



# Non-thermal processing of plant-based proteins: comparison of cold plasma treatment with high hydrostatic pressure, ultraviolet radiation, and ultrasonication treatments

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## Abstract

The current trend toward plant-based protein intake over animal protein offers a new pathway for agricultural practices to reduce environmental impact and greenhouse gas emissions. However, for their widespread use, it is of great importance to improve their techno-functional properties and digestibility. Novel non-thermal technologies that modify plant-based proteins involve structural changes. These changes are followed by adjustments to their functional characteristics, which are then used to create a system of protein ingredients for food formulation. Classical thermal methods are based on the utilization of heat and are expensive and environmentally unfriendly due to their relatively high energy requirements. Non-thermal and innovative techniques like cold plasma (CP), high hydrostatic pressure (HHP), ultraviolet radiation (UVR), and ultrasonication (US) are proven eco-friendly methods. Additionally, non-thermal methods are effective in improving the techno-functional properties of proteins and generally preserving nutritional value. This review discusses and compares the impact of CP, HHP, UVR, and US applications on various plant-based proteins, their structural alteration, and the subsequent changes in protein function and in vitro digestibility.

**Keywords** Protein modification · Non-thermal techniques · Plant-based proteins · Cold plasma · Ultrasonication · High hydrostatic pressure · Ultraviolet radiation

## Introduction

It is estimated that the global population will reach approximately 9.1 billion people by 2050. This population growth necessitates a 70% increase in food production [1]. In addition, malnutrition, which can lead to severe health issues, especially in developing countries, is often linked to inadequate protein and micronutrient intake. Similarly, developed countries' modern dietary habits result in low protein intake,

and cause malnutrition issues [2]. Considering the predicted rise in both global population and food demand, there has been a growing trend towards protein-rich diets in recent years, especially alternative protein sources [3].

Animal-based proteins have been utilized in the food industry due to their advantageous characteristics, such as high production levels and well-balanced concentrations of essential amino acids [4]. Notwithstanding these benefits, there is an increasing trend towards the consumption of non-animal protein in the diet due to vegetarianism and veganism, especially in Western countries. Moreover, animal-based proteins present several environmental drawbacks; they are expensive, limited in availability, and may negatively impact health, contributing to conditions such as obesity and high blood pressure [5]. Consuming non-animal protein in the diet, driven by health problems, it has been suggested that plant-based proteins could potentially meet the demand for protein [6]. Driven by this growing demand for protein, plant-based proteins have drawn increasing interest from the food industry over animal-based proteins

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in terms of sustainability, lower cost, accessibility, and customer acceptance [7]. Furthermore, their cultivation requires significantly less land and produces fewer greenhouse gases compared to animal breeding, therefore resulting in reduced deforestation and a potential mitigation of climate change.

Plant-based proteins are utilized as functional additives in foods, serving as emulsion and foam stabilizers, thickening and gelling agents, and oil- and water-binding agents [5]. However, their poor functionality, digestibility, and presence of anti-nutritional compounds with strong off-taste restrict their applications in food formulations [7]. Therefore, plant-based proteins can be modified to improve their techno-functional properties, making them versatile ingredients for food systems [5]. According to Avilés-Gaxiola et al. (2018), some of the modification techniques can also be successful in minimizing or eliminating the negative effects of anti-nutrients by enhancing functional properties of plant-based proteins [8].

The physical and chemical characteristics of proteins significantly influence their techno-functional properties throughout production, storage, and consumption. Modifying protein functionality typically involves applying physical, chemical, and enzymatic processes at different levels: the molecular level, the mesoscale (where molecules form structures smaller than 300  $\mu\text{m}$ , such as protein aggregates or emulsion droplets), and the macroscale, which pertains to the overall development of protein ingredients [7]. This process known as “protein modification” involves altering molecular structure or specific chemical groups to improve functional and bioactive properties [5]. A modification may alter the protein's molecular flexibility, size, surface charge, and hydrophobic/hydrophilic ratio, potentially enhancing existing functionality or creating new ones completely. Novel and green non-thermal technologies such as cold plasma (CP), high hydrostatic pressure (HHP), ultrasonication (US), and ultraviolet radiation (UVR) are employed to modify proteins for food formulation. These techniques offer lower levels of anti-nutritional factors and nutrient loss, enhance technical and functional properties, and prevent damage to heat-sensitive phytochemicals compared to thermal technologies [7]. On the other hand, unsuitable treatment conditions may cause the loss in desired techno-functional properties or the decrease in nutritional quality. Therefore, to obtain an efficient protein modification, process conditions of protein modification should be carefully chosen and controlled [9]. Moreover, reactive oxygen species (ROS) produced during cold plasma treatment are necessary to activate transduction pathways linked to synaptic alterations in case of their controlled production. However, excess accumulation of ROS can cause oxidative damage in the mammalian central nervous system [10, 11]. Therefore,

further investigation is required to provide information regarding the toxicity of plasma-treated food products.

CP is a non-thermal technique that has attracted attention in recent years. On the other hand, HHP, US, and UVR are the most used non-thermal techniques in food science and technology. Although there are previous review studies examining the effects of cold plasma on plant-based foods, to the best of our knowledge, there has not yet been any study on comparing CP with HHP, US, and UVR in terms of techno-functional properties and *in vitro* digestibility of plant-based proteins [12]. Therefore, the present review aims to provide an overview of these non-thermal technologies on plant-based protein modification and their effects on functionality and digestibility and to provide a comparative analysis in terms of changes in functional properties and *in vitro* digestibility upon modification.

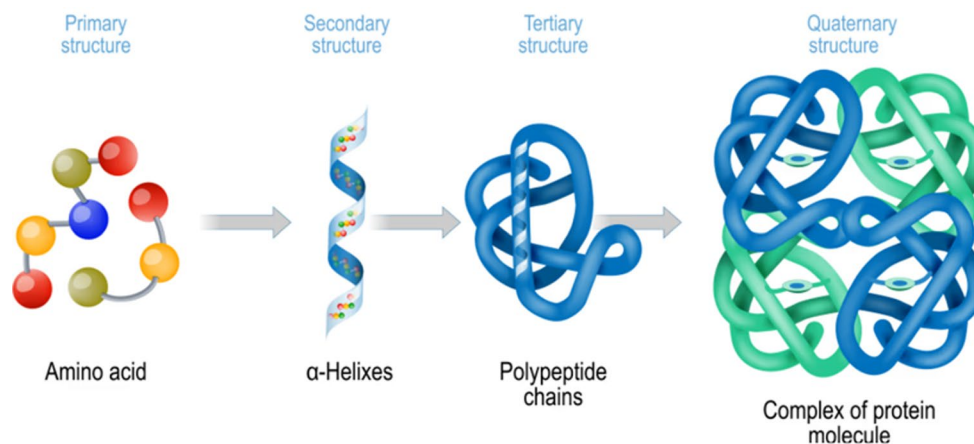
## Protein structure and functionality

The main structure of a protein consists of amino acids linked by peptide bonds. These amino acids are among the 20 naturally occurring ones, each containing an amino group ( $-\text{NH}_2$ ) and a carboxyl group ( $-\text{COOH}$ ), which participate in peptide bond formation [13]. The protein folds with hydrophobic amino acids in the protein structure's core and hydrophilic amino acids on its surface due to the varying degrees of water affinity of these amino acids, and so the free energy is minimized. Locally, this folding is shown by the creation of secondary structural elements like  $\alpha$ -helices and  $\beta$ -sheets, which are stable because they maximize hydrogen bond production while simultaneously reducing strain on the comparatively stiff amide connections. The peptide chain's irregularly folded, three-dimensional pattern, which is typically the native protein structure, is referred to as the tertiary structure due to the hydrophobic effect (Fig. 1) [14].

The primary, secondary, tertiary, and quaternary structures of proteins determine both their biological properties and processing behavior. Protein structural conformation is also influenced by carbon-bonded sulfhydryl ( $-\text{C}-\text{SH}$  or  $\text{R}-\text{SH}$ ), thioethers ( $-\text{C}-\text{S}-\text{C}-$ ), and carbonyl ( $-\text{CHO}$ ) functional groups [13, 15].

The common techno-functional characteristics of proteins are as follows: (i) solubility, water absorption capacity, swelling, thickening, syneresis, viscosity, wettability, etc. by hydration; (ii) emulsification, foaming, protein-lipid interactions formation, oil absorption capacity, flavor binding, etc.; by surface properties such as hydrophobicity, hydrophilicity, or surface charges; (iii) gelation, texture, viscosity, stickiness, gumminess, chewiness, hardness, cohesiveness, elasticity, adhesiveness, brittleness, network cross-binding, aggregation, etc.; determined by amino acid

**Fig. 1** Primary, secondary, tertiary, and quaternary structures of protein molecules (Source: Vectorstock)



sequence, composition, or size, and color, flavor, odor, texture, mouthfeel, smoothness, grittiness, turbidity, etc. by sensory and textural attributes [16]. The functional features of proteins have a significant impact on their activity in food. The structural properties of proteins (i.e., amino acid composition and sequence, structure, molecular size, and conformation) and their techno-functional properties such as water/oil absorption capacity, solubility, viscosity, emulsion, and foaming abilities must be considered together to fully understand their functions [9]. In this context, for instance, protein hydrophobicity or the ratio of hydrophobic/hydrophilic groups, extraction method applied, and post-extraction processes affect functional properties of proteins [3]. Across studies by Basak and Annature (2022), Ji et al. (2018), and Zhu et al. (2017), reduced surface hydrophobicity consistently correlated with increased water absorption and emulsification capacity, highlighting the role of protein unfolding and SH group exposure [17]. The increase in the SH group is attributed to the breaking of disulfide bonds following hydration of disulfide (S–S) bonds in proteins. In contrast, cross-linking of SH groups to form intermolecular S–S bonds between protein subunits results in a reduction in SH groups [18]. Overall, these opposing reactions indicate that processing conditions may significantly affect the balance between reduced and oxidized sulfur groups, thereby affecting protein structure and functionality.

### Protein modification with CP, HHP, UVR, and US treatments

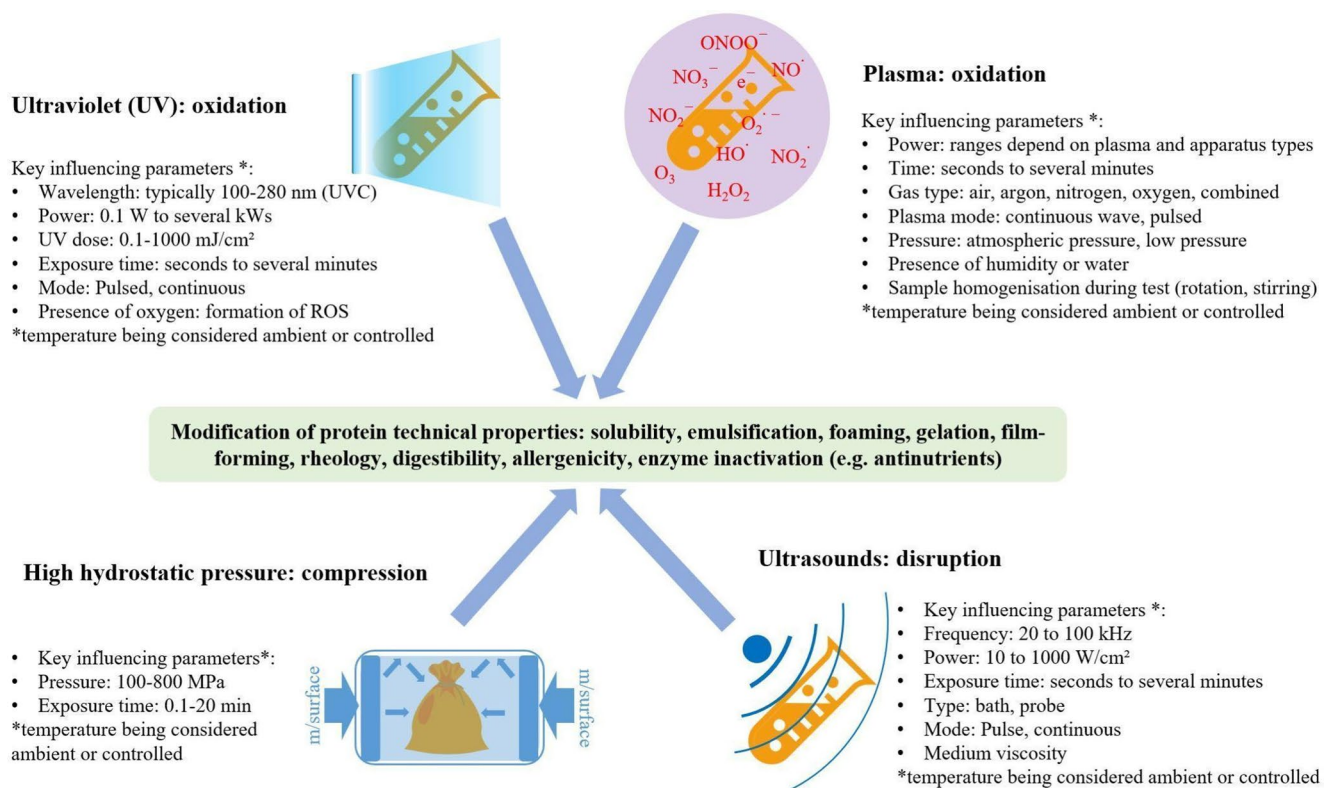
Protein modification is thought to be an alternate strategy for enhancing both the nutritional value and functional characteristics of proteins. The modification aims to alter protein conformation and intermolecular interactions [19]. These methods typically lead to unfolding, disaggregation, or irreversible denaturation of the protein structure, as well as changes in molecular size and surface properties, protein conformation, and spatial distribution of functional groups.

Chemical, physical, and biological/enzymatic modification techniques are used to alter proteins. Among these, physical modification techniques have gained prominence due to their non-thermal nature and minimal impact on nutritional integrity [20, 21]. Modifications may enhance nutritional attributes (e.g., amino acid bioavailability) and techno-functional properties (e.g., solubility, emulsification, gelation). In this review, commonly used physical approaches are discussed in the following instances. Figure 2 summarizes key parameters influencing protein modification and the resulting functional outcomes across CP, HHP, UVR, and US treatments.

### Cold plasma treatment

The cold plasma (CP) is one of the most innovative non-thermal techniques and can be used in the modification of proteins. The CP is described as a “green” method because it is energy efficient, cost-effective, sustainable, and uses fewer solvents and chemicals [22]. Plasma, the fourth state of matter, arises when a gas becomes ionized under high-energy conditions. Plasma is produced when a gas that is present or flowing between two electrodes with a large electrical potential difference is subjected to electrical energy. This results in gas ionization because of free electrons colliding with the gas molecules. CP is the name given to the ionized gas that is created by relatively low energy (1– eV) and electron density (up to  $10^{10} \text{ cm}^{-3}$ ) [23]. The first stage of plasma production consists of ionization, dissociation, and excitation steps. Secondary plasma processes include the production of ROS and reactive nitrogen species (RNS) [24]. CP systems include dielectric barrier, corona, microwave, sliding arc, radio frequency, and jet discharges, using air or other gases such as oxygen, nitrogen, argon, helium, or their mixtures [25].

Beyond its physical characteristics, CP has gained attention for its versatile applications in food science. The CP application can increase the usage possibilities of proteins as



**Fig. 2** Key parameters affecting protein alteration and their functionality in cold plasma, high hydrostatic pressure, ultraviolet radiation, and ultrasound treatments

food additives in the food industry by improving their functional properties, such as water-binding and gelling properties [26, 27]. Furthermore, CP can be utilized for microbial inactivation [28, 29], improving the quality [30] and sensory attributes [31] of food, modification of food package material [32, 33], allergy degradation [34], and enzyme degradation [35]. Finally, CP offers many advantages, including preserving nutrient content, no sensory alteration, and physical, structural, and compositional integrity [36].

### High hydrostatic pressure treatment

High hydrostatic pressure (HHP) is a non-thermal technique that enhances protein functionality through the application of pressures between 100 and 1000 MPa [26]. Foods, whether packaged or unpackaged, are subjected to these pressures to achieve high hydrostatic pressure, also known as ultrahigh pressure. While the secondary, tertiary, and quaternary structures may unfold and dissociate and are impacted to varying degrees, the primary structure of proteins stays the same because HHP affects the non-covalent bonds (ionic, hydrophobic, and hydrogen bridges) of proteins [37, 38]. The alterations in the protein structure result from the breaking of weak hydrogen bonds and van der Waals forces [38]. Protein denaturation and unfolding caused by high pressure

exposed core sulfhydryl groups (previously buried sulfhydryl groups), reducing solubility and increasing surface hydrophobicity, ultimately leading to protein aggregation or gelation.

Beyond its biochemical effects, HHP systems are engineered to accommodate both packaged and unpackaged foods. Industrial HHP systems consist of a high-pressure production mechanism, a temperature control mechanism, product placement, transportation systems, and a high-pressure vessel. Either the direct or indirect compression method is used to generate pressure in HHP applications. In the indirect compression process, a food pressure pump compresses a container to achieve the desired pressure. In industrial settings, this method is applied to packaged goods. In direct systems, a piston applies compression, generating pressure inside the container [39].

### Ultraviolet radiation treatment

Ultraviolet radiation (UVR) is a cost-effective, non-thermal technique used to cross-link proteins and enhance their functional properties [40]. UVR offers a physical, eco-friendly alternative to chemical treatments [41]. UVR is a non-ionizing portion of the electromagnetic spectrum between visible light and X-rays. There are four primary forms of UVR,

which range in wavelength from 100 to 400 nm: Ultraviolet (UV)-A (315–400 nm), UV-B (280–315 nm), UV-C (200–280 nm), and vacuum-UV (100–200 nm). UV-C radiation uses safe, non-toxic physical energy and is a non-thermal, chemical-free technique [42]. The effectiveness of UVR treatment depends not only on wavelength but also on several operational and product-specific factors. Process parameters (wavelength, exposure duration, light source, etc.), product type (chemical composition, viscosity, turbidity, etc.), and equipment (conformation and geometry) all affect how effective process conditions are when applying UVR [42]. Tryptophan, tyrosine, and phenylalanine are aromatic residues that absorb light, which is the primary cause of protein modifications in response to UVR. Compared to other aromatic residues, tryptophan has a higher probability of undergoing changes due to irradiation [43, 44].

Proteins are highly responsive to the production of primary free radicals (hydrogen atoms, hydroxyl radicals, and hydrated electrons) in the presence of UV radiation. Due to their significance in biological oxidative diseases, these reactions have been thoroughly explored [45]. While extensively studied in biomedical contexts, these radical-mediated reactions also influence protein behavior in food systems. According to Kuan et al. (2013), since UVR induces the polymerization of protein chains, it can alter protein functionality, including fluctuations in viscosity and solubility [46]. Moreover, intramolecular disulfide bonds may be disrupted, leading to the formation of new intermolecular disulfide bonds or protein aggregates after treatment.

### Ultrasound treatment

In ultrasound application, one of the new economical and environmentally friendly non-thermal method uses sound waves in the range of 20–1000 kHz. The propagation of these waves in the solvent results in the formation of microscopically small gaps or bubbles. Bubbles filled with gas or vapor grow until they reach a critical size and collapse, releasing energy through cavitation, and when they reach a critical volume that cannot absorb any more energy, they implode. These internal explosions occurring in cavitation bubbles cause energy accumulation in the region where they occur and create high-energy shear waves and turbulence in the cavitation region due to the high temperature and pressure. These mechanical effects not only disrupt cellular integrity but also influence the released protein structure and behavior in solution [47, 48].

The main way that US alters protein functioning is by heating and shearing localized hydrodynamically in solution, which changes the structure of the protein molecules. According to O'Sullivan et al. (2017), the only known mechanisms by which high-power ultrasound waves alter protein

structure are hydrodynamic shearing, which reduces native protein particles into small nanosized, dispersed nanoparticles [49]. Shearing often has an impact on the distribution and size of protein particles rather than the primary structure or molecular weight of the protein [49]. The severance of noncovalent interactions (e.g., hydrophobic, electrostatic, and hydrogen forces) that promote protein aggregation is the cause of high-power US reductions in native protein size.

## Comparison of CP with HHP, UVR, and US

### Protein structure

Proteins include thiol groups in the form of disulfide bonds (S–S) in oxidized cysteines or free sulfhydryl (SH) groups in cysteine residues. S–S and SH groups are crucial for the stability, three-dimensional conformation, and technical characteristics of proteins [27, 50]. Table 1 gives a comparison of various non-thermal emerging processing methods and their impacts on protein structure and techno-functional properties. Different alterations in the side chains of sulfur-containing amino acids can be brought on by plasma modification. In this regard, one of the first events that can be observed is the conversion of sulfhydryl groups into disulfides and other oxidized S-species [51]. Plasma treatment can cause an increase in the disulfide bond number of plant-based proteins, altering their secondary structure and rheological characteristics [5]. Protein aggregation and structural modification depend on these changes [52]. For instance, in Mehr and Koocheki's (2020) study, following 30 and 60 s of plasma treatment, the free-SH groups of the grass pea protein isolate decreased by 33.5% and 45.5%, respectively [52]. The rapid decrease in free -SH groups after 30 s of plasma treatment may be due to the substantial availability of -SH groups on the surface of protein particles to react with the reactive chemical species of plasma. However, the rate of oxidation reduces as the amount of accessible free SH groups falls when the treatment period is increased to 60 s [52]. Protein–protein interactions are controlled by  $\beta$ -sheets, whereas  $\beta$ -turns are in charge of keeping proteins hydrated. In the Held et al. (2019)'s study,  $\beta$ -turns for soft wheat flour increased considerably following plasma treatment [53]. According to Held et al. (2019), a decrease in  $\beta$ -sheets indicates a decreased possibility of protein aggregation, which hinders the formation of interconnected networks that impair gas holding capabilities [53].

Zhang et al. (2020) found that soybean protein had more random coils and fewer  $\alpha$ -helices. However, the  $\alpha$ -helix structure of grass pea protein isolate increased dramatically from 46.72% to 47.35% with an increase in exposure

**Table 1** A concise comparison of various non-thermal emerging processing methods and their impacts on protein structure and techno-functional properties [14, 17, 20, 21, 36, 50, 58, 62, 66, 67]

Non-thermal emerging technology	Process parameters	Mechanism	Effects on protein structure	Effects on functional properties of proteins
Cold plasma	Time, temperature, power, frequency, rotation, type of gas, and gas flow rate	Several high-energy radicals, including nitric oxide, atomic oxygen, superoxide, and hydroxyl radicals, can break covalent bonds and initiate various chemical reactions	High-energy reactions can cleave peptide bonds and oxidize amino acid side chains, potentially promoting protein–protein interactions. Secondary structures altered	Water/oil holding capacities, emulsifying and gelling properties of proteins were improved
High hydrostatic pressure	Pressure, temperature, and time	Protein unfolding can result from water penetrating the protein matrix, and this is influenced by the specific conditions and the protein system involved	Protein denaturation and aggregation occur, with these effects depending on the applied pressure	The process enhances emulsifying and foaming capabilities and improves protein solubility
Ultrasonication	Amplitude, frequency, intensity, energy density, time and temperature	Acoustic cavitation, involving the formation and collapse of air bubbles, triggers chemical reactions and physical effects that impact the structure and techno-functional properties of proteins	The process alters the secondary and tertiary structures, increases surface hydrophobicity and free sulfhydryl groups	The emulsifying and gelling properties of proteins enhanced
UV irradiation	Wavelength, exposure duration, light source	Protein molecules can directly absorb radiation energy, leading to alterations in their structure	In aqueous solutions, radiation-induced protein denaturation can generate hydroxyl radicals and other radicals. Superoxide anion radicals can modify the primary structure of proteins	The protein functionality, including solubility and viscosity, could be altered

duration at 9.4 kVpp [54]. This was accompanied by a considerable decrease in the level of unordered coil structure (from 22.42 to 21.68%). Following a short-term plasma treatment, grass pea protein isolate's conformation changed to a more stable and ordered secondary structure, as seen by a shift from the random coil structure to the  $\alpha$ -helix and  $\beta$ -sheet structures [52].

HHP treatment provides protein modification by causing protein aggregation or gelatinization because of the disruption of hydrophobic and electrostatic interactions. Depending on the pressure, temperature, and duration of the application, these effects could change [55]. While the secondary, tertiary, and quaternary structures may unfold and dissociate and are impacted to varying degrees, the primary structure of proteins stays unchanged because HHP affects the non-covalent bonds (ionic, hydrophobic, and hydrogen bridges) of proteins [37]. Van der Waals forces and weak hydrogen bonds are thought to be the cause of these structural alterations in the protein molecules [38]. The hydrophobic and electrostatic interactions that stabilize the quaternary and tertiary structures of globulin proteins are strongly influenced by static high pressure (> 100 MPa). Additionally, because hydrogen bonds are less sensitive to

pressure, secondary structural changes that are stabilized by them occur at higher static pressures (> 200 MPa) [56]. For example, Chen et al. (2019) found that the pressure range of 0.1–200 MPa may not alter the secondary structure of soy protein [57]. However, the slight increase in  $\beta$ -sheet at 300 MPa changed the secondary structure, and the significant increase in  $\beta$ -sheet was accompanied with a noticeable loss of  $\alpha$ -helix. Higher pressure (400 MPa) considerably altered the secondary structure [57]. Zhang et al. (2012) found that HHP treatment altered the local tertiary and quaternary structures of ferritin in soybean seeds. Furthermore, these alterations are reversible between 100 and 300 MPa, while irreversible denaturation may result from pressures greater than 300 MPa [38]. Tabilo-Munizaga et al. (2014) showed that the loss of  $\alpha$ -helix structure in wine proteins at high pressure settings may be caused by modifications in hydrogen bond stability and electrostatic interactions [37].  $\beta$ -sheet regions are less susceptible to pressure and less likely to deform than  $\alpha$ -helices [58] According to Khan et al. (2015), at pH 3, HHP treatment altered sweet potato protein's secondary structure. At 200 MPa, the  $\beta$ -sheet content increased to 67.00% while the  $\alpha$ -helix concentration decreased to 3.90% [58]. The  $\beta$ -sheet content increased

to 50.00% at 400 MPa and 57.40% at 600 MPa, while the  $\alpha$ -helix content decreased to 10.70% at 400 MPa and 9.60% at 600 MPa at the higher HHP treatment levels [58]. The quantity of free and/or available (reactive) SH groups may fluctuate because of HHP [27, 50]. HHP-induced unfolding exposes previously buried SH groups, altering the balance of free thiols and disulfide bonds. Protein unfolding at pressures ranging from 100 to 500 MPa permits the exposing of inaccessible SH groups in HHP applications [27].

In Kumar et al. (2020)'s study, it was found that the intensity of the peak in the amide I and amide II regions for the proteins gluten and gliadin had changed significantly when UVR was applied to wheat flour [41]. Additionally, the spectrum showed higher amide I peaks for flour that had undergone mild treatments compared to samples that were either untreated or severely treated. While  $\beta$ -sheet and  $\beta$ -turns increased but  $\alpha$ -helix reduced with mild treatment, it was shown that all major conformations in wheat flour changed negligibly with extreme treatment [41]. Radiation causes the folding of peptide chains, intramolecular disulfide chains, and secondary binding forces such as ionic, hydrophobic, or hydrogen bonds, or the bonds that hold many subunits together to form a functional protein. Proteins exposed to radiation may undergo permanent changes such as deamination, decarboxylation, oxidation of sulfhydryl groups, reduction of disulfide linkages, modification of amino acid moieties, valance change of coordinated metal ions, peptide-chain cleavage, and aggregation [46]. These conformational changes may arise from several processes, including the formation of intermolecular disulfide bonds involving newly generated sulfhydryl groups created during the hydration of a few broken S–S bonds. They may also result from the cleavage of existing disulfide bonds to release additional sulfhydryl groups or from crosslinking reactions between sulfhydryl and disulfide groups. The functionality of plant-based proteins has been altered because of UV-induced conformational changes. With a radiant energy of 112.8 kcal/mol of photons, a photon linked to UV-C radiation is powerful enough to alter O–H, C–C, C–H, C–N, H–N, and S–S bonds if it is absorbed by molecules [59].

US typically modifies proteins by rupturing and re-forming covalent or non-covalent connections with or within protein molecules. The effect of cavitation in aqueous media can enhance the interaction between proteins and water molecules [60]. The impact of ultrasonic treatment on the structural change of plant and animal proteins has already been reviewed in several publications [49, 60–63]. For instance, in the study of Arzeni et al. (2012), soy protein's functional characteristics, such as gelation, viscosity, and solubility, were altered by high-intensity ultrasound application [64]. These changes are thought to be closely related to molecular modifications, particularly an increase in hydrophobicity

and a variation in particle size [64]. High-power ultrasound at different intensities and durations (18.4, 29.58, and 73.95 W/cm<sup>2</sup> for 5, 12.5, and 20 min) was examined in Nazari et al.'s (2018) study to determine how it affected the functional properties of millet protein concentrate [65]. The findings indicated that the solubility of all ultrasound-treated millet protein concentrate was noticeably higher than that of the native millet protein concentrate [65].

## Techno-functional properties

### Protein solubility

Recent studies of CP, HHP, UVR, and US on processing conditions and changes in functional properties of plant-based proteins are given Table 2. Solubility is an important property of proteins that is the main condition to exhibit many other functional properties in case they are soluble. Understanding a protein's solubility property can significantly influence how well it functions as an ingredient in food formulations [3, 68]. At this point, the introduction of specific hydrophilic groups as a result of the reactive species produced by plasma enhances the contact and bonding of water molecules to the protein surface; thus, CP can aid in increasing the solubility profile of the protein isolates. Additionally, the increase in protein solubility with increasing voltage and application time has been associated with the surface charges and particle sizes of proteins [17]. Across multiple studies, CP consistently enhanced protein solubility, although the magnitude depended on voltage, frequency, and treatment time. For instance, Zhang et al. (2020) observed a 282% increase in soy protein isolate solubility after 120 Hz for 3 min, attributing this to improved water–protein interactions during plasma exposure [54]. Similarly, Bußler et al. (2015) reported a 191% improvement in solubility for *Pisum sativum* proteins under 8.8 kV for 10 min [69].

Moderate enhancements were also noted in zein proteins. Dong et al. (2018) found that 75 V CP treatment raised solubility by 25% at pH 7.0, alongside other functional upgrades such as 24% higher hydrophilicity (65 V, 60 s) and 28.57% greater tensile strength in zein films (65 V, 30 s) [51]. Likewise, Mehr and Koocheki (2020) showed that although surface hydrophobicity of grass pea protein initially increased after 30 s of treatment, overall solubility rose markedly with higher voltage and longer exposure times [52]. This behavior aligns with the principle that enhanced solubility corresponds to fewer hydrophobic residues and higher surface charge [70].

Similarly, in HHP treatments, protein solubility is affected at different levels depending on the applied pressure level. For example, solubility of proteins generally decreases at

**Table 2** A summary of the recent studies of cold plasma, high-pressure processing, ultrasonication, and ultraviolet radiation on the characteristics and functionality of plant-based proteins

Protein source	Technology	Processing conditions	Changes in functional properties	References
Kidney bean protein	High-pressure processing	Protein dispersions were treated at different pressures (200, 400 and 600 MPa) for 15 min	Water holding capacity, foaming capacity, and emulsifying property improved	[82]
Cow pea protein	High-pressure processing	Protein solutions were subjected to 200, 400, or 600±5 MPa for 5 min	Water holding capacity and gelation increased	[83]
Arachin from defatted peanut cakes	High-pressure processing	Pressure treatment was applied at 200, 300, 400, 500 and 600 MPa	The solubility and emulsifying properties of protein improved	[75]
Soybean protein isolate	High-pressure processing	The samples were treated at 60 °C under 0.1, 100, 200, and 300 MPa for 3 days by HHP	The protein solubility of soybean protein glycosylated by flaxseed gum reached 86.5% at high pressure (200 MPa)	[76]
Rice bran proteins	High pressure processing	100–500 MPa, 10 min	The solubility and oil absorption capacity improved at 100 and 200 MPa	[84]
Rapeseed protein isolate	High pressure processing	Treatment was operated at 200, 400, and 600 MPa for 15 min at 25 °C	The least gelation concentration of rapeseed isolate reduced from 15 to 6% after 600 MPa	[85]
Peanut protein isolates	High-pressure processing	Treatment was applied from 50 to 200 MPa for 5 min	The hardness of induced gels and the water and oil holding capacities of proteins significantly increased	[86]
Soy protein isolate	High-pressure processing	Treatment applied from 0 to 300 MPa in 100 MPa intervals	The foam overrun of soy protein increased from 85 to 114% at 100 MPa	[87]
Hazelnut meal protein	High-pressure processing	The process was performed at various pressures (0–150 MPa)	The particle size reduced, and water solubility, foaming and emulsifying properties improved	[88]
Faba bean protein	High-pressure processing	The protein solutions were treated at 103 MPa and 207 MPa for 6 cycles	The solubility and foaming capacity of faba bean protein improved. Tertiary and quaternary structures altered	[56]
Pea protein	High-pressure processing	The protein dispersions were treated at 200, 400 and 600 MPa	The foaming and emulsion properties improved at pH 3.0	[74]
Kidney bean protein isolate	High-pressure processing	The samples were treated at 300–600 MPa for 15 min	The water holding capacity, foaming and emulsion properties increased	[89]
Chickpea, kidney bean and soybean	Ultrasonication	Proteins treated at 2.5 W/cm <sup>3</sup> and 4.5 W/cm <sup>3</sup> for 5 min and 20 kHz with 750 W max power	The protein yield increased, partial protein unfolding and changes in secondary structure occurred	[90]
Soy and rice protein isolate, pea protein concentrate	Ultrasonication	Samples treated at different times (120, 360 and 600 s) and ultrasonic power (562.5, 637.5 and 712.5 W)	The impact of ultrasound was significantly less noticeable in most protein dispersions at a 5% concentration	[91]
Soybean β-conglycinin and glycinin fractions	Ultrasonication	20 kHz at 400 W for 5, 20, or 40 min	The increase in solubility, emulsifying activities, emulsion stability, and surface hydrophobicity occurred	[81]
Soy, pea and rice protein isolate	Ultrasonication	Sonication at ~ 34 W cm <sup>-2</sup> for 2 min	The emulsion properties improved for protein isolates by reducing the emulsion droplet size	[49]
Soy protein isolate	Ultrasonication	20 kHz at 50–55 W cm <sup>-2</sup> , 40% amplitude for 2, 6, 12 or 18 min	Longer duration of high-intensity ultrasound improved the emulsion stability of different oil-in-water emulsions	[92]
Black bean protein isolate	Ultrasonication	20 kHz ultrasonication applied at various powers (150, 300, or 450 W) and for different durations (12 or 24 min)	Surface hydrophobicity and protein solubility of the protein dispersions enhanced	[78]
Wheat gluten	Ultrasonication	Ultrasonication at power settings of 540, 720 and 900 W (corresponding to 60, 80, and 100% of maximum power) for 10 min at 25 °C	The foam capacity stability of ultrasonicated wheat gluten proteins gradually increased as the treatment power increased	[93]
Millet protein concentrate	Ultrasonication	Varying intensities and times (18.4, 29.58, and 73.95 W/cm <sup>2</sup> for 5, 12.5 and 20 min, respectively) were applied	The solubility and emulsion properties in all ultrasonicated proteins higher than those of the native millet protein	[65]
Soybean glycinin	Ultrasonication	ultrasound was applied at 20 kHz at 80 W cm <sup>-2</sup> from 0 to 40 min	Ultrasonication increased emulsion stability and decreased the turbidity	[30]

**Table 2** (continued)

Protein source	Technology	Processing conditions	Changes in functional properties	References
Soy protein isolate	Ultrasonication (Swirling cavitation)	Treatments were applied for different durations (0–60 min) and pressure (0.2–0.6 MPa) at 400 W	Treatment decreased the particle size and improved solubility and emulsifying properties	[94]
Pea protein isolate	Ultrasonication	Treatments was applied at 20 kHz, at varying amplitude 30%, 60%, 90% for 30 min	The foaming ability and stability remarkably increased	[95]
Soy protein isolate	Ultrasonication	Treatment was conducted at 25 kHz and different power level (200 W, 400 W and 600 W) for 15 min	The emulsifying capacity and stability remarkably improved against creaming during quiescent storage. Solubility was nearly doubled	[67]
Tamarin seed protein isolate	Ultrasonication	Treated different power level (100 W and 200 W) for 15 min or 30 min	The solubility, emulsifying and foaming properties, water and oil holding capacity were enhanced	[96]
Sunflower protein	Ultrasonication	Samples were sonicated at a frequency of 20 kHz (500 W and 25% amplitude) for 5, 10, 20 and 30 min	All functional properties were significantly enhanced except water holding capacity	[97]
Barley fractions and barley protein isolate	Ultrasonication	Process operated at 20 kHz and 100% amplitude of 100% for 2.5 min followed by cooling and another 2.5 min	The protein solubility and colloidal stability of samples improved	[80]
Potato protein and fractions	Ultrasonication	The potato protein and fraction (patatin) treated at 600 W for 10, 15, and 20 min	The protein emulsification and properties, foaming ability and solubility improved after 20 min ultrasonication, but foam stability significantly decreased	[98]
Chickpea protein isolate	Cold plasma treatment	Using air as the process gas (30 L/min), 30 s with a 4 cm nozzle-to-liquid distance and at around 30 °C	Treatment at pH 12, yielded the optimal functional profile characterized by peak performance in both solubility and surface hydrophobicity	[99]
Sunflower seed protein	Cold plasma treatment	4 mm electrode gap, 29.5 × 21 cm processing tray, a direct current of 1 ± 0.2 A, 50 W voltage and treatment times for 0, 1, 2, 3, 4, and 5 min	The solubility of sunflower protein exhibited an important increase, rising from 2.65 to 7.3%	[100]
Walnut protein isolate	Cold plasma treatment	Treatment was performed at 80 V and 1.0 ± 0.2 A with a 50 Hz pulse frequency for durations of 0 (control), 30, 60, 90, 120, and 150 s at 8 mm of the quartz dielectric plate	The treatment yielded comprehensive functional gains: solubility (11.42%), emulsification (activity: 0.50 → 2.75 m <sup>2</sup> /g; stability: 1.22 → 30.50 min), and binding capacities (water: 0.45 → 1.21 g/g; oil: 2.54 → 7.46 g/g)	[101]
Pea protein concentrates	Cold plasma treatment	Operating parameters included 0–30 kV, for 10 min total in 2-min intervals	The gelation capacity of pea protein enhanced by cold plasma and pH-shifting. High solubility and robust gels at 70 °C were achieved for aggregates	[102]

low pressure values such as 200 MPa due to the formation of insoluble aggregates and polypeptides of higher molecular weight [26]. On the other hand, protein solubility increases due to the transformation of insoluble aggregates into soluble aggregates of lower molecular weight when pressure values are increased up to 600 MPa [26]. Similarly, gradual decreases in the insolubility of soy protein isolates, when the pressure was increased from 200 to 600 MPa [71]. In the study of Puppo et al. (2004), solubility of soybean protein isolates (at pH 3) increased over 200 Mpa [72]. Similarly, in the study of Qin et al. (2013), when pressure was increased from 300 to 600 MPa, solubility of walnut protein isolates increased [73]. Conversely, Cao et al. (2017)'s study including application of HHP to proteins extracted from pine nuts, protein solubility increased at 200 MPa pressure values [74]. According to Qin et al. (2013), this could be connected to the exposure of embedded hydrophobic groups to an aqueous environment and the development of greater molecular weight aggregates as a result of protein–protein interaction

[73]. For instance, when pressure was above 300 MPa, the solubility of arachin from defatted peanuts dramatically declined, primarily because of the formation of insoluble aggregates from increased exposure of hydrophobic regions [75]. In contrast, glycosylated soybean protein isolate's solubility was enhanced by high pressure treatment at 200 MPa as compared to the untreated protein [76].

Cross-links, which primarily include aromatic amino acid residues, are known to develop when proteins are exposed to UVR [77]. SH/S–S exchange reactions and SH oxidation lead to protein aggregation reduced solubility. For instance, Kumar et al. (2021), sulfhydryl group level decreases with radiation applied up to a certain exposure time for a certain radiation power [59]. Kumar et al. (2021)'s study, cross-linking of free SH groups to form S–S bonds between protein subunits reached a conclusion regarding the aggregation of proteins in flour, which is also supported by a decrease in the solubility of proteins. In another study conducted by Kumar et al. (2021), It may be concluded

that the content of albumin in the aqueous extract from the treated flour decreases because of high radiation power treatment [41]. The decrease in albumin concentration indicated reduced water solubility, likely resulting from UVR-induced unfolding of secondary and tertiary structures that increases hydrophobic exposure. Additionally, intense UVR treatment of flour can generate weak acids through photochemical starch hydrolysis, increasing molecular polarity and further reducing protein solubility in water [59]. High power ultrasound application has recently been recognized as an alternate technique to increase the solubility of proteins by altering the structure, revealing more hydrophilic groups that are buried in the internal molecular structure, and reducing the particle sizes [60]. In an ultrasound application, the breakdown of internal hydrophobic connections within protein molecules and the acceleration of molecular motion led to protein aggregation. Consequently, the surface hydrophobicity and solubility of black bean protein dispersions improved [78]. Conversely, medium-power ultrasonic treatment improved the surface hydrophobicity and solubility of black bean protein dispersions by breaking them down into small soluble protein aggregates by cavitation forces [78]. According to Martínez-Velasco et al. (2018), applying the ideal amplitude and time significantly improved the solubility of faba bean protein dispersions while lowering their particle size,  $\zeta$ -potential, and surface tension [79]. While the sample remained naturally stable at alkaline pH, ultrasonication enhanced the barley protein isolate solubility at all pH levels and its colloidal stability at acidic and neutral pH [80]. The solubility and surface hydrophobicity of soybean  $\beta$ -conglycinin and glycinin fractions were found to increase with high-intensity US (20 kHz at 400 W for 5, 20, or 40 min) [81].

### Water–oil absorption capacity

Water absorption capacity (WAC) of proteins is one of the most important characteristics, and proteins with high WAC can be added to foods during food processing applications to improve their mouthfeel, thickening, and viscosity properties. On the other hand, oil absorption capacity (OAC) is refers to the ability of proteins to hold oil physically and is an important functional property, especially for meat, sausage, and mayonnaise [103]

Water is attracted by charged components such as proteins, bound water is localized near non-aqueous components and has a reduced mobility [104]. Water absorption capacity of proteins can be explained by their structural properties. For instance, Ji et al. (2018) found that a decrease in  $\beta$ -sheets, which are primarily in charge of proteins' hydrophobicity, increased the peanut protein isolate gel's capacity to absorb water [105]. Greater hydrogen bond connections between

amide and water molecules are implied by an increase in  $\alpha$ -helices, which lengthen the protein molecule and enhance WAC. Reduced hydrophobicity and increased hydrophilicity further enhance WAC in protein gels [56]. However, the mechanism of oil absorption capacity (OAC) depends on interfacial interactions, which aid in retaining the oil that has been absorbed, and hydrophobic proteins are essential for this functional property. The amount, type, and amino acid compositions—in particular, hydrophobic residues that interact with lipids' hydrocarbon chains—are all related to the OAC. The higher ability of more hydrophobic proteins to bind lipids suggests that side chains of non-polar amino acids bind to lipid chains [106].

According to Bußler et al. (2015), plasma-induced chemical modification of proteins on the surface of protein-rich pea flour may have altered the hydrophilic/hydrophobic balance, which in turn could have affected the flour's ability to bind oil and water [69]. For instance, in Ji et al.'s (2018) study, CP application caused protein gels to exhibit high WAC and reduced surface hydrophobicity due to the enlarged surface area and greater number of hydrophilic groups in peanut protein isolates; moreover, the gelation value of CP-treated samples was  $\sim 18\%$  higher than that of untreated isolates [105]. Experiments with a pea protein isolate further demonstrated that plasma-induced structural changes, reflected in a 191% rise in solubility, were responsible for WAC and OAC values in pea flour reaching 113% and 116%, respectively [69].

Similarly, HHP treatment from 50 to 200 MPa for 5 min gradually raised the WAC and OAC of peanut protein isolates [85]. In Cao et al.'s (2017) work on pine nut proteins, WAC and OAC were likewise elevated at 400 MPa [107]. OAC values climbed by 43.65, 45.98, 50.01, 50.31, and 59.66% under pressures of 50, 80, 100, 150, and 200 MPa, while WAC values showed corresponding improvements of 1.48, 2.09, 7.33, 9.51, and 29.46% [85]. As Li et al. (2011) demonstrated for soy proteins, WAC can be enhanced at lower pressure–time combinations but often declines at higher levels [50].

In UVR-induced protein modification, WAC rose steadily with radiation power and exposure duration, from  $75.6 \pm 1.13\%$  in untreated wheat flour to  $85.1 \pm 0.64\%$  at maximum settings [41]. This enhancement was attributed to protein unfolding, which expose hydrophilic groups such as hydroxyl ( $-\text{OH}$ ) and sulfhydryl ( $-\text{SH}$ ) groups capable of binding water [41]. In the study by Fathi et al. (2018), it was reported that edible films derived from sesame protein isolates displayed greater density and hydrophobicity following UVR treatment, whereas moisture content and solubility declined; UVR exposure also improved the structural and morphological characteristics of the films [40]. The formation of covalent bonds within the film matrix was cited as

the principal factor underlying the enhanced surface hydrophobicity [40].

Ultrasonication also influences WAC and OAC. Omura et al. (2021) observed that ultrasound generally reduced the WAC of soy and pea protein isolates, except under specific conditions (5.0% w/v, lower power and shorter time), where WAC rose slightly [108]. In rice protein isolates, all treatments resulted in marginally higher WAC relative to the control, likely due to the formation of poorly soluble aggregates capable of interacting with water or oil. Hu and Li (2022) reported that US pretreatment had no notable effect on the OAC of soy protein isolate nanofibrils [109]. In jackfruit protein isolates, ultrasound at 200, 400, and 600 W led to a decline in WAC, whereas OAC values were significantly higher than the control [110]. Additionally, a negative correlation between WAC and OAC was noted after ultrasonication. Conversely, okara proteins extracted via combined ultrasonication and alkali treatment exhibited improvements in both WAC and OAC [111].

### Emulsification and foaming properties

The economic value of food proteins is also significantly influenced by their emulsification and foaming qualities. The emulsification characteristics of dietary proteins are described by the terms emulsion stability (ES) and emulsion activity index (EAI). The ability of proteins to aid in the emulsion's creation and stabilization is known as the EAI. On the other hand, ES is defined as a protein's ability to stabilize an emulsion without affecting its structure over a period [112]. Additionally, foaming properties are explained by foaming capacity (FC) and foaming stability (FS) [113]. FC is defined as the potential of a protein to form foam when gas is introduced into protein solution, whereas FS is determined by measuring a decrease in foam volume over a measured period [112, 114]. Proteins' capacity to rearrange and modify their conformation, adsorb at the oil/air–water interface, and produce an interfacial, cohesive, viscoelastic layer are the basis for their foaming and emulsifying qualities. Hydrophobic/hydrophilic amino acid rearrangement, charge distribution, and structural flexibility all have a significant impact on foaming/emulsifying capabilities [15].

Improvement in emulsifying ability and interfacial activity may arise from CP treatment partial unfolding and breakdown of protein aggregates. The stability and activity of emulsions are positively impacted by electrostatic repulsions within the protein caused by the high negative charge introduced by plasma [52]. Short-duration CP treatments have been shown to produce moderately unfolded proteins with more flexible structures that may self-regulate at the interfaces of oil, water, and air. Zhang et al. (2021) found that better emulsification and foaming properties were

obtained in low-frequency (80 Hz) cold plasma application to soy protein compared to higher-frequency (120 Hz) application [115]. Similarly, after CP treatment, emulsion activity and foam capacity improved by 25, 27, and 23%, respectively [116]. In another study carried out by Zhang et al. (2021), it was noted that samples treated at 120 Hz exhibited the maximum foaming stability, whereas lower CP frequencies (80 Hz) were more effective in altering the soy proteins' emulsifying and foaming capacity [115]. However, Mehr and Koocheki (2020) asserted that high native charge introduced by plasma have a major impact on the droplet particle size of emulsions [52]. They observed that grass pea protein treated with plasma at 9.4 kVpp developed a softer structure and adsorbed the least quantity onto the oil droplets. Consequently, this treatment fails to create robust interfacial layer or prevent droplet flocculation during homogenization, which explains the larger particle sizes observed at emulsion at applied conditions. However, because of the improved protein adsorption and the development of a thick and elastic interfacial coating surrounding oil droplets, the emulsion stabilized with grass pea protein isolate treated at 18.6 kVpp for 60 s had smaller droplets [52]. Moreover, significant plasma-induced oxidation results in insoluble protein-to-protein complexes that adversely affect their interfacial properties. Therefore, while overoxidation of soy protein isolate led to molecular precipitation or aggregation via covalent or non-covalent interactions, a minor loss of ordered structures enhanced the functional efficiency of dietary proteins [15].

HHP treatment at 200 MPa could induce an increase in the EAI values of various soy protein isolates. However, applying higher pressures (400 and 600 MPa) did not lead to a significant change in EAI [26, 71]. According to Barbhuiya et al. (2021), the HHP process can reduce droplet size in oil-in-water emulsions, which can help stabilize and slow down the creaming rate [26]. Similarly, Fernandez-Avila et al. (2015) studied on the effect of HHP on physicochemical properties of soy protein isolate oil-in-water emulsion and found that the emulsions treated by HHP at 100 MPa and 200 MPa showed smaller particle size and higher stability [117]. According to Avramenko et al. (2013), HHP treatment reduced particle size and improved rheological properties of lentil protein emulsion [118]. The EAI of HHP-treated sweet potato proteins varied with pH and pressure. At pH 3, EAI increased significantly at 200 and 600 MPa but remained unchanged at 400 MPa. At pH 6, it increased notably at 400 and 600 MPa. However, at pH 9, no significant change was observed at 200 and 400 MPa, while a significant decrease occurred at 600 MPa. In the same study, the lower ES value was observed at 600 MPa for pH 3, which was probably the cause of the decrease in molecular flexibility of protein resulting from aggregation of the HHP treatment [58].

These results might suggest that unfolding of proteins and exposure of hydrophobic groups by HHP treatment led to a decrease in surface tension, which improved ES. HHP increased the surface hydrophobicity of rapeseed and potato proteins by four times but decreased the solubility of pea and cumin protein isolates [86]. Following HHP treatment, the rice bran protein's emulsifying qualities, foaming, and surface hydrophobicity were all improved [84]. According to Ahmed et al. (2018), considerable secondary structural alteration at pressures higher than 600 MPa greatly improved the kidney bean protein's ability to absorb water at the air–water and oil–water interfaces [82]. Furthermore, the protein solution's pH has a significant impact on how functional characteristics, including emulsification and heat stability, are altered. For example, compared to treatment at pH 10, cowpea protein treated with HHP at pH 8 exhibited superior gel-forming qualities and water absorption capability [83]. Zhu et al. (2017) used a HHP system in another work to investigate the functional characteristics of rice bran proteins [84]. Solubility and OAC were significantly improved by HHP at 100 and 200 MPa but foaming and water absorption capacities peaked at 500 MPa. Samples treated with HHP had a reduced least gelation concentration and noticeably greater emulsifying activity and foam stability. Furthermore, the rice bran protein's emulsion stability and surface hydrophobicity increased with pressure up to 400 MPa before somewhat declining at 500 Mpa [84].

Kuan et al. (2011) stated that the effect of UVR on protein structure and conformation and certain emulsifying and foaming properties can be anticipated [46]. Jambrak et al. (2009) state that surface hydrophobicity would be impacted by protein denaturation and conformational structural changes, which would then improve the oil-in-water emulsion system's adsorption [119]. The emulsifying and foaming qualities may be diminished because of the decrease in hydrophobicity depending on exposure intensity. Kuan et al. (2011) stated that the oil droplet size of UV-irradiated samples was smaller than that of the control sample (without UVR application) under specific conditions and decreased with increasing UV exposure time [46]. Few studies have explored UVR effects on plant-based protein emulsification; existing data are primarily from animal-based systems. The foaming ability and foaming stability of egg white protein increased with increasing UVR exposure time. The emulsifying and foaming capabilities were improved because of the conformational modifications of proteins [46]. Additionally, increased surface hydrophobicity can result in larger localized hydrophobic domains, which can improve foam stability and foaming capacity, as well as a decrease in protein surface tension for improved adsorption at the interface [120].

High-intensity ultrasound is used for emulsions stabilized by plant and animal-based proteins, which is considered an effective emulsification technology for emulsion production. The various types of oils employed and the length of time the ultrasonication treatment is applied affect the physicochemical characteristics of emulsions, according to a study by Taha et al. (2018) [92]. It is noteworthy that the emulsifying qualities of pea protein concentrate were markedly improved by the high-intensity ultrasonic treatment at 562.5 W for 426.66 s at a pH of 6.8. On the other hand, at pH 2.8, pea protein concentrate demonstrated both enhanced water dispersibility and superior emulsifying qualities, irrespective of the ultrasonication treatment used [121]. O'Sullivan et al. (2016) found that when soy, pea, and rice protein isolate solutions were ultrasonically treated at 20 kHz and approximately 34 W/cm<sup>2</sup> for two minutes, the emulsion characteristics improved for protein isolates by decreasing the size of the emulsion droplets [91]. The disruption of hydrophobic and electrostatic interactions, which keep protein aggregates in solution, brought on by the high levels of hydrodynamic shear and turbulence from ultrasonic cavitation, explains the observed decrease in protein size of plant protein isolates [92].

Ultrasonicated wheat gluten at 100% power level increased its foam capacity from 70% of untreated wheat gluten to the maximum value of 160%. Higher ultrasound power levels were shown to significantly increase the foam capacity of wheat gluten that had been ultrasonically treated. Furthermore, foam stability rose as ultrasonic power increased, peaking at 83% stability at 100% power. It was also noted that ultrasonicated wheat gluten significantly improved its ES and EAI [122]. Compared to the natural millet protein, the ultrasonicated millet protein content exhibited significantly greater solubility, emulsion activity, and stability. After low-intensity ultrasound treatments (82.37±5.51 mL), the foaming capacity of native millet protein concentrate (271.03±4.51 mL) declined; however, it increased dramatically with high-intensity ultrasound treatments (749.7±2 mL). Furthermore, following ultrasonication treatments, emulsion activity and stability significantly enhanced [65]. According to Xiong et al. (2018), the structure and foaming characteristics of pea protein isolate were examined in relation to the impacts of high-intensity ultrasound at 20 kHz, at amplitudes ranging from 30 to 90%, for 30 min [95]. The FC of pea protein isolate increased from 146 to 200%. Pea protein's FS increased from 58 to 73%. The partial unfolding of the protein molecules accounted for the improved mechanism [95].

## Gelation and rheological properties

The ability of food proteins to gel is a crucial functional characteristic in foods such as puddings, jellies, and meat products, where texture, flexibility, and water retention are important. In many food preparations, proteins are essential gelling elements. The fundamental process of gelation uses these dietary proteins to create gels or products containing gels that have important rheological characteristics, such as appearance and gel point. Gelation arises from protein–protein and protein–solvent interactions that form a three-dimensional network [46]. Moreover, the formation of a gel depends on protein, salt concentration, and pH [95]. Additionally, as proteins unfold, hydrophobic amino acid residues become visible, creating attractive forces between the hydrophobic areas of different proteins. These connections may be stabilized by other mechanisms (via hydrophobic contacts, Van der Waals, S–S bonds, and H-bonds), leading to irreversible aggregation [123, 124].

It is suggested that CP modification is a viable and effective way to enhance gelling qualities of food products [102]. After atmospheric CP treatment at 30 kV for 10 min, the gel-forming capabilities of pea protein isolates were examined in a recent work [54]. In this study, pea proteins without CP could form gels at temperatures higher than 95 °C, while those with CP could form gels in the temperature range of 70–90 °C, which are temperatures below the denaturation temperature. Additionally, after cold plasma application to pea protein, Zhang et al., (2021) observed that there was an improvement in the mechanical properties of the gels because of hydrophobic interactions and the increase in the amount of free SH groups [54].

HHP weakens or improves the gelation property of proteins, depending on applied pressure [38]. For example, when HHP was applied to pine nut protein isolates with poor gelation behavior, heat-induced gel strength increased, and HHP application improved functional properties of pine nut proteins [107]. Moreover, Kumar et al. (2021) investigated the effect of UVR on SH groups and viscosity of proteins in wheat flour with a mild UVR exposure at 12 and 36 W power and application times of up to 25, 50, 75, and 100 s [59]. Also, Kumar et al. (2021) observed the viscosity decreased with lower UVR applications, while an increase in viscosity occurred with more intensive UVR application [59]. Protein characteristics can be altered by ultrasonic waves, which also increase the gelling capability of proteins. However, the operating parameters—such as frequency (both high and low), duty cycle, power, treatment period, and treatment environment—determine the extent of alteration. Changes in gelation may be primarily caused by modifications to secondary and tertiary protein structures [125]. Ultrasound can improve surface hydrophobicity

and create novel intramolecular and intermolecular protein interactions, thereby improving gelation properties by partially unfolding the protein structure and breaking covalent and non-covalent bonds [97, 126]. According to Wang et al. (2023), ultrasound-treated mung bean protein exhibited a denser and more homogeneous network structure, especially at 300 W compared to the control due to disulfide bonds and hydrophobic interactions according to results of scanning electron microscopy analysis [127].

## In vitro protein digestibility

Both in vitro and in vivo digestive models can be used to evaluate the digestibility of proteins [128]. Because in vivo models are costly, time-consuming, and have concerns about animal welfare, most of the research on protein digestion uses in vitro digestibility models that mimic human digestive circumstances. These models are speedier, less expensive, replicable, and devoid of animal welfare-related ethical issues. These models should include suitable pH values, ionic compositions, enzyme activity, bile salt levels, incubation durations, and flow conditions for various gastrointestinal regions to accurately replicate human gastrointestinal circumstances [129]. Food proteins hydrolyze into peptides and amino acids when they come into contact with digestive enzymes [130].

To break up large food particles into smaller ones and increase the enzymatic surface area for further digestion, this process begins with mechanical alterations in the mouth and continues in the stomach. To facilitate their absorption into the circulation, different proteases break down polypeptides into peptides and free amino acids during the enzymatic breakdown of proteins, which starts in the stomach and continues through the small intestine and colon [128]. Protease access to peptide bonds is impacted by the 3D structure and protein aggregation state, which in turn affects protein digestibility [131].

Antinutritional elements such as trypsin inhibitors and phytates are the cause of many plant proteins' decreased digestibility in the digestive system [129]. By restricting enzyme access or creating complexes that decrease protease activity, these antinutritional substances can attach to proteins or gastrointestinal proteases and impede digestion [130]. By modifying molecular structures, aggregation states, or the release of proteins from food matrices, heat and non-thermal processing techniques can improve protein digestibility [132].

The term "protein digestibility" describes the ease with which proteins are digested by gastrointestinal tract enzymatic activity, which is impacted by interactions with other food ingredients. Amino acid sequences, bioavailability,

structural alterations, and the existence of antinutritional substances all affect how differently distinct plant proteins digest. The nutritional and functional qualities of plant proteins can be improved by non-thermal technologies such as CP, HHP, UVR, and US, which may also increase digestibility [14, 129]. Protein digestibility, which quantifies a protein's susceptibility to proteolysis, is a key determinant of its nutritional bioavailability with better health outcomes are linked to easily digestible proteins. Comprehensive review studies have documented a variety of physical, chemical, and enzymatic modification techniques to achieve the necessary functionality in terms of digestibility, particularly for plant proteins [129, 133]. Non-thermal processing significantly influences the digestibility of plant proteins by promoting structural modifications and enhancing hydrolysis within the gastrointestinal system. By inducing alterations such as protein unfolding, crosslinking, and aggregation, these modifications can effectively reshape the structural characteristics of proteins.

To improve the taste, shelf life, functionality, nutraceutical, and organoleptic qualities of food, a range of processing technologies have been used, such as CP, HHP, UVR and US. As covered in this part, the effects of various non-thermal processing conditions on the changes in vitro digestibility of plant protein have been brought to broad insight by recent studies (Table 3).

Studies investigating the effect of cold plasma technology on the digestibility of plant-based proteins are limited in the literature. However, a few studies can be summarized in this section. According to a study by Sadhu et al. (2017), amylase activity of mung beans was higher at 12 and 24 h, with a 25.2% increase in samples treated with 60 W plasma for 20 min. Protease activity also significantly increased in all plasma-treated samples, leading to higher soluble protein content (from 18.5 to 26.6 mg/g) and improved digestibility [134]. This enhancement is likely due to protein structural modifications caused by plasma treatment. In addition, barrier discharge plasma treatment effectively inactivated soybean trypsin inhibitors in soymilk in a study by Li et al. (2017) [50]. Following exposure to cold plasma at 51.4 W for 21 min, trypsin inhibitor activity in soymilk decreased by 86.1%. This reduction in trypsin inhibitors may contribute to improved plant protein digestibility [50].

At 600 MPa of pressure, antinutritional components were found to diminish. Whole legume protein digestibility increased as a result, rising 4.3% for split peas and 8.7% for white beans. A greater rate of hydrolysis and the greatest release of bioactive peptides (51.26% in the 500–1000 Da range) result from HHP's improvement of proteolysis accessibility. Protein denaturation and the creation of new protein–protein connections from the denatured strands are also responsible for the higher digestibility of

proteins treated with HHP [135]. However, HHP treatment reduced the amount of random coil in sweet potato protein while increasing the number of  $\beta$ -sheets. According to the study, the main factor affecting in vitro digestibility may be modifications in the protein structure of sweet potatoes. After 30 min of HHP treatment, the native sweet potato protein's digestibility rose from 53.83 to 59.06%. Furthermore, heat treatment improved in vitro protein digestibility and changed the secondary structures of sweet potato proteins more effectively than HHP treatment [136]. After treatment, a decrease in antinutritional factors is typically associated with an increase in plant protein digestibility [137]. Higher-power treatments were associated with lower band intensities at the SDS-PAGE analysis. The gastric digesta revealed that the higher-power treatments were associated with the reduced intensities of major native protein bands like vicilin and convicilin [121]. The strong physical forces and extremely reactive free radicals produced by sonic cavitation can damage protein molecules' backbone and side chains, changing their secondary structures and microstructures. The structural alterations brought about by ultrasonication might improve the accessibility of enzymes for the breakdown of proteins during digestion. Ultrasound pretreatment of proteins prior to enzymatic hydrolysis makes the structure more susceptible to fragmentation [138]. According to Vanga et al. (2020a), ultrasonication improved soymilk's protein digestibility while dramatically reducing trypsin inhibitor activity by up to 52% [139]. Furthermore, soymilk proteins'  $\alpha$ -helix and  $\beta$  sheet structures were decreased and increased, respectively, after 16 min of ultrasonic treatment. The same group's second study found that when almond milk proteins were exposed to pulsed high-intensity ultrasound, their secondary structural modifications were correlated with their in vitro digestibility [140]. Longer ultrasonic treatments (12–16 min) were found to boost digestibility. A drop in  $\beta$ -sheet structures and a commensurate rise in  $\alpha$ -helix structures were credited with this improvement. Additionally, a study examined the effects of two distinct pressurization instances at different time intervals on white beans and whole split peas, concentrating on antinutritional components like oligosaccharides, phytic acids, and trypsin inhibitors and their impact on in vitro digestibility [141]. Mao et al. (2020) investigated the effects of ultrasonic treatment on the in vitro digestibility and functional characteristics of potato proteins [142]. The potato protein's digestion rate and digestibility enhanced following ultrasonication at mild temperatures (40 °C) with pH shifting. The digestibility of the modified chickpea protein isolate was lower than that of the control sample when the ultrasonic treatment was light, presumably because of modifications in the hydrophobicity of protein surfaces. However, longer ultrasonic treatment periods at 200 or 400 W increased the chickpea protein

**Table 3** An overview of the latest research on the protein *in vitro* digestibility using cold plasma, high pressure processing, ultrasonication, and ultrasound treatments.

Protein source	Technology	Processing conditions	Changes in <i>in vitro</i> digestibility	References
Split pea and whole white bean	High pressure processing	Treatment was applied to various conditions (100–600 MPa, 30–60 min and 20–60 °C)	Protein digestibility was improved by up to 4.3% in peas applying 600 MPa at 60 °C regardless of time and 8.7% in beans treated at 600 MPa at 60 °C for 60 min compared to untreated samples	[141]
Bean, lupin and pea protein	High pressure processing	The samples were pressured at 20 °C for 30 min at 300 and 600 MPa	<i>In-vitro</i> gas production decreased in the HPP treated samples (300–600 MPa, 20 °C, 30 min) compared to untreated proteins	[143]
Pea proteins	High pressure processing	The pea protein solutions were treated at pressures of 70, 100, and 150 MPa for 1 and 3 passes	The 70 MPa with 3 passes presented the highest digestibility at the end of gastric phase that was comparable with control whereas the 150 MPa with 3 passes exhibited the lower degree of hydrolysis compared to both control and the treated samples with 70 MPa for 3 passes. HHP does not impair pea protein gastrointestinal fate	[144]
Soybeans	High pressure processing	The samples were pressured for either 5 and 10 min at 300, 450 and 600 MPa	The treatment significantly enhanced free amino acid content up to 2.75 times at 600 MPa when compared to control and reduced anti-nutrition factors	[19]
Sweet potato protein	High pressure processing	Treatment was applied at 200, 400 and 600 MPa	HPP did not have a significant effect on <i>in vitro</i> protein digestibility of sweet potato protein compared to control. On the other hand, thermal treatment was more effective at changing potato protein secondary structures and improving <i>in vitro</i> protein digestibility compared to HPP and untreated samples	[136]
Pea protein isolate	High pressure processing	The samples operated at 600 MPa for 5 min	The HHP followed by re-heating showed the highest rate of <i>in vitro</i> gastric digestion compared to untreated pea protein isolate	[145]
Lentil and faba bean protein concentrate	High pressure processing	The samples were treated at 600 MPa for 4 min	HPP resulted in comparable or greater gastric digestibility compared to untreated samples, but HPP didn't impact overall <i>in vitro</i> protein digestibility	[146]
Pea protein concentrate	Ultrasonication	The samples operated at 20 kHz, with five power/time combinations, providing the same energy input (240 kJ)	The pH adjustment and ultrasound pre-treatments did not affect <i>in vitro</i> protein digestion compared to untreated proteins	[121]
Potato protein	Ultrasonication	Treatments were operated at different frequencies (20, 28, 40, and 60 kHz) and ultrasonication time (0, 2, 5, 10, 20, and 30 min)	The digestibility rate increased by 16.0% and 30.8% during gastric and intestinal digestion with mild thermal (40 °C) conditions compared to untreated proteins	[142]
Lentils, chick-peas, peas, and soybeans	Ultrasonication and high hydrostatic pressure (HHP)	Ultrasonication at 47 kHz for 1.5 or 3 h, and HHP at 621 MPa for 0.5 or 1 h	Protein digestibility increased when soaking with HHP for 1 h from ~82% to ~85–86% in peas compared to untreated (raw/standard soaked) In contrast, ultrasound or HHP treatments alone during soaking generally had insignificant effects on digestibility compared to controls, with the significant improvements only when pressure-assisted soaking was followed by heat treatment	[147]
Buckwheat	Ultrasonication	20 kHz, pulsed on-time 10 s, off-time 5 s, the amplitude of 60%, and duration of 10 min	Improved the protein surface activity and overall digestibility of buckwheat protein (58.2%) compared to digestibility of untreated samples (41.4%)	[138]
Soymilk	Ultrasonication	Treatments were applied for different durations (1–16 min) at 25 kHz and 400 W	The reduction of trypsin inhibitors' activity and improving (non-statistically significantly) the digestibility of soymilk through changing the secondary structures of proteins compared to untreated soymilk	[139]
Faba bean protein	Ultrasonication	20 kHz ultrasonication applied at different amplitudes (50 and 70%) and durations (15 and 30 min)	Sonication decreased the <i>in-vitro</i> digestibility of protein compared to untreated proteins	[79]
Almond milk protein	Ultrasonication	Treatments were applied for different durations (1–16 min) at 25 kHz and 400 W	A minor increase in protein digestibility was observed with an increase in ultrasound treatment time from 83.2% to 85.1% compared to untreated protein	[140]
Quinoa	Ultrasonication	Power density and time were in the range of 0.26–0.66 W/mL and 10–40 min, respectively	The process led to small enhancement <i>in vitro</i> digestibility of quinoa protein (68.4%) compared to untreated <i>in vitro</i> digestibility of quinoa protein (51.3%)	[148]

**Table 3** (continued)

Protein source	Technology	Processing conditions	Changes in vitro digestibility	References
Rice bran	Ultrasonication	Treatment operated at 20 kHz and 23, 30, and 40% amplitude for 30 min	Ultrasonication at 40% amplitude enhanced protein digestibility of rice bran from 26.11% to 44.85% compared to untreated samples	[149]
Chickpea protein isolate	Ultrasonication	Treatment was at different levels of ultrasonic power (200, 400, 600 W) and treatment time (0, 10, 15 and 30 min)	The in vitro digestibility increased with the prolongation of ultrasonication time at 200 W or 400 W, and the sample (600 W/15 min) showed the highest digestibility (a 2.9% increase compared to untreated proteins)	[66]
Potato protein and fractions	Ultrasonication	The potato protein and fraction (patatin) are treated at 600 W for 10, 15, and 20 min	Digestibility of potato proteins was enhanced nearly 12% compared to untreated proteins	[98]
Soybean protein isolate	Ultrasonication	The protein dispersion was sonicated for 10 min at frequency of 20 kHz with a maximum power of 750 W, using different amplitude (20, 40, 60 and 80%) with a pulse duration of 5 s on / 5 s off	In vitro digestion rates of 80% amplitude ultrasound were the highest, indicating improved digestibility compared to untreated proteins	[109]

isolate's digestibility. Because the proper ultrasonic conditions changed the protein conformation, exposing cleavage sites for digestive enzymes, the protein isolate sample treated at 600 W for 15 min showed the highest digestibility [66]. Similar results were also reported by Hussain et al. (2021), who found that during the in vitro digestion of potato protein, the duration of high-intensity ultrasound treatment increased, the pH decreased significantly and quickly, and the protein digestibility increased [98]. The protein that was untreated had the lowest digestibility at 84%, while the protein that was treated for 20 min showed the largest pH reduction and the highest digestibility at 96%. The 15-min treatment came in second at 92%. Throughout the simulated gastrointestinal digestion, soybean protein nanofibrils pretreated with 80% amplitude ultrasound showed the highest digestion rate, and the digestibility of the isolate of soybean protein treated with ultrasound was consistently higher than that of untreated samples [109]. These impacts affect the digestion and functioning of proteins. Buckwheat protein's protein surface activity and digestibility increased from 41.4 to 58.2% after receiving ultrasound therapy at 20 kHz with a pulsed on-time of 10 s, off-time of 5 s, amplitude of 60%, and duration of 10 min [138]. By changing the secondary structures of proteins, ultrasonication has demonstrated the ability to decrease the action of trypsin inhibitors and increase digestibility of soymilk [139].

There are some studies regarding UVR treatment for animal products like  $\beta$ -lactoglobulin and casein [123, 124]. The core mechanism involves the photoinduced cleavage of disulfide bonds, initiated by the excitation of tryptophan residues. This cleavage induces the reorganization of secondary structure of protein by increasing the  $\beta$ -sheet and  $\alpha$ -helix structures while reducing  $\beta$ -turns. The structural changes directly correlated with a marked decrease in the gastric digestion resistance of protein. UVR-treated animal

proteins yielded a more diverse peptide profile upon digestion. However, most studies on plant proteins focus on UV-C's role in microbial safety or antinutrient reduction. Therefore, the application of UVR as a standalone or combinatorial physical pretreatment to specifically improve the proteolytic susceptibility of plant proteins constitutes a promising but underexplored research frontier, warranting further systematic study [123, 124].

## Conclusion and future scope

Plant proteins are increasingly gaining popularity as innovative food ingredients due to their practical qualities, as well as their ethical and sustainable aspects. However, compared to animal proteins, their use is restricted due to their limited techno-functional performance and undesirable sensory attributes. Non-thermal methods are gaining recognition as sustainable technologies for modifying the functionality of proteins and preserving natural flavor profiles of food without chemical additives. Without the use of chemicals, the processing procedures presented have demonstrated the ability to improve the functional, rheological, structural, thermal, physicochemical, digestibility, and nutritional qualities of plant proteins.

The collective findings assessed in this study reveal that the efficiency of cold plasma and other non-thermal techniques in modifying protein functionality is not universal but is determined by a complex interplay. Key determinants include target protein source, time of exposure, power, gas type for plasma, pressure for HHP, and environmental context of food matrix.

High hydrostatic pressure enhances the surface hydrophobicity, solubility, oil absorption capacity, in vitro protein digestibility, while also decreasing the sedimentation

of different plant proteins. It also raises the amount of free sulfhydryl groups and reveals hidden functional groups. The mechanical strength and interfacial characteristics is improved by cold plasma. Protein-enriched foods or techno-functional food ingredients can be developed from cold plasma and non-thermally processed plant proteins. Future studies should focus on pilot plant scale and continuous operation mode to better understand the commercial viability of these processed proteins, as most of the research has been done at the lab scale using batch-type instruments. Moreover, digestibility and toxicity of CP-treated proteins need to comprehensively be investigated in further studies.

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## Declarations

**Conflict of interest** Authors declare that they have no conflicts of interest related to this review study. The authors declare that grammar and language correction tools were used to enhance the readability of the article.

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