RECIUJES
Revista Científica da Universidade José Eduardo dos Santos
e-ISSN: 3006-9688 | Edição Especial AC-2025 | Vol. 06 | n.º 01 | 2026

PHYSICAL AND CHEMICAL PROPERTIES OF PLANT-BASED PROTEINS: STUDY OF GLUTELIN

PROPRIEDADES FÍSICAS E QUÍMICAS DE PROTEÍNA DE ORIGEM VEGETAL: ESTUDO DA GLUTELINA

Alcides Morais Lofa[1,2]*, Luvovádio Francisco Marcelo[1], Ricardo B. Ferreira[2]

[1] Faculdade de Medicina Veterinária, Universidade José Eduardo dos Santos. Huambo – Angola

[2] LEAF, Centro de Investigação Terra, Instituto Superior de Agronomia, Universidade de Lisboa

*lofaalcides25@gmail.com

RESUMO

Proteins are essential macromolecules that perform structural, metabolic, storage, and catalytic functions in cells. The growing global population has intensified the search for sustainable alternatives to animal proteins, highlighting plant proteins as promising candidates. However, their successful application depends strongly on their physicochemical and functional properties, underscoring the need for detailed characterization. Plant proteins are classified according to solubility into albumins, globulins, prolamins, and glutelins, which are soluble in water, saline, alcoholic, and alkaline/acidic solutions, respectively. Among these fractions, glutelins remain comparatively understudied despite their importance as seed storage proteins. This review aimed to compile and critically evaluate current knowledge regarding the physicochemical characteristics of glutelin fractions isolated from plant sources. Glutelins are encoded by multigene families and consist of heterogeneous acidic (30–36 kDa) and basic (19–22 kDa) subunits derived from a 57 kDa precursor typically stored in protein bodies (PB II). Their low water solubility arises from strong hydrophobic interactions and disulfide (SH–SS) linkages. Functional properties such as water absorption, emulsifying capacity, and gel formation are key determinants of texture, structure, and shelf life in food systems. Physical, chemical, and enzymatic modifications have shown promise in improving glutelin solubility, emulsification, and foaming properties.

Keywords: Protein, solubility, nutrition.

ABSTRACT

As proteínas são macromoléculas essenciais que exercem funções estruturais, metabólicas, de armazenamento e catalíticas nas células. O aumento da população mundial tem incentivado a procura por alternativas sustentáveis às proteínas de origem animal, destacando as proteínas vegetais. Entretanto, o desempenho dessas proteínas depende fortemente de suas propriedades físico-químicas e funcionais, o que ressalta a importância de caracterizá-las adequadamente. As proteínas vegetais são classificadas de acordo com sua solubilidade em albuminas, globulinas, prolaminas e glutelinas, solúveis em água, soluções salinas, alcoólicas e alcalinas/ácidas, respectivamente. Entre essas frações, as glutelinas permanecem relativamente pouco estudadas, apesar de seu papel relevante como proteínas de reserva. Assim, esta revisão buscou compilar e analisar criticamente o conhecimento disponível sobre as características físico-químicas das glutelinas vegetais. Essas proteínas são codificadas por famílias multigênicas e constituídas por subunidades ácidas (30–36 kDa) e básicas (19–22 kDa), derivadas de um precursor de 57 kDa, geralmente armazenado no corpo proteico PB II. Devido às intensas interações hidrofóbicas e à presença de ligações dissulfeto (SH–SS), apresentam baixa solubilidade em água. Propriedades como absorção de água, emulsificação e formação de gel são determinantes para aplicações tecnológicas. Estratégias físicas, químicas e enzimáticas têm mostrado potencial para melhorar a solubilidade, a capacidade emulsificante e a formação de espuma das glutelinas.

Palavras-chave: Proteína; solubilidade, nutrição.

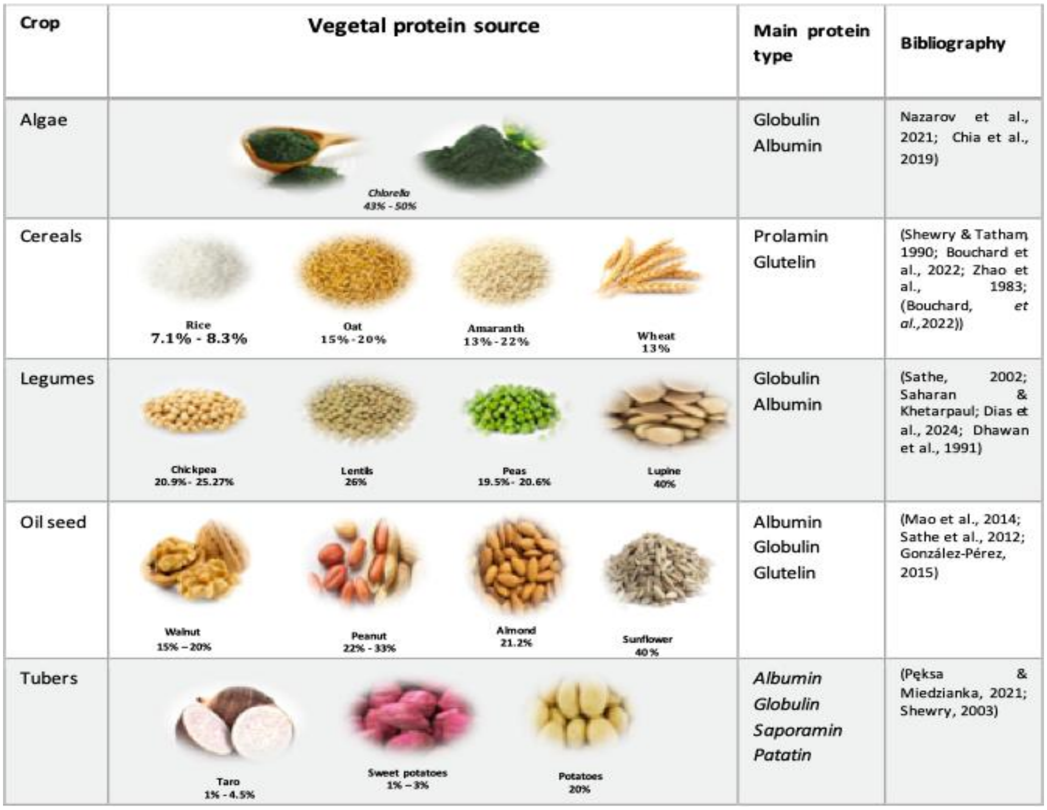
Introduction

Proteins are essential biological macromolecules responsible for most cellular functions, often acting in coordination with other biomolecules. They can be regarded as natural biomolecular devices exhibiting specific structural and functional properties (Sun *et al*., 2023). Proteins perform numerous enzymatic, structural, and regulatory roles and serve as nutrient reservoirs to sustain metabolic demands (Rasheed *et al*., 2020; Sun *et al*., 2023) Among all nutrients, proteins are indispensable for humans because they play a vital role in tissue formation, cellular repair, and metabolic regulation (Nehete *et al.*, 2013).

The global demand for protein is rapidly increasing. With continuous population growth, one of the greatest challenges is ensuring access to cost-effective protein sources that meet nutritional, environmental, and social sustainability standards (Sá *et al*., 2020; Guan *et al*, 2022; Gao *et al*., 2023). Consequently, there is an urgent need to identify and develop new, sustainable sources of food proteins.

Animal-derived proteins, while nutritionally valuable, are costly in terms of market price, land use, and environmental impact. The surge in consumer demand for plant-based proteins has been driven by both perceived health benefits and the lower environmental footprint of plant-derived foods compared to animal-based alternatives (Bouchard *et al*, 2022). The low efficiency of converting plant biomass into animal protein, combined with high production costs, further amplifies the need for alternative, plant-based protein sources (Gao *et al*., 2023; Kumar(a) *et al.*, 2022). Thus, identifying and exploiting plant proteins of high nutritional quality to complement or replace animal proteins has become a major research and industrial priority (Li *et al*., 2022). Consumer confidence in plant-based proteins has also grown considerably (Gonzáles-Pérez & Arellano, 2009; Pam *et al*., 2020). Currently, plant sources account for approximately 57% of global protein supply, while meat contributes 18%, dairy 10%, fish and shellfish 6%, and other animal-derived products 9% (Henchion *et al*.,2017). The functionality of plant proteins is crucial not only for human nutrition but also for animal feed formulations and biofuel production (Bean *et al.*, 2011).

Recent studies have demonstrated that proteins extracted from pulses and whole grains contain bioactive peptides with disease-preventive properties. Traditional plant protein sources such as soybeans, beans, and peas have long been utilized by humans. However, more recent research focuses on exploring novel protein sources including plants, algae, and entomoproteins (Sá *et al*., 2020; Djouadi *et al.*, 2022). The major plant-derived protein sources under scientific and industrial investigation include legumes, algae, cereals (and pseudocereals such as quinoa, amaranth, and buckwheat), seeds, roots, leaves, almonds, nuts, fruits, and agro-industrial by-products (Table 1) (Sá *et al*., 2020).



Crop	Vegetal protein source	Main protein type	Bibliography
Algae	Chlorella 43% - 50%	Globulin Albumin	Nazarov et al., 2021; Chia et al., 2019)
Cereals	Rice 7.1% - 8.3% Oat 15%-20% Amaranth 13%-22% Wheat 13%	Prolamin Glutelin	(Shewry & Tatham, 1990; Bouchard et al., 2022; Zhao et al., 1983; (Bouchard, *et al.*,2022))
Legumes	Chickpea 20.9%- 25.27% Lentils 26% Peas 19.5%- 20.6% Lupine 40%	Globulin Albumin	(Sathe, 2002; Saharan & Khetarpaul; Dias et al., 2024; Dhawan et al., 1991)
Oil seed	Walnut 15% – 20% Peanut 22% - 33% Almond 21.2% Sunflower 40%	Albumin Globulin Glutelin	(Mao et al., 2014; Sathe et al., 2012; González-Pérez, 2015)
Tubers	Taro 1% - 4.5% Sweet potatoes 1% – 3% Potatoes 20%	*Albumin* *Globulin* *Saporamin* *Patatin*	(Pęksa & Miedzianka, 2021; Shewry, 2003)

Table 1- The potential of different crops for protein production. The potential for protein production was evident in the algae.

Various crops exhibit high protein production potential (Table 1), particularly algae, sunflower, and, legumes with protein contents of approximately 45%, 40%, and 25%, respectively. Legume proteins possess the additional advantage of being water- or salt-soluble, which simplifies their extraction (Dias *et al*., 2024). The effectiveness of proteins in food applications depends not only on their nutritional quality but also on their functional properties (Kumar(b) *et al*., 2022)

The functional properties of proteins—those determining their performance in food systems—are largely governed by physicochemical characteristics, including molecular weight, size, shape, flexibility, amino acid composition, net charge, charge distribution, and hydrophobicity (Pam *et al*., 2020; Kumar(b) *et al.*, 2022). To comprehend the physical-chemical characteristics of plant proteins, it is necessary to undertake their classification.

Plant proteins are traditionally categorized into housekeeping and storage proteins according to their physiological roles. While housekeeping proteins participate in metabolic processes, storage proteins act as nitrogen and energy reserves for germination and early seedling development (Jain, 2023; Derbyshire *et al*., 1976).

Proteins may be categorized based on evolutionary relationships, chemical structure, mechanisms of action, biological function, cellular localization, or the separation technique employed during purification (Boulter & Derbyshire, 2024). According to Rasheed and colleagues, (2020), the earliest classification attempts for plant proteins were based on extractability and solubility, with the first systematic study of seed storage proteins conducted by T. B. Osborne, following the guidelines of the American Committee on Protein

Nomenclature (*American Physiological Society*. 1907). This nomenclature distinguishes proteins as simple, conjugated, or derived. Simple proteins are further subdivided into four primary categories (Osborne, 1924), according to their solubility in water, salt, alcohol, or acidic/alkaline solutions (Rosa *et al*., 2000, Lima *et al*., 2016), corresponding to the four classic fractions: albumins, globulins, prolamins, and glutelin, respectively (Chena *et al.*, 2018; Wang *et al.*, 2021). Although widely used, the Osborne solubility classification is considered crude due to overlapping solubility ranges among protein types, Osborne framework remains the foundation for seed protein categorization. Despite these attempts at more modern classification systems, Osborne classification (Table 2) is still the most widely used system, mainly used for protein extraction and purification procedures (Rasheed *et al*., 2020).

The increasing global demand for proteins, driven by population growth, is stimulating the search for new and sustainable protein sources. Plants provide a promising alternative for producing proteins with functional characteristics similar to those of animal proteins, yet with lower economic and environmental costs. Glutelin functions primarily as a seed storage protein (SSP). Plant storage proteins serve as essential reservoirs of amino acids, nitrogen, carbon, and sulfur required for seedling germination, and they constitute a major dietary source of protein for both humans and animals. Given their structural diversity and broad range of techno-functional properties—including solubility, thermal stability, aggregation behavior, and molecular weight distribution—characterizing the physicochemical attributes of plant glutelin is essential for advancing their application in food, nutraceutical, and biomaterial industries. In this context, the present review aimed to compile and critically evaluate current knowledge regarding the physicochemical characteristics of glutelin fractions isolated from plant sources.

Materials and Methods

This narrative review was conducted with the objective of compiling and synthesizing the available scientific knowledge on plant-derived proteins, with a specific focus on glutelins. A comprehensive literature search was performed between October 2024 and September 2025 in the following databases: ScienceDirect, Scopus, Web of Science, PubMed/Medline, and Google Scholar. The search strategy employed the following descriptors and boolean combinations, adapted for each database: “plant protein” **OR** “seed storage protein” **AND** “plant protein solubility” **OR** “glutelin plant protein” **OR** “molecular weight plant protein.”

Eligible documents included peer-reviewed original research articles, review papers, and book chapters addressing the physicochemical properties of glutelin proteins of plant origin. Studies without full-text availability, duplicated records, and publications lacking relevance to the review objectives were excluded.

Article selection was performed manually by the authors through title and abstract screening, followed by full-text evaluation. Extracted information was categorized into thematic domains, including:

(1) physicochemical composition of plant glutelins;
(2) structural and molecular characteristics;
(3) solubility behavior under different environmental conditions;
(4) functional properties relevant to food applications.

Results and Discussion

Characterization of Plant Proteins

Protein Type	Solubility	Characteristics	Examples in Plants
Albumins	Water	Globular, coagulable by heat	2S-type; e.g., Leucine, Legumalin, Phaselin, Ricin
Globulins	Salt solution	Globular, higher molecular weight than albumins Intra-molecular	7S vicilin-type (peas, soy etc.) 11S legumin-type (brassicas, oat, rice)
Prolamins	Alcohol/water mixtures (e.g., 70% ethanol)	Intra-molecular disulfide bonds, High proportion of proline and glutamine, repetitive motifs in central domains	Gliadins, Zein, Hordein, Secalin
Glutelins	Alkaline solutions	Intermolecular disulfide bonds, High proportion of proline and glutamine, repetitive motifs in central domains	Glutenins in wheat Glutelins in rice

Table 2- Types, characteristics (based on Osborne fractionation), and presence of plant proteins adopted by (Rasheed *et al*., 2020).

Compared with animal proteins, plant proteins offer substantial sustainability benefits and a lower environmental footprint, making them attractive for food system innovation. They can serve as delivery matrices for bioactive compounds, enhancing the stability, solubility, and bioavailability of functional ingredients through various encapsulation and distribution systems (Guan *et al*, 2022).

The functional properties of proteins determine their performance in food applications. These include properties such as the amino acid composition, molecular weight distribution, tertiary and quaternary structural morphology, thermal stability, water and oil absorption capacity, and in vitro digestibility (Kumar(b) *et al*., 2022). For example, Lupinus species are protein-rich and exhibit diverse biological activities, including anticancer, antibacterial, antifungal, and antioxidant properties. (Duarte *et al*., 2022; Pinheiro *et al*., 2017; Joana *et al*., 2022).

Amino acid composition of *Fagopyrum esculentum* (buckwheat) flour proteins

Globulins contained high levels of methionine and lysine, while prolamins were enriched in histidine, threonine, valine, isoleucine, and leucine. Glutelins were abundant in histidine, threonine, valine, isoleucine, leucine (Guo, & Yao), as well as methionine and cysteine (Bouchard, *et al.,*2022).

The solubility behavior of albumin and glutelin fractions exhibited a typical U-shaped pattern with respect to pH, reaching minimal solubility near pH 4.0. This phenomenon is attributed to the reduction in net surface charge at the isoelectric point, where electrostatic repulsion among protein molecules is minimized, leading to aggregation and reduced solubility (Chena *et al.*, 2018). At pH levels above or below this point, the net charge on protein molecules increases, thereby enhancing solubility due to increased intermolecular repulsion.

According to Ispano and collaborators (Shewry *et al.*, 2002), the Pfam database (http://www.sanger.ac.uk/Software/Pfam/) provides a comprehensive classification system for plant protein families based on structural and evolutionary relationships. Historically, however, the classification of plant proteins was pioneered by Osborne and contemporaries, who categorized them based on solubility and extractability in various solvents—namely water (albumins), dilute saline (globulins), alcohol/water mixtures (prolamins), and dilute acid or alkali (glutelins)—a system still widely employed today (Figure 2).

Functionally, most proteins from the albumin fraction participate in metabolic processes, including enzymatic reactions and protease or amylase inhibition, and often include lectins. In contrast, globulins, prolamins, and glutelins function primarily as seed storage proteins (SSPs) that provide nitrogen and carbon sources during germination (Boye *et al*., 2010).

Albumins

Albumins are defined as water-soluble, globular proteins that are coagulable by heat. The most well-known examples include serum albumin, the major protein in human blood, and ovalbumin from egg white. In plants, albumins occur as 2S seed storage proteins (Youle, & Huang, 1981). Various crops contain albumin fractions with distinct compositions: leucine-rich albumins in barley (*Hordeum vulgare*), wheat (*Triticum*), and rye (*Secale cereale*); legumelin in pea, soybean, and cowpea; phaselin in kidney bean; and ricin in castor bean (*Ricinus communis*) (Shewry & Pandya, 1999). Amino acid composition of *Fagopyrum esculentum* (buckwheat) flour proteins revealed that albumins were particularly rich in histidine, threonine, valine, phenylalanine, isoleucine, leucine, and lysine.

Albumins play important roles in the metabolic processes of maturing plants and contribute to the synthesis of defense compounds (Guo & Yao, 2006; Bean *et al*., 2011). As a major seed storage protein group widely distributed among both monocotyledonous and dicotyledonous species, albumins are stored within protein bodies of developing seeds. During germination and seedling growth, plants mobilize these proteins as sources of amino acids and carbon skeletons. In addition, 2S albumins serve a protective role, acting as defensive agents against fungal invasion (Moreno, & Clemente, 2008).

Based on their sedimentation coefficients, plant storage albumin is classified into 2S albumins with 8–10 kDa (small subunit) and 12–15 kDa (large subunit), giving a total molecular weight between 18 and 25 kDa. The 2S albumins are characterized by a high nitrogen content, primarily from glutamine, glutamate, asparagine, aspartate, and arginine. Notably, they share a high cysteine and methionine composition, distinguishing them from globulins Derbyshire *et al*., 1976). In most species, cysteine represents 6–13% of total amino acids, while methionine ranges from 1.6–3.8%. An exception is peanut albumin, which is deficient in both cysteine and methionine, whereas the Brazil nut (Bertholletia excelsa) 2S albumin contains an exceptionally high 17% methionine (Youle, & Huang, 1981).

Globulins

Globulins are globular proteins with higher molecular weights than albumins. They are soluble in dilute salt solutions but insoluble in water. In humans, serum γ-globulin represents the most well-known member of this group (Rasheed *et al*., 2020). In plants, globulins function as storage proteins in both dicots and monocots, making them one of the most prevalent storage protein groups (Franco *et al*., 1997). Globulins are also particularly rich in methionine and lysine (Guo & Yao, 2006).

Based on their sedimentation coefficients, plant storage globulins are classified into two principal categories: 7S (vicilin-type or β-conglutins) and 11S (legumin-type or α-conglutins). The 7S globulins (150–200 kDa) are predominant in peas and soybeans, while the 11S globulins (300–450 kDa) represent the major storage proteins in legumes such as *Lupinus* and *Brassica* species (Rasheed *et al*., 2020). Similar to albumins, globulins also play essential roles in plant metabolism and defense responses (Guo & Yao, 2006; Bean *et al.*, 2011). Bioactive peptides derived from globulins have demonstrated antifungal (Monteiro *et al.*, 2015; Carreira *et al.*, 2018), antibacterial (Abdel-Shafi *et al*., 2019), and anticancer properties, highlighting their functional versatility and potential in food and pharmaceutical applications.

Prolamins

Prolamins and glutelins are hydrophobic seed storage proteins that accumulate in protein bodies or small vacuoles. They are characterized by low water solubility and high levels of proline and glutamine ((Gonzáles-Pérez & Arellano, 2009; Bean *et al.*, 2011). Osborne first identified this unique class of alcohol-soluble cereal proteins and coined the term "prolamins" to reflect their high proline and amide nitrogen content (later recognized as derived from glutamine).

In most cereals, prolamins constitute the dominant class of seed storage proteins, followed by glutelin. Prolamins are enriched in histidine, threonine, valine, isoleucine, and leucine (Guo & Yao, 2006). In various cereals, prolamins are referred to by specific names derived from their Latin generic names: *gliadins* in wheat (*Triticum aestivum*), *zeins* in maize (*Zea mays*), *hordeins* in barley (*Hordeum vulgare*), *secalins* in rye (*Secale cereale*), *kafirins* in sorghum (*Sorghum bicolor*), and *pennisetins* in pearl millet (*Pennisetum glaucum*) (Bean *et al.*, 2011; Shewry & Tatham, 1990; Bibi *et al*., 2021). The 11- and 156-residue N- and C-terminal regions of prolamins flank a repetitive domain composed of eight conserved hexapeptides (Pro-Pro-Pro-Val-His-Leu). This sequence confers hydrophilic characteristics that paradoxically contribute to reduced solubility in water (Shewry & Tatham, 1990).

Cereal prolamins exhibit insolubility in water or dilute salt solutions, even when reduced, but become soluble in alcohol/water mixtures. Their solubility characteristics are attributed to repetitive amino acid sequences (as in α-zeins and Triticeae prolamins) or methionine-rich domains (in β- and γ-zeins). In contrast, δ-zeins are water-soluble when reduced but can form insoluble polymers through interchain disulfide bonding (Shewry & Tatham, 1990).

Prolamins possess distinctive amino acid profiles, typically enriched in proline, glutamine, methionine, aromatic amino acids, and glycine. These residues are critical to maintaining the structural integrity and solubility of the protein (Shewry & Tatham, 1990).

Based on molecular weight, prolamins are categorized into three primary groups: (a) High-molecular-weight (HMW) prolamins, (b) Medium-molecular-weight (MMW) prolamins, and (c) Low-molecular-weight (LMW) prolamins— the most abundant fraction in major cereal crops (Shewry & Tatham, 1990; Wieser, 2008).

Glutelin

Glutelin and prolamin are hydrophobic seed storage proteins that accumulate in protein bodies or small vacuoles. Rice and oats, containing higher concentrations of glutelin and globulins

(Bouchard, *et al.,*2022). Glutelin, the predominant protein in rice, is primarily composed of glutamine, asparagine, arginine, glycine, and alanine (Zhao *et al*., 2020), histidine, threonine, valine, isoleucine, and leucine. SDS–PAGE analysis under both reductive and non-reductive conditions confirms the presence of disulfide bonds across the major protein fractions (Guo & Yao, 2006).

Glutelin Proteins Characterization

Solubility of Glutelin Proteins

Approximately half of the global dietary protein supply originates from cereal seeds, where most proteins accumulate within protein bodies (PBs) in the endoplasmic reticulum (ER) (Pedrazzini *et al*., 2016). Two types of PBs—low- and high-density forms—develop concurrently in wheat endosperm cells. Gliadins are found in both types, whereas high-molecular-weight glutenins localize primarily in dense PBs, suggesting aggregation within the rough ER (Rubin, *et al.*, 1992).

mRNA localization within the ER governs subcellular protein targeting and storage organelle biogenesis. Distinct localization patterns of storage protein mRNAs define the deposition of corresponding proteins within specific endosomal compartments of cereal grains (Zhang *et al.*, 2022). In rice, proglutelins and α-globulins are synthesized separately in the cisternal ER and PB-ER, then co-transported via the Golgi apparatus into protein storage vacuoles (PB-II) (Li et *al.*, 2022).. Takahashi and collaborators, 2004 further proposed a direct transport pathway from the ER to protein storage vacuoles through precursor-accumulating vesicles. Protein accumulation begins approximately five days after flowering, with a 57 kDa polypeptide precursor giving rise to 22–23 kDa (acidic) and 37–39 kDa (basic) subunits, alongside a 26 kDa globulin component. Smaller 10–16 kDa prolamin peptides appear later during PB formation (Li et *al.*, 2022).

At the primary structure level, glutelin are rich in glutamine, asparagine, and hydrophobic amino acids such as leucine and isoleucine (Zhao *et al*., 2020). The high content of amide side chains from glutamine and asparagine promotes intermolecular hydrogen bonding and protein aggregation, reducing solubility under neutral pH. The secondary structure of glutelins is composed primarily of α-helices and β-sheets, stabilized by hydrogen bonds, which influence their conformational rigidity and their limited flexibility in aqueous environments (Pedrazzini *et al*., 2016).

The tertiary structure of glutelins is stabilized by extensive hydrophobic interactions and disulfide bridges (–S–S–) between cysteine residues. These bonds create compact, polymeric conformations that make the protein more thermally stable but also less soluble in water (Li et al., 2022). This compact folding limits solvent penetration, leading to aggregation and precipitation at isoelectric pH values (pH 4–5) Figure 1.

The quaternary structure of glutelins, particularly in cereal species such as wheat and rice, involves the association of multiple polypeptide subunits (typically one acidic and one basic) linked by disulfide bonds. In rice, glutelin form protein bodies (PB-II) within the endosperm, where they aggregate into large complexes via hydrophobic and covalent interactions (Kumamaru et al., 2010).

Protein solubility is strongly influenced by extrinsic factors such as ionic strength, solution composition, pH, and temperature. Low solubility remains a critical challenge across several fields, including structural genomics, protein engineering, human disease studies, and industrial applications. It also limits the stability and efficacy of protein-based pharmaceuticals during preparation, sterilization, and storage (Trevino *et al.*, 2008).

In food systems, protein solubility is directly associated with functional properties such as emulsification, foaming, gelation, and rheological behavior. However, many plant-based proteins exhibit poor aqueous solubility, largely due to complex interactions between environmental conditions and heterogeneous fraction compositions (Gao *et al.*, 2023). Glutelin, the predominant protein in rice, is primarily composed of glutamine, asparagine, arginine, glycine, and alanine. The amide groups of glutamine and asparagine facilitate protein aggregation, contributing to low solubility and poor surface-active behavior under neutral pH (Paraman *et al*., 2007; Zhao *et al*., 2020). The amide side chains and hydrogen-bonding capacity of these amino acids reduce molecular flexibility, leading to aggregation. Furthermore, hydrophobic and sulfhydryl-disulfide (SH–SS) interactions are major contributors to glutelin insolubility, even under high degrees of hydrolysis. Exposure of buried hydrophobic regions during heat denaturation or enzyme inactivation promotes aggregation and cross-linking, which diminishes both solubility and emulsifying capacity (Paraman *et al.*, 2007). The underlying mechanisms likely involve disulfide bond formation and complex non-covalent interactions, although these remain to be fully elucidated (Mao *et al*., 2012).

Variations in surface hydrophobicity among proteins are largely due to differences in amino acid composition and exposure of aromatic and aliphatic residues during extraction and processing. The solubility profiles of albumin and glutelin typically exhibit a U-shaped curve relative to pH, with minimum solubility near pH 4.0, where the net surface charge is lowest and protein–protein interactions dominate (Chena *et al.*, 2018). The solubility of the glutelin protein is a pressing challenge that needs to be understood to promote the use of this protein fraction.

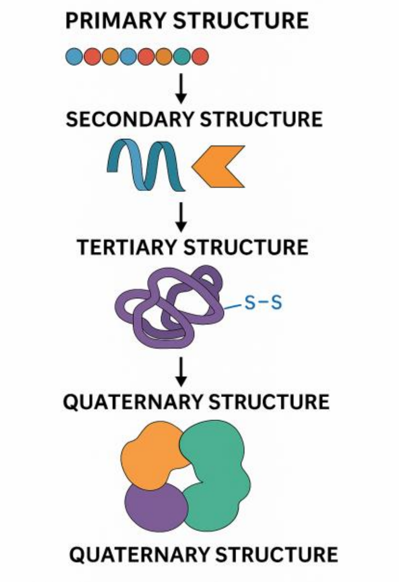


Figure 1. AI generated plant protein structures

Structural Modifications and Functional Improvement

Protein functionality depends not only on intrinsic characteristics such as amino acid composition, molecular weight, and tertiary structure but also on environmental parameters including ionic strength, pH, and temperature during processing (Zhao *et al*., 2019; Wang *et al.*, 2021). Protein solubility affects industrial food functionality, as it determines properties such as water absorption capacity, emulsion stability, and gel formation (Wang *et al.*, 2021). Water absorption, emulsion stability, and gel formation are crucial protein functions that dictate the texture, structure, and shelf life of many food products. Water absorption affects hydration and moisture retention, preventing dryness. Emulsion stability relies on proteins forming protective barriers at oil-water interfaces, while gel formation creates a three-dimensional network that provides structure and texture (Shen et al., 2022; Silva et al., 2022).

Several strategies have been developed to enhance glutelin solubility and functionality, including the addition of chemical additives (e.g., 50 mM L-arginine and L-glutamic acid), fusion peptides, and targeted hydrophobic or hydrophilic surface mutations (Trevino *et al.,* 2008).

Since the 1950s, researchers have employed structural modification techniques—both enzymatic and genetic—to tailor plant proteins for improved functionality and nutritional performance in food systems (Sun *et al.*, 2019). Modifications in physicochemical and structural attributes directly affect properties such as solubility, emulsification, and digestibility (Kumar(a) *et al.*, 2022). Experimental evidence indicates that thermal treatment (100 °C for 60 min at pH 7) can triple glutelin solubility and double its foaming capacity, accompanied by a reduction in aggregate size (~100 nm), decreased disulfide bond content, and increased surface charge and hydrophobicity (Zhao *et al*., 2019).

Additionally, enzymatic hydrolysis using pepsin in defatted walnut meal glutelin protein improved its surface hydrophobicity, solubility, emulsifying and foaming capacities, and water absorption, confirming that structural reorganization enhances functional behavior (Wang *et al.*, 2021; Sun *et al.*, 2019).

Conclusion

This comprehensive analysis highlights the biochemical complexity and functional diversity of plant storage proteins, particularly albumins, globulins, prolamins, and glutelins, which collectively constitute the foundation of protein reserves in seeds. Each class demonstrates distinctive solubility profiles, amino acid compositions, and physiological roles, reflecting their adaptation to specific plant metabolic and structural requirements.

Functionally, modifying intrinsic properties through physical (thermal), chemical, or enzymatic treatments has shown promising results, enhancing solubility, emulsifying, and foaming behaviors—critical for the food and nutraceutical industries. Moreover, the identification of bioactive peptides, including anti-inflammatory and anticancer fractions, underscores the biomedical relevance of these proteins beyond their nutritional roles.

In conclusion, a deeper understanding of the structural organization, solubility dynamics, and interrelationships among seed storage proteins can guide the development of novel plant-based protein sources with tailored functional and health-promoting attributes, contributing to sustainable nutrition and biotechnology innovation.

Bibliography

Abdel-Shafi, S. Al-Mohammadi, A. Osman, A. Enan, G. Abdel-Hameid, S. and Sitohy, M. (2019). "Characterization and antibacterial activity of 7S and 11S globulins isolated from cowpea seed protein," *Molecules*, vol. 24, no. 6, pp. 1–15: 10.3390/molecules24061082.

American Physiological Society. (1907): https://doi.org/10.1126/science.27.692.554.

Bean, S. Ioerger, B. Smith, B. and Blackwell, D. (2011), "Sorghum Protein Structure and Chemistry: Implications for Nutrition and Functionality," in *Advances in Cereal Science: Implications to food processing and health promotion*, J. A. Awika, S. R. Bean, and V. Piironen, Eds., American Chemical Society, pp. 131–147: http://dx.doi.org/10.1021/bk-2011-1089.ch007.

Bibi, R. Mokrane, H. Khaladi, K. and Amoura, H. (2021). "Improvement of Pearl millet (Pennisetum glaucum (L.) R. BR) prolamin extractability: Chromatographic separation, characterization and functional properties," *Journal of microbiology, biotechnology and food sciences*, vol. 11, no. 2, p. 3664 - 3674: 10.15414/jmbfs.3674.

Bouchard, J. Malalgoda, M. Storsley, J. Malunga, L. Netticadan, T. and Thandapilly, S. (2022). "Health Benefits of Cereal Grain-and Pulse-Derived Proteins," *Molecules*, vol. 27, no. 12, pp. 1–26: 10.3390/molecules27123746.

Boulter, D. and Derbyshire, E. (2024). "The general properties, classification and distribution of plant proteins," in *Plant Proteins*, 1st ed., vol. 1, Nottigham: Norton, G., 1977, pp. 333–24. Accessed: Feb. 25, [Online]. Available: https://www.sciencedirect.com/book/9780408709187/plant-proteins

Bozhkova, M. and Dencheva, A. (1995). "Ultrasound influence on Scenedesmus acutus productivity and protein content," *BULG. J. PLANT PHYSIOL*, vol. 21, no. 1, pp. 58–67.

Carreira, A. Ferreira, J. Pereira, I. Ferreira, J. Filipe, P. Ferreira, R and Monteiro, S (2018). "Blad-containing oligomer: A novel fungicide used in crop protection as an alternative treatment for tinea pedis and tinea versicolor," *J Med Microbiol*, vol. 67, no. 2, pp. 198–207: 10.1099/jmm.0.000675.

Chena, J. Mua, T. Zhanga, M. Goffinb, D. Maa, H. Liua, X. and Zhanga, D. (2018). "Structure, physicochemical, and functional properties of protein isolates and major fractions from cumin (Cuminum cyminum) seeds," *Int J Food Prop*, vol. 21, no. 1, pp. 685–701: 10.1080/10942912.2018.1454467.

Chia, S. Chew, K. Zaid, H. Chu, D. Tao, Y. and Show, P. (2019). "Microalgal Protein Extraction From Chlorella vulgaris FSP-E Using Triphasic Partitioning Technique With Sonication," *Front Bioeng Biotechnol*, vol. 7: 10.3389/fbioe.2019.00396.

Derbyshire, E. Wright, J., and Boulter, D. (1976). "Legumin and vicilin, storage proteins of legume seeds," *Phytochemistry*, vol. 15, no. 1, pp. 3–24: 10.1016/S0031-9422(00)89046-9.

Dhawan, K. Malhotra, S. Dahiya, B. and Singh, D. (1991). "Seed protein fractions and amino acid composition in gram (Cicer arietinum)," *Plant Foods for Human Nutrition*, vol. 41, no. 3, pp. 225–232: 10.1007/BF02196390.

Dias, F. Yang, J. Pham, T Barile, D. and Moura-Bell, J. (2024). "Unveiling the contribution of Osborne protein fractions to the physicochemical and functional properties of alkaline and enzymatically extracted green lentil proteins," *Sustainable Food Proteins*: 10.1002/sfp2.1026.

Djouadi, A. Sales, J. Carvalho, M. and Raymundo, A. (2022). "Development of Healthy Protein-Rich Crackers Using Tenebrio molitor Flour," *Foods*, vol. 11, no. 5: 10.3390/foods11050702.

Duarte, C. Mota, J. Assunção, R. Martins, C. Ribeiro, A. Lima, A. Raymundo, A. Nunes, M. Ferreira, R. Sousa, I. (2022). "New Alternatives to Milk From Pulses: Chickpea and Lupin Beverages With Improved Digestibility and Potential Bioactivities for Human Health," *Front Nutr*, vol. 9: 10.3389/fnut.2022.852907.

Franco, E. Ferreira, R. Teixeira, A. (1997). "Utilization of an improved methodology to isolate lupinus albus conglutins in the study of their sedimentation coefficients" *J Agric Food Chem*, vol. 45, no. 10, pp. 3908–3913: 10.1021/jf9702813.

Freitas, R. Teixeira, A. and Ferreira, R (2004). "Characterization of the Proteins from Vigna unguiculata Seeds," *J Agric Food Chem*, vol. 52, no. 6, pp. 1682–1687: 10.1021/jf0300588.

Gao, K. Rao, J. and Chen, B. (2023). "Plant protein solubility: A challenge or insurmountable obstacle," *Adv Colloid Interface Sci*, p. 103074: 10.1016/j.cis.2023.103074.

Gonzáles-Pérez, S. and Arellano, J. (2009). "Vegetable protein isolates," in *Handbook of hydrocolloids Vegetable*, 2nd ed., G. Phillips and P. Williams, Eds., Salamanca - Spain: Elsevier, pp. 383–419. : 10.1533/9781845695873.383.

González-Pérez, S. (2015). "Sunflower Proteins," in *Sunflower*, Elsevier, pp. 331–393. : 10.1016/B978-1-893997-94-3.50018-0.

Guant, T. Zhiheng, Z. Li, X. Cui, S. Julian, D. Clements, M. Chen, L. Long, J. Jiao, A. Qiu, C. and Jin, Z. (2022). "Preparation, Characteristics, and Advantages of Plant Protein-Based Bioactive Molecule Delivery Systems," *Foods*, vol. 11, no. 11: 10.3390/foods11111562.

Guo, X. and Yao H. (2006). "Fractionation and characterization of tartary buckwheat flour proteins," *Food Chem*, vol. 98, no. 1, pp. 90–94: 10.1016/j.foodchem.2005.05.055.

Henchion, M. Hayes, M. Mullen, A. Fenelon, M. and Tiwari, B. (2017). "Future protein supply and demand: Strategies and factors influencing a sustainable equilibrium," *Foods*, vol. 6, no. 7, pp. 1–21: 10.3390/foods6070053.

Hojilla-Evangelista, M. Selling, G. Hatfield, R. and Digman, M. (2017). "Extraction, composition, and functional properties of dried alfalfa (Medicago sativa L.) leaf protein," *J Sci Food Agric*, vol. 97, no. 3, pp. 882–888: 10.1002/jsfa.7810.

Ismail, B. Senaratne-Lenagala, L. Stube, A. and Brackenridge, A. (2020). "Protein demand: Review of plant and animal proteins used in alternative protein product development and production," *Animal Frontiers*, vol. 10, no. 4, pp. 53–63: 10.1093/af/vfaa040.

Jain, A. (2023). "Seed Storage Protein, Functional Diversity and Association with Allergy," *Allergies*, vol. 3, no. 1, pp. 25–38: 10.3390/allergies3010003.

Kawakatsu, T. Yamamoto, Hirose, M. Yano, S and Takaiwa, F. (2008). "Characterization of a new rice glutelin gene GluD-1 expressed in the starchy endosperm," *J Exp Bot*, vol. 59, no. 15, pp. 4233–4245, 2008: 10.1093/jxb/ern265.

Kumamaru, T., Ogawa, M., Satoh, H., Okita, T.W. (2007). Protein Body Biogenesis in Cereal Endosperms. In: Olsen, OA. (eds) Endosperm. Plant Cell Monographs, vol 8. Springer, Berlin, Heidelberg. https://doi.org/10.1007/7089_2007_115

Kumar[(a)], M. Tomar, M. Potkule, J. Reetu, C. Punia, S. Dhakane-Lad J. Singh, S. Dhumal, S. Chandra P. Bhushan, B. Anitha, T. Alajil, O. Alhariri, A. Amarowiczm, R. Kennedy, F. (2022). "Functional characterization of plant-based protein to determine its quality for food applications," *Food Hydrocolloids*, vol. 123. Elsevier B.V.: 10.1016/j.foodhyd.2021.106986.

Kumar[(b)], M. Tomar, M. Punia, S. Dhakane-Lad, J. Dhumal, S. Changan, S. Senapathy, M Berwal, M Sampathrajan, V. Sayed, A. Chandran, D. Pandiselvam, R. Raism, N. Mahato, D. Udikeri, S. Satankar, V. Anitha, T. Radha, R. Singh, S Amarowicz, R. Kennedy, J. (2022). "Plant-based proteins and their multifaceted industrial applications," *Lwt*, vol. 154, no. October 2021, p. 112620: 10.1016/j.lwt.2021.112620.

Li, Y. Zhang, C. Liang, Y. Wang, L. and Xiong, W. (2022). "Improving the Solubility of Rice Glutelin by High Temperature Cooking," *SSRN Electronic Journal*, pp. 1–18: 10.2139/ssrn.4129730.

Li, Y. Zhang, C. Liang, Y. Wang, L. Xiong, X. (2022). "Solubility and conformational characterization of rice glutelin after high temperature treatment," *Int. J. Biol. Macromol.* Vol. 223, pp. 1720-1726: 10.1016/j.ijbiomac.2022.10.100

Lima, A. Mota, J. Monteiro, A. and Ferreira, R. (2016). "Legume seeds and colorectal cancer revisited: Protease inhibitors reduce MMP-9 activity and colon cancer cell migration," *Food Chem*, vol. 197, pp. 30–38: 10.1016/j.foodchem.2015.10.063.

Mao, X. Hua, Y. and Chen, G. (2014). "Amino Acid Composition, Molecular Weight Distribution and Gel Electrophoresis of Walnut (Juglans regia L.) Proteins and Protein Fractionations," *Int J Mol Sci*, vol. 15, no. 2, pp. 2003–2014: 10.3390/ijms15022003.

Mao, X. Tong, P. Gualco, S. and Vink, S. (2012). "Effect of NaCl addition during diafiltration on the solubility, hydrophobicity, and disulfide bonds of 80% milk protein concentrate powder," *J Dairy Sci*, vol. 95, no. 7, pp. 3481–3488: 10.3168/jds.2011-4691.

Monteiro, S. Carreira, A. Freitas, R. Pinheiro, A. and Ferreira, R. (2015). "A nontoxic polypeptide oligomer with a fungicide potency under agricultural conditions which is equal or greater than that of their chemical counterparts," *PLoS One*, vol. 10, no. 4, pp. 1–23: 10.1371/journal.pone.0122095.

Moreno, F. and Clemente, A. (2008). "2S Albumin Storage Proteins: What Makes them Food Allergens?," *Open Biochem J*, vol. 2, pp. 16–28: 10.2174/1874091x00802010016.

Mota, J. Casimiro, S. Fernandes, J. Hartmann, R. Schemitt, E. Picada, J. Costa, L. Marroni, N. Raymundo, A. Lima, A. Ferreira, R. (2022). "Lupin Protein Concentrate as a Novel Functional Food Additive That Can Reduce Colitis-Induced Inflammation and Oxidative Stress," *Nutrients*, vol. 14, no. 10, p. 2102: 10.3390/nu14102102.

Nazarov, N. Vyshtakalyuk, A. Zobov, V. and Semenov, V. (2021). "The study of the toxicity of the L-ascorbate 1-(2-hydroxyethyl)-4,6-dimethyl-1,2-dihydropyrimidine-2-one on Chlorella vulgaris," p. 020023. : 10.1063/5.0069038.

Nim, Y. Sun, S. and Wong K. (2017). "Using homology modeling to understand the structural basis of specific interaction of a plant-specific AtSar1a-AtSec23a pair involved in protein ER export," in *Methods in Molecular Biology*, vol. 1662, Humana Press Inc., pp. 59–73. : 10.1007/978-1-4939-7262-3_5.

Osborne, T. (1924). “The vegetable proteins, by Thomas B. Osborne,” : 10.5962/bhl.title.18912.

Paraman, I. Hettiarachchy, N and Schaefer, C. (2007). “Glycosylation and deamidation of rice endosperm protein for improved solubility and emulsifying properties,” *Cereal Chem*, vol. 84, no. 6, pp. 593–599: 10.1094/CCHEM-84-6-0593.

Pedrazzini, E. Mainieri, D. Marrano, C. and Vitale, A. (2016). “Where do Protein Bodies of Cereal Seeds Come From?,” *Front Plant Sci*, vol. 7: 10.3389/fpls.2016.01139.

Pęksa A. and Miedzianka, J. (2021). “Potato Industry By-Products as a Source of Protein with Beneficial Nutritional, Functional, Health-Promoting and Antimicrobial Properties,” *Applied Sciences*, vol. 11, no. 8, p. 3497: 10.3390/app11083497.

Pinheiro, A. Carreira, A. Prescott, T. Ferreira, R. and Monteiro, S. (2017). “Bridging the gap to non-toxic fungal control: Lupinus-derived blad-containing oligomer as a novel candidate to combat human pathogenic fungi,” *Front Microbiol*, vol. 8, no. JUN, pp. 1–12: 10.3389/fmicb.2017.01182.

Rasheed, F. Markgren, J. and Hedenqvist, M. (2020). “Modeling to Understand Plant Protein,” *Molecules*, vol. 25, no. 4, pp. 873–889: 10.3390/molecules25040873

Rosa, M. Ferreira,R. and Teixeira, A. (2000). “Storage proteins from Lathyrus sativus seeds,” *J Agric Food Chem*, vol. 48, no. 11, pp. 5432–5439: 10.1021/jf000447r.

Rubin, R. Levanony, H. and Galili, G. (1992). “Evidence for the Presence of Two Different Types of Protein Bodies in Wheat Endosperm,” *Plant Physiol*, vol. 99, no. 2, pp. 718–724: 10.1104/pp.99.2.718.

Sá, A. Moreno, Y. and Carciofi, B. (2020). “Plant proteins as high-quality nutritional source for human diet,” *Trends Food Sci Technol*, vol. 97, no. pp. 170–184: 10.1016/j.tifs.2020.01.011.

Saharan K. and Khetarpaul, N. (1994). “Protein quality traits of vegetable and field peas: Varietal differences,” *Plant Foods for Human Nutrition*, vol. 45, no. 1, pp. 11–22: 10.1007/BF01091225.

Sathe, S. (2002). “Dry Bean Protein Functionality,” *Crit Rev Biotechnol*, vol. 22, no. 2, pp. 175–223: 10.1080/07388550290789487.

Sathe, S. Kshirsagar, H. and Sharma, G. (2012). “Solubilization, Fractionation, and Electrophoretic Characterization of Inca Peanut (*Plukenetia volubilis* L.) Proteins,” *Plant Foods for Human Nutrition*, vol. 67, no. 3, pp. 247–255: 10.1007/s11130-012-0301-5.

Shen, Y., Hong, S., & Li, Y. (2022). Pea protein composition, functionality, modification, and food applications: A review. In J. Wu (Ed.), Advances in Food and Nutrition Research (Academic P, pp. 71–127). Academic Press. https://doi.org/https://doi.org/10.1016/bs.afnr.2022.02.002.

Shewry, P. (2003). “Tuber Storage Proteins,” *Ann Bot*, vol. 91, no. 7, pp. 755–769: 10.1093/aob/mcg084.

Shewry, P. and Pandya, M. (1999). “The 2S Albumin Storage Proteins,” in *Seed Proteins*, R. Shewry, P.R., Casey, Ed., Dordrecht: Springer, pp. 563–586. : 10.1007/978-94-011-4431-5_24.

Shewry, P. and Tatham, A. (1990). “The prolamin storage proteins of cereal seeds: structure and evolution,” *Biochem. J.*, vol. 267, no. 1, pp. 1–12: 10.1042/bj2670001.

Shewry, P. Beaudoin, F. Jenkins, J. Griffiths-Jonesf, S. Mills, E. (2002). “Plant protein families and their relationships to food allergy,” *Curr. Opin. Allergy Clin. Immunol* , pp 906-910: 10.1042/bst0300906.

Silva, C. M., Felberg, I., Lima, J. R., Gouvêa, L. de P., Galdeano, M. C., Caldeira, R. F., & Azevedo, T. de L. (2022). Guia para Caracterização Tecnológica-Funcional de Ingredientes Proteicos para o Mercado de Produtos de Origem Vegetal. Embrapa Agroindustria de Alimentos, 145(1516–8247), 27.

Sun, Q. Ma, Z. Zhang, H. Ma, S. and Kong, L. (2019) “Structural characteristics and functional properties of walnut glutelin as hydrolyzed: effect of enzymatic modification,” *Int J Food Prop*, vol. 22, no. 1, pp. 265–279: 10.1080/10942912.2019.1579738.

Sun, X. Abioye, R. Acquah, C. and Udenigwe, C. (2023). “Application of Ultrasound Technology in Plant-Based Proteins: Improving Extraction, Physicochemical, Functional, and Nutritional Properties,” in *Green Protein Processing Technologies from Plants*, A. J. Hernández-Álvarez, M. Mondor, and M. G. Nosworthy, Eds., Springer, Cham, pp. 265–289. : https://doi.org/10.1007/978-3-031-16968-7_11.

Takahashi, H. Rai, M. Kitagawa, T. Morita, S. Masumura, T. and Tanaka, K. (2004). “Differential localization of tonoplast intrinsic proteins on the membrane of protein body type II and aleurone grain in rice seeds,” *Biosci Biotechnol Biochem*, vol. 68, no. 8, pp. 1728–1736: 10.1271/bbb.68.1728.

Takaiwa, F. Kikuchi, S. and Oono, K. (1987). “A rice glutelin gene family - A major type of glutelin mRNAs can be divided into two classes,” *MGG Molecular & General Genetics*, vol. 208, no. 1–2, pp. 15–22: 10.1007/BF00330416.

Tanaka, K. Sugimoto, T. Ogawa, M. and Kasai, Z. (1980). “Isolation and characterization of two types of protein bodies in the rice endosperm,” *Agric Biol Chem*, vol. 44, no. 7, pp. 1633–1639: 10.1080/00021369.1980.10864167.

Trevino, S. Scholtz, J. and Pace, C. (2008). “Measuring and increasing protein solubility,” *Journal of Pharmaceutical Sciences*, vol. 97, no. 10. John Wiley and Sons Inc., pp. 4155–4166: 10.1002/jps.21327.

Wang, J. Wang, G. Chen, N. An, F. Zhang, R. Zhang, Y. Rahmana, M. and Zhang, Y. (2021). “Characterization of structural, functional and antioxidant properties and amino acid composition of pepsin-derived glutelin-

1 hydrolysate from walnut processing by-products," *RSC Adv*, vol. 11, no. 31, pp. 19158–19168: 10.1039/d1ra00657f.

Wieser, H. (2008). "Detection of gluten," in *Gluten-free cereal products and beverages*, 8th ed., E. K. (Department of F. and N. S. U. C. C. I. Arendt and F. D. (Department of F. and N. S. U. C. C. I. Bello, Eds., Journal of Cereal Science, pp. 47–72. : https://doi.org/10.1016/B978-0-12-373739-7.X5001-1.

Xiang, Z. (2006). "Advances in homology protein structure modeling," *Curr Protein Pept Sci*, vol. 23, no. 1, pp. 1–7: 10.2174/138920306777452312.

Xiang, Z. (2006). "Advances in Homology Protein Structure Modeling," *Curr Protein Pept Sci*, vol. 7, no. 3, pp. 217–227: 10.2174/138920306777452312.

Youle, R. and A. Huang, H. (1981). "Occurrence of Low Molecular Weight and High Cysteine Containing Albumin Storage Proteins in Oilseeds of Diverse Species," *Am J Bot*, vol. 68, no. 1, pp. 44–48: 10.1002/j.1537-2197.1981.tb06354.x.

Zhang, L. Si, Q. Yang, K. Zhang, W. Okita, T. and Tian, L. (2022). "mRNA Localization to the Endoplasmic Reticulum in Plant Endosperm Cells," *Int J Mol Sci*, vol. 23, no. 21: 10.3390/ijms232113511.

Zhao, M. Xiong, W. Chen, B. Zhu, J. and Wang, L. (2019). "Enhancing the solubility and foam ability of rice glutelin by heat treatment at pH12: Insight into protein structure," *Food Hydrocoll*, vol. 103, p. 105626, 2020: 10.1016/j.foodhyd.2019.105626.

Zhao, M. Xiong, W. Chen, B. Zhu, J. and Wang, L. (2020) "Enhancing the solubility and foam ability of rice glutelin by heat treatment at pH12: Insight into protein structure," *Food Hydrocoll*, vol. 103, no. August 2019, p. 105626: 10.1016/j.foodhyd.2019.105626.

Zhao, W. Gatehouse, J. and Boulter, D. (1983). "The purification and partial amino acid sequence of a polypeptide from the glutelin fraction of rice grains; homology to pea legumin," *FEBS Lett*, vol. 162, no. 1, pp. 96–102: 10.1016/0014-5793(83)81056-4.