Research progress in soybean lipophilic protein (LP): Extraction, structural, techno-functional properties, and high-performance food applications

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Abstract

Background

Soybean lipophilic protein (LP), a vital component of soybean protein isolates (SPI), is distinguished by its high lipid content, particularly in phospholipids, making it a promising functional nanomaterial for efficient transport of nutrients and drugs. Although plenty of research has focused on SPI's additional storage proteins, including glycinin (11S) and *β*-conglycinin (7S). A better understanding of LP's structure and techno-functional properties is also crucial for SPI's comprehensive applications.

Scope and approach

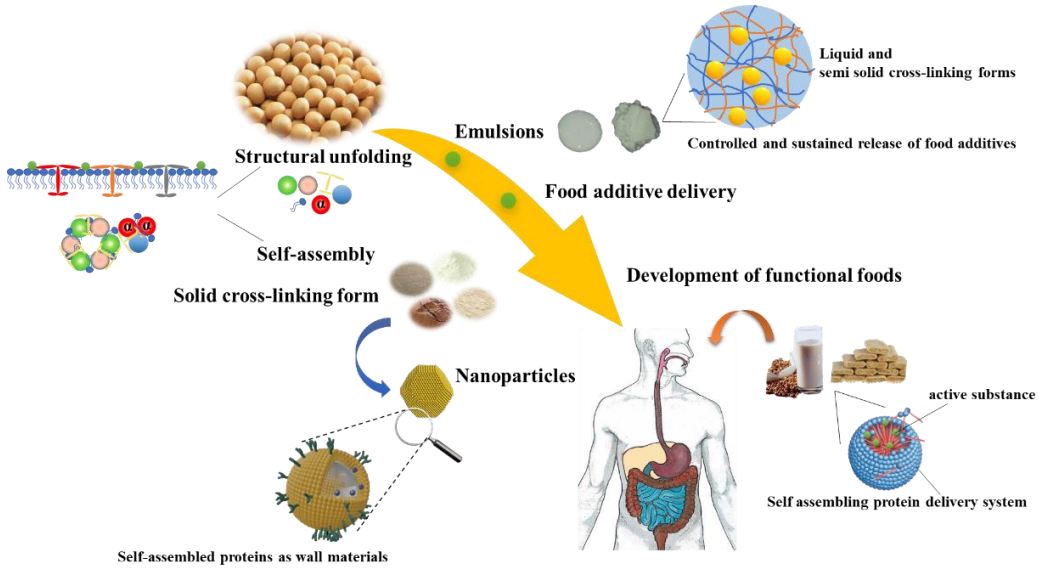
This article provides a comprehensive review of LP, covering its extraction, distribution, structure, function, and applications, as well as comparisons with 11S and 7S. The current methods for isolation and purification of LP from soybeans were discussed, and possible methods for extraction of LP from plant seeds from different sources were proposed. Additionally, this article introduces various methods for identifying LP using microstructural properties, such as molecular structure, microstructure, and self-assembly characteristics, and provides an overview of the techno-functional properties and potential application areas of LP.

Key findings and conclusions

LP is a natural composite component consisting of proteins and phospholipids. The properties of SPI must be interpreted simultaneously with those of LP, 11S, and 7S proteins, both in terms of structural and techno-functional characteristics. In addition, the special structural and functional properties of LP are expected to be widely applied in fields such as dairy products, meat substitutes (animal-derived protein substitutes), nutrient transport, edible plastic wrap, etc. The exploration of LP's structure and functionalities offers potential advancements in developing novel functional foods and wide applications in the food industry.

Keywords: Soybean lipophilic protein; Glycinin; *β*-conglycinin; Extraction; Applications; Physicochemical properties.

Graphical abstract



1. Introduction

Soy protein isolate (SPI), a high-quality plant protein, has long been considered a combination of two storage proteins, *β*-conglycinin (7S) and glycinin (11S). However, Samoto et al. (2007) improved the nitrogen solubility index by heat treatment of defatted soybean flour and isolated the third soybean lipophilic protein (LP) with high lipid content (especially phospholipids) in addition to 7S and 11S. Through Kjeldahl nitrogen determination, gel electrophoresis, and thin layer chromatography analysis, it was found that SPI is composed of 7S, 11S, and LP, accounting for 23.21%, 46.18%, and 31.30% of the total content, respectively (Samoto et al, 2007). This discovery prompted researchers to increase the exploration of LP and effectively compare the characteristics of LP with 11S/7S in order to reconsider the functional characteristics of SPI.

The most obvious difference between LP and 7S/11S is that the LP subunit contains 18 kDa and 24 kDa bands. And the 18 kDa and 24 kDa bands are typical subunit bands of oleosome surface proteins. Wu et al. (2011) compared the lipid composition of LPs with soybean phospholipids and found that their composition and content were basically consistent, further indicating that the membrane protein and soybean phospholipid complex at the oleosome interface is one of the important sources of LPs. Among them, the higher phospholipid content gives LP excellent interfacial properties, especially at the oil-water interface, so LP can be widely used as an excellent emulsifier (Gao et al., 2013; Zhong et al., 2020). Research has also confirmed that its effect is significantly better than 11S and 7S. In addition, the oleosome interface membrane protein is a hydrophobic protein, which endows LP with rich hydrophobic domains that can be used to encapsulate hydrophobic nutrients, thus making up for the shortcomings of most plant proteins lacking hydrophobic domains. However, the problem of poor solubility caused by the extensive hydrophobic domain of LP also limits its research progress.

Although there is currently a relative lack of research on the functional properties of LP outcomes, researchers have not given up on LP research. Considering that the compositions of oleosome interface membrane proteins and LP subunits are highly

similar (Wu et al., 2011), some researchers have further analyzed LP by understanding the structure and properties of oleosomes. A growing number of researchers are attempting to extract and purify LP in order to independently investigate its structural and functional characteristics as a unique natural protein-phospholipid complex (Li et al, 2020a; Zhong et al, 2022a; Zhu et al, 2022). The results show that the extensive hydrophobic domain and high-order complexity in the molecular structure of LP endow it with rich allosteric regulatory properties, and self-assembled LP even shows the ability to improve existing structures or create new structures (Zhong et al., 2022c). This has further increased scholars' enthusiasm for LP research. Figure 1A shows that LP research has been on an upward trend in recent years.

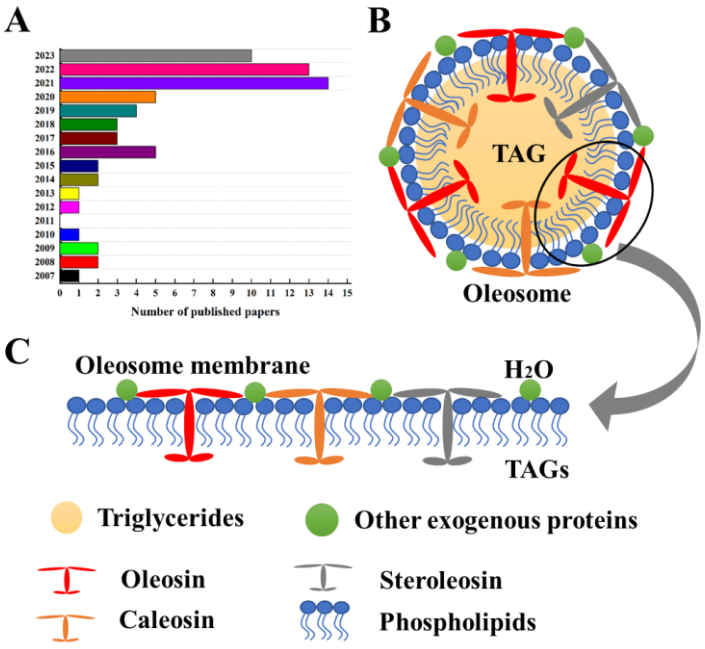


Figure 1. (A) Search number of scientific papers published on LP-related topics from 2007 to 2023; (B) Oleosome structure; and (C) Membrane structure at oleosome interface. "TAG" stands for triglyceride.

Therefore, in this review, based on the current research status of LP, 11S, and 7S, the distribution, preparation methods, and structural characteristics of LP are fully summarized, and the functional characteristics of LP are discussed from a structural perspective. The comparison of the structural and functional characteristics of LP, 7S, and 11S protein components provides a theoretical basis for a more comprehensive

analysis of SPI. Based on the unique structural and techno-functional characteristics of LP, further research and application opportunities as a food raw material remain to be explored.

2 Distribution of LP

Researchers have found that the most significant difference between LP and 7S/11S is that LP contains 18 kDa and 24 kDa bands, which may be subunits of oil-binding proteins (Matsumura, Sirison, Ishi, & Matsumiya, 2017). Based on the thin-layer chromatography, it was found that the lipids in LP were composed of phosphatidylethanolamine (PE), phosphatidylcholine (PC), and phosphatidylinositol (PD) (Samoto et al, 2007). The comparison of the three phospholipid components in soybean oleosome membrane proteins revealed a high degree of similarity, suggesting that a significant amount of the LP components originated from soybean oleosome membrane proteins. Wu et al. (2011) compared the lipid composition and soybean phospholipid portion within LP and found that the composition and content were basically consistent, further indicating that the binding protein of oleosomes is an important source of LP. Among them, due to the high hydrophobicity of the binding protein of the oleosome, it tightly binds to soybean phospholipids. In the process of normal n-hexane low-temperature degreasing, phospholipids are still retained in soybean meal and soybean milk liquid and cannot be removed. As a result, it can be currently inferred that a significant portion of soybean oil-binding proteins (LP) is derived from the binding of LP with soybean oil and phospholipids present on the surface of the oleosome. This indicates that LP serves as a primary component of the membrane proteins found in oleosomes (Figure 1B, C).

3 LP extraction methods

The 7S and 11S extraction methods have been continuously improved with the deepening of research, as shown in Figure S1 (Liu et al., 2007). These proteins are believed to account for approximately 60-70% and 30-40% of SPI, respectively (Deak, Murphy, & Johnson, 2006). However, Samoto et al. (2007) found that after heat treatment of defatted soybean meal, when the nitrogen solubility index (NSI) was

adjusted to 75%, another component, LP, was separated from it, except for 7S and 11S (Figure S1B), and its purity was verified by later experiments to be 88-92%. Zhu et al. (2022) reported that soybean meal was dissolved in Tris HCl buffer (0.04-0.06 M, pH 8.0-9.0) to further increase the NSI. The best extraction temperature of 47 °C, extraction period of 62 minutes, extraction pH of 8.5, and Tris HCl concentration of 0.05 M were determined using the response surface approach. The maximum extraction yield for LP was 11.8% under these circumstances (Figure S1B). The protein content of the LP components obtained by these extraction methods is about 76%, lower than 87% of 7S and 93% of 11S. However, the lipid content of LP is about 11.7%, much higher than 0.8% of 7S and 3.3% of 11S (Zhu et al., 2022). As the extraction temperature further increased, the yield of LP gradually decreased, while the yields of 7S and 11S remained unchanged. According to the findings of Samoto et al. (2007), the solubility of LP improves with increasing temperature. The hydrophobic region and phospholipids are completely exposed and can readily interact with other components (11S or 7S), leading to adsorption on the surface of other protein molecules, resulting in the formation of aggregates that hinder LP extraction and separation.

In fact, the soybean protein components separated by the conventional alkaline dissolution and acid precipitation methods also contain LP. Although LP components are not easily detected using Coomassie brilliant blue staining due to their insensitivity. The inclusion of 7S and 11S components in LP, along with its high phospholipid concentration, makes it difficult to identify in electrophoresis images. It can be reasonably concluded that the isolated proteins from soybean or other plant seeds are first extracted, and then the NSI of the isolated proteins is adjusted by changing the solvent in combination with heat treatment and pH conditions to obtain the corresponding LP components (Figure 2). In addition, based on the analysis of existing research results, the main components of LP are hydrophobic oleosome binding proteins (OBBPs) and phospholipids on the oleosome surface, which are the main components of oleosome membrane proteins. Therefore, extracting oleosomes from soybean or other plant seeds first and then obtaining corresponding lipophilic protein components through degreasing and purification treatment is also a worthwhile

extraction method for further research (Figure 2), and these methods are expected to further improve the yield of LP.

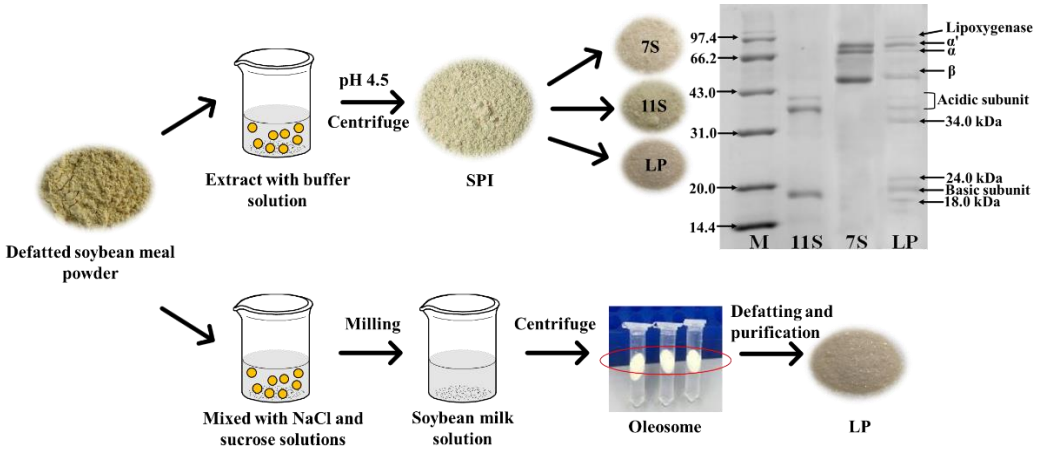


Figure 2. Method for extracting LP from SPI or oleosome and SDS-PAGE of 11S, 7S, and LP.

4 Structure and Identification of LP

4.1 Molecular properties

The molecular structure information of LP seems to be poorly understood compared to 11S and 7S. The most obvious difference is that LP has two specific subunits, 18 kDa and 24 kDa, and also shows a specific subunit band of lipoxygenase (about 90 kDa) (Figure 2). It is worth noting that the molecular weight of oil body proteins is 15-26 kDa, which contains special subunit bands of LP. Therefore, in future research, the structural and functional characteristics of oleosome proteins can be combined to comprehensively analyze the molecular structural characteristics of LP.

The LP score includes a group of proteins, including residual fragments of 11S, residual fragments of 7S, and OBBPs (mainly oleosin, caleosin, and steroleosin) (Matsumura, Sirison, Ishi, & Matsumiya, 2017). Samoto et al. (2007) showed that LP is one of the protein components of the oleosome membrane. The oleosome has an outer phospholipid monolayer and an inner micellar structure filled with triglycerides (TAGs) inside. The types of mosaic proteins in the oleosome can be divided into three types, namely oleosin (OL), caleosin, and steroleosin (Tzen, Cao, Laurent, Ratnayake, & Huang, 1993). Among them, OL is anchored in a hairpin-like structure, and its

hydrophilic part is retained outside the oleosome, providing extremely high stability to prevent fusion (Nikiforidis, 2019) (Fig. 1), and its content accounts for more than 90%. Research indicates that OL and caleosin, as structural proteins, might be crucial for maintaining oleosome stability and regulating oleosome size. This could enhance seed quality and have significant research implications for oil content, lipid storage, and protein extraction. Steroleosin is an enzyme present on the surface of the oleosome and may be related to the synthesis of steroid compounds (Nikiforidis et al., 2013).

4.2 Microscopic morphology

The use of electron microscopy, spectroscopy, and other methods to characterize the microstructure of proteins is also widely used. The soybean protein solution is a monodisperse suspension, and the molecular size and particle size distribution of the protein can be identified by dynamic light scattering (DLS). The size distribution of 11S and 7S is about 0.49 and 0.31 μm, respectively (Jia, Yan, Huang, Zhu, Qi, & Li, 2022). The LP particle size distribution is broad, ranging from a few microns to a few hundred microns in diameter. Large particles in the LP sample range from ten to hundreds of μm, whereas smaller particles, constituting about 50% of all particles, measure between 1-10 μm. The surface properties of LP are probably most significantly influenced by these smaller particles, as larger particles find it challenging to adsorb effectively at the interface. Correspondingly, the average area volume diameter (d) of LP is 19.2 ± 6.9 μm, indicating that LP molecules, instead of being completely soluble, disperse as aggregates in water (Matsumura, Sirison, Ishi, & Matsumiya, 2017; Samoto, et al., 2007; Zhong et al., 2022a).

Interestingly, spectroscopic methods revealed notable distinctions in the secondary and tertiary structures of proteins between 11S, 7S, and LP. For instance, the α-helix structure content was approximately 23% and 26% in 7S and 11S, respectively, as reported by (Jia, Yan, Huang, Zhu, Qi, & Li, 2022), while LP exhibited a content of 30%, according to (Zhong et al., 2022a). Studies have demonstrated a direct relationship between the α-helix structure content and the emulsification properties of soy protein. This association may explain why emulsions made from LP exhibit greater stability compared to those made from 11S and 7S proteins (Zhong et al., 2022a).

Endogenous fluorescence spectroscopy research shows that the tertiary structure of 7S and 11S proteins has maximum emission wavelengths (λ Max) of 300 nm and 340 nm, respectively, for Tyr and Trp residues. LP, on the other hand, exhibits a λ Max of around 270 nm (Zhong et al., 2022a). The locations of Tyr and Trp residues in various soybean protein molecular structures varied greatly, leading to variations in the strategies needed to reveal hydrophobic amino acid residues (Jiang et al., 2015). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are two techniques commonly employed in the characterization of protein molecules, allowing for the examination of their microstructure. SEM enables the analysis of the protein's surface, while TEM provides insights into its internal composition. The comparison shows that 11S is a mixture of block and cylindrical shapes with a rough surface, 7S has a smooth rod-shaped structure on the surface, and LP has an irregular sheet-like structure with a rough surface (Figure 3A-C). This is consistent with existing research results (Yang et al., 2021; Jia et al., 2022), and the microscopic morphology is inseparable from the molecular structure composition. 7S is a trimer composed of three subunits (α, α', and β). When stacked and aggregated in space, it is easy to form a rod-like structure. The 11S subunit is polymorphic and is a hexamer composed of different subunits. Similarly, the LP molecular structure not only contains a variety of subunit structures but also contains phospholipids. Subunit interactions with phospholipids can lead to changes in protein conformation, affecting surface roughness and micromorphology (Jia et al., 2022).

It is worth anticipating that the LP treated with ultrasound shows a distinct bilayer structure (Figure 3D-E) under TEM imaging. The hydrophilic and hydrophobic layers are clearly visible and appear to be adsorbed together, as indicated by the red arrows. In a study conducted by Gao et al. (2013), it was found that turbid LP dispersions (0.5%, w/v, pH 7.0) turned transparent after being sonicated in a 30% power water bath for 5 minutes. It is evident that the water dispersibility of LP is significantly enhanced following ultrasonic treatment. Furthermore, following ultrasonic treatment, the findings from dynamic light scattering, atomic force microscopy, and surface hydrophobicity measurement were contrasted with the initial LP sample. The analysis

revealed that ultrasonic treatment led to a reorganization of the hydrophobic and hydrophilic regions within the LP, with surface hydrophobic patches transitioning to the interior. Considering the significant phospholipid content in the LP component, it is reasonable to assume that the LP created through sonication possesses a core-shell configuration. This structure involves the hydrophobic surface proteins of LP being supported or enveloped by amphiphilic phospholipids, consequently enhancing the water dispersibility of LP. The distinctive structural features of LP have garnered increasing attention from researchers. In the future, the self-assembly characteristics of LP and its structure-activity relationship may attract significant research attention.

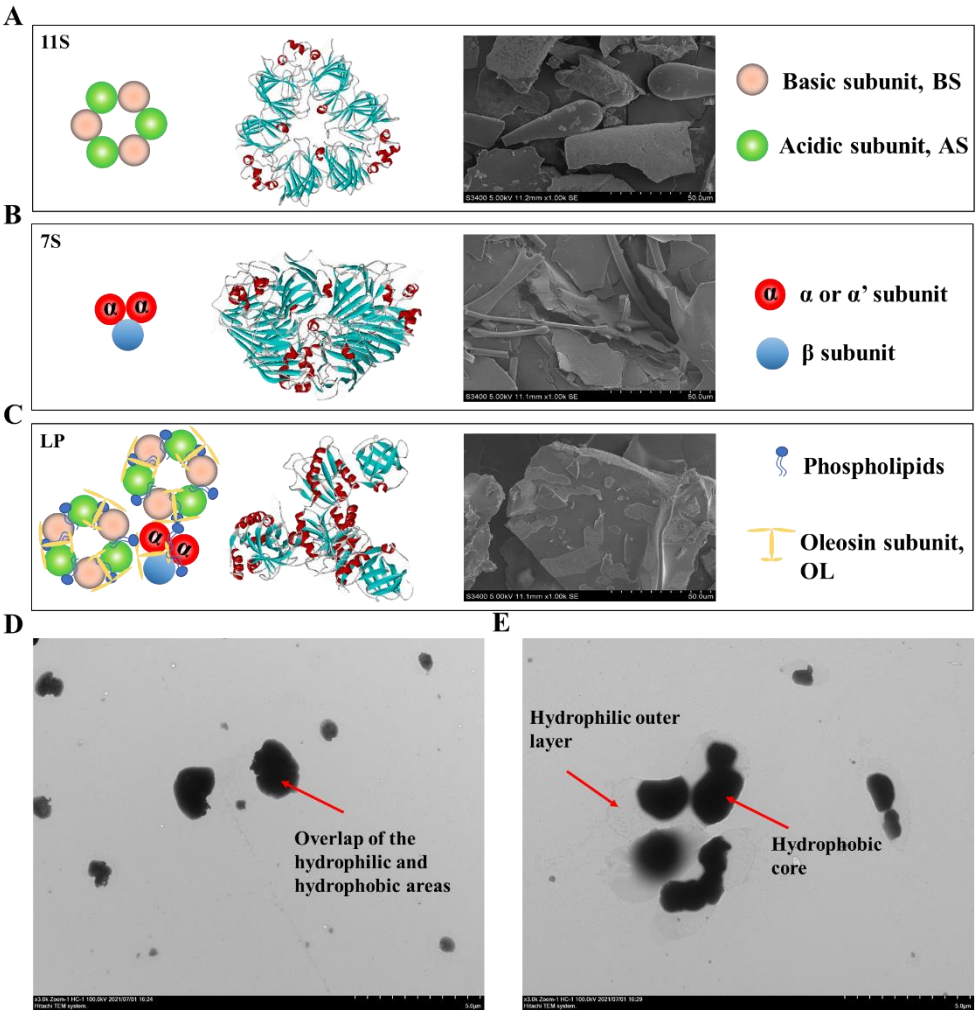


Figure 3. Structural analysis of 11S, 7S, and LP, including simulated structure, molecular structure, and scanning electron microscopy structure (A-C) and Transmission electron microscopy of LP: (D) Without ultrasound treatment; (E) After ultrasonic treatment.

4.3 Self-assembly characteristics

Soy proteins have the ability to undergo self-assembly, resulting in the formation of diverse structures such as emulsions, micelles, dendrimers, and particles (Figure S2). These structures can be tailored for specific applications in the food industry, including the controlled release of biosensors, the creation of coatings and emulsions, the incorporation of continuously active food additives, and the production of functional foods. At the same time, proteins can also undergo self-assembly with biological macromolecules such as phenols, carbohydrates, or other proteins. The interactions between these macromolecules primarily consist of hydrogen bonds, hydrophilic bonds, and ionic bonds (Tomadoni, Capello, Valencia, Science, & Technology, 2020).

At present, numerous investigations have been conducted to examine the self-assembly properties of 11S and 7S proteins. These investigations encompass various experimental conditions such as heating, enzymatic hydrolysis, pH modulation, treatment with urea or ethanol, reduction, and static high-pressure treatment, as outlined in Table 1. In addition to assembling proteins separately, multifunctional nanostructures can be formed by co-assembly with polysaccharides through various processes (such as heating or ultrasonic treatment) (Table 1). In contrast, there are relatively few studies on LP, but they have gradually increased in recent years (Table 1), and more and more researchers are interested in the unique structural and functional characteristics of LP. In future research, more studies on LP can be reported and combined with the research results of 11S and 7S in order to achieve a more comprehensive interpretation of the physical and chemical properties of SPI.

Table 1. A summary of the induction methods and assembly morphology of 11S, 7S, and LP protein self-assembly.

Proteins	Self-assembly induction methods	Mechanism of self-assembly	References
11S	0.05 M sodium chloride and heat at 80 ℃ for 20 min	hollow spherical structure	(Zhao, Guo, Ding, Ye, & Liu, 2020)
11S	pH, ionic strength and aragonite	Arag is a shell, and 11S/Arag	(Liu, Tian,

	(Arag)	is hexagonal.	Kumar, & Zhang, 2009)
11S	pH	Particle size and arrangement of hexamers	(Pizones Ruiz-Henestrosa, Martinez, Patino, & Pilosof, 2012)
11S	pH	Reversible aggregation	(Chen, Zhao, Chassenieux, & Nicolai, 2016)
11S	D-galactose (DG), heat	High temperature treated S-11S and DG bind to cysteine and phenylalanine sites through ionic and disulfide bonds, and self-assemble into more stable colloidal particles.	(Liu et al., 2022)
11S/7S	folic acid (FA)	Regular dispersion and aggregation	(Ochnio, Martínez, Allievi, Palavecino, Martínez, & Pérez, 2018)
7S	dextran	Spherical nanogels with core-shell structure	(Feng et al., 2015)

7S	pH, ionic strength and hydroxyapatite (HAp)	HAp is a shell, and 7S/HAp is disc-shaped.	(Liu, Tian, Kumar, & Zhang, 2009)
7S	pH and heat treatment	Protein fibril	(Tang, Wang, & Huang, 2012)
7S	ethanol	Structure expansion and aggregation	(Liu, Li, Zhang, & Tang, 2019)
7S	pH and chitosan (CS)	spherical particle	(Liu, Liang, Wei, Liu, & Liao, 2015)
7S	pH and sodium alginate (SA)	spherical particle	(Liu, Liao, Wei, Zhang, & Wang, 2019)
7S	High-intensity ultrasound (HIU), pH and ionic strengths	Unfolding/refolding and aggregation behavior of proteins	(Geng, Liu, Hu, Qin, Taha, & Zhang, 2022)
LP	ultrasound	Colloidal particles	(Gao et al., 2013; Li et al., 2020a)
LP	pH and heat treatment	Core-shell structured nanoparticles	(Zhong, et al., 2022b)
LP	Sodium dodecyl sulfate (SDS)	Network-like protein structure	(Zhong et al,

			2022a)
LP	Dithiothreitol (DTT)	Hollow core-shell structure	(Zhong et al, 2023a)
LP	Ethanol	Protein aggregates	(Zhong et al., 2023b)

5 Functional characteristics of LP

The molecular structure of SPI determines its functional characteristics, and any factor that can change the molecular structure of SPI can lead to changes in its functional characteristics. Among them, there are significant differences in the functional characteristics of soybean 7S and 11S components. The 7S component has good water solubility and emulsification, while the 11S component has good gel properties due to its high sulfur amino acid content. Alkaline environments can dissociate 11S, expand with the destruction of disulfide bonds, increase viscosity, and finally lead to the formation of gel (Parmar, Sharma, Sharma, & Singh, 2022). As the third storage protein of SPI, the unique structure of LP components can also cause functional changes, which becomes one of the reasons affecting the functional characteristics of SPI. Therefore, it is necessary to perform more research on LP functional characteristics.

5.1 Solubility

Soybean protein has some shortcomings. The solubility of SPI is often characterized by a low level, and it is common for undesirable tastes associated with beans to stick to the soy protein (Wu, Wang, Yang, Yin, Teng, & Zheng, 2011), which prevents protein expansion and cannot be used in other types of food (such as beverages). The prevailing consensus suggests that the limited solubility of SPI can be attributed to protein denaturation that occurs throughout the manufacturing process, particularly after thermal treatments like sterilizing and spray drying (Sirison et al., 2017).

It is important to acknowledge that the LP component comprises a collection of

304 protein components that are associated with lecithin. This component consists of
305 roughly 8-10% polar lipids, primarily phospholipids (PLs). The PLs have the ability to
306 create lipid-protein complexes with OBBP and are resistant to removal through hexane
307 degreasing (Gao et al., 2013). This characteristic may have a significant impact on the
308 solubility of LPs. Sirison et al. (2017) studied the solubility comparison of soybean
309 lipophilic protein (LP) with other soybean protein components. LP, 7S, 11S, and SPI
310 were prepared under conditions to avoid thermal denaturation. Under all test conditions,
311 the changes in pH value and ionic strength, and the solubility of LP (less than 20% at
312 room temperature), were less than other soybean protein fractions. When the
313 temperature is increased to 90 °C, the solubility of LP continues to increase (up to 40%).
314 The reason for this phenomenon is attributed to the robust intermolecular connections
315 among protein molecules in LP, which hinder their dissociation in aqueous
316 environments. However, the introduction of heat energy induces molecular mobility
317 inside proteins, thereby somewhat mitigating the cohesive forces between protein
318 molecules. The solubility of 7S and 11S showed a decreasing trend with increasing
319 temperature, decreasing from approximately 82% to 78% and 85% to 70%, respectively
320 (Sirison et al., 2017). The reduced solubility of proteins 11S and 7S can be attributed to
321 their spherical shape and inherent tight conformation. Upon heat denaturation, the
322 exposure of hydrophobic amino acid residues inside may lead to the formation of
323 aggregates, further diminishing the solubility of these proteins (Sirison et al., 2017).
324 The temperature-dependent changes in SPI solubility may reflect the equilibrium
325 between 11S, 7S, and LP. The dissolving behavior of LP was investigated in relation to
326 its source and composition using SDS-PAGE, phospholipid content measurement, and
327 Fourier transform infrared spectroscopy (Figure S3; relevant data have not yet been
328 published). Regardless of temperature and aqueous conditions, the solubility of SPI
329 shows moderate values (45-67%), which means that the solubility of SPI is determined
330 by the equilibrium of these three protein components (Sirison, et al., 2017). Among
331 them, increasing the solubility of LP seems to be more advantageous for further
332 improving the solubility of SPI in the future.

333 **5.2 Foaming properties**

The need to evaluate the foaming performance of LP arises from the growing popularity of soybean protein among both the food industry and consumers, as noted by Singh et al. 2008). This popularity is due to its role as a substitute for animal proteins like milk protein (Huppertz, 2010) and egg white protein (Sui et al., 2016), driven by the increasing demand for plant-based proteins for environmental sustainability, health promotion, and veganism. Soybean protein, known for its excellent gelling, emulsifying, and foaming properties, as well as water and fat retention capabilities (Sui et al., 2016), is thus widely used in various food products and warrants further exploration for more applications, including its use in creating bubbles.

In another interesting research, Sirison et al., (2021) compared the surface foaming characteristics of LP with those of 7S and 11S. The soybean protein samples were prepared using gentle methods to prevent thermal denaturation, enabling the examination of the impact of heat treatment on the surface foaming properties of the proteins. The surface foaming performance of LP is superior to that of other soybean protein fractions when heat treatment is not applied. This suggests that the phospholipid-protein complex present in LP has the potential to generate tiny bubbles that exhibit excellent drainage stability. The initial surface foaming performance of the unheated 7S and 11S was comparatively lower. However, the application of heat treatment resulted in a significant enhancement of the surface and foaming performance of both 7S and 11S. This improvement can be attributed to the alterations in particle size, Zeta potential, and surface hydrophobicity of 7S and 11S generated by thermal treatment. On the other hand, the foaming capacity of LP exhibits a decrease, but the foam's stability is enhanced with the application of heat, hence preserving the volume of foam until the last stage of examination. Heating may cause denaturation and exposure of hydrophobic amino acid residues of phospholipid-free proteins in LP. This promotes competitive adsorption between phospholipid-free proteins and phospholipid-protein complexes and prevents the formation of fine bubbles (100 μm^2) formed only by phospholipid-protein complexes. Denatured “free protein molecules” appear to be involved in the generation of bubbles, but it is also possible to form medium-sized bubbles (200 μm^2) due to lower amphipathicity and less flexible

conformation, which is characteristic of phospholipid-protein complexes (Sirison et al., 2021). The enhanced stability of the foam can be attributed to the synergistic impact of two factors: the delayed release of gas from the lamellar phase of smaller bubbles and the heightened resistance to the rupture of larger bubbles.

The high surface activity of LP, despite its main particle diameter being 1 to 10 μm, is notable, suggesting that these "particles" might act as a soft component to effectively cover the interface and reduce surface tension, as discussed by Tang (2017, 2020). This observation indicates a need for further research to clarify the relationship between particle size and the capacity to adsorb or cover the LP particle interface to minimize surface tension. The dispersion state of LP components, particularly their particle size distribution, may also contribute to their surface activity. Gao et al. (2013) observed that LP nanoparticles, which are comprised of phospholipid protein molecules, exhibit enhanced membrane properties at the air-water interface. Specifically, the LP nanoparticles were shown to create a membrane that is both more rigid and elastic, characterized by the presence of hydrophobic peptide aggregates and lipids. This could be due to the flexible structure and amphiphilic properties of LP components, as earlier results show that a complex of SPI and phospholipids exhibits enhanced emulsifying activity compared to individual components. The phospholipids in the LP fraction likely add amphiphilicity and flexibility to peptide molecules through complexation, enhancing their adsorption and structural adaptability at the interface (Figure 4A, B). This is supported by previous reports indicating that most phospholipids in LP combine with peptides to form complexes (Sirison et al., 2017). In accordance with Damodaran (2017) findings, the protein adsorption mechanism occurring at the air-water interface can be delineated into three consecutive phases: the migration of protein molecules from the bulk phase to the interface and subsequent attachment, the infiltration of amino acid residues into the air phase through molecular unfolding, and the reorganization of adsorbed protein molecules. It is worth noting that the surface activity and viscoelastic properties of these proteins play a pivotal role in the formation and durability of foam.

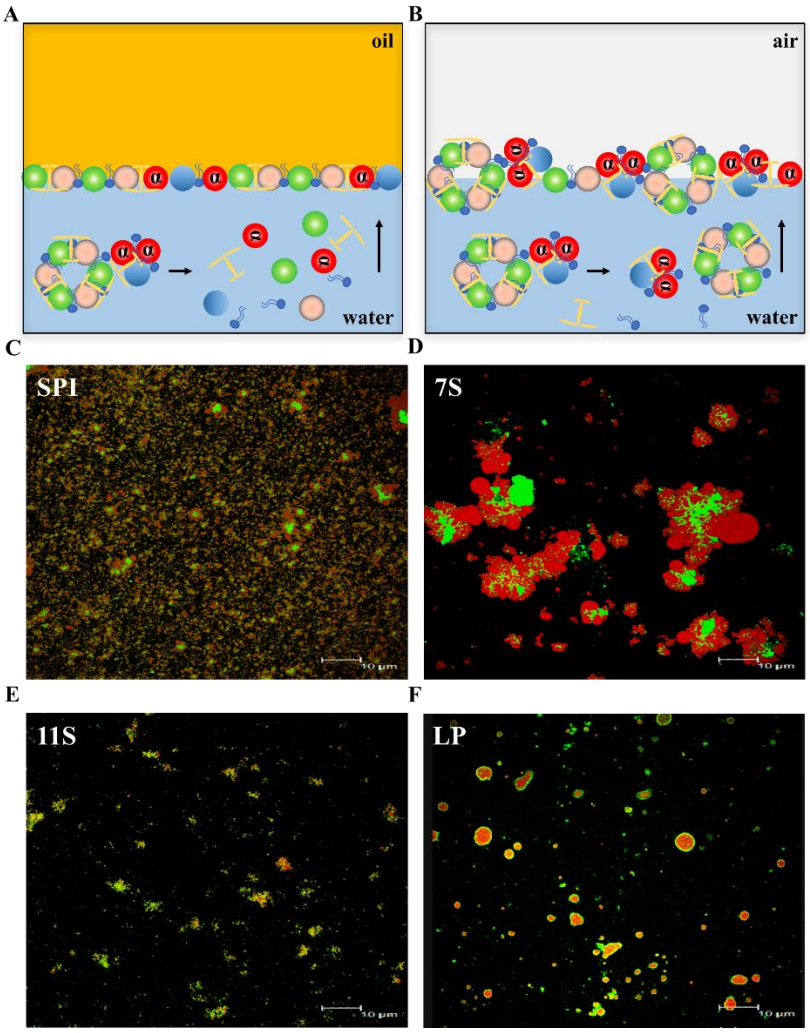


Figure 4. shows the adsorption analysis of LP at the oil-water and gas-water interfaces (A, B) and laser confocal microscopy images of soybean protein (C) SPI, (D) 7S, (E) 11S, and (F) LP.

The potential role of LP particles in foaming ability is highlighted, particularly due to their size range of 1-10 μm, as they might effectively cover the newly formed air-water interface and generate bubbles through mechanisms like "Pickering" or "Mickering" stabilization (Tang, 2017; Tang, 2020), as discussed in Section 3.1. In addition to its advantageous molecular structure, the phospholipid-protein complex, which serves as the primary constituent of LP, plays a vital role in the swift absorption and synthesis of membranes at the air-water interface due to its amphiphilic and adaptable conformation. However, unheated 7S and 11S, characterized by rigid spherical conformations, face challenges in adjusting to the adsorption processes. These processes entail molecular unfolding at the newly formed air-water interface and rearrangement of adsorbed molecules. LP, owing to its excellent surface activity, can

swiftly adsorb onto the air-water interface by incorporating air into the solution, stabilizing the interface through infiltration and rearrangement, thus generating fine bubbles and preventing immediate protein coagulation, as found in previous studies (Damodaran, 2017; Wang et al., 2021). Correspondingly, in a dynamic foam analyzer study, LP exhibited the fastest reduction in surface tension to the lowest level and the highest foaming ability (84.7 ± 3.1), followed by 7S (70.4 ± 1.6) and 11S (63.9 ± 7.7) in terms of foaming ability and surface activity. Therefore, the unheated soybean protein fractions' foaming ability is likely governed by their surface activity. This is consistent with the observations that surface tension is a result of the adsorption of protein molecules, while the surface modulus is closely related to the development of elastic protein membranes at the interface as a result of the interaction of infiltrating and rearranging adsorbed protein molecules.

These findings underscore LP's significant potential for application in various food products, including bubbles, as heating LP improves its foaming ability independently of changes in particle size distribution, as evidenced by the stability in the size distribution of LP particles upon heating. The process entails the competing adsorption phenomenon between proteins lacking phospholipids and complexes of phospholipids and proteins, hence impeding the development of tiny bubbles that are generally generated only by phospholipid protein complexes. Denatured "free protein molecules" participate in bubble formation upon heating, but their less amphiphilic properties and weaker conformation lead them to form medium-sized bubbles, a trait usually associated with phospholipid protein complexes. This phenomenon arises owing to the application of heat, which induces denaturation and exposes hydrophobic amino acid residues in proteins devoid of phospholipids (Sirison et al., 2021). Consequently, the surface hydrophobicity of these proteins is enhanced, and this may be quantified.

5.3 Emulsification or emulsifying ability

The primary protein found in LP is the oleosin-phospholipid complex, which serves as a fundamental constituent of oil bodies and exhibits a specific attraction for oil (Matsumura, Sirison, Ishi, & Matsumiya, 2017). Therefore, LP is expected to have high surface activity. According to the research results, the introduction of a concentration

of 0.0001% LP into water resulted in a notable reduction in surface tension by around 10 mN/m (Matsumura et al., 2017). Conversely, the inclusion of an equivalent quantity of 7S and 11S did not provide any discernible alterations in surface tension (Matsumura et al., 2017). The data presented in this study demonstrate that the surface activity of LP is significantly higher compared to that of 7S and 11S. This suggests that LP holds promise as a very effective emulsifier in various applications (Matsumura et al., 2017). Gao et al. (2013) compared the emulsification properties of LP with 7S and 11S. LP emulsion is not only resistant to coalescence but also resistant to flocculation and oxidation (Deleu et al., 2010). Nile blue and Nile red were used to stain proteins and lipids, respectively. Subsequently, the samples were observed using a laser confocal microscope, with proteins appearing in green and lipids in red (Figure 4C-F). The freshly prepared emulsion stood for 1 h, and the LP emulsion showed an obvious oil-in-water (O/W) structure, while 7S, 11S, and SPI all showed different degrees of coalescence. This indicates that LP can stabilize the oil-water interface and form a stable emulsion system when added separately, while 11S and 7S have similar interfacial properties to most spherical proteins, forming flexible and viscous interfacial structures and inevitably forming interfacial networks with pores or small pieces (Mackie, Ridout, Moates, Husband, & Wilde, 2007).

The addition of a suitable small-molecule surfactant (such as Tween 20) can penetrate the plaque, thereby expanding their structural domains at the interface and replacing protein networks, forming a more stable interface structure (Waschatko, Junghans, & Vilgis, 2012). At the same time, the study showed that the LP formed a complex interface after ultrasonic pretreatment, in which the phospholipid covered the pores or defects in the network structure, which could further stabilize the emulsion. Ultrasonic treatment led to the recombination of membrane proteins at the interface of the LP-based emulsion, which could retard the permeation and adsorption of Tween 20, thus preventing the occurrence of interfacial substitution. As the main constructive interfacial stabilizer, LP with strong hydrophobicity cannot be easily substituted by surfactants, which shows the integrity of oil droplets and prevents emulsion oxidation. Simultaneously, when exposed to elevated levels of salt and surfactant (specifically

Tween 20), LP emulsions exhibit remarkable resistance to heat influences, whereas 11S and 7S emulsions demonstrate flocculation and significant oil separation.

In recent years, research on the emulsification characteristics of LP has also been continuously emerging. Gao et al. (2014) employed a low-power ultrasonic pretreatment technique on LP to facilitate the preparation of an emulsion suitable for the encapsulation of conjugated linoleic acid. The study discovered that the loading capacity of low-protein (LP) samples subjected to ultrasound pretreatment was greater compared to LP samples that did not undergo ultrasound pretreatment. Additionally, the ultrasound-pretreated LP samples were able to achieve a sustained release of conjugated linoleic acid. This indicates that proper mechanical force pretreatment of LP can lead to the reorganization of protein phospholipid components and improve the formation of an emulsified delivery system. Similarly, Li et al. (2020a) discovered that the utilization of ultrasound in the pretreatment of the LP emulsification system exhibits promise as a viable vehicle for the delivery of hydrophobic bioactive constituents, such as vitamin E. In addition, the stability of LP nanoemulsion is higher than that of small molecule surfactants and other soy protein-stabilized emulsions, but protein emulsions often show pH-sensitive characteristics (Ravindran, Williams, Ward, & Gillies, 2018).

Protein emulsions are employed in various pH conditions, wherein acidic constituents frequently induce turbidity and precipitation in protein beverages and subsequently undergo pH fluctuations during transit via the gastrointestinal tract in humans. Therefore, the characterization of the emulsion's pH stability is particularly important, especially in the study of acidic pH stability. In our earlier study, it was determined that the pH level has a significant impact on the stability, rheological characteristics, and encapsulation effectiveness of the nanoemulsion produced using ultrasonically modified LP. Furthermore, an in-depth analysis was conducted to elucidate the underlying mechanisms responsible for these observed effects (Zhong et al., 2022b). In these studies, we tried to use hydroxypropyl methylcellulose (HPMC) and LP to have non-covalent interactions to improve the pH stability of the emulsion. The results showed that the addition of an appropriate amount of polysaccharide could be used as more "bridges" between emulsion droplets, thus further improving the

stability of LP lotion, including pH stability. Li et al. (2021) also obtained similar results. The latest study by Sirison et al. (2023) shows that the emulsion formed by 0.5% LP and 50% oil initially turned into semi-solid at pH 5.7 through gradual acidification in the form of liquid at pH 7.2, and then returned to the liquid through further acidification below pH 4.2. The observed significant alteration can be ascribed to a lack of adequate electrostatic repulsion among droplets throughout the pH range of 5.7 to 4.2, as indicated by ζ-potential analysis.

Similarly, Qi et al. (2017) explored the influence of different pH values on the stability of membrane protein-stabilized natural OB emulsion. It was found that the aggregation of oil bodies at different pH values is attributed to the synergistic effect between conformational changes and the interaction characteristics of OBBPs. The results of this study demonstrate that LP has the capability to generate a stable emulsion with a wide range of adjustable rheological properties. This highlights the promising prospects of utilizing LP in the development of food emulsions with varying textures, spanning from liquid to semi-solid states. Sun et al. (2022b) successfully generated a high internal phase emulsion (HIPPE) characterized by a gel-like network structure. This was achieved by employing LP, 11S, and 7S proteins, with the experimental parameters set at a pH of 2, an LP concentration of 1.5%, and an oil phase volume fraction of 80%. Among the various options considered, it can be concluded that LP-stable HIPPE has the most stable structure and possesses superior oxidation stability. The results of simulated in vitro digestion experiments indicate that HIPPE, when manufactured under acidic circumstances, exhibits favorable stability in gastric juice and effectively delays the release of lipids. These results can be used to develop delivery systems for functional food ingredients based on LP-stabilized emulsions for targeted and sustained release of nutrients. Similarly, we can also speculate that the highly tunable rheological property of LP emulsion is not only related to the electrostatic interaction caused by pH change but may also be related to multiple factors such as the ratio of the oil phase to the water phase, processing conditions, etc. There are still many interesting phenomena and mechanisms waiting to be discovered and analyzed in the future.

5.4 Gel properties

The physical and chemical characteristics of proteins, including gelation, foaming, and emulsification, frequently rely on the existence of salts and variations in pH (Nicolai, & Chassenieux, 2019). Different protein components may exhibit different gelling behaviors. The gelation process in soybeans has been observed to exhibit distinct driving modes for 7S and 11S proteins, resulting in the formation of gels with varying textural characteristics. According to the literature, the production of 7S gel is mostly attributed to the significant involvement of hydrogen bonding and hydrophobic interaction. However, the formation of 11S gel is predominantly influenced by electrostatic interaction and disulfide bonding. In general, the 7S/11S ratio has a notable impact on gel structure. It has been observed that the presence of 11S is particularly influential in gel formation. Furthermore, an increase in the proportion of 11S results in enhanced gel firmness and accelerated gelation kinetics (Zhou et al., 2019). The microstructure studies by CLSM or SEM also show that 11S produces rougher and more uneven structures than 7S (Zhou et al., 2019). Pang et al. (2021) used the same amount of 7S or 11S protein to replace 10%, 20%, or 40% of the protein in soybean milk powder. The effect of soybean protein components on acid in soybean milk gel was studied. The results showed that 7S and 11S could effectively improve the gel strength of soybean milk and improve the water retention of the gel when the incorporation rate was ≥ 20%. Many results have been reported on the gel properties produced by pH, salt, or heat-induced 11S, 7S, and 11S/7S ratios, but the characterization of LP gel properties is still limited (Wu, Hua, Chen, Kong, & Zhang, 2016; Wu, Hua, Chen, Kong, & Zhang, 2017; Geng, Liu, Hu, Qin, Taha, & Zhang, 2022). Sirison et al. (2023) found that the LP emulsion showed a reversible liquid-solid transition by acidification in the weak acid pH range of pH 7.2 to 4.1, and the emulsion gel state in the pH range of 5.6 to 4.5. Similarly, Sun et al. (2022a) also successfully prepared LP-based HIPPE gel at pH 2, which showed a more stable structure than 7S and 11S stable HIPPE gel. This indicates that pH adjustment is expected to be the formation condition of LP gel. In addition, Zhong et al. (2020) provided a detailed account of the manufacturing process, physical characterization, and investigation of

nutrient release behavior during in vitro digestion of a thermosensitive emulsion gel composed of LP-hydroxypropyl methylcellulose (HPMC) and calcium chloride (LHC-Ca). The analysis of the infrared spectra, X-ray diffraction (XRD), and ^{1}H NMR spectra revealed that the introduction of $CaCl_2$ induced alterations in the crystalline structure of the LP-HPMC composite. Additionally, it resulted in a reduction in the hydrogen content of the composite, thus leading to a substantial decrease in the emulsion gel temperature. As a result, a thermosensitive emulsion gel was formed. The aforementioned results have the potential to contribute to the advancement of functional thermosensitive emulsion gel carriers that are founded on LP-HPMC emulsion. These carriers can be utilized for the purpose of targeting and sustaining release of nutrients. In order to better interpret the gel properties of SPI, it is particularly important to increase the analysis of LP gel properties in future research, and reasonably adjust the addition ratio of LP/11S/7S to explore its effect on the gel properties of SPI.

5.6 Physiological characteristics

Soybean products are extensively consumed on a global scale and are garnering growing interest owing to their abundant nutritional content and physiological attributes (Liu et al., 2022). Previous studies have indicated that soybean protein possesses potential benefits in the management of hypercholesterolemia and cardiovascular illnesses, and can be more effective than animal protein in reducing high cholesterol (HCE) (Sirison et al., 2017). Numerous studies have demonstrated that the ingestion of soy-based food products has been found to significantly decrease the concentration of total cholesterol (TC) in the bloodstream, thereby imparting positive effects on cardiovascular well-being in both human subjects (Blanco Mejia et al., 2019) and animal models (Takahashi, & Konishi, 2011).

As the body of study has expanded, scholars have discovered that the HCE of soy protein is linked to its 7S and 11S fractions. Lovati et al. (1998) demonstrated that 7S soy glycine is more efficacious in enhancing the expression of low-density lipoprotein (LDL) receptors and promoting LDL breakdown in cultured HepG11 human liver cancer cells compared to 2S soy glycine. In addition, 7S can reduce plasma triglyceride (TG) concentrations in humans and hyperlipidemic rats. Nevertheless, the substitution

of soy protein isolate (SPI) with concentrated 7S or 11S globulin in the dietary regimen did not yield any discernible enhancements in the plasma lipoprotein profile or cardiovascular biomarkers of the primate subjects (Adams, Anthony, Chen, & Clarkson, 2008).

Due to the unique structure of LP, some researchers have conducted some research to indicate that LP has good physiological functions. Kanamoto et al. (2007) fed five-week-old male Sprague-Dawley mice with casein, LP, and their partial hydrolysates, respectively. It was found that the blood cholesterol and triglycerides of Sprague Dawley mice fed LP and peptides were lower than those fed casein, and the mice fed LP protein peptides had better effects, which is consistent with the previous speculation. The difference in bile acid and total lipid content in feces is not significant. Therefore, it can be assumed that LP and its peptides can directly alter lipid metabolism and affect blood cholesterol and triglyceride levels. Nonetheless, there was no statistically significant disparity observed in the messenger RNA (mRNA) levels of liver β-hydroxy-β-methylglutaryl CoA reductase, cholesterol 7 α-hydroxylase, and low-density lipoprotein. In the present study, an obesity model was established by subjecting mice to a high-fat diet. Subsequently, the mice were administered LP and its peptides, and it was seen that the administration of both LP and its peptides had preventive effects against metabolic syndrome (Kanamoto, Kimura, & Okamura, 2007). Simultaneously, with the decrease of triglycerides and total bile acid in the blood of mice, the amount of bile acid in the feces increases. In addition, when feeding LP and its peptides together with a high-fat diet containing 2% cholesterol for 4 weeks, the blood cholesterol levels of mice did not significantly increase (Sato, Shimizu, Inoue, & Nakata, 2014). Due to the fact that LP and its separation methods were not discovered until 2007, research on structural-functional characteristics has gradually increased in recent years. The research on the physiological activity of LP by researchers is still in its initial stage and has yielded some positive results, but the relevant mechanism of action is still blank. What can be confirmed at present is that a considerable portion of it comes from soybean oil-binding proteins and phospholipids on the oil surface. Due to its strong hydrophobicity and poor aqueous dispersibility, the study of many properties has

become difficult.

6 Applications

The growing popularity of soybean protein and other plant proteins in the food industry and among consumers, as noted by Singh, Kumar, Sabapathy, & Bawa (2008), is attributed to the increasing demand for plant proteins driven by environmental sustainability, health promotion, and the rise of vegetarian diets. These proteins are being utilized as food colloidal particles, soft microgels, and emulsifiers, replacing animal proteins like milk protein and whey protein (Huang et al., 2017). Besides their fundamental role in preventing droplet aggregation, these new food ingredients offer various additional functions, including exceptional physical and chemical stability. Consequently, the utilization of proteins in the food industry is evolving in line with their diverse functional properties (Figure 5).

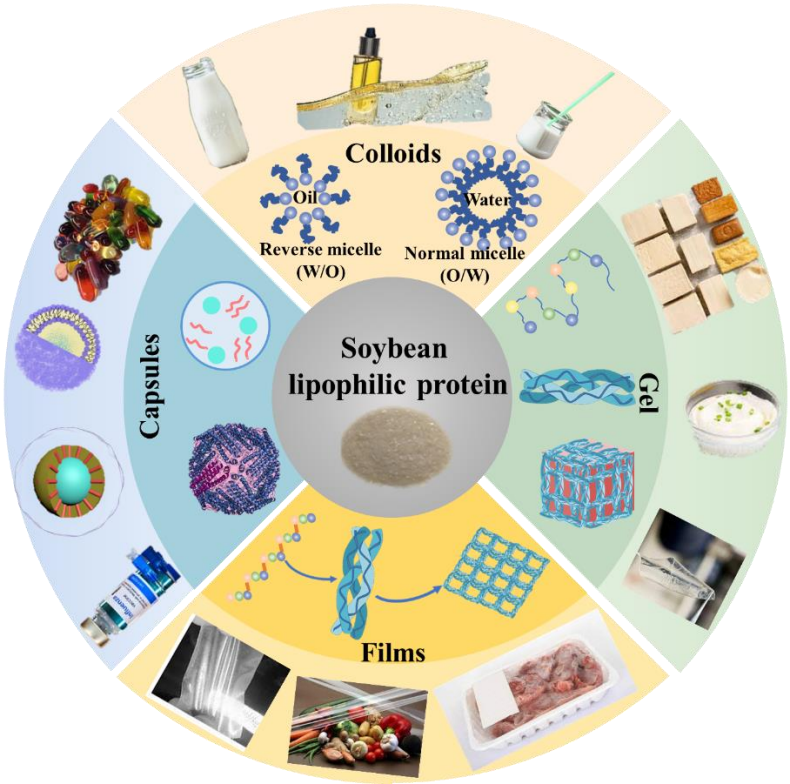


Figure 5. The relationship between the structural functional characteristics and applications of LP.

6.1 Dairy food substitutes and plant-based beverages

Globally, high plant-based protein milk beverages (including brewed protein

633 powders) that help induce satiety, control appetite, and maintain lean body mass are on
634 the rise (Mathur, Kumari, Grewal, Panghal, Rani, & Kargwal, 2020). Table S1 presents
635 an overview of the primary conventional dairy alternatives derived from soy protein.
636 The nutritional composition of these food items can serve as a valuable point of
637 reference for the animal-free food sector. As introduced in 4.6, all the 11S, 7S, and LP
638 in SPI have different nutritional and functional characteristics. Many European funding
639 organizations are launching project calls to develop innovative, healthy vegetarian
640 foods with excellent nutritional properties that are attractive to consumers and easily
641 accessible. Legumes remain the optimal alternative for substituting dairy products due
642 to their notable protein content and capacity to function as a favorable medium for the
643 fermentation of lactic acid bacteria (Mefleh, Summo, Faccia, Caponio, & Pasqualone,
644 2023). Fermentation can effectively eliminate the flavor and anti-nutritional
645 shortcomings of beans. Furthermore, in the context of substituting animal-based dairy
646 products, there exists a considerable need for plant proteins that possess desirable
647 emulsifying and foaming characteristics. The ideal emulsifier strongly adsorbs on the
648 surface of newly formed droplets at the interface, thereby preventing droplets from
649 coalescing with the surrounding environment (Kim, Wang, & Selomulya, 2020). In the
650 previous review of the functional characteristics of LP, it was shown that, compared
651 with SPI, 11S, and 7S, LP has strong emulsifying and foaming properties, as well as
652 excellent adsorption capacity at the oil-water and gas-water interfaces, and possesses
653 the basic structural-functional conditions as a substitute for dairy products. Therefore,
654 it is reasonable to assume that LP can serve as a substitute raw material for dairy
655 products. It can also be mentioned that the structural and functional characteristics of
656 soybean protein as a substitute for various types of animal foods are closely related to
657 the presence of LP components.

658 **6.2 Meat-based food substitutes (vegetarian meat)**

659 Replacing meat with meat substitutes can effectively mitigate the harmful
660 environmental consequences associated with our eating habits (Santo, Kim, Goldman,
661 Dutkiewicz, & Nachman, 2020). Therefore, the utilization of soy protein alternatives
662 as direct substitutes for meat products in food consumption patterns is increasing (Van

Mierlo et al., 2022). It is reported that 7S and 11S are denatured at about 70 °C and 90 °C, respectively. Therefore, during the preparation of ordinary meat products, conventional soy protein does not allow sufficient structural changes, that is, to limit its gel. It is worth noting that the solubility of LP gradually increases with increasing temperature (Sirison et al., 2017). Properly combined with pH adjustment, it can also significantly enhance its gel characteristics and achieve the reversible transformation of lotion gel (Sirison et al., 2023). Similarly, researchers address issues such as protein denaturation or nutrient loss by changing certain pre-denaturation conditions (such as temperature and pH) during the preparation process of soybean proteins, affecting their functional properties. This modification can also allow proteins to interact with other components (Zhang, Huang, Chen, Wang, & Wang, 2021). Lin & Mei (2000) pointed out that soybean protein self-assembly caused by an appropriate temperature increase can improve the emulsifying ability and stability of meat products, and the emulsifying properties of soybean products are also affected by their fiber content. According to the findings of Koshy et al. (2022), SPI subjected to acidic pH treatment exhibits a high protein content and a low fiber content, while also demonstrating favorable emulsifying properties.

It is worth noting that existing research mainly focuses on the use of SPI as a meat product substitute to enhance the physicochemical, textural, and structural properties of "artificial meat" (Santo et al., 2020; Youssef & Barbut, 2011; Chiang et al., 2021). However, there is relatively little research on the use of 11S and 7S components alone to prepare vegetarian meat, and research on the use of LP components for vegetarian meat preparation is even blank. On the one hand, it may be that using a certain protein component alone to prepare vegetarian meat does not meet the nutritional requirements; on the other hand, the functional characteristics cannot meet the requirements of meat viscosity, elasticity, and stretchability. However, due to LP's excellent high-temperature resistance and the reversible transition state of lotion gel induced by a suitable pH (4.2~5.7) (Sirison et al., 2023), it is reasonably speculated that LP can be used as one of the raw materials of meat substitutes, and more basic research in this area is needed.

6.3 Nutrient delivery system

693 Soybean protein has attracted increasing interest due to its nutritional richness and
694 excellent functionality in the production of nutrition delivery carriers (Amagliani, &
695 Schmitt, 2017). Moreover, based on the high tunability in soybean protein complex, a
696 variety of nutrient delivery carriers can be developed using protein components (SPI,
697 7S, 11S, LP, etc.) particles with different structures and functional properties, including
698 particles, nanoparticles, nanofibers, hydrogels, emulsions, Pickering emulsions, and
699 nano-emulsions. Recently, Chang et al. (2022) reviewed the application of emulsion
700 based on soybean protein particles as a delivery carrier for nutrition and health care
701 products. Tang et al. (2019) reviewed the application of SPI, 7S, and 11S nanostructures
702 as bioactive substance delivery systems. With the further increase in research, some
703 researchers also reviewed Pickering emulsion based on soybean protein particles and
704 its various food applications (Shi, Feng, Wang, & Adhikari, 2020; Tang, 2019).
705 However, little is known about the research on the structure, physicochemical
706 properties, stability, and other aspects of LP delivery systems. In order to fully realize
707 the potential of SPI particle-based oral delivery systems, it is crucial to review and study
708 the relevant characteristics of SPI component delivery systems.
709 Table 2 summarizes the current research progress in using LP as a carrier to deliver
710 nutrients. Researchers have exploited the special structure of the LP natural protein
711 phospholipid complex to induce high emulsification, encapsulate functional oils
712 (conjugated linoleic acid (CLA)), and lipid-soluble nutrients (Vitamin E) (Gao et al.,
713 2014; Li et al., 2020a). The results showed that nanoparticles (LPP) prepared by
714 ultrasonic pretreatment of LP not only improved the solubility of LP, but also served as
715 a new type of CLA and vitamin E delivery carrier with unique characteristics, including
716 high loading capacity, antioxidant activity, and stability. The delivery system can also
717 continuously release CLA and vitamin E in the simulated gastrointestinal tract (GIT).
718 With the deepening of research, researchers have begun to explore the encapsulation
719 effect of carrier systems formed by the combination of LP with HPMC, methylcellulose
720 (MC), or small-molecule surfactants (Tween 20) on nutrients. The results show that
721 appropriate concentrations of HPMC, MC, and Tween 20 are beneficial for further
722 improving the stability of LP emulsification carrier systems (Gao, et al., 2014; Li, et al.,

2021; Li et al., 2020b).

In recent years, the production of LP self-assembly behavior induced by environmental factors (pH and temperature) and inducers (ethanol, SDS, DTT, etc.) has been widely reported as a nutrient delivery system (Matsumura, Sirison, Ishi, & Matsumiya, 2017; Sun et al., 2022a; Sun et al., 2022b; Zhong, Sun, Song, Liao, Qi, & Li, 2023a; Zhong, et al., 2022c; Zhong et al., 2022a). The results showed that adjusting the induction conditions appropriately, including the concentration of inducers, induction time, and induction method, combined with the high adjustability of LP structure, can achieve partial or completely reversible recombination of LP's own subunit structure, thereby expanding the internal hydrophobic structure of LP and encapsulating some or all of the hydrophobic nutrients (resveratrol) inside the LP molecular structure, rather than binding to the surface. It can significantly improve the physicochemical stability and bioavailability of resveratrol. Therefore, LP can be an ideal material for nanocarrier systems, and SPI is widely used as an excellent nanocarrier system, which may also be the result of the joint promotion of the three components 11S, 7S, and LP.

Table 2. Research progress in LP as a carrier for loading nutrients and bioactive compounds

Nutrients	The form of nanocarriers	Advantages and disadvantages	References
Resveratrol	Nanoparticles and emulsion (different oil phase mass fractions)	• LP nanoscale adjustable with ethanol concentration ([E]) • Stability of emulsion induced by appropriate concentration [E] to form LP	(Zhong, et al., 2023b)

		• Enhanced stability of resveratrol ➢ The size of LP particles induced by [E] is relatively large	
Curcumin	Emulsion	• Enhanced solubility of curcumin • Enhanced bioavailability of curcumin • A favorable intestinal environment can promote the absorption of curcumin ➢ Curcumin has low encapsulation efficiency and binds to the surface of LP molecules	(Zeng et al., 2023)
Vitamin B12	Nanoparticles	• Enhanced stability of vitamin B12 • Improving the intestinal digestion rate of vitamin B12 • Improving the photosensitivity of vitamin B12	(Li, Gao, Wu, Geng, Teng, & Li, 2023)

		➢ Particle size too large	
Resveratrol	Nanoparticles	• Enhanced stability of resveratrol • The core-shell structure of LP can encapsulate resveratrol within the LP molecular structure ➢ Organic solvents are difficult to clean and are prone to residue	(Zhong et al., 2023a)
Vitamin E	Nanoemulsion	• LP has strong emulsifying properties • High encapsulation efficiency of vitamin E ➢ The form of emulsion is not resistant to storage	(Wang et al., 2021)
Vitamin E	Heat-sensitive emulsion gel	• Vitamin E sustained-release • Temperature sensitive carrier ➢ Preparation is relatively complex	(Zhong, Xie, Zhang, Sun, Qi, & Li, 2020)
Vitamin B12	W/O/W emulsion	• Enhanced encapsulation	(Li, et al., 2021)

		efficiency of vitamin B12 ➢ Preparation is relatively complex	
Resveratrol and Vitamin D3	Pickering emulsion	• Implementing co-encapsulation of resveratrol and vitamin D3 • Significant improvement in bioavailability ➢ The form of emulsion is not resistant to storage	(Zhong, et al., 2022a)
Resveratrol	Nanoparticles	• SDS induces the formation of a network-like protein structure, increasing the encapsulation capacity of resveratrol • LP undergoes reversible recombination to encapsulate resveratrol within the molecule • Significantly	(Zhong et al., 2022c)

		improved stability of resveratrol ➢ Organic solvents are difficult to clean and are prone to residue	
Epicatechin	Nanoparticles	• Improved emulsifying and antioxidant properties ➢ Lack of characterization of stability and bioavailability	(Sun et al., 2023)
Conjugated linoleic acid	Nanoemulsion	• Significantly enhanced antioxidant activity • Enhanced load capacity ➢ The form of emulsion is not resistant to storage	(Gao et al., 2014)
Lycopene	Coarse emulsion, nanoemulsion, emulsion gel and high internal phase Pickering emulsion (HIPPE)	• At acid-induced conditions, HIPPE has the best encapsulation effect on lycopene	(Sun et al., 2022b)

	• Enhanced stability in the gastric environment ➢ The form of emulsion is not resistant to storage

740

741 **6.4 Packaging Film**

742 The utilization of SPI in the development of protein packaging films has garnered
743 significant attention in academic research. This interest can be attributed to its cost-
744 effectiveness, favorable film-forming properties, environmentally sustainable nature,
745 and widespread availability (Wu Sun, Huang, Duan, Xiao, & Le, 2019). Nevertheless,
746 it should be noted that films based on SPI exhibit several limitations, including elevated
747 water solubility and inadequate mechanical and barrier characteristics. These
748 drawbacks pose challenges to its utilization within the food packaging sector (Xiao, Liu,
749 Kang, Wang, & Xu, 2020). Therefore, additional studies on the SPI structure are
750 necessary to address these limitations by analyzing the relevant film-forming properties
751 of each component (Liu et al., 2021; Mostafa, Airouyuwaa, Hamed, Wang, & Maqsood,
752 2023).

753 Table S2 summarizes other recently reported biological nanocomposites based on
754 soy protein for food packaging applications. It is worth noting that there are few reports
755 on the preparation of edible films for 11S, 7S, and LP components, as the preparation
756 of edible films often requires multiple functional characteristics such as excellent heat
757 resistance, bacterial resistance, resistance to different solvents, mineral oil, gas barrier,
758 and moisture resistance. This requires that the selected protein-based raw materials
759 have relatively comprehensive structural and functional properties, but the presence of
760 11S, 7S, and LP components simultaneously endows SPI with excellent ductility and
761 film-forming properties. For example, 7S has excellent emulsification, 11S has strong
762 gel properties, and LP, as a natural protein phospholipid complex, has strong heat

resistance, hydrophilic and lipophilic domains, and a strong interfacial adsorption capacity. However, these three components are used as raw materials alone, and the related film-forming characteristics have not been well studied.

7 Future research prospects

In recent years, LP has garnered considerable attention from both the academic and industrial sectors within the food industry. As the newly discovered third storage protein of SPI, alongside the 11S and 7S components, LP possesses a distinctive structural composition and impressive physiological characteristics. This has positioned LP as a promising and sustainable substitute for emulsifiers in a wide range of emulsification systems. Notably, the phospholipid protein components found in LP are regularly adsorbed at the oil-water interface layer, further enhancing its functionality and applicability. Furthermore, the distinctive structure of LP presents an opportunity for colloidal scientists to explore and create a wider range of colloidal products with diverse functional properties. However, the multifaceted functional characteristics of LP have yet to be fully comprehended, thereby restricting its potential applications. Moreover, ensuring the purity of LP remains a challenge, as conventional degreasing methods are unable to effectively eliminate phospholipid components, leading to the presence of residues from the 11S and 7S subunits. In the future, the utilization of PCR amplification technology holds promise for removing phospholipids and enabling the targeted design of high-purity LP. However, the understanding of the structure-function relationship of LP remains limited, and there is a need for further exploration in order to fully comprehend its potential applications. It is crucial to pay closer attention to unlocking the full range of its functional capabilities and expanding its applicability in various fields.

8 Conclusion

LP is a natural composite component consisting of proteins and phospholipids. The protein component of LP includes residual fragments of 11S, residual fragments of 7S, and oil-bound proteins. On the other hand, the phospholipids present in LP include PE,

PC, and PD. LP demonstrates remarkable properties such as excellent emulsifying, foaming, and interfacial adsorption capabilities. LP can dissociate into individual subunits under specific conditions and exhibit self-assembly into various polymer forms within the natural environment, resulting in distinct functional characteristics. This article provides a comprehensive review of LP, covering its extraction, distribution, structure, function, and applications. The current methods for isolation and purification of LP from soybeans were discussed, and possible methods for extraction of LP from plant seeds from different sources were proposed. This article introduces various methods for identifying LP using microstructural properties, such as molecular structure, microstructure, and self-assembly characteristics, and provides an overview of the functional properties and potential application areas of LP. In order to better interpret the structural functional characteristics and mechanism of SPI, the review also supplemented the relevant contents of 11S and 7S and conducted a comparative analysis with LP. Research has shown that the properties of SPI must be interpreted simultaneously with those of LP, 11S, and 7S proteins, both in terms of structural and functional characteristics. In addition, the special structural and functional properties of LP are expected to be widely applied in fields such as dairy products, meat substitutes (animal-derived protein substitutes), nutrient transport, edible plastic wrap, etc. However, there is still a need for further research to understand the mechanism by which LP functions in these applications and to establish a clear relationship between its structure and function. The properties of LP, such as encapsulation, nutrient delivery, or drug delivery, are determined by its structure. Therefore, through careful structural analysis, LP can be modified to induce self-assembly and form diverse molecular structures. This opens up the possibility of creating new variants of LP with unique properties, thereby expanding their application fields and increasing their economic value.

Acknowledgments

The authors would like to acknowledge the assistance provided by the Natural Science Foundation of China (Grants No. 32072355).

Conflict of interest

The authors confirm that they do not have any financial or other conflicts of interest associated with this work.

Credit/authorship contribution statement:

The author and all co-authors have participated in and contributed to the writing and development of the review manuscript presented in this work.

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