Application of emerging technologies to obtain legume protein isolates with improved techno-functional properties and health effects

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Word count: 22040

Short version of title: Improving legume protein isolates

Abstract

Current demand of consumers for healthy, and sustainable food products has led the industry to search for different sources of plant protein isolates and concentrates. Legumes represent an excellent non-animal protein source with high protein content. Legume species are distributed in a wide range of ecological conditions, including regions with drought conditions, making them a sustainable crop in a context of global warming. However, their use as human food is limited by the presence of anti-nutritional factors, such as protease inhibitors, lectins, phytates, and alkaloids, which have adverse nutritional effects. Anti-technological factors, such as fiber, tannins, and lipids, can affect the purity and protein extraction yield. Although most are removed or reduced during alkaline solubilization and isoelectric precipitation processes, some remain in the resulting protein isolates. Selection of appropriate legume genotypes and different emerging and sustainable facilitating technologies, such as high-power ultrasound, pulsed electric fields, high hydrostatic pressure, microwave and supercritical fluids, can be applied to increase the removal of undesirable compounds. Some technologies can be used to increase protein yield. The technologies can also modify protein structure to improve digestibility, reduce allergenicity, and tune technological properties. This review summarizes recent findings regarding the use of emerging technologies to obtain high-purity protein isolates and the effects on techno-functional properties and health.

Keywords: legumes; innovative technologies; anti-nutritional factors; protein extraction; allergenicity

1. Importance of legumes in human diet as a sustainable protein source

Legumes belong to the Fabaceae family, with approximately 20,000 species divided into 700 genera (Smýkal et al., 2015). Within legumes, those with dry edible seeds are known as pulses, while others harvested green are classified as vegetables. Legumes for human consumption include soybean (*Glycine max* (L.) Merr.), beans (*Phaseolus spp.*), peas (*Pisum sativum* L.), fava beans (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris*) and lupine (*Lupinus albus* L.), among other crops (FAO, 1994). Legumes are the second family in agronomic importance, representing approximately 15% of arable land worldwide (Watson et al., 2017). Moreover, legumes are key crops for sustainable agriculture, mostly because of their ability to adapt to a wide range of ecological conditions and their capacity to fix atmospheric nitrogen in symbiosis with soil bacteria, in a process known as biological nitrogen fixation (BNF).

Proteins are macronutrients of high nutritional and health importance, which are essential in human and animal diets. Legumes have historically been one of the main sources of proteins in the human diet because of their high-protein content and other agronomic advantages that have led to their cultivation since the Neolithic period (Huebbe & Rimbach, 2020). Indeed, legumes are the most suitable plants for use as an alternative to animal protein, providing approximately 33% of the protein requirements in the human diet (Bessada et al., 2019). However, since the middle of the 19th century, meat has displaced legumes in diets historically based on legume proteins, such as the Mediterranean diet. Hence, consumption and cultivation of legumes have decreased in recent decades in many countries (Varela-Moreiras et al., 2013; Zander et al., 2016). The shift in the habit of protein consumption has consequences for human health, because there is an increasing risk of cancer, diabetes, cardiovascular disorders, and

premature death associated with animal protein intake (Arnett et al., 2019; De Oliveira Mota et al., 2019).

The growing consumer interest in vegan and vegetarian products can be in part attributed to the awareness of healthy dietary habits and an increasing concern for animal rights and welfare (Norman & Klaus, 2020). Additionally, given that an average of 4.9 kg of vegetable protein is needed to obtain 1 kg of meat, livestock farming has put additional pressure on natural resources (Chéreau et al., 2016). Moreover, intensive livestock farming is in part responsible for the increase in greenhouse gas (GHG) emissions making the production of animal protein unsustainable (Kumar et al., 2017). Consequently, it has been predicted that a 50% reduction in meat production could reduce GHG emissions from agriculture by 25% to 40% (Zander et al., 2016). Furthermore, BNF is directly related to other beneficial environmental effects of legumes because this process reduces the need for synthetic fertilizers, which reduces the GHG emissions required for their production and transport (El Mujtar et al., 2019), and contributes to the increase in nitrogen use efficiency in agricultural systems (Anas et al., 2020).

Regardless of the motivations, the increasing interest in plant-based products has led to an increased production of legume protein concentrates (PCs, 40-70% protein) and protein isolates (PIs, 80-90% protein) because of their functional and nutritional properties (Klupšaitė & Juodeikienė, 2015; Khazaei et al., 2019). Indeed, the global plant-based meat market was valued at approximately USD 11.92 billion in 2018 and it is expected to generate approximately USD 21.23 billion by 2025 (Zion Market Research, 2019). However, the development of plant-based protein-rich products should consider different aspects, including i) selection of appropriate species, ii) design of efficient, safe, and environmentally friendly protein extraction processes to obtain PCs

149 and PIs, and iii) improvement of sensory, technological, and nutritional properties of
150 plant-based foods. Herein, we review the potential of different emerging technologies
151 that could be applied to obtain PCs and PIs from legumes. This review focuses on the
152 most relevant findings during the past 5 years regarding safety, nutritional value,
153 sensory quality, and technological properties of legume PIs as affected by emerging
154 technologies.
155
156 **1.1. Nutritional characteristics of legumes**
157
158 Legumes are now emerging as an excellent source of nutrients. For this reason, FAO
159 declared 2016 as the International Year of Pulses, to heighten their inclusion in a
160 sustainable food production strategy designed to achieve food security and adequate
161 nutrition (FAO, 2016). Remarkably, the amount of protein in legumes is one of the
162 highest in the plant kingdom, ranging from 20% in peas to 40% in lupines, with most
163 being storage proteins globulins (legumin and vicilin), albumins, and glutelins (Bessada
164 et al., 2019). With regards to their amino acid profile, legumes have high lysine, leucine,
165 aspartic acid, and arginine content but are usually poor in sulfur-containing amino acids
166 (methionine and cysteine) and tryptophan (Bessada et al., 2019). Furthermore,
167 digestibility or other health-related properties of legume proteins can be reduced by the
168 presence of other seed compounds, the so-called anti-nutritional factors (ANFs), which
169 can be classified as protein and non-protein compounds. Proteinaceous ANFs include
170 lectins and protease inhibitors (trypsin and chymotrypsin) that prevent protein digestion
171 in the gastrointestinal tract and reduce amino acid intake. Non-protein ANFs include
172 phenolic compounds (*e.g.,* tannins), saponins, and alkaloids, which play important roles
173 in plant protective mechanisms, and phytates that reduce the bioavailability of essential

minerals, such as iron. However, depending on their chemical structure, effects of concentration, exposure time, and interaction with other dietary components, ANFs can also be considered pro-nutrients with multiple health benefits, such as anti-inflammatory, anti-cholesterol, antioxidant, and anticarcinogenic activities (Cabezudo et al., 2021).

Some studies have shown that the amount of ANFs, such as tannins and protease inhibitors, decrease during seed germination, improving protein quality, and consequently, the digestibility of legume proteins (Ohanenye et al., 2020). Additionally, postharvest seeds treatments such as dehulling, fermentation, cooking, soaking and roasting affect their nutritional composition (James et al., 2020; Besada 2019). Thus, the exploration of seed germination and postharvest treatments could contribute to the increased utilization of legumes as an alternative to animal protein for the human diet. Additionally, the reduction of iron bioavailability by the presence of phytate should be considered to prevent iron deficiency in a legume protein-based diet. In this regard, legumes can also provide a heme-iron pigment, leghemoglobin, which is synthesized in the root nodules where nitrogen fixation takes place. This pigment can be exploited as an additive to legume PIs to overcome the presence of certain ANFs (phytate and polyphenols) that reduce the bioavailability of iron. Moreover, leghemoglobin has been used as a color additive mimicking the organoleptic properties of meat heme proteins (Sha & Xiong, 2020; FDA, 2019).

1.2. Genomic resources in plant breeding for legume selection

Protein quality and levels of ANFs differed significantly among cultivars of the same legume species. In fact, the different protein composition in yellow peas cultivars has been shown of great importance in obtaining pea protein isolates with desirable

functionality (Cui et al., 2020). Additionally, agricultural properties and adaptability to different climates and soil types should be considered when selecting optimal cultivars for human consumption, such as obtaining low alkaloid lupine (sweet lupine) varieties that humans can consume. Moreover, the high protein levels, together with the diversity of lupine species and their capacity to grow under diverse soil and climatic conditions, make these legumes an interesting alternative for the sustainable production of plant-based foods (Swiecicki et al., 2000). The use of legume genotypes adapted to local soil and climatic conditions will contribute to the development of sustainable food systems, with special attention given to necessary adaptations to climate change.

Genomics-assisted breeding (GAB) has been successfully used to combat biotic and abiotic stress in both cereals and legumes (Kole et al., 2015) and to improve the nutritional quality traits in agricultural crops (Chandra et al., 2020). Additionally, other genomic resources, such as genome assemblies and germplasm sequencing, have been reviewed for six major legumes (soybeans, groundnuts, cowpeas, common beans, chickpeas, and pigeon peas) (Thudi et al., 2021). Consequently, advances in next-generation sequencing (NGS), in addition to precision phenotyping technologies, are important for the selection of varieties with specific traits to make legumes a real alternative to animal protein (Giovanni & Murray, 2018; Yang et al., 2020). Therefore, great effort must be made to optimize the production and processing technologies to satisfy the food protein demand, from the selection of legume varieties with high-protein content and quality to the development of technologies to improve the production of healthy and sustainable food.

2. Facilitating emerging technologies for the removal of unwanted compounds and extraction of protein in legumes

Optimal protein isolation and purification procedures are vital for achieving high-quality PIs. However, several compounds such as ANF, located inside the cell-matrix, and the presence and characteristics of the legume cell walls, limit protein extraction (Byanju et al., 2020). Therefore, ANF should be removed to improve the protein digestibility and bioavailability of amino acids and iron. Furthermore, certain compounds that are typically present in legumes, such as triacylglycerides or carotenoids, must also be removed since they could affect the techno-functional properties, purity, and yield of PCs and PIs. In this regard, those compounds affecting techno-functional properties, yield or purity can be denominated anti-technological factors (ATFs). Some ANFs, such as tannins, could also be classified as ATFs because they may negatively affect various techno-functional properties, such as color, and affect the purity and yield of the PIs (Alu'Datt et al., 2014; Chéreau et al., 2016; Rahate et al., 2021). Table 1 summarizes chemical compounds present in legumes and their categorization based on ATF and ANF classifications.

The most important processes for obtaining legume PC and PI include dry-fractionation and wet-extraction processes. Dry-fractionation involve two processes, pin-milling and air-classification, where the different legume fractions are classified according to their size, density, and electrostatic properties (Klupšaitė & Juodeikienė, 2015; Assatory et al., 2019; Chéreau et al., 2016). This is a common, simple and sustainable method to produce PC; however, the purity of the protein fraction (fine and light fraction) is normally low (about 50% protein) and requires further processing for concentration (Khazaei et al., 2019; Klupšaitė & Juodeikienė, 2015). Moreover, high content of undesirable compounds (lipids, fibers, or ANFs) could be present in the enriched protein fraction (Schutyser et al., 2015). In contrast, wet-extraction processes are more convenient because of the higher purity, digestibility, and quality of the PIs

obtained (Khazaei et al., 2019; Boye et al., 2010). This can be attributed to the more efficient removal of ANFs and ATFs in wet-extraction processes compared to dry-fractionation (Vogelsang-O'Dwyer et al., 2020). The commonly applied method of wet-extraction for obtaining PCs and PIs involves different steps: (i) pretreatment of the seeds for cell wall disruption (altering chemical composition and structure of cellulose and hemicellulose), (ii) solubilization of proteins in an alkaline solution (pH>8), and (iii) selective protein precipitation by adjusting pH to the isoelectric point (approximately pH of 4.5) (Klupšaitė & Juodeikienė, 2015; Perović et al., 2020). Additional steps to separate the insoluble fractions (centrifugation or filtration) and prepare the final protein concentration (spray-drying or freeze-drying) are also required (Figure 2) (Khazaei et al., 2019). However, other procedures, such as reverse micelles prepared with hexane, surfactants, and water (Zhao et al., 2018), the salt-extraction method, using an appropriate salt solution at desired ionic strength for protein solubilization, and precipitation by dilution or ultrafiltration, have also been proposed (Klupšaitė & Juodeikienė, 2015). Protein yields are essential for industrial viability. However, several factors (cultivar, particle size, temperature, protein composition, lipid content, pH, and solubilizing agent) may influence the protein yield and the quality of the PIs (Aguilar-Acosta et al., 2020; Khazaei et al., 2019; Cui et al., 2020). Additionally, it is important to highlight that protein extraction is a complex process that includes important steps, such as the penetration of the solvent into the cells, redistribution of solvent into different cell compartments, and correct solubilization of the protein (Aguilar-Acosta et al., 2020).

The use of water-based solvents in wet-extraction processes allows the reduction and/or withdrawal of water-soluble ATFs and ANFs, such as α-galactosides (Vogelsang-O'Dwyer et al., 2020). However, not all compounds can be removed, and

other relevant ANFs and ATFs, such as phytic acid, remain in certain amounts (Mondor et al., 2009). Moreover, further processing of PIs, including baking, cooking, or extrusion, has been demonstrated to have a mild effect on the reduction of various ANFs and ATFs (Sánchez-Velázquez et al., 2021). Therefore, a multidisciplinary approach for minimizing and extracting ANFs and ATFs before the solubilization of the protein is a necessary step for obtaining PIs with high technological and nutritional properties (Figure 1). Additionally, further use of the separated fractions can be considered because of the functional-related properties of most of these compounds (Table 1).

Emerging technologies seek to intensify conventional extraction processes or provide new extraction procedures to enhance the process kinetics with less energy consumption and minimum use of solvents while maintaining or improving the functional properties of the extracted molecules (Bessada et al., 2019; Maroun et al., 2018). Mechanisms controlling solid-liquid extraction can be separated into those affecting (i) internal solids and (ii) external solvent transport. Transport mechanisms inside the solid particles encompass solvent diffusion into the matrix cells, solute solubilization, and diffusion of the solute into the particle surface. External transport is related to convective mechanisms, including solvent entry into the particle and migration of the extracted solute from the surface of the particle into the bulk solvent (Aguilar-Acosta et al., 2020). Furthermore, traditional extraction techniques are highly intensive in terms of time, use of solvents, and high temperatures, which could negatively affect not only the activity of the extracted compounds but also the protein matrix (Navarro del Hierro et al., 2018).

A common aspect of all extraction processes is that the cell wall is the main barrier to protein separation because proteins cannot cross it because of their high-molecular-

299 weight (Voudouris et al., 2017). Although the milling processing collapses the cell wall
300 and favors the liberation of protein matrices and starch granules, the use of emerging
301 technologies could be a promising alternative to improve protein extraction yields from
302 legumes (Aguilar-Acosta et al., 2020; Chemat et al., 2020). Emerging technologies must
303 be driven to improve internal and/or external mass transport mechanisms by considering
304 both target solutes and solvents without negatively affecting the structural constituents.
305 Novel extraction techniques attempt to ease the removal of molecules strongly bound to
306 the solid matrix under milder processing conditions (temperature, pH, or pressure) and
307 reduce the use of solvents or replacing them by more sustainable solvents and with
308 lower toxicity (Panja, 2018). Various eco-emerging technologies, also called green
309 technologies, such as high-power ultrasound (HPU), supercritical fluids (SFs), pulsed
310 (PEFs) and moderate electric fields (MEFs), high hydrostatic pressure (HHP), and
311 microwaves (MWs), have been extensively used to intensify the extraction of natural
312 compounds from vegetable matrices. Thus, a compilation of recent applications of
313 emerging technologies to improve the removal of different ANFs and ATFs in legumes
314 is shown in Table 2. Most previous literature has considered ANF and ATF removal as
315 independent processes and has sought alternative uses for these fractions. However, an
316 integrated analysis of ANF and ATF reduction or removal by extraction, as a previous
317 and necessary step, for the isolation of legume proteins, remains a quite unexplored
318 field to date. As stated above, innovative extraction techniques have attracted growing
319 interest in the food industry because they improve compound recovery and shorten the
320 extraction time, reducing energy and solvent consumption (Aguilar-Acosta et al., 2020;
321 Chemat et al., 2020). The application of different emerging technologies and their
322 optimal processing conditions for legume protein extraction are summarized in Table 3.
323 However, it is also important to note that applying these technologies during protein

extraction processes can modify protein microstructure and therefore exert different effects on the functional properties of PCs and PIs (Aguilar-Acosta et al., 2020; Ochoa-Rivas et al., 2017). Thus, the following sections present emerging sustainable technologies to remove undesirable compounds of legumes to improve purity, yield and overall quality properties of PIs.

2.1. Pulsed Electric Fields

2.1.1. Removal of anti-nutritional and anti-technogical factors

PEF-assisted removal is one of the most prominent technologies used in the recent literature for extraction purposes. PEF processing is based on electric field strengths above 1 kV/cm applied as short duration pulses in the range of μs or ms. PEFs cause electroporation of cell membranes, increasing permeability. PEF processing is mostly applied as a pretreatment to facilitate internal mass transport mechanisms (Puértolas et al., 2017). The importance of electric field strength lies in the electroporation effect on the cell membranes (Chemat et al., 2020). Electroporation causes structural modifications in vegetable cells (Puértolas et al., 2017), increasing permeability by creating microchannels that facilitate mass transfer of both solutes and solvents (Sarkis et al., 2015). The smaller the cell size, the higher the electric field level required for irreversible electroporation. Although the heat generated by the Joule effect during treatment can be moderate, PEF use is considered a non-thermal treatment, contributing to better preservation of thermolabile constituents (Chemat et al., 2020). Thus, PEF pretreatment has been demonstrated to effectively extract natural components, such as polyphenols and carotenoids, from very different matrices (Maroun et al. 2018). However, to our knowledge, this technology has not been used to extract ANFs and ATFs from legumes.

2.1.2. Extraction of proteins

PEF technology has been used to facilitate the extraction of various intracellular compounds, including proteins (Chemat et al., 2020; Voudouris et al., 2017; Zhang et al., 2021). However, there is a lack of knowledge regarding the effects of PEF on proteins (Zhang et al., 2021). Furthermore, no recent studies have used this technology for protein extraction from legumes. Nonetheless, the application of PEF improved protein extraction from sesame cake (Sarkis et al., 2015). Moreover, PEF is a non-thermal technique that could increase the yield and quality of the extracted proteins (Chemat et al., 2020). Another important advantage of PEF is the homogeneity of the method because all tissues (the electric field is distributed through all cells) are treated, compared to other techniques that only treat the surface (Siemer et al., 2018). Additionally, this technique was applied as a pretreatment followed by enzymatic hydrolysis because it facilitates enzyme access to the cells to cleave intracellular proteins (Zhang et al., 2021). Because of these aspects, PEF is a promising technique that could enhance legume protein extraction and reduce processing times.

2.2. Moderate Electric Fields

2.2.1. Removal of anti-nutritional and anti-technogical factors

Electrotechnologies also include MEF processing (Gavahian et al., 2018). MEF processing operates with lower field strengths (<1 kV/cm) than PEF (Rodrigues et al. 2020a). Thus, in MEF applications, the electroporation effect linked to the electric field is lower than in PEF treatments. On the other hand, MEF is applied continuously during the extraction process, which involves high-energy release (kJ/kg) into the medium. Thus, the concurrent presence of joint cell electroporation and considerable volumetric ohmic heating occurs (Gavahian et al., 2018). However, in many MEF applications

designed to extract natural components, such as carotenoids present in microalgae (Jaeschke et al., 2019), the temperature is controlled to avoid the thermal degradation of biomolecules. Moreover, several studies have reported the synergistic effects of combined electroporation and ohmic heating of vegetable cells for extraction purposes (Pereira et al., 2016). Thus, Pare et al. (2014) reported a positive effect of MEF application, coupled with an enzymatic treatment, in the extraction of oil from soybean seeds (70–90 °C, water solvent, 1:4 w/v, 50 Hz, 96 V/cm, 10 min), keeping the free fatty acids below an acceptable limit (3%).

2.3. Microwaves

2.3.1. Removal of anti-nutritional and anti-technogical factors

MW extraction, which uses electromagnetic waves with frequencies between 300 MHz and 300 GHz, is an interesting alternative to conventional extraction techniques. MW-assisted extraction is based on the interaction between the electromagnetic field and cell-matrix, which causes the rotation and alignment of some sensitive molecules with the electromagnetic field (Dalmoro et al., 2015). This alignment provokes molecular friction that allows selective and efficient heating in both solvent and matrix particles. Therefore, MWs provide shorter processing times and increased savings of solvents compared to conventional extraction (Zuluaga et al., 2020). Furthermore, heating of the water molecules inside the plant matrix expands cellular materials and facilitates the release of the cell contents when the structure is broken (Maroun et al., 2018). MWs have been widely used to extract various phytochemicals, such as tannins, alkaloids, and saponins, from different plant sources (Xiaokang et al., 2020). Dalmoro et al. (2018) showed that MW pretreatment (60–75 °C 1000 W, 2.45 GHz, 1 min) reduced the tannin content with minimum impact on the structure of legume seeds.

399 Moreover, Maroun et al. (2018) reported that MWs could facilitate the selective
400 extraction of polyphenols and shorten the time required for essential oil extraction from
401 plant cells 6-fold compared to traditional methods. Zuluaga et al. (2020) proposed an
402 optimized MW extraction process for inositols from pods (120 °C, 1200 W, water
403 solvent, 16.5 min) and seeds (90 °C, 1200 W, water + ethanol (17%) solvent, 21.5 min)
404 of different legumes, which was followed by a microbial-based treatment to further
405 remove interfering soluble sugars.

406 **2.3.2. Extraction of proteins**

407 The use of MW-assisted extraction alone or combined with the HPU-assisted
408 technique to enhance protein extraction from peanut flour has been investigated (Ochoa-
409 Rivas et al., 2017). In this study, the use of MW or HPU improved the extraction yield,
410 but the sequential application of both extraction techniques did not exhibit a synergistic
411 effect. With MWs, the application of higher power and longer extraction times
412 improved the extraction yields. The optimized conditions for the MW-assisted
413 extraction of protein were 725 W for 8 min. Moreover, combined extraction (MW and
414 HPU) yielded higher protein extraction than the use of MWs alone but did not differ
415 from the yield obtained from HPU alone. Therefore, the authors concluded that the
416 ultrasound technique was the most appropriate for extracting proteins from peanuts
417 (Ochoa-Rivas et al., 2017). Additionally, these technologies did not modify the protein
418 isolate microstructure, although the secondary structure was affected.

419

420 **2.4. Supercritical Fluids**

421 **2.4.1. Removal of anti-nutritional and anti-technogical factors**

422 SF extraction is an emerging technique that has attracted growing attention in the
423 food industry in recent decades. It is considered a green technology because of the

utilization of non-toxic non-polar solvents, which results in more sustainable processing, and reduced energy use and environmental pollution (Khawli et al., 2019). An SF is any substance at a temperature and pressure above its critical point. Under this condition, the density of an SF is close to that of a liquid, and the viscosity is similar to that of a gas. These characteristics make SFs highly suitable for extraction purposes. Carbon dioxide (CO_2) is the most widely used SF solvent in food applications because it is generally recognized as safe (GRAS) (Wrona et al., 2017). The CO_2 critical conditions are a temperature of 31 °C and 7.38 MPa pressure. Thus, the moderate temperatures applied in supercritical carbon dioxide (SC-CO_2) extraction allow the maintenance of the integrity of thermolabile compounds (Maroun et al., 2018). Furthermore, because of the nonpolar nature of CO_2, SC-CO_2 can be used to extract non-polar compounds, such as oils or carotenoids, and relatively low-polarity molecules, such as alkaloids, polyphenols, and saponins (Chemat et al., 2020; Khawli et al., 2019).

The selectivity for lipophilic compounds can also be adjusted by using a co-solvent to either increase or decrease the polarity of CO_2. Ethanol is the most frequently used co-solvent because it is considered a non-toxic solvent. The combination of CO_2 with ethanol as a co-solvent has been widely studied for the extraction of phenolic compounds from multiple plant matrices (Khawli et al., 2019). In legumes, Buszewski et al. (2019) showed that SC-CO_2 (16% ethanol) extraction increased polyphenol removal from germinated lupine seeds compared to conventional extraction processes. Moreover, *t*-resveratrol from peanut kernels was removed using SC-CO_2 (3% ethanol), exhibiting greater selectivity than conventional methods (Jitrangsri et al., 2020). In addition to phenols, SC-CO_2 modified with 10% ethanol can also improve alkaloid extraction yield (Nossack et al., 2000).

Given that high oil content limits the extraction of proteins, it is important to remove lipophilic compounds when obtaining PIs (Nadar et al., 2018). Additionally, SC-CO_2 extraction can be of interest for removing off-flavors (beany, grassy, earthy) because most are linked to the oxidation of the lipid fraction (Xu et al., 2020). Similarly, enzymatic browning is another common problem that can occur during legume processing. In this case, the reaction occurs between phenolic compounds that bind to proteins, especially under conditions of oxidative stress, which causes a loss in the quality of the extracted proteins, and in many cases, changes in the properties of these proteins. Additionally, it must be considered that, depending on the legume, high levels of ANFs can remain in the final PIs; thus, special attention must be paid to these compounds (Voudouris et al., 2017).

SC-CO_2 extraction has been widely used to remove oil from legumes and other ATFs and ANFs, avoiding large amounts of toxic organic solvents used in extraction processes (Schutyser et al., 2015). The use of SF is nowadays expensive and thus it is justified when obtaining high value products such essential oils and other phytochemicals for cosmetic and pharmaceutical uses. However, considering the protein-lipid interactions and the harsh conditions applied during alkaline solubilization and isoelectric precipitation, removing hydrophobic compounds before this step is advisable. Therefore, this technique may play a fundamental role in the pretreatment of legumes and allows the process to start with an initial material rich in proteins and free of compounds that may affect its subsequent protein extraction.

2.5. High Hydrostatic Pressure

2.5.1. Removal of anti-nutritional and anti-technogical factors

HHP consists of applying elevated pressure (between 100 and 1000 MPa) on extractable materials. The high pressure causes matrix changes in plant materials and maximizes permeabilization of cell membranes because of deprotonation of charged groups and dissociation of salt bridges and hydrophobic bonds. Therefore, this methodology can be applied as a pretreatment or during the extraction process. Both strategies will improve internal mass transport and the extraction of different bioactive compounds from plant cells (Grassino et al., 2020). Baier et al. (2015) used HHP (20 °C, water solvent, 1:1 w/v, 400 MPa, 10 min) as a pretreatment for pea seeds to improve further separation of proteins and oligosaccharides. The extension of the HHP effect is dependent on the molecular size of the extracted solute.

2.6. High Power Ultrasounds

2.6.1. Removal of anti-nutritional and anti-technogical factors

HPU addresses mechanical waves at high frequencies (>20 kHz) to modify products or processes. In liquid media, cavitation of air bubbles is the main phenomenon associated with HPU. Cavitation releases a large amount of mechanical and thermal energy, which positively affects the extraction of biomolecules from a solid matrix (Gharibzahedi & Smith, 2020; Maroun et al., 2018) because it may affect both internal and external mass transport. Cavitation, pressure variation, and oscillating particle velocity, induce an increase in solvent turbulence, facilitating convective flow, which encompasses solvent penetration into the solid matrix and solute solubilization in the bulk fluid. Moreover, mechanical stress caused by HPU may induce structural effects in the solid matrix, affecting its integrity and increasing concurrent internal solute and solvent transport. Therefore, the use of HPU for the intensification of polyphenol and other bioactive compounds extraction from vegetal-solid matrices in liquid media has

been extensively studied (Chemat et al., 2020). HPU can increase the extraction rate, reduce solvent use, and modify extract composition. For instance, HPU has been employed to extract saponins from lentils, fenugreek, and lupine (75 °C, water solvent, 1:10 w/v, 60% amplitude, 15 min) (Navarro del Hierro et al., 2018). Hayta and İşçimen (2017) obtained the highest extraction yield of antioxidant compounds from chickpeas at 25 °C, water solvent 0.40 w/v, 36.16% amplitude (power), and 20.17 min of HPU treatment. Zhang and Wang (2016) found that water + ethanol (40%) solvent (1:20 w/v ratio) at 25 °C for 30 min with three rounds of extraction treatment represented the optimal conditions for maximizing the polyphenol extraction from red beans (*Vigna angularis*). Moreover, Miano et al. (2019) claimed that employing this technology (25 °C, water, 25 kHz, 41 W/L, 300 min) for the hydration of lupine seeds before alkaloid extraction improved the removal yield of these ANFs by up to 21% compared to that from conventional hydration. Aguilar-Acosta et al. (2020) demonstrated that HPU treatment (63 °C, water solvent, 1:10 w/v, 100% amplitude, 10 min) reduced the alkaloid concentration by 50% in lupine compared to that from conventional extraction. Additionally, a 50% improvement in polyphenol extraction from yellow soybeans using HPU (25 °C, pure acetone solvent, 30% amplitude, 10 min) was demonstrated by Đurović et al. (2018). Therefore, by optimizing the treatment conditions (temperature, solvent type, solute/solvent ratio, supplied power, and time) for each legume and unwanted compounds, HPU can significantly improve the extraction yield of desired compound and shorten the extraction time.

Previous studies on the use of the emerging technologies described above have demonstrated their potential for ANF and ATF removal from legumes (Table 2) (Patonay et al., 2019; Romero-Díez et al., 2019). However, further research should be conducted to analyze their use in an integrated process to isolate the protein fraction and

their impact on the techno-functional and health-related properties of the PIs. Additionally, these emerging technologies should not be considered individually because their combination could be beneficial for removing unwanted compounds present in legumes. In this regard, Đurović et al. (2018) reported that the combined application of HPU and MWs resulted in a synergistic effect, leading to increased extraction of phenolic acids from yellow soybeans.

2.6.2. Extraction of protein

Ultrasound-assisted extraction is one of the most efficient technologies for greater protein extraction (Tassoni et al., 2020). Ultrasound increases the protein extraction yield because of cavitation, which causes structural damage and favors the release of proteins into the solvent (Byanju et al., 2020). It reduces particle size and improves the mixing between protein and solvent and, in consequence, solubilization (Chemat et al., 2020). Therefore, HPU could be used as either a pretreatment that facilitates the release of the legume proteins and/or improves the solubilization during extraction. However, it is important to highlight that sonication can modify protein structure (Byanju et al., 2020).

The use of HPU in the protein extraction of Ganxet beans was investigated by Lafarga et al. (2018), who optimized the pH and solvent concentration to maximize protein extraction. According to the experimental results, the use of ultrasound improved protein extraction yields. Ultrasonication for 60 min using 0.4 M NaOH as the solvent presented the maximum extraction conditions. As explained above, the cavitation phenomenon promotes both cell wall disruption and higher diffusion of the solvent into the cell material, which enhances mass transfer (Ochoa-Rivas et al., 2017). Moreover, strong alkali conditions and higher NaOH concentrations than the other conditions tested in this study also favored protein solubility and cell wall disruption

549 (Lafarga et al., 2018). Finally, neither increased NaOH concentration nor the use of
550 ultrasound resulted in protein degradation or fragmentation. Therefore, the use of an
551 HPU-assisted technique in combination with sequential alkaline extraction and acid
552 precipitation resulted in a highly efficient procedure to recover proteins from Ganxet
553 beans (Lafarga et al., 2018).

554 The HPU-assisted extraction of proteins from defatted peanuts also reduced material
555 particle size and increased protein yield while reducing the extraction time compared to
556 conventional extraction (Nguyen & Le, 2019). Interestingly, these authors reported that
557 an increase in pH reduced protein yield. They concluded that an increase in pH also
558 increased the viscosity of the solvent/material mixture, which reduced the cavitation
559 phenomenon, thereby decreasing protein yield. Additionally, an increase in ultrasonic
560 power above 30 W/g, extraction time more than 15 min, or temperature above 50 °C did
561 not affect or diminish the extraction of proteins. This confirms that mild extraction
562 conditions are better than extremes (very low or very high parameters); thus, a correct
563 selection of the extraction parameters is necessary to optimize the process and maximize
564 protein yield (Nguyen & Le, 2019). Comparable results were reported by other authors,
565 who observed that the extraction efficiency of peanut protein improved with the use of
566 an HPU-assisted procedure in comparison with alkaline extraction (Sun et al., 2021). In
567 this study, the application of 3.17 W/cm^3 at 35 °C for 30 min was the best condition for
568 protein extraction. Similarly, these authors reported that prolonged ultrasound time
569 promoted the aggregate formation of peanut protein molecules, whereas the application
570 of temperatures higher than 35 °C reduced the yield, which could negatively affect
571 protein extraction (Sun et al., 2021).

572 HPU technology has also been applied to lupine protein extraction (Aguilar-Acosta
573 et al., 2020). Different results were obtained depending on the lupine cultivar used. For

574 *L. mutabilis*, the application of ultrasound for 10 min had a beneficial effect on protein
575 yield, but longer sonication times (15 min) negatively influenced the yield. This could
576 be related to extreme protein damage caused by the ultrasound, promoting protein
577 aggregation and decreasing its solubilization (Aguilar-Acosta et al., 2020; Nguyen &
578 Le, 2019; Sun et al., 2021). This aggregation effect could also have positive
579 implications in the acid-precipitation stage, which is less explored in ultrasonic
580 intensification. However, ultrasound did not significantly affect the protein extraction of
581 *L. angustifolius*, but it is important to highlight that an average increase of
582 approximately 10% in protein yield was observed with HPU treatment for 15 min.
583 Additionally, *L. angustifolius* had a lower protein yield than *L. mutabilis*, which could
584 be related to the differences in the flour particle size and protein composition or
585 structure (Aguilar-Acosta et al., 2020).

586 Similarly, HPU as a pretreatment for kidney beans and soybeans improved the
587 protein extraction yields from soy flakes, and to a lesser extent, soybean flour and
588 kidney beans (in both cases, HPU-assisted extraction increased protein yields by
589 approximately 7%, although this was not significant) (Byanju et al., 2020). In contrast,
590 the sonication treatment reduced the extraction yield in chickpeas, which is attributable
591 to the high-lipid content of this legume, which reduces protein dissolution during the
592 extraction step because of protein-lipid interactions. In addition, a high oil content limits
593 the extraction of proteins because lipid-protein cross-links are generated, which reduces
594 the access of the solvent to the proteins in the cell matrices (Byanju et al., 2020). This
595 shows that the correct removal of lipids in the early stages is very important, not only to
596 prevent a reduction in the extraction of proteins but also to minimize the appearance of
597 off-flavors (Xu et al., 2020). Another possible explanation for the reduced extraction is
598 the high carbohydrate content of chickpeas, which could create a gel that negatively

affects protein accessibility. These authors also noted that the protein band patterns for both HPU-assisted samples and untreated legumes were similar, which indicated that the peptides did not undergo alterations. Moreover, the use of HPU did not affect the secondary structure of proteins extracted from soybean flakes, soybean flour, and chickpeas while unfolding and destabilizing the protein structure of kidney bean protein (Byanju et al., 2020).

In another study, the authors intensified soy protein extraction using HPU treatment (lab-scale experiment) of protein slurry and okara (the insoluble residue) (Preece et al., 2017a). In the soy protein slurry, the application of ultrasound (from 1 to 15 min) improved protein extraction, but there was no benefit in performing HPU-assisted extraction for more than 5 min because the maximum yields were already achieved. The same trend was observed in okara, with similar protein yield values between 5 and 15 min of ultrasound application, which did not justify using this treatment for more than 5 min. Therefore, in the lab-scale experiment, the authors concluded that ultrasound treatment increased protein extraction (Preece et al., 2017a). However, when the same authors used a pilot-scale extractor, they observed that HPU-assisted extraction of proteins from soybean processing materials was not recommended for industrial use (Preece et al. 2017b). In this case, although HPU improved the protein extraction yield during okara solution treatment, they concluded that considering the entire soybase production process, the results obtained using ultrasound treatment were comparable to traditional processes applied to okara at the pilot scale. Therefore, considering the life of the ultrasonic probe and the energy input, the authors did not consider ultrasound the most beneficial operation to improve protein extraction (Preece et al. 2017b). However, for mild ultrasonic applications, ultrasonic baths could also be considered for

applications because it minimizes surface erosion and the migration of metal ions to the solvent.

2.7. Enzyme-assisted extraction

2.7.1. Extraction of proteins

As mentioned above, the presence of different polysaccharides (cellulose, hemicellulose, or pectin) in the cell wall negatively affects the extraction of proteins from plant sources with conventional extraction techniques (Nadar et al., 2018). Additionally, protein extraction from the inner cell is limited by the high-molecular-weight of proteins. Therefore, both carbohydrates and proteases can be used to improve protein extraction from legumes (Voudouris et al., 2017). The selective activity of the enzymes that hydrolyze carbohydrates, under optimal conditions of temperature and pH, allows degradation of the plant cell wall, releasing the intracellular compounds of interest (Nadar et al., 2018). In addition, proteases partially degrade high-molecular-weight proteins into smaller proteins, and consequently, more soluble proteins (Baker & Charlton, 2020). Enzyme-assisted extraction is an extraction procedure that consumes little energy. It exhibits a rapid extraction rate while reducing the need to use solvents compared to traditional methods, and the selection of enzymes with synergistic effects can improve extraction yields (Nadar et al., 2018). Although protein extraction efficiency using an enzyme-assisted process is lower than that of chemical extraction processes (e.g., alkaline extraction), it would be interesting to have a pretreatment (cell wall disruption) followed by conventional chemical extraction. Consequently, it is clear that rather than an extraction process, it could be a very useful tool in conjunction with alkaline extraction. Additionally, enzyme-assisted extraction combined with other techniques, such as ultrasound, MW, or high-pressure extraction, has been accepted as a

powerful technique for extracting plant compounds (Nadar et al., 2018). However, enzymes are associated with drawbacks, such as high price or scaling-up for optimization (Baker & Charlton, 2020). Therefore, the synergy of enzyme-assisted extraction with other emerging techniques could be used to overcome these drawbacks.

A comparison between alkaline extraction, enzyme-assisted extraction, and a combination of protein recovery from defatted soy grit was investigated (Perović et al., 2020). The extraction time (1, 2, and 3 h) during alkaline extraction increased the protein yield. In contrast, in enzyme-assisted extraction, the use of both individual enzymes (cellulase, pectinase) and enzyme complexes (a commercial mixture of enzymes) improved protein extraction with the highest protein yield achieved by the commercial enzyme complexes. Contrary, xylanase did not affect protein yield. This occurred because protein cocktails enhanced protein extraction compared to individual enzyme treatments (Perović et al., 2020), probably because of a synergistic effect. Finally, an enzyme-assisted pretreatment followed by alkaline extraction (with an enzyme mixture) improved protein yield. In this case, the application of a combined enzymatic (1 h) and alkaline (1 h) extraction resulted in the highest protein yield and the shortest processing time. Therefore, the application of the enzymatic procedure improved protein extraction and reduced the alkaline extraction time, which positively affected the functional properties of the protein isolate (Perović et al., 2020).

As a general conclusion, the application of sustainable and emerging technologies, such as pretreatment (removing undesirable compounds or disrupting cell walls) or during extraction (increasing solvent-protein mixture or facilitating protein solubilization) of legume proteins has multiple advantages, such as reducing solvent use, processing time, waste production, and energy expenditure.

673

674 ## 3. Potential uses of emerging technologies for protein functionalization and
675 structuring

676 Proteins are versatile components that establish complex interactions with other
677 food constituents and their environment. Their physicochemical properties (e.g., charge,
678 surface hydrophobicity, molecular weight), function, and structure at different scales
679 influence the appearance, flavor, and color of foods (Foegeding, 2015; Mirmoghtadaie
680 et al., 2016). As a consequence of alkaline solubilization and isoelectric precipitation,
681 the physicochemical and techno-functional properties (water absorption, oil binding,
682 viscosity, gelling properties, and ability to form emulsions and foams) of proteins may
683 change, leading to PCs and PIs with decreased technological functionality (Chéreau et
684 al., 2016).

685 The increasing demand for plant-based products has led to the employment of high-
686 quality plant ingredients with tailored functionalities, which can be achieved using
687 different processing strategies (Zha et al., 2019). Recent reviews have focused on the
688 effects of different physical, chemical, and biological processing techniques on the
689 functionality of plant proteins (Akharume et al., 2021; Gharibzahedi & Smith, 2020,
690 2021). Thus, various physical technologies can be used not only to assist extraction
691 processes to obtain PCs and PIs with better yields but also to improve their techno-
692 functional properties. Furthermore, these technologies can be applied at different
693 processing points to functionalize and structure proteinaceous ingredients after their
694 extraction processes (Manassero et al., 2018a; Wang et al., 2020a). Moreover, they can
695 be combined with other processes, such as enzymatic hydrolysis, to obtain functional
696 food ingredients (Al-Ruwaih et al., 2019). In Table 4, we summarize the most important

techno-functional properties of proteins and how they can be improved using emerging technologies to obtain tailored protein ingredients from legumes.

3.1. Solubility

Solubility is one of the most important techno-functional properties of proteins because it directly impacts other functional properties. Thus, enzymatic hydrolysis is considered one of the most relevant methods for modifying tailor-made protein preparations and is typically used to improve the solubility and surfactant properties of proteins (Chéreau et al., 2016; García Arteaga et al., 2020). However, several emerging technologies have also shown promising results.

PEF treatments cause partial unfolding of proteins, enhancing interactions between other protein molecules and the surrounding media. At lower treatment intensities, egg white protein changes lead to increased solubility because of enhanced interactions with water. At higher treatment intensities, PEF induces total unfolding, denaturation, and the formation of insoluble protein aggregates with disulfide bonds as the dominant binding forces and a lower contribution of noncovalent bonds compared to that of thermally-induced protein aggregates (Zhao et al., 2009). Similarly, Li et al. (2007) found that the solubility of soybean PIs increased with increasing PEF strength, with the greatest increase at 30 kV/cm and 288 μs of treatment time. Above these conditions, the increase in solubility was lower because of protein denaturation and aggregation. In another work at lower field strength (1.65 kV/cm) but much longer treatment time (20,000 MEF pulses of 5 μs, total treatment time of 0.1 s) with pea PCs at pH 6, the treatment decreased protein solubility, whereas no effect was obtained on pea PCs at pH 5 (Melchior et al., 2020). Thus, the effects of PEF are dependent on protein nature and pH.

In general, HPU treatment improves the solubility of PI (Table 4) as a result of cavitational forces that lead to the partial unfolding of proteins, changes in their secondary and tertiary structures, and structural reorganization from large and irregular aggregates to small and uniform particles (Gharibzahedi & Smith, 2020). The increase in solubility has been attributed to a higher exposure of hydrophilic regions of proteins that enhance protein-water interactions (Gharibzahedi & Smith, 2020; Mirmoghtadaie et al., 2016). Additionally, a higher ultrasound intensity or treatment time induces greater exposure of internal hydrophobic regions of insoluble protein aggregates, which can be solubilized because of the formation of smaller aggregates stabilized by hydrophobic interactions, hydrogen bonds, and van de Waals forces (Gharibzahedi & Smith, 2020; Mirmoghtadaie et al., 2016). Furthermore, HPU treatment at 20 kHz for 15 min induced the greatest solubility increase in soy protein concentrate, whereas the greatest effect on protein isolates (PIs) was obtained at 40 kHz (Jambrak et al., 2009). Sonication of defatted soy flakes at an amplitude of 84 μm for 2 min also improved the solubility by 34% (Karki et al., 2009). Similarly, soy protein isolate solubility increased after 550 W treatment, which was related to a decrease in particle size (Ren et al., 2020).

In pea PIs, HHP treatments have been reported to cause a slight decrease in solubility (Chao et al., 2018). However, in other studies using PIs from different legumes, the solubility increased, thereby suggesting that this effect may depend on various conditions, such as the applied pressure, pH, and composition of the media in which proteins are dispersed (Li et al., 2011; Piccini et al., 2019). Overall, pressurization may improve the solubility of PIs by splitting aggregates while causing partial denaturation of proteins (Gharibzahedi & Smith, 2021; Manassero et al., 2018b). Alternatively, HHP can be used under mild pressure conditions (100–400 MPa) to increase the solubility of soybean PIs by binding phenolic compounds and inducing

747 glycation reactions with polysaccharides (e.g., flaxseed gum) (Chen et al., 2019; Liu et
748 al., 2020).
749
750 **3.2. Water and oil absorption capacities**
751 Water absorption capacity (WAC) is crucial in viscous foods, such as soups and
752 baked foods. The ability of proteins to imbibe water without dissolving helps provide
753 body, thickening, and viscosity (Sreerama et al., 2012). Oil absorption capacity (OAC)
754 plays an important role in many textural and quality properties of foods, including
755 flavor absorption and dough quality. These interactions are mainly attributed to the
756 physical entrapment of lipids, led by interactions with protein nonpolar side chains,
757 which are particularly numerous in proteins of plant origin. Therefore, the WAC and
758 OAC of proteins depend on the nature and physical modifications caused by food
759 processing (Li et al., 2007; Shevkani et al., 2019).
760 From the limited available data, it can be suggested that PEF can improve WAC or
761 OAC because of water or oil entrapment within a protein network resulting from the
762 formation of aggregates stabilized by disulfide bonds (Zhang et al., 2017). Melchior et
763 al. (2020) found that a PEF treatment of 1.65 kV/cm at 0.1 s of total treatment time
764 increased the WAC of pea PCs and both WAC and OAC of gluten concentrate at pH 6,
765 although the OAC decreased in pea PCs at pH 5. Therefore, the effect of PEF depended
766 particularly on the protein type and pH of the suspension.
767 Concerning HPU, the extension of treatment time improved WAC, attributed to
768 increased solubility and decreased particle size of the PIs (Wang et al., 2020a). The
769 same explanation was provided for the improved WAC in pea PIs obtained using the
770 HPU-assisted alkali method compared with that of the control method (Wang et al.,
771 2020b). These authors also reported an improvement in the OAC by HPU, which could

772 be caused by the exposure of hydrophobic groups or regions. However, in soy PIs, the
773 WAC did not improve as a result of HPU, although the OAC increased because of the
774 exposure of hydrophobic groups upon sonication and heating (Paglarini et al., 2019).

775 MW treatments cause an increase in temperature, generating similar effects as
776 conventional heating (Gomaa et al., 2013). However, canola seed pretreatment with
777 MWs or ultrasound led to PIs with improved WAC and OAC (Li et al., 2021). MW and
778 HPU pretreatments could have unfolded the protein molecules and increased the
779 exposure of hydrophilic amino acids and negative charges, leading to the increased
780 WAC, which together with increased exposure of hydrophobic and nonpolar side chains
781 led to a higher OAC.

782 Different legume PIs resulted in increased WAC after HHP exposure at moderate
783 pressures (300–400 MPa) (Gharibzahedi & Smith, 2021; Peyrano et al., 2016). The
784 unfolded conformation resulting from HHP treatments might provide linkages between
785 the protein subunits in a flexible network to entrap water molecules.

786

787 **3.3. Gelation properties**

788 The ability to gel is another important techno-functional property to consider and is
789 related to the capability of proteins to form a tridimensional network. Gels can be
790 induced by temperature, the addition of salt, a change in pH, and the addition of
791 enzymes or chemical cross-linkers. Cooked meat products are examples of heat-induced
792 gels, whereas cheese and yogurt are examples of cold gelation processes. However,
793 several facilitating technologies may also cause changes in the gelling properties of
794 proteins (Nunes & Tavares, 2019). In general, higher surface hydrophobicity and free
795 thiol groups favor the formation of protein aggregates and gels (Wu et al., 2020).

796 The cavitation effects of HPU treatments on protein solutions enhance solubility,
797 reduce particle size, and induce partial protein unfolding with increased exposure of
798 sulfhydryl and hydrophobic groups, which facilitate the formation of protein-protein
799 interactions to form dense, uniform, and stable gel structures with high WAC and OAC
800 (Gharibzahedi & Smith, 2020). In soybean PIs, ultrasound pretreatment (20 kHz, 400
801 W, 5 min) enhanced the WAC of the resulting gels induced by calcium sulfate (Hu et
802 al., 2013). Moreover, sonicated soybean PIs with soybean oil, inulin, and carrageenan
803 formed an emulsion gel with increased OAC (Paglarini et al., 2019). However, HPU
804 treatments can improve pea PI yields and reduce the gelling concentration of the
805 resulting PIs (Wang et al., 2020b). Additionally, thermal-, acid-, and calcium-induced
806 gelation of soybean and chickpea proteins pretreated with HPU resulted in greater
807 gelling ability and greater gel hardness (Khatkar et al., 2020; Wang et al., 2020a; Wang
808 et al., 2020c). Factors, such as exposure time, can be crucial because ultrasound
809 treatments at 20 and 40 kHz for 15 min induced rapid gelling of soy PCs, whereas this
810 effect did not occur when PCs were treated for 30 min (Jambrak et al., 2009).
811 Protein gels can also be influenced by other emerging and sustainable technologies.
812 In this sense, the cold-set gels of whey protein aggregates formed during ohmic heating
813 combined with MEF were weaker, more elastic, and had higher water retention and
814 swelling capacity than those heated in a conventional heat exchanger (Rodrigues et al.,
815 2020b). In soybean PIs and wheat gluten mixtures pretreated with different MW power
816 and further cross-linked by the addition of transglutaminase, gel strength and firmness
817 improved (Qin et al., 2016). HHP treatments have also been shown to increase the
818 number of hydrophobic regions and free sulfhydryl groups in various PIs and PCs,
819 which may explain their improved rheological and gelling properties (Akharume et al.,
820 2021; Gharibzahedi & Smith, 2021). Pretreatments with HHP enabled stronger cowpea

821 PI heat-induced gels, which formed at lower temperatures (Peyrano et al., 2019).
822 However, when comparing the characteristics of heat- and HHP-induced pea PI gels, the
823 latter were softer than those obtained by thermal treatments and required higher protein
824 concentrations to gel (Peyrano et al., 2021; Sim et al., 2019). These differences occurred
825 because heat-induced gels had a higher proportion of strong linkages than did HHP-
826 induced gels (Peyrano et al., 2021).
827
828 **3.4. Emulsifying properties**
829 Salad dressings, butter, mayonnaise, and other food products depend on the ability
830 of proteins to form and stabilize oil-in-water and water-in-oil emulsions.
831 Very little data are available on the effects of PEFs and other emerging and
832 sustainable technologies on legume protein emulsions. However, Xiang et al. (2011)
833 found that PEF-treated soymilk viscosity increased with increasing electric field
834 intensity and the number of pulses. PEF pretreatment increased the emulsion capacity
835 and emulsion stability of canola PIs after oil extraction (Zhang et al., 2017).
836 The replacement of organic solvents, such as hexane, with SFs may also be
837 advantageous for the overall quality and functionality of defatted proteins, as reported
838 for canola seeds (Li et al., 2021), corn germ (Espinosa-Pardo et al., 2020), and soy flour
839 (Kang et al., 2017). Kang et al. (2017) also noted that defatted soy flours with SF CO_2
840 led to improved emulsifying properties compared to conventional extraction with
841 hexane, which could be caused by the higher protein content of the resulting PIs.
842 However, further studies are needed because the emulsifying properties of PI obtained
843 from other plant proteins were not improved by fat extraction with SF CO_2 (Abirached
844 et al., 2020; Li et al., 2021).

Ultrasound enhances the emulsifying properties because of a decrease in particle size and viscosity, which facilitates the adsorption of proteins to the oil-water interface and reduces interfacial tension (Gharibzahedi & Smith, 2020; Ren et al., 2020). Hence, HPU treatments (20–500 kHz, 15–30 min) increased the emulsifying activity and emulsion stability of soy PIs and PCs (Jambrak et al., 2009; Ren et al., 2020). de Oliveira et al. (2020) found an important effect of pH on the emulsifying properties of ultrasound-treated (562 W, 427 s) pea PIs, with improvements at pH 2.8 and 6.8. However, emulsification capacity could also accompany HPU treatments, as shown in defatted soy flakes treated at 20 kHz and 21 μm amplitude for 60 s (Karki et al., 2009).

PIs from MW-pretreated rice bran resulted in improved emulsifying properties (Khan et al., 2011). Similarly, MW-assisted alkaline extraction of peanut flour resulted in PIs with improved emulsifying properties (Ochoa-Rivas et al., 2017). These improvements could be related to MW-induced unfolding and grafting reactions between soy proteins and different saccharides (Guan et al., 2011).

As stated previously, the treatment of PIs from different legumes with HHP causes structural unfolding and partial denaturation, leading to higher exposure of hydrophobic groups (Gharibzahedi & Smith, 2021). These changes modify the interfacial properties of proteins and can explain the formation of smaller emulsified particles (Chao et al., 2018; Manassero et al., 2018b). The reported reduction in droplet size, high ζ-potentials, and the likely formation of rigid membranes could explain enhanced emulsion stability (Manassero et al., 2018a). However, structural changes and the modification of ζ-potential induced by HHP appear to be pH-dependent, which can explain controversial results (Manassero et al., 2018a; Manassero et al., 2018b). In the presence of tea polyphenols, the emulsifying properties of soybean PIs have also been improved by

applying mild pressure conditions (100–400 MPa) because of the binding of phenolic compounds (Chen et al., 2019).

3.5. Foaming properties

The ability of proteins to form stable foams is crucial in foods such as cakes, soufflés, whipped toppings, and ice creams. Although proteins are the most commonly employed foaming agents in the food industry, their ability to foam differs greatly. The presence of multiple hydrophobic sites facilitates protein interactions and the formation of an air-water interface (Sosa et al., 2020). Consequently, the higher concentration of protein with surface-active groups in soy flour defatted with SF CO_2 could explain the improved foaming capacity and stability compared to defatted flour with hexane (Kang et al., 2017). Additionally, SFs can also be used to encapsulate compounds and improve fat dispersion through the particles from the gas saturated solutions (PGSS) method (Saldanha Do Carmo et al., 2016). These authors found that using this engineering process, pea proteins led to improved foaming stability, which could be related to the effects of applied dynamic high-pressure homogenization and increased surface hydrophobicity of proteins.

The foaming properties of legume proteins can also be improved by HPU because of the increase in surface hydrophobicity induced by cavitation, thereby resulting in a reduction of surface tension at the air-water interface (Gharibzahedi & Smith, 2020; Xiong et al., 2018). However, divergent results have been reported in the literature. For instance, ultrasound treatments (20–500 kHz, 15–30 min) increased the foaming capacity and stability of soy PCs (Jambrak et al., 2009). Xiong et al. (2018) found that the foaming ability of pea PIs increased after ultrasound treatments (20 kHz, 30 min) while foaming stability increased with increasing amplitude after 10 min, it decreased

with greater time. Morales et al. (2015) found an increase in foaming capacity of soy PIs, which was related to a reduction of particle size, although foam stability was not affected. In another study, HPU treatment (20 kHz, 10 min) improved the foaming capacity but reduced the foam stability of soy PIs (Ren et al., 2020). Foaming capacity could also be decreased by HPU treatment, as shown by Karki et al. (2009) in defatted soy flakes treated at 20 kHz and 21 μm amplitude for 60 s, although no change in foaming stability was observed. In this case, sonication might have altered the ability of soy proteins to unfold at the interface, resulting in poor surface activity.

MW-assisted extraction of peanut proteins resulted in PIs with improved foaming activity but decreased foaming stability (Ochoa-Rivas et al., 2017). However, MW-assisted extraction has exhibited controversial results when comparing PIs from different plant proteins (Jiang et al., 2021; Sun et al., 2017). Wastewater from cooked legumes (aquafaba) contains high quantities of proteins with excellent foaming properties. The comparison between conventional cooking and the combined cooking and microwaving method of aquafaba from lima beans resulted in no differences in the foaming and texture properties of vegan cupcakes in which the formulation egg was replaced by aquafaba (Nguyen et al., 2020).

In HHP-treated pea PIs, exposure up to 400 and 600 MPa increased the foaming capacity, whereas foaming stability depended on protein concentration (Chao et al., 2018). In agreement with these results, the foaming capacity of soybean PIs has been reported to increase in the range of 200–300 MPa and 5–15 min (Li et al., 2011). Kidney bean PIs exposed to 300 MPa also exhibited better foaming capacity than the control, whereas no differences were found in foam stability (Al-Ruwaih et al., 2019). Therefore, HHP intensity and exposure time seem to influence foaming capacity and stability.

3.6. Texturization: extrusion

The presence of fibers is a characteristic of many meat products. Thus, various methods have been proposed to imitate the fibrous texture of meat (Kumar et al., 2017). However, the only industrially viable option to functionalize and structure plant-based materials into fibrous products is extrusion (Dekkers et al., 2018). In this process, proteins are plasticized/molten inside the barrel by a combination of heating, hydration, and mechanical deformation. Depending on the moisture content, we can differentiate between high-moisture (50–80%) extrusion, in which texturized proteins present a fibrous texture that is more similar to meat, and low-moisture (<30%) extrusion, which generally forms texturized proteins with a sponge-like structure and hard texture that are moisturized afterward (Akharume et al., 2021; Dekkers et al., 2018). In the latter case, protein-rich fractions of legumes can be used to make extrudates with decreased sectional expansion, increased density, and specific hardness with increasing protein content (from 30% to 50%), which could be counteracted by preconditioning of the protein-rich ingredients (Martin et al., 2020). Jebalia et al. (2019) found that rupture stress and strain of pea flour and pea starch-protein composites obtained by low-moisture (25–35%) extrusion were negatively correlated with their interface index. Therefore, a higher interface index of the pea flour composite was related to increased brittle behavior (Jebalia et al., 2019).

Regarding the production of meat-like products, the control of shear and heat during high-moisture extrusion of soy protein facilitates structuring similar to muscle tissue (Jones, 2016). The formation of meat-like anisotropic structures from soy PCs occurred with increased extrusion temperature (100–143 °C). Under these conditions, protein-protein interactions were not influenced, and the authors concluded that changes in

polysaccharides present in soy PCs could be responsible for the change in the rheological properties (Pietsch et al., 2019). The interaction between barrel temperature (120 and 150 °C) and feed moisture (20, 24%) affected the expansion ratio of chickpea flour extrudates. Greater expansion occurred at higher temperatures, negatively correlated with the hardness and bulk density (Wang et al., 2019). Several studies have described the development of meat analogs with fibrous structures using high-moisture extrusion of legume PIs and PCs (Vatansever et al., 2020). Other shearing devices have also shown promising results for physical structuring, but they require further development to produce fibrous textures at an industrial scale (Jones, 2016).

4. Health effects of the technologically obtained PIs

Consuming the recommended quantity of good-quality protein is essential for optimal human growth, development, and health (Wu, 2016). The effects of plant proteins, including legumes (peas, lupine, fava beans, and lentils), have recently been reviewed, confirming the health-promoting effects of these extracts on glycemic, appetite, cardiovascular, and muscular outcomes (Lonnie et al., 2020). The benefits of technological treatment of these protein sources to remove ANFs have already been stated. Furthermore, as summarized in Figure 3, the treatments performed during protein extraction and functionalization of PIs may lead to protein structure changes with potential benefits beyond their role as a macronutrient.

Individuals become sensitized to dietary food allergens via the gastrointestinal tract during ingestion. During the process of digestion, dietary proteins can be broken up and produce peptides that could exhibit potential antigenicity (Verma et al., 2013). In particular, legumes play an important role in food allergies, with increased sensitization to legumes among populations from Mediterranean and Asian countries and Western

969 countries in the last few years. Immunoglobulin E (IgE)-binding proteins have been
970 identified in most legumes and are responsible for reactions from mild skin irritations to
971 life-threatening anaphylactic shock in sensitized individuals after their ingestion or
972 inhalation. In soybeans, one of the most widely utilized legumes in the food and feed
973 industries, the two most important antigenic proteins are glycinin and β-conglycinin,
974 with reactions more prevalent in children (He et al., 2015). These macromolecules enter
975 the lymph and blood through gaps between the intestinal epithelial cells and have
976 considerable antigenic activity that stimulates the immune system, resulting in specific
977 antigen-antibody reactions and T lymphoid cell-mediated delayed hypersensitivity (He
978 et al., 2015). Examples can be found for other legumes; for instance, the major
979 allergenic proteins associated with lupine sensitization are Lup-1, which is a β-conglutin
980 (vicilin-like protein), and Lup-2, which is an α-conglutin (legumin-like protein)
981 (Bingemann et al., 2019; Lucas et al., 2015). Lupine allergy may cause acute and severe
982 reactions, including anaphylactic shock and fatality (Anzani et al., 2020). Despite this,
983 lupine allergy is still quite rare, and thus its inclusion should be interpreted as a
984 precautionary measure and not as a real limitation (Lucas et al., 2015). Applying the
985 above-mentioned emerging technologies on legume processing, such as HPU, MWs,
986 and HHP, may reduce allergenicity because of the alteration of secondary protein
987 structure (Pojić et al., 2018). Changes in conformational epitopes, which are no longer
988 recognized by IgE antibodies, cannot activate the immune response (Pojić et al., 2018).
989 Although the application of these technologies opens up new possibilities for reducing
990 allergenicity, there are still a limited number of studies on this topic (Pojić et al., 2018;
991 Verhoeckx et al., 2015). Additionally, the extraction and functionalization treatments
992 can affect legume allergenicity differently, depending on a wide range of factors,

993 including the duration of the process, intensity, and presence of a food matrix (Aguilera,
994 2019).

995 Changes in protein structure derived from the isolation and processing might lead to
996 a potential reduction of allergens and the release of bioactive peptides. Peptides are
997 obtained from protein cleavage through enzymatic hydrolysis, microbial fermentation,
998 and food processing (Chakrabarti et al., 2018). Most studies on the effects of bioactive
999 peptides have focused on hydrolysates obtained through enzymatic hydrolysis using
1000 different protein sources, enzymes, and/or conditions to obtain the hydrolysates. Thus,
1001 enzyme-assisted extraction can help deliver health-promoting bioactive peptides. The
1002 most studied bioactivities for food hydrolysates are angiotensin-I converting enzyme
1003 (ACE) and dipeptidyl peptidase-IV (DPP-IV) inhibition. ACE inhibitors are used as
1004 targets for hypertension treatment, and *in vitro* studies have shown the ACE-inhibitory
1005 activity of legume hydrolysates and derived peptides, such as soybeans (Xu et al., 2021)
1006 and mung beans (Yi-Shen et al., 2018). DPP-IV inhibitors are used to treat diabetes
1007 development, in which some food-derived peptides might play a role. For example, *in*
1008 *vitro* studies have shown the DPP-IV inhibitory capacity of some peptides from soy and
1009 lupine (Lammi et al., 2016) and pigeon pea hydrolysates (Boachie et al., 2019). Other
1010 enzyme-inhibitory activities have also been shown for legume hydrolysates. Enzymatic
1011 digestion of black beans, green peas, chickpeas, and lentils has shown 3-hydroxy-3-
1012 methylglutaryl-coenzyme A reductase (HMGR) and pancreatic lipase (PL) inhibitory
1013 activity, with different and synergistic effects (Moreno et al., 2020). Inhibition of
1014 protein glycation, which could be related to the prevention of complications in diabetes,
1015 has been suggested for lentils (Kuerban et al., 2020). *In vitro* α-amylase inhibition has
1016 been observed in pigeon peas (Olagunju et al., 2020). Black bean, green pea, chickpea,
1017 lentil (Moreno et al., 2020), and pigeon pea hydrolysates (Olagunju et al., 2020) also

possess antioxidant activity. *In vitro* studies in different cell lines suggested the antiproliferative effects of lentils, which suggested potential anticancer effects (Kuerban et al., 2020). Thus, there is a wide spectrum of enzymatic inhibitory activities of legume hydrolysates, which point to them as an interesting source of bioactive peptides.

However, caution must be taken when considering effects derived mainly from *in vitro* studies because gastrointestinal digestion, which may lead to the further processing of the peptides, and absorption of the active peptides, must be considered. However, it is important to note that proteins and protein hydrolysates may also act at the gastrointestinal tract level. The intestinal peptides interact with receptors that activate the secretion of enterohormones, such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) or peptide YY (PYY), which are involved in a wide range of physiological and metabolic processes, such as appetite regulation, gastric motility, and glucose homeostasis (Roura et al., 2019). *In vivo* satiety effects and *ex vivo* secretion of CCK and GLP-1 were observed in soy (Yang et al., 2020) and pea (Häberer et al., 2011) protein hydrolysates. Another intestinal target with whole-body repercussions is the microbiota. There is evidence that soy protein, its hydrolysates, and peptides impact gut microbiota, although there is still no consensus on specific effects (Ashaolu, 2020). In turn, changes in the microbiota could lead to alterations in the gut barrier and inflammation. There is compelling evidence supporting the biological relevance of peptides released by either natural or artificial means from several dietary sources that act at different levels of the intestinal barrier (Martínez-Augustin et al., 2014). Intestinal anti-inflammatory effects have been shown for soybean proteins (Guha & Majumder, 2019).

Altogether, there is evidence of intestinal action of legume hydrolysates, which may have systemic effects regardless of peptide absorption. The methods used to obtain the

hydrolysates have not been detailed in this review, but the diversity of protocols used shows that several enzymatic hydrolysis conditions could lead to bioactive protein-derivates. Additionally, from this brief review, it appears that within legumes, several species could be chosen to obtain beneficial effects. These studies highlight the additional benefits of enzyme-assisted protein extraction.

There is less evidence regarding other techniques of protein extraction. There is no evidence of the biological effects of legume proteins exposed to MW-assisted protein extraction. HPU did not lead to changes in the molecular weight of chickpea or kidney bean protein; however, it exerts different effects on the secondary structure of proteins depending on the legume type (Byanju et al., 2020). Ultrasound treatment improves the release of bioactive peptides by enzymatic hydrolysis (Ashraf et al., 2020) or fermentation (Ruan et al., 2020). Changes in secondary structure induced by HPU-assisted extraction could modulate protein digestibility, but these effects require confirmation. Additionally, PEF-assisted extraction leads to changes in the secondary structure that could modulate protein functionality. In this regard, peptides obtained from soy protein have been shown to improve their antioxidant activity after PEF treatment (Lin et al., 2016).

Overall, a limited number of works have addressed the use of emerging technologies for ANF and ATF removal, or protein extraction, and assessed their effects on health properties of legume PIs. Therefore, more studies are needed to fully understand these effects.

5. Conclusions

Legumes have emerged as a sustainable protein source with a promising future as an alternative to meat and meat-based products. The selection of legume species should

emphasize their adaptation to local climatic conditions because of their high relevance for low-input agriculture. Additionally, low contents of ANFs, or other unwanted or undesirable components must also be considered because of their impacts on the extraction yield and techno-functional and health-related properties. Thus, special attention should be given to lipid, alkaloid, tannin, and saponin contents. Emerging technologies, such as PEFs, HPU, MW, and SFs, could be considered reliable and sustainable alternatives to intensify the removal of undesirable compounds from the protein fraction. Moreover, the discarded fractions containing unsaturated oil, carotenoids, or polyphenols could be further exploited for their bioactive properties, which adds value to the overall process and contributes to a circular economy. The use of the aforementioned emerging technologies may also be used as pretreatment or in assisted solubilization to intensify protein separation. Consequently, the phenomena caused by these technologies may facilitate protein solubilization and disruption of cell walls, which enhance protein yield and reduce solvent requirement, processing time, waste production, and energy consumption. Furthermore, the techno-functional properties of the PIs, such as solubility, foaming, emulsion, gelling, water binding, and oil binding capacities may also be modified. Therefore, it is possible to obtain tailor-made PIs with specific techno-functional properties. To improve the health-related properties of PIs, other approaches should be addressed. For this purpose, proteolysis induced by enzymatic hydrolysis or microbial fermentation could be of paramount importance because it leads to improved digestibility, reduced allergenicity, and the release of bioactive peptides. These effects could similarly be obtained using emerging technologies, although further research is required in this area. Finally, the application of novel physical and enzymatic processes to obtain high-quality and functional PIs offers interesting possibilities that should be explored in more detail; importantly, these

PIs could be more easily accepted by consumers than those obtained utilizing chemical processes.

Acknowledgements

This work was supported by the project LUPIPROTECH (Project PID2020-114422RR) from the Spanish "Ministerio de Ciencia e Innovación" and the "Agencia Estatal de Investigación." The authors also thank GAIN (Axencia Galega de Innovación) for supporting this review (grant number IN607A2019/01). Paola Navarro-Vozmediano acknowledges the FPU PhD contract (FPU19/03497) granted by the Spanish "Ministerio de Educación y Formación Profesional."

List of abbreviations

ACE, angiotensin-I converting enzyme

ANF, anti-nutritional factor

ATF, anti-technological factor

BNF, biological nitrogen fixation.

DPP-IV, dipeptidyl peptidase-IV.

GHG, greenhouse gas.

HHP, high hydrostatic pressure.

HPU, high-power ultrasound.

MEF, moderate electric fields.

MW, microwave.

OAC, oil absorption capactiy.

PC, protein concentrate.

PEF, pulsed electric fields.

PI, protein isolate.

1119 SF, supercritical fluid.

1120 WAC, water absorption capacity.

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1923 **Table 1**

1924 Most relevant compounds with undesirable effects in legume protein isolates.

Compounds	ANF[1]	ATF[1]	Reason[2]	Alternative use	References
Phenolic compounds (including phenolic acids, coumarins, flavonoids and tannins)	X	X	↓Yield, ↓purity, color effects, protein binding, ↓amino acid bioavailability	Food antioxidant, nutraceutical	Adrar et al. (2019) Alu'datt et al. (2013) Alu'Datt et al. (2014) Corrêa & Rogero (2019) Farha et al. (2020) Mondor et al. (2009)
Polysaccharides (including dietary fiber)	X	X	↓Nutrient Absorption, ↓yield, ↓solubility	Animal feed, food ingredient, nutraceutical	Chéreau et al. (2016) Nadar et al. (2018) Vong & Liu (2016) Vioque et al. (2012)
Alkaloids	X	X	Toxicity, bitterness	Nutraceutical	Aguilar-Acosta et al. (2020) Chaves et al. (2016) Klupšaitė & Juodeikienė (2015)
Carotenoids, tocopherols, phytosterols		X	↓Yield, ↓purity, color effects	Food coloring, food antioxidant, nutraceutical	Albuquerque et al. (2020) Moreno-Valdespino et al. (2020)
Phospholipids		X	Protein-lipid interactions, off-flavors generation	Food ingredients, cosmetics, nutraceutical	Sánchez-Vioque et al. (1998)
Protease inhibitors	X		↓Digestibility	Nutraceutical	Carbonaro et al. (2015) Mohan et al. (2015)
Phytates	X	X	↓Mineral bioavailability, ↓yield, ↓solubility	Nutraceutical	Bessada et al. (2019) Mondor et al. (2004)
Saponins	X	X	↓Absorption lipids, toxicity, ↓yield, ↓purity	Food, cosmetic, nutraceutical	Bessada et al. (2019) Navarro del Hierro et al. (2018) Reichert et al. (2019) Singh et al. (2017)
lectins	X		↓Absorption, impaired growth, red blood cell agglutination	Agricultural, nutraceutical	Bessada et al. (2019)
Alpha-galactosides	X	X	Flatulence	Animal feed, food ingredient, nutraceutical,	Martínez-Villaluenga et al. (2008)

			bioenergy production	
Reducing sugars	X	↓Yield, Maillard reactions	Animal feed, food ingredients, bioenergy production	Mondor et al. (2009) Zha et al. (2019)
Triacylglycerides	X	↓Yield, ↓purity, off-flavor precursors, protein-lipid interactions, polymerization reactions	Oilseed	Xing et al. (2018) Xu et al. (2020) Byanju et al. (2020)
Minerals	X	↓Yield, protein interactions	Agricultural	Boye et al. (2010)

Abbreviations: ANF, anti-nutritional factor; ATF, anti-technological factor.

[1] Those compounds considered as ANF and ATF are indicated with X.

[2] ↓ Denotes a decrease

Table 2

Recent applications of emerging technologies to improve the removal of ATFs and ANFs from legumes.

Material	ANF/ATF	Sustainable technique	Temperature (ºC)	Solvent	S/S ratio	Maximum extraction conditions	References
Soybean	Oil	MEF + Enzyme-assisted	70 – 90 50	Water	1:4/1:2 w/v	96 V/cm, 50 Hz, 10 min, 90°C + cellulase enzyme, 16 h	Pare et al. (2014)
	Polyphenols, phenolic acids	HPU, HPU + MW	25 25/55 - 85	Pure acetone		20 kHz, 30% amplitude, 10 min + 85°C, 2 min, 75 W	Đurović et al. (2018)
Chickpea	Polyphenols	HPU	25	Water	0,40 w/v	40 kHz, 36.16% amplitude, 20.17 min	Hayta & İşçimen (2017)
Red bean	Polyphenols	HPU	25	Water + Ethanol (40%)	1:20 w/v	50 kHz, 100 W, 30 min	Zhang & Wang (2016)
Lentil	Saponins	HPU	75	Water	1:10 w/v	60% amplitude, 15 min	Navarro del Hierro et al. (2018)
Lupine	Alkaloids	HPU	25	Water		(Hydration pretreatment) 25 kHz, water bath, 41 W/L, 300 min	Miano et al. (2019)
	Alkaloids	HPU	63 - 77	Water	1:10 w/v	24 kHz, 100% amplitude, 10 min, 63°C	Aguilar-Acosta et al. (2020)
	Saponins	HPU	75	Water	1:10 w/v	60% amplitude, 15 min	Navarro del Hierro et al. (2018)
	Polyphenols	SF	40 - 80	CO_2 + Ethanol (16.1%)		16.1% co-solvent, 147 bar, 40 min, 73°C	Buszewski et al. (2019)
Peanut	t-resveratrol	SF	50 - 70	CO_2 + Ethanol (3%)		483 bar, 50 min, 70°C	Jitrangsri et al. (2020)

Pods	Inositols	MW	50 - 120	Water	1:20 w/v	1200 W, 16.5 min, 120°C	Zuluaga et al. (2020)
Seeds	Inositols	MW	50 - 120	Water + Ethanol (17%)	1:20 w/v	1200 W, 21.5 min, 90°C	Zuluaga et al. (2020)
Pinto bean	Tannins	MW	60 - 75	Water		(Pretreatment) 1000 W, 2.45 GHz, 1 min	Dalmoro et al. (2018)
Pea	Oligosaccharides	HHP	20	Water	1:1 w/v	400 MPa, 10 min	Baier et al. (2015)

Abbreviations: ANF, anti-nutritional factor; ATF, anti-technological factor; HHP, high hydrostatic pressure; HPU, high-power ultrasound; MEF, moderate electric field; MW, microwave; SF, supercritical fluid; s/s ratio, solid/solvent ratio.

1943

1944 **Table 3**

1945 Emerging technologies for legume protein extraction.

Material	Technique	Extraction conditions	S/S ratio	T (ºC)	pH (extraction)	pH (precipitation)	Protein concentration	Optimum conditions	Protein yield (%)	Ref.
Soy grit	Enzyme-assisted	Alkaline extraction (1, 2 and 3h)	1:10	50	8	4.5	Freeze drying	Enzymatic assisted + alkaline extraction (1+1h)	40.87-45.93%	Perović et al. (2020)
		Enzymatic extraction (3h)			5.5					
		Enzymatic + alkaline extraction (1+1h)			5.5/8					
		Enzymatic + alkaline extraction (1+2h)			5.5/8					
Ganxet beans	HPU	Alkaline extraction (15 min)	1:10	4	12.06-12.94 (NaOH 0.1, 0.3, 0.3 & 0.4M)	5.5	Freeze drying	Ultrasound assisted (40 kHz, 250W, 60 min) in alkaline conditions (0.4M NaOH; pH 12.95)	78.73%	Lafarga et al. (2018)
		Ultrasound (30 or 60 min) in alkaline conditions			12.04-12.97 (NaOH 0.1, 0.3, 0.3 & 0.4M)					
Peanut meal	HPU	Ultrasonic power (0-60 W/g, 0-20 min)	1:5-1:20	40-70	7-10	-	-	Ultrasound assisted (30 W/g, 15 min, pH 6.8); 1:20 s/s; 50ºC	87.7%	Nguyen & Le (2019)
Lupine	HPU	Ultrasound (0-15 min, 24 kHz, 85 W/cm^2) in alkaline conditions	1:10	-	9	4.5	-	Ultrasound assisted (85 W/cm^2) during 10 or 15 minutes (depending on the cultivar)	~70%	Aguilar-Acosta et al. (2020)

Soybean, chickpea and kidney bean	HPU	Ultrasound (5 min, 20 kHz, 2.5-4.5 W/cm^3) in alkaline conditions	1:10	60	8.5	4.5	Freeze drying	Ultrasound assisted (4.5 W/cm^3) except for chikpea that present higher protein yield the untreated samples	Soy flakes (30.6-33.45%) Soy flour (50%) Kidney bean (51.4%)	Byanju et al. (2020)
Soy slurry and okara	HPU	Ultrasound (20 kHz, 400 W, 0-15 min) in alkaline conditions	1:6	50 (initial)	-	-	-	Ultrasound assisted (20 kHz, 5 min)	Soy slurry (~55%) Okara (~67%)	Preece et al. (2017a)
Peanut flour	-	Alkaline extraction	1:10	50	9	4.5	Spray-drying	Ultrasound assisted (24 kHz, 100% amplitude, 15 min)	~65%	Ochoa-Rivas et al. (2017)
	MW	Microwave (145-750 W, 2-10 min) in alkaline conditions	1:10-1:25	Variable						
	HPU	Ultrasound (24 kHz, 20-100% amplitude, 15-40 min) in alkaline conditions	1:10							
	MW + HPU	Microwave (725 W, 8 min) + ultrasound (24 kHz, 100% amplitude, 15 min) in alkaline conditions	1:10							

Symbols and abbreviations: -, not specified; HPU, ultrasound-assisted; MW, microwave-assisted; S/S, solid/solvent ratio.

1948

1949 **Table 4**

1950 Recent findings regarding the main effects of pulsed electric fields, high hydrostatic pressure, high-power ultrasounds and microwaves on
1951 techno-functional properties of legume proteins.

Legume	Applied matrix	Technology	Conditions	Sol.	WAC	OAC	Gel.	EC/ES	FC/FS	References
Soybean	Defatted PI	PEF	0-40 kV/cm, 0-547 µs	↑↓[1]	-	-	-	-	-	Li et al. (2007)
Pea	5% PC dispersion	PEF	1.65 kV/cm, 400 Hz, 0.1-0.3 s, pH 5-6	=↓[1]	↑↓	=↓[1]	-	-	↑/↑	Melchior et al. (2020)
Soybean	3% PI dispersion	HPU	20 kHz, 550W, 60 W/cm^2, 5, 10, 20, and 30 min, <35˚C	↑	-	-	-	↑/↑	↑/↓	Ren et al. (2020)
Soybean	10% PC dispersion	HPU	20 kHz, 750 W, amplitude 20%–40%, 10–20 min	↑↓	↑↓	-	↑↓	-	-	Khatkar et al. (2020)
Soybean	1-6% PI dispersion	HPU	400 W, 105–110 W/cm^2, 10 min	-	-	-	↑	-	-	Wang et al. (2020)
Soybean	11-12% PI dispersion	HPU	20 kHz, 30-40 W, 60 µm, 30 min	↑	=	↑	↑	-	-	Paglarini et al. (2019)
Soybean	Emulsions with 1% PI	HPU	20 kHz, 50–55W/cm^2, 40% amplitude, 2, 6, 12 or 18 min, 23˚C	-	-	-	-	↑/↑	-	Taha et al. (2018)
Soybean	≈1% PI	HPU	20 kHz, 600 W, 5 min,	↑	-	-	-	-	-	Huang et al. (2017)

	dispersion		25˚C							
Soybean	0.1-10 % PI dispersion	HPU	20 kHz, 34 W/cm^2, 2 min	-	-	-	-	=/=	-	O'Sullivan et al. (2016a)
Soybean	0.1-3% PI dispersion	HPU	20 kHz, 34 W/cm^2, 2 min	-	-	-	-	↑/↑	-	O'Sullivan et al. (2016b)
Soybean	10% PI dispersion thereafter exposed to transglutaminase	HPU	20 kHz, 400 W, 105-110 W/cm^2, 5-40 min, 14–20 ˚C	↑	↑	-	↑	-	-	Zhang et al. (2016)
Soybean	6% PI dispersion	HPU	20 kHz, amplitude 20%, 75, 80 and 85 ˚C	-	-	-	-	-	↑/=	Morales et al. (2015)
Soybean	10% PI dispersion thereafter exposed to transglutaminase	HPU	20 kHz, 400 W, 0-40 min, <20˚C	-	-	-	↑	-	-	Hu et al. (2015a)
Soybean	3% β-conglycinin and glycinin dispersions	HPU	20 kHz, 400 W, 5-40 min	↑	-	-	-	↑/↑↓[2]	-	Hu et al. (2015b)
Soybean	1% glycinin	HPU	20 kHz, 80 W/cm^2, 5-40 min, different ionic strengths	↑↓[1]	-	-	-	↑↓[1]/↑	-	Zhou et al. (2016)

Pea	1:6-1:12 raw pea powder to obtain pea PI	HPU	Optimized extraction conditions 750 W, amplitude 33.7%, 13.5 min, 25˚C	↑	↑	↑	↑	↑/↑	↑/↑	Wang et al. (2020)
Pea	5% PI dispersion	HPU	20 kHz, amplitude 30, 60, 90%, 22-48 W/cm^2, 30 min	-	-	-	-	-	↑/↑=	Xiong et al. (2018)
Pea	3% PI dispersion	HPU	20 kHz, 6.8 W/L, 5 min, < 35˚C	↑	-	-	-	-	-	Jiang et al. (2017)
Pea	0.1-10% PI dispersion	HPU	20 kHz, 34 W/cm^2, 2 min	-	-	-	-	↑/↑	-	O'Sullivan et al. (2016a)
Pea	0.1–3% PI dispersions and 10% rapseed oil emulsion containing 0.1–3% PI	HPU	20 kHz, 34 W/cm^2, 2 min	-	-	-	-	=/↑	-	O'Sullivan et al. (2015)
Chickpea	8% PI dispersion	HPU	20 kHz, 300 W, 5, 10, and 20 min	↑	↑	-	↑	↑/↑	↑/=	Wang et al. (2020)
Faba bean	10% PI dispersion	HPU	Optimized conditions 20 kHz, amplitude 72.67%, 16.1 min	↑	-	-	-	-	↑/↑	Martínez-Velasco et al. (2018)
Peanut	10% defatted peanut flour to obtain PI	HPU	24 kHz, amplitude 100%, 15 min	↓	↑	=	-	↓	↑/↓	Ochoa-Rivas et al. (2017)

Peanut	Peanut PI grafted with maltodextrin through HPU-assisted Maillard reaction	HPU	25 kHz, 250 W, amplitude 95%, 10-100 min	↑	-	-	-	↑/↑	-	Chen et al. (2016)
Soybean	10% PI dispersion thereafter exposed to laccase	MW	0, 120, 240, 360, 480, or 600 W for 1 min	-	↑	-	↑	-	-	Mu et al. (2020)
Lima bean	Aquafaba dispersion	MW	Cooking (100˚C for 30 or 60 min) vs. Cooking (100˚C for 15 or 45 min) + 840 W for 15 min	-	-	-	=	-	=	Nguyen et al. (2020)
Peanut	10% defatted peanut flour to obtain PI	MW	725 W, for 8 min	↓	↑	=	-	↑	↑/↓	Ochoa-Rivas et al. (2017)
Soybean	10% soy PI + 1% wheat gluten dispersion thereafter exposed to transglutaminase	MW	0, 70, 210, 350, 560 or 700W, for 1min	↓	↑	-	↑	-	-	Qin et al. (2016)
Soybean	10% Soybean white flakes	HHP	100 MPa, 200 MPa and 300 MPa, for 3	↑	-	-	-	-	-	Liu et al. (2020)

	incubated with flaxseed gum		days, 60 °C							
Soybean	1% PI dispersion	HHP	600 MPa, 5 min, 20˚C, with added Ca	↑	↑	-	↑	-	-	Piccini et al. (2019)
Soybean	0.5 mmol/L soy PI + tea polyphenols	HHP	200, 300 or 400 MPa, 10 min	↑	-	-	-	↑/↑	-	Chen et al. (2019)
Soybean	0.5% and 1% PI dispersed at pH 5.9 and 7	HHP	600 MPa, 5 min, 20˚C, with added Ca	-	-	-	↑	↑/↑	-	Manassero et al. (2018a)
Soybean	0.5% and 1% PI dispersed at pH 5.9 and 7	HHP	600 MPa, 5 min, 20˚C, with and without added Ca	↑	-	-	-	-	-	Manassero et al. (2018b)
Soybean	1% PI dispersions adjusted to different pH	HHP	600 MPa, 10 min, 20˚C	↑	-	-	-	-	-	Manassero et al. (2015)
Lentil	5% PI dispersion	HHP	300 MPa, 15 min, 20˚C	-	↑	-	-	↑/↓	↓/=	Ahmed et al. (2019)
Lentil	5% PI dispersion exposed to HHP and thereafter hydrolyzed	HHP	300 MPa, 15 min, 20˚C	-	=	-	-	↓/↓	=/↓	Ahmed et al. (2019)

Lentil	2% PC dispersion	HHP	100, 200, 300, 400, 500, 600 MPa, 15 min, 40˚C	=↓[1]	-	-	-	-	-	Garcia-Mora et al. (2015)
Cowpea	Seeds exposed to HHP and thereafter milled	HHP	200, 400 or 600 MPa, 5 min, 20˚C	↓	↓	=	=	↑↓[1]/=	↑/↑	Sosa et al. (2020)
Cowpea	Different concentrations of PI obtained by different alkaline solubilization pH	HHP	400 or 600 MPa, 5 min, 20˚C	-	-	-	↑	-	-	Peyrano et al. (2019)
Cowpea	1% PI obtained by different alkaline solubilization pH	HHP	200, 400 or 600 MPa, 5 min, 20˚C versus thermal treatments	↓=	↑	-	↑	-	-	Peyrano et al. (2016)
Kidney bean	5% PI dispersion	HHP	300 MPa, 15 min	-	↑	-	-	↑/↑	↑/=	Al-Ruwaih et al. (2019)
Kidney bean	20-25% PI dispersion	HHP	200, 400 or 600 MPa, 15 min, 20˚C	-	↑	-	-	↑/↑	↓/↓	Ahmed et al. (2018)
Pea	0.25% PI dispersion	HHP	200, 400 or 600 MPa, 5 min, 23 ˚C, different pH	↓	-	-	-	↑/↑↓	=↑/↓	Chao et al. (2018)

Pigeon pea	Seeds exposed to HHP and thereafter milled	HHP	200, 400 or 600 MPa, 5 min, 20˚C	↓	↑↓[1]	↑	=	=/↑↓[1]	↓/↓	Sosa et al. (2020)
Dolichos bean	Seeds exposed to HHP and thereafter milled	HHP	200, 400 or 600 MPa, 5 min, 20˚C	↓	↑	=	=	↓/=	↑↓/↑↓	Sosa et al. (2020)
Jack bean	Seeds exposed to HHP and thereafter milled	HHP	200, 400 or 600 MPa, 5 min, 20˚C	↓	=↓[1]	=	=	↓/=	↓/↓	Sosa et al. (2020)

[1] Decrease at extreme conditions

[2] Glycinin decreases, whereas conglycinin increases

Symbols and abbreviations: -, not specified; =, no effect;↑, increase; ↓, decrease; EC/ES, emulsifying capacity/stability; FC/FS, foaming capacity/stability; Gel., Gelation; HHP, high hydrostatic pressure; HPU, high-power ultrasounds; MW, microwaves; OAC, oil absorption capacity; PC, protein concentrate; PEF, pulsed electric fields, PI, protein isolate; Sol., solubility; WAC, water absorption capacity.

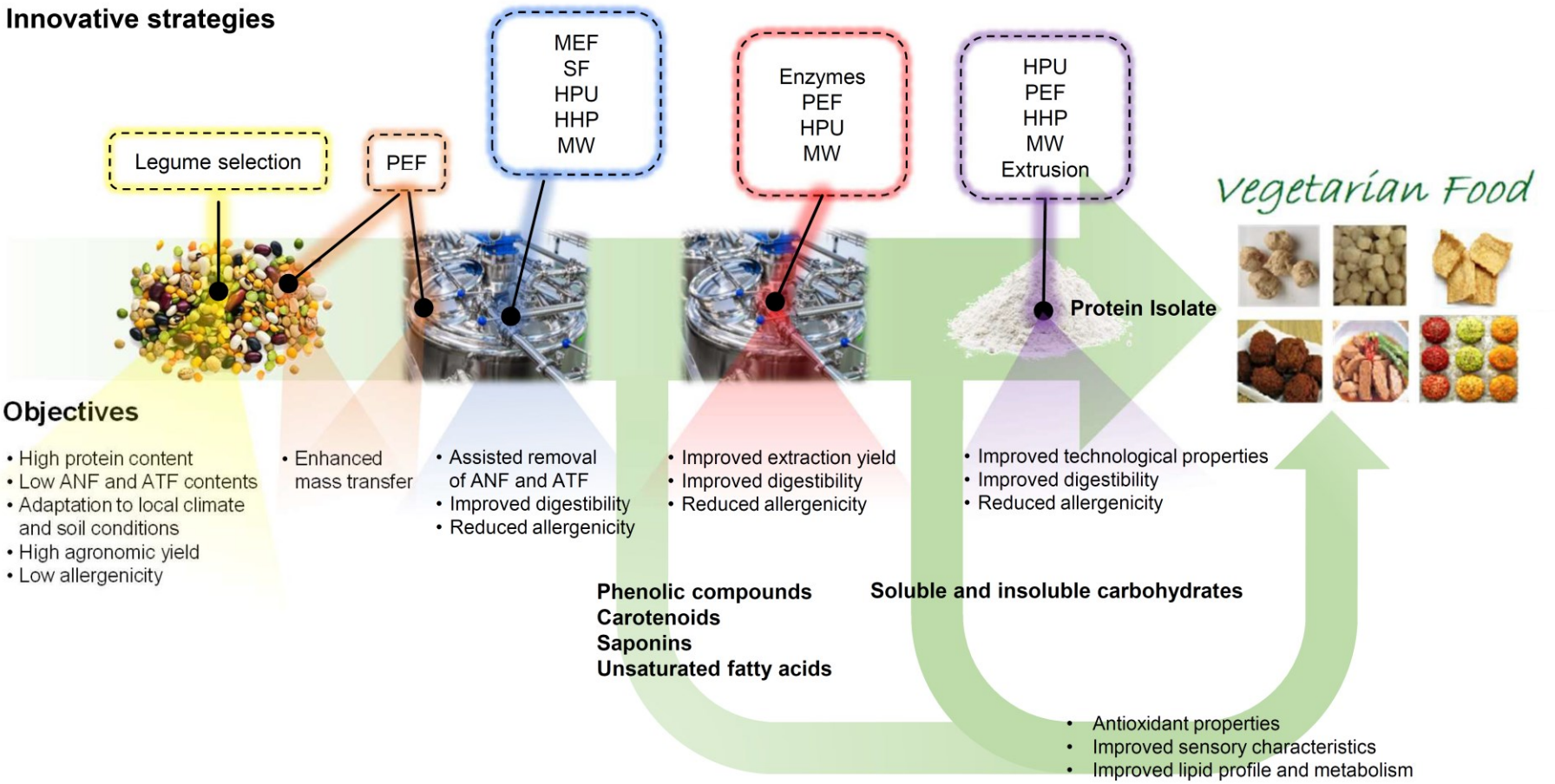


Figure 1:

Strategies and objectives of employing emerging technologies to obtain functional protein isolates.

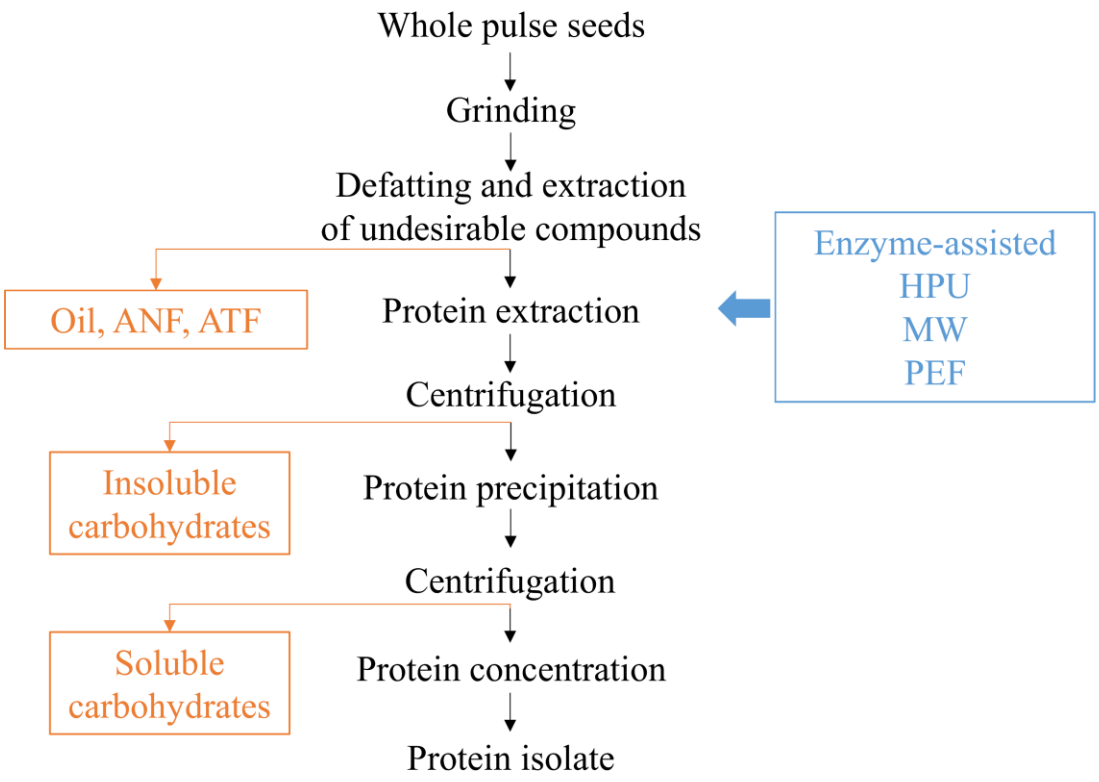
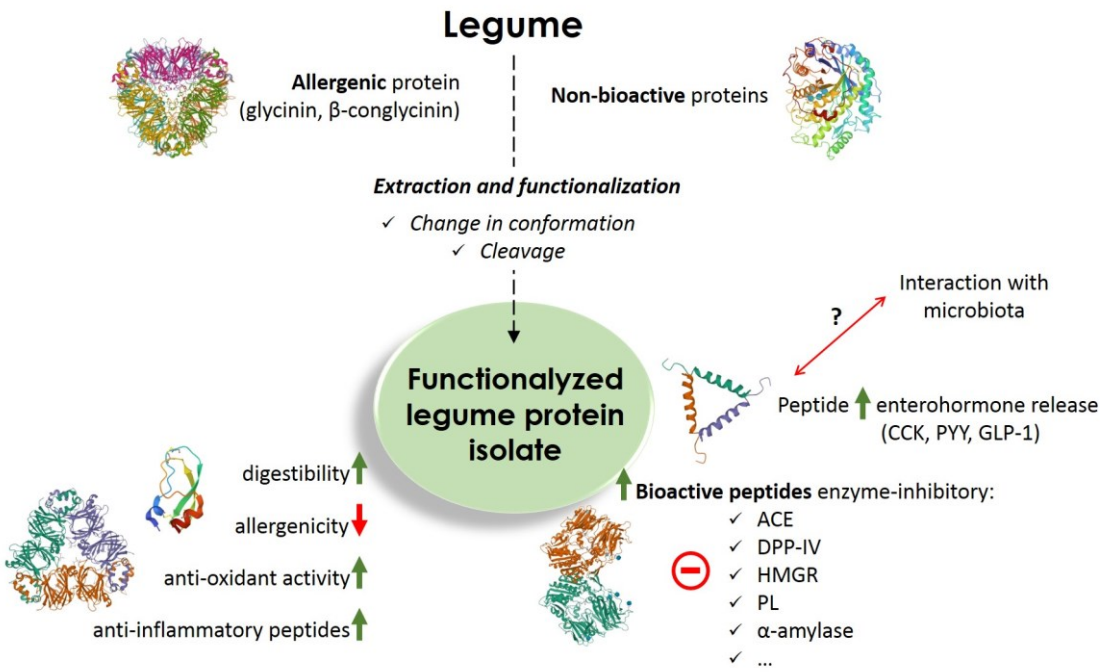


Figure 2

Scheme of the potential application of emerging technologies to improve protein recovery during wet-extraction processes.



1966

1967 **Figure 3**

1968 Summary of the mechanisms for the health effects of the technologically obtained legume

1969 protein isolates.

1970 CCK: cholecystokinin, PYY: peptide YY; GLP-1: glucagon-like peptide-1; ACE: angiotensin-I

1971 converting enzyme; DPP-IV: dipeptidyl peptidase-IV; HMGR: 3-hydroxy-3-methylglutaryl-

1972 coenzyme A reductase; PL: pancreatic lipase. Images from PDB-101.

1973

1974

Author Contributions

All authors were involved in writing the original draft. Ricard Bou, José J. Benedito, Rubén Domínguez, Miguel López-Gómez, Montserrat Pinent, Albert Ribas-Agustí, José V. García-Pérez, José M. Lorenzo and Ximena Terra participated in the conceptualization, review and editing of the manuscript.

Conflicts of Interest

No potential conflict of interest was reported by the authors.