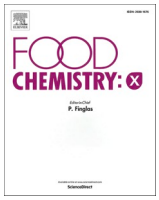
Food Chemistry: X 25 (2025) 102169

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Breaking barriers: Elevating legume protein functionality in food products through non-thermal technologies

Yuanyuan Wei [a], Delu Ning [b,c], Liping Sun [a], Ying Gu [a], Yongliang Zhuang [a,c], Yangyue Ding [a,*], Xuejing Fan [a,*]

[a] Faculty of Food Science and Engineering, Kunming University of Science and Technology, Kunming, 650500, China
[b] Yunnan Academy of Forestry and Grassland Sciences, Kunming 650201, China
[c] Yunnan Technology Innovation Center of Woody Oil, Kunming 650201, China

ARTICLE INFO

Keywords:
Legume proteins
Non-thermal technologies
Solubility
Emulsifying properties
Foaming properties

ABSTRACT

Legume proteins have recently gained significant interest in the food industry for their eco-friendliness and nutritional qualities. Research shows that the replacement of specific animal protein sources with legume proteins presents sustainability and economic benefit. Nonetheless, legume proteins frequently exhibit inferior functional properties and palatability compared to animal proteins. Various non-thermal technologies, including high hydrostatic pressure, ultrasound, cold plasma, pulsed electric field, and dynamic high-pressure microjet, had been investigated to enhance the functional properties of legume proteins without loss of nutritional and sensory properties. Although these technologies show potential, no systematic study has been conducted to summarize and compare their effects on different legume proteins. This review aims to fill this gap by addressing the most promising approaches of non-thermal technologies for the modification of functional properties of legume proteins. New insights are discussed, elaborating the effect of non-thermal technologies on the structural and functional behavior of proteins.

1. Introduction

Protein is one of the most important macronutrients in the human diet and plays important roles in the organism, particularly in the context of energy provision, growth and development promotion, and tissue repair. Deficiencies of relevant proteins may lead to malnutrition, wasting disorders, and a variety of metabolic diseases in the body (Wang et al., 2008). Human beings primarily obtain dietary protein through the consumption of animal-derived foods, such as meat, fish, eggs, and dairy products (Gharibzahedi & Smith, 2021). However, greenhouse gas emissions from livestock are one of the important contributors to global warming, and an increase in intensive livestock production is placing increasing pressure on the global supply of water, land, and energy resources consumed for raising, transporting, and slaughtering of livestock (Zhou, Hu, Xiang, & McClements, 2023). Conversely, with the increasing global population, human food demand is expected to increase by 70 % by 2050 (Gharibzahedi & Smith, 2021). These challenges have led to a commitment to finding healthy, environmentally friendly, and sustainable solutions for highly nutritious food, as well as identifying sustainable protein alternatives (Mulla, Subramanian, & Dar, 2022).

In recent years, plant-sourced proteins have become increasingly popular among consumers, driven by multiple factors such as health, environmental protection, sustainability, and ethics (Gharibzahedi & Smith, 2021), which has accelerated the redirection of dietary protein from animal sources to plant sources. Currently, legumes account for a

Abbreviations: HHP, high hydrostatic pressure; US, ultrasound; CP, cold plasma; PEF, pulsed electric field; DHPM, dynamic high-pressure microjet; SPIs, soy protein isolates; PP, pea protein; PPI, pea protein isolate; CPI, chickpea protein isolate; EA, emulsifying activity; ES, emulsifying stability; FC, foaming capacity; FS, foaming stability; EAI, emulsifying activity index; ESI, emulsion stability index; GPPI, grass pea (*Lathyrus sativus* L.) protein isolate; SPCs, soybean protein concentrates; DBD, dielectric barrier discharge; MAP, modified gas phase packaging; APPJs, atmospheric pressure plasma jets; ROS, reactive oxygen species; RNS, reactive nitrogen species; S-CPT, CP treatment for short periods of 30 and 60 s; L-CPT, CP treatment for long periods of 300 and 600 s; L-GPPIPT, long-term CP-treated GPPI; SVCOOK, sous vide cooking; HIPPEs, high internal phase Pickering emulsion; SPHMs, soya protein hydrolyzed microgel particles; LUT, lignan; PA, proanthocyanidins.

* Corresponding authors.
E-mail addresses: dingyangyue77@163.com (Y. Ding), fanxuejing@kust.edu.cn (X. Fan).

https://doi.org/10.1016/j.fochx.2025.102169
Received 3 August 2024; Received in revised form 22 October 2024; Accepted 6 January 2025
Available online 8 January 2025

notable portion of the global plant protein market, as shown in Table 1, and compared to animal proteins, legume proteins have advantages such as low cost, high yield, and low environmental pollution, and the sustainability, safety, and suitable functionality of legume proteins are at the focus of attention (Mulla et al., 2022). Legume proteins are regarded by some as underutilized in the human diet due to the following reasons:

i. Their lower nutritional value compared to animal proteins and deficiency in some specific amino acids (Rahate, Madhumita, & Prabhakar, 2021).

Table 1
A comprehensive comparison of legume proteins with animal proteins.

	Legume protein	Animal protein	Reference
Primary sources	Soy and pea proteins.	Egg albumin (ovalbumin), gelatin, and whey protein.	(Gharibzahedi & Smith, 2021)
Protein component	Lack cysteine and methionine.	Typically contain all of the essential amino acids required for humans.	(Shaghaghian et al., 2022)
Fat content	Generally low in fat and mostly unsaturated fatty acids.	Fat content varies greatly depending on the source, with high levels of meat fat and saturated fat and moderate levels of egg and dairy fat.	(Momen & Aider, 2023)
Cholesterol content	No cholesterol.	Some animal protein sources (e.g. eggs, meat) contain cholesterol.	(Jambrak, Lelas, Mason, Krešić and Badanjak, 2009)
Protein digestibility	75–80 % (may contain antinutritional factors)	90–95 %	(Kumar et al., 2022)
Biological value	80–98 %	79–104 %	(Berrazaga, Micard, Gueugneau, & Walrand, 2019)
Net protein utilization	61 %	73–92 %	(Berrazaga et al., 2019)
Protein digestibility-corrected amino acid score	74–93 %	91–100 %	(Berrazaga et al., 2019)
Functional properties	With a certain degree of emulsification, foaming, can be used in food processing to improve the texture of the product.	Good gelation, water retention, etc., which plays an important role in the processing of meat products.	(Wang et al., 2008)
Prices	Low cost of legume proteins.	Prices vary widely by type and may be more expensive overall relative to legume proteins.	(Nikbakht Nasrabadi, Sedaghat Doost, & Mezzenga, 2021)
Impact on the environment	The production process is relatively environmentally friendly, with less demand for resources and less pressure on the environment.	Production processes have a high environmental impact, such as greenhouse gases from animal feeding.	(Nikbakht Nasrabadi et al., 2021)
Suitable for people	Suitable for special groups such as vegetarians and people with cardiovascular disease.	Consumed by the general public, but for people with high blood fat and other people need to choose in moderation.	(Joshi, Timilsena, & Adhikari, 2017)

ii. The high molecular weight and low water solubility of legume proteins are major challenges in harnessing the functionality of these proteins (Alavi, Chen, & Emam-Djomeh, 2021).
iii. The recovery and separation of protein fractions come at a considerable financial expense.

In order to solve the above problems, some modification techniques, such as chemical, physical and enzymatic methods were performed to alter the functionality of legume proteins. Among them, common physical modification methods include heat treatments (e.g., drying, extrusion, roasting, steaming, and boiling) to alter the internal structure of plant proteins, resulting in denaturation and cross-linking of proteins (Ucar, Ceylan, Durmus, Tomar, & Cetinkaya, 2021). However, thermal treatments like extrusion, microwave heating and conventional heating may have complex effects on the behavior of proteins that ultimately hamper the nutritional and organoleptic properties of the proteins and cause a loss of protein bioactivity (Gharibzahedi & Smith, 2021). Because of the above drawbacks of the existing technologies, a novel, waste-less, low-temperature and eco-friendly modification method is of paramount importance. Non-thermal treatment is the innovative technology that has emerged with the potential to reduce environmental impacts and loss of sensory properties while maintaining the nutritional value and functionality of proteins. These technologies include high hydrostatic pressure (HHP) processing, ultrasound (US) processing, cold plasma (CP) processing, and pulsed electric field (PEF) processing (Li et al., 2020; Peyrano, Speroni, & Avanza, 2016; Qu, Chen, Wang, Xie, & Chen, 2023). Gharibzahedi and Smith (2021) demonstrated that applying HHP techniques at ultra-high pressures alters the spatial structure of proteins, resulting in enhanced water solubility and gel-forming ability. Similarly, US techniques can effectively enhance both nutrient absorption and functional attributes of legume proteins without affecting their bioactivity (Li et al., 2020). These non-thermal treatment methods have also been shown to reduce allergens and anti-nutritional factors, making legume proteins a safer and healthier food choice (Mulla et al., 2022).

Data for this review were collected using the Web of Science Core Collection; the search period was set from 2013 to 2023; the article type was limited to “Articles,” and the Web of Science category was set to “Food Science and Technology.” The results of our keyword co-occurrence analysis are shown in Fig. 1(A). These data can reveal the development trend of non-thermal technology in improving the functional properties of legume proteins. Articles that used HHP, US, CP, PEF, and dynamic high-pressure microjet (DHPM, also known as dynamic high-pressure microfluidization) to characterize the functional properties of soybean, pea, peanut, lentil, kidney bean, chickpea, and faba bean proteins, respectively, were collected, as shown in Fig. 1(B). In the current context of increasing global environmental pressures and food safety concerns, non-thermal technologies offer an effective alternative that not only improves the functionality and nutritional value of plant proteins but also contributes to sustainable development goals. As these novel techniques are increasingly gaining industrial attention, it is important to present updated research work on the impact of these technologies during potential industrial applications. To the best of our knowledge, a detailed review focusing on modifying the functional properties of legume proteins, specifically by using all the mentioned non-thermal technologies was not brought into the lime light. Hence, the current review intends to deliver a comprehensive and up-to-date overview of the modification of functional properties of legume proteins in relation to their structure. This overview will inform their role in the development of innovative products, such as 3D food printing, artificial meat, and sauces, and promote the integrated use of legume proteins within the food industry. The application of these technologies is expected to enable the exploitation of legume proteins in the global food supply chain (Yu et al., 2021). Future research and technology development should further explore and optimize these non-thermal methods to fully exploit their potential value in food science and

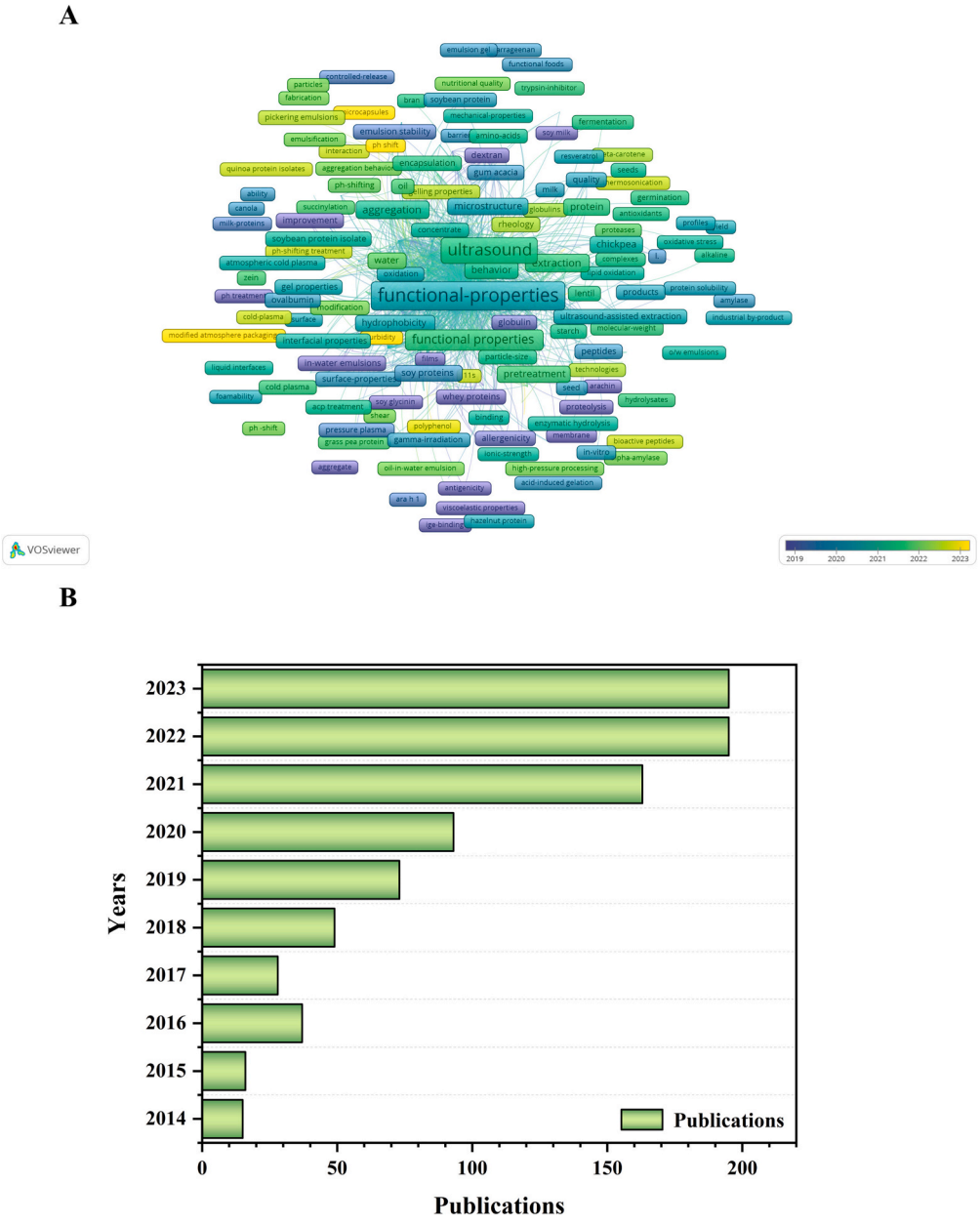


Fig. 1. An overview of co-occurrence analysis (A) using the VOSviewer program (version 1.6.20) and the publications of non-thermal technologies and functional properties of legume proteins in the last 10 years (B).

technology. This would be both a great step forward for the food industry and a strong response to global nutritional security challenges.

2. Legume protein

Legumes mainly include soy, peas, lentils, lupins, chickpeas, peanuts, and faba beans (Mulla et al., 2022). Fig. 2 illustrates that they can be a cost-effective and nutritious protein source.

2.1. Soy protein

2.1.1. Nutritional properties

Soy is rich in protein (40 %), lipids (20 %), carbohydrates (25 %), and crude fiber (5 %) and contains nutrients such as isoflavones, lecithin, minerals, and phytosterol (Xia, Pan, Cheng, Tian, & Huang, 2020). Soy protein contains all nine essential amino acids as well as a variety of bioactive peptides with the ability to reduce the risk of cardiovascular disease, high blood pressure, and certain cancers and act as antioxidants (Li et al., 2023). According to the Food and Drug Administration of the United States (FDA), incorporating soy protein into meals can lower cholesterol and the risk of cardiovascular disease (Sui, Zhang, & Jiang, 2021).

2.1.2. Soy protein composition

In soybean seeds, glycinin and β-conglycinin constitute the primary storage proteins (Zhang et al., 2021). According to their separation coefficients when subjected to ultracentrifuge sedimentation, the major storage proteins of soybean can be classified into four classes: 2S, 7S, 11S and 15S, with β-conglycinin (7S) and glycinin (11S) making up 70 % of the total protein of soy (Jia et al., 2022). Two of these protein fractions, 7S and 11S, consist mainly of 7S globulin and 11S globulin. The 7S globulin is a glycoprotein containing approximately 3.8 % mannose and 1.2 % glucosamine, and consists mainly of β-accompanied macroglobulin and γ-accompanied macroglobulin (Pomés et al., 2018). 11S

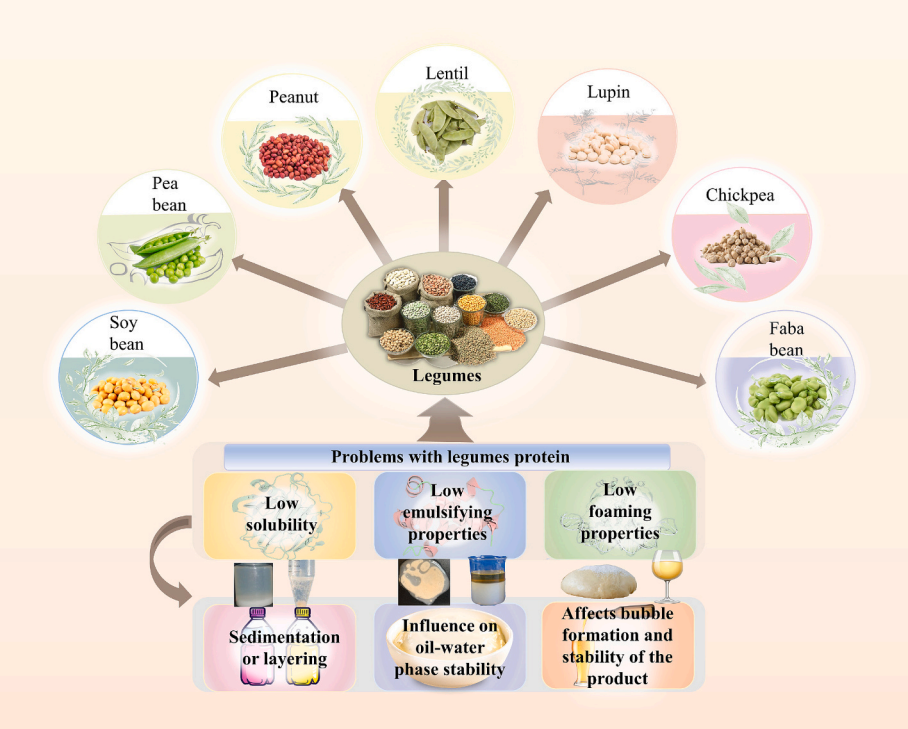


Fig. 2. Types of legumes and problems of legume proteins in the food industry.

globulin is also a glycoprotein, but its sugar content is relatively low at 0.8 %. The classification of soy proteins has been controversial in recent years, with some researchers pointing to the existence of a lipophilic protein in addition to 7S and 11S, with a lower protein content (76 %) than 7S (87 %) and 11S (93 %) and a higher fat content (11.7 %) than 7S (0.8 %) and 11S (3.3 %) (Ao, Liu, Wu, Zhao, & Hu, 2021).

2.1.3. Structure–function relationship

Research demonstrates that 7S globulin does not contain disulfide bonds either within or between peptide chains (Ippoushi, Tanaka, Wakagi, & Hashimoto, 2020), which results in reduced structural compactness of 7S globulin and reduced rigidity of the formed protein gels. Because many soya products are made from the gelling nature of soya proteins, the physicochemical properties of 7S globulin have a crucial impact on the application of soya proteins. The content of sulfur-containing amino acids (e.g., methionine and cysteine) in 7S globulin is significantly lower than that in 11S globulin, at only 16 % to 20 %. Meanwhile, the α subunit in 7S globulin has been shown to be one of the main factors triggering allergy to soy protein products in humans (Matsuo et al., 2020). A significant negative correlation ($r = -1$**, "**" denotes significant p-value less than 0.01) has been observed between the contents of 7S and 11S globulins, implying that adjusting the relative contents of 7S and 11S globulins may rectify the deficiencies of sulfur-containing amino acids in the storage proteins of soya bean seeds and reduce the risk of allergy in the consumption of soya bean protein products. Additionally, it was demonstrated that increased methionine residue levels might support improved soy protein emulsification. Compared to 7S globulin, 11S globulin showed higher emulsification activity and stability. The disparity in hydrophobic amino acid residues between 11S and 7S globulins, with the former exhibiting approximately 15 % higher levels, is a likely explanation for the observed phenomenon (Sui et al., 2021).

2.1.4. Applications

Soy protein isolates (SPIs) are widely used in the food and beverage industry because of their high yield, nutritional value, and functional properties. For example, SPIs are used in bakery products, nutritional bars, and sports nutrition products. Furthermore, because the taste of SPI is close to that of real meat, it can also be used as a meat substitute (Janardhanan, González-Diez, Ibañez, & Beriain, 2022; Yang et al., 2024).

2.2. Pea protein (PP)

2.2.1. Nutritional properties

Peas are the second largest source of edible legumes and are rich in protein (20–30 %), starch (55–68 %), and crude fiber (8–10 %), and low in fat (2 %) (Li et al., 2024). Pea protein (PP), a by-product of pea starch production, has an amino acid composition containing a high level of lysine, close to the standard pattern recommended by the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO). Research has shown that PP has a similar amino acid composition and primary sequence to soy protein, and the two have comparable functional properties and nutritional value (Qu et al., 2023). However, PP may reduce the incidence of obesity, atherosclerosis, and malignant tumors (Chao, Jung, & Aluko, 2018).

2.2.2. Pea protein (PP) composition

The main storage proteins in peas are albumin (18–25 %) and globulin (55–65 %). Globulins are soluble in salt solution and are classified according to their sedimentation coefficients as 11S globulins (legumin) and 7S globulins (vicilin and convicilin) with a ratio of 11S:7S close to 2:1 (Qu et al., 2023).

2.2.3. Structure–function relationship

In legumin, the hydrophobic groups are inside the molecule, whereas the hydrophilic groups are exposed to the outside. This structure allows the hydrophobic groups to have little contact with water, enhancing their stability. Vicilin usually carries sugar groups but lacks cysteine residues, which prevents the formation of important disulfide bonds (Barac et al., 2010). As a result, vicilin relies on hydrophobic interactions to maintain its structure. Compared to legumin, vicilin

exhibits better water solubility and superior interfacial properties. It forms a relatively greater hydrophilic surface, ensuring a more ordered and stable emulsion or gel structure (Mozafarpour, Koocheki, & Nicolai, 2022). The relative molecular mass of convicilin ranges from 71 to 75 kDa, and its secondary structure is similar to that of vicilin, predominantly consisting of a β-folded structure (Moll, Salminen, Schmitt, & Weiss, 2021). Notably, convicilin differs from vicilin in its amino acid composition, containing cysteine (Sha, Koosis, Wang, True, & Xiong, 2021).

2.2.4. Applications

PPs are used in many food applications. They can be used as a nutrient fortifier to enhance the nutritional balance of the human body and also to replace fat in food due to its rapid gel-forming properties in water, thereby reducing the risk of obesity (Chao et al., 2018). Additionally, PP exhibits good emulsifying properties and can be used as an emulsifier in dairy products, effectively reducing delamination and segregation. However, due to its relatively low charge and hydrophobic surface, PP tends to prefer the oil phase when adsorbed at the oil–water interface, which reduces the solubility and emulsification properties (He et al., 2021). Particularly near the isoelectric point of PP, the protein is prone to aggregation, which significantly impacts food quality and limits its application in the food industry (Li et al., 2024).

2.3. Peanut protein

2.3.1. Nutritional properties

Peanut is a rich source of plant-based protein (25–35 % of its content), accounting for about 11 % of the global protein supply. Peanut protein is rich in a wide range of amino acids, including all essential amino acids. It has a high utilization rate of 98 % and a digestibility rate of over 90 %, making it an easily digestible and absorbable source of protein (Yu et al., 2021). Due to its exceptional nutritional value, versatile functional properties, and relatively low cost, the abundant protein content in peanuts has gained significant attention as an appealing alternative.

2.3.2. Peanut protein composition

Peanut protein is an amphiphilic macromolecule composed of 10 % water-soluble protein and 90 % salt-soluble protein (Ji et al., 2018). The salt-soluble proteins are mainly composed of peanut globulins (arachin and conarachin with a ratio of 73:27) (Chen, Zhang, & Zhang, 2024). Structurally, arachin can be further subdivided into arachin I and arachin II, which have a similar amino acid composition and subunit pattern (Ji et al., 2018). Arachin I exists as a monomer with a sedimentation coefficient of 9S, whereas arachin II exists as a dimer with a sedimentation coefficient of 14S (Ji et al., 2019). These globular proteins are composed of six major subunits (S1–S6), which are bound in a non-covalent or covalent manner, and no disulfide bond exists.

2.3.3. Structure–function relationship

The complex structure of peanut proteins brings unique properties. As its main chain is formed by cross-linking different amino acids, peanut protein can be rich in both hydrophilic and lipophilic groups, showing the characteristics of nonionic surfactants (Chen et al., 2024).

2.3.4. Applications

In practical applications, making full use of the structural and functional properties of peanut proteins can provide new raw materials and solutions for food, cosmetics and other fields. The addition of peanut protein to pork meatballs improves the juiciness, color, and tissue status of the pork meatballs and further enhances the acceptability of the product (Wang et al., 2023). Peanut protein milk has total solids and protein content similar to bovine milk but contains a relatively higher content of phenolic compounds, which can prevent oxidative damage and coronary heart disease, stroke, and other diseases (Dai et al., 2022).

2.4. Lentil protein

2.4.1. Nutritional properties

Lentils are a rich source of protein (20.6–31.4 %) and carbohydrate (62–69 %, mainly starch) (Ahmed, Mulla, Al-Ruwaih, & Arfat, 2019). Additionally, their dietary fiber content surpasses that of beans and chickpeas (Jarpa-Parra, 2018). Lentils can induce short-term satiety and a hypoglycemic response, thus contributing to weight maintenance, especially due to the presence of β-glucans. Lentils also contain a number of active substances, including phenolic acids, flavanols, saponins, and condensed tannins, and have good antioxidant properties (Khazaei et al., 2019).

2.4.2. Lentil protein composition

Most of the protein in lentils is in the form of precipitated protein (80 %). Lentil protein consists of approximately 16 % albumin, 70 % globulin, 11 % glutenin, and 3 % prolamin (Ahmed et al., 2019). The molecular weights of albumin, glutenin, and alcohol-soluble glutenin have been estimated as 20, 17–46, and 16–64 kDa, respectively, consisting of about 13, 4, and 10 peptides, respectively (Jarpa-Parra, 2018). The 7S:11S ratio, is as high as about 3 in lentils, which is 12 times higher than that of peas and seeds. This suggests that lentils may fulfill the criteria for some specific uses (Maria Medeiros Theóphilo Galvão et al., 2024).

2.4.3. Applications

In the food industry, lentil protein concentrates have been used to replace eggs in the production of protein-rich doughnuts; lentil protein isolates have been used as emulsifiers for salad dressings and as stabilizers in nano emulsion systems (Khazaei et al., 2019). However, the anti-nutritional factors, slow hydrolysis times, low protein digestibility, and the potential for intestinal discomfort after consumption have limited the use of lentils in the food industry (Maria Medeiros Theóphilo Galvão et al., 2024).

2.5. Lupine protein

2.5.1. Nutritional properties

The main components of lupine are 8.30 % water, 5.66 % fat, 38.8 % protein, and 31.92 % dietary fiber (Naumann, Schweiggert-Weisz, Haller, & Eisner, 2019). In addition, it contains phytoestrogens, phytosterols, and other bioactive factors. Lupin is rich in a variety of essential amino acids, which help to strengthen the immune system (Ma, Habibi, & Sagis, 2024). Moreover, it is rich in lecithin, which helps to remove cholesterol from the walls of the blood vessels and prevent hardening of the blood vessels (Naumann et al., 2019).

2.5.2. Lupin protein composition

Lupin protein consists of glycosylated albumin and globulin in a ratio of 1:9. Albumin is a water-soluble protein containing disulfide bonds, and the main component is S-lupin globulin (Ma et al., 2024). Globulin consists mainly of α-lupine globulin (76.0 %) and β-lupine globulin (16.4 %), and a small amount of γ-lupine globulin (4.00–5.00 %); β-lupine globulin is the only lupine protein that does not contain disulfide bonds, which is a kind of oligomeric protein. γ-Lupine globulin, also known as 7S protein, is a more specific globulin that is soluble in water and salt solution (Lo, Kasapis, & Farahnaky, 2022).

2.5.3. Applications

Compared to soybeans, lupins stand out for their high dietary fiber and protein content and low market price (about 70–75 % of the cost of soybeans). However, the alkaloid-rich nature of lupin seeds somewhat limits its widespread use in the food industry (Lo et al., 2022). Nevertheless, lupin has significant applications in wheat products, such as bread, cakes, and biscuits. It not only enhances the essential amino acid content and water retention capacity of the product, thus extending the

shelf life, but it also increases the protein content, which in turn enhances the overall nutritional value of the food product (Ma et al., 2024). In addition, lupin can be used as a fat replacer and vegetable protein filler, adding flavor and nutrition to meat products, such as sausages.

2.6. Chickpea protein

2.6.1. Nutritional properties

Chickpeas are rich in high-quality proteins, fats, and amino acids, as well as a variety of micronutrients, making them an ideal source for human dietary needs (Wang et al., 2020). With a protein content of between 15 % and 30 %, chickpeas are not only rich in unsaturated fatty acids, but also contain a variety of vitamins (Wang, Wang, et al., 2023). In addition, chickpea also contains a variety of active substances, such as isoflavone, lectins, phenolic acids, and phytosterols, which endow chickpea with a wide range of biologically active functions, such as antioxidant, hypoglycemic, antifatigue, and memory improvement (Wang, Zhou, Li, Pan, & Du, 2024). Chickpea protein isolate (CPI) contains 18 amino acids, including 8 essential amino acids. The in vitro digestibility of chickpea protein ranges from 65.3 % to 79.4 %, and its protein efficacy ratio and bioavailability are much higher than those of other legumes (Wang, Zhang, Xu, & Ma, 2020).

2.6.2. Chickpea protein composition

Chickpea protein molecular weight is mainly between 17 and 95 kDa with numerous subunit bands, including salt-soluble globulin, water-soluble albumin, acid/alkali-soluble glutelin, and alcohol-soluble prolamin, which account for 64 %, 17 %, 17 %, and 1 % of the total protein, respectively (Tan, Li, Bai, & Gilbert, 2022).

2.6.3. Applications

Chickpea has a wide range of applications in food because of its numerous active substances and strong functional properties. As an exogenous additive in meat products, dairy products, beverages, and other food products, it can improve the product quality, nutritional value, and flavor (Wang et al., 2023). Adding chickpea flour to meat products can improve the water-holding capacity, emulsifying properties stability, and health functions of the meat products so as to balance people's daily dietary structure. Noordraven, Kim, Hoogland, Grauwet, and Van Loey (2021) added chickpea flour to instant soups, which resulted in a better thickening potential, increased content of proteins, minerals, and vitamins, and a slight improvement in the liquidity and nutritional value of the soup. Despite the favorable functional properties of chickpea protein and its common use as an emulsifier, gelling agent, and fat substitute in food items, its use in manufacturing is restricted because of the plant cell wall and the existence of anti-nutritional compounds (Wang et al., 2024).

2.7. Faba bean protein

2.7.1. Nutritional properties

Dried faba bean seeds are rich and varied in composition, with starch (58.3 %), protein (27.5–32.4 %), and dietary fiber (25.0 %) dominating (Alavi et al., 2021). Its protein content exceeds that of most legumes, such as peas, chickpeas, and lentils (Martínez-Velasco et al., 2018). In addition, the thiamin and riboflavin content of faba beans exceeds even that of some cereals and animal foods, making it recognized as an ideal provider of vitamin B_1 (Martínez-Velasco et al., 2018; Rahate et al., 2021). Faba beans are relatively low in fat (only 1.5 %) but have a superior fatty acid composition. Its content of unsaturated fatty acids is higher than saturated fatty acids, which makes faba beans superior to animal fats and milk fat in terms of fatty acid composition (Rahate et al., 2021). In addition to their high nutritional value, faba beans also contain phenolics and flavonoids with antioxidant activity (Alavi et al., 2021).

2.7.2. Faba protein composition

Faba beans contain almost twice as much protein as wheat grains, being composed of globulin (60 %), albumin (20 %), glutenin (15 %), and prolamins (8 %) (Rahate et al., 2021). It has been shown that 35 major protein bands are present in 35 different genotypes of faba bean extracts, with legumin and vicilin/convicilin accounting for 50 % and 27 % of the total proteins, respectively, as examined by Warsame, Michael, O'Sullivan, and Tosi (2020).

2.7.3. Applications

The ratio of legumin to vicilin/convicilin ranges from roughly 1:1 to 1:3 (Rahate et al., 2021). For this reason, faba beans are often used as an ideal ingredient for fortifying the protein content of various food products, such as bread, biscuits, and oil-in-water emulsions. However, the utilization of its components has been hampered by the presence of anti-nutritional factors and undesirable organoleptic properties (e.g., color and off-flavor) (Alavi et al., 2021).

3. Effect of non-thermal techniques on the functional properties of legume proteins

The functional properties of legume proteins have a significant impact on the texture, organoleptic quality, and processing of food products. In particular, protein solubility, emulsifying activity (EA) and stability (ES), and foaming capacity (FC) are key indicators for evaluating the potential of protein applications. The solubility of proteins is influenced by the pH of the medium and is usually maximized under conditions far from their isoelectric point (low acidic or high alkaline pH). In terms of emulsifying properties, EA measures the amount of oil that can be emulsified per unit of protein, whereas ES describes the ability of an emulsion to remain stable over time. Regarding the foaming properties, FC reflects the size of the interfacial area that can be created by agitating the protein, and foaming stability (FS) describes the ability of the foam to maintain its volume over a certain period of time (Wang et al., 2023). As mentioned above (Section 1), conventional heat treatment methods, although widely used in food processing, affect the structure and function of proteins (Ucar et al., 2021). Therefore, non-thermal techniques offer new ways to improve the functional properties of legume proteins. Fig. 3 shows the mechanisms by which the main non-thermal techniques may improve the functional properties of proteins. By physically modifying the protein molecules, these techniques not only maintain the nutritional value of the protein but may also enhance its functionality. The effect of each non-thermal technique on the functional properties of legume proteins is shown in Table 2 and Fig. 4.

3.1. High hydrostatic pressure (HHP)

HHP processing is an effective method for modifying the function of proteins of plant and animal origin. HHP is known to alter the molecular volume, disrupting and restricting the chemical bonding of protein molecules. This innovative non-thermal food processing technique is based on two principles (Pascal's principle and Le Chatelier's principle), which govern the quick and uniform application of high pressure to the food molecules, independent of the food form, simplifying the process and reducing energy consumption (Manassero, David-Briand, Vaudagna, Anton, & Speroni, 2018). HHP is able to adjust the secondary, tertiary, and quaternary structures of proteins without affecting the covalent bonds that maintain the flavor and nutrition of the food (Ross, Griffiths, Mittal, & Deeth, 2003). Applications of HHP in food processing involve deactivating microorganisms, such as bacteria, viruses, and parasites, as well as physically and chemically altering food structures, similar to low-temperature cooking, and extending shelf life through the partial inactivation of organisms or enzymes (Gharibzahedi & Smith, 2021).

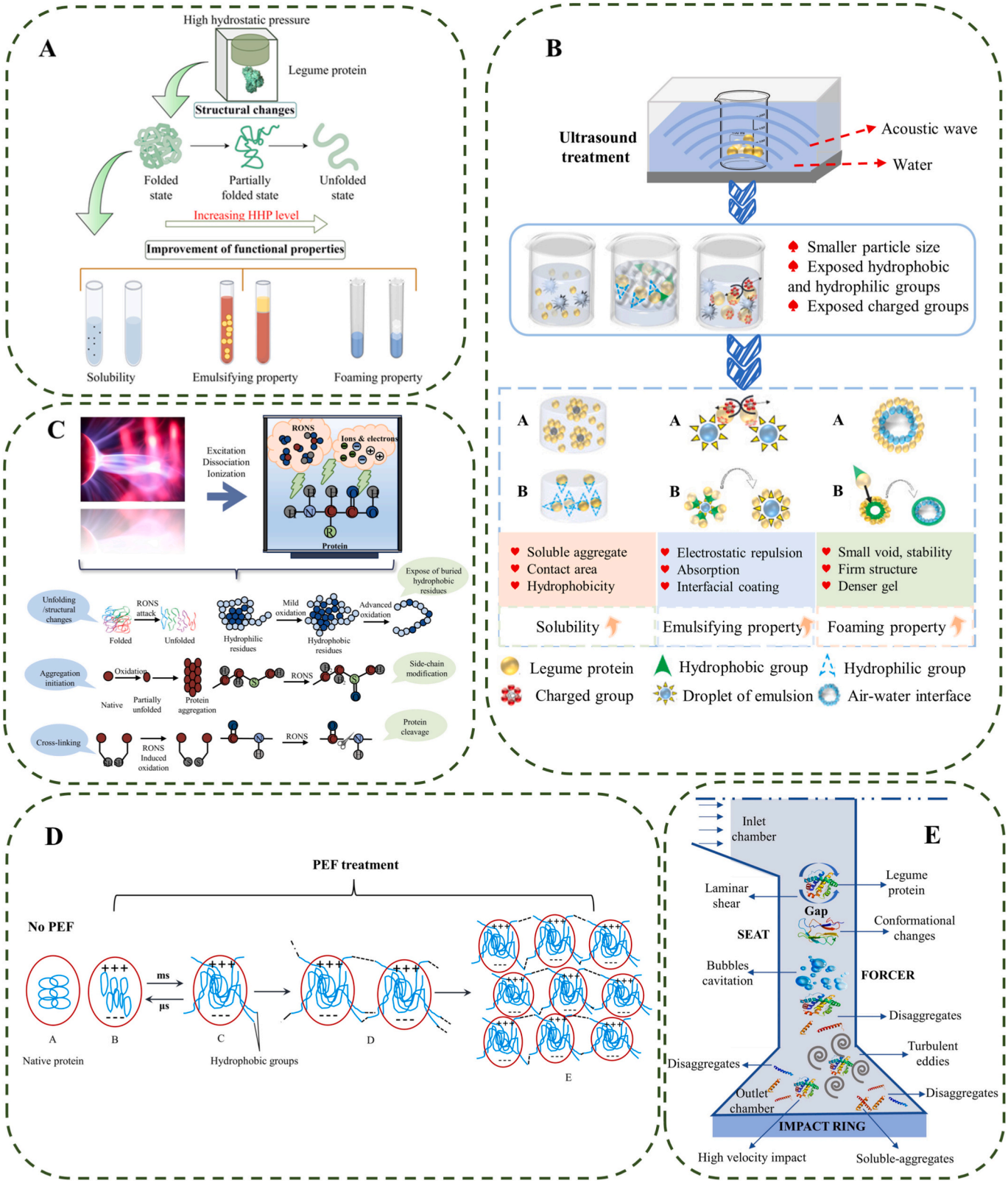


Fig. 3. Mechanism of action of (A) high hydrostatic pressure by FigDraw, (B) ultrasonic.
wave, (C) cold plasma on legume proteins, (D) pulsed electric field (Adapted from (Giteru, Oey and Ali, 2018)), and (E) dynamic high-pressure microjet.

3.1.1. Solubility

Solubility, a core functional attribute of proteins, is closely linked to the emulsifying properties and gelling properties of the protein. Although proteins are amphiphilic with both hydrophilic and hydrophobic functional groups, their solubility is affected by factors such as molecular weight, amino acid composition, net charge, and the method of protein isolation (Wang et al., 2021). HHP has been shown to change the solubility of legume proteins compared to their natural state.

Table 2
Summary of the impact of non-thermal technologies on the functionality of legume proteins.

Protein sources	Methods	Condition	Findings	Reference
SPIs	HHP	600 ± 5 MPa, 5 min.	Solubility↑	(Manassero et al., 2018)
SPI	HHP	600 ± 5 MPa, 5 min, The working pressure was reached at 5 MPa/s and released at 20 MPa/s	Solubility↑	(Piccini et al., 2019)
SPI	HHP	200 or 400 MPa, 10 min.	Solubility↑ EAI↑ ESI↑	(Tan et al., 2021)
SPI	HHP	200, 300, 400, and 500 MPa, 15 min/300 MPa for 5, 10, 15, and 20 min.	Solubility↑ EAI↑ ESI↓ FC↑ FS↓	(Li et al., 2011)
Soybean Protein	HHP	100, 200, 300, 400 and 500 MPa, 20 min.	Solubility↑	(Yang, Yang, Gao, & Chen, 2014)
Yellow field PPI	HHP	200–600 MPa, 5 min at 24 °C	Solubility- ES↑at 600 MPa FC↑ FS↑	(Chao et al., 2018)
Pea Protein Concentrate	HPP	600 MPa and 5 °C for 4 min, using a 55 L HPP unit.	Solubility↓ EAI↓ ESI↑ FE↑ FLS↑	(Hall & Moraru, 2021)
Cowpea protein isolates	HHP	200, 400, or 600 ± 5 MPa for 5 min	Solubility↓	(Peyrano et al., 2016)
Lentil Protein Concentrate	HPP	600 MPa, 5 °C, 4 min, using a 55 L HPP unit.	Solubility↓ EAI↓ ESI↑ FE↑ FLS↑	(Hall & Moraru, 2021)
Lentil protein isolate	High-pressure treatment	300, 450, and 600 MPa, 15 min.	EAI↑ ESI↓ FC- FS-	(Ahmed et al., 2019)
Lentil protein hydrolysate	High-pressure treatment	300, 450, and 600 MPa for 15 min	EAI- ESI- FC↓ FS-	(Ahmed et al., 2019)
Faba Protein Concentrate	HPP	600 MPa, 5 °C, 4 min, using a 55 L HPP unit.	Solubility↓ EAI- ESI↑ FE↑ FLS↑	(Hall & Moraru, 2021)
Red kidney bean protein isolate	High-pressure treatment	200 ± 10, 400 ± 10 and 600 ± 10 MPa, 20 min, 25 ± 2 °C.	Solubility↑ EAI↑ ESI↑	(Yin, Tang, Wen, Yang, & Li, 2008)
Red kidney bean protein isolate	High-pressure treatment	200, 400 and 600 MPa, 15 min at 23 °C.	EAI↑ ESI↑ FC↓ FS↓	(Ahmed et al., 2018)
Kidney beans protein isolates	HHP	300, 450, 600 MPa, 15 min.	EAI↑ ESI↑ FC↑ FS↓	(Al-Ruwaih et al., 2019)
Soy protein	High-intensity US	20 kHz, 400 W, 0, 5, 20 and 40 min.	Solubility↑	(Xia et al., 2020)
SPC	US	20, 40, 500 kHz, 15,30 min.	Solubility↑ EAI↑ ESI↑ FC↑ FS↑	(Jambrak et al., 2009)
SPIs	High-intensity US	20 kHz, 4.27 ± 0.71 W and 20 % of amplitude for 20 min at room temperature, at 75, 80 and 85 °C.	FC↑ FS-	(Morales, Martínez, Pizones Ruiz-Henestrosa, & Pilosof, 2015)
SPIs	US	0, 200, 400, and 600 W, 5 min, 25 °C.	Solubility↑ EAI↑ ESI↑	(Yan et al., 2021)
SPIs	US	20 kHz, 200, 400 or 600 W, 15 or 30 min.	Solubility↑	(Hu et al., 2013)
SPI nanoparticles	US	(0, 150, 300, 450, or 600 W) for 20 min, 20 kHz	EAI↑ ESI↑	(Hussain Badar et al., 2024)
GPPI	High-intensity US	24 kHz, 25, 50 and 75 % amplitudes, 5, 10 or 20 min.	Solubility↑ EAI↑ ESI↑	(Mozafarpour et al., 2022)
PPI	US	20 kHz, pH 2, 4, 10, or 12, 5 min.	Solubility↑	(Jiang et al., 2017)
PPI	US	750 W, 25 %–35 % amplitude, 5–15 min, pH 8–10.	Solubility↑ EAI↑ ESI↑	(Wang et al., 2020)

(*continued on next page*)

Table 2 (*continued*)

Protein sources	Methods	Condition	Findings	Reference
			FC↑ FS↑	
PPI	High-intensity US	10, 50, and 100 % amplitude, 30 and 70 MPa, 0, 1, 3, and 5 min.	Solubility↑ EC↑ EA↑	(Sha et al., 2021)
Lupin protein isolates	US	20 kHz, 500 W, 5, 10, 20 and 30 min, 10–15 °C.	Solubility↑	(Lo et al., 2022)
CPI	High-intensity US	20 kHz, 300 W, on-time 4 s, off-time 2 s, for 5, 10, and 20 min.	Solubility↑ EAI↑ ESI↑ FA↑ FS-	(Wang et al., 2020)
Faba bean protein isolate	High-intensity US	pH 7.4, 500/750 W, 20 kHz, 30 min.	Solubility↑ FA↑	(Martínez-Velasco et al., 2018)
Faba bean protein isolate	US	20 kHz, 1200 W, 10 and 20 min, pH 7, 10.	Solubility↑ FA↑ FS↑	(Alavi et al., 2021)
Fava bean protein isolate	US	400 W, 24 kHz, amplitudes of 60 %, 70 %, 80 %, and 90 %, 30 min.	Solubility↑ EAI↑ ESI↑ FA↑ FS↑	(Gulzar et al., 2024)
Black bean protein isolate	US	20 kHz, 0, 150, 300 and 450 W for 12 and 24 min.	Solubility↑ EAI↑ ESI↑ FA↑ FS↑	(Li et al., 2020)
Lentil protein isolate	US	700 W, 20 kHz,60 % or 70 % amplitude for 7 min.	Solubility↑	(Maria Medeiros Theóphilo Galvão et al., 2024)
Peanut protein isolate	US	20 kHz, 0, 120, 300, 480, 660, 840, 1020 W, 0, 1, 3, 5, 10, 20, 30 min.	EAI↑ ESI↑	(Zhang et al., 2014)
Mung bean protein isolate	High-intensity US	20 kHz, 360 W, 30 % amplitude, 5, 10, 20, and 30 min, 30, 50, and 70 °C.	Solubility↑	(Zhong & Xiong, 2020)
Ormosia protein	High-intensity US	20 min, 0, 125, 250, 375, 500 W.	Solubility↑ FC↑ FS↑	(Huang et al., 2024)
SPIs	PEFs	0–547 μs and 0–40 kV/cm.	Solubility↑	(Li et al., 2007)
SPIs	PEFs	5, 10, 20 kV/cm, 2 min, 1000 Hz and 40 μs, pH 3, 7, 11.	Solubility↑ EAI↑ ESI↑ FA↑ FS-	(Wang et al., 2023)
Pea protein concentrate	Moderate-intensity PEF	1.65 kV/cm, 5 μs, 400 Hz, 20,000 or 60,000 pulses.	Solubility↓ FC↑ FS↑	(Melchior, Calligaris, Bisson, & Manzocco, 2020)
Faba bean protein isolate	PEF	1.5 kV/cm, 20 μs, 20 Hz, 1000, 2000, 3000, and 4000 pulses.	Solubility↑ EAI↑ ESI↑ FA↑ FS↑	(Gulzar et al., 2024)
Mung bean protein isolate	High-intensity PEF	25 kV/cm, 0–400 pulses, 0–4 ms , pulse width of 10 μs.	Solubility↑ EAI↑ ESI↑	(Gulzar et al., 2023)
SPI	Microfluidization	120 MPa, Microfluidizer® processor model M-110EH, passed though the system three times.	Solubility↑ EAI↑	(Shen and Tang, 2012)
Pea albumin aggregates	Microfluidization	*Z*-shaped interaction chamber, 70, 90, 110 or 130 MPa.	Solubility- FC↑ FS↓at neutral PH FC- FS- at pH 5	(Djemaoune et al., 2019)
Pea globulin soluble aggregates	Dynamic high-pressure fluidization	Z-type chamber, 70 MPa and 130 MPa, passed through the system three times.	ESI↑	(Oliete et al., 2018)
Insoluble PP	Microfluidization	25, 50, 75, 100, 125, and 150 MPa, 1, 3, and 5 cycles, interaction chamber G10Z.	Solubility↑	(Moll et al., 2021)
PP	Industry-scale microfluidization	30, 60, 90 and 120 MPa.	Solubility↑	(He et al., 2021)
Peanut protein isolate	Microfluidization	40, 80, 120, and 160 MPa for 1 pass. pH 7.	Solubility↑	(Hu, Zhao, Sun, Zhao, & Ren, 2011)
SPI	CP	8 min for all three voltages (25, 30, and 35 kV).	Solubility↑ EA↑ ES↑ FA↑ FS↑	(Rout & Srivastav, 2024)
SPI	CP	DBD, 50 kV and 75 Hz, 180 s.	Solubility↑ EA↑ ES↑	(Li et al., 2023)

(*continued on next page*)

Table 2 (*continued*)

Protein sources	Methods	Condition	Findings	Reference
			FA↑ FS↑	
SPI-CMC	Plasma	DBD, 16, 18 and 20 kV, 5, 10 and 15 min.	Solubility↑ EAI↑ ESI↑	(Sharafodin, Soltanizadeh, & Barahimi, 2023)
SPI	Dielectric barrier discharge plasma	50 kHz, 50 W, 5, 10, and 15 min, 16, 18, and 20 kV, relative humidity of 32 ± 1 % and temperature of 22 ± 2 °C.	Solubility↑ EAI↑ ESI↑ FC↑ FS-	(Sharafodin and Soltanizadeh, 2022)
SPI	Atmospheric CP	DBD, 80, 100, and 120 Hz, 1, 2, 5, and 10 min, 40 kV.	Solubility↑ EAI↑ ESI↑ FC↑ FS↑	(Zhang et al., 2021)
GPPI	Cold atmospheric-pressure plasma	DBD, 20 kHz, 300 and 600 s, 9.4 to 18.6 kVpp.	Solubility↓	(Mehr & Koocheki, 2021)
PPI	CP	8 min for all three voltages (25, 30, and 35 kV).	Solubility↑ EA↑ ES↑ FA↑ FS↑	(Rout & Srivastav, 2024)
PPI	Cold atmospheric pressure plasma	SDBD, 8.8 kVpp, 3.0 kHz 10 min, conducted in triplicate.	Solubility↑	(Bußler et al., 2015)
PPI	Atmospheric pressure plasma jet	5, 15, 30, and 45 min, 2D-DBD, in triplicate.	Solubility↑ EC↑	(Bu et al., 2023)
PPI	Plasma	2D-DBD, O^3, N_xO_y, H_2O_2 and OH, 14.5 ± 0.1 W, 30 min.	Solubility↑ EC↑	(Bu et al., 2022)
PPI	CP	100 W, 0, 2.5, 5, 7.5, 10, 12.5, 15, and 20 min.	FA↑ FS↑	(Qu et al., 2023)
GPPI	CP	DBD, 9.4 and 18.6 kVpp, 30 and 60 s.	Solubility↑ CS↑	(Mehr & Koocheki, 2020)
GPPI	CP	DBD, 9.4 and 18.6 kVpp, 30, 60, 300, and 600 s.	FC↑ FS↑	(Mehr & Koocheki, 2023)
Peanut protein	CP	DBD, 70 W, 1–5 min.	Solubility↑	(Yu et al., 2021)
Peanut protein isolate	CP	DBD, 35 V, 2 ± 0.2 A, 1, 2, 3, and 4 min.	Solubility↑ ES↑	(Ji et al., 2018)
Peanut protein isolate	CP	DBD, 90 W, 0, 1, 2, 3, 4, and 5 min.	Solubility↑	(Yu et al., 2020)
Peanut protein isolate PPI-Dex	Atmospheric Pressure CP	DBD, 35 V, 2 ± 0.2 A, 0 (untreated), 0.5, 1.5, 2 and 3 min.	Solubility↑ ES↑	(Yu et al., 2020)
CPI	Atmospheric pressure plasma jet	30 L/min, 0, 10, 20, 30, 40 and 50 s.	Solubility↑ EAI↑ ESI- FC↑ FS↑	(Wang et al., 2023)
CPI	CP	30 L/min, 30 s.	Solubility↑ EAI↑ ESI↑ FC↑ FS↑	(Wang et al., 2024)

Note:↑: Increase, ↓: Decrece, —: No significant change.

Manassero, Vaudagna, Añón, and Speroni (2015) found that HHP treatment improves the solubility of proteins when using calcium-added samples, increasing their relative solubility by 450 % at 0.0050 mol/L. Manassero et al. (2018) and Piccini, Scilingo, and Speroni (2019) illustrated this further. However, it is worth noting that at pH 5.9, the solubility of the 5 g/L protein dispersion treated at 600 MPa for 5 min with calcium, followed by pressure (36.9 ± 0.4 %), was higher than that of the unpressurized dispersion (19.5 ± 0.4 %), but lower than that of the dispersion with calcium, followed by pressure (51.1 ± 1.2 %), suggesting that the order of calcium and HHP treatment has an effect on proteolysis, but not at pH 7.0 (Manassero et al., 2018). This indicates that the mechanism of HHP-induced structural changes is pH-dependent, suggesting an important role for electrostatic interactions. Li, Zhu, Zhou, and Peng (2011) also found that treatment in the range of 200 to 400 MPa increased the solubility of soybean proteins, which can be attributed to the transformation of insoluble aggregates to low-molecular-weight soluble aggregates, changes in the protein structure, and exposure of the hydrophobic regions of the proteins (Wang et al., 2021). Peyrano et al. (2016) treated cowpea protein isolates with HHP at 200–400 MPa at pH 8.0 and 10 and found a significant decrease in solubility, probably due to differences in protein type (11S or 7S globulin and/or albumin), properties, and conformational stability between the proteins (Wang, Zhou, et al., 2023). HHP treatment did not significantly affect the solubility of the proteins (1 % and 3 %) and even resulted in a decreasing trend of solubility with increasing treatment pressure at a protein concentration of 5 %, suggesting that the high concentration may be one of the reasons for the promotion of protein aggregation (Wang et al., 2008).

3.1.2. *Emulsifying properties*

A study of HHP treatment of SPI found that its emulsifying activity index (EAI) did not change significantly in the pressure range of 400–600 MPa, but its emulsion stability index (ESI) decreased significantly with the increase of pressure (Wang et al., 2021). This phenomenon can be attributed to the aggregation of protein molecules as a result of the HHP treatment, which in turn affects the molecular flexibility, leading to the aggregation of small oil droplets in the formation of the emulsion, thus decreasing the stability of the emulsifying properties

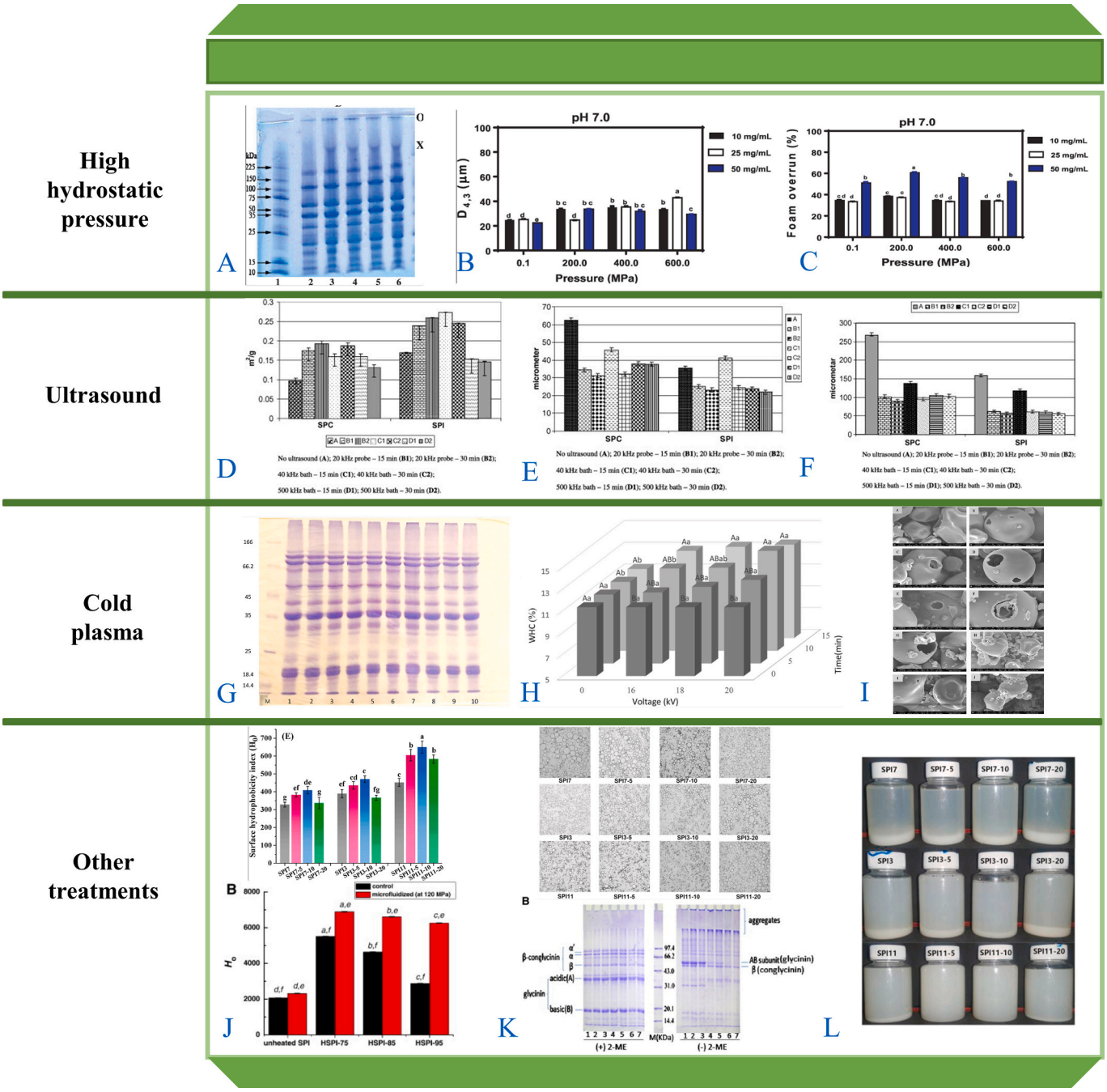


Fig. 4. Overview of the modification of functional properties of legume proteins by different non-thermal technologies. **(A to C)** Reduced SDS-PAGE analysis (O, origin; X, polymer), volume mean diameter (D[4,3]) of emulsions, and the foaming capacity of control (0.1 MPa) and high pressure-treated isolated pea protein. (Adapted from Chao et al. (2018)). **(D to F)** Specific surface area, volume–surface average diameter D[3,2], and D[4,3] of soy protein samples before and after ultrasound treatment. (Adapted from (Jambrak et al., 2009)). **(G to I)** SDS-PAGE analysis, water hold capacity of SPI suspension, and field emission scanning electron microscopy images with magnification of × 5000 after non-thermal atmospheric pressure DBD plasma. (Adapted from (Sharafodin and Soltanizadeh, 2022)). **(J to L)** surface hydrophobicity (H_o) at pH 7.0, non-reducing and reducing SDS-PAGE (B) profiles, and surface hydrophobicity of preheated and/or microfluidized SPI products. Optical microscopy of fresh emulsions and visual appearance of control and treated SPI samples. (Adapted from (Shen & Tang, 2012; Wang et al., 2023)).

(Hall & Moraru, 2021). On the contrary, a study of HHP treatment of pea isolates at different pH conditions revealed that HHP treatment at 400 MPa had a positive effect on EAI and ESI. The reason for the increase in EAI may be due to the increased exposure of hydrophobic sites of the protein and the unfolded protein structure under moderate pressure treatment (Tan et al., 2021). Similarly, Ahmed, Al-Ruwaih, Mulla, and Rahman (2018) and Al-Ruwaih, Ahmed, Mulla, and Arfat (2019) treated red kidney bean protein isolates with HHP and found that both the EAI and ESI increase with the pressure increase, up to about 400 MPa, after which the EAI decreases due to the formation of aggregates. Hall and Moraru (2021) treated broad bean protein concentrate, lentil protein concentrate, and PP concentrate with HHP (300 MPa for 15 min) and found that the EAI decreased significantly and ESI increased after HPP treatment compared to the control. This difference is probably due to differences in protein composition and structure.

3.1.3. *Foaming properties*

In a study by Li et al. (2011), HHP treatment of SPI was observed to

reach a maximum value of FC at 300 MPa and 15 min, beyond which FC decreased, whereas FS decreased with increasing treatment pressure and time. Chao et al. (2018) reached the same conclusion of decreased FS at different pH and protein concentrations and also found that FS values were greater at pH 3.0 and 7.0 than at pH 5.0. When red kidney bean protein isolates and concentrates were treated with HHP at 300 MPa, the FC values increased, and there was no significant change in the FS values of the red kidney bean protein isolates, but the FS values of the concentrates decreased significantly (Al-Ruwaih et al., 2019). The reason may be due to the production of smaller hydrophilic and/or charged peptides, which promotes their dispersion and adsorption at the interface, thus increasing FC and decreasing FS (Ahmed et al., 2019). At the same time, it was also found that with the increase of pressure applied during HHP treatment, the FS and FC of red kidney bean protein isolate showed a decreasing trend, and FC reached the lowest value of 42.1 % at 400 MPa; the explanation was that the increase of pressure partially denatures the molecular structure of the protein, thus affecting its functional properties (Ahmed et al., 2018). HHP improves the emulsifying properties of legume proteins, but the exact effect depends on the pressure, time, pH, and protein concentration of the treatment (Messens, Van Camp, & Huyghebaert, 1997).

3.2. Ultrasound (US)

US plays a vital role in the food industry as an environmentally friendly and efficient physical food processing technology (Yan, Xu, Zhang, & Li, 2021). Its frequency range is between 20,000 Hz and 100 MHz. These waves have directionality and penetrating power, but the propagation process is dependent on a medium, such as a solid, liquid, or gas, and cannot exist in a vacuum (Wang, Zhang, et al., 2020). When US propagates in a medium, it affects the medium mechanically, thermally, and biologically. These effects arise from three main actions: thermal, cavitation, and mechanical (Maria Medeiros Theóphilo Galvão et al., 2024). In particular, the role of food protein modification is mainly related to the transient cavitation phenomenon induced by US. Under the action of US, cavitation bubbles expand rapidly and collapse when they reach the resonance critical point, generating transient temperatures up to 5000 K and high pressures of up to 30 MPa (Tian, Wan, Wang, & Kang, 2004). This process induces a series of physical, chemical, and thermal effects, which modify food proteins. The application of this technology not only improves the efficiency and quality of food processing, but also provides consumers with safer and healthier food choices.

3.2.1. Solubility

US has been studied for its potential enhancement effect on protein solubility. Researchers have found that the solubility of pea protein isolate (PPI) was significantly increased to 77 % after sonication at 57–60 W/cm^2, 100 % amplitude, for 5 min, an effect that was significantly better than that of the 10 % and 50 % amplitude treatments. This phenomenon is thought to be due to the greater pressure and shear generated in the cavitation zone and the significant increase in the total acoustic energy applied under high-amplitude sonication (Sha et al., 2021). Similar trends are observed in other studies whereby the solubility of PPI increased with increasing amplitude and treatment time; however, there was a slight reduction in solubility after 20 min of sonication at 75 % amplitude (Mozafarpour et al., 2022). This was attributed to the stronger shear force resulting in more internal hydrophobic residues being exposed on the surface of the protein molecule, thus weakening the interaction between the protein and water and its solubility. The same results were found for SPI, lentil protein isolate, broad bean protein isolate, black bean isolate, and mung bean isolate (Gulzar, Martín-Belloso, & Soliva-Fortuny, 2024; Li et al., 2020; Maria Medeiros Theóphilo Galvão et al., 2024; Xia et al., 2020; Zhong & Xiong, 2020). Lo et al. (2022) treated lupin protein isolates at pH 5.0–9.0 by low-frequency US in the energy density range of 457–2746 J/mL and found that the solubility of the pH 9.0 samples was higher than that of the pH 5.0 samples, and that optimization of the maximum solubility of the pH 9.0 samples, which was increased by 15–20 % at an ambient pH ranging from 6.0 to 10, was achieved by US at 2746 J/mL. This can be attributed to the fact that the combination of alkaline conditions and sonication is effective in disrupting the original aggregates in the protein to form smaller micron-sized aggregates.

3.2.2. Emulsifying properties

The EAI of most legume proteins shows an increasing and then decreasing trend after US, with most of the maximum values obtained under US conditions of about 70 % amplitude, 300 W, and treatment time of 10–20 min. The EAI and ESI increased with time at low and medium power and decreased at high power, probably because high power accelerates the interactions of intermolecular disulfide bonds, leading to the formation of protein aggregates (Yu, Li, Sun, Yan, & Zou, 2022). However, Mozafarpour et al. (2022) found that the EAI and ESI of grass pea (*Lathyrus sativus* L.) protein isolate (GPPI) increased with increasing US amplitude and time, especially when the amplitude was between 50 % and 75 %. The increase in EAI may be attributed to an increase in protein solubility and surface hydrophobicity and a decrease in particle size after US treatment, facilitating the rapid diffusion and adsorption of the protein at the oil–water interface (Wang et al., 2020). The increase in ESI is attributed to the fact that cavitation at high US power disrupts the interactions between protein molecules and enhances the surface charge of protein molecules, thus effectively preventing droplet flocculation and increasing the ESI (Arredondo-Parada et al., 2020). Numerous researchers have discovered that this non-thermal technique enhances the EAI and ESI of legume proteins, such as SPIs, soybean protein concentrates (SPCs) (Jambrak et al., 2009), SPI nanoparticles (Hussain Badar et al., 2024), CPI (Wang, Wang, et al., 2020), faba bean protein isolate (Gulzar et al., 2024), and black bean protein isolates (Li et al., 2020). However, researchers have also found that US treatment may decrease the EAI and ESI of peanut protein isolates (Zhang et al., 2014), and the ESI of SPI treated with high-frequency US was found to be lower than that of untreated SPI (Karki et al., 2009).

3.2.3. Foaming properties

A previous study found that the FC of SPC and SPI reached maximum values at 20 kHz ultrasonic frequency for 30 min treatment, with SPC reaching 104 % and SPI reaching 153 %, respectively. However, FS gradually decreased after an initial increase; specifically, SPC reached a maximum value of 68.6 % FS at 40 kHz and 30 min treatment, whereas SPI reached a maximum value of 62.5 % FS at 40 kHz and 15 min treatment. The foaming properties of these proteins did not show significant changes at the ultrasonic frequency of 500 kHz (Jambrak et al., 2009). Alavi et al. (2021); (Gulzar et al., 2024; Wang, Wang, et al., 2020) discovered that US significantly increased the FC and FS of black bean protein isolates for reasons related to the exposure of hydrophobic regions, which increased individually with increasing power, but FC gradually decreased as the duration of treatment increased. The reason for the decrease in FS due to prolonged sonication at high power may be due to the denaturation of the protein. Wang, Zhang, et al. (2020) found that after sonication, the FC and FS of PPs increased by 19.5 % and 22.7 %, respectively, with improved foaming properties. PPs have smaller and more homogeneous bubbles after US, which results in better foaming properties, which may be due to the fact that the protein structure is partially unfolded, the protein particle size is reduced, and there is greater exposure of hydrophobic amino acid residues, allowing rapid formation of viscoelastic membranes and adsorption at the air–water interface (Higuera-Barraza, Del Toro-Sanchez, Ruiz-Cruz, & Márquez-Ríos, 2016).

3.3. Cold plasma (CP) treatment

CP treatment is a novel technique in food processing. Plasma is the

fourth state of matter. As the energy of the molecules in the system increases, solids change to liquids, and liquids change to gases (Ji et al., 2018). This leads to a change in the arrangement of the molecules. Ionized gases produced under atmospheric conditions or at low pressure are called CP (Waghmare, 2021). In addition to food preservation, CP can also be used to improve food quality parameters and protein properties. Additionally, CP technology finds applications in the textile as well as in the treatment of life-threatening diseases, such as cancer (Akhtar, Abrha, Teklehaimanot, & Gebrekirstos, 2022). Due to the positive effects on proteins and carbohydrates, CP treatment is beneficial for these food components. The plasma device consists of a vacuum chamber (in some cases), a radio frequency (RF) generator, an electrode, and a control unit. At atmospheric or vacuum pressure, it is possible to generate cooled plasma in the gas by discharging with a low-power input (Ucar et al., 2021). Typically, oxygen, nitrogen, and dry air are used as plasma gases in food processing application.

3.3.1. Solubility

Several studies determined that structural modifications induced by CP treatment can improve the solubility of plant proteins (Bu et al., 2023; Li et al., 2023). In addition to structural modifications, the formation of polar radicals (induced by high-energy ion bombardment) and/or the formation of new oxygen-containing groups through amino acid-activator interactions can also contribute to the solubility of proteins under CP treatment (Mollakhalili-Meybodi, Yousefi, Nematollahi, & Khorshidian, 2021). Bu, Nayak, Bruggeman, Annor, and Ismail (2022) treated PPI with different plasma species (O_3, N_xO_y, H_2O_2, and OH•) and found that the solubility of PPI samples treated with O_3 and OH• showed significantly higher solubility under non-heated conditions than the pH 2.0 PPI control and comparable solubility to the pH 7.0 PPI control. The authors suggested that this solubility increase could be explained by the formation of soluble aggregates and the retained high surface charges, which may have counteracted the observed increase in surface hydrophobicity. Yu et al. (2021) found that the binding of peanut protein isolate to sesbania gum was promoted after CP treatment, and its solubility increased from 610.00 to 1580.00 g/L. However, the solubility of this glycosylated product decreased when the CP treatment time was longer than 3 min, which may be because hydrophobic interactions caused the conjugates to aggregate and the CP treatment process caused over-oxidation of the conjugates by O3, leading to protein aggregation and reduced surface hydrophobicity (Sparavigna, 2008). Similarly, it has been shown that the solubility increased with increasing CP treatment time until reaching an optimum value (1.34 mg/mL) at 3 min. This increase in solubility was associated with an increase in polarity due to covalent bonding with hydrophilic sugars, thus preventing the aggregation process under unfavorable conditions (Yu et al., 2020).

Dielectric barrier discharge (DBD) plasma was used to modify soybean protein. Li et al. (2023) used modified gas phase packaging (MAP)-assisted DBD-CP to treat SPI and treated SPI powder with DBD-CP under the condition of air with an oxygen ratio of 20–60 %, respectively. They found that the soluble protein of SPI was 27.9 % more in the MAP than in the air package SPI when the oxygen concentration reached 60 %. In contrast, with the increase of plasma treatment time and voltage, the surface hydrophobicity of GPPI nanoparticles increased, whereas the solubility decreased, which the authors suggested may be related to the higher tyrosine cross-linking content, the spread of their secondary and tertiary structures, and their low ζ-potential value (Mehr & Koocheki, 2021). Therefore, while CP is an effective technique for improving the solubility of legume proteins, the conditions and processing parameters should be optimized to fully explore the potential of this technique to improve the functional properties of proteins.

3.3.2. Emulsifying properties

CP treatment can improve the emulsion stability of legume proteins by altering the protein structure and increasing the interfacial activity, but the outcome is highly dependent on the treatment condition; overly strong or prolonged treatments may lead to a decrease in protein functionality. Mehr and Koocheki (2020) reported that the ES of GPPI improved to varying degrees with increasing CP treatment voltages (9.4–18.6 kVpp) and treatment times (30–60 s). This may be due to better protein absorption and the formation of a dentate and elastic interfacial film around the oil droplets, which lead to a homogenous dispersion of small oil droplets, and a more stable emulsion. Additionally, Wang, Zhou, et al. (2023) found an increase in the EAI of CPI (from 28.39 to 34.60 m^2/g) after a 30 s treatment with atmospheric pressure plasma jets (APPJs), but a decrease after a 50 s treatment. This change is because the APPJ treatment results in the constant bombardment of proteins by reactive oxygen species (ROS) and reactive nitrogen species (RNS), which exposes the hydrophobic groups and amino acids within the protein, thereby improving its ability to bind to the oil droplets. In addition, during CP processing for 2 min, the stability of the peanut protein isolate emulsion increased steadily and reached its maximum value at that point. After that, the stability declined, which may be related to the average diameter of the peanut protein isolate micelles (Ji et al., 2018). A study by Mehr and Koocheki (2021) determined that CP-treated GPPI nanoparticles with higher di-tyrosine cross-linking content and lower free SH group content had better adsorption capacity at the oil–water interface and formed a stable interfacial film; furthermore, prolonging the CP treatment time could improve their emulsifying properties. A recent study observed that pH transfer and integrated treatment of CP improved the emulsifying properties of CPI. Specifically, EA increased from 57.47 ± 0.06 to 71.95 ± 0.19 m^2/g when the pH was shifted to 12, followed by a 30 s CP treatment (Wang et al., 2024). However, a high processing voltage and long processing time may cause the protein solution to form aggregates, which is not conducive to the flexibility of the molecule. Shaghaghian, McClements, Khalesi, Garcia-Vaquero, and Mirzapour-Kouhdasht (2022) observed that when the treatment voltage reached 20 kV, the EAI and ESI of the emulsion decreased significantly. Based on these results, it was speculated that the difference globular proteins (7S and 11S) in SPI behave differently to CP treatment. CP may decompose 7S and deform some of the monomers, and the hidden hydrophobic regions within the molecule are attracted to the surface and increase the active surface area. In contrast, 11S will form aggregates and become less hydrophobic.

3.3.3. Foaming properties

One study exposed PPI to a CP synergistic tartaric acid treatment for 0–20 min at a treatment power of 100 W. Increasing the treatment time from 0 to 10 min increased the bubble rate and bubble stability of PPI to 122.22 % and 101.82 % twice and four times as much as that of the control, respectively (Qu et al., 2023). CP-induced changes to the chemical and structural modification of proteins in GPPI could change the composition of the interfacial layer and form more stable nanoparticles (Mehr & Koocheki, 2020). Moreover, the erosive action of CP disrupted the PPI aggregates, increased the dissociation of tartaric acid from the proteins, exposed their hydrophobic groups, and enhanced their surface activity by introducing ionizing radicals (Bußler, Steins, Ehlbeck, & Schlüter, 2015). In addition, the nitric oxide derivative introduced by CP treatment can react with tartaric acid to generate inorganic acids (e.g., nitrate and nitrite) (Mehr & Koocheki, 2021), which accelerates protein deamination and contributes to the reduction of surface tension at the gas–liquid interface of the PPI solution. Mehr and Koocheki (2023) investigated the effect of CP treatment at two voltages of 9.4 and 18.6 kVpp for short periods of 30 and 60 s (S-CPT) and long periods of 300 and 600 s (L-CPT) on the foaming properties of GPPI. The results showed that the FC of GPPI increased significantly ($P < 0.05$) with the increase of treatment time and applied voltage under S-CPT, and for long-term CP-treated GPPI (L-GPPIPT) particles, the FC of L-GPPIPT/3009.4, L-GPPIPT/30018.6, and L-GPPIPT/60018.6 particles were increased by 2.75 %, 4.33 %, and 4.62 %, respectively, and the FC of GPPIPT/6009.4 particles was decreased by about 3.36 % compared with that of GPPI particles. Both S-CPT and L-CPT decreased the FS by

about 3.36 %, but both were higher than the control. Likewise, Wang, Wang, et al. (2023) reported that both the FC and FS of CPI increased greatly with increasing treatment time. Moreover, the FC significantly increased by 100.1 % after 30 s of APPJ treatment and decreased beyond that time due to the rise in carbonyl levels and reduction in free SH levels, which promote protein oxidation and protein aggregation (Yu et al., 2020). Sharafodin and Soltanizadeh (2022) found that the FC of SPI reached a maximum after 15 min of plasma treatment at 18 kV, but there was no significant change in the FS. The increase in FC may be attributed to the more pronounced disorganization of the protein structure during the prolonged treatment, which enhances protein adsorption at the water–air interface.

3.4. Other treatments

PEF technology has attracted widespread attention since it was first reported by German engineers in the 1960s. It uses short, high-voltage PEFs to precisely alter the molecular structure of proteins without causing other side effects (Gulzar et al., 2024) and is usually performed at room or low temperatures. The mechanism of protein modification induced by PEF treatment is that the free radicals generated on the surface of proteins disrupt the intermolecular forces, thus altering the structure and function of proteins (Wang et al., 2023). DHPM technology is another cutting-edge high-pressure homogenization technology. It creates supersonic jets through micron-sized orifices, causing fluids to undergo a rapid series of multiple integrated actions (Oliete, Potin, Cases, & Saurel, 2018). Compared with traditional methods, DHPM has higher energy density and shorter processing time, which can emulsify, homogenize, and refine materials while maintaining a low temperature and short processing time to reduce nutrient loss (He et al., 2021). DHPM does not require the addition of exogenous chemicals and is widely used to improve the emulsifying properties of materials and modify macromolecules (Djemaoune, Cases, & Saurel, 2019).

3.4.1. Solubility

It has been shown that PEF improves the solubility of faba bean (Gulzar et al., 2024), mung bean (Gulzar et al., 2023), and soya bean (Li, Chen, & Mo, 2007). Although the exact mechanism by which PEF improves protein solubility is unknown, the accepted hypothesis involves peptide dipole moment polarization and unfolding to affect hydration/solubility (Giteru, Oey, & Ali, 2018). Treatment with PEF and pH changes at a moderate PEF strength of 10 kV/cm and an alkaline environment (pH 11) promotes the unfolding of protein structures and induces the formation of smaller aggregates, resulting in an increase in the solubility of SPI from 26.06 % to 70.34 % (Wang, Zhou, et al., 2023). The solubility of SPI has been shown to reach 82 % following PEF treatment at an intensity of 30 kV/cm and treatment time of 288 μs (Li et al., 2007). However, different results have been obtained by Melchior et al. (2020), who found that a significant decrease in solubility was observed when PPI was treated by applying a medium-intensity PEF (1.65 kV/cm), irrespective of pH conditions. These findings were attributed to the formation of insoluble protein aggregates and/or protein unfolding (Zhong, Hu, Zhao, Chen, & Liao, 2005). Conversely, a 5 % (*w*/w) insoluble PP dispersion (pH 7.0) homogenized at 25–150 MPa for 1–5 cycles showed an increase in solubility from 23 ± 1 % to 86 ± 4 %. The authors concluded that the dissociation of large protein aggregates into smaller particles after microfluidics leads to more protein–water interactions and thus increased solubility (Moll et al., 2021). He et al. (2021) treated PPI using different pressures (30–120 MPa) and found that PP solubility increased 3.78-fold at 120 MPa. Based on these findings, the authors speculated that at high-pressure levels, PPs are subjected to strong shear, high-velocity impacts, transient pressure drops, cavitation forces, and additional thermal effects, which may alter the structure of PPs (disruption of morphology, reduction in particle size, unfolding of proteins), leading to an increase in proteolysis. Similar results were obtained in another study, where the solubility of SPI was greatly increased by microfluidics (120 MPa) treatment under neutral conditions (Shen & Tang, 2012). However, Djemaoune et al. (2019) showed that microfluidization in the pressure range of 70–130 MPa at pH 5.0 did not affect the solubility of pea albumin particles, whereas at pH 3.0, the higher the applied pressure, the higher the solubility, with peaks of 48.3 ± 0.2 % (110 MPa) and 48.7 ± 0.4 % (130 MPa), respectively.

3.4.2. Emulsifying properties

The EAI and ESI of PEF-treated faba bean protein isolates have been shown to be significantly higher compared to the control group, with a maximum of 54.65 m^2/g and 45.55 min at 2000 pulses, respectively. PEF alters the interfacial properties by promoting protein unfolding and potentially clustering the hydrophobic regions, facilitating the initial emulsifying properties by increasing the availability of the interfacial peptides, but also improves the long-term stability due to the reorganization of the hydrophobic–hydrophilic character (Gulzar et al., 2024). Wang et al. (2023) treated SPI with a PEF intensity of 10 kV/cm, which led to an increase in the EAI and a slight but non-significant increase in the ESI, but the highest EAI and ESI (increases of 90.05 % and 34.88 % compared to native SPI at pH 7.0, respectively), were observed when the treatment was applied under alkaline conditions (pH 11). These outcomes were mainly due to the higher flexibility, surface hydrophobicity, and free SH content of the modified SPI, which resulted in improved interfacial activity.

Microfluidization improves the ES of pea globulin aggregates by reducing flocculation, agglomeration, and emulsion formation, especially at high microfluidization pressures of 130 MPa. This stability was attributed to the gel-like structure formed in the emulsion (Oliete et al., 2018). The EAI of SPI was improved by microfluidization treatment at a pressure of 120 MPa under neutral conditions without thermal pretreatment, but its effect on particle size and ES depended on the temperature of the thermal pretreatment (Shen and Tang, 2012).

3.4.3. Foaming properties

Native SPI tends to have a weak FA of 98.7 %. PEF treatment alone only increased the FA of the SPI by 18.7 %, probably because the PEF-treated SPI still showed a relatively rigid structure. However, after a combination of PEF and pH treatment, the FA reached 202.3 %, which was 104.96 % higher than that of the untreated SPI. The synergistically modified SPI had a smaller particle size and a more hydrophobic surface, which facilitated adsorption at the air–water interface and improved air bubble trapping efficiency (Wang et al., 2023). In another study, the foam performance of broad bean protein isolate subjected to individual PEF treatments (under 2000 pulses) was significantly improved by nearly twofold. Meanwhile, the PEF-treated broad bean protein isolate had good FS, which was attributed to the moderate conformational changes induced by the pulses, which contributed to the rapid reduction of the surface tension and thus improved foam generation and expansion (Gulzar et al., 2024).

Modification of pea albumin aggregates using DHPM treatment at pH 7.0 significantly increased the FC, whereas the FS was insignificantly changed by microfluidization at pH 3.0 and 5.0, and was significantly decreased at pH 7.0. The microfluidization treatment at different pH values favored the surface tension of pea albumin aggregates at the air–water interface, which was mainly attributed to the diminution in the size and increase in the flexibility of the protein particles (Djemaoune et al., 2019).

4. Application of non-thermal technology for the modification of legume proteins

4.1. Fat substitution

Plant-based foods are finished food products made from plants that have been used as a source of proteins or fats. The use of various legume

proteins processed by non-thermal techniques as fat replacements in the food industry is a promising nutritional strategy. Legumes, especially soya beans, are rich in high-quality plant proteins that are close to animal proteins in terms of quantity and inter-ratio of essential amino acids, affording them a high nutritional value. At the same time, legume proteins are relatively low in fat, which makes them ideal fat replacements. Janardhanan et al. (2022) highlighted that pork backfat patties cooked using HPP treatment alone (350 MPa, 10 min) or combined with sous vide cooking (HPP + SVCOOK) had the highest fatness, flavor, chewiness, and the lowest crispiness. There was little variation in physicochemical and organoleptic parameters among the HPP + SVCOOK patties. The fat in the patties could be successfully replaced with either the HPP + SVCOOK soya or hydrogel emulsions.

Research shows that 5 % (*w*/w) for US and pH transfer of PPI-stabilized high internal phase emulsions (HIPEs) have the potential to be used as three-dimensional printing inks. The HIPEs constructed by Zhang, Zhao, Li, Kong, and Liu (2023) could be used to develop high-viscosity and scalable products (e.g., fat simulation) in the food industry.

US can significantly improve the structure and function of fat substitutes prepared with legume proteins. For example, Akhtar and Masoodi (2022) introduced Pickering emulsions stabilized by SPI-maltodextrin-pectin complexes with Himalayan walnut oil for the US-assisted emulsifying properties of a new type of mayonnaise, which greatly reduced the peroxide value of the mayonnaise formulation (2.65 meqO_2/kg) when compared to the free oil (8.33 meqO_2/kg) after prolonged storage, which improved the physical stability of the final mayonnaise. Conversely, Li et al. (2024) discovered that compared to PPI-based high internal phase Pickering emulsion (HIPPEs), HIPPEs made from US-treated PPI and mung bean starch complexes provide some references for HIPPEs in the food industry for the development and creation of novel functional food products, such as fat substitutes (mayonnaise, salad dressings, and sausages). Briefly, modified legume proteins may be effective fat substitutes for specific food applications, as shown in Table 3.

4.2. Delivery systems

Modified soy proteins have multiple advantages as delivery systems for bioactive compounds, including biocompatibility, degradability, targeting, and excellent controlled release. Quercetin is a flavonoid found in numerous fruits, vegetables, and grains. This compound shows a wide range of potential health benefits in terms of antioxidant, anti-tumor, antiviral, and anti-aging properties. Nevertheless, it is extremely sensitive to external environmental factors such as pH, temperature, and oxygen and has poor stability. In addition, its low solubility, inefficient absorption, and limited bioavailability limit its wide application in the food industry and other industries (Yang et al., 2024). Yang et al. (2023) successfully prepared soya protein hydrolyzed microgel particles (SPHMs) at various pH conditions with and without US, which significantly influenced the rheological characteristics, interfacial microstructure, and physical stability of an oil-in-water Pickering emulsion. In particular, at pH 3.0 and 9.0, followed by US treatment, the quercetin encapsulation efficiency reached 89.45 %, its bioaccessibility reached 65 % during in vitro simulated intestinal digestion for 2 h. Especially at pH 9.0, the potential of US-treated SPHMs for application in dairy-based functional foods was further stimulated.

Li et al. (2024) found that HIPPEs prepared from complexes of US-treated PPI and mung bean starch showed better solid appearance and storage stability compared to PPI-based HIPPEs. The complexes not only exhibit good loading and packaging ability but can also be used as a delivery system for hydrophobic bioactive compounds (e.g., astaxanthin, curcumin, and β-carotene). In related work, US-pretreated SPIs were combined with lignans to form a novel SPI-lignan (SPI-LUT) nano-delivery system. The experimental results revealed that appropriate US pretreatment may increase the system's solubility, loading amount, and encapsulated rate to 90.56 %/mg, 2.51 μg, and 89.72 %, respectively. Appropriate US pretreatment enhanced the LUT release and 2,2-Diphenyl-1-picrylhydrazyl removal of the SPI-LUT nano-delivery system to 89.40 % and 55.63 %, respectively, according to digestion and antioxidant tests (Sun et al., 2022). In addition, it was found that the gel network formed was more dense, uniform, and stable when US pretreatment was applied. These improvements in physical and chemical properties not only effectively inhibited the swelling behavior but also improved the riboflavin encapsulation efficiency and delivery capacity of transglutaminase-catalyzed SPI gel (Zhang et al., 2022).

Table 3
Application of non-thermal technology for modification of legume proteins.

Sources	Methods	Application	Findings	Reference
Soy protein	HHP (350 MPa, 10 min)	Hybrid patties	The highest intensities for fatness, flavor, chewiness, and the lowest for friability.	(Janardhanan et al., 2022)
CPI	HHP (100 to 600 MPa)	Production of bioactive peptides	The highest yield of antioxidant peptides (51.26 %) was obtained by hydrolysis under high pressure at 200 MPa for 20 min.	(Zhang, Jiang, Miao, Mu and Li, 2012)
Kidney bean protein isolate	HP (300–600 MPa, 15 min)	Production of bioactive peptides	Proteolysis of KBPI at 300 MPa for 15 min produced the highest degree of hydrolysis (23.9 %) and the antioxidant activities (30.1 % DRSA).	(Al-Ruwaih et al., 2019)
SPI	HHP (250 MPa and room temperature for 30 min)	Ice cream	The expansion and melting rates were significantly improved.	(Yan et al., 2022)
SPI	HHP (300 MPa for 5 min)	Beef patties	The toughness of treated SPI beef patties was lower than that of regular HHP-treated beef patties.	(Bernasconi et al., 2020)
SPI	HHP (400 MPa for 10–30 min)	Yogurt	HHP treatment greatly increased the water holding capacity and emulsifying activity index at pH 3 of SPI.	(Wang et al., 2021)
PPI	US (20 kHz, 500 W, 10 min)	3D printing inks	The apparent viscosity, storage modulus, elasticity index, and macroscopic viscosity index increased gradually.	(Zhang et al., 2023)
SPI	US (40 kHz, 10 min)	Mayonnaise	Higher storage modulus (G′) than loss modulus (G″), the high oxidative stability, homogenous emulsion systems.	(Akhtar & Masoodi, 2022)
PPI	US (30, 45 or 60 min)	Delivery system	The highest β-carotene retention rate of 73.58 %.	(Li et al., 2024)
Soy protein hydrolysate	US (240 W and 20 kHz for 30 min)	Delivery system	The encapsulation rate of quercetin was enhanced to 89.45 % and bioaccessibility to 65 %.	(Yang et al., 2023)
SPI	US (12 min)	Delivery system	The encapsulation efficacy, loading amount and solubility to 89.72 %, 2.51 μg/mg and 90.56 %.	(Sun et al., 2022)
SPI	US (400 W, 30–120 min)	Delivery system	Significantly enhanced the gel strength, storage modulus, loss modulus, consistency, and thermal stability, but decreased the flow behavior index of TCSG.	(Zhang et al., 2022)
SPI	CP (50 W for 60 s)	Soybean oils	Soybean oils can be stabilized by SPI-PA complexes to form HIPPEs with a lipid oxidation inhibition rate of > 65 %, creaming index (CI) > 80 %.	(Gong, Guo, Wang, Huang and Zhu, 2023)

4.3. Production of bioactive peptides

The structure and function of legume proteins can be optimized through non-thermal technical modifications to better exploit their bioactivity. Especially when this modified legume protein is used to produce bioactive peptides, its advantages are more prominent. It has been found that enzymatic hydrolysis after HHP treatment can effectively improve the digestibility of proteins, thus increasing the yield of bioactive peptides (Marciniak, Suwal, Naderi, Pouliot, & Doyen, 2018). Zhang et al. (2012) pretreated CPI before hydrolysis by HPP at 100–600 MPa. It was discovered that the hydrolysis rate was greatly increased when the pressure was above 300 MPa, and the molecular weight determination of the enzymatic hydrolysis products showed that high-pressure-induced hydrolysis significantly increased the number of low-molecular-weight peptides. The highest yield of antioxidant peptides (51.26 %) was obtained by hydrolysis under HPP at 200 MPa for 20 min. A similar result was obtained by Al-Ruwaih et al. (2019), the antioxidant potential of the hydrolysates was enhanced with HPP assistance. It should be noted that the yield of the produced bioactive peptides may be influenced by the treatment conditions. Boukil, Suwal, Chamberland, Pouliot, and Doyen (2018) found that the degree of hydrolysis rose dramatically, and the release of peptides with antioxidant and Angiotensin Converting Enzyme inhibitory activity was greatly boosted by HHP when the pressure was increased from 0.1 to 200 MPa.

4.4. Other applications

Yan et al. (2022) examined the synergistic modification of soya proteins using HHP technology (250 MPa, room temperature conditions) and phospholipids (150 μg). They showed that ice creams produced using the modified soya proteins in place of milk powders received a high acceptance in organoleptic evaluations and that the overall quality of the ice creams was similar to that of traditional milk ice creams. Another study investigated the combined application of SPI addition and HHP treatment (300 MPa, 5 min) for replacing 20 % of meat protein in beef patties. An evaluation of the patty process, color, and texture indicated that the resilience of processed SPI beef patties was inferior to that of conventional HHP-treated beef patties (Bernasconi, Szerman, Vaudagna, & Speroni, 2020).

An SPI-PA complex formed by combining proanthocyanidins (PA) with CP-treated SPI could effectively stabilize soybean oil and form a high *endo*-picoline emulsion, showing more than 65 % inhibition of lipid oxidation and more than 80 % creaming index, with rheological properties superior to those of the conventional emulsion system (Gong et al., 2023). Other research has shown that the addition of 8 % SPI to yogurt treated with HHP at 281 MPa with a holding time of 19 min enhanced the water-holding capacity and rheological properties of the yogurt, resulting in enhanced water retention, lighter color, and better flavor due to a reduction in volatile components associated with strong flavors (Wang et al., 2021). These findings further confirm the promise of non-thermal technologies for a broad range of applications in areas such as food processing.

5. Conclusions and future prospects

Legume proteins are receiving increasing attention globally due to their plant-based quality as an ingredient. In this review, the theory (work principle) of US, HHP, CP, PEF, and DHPM non-thermal technologies, and their applications in the modifications of structural and functional properties of legume protein are exhaustively elaborated and discussed. These techniques have been shown to improve the solubility, emulsifying properties, and foaming properties of legume proteins while retaining their nutritional value and natural structure to meet the demand for high-quality food in the modern food industry. US technology destroys the structure of legume proteins through its multiple effects and improves their solubility and ES. HHP technology regulates the structure and function of legume proteins without significantly altering the temperature. CP technology improves the functionality of legume proteins by using the interaction between active particles and legume proteins. PEF and DHPM technologies precisely regulate the structure and molecular properties of legume proteins through electric fields and high-pressure jets. These non-thermal technologies have been widely used in the modification, processing, and new product development of legume proteins, optimizing their functional properties and enriching the protein sources for the food industry. At the same time, these technologies are easy to operate, consume low energy, and are environmentally friendly, which is in line with the green development requirements of the modern food industry.

Although non-thermal technologies have achieved significant results in improving the functional properties of legume proteins, they are still facing many challenges and problems.

i. Different technologies have different mechanisms and effects on protein structure, so their synergistic effects need to be explored in depth to maximize the functionality of legume proteins.
ii. There is also a need to investigate the effects of non-thermal treatments on the long-term stability of legume proteins to enhance their safety.
iii. Due to the high cost of specialized equipment, the expense of high energy consumption and maintenance, and the relatively low production efficiency, much of the research on the use of non-thermal technologies to improve legume proteins is still at the laboratory stage and should be scaled up for industrial production in the future.
iv. The modification effects of non-thermal technologies are limited. Therefore, green, environmentally friendly, and sustainable preparation systems can be explored by integrating a combination of physical, chemical, and enzymatic methods. This approach aims to enhance the modification effects and reduce costs.
v. In the future, the development of miniaturized and high-efficiency equipment is essential to reduce equipment costs, enhance flexibility, and broaden applicability. Concurrently, improving the processing efficiency of such equipment will be crucial to satisfy the demands of mass production.

CRediT authorship contribution statement

Yuanyuan Wei: Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Delu Ning:** Supervision, Resources, Formal analysis. **Liping Sun:** Supervision, Resources, Formal analysis. **Ying Gu:** Supervision, Resources, Formal analysis. **Yongliang Zhuang:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Yangyue Ding:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Xuejing Fan:** Visualization, Methodology, Investigation, Conceptualization.

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.

Acknowledgements

The author gratefully acknowledges the Yunnan Major Scientific and Technological Projects (202402AE090031), Yunnan Fundamental Research Projects (Grant No.202401AT070413), Special Project for High-level Scientific and Technological Talents and Innovation Teams of Yunnan Province (202405AS350005).

Data availability

No data was used for the research described in the article.

References

Ahmed, J., Al-Ruwaih, N., Mulla, M., & Rahman, M. H. (2018). Effect of high pressure treatment on functional, rheological and structural properties of kidney bean protein isolate. *LWT, 91*, 191–197. https://doi.org/10.1016/j.lwt.2018.01.054

Ahmed, J., Mulla, M., Al-Ruwaih, N., & Arfat, Y. A. (2019). Effect of high-pressure treatment prior to enzymatic hydrolysis on rheological, thermal, and antioxidant properties of lentil protein isolate. *Legume Science, 1*(1), Article e10. https://doi.org/10.1016/j.lwt.2018.01.054

Akhtar, G., & Masoodi, F. A. (2022). Structuring functional mayonnaise incorporated with Himalayan walnut oil Pickering emulsions by ultrasound assisted emulsification. *Ultrasonics Sonochemistry, 86*, Article 106022. https://doi.org/10.1016/j.ultsonch.2022.106022

Akhtar, J., Abrha, M. G., Teklehaimanot, K., & Gebrekirstos, G. (2022). Cold plasma technology: Fundamentals and effect on quality of meat and its products. *Food and Agricultural Immunology, 33*(1), 451–478. https://doi.org/10.1080/09540105.2022.2095987

Alavi, F., Chen, L., & Emam-Djomeh, Z. (2021). Effect of ultrasound-assisted alkaline treatment on functional property modifications of faba bean protein. *Food Chemistry, 354*, Article 129494. https://doi.org/10.1016/j.foodchem.2021.129494

Al-Ruwaih, N., Ahmed, J., Mulla, M. F., & Arfat, Y. A. (2019). High-pressure assisted enzymatic proteolysis of kidney beans protein isolates and characterization of hydrolysates by functional, structural, rheological and antioxidant properties. *LWT, 100*, 231–236. https://doi.org/10.1016/j.lwt.2018.10.074

Ao, L., Liu, P., Wu, A., Zhao, J., & Hu, X. (2021). Characterization of soybean protein isolate-food polyphenol interaction via virtual screening and experimental studies. *Foods, 10*(11), 2813. https://doi.org/10.3390/foods10112813

Arredondo-Parada, I., Torres-Arreola, W., Suárez-Jiménez, G. M., Ramírez-Suárez, J. C., Juárez-Onofre, J. E., Rodríguez-Félix, F., & Marquez-Rios, E. (2020). Effect of ultrasound on physicochemical and foaming properties of a protein concentrate from giant squid (Dosidicus gigas) mantle. *LWT, 121*, Article 108954. https://doi.org/10.1016/j.lwt.2019.108954

Barac, M., Cabrilo, S., Pesic, M., Stanojevic, S., Zilic, S., Macej, O., & Ristic, N. (2010). Profile and functional properties of seed proteins from six pea (Pisum sativum) genotypes. *International Journal of Molecular Sciences, 11*(12), 4973–4990. https://doi.org/10.3390/ijms11124973

Bernasconi, A., Szerman, N., Vaudagna, S. R., & Speroni, F. (2020). High hydrostatic pressure and soybean protein addition to beef patties: Effects on the formation of mixed aggregates and technological parameters. *Innovative Food Science and Emerging Technologies, 66*, Article 102503. https://doi.org/10.1016/j.ifset.2020.102503

Berrazaga, I., Micard, V., Gueugneau, M., & Walrand, S. (2019). The role of the anabolic properties of plant-versus animal-based protein sources in supporting muscle mass maintenance: A critical review. *Nutrients, 11*(8). https://doi.org/10.3390/nu11081825

Boukil, A., Suwal, S., Chamberland, J., Pouliot, Y., & Doyen, A. (2018). Ultrafiltration performance and recovery of bioactive peptides after fractionation of tryptic hydrolysate generated from pressure-treated β-lactoglobulin. *Journal of Membrane Science, 556*, 42–53. https://doi.org/10.1016/j.memsci.2018.03.079

Bu, F., Feyzi, S., Nayak, G., Mao, Q., Kondeti, V. S. S. K., Bruggeman, P., & Ismail, B. P. (2023). Investigation of novel cold atmospheric plasma sources and their impact on the structural and functional characteristics of pea protein. *Innovative Food Science & Emerging Technologies, 83*, Article 103248. https://doi.org/10.1016/j.ifset.2022.103248

Bu, F., Nayak, G., Bruggeman, P., Annor, G., & Ismail, B. P. (2022). Impact of plasma reactive species on the structure and functionality of pea protein isolate. *Food Chemistry, 371*, Article 131135. https://doi.org/10.1016/j.foodchem.2021.131135

Bußler, S., Steins, V., Ehlbeck, J., & Schlüter, O. (2015). Impact of thermal treatment versus cold atmospheric plasma processing on the techno-functional protein properties from Pisum sativum 'Salamanca'. *Journal of Food Engineering, 167*, 166–174. https://doi.org/10.1016/j.jfoodeng.2015.05.036

Chao, D., Jung, S., & Aluko, R. E. (2018). Physicochemical and functional properties of high pressure-treated isolated pea protein. *Innovative Food Science & Emerging Technologies, 45*, 179–185. https://doi.org/10.1016/j.ifset.2017.10.014

Chen, L., Zhang, S.-B., & Zhang, S.-Y. (2024). Effects of pH-shifting and ultrasound on the structural and emulsifying properties of peanut globulin fractions. *Food Chemistry: X, 22*, Article 101390. https://doi.org/10.1016/j.fochx.2024.101390

Dai, T., Shuai, X., Chen, J., Li, C., Wang, J., Liu, W., & Wang, R. (2022). Whole peanut milk prepared by an industry-scale microfluidization system: Physical stability, microstructure, and flavor properties. *LWT, 171*, Article 114140. https://doi.org/10.1016/j.lwt.2022.114140

Djemaoune, Y., Cases, E., & Saurel, R. (2019). The effect of high-pressure microfluidization treatment on the foaming properties of pea albumin aggregates. *Journal of Food Science, 84*(8), 2242–2249. https://doi.org/10.1111/1750-3841.14734

Gharibzahedi, S. M. T., & Smith, B. (2021). Effects of high hydrostatic pressure on the quality and functionality of protein isolates, concentrates, and hydrolysates derived from pulse legumes: A review. *Trends in Food Science & Technology, 107*, 466–479. https://doi.org/10.1016/j.tifs.2020.11.016

Giteru, S. G., Oey, I., & Ali, M. A. (2018). Feasibility of using pulsed electric fields to modify biomacromolecules: A review. *Trends in Food Science and Technology, 72*, 91–113. https://doi.org/10.3390/foods10071594

Gong, W., Guo, X.-L., Wang, S.-J., Huang, H.-B., & Zhu, X.-M. (2023). Construction of high internal phase Pickering emulsions using cold plasma modified soy protein isolate-proanthocyanidin complex. *Food Research International, 167*, Article 112664. https://doi.org/10.1016/j.foodres.2023.112664

Gulzar, S., Martín-Belloso, O., & Soliva-Fortuny, R. (2024). Tailoring the techno-functional properties of fava bean protein isolates: A comparative evaluation of Ultrasonication and pulsed electric field treatments. *Foods, 13*. https://doi.org/10.3390/foods13030376

Gulzar, S., Tagrida, M., Patil, U., Ma, L., Zhang, B., & Benjakul, S. (2023). Mung bean protein isolate treated with high-intensity pulsed electric field: Characteristics and its use for encapsulation of Asian seabass oil. *Journal of Microencapsulation, 40*(5), 330–344. https://doi.org/10.1080/02652048.2023.2198030

Hall, A. E., & Moraru, C. I. (2021). Structure and function of pea, lentil and faba bean proteins treated by high pressure processing and heat treatment. *LWT, 152*, Article 112349. https://doi.org/10.1016/j.lwt.2021.112349

He, X., Chen, J., He, X., Feng, Z., Li, C., Liu, W., & Liu, C. (2021). Industry-scale microfluidization as a potential technique to improve solubility and modify structure of pea protein. *Innovative Food Science and Emerging Technologies, 67*, Article 102582. https://doi.org/10.1016/j.ifset.2020.102582

Higuera-Barraza, O., Del Toro-Sanchez, C., Ruiz-Cruz, S., & Márquez-Ríos, E. (2016). Effects of high-energy ultrasound on the functional properties of proteins. *Ultrasonics Sonochemistry, 31*, 558–562. https://doi.org/10.1021/jf201781z

Hu, H., Wu, J., Li-Chan, E. C. Y., Zhu, L., Zhang, F., Xu, X., & Pan, S. (2013). Effects of ultrasound on structural and physical properties of soy protein isolate (SPI) dispersions. *Food Hydrocolloids, 30*(2), 647–655. https://doi.org/10.1016/j.foodhyd.2012.08.001

Hu, X., Zhao, M., Sun, W., Zhao, G., & Ren, J. (2011). Effects of microfluidization treatment and transglutaminase cross-linking on physicochemical, functional, and conformational properties of peanut protein isolate. *Journal of Agricultural and Food Chemistry, 59*(16), 8886–8894. https://doi.org/10.1021/jf201781z

Huang, D., Xu, Y., Zhang, W., Liu, Y., Zhang, T., Liu, H., & Li, D. (2024). Enhancement of foaming property of ormosia protein: Insights into the effect of high-intensity ultrasound on physicochemical properties and structure analysis. *Food Hydrocolloids*. , Article 109902. https://doi.org/10.1016/j.foodhyd.2024.109902

Hussain Badar, I., Wang, Z., Chen, Q., Liu, Q., Ma, J., Liu, H., & Kong, B. (2024). Ultrasonic enhancement of structural and emulsifying properties of heat-treated soy protein isolate nanoparticles to fabricate flaxseed-derived diglyceride-based Pickering emulsions. *Food Chemistry, 442*, Article 138469. https://doi.org/10.1016/j.foodchem.2024.138469

Ippoushi, K., Tanaka, Y., Wakagi, M., & Hashimoto, N. (2020). Evaluation of protein extraction methods for β-conglycinin quantification in soybeans and soybean products. *LWT, 132*, Article 109871. https://doi.org/10.1016/j.lwt.2020.109871

Jambrak, A. R., Lelas, V., Mason, T. J., Krešić, G., & Badanjak, M. (2009). Physical properties of ultrasound treated soy proteins. *Journal of Food Engineering, 93*(4), 386–393. https://doi.org/10.1016/j.jfoodeng.2009.02.001

Janardhanan, R., González-Diez, M., Ibañez, F. C., & Beriain, M. J. (2022). Comparison of high hydrostatic pressure processed plus sous-vide cooked meat-based, plant-based and hybrid patties according to fat replacement. *Foods, 11*(22), 3678. https://doi.org/10.3390/foods11223678

Jarpa-Parra, M. (2018). Lentil protein: A review of functional properties and food application. An overview of lentil protein functionality. *International Journal of Food Science and Technology, 53*(4), 892–903. https://doi.org/10.1111/ijfs.13685

Ji, H., Dong, S., Han, F., Li, Y., Chen, G., Li, L., & Chen, Y. (2018). Effects of dielectric barrier discharge (DBD) cold plasma treatment on physicochemical and functional properties of peanut protein. *Food and Bioprocess Technology, 11*, 344–354. https://doi.org/10.1007/s11947-017-2015-z

Ji, H., Han, F., Peng, S., Yu, J., Li, L., Liu, Y., & Chen, Y. (2019). Behavioral solubilization of peanut protein isolate by atmospheric pressure cold plasma (ACP) treatment. *Food and Bioprocess Technology, 12*, 2018–2027. https://doi.org/10.1007/s11947-019-02357-0

Jia, Y., Yan, X., Huang, Y., Zhu, H., Qi, B., & Li, Y. (2022). Different interactions driving the binding of soy proteins (7S/11S) and flavonoids (quercetin/rutin): Alterations in the conformational and functional properties of soy proteins. *Food Chemistry, 396*, Article 133685. https://doi.org/10.1016/j.foodchem.2022.133685

Jiang, S., Ding, J., Andrade, J., Rababah, T. M., Almajwal, A., Abulmeaty, M. M., & Feng, H. (2017). Modifying the physicochemical properties of pea protein by pH-shifting and ultrasound combined treatments. *Ultrasonics Sonochemistry, 38*, 835–842. https://doi.org/10.1016/j.ultsonch.2017.03.046

Joshi, M., Timilsena, Y., & Adhikari, B. (2017). Global production, processing and utilization of lentil: A review. *Journal of Integrative Agriculture, 16*(12), 2898–2913. https://doi.org/10.1016/S2095-3119(17)61793-3

Karki, B., Lamsal, B. P., Grewell, D., Pometto, A. L., Van Leeuwen, J., Khanal, S. K., & Jung, S. (2009). Functional properties of soy protein isolates produced from ultrasonicated defatted soy flakes. *Journal of the American Oil Chemists' Society, 86*, 1021–1028. https://doi.org/10.1007/s11746-009-1433-0

Khazaei, H., Subedi, M., Nickerson, M., Martínez-Villaluenga, C., Frias, J., & Vandenberg, A. (2019). Seed protein of lentils: Current status, progress, and food applications. *Foods, 8*(9), 391. https://doi.org/10.3390/foods8090391

Kumar, M., Tomar, M., Punia, S., Dhakane-Lad, J., Dhumal, S., Changan, S., & Kennedy, J. F. (2022). Plant-based proteins and their multifaceted industrial applications. *LWT, 154*, Article 112620. https://doi.org/10.1016/j.lwt.2021.112620

Li, H., Zhu, K., Zhou, H., & Peng, W. (2011). Effects of high hydrostatic pressure on some functional and nutritional properties of soy protein isolate for infant formula. *Journal*

of Agricultural and Food Chemistry, 59(22), 12028–12036. https://doi.org/10.1021/jf203390e

Li, L., Zhou, Y., Teng, F., Zhang, S., Qi, B., Wu, C., & Li, Y. (2020). Application of ultrasound treatment for modulating the structural, functional and rheological properties of black bean protein isolates. *International Journal of Food Science and Technology, 55*(4), 1637–1647. https://doi.org/10.1111/ijfs.14428

Li, Q., Shen, F., He, X., Xing, C., Yan, W., Fang, Y., & Hu, Q. (2023). Modification of soy protein isolate using dielectric barrier discharge cold plasma assisted by modified atmosphere packaging. *Food Chemistry, 401*, Article 134158. https://doi.org/10.1016/j.foodchem.2022.134158

Li, S., Zhu, Y., Hao, X., Su, H., Chen, X., & Yao, Y. (2024). High internal phase Pickering emulsions stabilized by the complexes of ultrasound-treated pea protein isolate/mung bean starch for delivery of β-carotene. *Food Chemistry, 440*, Article 138201. https://doi.org/10.1016/j.foodchem.2023.138201

Li, Y., Chen, Z., & Mo, H. (2007). Effects of pulsed electric fields on physicochemical properties of soybean protein isolates. *LWT, 40*(7), 1167–1175. https://doi.org/10.1016/j.lwt.2006.08.015

Lo, B., Kasapis, S., & Farahnaky, A. (2022). Effect of low frequency ultrasound on the functional characteristics of isolated lupin protein. *Food Hydrocolloids, 124*, Article 107345. https://doi.org/10.1016/j.foodhyd.2021.107345

Ma, X., Habibi, M., & Sagis, L. M. C. (2024). Interfacial and foaming properties of soluble lupin protein isolates: Effect of pH. *Food Hydrocolloids, 155*, Article 110228. https://doi.org/10.1016/j.foodhyd.2024.110228

Manassero, C. A., David-Briand, E., Vaudagna, S. R., Anton, M., & Speroni, F. (2018). Calcium addition, pH, and high hydrostatic pressure effects on soybean protein isolates—Part 1: Colloidal stability improvement. *Food and Bioprocess Technology, 11*, 1125–1138. https://doi.org/10.1007/s11947-018-2084-7

Manassero, C. A., Vaudagna, S. R., Añón, M. C., & Speroni, F. (2015). High hydrostatic pressure improves protein solubility and dispersion stability of mineral-added soybean protein isolate. *Food Hydrocolloids, 43*, 629–635. https://doi.org/10.1016/j.foodhyd.2014.07.020

Marciniak, A., Suwal, S., Naderi, N., Pouliot, Y., & Doyen, A. (2018). Enhancing enzymatic hydrolysis of food proteins and production of bioactive peptides using high hydrostatic pressure technology. *Trends in Food Science and Technology, 80*, 187–198. https://doi.org/10.1016/j.tifs.2018.08.013

Maria Medeiros Theóphilo Galvão, A., Lamy Rasera, M., De Figueiredo Furtado, G., Grossi Bovi Karatay, G., M. Tavares, G., & Dupas Hubinger, M. (2024). Lentil protein isolate (Lens culinaris) subjected to ultrasound treatment combined or not with heat-treatment: Structural characterization and ability to stabilize high internal phase emulsions. *Food Research International, 183*, Article 114212. https://doi.org/10.1016/j.foodres.2024.114212

Martínez-Velasco, A., Lobato-Calleros, C., Hernández-Rodríguez, B. E., Román-Guerrero, A., Alvarez-Ramirez, J., & Vernon-Carter, E. J. (2018). High intensity ultrasound treatment of faba bean (Vicia faba L.) protein: Effect on surface properties, foaming ability and structural changes. *Ultrasonics Sonochemistry, 44*, 97–105. https://doi.org/10.1016/j.ultsonch.2018.02.007

Matsuo, A., Matsushita, K., Fukuzumi, A., Tokumasu, N., Yano, E., Zaima, N., & Moriyama, T. (2020). Comparison of various soybean allergen levels in genetically and non-genetically modified soybeans. *Foods, 9*(4), 522. https://doi.org/10.3390/foods9040522

Mehr, H. M., & Koocheki, A. (2020). Effect of atmospheric cold plasma on structure, interfacial and emulsifying properties of grass pea (Lathyrus sativus L.) protein isolate. *Food Hydrocolloids, 106*, Article 105899. https://doi.org/10.1016/j.foodhyd.2020.105899

Mehr, H. M., & Koocheki, A. (2021). Physicochemical properties of grass pea (Lathyrus sativus L.) protein nanoparticles fabricated by cold atmospheric-pressure plasma. *Food Hydrocolloids, 112*, Article 106328. https://doi.org/10.1016/j.foodhyd.2020.106328

Mehr, H. M., & Koocheki, A. (2023). Effects of short-term and long-term cold plasma treatment on the color, structure, and Pickering foaming properties of grass pea protein particles. *Food Hydrocolloids, 143*, Article 108846. https://doi.org/10.1016/j.foodhyd.2023.108846

Melchior, S., Calligaris, S., Bisson, G., & Manzocco, L. (2020). Understanding the impact of moderate-intensity pulsed electric fields (MIPEF) on structural and functional characteristics of pea, rice and gluten concentrates. *Food and Bioprocess Technology, 13*, 2145–2155. https://doi.org/10.1007/s11947-020-02554-2

Messens, W., Van Camp, J., & Huyghebaert, A. (1997). The use of high pressure to modify the functionality of food proteins. *Trends in Food Science & Technology, 8*(4), 107–112. https://doi.org/10.1016/S0924-2244(97)01015-7

Moll, P., Salminen, H., Schmitt, C., & Weiss, J. (2021). Impact of microfluidization on colloidal properties of insoluble pea protein fractions. *European Food Research and Technology, 247*(3), 545–554. https://doi.org/10.1007/s00217-020-03629-2

Mollakhalili-Meybodi, N., Yousefi, M., Nematollahi, A., & Khorshidian, N. (2021). Effect of atmospheric cold plasma treatment on technological and nutrition functionality of protein in foods. *European Food Research and Technology, 247*, 1579–1594. https://doi.org/10.1007/s00217-021-03750-w

Momen, S., & Aider, M. (2023). Production of highly soluble and functional whey/canola proteins through complexation using alkaline electro-activation. *Food Hydrocolloids, 137*, Article 108395. https://doi.org/10.1016/j.foodhyd.2022.108395

Morales, R., Martínez, K. D., Pizones Ruiz-Henestrosa, V. M., & Pilosof, A. M. R. (2015). Modification of foaming properties of soy protein isolate by high ultrasound intensity: Particle size effect. *Ultrasonics Sonochemistry, 26*, 48–55. https://doi.org/10.1016/j.ultsonch.2015.01.011

Mozafarpour, R., Koocheki, A., & Nicolai, T. (2022). Modification of grass pea protein isolate (Lathyrus sativus L.) using high intensity ultrasound treatment: Structure and functional properties. *Food Research International, 158*, Article 111520. https://doi.org/10.1016/j.foodres.2022.111520

Mulla, M. Z., Subramanian, P., & Dar, B. N. (2022). Functionalization of legume proteins using high pressure processing: Effect on technofunctional properties and digestibility of legume proteins. *LWT, 158*, Article 113106. https://doi.org/10.1016/j.lwt.2022.113106

Naumann, S., Schweiggert-Weisz, U., Haller, D., & Eisner, P. (2019). Retention of primary bile acids by lupin cell wall polysaccharides under in vitro digestion conditions. *Nutrients, 11*(9), 2117. https://doi.org/10.3390/foods10112622

Nikbakht Nasrabadi, M., Sedaghat Doost, A., & Mezzenga, R. (2021). Modification approaches of plant-based proteins to improve their techno-functionality and use in food products. *Food Hydrocolloids, 118*, Article 106789. https://doi.org/10.1016/j.foodhyd.2021.106789

Noordraven, L. E., Kim, H.-J., Hoogland, H., Grauwet, T., & Van Loey, A. M. (2021). Potential of chickpea flours with different microstructures as multifunctional ingredient in an instant soup application. *Foods, 10*(11), 2622. https://doi.org/10.3390/foods10112622

Oliete, B., Potin, F., Cases, E., & Saurel, R. (2018). Modulation of the emulsifying properties of pea globulin soluble aggregates by dynamic high-pressure fluidization. *Innovative Food Science and Emerging Technologies, 47*, 292–300. https://doi.org/10.1016/j.ifset.2018.03.015

Peyrano, F., Speroni, F., & Avanza, M. V. (2016). Physicochemical and functional properties of cowpea protein isolates treated with temperature or high hydrostatic pressure. *Innovative Food Science & Emerging Technologies, 33*, 38–46. https://doi.org/10.1016/j.ifset.2015.10.014

Piccini, L., Scilingo, A., & Speroni, F. (2019). Thermal versus high hydrostatic pressure treatments on calcium-added soybean proteins. Protein solubility, colloidal stability and cold-set gelation. *Food Biophysics, 14*, 69–79. https://doi.org/10.1007/s11483-018-9558-z

Pomés, A., Davies, J. M., Gadermaier, G., Hilger, C., Holzhauser, T., Lidholm, J., & Radauer, C. (2018). WHO/IUIS allergen nomenclature: Providing a common language. *Molecular Immunology, 100*, 3–13. https://doi.org/10.1016/j.molimm.2018.03.003

Qu, Z., Chen, G., Wang, J., Xie, X., & Chen, Y. (2023). Preparation, structure evaluation, and improvement in foaming characteristics of fibrotic pea protein isolate by cold plasma synergistic organic acid treatment. *Food Hydrocolloids, 134*, Article 108057. https://doi.org/10.1016/j.foodhyd.2022.108057

Rahate, K. A., Madhumita, M., & Prabhakar, P. K. (2021). Nutritional composition, anti-nutritional factors, pretreatments-cum-processing impact and food formulation potential of faba bean (Vicia faba L.): A comprehensive review. *LWT, 138*, Article 110796. https://doi.org/10.1016/j.lwt.2020.110796

Ross, A. I. V., Griffiths, M. W., Mittal, G. S., & Deeth, H. C. (2003). Combining nonthermal technologies to control foodborne microorganisms. *International Journal of Food Microbiology, 89*(2–3), 125–138. https://doi.org/10.1016/S0168-1605(03)00161-2

Rout, S., & Srivastav, P. P. (2024). Modification of soy protein isolate and pea protein isolate by high voltage dielectric barrier discharge (DBD) atmospheric cold plasma: Comparative study on structural, rheological and techno-functional characteristics. *Food Chemistry, 447*, Article 138914. https://doi.org/10.1016/j.foodchem.2024.138914

Sha, L., Koosis, A. O., Wang, Q., True, A. D., & Xiong, Y. L. (2021). Interfacial dilatational and emulsifying properties of ultrasound-treated pea protein. *Food Chemistry, 350*, Article 129271. https://doi.org/10.1016/j.foodchem.2021.129271

Shaghaghian, S., McClements, D. J., Khalesi, M., Garcia-Vaquero, M., & Mirzapour-Kouhdasht, A. (2022). Digestibility and bioavailability of plant-based proteins intended for use in meat analogues: A review. *Trends in Food Science & Technology, 129*, 646–656. https://doi.org/10.1016/j.tifs.2022.11.016

Sharafodin, H., & Soltanizadeh, N. (2022). Potential application of DBD plasma technique for modifying structural and physicochemical properties of soy protein isolate. *Food Hydrocolloids, 122*, Article 107077. https://doi.org/10.1016/j.foodhyd.2021.107077

Sharafodin, H., Soltanizadeh, N., & Barahimi, M. S. (2023). Conjugation of soy protein isolate with carboxymethyl cellulose through dielectric barrier discharge (DBD) plasma. *Food Chemistry, 407*, Article 135059. https://doi.org/10.1016/j.foodchem.2022.135059

Shen, L., & Tang, C.-H. (2012). Microfluidization as a potential technique to modify surface properties of soy protein isolate. *Food Research International, 48*(1), 108–118. https://doi.org/10.1016/j.foodres.2012.03.006

Sparavigna, A. (2008). *Plasma treatment advantages for textiles. arXiv preprint*. arXiv:0801.3727. https://doi.org/10.48550/arXiv.0801.3727.

Sui, X., Zhang, T., & Jiang, L. (2021). Soy protein: Molecular structure revisited and recent advances in processing technologies. *Annual Review of Food Science and Technology, 12*, 119–147. https://doi.org/10.1146/annurev-food-062220-104405

Sun, F., Li, B., Guo, Y., Wang, Y., Cheng, T., Yang, Q., & Wang, Z. (2022). Effects of ultrasonic pretreatment of soybean protein isolate on the binding efficiency, structural changes, and bioavailability of a protein-luteolin nanodelivery system. *Ultrasonics Sonochemistry, 88*, Article 106075. https://doi.org/10.1016/j.ultsonch.2022.106075

Tan, M., Xu, J., Gao, H., Yu, Z., Liang, J., Mu, D., & Zheng, Z. (2021). Effects of combined high hydrostatic pressure and pH-shifting pretreatment on the structure and emulsifying properties of soy protein isolates. *Journal of Food Engineering, 306*, Article 110622. https://doi.org/10.1016/j.jfoodeng.2021.110622

Tan, X., Li, C., Bai, Y., & Gilbert, R. G. (2022). The role of storage protein fractions in slowing starch digestion in chickpea seed. *Food Hydrocolloids, 129*, Article 107617. https://doi.org/10.1016/j.foodhyd.2022.107617

Tian, Z. M., Wan, M. X., Wang, S. P., & Kang, J. Q. (2004). Effects of ultrasound and additives on the function and structure of trypsin. *Ultrasonics Sonochemistry, 11*(6), 399–404. https://doi.org/10.1016/j.ultsonch.2003.09.004

Ucar, Y., Ceylan, Z., Durmus, M., Tomar, O., & Cetinkaya, T. (2021). Application of cold plasma technology in the food industry and its combination with other emerging technologies. *Trends in Food Science & Technology, 114*, 355–371. https://doi.org/10.1016/j.tifs.2021.06.004

Waghmare, R. (2021). Cold plasma technology for fruit based beverages: A review. *Trends in Food Science & Technology, 114*, 60–69. https://doi.org/10.1016/j.tifs.2021.05.018

Wang, C., Yin, H., Zhao, Y., Zheng, Y., Xu, X., & Yue, J. (2021). Optimization of high hydrostatic pressure treatments on soybean protein isolate to improve its functionality and evaluation of its application in yogurt. *Foods, 10*(3), 667. https://doi.org/10.3390/foods10030667

Wang, F., Zhang, Y., Xu, L., & Ma, H. (2020). An efficient ultrasound-assisted extraction method of pea protein and its effect on protein functional properties and biological activities. *LWT, 127*, Article 109348. https://doi.org/10.1016/j.lwt.2020.109348

Wang, J., Zhou, X., Ju, S., Cai, R., Roopesh, M., Pan, D., & Du, L. (2023). Influence of atmospheric pressure plasma jet on the structural, functional and digestive properties of chickpea protein isolate. *Food Research International, 174*, Article 113565. https://doi.org/10.1016/j.ifset.2024.103607

Wang, J., Zhou, X., Li, J., Pan, D., & Du, L. (2024). Enhancing the functionalities of chickpea protein isolate through a combined strategy with pH-shifting and cold plasma treatment. *Innovative Food Science & Emerging Technologies*. , Article 103607. https://doi.org/10.1016/j.ifset.2024.103607

Wang, R., Wang, L.-H., Wen, Q.-H., He, F., Xu, F.-Y., Chen, B.-R., & Zeng, X.-A. (2023). Combination of pulsed electric field and pH shifting improves the solubility, emulsifying, foaming of commercial soy protein isolate. *Food Hydrocolloids, 134*, Article 108049. https://doi.org/10.1016/j.foodhyd.2022.108049

Wang, X., Zhao, Y., Wang, T., Li, Y., Wang, C., Zhao, X., & Wang, H. (2023). The structural and functional properties changing of peanut protein isolate (PPI) via different treatments and their application in pork meatballs production. *LWT, 184*, Article 115046. https://doi.org/10.1016/j.lwt.2023.115046

Wang, X.-S., Tang, C.-H., Li, B.-S., Yang, X.-Q., Li, L., & Ma, C.-Y. (2008). Effects of high-pressure treatment on some physicochemical and functional properties of soy protein isolates. *Food Hydrocolloids, 22*(4), 560–567. https://doi.org/10.1016/j.foodhyd.2007.01.027

Wang, Y., Wang, Y., Li, K., Bai, Y., Li, B., & Xu, W. (2020). Effect of high intensity ultrasound on physicochemical, interfacial and gel properties of chickpea protein isolate. *Lwt, 129*, Article 109563. https://doi.org/10.1021/acs.jafc.0c02927

Warsame, A. O., Michael, N., O'Sullivan, D. M., & Tosi, P. (2020). Identification and quantification of major faba bean seed proteins. *Journal of Agricultural and Food Chemistry, 68*(32), 8535–8544. https://doi.org/10.1021/acs.jafc.0c02927

Xia, W., Pan, S., Cheng, Z., Tian, Y., & Huang, X. (2020). High-intensity ultrasound treatment on soy protein after selectively Proteolyzing Glycinin component: Physical, structural, and aggregation properties. *Foods, 9*(6), 839. https://doi.org/10.1016/j.lwt.2022.113223

Yan, G., Cui, Y., Lia, D., Ding, Y., Han, J., Wang, S., & Zheng, H. (2022). The characteristics of soybean protein isolate obtained by synergistic modification of high hydrostatic pressure and phospholipids as a promising replacement of milk in ice cream. *LWT, 160*, Article 113223. https://doi.org/10.1016/j.lwt.2022.113223

Yan, S., Xu, J., Zhang, S., & Li, Y. (2021). Effects of flexibility and surface hydrophobicity on emulsifying properties: Ultrasound-treated soybean protein isolate. *LWT, 142*, Article 110881. https://doi.org/10.1016/j.lwt.2021.110881

Yang, H., Yang, A., Gao, J., & Chen, H. (2014). Characterization of physicochemical properties and IgE-binding of soybean proteins derived from the HHP-treated seeds. *Journal of Food Science, 79*(11), C2157–C2163. https://doi.org/10.1111/1750-3841.12665

Yang, J., Zhu, B., Lu, K., Dou, J., Ning, Y., Wang, H., & Jiang, L. (2023). Construction and characterization of Pickering emulsions stabilized by soy protein hydrolysate microgel particles and quercetin-loaded performance in vitro digestion. *Food Research International, 169*, Article 112844. https://doi.org/10.1016/j.foodres.2023.112844

Yang, S., Lian, Z., Cheng, L., Liu, X., Dai, S., Tong, X., & Jiang, L. (2024). Insight into succinylated modified soy protein isolate-sodium alginate emulsion gels: Structural properties, interactions and quercetin release behavior. *Food Hydrocolloids, 151*, Article 109857. https://doi.org/10.1016/j.foodhyd.2024.109857

Yin, S.-W., Tang, C.-H., Wen, Q.-B., Yang, X.-Q., & Li, L. (2008). Functional properties and in vitro trypsin digestibility of red kidney bean (Phaseolus vulgaris L.) protein isolate: Effect of high-pressure treatment. *Food Chemistry, 110*(4), 938–945. https://doi.org/10.1016/j.foodchem.2008.02.090

Yu, C., Li, S., Sun, S., Yan, H., & Zou, H. (2022). Modification of emulsifying properties of mussel myofibrillar proteins by high-intensity ultrasonication treatment and the stability of O/W emulsion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 641*, Article 128511. https://doi.org/10.1016/j.colsurfa.2022.128511

Yu, J.-j., Ji, H., Chen, Y., Zhang, Y.-F., Zheng, X.-C., Li, S.-H., & Chen, Y. (2020). Analysis of the glycosylation products of peanut protein and lactose by cold plasma treatment: Solubility and structural characteristics. *International Journal of Biological Macromolecules, 158*, 1194–1203. https://doi.org/10.1016/j.ijbiomac.2020.04.255

Yu, J.-J., Chen, G.-Y., Zhang, Y.-F., Zheng, X.-C., Jiang, P.-Y., Ji, H., & Chen, Y. (2021). Enhanced hydration properties and antioxidant activity of peanut protein by covalently binding with sesbania gum via cold plasma treatment. *Innovative Food Science and Emerging Technologies, 68*, Article 102632. https://doi.org/10.1016/j.ifset.2021.102632

Yu, X., Huang, S., Nie, C., Deng, Q., Zhai, Y., & Shen, R. (2020). Effects of atmospheric pressure plasma jet on the physicochemical, functional, and antioxidant properties of flaxseed protein. *Journal of Food Science, 85*(7), 2010–2019. https://doi.org/10.1111/1750-3841.15184

Zhang, J., Zhao, S., Li, L., Kong, B., & Liu, H. (2023). High internal phase emulsions stabilized by pea protein isolate modified by ultrasound combined with pH-shifting: Micromorphology, rheology, and physical stability. *Foods, 12*(7), 1433. https://doi.org/10.1016/j.foodhyd.2022.107782

Zhang, P., Bao, Z.-Y., Wang, H., Tu, Z.-C., Sha, X.-M., & Hu, Y.-M. (2022). Ultrasonic pretreatment improved the physicochemical properties and riboflavin delivery ability of transglutaminase-catalyzed soy protein isolate gel. *Food Hydrocolloids, 131*, Article 107782. https://doi.org/10.1016/j.foodhyd.2022.107782

Zhang, Q., Cheng, Z., Zhang, J., Nasiru, M. M., Wang, Y., & Fu, L. (2021). Atmospheric cold plasma treatment of soybean protein isolate: Insights into the structural, physicochemical, and allergenic characteristics. *Journal of Food Science, 86*(1), 68–77. https://doi.org/10.1016/j.foodhyd.2021.107373

Zhang, Q.-T., Tu, Z.-C., Xiao, H., Wang, H., Huang, X.-Q., Liu, G.-X., & Lin, D.-R. (2014). Influence of ultrasonic treatment on the structure and emulsifying properties of peanut protein isolate. *Food and Bioproducts Processing, 92*(1), 30–37. https://doi.org/10.1016/j.fbp.2013.07.006

Zhang, S., Du, H., Ma, Y., Li, H., Kan, G., & Yu, D. (2021). Linkage and association study discovered loci and candidate genes for glycinin and β-conglycinin in soybean (Glycine max L. Merr.). *Theoretical and Applied Genetics, 134*(4), 1201–1215. https://doi.org/10.1007/s00122-021-03766-6

Zhang, T., Jiang, B., Miao, M., Mu, W., & Li, Y. (2012). Combined effects of high-pressure and enzymatic treatments on the hydrolysis of chickpea protein isolates and antioxidant activity of the hydrolysates. *Food Chemistry, 135*(3), 904–912. https://doi.org/10.1016/j.foodchem.2012.05.097

Zhong, K., Hu, X., Zhao, G., Chen, F., & Liao, X. (2005). Inactivation and conformational change of horseradish peroxidase induced by pulsed electric field. *Food Chemistry, 92*(3), 473–479. https://doi.org/10.1016/j.foodchem.2004.08.010

Zhong, Z., & Xiong, Y. L. (2020). Thermosonication-induced structural changes and solution properties of mung bean protein. *Ultrasonics Sonochemistry, 62*, Article 104908. https://doi.org/10.1016/j.ultsonch.2019.104908

Zhou, H., Hu, X., Xiang, X., & McClements, D. J. (2023). Modification of textural attributes of potato protein gels using salts, polysaccharides, and transglutaminase: Development of plant-based foods. *Food Hydrocolloids, 144*, Article 108909. https://doi.org/10.1016/j.foodhyd.2023.108909