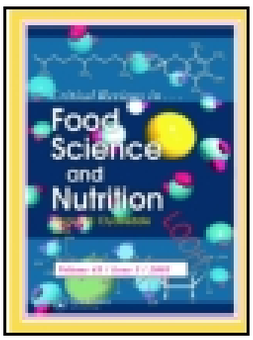

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REVIEW

Food processing for the improvement of plant proteins digestibility

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ABSTRACT

Proteins are essential macronutrients for the human diet. They are the primary source of nitrogen and are fundamental for body structure and functions. The plant protein quality (PPQ) refers to the bioavailability, digestibility, and amino acid composition. The digestibility specifies the protein quantity absorbed by an organism relative to the consumed amount and depends on the protein structure, previous processing, and the presence of compounds limiting the digestion. The latter are so-called antinutritional factors (ANF), exemplified by phytates, tannins, trypsin inhibitors, and lectins. Animal proteins are known to have better digestibility than plant proteins due to the presence of ANF in plants. Thus, the inactivation of ANF throughout food processing may increase the PPQ. New food processing, aiming to increase the digestibility of plant proteins, and new sources of proteins are being studied for the animal protein substitution. Here, it is presented the impact of processing on the protein digestibility and reduction of ANF. Several techniques, such as cooking, autoclaving, germination, microwave, irradiation, spray- and freeze-drying, fermentation, and extrusion enhanced the PPQ. The emerging non-thermal technologies impact on protein functionalities but require studies on the protein digestibility. How to accurately determine and how to improve the protein digestibility of a plant source remains a scientific and technological challenge that may be addressed by novel or combining existing processing techniques, as well as by exploring protein-enriched by-products of the food industry.

KEYWORDS

Protein quality; in vitro digestibility; antinutritional factors; thermal processing; food security

Introduction

Proteins play an essential role as structural and functional components to maintain growth and other physiological functions in humans (Boye, Wijesinha-Bettoni, and Burlingame 2012; Adenekan et al. 2018). In recent years, the demand for protein supply has increased due to the growth of the world population and the popularity of diets that are higher in protein (Berryman et al. 2018). As the main nitrogen source in the human diet, proteins consist of amino acids linked by peptide bonds. The amino acids are vital for maintaining the function of all organs, brain, hormones, muscles (including heart muscles), biological fluids (blood) and immune system (Boye, Wijesinha-Bettoni, and Burlingame 2012; Adenekan et al. 2018). Dietary reference intakes (DRI) proposed by the Institute of Medicine (IOM 2005) suggests the ingestion of 0.8 to 1.0 grams of protein by body-weight (g/kg) per day is a reasonable amount that adults should eat as part of a complete diet, and the minimal level of 0.66 g/kg to avoid deficiency.

Food protein quality is an important criterion for the adequate nutrition and maintenance of good health (Habiba 2002; Boye, Wijesinha-Bettoni, and Burlingame 2012). Protein quality refers to the amino acids profile, its bioavailability, and digestibility allowing the absorption of the amino acids. Thus, protein quality evaluation determines the capacity of a food protein to satisfy metabolic demands for amino acids and nitrogen (Boye, Wijesinha-Bettoni, and Burlingame 2012; Gilani, Xiao, and Cockell 2012; Han, Chee, and Cho 2015; Pencharz, Elango, and Wolfe 2016). Protein digestibility measurements indicate the quantity of protein that is hydrolyzed by the digestive enzymes and absorbed by the organism relative to the consumed protein amount (Mechi, Caniatti-Brazaca, and Arthur 2005; Berno, Guimarães-Lopes, and Canniatti-Brazaca 2007; López et al. 2018). Then, it is a kind of efficiency and affects the quantity of protein required in the human diet.

Food protein digestibility is a measure of the susceptibility of a protein to proteolysis (Duodu et al. 2003) and depends on the protein structure, thermal processing intensity, and presence of some compounds that are prejudicial to protein digestion, the so-called antinutritional factors. The reduction or elimination of these compounds is important to improve the biological utilization of plant proteins, which generally have lower digestibility (75–80%) when compared to animal proteins (90–95%) (such as meat,

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poultry, egg, and milk). Additionally, plant proteins present lower enzyme accessibility due to rigid cell walls and seed coats (Habiba 2002; Berno, Guimarães-Lopes, and Canniatti-Brazaca 2007; Kniskern and Johnston 2011; Annor et al. 2017).

Besides the presence of the antinutritional factors, from a nutritional point of view, some plant proteins may be considered inferior to animal proteins due to their deficiency in the essential amino acid composition (Multari, Stewart, and Russell 2015). Generally, cereals contain lysine deficiency, while legumes have low levels of sulfur amino acids (methionine and cysteine) (Nosworthy, Neufeld, et al. 2017; Vendemiatti et al. 2008; Millward 1999). However, in comparison to animal-based proteins, plant foods are a rich source of fiber, carbohydrates, oligosaccharides, and polyunsaturated fatty acids. Furthermore, many studies point out that plant proteins consumption are associated by a significant decrease of cardiovascular diseases (CVD), type II diabetes mellitus, obesity, and low-density lipoprotein (LDL) cholesterol, while animal-based protein consumption tends to increase the risk of these health issues due to its lipidic profile (Neacsu, McBey, and Johnstone 2016; Guasch-Ferré, Zong, et al. 2019). The intake of animal protein from red meat is associated with the consumption of saturated fats, and protein substitution may contribute to healthier aspects of the human diet, reducing the chances of chronic and cardiovascular diseases (Guasch-Ferré, Satija, et al. 2019; Guasch-Ferré, Zong, et al. 2019). Therefore, the intake of plant sources is encouraged, while the intake of animal-based sources (particularly red and processed meat) is discouraged in some diets (Guasch-Ferré, Zong, et al. 2019).

There are many studies about the application of food processing on plant proteins aiming to improve their quality, mainly using thermal techniques. Some examples are cooking (Park, Kim, and Baik 2010; Annor et al. 2017; Kamela et al. 2016), autoclaving (M. Sun et al. 2012; Kalpanadevi and Mohan 2013), microwave heating (M. Sun et al. 2012; Shimelis and Rakshit 2005), germination (Alonso, Aguirre, and Marzo 2000; Kalpanadevi and Mohan 2013), irradiation (Mechi, Caniatti-Brazaca, and Arthur 2005; Siddhuraju, Makkar, and Becker 2002), drying (Tang 2007), fermentation (Dias et al. 2010; Espinosa-Páez et al. 2017), and extrusion (Wu et al. 2015; Y. Wang et al. 2008).

Some projections are that the world's population reaches 10 billion people by 2050 (Nadathur, Wanasundara, and Scanlin 2017), which suggests that the food protein supply may be scarce. Besides, livestock farming for producing animal proteins has negative environmental impacts, such as climate change, freshwater depletion, and biodiversity losses; as well as there are limited supply and increasing costs of animal proteins in developing countries (Sun et al. 2012).

The above issues motivate studies focusing on the consumption of proteins from new sources as alternatives to replace animal-based protein (Youssef 1988). Plant proteins, such as vegetables, seeds, grains, leaves, legumes, and cereals, and sources from algae and insects are currently being evaluated (Nadathur, Wanasundara, and Scanlin 2017; Adenekan et al. 2018; López et al. 2018). Alternative sources with high protein quality may be fundamental for the specific requirement of some groups, such as athletes and older people, or for supplying the protein deficit in some regions of the world.

In the last few years, there has been an increasing interest in searching for protein sources with high nutritional value and adequate functionality in food industry processes and applications, such as solubility, emulsification, foaming, gelation, viscosity, water-holding, and oil-holding capacities. Plant proteins could be used for this purpose, and several studies have been carried out to evaluate the functionalities of proteins from plant sources (Chew, Casey, and Johnson 2003; Mohamed et al. 2009; López et al. 2018).

New processing developments are primarily motivated by increasing consumers' demand for high-quality food products. The critical challenges in the plant protein field are the utilization of sources with nutritional value similar to animal-based proteins and the development of novel food processing techniques for enhancing the nutritional quality of traditional plant protein sources. Furthermore, the target is the development of delicious, nutritious, healthy, affordable, and convenient alternative protein products for consumers' acceptance in terms of cultural and sensory attributes (e.g., appearance, taste, texture, and flavor).

Thus, owing to the broad scope of this field, this critical review explored the knowledge of how different techniques of food processing can impact the nutritional quality of the main sources of plant proteins in terms of protein digestibility. Also, this work showed the influence of the antinutritional factors in plant proteins while impairing digestibility, as well as aimed to present the standard tests for digestibility evaluation *in vitro* and *in vivo*.

Plant proteins quality

The protein quality, or nutritional value, is the protein capacity to replace the nitrogen that the organism inevitably loses as a consequence of the metabolism in the biological processes (Aguilar et al. 2015). The nutritional value of a food product is not only dependent on the ingested protein amount, but is also influenced by age, health status, physiological status, and energy balance. Additionally, it depends on the presence and bioavailability of essential amino acids, and the protein digestibility, which leads to growth and health maintenance in humans (Potier and Tomé 2008; Aguilar et al. 2015; Arribas et al. 2017).

Nutritional composition of plant proteins

The consumption of healthier foods increased due to the consumers' awareness for the importance of diet, the benefit and health promotion beyond nutrition (Aguilar et al. 2015). Currently, several plant proteins (e.g., legumes,

cereals, seeds, grains, and leaves) have been intensely studied due to their rich composition (e.g., protein, fibers, minerals, and other bioactive compounds), aiming to develop novel food products with improved nutritional properties (Hughes et al. 2011; Arribas et al. 2017; Coda et al. 2017). Plant proteins have been widely used as nutritional supplements and functional agents in foods systems (Nosworthy and House 2017; Opazo-Navarrete, Schutyser, et al. 2018). Among plants, vegetables are the most abundant and cheapest sources of proteins (Siddhuraju, Makkar, and Becker 2002; Kamela et al. 2016), while legumes are the earliest food crops cultivated by the mankind and are rich in nutrients, with a high content of proteins, fibers, carbohydrates, and are a remarkable source of minerals and vitamins (Bhatty, Gilani, and Nagra 2000; Dias et al. 2010; Hussain et al. 2012). Furthermore, legumes and some cereals are gluten-free, a good alternative for celiac people and vegetarian diets (Aguilar et al. 2015; Arribas et al. 2017).

There are numerous options for plant proteins sources. They have high variability in nutritional aspects and functionality. Many recent studies regarded plant proteins with high nutritional quality in terms of amino acid composition (the higher content of essential amino acids, the higher the quality) and protein digestibility (the higher the digestibility, the higher the quality), such as soybean (*Glycine max*) (Sánchez-Vioque et al. 1999; Wang et al. 2010; López et al. 2018), bean (*Phaseolus vulgaris*) (Dias et al. 2010; Espinosa-Páez et al. 2017), pea (*Pisum sativum*) (Park, Kim, and Baik 2010), chickpea (*Cicer arietinum* L.) (Singh 1985; Clemente et al. 1998; Potier and Tomé 2008; Wang et al. 2010), lupin (Chew, Casey, and Johnson 2003), rice (*Oryza sativa*) (Han, Chee, and Cho 2015), flaxseed (or linseed) (*Linum usitatissimum*) (Hussain et al. 2012; Anaya et al. 2015), amaranth (*Amaranthus* L.) (Aguilar et al. 2015), chia (*Salvia hispanica*), quinoa (*Chenopodium quinoa*) (López et al. 2018), and sesame seed (*Sesamum indicum*) (El-Adawy 1995).

Many authors point out plants as a generally relatively low protein nutritional value source due to its deficiencies in some essential amino acids, like legumes are usually deficient in the sulfur amino acids (methionine and cysteine), while cereals mostly have low levels of lysine (Gabert et al. 1995; Millward 1999; Sun and Liu 2004; Pires et al. 2006; Vendemiatti et al. 2008; Nosworthy, Neufeld, et al. 2017). Besides, plant protein deficiency is also attributed to the presence of compounds commonly called antinutritional factors, once they inhibit protein bioavailability (Van Der Poel 1990; Yañez et al. 1995). Many authors have been demonstrated that the presence of these compounds decreases the digestibility of the proteins, and they may be heat-labile or heat-stable (Saharan and Khetarpaul 1994; Siddhuraju, Makkar, and Becker 2002; Espinosa-Páez et al. 2017; Zhang et al. 2017). Some examples of these compounds are the proteases inhibitors (trypsin and chymotrypsin), lectins, phytates, fibers, and polyphenols (tannins) (Shimelis and Rakshit 2005; Park, Kim, and Baik 2010; Aletor 2012; Bartkiene, Juodeikiene, and Vidmantiene 2012; Kalpanadevi and Mohan 2013).

The amino acid deficiency issue could be solved through protein complementation, such as the mixtures of cereals and legumes that are being evaluated as an effective strategy to achieve the best possible quality plant protein mixtures (Pencharz, Elango, and Wolfe 2016). Furthermore, as presented in the next sections, the presence of the so-called antinutritional compounds can be decreased or their action inhibited by food processing. In this way, many plant sources can be used as a high-quality protein.

The so-called antinutritional factors

The usefulness of plants as a protein source for the human body is limited by the presence of some compounds, which have some detrimental physiological effects on the protein digestion, thereby limiting its nutritional value (Gupta 1987; Giami 2004). Several authors use the term "antinutritional factors" to design these compounds that reduce the nutritional value of the consumed food. The most common examples are the protease inhibitors, phytates, polyphenols, fibers, haemagglutinins (lectins), and non-starch polysaccharides (NSP). They have been reported to adversely affect the protein and amino acid digestibility (Alonso, Aguirre, and Marzo 2000; Gilani, Xiao, and Cockell 2012; Anaya et al. 2015; Shi et al. 2017) by reducing its bioavailability and interfering with metabolic processes, provoking deleterious effects on the gastrointestinal tract physiology (Boye, Wijesinha-Bettoni, and Burlingame 2012; Kamela et al. 2016; Tuśnio et al. 2017; Adenekan et al. 2018). The factors affecting protein digestibility may be categorized into two main groups: exogenous factors (presence of antinutritional factors) and endogenous factors (cross-linking, hydrophobicity, and changes in protein secondary structure) (Duodu et al. 2003). The antinutritional factors inhibit protein digestion and increase endogenous nitrogen losses into the feces, causing a decrease in the protein digestibility and an increase in protein requirements (Schaafsma 2012). These antinutritional factors can bring significant effects on gastric metabolism. Protease inhibitors can affect the protein globular structure, hindering the action of digestive enzymes in the small intestine, reducing the digestibility. Polyphenols can form complex with digestive enzymes, inactivating the digestion activity, and decreasing protein digestibility. Other examples of these compounds' effects on the gastric metabolism are present in Table 1.

Several processing techniques may be considered to overcome the adverse factors, once they could improve the protein digestibility of plant proteins and, therefore, their utilization by the human body (Siddhuraju, Vijayakumari, and Janardhanan 1996; Alonso, Aguirre, and Marzo 2000; Coda et al. 2017). Many reports showed that heat treatments reduced the content of some of these compounds (heat-labile) and improved the nutritional value of plant proteins (Gupta 1987; Van Der Poel 1990; Mansour et al. 1993; Yañez et al. 1995; Siddhuraju, Vijayakumari, and Janardhanan 1996; Delfino and Canniatti-Brazaca 2010; Tuśnio et al. 2017).

Table 1. Major effects on the gastric physiology of the so-called antinutritional factors (ANFs) present in plant proteins.

Antinutritional factors (ANFs)	Plant protein source	Major effects	References
Protease inhibitors Trypsin-chymotrypsin inhibitor	Grains, legumes and seeds Soybean, kidney bean, pea, fababean, cowpea, chickpea, karkade, pigeon pea, bean	Activity reduction of (chymo)trypsin Decreased digestion by animals, depressing their growth Reduces food intake Affects protein globular structure, hindering the action of digestive enzymes in the small intestine Increases the pancreatic secretion of trypsin Substancial reduction in protein and amino acid digestibility and protein quality	Van Der Poel 1990; Boisen and Eggum 1991; Mnembuka and Eggum 1995; Abu-tarboush and Ahmed 1996; Sánchez-Vioque et al. 1999; Habiba 2002; Salgado et al. 2002; Siddhuraju, Makkar, and Becker 2002; Gilani, Xiao, and Cockell 2012; Anaya et al. 2015; Coda et al. 2017; Tuśnio et al. 2017
Polyphenols Tannins	Legumes and cereals Sorghum, fababean, pea, pigeon pea, cowpea, chickpea, bean	Forms complex with enzymes or protein Oxidation to quinones, forming peroxides and could bring oxidation of several amino acids Increases the degree of cross-linking Increases the secretion of endogenous protein Reduces protein digestibility Decreases protein solubility Alters organoleptic and functional properties Inactivation of digestive enzymes Induces the increase in proline-rich proteins in the saliva	Bressani, Hernandez, and Braham 1988; Van Der Poel 1990; Boisen and Eggum 1991; Yadav and Khetarpaul 1994; Alonso, Aguirre, and Marzo 2000; Andrabi et al. 2005; Delfino and Canniatti-Brazaca 2010; Mariscal-Landín, Souza, and Avalos 2010; Gilani, Xiao, and Cockell 2012; Salgado et al. 2012; Marpalle et al. 2015; Annor et al. 2017; Tuśnio et al. 2017; Adenekan et al. 2018
Phytic acid Phytates	Cereals and legumes Pea, pigeon pea, soybean, fababean, sorghum, cowpea, chickpea, bean	Forms complex with anions and protein Depresses absorption of minerals Substancial reduction in protein and amino acid digestibility and protein quality Decreases protein solubility Causes protein resistant to proteolytic digestion Inhibit proteolytic enzymes	Van Der Poel 1990; Yadav and Khetarpaul 1994; Abu-tarboush and Ahmed 1996; Siddhuraju, Vijayakumari, and Janardhanan 1996; Alonso, Aguirre, and Marzo 2000; Siddhuraju, Makkar, and Becker 2002; Duodu et al. 2003; Gilani, Xiao, and Cockell 2012; Albarracín, José González, and Drago 2015; Albarracín et al. 2015; Shi et al. 2017; Tuśnio et al. 2017; Adenekan et al. 2018
Fiber	Legumes and cereals Soybean, pea, carob, millet, flaxseed	Proteolysis hampered Affects gastric emptying, gastric filling and energetic dilution capacity Interaction between proteins and polysaccharides Reduces enzyme activity in the lumen	Gupta 1987; Van Der Poel 1990; Boisen and Eggum 1991; Salgado et al. 2001; Marpalle et al. 2015; Annor et al. 2017; Arribas et al. 2017
Haemagglutinins Lectins	Legumes Pea, chickpea, bean	Binds to glycoproteins on red blood cells and in the intestinal mucosa, causing cells agglutination and damage of epithelium Immunological reactions Metabolism toxicity Decreases nutrients digestibility Induces changes in epithelial cell metabolism	Singh 1985; Gupta 1987; Van Der Poel 1990; Boisen and Eggum 1991; Siddhuraju, Makkar, and Becker 2002; Park, Kim, and Baik 2010; Gilani, Xiao, and Cockell 2012; Tuśnio et al. 2017
Non-starch polysaccharides (NSP)	Legumes and cereals Soybean, canola, pea, sorghum	Adsorbs amino acids and peptides released during protein hydrolysis Reduces protein digestibility	Cowan et al. 1996; Salgado et al. 2002; Duodu et al. 2003; Meng and Slominski 2005

Although many authors presented the mentioned compounds as detrimental and disadvantageous for the digestibility of proteins, as shown in Table 1, the use of the term "antinutritional factors" is inadequate because these compounds also have other benefits for human health. For example, studies show that increased fiber intake benefits many gastrointestinal disorders, lowers blood pressure and serum cholesterol levels and may enhance the immune function (Anderson et al. 2009; Lambeau and McRorie 2017). Also, several clinical studies suggest that plant polyphenols have some biological activities, such as antioxidant, anti-inflammatory, antibacterial, anticancer, anti-diabetic, and reduce the risks of cardiovascular diseases (Fang and Bhandari 2010; Annor et al. 2017). Furthermore, pieces of evidence indicate that the phytic acid and phytate display a wide range of bioactivities including antioxidant, anticancer, cardiovascular protective, and inhibition effects for the kidney stone formation (Aider and Barbana 2011; Rizzo and Baroni 2018). However, regarding protein digestibility, these compounds should be removed to enhance the protein quality of a plant source.

Protein quality evaluation methods

The amino acid content is a critical determinant of nutritive value and most of the protein quality evaluation methods

are directly or indirectly related to the efficacy that they can satisfy amino acid requirements for humans (Hussain et al. 2012). Amino acids are classified as: (a) indispensable or essential: those which are not synthesized by the human body and, therefore, must be obtained by the food intake (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine); and (b) dispensable or non-essential: those which the body can produce (asparagine, glutamine, glutamic acid, alanine, serine, cysteine, tyrosine, glycine, arginine, proline, aspartic acid) (Schaafsma 2005; Boye, Wijesinha-Bettoni, and Burlingame 2012). The digestibility, combined with essential amino acid composition, is regarded as the most determinant factor of protein nutritive value (Wu et al. 2015) and determines the availability of physiologically active amino acids and peptides, which is affected by processing treatments and the compounds present in the food matrix (Marambe, Shand, and Wanasundara 2013).

There are some methods to evaluate protein quality. Table 2 summarizes the methods frequently used for the determination of in vitro and in vivo protein digestibility.

The in vitro technique can be designed to use specific enzymes either to give maximal digestibility values and to measure the initial rate of hydrolysis. The applicability of the results depends on a high correlation with in vivo values obtained under standardized conditions using the identical material. Therefore, it is suggested for the in vitro evaluation the use of the same pull of enzymes that occur in the digestive tract (Boisen and Eggum 1991).

The *in vitro* protein digestibility (IVPD) gives information on protein stability and how they withstand digestive processes (Coda et al. 2017). The IVPD is an important parameter for the evaluation of a protein nutritional potential but can overestimate the actual nutritional value, since it disregards the biologically unavailable amino acids (Aguilar et al. 2015). The bioassays with animals to determine true protein digestibility are expensive and time-consuming procedures. Then, different in vitro digestibility methodologies have been developed in the last century (Giami 2004; Boye, Wijesinha-Bettoni, and Burlingame 2012; López et al. 2018). In comparison to in vivo methods, the in vitro assays are more reliable, faster, simpler, and are a commercial alternative when the quality of proteins (Clemente et al. 1998). The compounds present in plant proteins that depress the digestibility in vivo have a much smaller effect on measurements in vitro (Boisen and Eggum 1991; Boye, Wijesinha-Bettoni, and Burlingame 2012). However, the in vitro protein digestibility (IVPD) method gives an estimation of protein quality and can be used to rapidly screen samples and evaluate the effects of various food processing methods on protein quality (Giami 2004).

The in vitro methods describe the protein digestibility, whereas the in vivo methods do not only evaluate the digestibility but the protein quality as well. Methods frequently used for nutritional quality assessment and determination of in vivo protein digestibility, described in Table 2, include protein efficiency ratio (PER), net protein ratio (or retention) (NPR), net protein utilization (NPU), biological value (BV), true digestibility (TD), protein digestibility corrected amino acid score (PDCAAS) and digestible indispensable amino acid score (DIAAS) (Boye, Wijesinha-Bettoni, and Burlingame 2012; Mathai, Liu, and Stein 2017). These methods are different, and the results are not directly comparable, but all can be used to indicate the protein quality of a protein source.

The first method adopted for routine evaluation of the food protein quality was the protein efficiency ratio (PER). It involves feeding a test protein diet and a casein control diet to rats, calculating the ratio of the weight gain and the amount of protein consumed (Schaafsma 2012; Gilani 2012). Rats have a higher need for sulfur amino acids than humans; thus, the PER method overestimates the requirements and underestimates the quality of some proteins, especially plant proteins (Boye, Wijesinha-Bettoni, and Burlingame 2012). If the protein has poor quality, the error of PER increases, showing PER as non-proportional to protein quality (Gilani 2012).

The net protein ratio (NPR) is a biological method that overcomes the major weakness in the PER assay by adding the weight loss of rats fed a non-protein diet to the weight gain of rats fed the test protein in the calculation, assuming that the protein required is equivalent to the protein needed for maintenance (Gilani 2012). The NPR measures the efficiency of protein utilization by using growing rats; however, it often underestimates the protein quality of plant foods, since growing rats require higher amounts of certain essential amino acids than humans (Sousa et al. 2011). The net protein utilization (NPU) is defined as the retained fraction of nitrogen intake and allows evaluating the effectiveness of the protein for normal growth and development (Aguilar et al. 2015). The NPU provides a quantity of overall protein utilization and reflects the proportion of ingested protein retained (Boye, Wijesinha-Bettoni, and Burlingame 2012).

The biological value (BV) of a protein represents the fraction of absorbed nitrogen that is retained by the organisms to maintain the integrity of the tissue, the development, and the growth (Schaafsma 2012; Aguilar et al. 2015; Hussain et al. 2012). The BV provides a measure of how well the absorbed amino acid profile matches that of the requirement (Boye, Wijesinha-Bettoni, and Burlingame 2012). The true protein digestibility (TD) is an important variable in consideration of the nutritional adequacy of a protein source since it represents the portion of nitrogen of the diet that is available for maintenance and growth functions (Aguilar et al. 2015). The TD is measured through the rats' balance assay by measuring nitrogen in food and feces (Hussain et al. 2012). The nitrogen balance (NB) provides a measure of body nitrogen retention based on directly measuring daily nitrogen intake minus nitrogen excreted (Boye, Wijesinha-Bettoni, and Burlingame 2012).

The PDCAAS is the index recommended by the FAO/WHO (1991) to evaluate the nutritional quality of the proteins and estimate more reliably the protein value of food for human consumption (Sousa et al. 2011; Gilani 2012; Aguilar et al. 2015), accounting the protein digestibility and indispensable amino acid profile (Kniskern and Johnston

Table 2. Methods frequently used for determination of in vitro protein digestibility and in vivo protein quality.

Protein quality evaluation methods	Observations	Calculation	References
In vitro			
In vitro protein digestibity (IVPD)	Estimation of protein quality; rapid and low cost procedure; overestimates the true nutritional value, since it disregards the concentration and availability of amino acids	Akeson and Stahmann (1964) → pepsin and pancreatin $IVPD\ (\%) = \frac{Digested\ protein}{Total\ protein} \times 100$ Hsu et al. (1977) → trypsin, chymotrypsin and peptidase $IVPD\ (\%) = 210.464 - 18.103.pH_{10min}$	Akeson and Stahmann 1964; Ramachandra and Monteiro 1990; Mansour et al. 1993; Bishnoi and Khetarpaul 1994; Yadav and Khetarpaul 1994; Preet and Punia 2000; Marpalle et al. 2015; Coda et al. 2017 Hsu et al. 1977; Clemente et al. 1998; Sánchez-Vioque et al. 1999; Habiba 2002; Lqari et al. 2002; Shimelis and Rakshit 2005; Wang et al. 2008; Park, Kim, and Baik 2010; Pastor-Cavada et al. 2010; Aletor 2012; Bartkiene, Juodeikiene, and Vidmantiene 2012; Salgado et al. 2012; Zhang et al. 2017
In vitro protein digestibility - corrected amino acid score (IVPDCAAS)	Rapid and low cost assay; does not rely on animal experimentation; replacement for currently recommended *in vivo* rats bioassays	$AAS = \frac{Content\ of\ first\ limiting\ amino\ acid\ in\ a\ test\ protein\ (mg/g)}{Content\ of\ corresponding\ amino\ acid\ in\ a\ reference\ protein\ (mg/g)}$ $IVPDCAAS = IVPD\ (\%) \times AAS$	Nosworthy, Franczyk, Medina, et al. 2017; Nosworthy, Franczyk, Zimoch-Korzycka, et al. 2017; Nosworthy and House 2017; Nosworthy et al. 2018a; 2018b
In vivo			
Protein efficiency ratio (PER)	Ratio of the rat weight gain and the amount of protein consumed; first method adopted; overestimates the requirements for humans and underestimates the quality of some proteins	$PER = \frac{Body\ weight\ gain\ in\ mass\ (g)}{Protein\ intake\ (g)}$	Giami 2004; Aletor 2012; Boye, Wijesinha-Bettoni, and Burlingame 2012; Gilani 2012; Schaafsma 2012; Han, Chee, and Cho 2015; Coda et al. 2017
NET protein ratio (or retention) (NPR)	Overcomes the major weakness in the PER assay by adding the weight loss of rats fed a non-protein diet; underestimates the protein quality, since rats require higher amounts of amino acids than humans	$NPR = \frac{Weight\ gain\ of\ test\ rat - Weight\ loss\ of\ protein-free\ diet\ test\ rat}{Protein\ consumed\ by\ rat}$	Giami 2004; Sousa et al. 2011; Aletor 2012; Boye, Wijesinha-Bettoni, and Burlingame 2012; Gilani 2012; Han, Chee, and Cho 2015
Net protein utilization (NPU)	Proportion of nitrogen intake (ingested protein) that is retained; measure of overall protein utilization	$NPU\ (\%) = \frac{I - (F - M) - (U - E)}{I} \times 100$ Where: I = Nitrogen intake in the test group; F = nitrogen excreted in the feces; U = nitrogen excreted in the urine; M = endogenous fecal nitrogen excreted by protein-free group (basal diet); E = endogenous urinary nitrogen excreted by protein-free group (basal diet);	Saharan and Khetarpaul 1994; Chew, Casey, and Johnson 2003; Montoya et al. 2008; Aletor 2012; Boye, Wijesinha-Bettoni, and Burlingame 2012; Gilani 2012; Sun et al. 2012; Aguilar et al. 2015; Han, Chee, and Cho 2015
True digestibility (TD)	Represents the portion of diet nitrogen that is available for maintenance and growth functions	$TD\ (\%) = \frac{I - F - Fk}{I} \times 100$ Where: I = Protein intake of rats fed test diet; F = Protein excreted in feces of rats fed test diet; Fk = Protein excreted in feces of rats fed protein-free diet	Saharan and Khetarpaul 1994; Chew, Casey, and Johnson 2003; Giami 2004; Hughes et al. 2011; Aletor 2012; Boye, Wijesinha-Bettoni, and Burlingame 2012; Hussain et al. 2012; Aguilar et al. 2015; Han, Chee, and Cho 2015;
Biological value (BV)	Proportion of the absorbed nitrogen retained for maintenance and growth, taking into consideration the metabolic nitrogen loss	$BV\ (\%) = \frac{NPU}{TD} \times 100$	Saharan and Khetarpaul 1994; Aletor 2012; Boye, Wijesinha-Bettoni, and Burlingame 2012; Gilani 2012; Hussain et al. 2012; Schaafsma 2012; Aguilar et al. 2015; Han, Chee, and Cho 2015; Coda et al. 2017
Protein digestibility corrected amino acid score (PDCAAS)	Based on the ratio of the first-limiting essential amino acid in the test protein to the reference; underestimates the value of high-quality proteins and overestimates the value of low-quality proteins; chemical scores exceeding 100 % are truncated	$AAS = \frac{Content\ of\ first\ limiting\ amino\ acid\ in\ a\ test\ protein\ (mg/g)}{Content\ of\ corresponding\ amino\ acid\ in\ a\ reference\ protein\ (mg/g)}$ PDCAAS (%) = Amino acid score (AAS) × True digestibility (TD) × 100	Sarwar 1997; Gilani and Sepehr 2003; Schaafsma 2005; Kniskern and Johnston 2011; Sousa et al. 2011; Boye, Wijesinha-Bettoni, and Burlingame 2012; Gilani 2012; Schaafsma 2012; Sun et al. 2012; Aguilar et al. 2015; Han, Chee, and Cho 2015; Pencharz, Elango, and Wolfe 2016
Digestible indispensable amino acid score (DIAAS)	Tests with pigs for an appropriate estimation for humans, avoiding the flaws of the PDCAAS procedure	DIAAS (%) = Lowest value of digestible indispensable AA reference × 100	Pencharz, Elango, and Wolfe 2016; Mathai, Liu, and Stein 2017

2011). It is based on the ratio of the first limiting essential amino acid in the test protein to the reference protein. Although high-quality proteins have a higher PDCAAS, the values are always truncated at 100 %, so the highest PDCAAS value that any protein can achieve is 1.0, meaning that after digestion of the food protein, one unit of protein provides 100 % of the indispensable amino acids required by the 2–5 years old child (M. Sun et al. 2012; FAO 2013). The limiting amino acid score is multiplied by true digestibility, which gives a value for protein quality corrected for digestibility (Hughes et al. 2011; Boye, Wijesinha-Bettoni, and Burlingame 2012).

The PDCAAS is a useful routine assay for the protein quality assessment of proteins in mixed diets for normal healthy subjects, despite several limitations and disadvantages (Schaafsma 2012). Studies showed it generally underestimates the value of high-quality proteins and overestimates the value of other proteins. Also, it may be unsuitable for the protein quality prediction of plant proteins, which could present antinutritional factors (Sarwar 1997; Gilani 2012; Schaafsma 2012).

Currently, FAO recommends an amino acids evaluation procedure called digestible indispensable amino acid score (DIAAS) at the small intestine (ileum) of pigs, as an appropriate and more accurate estimation for humans, avoiding the flaws of the PDCAAS procedure (evaluation in rats) (Mathai, Liu, and Stein 2017), like the truncation (Pencharz, Elango, and Wolfe 2016). The pigs have been widely promoted as a useful model for human nutrition studies due to a physiologically and anatomically similar digestive tract; then, pigs have been suggested as being a better model than rats for predicting protein digestibility for the adult human (Deglaire et al. 2009).

DIAAS values are the percent of the dietary requirement for each essential amino acid met by ingestion of 0.66 grams by weight (kg) per day of protein. The DIAAS scores for animal proteins (milk, eggs, beef) are above 100 %, whereas plant proteins are generally below 80 % (exception of soy) (Pencharz, Elango, and Wolfe 2016).

Despite the studies emphasize the in vitro assays for evaluating the in vitro protein digestibility, the FAO considers in vivo measurements with animal models more meaningful, and DIAAS has been endorsed as the best standard method for protein nutritional quality (Loveday 2019; FAO 2013).

A great diversity of static *in vitro* digestion methods under different experimental conditions proposed in the literature makes a comparison between those studies impossible. Consequently, an internationally harmonized static model (INFOGEST protocol) has been developed (Dupont and Mackie 2015; Brodkorb et al. 2019; Egger et al. 2016), aiming to consolidate conditions for simulated food digestion, analyzing the digestion products (e.g., amino acids, simple sugars, fatty acids) and evaluating the release of the micronutrients from the food matrix (Brodkorb et al. 2019). The major advantages of the INFOGEST method are reproducibility, simplicity, and low-cost assessment (Brodkorb et al. 2019), and it has been used to study the digestion of milk, egg, and pasta (Dupont and Mackie 2015). To the present moment, INFOGEST protocol has not been used on plant proteins. However, in the future, this static in vitro simulation could properly evaluate plant protein digestibility and determine the influence of food processing on the nutritional quality of alternative proteins.

Woolf, Fu, and Basu (2011) developed an algorithm called vProtein, available online (http://www.vprotein.com), that matches plant foods based on the amino acid composition. Despite it corroborates for the potential of computational tools to identify sources to satisfy human protein needs, it does not account for protein digestibility (Woolf, Fu, and Basu 2011; Loveday 2019).

This section presents the methods in vitro and in vivo found in the literature used for protein evaluation of plant proteins, but this review is not focused on the existing methods of clinical trials that are performed in humans. The results of some studies reviewed here may not be comparable since it eventually uses different protocols for protein digestibility evaluation. It is necessary to move toward standardized protocols to efficiently compare the impact of food processing on plant protein digestibility.

Effects of food processing on protein digestibility

Many food processing techniques, such as cooking, dehulling, soaking, germination, microwave, irradiation, fermentation, and extrusion have been demonstrated as improving the nutritional quality of plant proteins (Gupta 1987; Saharan and Khetarpaul 1994; Clemente et al. 1998; Siddhuraju, Makkar, and Becker 2002; Shimelis and Rakshit 2005; Boye, Wijesinha-Bettoni, and Burlingame 2012; Sun et al. 2012; Tuśnio et al. 2017) and eliminating/inactivating the compounds that may prejudice protein digestibility (Khalil and Mansour 1995; Bhatty, Gilani, and Nagra 2000; Kamela et al. 2016). Each thermal or non-thermal process used can bring different results concerning protein digestibility, as can be observed in Tables 3 to 6. During food processing, protein products are treated with heat for a variety of purposes, such as sterilization, enhancement of flavor or texture, destruction of toxic or some prejudicial heat-labile compounds, improvement of desirable physical, and functional properties (Sarwar 1997; Gilani and Sepehr 2003).

Although the use of processing is beneficial in terms of protein quality by inactivating the compounds that lowers the protein digestibility of plant proteins, the chemical changes produced by the heating process can also decrease nutritional benefits by degrading some heat-labile micronutrients, like reducing the assimilation of some vitamins and minerals and provoke the generation of some toxic compounds (Canniatti-Brazaca 2006). Some detrimental effects of thermal processing can occur, such as protein degradation, as a consequence of Maillard reaction, impacting essential amino acids bioavailability. The non-enzymatic browning has been presumed to affect the quality of the protein due to the blockage of amino acids and the product formed has proteolytic inhibitor activity that reduces the IVPD (Shimelis and Rakshit 2005). Carbonyls may react with other amino acids or polymerize into brown melanoidins, which adversely impacts lysine

Table 3. Influence of thermal processing on the protein digestibility of plant proteins.

Source of plant protein	Food processing	Protein quality evaluation method	Results	Reference
Soybean (*Glycine max*)	Irradiation (10 kGy)	IVPD (%)	89.3	Lee et al. 2012
	Autoclaving (123 °C, 20 min)		81.3	
	Peeling and cooked (100 °C, 30 min)	IVPD (%)	89.8 ± 0.1	Berno, Guimarães-Lopes, and Canniatti-Brazaca 2007
	Defatted flour	IVPD (%)	79.8	Siddhuraju, Makkar, and Becker 2002
	Defatted flour and irradiation (1 kGy)		81.2	
	Defatted flour and irradiation (5 kGy)		82.3	
	Defatted flour and irradiation (10 kGy)		84.2	
Bean (*Phaseolus vulgaris* L.)	Raw	IVPD (%)	92.8	Delfino and Canniatti-Brazaca 2010
	Autoclaving (121 °C, 10 min)		92.3	
	Raw after 6 months storage		95.3	
	Autoclaving after 6 months storage		97.9	
	Microwave (800 W, 2450 MHz, 1 min)	IVPD (%)	81.8	Shimelis and Rakshit 2005
	Microwave (800 W, 2450 MHz, 3 min)		85.8	
	Raw	IVPD (%)	84.0 ± 0.3	Mechi, Caniatti-Brazaca, and Arthur 2005
	Autoclaving (121 °C, 10 min)		84.2 ± 0.3	
	Cooked and irradiation (2 kGy)		82.2 ± 0.1	
	Cooked and irradiation (6 kGy)		84.4 ± 0.5	
	Cooked and irradiation (10 kGy)		82.3 ± 0.8	
	Raw	IVPD (%)	68.1 ± 0.4	Alonso, Aguirre, and Marzo 2000
	Germination (25 °C, 72 h)		78.0 ± 0.3	
	Autoclaving (9 psi, 112 °C, 30 min)	PER (ratio)	1.9 ± 0.3	Yañez et al. 1995
		NPR	3.3 ± 0.4	
	Autoclaving (121°C, 15 min)	TD (%)	68.0	Van Der Poel 1990
Pea (*Pisum sativum* L.)	Raw	IVPD (%)	80.1	Boye, Wijesinha-Bettoni, and Burlingame 2012
		PDCAAS (%)	46	
	Autoclaving (15 psi, 121 °C, 20 min)	IVPD (%)	88.3	
		PDCAAS (%)	67	
	Microwave (1200 W, 15 min)	IVPD (%)	90.9	
		PDCAAS (%)	92	
	Raw	IVPD (%)	83.5	Park, Kim, and Baik 2010
	Cooking (98 °C, 30 min)		86.8	
	Raw	IVPD (%)	73.5 ± 1.3	Habiba 2002
	Cooking (100 °C, 40 min)		78.3 ± 1.2	
	Autoclaving (121 °C, 15 min)		78.3 ± 1.4	
	Microwave (2450 MHz, 12 min)		75.5 ± 1.2	
	Autoclaving (15 psi, 10 min)	IVPD (%)	86.3 ± 0.1	Bishnoi and Khetarpaul 1994
	Germination (48 h)		82.7 ± 0.1	
	Uncooked flour	PER (ratio)	2.3 ± 0.2	Saharan and Khetarpaul 1994
		TD (%)	66.7 ± 2.2	
		BV (%)	62.9 ± 2.8	
		NPU (%)	42.1 ± 3.0	
		NPR	50.0 ± 1.4	
	Autoclaved (15 psi, 15 min) flour	PER (ratio)	2.5 ± 0.2	
		TD (%)	70.5 ± 1.7	
		BV (%)	67.2 ± 3.1	
		NPU (%)	47.4 ± 3.1	
		NPR	51.2 ± 2.0	
Finger millet (*Eleusine coracana*)	Raw	IVPD (%)	79.0	Annor et al. 2017
	Cooked		84.7–86.3	
	Germination (30 °C, 48h)		92.0	
Cowpea (*Vigna unguiculata* L.)	Raw	IVPD (%)	71.3 ± 0.1	Kalpanadevi and Mohan 2013
	Germination (30 °C, 48h)		79.5 ± 0.3	
	Cooking (100 °C, 30 min)		78.7 ± 0.1	
	Autoclaving (103.4 kPa, 30 min)		80.1 ± 0.5	
	Germination (30 °C, 96h) + autoclaving		84.9 ± 0.3	
	Raw	IVPD (%)	81.6	Boye, Wijesinha-Bettoni, and Burlingame 2012
		PDCAAS (%)	75	
	Autoclaving (15 psi, 121 °C, 20 min)	IVPD (%)	89.7	
		PDCAAS (%)	97	
	Microwave (1200 W, 15 min)	IVPD (%)	92.2	
		PDCAAS (%)	84	
	Autoclaving (5 psi, 30 min)	IVPD (%)	76.1	Laurena et al. 1987
	Autoclaving (10 psi, 30 min)		77.8	
	Autoclaving (15 psi, 30 min)		80.1	
Amaranth (*Amaranthus hybridus*)	Sun-dried and unsliced	TD (%)	84.4 ± 8.9	Kamela et al. 2016
		PER (ratio)	1.1 ± 0.2	
	Cooked (100 °C, 10 min) and sliced	TD (%)	92.0 ± 8.4	
		PER (ratio)	1.9 ± 0.3	
Amaranth (*Amaranthus cruentus*)	Dried seeds flour	TD (%)	75.4 ± 7.5	Aguilar et al. 2015
		BV (%)	44.5 ± 15.3	
		PDCAAS (%)	36.2 ± 1.3	

(*continued*)

Table 3. Continued.

Source of plant protein	Food processing	Protein quality evaluation method	Results	Reference
Corn (*Zea mays*)	Raw flour	IVPD (%)	88.3	Duodu et al. 2003
	Cooked (100 °C, 20 min) flour		90.7	
Sweet potato (*Ipomoea batatas* L.)	Raw	IVPD (%)	52.8 ± 0.7	Sun et al. 2012
	Cooking (100 °C, 60 min)		85.7 ± 1.4	
	Microwave (700 W, 3 min)		94.1 ± 1.8	
	Drying (130 °C, 60 min)		54.7 ± 0.4	
	Autoclaving (127 °C, 20 min)		99.2 ± 0.1	
	Autoclaving (127 °C, 20 min)	NPU (%)	92.0 ± 1.0	Sun et al. 2012
		TD (%)	95.1 ± 3.1	
		PDCAAS (%)	70.0 ± 0.1	
Chickpea (*Cicer arietinum*)	Raw	PER (ratio)	1.5 ± 0.1	Bhatty, Gilani, and Nagra 2000
		TD (%)	64.6 ± 0.4	
		NPU (%)	36.7 ± 1.1	
	Cooking (100 °C, 40 min)	PER (ratio)	0.8 ± 0.1	
		TD (%)	77.9 ± 0.7	
		NPU (%)	38.4 ± 0.4	
	Raw	IVPD (%)	71.8 ± 1.0	Clemente et al. 1998
	Autoclaving (120 °C, 50 min)		83.5 ± 0.1	
Fababean (*Vicia faba* L.)	Raw	IVPD (%)	70.8 ± 0.2	Alonso, Aguirre, and Marzo 2000
	Germinated (25 °C, 72 h)		78.1 ± 0.2	
	Raw	IVPD (%)	64.6 ± 1.2	Khalil and Mansour 1995
		PER (ratio)	2.4	
	Cooking (45 min)	IVPD (%)	71.2 ± 1.2	
		PER (ratio)	2.7	
	Autoclaving (121 °C, 30 min)	IVPD (%)	73.7 ± 1.4	
		PER (ratio)	2.6	
	Germination (25 °C, 72h)	IVPD (%)	72.2 ± 1.3	
		PER (ratio)	2.6	
Potato (*Solanum tuberosum*)	Cooking + freeze-drying	IVPD (%)	100	Boye, Wijesinha-Bettoni, and Burlingame 2012
Kidney bean (*Phaseolus vulgaris*)	Raw	IVPD (%)	78	Boye, Wijesinha-Bettoni, and Burlingame 2012
		PDCAAS (%)	60	
	Autoclaving (15 psi, 121 °C, 20 min)	IVPD (%)	86.1	
		PDCAAS (%)	77	
	Microwave (1200 W, 15 min)	IVPD (%)	88.6	
		PDCAAS (%)	64	
Oat (*Avena sativa*)	Autoclaving (121 °C, 15 min) + freeze-drying	IVPD (%)	90.0	Boye, Wijesinha-Bettoni, and Burlingame 2012
Buckwheat (*Fagopyrum esculentum*)	Ultrasonic extraction and spray-drying	IVPD (%)	74.2 ± 2.3	Tang 2007
	Ultrasound, spray-drying and defatting		81.3 ± 1.5	
	Ultrasound, freeze-drying and defatting		79.6 ± 1.2	
Velvet bean (*Mucuna pruriens*)	Raw	PER (ratio)	0.7	Siddhuraju, Vijayakumari, and Janardhanan 1996
		BV (%)	58.7	
		TD (%)	48.5	
		NPU (%)	28.5	
	Autoclaving (120 °C, 30 min)	PER (ratio)	1.4	
		BV (%)	74.5	
		TD (%)	81.6	
		NPU (%)	60.8	

availability and protein digestibility. High processing temperatures may also induce cross-linking, protein-protein interactions and racemization of amino acids (Chiesa and Gnansounou 2011; Patto et al. 2015).

Several studies evaluated that food processing impacts on the protein digestibility by influencing on the protein structure and also affecting food composition and nutritional properties. Nevertheless, going through the processes, there is no investigation uncoupling the effects on the protein itself and the close environment, not being possible to detach how each processing consequence impacts on the protein digestibility.

Cooking

Cooking plays an important role in legumes palatability and influences the bioavailability of nutrients, enhancing the digestibility and nutritional value (Singh 1985; Siddhuraju, Vijayakumari, and Janardhanan 1996; Dias et al. 2010; Kalpanadevi and Mohan 2013). The low protein digestibility in uncooked proteins is due to the presence of the heat-labile compounds already discussed in this review; therefore, cooking has many effects on protein digestibility, such as protein denaturation (Clemente et al. 1998; Fawale et al. 2017) or reduced resistance of protein to enzyme attack, and proteins may interact with non-protein components, thereby affecting their digestibility (Olivos-Lugo, Valdivia-López, and Tecante 2010; Fawale et al. 2017). The cooking process increases the leaching out of the unfavorable compounds and the destruction of protease inhibitors (Bishnoi and Khetarpaul 1994; Fawale et al. 2017), while it improves the digestibility of peas, beans, millet, cowpea and chickpea (Table 3) (Clemente et al. 1998; Habiba 2002; Delfino and Canniatti-Brazaca 2010; Park, Kim, and Baik 2010;

Table 4. Influence of fermentation on the protein digestibility of plant proteins.

Source of plant protein	Food processing	Protein quality evaluation method	Results	Reference
Bean (*Phaseolus vulgaris* L.)	Unfermented bean flour	IVPD (%)	40.0 ± 1.7	Espinosa-Páez et al. 2017
	Fermented with *Pleurotus ostreatus* (70 °C)		48.1 ± 0.8	
	Unfermented flour	IVPD (%)	54.4	Dias et al. 2010
	Flour treated with protease from *Bacillus sp.* (28 °C, 5 h)	81.6		
Finger millet (*Eleusine coracana*)	Fermented	IVPD (%)	71.2–83.7	Annor et al. 2017
Oat (*Avena sativa*)	Unfermented oat flour	IVPD (%)	63.3 ± 1.7	Espinosa-Páez et al. 2017
	Fermented with *Pleurotus ostreatus* (70 °C)		70.0 ± 0.3	
Corn (*Zea mays*) and Soybean (*Glycine max*)	Unfermented meal	IVPD (%)	78.4 ± 2.0	Shi et al. 2017
	Fermented with *B. subtilis* and *E. faecium* (37 °C, 24 h)		86.3 ± 2.2	
Soybean (*Glycine max*)	Untreated flour	IVPD (%)	75.3 ± 1.2	Bartkiene, Juodeikiene, and Vidmantiene 2012
	Fermented with *Pediococcus acidilactici* flour (30 °C, 72 h)		88.7 ± 0.9	
	Unfermented	IVPD (%)	83.0	Ojokoh and Yimin 2011
	Fermented with *Bacillus natto* (25 °C, 48 h)		90.0	
Kariya (*Hildergardia barteri*)	Raw and unfermented flour	IVPD (%)	63.7	Fawale et al. 2017
	Raw and fermented flour (30 °C, 96 h)		82.1	
	Cooked (100 °C) and fermented flour (30 °C, 96 h)		85.5	
Cowpea (*Vigna unguiculata* L.)	Fermented with *Saccharomyces cerevisiae* (25 °C, 24 h)	IVPD (%)	84.3	Boye, Wijesinha-Bettoni, and Burlingame 2012
		PDCAAS (%)	81	
Flaxseed (*Linus usitatissimum*)	Untreated flour	IVPD (%)	64.1 ± 0.3	Bartkiene, Juodeikiene, and Vidmantiene 2012
	Fermented with *Pediococcus acidilactici* flour (30 °C, 72 h)		72.7 ± 0.5	
Pea (*Pisum sativum* L.)	Fermented with *Saccharomyces cerevisiae* (25 °C, 24 h)	IVPD (%)	82.9	Boye, Wijesinha-Bettoni, and Burlingame 2012
		PDCAAS (%)	82	
Kidney bean (*Phaseolus vulgaris*)	Fermented with *Saccharomyces cerevisiae* (25 °C, 24 h)	IVPD (%)	80.9	Boye, Wijesinha-Bettoni, and Burlingame 2012
		PDCAAS (%)	81	
Pumpkin seeds (*Telfairia occidentalis* Hook)	Unfermented	IVPD (%)	58.2 ± 0.8	Giami 2004
	Natural fermentation (24 h)		68.4 ± 0.7	
	Natural fermentation (5 d)		78.2 ± 0.2	
Black gram (*Vigna mungo*)	Unfermented	IVPD (%)	53.0 ± 0.5	Yadav and Khetarpaul 1994
	Natural fermentation (25 °C, 18 h)		70.1 ± 0.5	
	Natural fermentation (30 °C, 18 h)		73.8 ± 0.6	
	Natural fermentation (35 °C, 18 h)		79.3 ± 0.6	

Kalpanadevi and Mohan 2013; Annor et al. 2017). The processing parameters for plant protein cooking are usually set to around 100 °C and the time varies from 10 to 60 min (Table 3).

Studies showed that the IVPD increases in cooked peas (100 °C, 40 min) when compared with raw peas as a consequence of the complete elimination of the trypsin inhibitor, the reduction of tannins and phytic acid contents, as well as the effect of heat on the three-dimensional structure of pea proteins (Habiba 2002).

On the other hand, overheating proteins may depress digestibility and amino acid availability, causing a slower release of amino acids from the protein and decomposition of essential amino acids. Therefore, a safe heating process is critical to the processing of plant proteins to establish maximum nutritional value (Van Der Poel 1990). Heat-induced conformational changes in protein, such as an aggregation of protein through increased hydrophobicity and disulfide bond formation, could also impair susceptibility of protein to proteolysis (Berno, Guimarães-Lopes, and Canniatti-Brazaca 2007; Park, Kim, and Baik 2010). Also, heat processing can cause a decrease in protein digestibility, via non-enzymatic browning, thermal cross-linking and the formation of toxic compounds and complexes between proteins and tannins/phytates (Habiba 2002).

Autoclaving

Autoclaving is a high-pressure cooking method and can be a sterilization step using steam to eliminate microorganisms.

Table 5. Influence of extrusion on the protein digestibility of plant proteins.

Source of plant protein	Food processing	Protein quality evaluation method	Results	Reference
Flaxseed (*Linus usitatissimum*)	Extrusion Experimental design varying screw speed, moisture, temperature and feed rate	IVPD (%)	73.1–77.0	Wu et al. 2015
	Extrusion (95–100 °C)	NPU (%) TD (%) BV (%) NPR	58.4 ± 6.5 73.0 80.0 ± 8.7 3.2 ± 0.3	Giacomino et al. 2013
	Extrusion Experimental design varying screw speed, moisture, temperature and feed rate	IVPD (%)	69.5–77.4	Wang et al. 2008
Canola (*Brassica sp.*)	Raw Extrusion (110 °C)	IVPD (%)	79.5 78.1–81.3	Zhang et al. 2017
Red sorghum (*Sorghum spp*)	Raw Extrusion (182 °C, 14 % moisture)	IVPD (%)	53.2 ± 2.0 70.0 ± 0.2	Llopart et al. 2014
Corn (*Zea mays*)	Extruded flour (79.4 °C)	IVPD (%)	80.9	Boye, Wijesinha-Bettoni, and Burlingame 2012
Soybean (*Glycine max*)	Extrusion	IVPD (%)	88.8 ± 0.7	Berno, Guimarães-Lopes, and Canniatti-Brazaca 2007
Fababean (*Vicia faba* L.)	Extrusion (156 °C, 25 % moisture)	IVPD (%)	87.4 ± 0.2	Alonso, Aguirre, and Marzo 2000
Bean (*Phaseolus vulgaris*)	Extrusion (156 °C, 25 % moisture)	IVPD (%)	83.0 ± 0.3	Alonso, Aguirre, and Marzo 2000
	Extrusion (150 °C, 16 s)	TD (%)	79.0	Van Der Poel 1990

Studies showed that autoclaving process not only provides the same wet heat for chemical modification of the antinutritional factors, but also its simultaneous removal by leaching, considering its effects on digestibility and the overall nutritional quality (Laurena et al. 1987). Several authors have studied the effect of autoclaving on the IVPD of various plant proteins (Table 3) (Laurena et al. 1987; Van Der Poel 1990; Khalil and Mansour 1995; Habiba 2002; Boye, Wijesinha-Bettoni, and Burlingame 2012; Lee et al. 2012; Sun et al. 2012; Kalpanadevi and Mohan 2013). The processing parameters for autoclaving plant proteins vary from 5 to 15 psi; 112 to 127 °C; and 10 to 50 min (Table 3). Autoclaving (121 °C, 30 min) with pressurized steaming at 15 psi resulted in the highest reduction of polyphenols (80–83 %) and the greatest improvement in the IVPD (34 %). Autoclaving treatment (121 °C, 60 min) reduced the undesirable compounds of rapeseed products from 9 to 43 % for phytic acid and from 41 to 67 % for tannins (Mansour et al. 1993).

Microwave

Microwave energy is non-ionizing radiation and uses electromagnetic waves of frequency in the range from 300 MHz to 300 GHz leading to instantaneous heat generation within the product due to molecular motion (migration of ions and the rotation of dipoles) (Chandrasekaran, Ramanathan, and Basak 2013; Divekar et al. 2017). The radiation energy disrupts hydrogen bonds and enables the migration of dissolved ions, also affecting the secondary protein structure, which improves some functional properties (emulsifying, foaming, oil- and water-holding capacity) and enhances the IVPD (Pojić, Misan, and Tiwari 2018).

Microwave heating is considerably effective in the inactivation of the protease inhibitors in selected legumes. Results indicate that microwave can be used to effectively reduce some other detrimental compounds, enhancing protein quality (Jourdan, Noreña, and Brandelli 2007; Vagadia, Vanga, and Raghavan 2017; Vagadia et al. 2018). The improvement depends on the heating duration, once the best reduction of these compounds was obtained with 3-minute microwave exposure (800 W at 2450 MHz) for beans (Table 3) (Shimelis and Rakshit 2005). However, adverse effects may occur with prolonged heating, like the occurrence of non-enzymatic browning (Maillard reaction).

Germination

Germination takes place when dry seeds (dormant) uptake water, resulting in the elongation of the embryonic axis (Albarracín et al. 2015). During germination, hydrolytic enzymes lead to biochemical changes, structural modification, and can increase the nutritional value, decreasing the so-called antinutritional factors by enzymatic activity or leaching (Singh 1985).

The improvement of protein digestibility after germination was attributed to a reduction of polyphenols and phytic acid in the germinated seedling and an increase in soluble proteins due to the action of proteolytic enzymes, which were also effective in hydrolyzing protein-polyphenol complexes in the seed (Bishnoi and Khetarpaul 1994; Boye, Wijesinha-Bettoni, and Burlingame 2012). Several germinated plant proteins, like beans, peas, millet, cowpea, and fababean were studied for evaluation of the IVPD (Table 3) (Bishnoi and Khetarpaul 1994; Khalil and Mansour 1995; Alonso, Aguirre, and Marzo 2000; Kalpanadevi and Mohan

Table 6. Influence of isolation and concentration procedures on the protein digestibility of plant proteins.

Source of plant protein	Food processing	Protein quality evaluation method	Results	Reference
Soybean (*Glycine max*)	Protein isolate	IVPD (%)	90.6 ± 0.2	Sánchez-Reséndiz et al. 2018
	Flour	PDCAAS (%)	86.0	Mathai, Liu, and Stein 2017
	Protein isolate		93.0	
	Protein isolate	IVPD (%)	79.1 ± 0.3	Aletor 2012
	Protein isolate	PDCAAS (%)	100	Hughes et al. 2011
	Protein concentrate		100	
	Protein isolate	PDCAAS (%)	100	Schaafsma 2005
	Protein concentrate	IVPD (%)	66.9	Youssef 1988
Flaxseed (*Linum usitatissimum* L.)	Protein isolate	TD (%)	93.2 ± 2.0	Anaya et al. 2015
	Protein isolate	IVPD (%)	68.0 ± 1.8	Marambe, Shand, and Wanasundara 2013
Chickpea (*Cicer arietinum*)	Protein isolate	IVPD (%)	87.5	Wang et al. 2010
	Protein isolate	IVPD (%)	96.1	Sánchez-Vioque et al. 1999
Lupin (*Lupinus angustifolius*)	Protein concentrate	TD (%)	98.3 ± 1.3	Chew, Casey, and
	(Isoelectric precipitation)	NPU (%)	45.4 ± 16.8	Johnson 2003
	Protein concentrate	TD (%)	98.2 ± 1.7	
	(Ultrafiltration)	NPU (%)	45.9 ± 23.9	
	Flour	IVPD (%)	80.0 ± 0.2	Lqari et al. 2002
	Protein isolate		93.9 ± 4.7	
Amaranth	Flour	IVPD (%)	73.0–76.2	López et al. 2018
(*Amaranthus hybridus*)	Protein concentrate		78.7–82.0	
Quinoa (*Chenopodium quinoa*)	Quinoa protein isolate	IVPD (%)	75.9–78.1	López et al. 2018
Pigeon pea (*Cajanus cajan*)	Legume flour	TD (%)	80.1 ± 0.1	Adenekan et al. 2018
		PER (ratio)	1.5 ± 3.3	
	Protein isolate	TD (%)	95.2 ± 0.3	
		PER (ratio)	1.7 ± 0.1	
Peanut (*Arachis hypogaea*)	Phosphorylated protein isolate	IVPD (%)	95.3 ± 0.1	Sánchez-Reséndiz et al. 2018
Pea (*Pisum sativum* L.)	Protein concentrate	PDCAAS (%)	71.0	Mathai, Liu, and Stein 2017
Sunflower (*Helianthus annuus* L.)	Protein concentrate (Isoelectric precipitation)	IVPD (%)	95.4 ± 0.3	Salgado et al. 2012
Potato (*Solanum tuberosum*)	Protein isolate	IVPD (%)	86.5 ± 0.5	Aletor 2012
Cassava (*Manihot esculenta*)	Protein isolate	IVPD (%)	86.3 ± 0.3	Aletor 2012
Chia (*Salvia hispanica* L.)	Defatted flour	IVPD (%)	28.4 ± 0.2	Olivos-Lugo, Valdivia-López,
	Protein isolate		49.4 ± 1.6	and Tecante 2010
Foxtail millet (*Setaria italica*)	Protein concentrate	IVPD (%)	81.0 ± 1.5	Mohamed et al. 2009
Karkade (*Hibiscus sabdariffa*)	Defatted flour	IVPD (%)	82.1 ± 0.1	Abu-tarboush and
	Protein isolate		87.1 ± 0.1	Ahmed 1996
Sesame (*Sesamum indicum*)	Protein isolate	IVPD (%)	80.6	El-Adawy 1995
	Protein concentrate		78.5	
Rapeseed (*Brassica napus*)	Protein concentrate	IVPD (%)	83.5	Mansour et al. 1993
		TD (%)	82.1	
	Protein isolate	IVPD (%)	89.6	
		TD (%)	90.4	

2013; Annor et al. 2017). The processing parameters for the germination of plant proteins range from 25 to 30 °C during 48 to 96 h (Table 3).

Irradiation

Food irradiation is a safe technology in which the food is exposed to ionizing radiation in a particular environment for a specific time and under process-controlled conditions. The radiation absorbed dose is measured in Grays (1 Gy = 1 J/kg). The maximum irradiation of 10 kGy is the dose recommended for not inducing radioactivity and is safe for human health, besides it can avoid the diseases caused by microorganisms of public health importance (Costa, Deliza, and Rosenthal 1999). The energy is enough to break chemical bonds, causing physical and sensory changes.

Ionizing radiation treatment could serve as a processing method for inactivation or removal of some unfavorable compounds, showing that this method is a promising technique (Siddhuraju, Makkar, and Becker 2002). Some authors have evaluated that food irradiation positively impacts on the protein quality (Boye, Wijesinha-Bettoni, and Burlingame 2012; Lee et al. 2012). However, irradiation also could have a negative effect on the digestibility, probably due to the destruction of certain amino acids (such as aromatic and sulfur amino acids) in aqueous media under the influence of gamma radiation. Studies showed that the protein digestibility in raw beans decreased with the increase of the radiation dose used (Mechi, Caniatti-Brazaca, and Arthur 2005).

Drying

There are various drying methods used for removing water from the food, including sun-, hot air-, spray-, freeze-, and vacuum-drying (Sun-Waterhouse, Zhao, and Waterhouse 2014; Barba et al. 2015; Monteiro, Carciofi, and Laurindo 2016; Tontul et al. 2018). The spray-drying is a downstream unit operation frequently used in the food industry to extend the food shelf-life. Liquid foods are pumped through the nozzle of the spray-dryer and brought into a countercurrent flow of hot air, which causes vaporization of moisture leaving a shelf-stable powdered particulate material. Spray-

drying is often used to dry whey protein, soybean protein and a variety of other products. This process utilizes high temperatures, reducing the heat-labile compounds present in plant proteins, and affecting the digestibility and protein functional properties (Tang 2007; Boye, Wijesinha-Bettoni, and Burlingame 2012).

The freeze-drying is a drying process employed to convert most of the water into ice, then remove the ice by sublimation and eliminate the unfrozen water by desorption. The protein concentration is an important parameter because it can lead to high ionic strength, increasing protein-protein interactions and aggregations (Sun-Waterhouse, Zhao, and Waterhouse 2014).

Some studies show how spray- and freeze-drying processes can influence the quality of plant proteins (Table 3) (Boye, Wijesinha-Bettoni, and Burlingame 2012; Sun et al. 2012; Aguilar et al. 2015; Kamela et al. 2016). Few studies that use drying methods intending to improve the protein digestibility. However, most studies use this technology to enhance the functional properties of proteins, such as protein solubility, water-holding capacity, emulsifying, and foaming properties. Zhao et al. (2013) described the influence of freeze-drying (−53 °C, final moisture content of 35.6 g/kg moisture content) and spray-drying (185 °C, final moisture content of 77.8 g/kg) on the improvement of functional properties of rice protein isolate. Tang (2007) studied effect of the drying method (i.e., spray-drying at 130 °C and freeze-drying at 4 °C) for functional properties of buckwheat protein. Berghout et al. (2015) also evaluated the impact of freeze-drying (−20 °C) on the lupin protein isolate functional properties.

Fermentation

The fermentation process involves the use of various microorganisms' sources like bacteria and yeasts. It is a technological alternative for improving the nutritional value of a great variety of legumes and cereals. Fermented foods are potential ingredients for the elaboration and fortification of products for human nutrition, with acceptable sensory properties like unique flavor, aroma and texture attributes that are highly appreciated by the consumer. The microorganisms used in fermentation synthesize enzymes, which hydrolyze food constituents and contribute to the development of products with desirable organoleptic properties (Boye, Wijesinha-Bettoni, and Burlingame 2012).

This low cost biotechnology process is a simple way to achieve a nutritionally enhanced ingredient, increasing protein availability due to the partial denaturation of storage proteins, together with the reduction of undesirable compounds by microbial enzymes, causing chemical changes and functionality of foods (Giami 2004; Boye, Wijesinha-Bettoni, and Burlingame 2012; Coda et al. 2017; Espinosa-Páez et al. 2017; Fawale et al. 2017). During fermentation, insoluble proteins undergo structural changes, which make them more accessible to pepsin attack, rather than being broken down into smaller sub-units (Giami 2004). Fermentation can play an important role in improving the digestibility and bioavailability of nutrients (Yadav and Khetarpaul 1994; Bartkiene, Juodeikiene, and Vidmantiene 2012; Fawale et al. 2017). Table 4 presents examples of the influence of fermentation on the protein digestibility of plant proteins. The processing parameters of fermenting plant proteins vary from 25 to 70 °C throughout 5 to 96 h (Table 4).

Extrusion

Extrusion is one of the most efficient and versatile food processing technologies that can be used to produce dehydrated foods (Ojokoh and Yimin 2011). It is a hydrothermal process used in food texturization for producing pre-cooked cereals and legumes, suitable for making a wide variety of products, such as cream soups, baby food, snacks, breadcrumbs, modified starches, noodles, powdered drinks, meals nutritionally improved, and textured vegetable protein (Boye, Wijesinha-Bettoni, and Burlingame 2012; Llopart et al. 2014; Albarracín et al. 2015). A significant technological advantage of extrusion is that the product is simultaneously cooked and dried, resulting in low-moisture shelf-stable extrudates.

This process leads to the denaturation of proteins, exposing enzyme access sites, and partial or total destruction of thermo-labile compounds and is effective to improve the IVPD, impacting protein quality (Alonso, Aguirre, and Marzo 2000; Ojokoh and Yimin 2011; Boye, Wijesinha-Bettoni, and Burlingame 2012; Giacomino et al. 2013; Arribas et al. 2017; Tuśnio et al. 2017; Zhang et al. 2017). Besides, it has relatively high efficiency, versatility, production capacity, low operating costs and requires shorter cooking time than other heating processes and can be used on a large scale (Giacomino et al. 2013; Tuśnio et al. 2017).

Extrusion variables, such as the temperature, screw speed, screw configuration, and feed ratio are determinants to mechanical and thermal energy inputs, and to residence time, which influence the degradation and interaction of components and hence the product attributes (Arribas et al. 2017; Zhang et al. 2017). During extrusion, mechanical shear pressure also plays an important role in the disruption of the protein bodies, results in the changes to the physical, chemical and nutritional quality of the extruded food products, improving protein digestibility (Arribas et al. 2017; Zhang et al. 2017). The degree of protein hydrolysis, extent, and type of changes to protein are induced by the process conditions used during extrusion (degradation, dissociation, aggregation or cross-links covalent bonds) (Zhang et al. 2017).

Studies showed that the extrusion processing increased the IVPD in blends of rice, pea, and carob flour when compared to the corresponding non-extruded formulations, reaching values from 88 to 95% (Arribas et al. 2017). It was optimized the extrusion process parameters for flaxseed protein using response surface methodology (RSM) and the appropriate conditions were determined (temperature of 134.3–156.1 °C, screw speed of 114–165.7 rpm, feeding speed of 34.39–45.95 kg/h, and moisture content of 17.37–22.43%) (Wu et al. 2015). Another study evaluated the effects of

processing parameters on the protein digestibility of the extruded flaxseeds. The surface response graphs showed that the maximum IVPD was obtained at the extrusion conditions: screw speed of 120 rpm, moisture content of 10%, temperature of 120 °C, and feed rate of 91.4 kg/h (Wang et al. 2008). Table 5 presents more examples of the influence of extrusion on the protein digestibility of plant proteins. The processing parameters for the extrusion of plant proteins ranged between 95 and 182 °C.

Protein concentration and isolation

Physical processes and dry or wet fractionation are very effective to improve the protein quality (Yang et al. 2012) and can produce plant proteins ingredients, like flours (20 to 30% of protein content), enriched flours (30 to 50%), concentrates (50 to 80%), and isolates (>90%) (Stone et al. 2019).

Producing protein isolates from vegetal sources is of great interest due to their functional components of high protein processed food products with sensory and nutritional properties (Mohamed et al. 2009). A proper isolation technique ensures highly purified protein and the protein isolate is an excellent dietary supplement and beneficial for physical strength performance and weight management (Adenekan et al. 2018).

Wet fractionation is the mainstream and most commonly used method to extract proteins in the industry to obtain high levels of plant protein (concentrates/isolates), including alkaline extraction followed by isoelectric precipitation (pH adjusted to the protein's isoelectric point, i.e., zero net charge) (Lqari et al. 2002; Chew, Casey, and Johnson 2003; Adenekan et al. 2018; López et al. 2018; Tontul et al. 2018). The thermal treatment and alkalization applied during protein extraction may account for the enhanced digestibility reported for the isolate, when compared with the protein flour (Sánchez-Vioque et al. 1999; Olivos-Lugo, Valdivia-López, and Tecante 2010; Pastor-Cavada et al. 2010; López et al. 2018). However, this methodology has major drawbacks due to high processing costs and the great environmental impact with the requirement of a high amount of water and energy.

Some milder processing techniques are used to overcome these disadvantages. Dry fractionation (milling/air classification) is an effective technique used to produce successfully protein concentrates applied to legumes (e.g., pea, bean and lentil) and some cereals (e.g., barley and wheat) (Schutyser and van der Goot 2011; Schutyser et al. 2015; Opazo-Navarrete, Freire, et al. 2018).

Furthermore, membrane separation (ultrafiltration) has been identified as an alternative process to isoelectric precipitation for the manufacture of purified protein ingredients from legumes, resulting in improved protein recovery and improved physical-functional properties in terms of protein solubility and gel elasticity (Chew, Casey, and Johnson 2003; Berghout et al. 2015). Also, the processing conditions (type mode, extraction temperature, time, protein:water ratio, equipment) can easily affect the protein functionality of the final powdered ingredient (Samaranayaka 2017; Stone et al. 2019).

As mentioned, isolation procedures have a profound influence on the structural and functional properties of the proteins. Processing causing changes to quaternary and tertiary structure of protein, during the partially denaturation, making the protein susceptible and accessible to the attack of digestive enzymes (proteases), increasing digestibility (Sánchez-Vioque et al. 1999; Lqari et al. 2002; Olivos-Lugo, Valdivia-López, and Tecante 2010; Pastor-Cavada et al. 2010; Stone et al. 2019). Also, the improved protein digestibility is a result of some changes in amino acids composition (e.g., lysine and sulfur amino acids) (Yang et al. 2012). The higher digestibility of the plant protein isolates may be linked to reduce and eliminate some unfavorable compounds. Protein isolates presented a drastic reduction in the levels of tannins and phytates in comparison of legume flours or raw seeds, showing the efficiency of the protein isolation for reducing these compounds (Sánchez-Vioque et al. 1999; Adenekan et al. 2018).

Mansour et al. (1993) evaluated the composition of rapeseed products and the isolation technique eliminated 74–92% of phytic acid and 100% of tannins and trypsin inhibitor for protein concentrate and isolate.

The IVPD of the sunflower protein concentrates obtained with isoelectric precipitation was determined (95.4%), using casein digestibility as a reference (100%) (Salgado et al. 2012). The removal of protease inhibitors in protein isolates increases the IVPD of lupin isolates (93.9%) when compared to the flour (80%), obtaining protein isolates of acceptable nutritional value with a high protein digestibility and low content of undesirable substances (Lqari et al. 2002). Table 6 presents examples of the influence of isolation and concentration procedures on the protein digestibility of plant proteins.

Enzymatic hydrolysis

The protein hydrolysis consists of the cleavage of peptide bonds that breaks proteins into smaller peptides and free amino acids, and exposing of hydrophobic groups, which increase the digestibility and functional properties of proteins (Chen et al. 2011; Day 2013; Contreras et al. 2019; Liceaga and Hall 2019). Chemical hydrolysis (by acids or alkali) has major disadvantages, which can form detrimental amino acid residues (e.g., lysinoalanine) and yield products with reduced nutritional qualities (Potier and Tomé 2008; Patto et al. 2015). The enzymatic hydrolysis of proteins is an alternative previously used in the food industry to improve the biological value and functional properties of these molecules (Dias et al. 2010).

Protein hydrolysates (also dominated by di- and tri-peptides) by an enzymatic treatment (e.g., cellulases, hemicellulases, proteases) may provide enhancing protein availability, increasing the digestibility by the enzymatic decrease of undesirable compounds present in plant proteins (Potier and Tomé 2008; Dias et al. 2010; Fawale et al. 2017). Proteases (or peptidases) (e.g., Alcalase™ and

Flavourzyme™) have been used to enhance the nutritional value of the products by modifying protein structures (Kapravelou et al. 2013; Patto et al. 2015). Studies indicated that protein hydrolysis could reduce the protein antigenicity, rising the tolerance and producing peptides that not activate in vitro IgE antibody binding activity, declining the allergenicity (Patto et al. 2015).

Dias et al. (2010) studied the enzymatic hydrolysis (using two proteases) of four cultivars of beans and results showed the increase on the protein digestibility by favoring the absorption of amino acids and short-chain peptides, which improves the nutritional quality.

Several studies showed that hydrolysis of protein isolates under controlled conditions have technological advantages and show positive effects on protein functionality. Results indicated that protein enzymatic hydrolysis was successful for increasing protein solubility, foaming capacity and stability, and gelation capacity (Moure et al. 2006; Potier and Tomé 2008; Zhang et al. 2012; Sun-Waterhouse, Zhao, and Waterhouse 2014; Patto et al. 2015; Voudouris et al. 2017).

However, there are some challenges regarding the application of protein hydrolysates, since the protein hydrolysis may results in the formation of hydrophobic peptides, which causes the development of bitterness and off-flavors, impacting negatively on taste, limiting the use of protein hydrolysates in food products (Longo and Sanromán 2006; Jiang et al. 2010; Sun-Waterhouse, Zhao, and Waterhouse 2014; Amagliani et al. 2017; Liceaga and Hall 2019).

Concluding remarks

It is clear that a better correlation between the standard protocols of in vitro and in vivo procedures and the actual human body digestion is essential and not fully established. Nevertheless, as clinical trials are much harder to be done, time-consuming, more expensive, and ethically contestable, those standard protocols are important signs towards the actual protein digestibility into the human body, and more efforts have to be done in this field aiming more reliable results. Besides, they are instrumental when estimating the overall processing impact on the plant protein digestibility.

In many cases, the most affordable food plants providing proteins lack some essential amino acids and present relatively low protein digestibility due to the presence of some compounds that prejudice protein digestion, the so-called antinutritional factors. Conventional techniques based on thermal processing are well established to reduce or eliminate these compounds and increase the measured digestibility of the plant proteins.

However, the term antinutritional factors should be revised. Although these compounds impair protein digestibility, they possess several biological activities and benefits for promoting human health. Thus, they should not be degraded or discarded, as many authors reported; techniques should be taken only to dissociate them from the proteins or to recover these compounds for other beneficial applications.

Knowledge linking each plant source to each processing technique under a chosen parameter setup is a key factor in obtaining higher-quality proteins. However, as presented above, one should consider that a plant protein source may lose other nutritional properties when enhancing the protein digestibility and processing selection requires more in-depth evaluation in this direction too.

Regarding the processes, there are at least three worth alternative strategies to be explored, as it follows. Techniques using mild conditions may lead to a balance between nutritional aspects, beyond protein digestibility, and feasible processes with reduced environmental impact. Also, eco-innovative processing techniques, such as the non-thermal emerging methods (e.g., pulsed electric field, ultrasound, and high-pressure), seem to be promising to increase protein nutritional value and techno-functionalities since they have been reported to affect protein structure and food composition under gentle temperatures. However, the literature is scarce in studies correlating these technologies and plant protein digestibility. Third, only one processing method may not produce the desired removal of all unfavorable compounds, and a few demonstrated combining methods as a promising alternative to this end. It may preserve nutritional features other than improving protein quality.

It has been demonstrated that efforts are needed for developing novel techniques and processing setups for enhancing the nutritional quality of traditional protein sources. Also, evaluating new and sustainable protein resources is a prospect, including agro-industrial by-products and wastes, such as the residues from oilseeds extraction industries that contain a high protein content, often discarded or used as feedstock for animal feed. Another worth aspect is to evaluate plant sources blending and product formulation to obtain a food rich in protein that covers the required essential amino acid spectrum with an improved nutritious feature. A developed solution, coupling the plant protein source and a processing technique, needs to fit the environmental, economic, and health requirements, as well as the consumers' sensory (e.g., appearance, flavor, and texture) and cultural aspects, included and not limited to tradition, religion, and animal welfare.

On a worldwide basis, there is a constant requirement for protein quality and availability, covering the food security obligation. Plant protein digestibility and bioavailability are critical aspects when aiming to meet human nutritional needs into a scenario of increasing the world's population and constrained environmental resources, especially when looking for animal-based protein substitution. How to accurately determine and how to improve the protein quality of a plant source remains a scientific and technological challenge that should be addressed shortly.

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