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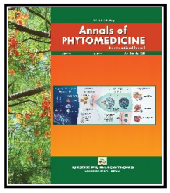

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A comprehensive review of green technology for plant-based protein extraction

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Abstract

The shift toward plant-based foods as alternatives to animal-derived products is driven by environmental concerns, health implications, and the increasing strain on global food resources. As the global population grows, the demand for protein sources rises, posing challenges to food security. Although, plant-based foods provide a sustainable and versatile alternative, they often lack certain essential nutrients found in animal products. To bridge this gap, advanced food processing technologies must improve their nutritional quality, functionality, and consumer appeal. This review explores innovative protein extraction techniques that enhance the efficiency and sustainability of plant-based food production, making these alternatives more viable for large-scale consumption. One of the key challenges in plant-based food production is the significant amount of food waste generated, which, if properly utilized, could serve as a valuable resource for protein extraction. This review examines various methods for recovering proteins from plant-based food waste, highlighting their role in addressing food insecurity, protein-energy malnutrition, and environmental sustainability. The advanced technique optimize pH, temperature, extraction time, and solvent use to maximize protein yield and minimize environmental impact. Enzyme-assisted extraction employs specific enzymes to break down cell walls and improve protein solubility, while ultrasound-assisted extraction leverages cavitation forces to enhance protein release. Reverse micelle and supercritical fluid extraction utilize surfactants and CO_2, respectively, to achieve high-purity protein separation. PEF technology enhances protein recovery through non-thermal electroporation, not only improving protein extraction efficiency but also enhancing the functional properties of the recovered proteins, making them more suitable for use in functional foods and dietary applications. By transforming plant-based food waste into a valuable protein source, these advancements contribute to global sustainability efforts, supporting food security, health, and environmental conservation initiatives.

1. Introduction

Food wastage happens in throughout the supply chain, agricultural productivity, post-harvest handling, storage, industries, manufacturing and packaging up to distribution to consumers. Plant-based waste, rich in carbohydrates, proteins, lipids, micronutrients, minerals, and bioactive compounds, represents a valuable reservoir for repurposing into protein-rich foods. This approach addresses food waste management and nutritional insecurity by extracting proteins *via* conventional or innovative techniques for use as functional or therapeutic ingredients to combat protein-energy malnutrition across demographics (Ravindran *et al*., 2016; Baiano *et al*., 2014).

Global hunger remains a critical issue, with 45% of the 8.1 billion global population experiencing food insecurity (World Food Program). Among 125 countries; India, rated as 111th in Global Hunger Index 2023 (Score: 27.3), exemplifies severe challenges with hunger and malnutrition consists 35.5% under five stunted, 18.7% wasted and 13.7% undernourished due to food price hikes, climate change and undermining the prospects of reaching SDG 2: Zero hunger by 2030 (GHI 2024). Once a year, about 1.25 billion tons of foodstuffs are wasted worldwide, equating to 280-300 kg per capita, exacerbating malnutrition for 783 million people. Projections suggest undernourishment could rise to 841 million by 2030 (UNEP) (Chen *et al*., 2020). Meeting the anticipated 40% increase in demand for cereals, legumes and oilseeds by 2050 driven by population growth, requires alignment with sustainable development goals (SDGs) targeting zero hunger and improved health (Boliko *et al*., 2019).

In the 21st century, global demand for high-protein foods such as livestock, dairy products, cereals, pulses, nuts, and oilseeds is projected to rise significantly faster than cereals, driven by population growth (Westhoek *et al*., 2014). This trend is expected to accelerate the protein rich food improvement as environmental responsive and sustainable nutrient.

Dietary proteins are vital for tissue synthesis, metabolic regulation, immune function, and nutrient transport. The recommendation of about 30% (0.8 g/kg weight of body) must be from protein sources which is based on the AMDR (acceptable macronutrient distribution

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range). While plant proteins exhibit 80% digestibility, leads to rising of protein intake for specifically about 1.2-2.0 g/kg for athletes or during pregnancy (Wu *et al*., 2022; Brestensky *et al*., 2019). Protein quality, accessed *via* amino acid composition (EAA), biological value (BV), protein efficiency ratio (PER) and protein digestibility corrected amino acid score (PDCAAS), depends on digestibility, anti-nutritional factors such as phytate and tannins (Brestensky *et al*., 2019; Sa *et al*., 2024).

High-income nations increasingly adopt diets high in fats and sugars (HFHS), prioritizing high intake of processed red meat (animal-based proteins), a trend linked to chronic disease risks such as cardiovascular disease, hypertension, colorectal cancers, pancreatic cancer, liver cancer (Poorolajal *et al*., 2024). Current dietary guidelines advocate shifting toward plant-based sources without excluding animal products, emphasizing balanced nutrition (Pointke *et al*., 2022). This transition aligns with research on sustainable protein production and holistic health impacts, underscoring the need to integrate food waste valorization into strategies addressing hunger, environmental sustainability, and public health.

Plant-based protein extracts are increasingly recognized as high-quality alternatives to animal-derived proteins for human consumption. Unlike animal proteins, which are often accompanied by higher levels of saturated fats and cholesterol, plant proteins are naturally low in fat and free of cholesterol, making them beneficial in reducing the risk of cardiovascular diseases, obesity, and metabolic disorders (Li *et al*., 2020). Extracts from oilseeds, legumes, and cereals provide essential amino acids along with bioactive compounds such as dietary fiber, polyphenols, and antioxidants that further enhance human health (Gorissen *et al.,* 2018). While animal proteins are complete in their amino acid composition, their regular consumption may increase lipid accumulation in the body, contributing to non-communicable diseases like type 2 diabetes and atherosclerosis (Song *et al.,* 2016). In contrast, plant proteins are associated with lower saturated fat intake and contain beneficial unsaturated fatty acids, particularly omega-3 and omega-6, which improve overall nutritional quality (Wang *et al.,* 2024). However, plant proteins do present limitations such as amino acid deficiencies (low lysine in cereals and low methionine in legumes) and the presence of anti-nutritional factors that reduce digestibility and mineral absorption (Kumar *et al.,* 2020).

These limitations can be overcome through advanced food processing technologies that enhance the quality, functionality, and consumer appeal of plant-based proteins. Techniques such as alkaline isoelectric precipitation, enzyme-assisted extraction, and ultrafiltration improve protein purity and reduce anti-nutritional compounds, while microbial fermentation not only enhances digestibility but also enriches proteins with micronutrients like vitamin B12 (Devappa *et al.,* 2020). Blending different plant sources, such as cereals and legumes, ensures a balanced amino acid profile comparable to animal proteins (Gorissen *et al.,* 2018; Mathai *et al.,* 2017). Novel technologies including extrusion, reverse micellization, and air classification improve texture, solubility, and sensory quality (Lam *et al.,* 2018).

The Bio-E3 concept (Eco-friendly, Efficient, and Economical) is an emerging approach in plant-based protein extraction that emphasizes sustainable, high-yield, and cost-effective processing techniques. This concept integrates innovative extraction methods such as ultrasound aided extraction (UAE), enzyme-assisted extraction (EAE), pulse electric field (PEF) technology, and supercritical fluid extraction (SFE) to maximize protein recovery while minimizing environmental impact. By reducing solvent usage, energy consumption, and processing time, Bio-E3 promotes the development of clean-label, functional plant proteins suitable for food and nutraceutical applications. Studies suggest that enzyme-assisted methods, combined with ultrasound or high-pressure treatments, can enhance protein solubility and digestibility, further improving the extracted protein functional properties (Zhang *et al*., 2022; Kumar *et al*., 2021). This sustainable approach aligns with the circular bio-economy by valorizing foodstuff leftovers, contributing to comprehensive efforts in nutritional security and environmental conservation. Importantly, plant proteins also address environmental and ethical concerns, as they require fewer natural resources and generate lower greenhouse gas emissions than animal protein production. This dual benefit of nutritional enhancement and sustainability makes plant protein extracts a highly valuable component in modern diets and functional food innovations (Poore and Nemecek, 2018).

2. Sources of protein from foodstuff leftovers

Globally, the processed plant-based foods generates approximately 2.5 billion tons of food (Godhavn, 2024) leftovers every year, which are often thrown away as surplus, considered as ecological contaminants, results in major economic fatalities and environmental influences, contributing extensively to greenhouse gas emissions and resource depletion (Malek *et al*., 2018). These by-products (cereal, legume, oilseeds and nuts), including leaves, roots, seeds, fruit peels, bran, husk and oilseed cake meals, as illustrated in Figure 1, are typically disposed of by the food and agricultural industries despite their potential value. Valorizing these materials could reduce waste and create economic opportunities (Cecilia *et al*., 2019). Plant-based food waste is rich in carbohydrates, crude proteins, crude fats, crude fibers, phytocompounds (such as nitrogen containing compounds alkaloids, phenolic compounds, anthocyanin, lycopene), and anti-nutritional factors, making it a valuable resource for functional food ingredients (Parniakov *et al*., 2016). By employing various extraction techniques to recover these compounds, significant contributions can be made toward global food sustainability, environmental preservation, food security, and economic improvement (Rahaman *et al*., 2021).

According to Liu *et al.* (2020), food waste production is higher in developed countries, accounting for 56% of the total, with 40% occurring during the consumption stage. Plant-based food waste, as outlined in Table 1, is recognized as a source of high protein and essential amino acid profile, making it suitable for protein extraction (Kamal *et al*., 2021). For instance, wheat bran, containing 11% protein, is a viable source with high levels of lysine, arginine, tryptophan, tyrosine, and cysteine (Jimenez Pulido *et al*., 2022). Oilseed meals, such as rapeseed cake, sesame meal, and coconut meal, are also promising, with protein content ranging from 21% to 37%. Similarly, soybean hulls and flaxseed meal, with 21% protein (dry basis), are considered superior sources. In contrast, papaya seeds, pumpkin kernel meal, hop, and buckwheat bran, despite having crude protein content exceeding 20%, exhibit a lower nutritional profile (Kamal *et al*., 2021).

The various plant-based food waste sources potential for protein extraction based on their crude protein content (per 100g). Sesame cakes (37.45 g) and rapeseed press cake (36 g) contain the highest protein concentrations, making them ideal candidates for extraction.

Buckwheat bran (27.8 g) and papaya seeds (25.9 g) also present promising protein yields. Other valuable sources include flaxseed meal (20.9 g), soybean hull (21 g), brewer's dry grain (19.96 g), and coconut cake (22 g), all of which could be effectively utilized in protein extraction processes. Meanwhile, lower-protein sources like almond husk (3.27 g), cane molasses (3 g), whey permeate waste (0.25 g) may have limited direct applications but could still contribute to specific extraction techniques (Kumar *et al*., 2022).

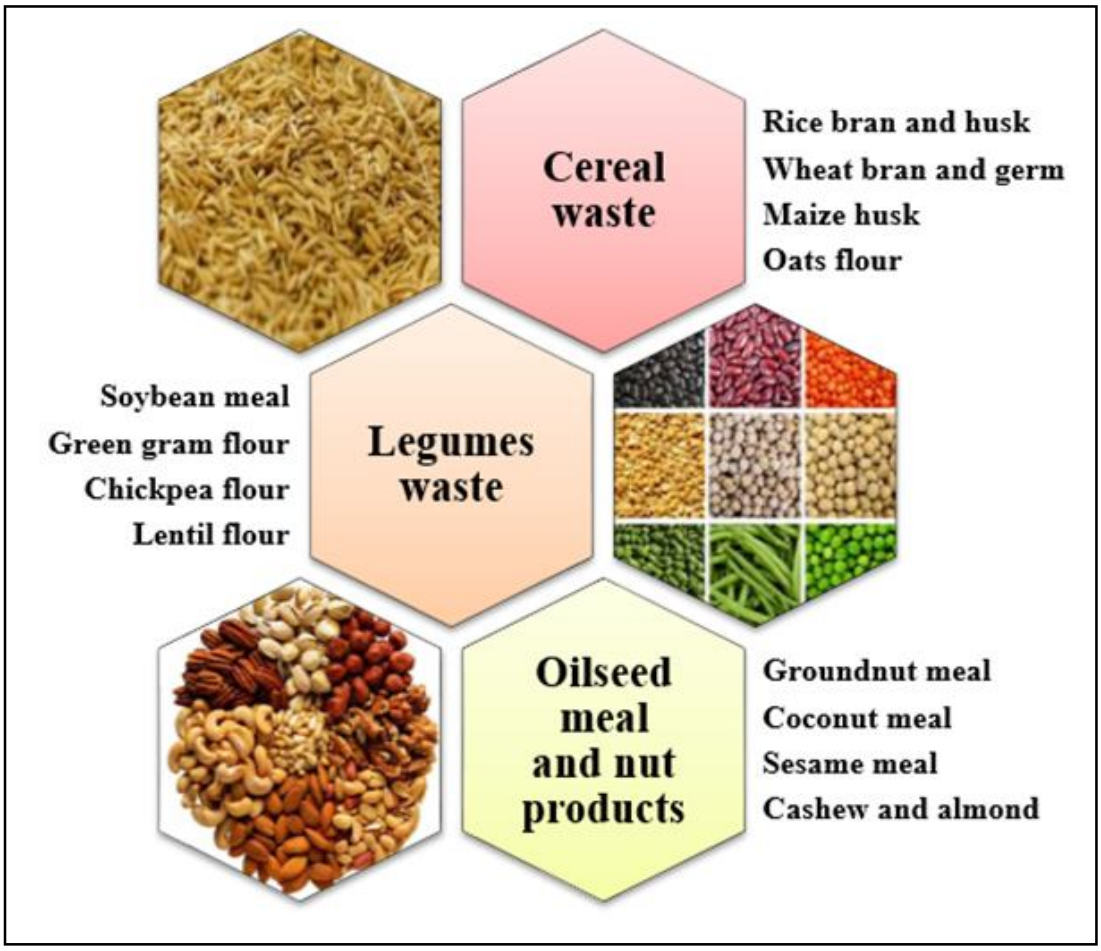

Figure 1: Source of plant based food waste.

By optimizing extraction methods for these high-protein food waste sources, sustainable protein recovery can be enhanced, reducing waste and supporting the advance of ingredients from plant based protein for food and feed applications. Protein-rich plant-based food waste is economically sustainable, easily recoverable, applicable to functional foods and supplements. The recovery of proteins from these wastes can be achieved through advanced technologies such as membrane separation, adsorption, enzyme-assisted extraction, and conventional methods like alkaline or acid precipitation (Pattnaik *et al*., 2021).

2.1 Protein extraction from food waste

2.1.1 Enzyme assisted extraction (EAE)

Enzyme assisted extraction (EAE) is a sustainable and environmentally friendly bioprocessing technique boost high extraction of protein. This method operates under mild conditions, generating minimal waste and consuming less energy than conventional physical extraction and chemical extraction. A variety of carbohydrases, endoproteases, β-glucosidases, phytases, cellulases, pectinases, xylanases, and alcalases are utilized to facilitate protein solubilization by hydrolyzing plant cell wall components like cellulose, hemicellulose, pectin, and intracellular proteins. Optimization of enzymatic treatment parameters such as enzyme-to-substrate ratio, pH, temperature, influence protein extraction, protein yield and recovery formulated using statistical modeling. In addition to improving extraction efficiency, EAE, particularly when utilizing proteases, may enhance the dietary quality (protein bioavailability), functional properties and nutraceutical compounds of recovered proteins. EAE with physical pre-treatment methods, such as ultrasonic, microwave-assisted techniques, can improve cell wall disruption, thereby increasing extraction efficiency. This combined approach may able to accelerate the economic viability of reduced enzyme requirements and energy consumption, ultimately lowers overall extraction costs (Kleekayai *et al*., 2023).

Zhang *et al*. (2019) recover ultra-filtered (UF) protein by enzyme aided extraction (EAE) of soybeans. The protein-rich skim fraction (SF), a by-product during enzyme aided protein extraction (EAE), containing 61% protein. Therefore, developing an efficient protein

recovery method for this fraction is significant. Dual polyethersulfone (PES) membranes with molecular weight of 3 to 5 kDa was employed. Finally, 5 kDa molecular weight membrane exhibited superior filtration, indicates higher permeate flux and reduced impurity rejection. Among the proteases tested, alcalases 2.4 l and protex 6 l showed greater hydrolytic activity compared to flavourzymes and protex 7 l, leads to enhanced filtration flux and lower retention coefficient of protein. The protein recovery exhibited an amino acid composition comparable to soy protein concentrate (SPC), suggestively reducing anti-nutritional factors, indicating its improved protein quality. These findings suggest that higher protein recovery from the EAE-UF of soybeans is a viable approach for simultaneously removing undesirable components from the final protein product.

Yu *et al*. (2020) aim to develop an environmentally sustainable ultrasound-assisted enzymatic process for protein extraction from brewer's spent grain (BSG) then to characterize their physicochemical properties. Increased Alcalases enzyme usage 1 µl enhance protein recovery efficacy from 30% to 60%. Pre-treatment method such as ultrasound also improves protein recovery up to 70%. Notably, ultrasound pre-treatment significantly reduced enzyme consumption 70% and shortened enzyme incubation time 50%, highlighting its process efficiency. The obtained protein concentrates exhibited molecular weights below 15 kDa and improved solubility in pH (1.0-11.0), with ultrasound pre-treatment further enhance protein solubility >90%. Proline and glutamic acid were identified as predominant amino acids in the protein concentrates. These findings indicate that enzymatic process coupled with ultrasound pre-treatment, is a sustainable and effective method for protein recovery from BSG, offering a promising approach for value-added utilization of this agro-industrial by-product.

Xu *et al.* (2021) investigate the enzymatic hydrolysis of protein isolates from lentil, chickpea and pigeon pea using alcalases and bromelain enzymes, with a focus on characterizing the soluble fractions of the resulting hydrolysates. The hydrolysates were compared to their non-hydrolyzed counterparts and whole protein hydrolysates. Protein isolates treated with alcalases exhibited a higher protein hydrolysis, as indicated by an increased presence of smaller peptides. Additionally, all hydrolysates demonstrated lower surface hydrophobicity. Bromelain treatment enhanced the water absorption and oil-binding capacity of the lentil, chickpea and pigeon pea. Antioxidant properties of PPI and LPI hydrolysates exhibited greater radical scavenging activities against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO) radicals compared to CPI. The soluble fractions of bromelain-treated hydrolysates inhibited DPPH radical activity within 20.5% to 32.3%. Both Alcalase and bromelain hydrolysates demonstrated comparable NO radical scavenging capacities. Compared to bromelain, alcalase treated hydrolysates exhibited superior oxygen radical absorbance capacity (ORAC) treated with bromelain. Furthermore, at a concentration of 2 mg/ml, the soluble fractions of bromelain protein hydrolysates suppressed the production of the pro-inflammatory molecule NO in lipopoly-saccharide (LPS)-induced macrophages by 50% to 59%. These findings highlight the potential of enzymatic hydrolysis as an effective approach for generating bioactive protein-derived compounds with effective functional and nutraceutical properties from pulses.

Vasquez *et al*. (2019) represent a promising source of protein-based seaweeds because of substantial protein content, still polysaccharides can impede protein extraction effectively. From this study optimized enzyme-assisted extraction (EAE) approach done by cellulases to improve recovery of protein in the red seaweed *Chondracanthus chamissoi* and brown seaweed *Macrocystis pyrifera*. Comparative analysis of protein yield of enzymatic and non-enzymatic methods disrupts the cellulases sensitive polysaccharides significantly improve protein extraction efficacy. The optimized conditions yield protein extraction efficiencies of 75% for *M. pyrifera* (18 h, enzyme-to-seaweed ratio of 1:10) and 36% for *C. chamissoi* (12 h, enzyme-to-seaweed ratio of 1:10). Furthermore, both protein extracts exhibited antioxidant activity, while the extract from *M. pyrifera* demonstrated potential antihypertensive properties. These findings provide a strong foundation for further investigations into seaweed-derived protein extraction as efficient for the development of nutraceutical foods. The proposed enzyme-assisted technology aids an effective, eco-friendly and sustainable methodology to recover protein in seaweed biomass.

2.1.2 Ultrasonic-assisted extraction (UAE)

Currently, the food industry extensively utilizes high-power sonication (HPS), defined by 17-99 kHz frequency and electricity usage up to 999 W/cm^3. HPS operates by generating increased shear capacity and macro turbulence for the formation of cavitation bubbles and it collapses during higher temperatures (4000 K) and pressures (999 atm).

Increased shear capacity and macro turbulence generated by ultrasound (US) are responsible for the disintegration of membranes and cell walls in plant materials, resulting in greater transfer of extraction solvents into lysed cells, which leads to improved extraction yields of molecules associated with cell wall components and intracellular compounds (Zhang *et al.,* 2011). In addition to their cavitation mechanism, the US can boost extraction yields by decreasing the size of substrate particles by a factor of 10 (Karki *et al.,* 2010). Effectiveness in extraction by the US can modify the physico chemical and functional properties of proteins by disrupting hydrophobic interactions and hydrogen bonds that stabilize structures of proteins (Xiong *et al.,* 2018). Despite the advantageous technological and environmental aspects of the US, its use in the plant-based protein sector is quite limited. By leveraging its beneficial cavitational and shear capacity, the US as an eco-friendly pre-treatment approach combined with isoelectric alkaline precipitation method to enhance the protein extraction of protein rich plant based food leftovers, although maintain minimal impacts on the physico chemical and functional properties of the extracted proteins.

Several factors influence the efficiency of ultrasound-assisted protein extraction, including ultrasound frequency, intensity, treatment time, solvent type, temperature, and raw material characteristics. Higher ultrasound intensity and prolonged treatment times generally enhance cell wall disruption, leading to improved protein release. However, excessive exposure may cause protein denaturation and affect functional properties. The choice of solvent also plays a crucial role, as alkaline or enzymatic solutions can further aid in protein solubilisation. Temperature control is essential to prevent heat-induced structural damage while maximizing extraction efficiency. Additionally, the composition, moisture content and particle size, of the plant protein impact the effective ultrasound treatment. Optimizing these factors ensures a maximizing protein recovery and maintaining desirable functional properties (Ampofo *et al*., 2022).

Ultrasound treatment significantly impacts the physicochemical and functional properties of plant-based proteins by altering their structural and physicochemical characteristics. Through cavitations and shear forces, ultrasound disrupts peptides hydrogen bonds and hydrophobic connections, prominent to improved protein solubility, emulsification, foaming, and gelation properties. Enhanced solubility improves protein dispersion in aqueous systems, making it more suitable for food formulations. Ultrasound can also improve emulsifying and foaming capacities by altering protein conformation, increasing surface hydrophobicity, and reducing particle size, which enhances interfacial stability. Additionally, controlled ultrasound application can modify gelation behaviour, influencing the oil holding capacity and water holding capacity and stability of plant-based protein gels. However, excessive ultrasound exposure may lead to protein aggregation or denaturation, negatively affecting functional performance. Optimizing ultrasound parameters allows for targeted improvements in protein functionality, creating as a valued tool in developing plant-based food products with desirable textural and sensory attributes (Flores *et al*., 2019).

Dabbour *et al.* (2018) aimed to enhance protein extraction from sunflower meal by optimizing ultrasonic-assisted extraction (UAE) conditions using the Box-Behnken design. The optimization process was based on maximizing protein recovery and protein content in sunflower meal. The optimized factors for protein extraction were identified as a power of 250 W/l, a temperature of 40°C, and an extraction duration of 20 min. Under these conditions, the recorded protein yield was 55.10%, with a power 0.15 kW/h. These experimental values closely aligned with the predictions generated by mathematical models. At the optimal UAE conditions, the functional and physico chemical properties of the extracted protein concentrates were assessed. The protein content was determined to be 934.92 g/kg, while the measured bulk density, water-holding capacity, oil-holding capacity and particle size were 0.372 g/ml, 0.985 g water/g protein, 2.06 g oil/g protein and 627.6 nm correspondingly. The maximum solubility, emulsion stability (ES) and emulsifying activity (EA) were achieved at pH 9.0, with values of 74.59%, 50.45% and 52.45%, respectively. These findings indicate that UAE is an active technique for improving the protein recovery efficiency, functional properties of sunflower meal protein isolates.

Nguyen *et al.* (2019) applied ultrasound-assisted extraction to a mixture of defatted peanut meal and water to enhance protein recovery. The influence of key ultrasonic processing parameters, including solid: liquid ratio, pH, ultrasonic frequency, power, temperature and time for protein extraction was systematically examined. The outcomes demonstrated that the ultrasound assisted extraction not only reduced particle size but also improve protein yield by 19% compared to conventional extraction methods. Optimum extraction parameters were determined in a fat removed groundnut solid: liquid ratio of 1:10 (w/v), an ultrasonic power of 40 W/g, pH of 7, temperature of 40°C, and an ultra-sonication time of 20 min. Under these conditions, the maximum protein yield reached 87.7 ± 0.7%. These conclude that the ultrasound-assisted extraction is a hopeful green technique to improve groundnut oilseed cake protein yield, offering potential advantages for the plant-based protein.

Karki *et al.* (2010) explored pre-treatment method (high-power ultrasound) enhance both protein and sugar extraction from fat removed soybean flakes. The flakes were dispersed in water and subjected to sonication at 15, 30, 60, and 120 seconds using a portable ultrasound unit. Ultrasonic amplitudes of 21 to 84 µm were applied, corresponding to power densities of 2.56 W/ml. Scanning electron microscopy revealed significant structural disruption in the sonicated soy flakes, with particle size reductions of closely 10-fold at higher amplitudes. The most pronounced effects were observed at the highest amplitude with a 120 second sonication, where total sugar release increased by 50% and protein yield by 46% compared to non-sonicated controls. Additionally, experiments were conducted with and without cooling to assess the impact of heat generated during sonication. The results indicated that temperature variations had no substantial influence on carbohydrate and protein recovery. Overall, ultrasonic pre-treatment proved to be an effective method for improving soy protein extraction efficiency, potentially reducing production costs and enhancing the economic viability of soy-based protein processing.

2.1.3 Reverse micelles extraction (RME)

Reverse micelle (RM) extraction is an advanced technique for isolating proteins with high purity and efficiency. This method employs nanometer-sized (1-10 nm) surfactants to create micelles in organic solvents, allowing selective solubilization and recovery of target proteins from plant-based sources (Rashman *et al.,* 2025). RM extraction offers advantages such as mild processing conditions, reduced solvent usage, and high extraction yields while preserving protein functionality. The process involves the formation of reverse micelles, where proteins are transported across the micelle interface due to electrostatic interactions, leading to effective separation. According to Sun *et al.* (2019), RME minimizes thermal degradation and denaturation, making it ideal for heat-sensitive bioactive compounds. Additionally, its application in food processing ensures enhanced protein purity, improved solubility, and better emulsification properties, making extracted proteins suitable for functional foods and nutraceuticals. The optimization of parameters like surfactant type, solvent selection, and pH conditions helps to enhance scalability and economic feasibility.

Protein extraction using the reverse micelles (RMs) technique typically involves two main steps: forward and backward extraction (Sun *et al.,* 2008). In forward extraction, solubilisation of protein into the aqueous cores of reverse micelle. In the next process backward extraction step facilitates the protein yield of from the micelles (Leser *et al.,* 1993). The key parameters influencing forward extraction include W€ (water-to-surfactant ratio), pH, ionic strength, extraction duration, temperature, and the ratio of raw material to RM solution (w/v) (Sun *et al.,* 2008). Meanwhile, the optimal parameters affecting backward extraction involve the solvent ratio, centrifuge time, temperature, pH and ionic strength of the aqueous buffer used to retrieve proteins from the micelles (Zhao *et al.,* 2011).

Zhao *et al.* (2018) conducted the comparative analysis to evaluate the proximate composition, functional and sensory properties of proteins isolates of soybean using reverse micelles (RMs) versus those obtained *via* alkaline extraction and alkaline isoelectric precipitation (AEIP). The findings demonstrated that soybean proteins isolated through RMs exhibited superior techno-functional properties, including a higher protein solubility index (96.9%), oil absorption stability (2.57 g/g), foaming capacity (131.65%), foaming stability (84.33%), emulsifying capacity (81.71%), and emulsifying stability (82.26%) compared to their AEIP. However, the water-

holding capacity of RMs-extracted proteins was reduced by 8.82% relative to AEIP-extracted proteins. A comparative assessment of amino acid composition revealed that while certain individual amino acid levels were similar across both extraction methods, proteins derived from RMs exhibited higher total amino acid content (82.50%), essential amino acid (EAA) content (27.91%), amino acid score (AAS) (115), and biological value (BV) (92.67) in contrast to AEIP-derived proteins. Overall, these findings highlight that reverse micelle extraction is a favorable technique for increasing the proximate composition, functional property and sensory properties of soybean proteins, creating it a viable substitute to conventional extraction techniques.

Zhang *et al*. (2017) aimed optimization of protein extraction from grape seeds *via* the reverse micelles technique. Employing response surface methodology (RSM), they systematically evaluated factors influencing the extraction process. Initially, steepest ascent methods and plackett-burman design (PBD) identify key variables: pH, acetyl tri-methyl ammonium bromide (CTAB) concentration, sodium chloride concentration, and protein content. Subsequent optimization using the box-behnken design (BBD) determined the optimal conditions: CTAB concentration of 38 mmol/l, pH 6, NaCl concentration of 0.01 mol/l, and protein content of 2.1 mg/ml. Under these parameters, the forward extraction protein yield achieved was 83%. This research highlights the efficacy of reverse micelles extraction as a potent method for protein recovery from grape seeds.

A study by Zhao *et al.* (2019) investigated how different extraction methods, reverse micelles (RMs) and alkaline alkaline isoelectric precipitation (AEIP) affect the structural properties of walnut protein compounds, including globulin, prolamin, glutelin and albumin sulfhydryl (SH) and disulfide (DS) Bond Contents. Compared to AEIP, RMs extraction resulted in significantly higher SH contents in glutelin, prolamin and globulin fractions ($p<0.05$), while the albumin fraction exhibited a decrease. DS bond contents were similar between methods for albumin, globulin, and glutelin, except for an increase of 2.57% in prolamin extracted *via* RM. Fourier transform infrared (FTIR) spectroscopy revealed that RMs extraction led to increased structure of α-helix in globulin, β-sheet in prolamin, and unordered structures in globulin, prolamin, and glutelin. Additionally, turn structures were more prevalent in glutelin, albumin and prolamin protein fractions obtained through RM compared to AEIP. Scanning electron microscopy (SEM) indicated that RM extraction influence four protein fraction structure surface, suggesting alterations at the microscopic level. These findings suggest that the selection of protein recovery technique significantly impacts structural characteristics of the walnut protein fractions, which may, in turn, affect their functional properties in food applications.

2.1.4 Subcritical/supercritical fluid extraction

Subcritical fluid extraction involves use of water or other solvents (ethanol) at temperatures and pressures below their critical points but above their boiling points under atmospheric conditions. For water, this means temperatures between 101°C to 375°C and pressures to maintain the liquid state (typically 10-100 bars). In this subcritical state, water exhibits enhanced solvent properties, such as reduced polarity and increased diffusivity, making it an effective medium for extracting polar and semi-polar compounds, including proteins. In subcritical water extraction (SWE), plant material (seeds, leaves, or legumes) is mixed with water and subjected to controlled heating and pressurization. The altered properties of subcritical water allow it to penetrate plant cell walls, solubilize proteins, and release them into the liquid phase. The process can be tuned by adjusting temperature, pressure, and extraction time to optimize protein yield and minimize degradation. Lower temperatures (120-180°C) are often used to preserve protein structure, as excessively high temperatures can lead to denaturation or hydrolysis into smaller peptides (Smith *et al.,* 2022).

Supercritical fluid extraction (SFE) uses carbon dioxide (CO_2), above its critical temperature (31°C) and pressure (74 bar). In this supercritical stage, CO_2 exhibits liquid and gas at high density like a liquid for solvating power and low viscosity like a gas for rapid diffusion. This makes it an excellent solvent for separating proteins, especially when modified with co-solvents. In SFE, supercritical CO_2 is pumped through a vessel containing ground plant material. While CO_2 alone is better suited for non-polar compounds (lipids), adding a polar co-solvent like ethanol or water (typically 5-20% by volume) enhances its ability to extract polar proteins. The fluid dissolves the target compounds, and upon depressurization, the CO_2 evaporates, leaving behind a concentrated protein extract. The process is often conducted at moderate temperatures (40-60°C), which helps preserve protein integrity (Garcia *et al.,* 2023).

Park *et al.* (2019) investigate the physicochemical properties and phytocompounds derived from laver (*Pyropia yezoensis*) protein hydrolysates produced through subcritical water hydrolysis (SWH). The process was conducted at 120-230°C temperature under pressure 30 bars, with boiling water and ethanol extractions performed for comparison. The protein hydrolysates obtained at 180-210°C exhibited considerably higher bioactive compounds, such as total phenolic (14.230 mg gallic acid/g), proteins (20.12 g/100 g), and reducing sugars (158.38 mg glucose/g). These hydrolysates also demonstrated superior radical-scavenging activities, with DPPH assays showing maximum values of 16.63 mg trolox/g, outperforming hot water and ethanol extracts. The findings highlight SWH as an efficient, eco-friendly method for enhancing the extraction of phytocompounds from *P. yezoensis,* suggesting its potential for developing functional food ingredients with antioxidant properties.

The study by Zhu *et al.* (2010) explores amino acids composition from bean dregs through hydrolysis in subcritical water, an environmentally friendly technique leveraging water's unique solvent properties under elevated temperature and pressure conditions. The experiments were conducted 180°C to 280°C temperature and pressures between 2.0 and 10.0 MPa, with reaction times varying from 10 to 60 min. The results revealed that optimal hydrolysis occurred at 240°C and 6.0 MPa for 30 min, yielding a maximum amino acid content of 15.6 g/100 g dry bean dregs, with key amino acids such as aspartic acid, glutamic acid and leucine identified *via* high-performance liquid chromatography (HPLC). The study found that greater temperatures and longer reaction times increased amino acid yield up to a point, beyond which degradation into smaller peptides or organic acids occurred. Compared to traditional acid or enzymatic hydrolysis, subcritical water hydrolysis offered advantages in efficiency and reduced chemical waste, demonstrating its potential as a sustainable method for valorizing bean dregs, a byproduct of soybean processing, into valuable amino acid-rich food products.

Jokic *et al.* (2011) investigate the efficacy of supercritical fluid extraction (SFE) using carbon dioxide (CO_2) with ethanol as a co-solvent to extract proteins from soybean meal, a widely available plant-based protein source. The extraction was performed at varying pressures (200-400 bar), temperatures (40-60°C), and co-solvent concentrations (5-15% ethanol) to optimize protein yield and functionality. Results showed that the highest protein yield of 18.5 g/100 g soybean meal was achieved at 300 bar, 50°C, and 10% ethanol, with a notable retention of protein solubility (85%) and emulsifying capacity compared to conventional solvent extraction. The addition of ethanol enhanced the solubility of polar protein fractions, overcoming the limitations of pure supercritical CO_2, which is less effective for polar compounds. The extracted proteins were analyzed using SDS-PAGE and FTIR, confirming minimal denaturation and preservation of native structures. Compared to hexane-based methods, SFE reduced residual solvent content and environmental impact, positioning it as a sustainable alternative for producing high-quality soy protein isolates for food applications. These findings underscore the potential of SFE as a green technology for valorizing soybean meal into functional protein ingredients.

2.1.5 High pressure assisted extraction (HPAE)

High-pressure assisted extraction (HPAE) is an advanced, eco-friendly technique that utilizes elevated pressures (typically 100-600 MPa) increase the extraction of proteins. HPAE leverages high hydrostatic pressure to disrupt plant cell walls components, facilitates the release of proteins compounds without relying heavily on thermal energy or large volumes of organic solvents. This non-thermal approach improves extraction efficiency, increases protein yield, and preserves physicochemical and functional properties of the recovered proteins. HPAE can be applied alone or in combination with enzymatic hydrolysis to optimize outcomes, depending on the plant matrix and desired protein characteristics. Commonly used in the food industry for processing legumes, oilseeds, and by-products like soybean meal or pea flour, HPAE aligns with the growing demand for green technologies that minimize environmental impact while meeting the nutritional needs of a rising global population (Rashwan *et al.,* 2025).

Al-Ruwaih *et al.* (2019) investigate the use of high-pressure assisted enzymatic proteolysis (HPAE) to enhance protein extraction and hydrolysis from kidney bean isolates, analysing their physical, chemical, rheological, functional and antioxidant properties. Applying pressures of 200, 400, and 600 MPa for 15 min alongside enzymatic hydrolysis alcalases significantly improved hydrolysis degree, reaching 28.6% at 600 MPa compared to 15.2% at ambient pressure, due to pressure-induced protein unfolding and increased enzyme accessibility. Functionally, the hydrolysates showed higher solubility (92% at 600 MPa vs. 78% at 0.1 MPa) and emulsifying activity (EAI increased from 45 to 62 m^2/g), attributed to exposed hydrophilic and hydrophobic regions. Structural analysis *via* FTIR revealed shifts in secondary structures, with reduced β-sheets and increased random coils, enhancing digestibility. Rheological properties improved, with viscosity dropping from 0.12 to 0.07 Pa at 600 MPa, making the hydrolysates suitable for liquid foods. Antioxidant activity also rose, with DPPH radical scavenging reaching 68% at 600 MPa a 40% increase over the control due to bioactive peptide release. The study demonstrates that HPAE at 600 MPa optimizes protein hydrolysis, yielding superior functional and bioactive properties for nutraceutical and food applications.

2.1.6 Deep eutectic solvents (DES) extraction

Deep eutectic solvents (DES) are eco-friendly solvents formed by a hydrogen bond acceptor (HBA) such as choline chloride and hydrogen bond donor (HBD) such as glycerol, urea. These solvents exhibit low volatility, high biodegradability, and tuneable physicochemical characteristics, making them ideal for green chemistry applications. DES has gained significant attention in protein extraction from plant-based foods efficiently degrade cell wall compounds and solubilize proteins while maintaining structural integrity and functionality. Unlike conventional solvents, DES can be tailored for specific applications by varying its components, allowing for selective extraction with minimal environmental impact. Their low toxicity and potential for recycling further enhance their appeal as sustainable alternatives in food processing and biotechnology. Research continues to explore novel DES formulations to optimize extraction efficiency, reduce costs, and facilitate industrial adoption (Rashwan *et al.,* 2025).

Zhang *et al.* (2023) investigated the efficiency of choline chloride (ChCl) based deep eutectic solvents (DES) for protein extraction from soybean meal, comparing different hydrogen bond donors (HBDs), glycerol, urea, and lactic acid to optimize yield and functionality. Results demonstrated that lactic acid-based DES (ChCl: LA) achieved the highest protein extraction yield (85.2%) due to its effective disruption of cell wall structures and enhanced protein solubility. The extracted proteins retained high purity and exhibited superior functional properties, including improved emulsifying activity, solubility, and thermal stability, compared to conventional alkaline extraction methods. Sodium dodecyl sulphate polyacrylamide gel electrophoresis and Fourier transform infrared spectroscopy used to confirm minimal protein denaturation, preserving protein structural integrity. Additionally, DES extraction reduced anti-nutritional factors such as phytic acid, enhancing nutritional quality. Process optimization revealed that a 1:2 ChCl: LA molar ratio, 30% water content, and 50°C extraction temperature maximized efficiency. These findings highlight DES as a sustainable, high-performance alternative for plant-based protein recovery, offering both environmental and functional advantages over traditional extraction techniques.

2.1.7 Pulse electric field (PEF) technology

Pulsed electric field (PEF) emerging non-thermal, energy-efficient technology to enhance protein extraction from plant-based food matrices. By applying short, high-voltage electric pulses (typically 1-20 kV/cm), PEF induces electroporation, reversibly or irreversibly permeabilize cell wall compounds and facilitate proteins release as illustrated in Figure 2. Studies demonstrate that PEF pre-treatment significantly improves protein extraction yields from sources such as soybean, pea and algae while preserving protein functionality, including solubility, emulsification and thermal stability. PEF reduces processing time, energy consumption, and solvent use. Optimization parameters, *viz*., electric field strength, pulse duration, and specific energy input, play critical roles in extraction efficiency (Gomez *et al.,* 2022). Recent advancements highlight PEF's compatibility with downstream processes, such as enzymatic hydrolysis or membrane filtration, to further refine protein isolates. With its scalability and minimal thermal degradation effects, PEF presents a promising sustainable alternative for the food industry for plant-based proteins.

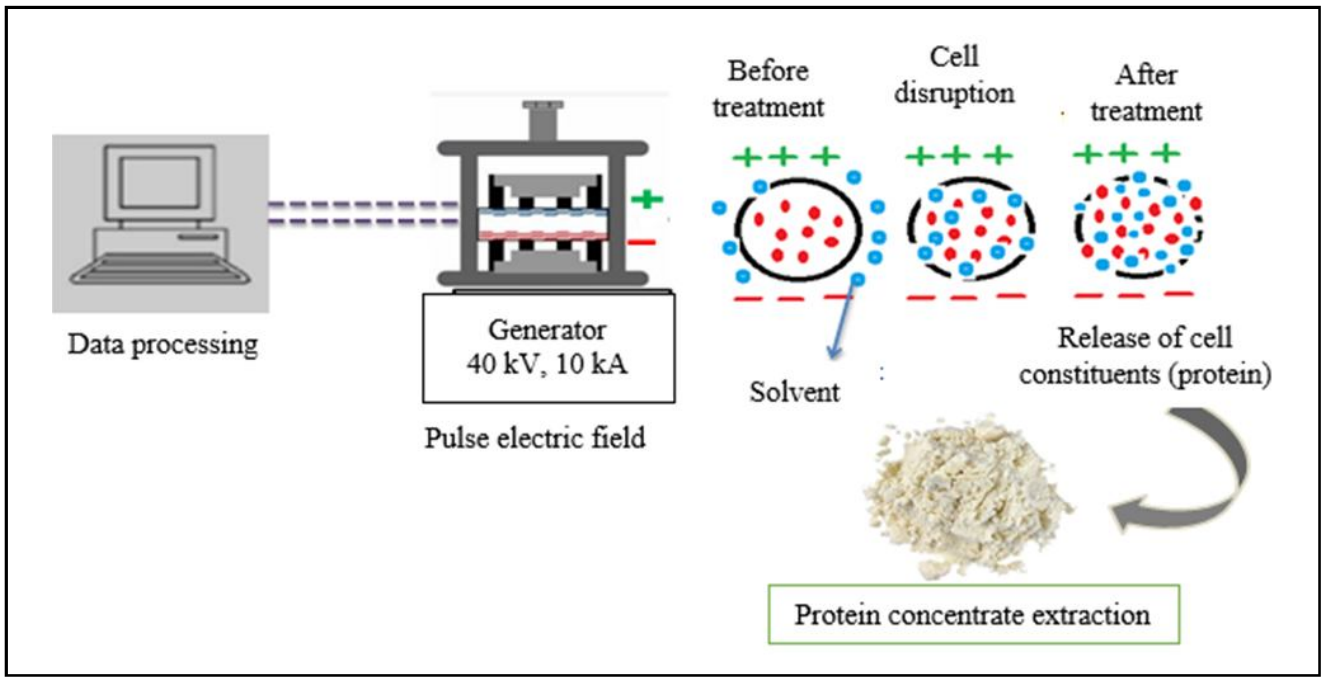

Figure 2: Pulse electric field (PEF) protein extraction method.

Smith *et al.* (2023) investigate the pulse electric field (PEF) technology optimization for protein extraction from soybean (*Glycine max*) and pea (*Pisum sativum*) flours, focusing on yield, functionality, and process sustainability. A systematic evaluation of parameters such as electricity 15 kV/cm; pulse number: 50-200; specific energy input: 10-50 kJ/kg) was conducted to determine optimal extraction conditions. Results demonstrated that PEF pre-treatment at 10 kV/cm with 100 pulses and 30 kJ/kg specific energy increased protein extraction yields by 32.5% and 28.7% for soybean and pea proteins respectively Spectroscopic (FTIR) and electrophoresis (SDS-PAGE) analyses confirmed the preservation of native protein structures, while functional characterization revealed enhanced solubility (up to 85%), emulsifying activity (45% improvement), and thermal stability. 40% reduction in energy consumption while using pulse electric field when compared to alkaline extraction methods.

Zhang *et al.* (2024) introduce an innovative synergistic approach combining deep eutectic solvents (DES) and pulsed electric field (PEF) technology for efficient protein extraction from plant matrices. Choline chloride-glycerol DES (1:2 molar ratio) coupled with PEF pre-treatment (10 kV/cm, 100 pulses), we achieved a remarkable 92.3% protein extraction yield from defatted sunflower meal, representing a 45% increase over conventional alkaline extraction. The hybrid DES-PEF method demonstrated unique advantages are PEF-induced electroporation enhanced solvent penetration and protein release, while DES effectively solubilized proteins with minimal denaturation. Characterization of the extracted proteins revealed superior functionality, including 89% solubility (pH 7.0), 38% improved emulsifying activity index, and preserved secondary structures as confirmed by circular dichroism spectroscopy. Process optimization through response surface methodology identified optimal conditions (DES hydration 30%, PEF energy input 25 kJ/kg, extraction time 30 min) that maximized yield while minimizing energy consumption. The environmental impact assessment showed a 60% reduction in chemical waste generation compared to traditional methods. This DES-PEF synergistic approach presents a breakthrough in sustainable protein extraction, offering high efficiency, enhanced protein functionality, and reduced environmental footprint for the plant-based food industry.

3. Conclusion

Plant protein technology plays a crucial role in addressing the growing demand for sustainability of protein. While plant-based proteins (PBPs) are abundant and affordable than animal proteins, their direct consumption remains limited due to their primary use as animal feed. The physicochemical, functional, sensory, rheological properties of PBPs are influenced by environmental factors like pH and ionic strength. Advanced extraction methods includes enzyme-assisted, ultrasound-assisted, microwave, pulsed electric field (PEF), and high-pressure technique can achieve up to 86-95% protein yield with enhanced functionality. These innovative approaches not only improve extraction efficiency but also enable the development of non-dairy alternatives like plant-based yogurts and cheeses, as well as 3D-printed foods, meat analogs, and bio-based materials like bioplastics and nanoparticle synthesis. Additionally, PBPs exhibit valuable bioactivities, including antioxidant, antimicrobial, and antidiabetic properties, further expanding their applications in food and nutraceutical industries. The valorization of plant-based food waste through sustainable protein extraction offers solutions to food insecurity, malnutrition, and environmental challenges. While traditional methods such as alkaline extraction and isoelectric precipitation remain widely used, emerging techniques like reverse micelle extraction, supercritical fluid extraction, and PEF technology provide eco-friendly, high-purity, and energy-efficient alternatives. The successful integration of these technologies into industrial processes can reduce food waste, lower production costs, and enhance protein functionality for various applications. However, further research is needed to optimize extraction parameters, scale

up production, and assess the environmental and economic impact of these methods. Collaboration among academia, industry, and policymakers is essential to advance sustainable food systems and aims to achieve worldwide protein sustainability and acceptability for all age groups based on their protein requirements.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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