume proteins, often depends on the removal of these inhibitors. Therefore, this chapter focuses on the factors affecting the efficient digestion of proteins, with emphasis on ANFs and methods to eliminate them. Enzymatic treatment is an effective method to solve the problems encountered. Exogenous enzymes, act as digestive aids and help improve protein digestibility *in vivo*, where digestion is impaired due to insufficient digestive enzymes. Enzymes provide an environment-friendly alternative to energy-intensive processes in the food industry. Complete digestion of legumes will prevent wastage and enhance food security, besides contributing to sustainability.

Keywords: protein digestibility, antinutritional factors, trypsin inhibitors, enzymes, proteases

1. Introduction

The origin of legumes in the diet of human beings, dates back to ancient times. The discovery of what is believed to be a pigeonpea in an Egyptian tomb, lead us to believe that lentils were used as food thousands of years ago. What was part of the diet of the ancient Aztec, Inca, Greek, Egyptian and Indian Vedic cultures, continues to hold importance even in today's modern world. For centuries now, legumes have been consumed by people all over the world. Globally, grain legumes occupied 81 million ha with production of more than 92 million tonnes. Major grain legume producing countries are India, China, Myanmar, Canada, Australia, Brazil, Argentina, USA and Russia [1]. Among the legumes consumed by humans, soyabean is by far the most widely used. Referred to as "poor man's food", pulses and beans are part of the staple diet among the low-income population, as they are used as a main source of protein, instead of animal meat, which has traditionally been more expensive and not as easily available. They have emerged as effective tools in the fight against global malnourishment. Most health organizations recommend consuming vegetable protein on a regular basis, as it has been shown to lower blood cholesterol levels, the risk of coronary heart disease, and diabetes [2]. According to Dietary guidelines for Americans, U.S. Department of Agriculture, and U.S. Department of Health and Services, legumes should be included several times a week, in the diet.

Apart from being used as a protein source for the diet, legumes have other health benefits that are only now being realized. However, the digestibility of plant seed proteins is low, as compared to animal proteins. Hence it is important to understand the factors influencing the complete breakdown of proteins into their constituent components, so that remedial measures can be undertaken to maximize their use in food or in food applications.

2. Health benefits of legumes

The nutritional value of legumes was recognized in ancient cultures. Fava beans were used in recipes, in what is claimed to be the oldest cookbook during the Roman civilization. For human nutrition, approximately 20 leguminous species are employed as dry grains. Among the legumes used, the most common one is soyabean, followed by lentil, chickpea, and cowpea, with soyabean being the most important, due to its high protein content (**Table 1**).

Sr. no	Legumes	Crude protein % (w/w)	Reference
1.	Soyabean	38	[3, 4]
2.	Lentil	26	[5]
3.	Chickpea	22	[3, 5]
4.	Cowpea	25	[6]

Table 1.
Crude protein content of commonly used legumes.

In comparison to animal protein sources, legume seeds are high in dietary fiber, which is good for gut health [7]. They possess high nutritional value. Legumes are rich sources of good quality proteins, calories, certain minerals, fibers, vitamins and are cholesterol free. Thus, legumes have the potential to increase the nutritional quality of foods, and hence, efforts are underway for their integration into novel food preparations with improved nutritional and functional qualities [8].

Proteins or peptides derived from legumes have played a significant role, beyond simply providing amino acids for growth and tissue repair. The role of legume proteins in the general growth and maintenance of living organisms is well documented. However, little is known about the beneficial effects of peptides derived from legumes.

2.1 Antioxidant property of peptides

Bioactive peptides are amino acid sequences, that exert beneficial effects in the body to improve human health beyond their nutritional values [9, 10]. These peptides can have antioxidative, antimicrobial, anticarcinogenic, or anti-inflammatory activities, based on their sequence and size. The antioxidant activity can be defined as metal chelating or radical scavenging properties, which have a direct or indirect impact on the inhibition of free radical generation. Intake of such bioactive peptides can minimize the risk of chronic diseases [11, 12].

Environment-friendly processes like enzymatic hydrolysis are preferred to chemical hydrolysis as it results in bioactive peptides [13, 14]. The most frequently used commercial proteolytic enzymes are papain, pancreatin, trypsin, chymotrypsin, and bromelain. The high antioxidant activity of soy hydrolysate obtained after proteolytic digestion has been well documented earlier [15]. In a recent study, an enzyme formulation, PepzymeAG has been shown, to not only improve the digestion of pea protein, but also result in peptides with antioxidant and antidiabetic properties [16]. Peptides are therefore gaining importance and their use is expanding, in nutraceuticals and pharmaceuticals products.

Antioxidant properties of peptides can be assessed by different methods viz. DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) etc. Purification and identification of specific peptides and/or amino acids with antioxidant activities are needed to expand their application in the food and pharmaceutical industries.

3. Digestion of proteins into amino acids

Proteins cannot be absorbed by the digestive tract as is. Several proteolytic enzymes are at play in the digestive system of mammals. In the acidic condition of the stomach, pepsin has optimum activity. The pancreas secrete proteolytic enzymes, like trypsin and chymotrypsin, which function at a higher pH of 8–9. Due to the action of these proteolytic enzymes, proteins are digested into oligopeptides and then amino acids (**Figure 1**). The amino acids available are of great importance in nutrition.

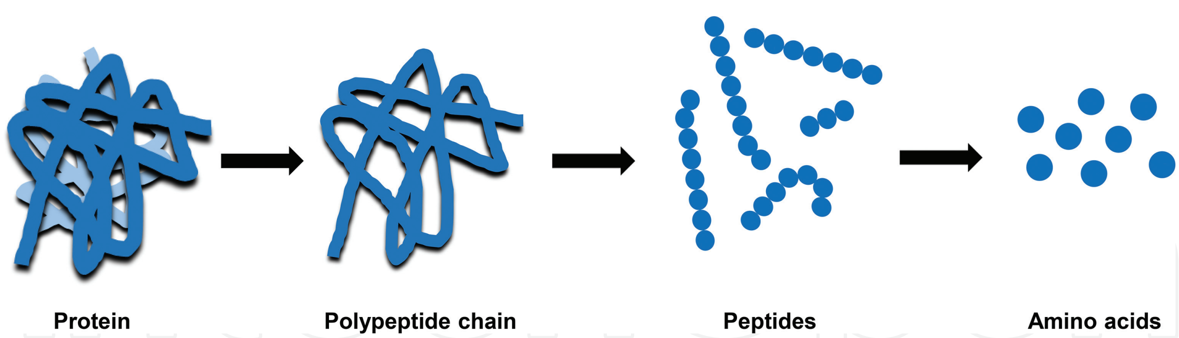

Figure 1.
Digestion of proteins into peptides and amino acids.

3.1 Essential amino acids

An essential amino acid, or indispensable amino acid, is an amino acid that cannot be synthesized from scratch by the organism fast enough to supply its demand, and must therefore come from the diet. The essential amino acids (EAAs), (e.g., arginine, methionine, lysine, leucine, isoleucine, tryptophan, valine, threonine, phenylalanine, and histidine) cannot be produced endogenously, so 10–20 mg/kg body weight of each must be obtained in the diet each day from consumed protein [17]. Moreover, dietary protein sources must provide the whole range of EAAs since proteins deficient in one or more EAAs generate an unpleasant eating response, resulting in a

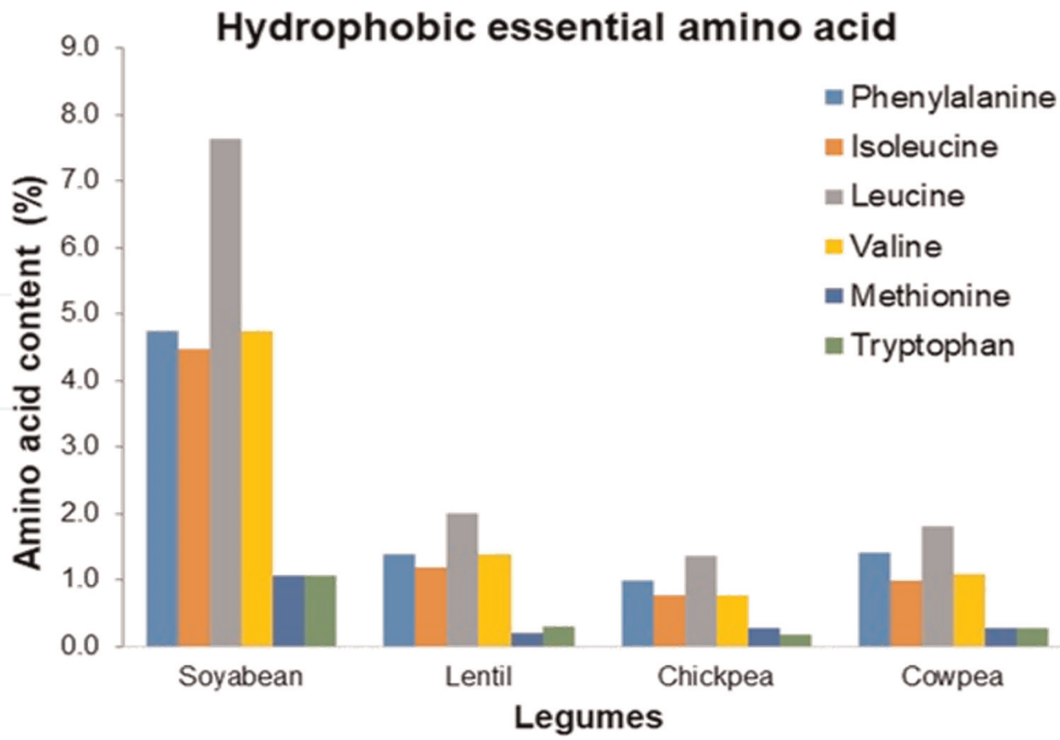

Figure 2.
Hydrophobic essential amino acid profile in different legumes.

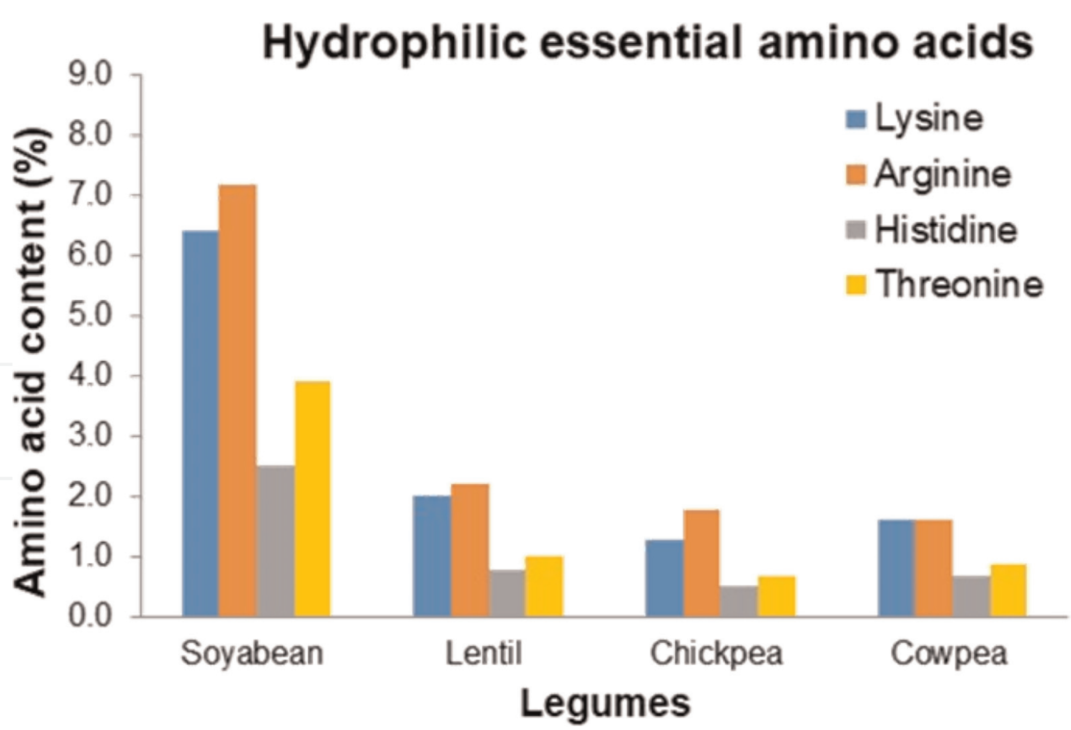

Figure 3.
Hydrophilic essential amino acid profile in different legumes.

considerable decline in diet consumption. **Figures 2** and **3** illustrate all the hydrophobic and hydrophilic essential amino acids, present in easily accessible legumes such as soyabean, chickpea, cow pea, and lentils.

The following percent of their contents in soyabean grain are documented in the reference literature: Leucine is approximately 7.6% of crude protein and lysine is about 6.3% of crude protein whereas valine, isoleucine and phenylalanine are around 4.7% each, of crude protein [18]. When compared to animal proteins, soyabean protein has a lower amount of sulfur containing amino acids. Legume proteins, with the exception of soyabean (*Glycine max*), are considered incomplete due to a lack of key sulfur-containing amino acids (methionine and cysteine). To compensate for this deficiency, legume proteins are supplemented with cereal proteins, which are low in lysine but high in methionine and cysteine [3].

3.2 Protein digestibility evaluation method

Although legume-derived proteins are nutritionally adequate, their protein quality and digestibility remain an issue [19]. WHO recommends that in order to support optimal health and growth, humans should consume high quality proteins [20]. Protein quality is the ability of dietary proteins to fulfill the metabolic needs of the body, thus quality matrices are governed by the content of limiting amino acids in food and their digestibility [21]. Limiting amino acids are those amino acids that do not meet the minimal requirement of the body and need to be included in a diet.

Different regulatory bodies across the world (US-FDA, Canadian food inspection agency) use protein quality information to determine 'Protein Digestibility'. From a consumer point of view, protein quality claims can influence the perception of health benefits of the product. Therefore, nowadays, commercial protein powders often provide protein content claims in the form of a digestibility score.

Protein digestibility can be defined as the fraction of protein that is available for absorption after it is ingested. It is a measure of the bioavailability of the protein. High digestibility is dependent on the hydrolysis of peptide bonds that are characteristic of proteins. The digestibility of plant proteins is lower ($<80\%$) than animal proteins ($\geq 90\%$) [22]. A joint FAO/WHO (Food and Agricultural organization/ World Health Organization) expert consultation committee proposed the first method for protein quality evaluation in 1990, the Protein digestibility-corrected amino acid score (PDCAAS).

$$\text{PDCAAS\%} = \frac{(\text{mg of first limiting amino acid in 1 g test protein})}{(\text{mg of the same amino acid in 1 g reference protein})} \times \text{TD} \times 100$$

Here, true digestibility $(\text{TD}) = \frac{\text{I}-\text{F}-\text{Fk}}{\text{I}}$

I = protein intake of rats fed Test diet

F = protein excreted in feces of rats fed Test diet

Fk = protein excreted of rats fed protein-free diet.

However, in 2011, FAO/WHO made a recommendation that the new protein quality measure (digestible indispensable amino acid score; DIAAS) replace the old PDCAAS.

$$\text{DIAAS\%} = \frac{(\text{mg of digestable dietary indispensable amino acid in 1 g of the dietary protein})}{(\text{mg of the same dietary indispensable amino acid in 1 g of the reference protein})} \times 100$$

There are many reasons why this shift has been recommended, two of them were the superior scoring method and the accurate sampling method [23–25]. For instance, the PDCAAS score of Whey Protein Isolate (WPI) and Soy Protein Isolate (SPI) is 1 and 0.98 (no significant difference). But the DIAAS score of WPI's is 1.09 and for SPI it reduces to 0.90. This gives a clear distinction of protein quality and in turn helps to make informed decisions. Knowledge of the IAA (Indispensable amino acid) content from protein sources, is not sufficient to accurately determine the requirement of the type of amino acid, because it varies with respect to physiological conditions, age etc. Therefore, FAO concludes that the current data of digestibility is insufficient and suggests that additional data is required, on ileal digestibility of human foods, determined in animal as well as human models [25].

Other alternate ways to determine protein digestibility, such as *in vitro* digestion methods that are less time consuming, controllable and easy to perform are INFOGEST, and *in vitro* PDCAAS [26, 27]. In a recent study, INFOGEST was used to study the digestion of pea proteins using enzymes under simulated gastrointestinal conditions [16].

4. Factors affecting protein digestibility (extrinsic and intrinsic factors)

The full benefits of legumes depend on how easily the proteins are digested. Proteins are polymers of amino acids. Amino acids are linked together by a peptide bond formed between the amino group of one amino acid and the carboxyl group of the adjacent amino acid. The sequence of amino acids defines its primary structure. The organization of amino acids into secondary and tertiary structures is what defines the ultimate protein structure, an attribute that is unique and dependent on the primary structure. The polypeptide chain is not linear, but adopts a three-dimensional structure and can be interlinked via disulphide bonds, making for a stable structure. Breakdown of this structure is required before peptides or amino acids can be released, either by internal digestion or by processing methods.

Different legumes contain different types of proteins. Hence, the increase in digestibility of legume proteins varies, depending on the type of protein they contain. When compared to animal proteins, the digestibility of legume protein is low. Among legumes, there are variances in the digestibility of proteins, with ease of digestibility increasing in the following order: soyabean, lentil, chickpea and common bean [7, 8]. Protein structure and functionality, compartmentalization, the permeability of cell walls, the protective seed coat, and enzyme accessibility are all important aspects of this trait.

The digestibility of proteins, can be influenced by several factors that can be classified as extrinsic factors or intrinsic factors. Extrinsic factors include pH, temperature, ionic strength conditions, and the food matrix, as well as the presence of secondary molecules present in the environment of the protein. Intrinsic factors are those factors that contribute to the inherent property of the protein, and impart its characteristics. These include protein amino acid sequence and protein structural characteristics. Furthermore, growth conditions (e.g., drought and heat stress) can influence both internal and exterior elements throughout plant development [28, 29]. The pre-harvest characteristics influencing plant protein digestibility, on the other hand, are beyond the scope of this chapter.

4.1 Extrinsic factors

Extrinsic factors can affect the digestibility of legume proteins: these include interaction with other compounds such as carbohydrates, lipids, and antinutritional factors like tannins, phytates, trypsin inhibitors and lectins. These are described in detail, in another section in this chapter. In the seed matrix, proteins are complexed with other compounds like phenolic compounds and carbohydrates, causing a physical entrapment of cellular structures that shield the proteins from the action of proteases. Drulyte et al. suggested that cell wall rigidity and fiber content may influence protein digestibility. Particle size reduction disrupts the cell wall integrity; thus, the reported improvement of digestibility attributed to milling could also be due to the alteration of the cell wall, which enhances legume seed protein digestibility [30]. In a study, by Melito et al., physical or enzymatic removal of the cell walls enhanced legume digestion by up to 50%. This shows that physiological barriers such as the cell wall have an impact on protein digestion [31].

4.2 Intrinsic factors

The low digestibility of legume proteins is attributed to their amino acid composition and protein quaternary structure. Proline-rich regions often diminish protein chain flexibility and are renowned for their high resistance to peptidase hydrolysis. Legume seed storage proteins are classified into globulins, albumins, glutelins, and prolamins according to their solubility properties in water, salted water, or ethanol/water solutions. Among these, globulins and albumins are the most abundant proteins found in legumes. Albumins are soluble in water. Examples of albumin proteins are Kunitz trypsin inhibitor and Bowman-Birk trypsin/chymotrypsin inhibitors [8].

Globulins are extracted in salt solutions, and represent approximately 70% of legume seed proteins. They consist mainly of the 7S proteins called vicilins, and 11S proteins called legumins, [32]. Soyabean protein contains three major fractions such as 2S, 7S, and 11S. In soyabean, 11S and 7S fractions represent approximately 70% of total protein. 11S fraction consists only of glycinin, which typically exists as a hexamer and 7S fraction majorly consists of β-conglycinin. The molecular weight of seed proteins ranges from 8 to 600 kDa [33]. Albumins have a molecular weight ranging from 50 to 80 kDa. These proteins generally exist in oligomeric form. The 7S globulins are typically trimers of molecular weight about 150 kDa, while the 11S proteins form hexamers of molecular weight about 350–400 kDa, or higher association of subunits, such as the 15–18S globulins found in soyabean globulins [34].

One of the factors influencing the stability of proteins, is their secondary structure. In legumes proteins, the predominant secondary structure is the β-sheet conformation, as compared to the α-helix structure [35]. This β-sheet conformation contributes to its resistance to proteolysis in the gastrointestinal tract. The β-sheet structure of legume proteins is a contributing factor to aggregation which occurs during the processing of legume proteins. Protein aggregation affects the biological value and technological usefulness of the raw materials when used in food production. Another contributing factor to increased stability is the presence of disulphide bonds, formed between the polypeptide chains. Globulins showed better *in vitro* digestibility than albumins due to the presence of lower cysteine content and hence less number of disulphide bond as compared to albumins [7, 36].

The amino acid sequence of a protein determines the type of peptides formed on digestion. Peptidases have a high specificity for hydrolysing peptide bonds that are next to a specific type of amino acid. In processing, the sequence of peptides formed, depends on the legume protein used, and the specificity of the enzyme used. This determines the antioxidant activity of the resultant peptide. **Figure 4** shows an outline for the production of peptides, produced by enzymatic methods.

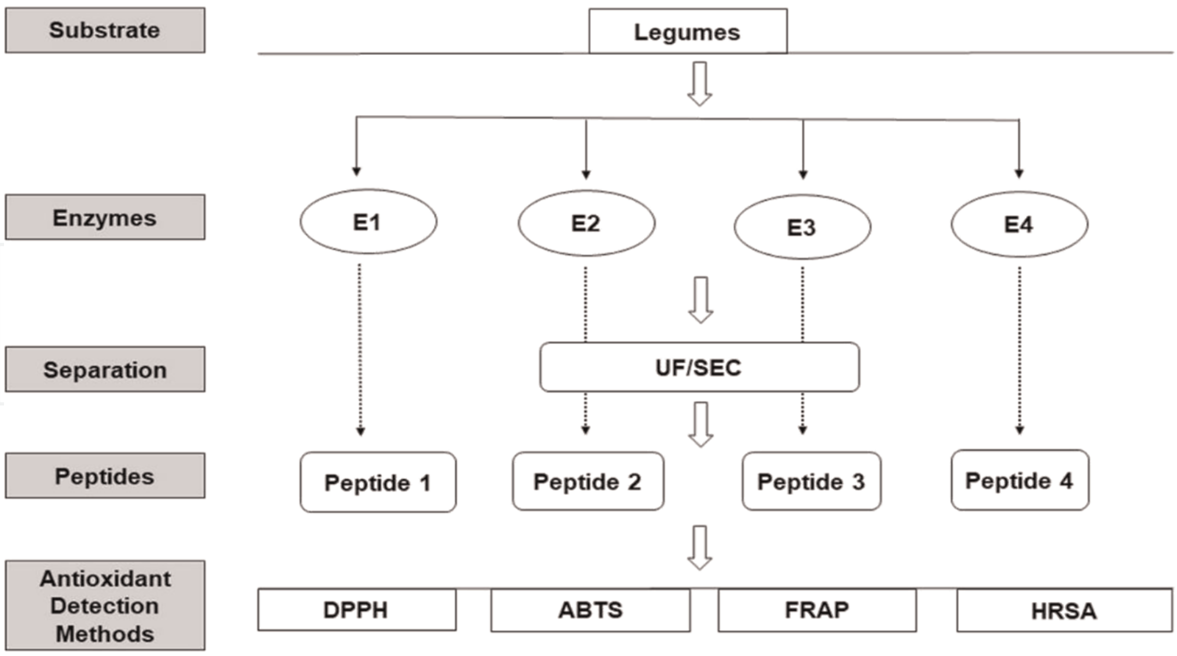

Figure 4.
Digestion of proteins into peptides by specific enzymes and selection for antioxidant property. Abbreviations: *E (different enzymes), UF (ultrafiltration), SEC (size-exclusion chromatography), DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis,3-ethyl benzoline-6-sulfonic acid), HRSA (hydroxyl radical scavenging activity).*

5. Enhancement of protein digestibility using enzymatic treatment

Protein hydrolysis includes the breakage of peptide bonds, resulting in smaller peptides and free amino acids, which improves digestibility and functional characteristics [37]. Chemical hydrolysis (by acids or alkalis) has significant drawbacks since it can produce harmful amino acid residues (e.g., lysinoalanine) and produce goods with lower nutritional quality [38, 39]. Protein enzymatic hydrolysis by enzymes was previously used in the food industry to enhance the biological value and functional qualities of these molecules [40]. Protein hydrolysates produced by enzymatic treatment (e.g., cellulases, hemicellulases, proteases) may improve protein availability and digestibility by reducing undesired compounds found in legumes [38, 40, 41]. Proteases (or peptidases) have been used to improve product nutritional value by modifying protein structures [39, 42]. Protein hydrolysis has been shown to lower protein antigenicity, increase tolerance, and create peptides that do not stimulate *in vitro* IgE antibody binding activity, therefore decreasing allergenicity.

Protein enzymatic hydrolysis was found to be effective in enhancing protein solubility, foaming capacity and stability, and gelation capability [38, 39]. However, there are some challenges with the application of protein hydrolysates, because protein hydrolysis can result in the formation of hydrophobic peptides, which causes the development of bitterness and off-flavors, negatively impacting taste and limiting the use of protein hydrolysates in food products [37].

To improve the availability of protein in fava beans, enzymatic treatments were performed in four cultivars (ON, OPNS, TAL and VC3). The greatest change was observed in the OPNS cultivar treated with protease, which increased its digestibility from 54.4% (control treatment) to 81.6% [40]. Legume preparations when treated with pepsin/pancreatin in an *in vitro* digestion simulation, have resulted in 20–46% increase in the degree of hydrolysis [43].

6. Problems encountered in the digestion of legumes

6.1 Insufficient digestive enzymes

Digestive enzymes are synthesized by the stomach, small intestine, and pancreas. The pancreas have an essential role in the digestion, absorption, and metabolism of carbohydrates, fats, and proteins hence is the enzyme "powerhouse" of digestion. Insufficient secretion of digestive enzymes by the pancreas is called exocrine pancreatic insufficiency. Some enzyme insufficiencies are genetic, hereditary and congenital or develop over time, and with age. Any impairment of digestive enzymes over a prolonged period results in deficiencies of vitamins and minerals, gastrointestinal irritation, malnutrition, and complications, leading to poor quality of life.

Impaired enzyme-related digestion can be alleviated by prescription digestive enzymes. These over-the-counter digestive enzyme supplements are used to treat health issues such as acid reflux, gas, bloating and diarrhea. Enzyme supplements, like VegPeptase™ can be used to improve the digestibility of legumes. These supplements aid in better digestion of "hard-to-digest" proteins in food and absorption of nutrients. Pancreatic enzyme replacement therapy is the most popular and the only FDA-approved enzyme replacement therapy (PERT). PERT is the use of medications that contain enzymes to replace what the pancreas is deficient in producing. These medications contain proteases, amylases and lipases. Microbial sources of enzymes viz. cellulase, protease, and lipase can be used to improve digestion and access the required nutrients, when shifting to a plant-based diet. Similarly, plant-sourced enzymes like bromelain (from pineapple) and papain (from papaya) are proteolytic enzymes, which are included in many digestive formulas. They have an additional use as systemic enzymes and against inflammation. This helps people follow a less restricted diet on a long term basis.

6.2 Antinutritional factors (ANFs)

One of the main factors affecting the protein digestibility of legumes is the presence of antinutritional factors. Antinutritional factors are compounds that are known to affect the digestibility and thus impair the nutritional quality of various foods, including legume food proteins [44]. These antinutritional factors are present in unprocessed food or foods, as a result of processing (e.g., Maillard reaction products in soyabean-based products) [45]. Major antinutritional factors, which are found in legumes include saponins, tannins, phytic acid, gossypol, lectins, protease inhibitors, amylase inhibitors, and goitrogens [46]. These antinutritional factors cause unfavorable effects when consumed in large quantities. They are also known to cause allergic responses in some individuals, which is a cause for concern [47]. Thus, the exclusion or deactivation of these antinutritional factors and allergenic compounds can promote protein digestibility.

Among the ANFs found in legumes, the following are known to interfere with protein digestion in humans and animals: protease inhibitors (trypsin inhibitors), tannins, lectins, and phytic acid (**Figure 5**).

6.2.1 Protease inhibitors (trypsin inhibitors)

One of the main ANFs found in legumes are protease inhibitors. They are small proteins, which have evolved as defense strategies in plants [48]. As the name

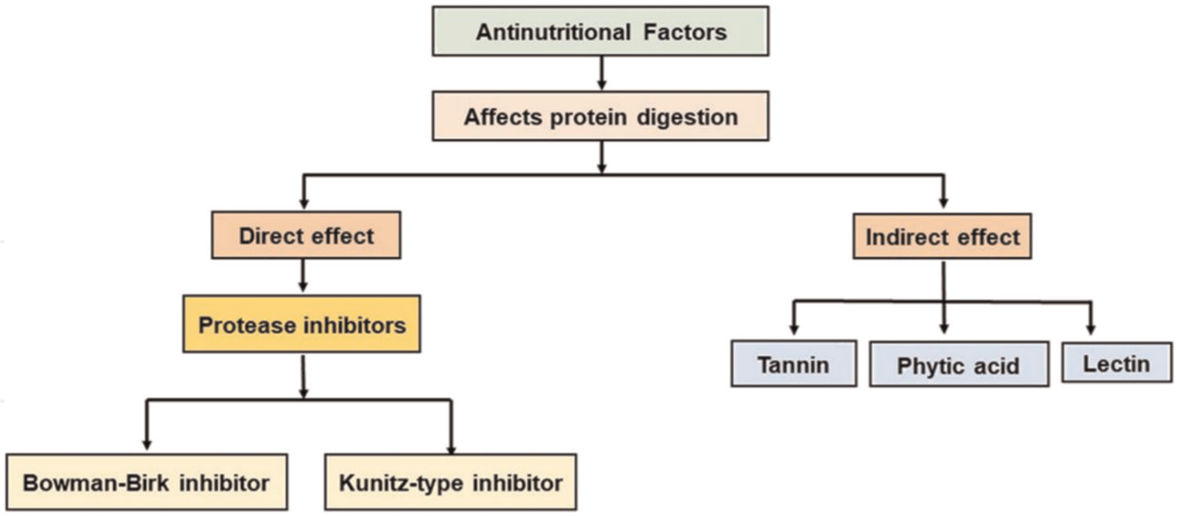

Figure 5.
Antinutritional factors that interfere in protein digestion.

suggests, these inhibitors inhibit the action of proteases in mammals, thus impairing protein digestion [49] and affecting the nutritional value of foods [50].

Trypsin and chymotrypsin are the main proteases, in the lumen of the upper gastrointestinal tract, where they exercise their digestive functions [51]. The presence of trypsin inhibitors in the diet leads to the formation of irreversible enzyme-trypsin inhibitor complexes. These complexes are indigestible, even in the presence of high amounts of digestive enzymes [52]. Trypsin inhibitors block the active site of trypsin/chymotrypsin, through the N- or C-terminus and exposed loop [51], effectively preventing these enzymes from acting on the protein substrate (**Figure 6**). Therefore, when legumes are eaten raw or without being cooked properly, they upset digestive functions and cause diarrhea or excessive gas [52]. In such cases, even

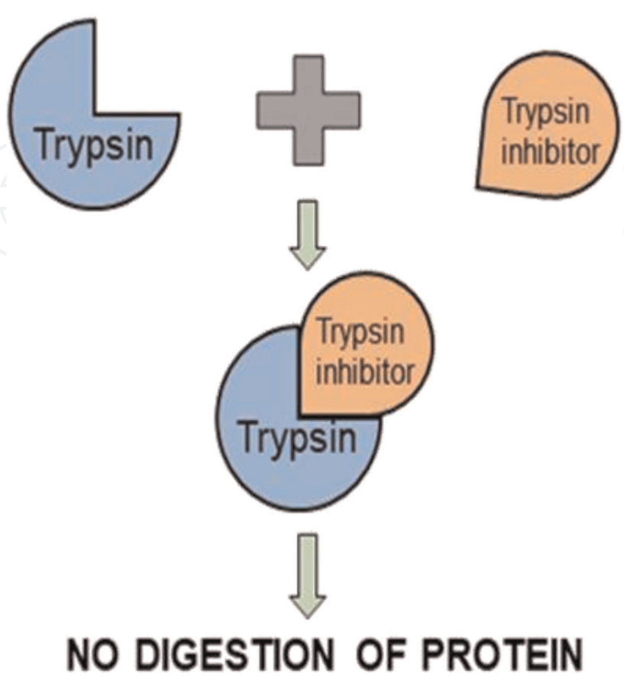

Figure 6.
Trypsin inhibitor competitively binds with trypsin, and prevents it from digesting the protein.

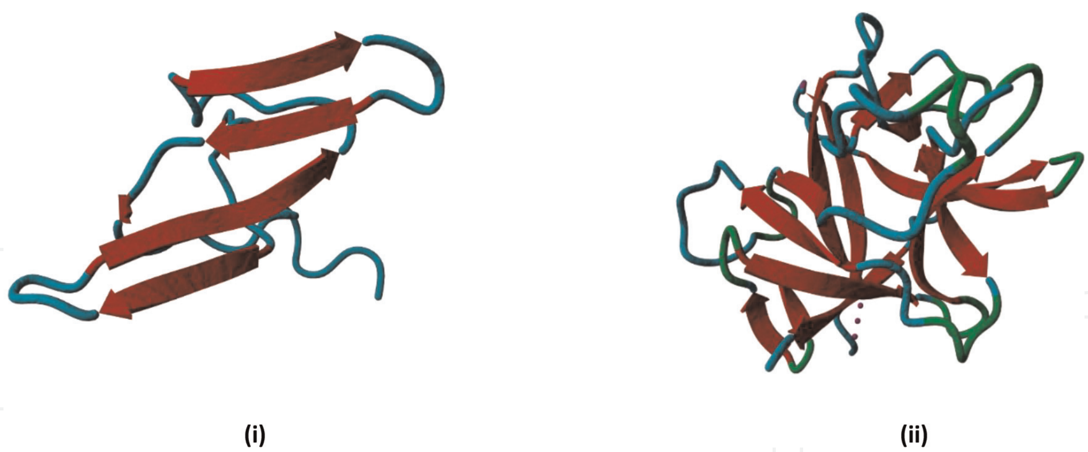

Figure 7.
Protein structure of trypsin inhibitors, (i) Bowman-Birk inhibitor (BBI), and (ii) Kunitz-type inhibitor, from soyabean. Images are prepared using PDB ids, 1BBI and 6NTT, using YASARA, version 22.9.24. The beta sheets are depicted in red.

Antinutritional factors	Concentration in raw soyabean seeds
Protease inhibitors	25–50 mg/g
Glycinin	150–200 mg/g
β-conglycinin	50–100 mg/g
Lectins	2100–3500 ppm
Phytic acid	6 mg/g

Table 2.
Antinutritional factors in raw soyabean seeds [58].

though the intake of protein is high, the complete mobilization of amino acids is prevented.

In legumes, the trypsin inhibitor content ranges from 3 to 84 U/mg, while the chymotrypsin inhibitor content varies from 0 to 17 U/mg [53, 54]. The prominent trypsin inhibitors in legumes are the Bowman-Birk inhibitor and Kunitz-type inhibitor (**Figure 7**) [55–57]. Kunitz-type inhibitor (molecular weight 18–24 kDa) and Bowman-Birk inhibitors (molecular weight 7–9 kDa) are both capable of inhibiting trypsin and chymotrypsin enzymes. In soyabeans, glycinin and β-conglycinin constitute 65–80% of the protein fraction or 25–35% of the soya seed weight (**Table 2**) [59]. Because of their predominant beta-barrel structure, they are very stable.

6.2.2 Lectins

Lectins are proteins that have specificity for carbohydrates. When combined with the glycoprotein components of red blood cells, they cause agglutination of the cells. Lectins bind to epithelial membrane of glycoproteins, such as brush-border membrane enzymes, gangliosides, glycolipids, receptors, secreted mucins, and transport proteins [60]. They disturb intestinal permeability and interfere with the absorption of digestive end products in the small intestine [61]. Protein digestion is affected, leading to nitrogen loss; the undigested and unabsorbed proteins in the small intestines reach the colon where they are fermented to short chain fatty acids and release gases leading to gastrointestinal disorders. The affected intestinal permeability allows the entrance of the bacteria and their endotoxins into the bloodstream, causing a toxic response. Moreover, lectins may also be internalized directly and cause systemic effects. They can disrupt protein, carbohydrate, and lipid metabolism [62]. Lectins are also resistant to heat and digestive processes, during their intestinal passage their activity is retained [63].

6.2.3 Phytic acid

Phytic acid (myo-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate) (**Figure 8a**), is a secondary compound found in plant seeds of legumes [64]. Generally, phytates contain about 50–80% of the total phosphorus present in the seeds [65]. Due to its chelating property, phytic acid complexes with metal ions, like iron, magnesium and calcium, reducing their bioavailability, and resulting in mineral ion deficiencies in human nutrition [66, 67]. In addition, phytic acid interferes with the digestion of proteins. In both acidic and basic pH, phytic acid forms a complex with proteins and alters the protein conformation. It also binds trypsin and thus affects the action of trypsin on proteins [68–70].

6.2.4 Tannins

Tannins are located in the layer between the external tegument and the aleuronic layer inside the seeds, protecting the plant embryo from mechanical and oxidative damage and maintaining its dormancy [71]. They are also present in plant leaves, fruits, and bark [72].

The consumption of tannins can cause hardening of the gastrointestinal mucosa, resulting in reduced nutrient absorption. Tannins affect protein digestibility, by forming reversible and irreversible complexes between the hydroxyl group of tannins (**Figure 8b**) and the carbonyl group of proteins, leading to a decrease in essential amino acid availability [28, 73, 74]. These complexes are relatively large and hydrophobic in nature [75]. The breakdown products constitute a large number of compounds, which can be toxic. In the oral cavity, tannins bind to proline-rich proteins in saliva, and this helps to protect dietary and endogenous protein. However, in the

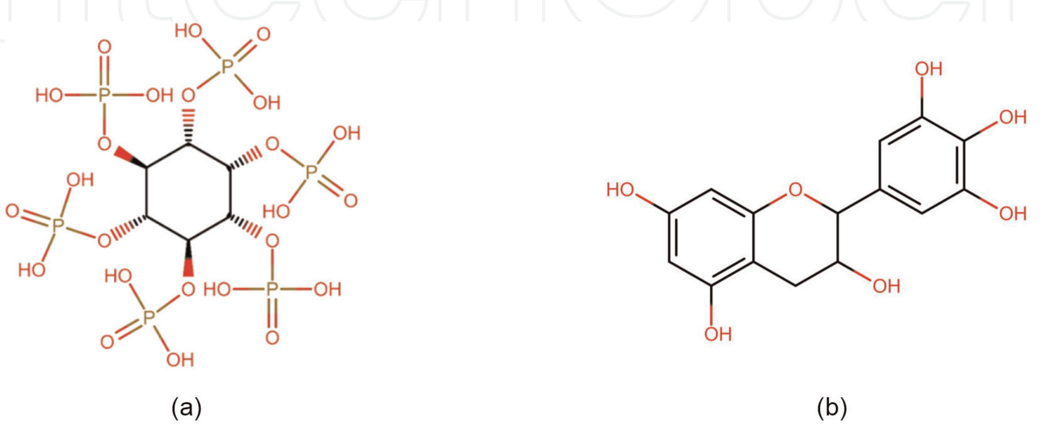

Figure 8.
(a) Chemical structure of phytic acid (b) Chemical structure (basic unit) of tannin. (chemical structures are prepared using Marvin JS).

absence of sufficient salivary secretion, tannins are then free to interact with digestive enzymes [76, 77]. Tannins are known to inhibit the digestion of proteins by 28% [46].

6.3 Methods to eliminate antinutritional factors

Some of the common methods employed to diminish or eliminate antinutritional factors include soaking, heating, cooking, germination, fermentation, extraction, irradiation, and enzymatic treatment [78]. The application of a single technique is frequently insufficient for effective treatment and so combinations of methods are usually employed. These treatments can be classified based on the processing techniques—physical, chemical, biological and enzymatic.

6.3.1 Physical treatment

Soaking overnight is the most common method used to reduce the antinutritional content in legumes and improve their nutritional value. Most of the antinutrients in these foods are found in the upper layer. Since many are water-soluble, they can be eliminated by prolonged soaking. In legumes, soaking has been found to decrease phytate, protease inhibitors, lectins, and tannins. Soaking is typically used in combination with other methods, like thermal treatment, germination, and fermentation.

Thermal treatments, like cooking, boiling, autoclaving and microwave cooking are the most popular methods for processing legumes, because it improves protein digestibility. Processing by heat is an effective technique to limit ANFs and improve nutrient digestibility in legumes [79]. Heating results in denaturation of the protein, an increase in surface area and exposure of cleavage sites that are otherwise inaccessible to protease enzymes [80]. Thus, a reduction in the concentration of ANFs, due to heat treatment is responsible for improved protein digestibility [81].

However, not all heat treatment is advantageous. Excessive or intensive heating may result in the degradation of heat sensitive amino acids and micronutrients and limit their bioavailability [30]. It may also lead to the formation of new products called neoantigens, which can elicit an allergic response. These neoantigens result from the Maillard reaction, by interaction of proteins with sugar residues upon heating [33]. Allergenic legume proteins elicit an allergenic response by surviving the acidic gastric conditions and action of digestive proteases. However, many are resistant to heat. Allergenic proteins in peanut are heat-resistant, while those in soya are partially heat-stable.

6.3.2 Biological treatment

During the germination of legume seeds, enzymes like amylase, protease, and lipase are activated to degrade starch, storage-protein and proteinaceous antinutritional factors. Germination is reported to suppress the amount of phytate, tannins, and trypsin inhibitors in different legume seeds [82], thus improving protein digestibility.

Fermentation is a traditional technique, where microorganisms facilitate enzymatic reactions that reduce the antinutrient content and thus increase the digestibility of plant proteins [83–85]. During this process, hard-to-digest proteins, like glycinin and β-conglycinin, of soyabean, are hydrolyzed to bioactive peptides. This results in improved solubility and hence higher protein digestibility of complex storage proteins [86]. This reduces the levels of undigested proteins that can cause food allergies [87]. Unfortunately, the microorganisms involved in the fermentation process can also utilize amino acids and proteins, resulting in the loss of amino acids and proteins [85]. Therefore, due to lack of specificity and optimum conditions, which could lead to maximum protein digestibility with minimal loss of protein, the use of this technique remains unpredictable. Future food processing methods may need to incorporate techniques that reduce these antinutritional factors, and are economically feasible, for both the environment and customers.

6.3.3 Enzymatic treatment

The universal use of enzymes in food and feed processing is due to their unmatched specificity, operating under mild conditions of pH, temperature and pressure while displaying high activity, high turnover numbers and high biodegradability [88]. Thus, the application of enzymes is considered as a promising approach for plant protein modifications. Major groups of enzymes used in food applications are proteases, amylases, and lipases for the manipulation of proteins, starch, and lipids, respectively. Proteases can enhance protein digestibility by reducing the amount of trypsin inhibitors [38, 40, 41] and lectins. Phytase may also be applied in the industrial processing of soyabean to prepare certain foods for human consumption. Phytases have gained attention in human nutrition, especially to counteract zinc and iron deficiencies [89], by improving their bioavailabilities [90]. Saito et al. have developed a novel process for removal of the major soyabean storage proteins β-conglycinin and glycinin, using phytase added to defatted soy milk at pH 6 with incubation at 40°C [91]. Phytic acid reduction by bioprocessing as a tool for improving the *in vitro* digestibility of fava bean flour has been demonstrated by Rosa-Sibakov et al. The improvement in protein digestibility was dose dependent and correlated to phytic acid content reduction, which explains the influence of enzymatic phytase treatment and LAB (lactic acid bacteria) fermentation on food digestibility, protein quality and protein solubility [92].

Food security is a global issue; hence increasing the nutritional value of food that is underutilized, will be an important part of the solution. Therefore, it will be interesting to explore the potential of enzymes in legume processing for human and animal health.

6.4 Legumes in animal nutrition

Legumes are used as a protein source in animal nutrition. Soyabean is the most important protein source in poultry and swine diets. Legumes are increasingly being used as a sustainable replacement for fish meals in aquafeed and pet diets. Globally, approximately 98 percent of soyabean meal is used as animal feed. Among the most significant ANFs in animal nutrition, are the trypsin inhibitors, found in raw soyabeans. By interfering with trypsin and chymotrypsin activity, they impair digestion in monogastric animals and some young ruminant animals [93]. Other young monogastric, such as swine, have also responded to soyabean meal, with reduced growth performance [94, 95]. Trypsin inhibitors have deleterious effects on animals. They result in stunted growth, reduced feed efficiency and pancreatic hypertrophy [93]. Lectins attach to mucosa cells damaging the intestinal wall and reducing the absorption of nutrients [63]. Glycinin and β-conglycinin are two allergenic soyabean proteins that are not digested easily. Glycinin damages intestinal morphology, causing intestinal atrophy and necrosis [94, 96, 97]. β-conglycinin causes a hypersensitive immune response and negatively affects the growth performance of animals [98, 99]. Other antinutritional factors like tannins cause decreased feed consumption in animals, as they bind dietary protein and digestive enzymes to form complexes that are not readily digestible [100], reducing palatability and growth rate [101]. Higher concentrations of undigested protein, result in fermentation in the distal intestinal tract of poultry, and are attributed to the proliferation of pathogenic bacteria such as *Clostridium perfringens* [102–105], leading to diseases like coccidiosis and necrotic enteritis. Coccidiosis is the most frequently reported and economically important poultry-related disease worldwide [106].

6.4.1 Use of enzymes in animal feed

Monogastrics lack endogenous enzymes to break down soyabean anti-nutrients [107, 108]. Animal feed is not processed and hence ANFs that would normally be reduced in human nutrition by pre-processing, are not eliminated before they are consumed. Moreover heat treatment greatly reduces the nutritional value of the feed. Hence, an effective treatment to counteract these ill effects of ANFs, is the use of exogenous enzymes, added as feed additives to soyabean meal (SBM). Since trypsin inhibitors are proteins, they can be broken down and eliminated by the action of proteases. In an interesting study, protease inclusion in broiler diets, led to improved nutrient digestibility and upregulation of growth-related genes [109]. Enzyme supplementation of proteases (e.g., DigeGrain Pro 6), thus improves growth performance, by increasing protein digestibility. This results in better utilization of the protein content in the feed, leading to minimum wastage.

6.4.2 Use of legumes as an alternative to fish meal

Fish meal, due to its high protein content and palatability is the primary choice of feed in aquaculture [110, 111]. Small fishes like sardines and anchovies are extensively used for fishmeal, leading to overfishing and depletion of fish stocks in the oceans. In addition to not being a sustainable source of feed ingredient, fishmeal is associated with high cost, and hence alternative sources of protein and energy need to be investigated. Hence, recent research has focussed on the evaluation of plant proteins like soyabean meal, lupin meal, and various legumes (cowpea, green mung bean, rice bran) [112–115] as ingredients in feeds for aquatic animals. In diets where fishmeal was replaced by SBM (30%) in the feed of European seabass, optimum growth and feed utilization was maintained. No case of enteritis was observed in histological analysis, and nutritional status was similar as with fish meal [116]. Soy white flakes, a product obtained during soybean processing, was used to prepare aquafeed with suitable properties (lower water absorption and higher solubility indices, high durability, lower bulk density) [117]. Fermentation of SBM by a bacterial strain *Shewanella sp. MR-7*, prior to feeding, led to improved performance and alleviation of soy-related inflammation, caused due to ANFs [118]. In another study, the use of protease allowed slightly lower protein content to be used in the feed of Nile tilapia. Growth parameters, feed intake and feed conversion efficiency was unaffected. As an added benefit, water quality was improved due to lower ammonia and nitrite content [119].

Thus the replacement of fish meal with SBM, when coupled with protease treatment can avoid problems associated with trypsin inhibitors, use proteins efficiently and prevent excretion of undigested products that lead to contamination of water.

7. Conclusion

In 2022, the world population touched 8 billion and is estimated to reach 9.7 billion by 2050 [120]. An increase in legume production by ∼25% is needed to fulfill the protein demand of the world's population. Legumes have the additional advantage of having a low GHG footprint. However, efficient processes, both *in vitro* and *in vivo* must be employed in order to unlock the potential of legumes in nutrition. The use of enzymatic treatment, not only offers a greener alternative but also added health benefits. In spite of several health benefits, a considerable number of people are reluctant to include legumes in their daily diet. To increase the popularity of legumes in the diet, future research must focus on processes that improve the taste and texture of legume preparations, without stripping them of vital nutrition. The problem of low content of essential amino acids like methionine, can be circumvented by genetic engineering of legumes to increase the synthesis of amino acids like methionine, through metabolic engineering or through the engineering of legume proteins so that they contain higher concentrations of methionine.

The use of legumes coupled with enzymatic treatment in animal feed, will prevent unnecessary use of antibiotics and culling of animals due to disease, while improving their overall health, and result in economic benefits. Recently, the food systems have been threatened by the three C's, i.e., climate change, conflict, and Covid-19 pandemic [121]. The solution then lies, in maximizing the use of resources. Rather than following the mantra "more is better", optimum use of resources, is the need of the hour. Large production volatility and lesser profitability, relative to other crops are barriers to expanded legume use. A future transition to using legumes as a primary source of dietary protein may be made possible by increased consumer knowledge and investment in growing new varieties of legumes. Moreover, breeding of drought resistant varieties will enable legumes to be grown locally, and avoid dependence on supply chains. Overall, improving the protein digestibility of legumes will allow complete utilization of its nutritional components, prevent the wastage of food, and contribute to sustainability.

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Conflict of interest

The authors declare no conflict of interest.

Abbreviations

ANFs	antinutritional factors
ABTS	2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
BBI	Bowman-Birk inhibitor
DIAAS	Digestible indispensable amino acid score
DPPH	2,2-diphenyl-1-picrylhydrazyl

EAA	essential amino acid
FAO	Food and Agricultural Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FDA	food and drug administration
FRAP	ferric reducing antioxidant power
GHG	greenhouse gases
HRSA	hydroxyl radical scavenging activity
INFOGEST	an international network of excellence on the fate of food in the gastrointestinal tract
IgE	immunoglobulin type E
IAA	indispensable amino acid
LAB	lactic acid bacteria
ON	Ouro Negro
OPA	o-phthalaldehyde
OPNS	OP-NS-331
PDCAAS	protein digestibility corrected amino acid score
PERT	pancreatic enzyme replacement therapy
Ppm	parts per million
mg/g	milligram per gram
SBM	soyabean meal
SPI	soy protein isolate
SEC	size exclusion chromatography
TAL	Talisma
TCA	trichloroacetic acid
TD	true digestibility
UF	ultrafiltration
UN	United Nations
U/mg	Unit per milligram
VC3	VC-3
WHO	World Health Organization
WPI	Whey Protein Isolate

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