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Review

Plant Protein Heat-Induced Gels: Formation Mechanisms and Regulatory Strategies

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Abstract: With increasing awareness of human health, proteins from plant sources are being considered as alternatives to those from animal sources. The market for plant-based meat substitutes is expanding to satisfy the growing consumer demand. However, the functional properties of natural proteins frequently do not satisfy the needs of the modern food industry, which requires high-quality properties. Research on improving the functional properties of proteins is currently a popular topic. Based on the gel properties of proteins, this study focused on the formation mechanism of heat-induced protein gels, which will be helpful in expanding the market for plant protein gel products. Regulatory strategies for heat-induced gels were reviewed, including protein composition, pH, ionic strength, other food components, and processing techniques. The effects of other food components (such as polysaccharides, proteins, polyphenols, and liposomes) are discussed to provide insights into the properties of plant protein gels. Studies have shown that these factors can effectively improve the properties of plant protein gels. In addition, the development and application potential of emerging processing technologies that can contribute to safe and effective applications in actual food production are discussed. For the future, plant protein gels are playing an irreplaceable role in the new direction of future food.

Keywords: plant protein gels; formation mechanisms of heat-induced gels; regulatory strategies; development prospect

Citation: Ma, Y.; Chen, F. Plant Protein Heat-Induced Gels: Formation Mechanisms and Regulatory Strategies. *Coatings* **2023**, *13*, 1899. https://doi.org/10.3390/coatings13111899

Academic Editor: Sorin Avramescu

Received: 14 September 2023
Revised: 1 November 2023
Accepted: 3 November 2023
Published: 6 November 2023

1. Introduction

Meat is an indispensable food resource because of its nutritional value and unique taste. It is estimated that meat consumption will increase by more than 50& by 2050 [1]. However, the increasing demand for meat will lead to a series of negative impacts on environmental pollution, global warming, animal welfare, and human health [2]. Therefore, plant-based meat substitutes are increasingly being introduced to the market because of their greater sustainability and ethics [3]. Plant-based meat substitutes may include proteins, lipids, binding (starch or gelling) agents, flavours, and colour materials [4]. Among these, proteins are the most important. The molecular structure of proteins can be divided into four levels, which are primary structure, secondary structure, tertiary structure, and quaternary structure. Proteins can be transformed in multiple structures to cause complex conformations, which perform their biological functions [5]. Proteins not only have nutritional value but also functional properties such as solubility, gelling, water- and oil-holding capacities, emulsification, and foaming, which can satisfy the needs of specific foods. Gelation is one of the most important functional properties of protein, as it provides the texture and structure of food. The properties of protein gels affect the texture, flavour, taste, stability, nutrition, and health properties of food, and play an important role in maintaining food quality [6]. However, plant protein is inferior to animal protein in terms of gel properties. With the increasing of the awareness of sustainability and physical

health, plant protein gels as a substitute for animal protein gels in the development of new health food has become a research hotspot. In recent years, studies on plant proteins as substitutes for animal proteins have mainly focused on their gelling properties. Hence, we reviewed recent research on this functionality: the capacity of plant proteins to form gels during heating.

Protein gelation is caused by the unfolding and aggregation of protein structures, which balances the attractive and repulsive forces required to form a three-dimensional network [7]. Protein gels are categorised according to different classification standards. Protein gels can be classified as opaque or translucent depending on the shape of the protein molecule. Opaque gels, also known as coagulating gels, have a strong water-holding capacity, which is the result of a random aggregation of proteins. Even if reheated, they do not return to the state before the gel. Translucent gels, also known as reversible gels, have high elasticity and strong water-holding capacity and are the product of an ordered combination of protein molecules. After heating, the mixture can be transformed back into the state before the gel. Tani et al. [8] also found that fibrous proteins (e.g., gelatine) form transparent gels; however, most globular proteins (e.g., soy protein) form opaque gels.

According to the methods used for protein gel formation, they are divided into heat-induced and cold-induced gels. The heat-induced gels were made at high protein concentrations and high temperatures. Generally, the preparation of heat-induced gels consists of three different steps: denaturation, aggregation, and gelation [9]. The preparation conditions of cold-induced gels are relatively mild. The protein solution should be heated above the denaturation temperature to avoid the formation of heat-induced gels, and the protein concentration needs to be below the critical gel concentration. Then, the cold-induced gels are formed by adding salts, acids, or enzymes and so on after cooling [10]. Compared with heat-induced gels, the preparation of cold-induced gels was more complex. Heat-induced gels are the most common form of gel formation. Recently, heat-induced gels of plant-based proteins have been extensively studied and used in the food industry [11]. In this study, the formation mechanism and regulatory strategies of plant protein heat-induced gels were reviewed, providing a new idea for developing novel plant-based gel products.

2. Formation Mechanism of Heat-Induced Protein Gel

Generally, the basic formation principles of heat-induced gels involve denaturation, dissociation, aggregation, and cross-linking [12]. The formation of a thermal gel network is very complex, involving protein–protein and protein–water interactions as well as attraction and repulsion between adjacent polypeptide chains [13]. Soy protein isolate (SPI) is a typical plant-based globulin whose thermal gelation process is divided into three steps. As exhibited in Figure 1, in the first step, when the temperature reaches the denaturation temperature of the SPI, the natural protein denatures, leading to the dissociation of the subunits and exposure of hydrophobic groups to the surface of the molecule. Meanwhile, the hydrophobic interactions and electrostatic forces increase. In the second step, disulfide bonds play a crucial role in the aggregation of protein molecules and stabilisation of the network of heat-induced gels [14,15]. In the third step, hydrogen bonds are regenerated between the aggregates during cooling, promoting protein cross-linkages to form a stable three-dimensional network [16]. Protein denaturation is a critical condition for protein gel formation [17]. This thermal gel formation mechanism also applies to most globulin gels. A study on peanut protein isolate (PPI) gels revealed that heating promoted the protein–protein interactions via hydrophobic associations and disulfide bridging, resulting in easier cross-linking and the formation of a denser PPI gel network [18]. Wang et al. [19] reported that ionic and hydrogen bonds did not significantly contribute to heat-induced wheat gluten gel formation as the temperature increased from 25 to 90 °C. In addition, they also found that hydrophobic interactions continuously increased with increasing gelation temperatures up to 70 °C, suggesting that they played an important role in heat-induced wheat gluten gel formation. Results of experiments on SPI gel forces revealed that electrostatic interaction and disulfide bonds play a dominant role in the formation of glycinin (11S) globulin gels,

while hydrogen bonding was prominent in β-conglycinin (7S) globulin gels [20]. Although heat-induced gels are an important form of plant protein gel products, they have certain limitations in practical production. Because the formation mechanism of heat-induced gels is extremely complex, several factors that affect the formation and quality are showed in Figure 2, such as the protein composition, ions, pH, glycosylation, enzymes, process technology, and the synergy between these factors. These are discussed in this paper.

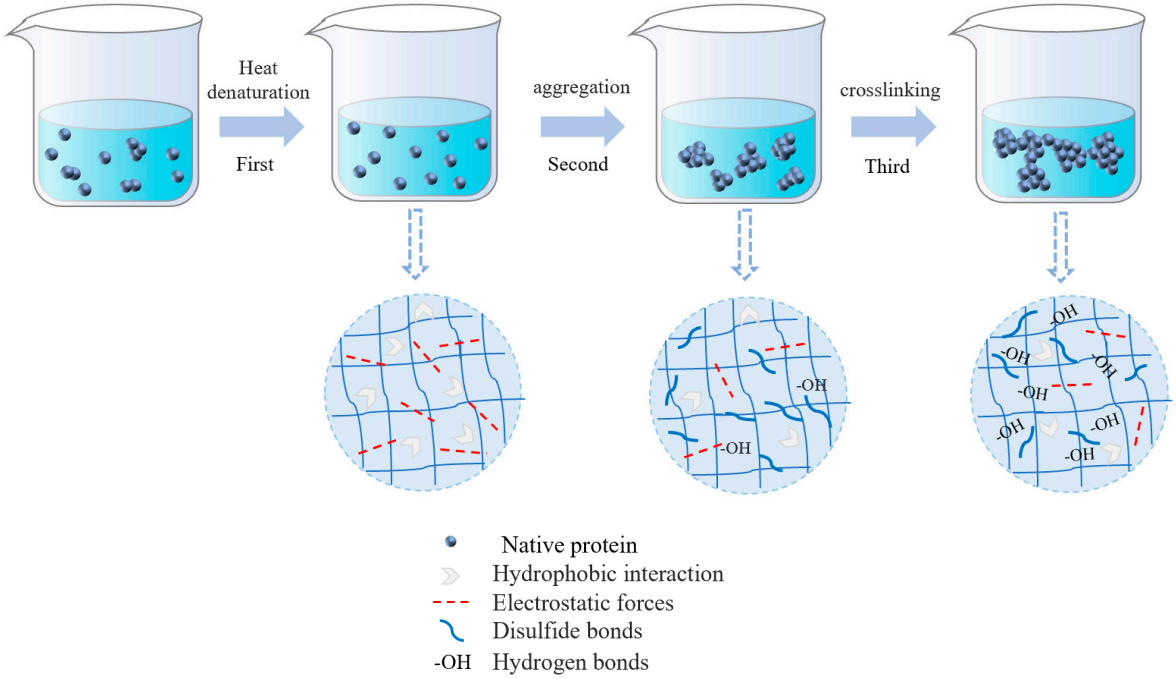

Figure 1. The formation process of plant protein heat-induced gels.

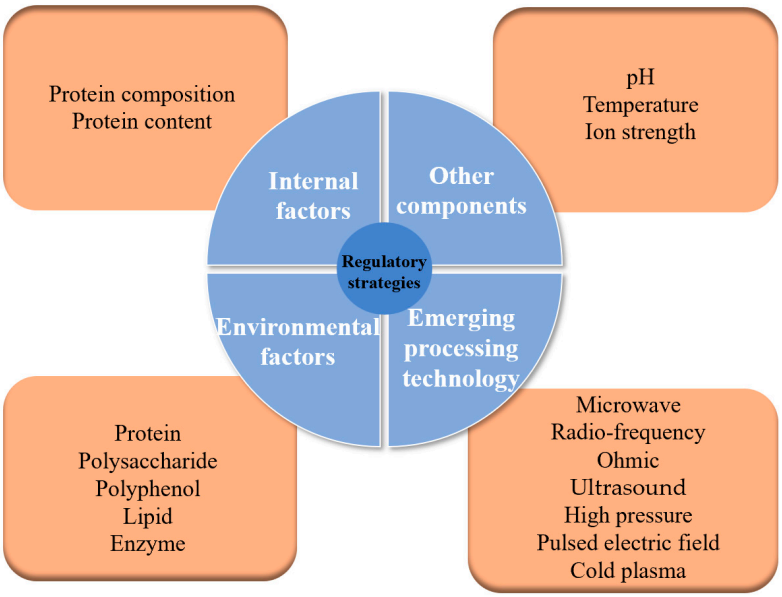

Figure 2. The factors affecting the formation and quality of heat-induced gels.

3. Regulatory Strategies of Heat-Induced Protein Gels

3.1. Regulation of Internal Factors

Protein Composition and Content

Several types of plant proteins and their sources are widely available. Most plant proteins are globular proteins. Their sources and protein compositions determine their

functional properties. For example, SPI is a mixture of various proteins, with 11S and 7S as the main proteins (generally 70% of total protein content of SPI) [21]. The former, 11S, is a hexamer composed of three acidic and three basic subunits formed through disulfide bonds [22]. The latter, 7S, is composed of three subunits α (72 kDa), α′ (76 kDa), and β (53 kDa), which are connected by non-covalent bonds [23]. The subunits, content, and ratio of 7S and 11S in SPI closely define the texture of the gel. Takashi [24] found that the gel hardness of 11S globulin was greater than that of 7S at the same concentration, and the gel properties improved with an increase in 11S content. It has been reported that SPI solutions can form a gel with a concentration of 8–16 g/dL, and the gel strength increases with an increase in concentration. PPI are also regarded as plant proteins with high nutritional value because they contain all 20 amino acids [25]. PPIs contain water-soluble and salt-soluble proteins according to their solubility, with the former accounting for 10% of the total peanut protein and the latter accounting for 90% [26]. The salt-soluble proteins comprise arachin (14S), conarachin I (7.8S), and conarachin II (2S). Arachin is present in the highest proportion, accounting for approximately 75% of the salt-soluble proteins [27,28]. The unbound fluid content decreased with increasing peanut protein concentration, and the gel strength was higher than that of a protein solution at a low concentration. However, at higher concentration, the gel strength decreased because of the disordered gel network structure [29]. Wheat gluten protein (WGP) is also an important source of protein in human food and contains approximately 15 amino acids [30]. WGP constituents are divided into globulin, albumin, glutenin, and gliadin based on their solubilities. Gliadin is an oligomeric spherical protein with a molecular weight of 30–80 kDa. It interacts with other proteins via hydrogen bonding, hydrophobic interactions, and electrostatic interactions to form a compact three-dimensional network structure [31]. Glutenin is a fibrous protein with a molecular weight of 10–1000 kDa. The structure of glutenin is stabilised mainly by the S-S bonds formed between molecules [32]. It has been reported that the glutenin composition, ratio of glutenin to melolin, molecular weight, and concentration of gluten affect the gel strength of wheat products [33]. Compared with SPI and PPI, WGP increased the viscoelasticity of the product at low concentrations. Therefore, the composition and concentration of proteins affect gel properties during food processing.

3.2. Regulation of Environmental Factors

3.2.1. pH

Proteins are sensitive to changes in pH. Treatment with pH can change the attractive and repulsive forces of protein molecules and the ability of protein molecules to bind to water molecules, thus affecting gel formation. For instance, Li [18] investigated the effects of pH treatments (pH 2, 4, 10, and 12) on the structural and heat-induced gel properties of PPI. The results indicated that the gel strength of PPI10 was significantly improved by exposure of more active sites, which promoted the protein–protein interactions via hydrophobic associations and disulfide bridging upon heating. The gel strength was poor at pH 4 because of the poor solubility near the isoelectric point of PPI. However, PPI2 and PPI12 lost their gel-forming abilities. Sun et al. studied the effect of alkaline pH-shifting bonded with mild heat treatment on the structure and gel functionalities of SPI [34]. The results revealed that the solubility and surface hydrophobicity of SPI increased from pH 7.0 to 12.0, and transglutaminase (TGase) cross-linking-modified SPI exhibited significantly increased gel hardness. In addition, SPI subjected to extreme pH treatment displayed deterioration of gel properties owing to excessive unfolding of protein chains and destruction of conformation [35]. These studies indicate that pH treatment may be a convenient and economical method for preparing plant protein gels.

3.2.2. Temperature

Heating causes the denaturation and unfolding of protein molecules, leading to the exposure of concealed active sites, which enhances intermolecular interactions. In general, lower temperatures favour hydrogen bonding, whereas higher temperatures

favour hydrophobic interactions [36]. An appropriate heat treatment temperature can change the natural conformation of proteins, effectively regulate the aggregation degree and morphology of aggregates, and achieve orderly aggregation of the protein gel network. For example, preheating at different temperatures of 70–100 °C for an appropriate time promoted protein denaturation and stronger tendency to aggregation, leading to a higher elastic modulus and hardness of the gel, effectively improving the gelling ability of SPI [37]. The effects of preheating (100 °C, 30 min) or ultrasonic treatment on the heat-induced aggregation and gelation behaviour of SPI were studied by Wang et al. [38]. The results indicated that the ability of SPI to form a gel was influenced by the degree to which proteins unfolded and the particle size of proteins after pre-treatment. The gelling ability of preheated samples was higher than that of control samples. Sun et al. [36] compared the effects of alkaline pH-shifting alone with those using a combination of alkaline pH-shifting and mild heating (50 °C, 1 h) on the physicochemical properties of SPI. The results revealed that combined treatment exposed more hydrophobic groups, unfolded the protein spatial structure, and reduced the particle size, resulting in increased gel hardness and a tighter gel network structure. The above results suggest that the heating temperature has a positive effect on the gel properties of plant proteins.

3.2.3. Ion Strength

Ionic strength can also affect gel formation by improving the electrostatic barrier and reducing the electrostatic repulsion between molecules. Ions can change the ionisation of protein functional groups and the thickness of the double electric layer, affect protein–protein and protein–water interactions, and affect the network structure of the protein gel. An appropriate ionic strength may positively affect the gel properties of proteins [39]. Nakamura et al. [40] found that although an orderly and tight three-dimensional network structure was formed at low ion concentrations, the gel structure was disordered and uneven at high ion concentrations. Several studies confirmed these findings. For example, Gao et al. [41] studied the effects of ionic strength on the properties of heat-treated cottonseed protein gels. The results revealed that more proteins could be wrapped in the gel network at a low salt concentration (0.2 mol), and that the change in salt ion concentration significantly affected the three-dimensional network structure of the heat-induced protein gels. Zhang et al. [42] indicated that adding appropriate concentrations of salt ions could induce a more compact WGP gel structure. However, once the threshold concentration was exceeded, the stability of the gel network decreased. The effects of environmental factors on heat-induced gels are summarised in Table 1.

Table 1. Effects of environmental factors on the quality of plant protein gel.

Sample	Aim of the Study	Influence Factor	Results	References
Soybean protein hydrolysate	Gelation characteristics	pH 3, 5, 7, 9	The most superior SPH gel was formed at pH 7 than acidic and alkaline conditions	[43]
Peanut protein isolate	Gel properties and protein structure	pH-shifting (2, 4, 10, 12)	Gel strength of PPI10 were significantly improved	[18]
Soy protein isolate	Textural properties	Extreme acid pH-shifting (1.5)	SPI structural unravelling at acidic pH-shifting	[35]
Soy protein isolate	Textural properties	Preheating treatment (70–100 °C)	Elastic modulus and hardness of the gel are higher than control	[37]
Soy protein isolate	Gelation behaviour	Preheating (100 °C, 30 min)	The gelling ability of preheated samples was higher than the control samples	[38]

Table 1. *Cont.*

Sample	Aim of the Study	Influence Factor	Results	References
Soy protein isolate	Physicochemical properties	Mild heating (50 °C, 1 h)	Preheating increases the gel hardness and makes the gel network tighter	[34]
Mung bean protein and wheat gluten	Gel characteristics	Na^+ and Ca^{2+} (0–0.005 g/mL)	Low concentration (0.001–0.002 g/mL) could improve the texture properties of composite protein gel and high concentration could disrupt the network structure	[39]
Pea protein	Gelation behaviour	pH (3, 7, 9) and ionic strength (0.1 M, 0.9 M)	Salt addition at acidic pH could protect the protein from acid denaturation	[44]
Faba bean	Gelation behaviour properties	NaCl concentrations (0.1 M, 0.2 M, 0.3 M)	The effect of NaCl concentration showed opposing trends for 7S and 11S	[45]

3.3. Regulation of Other Components

Protein gelation plays an important role in food processing because of the generation of pleasant textures in foods. In the gel network, cross-linking between the polypeptide chains of proteins is driven by covalent bonds (disulfide bonds), non-covalent bonds (hydrophobic interactions, hydrogen bonds, ionic interactions), or a combination of these mechanisms [46]. The protein gel itself does not exhibit good stability in a complex external environment, which greatly limits its application in the food industry [47]. Therefore, substances that change protein structure and their interactions may affect protein gelation. It has been indicated that functional characteristics of the protein gel can be favourably improved with other constituents in industrial production [48].

3.3.1. Protein–Protein Interaction

The presence of numerous hydrophobic groups in vegetable proteins leads to low gel solubility. The gel properties of vegetable proteins are inadequate for their use as the only ingredients, and they are always used with other proteins in food production. Protein–protein interactions play an important role in the textural properties of several foods, affecting the form, texture, and flavour of products. Vegetable proteins can act on animal proteins to form gels with denser three-dimensional networks that can be used to develop products with higher nutritional value [49]. Vegetable proteins affect the textural properties by interfering with the gelation of animal proteins. Luo et al. [50] studied the effect of SPI on the properties of silver carp surimi gels under different conditions and found that it could create a better composite gel by changing the microstructure of the surimi gel. Wang et al. [51] found that rice protein and whey protein isolate (RP-WPI) complexes acquired considerable surface charges, resisting aggregation of the protein bodies, with the solubility of RPs increasing to greater than 50%. Research has shown that non-covalent bonds (hydrogen bonds and hydrophobic interactions) are the most important forces in stabilising animal protein–plant protein complex gels. However, certain studies have also reported that wheat gluten proteins, as plant proteins, do not cross-link with myofibrillar proteins and the interaction between the two proteins must be promoted through TGase to improve their gel properties [52].

In addition, interactions between vegetable proteins can improve gel quality. For instance, SPI is frequently added to pasta products to balance the amino acids in WGP [53]. A few researchers reported that the form of the SPI determines the degree of interaction. The polymer between globulin and WGP is formed by a disulfide bond, while that between β-soy globulin and WGP is formed by a covalent bond [54–56]. However, owing to the lack of cross-linking between plant proteins, a few studies introduced cross-linking agents to improve the gel quality. Zhang and Chen [42] combined lysine-rich potato protein isolate

and wheat protein using a salt ion cross-linking mechanism, which not only balanced the structure of various essential amino acids, but also perfectly complemented each other in nutritional value and contributed to the formation of better gels. Therefore, it is essential to promote cross-linking between proteins to improve the structural and mechanical properties of the product.

3.3.2. Protein–Polysaccharide Interaction

The binding modes of proteins and polysaccharides are non-covalent (electrostatic interactions, hydrophobic interactions, van der Waals forces, hydrogen bonding, etc.). The amino group of the amino acid side chain and the carbonyl group of the reducing end of the polysaccharide form covalent bonds to cross-link and promote the formation of protein–polysaccharide covalent complexes, respectively (Figure 3) [57]. Protein–polysaccharide interactions are crucial for the development of plant-based food products [58]. During protein gelation, protein denaturation exposes more functional groups (e.g., sulfhydryl, hydrophobic, and amine groups), which increases the number of potential interaction sites [59]. The incorporation of polysaccharides can alter the inter- and/or intramolecular interactions involved, thereby affecting the gelling function of proteins and contributing to desirable textural and sensory properties [60].

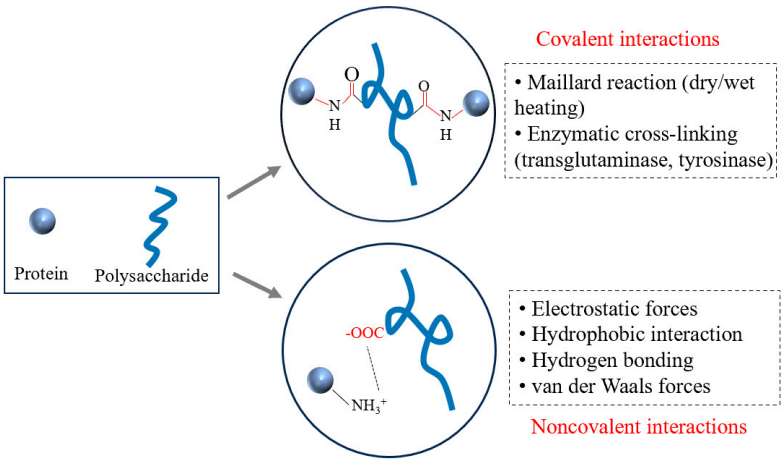

Figure 3. Interactions between proteins and polysaccharides.

Protein–polysaccharide gels with improved functional properties have attracted significant attention worldwide for novel food development [61]. Examples include those from soy, peanuts, and peas, which are the most studied in terms of food gelation. Zhou et al. [62] evaluated the impact of κ-carrageenan (KC) on the gelling behaviour of SPI. The results indicated that as the SPI:KC ratio increased from 100:0 to 95:5, KC induced complex gel formation, facilitating molecular interactions; however, excessive KC (SPI:KC = 90:10) over-compacted the gel. Jiao et al. [63] studied the effects of electrostatically charged and neutral polysaccharides on the rheological characteristics of PPI. They found that PPI–polysaccharide interactions could better control these complexes in manufactured foods. Certain researchers have focused on the effects of dietary fibres on the gel properties of proteins. Previous studies have shown that the addition of dietary fibre could enhance gel strength and WHC by forming a stable network structure [64,65] and reported that 0.1–0.5% inulin had a filling effect in the gaps of the oat protein network, significantly improving the gel properties of oat protein. Niu et al. [66] also reported that PPI-oat dietary fibre mixture gels had higher gel strength and WHC than PPI gels. Additionally, the use of inducers can further promote the cross-linking of protein polysaccharides and facilitate the preparation of more stable gel structures. Yu et al. [67] investigated the effects of TGase induction and TG-$MgCl_2$ co-induction on the gel structure and properties of SPI-maltodextrin (MD). The results revealed that all inductions led to covalent bond cross-linking of the

MD-glycosylated soybean isolate (MGSI) during gel formation. A combined treatment with enzymes and $MgCl_2$ may be an effective way to develop plant protein–polysaccharide gels.

3.3.3. Protein–Polyphenol Interaction

Proteins and polyphenols coexist in food matrices, and their interactions with each other are frequently classified as covalent or non-covalent (hydrophobic interactions as well as hydrogen bonding) [68]. The interaction between proteins and polyphenols is shown in Figure 4. Protein–polyphenolic interactions affect the structural, functional, and gel properties of proteins [69,70]. Protein–polyphenolic interactions also influence the antioxidant activity of polyphenols [69,71].

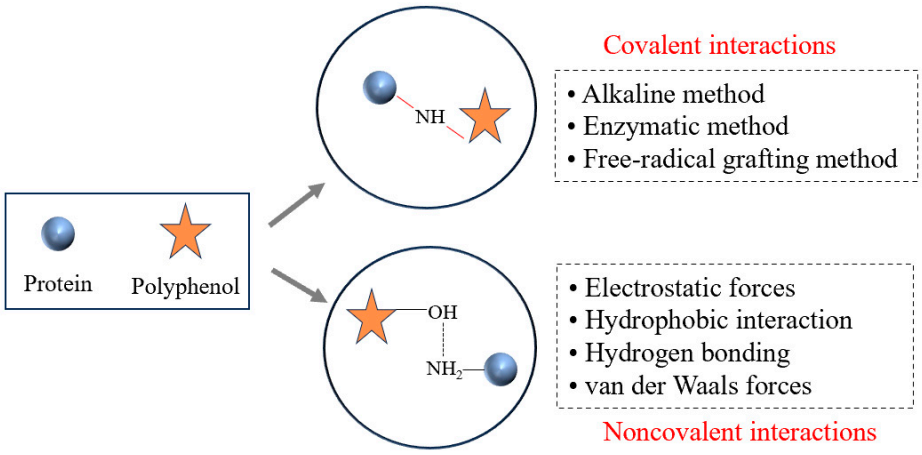

Figure 4. Interactions between proteins and polyphenols.

Recently, numerous studies have explored protein–polyphenol interactions and the functional properties of their complexes. For example, the phenol groups on proanthocyanidins can form hydrogen bonds with the hydroxyl groups on gelatine proteins and promote noncovalent interactions between them [72]. However, covalent reactions between phenolic compounds and proteins mainly depend on the structure of the phenolic compounds. Basic protein–polyphenol complexes were obtained by covalent binding of the protein and polyphenol under basic oxygen conditions, which can form a C-S or C-N bond [73]. Sui et al. [74] studied the impact of SPI–anthocyanin interactions on the functional and conformational changes in proteins. These results suggest that anthocyanins are more likely to form covalent interactions with the SPI. The addition of anthocyanins changed the secondary structure of SPI and significantly improved its emulsifying, foaming, and gel properties. Xu et al. [75] covalently combined microwaves, alkali polyphenols (ferulic acid), and microwave–alkali polyphenols to modify SPI gels. After the three modifications, a gel system with stronger elasticity was formed, and the texture, water-holding capacity, and hydration properties of the emulsion gel increased significantly. The results indicated that microwave pre-treatment combined with the covalent binding of polyphenols using an alkaline method is an effective method for soybean protein emulsion gel modification. Guo et al. [9] also found that SPI–tannic acid interactions improved the rheology and textural properties of gels, providing a reference for the covalent bonding of proteins and polyphenols to prepare gels. Therefore, protein–polyphenolic complex gels have great potential in the design and application of plant-based foods.

3.3.4. Protein–Lipid Interaction

The addition of lipids to the protein also contributes to the formation of the gel network structure. In a gelled protein matrix, fat globules act as filler particles, depending on their interactions during gelation. Shimada and Matsushita [76] reported that the effect of oil on gel formation occurs via protein–oil interactions after protein denaturation. Lipids, as fillers, affect the hardness and other properties of gel [77,78]. Research has shown that the

gel properties of SPI were significantly enhanced when it was treated with acidified oil. The composition, content, shape, and particle size distribution of fatty acids in lipids affect the protein–oil interactions and rheological behaviour of protein gels. Ningtyas et al. [77] investigated the effect of different types of fats (anhydrous milk fat, palm stearin, and soybean oil) and concentrations (0.5%, 1%, and 2%) in SPI gel on their gelation behaviour and textural properties. The results indicate that the physical state of the fat introduced in the mixture plays a major role in influencing the textural differences of the SPI gels, and in turn, has important consequences on the sensory attributes. The main fatty acids in palm stearin are palmitic (53%), oleic (28%), and linoleic acids, which can influence gel properties [79].

Recently, increasing attention has been paid to research on fat substitutes in protein–lipid coexistence systems with improvements in living standards. Ghoi et al. [80] found that sausages with 2% rice bran fibre have excellent textural properties and improved flavour, similar to sausages with 30% fat content. The interaction between proteins and lipids is a basic problem that affects the texture and quality of food in protein gel systems. Therefore, the analysis of protein–lipid interactions provides a theoretical basis for the development of high-quality food.

3.3.5. Protein–Enzyme Interaction

Enzyme-induced gels undergo intramolecular and intermolecular cross-linking and breaking via enzymatically catalysed reactions, leading to the formation of protein gels. TGase can catalyse acyl transfer reactions between γ-carboxamide groups of glutamine residue and ε-amino groups of lysine residue to form ε-(γ-glutamyl) lysine isopeptide bonds, which result in the covalent cross-linking of protein molecules and promote the formation of gels [81]. Currently, TGase is added to soybean, meat, dairy, and aquatic products, and other foods to improve their gel texture and functional properties [82]. Sun and Arntfield [83] found that TG formed cross-links among pea protein polypeptide chains, which enhanced both the strength and elasticity of the gel and improved the gelation properties of heat-induced gels. It has also been reported that TGase can solve the problem of syneresis and enhance WHC in yoghurt [84]. Huang [85] studied the degree of hydrolysis (DH), sulfhydryl content (SH), surface hydrophobicity (H0), secondary structural constitution, and microstructure of TGase-treated soybean protein (SPI, 7S, and 11S), which provides adequate technical support and a theoretical basis for soybean protein gel products. In addition, protein glutaminase (PG) is an enzyme that catalyses the deamidation of glutamine residues in proteins and can be used to expose hydrophobic groups and increase surface hydrophobicity. Thus, PG improves the strength of heat-induced gels [86]. According to recent research, other enzymes (alkaline proteases, papain, and trypsin) can also improve the gel properties of plant proteins.

3.4. Regulation of Emerging Processing Technology

Alternatively, certain emerging food-processing technologies, such as ultrasound, microwave, high pressure, ohmic heating, pulsed electric fields, and cold plasma, have been investigated for obtaining optimal protein quality [87,88]. The process conditions and other food components are reviewed and summarised above. Therefore, this section focuses on emerging processing technologies that are non-thermal or performed at mild temperatures/short times to improve the gel properties of plant proteins.

3.4.1. Microwave-Assisted Process

Microwave heating (MH) uses non-ionising electromagnetic waves from 300 MHz to 300 GHz and wavelengths ranging from 1 mm to 1 m [89]. MH can disrupt H-bonds and unfold the side chain groups of proteins to expose more active sites, enhancing the interaction within and between protein molecules and changes in protein structure, thereby improving the gel properties of proteins [90]. Liu et al. [91] studied the effect of microwave treatment on SPI gel properties and found that microwave treatment promoted SPI gels

to have better viscoelasticity and micronetwork structures than traditional heat-induced gels. Khan et al. [92] also reported that microwave treatment promotes the unfolding of rice bran protein structure and improves the gel properties of the protein. Thus, MH is an effective and feasible method for improving the properties and microstructures of protein gels.

3.4.2. Radio-Frequency-Assisted Process

Radio-frequency heating (RFH) is different from MH, with frequencies between 300 kHz and 300 MHz [93]. This could cause the polar molecules inside the food to rotate and the charged particles to reciprocate, thereby increasing the food temperature. RFH is widely used in the food industry as an emerging electromagnetic heating technology owing to its rapid and uniform heating, large penetration depth, and low energy consumption. Boreddy and Subbiah [94] reported that RFH can improve the gel properties of protein powders. RFH was used to sterilise soybean milk by Uemura et al. [95], who found that tofu treated with RFH had a higher gel strength than that treated with traditional heating.

3.4.3. Ohmic-Assisted Process

Ohmic heating (OH) is also an advanced thermal processing method. It uses electricity passed through food materials to produce Joule heat to achieve a heating effect [96]. OH can shorten the processing time and retain food quality compared with traditional heating methods [97]. The OH method is a rapid and effective method for gel preparation. In recent years, OH has attracted increasing attention for improving the textural quality of food. For instance, Ghoi et al. [98] reported the effect of OH on the quality characteristics of Alaskan cod surimi gels and obtained higher elasticity and hardness in the gel. The effects of moderate electric fields (MEF) inherent in OH on the structural and gelling properties of pea protein isolate are as follows. The OH-treated gels had the highest water holding capacity and uniform three-dimensional network structure [99]. Thus, OH might be an effective technology for changing protein structure and producing protein gels. There are opportunities for new applications for this emerging technology.

3.4.4. Ultrasound-Assisted Process

Ultrasound (US) is a non-thermal technology, which is applied in two frequency ranges: high (100 KHz–1 MHz, power < 1 W cm^{-2}) and low (16–199 KHz, power in the range of 10–100 W cm^{-2}). The effect of US on food is attributed to thermal effects, cavitation, and mechanical action, which promote the unfolding of protein structures, leading to changes in the functional characteristics of proteins, such as reduced protein particle size and viscosity, increased surface hydrophobicity, and improved solubility, gelling properties, emulsifying properties, and foaming properties [100–102]. In recent years, several studies have demonstrated that US treatments can improve the heat-induced gel properties of plants [103] and myofibrillar proteins [104]. Hu et al. [105] reported that the gel properties of SPI have a favourable influence on medium-amplitude and short-term US. Resendiz et al. [106] indicated that ultrasonic treatment promoted the movement of protein molecules, causing protein aggregation, which reduced the minimum gelling concentration of jackfruit (*Artocarpus heterophyllus*) seed protein isolates. Chen et al. [101] investigated the effect of ultrasonic treatment on peanut protein. The results demonstrated that the tertiary and quaternary structures of the peanut proteins were destroyed, which changed their protein conformation and improved the physicochemical properties. Ultrasonic treatment is a rapid and efficient method for developing new products with unique gel properties [107]. Table 2 presents the effects of ultrasonic treatment on plant proteins.

Table 2. Examples of US application on plant proteins.

Sample	Aim of the Study	Processing Conditions	Results	References
Soybean protein isolate	Gelation properties	20 kHz 150–450 W	Under 300 W, the gel hardness reached a maximum of 998.9 g, with water-binding capacity of 87%	[108]
Soybean protein isolate	Physicochemical characteristic	20 kHz at 400 W for 5, 20, or 40 min	HUS induced structural changes in SPI molecules and improved WHC and gel strength	[105]
Black bean protein isolates	Functional and structural properties	20 kHz at 150, 300, or 450 W and 12 or 24 min	Varying the ultrasonic treatment conditions can generate BBPI samples exhibiting distinct structural properties	[109]
Walnut protein isolate	Physicochemical and functional properties	200, 400, or 600 W and 15 or 30 min	sonication increased the water solubility, decreased the number of large aggregates, and improved the emulsifying properties of the walnut proteins	[110]
Jackfruit seed protein isolate	Technofunctional properties and structure	20 kHz at 200, 400, and 600 W for 15 min	200 W and 400 W improved the oil holding capacity (OHC) and emulsifying capacity (EC), but the emulsifying activity (EA) and emulsion stability (ES) increased at 400 W and 600 W. The least gelation concentration (LGC) decreased at all powers	[106]
Soybean protein isolate	Properties of SPI gel	The different papain-hydrolyzed SPI was treated with ultrasound (300 W, 20 min)	The gel strength and WHC were higher in the SPI gel treated with a combination of ultrasound and enzyme than that treated with ultrasound or enzyme alone	[111]
Soybean protein isolate	Structure and properties gels of SPI	400 W for 5 and 15 min at 80 °C	Ultrasound heat treatment of SPI produced GSCG faster and increased the storage/elastic modulus (G′), gel strength, and water-holding capacity of GSCG	[112]
Peanut protein isolate	Physical and structural properties of the mixed gel system	360 W for 30 min	The gel strength and water-holding capacity (WHC) of PPI gels and peanut protein isolate and oat dietary fibre mixture gels were enhanced after ultrasonic treatment	[66]

3.4.5. High-Pressure-Assisted Process

High-pressure processing (HPP), also known as high hydrostatic pressure (HHP) or high isostatic pressure (HIP), is a non-thermal technology that uses hydrostatic pressures of up to 1000 MPa [113]. HPP increases the surface hydrophobicity, affects the intermolecular forces (hydrogen bonds, hydrophobic interactions, electrostatic interactions, and disulfide bonds), and causes protein denaturation, aggregation, and gelation [114]. A few studies have shown that HPP technology can be used to improve plant protein properties and maintain the original nutrition, colour, and flavour of food and is considered a safe physically based technology [115]. Therefore, HPP is widely used in food gel systems. Zhang et al. [116] studied the effects of HPP on tofu gel properties and reported that the

texture of the HPP-induced tofu gel was affected by the application level and treatment time. These gels had a smooth appearance, homogeneous texture, light-yellow colour, and fewer off-flavours at 500 MPa for 20 min. This may be because HPP disrupts hydrophobic and electrostatic interactions, resulting in the denaturation of soy proteins to produce a cross-linked network.

High-pressure homogenisation (HPH) refers to a fluid subjected to a very high shear stress owing to a large pressure gradient. Cavitation, shearing, and turbulence can break down suspended particles or globules, which significantly affect protein conformation [117]. HPH is a commonly used processing method for plant protein modification because of its shorter processing time, lower pressure, and minimal effects on nutritional and sensory qualities [118]. Sun et al. [119] evaluated the conformational changes and rheological properties of SPI gels pre-treated with HPH at different pressures and cycles. They found that the best ability of the gel to withstand small strains (<3%) was demonstrated in a single HPH cycle under 100 MPa, with satisfactory stability to withstand large-amplitude oscillatory shear in three HPH cycles under 100 MPa. The gels eventually exhibited "soft gel" and shear thinning behaviour with HPH pre-treatment. Jiao et al. [63] reported that HPH can change the gelling time of polysaccharide–PPI mixture gels and induce gel-weakening behaviour in the PPI–xanthan gum mixture when the temperature is increased.

HPH typically reaches 100 MPa [120], whereas HPP can reach pressures ten times higher. Another difference between HPP and HPH is that HPP is governed by the ordering principle [121], whereas HPH perturbs the protein structures [122]. Table 3 lists the applications of high-pressure technology to plant proteins.

Table 3. Examples of HP application on plant proteins.

Sample	Aim of the Study	Processing Conditions	Results	References
Cowpea protein isolate	Gelation properties	400 and 600 MPa for 5 min	HHP-induced gels were less hard and adhesive than heat-induced ones.	[121]
Soybean protein isolate	Rheological properties	40, 60, 80, and 100 MPa, and the cycles 1, 2, and 3	The gels eventually exhibited "soft gel" and shear thinning behavior with HPH pretreatment.	[119]
Sweet potato protein	Gelation Properties	400 MPa 30 min 25 °C	Textural properties of SPP gels were improved by sulfur-containing amino acids and HHP.	[123]
Mung bean, chickpea, pea, lentil, and faba bean proteins	Rheological analyses	600 MPa, 5 min, 5 °C	HPP formed viscoelastic gels ($G' > G''$) for all plant protein samples with comparable gel strength to commercial dairy yogurts.	[124]
Potato protein isolate	Gelation Properties	300–500 MPa	Gel hardness increased with both gelation temperature and pressure, while water-holding capacity was lower for the pressure-induced gels.	[125]
Peanut protein isolate	Rheological characteristics	600 Mpa 2 min	High-pressure homogenisation changed the gelling time.	[63]
Soybean protein isolate	Gelation Properties	500 MPa 20 min	Tofu gel could form with strength at 500 MPa for 20 min.	[116]
Faba bean protein	Functional properties	103 MPa and 207 MPa for 6 cycles	HPH resulted in certain level of protein unfolding with increased surface hydrophobicity.	[126]
Soybean protein isolate	Gelation properties	400–600 MPa	HP and subsequent calcium incorporation to form self-standing cold-set gels.	[127]

3.4.6. Pulsed-Electric-Field-Assisted Process

The pulsed electric field (PEF) is a promising non-thermal food processing technology. It is generally believed that the action mechanism of PEF in food production and processing is mainly based on two theories: electroperforation and electrical breakdown [128]. The electroperforation effect implies that an additional electric potential is induced when food is exposed to an external electric field. The action of the PEF causes ions to move along the direction of the force line by applying an electric field, which leads to the accumulation of ions on the membrane and causes cell polarisation. When the electric field intensity exceeds the critical threshold of the transmembrane potential, it causes electrical breakdown of the cell membrane or viscoelastic deformation [129,130]. Another mechanism is that PEF increases the transmembrane potential and the Joule thermal effect, reducing the energy required for forming hydrophobic pores; less energy can form hydrophilic pores and maintain a more stable cell structure [130,131]. Compared with traditional heat treatment technology, PEF treatment offers lower temperature, shorter time, and less energy loss, maintaining the sensory properties and nutritional value of food to the greatest extent [132].

PEF technology has a wide range of applications such as sterilisation, enzyme destruction, freezing, and extraction of bioactive compounds [133]. In recent years, the application of PEF has focused on the structural modification of proteins. PEF promotes the exposure of active groups (hydrophobic and sulfhydryl groups) within the protein, resulting in changes in protein structure and functional properties. Ashutosh et al. [134] indicated that a higher electric field strength of 3 V/nm significantly affected protein conformation. Sun et al. [135] investigated the effects of PEF treatment on the functional properties of glycosylated WPIs and reported that, compared with the initial WPI, its secondary structure was destroyed, with significant improvements in its solubility and emulsifying properties. Dong et al. [136] studied the effect of PEF at different intensities (8, 18, and 28 kV/cm) on the conformation and gelation properties of myofibrillar proteins (MPs). They found that optimised PEF treatment (18 kV/cm) could induce MPs with a relatively small particle size, contributing to the production of a more homogeneous gel structure. However, the effect of the pulsed electric field on the gel properties of plant proteins was less pronounced.

3.4.7. Cold-Plasma-Assisted Process

Cold plasma is frequently considered as the fourth state in addition to solids, liquids, and gases. Cold plasma comprises UV photons, electrons, ions, neutral atoms, free radicals including OH* and OOH*, reactive oxygen species (such as O_3, O, H_2O_2, and $^1O_2$), and reactive nitrogen species (including NO, NO_2, NO_3, N_2O_3, N_2O_4, and ONOO), which can efficiently deactivate bacteria, yeasts, and moulds by damaging nucleic acids, lipids, and proteins [137]. This treatment process is solvent-free, efficient, and environmentally friendly [138,139]. Furthermore, cold plasma can introduce new oxygen-containing groups (e.g., -COOH, -CO, and -OH) and change the properties of the original groups present on the food surfaces [140]. Cold plasma can change the residue composition of the protein and promote the unfolding of its structure. This was demonstrated by Li [141] in his work on the effect of atmospheric-pressure cold plasma on the solubilisation of peanut protein isolates. Additionally, cold plasma can induce aggregation and cross-linking between protein molecules, promote the formation of disulfide bonds, and improve the protein gel properties. Frías et al. [142] studied the effects of cold plasma on the inactivation of pathogenic microorganisms in tofu and its functional quality. The results demonstrated that cold plasma was effective in controlling microbial growth and created a product with better textural properties and less hardness and springiness than thermally treated commercial tofu. These studies demonstrated that cold plasma is not only a valuable antimicrobial technology but can also be used to modify the surface structure of food and change its physical and chemical properties.

4. Conclusions

The rise in plant-based foods has made heat-irreversible plant-based protein gel materials even more important. The use of plant proteins to replace animal proteins has received considerable attention in the food industry owing to their better sustainability and lower environmental impact. Compared with animal-derived protein gels, such as egg white protein and myofibrillar fibres, plant proteins have poor thermal gelatability, which greatly limits its application in the food industry. Therefore, research is focused on the regulation of plant proteins to improve their gel properties. This study reviewed studies on the regulation of plant protein gel properties based on environmental factors, food components, and emerging processing technologies. Studies have shown that these methods can effectively improve the properties of plant protein gels. Although these studies have achieved considerable results in the food industry, they lack sufficient depth to ensure the repeatability of the experiments and their application in the production process. For the future, the properties of protein gels are closely related to the processing, flavour, texture, nutrition and health properties of food. With the development of the food industry and the increase in health awareness, plant protein gels are playing an irreplaceable role in the new direction of future food, such as animal protein substitutes, low-salt foods, low-sugar foods, low-fat foods, elderly foods, 3D printing, precision nutrition, and so on. It is very important to gain a deeper understanding of gelation mechanisms and regulatory strategies, which is essential for improving the quality of the gel products and developing novel plant-based gel products.

Author Contributions: Y.M.: conceptualisation, formal analysis, investigation, validation, writing the original draft, and visualisation. F.C.: supervision, project administration, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the National Natural Science Foundation of China [grant number U21A20270] and the National Key Research and Development Program of China [grant number 2022YFD2101403].

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data availability is not applicable to this article as no new data were created or analysed in this study.

Conflicts of Interest: The authors declare no conflict of interest.

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