Review

Next-generation extraction technologies for plant proteins: Enhancing yield, functionality, and sustainability

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Abstract

Plant protein extraction is vital in biotechnology, pharmaceuticals, and functional foods, where protein functionality directly influences downstream applications. Conventional extraction methods are limited by long processing times, high solvent consumption, and potential degradation of protein bioactivity, necessitating more efficient and protein-preserving approaches. This review (encompassing studies published from 2015 to 2025) evaluates advanced extraction technologies with emphasis on efficiency, sustainability, and preservation of protein functionality. Key strategies include ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and enzyme-assisted extraction (EAE) methods, as well as pulsed electric fields (PEF), supercritical fluid extraction (SFE), ionic liquid extraction (ILE), deep eutectic solvents (DES), pressurized liquid extraction (PLE), three-phase partitioning (TPP), detergent-assisted adsorption, and reversed micellar extraction (RME). These approaches employ physical, chemical, or enzymatic mechanisms to improve efficiency, minimize solvent use, and better preserve protein integrity. UAE, MAE, and EAE increase protein yields and improve functional traits such as solubility, emulsification, and antioxidant activity, while PEF and SFE mainly enhance solubility and purity. ILE and DES are notable for their environmental sustainability, whereas PLE, TPP, and RME combine high yields with faster processing and improved bioactivity preservation. These techniques have demonstrated effectiveness across diverse plant matrices, highlighting their versatility for both research and industrial applications. Emerging trends emphasize the optimization of extraction processes to balance high yields with protein bioactivity, offering valuable insights for developing efficient, sustainable, and environmentally friendly methods in plant-based protein production.

Keywords: Protein extraction, Ultrasound-assisted extraction, Microwave-assisted extraction, Enzymatic-assisted extraction, Pulsed electric field, Supercritical fluid extraction, Ionic liquids, Deep eutectic solvents

1. Introduction

Plant proteins are increasingly valued as sustainable alternatives in food, pharmaceutical, and biotechnological applications, yet their extraction remains technically challenging. Unlike downstream protein processing, recovering proteins from complex plant matrices often presents greater difficulties. Conventional approaches, including alkaline solubilization and solvent-based extraction, frequently result in suboptimal yields, protein denaturation, or degradation. In addition, their heavy reliance on chemical reagents raises concerns about environmental impact, safety, and cost-effectiveness, making them unsuitable for large-scale industrial use.

To address these limitations, a range of advanced extraction methods has been developed and optimized over the past decade. Techniques such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pulsed electric fields (PEF), supercritical fluid extraction (SFE), and deep eutectic solvents (DES) have demonstrated the ability to improve protein yield, purity, and functional properties. Beyond enhancing efficiency, these methods offer greater sustainability by reducing resource demands and environmental burden. For example, UAE of chickpeas required only 66.2 kJ/kg protein compared with 1,941 kJ/kg for conventional alkaline extraction (CAE),[1] while MAE

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of foxtail millet achieved 30% protein recovery in just 66 s, far faster than maceration.[2] Supercritical carbon dioxide (CO_2)–ethanol (EtOH) extraction of lupin preserved protein integrity with less solvent use than Soxhlet extraction,[3] PEF treatment increased rapeseed juice yield from 34% to 81% while doubling protein content.[4] Similarly, ultrasound-assisted DES extraction reached a 46% protein yield within 120 s,[5] highlighting the potential of these approaches to lower chemical inputs, reduce energy consumption, and support industrial scalability.

This review synthesizes recent advances in plant protein extraction between 2015 and 2025, highlighting the mechanisms, advantages, and potential for large-scale application of novel methodologies. It also examines optimization strategies and research trends, providing guidance for researchers and industry professionals seeking to maximize protein yield and bioactivity while advancing sustainable protein production to meet future demand.

2. Tradition protein extraction methods

Protein extraction from plant sources plays a fundamental role in the food, pharmaceutical, and biotechnological industries. Traditional extraction methods have been widely applied due to their low cost, operational simplicity, and, in some cases, lower environmental burden compared to modern solvent-intensive processes. These methods are generally categorized as solvent-based, enzyme-assisted, alkaline/acid-based chemical, and physical non-chemical approaches. Each approach exhibits distinct advantages and limitations with respect to protein yield, purity, scalability, and environmental impact. Despite their inherent limitations, conventional methods remain relevant for specific applications, offering a practical compromise between extraction efficiency, cost, and sustainability.

2.1. Solvent-based extraction method

Solvent-based extraction is widely used for isolating proteins from diverse plant matrices due to its simplicity, low cost, and comparatively high efficiency. The method relies on differences in protein solubility, enabling selective separation from non-protein cellular macromolecules. A common approach is ammonium sulfate precipitation, where high salt concentrations induce protein aggregation while largely preserving structural integrity and functionality. Organic solvents (e.g., acetone, EtOH, methanol) precipitate proteins by reducing solvation layers, promoting aggregation and separation. Such techniques are frequently applied as preliminary steps to concentrate proteins before downstream purification. Although regarded as conventional, these methods remain applicable across numerous plant species and contexts. Solvent extraction continues to be a viable approach for both laboratory research and large-scale industrial protein production.

However, many organic solvents are toxic and flammable, creating safety hazards and environmental concerns. Protein exposure to harsh solvents can cause denaturation, resulting in the loss of biological activity. Residual solvents may compromise the safety and suitability of protein isolates for food and pharmaceutical applications. Additional purification steps are often required to remove residual solvents and impurities, increasing process complexity and cost. Protein yield and quality strongly depend on solvent selection and extraction conditions, underscoring the need for optimization to ensure reproducibility and industrial applicability.

2.2. Enzymatic extraction method

Enzymatic extraction uses specific enzymes to degrade cell walls or membranes, enabling the release of intracellular proteins from plants or microbial matrices. Proteases and cell wall-degrading enzymes hydrolyze structural polysaccharides and proteins, thereby enhancing protein release from the cellular matrix. This approach is especially effective for plant cells with rigid cell walls and for microbial cells where mechanical or chemical disruption is insufficient. A major advantage is the preservation of protein structure and functionality, as the method minimizes exposure to harsh chemicals and high temperatures. Enzymes such as cellulases and pectinases hydrolyze plant polysaccharides, further enhancing protein recovery. Thus, enzymatic extraction is particularly valuable when maintaining protein bioactivity and structural integrity is essential.

However, enzyme selection is critical, as efficiency and potential side effects depend on enzyme specificity and plant matrix composition. A primary limitation is the high cost of enzymes, which can impede large-scale industrial applications. Careful enzyme selection is also required to maintain protein integrity. Incomplete extraction may occur when proteins remain tightly bound to matrix components. Enzymes are active only within specific pH and temperature ranges; deviations from optimal conditions can reduce efficiency or induce protein denaturation. Excessive protease activity may also cause over-hydrolysis, generating small peptide fragments and compromising intact protein recovery. Therefore, post-extraction steps are often necessary to inactivate or remove residual enzymes. At low enzyme concentrations (e.g., 0.25%), protein yields may fall below those achieved with commercial extraction systems. Although protein purity is generally high, yields and quality vary considerably with plant source, enzyme type, and processing conditions. Such variability poses challenges for achieving consistent and reproducible extraction outcomes.

2.3. Chemical extraction method

Chemical extraction methods are widely employed for isolating plant proteins due to their effectiveness and versatility in disrupting plant tissues to release intracellular proteins. These methods typically involve pH adjustment, salt addition, or the use of detergents to separate proteins from other cellular components. Alkaline extraction is commonly applied, using sodium hydroxide or potassium hydroxide to solubilize proteins before isolation. Proteins can subsequently be precipitated by adjusting the pH to their isoelectric point, at which they become insoluble and separate from the solution. Acid extraction follows a similar principle but is more suitable for proteins stable under acidic conditions. Salt extraction relies on high concentrations of salts, such as ammonium sulfate, to precipitate proteins based on differential solubility. Detergents can disrupt cell membranes, facilitating the release of intracellular proteins. In some cases, enzymes are added to assist in cell wall degradation and improve protein yield. These techniques are versatile and widely applied in food, pharmaceutical, and biotechnology industries, particularly for isolating proteins from complex plant matrices.

Despite their effectiveness, chemical methods present important limitations. Strong acids, bases, or salts can denature proteins, alter their structure, and compromise functionality and nutritional value. Residual chemicals may remain in the protein preparations, rendering them unsuitable for food or pharmaceutical applications. Moreover, these methods require strict pH control and extensive purification, which increases processing time, cost, and operational complexity. For instance, salt-based extraction necessitates multiple washing steps to remove residual salts, whereas detergents may introduce additional contaminants. Environmental concerns also arise, as chemical waste must be properly managed to prevent ecological harm.

2.4. Non-chemical extraction method

Non-chemical (physical) extraction methods rely on mechanical processes to separate proteins from plant matrices without the use of chemical solvents. Common approaches include milling, sieving, and air classification, which help fractionate proteins from non-protein components such as starch, fiber, and lipids. Comminution is often the first step, reducing particle size to enhance the efficiency of subsequent protein separation. After comminution, physical separation methods are applied to obtain protein-enriched fractions. Because they avoid harsh solvents, these methods are generally more environmentally sustainable and cost-effective. However, a major limitation is the lower purity of protein preparations, as residual non-protein components often remain in the isolates.

3. Need for advanced novel methods

Although widely used, conventional protein extraction methods have significant limitations that affect efficiency, protein quality, and scalability. Non-chemical techniques, such as milling and sieving, often produce proteins of lower purity and yield, as they remain mixed with starch, fiber, and other plant constituents. Achieving higher yields and purity typically requires additional processing, increasing both time and cost. Solvent-based methods can be effective but rely on toxic, flammable chemicals such as acetone and methanol, raising safety, environmental, and disposal concerns. Fluctuations in pH or temperature can denature proteins, reducing their biological activity—a critical concern for food and pharmaceutical applications. Enzymatic methods, while preserving protein function, are often too costly for large-scale production. Proteins obtained through conventional methods often exhibit lower stability and require special handling or preservatives, further complicating processing.

These challenges highlight the need for advanced extraction techniques. Modern techniques (Figure 1) improve efficiency while reducing energy consumption, processing time, and overall costs. They enhance protein yield, purity, and functionality by minimizing harsh chemicals, optimizing conditions, and preventing denaturation. From an environmental perspective, these methods are safer, generating less waste and reducing reliance on harmful solvents. Techniques such as DES extraction and pressurized liquid extraction (PLE) are suitable for large-scale applications, whereas PEF and ionic liquid (IL) extraction offer greater flexibility for diverse industrial settings. These methods can be adapted to different plant matrices, improving protein quality and reducing variability. Overall, advanced extraction techniques address the shortcomings of conventional methods, offering more efficient, sustainable, and cost-effective solutions.

4. Novel and advanced extraction techniques

4.1. UAE

UAE uses acoustic cavitation—rapid bubble formation and collapse—to perforate plant cell walls and enhance mass transfer of intracellular proteins (Figure 2). Properly tuned (frequency, power density, pulse duty cycle, temperature, and solid-to-liquid ratio), UAE typically shortens extraction time and reduces energy input relative to conventional stirring or soaking, while maintaining protein quality. For instance, ultrasound-assisted alkaline extraction (UAAE) of cherimoya seed protein (pH 10.5, 41.8°C, 26.1 min) increased yield by 6% and protein content by 12%.[6] Similarly, UAE of chickpea protein improved yield from 55.1% to 66.1%, reduced extraction time from 60 to 10 min, and lowered energy input

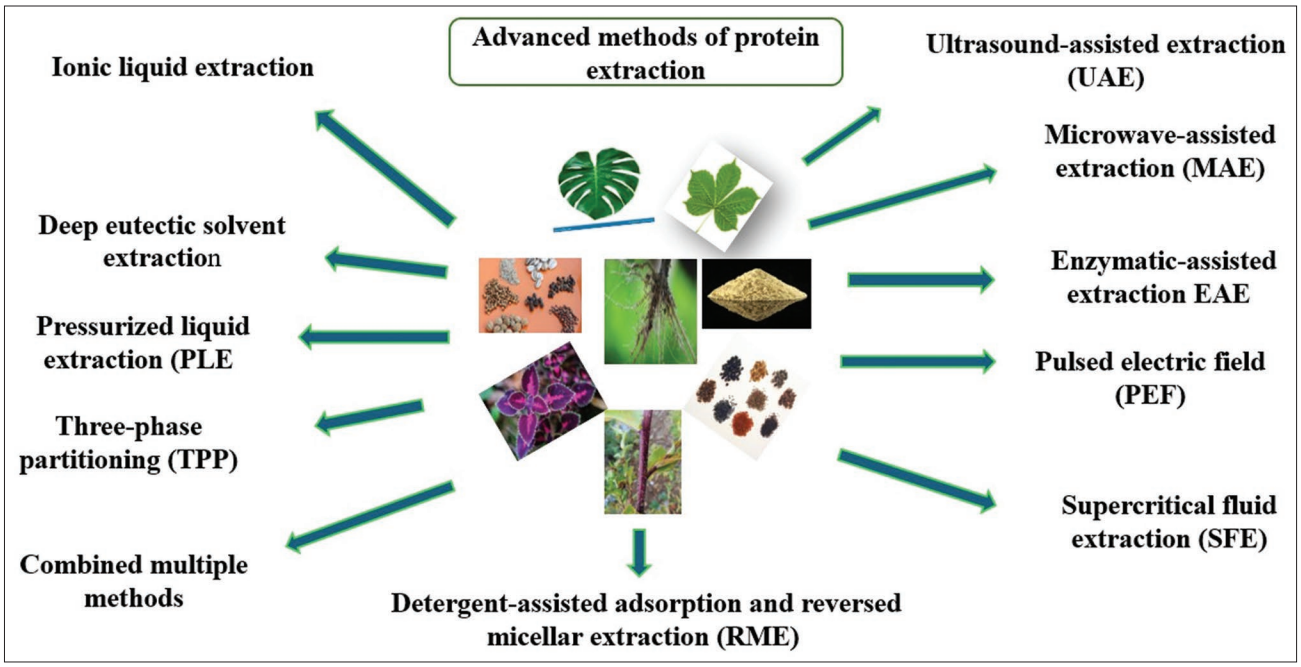

Figure 1. Advanced methods of protein extraction

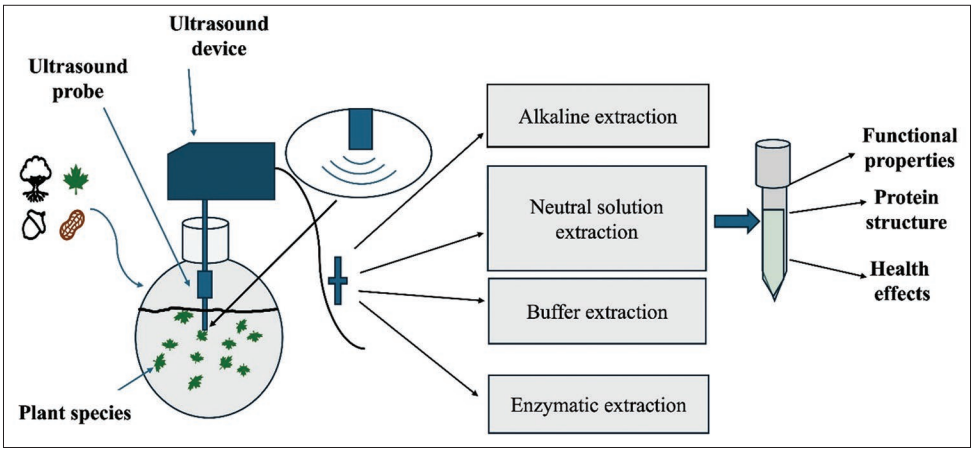

Figure 2. Mechanism of ultrasound-assisted extraction

from 1,941 to 66.2 kJ/kg protein, without affecting amino acid composition or structure.[1] These case studies reflect a broader trend where UAE achieves yield gains of ~5–15% and energy savings of up to 30-fold compared with CAE. At the same time, over-sonication or prolonged exposure to high alkaline pH can cause partial unfolding or side reactions; therefore, UAE's value lies in accelerating diffusion and cell disruption under milder conditions rather than relying on harsher chemistries. Compared with other "intensification" methods such as microwave- or enzyme-assisted extraction (EAE), UAE is broadly compatible with aqueous/alkaline media, can be retrofitted to existing tanks (probe or flow-through horns), and scales through multiple transducers, making it attractive for industrial plant protein processing.

4.1.1. UAAE

Over the past decade, UAAE has proven to be a highly effective method for plant protein recovery, consistently outperforming CAE in yield, functionality, and structural integrity.[1,6-20] UAAE has been successfully applied across diverse matrices, including chickpeas,[1] cherimoya seeds,[6] lupin species,[7] Moringa seeds,[10] evening primrose seed cake,[12] walnuts,[14] pumpkin seed press cake,[16] duckweed,[17] pea protein[18] pistachio meal,[19] and rice peptides.[20] Across these systems, it often shortens extraction time (10–120 min) and increases yields by 15–55% compared with CAE. For instance, chickpea yield improved from 18% (CAE) to 27% with 30 min sonication at 20 kHz[1]; pea protein reached ~85% under optimal pH 9.6 with 60 min UAAE[18]; walnut protein

yield rose from 50% to 65% within 30 min[14]; and Moringa protein increased by ~20% in 45 min.[10] These improvements stem from cavitation effects that disrupt cell walls, reduce particle size, and enhance solvent penetration.[10,14,16,20]

Beyond yield, UAAE substantially improves functional properties critical to food applications, including solubility, water- and oil-holding capacities, emulsifying activity, and foaming.[1,6-8,10-12,14,16-19] Pea protein solubility increased from ~55% (CAE) to ~78% at pH 9.6 after 60 min UAAE,[18] while chickpea solubility rose from 47% to 62% after 30 min.[1] Walnut protein showed a 25% increase in water-holding and a 20% increase in oil-holding capacity.[14] Similarly, Moringa emulsifying activity index (EAI) increased from 38 to 55 m^2/g,[10] and pumpkin seed protein foaming capacity improved from 32% to 50% with 20–30 min UAAE.[16] Structural studies confirm these enhancements are linked to partial unfolding: α-helix content decreases (e.g., 28% → 21% in Moringa[10]) while β-sheets increase (e.g., 22% → 28% in pea protein[18]), with minor shifts in fluorescence and hydrophobicity also reported.[6,10,14,18,19] These molecular changes strengthen protein-solvent interactions and interfacial activity, underpinning functional improvements.

Modified UAAE strategies highlight its adaptability for tailoring protein ingredients. High-pressure assisted UAE improved yield and solubility through intensified mass transfer[13]; polysaccharide-assisted UAE enhanced emulsification and foam stability through protein–polysaccharide interactions[15]; and coupling UAE with isoelectric precipitation enabled selective recovery of bioactive fractions.[9] Key extraction parameters—pH (optimal 9–11), frequency (20–35 kHz), amplitude, time, solid-to-liquid ratio, and power density—critically influence yield and protein properties.[1,6-8,10,12,14,16-20] Importantly, UAAE can also reduce energy demand: Chickpea extraction required only 66.2 kJ/kg protein versus 1,941 kJ/kg for CAE.[1] Taken together, these findings establish UAAE as a flexible, energy-efficient, and scalable method for producing functional plant protein ingredients, supplements, and foods.[7,10,14,16,18]

4.1.2. Ultrasound-assisted acidic extraction (UAAcE)

UAACE has shown strong potential for the simultaneous recovery of proteins and bioactive compounds, demonstrated by its application to purple sweet potato (*Ipomoea batatas* cv. E-Shu No.8).[21] Under optimized conditions (40 min ultrasound at 45 kHz, 178 W, followed by 80°C thermal extraction), the method yielded 0.753 mg/g protein, 3.877 mg/g polyphenols, and 0.293 mg/g anthocyanins—substantially higher than conventional solvent extraction.[21] Importantly, UAAcE increased the diversity of recovered anthocyanins (13 vs. 6 in conventional extraction), reflecting the ability of ultrasound-induced microfractures to enhance solvent penetration and release valuable compounds.[21]

The process is sensitive to operating conditions, with temperature emerging as the dominant factor, while pH and EtOH concentration played secondary roles.[21] This highlights the importance of careful parameter optimization to balance protein yield and bioactive compound recovery. Compared with alkaline- or enzyme-assisted ultrasound, UAAcE is particularly effective for polyphenol and anthocyanin enrichment, making it well-suited for applications where nutritional and functional components are desired.

From an industrial perspective, UAAcE offers efficiency advantages through one-step co-extraction, reduced solvent and energy consumption (specific energy: 8,406.733 J/mg), and shorter processing times.[21] However, protein yield remains moderate compared with enzyme-assisted ultrasound, suggesting hybrid strategies combining acidic solvents with enzymatic hydrolysis may further improve performance. Overall, UAAcE represents a versatile and sustainable extraction approach with clear potential for scale-up, especially for functional food and nutraceutical production where both proteins and bioactive compounds are targeted.

4.1.3. Ultrasound-assisted neutral solution extraction (UANSE)

UANSE represents a tunable, low-chemical intensity platform where cavitation balances cell disruption, unfolding, and re-aggregation. Reported benefits across roots, leaves, seeds, and press cakes include markedly shorter extraction times—~7× faster in[22]—and improved solubility and interfacial properties that support emulsification and hydrolysis (e.g., emulsifying stability/EAI boosts in lupin,[23] high solubility in *Wolffia*[24]). At the same time, studies highlight matrix-specific trade-offs: Press cakes showed reduced oil binding and foaming despite improved digestibility and antinutrient reduction[25]; excessive power or heat promoted aggregation that capped yields[26,27]; and structural shifts (α-helix↓, random coil↑, disulfide formation) that aid emulsification could penalize certain techno-functions. Clear process windows emerge at moderate intensities and duty-cycled power at 20–40°C, which maximize soluble release and hydrolysis readiness, while high underpotential deposition and temperature risk over-denaturation and cross-linking. For bioactivity targets, neutral water plus low-temperature sonication preserves functionality (e.g., antidiabetic fractions in *Momordica*[28]), while coupling ultrasound with fractionation or mild hydrolysis[24,29] converts structural loosening into application-ready peptides.

From an industry perspective, UANSE is compelling for clean-label, food-grade workflows that avoid alkali or acid residues and valorize side-streams such as almond/coconut press cakes,[25] buckwheat,[30] and pecan[29] Compared with buffer-assisted ultrasound (UABSE), UANSE induces milder structural changes and offers broader compatibility for beverages, plant-based dairy, and enzyme-enabled

fortification. In contrast, UABSE can deliver stronger functional shifts (e.g., color removal, emulsification) but with added salts and possible digestibility losses. Actionable design rules have emerged: Use neutral water and moderate power for mainstream isolates and beverages; add brief EtOH co-solvent when pigment or polyphenol removal is needed[31]; employ ultrasound-assisted enzymatic steps when solubility and digestibility are key[29]; and avoid long exposures at high underpotential deposition/temperature.[26,27] To enable scale-up, future studies should prioritize standardized reporting of energy per kg, continuous-flow sonication performance, and application trials in foods such as dairy and meat analogues. In sum, UANSE is not merely "another extraction method" but a parameterizable manufacturing tool, with optimal conditions defined by whether the goal is clean-label functionality or targeted bioactive enrichment.

4.1.4. Ultrasound-assisted buffer solution extraction

Ultrasound-assisted buffer solution extraction has emerged as a versatile method for recovering plant proteins with enhanced solubility, functionality, and structural control, using buffer systems such as phosphate-buffered saline (PBS), Tris-hydrochloride (HCl), and sodium chloride (NaCl). Beyond yield improvement, UABSE tailors protein structure, particle size, and interfacial behavior to generate high-value functional proteins. For example, probe-type ultrasound in PBS increased the solubility of *Cyperus esculentus* protein from 41.29% to 67.53%, while reducing particle size below 220 nm, improving emulsification for dairy alternatives and high-protein beverages.[32] However, the same study showed that excessive power (>500 W) caused re-aggregation, underscoring the need for energy optimization in industrial applications. In contrast, bath-type ultrasound in Tris-HCl nearly doubled yields for quinoa, lentils, and black beans while preserving primary structure,[33] highlighting its compatibility with large-scale production where minimal denaturation is required.

Sodium chloride-assisted UABSE strategies offer another dimension by leveraging ionic strength to adjust both functionality and bioactivity. For example, soybean oleosin extraction improved emulsification and antioxidant capacity by ~242% but reduced digestibility (–22.1%) due to disulfide bond formation,[34] suggesting potential for sustained-release nutraceuticals rather than conventional foods. Sunflower meal protein processing improved purity (93.21%), solubility (56.86%), and color (L = 81.37), aligning with clean-label applications in spreads and bakery products.[35] Collectively, these studies show that buffer composition directs the functional profile: PBS and Tris-HCl favor neutral, minimally modified proteins for general formulations, while NaCl-based systems enable targeted modifications such as antioxidant enrichment or color enhancement, albeit with digestibility trade-offs. Moving forward, UABSE holds strong potential to support the plant-based protein sector through tunable processing, but future work should prioritize energy-efficient continuous systems, integration with enzymatic treatments to restore digestibility, and application-specific optimization balancing yield, techno-functionality, and nutritional quality.

4.1.5. Ultrasound-assisted enzymatic extraction (UAEE)

UAEE combines the catalytic specificity of enzymes with the disruptive effects of ultrasound, consistently outperforming enzyme-only or ultrasound-only approaches. Across diverse plant matrices, UAEE has delivered higher yields, improved solubility, and functional enhancements while generating proteins with superior digestibility and bioactivity. For instance, coupling ultrasound with α-amylase on rice dreg powder increased yield from 53.59% to 89.58% and purity from 77.47% to 92.99%, alongside improved foaming and emulsifying capacity.[36] Similar synergistic outcomes were seen in cellulase-assisted UAEE of mulberry leaves (171% yield increase over alkaline extraction[37]), Alcalase-assisted UAEE of sesame bran (87.9% recovery with bioactive peptide-rich hydrolysates[38]), and UAEE with alkaline proteinase in pecans, which improved solubility and thermal stability for stable emulsions.[29] Collectively, these results highlight UAEE's ability to improve efficiency and simultaneously tailor protein structures and techno-functional attributes for targeted food applications.

UAEE performance depends strongly on enzyme selection, substrate type, and process conditions, offering flexibility but also requiring optimization. For example, different *Bacillus* proteases applied to Ramon seed flour produced proteins with distinct functionalities ranging from enhanced emulsifying capacity to antioxidant or anti-inflammatory activity, demonstrating the potential to design processes around desired end uses.[39] However, excessive sonication can compromise protein integrity, as observed with cellulase-assisted UAEE of mulberry leaves, underscoring the need to balance yield with quality.[40] Compared with CAE, which often achieves high recovery at the expense of functionality, UAEE reduces chemical intensity and better preserves bioactivity. Its scalability, alignment with clean-label and sustainability demands, and potential to generate customized functional proteins make it attractive for plant-based dairy, meat analogues, and nutraceuticals. Future progress will depend on refining enzyme–ultrasound combinations for specific raw materials and demonstrating cost-effectiveness at an industrial scale.

4.2. MAE

MAE has emerged as a rapid, sustainable, and efficient approach for extracting plant proteins, consistently delivering

higher yields and shorter processing times compared with conventional techniques. By generating internal heating, microwave irradiation disrupts plant cell walls and promotes solvent penetration, enhancing protein release, reducing solvent usage, and decreasing overall extraction time (Figure 3). Protein yield is highly dependent on microwave power; for instance, foxtail millet processed at 960 W for 66 s with distilled water at pH 10 achieved 30.02 ± 0.97% recovery, significantly outperforming conventional maceration and demonstrating MAE's efficiency for industrial protein extraction.[2] Similarly, microwave-assisted alkali extraction of pumpkin seeds at 416 W, a 1:10 solid-to-solvent ratio, and pH 11 yielded 55% protein with enhanced functional properties, including improved solubility, emulsification, foaming capacity, and antioxidant activity, highlighting its applicability for functional food ingredients.[41] A lower-power MAE approach (100 W) with ultra-pure water, adjusting the pH from 8 to 3 to precipitate proteins, successfully recovered proteins while enhancing antimicrobial activity and increasing phenolic and flavonoid content, demonstrating MAE's capacity to produce bioactive-enriched extracts.[42]

Using natural DES (NADES), MAE at 240–800 W with solid-to-liquid ratios of 10–60 mL/g effectively extracted both proteins and bioactive compounds, including phenolics and flavonoids, offering a sustainable alternative to conventional solvent extraction.[43] Microwave-assisted enzymatic hydrolysis at 55 ± 2°C for 1 h using Alcalase and Flavourzyme generated <3 kDa peptides from chia seed mucilage with potent antimicrobial activity, illustrating MAE's ability to enhance functional bioactivity beyond simple protein extraction.[44] High-power MAE at 1,200 W and 60°C for 20 min with a methanol/water (90:10, v/v) mixture preserved the structure of highly stable cyclotides while achieving yields comparable to maceration, highlighting MAE's suitability for sensitive peptide recovery and potential pharmaceutical applications.[45] Collectively, these studies demonstrate MAE's versatility, where adjustments in microwave power, pH, solvent type, and processing time allow optimization of protein yield, functional properties, and bioactive compound recovery, making it highly relevant for nutraceutical, functional food, and pharmaceutical applications.

4.3. Enzyme-assisted extraction

Enzyme-assisted extraction is a mild and efficient method for recovering plant proteins, as enzymes such as cellulase and protease hydrolyze cell wall components, enhancing protein release while preserving protein structure (Figure 4). This approach reduces extraction time and improves environmental sustainability by limiting the use of harsh chemical solvents, making it suitable for industrial-scale and eco-friendly applications. For example, Celluclast® 1.5L applied at 55°C and pH 5.0 for 15 min extracted 1.87–6.64 mg/g of protein from olive leaves, demonstrating both method efficiency and variability across plant matrices.[46] Similarly, alkaline protease-assisted extraction at 50°C and pH 9.0 produced roughly 650 g of freeze-dried protein from bulk plant material, highlighting the method's scalability for industrial applications.[47] Protease-assisted extraction at 50°C and pH 7 for 10–30 min improved protein recovery, though specific yield values were not reported, indicating a need for more standardized quantification in future studies.[39] Cellulase treatment at 55°C for 60 min (20 U/g) achieved the highest reported protein recovery under the tested conditions, demonstrating that enzyme concentration and exposure time are key determinants of extraction efficiency.[48] In a related study, the same cellulase concentration yielded up to 5.1 mg/g of protein from a different plant substrate, illustrating the method's adaptability across diverse plant materials.[43,48] Overall, these studies highlight EAE's versatility and efficiency across multiple plant matrices, where enzyme selection—including cellulase, protease, and others—critically influences protein yield, digestibility, and

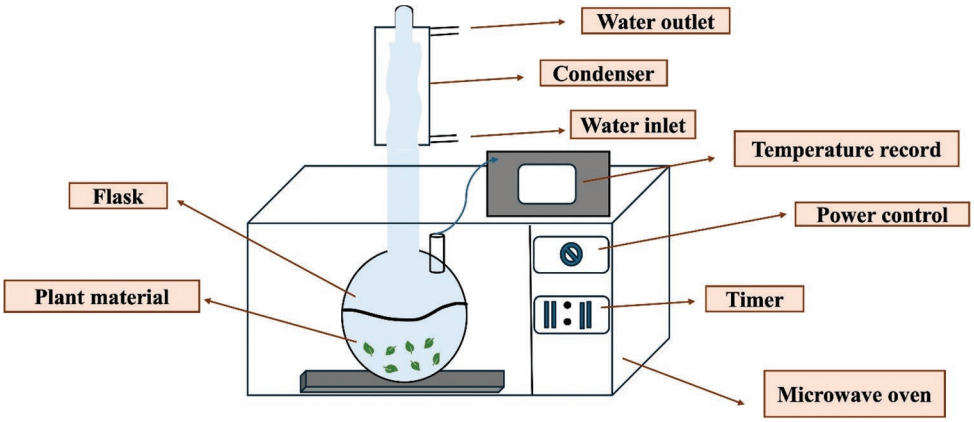

Figure 3. Microwave-assisted extraction

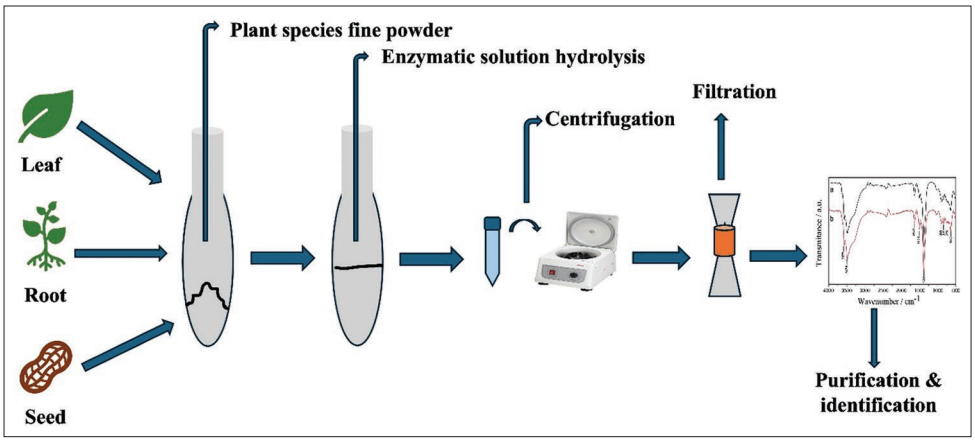

Figure 4. Mechanism of enzyme-assisted extraction

bioactive properties, which are important considerations for both research and industrial applications.

4.4. PEF

PEF extraction is transforming protein recovery by applying high-voltage pulses to induce electroporation in plant cell membranes, enhancing intracellular compound release while avoiding excessive heat or harsh chemicals, thus preserving protein integrity and functionality (Figure 5). One study optimized PEF at 8 kV/cm, with a 2 ms pulse duration and 10 bar pressure, to extract proteins and polyphenols from rapeseed stems,[4] achieving a remarkable increase in juice expression yield from 34% to 81%, doubling protein content from 0.07 to 0.14 g bovine serum albumin/100 g dry matter, and improving the consolidation coefficient from 2.2×10^{-8} to 9.0×10^{-8} m^2/s, while producing a well-dehydrated press cake suitable for bioenergy applications. In a follow-up study, PEF at 0.9 kV/cm was combined with ultrasound (325 W, 15 min) to extract chickpea protein isolate,[49] resulting in a 47.28% increase in protein yield compared to conventional extraction methods, highlighting the synergistic effect of combining PEF with mechanical agitation. Another study applied PEF at 1.6 kV/cm with 20 ms pulses at 100 Hz for semi-refined flaxseed protein extraction,[50] demonstrating that higher energy input improved protein recovery and purity, and that water versus alkaline extraction influenced protein conformation, suggesting PEF's role in modifying protein structural properties. The results demonstrate PEF's versatility: It can double protein yield and enhance juice concentration and press cake dehydration,[4] increase yield by nearly 50% when combined with ultrasound,[49] and improve protein recovery and structural quality depending on energy input and extraction medium.[50] Although the studies employ different parameters and extraction methods, they collectively highlight PEF's ability to enhance protein and polyphenol recovery efficiently and sustainably, reduce energy and solvent use, preserve protein functionality, and offer potential for industrial-scale applications in food, nutraceutical, and bioenergy sectors.

4.5. SFE

SFE is one of the most effective and straightforward techniques for extracting protein from plant materials. Several studies have focused on optimizing parameters such as solvent choice, temperature, pressure, and extraction time to maximize protein yield while maintaining protein functionality (Figure 6). In these studies, CO_2 was used as the main solvent, often combined with EtOH as a co-solvent to improve solubility and enhance extraction efficiency.[3,51,52] Temperature settings ranged from 40°C to 60°C, and pressure varied from 150 to 350 bar, with conditions tailored to different plant sources: 250 bar for lupin seeds and 350 bar for maize,[3,51] respectively. These studies consistently highlight the importance of preserving protein structure during extraction. For example, a study by Görgüç *et al*.[38] demonstrated that SFE followed by EtOH/CO_2 extraction produced lupin flour with 36% protein, maintained essential amino acids, and showed minimal structural changes as confirmed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and Fourier transform infrared spectroscopy (FTIR). Shah and colleagues[51] showed that maize proteins treated with SFE retained their α-helical structure and virus-like particle formation, enhancing immunogenicity compared to hexane or full-fat treatment. Meanwhile, Teixeira *et al*.[52] compared SFE with subcritical propane and conventional Soxhlet extraction in sapucaia nut flour and found that SFE preserved protein integrity better than Soxhlet, while subcritical propane also maintained structure but with slightly lower efficiency. Protein content across these methods was analyzed using techniques like the Dumas method and ammonium sulfate precipitation with diethylaminoethyl column chromatography, while structural changes were assessed through SDS-PAGE and FTIR. Besides SFE, subcritical propane extraction had minimal impact on protein content, whereas conventional

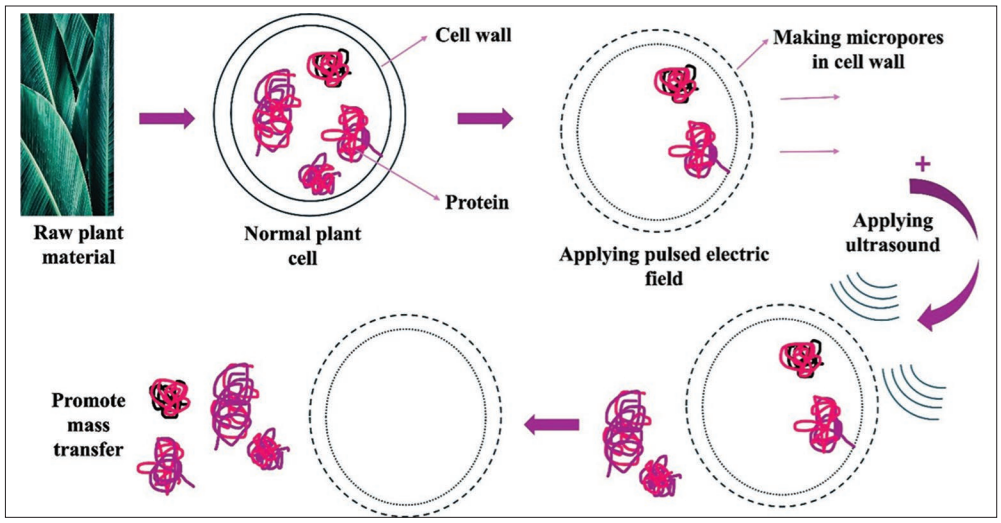

Figure 5. Mechanism of pulse electric field extraction

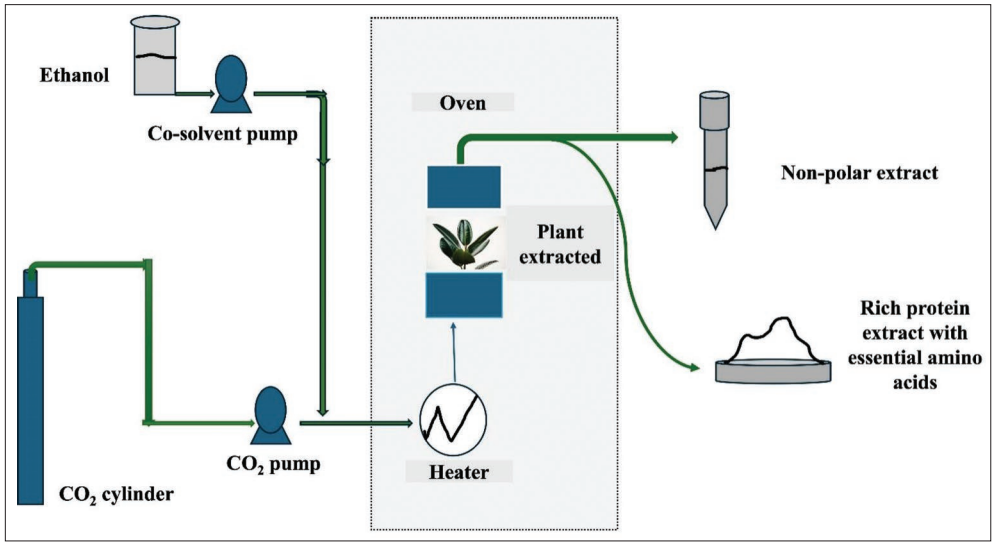

Figure 6. Mechanism of supercritical fluid extraction

Soxhlet extraction tended to disrupt protein structure more. Overall, these studies emphasize that adjusting extraction conditions such as temperature, pressure, and co-solvent proportion is critical to optimizing protein recovery while preserving functionality, demonstrating the flexibility and industrial potential of SFE for producing high-quality plant proteins for both food and pharmaceutical applications.

4.6. IL extraction

IL have emerged as versatile solvents for plant protein extraction due to their liquid state at room temperature, low vapor pressure, high thermal stability, and strong ability to solubilize both hydrophilic and hydrophobic molecules. Their tunability, biodegradability, and generally lower toxicity compared to conventional solvents make them promising tools for sustainable bioprocessing (Figure 7). However, extraction efficiency and protein quality vary greatly depending on the IL type and system design. For example, a hydrophilic IL ([C2mim][Ac])–methanol co-solvent applied to *Jatropha* biomass solubilized only ~12% of total protein, leaving ~86% in the delipidified biomass and retaining ~31% IL, underscoring economic and operational challenges.[53] In contrast, imidazolium-based ILs applied to selenium-enriched peanut leaves yielded up to 63.3% protein with [AMIM]Cl compared to 43.0% with [BMIM]Cl, with [AMIM]Cl maintaining protein secondary structures while [BMIM]Cl caused partial disruption.[54] These findings highlight a critical trade-off: Highly aggressive IL systems improve solubilization but risk damaging protein integrity, whereas milder IL preserves structure but reduce recovery.

A particularly promising development is the C12Im-Cl-assisted extraction of tobacco leaf proteins, which combined high yield (17.5 mg/g leaf powder) with preserved enzymatic digestibility and deep proteomic coverage (5,153 unique

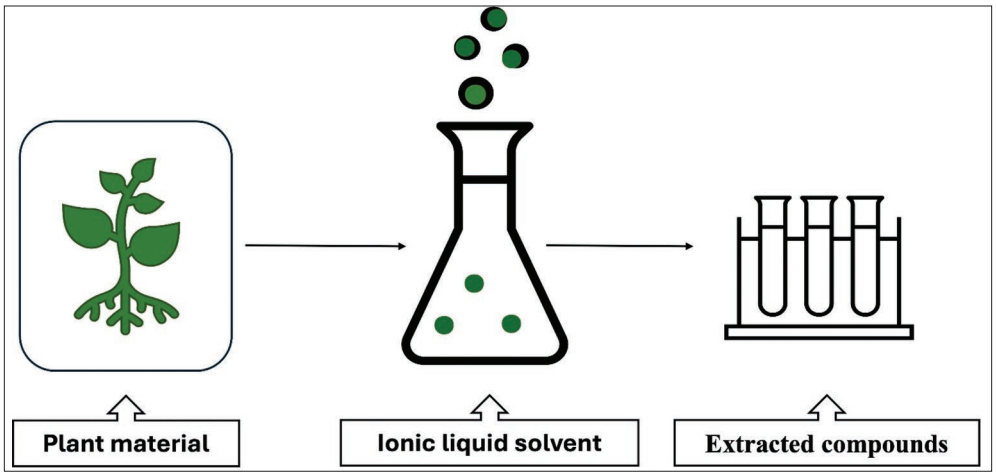

Figure 7. Ionic liquid extraction

phosphoproteins, 14,441 phosphopeptides, and 7,845 phosphorylation sites), clearly outperforming conventional urea-based methods.[55] Unlike [C2mim] [Ac] methanol or [AMIM]Cl systems, C12Im-Cl enabled efficient recovery of membrane-associated proteins while maintaining compatibility with downstream proteomic workflows. These results demonstrate that IL selection must be tailored not only for yield but also for the intended application, whether for protein concentrates, enzyme recovery, or advanced proteomics. Still, challenges such as IL removal, cost, and scalability remain significant hurdles for industrial implementation.

Compared to DES, IL offers greater versatility in extracting structurally complex proteins and enhancing proteomic depth, but often at the expense of higher recovery costs and a greater risk of structural disruption.[54,55,54] DES, on the other hand, typically provides better preservation of protein bioactivity and lower environmental impact.[19,56,57] Thus, IL and DES can be viewed as complementary: IL excels in analytical and proteomic applications, while DES is more suited to food, nutraceutical, and large-scale industrial protein recovery. Future innovation may lie in hybrid systems that combine the solubilization strength of IL with the biocompatibility of DES to achieve sustainable and application-specific extraction.

4.7. Deep eutectic solvent extraction

Recent advances highlight DES as a versatile and sustainable platform for plant protein extraction, providing a green alternative to conventional alkali- or solvent-based methods (Figure 8). The performance of DESs depends strongly on hydrogen bond donor, acceptor, and water content, which together shape protein yield, solubility, structural stability, and functional quality. For example, ChCl–1,3-propanediol–water achieved 62.5% recovery with intact SDS-PAGE bands and enhanced β-sheet content, prioritizing stability and recovery.[58] By contrast, ChCl–glycerol produced lower yields (15.87%) but superior techno-functionality, including high solubility at alkaline pH (67.8% at pH 13), strong emulsifying activity (EAI = 11.1 m^2/g, emulsion stability index = 70.9 min), robust foaming (94.4%/86.6%), and elevated amino acid content.[59] Similarly, ChCl–1,4-butanediol/water (DES15) balanced yield and function, extracting oat proteins with 55.72% protein content, 41.43% recovery, high solubility (~88.8% at pH 9), strong foaming (94.4%/86.6%), and improved thermal stability (temperature threshold ≈ 111°C) linked to higher α-helix content.[60] Collectively, these examples illustrate that DES composition can be tuned to prioritize yield, stability, or functional properties depending on application needs.

Beyond single-case systems, DES has shown strong performance across diverse plant matrices. In bamboo shoots, ChCl–levulinic acid DES outperformed sodium hydroxide extraction, yielding 39.16 ± 1.22 mg/g dry weight (DW) protein from tender shoots versus 23.88 ± 1.10 mg/g with alkali, while preserving stronger antioxidant properties such as 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid radical scavenging.[57] ChCl-urea further demonstrated broad applicability, extracting up to 79% protein from brewer's spent grain with >50 wt% protein concentrates.[19] Alternative carboxylate-urea DES (e.g., NaAcO-urea) improved recovery further, underscoring the adaptability of DES for different biomass sources. Importantly, these results position DES not only as high-yield extraction media but also as effective tools to preserve bioactive functionality, an advantage over harsh alkali methods that often compromise protein integrity.

Deep eutectic solvent extraction also enables the selective release of bioactive peptides with nutraceutical potential. ChCl–acetic acid systems applied to pomegranate peel generated hydrolysates with more diverse peptide profiles (23 vs. 14 peptides with Alcalase; 20 vs. 4 with thermolysin) than PLE, enriched in hydrophobic/aromatic amino acids that support antioxidant activity.[56] These DES-derived

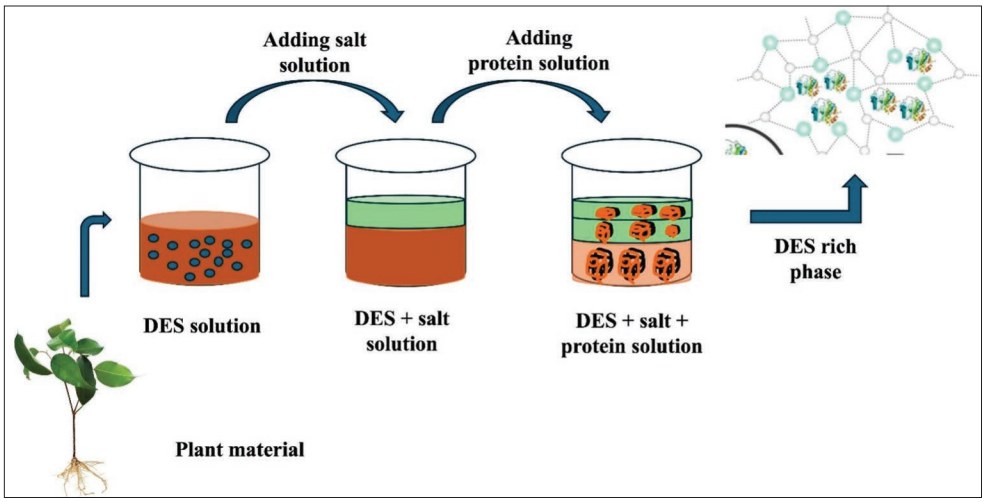

Figure 8. Mechanism of deep eutectic solvent (DES) extraction

peptides displayed stronger antihypertensive effects (half maximal inhibitory concentration = 23–28 μg/mL) compared to conventional methods (49–75 μg/mL). Such findings underscore a future role for DES in designing functional foods and nutraceuticals, where both yield and bioactivity are critical. While challenges remain—such as high viscosity and the need for co-solvents—DES technology offers a flexible, sustainable, and customizable approach for scaling protein extraction to industrial applications, particularly in contexts where structural preservation and targeted functionality are valued.

4.8. PLE

PLE has emerged as a highly effective method for extracting proteins from plant materials, as demonstrated in a recent comparative study[61] (Figure 9). PLE (ASETM 150; 1,500 psi) creates a tunable EtOH–water environment at elevated temperature and pressure, which lowers viscosity, improves matrix wetting, and accelerates mass transfer. This promotes the desorption/solubilization of protein–polysaccharide–phenolic complexes while avoiding prolonged heat exposure that drives glycation. In lime peels, PLE recovered ~66–69% of total protein in 7–11 min (5.9 ± 0.3 and 5.0 ± 0.2 g/100 g DW for Mexican and Spanish peels), versus ~12–15% for UAE-NaDES (1.00 ± 0.06; 1.14 ± 0.04 g/100 g) and ≤~1–3% for UAE–TrisHCl (typically ≤0.1–0.2 g/100 g) under their respective optimized conditions.[61] Matrix-specific PLE optima were required (Mexican: 22% EtOH, 129°C, 7 min, two static cycles; Spanish: 75% EtOH, 134°C, 11 min, one cycle), and 100% EtOH depressed yield in Spanish peels to 1.0 ± 0.1 g/100 g by extracting interferents—evidence from Dumas analysis suggests that cosolvent polarity, rather than a "more organic" composition, governs performance.

Compared with ultrasound in viscous DES or dilute buffer, short, hot, pressurized PLE pulses more effectively disrupt hydrophobic/hydrogen-bond networks, broadening proteome capture in a single cycle. This is supported by study design (Box-Behnken optimization with multi-response desirability balancing yield and A420) and analytics (SDS-PAGE showed broader banding for PLE versus UAE–TrisHCl; A420 tracked Maillard formation). Functionally, thermolysin hydrolysates from PLE proteins showed stronger •OH inhibition and ferric reducing antioxidant power activity than those from Alcalase/AlcalasePURE (angiotensin-converting enzyme inhibition <50% across enzymes), and yielded more/longer aromatic and aliphatic hydrophobic peptides—linking extraction choice to downstream bioactivity, not just yield.[61]

Operationally, PLE offers minute-scale cycles with food-grade solvents but requires pressure-rated hardware, heat management, and closed-loop EtOH recovery; UAE-NaDES remains attractive where low-temperature operation, greener solvent identity, or simpler equipment are prioritized, even at the cost of lower yield. A practical rule from a study by Sánchez-Elvira *et al*.[61]: Choose PLE when throughput and percentage of total protein recovered are critical, and the matrix tolerates ~120–140°C for several minutes; prefer UAE-NaDES for heat-sensitive targets or when solvent circularity is paramount. Limitations include matrix specificity (lime peels) and short-term datasets. Next steps include benchmarking against lower-viscosity/diluted DES, standardized multi-assay protein quantification, pilot-scale solvent/energy recovery, and food-use compliance testing.

4.9. Three-phase partitioning (TPP)

TPP is a liquid-liquid separation technique that combines a salting-out agent (typically ammonium sulfate) with an organic phase (commonly t-butanol) to generate an interfacial protein precipitate. It has gained traction for plant protein recovery due to its simplicity, scalability, and compatibility with the concurrent recovery of lipids and phenolics[62,63] (Figure 10).

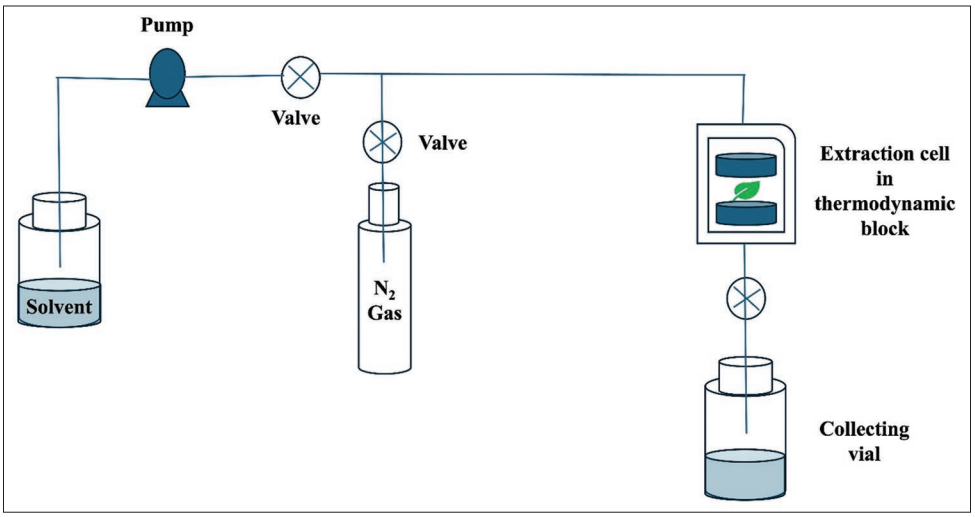

Figure 9. Mechanism of pressurized liquid extraction

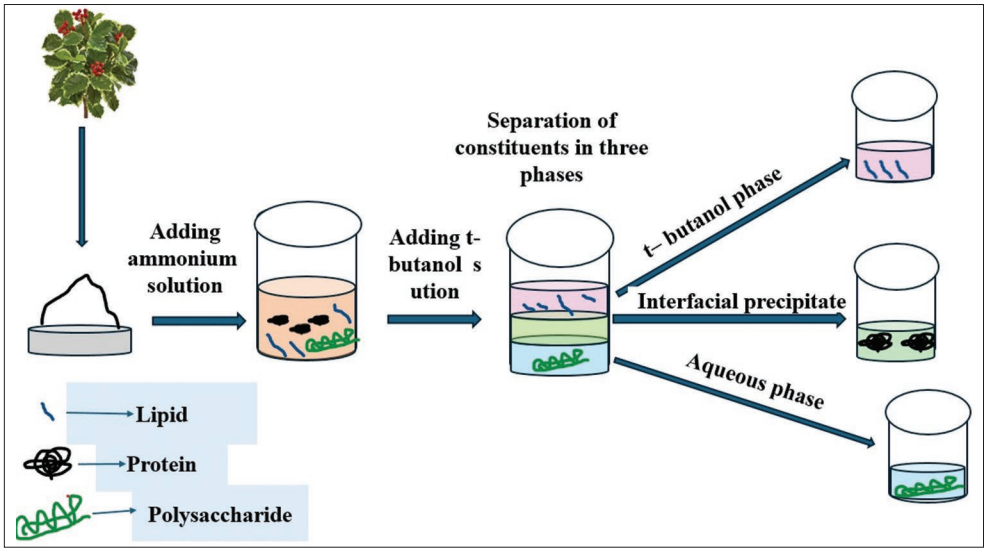

Figure 10. Mechanism of three-phase partitioning

Mechanistically, hydrophilic impurities preferentially remain in the salt-rich lower aqueous phase, lipophilic impurities partition into the upper t-butanol phase, and the target protein concentrates at the interface—features that enable effective decontamination and enrichment. Optimal performance requires tuning of the ammonium sulfate level (e.g., 15% w/v for borage polyphenol oxidase [PPO]; 30–50% w/v screened with a ~40% optimum in custard apple), the t-butanol: Aqueous ratio (commonly ~1:1 v/v for protein TPP, up to 1:2 v/v when co-recovering oil), pH (~6.5 for PPO), and temperature (25–26°C); otherwise enzymes may mispartition (e.g., PPO remained in the aqueous phase after the first cycle), and high salt can degrade proteins, reducing activity.[62,63]

Compared with CAE, which can require ~36 h for some plant matrices,[41] TPP often reduces processing time to ~2 h.[62] In borage, PPO recovered by TPP achieved a 3.59-fold purity increase with 68.75% activity recovery,[62] while a separate study reported ~59.42 ± 0.11% protein yield with a conventional plant extraction workflow.[41] Because these figures reflect different targets and matrices (enzyme activity recovery vs. bulk protein yield), they are not directly comparable but illustrate TPP's potential to close the efficiency gap when the target is activity-sensitive.

Both the custard apple seed[63] and borage PPO[62] studies used ammonium sulfate (reported as ~30–50% saturation; authors also report 30% and 15% on a % w/v basis) with t-butanol at ~1:1 (v/v, organic: Aqueous), underscoring TPP's operational flexibility. In borage, TPP selectively purified PPO with 68.75% activity recovery and a 3.59-fold purity increase.[62] In custard apple seed, TPP enabled simultaneous oil and protein recovery, yielding 17.48 g protein per 100 g seed powder (w/w) with conventional TPP (20 min mixing) and 18.46 g/100 g (↑5.7% relative) after ultrasound pre-treatment (40 kHz, 30 W, 75% duty cycle) applied for 150 s, thereby

cutting the extraction time from ~20 min to ~2.5 min (~87.5% reduction).[63] While Alici and Arabaci's study[62] centers on enzymatic activity retention, Panadare *et al*.[63] quantifies yield and demonstrates process intensification via ultrasound.

Overall, these results support the mechanistic expectation that partitioning is governed by molecular polarity and solubility, and that salt level, solvent ratio, pH, temperature, and pre-treatments (e.g., ultrasound) can be tuned to enhance recovery and/or activity retention. From an industrial perspective, TPP is promising for nutraceuticals, enzyme production, and functional ingredients; however, sustainable deployment hinges on closed-loop solvent recovery (t-butanol), management of high-salinity effluents, and confirmation of food-grade solvent use and residuals when the end-use is food.

4.10. Detergent-assisted adsorption and cationic reversed micellar extraction (RME)

Detergent-assisted adsorption combined with cationic RME is effective for isolating challenging plant proteins (Figure 11), particularly hydrophobic or membrane-bound enzymes such as apple PPO.[64] Non-ionic detergents (e.g., Triton X-100) minimize lipid–protein interactions to solubilize membrane proteins with limited denaturation, while cationic surfactants such as dodecyltrimethylammonium bromide form reversed micelles that sequester proteins within nanoscopic aqueous cores in an organic phase; subsequent back-extraction to an aqueous phase enhances recovery and purity.[64] Relative to conventional aqueous extractions, surfactant-mediated workflows reduce aggregation and better retain catalytic activity—advantages particularly relevant for hydrophobic or membrane-bound plant proteins.

In bench-scale studies with apple tissue, forward extraction at pH 7.2 with 100 mM dodecyltrimethylammonium bromide at 4°C, followed by back-extraction with 0.5 M KCl and 10% EtOH, yielded 99 ± 6% activity recovery and a 17 ± 2-fold purification.[64] For context, CAE of mulberry leaves yielded only ~8 mg/mL protein,[40] illustrating the modest recoveries often reported for aqueous methods. Although the different plant matrices and protein targets limit direct comparisons, such evidence highlights the efficiency gap that RME helps to address when extracting difficult proteins such as PPO.

Incorporating AG2-X8 anion-exchange resin removed inhibitory phenolics, compounds that can bind or oxidatively inactivate PPO, thereby improving stability and activity. For instance, combining 0.5 g resin/g apple with 0.02–0.1 wt% Triton X-100 increased PPO activity from 326 ± 30 U/g to 2,166 ± 74 U/g. No additional gains were observed beyond this range, reflecting a plateau response likely due to saturation of phenolic removal and/or micellar capacity.64 As shown by heat-coagulation extraction of alfalfa, conventional methods can yield substantial protein fractions (16.6 g/100 g), but with low purity (only 63.9 g/100 g protein, ~37% impurities).[65] In contrast, detergent-assisted RME of apple PPO achieved high activity recovery and purification fold.[64] While these studies differ in matrix and target protein, taken together they illustrate a common trend: conventional bulk methods often compromise purity, whereas advanced strategies can deliver both high recovery and functional retention.

Process interventions such as pressurized CO_2 and thermosonication reduced PPO activity to 16 ± 3% and 9 ± 1%, respectively,[64] suggesting tunable activity for applications such as enzymatic browning control. Structural preservation was inferred from retained solubility and size profiles (further spectroscopic confirmation would be valuable). Overall,

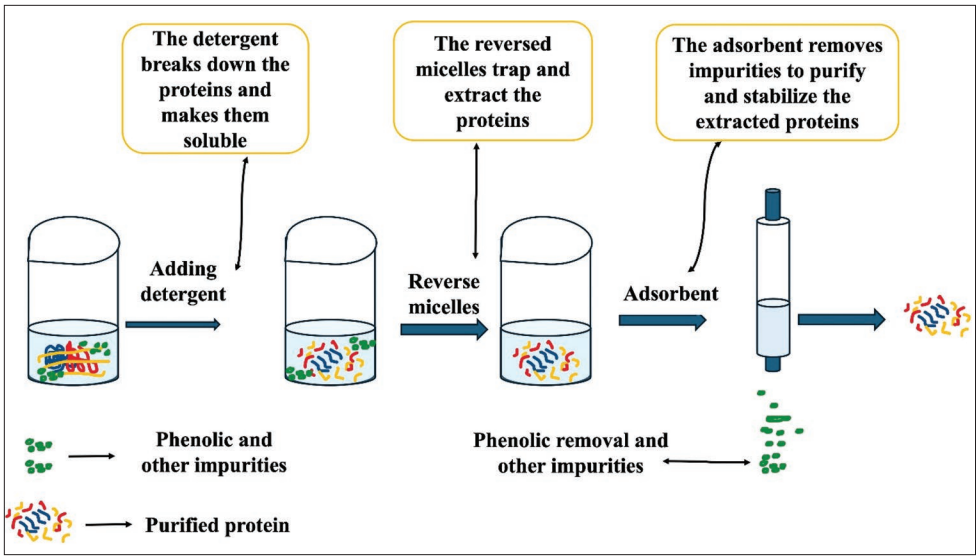

Figure 11. Mechanism of detergent-assisted adsorption and reversed micellar extraction

bench-scale evidence indicates that RME can deliver high recovery of functional proteins and can be coupled with greener elements (e.g., EtOH-assisted back-extraction, resin regeneration). However, translation will require pilot-scale validation of cost, energy, and surfactant recovery; rigorous verification of surfactant residue removal (and the use of food-grade alternatives) if the end-use is food; and head-to-head benchmarking against UAE, DES/PLE, and enzymatic workflows. Looking forward, RME holds strong potential for integration into the food, pharmaceutical, and biocatalysis industries, particularly where high-purity, functional proteins are required, and conventional methods fall short.

4.11. Combined methods for protein extraction from plants

Combining extraction methods can significantly improve both the yield and functional quality of plant proteins compared with single techniques (Figure 12). UAE paired with ammonium sulfate precipitation enhances solubility and recovery by disrupting cell walls and increasing mass transfer.[66,67] In rapeseed, UAE combined with two stages of conventional extraction achieved the highest yield of 9.81 g/100 g dry matter,[68] while UAE–MAE in Sichuan pepper improved recovery and enabled encapsulation with antimicrobial properties.[42] These examples illustrate that hybrid methods often provide synergistic benefits in both efficiency and functional outcomes beyond what individual approaches can achieve.

Other combinations highlight structural or functional optimization. Ultrasound-ultrafiltration-assisted alkaline isoelectric precipitation in alfalfa produced protein with high purity (91.1 g/100 g) and notable recovery.[65] EAE combined with conventional methods improved protein recovery in sugar beet leaves (38.09%) while preserving solubility and functional properties.[69] Together, these studies demonstrate that integrating physical disruption with selective precipitation or enzymatic hydrolysis can enhance protein quality while maintaining bioactivity, making these strategies well-suited for functional food ingredients.

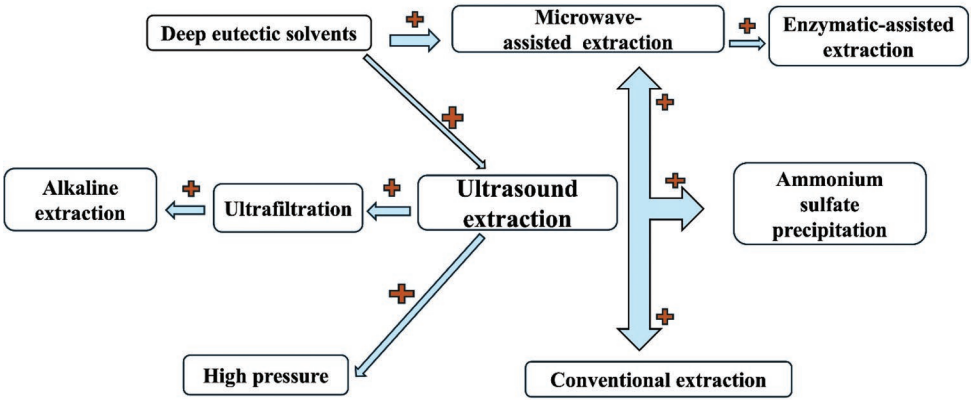

Figure 12. Method combination for protein extraction

Table 1. Novel protein extraction methods and their use in different plant parts

Plant material	Extraction method	Description	References
Seed	Ultrasound-assisted alkaline extraction	Uses sound waves and an alkaline solution to break open seed cells, improving protein extraction and yields	1,6-14,16,18-20,33
	Ultrasound-assisted neutral solution extraction	Uses sound waves and a neutral solution to gently extract proteins and other compounds from seeds	23,25-30
	Ultrasound-assisted buffer solution extraction	Uses sound waves and a pH-stable buffer to extract proteins while maintaining structural integrity and function	32,33
	Ultrasound-assisted NaCl solution extraction	Uses saltwater and sound waves to extract salt-soluble proteins from seeds	34,35
	Ultrasound-assisted enzymatic extraction	Combines sound waves with enzymes to break down seed cells and increase protein yield	29,36,38,39,44
	Microwave-assisted extraction	Uses microwave heating to break rapidly disrupt seed cells, enhancing protein release and reducing solvent use	2,41,42,44
	Enzymatic-assisted extraction	Uses enzymes like cellulases, proteases, and pectinases to degrade seed cell walls and release proteins	39,47

(*Cont'd...*)

Table 1. (*Continued*)

Plant material	Extraction method	Description	References
	Pulsed electric field	Applies short bursts of electricity to permeabilize seed cells membranes, boosting protein extraction efficiency	49,50
	Supercritical fluid extraction	Uses pressurized carbon dioxide to extract proteins and compounds from seeds without traditional solvents	3,51,52
	Ionic liquid extraction	Uses special liquid salts to dissolve seed components and extract proteins with reduced environmental impact	53
	Deep eutectic solvent extraction	Uses biodegradable, tunable solvents (often paired with physical methods) to extract proteins	58,60
	Three-phase partitioning	Separates seed extracts into three layers, isolating proteins and enzymes with high purity and yield	63
	Ultrasound-assisted extraction combined with conventional extraction	Uses sound waves to disrupt seed cells prior to conventional extraction, improving protein recovery	68
	Ultrasound-assisted extraction combined with microwave-assisted extraction	Combines ultrasound and microwave heating to accelerate and enhance protein extraction	42
Leaves	Ultrasound-assisted alkaline extraction	Uses sound waves and an alkaline solution to disrupt leaf cell walls and extract proteins like chlorophyll-binding proteins	17
	Ultrasound-assisted neutral solution extraction	Uses sound waves and a neutral solution to gently extract proteins and other bioactives from leaves	24,31
	Ultrasound-assisted enzymatic extraction	Combines sound waves with enzymes to break down leaf tissues and release proteins and other compounds	37,40
	Ultrasound-ultrafiltration-assisted alkaline isoelectric precipitation	Uses sound ultrasound, pH changes, and membrane filters to isolate proteins from leaf material	65
	Microwave-assisted extraction	Uses microwave heating to rapidly disrupt leaf tissues and release bioactive compounds and proteins	43,45
	Enzymatic-assisted extraction	Uses enzymes to degrade leaf cell walls, enhancing the extraction of proteins and bioactive compounds	43,46
	Ionic liquid extraction	Uses eco-friendly ionic liquids to extract proteins and antioxidants from leaves	54,55
	Ultrasonic cell disrupter-assisted Osborne method	Uses ultrasound to break open leaf cells, followed by the Osborne method to fractionate and purify proteins	67
Roots	Ultrasound-assisted neutral solution extraction	Uses sound waves and a neutral solution to extract proteins and water-soluble compounds from root tissues	22
	Ultrasound-assisted acidic extraction	Uses sound waves and an acidic solution to break down root cell walls and extract proteins	21
Stem	Microwave-assisted extraction	Uses microwave heating to break down stem tissues and improves protein extraction	43
	Enzymatic-assisted extraction	Uses enzymes to degrade rigid stem cell walls, facilitating protein release	48
	Pulsed electric field	Applies short electric pulses to increase stem tissue permeability and enhance protein recovery	4
	Deep eutectic solvent extraction	Uses green solvents to break down stem tissues and extract proteins	57
The whole plant	Three-phase partitioning	Uses organic solvents and salts to create three distinct layers, isolating and purifying proteins from whole plant material	62
Fruit	Ultrasound-assisted alkaline extraction	Uses sound waves and an alkaline solution to disrupt fruit tissues, improving protein recovery and reducing thermal damage	15
	Deep eutectic solvent extraction	Uses biodegradable solvents to extract proteins from fruit tissues	56
	Pressurized liquid extraction	Applies high pressure and temperature to recover bioactive compounds and proteins from fruit tissues	61
	Detergent-assisted adsorption and reversed micellar extraction	Uses detergents to form micelles that isolate proteins and other bioactive compounds from fruit tissues	64
No specific plant	Deep eutectic solvents combined with physical field technologies	Uses green solvents in combination with methods like ultrasound or microwaves to improve protein extraction from various plant tissues	5

Abbreviation: NaCl: Sodium chloride.

Emerging green hybrid technologies further expand the potential of these approaches. Ultrasound-assisted DES extraction achieved yields up to 46% within 120 s,[5] while high-pressure ultrasound in pearl millet recovered 63.43% protein under mild conditions.[13] Microwave-assisted enzymatic hydrolysis of chia seed proteins generated <3 kDa peptides

Table 2. Limitations of plant protein extraction methods

Extraction method	Key limitations	References
Ultrasound-assisted alkaline extraction	High pH combined with ultrasound may cause protein denaturation, loss of functional groups, and increased risk of Maillard reactions if sugars are present. Limited effectiveness for very dense tissues; scalability challenges due to non-uniform energy distribution	1,6-20,33
Ultrasound-assisted neutral solution extraction	Lower extraction efficiency compared to alkaline or enzymatic methods; may require longer sonication, increasing energy input and risk of localized heating; incomplete cell disruption in tough matrices	22-31
Ultrasound-assisted buffer solution extraction	pH-stabilized buffers preserve protein structure but may reduce extraction yield; buffer composition can affect solubility; energy-intensive for viscous matrices; cavitation-induced shear may fragment sensitive proteins	32,33
Ultrasound-assisted NaCl solution extraction	Limited to salt-soluble proteins; high salt concentrations can interfere with downstream applications; localized heating may denature proteins; incomplete extraction of non-salt-soluble proteins	34,35
Ultrasound-assisted enzymatic extraction	Requires cost and optimization of enzyme type, concentration, and conditions; risk of enzyme inactivation from prolonged sonication or localized heat; potential residual enzyme contamination; higher complexity (equipment and process) than UAE alone	29,36-40,44
Ultrasound-assisted acidic extraction	Moderate protein yield compared to enzyme-assisted ultrasound; highly sensitive to parameters (e.g., temperature, pH, solvent concentration); risk of protein denaturation at elevated temperatures; scalability often requires hybrid strategies (e.g., acidic+enzymatic)	21
Microwave-assisted extraction	Non-uniform heating may cause localized protein degradation; limited penetration in large or dense samples; requires specialized equipment; solvent selection is critical; potential hotspots may lead to partial denaturation	2,41-45
Enzyme-assisted extraction	High enzyme cost; long reaction times for some matrices; activity depends on pH and temperature; risk of incomplete hydrolysis or residual enzyme contamination; enzymes may denature if mishandled	39,43,47-48
Pulsed electric field	High equipment cost; limited penetration in dense tissues; energy efficiency depends on matrix conductivity; requires optimization for each plant type; less effective on rigid tissues	4,49,50
Supercritical fluid extraction	High capital and operational costs; requires precise temperature/pressure control; limited solubility for polar proteins without co-solvent; CO_2 removal may need additional steps; safety concerns for high-pressure systems	3,51,52
Ionic liquids	Often costly and potentially toxic; incomplete removal may affect downstream use; efficiency varies by IL type; may disrupt protein structure; disposal and recovery pose environmental issues.	53-55
Deep eutectic solvents	High viscosity can limit mass transfer; may require co-solvents; performance depends on DES composition and water content; limited industrial validation; long extraction times for dense biomass	5,56-58,60
Pressurized liquid extraction	Requires high-pressure equipment; heat-sensitive proteins may degrade; matrix-specific optimization needed; closed-loop solvent recovery essential for sustainability; higher operational complexity than conventional methods	61
Three-phase partitioning	Requires salts and organic solvents that must be recovered or properly disposed; sensitive to pH, temperature, and solvent ratios; industrial scalability requires further optimization; incomplete separation possible with complex matrices	62
Detergent-assisted adsorption and RME	Surfactant removal is critical for food/pharmaceutical applications; high cost and system complexity; limited large-scale validation; may pre-treatments; potential protein conformational changes due to surfactants	64
Combined/hybrid methods	Increased process complexity; higher capital and operating costs; multi-step optimization needed; potential cumulative stress on protein structure; limited industrial-scale feasibility; downstream compatibility must be verified	5,42,68

Abbreviation: IL: Ionic liquid; NaCl: Sodium chloride; RME: Reverse micellar extraction; UAE: Ultrasound-assisted extraction.

with strong antimicrobial activity against *Escherichia coli* and *Listeria monocytogenes*,[44] demonstrating the ability of hybrid methods to create bioactive peptides with nutraceutical and therapeutic potential. Collectively, these studies show that while conventional methods like alkaline precipitation remain simple and low-cost, hybrid techniques consistently provide higher efficiency, enhanced bioactivity, and more sustainable processing. Future work should prioritize optimization of sequential or multimodal methods and industrial-scale validation for broader application in functional foods and nutraceuticals.

5. Conclusion

Plant protein extraction has advanced significantly to meet the demand for efficient, sustainable methods that preserve protein functionality. Conventional approaches often struggle to maximize yield, minimize solvent use, and maintain bioactivity, driving the development of innovative techniques such as UAE, MAE, EAE, PEF, SFE, ILs, DES extraction, PLE, TPP, detergent-assisted adsorption, and RME. These methods enhance recovery, solubility, and bioactivity, while green solvents such as ILs and DES reduce environmental impact.

Despite these advances, important gaps remain. We have listed in Tables 1 and 2 the novel protein extraction methods and their use in different plant parts, and the limitations of various plant protein extraction methods, respectively. For IL- and DES-based extractions, systematic *in vivo* toxicity and biodegradability data are lacking, limiting regulatory acceptance and scale-up. Many methods also require further process optimization, cost reduction, and validation in continuous or industrial-scale systems to move beyond lab-scale success. In addition, protein quality and functionality after processing need to be consistently assessed under application-relevant conditions (e.g., in complex food matrices).

Looking ahead, the integration of artificial intelligence and machine learning into process modeling and optimization offers a powerful opportunity to accelerate development, reduce experimental costs, and identify optimal extraction parameters across diverse plant sources. Combining emerging green solvents with physical field technologies and digital optimization could provide the basis for highly efficient, scalable, and sustainable extraction platforms. Overall, these directions highlight a clear pathway toward bridging current limitations and achieving industrial-scale production of functional plant proteins for food, pharmaceutical, and biotechnological applications.

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Conflict of interest

The authors declare that they have no competing interests.

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Writing–review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability statement

Not applicable.

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