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Soy protein isolates: A review of their composition, aggregation, and gelation

Li Zheng[1] | **Joe M. Regenstein**[2] | **Linyi Zhou**[3] | **Zhongjiang Wang**[1]

[1] College of Food Science, Northeast Agricultural University, Harbin, China

[2] Department of Food Science, Cornell University, Ithaca, New York, USA

[3] School of Food and Health, Beijing Technology and Business University, Beijing, China

Correspondence

Zhongjiang Wang, College of Food Science, Northeast Agricultural University, Harbin, Heilongjiang, 150030, China.
Email: wzjname@neau.edu.cn

Linyi Zhou, School of Food and Health, Beijing Technology and Business University, Beijing, 100000, China.
Email: dorisneau@126.com

Joe M. Regenstein, Department of Food Science, Stocking Hall, Cornell University, Ithaca, NY, 14853-7201, USA.
Email: jmr9@cornell.edu

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Abstract

Considering that a series of complex issues such as environmental problems, sustainable development, animal welfare, and human health are on a global scale, the development of vegetable protein-based meat substitutes provides a potential solution to the disparity between meat consumption demand and supply. The research and development of vegetable protein-based meat substitutes have become a major commercial activity, and the market is expanding to meet the growing consumer demand. Soy protein isolates (SPI) are often used as a raw material for vegetable meat substitutes because of their potential to form fiber structures. Although significant initial success has been achieved, it is still a challenge to explain how the composition and aggregation of SPI influence gel properties and the mechanism(s) involved. This article reviews the latest research about SPI. The relationship between the composition, aggregation, and gelation properties of SPI is based on a through literature search. It focused on the application of SPI in heat- and cold-induced gels, given the diversified market demands. The research on cold gel has helped expand the market. The methods to improve the properties of SPI gels, including physical, chemical, and biological properties, are reviewed to provide insights on its role in the properties of SPI gels. To achieve environmentally friendly and efficient ways for the food industry to use SPI gel properties, the research prospects and development trends of the gel properties of SPI are summarized. New developments and practical applications in the production technology, such as for ultrasound, microwave and high pressure, are reviewed. The potential and challenges for practical applications of cold plasma technology for SPI gel properties are also discussed. There is a need to transfer the laboratory technology to actual food production efficiently and safely.

KEYWORDS

cold gels, gelation technology, glycine max, plant-based products, soy protein isolates, soya beans

1 | INTRODUCTION

Meat has long been an indispensable food resource with its good nutritional profile and a unique and attractive taste. However, the continuous increase in the demand for meat worldwide has led to worries about the supply (T. Y. Zhang, Dou, et al., 2021). Specifically, it is estimated that by 2050, meat consumption will increase by more than 50% (Bonny et al., 2017). As the demand for meat increases, a series of complex ethical and environmental issues related to meat need to be addressed (Kumar et al., 2017; Sha & Xiong, 2020). These issues are increased demand for land and water resources, ecological changes caused by the greenhouse effect, and animal welfare impacts (Clark et al., 2017; J. He et al., 2020; Sanchez-Sabate & Sabate, 2019). Vegetarian foods such as tofu, Chiba tofu, and plant-based meat substitutes have developed as partial solutions to these issues (Xu et al., 2020; L. Zhang, Hu, et al., 2021; L. Zheng, Wang, et al., 2020). Furthermore, the development of vegetarian foods is also a trend that vegetarians, animal welfare advocates, and healthy dieters support. From the livestock industry's perspective, these vegetable protein-based products should not be called "meat" because they do not come from animals. To avoid consumer confusion, the term "plant-based meat substitutes" is used throughout this article. At the same time, the industry experts and scholars working with governmental regulatory agencies need to standardize and harmonize a set of appropriate terms for use with products, so consumers are not confused but are still encouraged to purchase these products.

The composition of vegetarian protein-based meat substitutes may include proteins, lipids, binding (starch or gelling) agents, flavor, and color materials (T. Y. Zhang, Dou, et al., 2021). Among them, protein is usually the most important ingredient because it not only provides certain structural properties but also addresses nutritional needs (Joshi & Kumar, 2016). Studies have shown that replacing meat with high-quality plant proteins in the diet was beneficial to human health (Crimarcoa et al., 2020). Among many plant proteins, soy protein isolate (SPI) has many advantages, which makes it a good choice to replace meat protein. Specifically, SPI provides a good balance of amino acids because it contains all the essential amino acids (Thrane et al., 2017). In addition, SPI has good functional properties and low price (González et al., 2019; Kyriakopoulou et al., 2019; Niu et al., 2017). Therefore, SPI is often used in products such as sausages, ham, tofu, and reconstituted meat (Majzoobi et al., 2017; Rostammiry et al., 2017; L. Zheng, Regenstein, et al., 2020). A few specific examples of real products using SPI in plant-based meat substitutes are summarized in Table 1. Although SPI is economical, most plant-based meat substitute products (such as plant protein hamburgers) made from plants are currently more expensive than animal-based ground meat products. The range of processing and the high dependence on additives and functional ingredients make the cost of plant-based meat substitutes high. This leads to economic challenges for consumers, especially for some vegans. Further technological innovation is needed to reduce production costs while maintaining product quality. Optimized processing conditions, lowering the cost of proteins, the continuous development of economical but functional ingredients, and efficient production methods may improve the viability of such products.

The composition of vegetarian protein-based meat substitutes determines the color, flavor, and texture of the final product. To further understand vegetarian foods, more research on their basic composition is needed. However, there are few review articles about the influence of the composition and aggregation of SPI on gel properties, and the mechanism behind it has not been fully explored. This review aims to fill the gaps in this area. The composition-related information about SPI has been updated, and the relationship between composition and gel properties was systematically analyzed to provide a theory which will also be practically applicable. The influence of the aggregation state of cold- and heat-induced gels on gel properties has been summarized, again including practical applications. Ultrasound, microwave, and high-pressure (HP) methods for improving the gel properties have been updated, and finally, the opportunities and challenges have been discussed.

2 | OVERVIEW OF SPI

Based on the average dry matter, the protein content of soybeans is about 40%, and the oil content is about 20%. SPI is obtained by removing oil at lower temperature (Nishinari et al., 2014; Preece et al., 2017). SPI is the purest form of protein from soybean, with a protein content of more than 90% (Astawan & Prayudani, 2020; Yang & Li, 2020). Due to the high protein content of SPI, it is often used in food processing to improve food quality (Qin et al., 2017), including vegetarian food (X. Wang et al., 2018). In addition, SPI also has unique effects such as lowering blood lipids, cholesterol, and opposing the development of certain cancers (George et al., 2020; Y. Liu et al., 2017; Mercer et al., 2017). Thus, SPI is a high-quality plant protein and one of the few varieties of plant protein that can replace animal protein. SPI has a major role in producing the type of viscoelastic textural characteristics in processed vegetarian foods (e.g., tofu, Chiba tofu, and plant-based meat substitutes meat), which are of really different mouthfeel and chewiness from animal muscle products (e.g., ribs, steaks, and chops). The formation of the sensory texture of processed vegetarian foods largely depends on the gelling ability of SPI.

TABLE 1 Some examples of vegetable protein-based products using soy protein isolates (SPI)

Company	Products	Ingredients according to their website (major plant proteins used)	Website
Morning Star Farms	Burgers (veggie grillers prime® burgers; veggie grillers original burgers; vegan meat lovers burger; veggie lovers vegan burgers)	SPI, wheat gluten, soy flour	https://www.morningstarfarms.com
	Sausage (veggie original sausage patties; veggie sausage, egg and cheese vegetarian breakfast sandwich; veggie breakfast sausage links; veggie hot and spicy sausage patties; veggie maple flavored sausage patties)	SPI, wheat gluten, soy protein concentrate, soy flour	
	Chik'n (veggie BBQ chik'n nuggets, veggie Chik'n nuggets, veggie buffalo chik patties, veggie original chik patties, veggie zesty ranch chik'n nuggets, veggie sweet mustard chik'n nuggets)	SPI, soy protein concentrate, wheat flour, wheat gluten, potato starch, cornstarch, wheat starch	
	Meal starters (veggie chik'n strips, veggie chorizo crumbles™)	SPI, wheat gluten, soy flour	
Gardein	Chick'n and turk'y (ultimate plant-based chick'n tenders, plant-based turk'y roast)	Enriched wheat flour, SPI, wheat flour, vital wheat gluten, pea protein concentrate	https://gardein.com/
	Plant-based jerky (original ultimate plant-based jerky, teriyaki ultimate plant-based jerky, hot and spicy ultimate plant-based jerky)	SPI, vital wheat gluten, pea protein	
Impossible Foods	Impossible™ burger	Soy protein concentrate, 2% or less of: soy leghemoglobin, SPI, potato protein	https://www.impossiblefoods.com/
	Impossible sausage	Soy protein concentrate, 2% or less of: SPI, soy leghemoglobin	
	Impossible pork	Soy protein concentrate, 2% or less of: soy leghemoglobin, SPI	
	Impossible meatballs	Soy protein concentrate, 2% or less of: soy leghemoglobin, SPI, wheat flour, potato protein, hydrolyzed soy protein	
BOCA	Patties (original chik'n veggie patties, spicy chik'n veggie patties)	SPI, wheat flour, soy protein concentrate, yellow corn flour, wheat gluten, hydrolyzed corn protein	https://www.bocaburger.com/
	Chik'n nuggets	Wheat flour, SPI, soy protein concentrate, wheat gluten, yellow corn flour,	
Fry's Family Food	Burger, snacks, mince, sausages, nuggets	Vegetable protein (wheat [gluten], soya)	https://www.fryfamilyfood.com/za/
Rebellyous Foods	Plant-based patties, tenders, nuggets	Textured wheat protein, soy protein concentrate, enriched flour, wheat flour, 2% or less of: yellow corn flour, SPI	https://rebellyous.com/ourofferings/

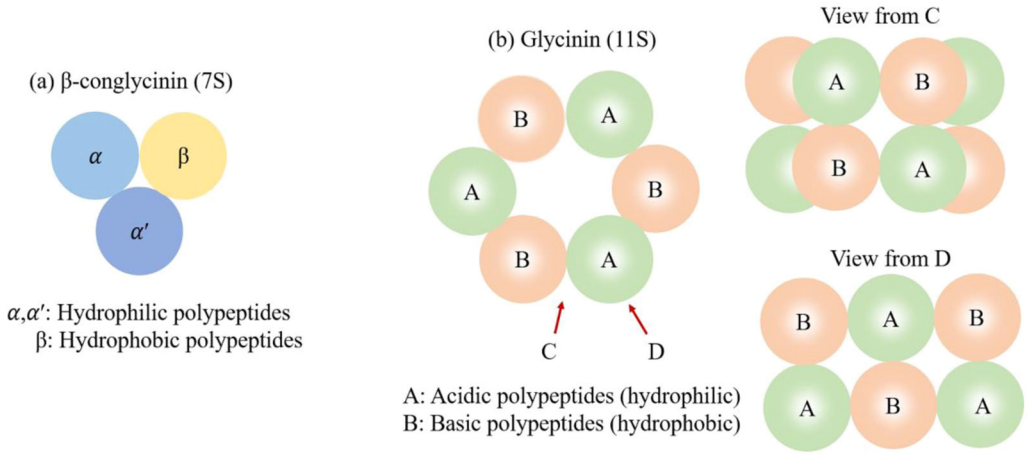

FIGURE 1 Schematic structures of soy protein 7S (a) and 11S (b) globulins. Adapted from Badley et al. (1975) and Sha and Xiong (2020)

Protein gels usually refer to a viscoelastic entity composed of chains or strands that are cross-linked into a continuous network structure and have the ability to immobilize water (Xiong, 2018). Acosta-Domínguez et al. (2021) evaluated the effect of a cryogenic treatment on the physical properties and gel properties of SPI. Compared with SPI, the cryogenically processed SPI has lower bulk, tap, and particle densities. These parameters indicated that there are hollow areas on the modified SPI surface, so the mass of modified SPI occupied a larger volume than SPI. The change in density comes from the size, structural characteristics, and distribution of the particles. When preparing soft, dense products, lower density values may be required. In terms of functional properties, the cryogenically processed SPI showed a higher gel capacity than SPI. In addition, the gelling properties of SPI are influenced by protein composition and aggregation.

2.1 | Composition and gel properties of SPI

SPI is a mixture of various proteins, which are characterized as 2S, 7S, 11S, and 15S according to their sedimentation coefficients, after high-speed centrifugation (Bennetau-Pelissero, 2019). Among them, 7S (β-conglycinin) and 11S (glycinin) are the main components, accounting for ~70% of the total crude protein (Boehm et al., 2018). 7S is a trimeric glycoprotein, which is composed of three subunits α (72 kDa), α' (76 kDa), and β (53 kDa) (Ippoushi et al., 2019). 11S is a hexameric glycoprotein, which is composed of five different subunits, each of which is an acidic (A) polypeptide linked to a specific basic (B) polypeptide through a disulfide bond (Wu et al., 2019). The AB subunit combines into two hexagonal rings forming a hollow cylinder through hydrogen bonding and electrostatic interactions. The structures of 7S and 11S are shown in Figure 1a and b, respectively (Badley et al., 1975).

The subunits, content, and ratio of 7S and 11S in SPI have a significant effect on the texture of a gel. Ramlan et al. (2004) studied the effect of the lack of α and α' subunits on the gelling properties of soy protein. The results showed that with the same experimental conditions, the effect of subunit composition on gel hardness was as follows: lack of α' subunit>7S full subunit>lack of α subunit. From scanning electron microscopy (SEM) observations, it was postulated that when the subunits are missing, the difference in the thickness of the protein component causes the density of the gel network structure to be different. F. Wang, Meng, et al. (2020) showed that based on the presence of the α subunit and 11S, the quality of the tofu can be inferred by using a tofu quality evaluation model. Fukushima (1991) showed that the $A_5A_4B_3$ subunit has a major role in the formation rate and transparency of the SPI gel. The A_3B_4 subunit is closely related to the hardness of the SPI gel, and the lack of the A_3 subunit may increase the hardness of the gel. Poysa et al. (2006) came to the same conclusion that the A_3 subunit of 11S improved the hardness of tofu. Bainy et al. (2008) also showed that the existence of the A_3 subunit hinders the development of gel network structure. The research on subunit-deficient soybean proteins is summarized in Table 2, confirming that different subunit compositions have different effects on gel properties. Because the subunit composition of 11S globulin is more complex than that of 7S globulin, the study of how this affects the mechanism of action of subunits in the gel formation process is more complex.

As early as 1990, Taira (1990) concluded that there was no significant correlation between the hardness of tofu and the ratio of 11S:7S. Other researchers believe that the 11S subunit content and the 11S:7S ratio are positively correlated with the hardness of tofu (S. T. Guo & Ono, 2005;

TABLE 2 The research progress with subunit-deficient soybean protein

Types of missing subunits	Method	Relationship with functional characteristics	References
Lack of α and α' subunits	SDS-PAGE; FT-TR; DSC; SEM	Lack of α' subunit increases gel hardness;the absence of α' subunit has a greater impact on gel hardness than the absence of α subunit	Ramlan et al. (2004)
Lack of α subunit	SDS-PAGE	The lower α subunit contributes to the chewiness of tofu gel products	F. Wang, Meng, et al. (2020)
Lack of A_3 subunit	SDS-PAGE	The lack of the A_3 subunit increases the hardness of the gel; the existence of the A_3 subunit hinders the development of gel network structure	Fukushima (1991); Poysa et al. (2006); Bainy et al. (2008)
Lack of A_5 subunit	Dynamic viscoelastic measurements	Tofu gel with missing A_5 subunit is found firmer	Nishinari et al. (1991)
Lack of A_4 subunit	SDS-PAGE	The removal of the A_4 subunit can further improve the texture hardness and water holding capacity of the tofu gel	James and Yang (2016)

Abbreviations: DSC, differential scanning calorimetry; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; SEM, scanning electron microscopy; FT-TR, fourier transform infrared spectroscopy.

Mujoo et al., 2003; F. Wang, Meng, et al., 2020). Many researchers have tried to correlate the composition with the rheological/textural properties of gels. The discrepancy seems to stem from the different compositions of SPI that affect the gelling ability, not only the 11S and 7S but also the 2S and 15S globulins. Tay et al. (2006) investigated the relationship between 2S and gel properties and compared gelation with the corresponding behavior of 7S and 11S. The results showed that in the initial stage of structure formation, 2S did better than 7S, and 11S showed the highest aggregation rate. However, over time, 7S produced a network that was stronger and had more water holding capacity (WHC) than 2S. The low molecular weight of the 2S chain was now a disadvantage with respect to the gel properties. The 15S fraction is a minor component (1%−11%) of soy protein. Many properties of 15S are similar to 11S, such as solubility and dissociation. Recent research shows that 15S is a dimer of 11S (Ni et al., 2021). Although there have been some inconsistencies among the reports mentioned above, the general consensus seems to be that the content of 7S and 11S is closely related to gel quality (F. Wang, Meng, et al., 2020).

Specifically, the hardness of gels is higher with increasing 11S content, while the springiness of gels is higher with increasing 7S content (Q. Zhang et al., 2018). The firmness/hardness of gels prepared using gluconate-delta-lactone (GDL) increases with an increasing ratio of 11S:7S (S. T. Guo & Ono, 2005; Onodera et al., 2009). Thus, after gel formation is completed, the gel hardness (strength) with 11S is stronger than that of 7S. In addition to the composition of SPI, in general, the aggregation state is also an important factor affecting gel strength.

2.2 | Aggregation and gel properties of SPI

Due to the complexity of soy protein, the gelation process depends on many factors that has challenged researchers. For globulins from different sources, the aggregation rules are similar to a certain extent. Most of the in-depth studies on the aggregation mechanism of soy protein components have been done using neutral or weak alkaline conditions and low protein concentrations.

Nakamura et al. (1984) proposed a gelation mechanism for 11S: when heated for a short period of time (15 s), six protein molecules form short chains, and each chain is unfolded but remains spherical (chain I). Chain I combines with itself to form a longer straight chain (chain II). Chain II combines with itself and/or with chain I to form branched and unbranched chains (chain III). Then, chain III forms a gel network structure. The whole process is shown schematically in Figure 2. Using differential scanning calorimetry (DSC) analysis, the denaturation temperature of 7S is ~75°C, and the denaturation temperature of 11S is ~90°C (Niu et al., 2018; Renkema et al., 2000). To clarify the role of 7S in the thermal aggregation of soy proteins, the structures of soluble and insoluble aggregates of 11S and 7S were studied using small angle X-ray scattering and dynamic light scattering (S. T. Guo & Ono, 2005; Jian et al., 2012). The results showed that the soluble aggregates of 7S have a limited size and a less compact conformation, while the insoluble aggregates of 11S have a denser core and less dense outer shell. The increase in the size of insoluble aggregates of 11S was terminated by 7S globulin, possibly due to the interaction of 7S globulin with the most insoluble basic peptide of the 11S globulin, which increased

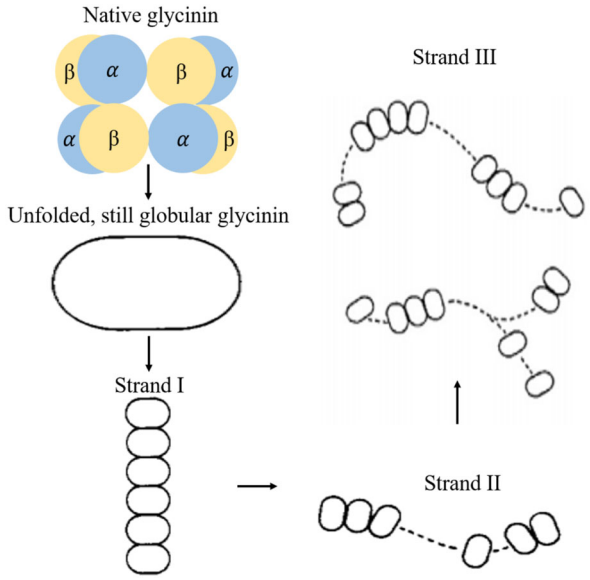

FIGURE 2 Formation of soluble aggregates in the course of gelation of glycinin (11S). Adapted from Nakamura et al. (1984)

its solubility (J. Guo et al., 2012). X. T. He et al. (2016) further showed that after heating, the β subunit of 7S forms a soluble aggregate with 11S, and in the heat treatment process, the α and α' subunits showed a greater ability to inhibit the thermal aggregation of basic peptides than the β subunit. The further study of the relationship between specific subunits of soy proteins and functional properties may still be beneficial.

The structure of protein aggregates affects the characteristics of the gel. The concentration of protein, pH, ionic strength, heat treatment conditions, and so forth all affect the structure of protein aggregates, which also have a significant impact on the properties of protein gels (N. Chen et al., 2018; N. Chen, Zhao, Chassenieux, et al., 2017; N. Chen, Zhao, Niepceron, et al., 2017). N. Chen et al. (2016) heated SPI in an aqueous solution at neutral pH to form flexible aggregates. Protein thermal aggregation and gelation rate were affected by temperature. After the temperature reaches the protein thermal denaturation temperature, within the temperature range studied (50−95°C), protein aggregation and gelation rate continued to increase with temperature. At a particular temperature, as the protein concentration increases, the aggregation rate increases sharply, and the time for gel formation is continuously shortened. Gels are formed at concentrations as low as 50 g/L and temperatures as low as 50°C. H. B. Zhao et al. (2016) studied the effects of different degrees of denaturation and aggregate sizes on the formation of gels induced by $CaSO_4$. They found that the hardness and WHC of the gel were positively correlated with the size of the aggregates and the degree of denaturation of 11S. At low pH, when the protein denaturation temperature is reached, higher concentrations of protein, especially proteins that easily form soluble aggregates, the proteins are prone to aggregation which improves their gel properties. These processing conditions can be used to produce soy proteins with high gelling ability that can be added to vegetarian products such as tofu, Chiba tofu, and plant-based meat substitutes meat to improve the elasticity and taste of the product, thereby improving their overall quality.

The interaction mechanism of 7S and 11S in the gel formation process can be summarized as follows: The soy protein solution is heated to dissociate the protein molecules into α, α', β, A, and B subunits. When the protein solution is further heated, the dissociated subunits interact with each other to form a soluble peptide chain. At the appropriate protein concentration, the β subunit interacts with the basic subunit B and precipitates. The oligomers formed by the interaction of α, and α' subunit and acidic subunit A are concentrated in the supernatant. The contribution of 7S and 11S to the formation of the gel network is different (Chao et al., 2017). They showed that the 7S heat-induced gel is more likely to break than the 11S heat-induced gel when the pH value and protein content were set. This may be related to the different molecular structures of the heat-induced gel (Renkema et al., 2001). With heating and coagulant, electrostatic interactions and disulfide bonds help form a stable 11S gel as a stable three-dimensional network structure. However, with the same conditions, the formation of 7S gel occurs using only hydrogen bonding (Utsumi et al., 1997). Therefore, the textural properties of heat-induced 11S gel are better than 7S gel.

The differences seem to arise from two factors that affect the gelling ability of SPI. The first factor is the composition and aggregation of SPI, which includes the other components (such as fat, phenolic compounds, and phytic acid). The other is the method of gel formation, including heat- and cold-induced gels. Once the composition and aggregation characteristics of the SPI are selected and determined to be suitable for making a high quality gel, process conditions remain to be considered and optimized.

3 | APPLICATIONS OF SPI GELS

In general, gelation of SPI is induced by the unfolding and subsequent aggregation of the proteins. Proteins can form various types of gel structures to provide different applications in food. Thus, the protein gels are divided into different categories according to different classification standards used in actual production. The first classification relies on the nature of the protein aggregates and is divided into transparent and opaque gels. Gels formed from filamentous aggregates are transparent because the

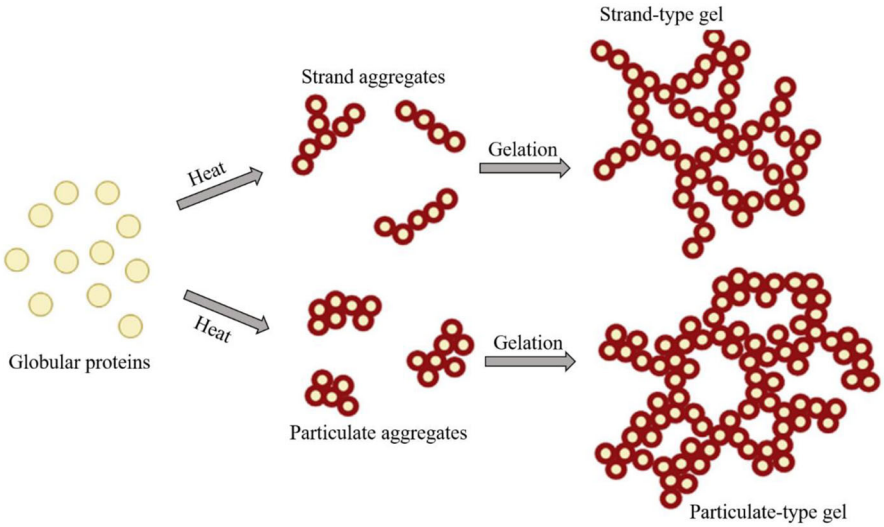

FIGURE 3 Strand and particulate gels formed from globular proteins. Adapted from Peng et al. (2016)

width of the filaments is too small for them to scatter light strongly, while gels formed from aggregates of particles are opaque because the larger size of the particles can strongly scatter light (Bryant & Mcclements, 1998). The study by Tani et al. (1995) also pointed out that the gels formed by fibrous proteins (e.g., gelatin) are transparent, where the fiber chains interact with each other through the "crystallization" or "point contact" connection regions; however, the gels formed by most globular proteins (e.g., soy protein) are usually opaque or translucent. Typical globular protein gels, according to different gelation conditions, such as protein concentration, pH, ionic strength, and heating program, show strands or particulate characteristics or both. As shown in Figure 3, the differences between the particulate and strand type gels are depicted (Peng et al., 2016). A network of heterogeneous particles or mixed type gels containing a large number of voids determines many important texture qualities of soy milk gel foods, such as viscosity, hardness, cohesiveness, and WHC (Cruz et al., 2009).

Another way to classify gels is by their thermal stability. They can be divided into thermoreversible gels and nonthermoreversible gels (Lopes da Silva & Rao, 2007). Among them, carrageenan and agar form a thermoreversible gel, while alginate forms an ionic nonthermoreversible gel. To explain the temperature dependence of the elasticity of thermoreversible gels, Nishinari et al. (1985) proposed a simple model. Thermoreversible gels are assumed to consist of two regions: a somewhat crystalline region composed of connecting regions and an amorphous region composed of long flexible chains. This is the consensus for carrageenan gels and other polysaccharide gels and polyvinyl alcohol gels.

The third classification scheme depends on the tissue state of protein gels, and these gels are divided into hydrogels and aerogels. Hydrogels are a three-dimensional solid network made of physically or chemically cross-linked hydrophilic polymer structures. Large amounts of water or other biological fluids can be entangled in this network (Abaee et al., 2017). Hydrogels can be formulated into different physical forms, such as coatings, films, microparticles, and nanoparticles. Aerogels are a light and porous solid material derived from gels and can be used for catalysis (Lefatle & John, 2018). A number of studies have shown that protein-based aerogels are becoming a promising sustainable biomaterial in the fields of medicine and biological sciences (Ahmadi et al., 2016; Fitzpatrick et al., 2018; Kleemann et al., 2019).

Finally, according to the process conditions for protein gel formation, they are divided into heat- and cold-induced gels. Heat-induced gelation is an important property for the preparation of soy products (Puppo & Aón, 1998). In recent years, the heat-induced gels of SPI have been intensively studied and are used in the food industry (N. Chen et al., 2016; D. Q. Lin et al., 2017; Renkema et al., 2002). The formation conditions for the cold-induced gel of SPI are relatively mild, which provides a pathway for the development of new foods (C. B. Zhao et al., 2020). These will be discussed in the next two sections.

3.1 | Heat-induced gels of SPI

SPI can be induced to form hydrogels using a variety of methods. Thermo gels have been the most studied (Y. Guo et al., 2020). Generally, the preparation of globular protein gels consists of three different steps: denaturation, aggregation, and gelation. In a typical thermal gel, these processes can occur simultaneously during the heat treatment

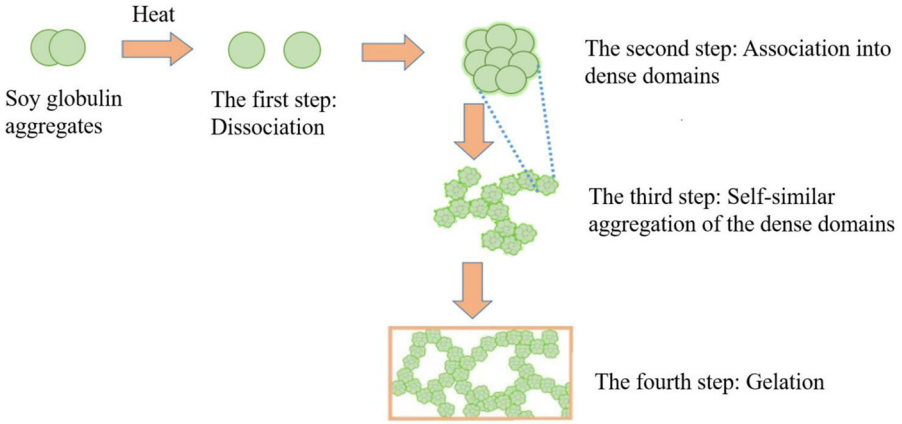

FIGURE 4 The four steps leading to gelation of soy protein isolates (SPI) in aqueous solutions during heating. Adapted from Nicolai and Chassenieux (2019)

(Vilela et al., 2011). Heat denaturation of soy protein is considered to be a prerequisite and necessary step for gel formation. Heating at a temperature higher than 65°C can form a three-dimensional network structure (Chao et al., 2017; Y. Guo et al., 2020). Nicolai and Chassenieux (2019) reviewed the recent studies of SPI gels and divided the gelation of SPI into four steps as shown in Figure 4. In the first step, small aggregates of natural globulin are dissociated during heating. In the second step, the proteins recombine into dense, approximately spherical particles with a radius between 30 and 50 nm. In the third step, these particles randomly stick together to form self-similar aggregates. The bonds between the proteins in these aggregates are strong enough to resist cooling and dilution. In the fourth step, the aggregates percolate and form a system spanning network.

Chemical, physical, and enzymatic treatments have been used to modify SPI to improve nutrition and specific functional properties. When SPI is denatured by heat treatment, the groups and side chains gradually expand, and the active site is exposed, which triggers the formation of macromolecular aggregates through the interaction between covalent (disulfide bonds) and noncovalent bonds (such as hydrogen bonds and electrostatic and hydrophobic interactions) between proteins. When the concentration of soy protein is high enough, a gel with a three-dimensional network is formed (Wan et al., 2021; J. Wang, Na, et al., 2020; W. Wang et al., 2021). Chien et al. (2014) prepared soy protein gels with different weight percentages (15, 18, and 20 wt%) along with heat treatments. The gel system can adjust the properties of the gel by changing the concentration of soy protein. The increase in protein concentration makes the structure of the gel smoother, more viscous, and less granular. Kangii et al. (1991) found that a firm, tough, and unfracturable soy protein gel was formed with higher heating temperatures and protein concentration. The elasticity of the gel was similar at all protein concentrations, but it was lower when heated at a higher temperatures. To form a rigid gel, it must be heated to >93°C. This may be related to the specific SPI and experimental conditions, so this work needs to be repeated with other samples of SPI. In addition, adding soy protein (1, 3, and 6 g/100 g) to an inulin gel resulted in a denser and more uniform gel network, with higher yield stress, texture (adhesiveness and hardness), and spread ability parameters (Florowska et al., 2020).

Some studies have also focused on the effects of ionic strength, salt, and pH on soy protein gels. As early as 1994, Nagano et al. (1994) pointed out that the 7S denaturation peak temperature detected in heated DSC shifts to a lower temperature as the pH value decreases. This was consistent with Renkema et al. (2002) who showed that in the presence of 0.2 M NaCl, the storage modulus of 12 wt% SPI increased with decreasing pH. Furthermore, the 7S denaturation peak temperature of SPI (using DSC) also moved to a lower temperature as the pH decreased. Wongprecha et al. (2000) found that the endothermic peak of DSC accompanying 11S denaturation shifted to a higher temperature as the concentration of added NaCl increased. Lakemond et al. (2003) using DSC showed that the denaturation temperature of 11S shifted to a higher temperature as the ionic strength increased. The rheological properties of heat-induced soy protein gels are also affected by the ratio of 7S/11S. Compared with gels rich in 7S, gels rich in 11S had a higher storage modulus. The reason that 11S produces a better gel than 7S is believed to be due to the difference in network structure and the strength of the interaction between the protein molecules (Renkema et al., 2001). Furthermore, Chao et al. (2017) studied the relationship between the rheological properties of heat-induced soy protein gels and the ratio of network proteins in the gel and the size of the protein aggregates in solution. The

results showed that the composition of non-network proteins includes undenatured 11S AB subunits, high levels of A and A_3 polypeptides, and low levels of 7S α and α' subunits. There was a positive correlation between the storage modulus of the gels formed at different temperatures and the network protein ratio. The higher the 11S/7S ratio the higher the storage modulus (G′) of the soy protein gel, which may be due to the formation of larger and tighter aggregates by the B peptide through hydrophobic bonds.

Therefore, the heat-induced gelation behavior of soy proteins can be changed by adjusting the heating temperature, heating time, ionic strength, pH, and protein concentration or the composition. As long as any one of these influencing factors changes, the gel properties of SPI will change. Thus, there is still a need for systematic basic research to better understand the role of each of these factors for effective prediction and control of these effects in actual production.

3.2 | Cold-induced gels of SPI

Hongsprabhas and Barbut (1997) reported the formation of cold-induced gels. Compared with SPI heat-induced gel, the preparation conditions for cold-induced gel are relatively mild. They can be made at low protein concentrations and low temperatures by adding additives such as salt, acid, and/or enzymes (Wan et al., 2021). In addition to having a structural support role in food systems, cold-induced gels also make up for the shortcomings of soy protein heat-induced gels in heat sensitive applications that harmed with a high temperature treatment. These have found good application prospects in the fields of food and medicine (Maltais et al., 2010). The preparation of soy protein cold-induced gels is more complicated, and their formation is usually divided into two steps. The first step is to prepare a heat denatured protein solution. Specifically, the protein solution should be heated to above the denaturation temperature to expose the protein molecules to reactive functional groups, and to avoid the formation of a thermal gel. The protein concentration of the system needs to be below the critical gel concentration. At the same time, the ionic strength must also be low enough; otherwise, protein aggregation may occur. The second step is mainly inducing cold-setting. Specifically, salt is added after cooling to reduce electrostatic repulsions and ultimately to promote gel formation (Bryant & Mcclements, 1998; Marangoni et al., 2000). The soy protein cold-induced gelation procedure is shown in Figure 5.

It is worth pointing out that gelation is a phenomenon; therefore, the definition and measurement of gelation depend on the observer's perspective and the technique(s) used to evaluate it. In some early studies (Hongsprabhas & Barbut, 1997; Ju & Kilara, 1998a; McClements & Keogh, 1995), the researchers suggested that the preheating (80°C, 30 min) of proteins is a necessary step in salt or acid-induced cold gelation. The reason is that during heating some denaturation and polymerization of proteins occurs, and the longer heating times (up to 30 min) also increased the hardness of the cold-set gels (Ju & Kilara, 1998b). Thus, when a gel is classified as a cold-set gel, heat treatment is allowed in its manufacturing process prior to gel setting. The results of Maltais et al. (2005) showed that thermally denatured SPI dispersions at 6%−9%, that is below the critical gelation concentration, can form a gel at room temperature by adding calcium chloride. According to the changes in the concentration of protein (6%, 7%, 8%, and 9%) and $CaCl_2$ (10, 15, and 20 mM), a variety of gels with different opacity, WHC, and rheological properties can be obtained. Increasing the concentration of $CaCl_2$ from 10 to 20 mM caused the gel opacity to increase, while increasing the protein concentration from 6% to 9% decreased its opacity. WHC is improved with the increase of protein concentration and the decrease of $CaCl_2$, and the elastic modulus is increased with the increase of protein and calcium chloride. This study represents the starting point for the development of soy protein cold-induced gels. The formation of cold-induced gels leads to the possibility of using food proteins as carriers of sensitive nutritional compounds and the development of innovative functional food ingredients (L. Chen et al., 2006). Maltais et al. (2009) studied the release of riboflavin from SPI gels with filamentous or particulate microstructures using gastric and intestinal conditions. They concluded that the calcium-induced cold-set soybean gel can be used as a carrier to capture biologically active molecules for transmission and absorption in the intestinal tract.

Besides salt additives, acid additives can also be used. GDL is an acid additive and has been used for soybean cold-induced gels. Studies have explored the effects of protein components, the amount of GDL added, and gelation conditions (time, temperature, additives, etc.) on the quality of acid-induced soy protein gels. The results showed that compared with other gels, the gel with a higher ratio of 11S to 7S had higher cohesiveness, gumminess, hardness, and *L* values (Tay & Perera, 2010). Schuldt et al. (2014) found that the addition of NaCl would delay the formation of acid-induced SPI gel, and the linear viscoelastic region of the gel decreased with the increase of NaCl. The presence of NaCl shielded electrostatic interactions and enhanced protein solubility resulting in lower gel stiffness. Based on the mechanism proposed for GDL gelation, it suggested that some other characteristics of soy protein, especially the charge state, have an important role in the acid-induced gelation process. Wan et al. (2021) preheated different concentrations of SPI to produce soluble

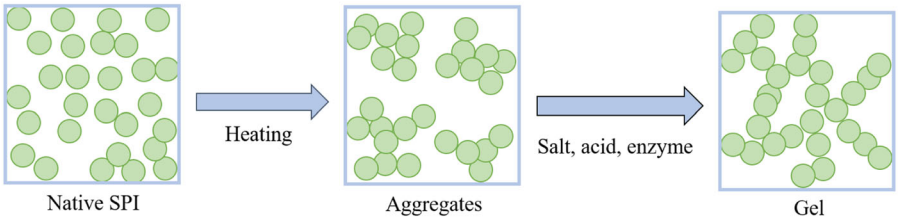

FIGURE 5 Soy protein isolates (SPI) cold-induced gel formation procedure. Adapted from Marangoni et al. (2000)

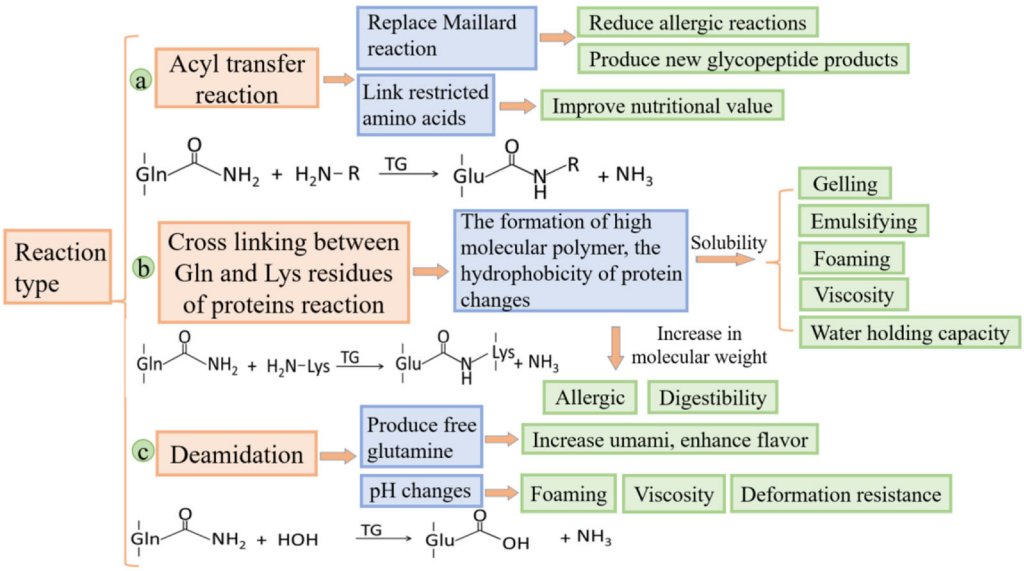

FIGURE 6 Transglutaminase (TGase)-catalyzed food protein modification reaction and its mechanism

SPI aggregates with different particle sizes and zeta potentials. In addition, these soluble SPI aggregates were used to prepare acid-induced gels, and the gelation process and gel properties were compared. The results showed that the soluble SPI aggregates with more surface charges formed acid-induced gels with finer networks. Soluble SPI aggregates can be adjusted to influence the quality of cold-set SPI gel.

Enzyme additives are also used in the preparation of cold-induced gels. Transglutaminase (TGase) can catalyze the acyl transfer reaction between the γ-hydroxylamine group of glutamine residues (Gln) and the ε-amino group of lysine residues (Lys), resulting in inter- or intramolecular cross-links (M. Zhang et al., 2020). TGase can catalyze three reactions: the acyl transfer reaction, the cross-linking reaction of Gln and Lys residues, and the deamidation reaction (Zink et al., 2016; Warisul & Khare, 2018). TGase has been used to improve the gelation and textural properties of soy protein-based products (Qin et al., 2016a). The TGase-catalyzed food protein modification reaction and its mechanism are shown in Figure 6. In soy products, 7S was more easily catalyzed and polymerized by enzymes, while 11S was not easily polymerized and can only polymerize acidic subunits in the protein, with limited polymerization of basic subunits (Zang et al., 2019). This is mainly because 7S contains a relatively high content of Gln and Lys. Some studies have found that the formation of SPI gels depends on the balance of inter- and intramolecular forces. TGase can induce the formation of intermolecular disulfide bonds during the cross-linking process of SPI, reducing the content of free sulfhydryl groups, thereby significantly improving the rheology of the SPI gels and resulting in a denser and more uniform three-dimensional gel network (Qin et al., 2017, 2016a). TGase cross-linked SPI also helps tofu form a longer-lasting gel and can preserve the structural properties of tofu for a longer period of time (M. K. Zhang et al., 2019). Furthermore, Xing's research team discovered during the process of developing "biological tofu" using soy protein/milk protein as raw materials that the addition of TGase can effectively control energy intake and reduce protein allergenicity (G. L. Xing et al., 2020; G. Xing et al., 2019). In general, the addition of TGase can protect the Lys in SPI from damage by chemical reactions, and the enzymatic reaction conditions are

mild, which helps to improve the springiness and WHC of protein gels. Compared with other additives, TGase is safer and may be more economical. The current applications research with TGase mainly focuses on improving the functional properties of proteins, and the research on nutritional value, flavor release, and adsorption of other compounds should be expanded.

4 | CURRENT METHODS TO IMPROVE GEL PROPERTIES OF SPI

As mentioned in Section 3.1, the gel properties of SPI could be improved using physical, chemical, and biological methods. Specifically, these can be divided into two categories, one is the internal factors, that is, the raw materials themselves (the genes and their composition), and the other is the environmental factors, which involve the application of different conditions (temperature, time, pH, acid, salt, enzyme, etc.) and technology (ultrasound, microwave, high pressure, and cold plasma). In general, the internal factors and process conditions have been reviewed and summarized above, so this part focuses on the application of technology to improve the gel properties of soy protein-based products.

4.1 | Application of ultrasound

Ultrasound can be divided into high-intensity (power 10–1000 W cm^{-2}, frequency 16–100 kHz) and low-intensity (power <1 W cm^{-2}, frequency > 100 kHz,) (Soria & Villamiel, 2010). High-intensity ultrasound (HIU) is considered a green and cost-effective technology, which is used in the food industry for food modification (T. Zheng et al., 2019; P. Zhang et al., 2016). Z. Zhang et al. (2017) pointed out that the impact of ultrasound on the liquid system is mainly due to cavitation and micro-streaming currents, which makes the liquid rapidly form bubbles and rupture, then high-shear energy waves and turbulence are generated. Cavitation and high-shear energy waves can promote the unfolding and partial denaturation of protein molecules, exposing the surface-active sites of the protein, thereby enhancing the heat-induced aggregation and gelation behavior of soybean protein with subsequent processing (Y. Wang et al., 2017).

Some studies (Hu et al., 2013; H. F. Lin et al., 2016; Zhou et al., 2016) have shown that HIU can beneficially change the structure and functional properties of food proteins. Hu's research team showed that with HIU at 20 kHz and 400 W for 20 min, the WHC, gel strength, and gel hardness of the GDL-induced SPI gel were improved, and the gel protein network structure was more compact (Hu et al., 2013). In addition, they also showed that in the cold-induced gel formed with TGase, the pretreatment of SPI with HIU significantly improved the gel strength, WHC, and gel yield. HIU caused the microstructure of the gel to be more uniform and denser, probably because the conformation changed and more non-covalent interactions between molecules may be formed (P. Zhang et al., 2016). Furthermore, Hu's team systematically studied the effects of HIU on the structure, and physical and chemical properties of SPI produced by different denaturation methods (natural SPI, alcohol denatured SPI, and heat and moisture denatured SPI). The results showed that heat-moisture denatured SPI and its cold gel showed higher sulfhydryl content, a denser gel and higher WHC than other SPI aggregates. For natural SPI, a longer period of HIU increased the sulfhydryl content, gel strength, and WHC (T. Zheng et al., 2019). HIU could be used to improve the physical, chemical, and functional properties of SPI. However, the physical and chemical properties of SPI changed according to the denaturation method or HIU time.

4.2 | Application of microwaves

Microwaves (frequency 300 MHz–300 GHz) are part of the electromagnetic spectrum and are considered a rapid, clean, convenient, and cost-effective method (Na et al., 2016; Qin et al., 2016b). The dielectric properties of food materials are the main factors affecting the interaction between microwaves and food. Ahmed et al. (2008) investigated the dielectric properties of SPI in the denaturation temperature range (20–90°C), pH (4.5, 6.6, and 10), and at different concentrations (5, 10, and 15 g/100 g water). Their results showed that the dielectric constant of SPI decreases with increased temperature (except at 90°C) and frequency, and increased with concentration. The association/dissociation behavior of the SPI dispersion changed due to the electrostatic attraction/repulsion between protein molecules when heated, which is believed to be the reason for the significant increase in dielectric parameters. In addition, microwave radiation can break bonds such as disulfide bonds and hydrogen bonds, resulting in the unfolding of protein structures (Banik et al., 2003). Microwave treatments have been used to improve the gel properties of proteins, including improving gel strength, increasing WHC, and reducing gel cooking loss. For example, H.-H. Liu and Kuo (2010) found that compared with traditional heating, microwave heating gives SPI a have higher viscoelasticity and a denser microstructure. The best microwave conditions are needed in the industrial optimization of gel products where the whiteness and strength of the gel are important. Qin et al. (2016b) used

microwaves at different powers to process a SPI–wheat gluten mixture and found that with increased microwave power, the gel strength, WHC, and storage modulus of the system gradually increased.

After microwave treatment of protein, its molecular spatial conformation has changed, which brings about changes in the physical and chemical properties and gel properties. Its effect depends on the type and concentration of protein, microwave power, microwave time, the interaction between components, and the pH of the system. After clarifying the influence of microwave on the structure and properties of food proteins, mathematical models of the relationship of microwaves on protein structure should be determined. The interaction between the gel system and other food ingredients with microwaving also needs additional work.

4.3 | Application of high pressure

HP treatment can cause protein denaturation and different degrees of aggregation or gelation, resulting in changes in textural properties in the expected direction, and the shelf life may be prolonged (Li et al., 2020). Tattiyakul and Rao (2016) have shown that HP technology can be used to replace long-term heat treatments in food processing and is considered a safe physically based technology. Thus, HP is widely used with food gel systems. H. Zhang et al. (2009) studied the characteristics of tofu gels made using HP. The mechanism of HP-induced gelatinization of tofu was also discussed. The results showed that HP (500 MPa, 20 min) could result in a strong tofu gel with a cross-linked network structure. These gels had a smooth appearance, less off-flavors, and a more uniform texture. The gelation of tofu may be mainly caused by the denaturation of soy proteins with HP, and the coagulant also promoted coagulation. HP treatment exposes the hydrophobic area of the native protein molecule to the solvent. Because the denatured soy protein is negatively charged, the hydrogen ions generated by the hydrolysis of GDL added to the system neutralized the net charge of the protein. Therefore, the hydrophobic interaction of the neutralized soy protein became more pronounced and induced aggregation leading to a cross-linked network. Zuo et al. (2016) studied the effect of HP heating on the yield and textural properties of tofu gels. The results showed that compared with the traditional tofu made by heating soy milk at 97°C with atmospheric pressure, the tofu made by heating with higher pressure and temperature (0.17 MPa, 115°C) had higher yields and significantly improved texture characteristics, including chewiness, springiness, and hardness. This may be due to the increased protein particle content of soy milk with high pressure and high temperature, thus forming a dense tofu network, which could retain more water and solids, thereby improving texture characteristics.

The effect of HP processing on the physical properties of SPI has also been studied. Guettler et al. (2013) analyzed the surface energy of autoclaved and potassium permanganate-treated raw soy materials (i.e., soy flour, SPI, soy hulls, and insoluble soy). The results showed that based on the polar surface energy, the most suitable soy materials compatible with hydrophobic polymer materials seemed to be SPI, soy hulls, and insoluble soy treated with potassium permanganate. Autoclaved SPI (23.6 mN/m) has the highest polar surface energy. The increased hydrophilicity of SPI can be explained by the disappearance of the hydrophobic region of the protein caused by heat treatment and protein aggregation. They also showed that the polar surface energy characteristics can be used as an indicator of the effect of potassium permanganate and autoclave treatments on soy materials.

Some researchers have studied the effects of HP treatments on different fractions (7S and 11S). Speroni and Anon (2013) studied the ability of SPI, 7S, and 11S to form a cold-induced gel using HP (400−600 MPa) and incorporating calcium. The results indicated that SPI formed a better gel with a higher WHC, 7S formed an aggregated gel with lower WHC, and 11S did not form a self-standing gel. The 7S retained ~30% of its natural structure after a 600 MPa treatment, and 11S was 100% denatured after a 400 MPa treatment, which is consistent with Puppo et al. (2004). However, H. Zhang et al. (2005) studied the effect of HP treatments on the protein in soy milk. They showed that 7S and 11S denatured at HP of 300 and 400 MPa, respectively. The conflicting conclusions may be related to how extensively the protein systems were purified. More work with SPI and HP is likely to be beneficial.

4.4 | Application of cold plasma

The non-thermal physical technologies that have been used for protein modification include cold plasma technology. Cold plasma technology was mostly used initially in the electronics industry to improve the surface coating performance of polymer materials. With the increased interest in non-thermal food processing, cold plasma technology applications have increased (Ekezie et al., 2017). Cold plasma is a type of plasma that can be produced close to room temperature (~30−60°C), and it is still an emerging technology (Coutinho et al., 2018). Bormashenko et al. (2021) studied the modifications of the surface characteristics of SPI using a cold plasma treatment, and the results showed that cold plasma treatment significantly reduced the apparent contact angle of the powder, indicating that

the powder became more hydrophilic. This may be due to an increase in plasma-induced surface oxidation. Cold plasma treatments can improve the wettability of SPI powders and improve the long-term stability, particularly for food suspensions without stabilizers.

The use of cold plasma technology also has a positive impact on the physical and functional properties of gel products. Frías et al. (2020) studied the effect of using cold atmospheric pressure plasma to treat sterile deionized water as a soaking solution for tofu gel products. The results showed that the tofu products had better texture characteristics and higher amounts of phenolic compounds. At the same time, the growth of microorganisms was effectively controlled, and the shelf life of tofu products was extended. Meinlschmidt et al. (2016) further showed that cold plasma treatment can effectively reduce the sensitization of SPI. These studies showed the potential benefits of cold plasma technology but additional factors such as analysis of the economics and equipment design are needed before industrial implementation.

5 | CONCLUSIONS AND FUTURE RESEARCH NEEDS

The population of world is expected to increase to 11.2 billion people by 2100 (Dupont & Fiebelkorn, 2020). According to the forecast of the OECD/FAO (2019), the total global meat production will increase to 364 million metric tons by 2028. In the United States alone, the per capita consumption of red meat and poultry in 2018 was close to a record at ~100 kg, and China's meat production also reached a record high, exceeding 45.4 billion kg according to data from the USDA (2019). The imbalance between human–animal–environmental supply and demand involves many important issues, including land use, fresh water consumption, greenhouse gas emissions, and animal welfare. It is increasingly recognized that the current methods of industrialized animal husbandry are unsustainable, and that cereal- and legume-based protein products can provide traditional meat consumers and vegetarians with valuable options. The development of vegetable protein-based products is sustainable, humane, and safe. The continued growth and demand of the fast-growing vegetable protein-based products market will provide ample opportunities for entrepreneurs and food scientists to further explore new innovations. There is a lot being written about plant-based foods including the history of its development, technology, opportunities, and challenges. People are now paying attention to their nutritional profile. Consumers are becoming aware that such foods are highly processed and not necessarily more healthful than meat. Because of the large amount of processing, plant-based meat substitutes will lose some naturally occurring nutrients. Plant-based meat substitutes may also contain more salt than meat products, which is challenging for low-salt diets and for promoting health. Preprocessing to inactivate certain anti–nutritional factors in soy-based materials and the use of some sodium chloride substitutes are possible solutions, which can be a bit more complicated. More work is needed to develop more in-depth details and explanations to allow for the better understanding of the potential of plant-based protein products. The emergence and development of plant-based foods has provided consumers whose center of the place eating was focused on animal-based foods with more choices, but this does not mean that consumers will only eat plant-based foods and abandon animal-based foods. The key to a balanced use of both types of food depends on the conditions in different countries and regions.

The rise of plant-based simulated meat has made thermally irreversible plant-based gelatinous materials a more important ingredient. SPI has good nutritional value, emulsification, filling, and cohesive functions. It is not only used as a raw material for textured protein in plant-based simulated meat but also can be used as a filler, binder, and gelling agent. Compared with animal-derived gel proteins such as egg white protein and myofibrils, its thermal gelation properties are not as good. Therefore, modifying SPI to improve its gel properties has become an important research focus. Although considerable success has been achieved in the research on internal factors (i.e., genes and composition) and procedural factors (protein concentration, coagulant type, temperature, and time) that affect the gel properties of SPI, these studies lack sufficient depth to guarantee the repeatability of experiments and its use in production processes. Therefore, there is an urgent need to further study SPI.

Further work with SPI and ultrasound, microwave, and HP including comparative work between these methods is needed along with work to clarify the interaction between the main proteins, 11S, and 7S as it is not economical to use each separately in most food products. It is also necessary to better understand the interaction with coagulants. All these studies then need to be applied to reduce the cost of production and improve product attributes to contribute to feeding the growing global population.

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AUTHOR CONTRIBUTIONS

Li Zheng wrote the original manuscript and designed the table and figures. Joe M. Regenstein completed proofreading and editing of the manuscript. Linyi Zhou provided suggestions and collected relevant papers. Zhongjing Wang obtained the funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Joe M. Regenstein https://orcid.org/0000-0002-2656-9815
Zhongjiang Wang https://orcid.org/0000-0001-7433-1871

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