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# The role of emergent processing technologies in tailoring plant protein functionality: New insights

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## ABSTRACT

**Background:** Plant proteins possess promising technological-functional properties that can be used for the development of innovative protein systems. Following the global requirements of environmentally friendly politics, “green” and cost-effective processing technologies, such as ohmic heating and high pressure processing are of great interest. These technologies have demonstrated their potential to modify protein structure and therefore their function, opening interesting possibilities for the design of functional food systems. However, these innovations must also include nutritional and health/wellness aspects, such as the interaction with other food components, and the behavior in the gastrointestinal tract (digestibility and bioavailability).

**Scope and approach:** This review addresses the most promising technological-functional attributes of plant proteins, as well as considerations and strategies needed for the development of innovative food systems. New insights will also be provided on how emerging processing technologies such as ohmic heating and high pressure processing can affect the behavior of proteins. The processing effects in proteins’ structure and in their technological-functional properties and ultimately in the biofunctional and nutritional aspects of foods made therefrom will be critically discussed.

**Key findings and conclusions:** Fundamental research regarding the relationship between structural modifications and functionality of more conventional proteins is still required. Furthermore, additional research is necessary on proteins from less studied sources, highlighting those displaying both functional and quality parameters of interest. Emergent processing technologies can help guaranteeing the quality and preservation of foods, as well as act as effective tools to develop technological-functional attributes of food proteins ensuring nutritional and health/wellness aspects.

## 1. Introduction

Due to economic development and urbanization in developed countries, the consumption of animal protein has reached levels that exceed the standard nutritional needs. A quite different scenario occurs in undeveloped countries where animal protein sources are scarce and protein-energy malnutrition is a serious concern (Kumar, 2016; Neacsu, McBey, & Johnstone, 2017). The world demand for the main sources of animal protein (in the form of meat, dairy and fish products) is expected to double by 2050, despite all the negative environmental, ethical, and public health impacts associated with their production and consumption (Neacsu et al., 2017). This, coupled with the continuous growth of the population which is expected to exceed 9 billion people by 2050, will lead to a continuous increase in the demand for food proteins that could

only be met by the consumption of novel and sustainable protein sources (Pojić, Mišan, & Tiwari, 2018; Worldometers, 2020). The demand for sustainable alternatives to animal protein sources led to a growing interest in plant-based proteins. The cultivation of such protein sources is considered less damaging to the environment comparing to the exploitation of animal protein, so they represent a more sustainable choice (Poore & Nemecek, 2018; Wu et al., 2014).

Plant proteins can be found in numerous sources, being distinguished mainly into pulse proteins (e.g., peas, beans, chickpeas, lentils and lupines), oilseed proteins (soybeans, peanut, flaxseed and rapeseed/canola), cereal proteins (wheat, corn, rice, oats, barley and sorghum) and also the so called “pseudocereal” proteins (quinoa, amaranth, chia and buckwheat) (Kyriakopoulou, Dekkers, & van der Goot, 2019). Among all the plant proteins, currently in use by the food industry, the

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**Table 1**

Examples of recent studies reported in the literature evaluating the technological-functional properties of plant proteins.

Protein source		Technological-functional properties					Reference
		Sol	WAC	OAC	Emul	Foam	
<b>Pulse</b>	Bean	X			X		Rahmati, Koocheki, Varidi, and Kadkhodae (2018)
	Chickpea	X			X		Mokni Ghribi et al. (2015)
	Lupin				X		Burgos-Díaz et al. (2016)
	Pea	X	X	X	X	X	Stone, Nosworthy, Chiremba, House, and Nickerson (2019)
	Pea	X	X	X	X	X	Zhao, Chen, Hemar, and Cui (2020)
<b>Oilseeds</b>	Cowpea					X	Peyrano, de Lamballerie, Speroni, and Avanza (2019)
	Soy	X	X	X	X	X	Stone et al. (2019)
	Soy	X	X	X	X	X	Zhao, Chen, et al. (2020)
	Canola						X Kim, Varankovich, and Nickerson (2016)
	Peanut	X	X				X Li et al. (2020)
<b>Cereal</b>	Peanut	X	X	X	X		X Zhang, Wang, Li, and Yan (2020)
	Oat		X				X Nieto Nieto et al. (2016)
	Oat				X		X Zheng, Li, and Liu (2020)
	Wheat	X	X	X	X	X	X Zhao, Chen, et al. (2020)
	Barley	X	X	X	X	X	X Stone et al. (2019)
<b>Pseudocereal</b>	Rice	X	X	X	X	X	X Zhao, Chen, et al. (2020)
	Chia	X	X	X	X		X López et al. (2018)
	Quinoa	X	X	X	X	X	X Dakhili, Abdolizadeh, Hosseini, Shojaee-Aliabadi, and Mirmoghtadaie (2019)
	Quinoa	X	X	X	X	X	X Elsahaimy, Refaay, and Zaytoun (2015)
	Amaranth	X	X	X	X	X	X López, Galante, Raimundo, Spelzini, and Boeris (2019)

Abbreviations: Sol: solubility; WAC: water absorption capacity; OAC: oil absorption capacity; Emul: Emulsifying; Foam: Foaming; Gel: Gelation.

majority are derived from soybeans and wheat. However, these protein sources are known for being part of “The Big Eight” allergens (Koeberl, Clarke, & Lopata, 2014). Therefore, other protein sources such as pea and rice have emerged over the last years as promising plant protein substitutes due to their recognized hypoallergenicity along with other advantageous attributes such as nutritional and biological value (Zhao, Chen, Hemar, & Cui, 2020).

The first attempt to divide plant proteins in their different fractions occurred in the beginning of the 20th century, when Thomas B. Osborne proposed a classification system for plant proteins based on their solubility and extractability in different solvent (Osborne, 1908). The proposed system allowed the general division of plant proteins in four main classes: albumins, globulins, prolamins and glutelins. Albumins consist in a water-soluble protein fraction that can coagulate if heated, whereas globulins are insoluble in water but soluble in diluted salt solutions (e.g., 0.1 mol L<sup>-1</sup> NaCl). Prolamins have the particularity of being resistant to heat and are both insoluble in water and saline solutions, although being soluble in concentrated (60–70% v/v) aqueous alcohol solutions. Glutelins are also insoluble in water but diluted acid or alkali solution can be used to promote their solubilization and extraction (Lafarga, 2018). Over the years, protein fractionation has been refined and it is already established that these four major plant protein classes are still constituted by a complex combination of proteins. Moreover, depending on the plant (or even on the species or sub-species), the proportion of each protein group and their molecular size varies substantially. For instance, pulses and oilseeds proteins are mainly composed by albumins and globulins, whereas prolamins and glutelins are predominant in cereals. Additionally, due to their molecular structures, each one of these protein fractions display distinctive properties that could be useful for food applications (Day, 2013; Loveday, 2019). Besides their recognized nutritional value and biological activity (e.g., anti-cholesterol, anti-hypertensive, anti-oxidant, anti-inflammatory, anti-carcinogenic and anti-microbial), they also possess promising technological-functional properties (e.g., gelling, emulsifying, and foaming) that can be tailored to develop innovative protein systems at different scales (nano, micro and macro). Therefore, novel valuable ingredients for incorporation in complex food matrices could be developed by using proteins derived from such alternative sources as raw materials (Fasolin et al., 2019; Lafarga, 2018; Moreno-Valdespino, Luna-Vital, Camacho-Ruiz, & Mojica, 2020; Sieniawska, Maciejewska-Turska, Świątek, & Xiao, 2020). Some plant proteins (e.g., globulins) present limited solubility, which hampers the development of new functional ingredients, since this

property is directly related with the ability to form gels, foams and emulsions. This limitation is often overcome by thermal processing of proteins, causing irreversible modifications in the proteins' structure. However, conventional thermal processing treatments are highly aggressive and frequently compromise the nutritional and sensorial profile of proteins (Aryee, Agyei, & Udenigwe, 2018; Nicolai & Chasseigneux, 2019).

Ohmic heating (OH) and high pressure processing (HPP), together with other novel technologies, are considered ‘emerging high-potential technologies for tomorrow’ (De Vries et al., 2018). These technologies, due to their established advantages in food safety, minimal loss of nutrients and energy efficiency are attracting increasing attention in the food scientific community, seeking to replace high cost and aggressive thermal treatments (De Vries et al., 2018; Han, Cai, Cheng, & Sun, 2018; Queirós, Saraiva, & da Silva, 2018). These novel technologies were primarily used to increase shelf-life and the nutritional value of foods, but more recently they have been gaining attention as tools to change proteins' structure and functional properties, with promising results in conventional proteins (Aryee et al., 2018). Nonetheless, to the best of our knowledge, there is still a significant lack of information regarding the potential impact of such emerging technologies on the structure and functional properties of plant proteins (Li, Ye, Tian, Pan, & Wang, 2018; Queirós et al., 2018).

This review aims at providing an overview of the most promising technological-functional properties of plant proteins. It also includes a state-of-the-art regarding OH and HPP technologies as well as the influence of their associated principles of operation: moderate electric fields (MEF - which are associated to OH) and high hydrostatic pressure (HHP - which is the main effective factor of HPP) on the structure and functional properties of plant proteins. Finally, we discuss new insights on how these emerging food processing technologies could impact proteins in complex food systems and the interaction between the processed proteins and other food components towards the development of novel, nutritious and healthy food.

## 2. Technological-functional attributes of plant proteins

The behavior of plant proteins within food systems is highly dependent on their technological-functional properties. In order to fully understand their behavior, protein structural features (i.e., amino acid composition, structure and conformation) must be considered and related with their technological-functional properties such as solubility,

water/oil holding capacity, viscosity, aggregation mechanisms and interfacial properties, responsible for foaming and emulsion abilities (Lafarga, 2018). Besides the protein's intrinsic factors, functionality is also reliant on environmental factors such as pH, temperature and ionic strength as well as on processing impact inherent to the selected methodology of extraction and/or purification (Aryee et al., 2018; Joshi, Adhikari, Aldred, Panozzo, & Kasapis, 2011). Novel processing technologies, such as OH and HPP, also have an impact in tailoring protein's functionality (Li et al., 2018; Queirós et al., 2018) and considering the scope of this review, such effects will be particularly addressed (see section 4). Technological functionality of plant proteins, which is fundamentally dependent on physical and chemical attributes, ultimately influences texture, appearance and stability of food matrices (Pojić et al., 2018). Recent studies can be found in the literature regarding the characterization of proteins isolated from a wide number of plants in terms of their technological-functional properties as shown in Table 1. An overview of the technological-functional properties of plant proteins and their related parameters will be presented as follows.

### 2.1. Protein solubility

Solubility is an important parameter that must be assessed since it is directly associated with important functional properties such as gelling, emulsifying and foaming (Shevkani, Singh, Chen, Kaur, & Yu, 2019). It is highly dependent on the balance of hydrophobic and hydrophilic residues present in the protein structure and on the protein's surface charge, and also on the solution's pH, ionic strength, solvent type and temperature (Lafarga, 2018). Some studies with pulse proteins reported the positive association between solubility and surface charge whereas a negative relation occurs when relating solubility with surface hydrophobicity (Karaca, Low, & Nickerson, 2011; Shevkani, Singh, Kaur, & Rana, 2015). Solubility of plant proteins is also affected by the system's pH. Namely, proteins are less soluble in a pH range around their isoelectric point, where the net charge of the protein is zero and protein-protein interactions favour aggregation due to the reduction of intermolecular electrostatic repulsion. In turn, their solubility is increased as the system's pH moves away from the isoelectric point (Bessada, Barreira, & Oliveira, 2019; Karaca et al., 2011; Shevkani et al., 2019). Recently, Zhao, Shen, Wu, Zhang, and Xu (2020) studied the pH related solubility of distinct plant proteins, i.e., pea, soybean, wheat and rice. Their isoelectric point was estimated to be around pH 4–6, 4–5 and 6–7 for pea, soybean and wheat, respectively, at which protein precipitation was almost complete. Moreover, for wheat protein, a perfect "U shape" curve was obtained in a pH/protein solubility graph indicating that both acidic and alkaline media, away from the isoelectric point, are suitable for protein's solubilization. Regarding soybean and pea proteins, the solubility profile was asymmetric, meaning that an alkaline medium is preferential to ensure a better protein solubility. On the other hand, rice proteins have shown to remain almost insoluble in the entire pH range (these results were in agreement with other studies reported in the literature) (Hefei Zhao, Chen, et al., 2020; Hoogenkamp, Kumagai, & Wanasundara, 2017). In order to define potential food applications, it is then crucial to understand the distinct solubility mechanisms of selected proteins (Bessada et al., 2019).

### 2.2. Water and oil/fat absorption capacities

Proteins are recognized for their ability to hold water and oil/fat. Water absorption capacity (WAC) is expressed as the amount of water absorbed by 1 g of protein. On the other hand, fat/oil absorption capacity (FAC/OAC) is specified as the quantity of fat/oil that 1 g of protein powder or flour is able to absorb. These are considered two particularly important parameters, having a profound effect on texture (e.g., juiciness, tenderness and mouthfeel) and flavor retention (Bessada et al., 2019; Shevkani, Kaur, Kumar, & Singh, 2015). Fat/oil absorption capacity is an extremely important parameter in the formulation of

foods in which fat/oil impacts the textural food attributes (e.g., meat replacers/derivatives or ground meals) (Aryee et al., 2018; Tiwari & Singh, 2012). On the other hand, WAC is a property that becomes crucial in foods with associated viscosity as soups, doughs and custards (Shevkani et al., 2019; Sreerama, Sashikala, Pratape, & Singh, 2012). The location of polar amino acids in protein-water interaction sites has been related with WAC, while the nonpolar amino acids in protein-oil interaction sites largely determines FAC (Bessada et al., 2019; Boye, Zare, & Pletch, 2010; Porras Saavedra, Güemes-Vera, SOTO, Martínez, & Yañez, 2013). Regarding pulse proteins, few studies reported large variations between the WAC and FAC/OAC of different pulses such as field pea, cowpea, kidney bean, chickpea, faba and lentil (Shevkani et al., 2019). Zhao and co-workers (2020) recently reported significant variations between WAC values of pulse, oilseed and cereal proteins. In this case, rice and wheat had the lowest WAC values (about 1 g/g), whereas WAC value for soybean and pea protein were about 3 g/g and 5 g/g, respectively. In addition, regarding OAC, all proteins in study presented similar values (Hefei Zhao, Chen, et al., 2020).

Thermal processing leads to protein denaturation, thus possibly causing the loss in their solubility, however the ability to retain water seems to be typically improved (Aryee & Boye, 2017; Aryee et al., 2018). In fact, every process that may cause structural rearrangement in proteins may impact the hydrophobic/hydrophilic balance on the protein's surface, thus impacting its solubility and WAC or OAC. Emergent processing technologies could then constitute a viable option to avoid the detrimental effects of conventional thermal processing while affecting such parameters.

### 2.3. Emulsifying and foaming properties

Emulsifying and foaming properties of proteins play a crucial role in influencing the structure, stability and sensorial attributes in foods such as mousses, toppings, margarine, beverages or ice cream (Aryee et al., 2018). Proteins are known for their amphiphilic nature (i.e., the presence of both polar and nonpolar amino acid residues). Their ability to be adsorbed at the interface of oil or air droplets allows the development of stable films and dispersions, thus acting as emulsifiers. The dispersion of oil droplets in an aqueous medium or the surrounding of air cells with a film leads to the formation of emulsions and foams, respectively (Bessada et al., 2019; Damodaran, 2005).

To measure the emulsifying properties of proteins, emulsifying activity index (EAI) and emulsifying stability index (ESI) must be determined. EAI consists in measuring the quantity of emulsified oil per 1 g of protein, whereas ESI determines the emulsion's resistance during a specific period (Boye, Zare, & Pletch, 2010; Burger & Zhang, 2019). Emulsifying capacity is directly influenced by both intrinsic protein attributes such as molecular weight, conformational stability, water solubility, amino acid composition and hydrophobicity-hydrophilicity ratio at the surface as well as by external parameters such as pH, temperature and ionic strength (Bessada et al., 2019). Due to their emulsification properties, soy proteins have been explored more than other plant proteins (Burger & Zhang, 2019; Day, 2013). Nevertheless, proteins from pulses, such as pea, lupin and chickpea, have demonstrated to possess emulsifying properties similar to soy proteins (Bessada et al., 2019; Burger & Zhang, 2019; van de Noort, 2017). Over the last decades a significant variety of emulsifying properties has been reported in different plant proteins. For instance, the EAI and ESI of soybean, pea, wheat and rice protein were determined at pH 7. The results indicated that soybean and pea protein displayed similar emulsifying properties (above  $100 \text{ m}^2 \text{ g}^{-1}$ ) therefore, it seems reasonable to substitute soybean with pea protein in meat and sausage products. In contrast, wheat and rice protein displayed emulsifying properties significantly less evident (below  $40 \text{ m}^2 \text{ g}^{-1}$  and  $20 \text{ m}^2 \text{ g}^{-1}$ , respectively) when compared with the others previously mentioned, thus, their use as emulsifiers appears to be limited at the studied pH (Zhao, Chen, et al., 2020). Other plant proteins such as those presented in rice bran displayed emulsifying properties

comparable to casein (Fabian & Ju, 2011; Hoogenkamp et al., 2017). Moreover, a recent study based on the phenomenon recognized as Pickering stabilization (where solid particles located at the interfacial surface of emulsion droplets protect them against coalescence by interfacial action), has successfully tailored zein and gliadin to behave as stabilizers in the preparation of stable oil-in-water emulsions (Liu, Huang, Chen, Deng, & Yang, 2019).

As mentioned before, plant proteins also possess foaming properties, which are commonly evaluated in terms of foaming capacity (FC) and foaming stability (FS). FC consists in measuring the volume (%) of incorporated air after whipping whereas FS is related to the foam stabilization (volume) during a specific period. There might be a relationship between protein's surface charge and foaming since a greater charge promotes the weakening of hydrophobic interactions, thus causing an increase in protein solubility, allowing them to quickly disperse on the interface and encapsulate air particles (Shevkani et al., 2019). Proteins such as pea and lupin, demonstrated to have good FC and FS, readily compared with egg and soybean proteins (Bessada et al., 2019; Wong, Pitts, Jayasena, & Johnson, 2013; Zhao, Chen, et al., 2020). The emulsifying and foaming capacity of plant proteins, plus their similar behavior to some of the more established emulsifiers, make of these proteins interesting alternatives for use in the food industry.

#### 2.4. Gelation properties

The ability to form gels under certain conditions is another essential protein functionality for the food industry, in particular for a variety of foods such as puddings, jellies and meat products where water retention, elasticity and texture are determinant attributes. Different physical or chemical methods, such as heat, pressure, chemical and enzymatic treatments may be applied to cause protein gelation (Aryee et al., 2018; Bessada et al., 2019). When protein unfolding occurs, hydrophobic amino acid residues become exposed, resulting in attractive forces between hydrophobic regions in different proteins. These interactions may be stabilized by other mechanisms (via disulphide bonds, hydrogen bonds, hydrophobic, van der Waals interactions) resulting in irreversible aggregation. The propagation of this phenomenon results in the formation of a three-dimensional network (if the protein concentration is high enough), thus forming a gel (Ramos et al., 2015). Many factors are responsible for influencing the protein's gel formation as well as final structure and associated mechanical properties (e.g., pH, temperature, ionic strength, molecular weight along with the processing treatment and respective conditions) (Lin et al., 2017). The measurement of the protein's gelling capacity is performed by the minimal protein concentration that allows gelation to occur (designated as "least gelation concentration") (Zhao, Chen, et al., 2020). Some reports on heat-induced gelation of plant globulins from different sources (e.g., soy, pea, canola, quinoa, lupin, cotton seed and chia) have demonstrated their potential to form gels with different features. Some studies had also reached the conclusion that plant globulins gels are formed during heating in an analogous way of other globular proteins as dairy proteins. However, further research is still required to fully understand the gel forming mechanisms and also the subsequent comparison between different plant globulins and dairy proteins in which the process is much more established (Nicolai & Chassenieux, 2019). Nonetheless, plant protein gels present interesting gelling capacities, suitable to specific applications and passible to be tuned during the production processes of the foods they are to be integrated in. Gels from lupin protein isolate (LPI) seem to be less strong when compared with a reference material such as soy protein isolate (SPI). Nonetheless, LPI gels constitute a good choice in the development of less viscous foods after the application of heat treatments (Berghout, Boom, & van der Goot, 2015; Bessada et al., 2019). The impact of a pH-shifting treatment (subjecting peanut protein isolate (PPI) to a pH 10 for 1 h, followed by a shift back to pH 7) in the structure and heat-induced gel properties of PPI, resulted in a gel with significantly improved breaking force and water holding capacity. Such

behavior was attributed to the decreased particle size, increased solubility, surface hydrophobicity and free sulfhydryl group content (J. Li et al., 2020). Thermal gelation of cowpea protein isolates and subsequent rheological characterization, demonstrated their versatility to be used as gelling agents. The introduction of a pH shifting treatment during protein extraction has also improved the mechanical properties of the obtained gels (Peyrano, de Lamballerie, Speroni, & Avanza, 2019).

The combination of proteins with polysaccharides has also been explored for developing innovative gels with distinctive microstructure and sensory features along with improved functionality (Chu, Yang, Li, Lin, & Zheng, 2019; Nieto; Haibo Zhao, Chen, et al., 2020; Nieto Nieto, Wang, Ozimek, & Chen, 2016; Yan, Yin, Li, Yadav, & Jia, 2020).

### 3. Emergent food processing technologies

#### 3.1. Ohmic heating (OH)

The demand for alternatives to conventional thermal technologies in the food industry has led to the introduction of electric field-based process. OH is a thermo-electrical processing technology based on the passage of an electrical current through a food with an associated electrical resistance (Pereira & Vicente, 2010). Its action can then be explained by the Joule's law, in which the passage of an electric current through a conductive (or semi-conductive) material generates heat. OH has the particularity of electrodes contacting directly with the foodstuff, and although a direct or alternating current can be applied to achieve OH, it is advisable to use an alternating current because it prevents or restricts electrochemical and electrolytic reactions (Jaeger et al., 2016). Due to the direct energy dissipation in the food, heating occurs at a fast rate and homogeneous dissipation takes place. This results in the achievement of the target processing temperatures in a reduced treatment time, preventing excessive thermal damage to foodstuff (i.e., loss of sensorial and nutritional attributes) (Knirsch, Alves dos Santos, Martins de Oliveira Soares Vicente, & Vessoni Penna, 2010; Pereira & Vicente, 2010; Sakr & Liu, 2014; Sastry, 2005; Silva, Santos, & Silva, 2017). Additionally, OH has demonstrated to be beneficial in reducing surface fouling-related issues or even thermal abuses on the product due to the absence of hot surfaces and the inherent lack of heat transfer limitations. It is also considered an environmentally friendly processing technology due to the associated high energetic efficiencies (>95%) and low maintenance costs (Kumar, 2018; Pereira & Vicente, 2010). Usually, OH occurs within the specifications of MEF, in which the electric field is applied between 1 and 1000 V cm<sup>-1</sup>, the waveform is typically sinusoidal due to the easier production and modulation, frequency ranges between 1 Hz and 1 MHz and the treatment time has no restrictions (Rodrigues, Fasolin, et al., 2020; Sastry, 2008). The distinction between OH and MEF is related with the intended effect: thermal action, which is related to OH or the electric field non thermal-effects (where heat generation is minimized or even restricted), linked with MEF. However, in practical applications, both effects occur simultaneously and it is not possible to strictly differentiate the thermal and electric effects (Rodrigues et al., 2019; Sastry, 2008).

Over the years, a wide number of potential applications of OH were presented, including low temperature, microbial inactivation, blanching, pasteurization, sterilization, dehydration, evaporation, fermentation, extraction, and also to heat food in long-duration space missions as well as in the military field (Kumar, 2018; Sastry et al., 2009). The industrial implementation of OH has been growing since the 1990s, with a steady increase in numbers of new manufacturers and commercial installations. As a matter of fact, the successful implementation of this technology made it possible to find commercially available food products that are currently being processed by OH. (Rodrigues et al., 2019; Sastry, 2008).

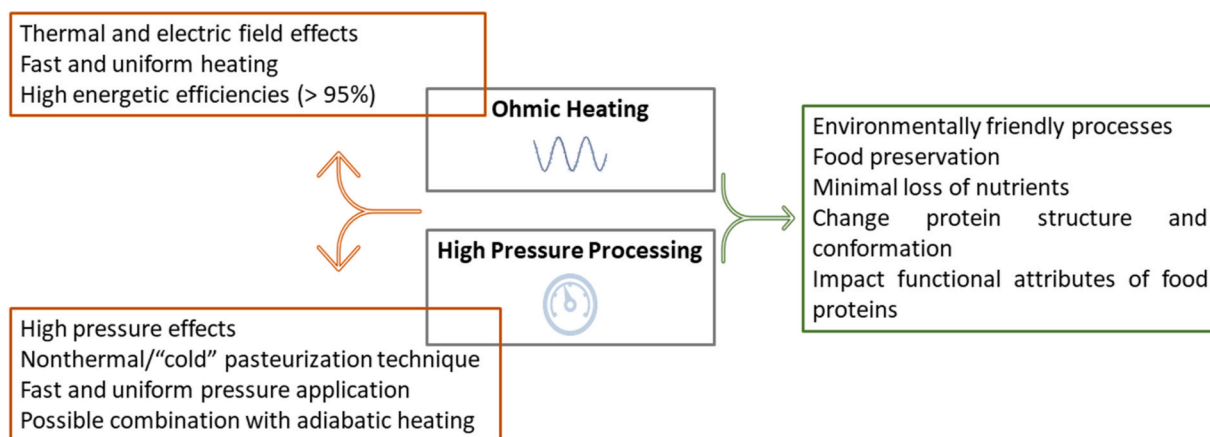


Fig. 1. Schematic framework of the main features of OH and HPP and their common effects on food processing.

### 3.2. High pressure processing (HPP)

HPP, also called high hydrostatic pressure processing or ultrahigh pressure processing, is a processing technology that has been used in the food industry to inactivate spoilage microorganisms and dangerous pathogens. In HPP, heat is substituted by pressure (ranging from 100 to 800 MPa), so it has been called as a nonthermal or "cold" pasteurization technique, avoiding the disadvantageous thermal effects on the sensorial and nutritional attributes of foods (Balasubramaniam et al., 2008, 2015; Muntean et al., 2016; Queirós et al., 2018). In order to foresee the impact of HPP on food it becomes necessary to understand some of the basic principles by which the behavior of foods under pressure is determined. The first one is known as the Le Chatelier's principle and describes the tendency of a system to shift towards the lowest volume by pressure application. The principle of microscopic ordering states the antagonistic forces of temperature and pressure on the molecular structure and chemical reactions, which means that when temperature remains constant, an increase in pressure leads to the increase of the ordering degree of molecules. At last, according to the isostatic principle, pressure and the correspondent effects are applied fast and homogeneously through the entire food product (with or without being packaged), no matter its volume, size, shape and composition (Balasubramaniam et al., 2015; Huppertz, Vasiljevic, Zisu, & Deeth, 2019). HPP can be processed at a temperature from below 0 °C (to reduce eventual effects of adiabatic heat) to above 100 °C (Pereira & Vicente, 2010). Cooling jackets, heat exchangers and recirculation of the cooling medium are used to control the processing temperature (Alexandre, Pinto, Moreira, Pintado, & Saraiva, 2019). Regarding food preservation, this nonthermal technology yields numerous advantages: homogeneous treatment due to the uniform pressure application within the food product; shelf lives of treated samples are similar or even higher to those subjected to thermal pasteurization and the preservation of the food quality parameters is maintained; heat impact is minimal; low contamination risk and low energy consumption. For these reasons HPP is considered as an environmentally friendly processing technology (Huang, Wu, Lu, Shyu, & Wang, 2017). Several factors are responsible for influencing the efficacy of HPP such as temperature, treatment time, food matrix characteristics, pressure level used and the nature of pressure (batch or continuous), type of microorganisms and packing material (Alexandre et al., 2019). Currently, this technology has been applied to the pasteurization of fruits, yogurts, sauces and salad dressings; pasteurization of meats and vegetables; decontamination of heat sensitive ingredients as vitamins and flavorings (Alexandre et al., 2019; Muntean et al., 2016).

Fig. 1 summarizes some relevant aspects of HPP and OH (as discussed in the previous subsection) as well as the common outputs generated through their application on food products/ingredients.

### 4. Impact of OH and HPP on protein functionalization

OH and HPP have established their potential in replacing conventional processing techniques by improving shelf-life (through microbial inactivation) and food quality (i.e., by avoiding or significantly reducing the negative changes in the sensorial and nutritional properties of foods) (Aryee et al., 2018). However, over the last few years, further research has been addressed on the potential effects of OH and HPP application in changing the inherent structure of proteins. This gave rise to the development of novel food ingredients such as gels and films with tailored functionality for different food applications. Currently, their ability to modulate proteins' structure and influence protein functionality is well described in the literature, particularly in the case of dairy proteins (Munir et al., 2019; Rodrigues et al., 2019). In fact, significant research has been developed regarding the denaturation and functional properties of whey proteins, due to their interesting technological properties and recognized biological value (Huppertz et al., 2019; Pereira et al., 2018). Promising results were obtained by the application of OH in modifying the denaturation and aggregation pathways of these bio-macromolecules, thus opening the possibility to develop innovative protein systems as gels, films and nano/micro structures with tailored functionality (Pereira, Teixeira, & Vicente, 2011; Rodrigues et al., 2015). The impact of MEF effects during a OH treatment on whey protein isolate (WPI) solutions was recently established, from molecular to macroscopic levels. The application of OH to WPI solutions and the control of its variables (i.e., voltage and frequency), not only affected the protein denaturation and aggregation pathways but also allowed to control the gelation process and to modulate molecular interactions, influencing the protein network formation and thus determining the final gel properties (Rodrigues, Fasolin, et al., 2020).

Protein functionalization can also be promoted by the application of HPP. Considerable work has been focused on the mechanisms of unfolding and aggregation of  $\beta$ -Lactoglobulin ( $\beta$ -Lg) during the application of HPP and after its release (Munir et al., 2019). This major whey protein fraction is known as the most sensitive to processing and thus largely dominates the HPP-induced denaturation, aggregation and gelation behavior of the whey protein systems (Huppertz et al., 2019). The effect of high pressure on the structure of whey proteins has been explained in detail as well as the proposed mechanisms underlying the pressure-induced unfolding and aggregation of  $\beta$ -Lg (López-Fandiño, 2006; Orlén, Olsen, & Skibsted, 2007; Stapelfeldt & Skibsted, 1999). The modification of their functional properties, such as gelation, foaming capacity and emulsifying activity has also been related with such pressure-induced structural changes (López-Fandiño, 2006).

Plant proteins have also demonstrated promising functional properties that often need to be improved, particularly by being submitted to conventional heat processing. Therefore, in the next subsections, the

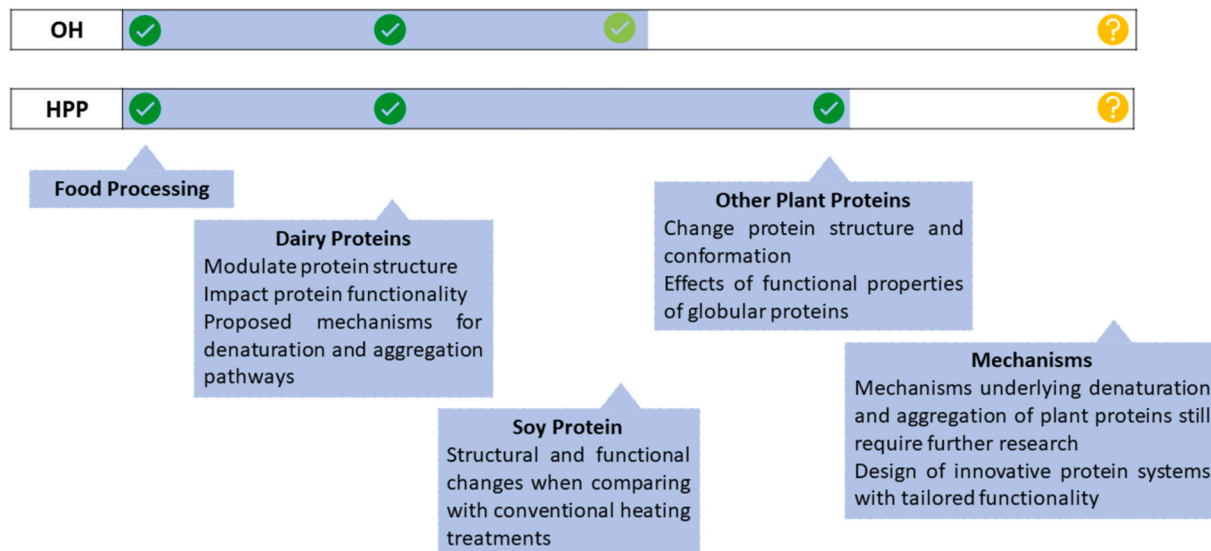


Fig. 2. Progress in research of plant protein functionalization under the effects of OH and HPP.

available information on how OH and HPP affect the structure and functionality of these proteins will be gathered and critically discussed.

#### 4.1. Ohmic heating

Currently, few reports can be found in the literature regarding the application of OH on plant protein-based foods and their effects on plant protein structure and functional properties. In contrast, extensive research has been developed on conventional proteins and correspondent based-foods, such as meat, eggs and dairy. Furthermore, the available information reported in the literature was mainly related to soy protein and its derived food products such as tofu. In fact, the OH processing of soymilk to induce its coagulation for production of tofu has shown promising results in the textural properties of the final product (Shimoyamada et al., 2015; Wang et al., 2007). In addition, an opposite relation was found between the voltage applied and the viscosity, precipitation and surface hydrophobicity of soy protein (Shimoyamada et al., 2015). Li et al. (2018), in more fundamental studies, explored the effects of OH on the structure and functional properties of protein in soybean milk. It was verified that significant changes occurred in the structure and functional properties of proteins, when compared to the traditional heating method (Li et al., 2018). It was also suggested that the modifications in total sulfhydryl content, surface hydrophobicity and functional properties such as foaming and emulsification capacity were induced by the application of electric fields inherent to OH (Cha, 2011; X.; Li et al., 2018). The formation of protein-lipid films by the application of OH to promote heating of soymilk was also explored. Lei, Zhi, Xiujin, Takasuke, and Zaigui (2007) have demonstrated the successful production of films with better yield, film formation rate, rehydration capacity and protein incorporation efficiency (Lei et al., 2007). Recently, it was also evaluated the impact of OH in the structural and thermo-physical properties of sunflower protein isolate (SPI). MEF during a OH treatment significantly reduced the average protein particle size, affecting homogeneity as well as the surface tension of SPI solutions. Moreover, the tertiary structure of SPI was changed and a decrease in the denaturation temperatures and enthalpies of SPI samples was also observed (Subaşı et al., 2021).

Even though there are now compelling evidences mostly on the modifications induced in soy proteins and soy protein-based foods by the use of OH, the mechanisms underlying their denaturation and aggregation still require further research. Particularly the impact of OH effects on soy protein from macroscopic to molecular scale must be established, thus allowing the control of their functional behavior and the

development of new food ingredients. Furthermore, additional efforts to determine the effects of OH on the structure and functional properties of other plant proteins remain essential. This field can then benefit from the more established knowledge of OH effects on conventional proteins and transfer the developed methodologies and protocols to accelerate its development in plant proteins (as shown in Fig. 2).

#### 4.2. High pressure processing

HPP-induced changes on plant proteins have been reported in the literature over the last few years. Furthermore, research has also been focused on how the application of pressure allows tailoring certain functional attributes such as the capacity to form aggregates or gels, or even, their ability to stabilize emulsions and foams, for different food applications (Queirós et al., 2018).

Pressure has a profound impact on protein denaturation and final conformation, by inducing modifications in their structure at different levels (Gharibzadeh & Smith, 2021; Huppertz et al., 2019; Messens, Van Camp, & Huyghebaert, 1997). In fact, only noncovalent bonds, e.g., hydrogen, ionic and hydrophobic bonds are affected by HPP while the covalent bonds, which have very low compressibility at high pressure, remain relatively intact (Considine, Kelly, Fitzgerald, Hill, & Sleator, 2008). Therefore, the protein's primary structure remains almost intact owing to the covalent bonds, while the secondary, tertiary and quaternary structures can be affected, because their ionic, hydrophobic, hydrogen bonds and the electrostatic interactions can be disrupted (Rendueles et al., 2011; Yang, 2016). Generally, the primary structure is only affected by pressures above 2 GPa and the secondary and tertiary/quaternary structures are mostly compromised by lower pressure values of >400 and 100–200 MPa, respectively (Knorr, Heinz, & Buckow, 2006; Winter, Lopes, Grudzielanek, & Vogtt, 2007). Recently, several studies were reviewed concerning the effect of HPP on the secondary structure of different plant proteins, having stated that the type of protein, environmental conditions and concentration highly affects the impact of pressure on the plant protein's secondary structure (Queirós et al., 2018).

HPP is now established as a nonthermal processing technology capable of modifying protein structure and affecting the physicochemical forces and interactions between protein molecules of dispersion system, including surface hydrophobicity, electrostatic interaction, disulphide linkages and hydrogen bonding (He, He, Chao, Ju, & Aluko, 2014; Wang et al., 2017). Thus, it is not surprising that, under specific conditions, HPP-induced modifications may lead to protein unfolding,

aggregation and ultimately to gel by the formation of a three-dimensional protein network system (De Maria, Ferrari, & Marasca, 2016; Ma et al., 2013; Zhao, Mu, Zhang, & Richel, 2018a). Recently, Queirós et al. (2018) reported that two distinct strategies were being used in the studies developed so far, one consists in study the formation of a gel by submitting the protein solution to HPP, the other is related with pre-treating the protein solution under HPP effects, below the concentration required to gelation and then recovering the HPP-treated solution to study the heat-induced gelation. Regarding the first reported strategy, it was highlighted the importance of protein concentration in the production of gels and their final rheological behavior. In the second case, pre-treatment generally decreases the minimum protein concentration that becomes necessary to allow the subsequent gelation by heating (Queirós et al., 2018).

When studying sweet potato protein gels induced by HPP, it was found a strong dependence of the rheological properties as a function of the system's pH. Gels formed by the application of 400 MPa, at three distinct pH values (pH 9.0, 6.0 and 3.0), would all constitute a reasonable option for developing novel food ingredients, but gels formed at the higher pH values presented better performances (Zhao et al., 2018a). In other studies conducted by the same authors it was demonstrated that a combination of HPP with a microbial transglutaminase treatment (Zhao, Mu, Zhang, & Richel, 2019) and the addition of salt ions (Zhao, Mu, Zhang, & Richel, 2018b) had a positive impact in the textural properties of the formed gels. Further research has continued to demonstrate the positive impact of HPP pre-treatment in the rheological behavior of heat-induced cowpea protein gels (Peyrano, de Lamballerie, Avanza, & Speroni, 2019) and wheat gluten gels (Wang et al., 2019). HPP also impacts the formation of pseudocereal protein-based films, having demonstrated a better uniformity, better mechanical properties, lower water vapor permeability and water solubility when compared to the non-treated protein system. In addition, HP-treated films presented improved functionality when compared to those that were thermally treated (Condés, Añón, & Mauri, 2015).

Chao, Jung, and Aluko (2018), recently reported an enhanced emulsion formation and foaming capacity of pea proteins when submitted to HPP treatment at a pH 3.0 (Chao et al., 2018). Other studies have also been performed on distinct plant proteins to assess the effects of HPP on other functional properties such as oil-in-water emulsifying ability and foaming properties. The obtained results are diverse, such as improvement, decrease and even the inexistence of changes in emulsifying and foaming properties. Such distinctive behavior appears to be resultant from the type of protein in study, solution conditions and processing conditions (e.g., the applied pressure), thus its interpretation must be considered case by case. In order to fully understand the structure/function relationship of these proteins under HPP, changes in surfactant properties and the interfacial adsorption processes must be further enlighten to achieve the desired emulsifying and foaming properties to the intended use (Baier & Knorr, 2015; Galazka, Dickinson, & Ledward, 2000; Khan, Mu, Sun, Zhang, & Chen, 2015; Li, Zhu, Zhou, & Peng, 2011; Qin et al., 2013; Queirós et al., 2018; Yin, Tang, Wen, Yang, & Li, 2008; Zhao, Zhou, Zhang, Ni, & Li, 2015; Zhou et al., 2016).

Despite the advances in this area of knowledge, the exact correlation between the modifications in protein structure and the change in functionality remains to be completely unveiled. Therefore, further research is required in order to allow the development of protein systems with tailored functionality, envisioning specific applications (as shown in Fig. 2).

## 5. Design of complex food systems

The established ability of OH and HPP in tailoring protein functionality, coupled with a minimized environmental impact, make of these emergent processing technologies valuable tools to the development of novel and highly functional multi-component food matrices. Such concept, although fundamental to fulfil the current global trends,

must be developed along with nutritional and health aspects. This is fundamental to ensure the development of functional food from new renewable sources, capable of delivering the nutritional needs and promote the general health and wellbeing of the population.

### 5.1. Interaction with other food components

In multi-component food systems, the interactions between different protein fractions, other components or additives inevitably occur, with a high potential for these interactions to affect the properties of the final food product (James Harper, 2014; Lin et al., 2017; Queirós et al., 2018). For that reason, it becomes necessary to understand the synergistic effects of the presence of other food constituents during OH and HPP action on the structure and functionality of proteins integrated in the food system. However, that knowledge is still scarce and only a few studies can be found in the literature.

During the last few years, it has been demonstrated that the presence of MEF during a OH treatment can affect the unfolding, denaturation and aggregation pathways of numerous globular proteins, mainly from animal sources. More recently, it was even demonstrated how MEF can be applied to control the gelation process of whey proteins (Rodrigues, Avelar, et al., 2020). However, with regard to their impact on plant proteins functionalization, this is an area that clearly requires further research, as reviewed in the previous subsection (4.1). Likewise, the influence of protein-protein interactions and of the interactions of proteins with other food constituents (such as polysaccharides and bioactive compounds, e.g., vitamins) is still not unveiled.

Regarding HPP, there are a few studies dealing with this topic. One of the first published reports dates back to 1994, in which it was demonstrated the influence of salts (NaCl, LiCl and KCl) and glycerol in the inhibition of the high pressure-induced dissociation of vicilin, the 7 S storage pea's protein (Pedrosa & Ferreira, 1994). Other authors have also observed that the presence of NaCl (Añón, de Lamballerie, & Speroni, 2011) and Ca (Speroni, Añón, & de Lamballerie, 2010) had an influence on soybean proteins subjected to HPP, being clearly dependent on the value of applied pressure and the salt/mineral concentration. The conjugation of plant proteins with polysaccharides to form fine complexes has been reported in the literature (Lin et al., 2017). Moreover, although still scarce, few studies can be found regarding the effects caused by the addition of polysaccharides to HPP-treated plant proteins, with indication to the existence of two distinct outcomes (Queirós et al., 2018). The first is related with an improvement in the emulsifying properties of vegetable proteins such as sweet potato protein (Khan, Mu, Ali, et al., 2015) and 11 S globulin of *Vicia faba* (Galazka, Dickinson, & Ledward, 1999), due to their interaction with guar gum and carrageenan, respectively. The other one with the decrease in protein aggregation during or after the application of HPP during a HPP treatment (Galazka et al., 2000; Galazka et al., 2000). Generally, polysaccharides appear to play a protective role against protein unfolding and protein-protein aggregation after HPP treatment, thus preventing the loss in protein's functionality (Galazka et al., 2000). The change in the structural and functional properties of soy protein isolate (SPI) by glycation with flaxseed gum (FG) through the Maillard reaction under HPP was hypothesized (Liu et al., 2020). By monitoring changes in SPI, the authors have reported an improvement in solubility of SPI glycosylated with FG, reaching 86.84% for the treatment of SPI-FG at pH 8 and 200 MPa. The control of glycation by HPP proved to be an effective strategy to improve SPI processability and applicability. The interaction between plant proteins and polyphenols has also been studied. However, the main focus of the studies has been related with the antioxidant properties or encapsulation and not so much with the eventual modification of proteins' properties due to the addition of the phenolic compounds investigated (Jin et al., 2021; Kadam, Palamthodi, & Lele, 2019; Pham, Wang, Zisu, & Adhikari, 2019; Ribnický et al., 2014; Tapal & Tiku, 2012; Xiang, Sun-waterhouse, Cui, Wang, & Dong, 2018). Nevertheless, there are evidences that the application of HPP during a HPP treatment to

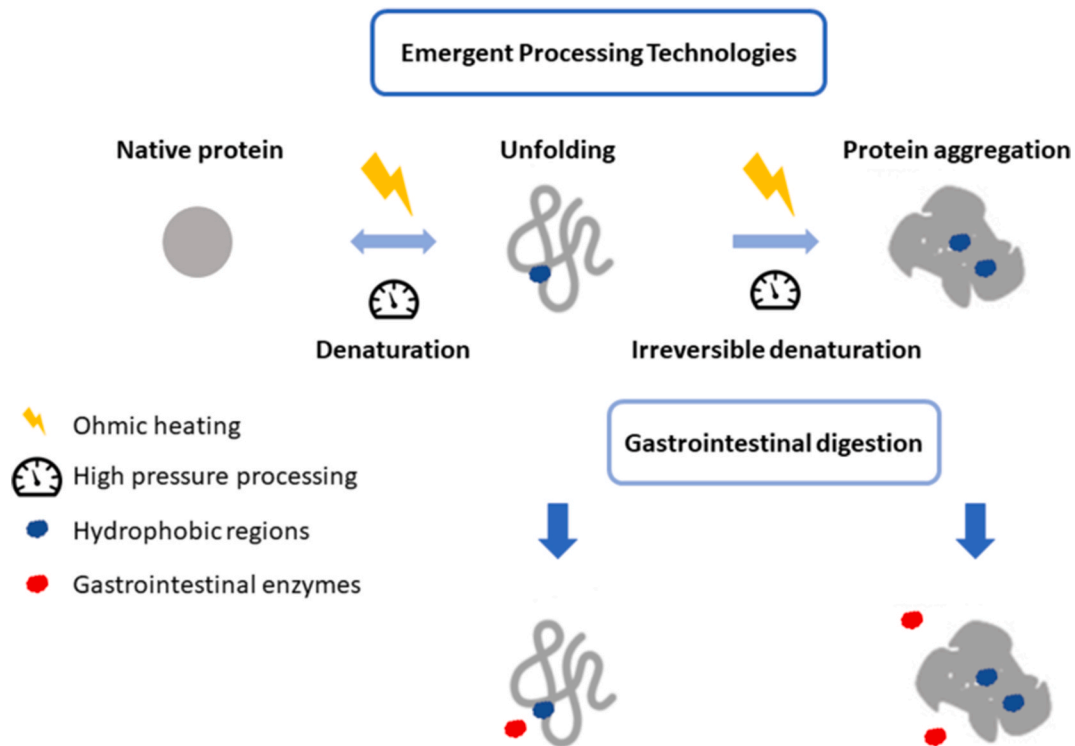


Fig. 3. Proposed influence of OH and HPP on protein gastrointestinal digestion.

modify proteins' properties may also influence the way proteins and polyphenols interact with each other, yet so far this has not been completely elucidated. In this context, the interactions between tea polyphenols (TP) and HPP-treated soybean protein (SP) were recently evaluated. The combination of TP and HPP treatment displayed a synergistic effect in solubility and emulsifying activity of SP, thus suggesting this interactions can be enhanced and bring advantages on the technological-functional properties of proteins (Chen, Wang, Feng, Jiang, & Miao, 2019).

## 5.2. Nutritional balance

Beyond the technological-functional role of proteins in foods that in many cases dictates food structure, stability and organoleptic profile, proteins are also dietary macronutrients with important structural and functional roles in the human body (Loveday, 2019; Sá, Moreno, & Carciofi, 2019). Nowadays, the consumers' awareness of the importance of diet and the promotion of health beyond nutrition has led to an increase of consumption of (perceived to be) healthier foods. For that reason, numerous plant protein sources (e.g., cereals, legumes, oilseeds, pulses and pseudocereals) have been comprehensively studied because of their composition rich in protein, fibers, minerals and other bioactive compounds with the aim of using them to the development of nutritionally improved food products (Arribas et al., 2017; Coda, Varis, Verni, Rizzello, & Katina, 2017).

From a nutritional point of view, proteins from plants may be considered inferior to those of animal origin since they often present a deficiency in some of the 9 essential amino acids composition (Arribas et al., 2017). Essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, threonine, and valine) correspond to those not being synthesized by the human body and thus, they only can be obtained by food intake (Lafarga, 2018). In general, cereal proteins possess low values of lysine while legume derived-proteins possess low levels of essential sulfur-containing amino acids (methionine and cysteine) (Neacsu et al., 2017; Pencharz, Elango, & Wolfe, 2016). Besides that, they often possess some compounds designated as

antinutritional factors, that are commonly considered responsible for plant protein deficiency. These so-called antinutritional factors are responsible for decreasing protein digestibility and inhibiting their bioavailability and thereby, limiting their nutritional value (Espinoza-Páez et al., 2017; Giami, 2004; Sá et al., 2019; Zhang, Liu, Ying, Sanguansri, & Augustin, 2017). They include antinutritional proteins such as lectins and trypsin inhibitors and antinutritional chemicals such as phytates, tannins, and polyphenols (Adenekan, Fadimu, Odunmbaku, & Oke, 2018; Alonso, Aguirre, & Marzo, 2000; Arribas et al., 2017; Drulyte & Orlien, 2019; Espinoza-Páez et al., 2017; Tušnio et al., 2017). The application of conventional processing techniques (e.g., cooking, autoclaving, germination, extrusion and fermentation) to reduce or eliminate these antinutritional compounds is well established. Nonetheless, such techniques, mainly those applying aggressive heat treatments, are often detrimental to other food constituents, so processing technologies that are applied under mild conditions are preferable (Sá et al., 2019). OH and HPP, alone or combined, may constitute a good alternative to the inactivation of such compounds under gentle temperatures, considering their already established success in modifying numerous food properties.

The issue related with the amino acid deficiency could be overcome by protein complementation, i.e., blending cereal proteins with legume proteins as a strategy to develop a more nutritious food product (Arribas et al., 2017; Pencharz et al., 2016). Moreover, as discussed in a previous subsection (5.1), the combination of plant proteins with other polymers (other proteins, from both animal or plant origin, or even polysaccharides) can be used to not only develop novel and highly functional complex food systems, but also to contribute to their balanced nutritional value (Lin et al., 2017).

## 5.3. Protein digestibility

Digestibility of food proteins is a measure of their susceptibility to be hydrolysed by the enzymes present in the gastrointestinal tract, being reliant on the structure of proteins, the presence of other food components, in particular antinutritional factors and the eventually applied



**Table 2**  
Changing the digestibility of plant proteins by the application of HPP.

Plant proteins	Approach	HPP conditions	Output	Reference
<b>Split pea protein</b>	Pre-treatment	100 or 600 MPa/30 or 60 Min/20 or 60 °C	Protein digestibility increased by up to 4.3% in peas when treated at 600 MPa and 60 °C, regardless of time	Linsberger-Martin, Weiglhofer, Thi Phuong, and Berghofer (2013)
<b>Kidney bean protein isolate</b>	HPP-assisted enzymatic hydrolysis	300–600 MPa/15 min	Improvement of alcalase activity and production of the highest degree of hydrolysis (23.9%) at 300 MPa	Al-Ruwaih et al. (2019)
<b>Red bean protein isolate</b>	Pre-treatment	400, 500, and 600 MPa/5 min/25 °C	IVPD <sup>a</sup> values for HPP 600 MPa treated samples increased from 79.3% to 83.8% compared with untreated samples	Lee et al. (2018)
<b>Flaxseed protein isolate</b>	HPP-assisted enzymatic hydrolysis	100 and 300 MPa/5 and 10 min	A pressure level of 300 MPa for 10 min improved the degree of hydrolysis by 1.7 times as compared to that of the control	Franck et al. (2019)
<b>Soy protein isolate</b>	Pre-treatment	200–300 MPa, 15 min, 20 °C	The % N release at the end of pepsin and trypsin digestion for samples treated at 300 MPa/15 min significantly increased by 6 and 7%, respectively, in comparison with the native protein	Li et al. (2011)
<b>White bean protein</b>	HPP pre-treatment	200 or 400 MPa/30 or 60 Min/20 or 60 °C	Protein digestibility increased by 8.7% in beans treated at 600 MPa at 60 °C for 60 min	Linsberger-Martin et al. (2013)

<sup>a</sup> IVPD (*In vitro* protein digestibility).

processing technology (Loveday, 2019; Sá et al., 2019).

OH and HPP are currently recognized as valuable tools in the maintenance of the nutritional and sensorial attributes of proteins along with impacting their structure. It is then anticipated that such eco-innovative processing technologies could not only affect functionality but also play an important role in affecting proteins' nutritional value. To our best knowledge, the available information in the literature, connecting the application of such emergent processing technologies to plant protein and how it will affect their behavior within the gastrointestinal tract, remains scarce, so further research is needed in order to

understand how these processes affect proteins' structure and its impact in gastrointestinal digestion and absorption. Fig. 3 illustrates the possible impact of OH and HPP on protein denaturation, three-dimensional structure of food proteins and therefore on their behavior towards gastrointestinal digestion.

Besides the profound influence on the functional and organoleptic attributes of food, proteins and peptides possess numerous bioactive properties, such as, antidiabetic, antihypertensive, cholesterol-lowering, antimicrobial, anti-cancer, immunomodulatory, anti-inflammatory, ability to bind vitamins and minerals among others with great importance for human health and body functions (Daliri, Oh, & Lee, 2017; Hernández-Ledesma, García-Nebot, Fernández-Tomé, Amigo, & Recio, 2014; Udenigwe & Aluko, 2012). However, bioactive peptides generally remain inactive within their polypeptide backbone and, during food digestion, partial hydrolysis by proteolytic enzymes allows their release and the generation of bioactivity (Chalamaiah, Yu, & Wu, 2018; Evangelho et al., 2017).

The digestibility of proteins and the release of bioactive peptides has been improved by the application of HPP as a pre-treatment followed by enzymatic hydrolysis or during enzymatic hydrolysis (Queirós et al., 2018) (see Table 2). A study conducted on pea protein isolate has shown the beneficial effect that HPP pre-treatment had on its digestibility since it has enhanced the degree and rate of proteolysis. It was suggested that the unfolding of globular pea proteins has occurred, allowing hydrophobic groups to be exposed to pepsin (Laguna, Picouet, Guàrdia, Renard, & Sarkar, 2017). HPP (300–600 MPa for 15 min) assisted enzymatic proteolysis (using Alcalase) of kidney bean protein isolate (KBPI) was performed by Al-Ruwaih, Ahmed, Mulla, and Arfat (2019). It was concluded that high pressure facilitated the enzymatic hydrolysis of KBPI. In fact, alcalase activity was improved and produced the highest degree of hydrolysis (23.9%) at 300 MPa, probably due to the unfolding of protein structure and the exposure of cleavage sites, facilitating protein hydrolysis. The same treatment has also increased the antioxidant activity of the hydrolysates (Al-Ruwaih et al., 2019). Franck et al. (2019) assessed the HPP (100 and 300 MPa for 5 and 10 min) assisted enzymatic hydrolysis of flaxseed protein isolate using trypsin. The degree of hydrolysis and antioxidant activity were significantly improved by an applied pressure of 300 MPa for 10 min. It was also suggested that the extent of hydrolysis and the obtention of smaller peptides through HPP-assisted enzymatic hydrolysis was related with the improvement in antioxidant capacity (Franck et al., 2019).

When considering OH processing, no available data on the digestibility of processed plant proteins could be found in the literature. However, it can be extrapolated from studies involving other conventional proteins sources (e.g., milk and egg) that this technology can have a significant impact on the proteins' behavior through the gastrointestinal tract. HPP and OH may bring the opportunity to improve the digestibility of plant proteins and the generation of bioactive peptides. Nevertheless, particularly in the case of MEF application during a OH treatment to plant proteins, research should be addressed to fully understand its implications.

## 6. Conclusions

Global nutritional trends have been shifting towards the consumption of novel and sustainable substitutes to animal-based proteins. Thus, the development of food alternatives that not only meet consumers' requirements towards animal protein substitution but are also able to accomplish the human requirements in terms of health/wellbeing and nutrition should be a priority for food researchers and industry.

Fundamental research concerning the relationship between structural changes and functionality of more conventional proteins such as soy, pea, kidney bean, cowpea, rice or lupin is still needed along with upcoming research on proteins from less studied sources that display both functional and quality parameters of interest to replace those from animal origin.

Feeding the world population sustainably also requires the use of eco-innovative and cost-effective processing technologies such as HPP and OH. Moreover, the application of pressure and electrical effects has been shown to have an important role in the functional attributes of food proteins, while ensuring the maintenance in their quality parameters and acting as effective tools to the preservation of food. There are currently strong indications on how plant proteins can be affected by HPP and OH. However, their tailoring to prepare a wide range of structures (e.g., micro or nanostructures, gels or films) for food incorporation/combination with other food components towards the design of complex food systems is far from being understood. Therefore, upcoming research regarding this topic is highly required.

Nutritional and health/wellness aspects must also be considered along with functionality. Future evaluation regarding the nutritional balance of novel multicomponent systems and the behavior of such processed proteins during their passage through the gastrointestinal tract (digestibility and bioavailability) is also of great interest.

#### Credit author statement

**Zita Avelar:** Conceptualization, Investigation, Writing - Original Draft. **Rui M. Rodrigues:** Conceptualization, Writing - Review and Editing, Supervision. **António A Vicente:** Writing - Review and Editing, Supervision. **Jorge A. Saraiva:** Writing - Review and Editing, Supervision.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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